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PREFACE

This book contains papers presented for the International Conference on Bio & Food Electrotechnologies (BFE2009), which is organized in 22-23 October 2009 with sponsorship from the International Society of Food Engineering, the French Society “Génie de Procédés Industriels”, the Regional Council of Picardy, the “Industries and Agro-Resources” Competitiveness Cluster and the University of Technology of Compiègne (UTC).

Recently, the electrotechnologies, based on effects of pulsed electric fields (PEF), ohmic heating (OH), DC electric field, and other methods, gained the real interest in relation to the processing of foods. These techniques allow inactivating the microorganisms, enhancing extraction from food plants and dehydration of biosolids. The PEF and pulsed OH preserve nutritional, functional, structural and sensory properties of liquid and solid foods better than conventional technologies.

The goal of this meeting is to bring together the scientific and industrial community interested in the recent advances and innovative ideas in the field of Bio and Food Electrotechnologies.

The primary topics of the conference are:

- Fundamental Principles, Electrophysical Properties of Bio & Food Materials (including basic mechanisms and effects of electric fields in biological tissues, electrophysical properties of biomaterials and modeling).
- Electric Field Treatments in Bio & Food Processing, Production of Safe and High Quality Products (pulsed electric fields, ohmic heating, moderate electric field processing, arc-discharge, electrosorption, electro-dehydration, other electrotechnologies, and combined treatments).
- Applications of Electrotechnologies (including testing and validation, development of electric field generators and treatment chambers, pilot and industrial scale realizations).

The program of BFE2009 includes more than 50 contributions, presented in this book and covers a wide range of topics from the most recent and still experimental processes and methods.

I am sincerely grateful to all the authors for submitting their papers in time and to our sponsors for their financial and technical support. A warm word of thanks to Professors Gustavo Barbosa-Canovas, Nikolai Lebovka and Sudhir Sastry co-chairs of the Scientific Committee, for their kind advises and collaboration.

My thanks to all the colleagues who have collaborated in the conference organization, and particularly to Dr. Jean-Louis Lanoiselle, Dr Elisabeth Van Heck and Prof. Nikolai Lebovka for their help in the edition of this book.

I hope that the efforts of the organizing committee and all the registrants and speakers, have produced a memorable conference.

E. Vorobiev

Compiègne, October 2009
Abstract:

The method known as electroporation, which consists of application of short electric pulses (micro to millisecond duration) with high amplitudes that cause cell membrane permeabilization, is widely used in medicine and biotechnology and it is gaining importance in food treatment. For its successful use proper selection of electric pulse parameters such as the amplitude of electric pulse, its duration, the number of applied pulses and their repetition frequency is crucial. Those parameters should be chosen according to the desired application and need to be optimized for different cell types. When the desired outcome of the treatment is introduction of small or large molecules or cell electrofusion, good cell viability after the treatment is important. On the contrary, when the main goal is efficient cell killing electric pulse parameters should cause irreversible cell damage. The effectiveness of electric pulse parameters is dependent also on characteristic of the electroporation medium and varies significantly according to the treated sample. The geometry of electrodes used is important as it affects electric field distribution in the treated sample and thereby the outcome of the treatment. Very complex situation is found in inhomogeneous biological tissues composed of different cell types with different characteristics and for such situation the theoretical modelling of electric field distribution is essential.

Electroporation is a universal method applicable to different cell types and used for different purposes such as electrochemotherapy for cancer treatment and emerging electro gene-therapy in medical practice, water and liquid food sterilization in food treatment, gene transfection or cell fusion of bacteria, yeast, and plant protoplasts or intact plant tissue in biotechnology. Lately shorter electric pulses of nanosecond duration and higher amplitudes were found to cause permeabilization of sub cellular structures. They are used to improve gene transfection in non-dividing cells. Understanding of the phenomena, its basic mechanisms and optimization of all the parameters that affect electroporation is a prerequisite for successful applications.
BIOIMPEDANCE IN FOOD PROCESSING

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Abstract:
Impedance measurement is a simple and inexpensive method to characterize material. Various food, like meat or vegetables, is composed of cells. They are surrounded by an insulating membrane while the cytosol and the extra-cellular fluids are electrolytes. Thus, the membrane behaves electrically like a capacitor while the electrolytes show resistive behaviour. Cells, especially their membranes, influence the impedance in a frequency range up to several MHz. At higher frequencies mostly the cytosolic content, i.e. macromolecules give rise to characteristic relaxation processes. The content of water can be assessed using microwave spectroscopy. The quality of food is often reflected in water loss or permeability changes of membranes. Using the impedance together with suitable models, quality assessment of food like for instance meat or fruits, becomes practicable and inexpensive. Moreover, the characteristic drop in impedance of incubation media is a measure for microbial contamination. The usage of specific culture media allows a high sensitivity and selectivity in microbial detection.

Key words: bioimpedance, food quality, drip loss, microbial detection, water content

1 Introduction

Bioimpedance denotes the passive electrical properties of biological material. Other methods, like conductometry or dielectric spectroscopy are closely connected to impedance spectroscopy. The difference is often only the use of different frequency ranges, equipment or ways of presenting the results. This paper should give a brief introduction to impedance measurements and the usage in food processing. For further reading [1] is recommended.

1.1. Passive electrical properties – the basics

The application of a voltage, $U$, between electrodes results in a current, $I$, depending on the conductive behaviour of the material in between, $I = GU$, where $G$ is the conductance. The reciprocal of the conductance is the resistance $R$: $R = 1/G = U/I$. If electrical measurements for characterization of materials are used, the results should be comparable. Both the resistance and the conductance depend on the geometry of the electrode system. This means, even if the same sample is measured with different electrodes, a different conductance or resistance is obtained. The geometry independent intrinsic behaviour of any matter is either the conductivity, $\kappa$, or the resistivity, $\rho$. For probe calibration a cell constant, $k$, is introduced. $k = l/A$, where $l$ is the distance between the electrodes and $A$ their surface area. The conductivity and the resistivity can be obtained by $\kappa = Gk$ and $\rho = R/k$ resp. $k$ is easily calculated for a simple geometry like for plate capacitors. In most practical applications, $k$ is obtained by calibration with a test medium of known conductivity. Therefore, the measurement chamber (or electrode system) is filled with the calibration medium (i.e. 0.01 M KCL, $\kappa = 0.14$ S/cm at 25 $^\circ$C ) and $k$ is calculated using the measured conductance. Despite $k$ being often regarded as constant, it depends on frequency due to the existence of parasitic capacitances.

If the material has capacitive behaviour, i.e. it is able to store electrical energy, its impedance depends on frequency. If a voltage, $U_0$, is applied to a serial combination of a resistor and an uncharged capacitor, the initial current is $I = U_0/R$ because all of the
transported charges are accumulated at the capacitor. Once the capacitor is fully charged \((U_{\text{capacitor}} = U_0)\), no further current flows, so it behaves like an insulator. The energy, \(W\), stored in a charged capacitor is \(W = 0.5CU_{\text{capacitor}}\), where \(C\) is the capacitance.

A polarity change causes the discharge and recharge resulting in a flow again. The marked difference to resistors is, that the electrical energy is not dissipated but stored in the capacitor. The total current increases with the speed of polarity change. Because this current flow results from the displacement of charge carriers and no real transport from one side to the other of the capacitor happens, it is called imaginary current. Given the current due to varying voltage across the capacitor, an imaginary resistance, \(X_c\), can be defined. In the special case of sinusoidal voltage, this capacitive resistance is \(X_c = \frac{1}{j\omega C}\), where \(\omega\) is the angular frequency, \(\omega = 2\pi f\). \(f\) is the frequency and \(j\) is the imaginary unit \(j = \sqrt{-1}\). The impedance for a serial combination of \(R\) and \(C\) is \(Z = R + \frac{1}{j\omega C}\). If the imaginary part is extended with \(j\), one obtains \(Z = R - \frac{1}{j\omega C} = R + X_c\). Often, the symbol for the magnitude of the imaginary part is \(X\), so that the impedance is \(Z = R + jX\). Another way to write the impedance is 
\[
Z = \text{Re}(Z) + j\text{Im}(Z) = Z' + jZ''.
\]

\(\text{Re}()\) and \(\text{Im}()\) denotes the real and the imaginary part of the impedance. The negative imaginary part of the impedance for capacitive objects means a negative phase shift, i.e. the voltage is delayed with respect to the current, which is due to the electrical energy stored in the capacitor. The opposite case, the inductive behaviour, is not important for biological objects and therefore beyond the scope of this paper.

The reciprocal of the impedance is the admittance \(Y\): \(Y = \frac{1}{Z} = G + jB\). It should be noted, that the conversion between impedance and admittance is simple at one frequency. Otherwise, \(R\) is not simply the reciprocal of \(G\) (rep. \(X\) and \(B\)). While \(Z = R + jX\) describes a serial combination of \(R\) and \(C\), the simplest model for \(Y = G + jB\) is a parallel combination.

For unstructured media or at very high frequency, the dielectric behaviour, i.e. the complex permittivity \(\varepsilon^*\), is simpler to interpret.

\[
\varepsilon^* = \frac{Yk}{j\omega\varepsilon_0} = \varepsilon' + j\varepsilon''
\]

\(\varepsilon_0\) is the permittivity of vacuum (\(\varepsilon_0 = 8.85 \times 10^{-12}\) As/Vm). The geometry factor \(k\) is required, because the permittivity is independent of the probe geometry.

Each complex number has a magnitude and a phase angle. The magnitude of the impedance is \(|Z| = \sqrt{R^2 + X^2}\) while the phase angle is \(\varphi = \arctan(X/R)\).

1.2. Presentation of passive electrical properties

The most popular form of presentation is the magnitude and phase angle or real and imaginary part \((Z, Y, \varepsilon)\) versus frequency (Fig.1 a,b). Fig 1 shows the impedance, \(Z\), of a serial combination of a 100 \(\Omega\) resistor and a 10 nF capacitor in parallel with a 1 k\(\Omega\) resistor. If the y-axis is in logarithmic scale as well, e.g. the attenuation vs. frequency, it is called Bode-plot.

An often used representation is the locus diagram (Wessel diagram) where the imaginary part is drawn versus the real part (Fig.1c). For materials with low conductivity or measurements in the GHz-range, the presentation in the permittivity plane becomes advantageous because of the high resolution at higher frequency. The locus diagram in the \(\varepsilon\)-plane is called Cole-plot and in case of materials like biological tissues, where the Cole-Cole model can be applied also Cole-Cole-plot [1].
Often, for simpler interpretation of the results, an equivalent circuit as model for the material under test (MUT) is used. The elements of this circuit can be obtained by fitting the spectrum to this model.

### 1.3. Methods for impedance measurement

The basic measurement method for passive electrical behaviour is the application of a current and the measurement of the voltage dropping across the object or vice versa, voltage applied and current measured. If the impedance spectrum is needed, a frequency sweep is performed with phase sensitive current and voltage measurement at each discrete frequency. Since the frequency is the parameter, this method works in frequency domain. The advantage is the opportunity to use selective amplifiers which greatly reduces the noise. The disadvantage is the long measurement time since at least ten periods for each frequency should pass until the system becomes stable. A much faster approach is the application of a broad bandwidth signal and measuring the answer as function of time (time domain). Owing to the wide bandwidth of the amplifiers, a high noise is present in the results which can be reduced by averaging. Finally, the spectrum is obtained by transformation of voltage and current into the frequency domain where the passive electrical behaviour can be calculated. Alternatively, the elements of an equivalent circuit or any other physical or mathematical model can be calculated directly in time domain.

One of the most demanding problems in assessment of electrical properties of biological material is electrode polarization. Especially at low frequency electrified electrodes build up a counter ion cloud where the mobility of charge carriers is low. This means a high impedance of the electrode which can easily exceed the impedance of the material between the electrodes. The use of non polarisable electrodes like silver-silver chloride (Ag/AgCl) or calomel electrodes is possible in some cases, like in medical applications but not practicable in food industry. Here the use of large electrodes can reduce the current density at the electrodes, thereby reducing the electrode polarization. An elegant way to reduce the influence of electrode polarization is the use of different electrodes for current application and voltage monitoring (Fig. 2).

Fig. 2. (a) Two and (b) four electrode configuration
It is impossible to measure the voltage drop across the MUT using the two electrode configuration because the voltage monitor always measures the sum of the voltage dropping at the object and the electrode. Using the four electrode interface, the voltage is monitored independently of the impedance of the current electrodes. If the same material and geometry is used for both voltage electrodes, any potential difference between the electrolyte and the electrode will be compensated if the input impedance of the voltage monitor is high. In practice, voltage monitors with input impedance on the order of $10^6 \Omega$ are used. In order to ensure optimal measurement condition, special front ends adapted to the MUT are required.

The passive electrical properties of a material can be assessed in transmission or reflection mode. While the transmission measurement uses the attenuation of the signal transmitted through the object (Fig.3), in reflection mode the energy reflected from the interface electrode-object is monitored.

![Diagram](image)

**Fig.3** Transmission and reflection at the material under test (MUT)

While sophisticated electrode systems (i.e. four electrode interface) with well adapted front ends are mostly used for impedance measurements in liquids or slurries, simple and rigid electrode systems (i.e. two electrode systems) are used for meat or vegetables. Reflection measurements are preferentially used at high frequency. Other than electrode systems for low frequency applications, reflection systems in the microwave region are favoured with antennas, thereby allowing a contactless measurement.

2. **Electrical impedance of biological matter**

For modelling the passive electrical behaviour of biological matter simple cells with more or less insulating membranes and ionic content within the cells but also in the extracellular medium are assumed. Due to a charge density at the membrane surface, cells are surrounds by a counter ion layer.

The permittivity of biological matter shows several distinct frequency dependencies [2]. At low frequency (mHz – 100 Hz), the lateral movement of ions along insulating membranes causes the $\alpha$-dispersion. The polarization of cell membranes yields the $\beta$-dispersion between 1 kHz and 10 MHz depending on the size of the cells and the conductivity of the surrounding electrolytes. In a frequency range up to GHz, mostly macromolecules give rise to the $\gamma$-dispersion. The $\delta$-dispersion is caused by water and ranges from 2 GHz up to 25 GHz.

With respect to impedance, food should be divided into structured and unstructured matter. Structured matter consists of living or dead cells with insulating membranes like for instance vegetable, meat, fish or living cultures. Juice, oil, bakery products or homogenized food does not exhibit a structure with insulating cell membranes. While structured food shows $\alpha$- and $\beta$-dispersion, these dispersions are absent in unstructured food the conductivity is almost constant up to several MHz.

2.1. **Membrane integrity**

Agricultural products consisting of cells with insulating cell membranes, like for instance fresh meat, show a characteristic impedance spectrum. An insulating membrane, facing good conducting electrolytes behaves electrically like a capacitor. At dc essentially all the current flows around the cells through the extra-cellular electrolytes while at higher frequency the membranes are electrically shorted and the current access the cell interior as well.

The use of the entire spectrum is often too complicated, thus a simpler parameter would be favourable. This can be simply the ratio of the resistance at high and low frequencies or a
parameter like $P_y$ \[3\]. $P_y = (R_0 - R_\infty)/R_0$ (Fig.4). It is the circumference of the $\beta$-dispersion normalized to the low frequency resistance. It is zero, if no cells are present and approaches one for 100 % densely packed cells with no extracellular space. Thus, the $P_y$ is a function of the volume fraction surrounded by insulating cell membranes. For practical applications it is scaled, for instance between 0 and 100.

\[\text{Fig.4 Schematic for structured food (with cells) between electrodes. Two pathways can be distinguished: the extracellular route ($R_e$) and the intracellular route ($R_i, C_m$) crossing the cell membrane. The lumped circuit is typical for modelling biological matter within the $\beta$-dispersion. The Wessels plot in the $\beta$-dispersion is theoretically a half circle but in real tissues it is depressed with respect to the real axis (dashed line). $R_0$ is the resistance below the $\beta$-dispersion and $R_\infty$ is above. $\omega_\beta$ is the characteristic frequency of the $\beta$-dispersion [1].}\]

If meat has a low drip loss, i.e., the juice spilling out within a defined time (24 h) is negligible, $P_y$ will be high. However, in meat tending to PSE (pale soft exudative) quality, the $P_y$ is much lower.

\[\text{Fig.5. $P_y$ of } M.\text{long.dors. (pig), stored at 4 °C for two different drip loss (data by L.Schöberlein, former Schlachtkombinat Leipzig, Germany)}\]

As seen in Fig.5, $P_y$ exhibits strong time dependence due to curing processes where the cell membranes become permeable. Therefore, the quality assessment of meat using the impedance is only reliable, if the time elapsed after stunning is known.

Similar to the quality assessment of meat, also other agricultural products like carrots or potatoes are characterized by their electrical properties.

2.2 Non structured product and measurement of water content

The $\beta$-dispersion is completely absent in unstructured media like juice, bread or slurries. Besides this, cell suspensions with a very low cell fraction ($p<10^5$ cells/ml) will not show a measurable $\beta$-dispersion. Since the impedance in a range between 1 kHz and 1 MHz is almost constant, only a single number rather than the spectrum makes sense. For detection of microbial contamination, a sample is incubated in culture media and the low frequency conductivity measured over time (Fig.6a). Despite the cells are not visible, the change of the ionic content of the culture media due to the metabolism of the cells is a measure for the number of cells.
Fig. 6 (a) conductivity changes at dc after incubation. (o – Salmonella Typhimurium in peptone water at 37°C, + and * - same medium but incubated with ham and ground pork, data by A.Mülverstedt, fzmb Bad Langensalza, Germany) (b) Dependence of the reflection factor at 2.54 GHz on the dry matter (milk powder, data by X.Nakos, iba Heiligenstadt, Germany).

The water content is a quality measure for bakery products or dried food. Using the absorbance of water in the microwave region, the contactless assessment of water content even for granular matter or slurries is possible (Fig.6b).

Moreover, the freshness of agricultural products influences the impedance in the microwave region. This has been used for assessment of the freshness of fish [4] or eggs.

3. Conclusion

Bioimpedance measurement is a simple and non-destructive way for the characterization of agricultural product for quality assessment but also for process control. The frequency range and measurements methods should be optimized for the application. Sophisticated data processing is used for retrieving parameters which directly correlate with quality or process parameters and makes impedance measurements a useful tool without need of highly trained technicians.

4. References

ELECTRIC FIELD ENHANCED EXTRACTION AND SEPARATION PROCESSES

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Abstract:

Extraction and separation processes for solid – liquid systems (diffusion, pressing, filtration, centrifugation, drying) are largely used in the food industry for sugar, fruit juices, wine and vegetable oil production; these methods are also widely applied for extraction and separation of different biomolecules (colorants, anti-oxidants, proteins, aromas, etc.) from food plant tissues. Pretreatment methods, both conventional (grinding, heating) and alternative (power ultrasound, enzymes, etc.), enhance effectively the extraction and separation processes but degrade and disorganise importantly the plant tissue structure. Unfortunately, denaturated tissue becomes easily permeable not just for target cell substances but also for undesirable species (cell debris, pectin, etc.) affecting extract quality. Consequently, very complicated purification is often required to separate the target molecule from impurities.

Pulsed electric field (PEF) of moderate intensities is a very promising treatment for selective extraction of different biomolecules from plant tissue. As it was recently demonstrated by different research teams, the PEF is less destructive for plant tissues than alternative mechanical fragmentation or hot water denaturation. Electroporated cell membranes and cell wall network may be selectively permeable for different intracellular substances. Consequently, usual industrial extraction and separation methods can be modified to minimize the transformation technologies and to preserve better the product quality. This work presents some recent research results concerning extraction and separation from food plants combined with PEF. It is demonstrated for different plant tissues that electroporation enhances mass transfer during pressure extraction (increase of tissue hydraulic permeability and pressure conductivity), aqueous extraction (increase of solute diffusivity), and drying (increase of humidity diffusivity). The modification of tissue textural properties by PEF is also discussed. Several examples are presented to demonstrate the selective extraction of intracellular compounds (colorants, sugar,...) by PEF. Some examples of potential applications of the PEF extraction/separation technologies and pilot-scale experiments are also demonstrated.

Key words: PEF treatment, plant tissues, extraction, separation
RECENT DEVELOPMENTS IN FOOD PRESERVATION
BY PULSED ELECTRIC FIELDS

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Abstract:

Electricity has been used for long time in food processing with preservation purpose. It is well known that electrical currents can be used to heat foods by different means –for instance, by immersing a resistive wire into the food product or even by directly passing electricity through the food matrix, which itself acts as an electrical resistor. However, when high-voltage/short-time bursts of electrical energy are applied on a food product, the objective of preservation can be achieved without a substantial increase in temperature, because the energy is dissipated between pulses and the effect on the product is due to mechanisms different from those occurring during thermal processing. As a result, high intensity pulsed electric fields (HIPEF) technology is able to inactivate pathogenic and alterative microorganisms and significantly reduce the activity and/or stability of deleterious enzymes with a low impact on the physical, chemical and sensorial properties of fluid foods. In addition, kinetic approaches to better control the processing variables and shelf-life studies have been proposed. The feasibility of using HIPEF to obtain safe, stable and fresh-like products has been demonstrated by different research groups and the scaling up of the technology to the industrial implementation is the next step to be attained. An update of the state of the art in the development of the HIPEF technology is presented and discussed.
Abstract:

In the present scientific work chitosan, produced from shells of crustaceans, was purified by means of electrofiltration. The application of electric field in this process is enabled by the high zeta potential value of the positively charged biopolymer, caused by the amino groups in its structure. Promising preliminary experiments demonstrated high purification under 1, 2 and 4 bar conditions and 40-80 V. Filter cakes were analysed by means of thermogravimetric methods. Results demonstrate that chitosan can be successfully concentrated up to 7 times by introducing the new method of electrofiltration.

Key words: electrofiltration, biopolymers, chitosan, zeta potential

1 Introduction

Downstream processing is the main cost-consuming element in the biotechnological production process due to the various separation steps involved. In addition to investment and operating costs each further purification step give rise to subsequent product loss. Therefore, it is economically essential in downstream processing to reduce the number of purification steps in order to reach desired purity, concentration and activity [1].

In downstream processing filtration is an efficient technique for volume reduction. However, biopolymers are difficult to filtrate for they form impermeable filter cakes with high specific resistance. Therefore, in industry, dead-end filtration is not suitable for biopolymer purification. Cross-flow filtration, as an alternative, also encounter disadvantages. On one hand biopolymers are products mostly sensitive to the occurring during cross-flow filtration shear forces and on other – obtaining the desired final concentrations is also a problem. Changing the structure of the filter cake by precipitation of the biopolymers with organic solvent is another option but in this case recycling of the precipitation agent generally requires a lot of energy [2].

A promising approach eliminating all mentioned problems is a hybrid processes called electrofiltration. The aim of this technique is to improve the filtration kinetic of products difficult to filter. Combination of several purification steps including membrane filtration and
electrophoresis in a dead-end process, with electric field parallel to the flow direction of filtrate, is achieved by a single technique known as electrofiltration. Biopolymers, which due to the present amino or carboxyl groups are usually charged molecules, are affected by both electrophoretic and hydrodynamic forces, reducing the thickness of the filter cake. As a result electrofiltration could reduce processing time to several minutes instead of hours by using conventional filtration [3].

2. Theoretical background

2.1 Basics of electrofiltration

The principle of electrofiltration is based on overlaying electric field on a standard dead-end pressure filtration. This field works parallel to the flow direction of the filtrate. The electric field induces an electrophoretic flux of charged biopolymers towards the oppositely charged electrode. When the electrophoretic force ($F_E$) overruns the hydrodynamic resistance force ($F_W$), charged particles migrate away from the filter medium resulting in significant reduction of the thickness of the filter cake on the membrane (Figure 1). Due to the electrophoretic migration of the charged biopolymers, the formation of a surface layer on the membrane is reduced. This enables the filtration of charged biopolymers [6]. In addition the migration of the counter ion atmosphere around the charged biopolymers leads to an electro-osmotic dewatering. Darcy’s law was modified considering electrophoretic and the electro-osmotic effect and is presented in eq. (1) [4]:

$$\frac{t_f}{V_L} = \frac{\eta \cdot \alpha_{av} \cdot c \cdot (E_{cr} - E)/E_{cr}}{2 \cdot (\Delta p_H + \Delta p_E) \cdot A^2} \cdot V_L \cdot \Delta p_E$$

where $\eta$ is the dynamic viscosity; $\alpha_{av}$ is the specific filter cake resistance; $c$ is biopolymer concentration in the dispersion; $E_{cr}$ is the critical electrical field strength, generating a force on the biopolymer surface that equals hydrodynamic resistance force to the electrophoretic force; $E$ is the electrical field strength; $\Delta p_H$ is the hydraulic pressure; $\Delta p_E$ is the electro-osmotic pressure; $A$ is the filter surface area; $V_L$ is the filtrate volume; $t_f$ is the filtration time.

![Fig. 1: Schematic overview of the filter chamber and the principle of electrofiltration.](image)

Electrofiltration could be analysed in three phases [6]. In first phase, no filter cake is formed on the membrane and as a result filtration flux is higher than the electrophoretic resistance force. In the second phase, formation of filter cake causes a decrease in filtration flux and resistance force. On the membrane on the anode side they act in the same direction
and on the cathode side - in opposite directions. At a certain process point both forces affecting the biopolymer (hydrodynamic resistance force and electrophoretic force) equilibrate. From this moment filter cake growth is terminated. The electric field strength is approximately equal to the critical electric field strength in eq. (1). In this phase filter cake size stays constant. That results in increased dewatering of the biopolymer dispersion. In the third phase, more biopolymer is deposited on the membrane on the cathode side. It is necessary only 1 hour for the collection of filtrate volume of 135 cm$^3$ with electrofiltration in comparison to 62.8 h in the pressure filtration.

In addition to the promising results concerning concentration of xanthan, electrofiltration of microbiologically produced polyhydroxybutyrate granules (PHB) - a biodegradable polyester, represents an attractive application showing very good results [5, 6]. Other promising fields include fractionation of the enzyme serinoprotease and the protein bovine serum albumin (BSA) [7].

2.2. Chitosan

The aminoglucan chitin is the second most common natural polysaccharide after cellulose, with the amount of ten gigatons in nature [8]. Due to its poor solubility technical applications of chitin are limited. The derivative chitosan, which is soluble in acids, is obtained from chitin by deacetylation. Chitosan can be produced from fungi, insects, crustaceans or from the cell walls of fungus micelle by extraction, deacetylation and precipitation or by enzymatic hydrolysis in the presence of a chitin deacetylase. The advantages of fungus derived chitosan are the all-year-long character of the production instead of seasonal one and the product is heavy metal free qualifying it as attractive for medical applications [9]. The polycationic, chelate-forming and dispersive properties of chitosan are derived from the free amino groups of the polysaccharide [10, 11]. The application fields include many technical areas such as waste water treatment, agriculture, food and biotechnology in the form of a flocculant or stabilizer, and also in cosmetic and medical fields as lipid adsorbents [12].

3 Materials and methods

3.1. Materials

For the electrofiltrations experiments 5 g/l low molecular weight chitosan (Sigma-Aldrich, Germany) dispersions with 1% v/v acetic acid and polyethersulfon membranes Supor® - 100 (Pall, East Hill, NY, USA) were used.

3.2. Characterisation techniques

Chitosan’s zeta potential at different pH values was determined with PenKem 501 (Collotec, Bedford Hill, NY, USA) and the Aquosizer 3000 HS (Malvern, Worcestershire, UK) equipment. In both cases the electrophoretic mobility of the colloids in glass capillaries was measured using the Smoluchowski equation, determining the zeta potential. Measurements were conducted under 25°C conditions.

Particle size distribution was determined by Aquosizer 3000 HS (Malvern, Worcestershire, UK).

Deacetylation Degree (DD) describes the percentage of free and reactive amino groups in chitosan molecule as well as the degree of chitin into chitosan conversion. DD was determined by acid-base titration - 250 mg chitosan dissolved in 50 ml 0.1 M HCl and titrated with 0.1 M NaOH solution and indicator methyl orange. Equivalence point is visualized by a colour change from orange to yellow. Calculation was made according to the equations (2) and (3) [13].
\[ DD[\%] = \frac{x}{x + \left(\frac{m_{\text{chitosan}} - x}{204}\right) \cdot 100} \]  

\[ x = \frac{(V_{\text{HCl}} \cdot C_{\text{HCl}} - V_{\text{NaOH}} \cdot C_{\text{NaOH}}) \cdot 161}{C_{\text{HCl}}} \]  

3.3. Electrofiltration procedure

Electrofiltration experiments were carried out in a lab-scale equipment designed in the Institute of Engineering in Life Sciences at the University of Karlsruhe.

The filter chamber is between the electrodes that generate the electric field. The cathode is made of stainless steel and the anode is made of titanium coated with iridium oxide. These materials have been selected in order to prevent electrodes corrosion and to decrease water electrolysis products. Between the membrane and the electrode a flushing chamber is situated supplied by a circulating buffer solution. The buffer function is to stabilize the pH, to remove the resulting electrolyte gases and to keep the temperature of the system constant by a cooling coil. The buffer solution from the anode and cathode sides is transported to a filtrate-collecting system and pumped by a two-headed peristaltic pump type 323U (Watson-Marlow, Wilmington, USA).

3.4. Analysis of the filter cakes

The concentration of chitosan was measured by thermo-gravimetric (TG) - analysis with TG 209 unit cell (Netzsch, Selb, Germany). Filter cake samples were heated in ceramic seal at a constant heating rate of 10 K/min. The process requires inert gas nitrogen at a constant flow rate of 50 ml/min [14]. The thermal degradation of chitosan in nitrogen is a one-step reaction.

4. Results and discussion

4.1. Characterisation

Physicochemical properties, such as molecular weight, viscosity and solubility of chitosan are influenced by DD. Values of 85% and higher are relevant for the degree of purity.

Chitosan has a positive charge with a zeta potential value of +65 mV which mediate and allow its electrofiltration.

Particle size distribution was between 0.989-1.217 µm with an average value of 1.097. As The membrane pore size of 0.1 µm was selected assuming that bigger pore sizes can result in blocking of the pores when two molecules pass through the membrane simultaneously.

The discussed parameters are presented in Table 1. Zeta potential value was measured at pH 4.

<table>
<thead>
<tr>
<th>DD (%)</th>
<th>Zeta Potential (mV)</th>
<th>PSD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>85</td>
<td>+65</td>
<td>1,097</td>
</tr>
</tbody>
</table>

Tab. 1: Characterisation results of chitosan samples

4.2. Influence of electric field

Experiments with different pressures and voltages were conducted in lab scale facility in order to optimize the operating conditions of chitosan and promising results were achieved. Figure 2 represents the results of electrofiltration of chitosan under 4 bar conditions with different voltages. In comparison to the conventional filtration electrofiltration of chitosan is much better regarding mass of filtrate.
Fig. 2: Mass of Filtrate versus filtration time plot of electrofiltration of chitosan under 4 bar with different voltages

With the pressure increase, the filtrate mass also increases, demonstrating that the high speed of the hydrodynamic force is dominating over the electrophoretic force. After a test period of 2500 s to 3000 s pressure is no longer relevant for the filtrate flux but the amount of voltage. By increasing the resistance of the filter cake, the hydrodynamic force decreases so does the filtration rate.

4.3. Thermogravimetric analyses

Comparative study of degradation curves of electrofiltration experiments with chitosan at 4 bar and 40V and 80V are presented on figure 3. No chitosan was found in the filtrate demonstrating the relevance of the chosen membrane pore size. Analyses of the filter cake revealed that chitosan was concentrated maximum 7 times.

Fig. 3: Thermogravimetric analytic plot of electrofiltration of chitosan under 4 bar with 40 and 80 V

With a heating rate of 10°C/min the thermal degradation of chitosan starts at temperature of approximately 200°C. By using 40 V an 7-fold concentration of chitosan in the filter cake from 0.5% to 3.5% was demonstrated. In experiments with 80 V only a 4.4-fold increase in the concentration was achieved. Although the mass of filtrate at 80 V was significantly higher than the one at 40 V, the chitosan concentration was lower. In this case the structure of the filter cake plays a significant role. At higher voltages the filter cake formed is less dense.

5. Conclusion and outlook

Electrofiltration of chitosan gives promising results at 4 bar and 80 V. Thermogravimetric analysis demonstrate that 7-fold concentration is achieved.
Improving the economic aspects of electrofiltration is essential. Challenges like high energy demand, electrode costs, salt concentrations in dispersion, poor electrical conductivity of membranes, affecting electrofiltration negatively are present. More profound understanding of the processes occurring inside the filter chamber will result in process and apparatus optimization and overcoming the specific problems of electrofiltration. Knowledge for the optimization of filter design could also be retrieved. Regarding this lab-scale experiments with dispersions containing biopolymers labeled with fluorescence markers are planned. A new filter chamber was constructed which gives the possibility to trace the loss of voltage on the membranes inside the filter chamber.

References

ELECTRIC FIELD INDUCED EFFECTS IN PLANT TISSUES

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Abstract:
The attractivity of bio-material treatment by direct and alternating electrical current, pulsed electric fields (PEF) and arc discharges was demonstrated by the long history of development of electrotechnologies in the twentieth century. However, our days gave rise to many new ideas of modern industrial applications of electrotechnologies. They are based on electroporation phenomenon and were initially only of academic interest in relation to properties of bio-membranes. This review discusses some fundamental aspects of pulsed electric field (PEF) application as a tool enhancing processing of plant tissues. PEF technique demonstrates good efficiency of plant tissue disintegration, moderate power consumption and presence of a near-isothermal regime for electric field strength within \( E = 100 \text{–} 600 \text{ V/cm} \) [1,2]. Moreover, PEF treatment is more advantageous as compared to other treatment techniques (such as mechanical, high temperature, freezing-thawing, acoustical, etc...), which are less efficient and may result in deterioration of quality, colour and vitamin constituents of plant products and denaturation of their intracellular components. The plant tissues exhibit large diversity of cell sizes, shapes and textures, as well as heterogeneity of structure, and many of PEF-induced effects in these materials are still poorly understood. Moreover, the definition of electropermeabilization degree is still controversial and depends on experimental technique (electrical conductivity, acoustical response, solute diffusion, etc...) used for its estimation. A critical discussion of electropermeabilization phenomenon in plants, plant cells and cell membranes, kinetics of their damage, synergetics of electrical and thermal treatments, power consumptions aspects and efficiency of plants damage as a function of pulsation protocol, is presented. The recent experimental examples of PEF applications for acceleration of pressing, drying, diffusion and freeing possesses in plant tissues are also presented and discussed.

Key words: PEF treatment, electroporation, plant tissues

References

OHMIC HEATING AND MODERATE ELECTRIC FIELD TECHNOLOGIES: PRESENT AND FUTURE

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Abstract:

Recent work on ohmic heating and moderate electric fields has provided new insights into the influences of electric fields in permeabilizing cellular materials. Particularly, under weak fields, evidence is accumulating that reversible permeabilization may be maintained for extended periods of time, while the cells continue their biological activity. Waveform patterns have also shown significant effects, with the presence of high-frequency harmonic oscillations accelerating production of bacteriocins during fermentation.

In food processing applications, a number of process improvements may be seen using the alternating fields associated with ohmic heating. These include the enhancement of mass transfer resulting in potential improvements in drying, juice expression, extraction from cellular materials, and acidification. An interesting development is an environmentally friendly produce peeling process, which reduces the use of caustic compounds by over an order of magnitude, while maintaining peel quality and yield at levels comparable to caustic peeling processes. The application of moderate electric fields is currently in use for inactivation of bacteria within hospital water supplies – this is being accomplished at a lower temperature than conventional thermal inactivation.

These and other studies in the area suggest a promising and widespread future for ohmic and moderate electric field processing in food and bioproducts processing.

Key words: ohmic heating, moderate electric field processing, cells, sterilization

1 Introduction

Ohmic or Joule heating occurs when current flows through a conductor, generating heat within it. In the nineteenth century, this approach was the basis for a number of patents, and it briefly achieved commercial application for milk pasteurization in the 1930s. Thereafter, it has been investigated continuously since the 1980s, and has achieved some commercial acceptance.

In the past decade, a number of new manufacturers have developed the technology, and the cost of ohmic heaters per kilowatt of electrical energy has declined greatly. Currently, there are a significant number of ohmic heating facilities in Europe, Japan, Mexico, South America and elsewhere, processing a variety of products, most notably fruit slices and large fruit pieces in syrup.

A key aspect in the development of less expensive equipment has been the advent of solid-state power supply technology, such as the Integrated Gate Bipolar Transistor (IGBT) around 1997, which have substantially altered the cost and footprint of heaters. A further benefit has been the near-elimination of electrolytic effects via frequency control. A parallel development from Electricité de France, has been the development of improved materials
(dimensionally stable anodes) which have enabled operation at the European power frequency of 50 Hz.

2. Ohmic heating for food sterilization and pasteurization

Although ohmic heating has received most attention as a sterilization or pasteurization treatment for particulates, it is far from being a mature technology. A great variety of designs are possible, and may be tailormade to individual applications. Much is left to the designer’s creativity, and the variety can only increase as the technology develops.

A key feature is that there is room for creativity both from the equipment designer’s and product developer’s perspective. Most ohmic heaters are built to operate over a range of electrical conductivities. These can either be high voltage, low-current devices (usually with electric fields in line with the flow), or low-voltage, high-current devices, with fields typically across the flow. The former are intended to operate with high electrical conductivity fluids, typically products such as soups, stews, or preparations containing sauces with sufficient salt to provide electrical conductivity. The latter are intended for relatively low electrical conductivity fluids, such as water or foods/beverages without ionic additives. Both types of designs are now commercially available, and it is possible to heat foods over a wide range of electrical conductivity. Further, for a given heater, it is possible to develop products that may be heated within it by changing (within product limits) the electrical conductivity of the ohmically processed component. Given that ionic food components enhance electrical conductivity, and nonpolar components, such as fats, decrease it, it is possible to modify formulations to fit a particular heater’s operational range. This design interplay between product and package is still in its infancy, but holds promise for future development.

3. Effect of ohmic heating and moderate electric fields (MEF) on microorganisms

At the time of reintroduction of ohmic heating in the 1980s, it was thought that electricity had no nonthermal effects on bacteria, and that the efficacy of the process was itself principally thermal. Close to the turn of the century (Sastry and Barach, [1]), it began to be recognized that some nonthermal effects might exist, but overall, the view remained that it was a thermal process and that there was no need to invoke any nonthermal effects in a process filing. One of the major difficulties in this regard has been the convincing demonstration that a given experiment has legitimately compared thermal and nonthermal effects. In such cases, it is critical that experiments be conducted in such a way that thermal histories of conventional and ohmic samples is matched as closely as possible. Many studies in this area have failed at this exacting requirement, and consequently cannot be considered reliable.

Palaniappan et al [2] reviewed the literature available at the time on the effect of electricity on microorganisms and concluded that much of the evidence was inconclusive. One difficulty in interpreting prior experimental data was the lack of adequate temperature control within experiments; thus it was not possible to conclude whether a given effect was due to thermal or electrical effects. Thereafter, it has been strongly recommended that studies purporting to study electrical effects ensure that control samples would be treated with the same thermal history as ohmic or MEF processed samples. This is a difficult task, but is possible to accomplish as shown in several papers. Thus in this review, we will specifically address whether or not a given study maintained equal temperatures.
Palaniappan et al [3] studied the death kinetics of yeast cells (*Zygo saccharomyces bailii*) under conventional and ohmic heating, and found no difference in the death rate. However, pretreatment by sublethal ohmic heating (now called MEF) caused a significant decrease in the subsequent death times of *E. coli* vegetative cells at specific temperatures. This study was conducted with identical temperature histories for both ohmic and conventional treatments. However, since that time, it has been realized that additional precautions that are needed to ensure that the results are not influenced by underprocessed bacteria hidden in spaces between the electrode and the test chamber wall (Fig. 1). This problem has been remedied in succeeding studies, resulting in more reliable data.

Cho et al [4] studied the inactivation of *Bacillus subtilis* spores in buffer solution under conventional and ohmic heating. They too, maintained identical temperature histories between ohmic and conventional cells, and used a treatment cell that overcame the limitations of earlier work. They found that thermal death times decreased significantly under ohmic heating conditions.

![Fig. 1: Illustration of microbial ingress into cavity formed between electrode and system walls. Microorganisms within the cavity may be underprocessed and dramatically alter total counts when mixed with the bulk fluid sample.](image)

Pereira et al [5] studied inactivation kinetics of *E. coli* in goat’s milk, and *Bacillus licheniformis* ascospores in cloudberry jam during ohmic and conventional heating under identical temperature histories. They found that the thermal death times were shortened under ohmic heating. These studies showed that the results obtained in buffer media still held within a food matrix.

More recently, Somavat et al. [6] have shown that the inactivation rates even of thermophilic bacteria such as *Geobacillus stearothermophilus* within tomato soup are accelerated by ohmic heating compared to conventional heating under identical thermal histories.

A study on yeast cells by Yoon et al [7] showed that intracellular materials from *Saccharomyces cerevisiae* were exuded during ohmic heating with field strengths from 10-20V/cm. The amount of exudation was found to increase with field strength and frequency. It was concluded that ohmic heating induced electroporation, causing irreversible damage to cell membranes.

Sun et al. [8] studied the death rates of *Streptococcus thermophilus* in milk, using conventional and combination (sublethal ohmic + conventional) treatments. Combination treatments were performed by ohmically heating samples from 10 C (50 F) to 42 C (108 F)
for twelve successive cycles followed by the same conventional heating treatment as the purely conventionally treated samples. Inactivation of *Streptococcus thermophilus* was significantly enhanced by the combination treatment, suggesting a nonthermal effect of electricity. Sun et al. [9], employing identical heating conditions, found that the decimal reduction times of viable aerobes and *Streptococcus thermophilus* 2646 in milk were both significantly reduced by ohmic compared to conventional heating.

Leizerson and Shimoni [10] reported that ohmic heating resulted in a high quality orange juice, which was sensorially indistinguishable from fresh juice while completely (in their tests) inactivating bacteria, yeast and molds, and reducing pectin esterase activity by 98%. Further studies by Leizerson and Shimoni [11] showed that the sensory shelf-life of ohmically processed juice was twice that of conventionally pasteurized juice. These results indicate the advantages of ohmic heating, but because of lack of identical temperature treatments, it is not possible to conclude if the stated advantages are due to rapid heating or to the electric field per se.

A study on mold (*Aspergillus niger*) in tomato by Yildiz and Baysal [12] suggested some enhancement by ohmic heating, although the methodology in the paper is not convincing, because of lack of temperature control during experiments; making it difficult to separate thermal and nonthermal effects.

Even when ohmic heating is counteracted by cooling, electric fields as low as 1-2 V/cm have been observed to influence bacterial cells. Cho et al [13] studying fermentation of Lactobacillus acidophilus, found the lag phase to be significantly altered by MEF treatment. Subsequently, Loghavi et al. [14, 15] have not only confirmed these results, but also shown the sensitive influence of waveforms and frequency. Finally, the most recent studies in our laboratory using combinations of cell-permeable and impermeable dyes, have shown that cells are permeabilized at field strengths as low as 2 V/cm, and that lower frequencies increase the extent of permeabilization. While these studies are not specifically related to microbial inactivation, they serve to show that even mild electric fields have significant effects on cells.

4. Effects of ohmic heating/MEF processing on enzymes

A few studies have addressed nonthermal effects of ohmic or moderate electric fields on enzymes. Castro [16] has shown that the presence of an electric field does not cause enhanced inactivation of alkaline phosphatase, pectinase and β-galactosidase. However, lipoxygenase and polyphenoloxidase kinetics were significantly affected by the electric field, reducing the time needed for inactivation.

Yildiz and Baysal [12] have also reported on improved inactivation of pectin methylesterase (PME) activity in tomato, but as mentioned above, lack of suitable temperature control renders their results less than convincing.

5. Other potential applications of ohmic and MEF processing

The number of potential applications of electrothermal processing is vast: the electric fields associated with ohmic heating have been found to have significant nonthermal effects on cells, paving the way to a wide range of improved food processes, known collectively as Moderate Electric Field (MEF) Processing. Examples of such applications have been potential improvements in blanching (Sensoy and Sastry [17]), extraction (Sensoy and Sastry
drying (Wang and Sastry [19]; Salengke and Sastry [20]), juice expression (Wang and Sastry [21]), frying (Salengke and Sastry [22]), detection of starch gelatinization (Wang and Sastry [23]), acceleration of fermentations (Cho et al. [13]; Loghavi et al. [14]), and peeling of produce (Wongsa-Ngasri and Sastry, [24]).

6. Conclusions

The effects of ohmic and moderate electric field processing on biological materials is still emerging, and promises to be a fertile area in the future. Although much research has gone into characterization of high intensity fields, with short pulse durations, or high frequencies, a wealth of information remains to be discovered at smaller fields and lower frequencies, making this area interesting for some years to come.

References

Acknowledgments

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Abstract:

Electroporation is an innovative method in food processing to support the extraction of substances from cells. By applying a pulsed electric field to the tissue the cell membranes are charged and pores are formed in the membranes fostering the extraction. Although this principle is common to all electroporation devices, due to the different properties of each material, the devices and processes have to be designed for each application individually. The publication gives an overview on the advantages of electroporation for the processing of mash, sugar beets, and energy crop, and also on the design of electroporation devices for these applications.

Key words: Electroporation, wine grapes, apples, sugar beets, energy crop.

1 Introduction

The extraction of substances from biological tissue is an important processing step in the preparation of many kinds of food. Sugar is extracted from sugar beets by means of an extraction process at 69°C to 73°C after a thermal denaturation at 70°C to 78°C. Unlike for the production of sugar from sugarcane, the required energy for heating is not delivered by the plants, instead oil or coal has to be burned for heating. The juice from fruits is extracted by pressing. But especially in the second pressing stage enzymes or thermal treatment may be used to foster the extraction. For the preparation of red wine the fermentation of the mash is the classic way to open the cell membranes and to extract the pigments and valuable substances. But it requires a 1 to 3 weeks for fermentation inclusive extraction. Another way is the thermovinification. The mash is heated up to approximately 80 °C and kept at this temperature for 2 minutes, before it is cooled back to less than 40 °C. This faster method consumes much energy, because especially in small heating devices the energy is not recuperated.

If an electric field is applied to a cell, the membrane is charged resulting in a field enhancement across the membrane, Fig. 1. At a voltage of approximately 0.5 - 1 V across the membrane pores are formed. When applying a field-sensitive dye (ANNINE-6) to the cell, staining the bi-lipid layer of the cell membrane, the charging process of the cell membrane can be observed as a change in wave length of the fluorescent light from the dye which is transferred into a change of intensity by filtering [1]. Fig. 2 shows a microscopic view of a protoplast before and during the application of an electric field with the cell membrane stained with ANNINE-6. In the experiment the pore formation is visible as a saturation effect limiting the voltage across the membrane. Due to the angular dependency of the field
enhancement across the membrane and the natural DC-potential across the cell membrane of -100 to -200 mV with rising voltage the pore formation starts at the negative pole of the cell. Small pores may close after the pulse application. If sufficient energy is applied the pores grow and irreversible pores are formed. Through these pores, substances may be extracted from the cell. Fig. 3 shows microscopic pictures of cells from the skin tissue of Lemberger wine grapes before and after electroporation. The pigments are stored inside a vacuole. For an extraction both membranes of the cell and the vacuole have to be opened.

Fig. 1: Space charge configuration across the membrane of a ball-like biological cell.

Fig 2: BY2-Protoplast (tobacco) before and during the application of an electric field with the cell membrane stained with ANNINE-6

Fig. 3: Microscopic photographs of peel tissue of Lemberger wine grapes before and after electroporation.

2 Applications of electroporation

2.1 Processing of sugar beets, wine grapes, and apples

Electroporation is considered to be an interesting alternative to open biological cells for an extraction process:

Experiments on the electroporation of sugar beets on-site in a sugar factory demonstrated a more energy-efficient extraction of sugar from electroporated sugar beets in combination with the method of alkaline extraction [2]. For the alkaline extraction lime milk is added to the cossettes resulting in a strengthening of the cell walls. As a consequence the juice can better drain out of the cells after opening of the cell membranes. Due to the better extraction the extraction temperature can be decreased from 72°C to 60°C without affecting the efficiency of the sugar extraction. A minimum temperature of 60°C prevents the growth of mesophilic bacteria in the thin juice. Moreover, the amount of water for the extraction can be reduced resulting in a considerable reduction of evaporation energy for the subsequent concentration process of the extracted sugar solution. The strengthened cell walls result in a better water extraction in the pressing stage for the extracted cossettes. Consequently, for drying the cossettes less evaporation energy is necessary. Due to an improved purity of the extracted juice less lime milk for purging is required. This results in additional savings for lime stone and coke for the production of the lime milk. The estimated consumption of electric energy for the electroporation is only 1 to 1.5 kWh per ton of sugar beets, which is only 3 % of the total electric energy consumption of a sugar factory.

The electroporation of mash from red wine grapes combines the advantages of a fast processing and a gentle treatment at low temperature [3]. The pigments are extracted from the skin tissue within less than 24 h after electroporation by diffusion in an aquatic extraction process. Table 1 shows the results of the chemical analysis of differently treated variants of must and wines from Pinot Noir- and Riesling grapes. The data of the heated (thermovinification) and electroporated variant from Pinot Noir grapes are comparable. For Riesling grapes the electroporation has been compared to mash only pumped through the device. As the chemical analysis showed, the electroporation of mash from white wine grapes enables the extraction of more yeast digestible nitrogen which promises advantages to avoid
the “atypical ageing note” of the wine. Furthermore, the content of total acid in the must and the wine is reduced.

The electroporation of mash from apples enables an increased yield of high quality unfiltered juice in the first pressing stage, table 2. In 2006, an electroporation device for apple mash with a throughput of 10 t/h has been installed on-site at a producer of apple juice. In total the increase by 6 % of unfiltered juice results in an increased profit compensating the costs for the electroporation device after only 1.5 years.

<table>
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<tr>
<th>Tab. 1: Chemical analysis of must and wine.</th>
<th>Tab. 2: Electroporation of apple mash.</th>
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2.2 Electroporation-assisted drying of green bio-mass

For the production of bio-fuel in a BTL-process dry bio-mass serves as source material. According to estimations, in Germany about 10% of fuel consumption could be covered by BTL-fuel from dry bio-mass. By the additional use of green bio-mass this fraction can be doubled. Crucial is an energy-efficient drying method for green bio-mass. The electroporation of the green plant material enables the extraction of a considerable amount of juice by pressing omitting the evaporation energy [4]. Fig. 4 shows the amount of extracted juice from grass after electroporation at $\hat{E} = 7$ kV/cm with different numbers of applied pulses and subsequent pressing at 48 daN/cm² for 15 minutes. After electroporation with sufficient energy the yield of juice could be doubled. Subsequently, the material has been dried in an oven at 105 °C. As an example drying curves for young maize are shown. The electroporated material dried much faster than the only pressed material. As the different steepness of the curves for 20, 40, and 80 pulses shows demonstrates, this is not only because of smaller initial water content but as well due to a better diffusion of the vapour through the electroporated material. The electric energy required for the electroporation is only small compared to the evaporation energy, Fig. 5. With electroporation approximately half of the evaporation energy for drying of non-electroporated material can be saved.

3 Electroporation devices

3.1 Batch-processing of energy crop

When electroporating mash or whole sugar beets in a continuous flow the electric contact to the electrodes is established by the juice or water, the plant material is immersed in. To omit the use of additional water for a drying process a portion of the juice from inside the plant material can be extracted by pressing to establish the electric contact to the electrodes, [5]. To prevent air from being suck between the electrodes the pressure has to be maintained during the pulse application [6].

For field tests, the electroporation device KEA-MOBIL has been equipped with an electroporation reactor for batch-processing, Fig 6. The system consists of a hydraulic press made from insulating material capable of applying a pressing force equivalent to up to 11 tons to the piston. The piston and the bottom of the test vessel both serve as electrodes. To enable a throughput of 50 kg per hour the device has been equipped with an automated feeding system. One test vessel is manually emptied and filled outside the device, while the content of another test vessel is treated. The use of a hydraulic press and the manual handling of the material enables studying the influence of different parameters like pressing force, number of applied
pulses, applied electric field and energy independent of each other for different sorts of plants like grass, green rye, maize, and lucerne, each having different properties for conveyance.

Fig. 4: Yield of juice after pressing and drying curves depending on the applied number of pulses (grass and young maize).

Fig. 5: Energy required for electroporation and evaporation (young maize, $E = 7$ kV/cm).

3.2 An electroporation device for mash from wine grapes

The electroporation of wine grapes requires an electric field strength in the order of 25 kV/cm to 35 kV/cm because of the small diameter of the vacuoles in the order of 5 µm. So the field strength may be higher than the breakdown strength of air under normal conditions (30 kV/cm). Fig. 7 shows a photo of the electroporation device for mash. Gas transported with the mash is partly removed by an automated de-gasing valve at the inlet of the electroporation reactor. To prevent a flash-over inside the reactor caused by remaining gas bubbles in the mash, the mash is pressurized to approximately 2 to 3 bar$_{abs}$. By the increased pressure the gas bubbles shrink and the breakdown strength of the gas is increased. To prevent the mesh from blocking for the pressure regulation a second pump is used rather than a throttling valve [7]. An air chamber decouples both pumps from each other, Fig. 8a. A quasi homogeneous field distribution inside the reactor guarantees a homogeneous treatment of the material, Fig. 8b. The shape of the electrodes has been calculated in such a way, that no field enhancement at the borders of the electrodes occurs. The electroporation reactor is operated in ground-symmetric operation. So the voltage between the electrodes and ground is only half of the voltage with one electrode grounded. Hence, the insulation distance can be reduced and the device becomes more compact than in unsymmetrical operation. Moreover, the center of the reactor is virtually nearly on ground potential omitting a considerable current flow out of the reactor. For safety reasons additional ground electrodes are provided in flow direction before and after the reactor safely preventing current from flowing out of the electroporation device. The electroporation reactor (Fig. 9) has been manufactured form polyethylene with electrodes from stainless steel. The electroporation reactor is fed by a 6-stage Marx-generator with a stage capacitance of 140 nF and a charging voltage of 50 kV/stage. So the total energy per pulse is 1.05 J. Due to the ground-symmetric operation it is grounded at its center. For a repetition rate up to 20 Hz the spark gaps are cooled by a flow of nitrogen gas. During the harvest in 2008 the electroporation device has been operated successfully on-site at two wineries and more than 5 m³ of mash has been treated.
3.3 An electroporation device for large throughput

In a sugar factory a large throughput of sugar beets of up to the order of 15000 t/d has to be treated. Fig. 10 shows a test-setup for electroporation at a throughput of 10 t beets per hour. Although due to the larger size of the cells of sugar beets only a specific energy of approximately 1.0-1.5 kWh/t at a field strength in the order of 3-5 kV/cm is required the estimated total current for a large electroporation device would be 72 kA [8]. Such a high current can not be delivered by a single Marx generator in repetitive operation. The inductivity of the pulse circuit, which is typically in the order of 10 µH to 25 µH, would limit the current. Moreover, the wear of the spark gaps scales more than proportional to the current. Hence, the electroporation reactor has to be fed by several Marx generators, which are triggered simultaneously [9]. During a seasonal campaign one Marx generator has to pulse up to 200 million times. For a homogeneous burn-up of the spark gap switches, they are equipped with a homogeneous field profile according to Borda or Rogowski. To omit an additional trigger electrode, which would be subjected to an increased wear, the Marx generators are triggered by over-volting their first spark gaps. According to Fig. 11 the over-voltage is coupled into the circuit by pulse transformers connected to trigger pulse generators replacing the ground-side charging coils. The polarity of the pulse is chosen in such a way, that the induced pulse is added to the charging voltage of the stage capacitor. For the initialization of the breakdown of the spark gap seed electrons are required. The time scatter until their appearance determines the jitter. To keep the jitter low, seed electrons can be generated by illuminating the electrodes with ultra violet light. This light can be easily generated by a corona ring surrounding the electrodes, Fig. 12a. The ring is designed in such a way, that a corona discharge in a small high-field area around the ring emits light to the electrodes. To obtain the stable light emission of a negative glow discharge the ring is electrically connected to the negative switching electrode. The electric field strength in the inter-electrode space between the ring and the switching electrodes has been designed to be sufficiently small to prevent a flash-over. Fig. 12b shows the measured switching times of a 7-stage Marx generator. For a charging voltage of 93 % of the self-breakdown voltage the jitter of the total switching time has been measured to 55 ns. Nevertheless, in some few cases the total switching delay is more than 150 ns. To prevent energy oscillations between the Marx generators due to delayed switching, every Marx generator is connected to a separate pair of electrodes inside the electroporation reactor. The oscillations are damped by means of the resistance between neighboured electrode systems. This resistance is defined by the shape of the area between two neighboured electrode systems and the specific conductivity of the material filling this area. The energy due to losses in this resistance is transferred to the mash.
4 Conclusion

Electroporation in industrial scale enables an efficient and gentle way to open cells for an extraction process. While an electroporation device for apple mash is already in operation at a producer for apple juice, the electroporation devices for mash from wine grapes and sugar beets require still some research and development for an optimized and reliable operation in an industrial environment.

References

PULSED ELECTRIC FIELD PRESERVATION OF HEAT SENSITIVE PRODUCTS – FOOD SAFETY AND QUALITY ASPECTS

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Abstract:
PEF-preservation of milk was investigated with focus on microbial inactivation while maintaining the native antimicrobial systems and avoiding denaturation of whey proteins occurring during traditional thermal treatment. Membrane damage and the occurrence of sublethally injured cells were studied by flow cytometry. The role of media complexity and the protective effect of food constituents in microbial inactivation were taken into consideration. The impact of pulsed electric fields on bioactivity and antimicrobial activity of valuable food constituents like Lysozyme, Lactoperoxidase or Lactoferrin were studied. Results showed residual activities above 90% after pulsed electric field treatment indicating the technology’s advantage of microbial inactivation with minor impact on food constituents.

Key words: PEF preservation, sublethal injury, protective effects, enzyme inactivation and retention

1 Introduction

Pulsed electric field treatment can be an attractive alternative to traditional thermal pasteurization since it combines gentle food preservation with short treatment times, continuous operation as well as ease of implementation into existing product flow.

Inactivation of most of the spoilage and pathogenic microorganisms has been shown but inactivation kinetics obtained from PEF treatment in buffer systems have only limited comparability with real food products since a significant protective effect of food constituents occurs.

Membrane damage and inactivation of microorganisms due to PEF, firstly considered as an all-or-nothing revealed a required differentiated approach even if the critical parameters for the electrical breakdown of cell membranes are exceeded. Membrane damage and sublethal injury is repairable under certain conditions and the extent to which cells repair their injuries depend on treatment intensity, microorganism and treatment medium pH. The impact of food constituents on PEF effectiveness and the occurrence of sublethal injuries are not fully elucidated.

Assured food safety and stability, along with a desired level of microbial inactivation requires accurately defined treatment intensity followed by a predictable microbial inactivation.

Since the application of non-thermal preservation technologies aims to destroy microorganisms while maintaining fresh-like physical and chemical characteristics of food products, the impact of the pulsed electric field treatment on bioactive food constituents with a low sensitivity against heat during thermal preservation is of certain interest.
The evaluation of the effect of PEF on bioactives is complex. Available reports are limited, different experimental setups and processing parameters make them difficult to compare and only few data are available regarding pulsed electric fields effects on other food constituents and food properties. The consideration of electric field side effects like temperature increase and the occurrence of electrochemical reactions is the most challenging aspect within this context.

2 Food safety aspects

To validate the protective effect of milk in comparison to PEF treatments in an aqueous buffer solution, inactivation of *Lb. rhamnosus* was performed in raw milk (4.3 % fat, 3.3 % protein, 4% lactose) and in Ringer solution both with a pH value of 6.6 and a conductivity of 4.6 mS/cm to guarantee the same electrical properties and therefore the same treatment parameters and process requirements to achieve a comparable PEF intensity. Inactivation of *Lb. rhamnosus* in Ringer solution and in raw milk depending on the total specific energy input is shown in figure 1. To further extend the information on the mechanism of inactivation, the occurrence of sublethal injuries was taken into account and analysed by using flow cytometry. The vital fraction in milk was reduced by 21% whereas a reduction of 86% was achieved in Ringer solution at similar treatment intensity. This protective effect also seems to help in preventing membrane damage as only a very small sublethal fraction of 2% was found in milk in comparison to 40% in Ringer solution.

![Fig. 1: Influence of complex treatment media (milk in comparison to Ringer solution RS) on the occurrence of sublethally damaged Lb. rhamnosus (PEF treatment in the micro batch system at 17 kV/cm, FCM data shown).](image)

In order to obtain a maximum of food safety, a direct transfer of cells from the vital to the lethal fraction is favourable. However, since the impact on food quality characteristics limits the applicable treatment intensities, a limited number of dead cells may result. In that case, a large sublethally injured fraction would have an important potential for subsequent complete inactivation by the application of additional hurdles such as suboptimal storage conditions.

3 Food quality aspects

The non-thermal inactivation of microorganisms by PEF is based on the electroporation of membrane structures. High intensity electric fields are unlikely to affect covalent chemical bonds but the electric field application and related side effects may show an impact on food compounds.
Due to the application of PEF, changes in the conformational state of proteins might cause changes in enzyme structure and activity. In general, the mechanisms involved in the inactivation of enzymes by PEF are not fully understood but possible mechanisms could entail: polarization of the protein molecule; dissociation of non-covalently linked protein sub-units; changes in the protein conformation so that hydrophobic amino acid or sulphhydryl groups are exposed; attraction of polarized structures by electrostatic forces, and hydrophobic interactions.

The observed effects of PEF on enzymes by different research groups appear to depend, besides on the enzyme, on the characteristics of the PEF treatment system used and on the electric process parameters. Figure 2 shows the impact of a pulsed electric field treatment of milk on the activity of major bioactive protein compounds.

![Figure 2: Impact of pulsed electric field treatment on enzymes and bioactive components in milk; (LF Lactoferrin, LPO Lactoperoxidase, ALP Alkaline Phosphatase).](image)

Electrophoresis of whey proteins showed in comparison to heat treated milk a significant reduced denaturation of immunoglobulin G (IgG) and bovine serum albumin (BSA). PEF treatment at high intensity decreased the content of native IgG and BSA of about 10% whereas a reduction of 65% IgG and 30% BSA occured after pasteurization (figure 3).

![Figure 3: Impact of pulsed electric field treatment on native protein determined by densitometry of IgG and BSA bands after electrophoresis of whey proteins from raw, PEF-treated (38 kV/cm, 22°C, different energy input in kJ/kg) and pasteurized (75°C, 30 s) milk.](image)

As the PEF effect on enzymes and other milk proteins remained small its application could be used to reduce the microbial count in milk for production of raw-milk-type cheese varieties. A shelf life assessment of PEF treated milk was carried out by monitoring microbial...
growth, showing that the antimicrobial effect of an activated lactoperoxidase-system is retained after a PEF treatment and the synergistic effect of PEF inactivation. The retained antimicrobial activity of native milk constituents effectively prevents microbial growth. Other possible applications include the pasteurisation of the above mentioned bioactive components after their isolation from milk for further use as bioactive proteins.

During a PEF treatment there are many factors which have an impact on the enzyme inactivation and loss of protein bioactivity, such as temperature, pH, electrochemical reactions, and effects of the pulsed electric field. Temperature increase at the electrodes is considered one of the major factors resulting in enzyme inactivation [2].

Electrochemical reactions may result in a partial electrolysis of the solution, in corrosion of the electrode material and in introduction of small particles of electrode material in the liquid [3, 4]. As known, enzymes are very sensitive to pH changes and local changes of pH can therefore have a significant impact on enzyme stability during PEF treatment.

Although enzymes do not contain membrane structures which are the target for inactivation based on electroporation, the possible impact of PEF treatment indicates that process modifications towards the inactivation of microorganisms and inactivation or retention of enzyme structures are necessary.

4 Conclusion

The transfer of inactivation results from model systems to real foods and the determination of appropriate PEF treatment parameters require the consideration of existing particularities. The occurrence of sublethal damage to microorganisms and the ability to recover and regain structural integrity, metabolic activity and culturability as well as the crucial impact of food constituents as protective factors against microbial inactivation by PEF are key aspects relevant for the evaluation of the microbiological safety of a product after PEF treatment.

The pulsed electric field effect on protein structures in milk was found to be minor and a high rate of retention of bioactivity was detected. However, depending on processing parameters and treatment conditions, pulsed electric field side effects like temperature increase, the occurrence of high local temperatures as well as the effect of electrochemical reactions has to be taken into account to maintain the desired food quality.

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Utilisation of conductive plastic packaging film for pulsed electric field (PEF) treatment

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Abstract:
Food treatment with pulsed electric fields (PEF) is a non-thermal preservation method that can preserve the fresh like characteristics and extend the shelf life of pumpable products. State of the art treatment systems often consist of a stainless steel treatment chamber and are followed by a hygienic packaging machine to avoid post-process contamination. These systems have as main disadvantage that stainless steel treatment chambers have a limited lifetime and hygienic packaging is necessary. Both disadvantages can be eliminated with PEF treatment after packaging. This paper describes the parameters that influence the loss in a conductive plastic package and how this loss can be minimised. Also the required power for so-called PEF-in-pack treatment is discussed.

Key words: PEF, in-pack, food preservation, packaging, optimisation

1 Introduction

Pulsed Electric Field (PEF) treatment is a non-thermal preservation method for liquid products that gains an increased interest from industry for several reasons: the products taste better and the fresh like characteristics are preserved [1], [2]. Most nowadays PEF system designs consist of a continuous flow treatment chamber with stainless steel electrodes, which treats the product before packaging. These systems have as main disadvantage that the stainless steel electrodes exhibit a certain wear and that the product can be re-contaminated during packaging. There has been a growing interest in so-called active-plastics for food packaging [3]. Some new packaging concepts fulfil additional functions, such as blocking undesirable molecules like oxygen or releasing substances like carbon dioxide. The traditionally used plastics are electrically non-conductive. With electrical conductive polymers new functions can be added, which could expand the number of applications further. Applications, like conductive sterility testing or other non-thermal electrical preservation methods of pre-packed food products become possible [4], [5] and [6]. In case of PEF treatment after packaging, post-process contamination is avoided and therefore hygienic packaging machines are no longer necessary. Food in non-conductive film packages (pouches) cannot be treated with conventional PEF techniques due to the dielectric properties of the packaging layer [2]. It has been shown that composite electrical conductive films can be used for PEF treatment [7]. During the manufacturing process of plastic conductive films some orientation of the filler particles can take place because of specific flow conditions, leading to anisotropy. This can give rise to different conductivities in parallel direction ($\sigma//$) and perpendicular direction ($\sigma\perp$). A larger parallel conductivity is unwanted; it will lead to more current and thereby more loss in the packaging film. On the other hand it is contradictory with the perpendicular conductivity, which needs to be large to get sufficient electric field in the liquid for PEF treatment.

This paper describes the influence of the film conductivity, food conductivity, and packaging dimensions on the power loss in the package during PEF treatment of pre-filled pouches. The film conductivity for minimum loss in the package has been derived and the required power for single and successive PEF-in-pack treatment has been investigated.
2 Film utilisation and optimisation

Conductive films can be used for PEF in two different ways. First, it can be used as electrode coverage in normal treatment chambers with two insulated electrodes. Or second, as a pre-filled heat sealed pouch, where the conductive film fulfils two functions; it acts as the electrode during PEF treatment and it has a packaging function during product life. Fig. 1 shows the pouch representation and its electrical equivalent for so-called PEF-in-pack treatment. The film is split up in a perpendicular resistance (electric field $\perp$ to film surface) and a parallel resistance (electric field // to film surface), $R_{pe}$ and $R_{pa}$ respectively. The resistance of the food is represented by $R_f$. How to minimise the loss in the package is the main question from optimisation point of view.

2.1 Electrode coverage

If the film is used as electrode coverage, the four sidewalls represented by $R_{pa}$ are non-conductive. Both electrodes are covered with the conductive film and are electrically in series with the food product. Only $R_{pe}$ and $R_f$, depicted in the equivalent circuit Fig. 1c, needs to be considered. The perpendicular film resistance and food resistance equals,

$$R_{pe} = \frac{2d}{xz\sigma_{\perp}}$$  \hspace{1cm} (1)

$$R_f = \frac{y}{xz\sigma_f}$$  \hspace{1cm} (2)

where $x, y$ and $z$ are the dimensions of the treatment volume [m] and $d$ the film thickness [m].

The film and food conductivity are $\sigma_{\perp}$ and $\sigma_f$ [S·m$^{-1}$] respectively. Which part of the applied power is dissipated in the film can be calculated with eq. (1) and (2) as follows

$$\frac{P_{elec}}{P_{tot}} = \frac{i^2R_{pe}}{i^2R_{pe} + i^2R_f} = \frac{\sigma_f}{\sigma_f + \frac{1}{2d}\sigma_{\perp}}$$  \hspace{1cm} (3)

A large electrode distance ($y$), a small film thickness ($d$), and a large perpendicular conductivity ($\sigma_{\perp}$) will lead to the lowest relative losses.

2.2 Pouch construction

The film can also be used to make a pre-filled food pouch like Fig. 1a.

Due to the additional loss in the four conductive sidewalls the highest film conductivity will not automatically lead to the lowest losses. PEF treatment of these type of pouches can be realised in various ways, e.g. by connecting electrodes as shown in the simplified 3D representation of Fig. 1b.
Fig. 1: a) Filled and sealed pouch; b) simplified 3D construction; c) electrical equivalent.

Besides $R_{pe}$ and $R_f$, the parallel resistance of these sidewalls, $R_{pa}$, needs to be considered.

$$R_{pa} = \frac{\gamma y}{(2x+2z)d} \sigma_y = \frac{\gamma y}{(2x+2z)d} a \sigma_\perp$$  \hspace{1cm} (4)

where $a$ is defined as the ratio between the parallel and perpendicular conductivity of the polymer film.

To optimise the energy efficiency of the PEF-in-pack treatment the total dissipated energy in all six walls of Fig. 1b need to be known. This dissipated energy equals

$$P_{pl\%} = \frac{P_{pe} + P_{pa}}{P_{tot}} = \frac{i_1^2 R_{pe} + i_2^2 R_{pa}}{i_1^2 R_{pe} + i_2^2 R_{pa} + i_f^2 R_f}$$  \hspace{1cm} (5)

Substitution of eq. (1), (2) and (4) in (5) results in the loss percentage in the pouch as function of the geometry, film and food parameters.

$$p_{pl\%}(x,y,z,d,a,\sigma_\perp,\sigma_f) = 1 - \frac{\sigma_f \sigma_\perp y^2 z}{(2d \sigma_f + \sigma_\perp y)(4ad^2 \sigma_f (x+z) + \sigma_f xyz + 2ad \sigma_\perp y (x+z))}$$  \hspace{1cm} (6)

The package conductivity for minimum losses can be found by

$$\frac{\partial p_{pl}}{\partial \sigma_\perp} = 0$$  \hspace{1cm} (7)

Solving this equation results in

$$\sigma_{\perp min}(x,y,z,d,a,\sigma_f) = \frac{\sqrt{a(x+z)(xyz + 4ad^2(x+z))}}{ay(x+z)} \sigma_f$$  \hspace{1cm} (8)

By substitution $\sigma_{\perp min}$ in eq. (6) the minimum value of the loss in the pouch can be calculated.

### 3 Influence of parameters

The parameter dependencies will be investigated with eq. (3) and eq. (6), which represents the relative film losses for the electrode coverage configuration and pouch construction. To get a realistic outcome (related to real food and film properties) the parameters have been varied within the following ranges:

- Package conductivity ($\sigma_\perp$): {0–1 S·m⁻¹};
- Food conductivity ($\sigma_f$): {0.1–1.6 S·m⁻¹};
- Anisotropy factor $a$: {0.25–4};
- Dimensions (x, y, z): {1–16 cm};
- Film thickness (d): {10–160 μm}
All depicted relative loss figures has one common graph for a food conductivity of 0.4 S·m⁻¹, an anisotropy factor, \( a = 1 \), dimensions, \( x = y = z = 1 \) cm and a film thickness of \( d = 80 \) μm. From this starting point the mentioned parameters will be varied to investigate their influence on the relative loss in package. This common graph is plotted bold in Fig. 2, 3, 4 and 5.

### 3.1 Loss in the package as function of package conductivity and food conductivity

Fig. 2 shows the loss in the package as function of the package conductivity for food conductivities from 0.1–1.6 S·m⁻¹ and is calculated with eq. (6). For these graphs the dimensions \((x,y,z)\), anisotropy factor \((a)\), and film thickness \((d)\) are fixed at \(1\times1\times1\) cm, 1, and 80 μm respectively. The minimum loss is 4.4%. For a food product with a conductivity of 0.4 S·m⁻¹, the package conductivity for minimum losses \((\sigma_{\text{min}})\) equals 0.28 S·m⁻¹, which can be calculated with eq. (8). Lower food conductivities result in a sharper minimum, which results in a higher sensitivity for package conductivity variations. So, small deviations in \(\sigma_{\perp}\) result in a faster increase of the losses and thereby a decrease in energy efficiency. In general, due to the asymmetric shape of the graphs the sensitivity (of \(\sigma_{\perp}\)) above \(\sigma_{\text{min}}\) is less than below \(\sigma_{\text{min}}\).

3.2 Loss in the package as function of the package conductivity and anisotropy

Thermoplastic molecules gain some orientation during film manufacturing, which makes the macroscopic properties of stretched polymer film such as conductivity anisotropic. Fig. 3 shows the loss in the package as function of package conductivity for different anisotropy factors \(a\). For these graphs the dimensions \((x,y,z)\), food conductivity \((\sigma_f)\), and film thickness \((d)\) are fixed at \(1\times1\times1\) cm, 0.4 S·m⁻¹, and 80 μm respectively.

As expected for larger values of \(a\), (lower \(R_{pa}\)) the losses will increase and the optimum film conductivity for minimum losses will decrease. For a food product of 0.4 S·m⁻¹ and \(a = 2\), the package conductivity for minimum losses equals 0.2 S·m⁻¹, instead of 0.28 S·m⁻¹ for \(a = 1\). Fig. 3 also shows a special case for \(a = 0\). In this case the four sidewalls are non-conductive and this graph is equal to eq. (3), where the film is only used as electrode coverage.
3.3 Loss in the package as function of the package conductivity and package dimensions

Fig. 4 shows the relative loss as function of the package conductivity for several dimensions. The graph for \( x, y \) and \( z = 1 \) cm is the same as shown in Fig. 2 and Fig. 3.

By increasing the package in all three dimensions from \( 1 \times 1 \times 1 \) to \( 16 \times 16 \times 16 \) cm (i.e. volumes from 1 mL up to 4 L) the relative losses decrease drastically. The same behaviour can be obtained in Fig. 5, where a decrease in film thickness leads to lower losses. Via the derived formulas the film conductivity for minimum losses can be calculated. In general for larger anisotropic factors \( (a) \) the losses increase, larger pouches and thinner films result in relative lower losses. On the other hand larger pouches will need more peak power.

4 Required electrical power

4.1 Single treatment

Fig. 4 shows the loss in the package for larger packages that are treated as a whole. Upscaling to a larger volume can be realised by expansion in the \( x, y \) or \( z \) direction. The parameter \( y \), which is the distance between the electrodes, has influence on the needed pulse voltage. At a distance of 1 cm and an average electric field of 3 kV\cdot mm\(^{-1}\) 30 kV is needed. Expansion in the \( x \)- and \( z \)-direction results in a larger conductive area and thereby larger pulse currents.

Fig. 4: Loss % in the package as function of the package conductivity for different dimensions. Thickness \( d=80 \mu m \), \( a=1 \), conductivity. Anisotropy factor \( a=1 \), \( \sigma_f=0.4 S/m \), \( x=y=z=1 \) cm.

By neglecting the relative small loss in the package, the power that is required to realise a uniform electric field, \( E \), approximates

\[
 P_p \approx \sigma_f E^2 V_t
\]

where, \( V_t \), is the treatment volume [2]. To keep the pulse voltage and current within the (arbitrary) boundaries of 100 kV and 1000 A \( (P_p = 100 \text{ MW}) \) the maximum packaging volume for 0.4 S\cdot m\(^{-1}\) is approximately 28 cm\(^3\). Via the maximum pulse voltage and electric field (100 kV and 3 kV\cdot mm\(^{-1}\) this will results in a cubical package of \( \sim 3 \times 3 \times 3 \) cm. The neglected loss in this package film can be calculated with eq. (6) and equals 1.6%.
4.2 Successive treatment

For larger packages within the mentioned power constraint, the package cannot be treated as a whole and therefore parts of the package need to be treated successively. Successive treatment can be realised with electrodes that slide along the package as depicted in Fig. 6a. During successive treatment there is conductive liquid outside the treatment volume that carries extra current, Fig. 6b. This fringing current will cause additional losses that need to be supplied by the pulse generator.

If the electrodes are positioned at the edge of the package \( (d_x = 0) \), the additional current can only flow at one side of the electrode configuration. In the middle of the package, the current will flow on both sides. So during successive treatment the required power becomes larger than the value calculated with eq. (9) and depends also on the electrode position, \( d_x \). Fig. 7 shows the power multiplication factor for an infinite long package \( (l_p = \infty) \) with a square footprint of 3×3 cm as function of the electrode position. The theoretical value, which is calculated with eq. (9), is equal to \( k_p = 1 \). Each depicted point in Fig. 7 represents a 3D FE calculation of the power in the total structure of Fig. 6 (i.e. package and food together).

For all simulations the food conductivity was equal to the film conductivity, 0.4 S·m⁻¹, and the average field strength was kept constant at 3 kV·mm⁻¹. A higher aspect ratio defined by \( w/h \) results in a relatively lower additional power needed for successive treatment. For the depicted aspect ratios of 0.5, 1 and 2 the theoretical peak power equals 48.6, 97.2 and 194.4 MW respectively. For \( w/h = 1 \) and \( d_x = 0–3 \) cm, the extra needed power is between 14% and 29%. For \( d_x > 3 \) cm, which is approximately the package height, the power becomes constant.

Fig. 6: Successive treatment with moving electrodes(a); Side-view with electrodes, additional losses (in grey) and parameter definition(b)

5 Discussion

This paper focuses on the electrical properties of a conductive film for PEF-in-pack. Besides that, the question; “how to get sufficient electric field inside a moving conductive pouch” is not investigated. PEF inactivation with composite conductive film electrodes in a static set-up is demonstrated in [7]. For moving constructions additional research is necessary. The local electric field distribution, development of food grade films and the pouch-electrode interface need special attention [5].
PEF in pack treatment of larger food packages, typically above 30 mL, need to be done in parts to avoid too high peak power values. To keep the peak power within certain boundaries for a real food package, e.g. a ½ litre bag-in-pack that measures ~10×7×7 cm, a rectangular treatment aperture \( V_t \) of 2×2×7 cm \((x,y,z)\) can be considered. Treatment is possible when the flexible pouch is locally compressed (in y-direction) and travels in the x-direction through a local constriction. In this case the electrodes need to act as a wringer in which the food will be PEF treated successively.

6 Conclusions

The film conductivity for minimum loss in a food pouch for PEF has been derived and the dependencies for film, food, and geometry parameters have been investigated. In general can be concluded that the film conductivity for the lowest relative loss in the package is close to the conductivity of the food product to be treated, typically between 0.1–2 S·m⁻¹. The lowest relative losses are obtained at low anisotropy factors \( \sigma//\sigma_\perp \), larger packages and thin films.

To limit the increasing peak power in larger packages, successive treatment of parts with moving electrodes, is needed. For successive treatment slightly more power is needed than the theoretical value \( \sigma E^2 V \) and the needed power depends on the position of the electrodes. For a package with a footprint of 3×3 cm the extra needed power has been determined and is typically between 5–65% for electrode aspect ratios between 2–0.5.

References


EFFECT OF ELECTRICITY ON THERMOPHILIC BACTERIAL SPORES: INACTIVATION KINETICS OF GEOBACILLUS STEAROTHERMOPHILUS SPORES UNDER THE EFFECT OF PULSED OHMIC HEATING

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Abstract:
Literature reveals a limited understanding of the effect of electricity on bacterial spores during ohmic heating. Absence of a standard bio-validation method with precision comparable to those available for conventional heating could be a major factor contributing to this inadequate understanding. The aim of this study was to determine the kinetics of inactivation of Geobacillus stearothermophilus spores (ATCC 7953) using an improved universal capillary cell (UCC) ohmic device. Important features of the micro size UCC device (volume 37µL) developed for the experiments were- a) capability to test multiple cells at a time, b) identical time-temperature histories for both ohmic treatment and conventional control, c) provision of rapid cooling under pressurized conditions (less than 10 seconds for a reduction of greater than 100°C), and d) elimination of the potential sources of error which were inherent to most of the previous studies on ohmic heating, including heating uniformity, matching time-temperature histories, isolation of non-thermal effects and elimination of errors due to heat losses from the device.

Ohmic heating of tomato soup inoculated with $10^8$ spore cfu/mL done at frequency of 60 Hz and 10 kHz was compared with conventional heating at 121°C, 125°C and 130°C for four different holding times (three replicates each). The study has shown that ohmic heating at 10 kHz has resulted in accelerated inactivation of thermophilic bacterial spores than conventional treatment. D-values obtained for conventional treatment were in good accordance with the generally reported range for this specific strain of G. stearothermophilus, hence confirming precision of the invented UCC.

Key words: universal capillary cell (UCC), ohmic heating, Geobacillus stearothermophilus spores, inactivation kinetics

1 Introduction
Ohmic heating is considered to kill microorganisms only through a thermal effect. Limited literature exists on additional non-thermal effects of electricity on microbial inactivation. In the absence of adequate understanding of spore inactivation kinetics, process severity requirements are equivalent to thermal sterilization [1]. New methods are needed for efficient bio-validation of processing techniques such as ohmic heating in order to realize the full potential of the process. The aim of this study was to develop a precise universal capillary cell (UCC) ohmic heating device to quantify additional non-thermal lethal effect of electricity during ohmic heating on Geobacillus stearothermophilus spores.

Past studies have investigated non-thermal effects of electricity on microorganisms such as yeast, vegetative bacterial cells and bacterial spores. Sublethal electrical treatments have been reported to reduce thermal requirement for inactivation of microorganisms [2] and to decrease lag period together with modification of metabolic properties during fermentation of lactic acid bacterium [3]. Intermittent ohmic heating has reported additional non thermal lethality of
**Bacillus subtilis** spores when compared with conventional heating [4]. A synergistic effect of heat and electrolysis under the influence of low amperage direct electric current on *Saccharomyces cerevisiae* have also been suggested by another study [5]. Results reflect, gradually evolving yet inadequate understanding of non-thermal lethality.

Absence of a standard ohmic device with precision comparable to those available for conventional heating could be a major factor contributing to this inadequate understanding. The designs of ohmic heaters used in different studies varied greatly with sizes ranging from 8mL to 100mL [4] [5]. These devices were considerably larger than the capillary sized devices (capacities ranging between 100µL to 1mL) used in conventional heating studies [1], and hence failed to ensure elimination of potential sources of errors related to heating uniformity, matching time temperature histories, isolation of non-thermal effects and elimination of errors due to heat losses from the device. These unresolved sources of error can be categorized under engineering problems and microbiological constraints.

**Engineering problems**
1. Large size of ohmic sample holders as compared to capillary sized devices (capillary tubes or TDT canisters) used for conventional inactivation studies.
2. Convection currents inside the sample container can affect uniformity of the heating profile.
3. Thermal stratification of the liquid media can occur.
4. Temperature difference between the heater wall and surrounding can create a cold layer near the surface. Temperature dependence of electrical conductivity of food materials can further exaggerate this difference.
5. Absence of a standard size and design of ohmic heater can cause additional variability in the results of different studies.

**Microbiological constraints**
6. Presence of micro-crevices around seals and o-rings can result in errors in the final survivor count.
7. Difficulties in obtaining matching time-temperature histories for a larger size ohmic heater and a small capillary conventionally heated control.
8. Thermophilic organisms (*Clostridium botulinum* and surrogates) require heating above 100°C, which necessitates application of external pressure. Spores can survive in the headspace area (if pneumatic pressure is used) and in between sliding parts (if a spring loaded device is used).
10. Conventionally, preference is given to a multiple-cell device to improve experimental control.

Hence the objectives of our study were to develop a precise UCC ohmic heating device which could eliminate all these sources of errors, and to study microbial inactivation kinetics of *G. stearothermophilus* spores under ohmic and conventional heating. Ohmic treatment was tested at two different frequencies (60 Hz and 10 kHz) considering that past studies have shown enhanced inactivation of vegetative microorganisms at lower frequency [3] [5] and reduced electrochemical reactions at higher frequency [6] [7].

**2 Materials and methods**

**Concept of a UCC**
Glass capillary tubes containing 37µL of inoculated food sample were sealed using end plugs. Multiple such UCC were treated by aligning parallel to the electric field inside an external ohmic heating chamber containing an iso-conductive salt solution. Conductivity of the end
plugs defined ohmic or conventional mode of heating of the UCC. Tomato alginate end plugs were used to prepare ohmic UCC, while a non conductive plastic sealant was used to seal conventional UCC (figure 1). Temperature was monitored by inserting a tiny thermocouple junction inside the ohmic and conventional UCC and used for their respective runs. Small size, similar preparation and treatment of both ohmic and conventional samples, elimination of temperature difference between the sample capillary and surrounding, and provision of a cooling section to rapidly cool the treated sample (figure 2) eliminated all potential sources of error as described earlier.

Fig. 1: A universal capillary cell (UCC)

Power supply unit
An Integrated Gate Bipolar Transistor (IGBT) chip was used in the circuitry as a switching device to generate a high-frequency pulsed square waveform. A function generator (Instek, Chino, CA, USA) was used to deliver 10 kHz or 60 Hz frequency input to the IGBT circuit. Temperature, frequency, voltage, current and time data were recorded by a data logger (Agilent Technologies Inc, Santa Clara, CA, USA).

Preparation of spore solution
*G. stearothermophilus* (ATCC 7953) was obtained from the American Type Culture Collection (Manassas, VA, USA). The strain was grown in tryptose broth (TB; Difco, Becton Dickinson and Co., Sparks, MD, USA). For spore preparation, aliquots of 100µl overnight cultures of *G. stearothermophilus* were spread onto nutrient agar (NA; Difco) supplemented with 10-ppm manganese chloride. Plates were incubated at 55°C until >90% of the cells were sporulated. Sporulation was verified by observing the refractile spores using phase-contrast microscopy. Spore suspension was prepared and heated at 85°C for 10 min to ensure the absence of vegetative cells. The spore suspension was stored at 4°C until used.

3 Results and discussion
Temperature distribution tests verified that ohmic UCC was indeed heated through an electric effect, by observing that temperature inside UCC always remained 1-2°C higher than the external chamber solution (figure 3). This temperature gradient in favor of the ohmic UCC showed that there was no heat flow (conduction or convection) from the chamber fluid to the sample, hence confirming that the sample was heated by pure ohmic heating.
Accelerated inactivation of *G. stearothermophilus* was reported under high frequency electric field than the conventional heating treatment. Ohmic heating at 10 kHz showed more inactivation of the bacterial spores than conventional heating. It is postulated that release of polar ionic materials like DPA from the spore core together with the spore coat proteins at high temperature assisted in increased lethal effect of high frequency electricity. D-values obtained for conventional treatment (2.08 min. at 121ºC, 0.60 min. at 125ºC and 0.07 min. at 130ºC) were in good accordance with the generally reported range for this specific strain, hence confirming precision of the invented device.

### 4 Conclusion

A precise UCC device for studying microbial inactivation kinetics under ohmic heating has been developed and successfully tested. The study has concluded accelerated inactivation of thermophilic bacterial spore under high frequency electric field potentially due to the release and vibrations of polar DPA at high temperature-frequency conditions.

### References


SIMULATION AND OPTIMIZATION OF PEF TREATMENT CHAMBER
GEOMETRY CONSIDERING DIFFERENT PROCESSING CONDITIONS

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Abstract:
The main purpose of the present work was to optimize the pulsed electric field (PEF) treatment chamber for continuous liquid food processing, considering flow rate characteristics, electric field strength and insertion of static mixing devices into the treatment chamber. Simulation of the PEF process considering different treatment chamber setups was performed using Finite Element Method (FEM) prior to experimental verification. A square wave pulse modulator (pulse width of 3 μs at an initial voltage of 18 kV) and a co-linear treatment chamber were used for the experiments. The Finite Element Method (FEM) analysis showed how the electric field strength depends on the treatment chamber geometry and how it can be improved by the insertion of static mixing devices, as well as, how the velocity and temperature profiles can be altered. The insertion of static mixing devices allows a more homogeneous treatment and also avoids temperature spots of the liquid close behind the insulator. Microbial inactivation was increased by 0.6 – 1.0 log-cycles due to the modification of the treatment chamber design.

Key words: PEF simulation, CFD, microbial inactivation.

1 Introduction
Pulsed electric field (PEF) treatment of liquid foodstuff is considered as a non-thermal alternative for inactivation of microorganisms. This processing technology consists of treatment with very short electric pulses (1 – 100 μs) at high electric field intensities (10 – 50 kV/cm) at moderate temperatures to affect the integrity of cell membranes by electroporation. This can be more or less effective, depending on the process factors (electric field strength, treatment time, fluid flow and temperature) and on the treatment chamber design, which is one of the most important factors. An appropriate design allows the implementation of a process at industrial scale and a uniform treatment of food with a minimum increase of temperature. Current flow occurs in the food, which leads to ohmic heating since electrical energy is dissipated. Hence, temperature increase is a PEF side effect that has to be considered when treatments of heat sensitive products are investigated.
The simulation of PEF allows us to understand in more details how the electric field strength, velocity and temperature distribution are and how these process factors impact the microbial and enzymatic inactivation.

The present work is focusing on the impact of the treatment chamber on the electric field strength, velocity and temperature profile and on the microbial inactivation.

The main objectives have been: investigation of the treatment chamber geometry impact on the electric field strength distribution and on the microbial inactivation, and investigation of the fluid flow rate impact on the microbial inactivation.

2 Material and Methods

For the investigations related to the treatment chamber geometry and the velocity effect on the microbiological inactivation, *Lactobacillus rhamnosus* E522 (VTT Biotechnology, Espoo, Finland) was used and suspended in Ringer solution (Merck KGaA, Darmstadt, Germany) adjusted at a conductivity of 2.3 mS/cm.

The pulse generating system consists of a high voltage power supply with 1 kV maximum voltage, six energy storage capacitor banks and six parallel IGBT units for rectangular pulse generation. Using a 1:50 pulse transformer at the secondary side up to 50 kV pulses can be achieved, the maximum current is limited to 200 A, the maximum repetition rate is 400 Hz, subjected to average power. Pulse width is adjustable between 3 and 8 μs (ScandiNova Systems, Uppsala, Sweden).

The finite element analysis and solver software package Comsol multiphysics (Comsol Inc., Burlington, USA) was used for calculation of electric field strength, temperature distribution and fluid flow, as well as, for analysis of coupled phenomena. Amongst the governing equations to be solved are the Navier-Stokes equations, describing movement in incompressible fluids, general equations for an energy balance, considering heat transfer through convection and conduction, finally, Poison’s equations to solve the electrical potential, based on charge conservation [1,2,3,4].

![Fig. 1: Effect of insertion of grids on the electric field strength, adapted from [3]. Initial voltage: 22 kV. Symbols in figure (i) insulator and (HV) high voltage electrode.](image1)

![Fig. 2: Inactivation of Lb. rhamnosus using a treatment chamber with four inserted grids in comparison to a treatment chamber without grid. Initial voltage of 18 kV. Treatment medium: Ringer solution at 2.3 mS/cm and inlet temperature 20ºC.](image2)
3 Results

The insertion of static mixing devices, like a grid, as used in [3] could have a strong impact on the electric field distribution and therefore on the microbial inactivation. Nevertheless, the main use of these devices is to generate a homogeneous velocity profile. The velocity profile in the treatment chamber (laminar or turbulent) provides information on the residence and treatment time of the liquid food. Since the fluid velocity differs depending on the location within the treatment chamber, there will be differences in the treatment time, i.e. at the centre of the treatment chamber, the liquid food will receive shorter treatment times, since the velocity at this point is higher. The following results describe how the insertion of grids alters the electric field strength, the microbial inactivation, the velocity profile and the temperature distribution. It is also shown how the mass flow itself can impact the effectiveness of the microbial inactivation.

As observed, the electric field strength is strongly affected by modification of the treatment chamber. The grids are made of metal and were set up as electrodes, as shown in figure 1. As expected, the alteration of the electric field strength has an impact on the microbial inactivation as well, as shown in figure 2.

The main idea of inserting grids is to improve the liquid mixture, which allows all of the particles into the fluid to receive the same treatment and also to avoid liquid recirculation. It could be in partly achieved by eliminating the parabolic profile of laminar flows (figure 3).

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**Fig. 3:** Impact of insertion of grids on the radial velocity profile. Radial coordinate 0,0 corresponds to the centre of the treatment chamber. Treatment chamber diameter: 4 mm.

**Fig. 4:** Temperature contour along the second insulator of the treatment chamber. Treatment chamber without grids (above) and chamber with 2 metal grids set up as electrodes (below). Simulation performed with an initial voltage of 18 kV, 46 Hz and 2,6 mS/cm.

**Fig. 5:** Inactivation of Lb. rhamnosus at 4.9 and 16 l/h, using Ringer solution adjusted at 2.3 mS/cm.
Changing the flow characteristics has a significant impact on the temperature distribution in the treatment chamber by eliminating recirculation and increasing mixture (figure 4). Besides the alteration of the flow characteristics and related temperature distribution, the mass flow itself can alter the microbial inactivation.

By increasing the flow velocity it is possible to produce turbulence and fluid mixture, which produces a more homogeneous treatment and also increases microbial inactivation, as shown in figure 5.

4 Conclusion

The simulation of PEF process with computational tools allows the design of adequate treatment chambers leading to improved efficacy of microbial inactivation and to reduced over-processing of the food.

The results of modelling show how an adequate treatment chamber design can increase the electric field strength and result in an increase of microbial inactivation. The gained improvement of microbial inactivation was in the range of one log cycle under the studied conditions.

The microbial inactivation due to increment of flow velocity shows that it is advisable to perform experiments at high flow rates, which is of industrial interest.

The insertion of grids in the electric field zone produces a homogeneous and more intense electric field. On the other hand, the insertion of grids produces mixture at relative low flow velocities, which produces a more homogeneous treatment and also increases the microbial inactivation. Furthermore, the generated mixture avoids the high temperatures generated in the zone near the insulators and leads to a more homogeneous temperature distribution in the treatment chamber. The latter has an important impact on the retention of heat sensitive compounds.

References


PULSED ELECTRIC FIELD-INDUCED CELL PERMEABILISATION OF POTATO TISSUE LEAD TO SUSTAINED METABOLIC CHANGES

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Abstract:
Metabolite profiling was used to characterize stress responses of potato tissue subjected to reversible electroporation, providing insights on how potato tissue responds to a physical stimulus such as pulsed electric fields (PEF), which is an artificial stress. Wounded potato tissue was subjected to field strengths ranging from 200 to 400 V/cm, with a single rectangular pulse of 1 ms. Electroporation was demonstrated by propidium iodide staining of the cells nucleae. Metabolic profiling of data obtained through GC/TOF-MS complemented with orthogonal projections to latent structures (OPLS) clustering analysis showed that 24 h after the application of PEF, potato metabolism shows PEF-specific responses characterized by the changes in the hexose pool that may involve starch and ascorbic acid degradation.

Key words: electroporation, stress response, cell membrane recovery

1 Introduction
In recent years, there has been an increasing interest in the use of pulsed electric field (PEF) technology due to its potential to induce non-thermal permeabilization of cell membranes. Depending on the cell properties (i.e. size, conductivity, shape and orientation) and electropulsation parameters (i.e. field strength, duration and number of pulses), the application of PEF may cause lethal damage to cells due to irreversible loss of cell membrane permeability properties, leakage of cytoplasmic contents and lysis (Aronsson et al. 2001).

By strict control of the electropulsation parameters, permeabilization may evade affecting the cell viability. In this non-lethal version of the PEF technique, physiological responses to PEF-induced stress are still largely unknown. When using PEF as stressor, interesting findings for the pharmaceutical field, such as the increased yields of a cytostatic compound in cell culture of Taxus chinensis (Ye et al. 2004), and for the food science and nutrition field, such as the increased concentration of antioxidants and phytoesters from oil seeds and fruits (Guderjan et al. 2005, 2007; Balasa 2007), encourage deeper metabolic studies that can help to a better understanding of the complex, dynamic metabolic behavior of plant tissues subjected to this novel (i.e. not present in nature) stress condition.

Metabolic profiling, which refers to the non-biased, comprehensive analysis of soluble cellular metabolites from a biological system (Dunn et al. 2005) has been applied to the study
of plant metabolism over the past several years (Wishart 2008). Global metabolic profiling is a research tool that can detect and monitor unidentified compounds as well as identified metabolites that play important roles in metabolism and physiology (Kaplan et al. 2004) and, in the context of this work, responses to stress.

We have performed metabolic profiling analysis using gas chromatography-mass spectrometry/time-of-flight (GC/TOF-MS) aiming at identifying compounds that exhibit PEF-specific responses when the electric pulses are applied to cause electroporation to the wounded potato tissue. This study allows discriminating between the potatoes response to PEF treatment against that of wounding alone, giving insights on to what extent PEF overlaps with other stresses. We suggest here that PEFs may cause effects in the metabolome of potato, which might be linked with cellular events following electroporation and recovery of cell membrane functionality.

2 Materials and methods

2.1 Preparation of samples and electrical treatments

Medium-sized potato tubers (13.0±3.0 cm in length, 7.0±0.5 cm diameter) harvested in the south of Sweden were manually washed and peeled. Slices, 15 mm thick were cut from the centre of each tuber. The slices were oriented perpendicular to the major tuber axis. Rectangular cross-section samples, 15 mm long and 6.0 mm wide, were obtained from the phloem parenchyma tissue of the slices using a pair of parallel sharp blades. Immediately after cutting, the sample was rinsed with distilled water (at 20ºC) and gently blotted with medical wipes to remove the excess of water from the sample surface.

Electric pulses were delivered to the rectangular samples through two parallel, flat stainless steel electrodes (35 mm long and 10 mm wide) separated 6 mm. Electric pulses were delivered axially to the tissue using a Cellect electromanipulation instrument (BioFusion SCI AB, Lund, Sweden). Samples were treated at varying voltages (120, 180, 240 and 300 V, which corresponds to the electric field strength in air of 200, 300, 400 and 500 V/cm) with 1 ms rectangular pulses.

2.2 Fluorescence microscopy

The effect of electrical treatments on cell membrane electroporation was tested by fluorescence microscopy. Rectangular samples of potato parenchyma were incubated for 20 h at 4ºC in a 25 µM solution of propidium iodide (Sigma, λ<sub>ex</sub> = 536 nm; λ<sub>em</sub> = 617 nm) in 10 mM PBS buffer, pH 7.5. After the incubation time, samples were PEF-treated. Untreated samples were used as negative controls. After treatment, a 1 mm thick cross section was cut from the rectangle and immediately placed in 5ml PBS buffer for 3 min under slight agitation to wash away the excess of dye and starch from its surface. Microscopic observations of the cross section were made with a Nikon inverted fluorescence microscope (Nikon Co., Kawasaki, Japan) at a magnification of 10X. Five slices from four different tubers were examined at each experimental condition.

2.3 Metabolic profile evaluation of the effect of electrical treatments

24 hours after PEF application, sample preparation, extraction, derivatization and GC/TOF-MS analysis of metabolites was performed as described by Gómez Galindo et al. (2009).

2.4 Processing of metabolites data

Non-processed MS data from GC/TOF-MS analysis were exported in NetCDF format to MATLAB 7.0 (Mathworks, Natick, MA, USA), as described by Gómez Galindo et al. (2009). The resolved MS spectra were matched against reference mass spectra using the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral
search program 2.0. Peaks were identified or annotated based on retention indices (RIs) and the reference mass spectra comparison to the following databases: commercial database from NIST v. 2.0, the Umeå Plant Science Centre (Umeå, Sweden) in house metabolomics database and the Max Plank Institute for Molecular Plant Physiology (Postdam, Germany) in house metabolomics database.

2.5 Statistical analysis for metabolic profiling

After peak annotation, the obtained data matrix after GC/TOF-MS analysis was normalized using the concentrations of the 11 internal standards. After normalization, the matrix was used for multivariate analysis. The orthogonal projections to latent structures (OPLS) method was performed as a supervised multivariate analysis with SIMCA-P+ 11.5 software (Umetrics AB, Umeå, Sweden).

In order to narrow down the variables (metabolites) most influential to the separation of the groups in the OPLS model, weight plots ($w* c[2]O$ vs. $w* c[1]P$) showing relationships between all factors and responses were used.

3 Results

3.1 Electroporation

The electroporation experienced by the samples at the applied PEF conditions is demonstrated by the penetration of propidium iodide in the cells and the staining of their nucleus (Fig 1), which can be clearly seen in the pictures as bright circles inside the cells.

3.2 Cluster analysis of metabolic profiles

After untargeted GC/TOF-MS analysis, the H-MCR process extracted 154 metabolite peaks and their mass spectra. Using the databases, 64 peaks were identified or annotated as known metabolites.

OPLS was applied to all metabolic peaks for all treatments (Fig 2). Data obtained at 400 V/cm were not included because cell death and microbial contamination were suspected. Twenty four h after the application of the treatments, 3 clusters were clearly observed, the metabolites from fresh tissue, those of the wounded tissue and those of the PEF-treated tissue, without any discrimination between the intensities of the applied electric field.

3.3 Specific responses to wounding and PEF-stress

The OPLS clustering was further analyzed as follows: (i) the fresh tissue (Time 0) differentiated from the tissue 24 h after wounding. (ii) The tissue 24 h after wounding from the tissue 24 h after it was wounded and PEF-treated. In this way, metabolites contributing to the described clustering, differentiating metabolic responses between wounding specific effects and PEF specific effects could be analyzed. As detailed in the Materials and methods.
section, weight and variable importance plots together with ANOVA analysis were used to select the discriminative metabolites.

Fig. 2. Orthogonal projections to latent structures (OPLS) analysis of metabolites from samples of potato parenchyma subjected to different treatments. Samples were either (■) untreated (Time 0), (●) wounded or (❖, ●, ▲) wounded and PEF-treated at varying voltages, as described in the Materials and methods section. Clustering analysis of samples is reported 24 h after treatments.

Metabolites showing to be significantly affected 24 h after wounding are highlighted with a circle in a simplified metabolic pathway map (Fig 3). Only known compounds are reported in Fig 3. Metabolites are annotated red for increased concentration and blue for decreased concentration.

Differences of metabolites obtained 24 h after the tissue was wounded and PEF treated are highlighted in Fig 3 as PEF specific effects. When the wounded tissue has been subjected to an electric field, its overall metabolic response is apparently the same as the wounding response regarding the changes in the amino acid pool and the tendency to increase the levels of sterol and galactosyl glycerol-like compounds. Interestingly, the metabolic response after PEF stress differentiates from wounding in the hexose pool. There is an accumulation of sucrose and fructose as well as an accumulation of threonic acid, a degradation product of ascorbic acid, which will eventually contribute to the hexose pool (Loewus 1999).

Another interesting deviation of the wounding specific response when PEF is applied is the high level of quinate and low level of chlorogenic acid. Chlorogenic acid constitutes up to 90% of the total phenolic content of potato tubers and is an important defence-related compound, protecting potatoes from attack by phytopathogens and insects (Friedman 1997), and it is accumulated in wound-healing potatoes as a part of their defence mechanism. Surprisingly, the concentration of chlorogenic acid of the PEF-treated tissue is similar to that of the fresh potato (Time 0). Visual assessment of the potato samples did not reveal differences in the levels of enzymatic browning between the wounded and the PEF treated tissue that could justify the differences in the levels of chlorogenic acid.

4. Discussion

Our results demonstrate that 24 h after the application of PEF, potato metabolism shows PEF-specific responses characterized by the changes in the hexose pool that may involve starch and ascorbic acid degradation. In potatoes, accumulation of sucrose and other hexoses has
been reported to be a common stress response when the stressor, such as cold temperatures and drought, targets the plasma membrane and may produce electrolyte leakage (Herppich et al. 2001; Blenkinsop et al. 2004). This sugar accumulation has been directly correlated with starch degradation (Blenkinsop et al. 2004). Increase in soluble sugars may play a role in osmoregulation and possibly also in the activation of respiratory metabolism (Espen et al. 1999). Osmoregulation might be very relevant for the case of PEF stress as cells in the tissue might experience a significant osmotic imbalance after pulsing but not after wounding, with different metabolic consequences.

With the application of PEF, opening of pores in the plasma membrane will result in the efflux and influx of polar molecules. After the pulse application, resealing process takes place in a time scale of seconds or minutes. After resealing, the cell membrane recovers its properties in a long term physiological process that may take from several hours to days (Teissie et al. 2005). This process might involve ATPase activity, which uses the chemical energy of ATP, helping the cells to take up the leaked ions against the concentration gradient (Arora and Palta 1991). This hypothesis of the high metabolic energy requirements during the recovery process is supported by our results suggesting that the wounded tissue subjected to PEF is actively mobilizing its carbon energy sources that might involve starch and ascorbic acid degradation, contributing to the hexose pool. The time scale of hours where the reported PEF-specific effects took place agrees with the time scale needed for the recovery process.

Fig. 3. Changes in the levels of metabolites in potato tissue caused by wounding and by the application of PEF to the wounded tissue. The changes in metabolite contents, specific for each kind of stressor, are highlighted in the simplified metabolic maps with a circle. Metabolites are annotated red for increased concentration and blue for decreased concentration.
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EFFECTS OF ELECTRIC MICRO-CURRENT ON WINE CHARACTERISTICS

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Abstract:
A new approach to modulate oxido-reduction potential (ORP) by continuous electric micro-current passing between platinum coated titanium electrodes during fermentation and maturation of red wine was examined. The application of constant current during wine fermentation stimulated the rate of sugar consumption and the production of ethanol, glycerol and succinic acid. Saccharomyces cerevisiae viable cells and the formation of acetic acid, acetaldehyde and higher alcohols were similar to that found in the control wine in which the redox potential was modulated oxygenating the mass. The sensory analysis revealed that wine produced with micro-current presented more accentuated typical varietal notes of Sangiovese with respect to the control wine. The effect of the application of micro-currents on the maturation of Sangiovese wines was followed by two different approaches: a) oxygen production by high electric voltage to simulate micro-oxygenation process; b) modulation of ORP without oxygen production. During wine maturation, micro-currents induced a greater polymerization and stabilization of colour compounds and a minor bitter and astringency taste than micro-oxygenation and barrel aging of the wine.

Key words: micro-current, titanium electrodes, alcoholic fermentation, wine maturation.

1 Introduction
Among the most interesting practices utilized today in the elaboration of equilibrated wines, controlled aeration or oxygenation of musts and wines are receiving particular attention [1, 2]. Numerous studies have shown how colour, aroma, flavour and in-mouth tactile sensation are markedly modified by the interaction between dissolved oxygen in the wine and various phenolic and odorous constituents formed and modified during fermentation and ageing [3, 4]. By assuming that the reagents which intervene in these redox reactions are electrons and positive charges, it could be possible to guide, regulate and control them by electrochemical means. Two electrodes into the must or wine separated by a membrane permeable to electric current stimulated oxidation reactions that characterise the fermentation and stabilization processes of wine-making [5-7]. The role of oxygen, which is indispensable in classic wine maturation process (aeration and micro-oxygenation), is brought into discussion. While the possibility of wine-making in the complete absence of oxygen makes it possible to safeguard both phenolic compounds responsible for colour and volatile compounds responsible for varietal and fermentation aroma; on the other hand, during the fermentation process oxygen is needed by the yeast to synthesize lipids essential for growth, for plasma membrane integrity, and the maintenance of high glycolytic and ethanol production rates [8].

The aim of this study is to evaluate the effect of the modulation of oxido-reduction potential (ORP) by micro-currents on: 1) the growth and fermentative activity of yeast during red wine production; 2) the maturation process of red wine with regard to colour and some taste characteristics (bitter and astringency). Two approaches have been followed: a) oxygen production by high electric voltage during wine maturation to simulate micro-oxygenation
process; b) modulation of ORP in absence of oxygen production to stimulate the reaction of phenol compound polymerization during wine stabilization

2 Materials and Methods

2.1 Electrochemical devices

1) Device for micro-oxigenation trial. The electrolytic cell was a 25-Hl plastic tank. A titanium net (total surface area 35 m²) was attached to a central axis in the centre of tank, which in turn was connected to an electric motor to permit it to slowly circulate within the tank. The tank was the anodic cell of the electrolytic system. Three smaller tanks (cathodic cells) were positioned laterally. The electrodes for these cells were composed of a series of titanium plates. The three cathodic cells were connected in series by way of a specifically-designed circuit and separated from the anodic cell by a semi-permeable membrane (type CR67HMR-F06 by Ionics, Massachuset USA). The external cathodes were filled with the same wine contained within the main tank. Electrodes, to measure Redox potential, were introduced into the main tank through two small holes on the side of the tank.

2) Device for oxi-do-reduction potential (ORP) modulation. A 7-L glass cell separated into two compartments by an ion-exchange membrane (type CR67HMR -F06 by Ionics, Massachuset, USA) was used. The anodic compartment contained a platinated titanium net with an area of 0.1 m² and was provided with various fittings for the electrical and redox potential probes, stirring rods, inert gas entry, and sampling ports. The cathodic compartment contained a titanium net and feed and discharged ducts for an electrolyte, preferably a sacrificial wine, which served to maintain an osmotic equilibrium across the membrane. The anodic compartment was connected by a peristaltic pump to the external container to allow a continuous circulation during treatments (alcoholic fermentation or maturation).

2.2 Fermentation trials

30 Kg of Sangiovese grapes from 2007 vintage from vineyards located in Toscany (Chianti Classico area) were crushed and destemmed and 20 Kg of must (reducing sugars 260 g/L, pH 3.4, titratable acidity 4.7 g/L) were placed in glass fermentors (30 L), fitted with fermentation lock and redox potential probe. Fifty mg/L of sulfur dioxide was added before inoculation with 250 mg/L of F83 (Laffort) S. cerevisiae rehydrated yeast. The grape skins were maintained down into fermenting must by a stainless steel net located just under the upper part of the liquid, reproducing the submerged cup practice. Fermentation temperature was maintained at 25 °C. Two trials were set up: fermentation subjected to micro-current treatment (3mA), in the absence of oxygen (trial M) and fermentation with daily oxygenation (delestage practice), as control (trial C). Each trial was performed in duplicate.

2.3 Wine maturation with micro-currents

Sangiovese wine from 2006 vintage after six weeks of the end of alcoholic fermentation was utilised.

2.3.1 Maturation with electrochemical micro-oxigenation

In this trials the device 1 was utilised applying a current of 0.75 A. This corresponds to 0.3 mA/L, which is the same as a 60 ppm/L/month oxygen dose. The treatment lasted 10 days (20°C), then the wine was racked and stored in stainless steel (18°C), until analysis. Two stainless steel tanks of 25 Hl capacity were filled with the same wine: one was a control and the other had a micro-oxigenator applied which delivered an oxygen dose of 5 ppm/L/month. Finally, three barriques were filled with the same wine. The described conditions lasted four months at 18°C and were done in duplicate.
2.3.2 Maturation with electrochemical modulation of ORP

A 30-L glass container was completely filled with wine and connected to the anodic compartment of device 2 as above described. To allow a continuous circulation of wine during electrochemical treatment, the flow was maintained at 30 liter/h to realise a complete cycle. A direct current of 0.37 mA was applied for 10 h, then the wine was racked and stored in stainless steel (18°C), until analysis. As control a 25 L glass container was filled with the same wine and stored at 18°C. The described conditions lasted two months and were done in duplicate.

2.5 Analyses

Saccharomyces cerevisiae viable cells were counted using WL medium (Oxoid, Milano, Italy). Glucose, fructose, succinic and acetic acid, ethanol and glycerol were analyzed by a Perkin Elmer series 200 HPLC, containing two columns Polyspher OA KC (Merck, France) in series. The columns were eluted at 95 °C with 0.00125 N of sulfuric acid at flow rate of 0.4 ml/min. Detection was by means of a Perkin Elmer series 200 Refractive Index. Ethyl acetate, acetaldehyde, and higher alcohols were analyzed by direct injection of the sample into GC following the method described by Bertuccioli [10]. Colour intensity and Hue were determined spectrophotometrically [11], free anthocyanins and polymeric anthocyanins were evaluated by HPLC [12]. Wines were sensorially evaluated for red fruit (cherry) floral (iris) and global aroma and for bitterness and astrincency taste by a panel of 12 trained judges [13].

3 Results

3.1 Modulation of ORP during fermentation

The intensity and relation between oxydation and reduction processes which took place during alcoholic fermentation can be measured by on-line ORP measurements. The lowest value (-100 mV) of ORP was observed for both trials at 48h, when S. cerevisiae cells reached maximum growth and started to produce ethanol.

![Graph](https://via.placeholder.com/150)

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Fig. 1: Evolution of redox-potential in micro-current (…) and control (→) fermentation.

A sharp increase of ORP values was observed in the control trial, in coincidence with the oxygenation of fermenting must. In general during the fermentation process the ORP values of electrostimulated fermentation were higher with respect to the control (figure 1). The application of micro-current did not show any effect on the evolution of viable cells of S.
cerevisiae (figure 2). The time-course of sugar consumption and ethanol formation showed that microcurrent stimulated the fermentative activity of yeast cells: the sugar was consumed faster and in a more complete way with respect to the control (figure 3).

![Graph of S. cerevisiae viable cells, sugar consumption, and ethanol formation.](image)

At the end of alcoholic fermentation the content of glycerol, succinic and acetic acid, as well as acetaldehyde, ethyl acetate and higher alcohols was measured (table 1). Significant differences were observed between the content of glycerol and succinic acid of electro-stimulated and control wines, whereas the content of acetic acid, acetaldehyde, ethyl acetate and higher alcohols was unaffected by the application of microcurrents.

<table>
<thead>
<tr>
<th>Wine sample</th>
<th>Glycerol g/L</th>
<th>Acetic acid g/L</th>
<th>Succinic acid g/L</th>
<th>Acetaldehyde mg/L</th>
<th>Amylic alcohol mg/L</th>
<th>isoamyl alcohol mg/L</th>
<th>n-propanol mg/L</th>
<th>Ethyl acetate mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10.5±0.20</td>
<td>0.38±0.03</td>
<td>1.8±0.2</td>
<td>50±6</td>
<td>68±5</td>
<td>40±6</td>
<td>28±6</td>
<td>35±3</td>
</tr>
<tr>
<td>M</td>
<td>11.6±0.18</td>
<td>0.42±0.02</td>
<td>2.6±0.1</td>
<td>60±4</td>
<td>70±3</td>
<td>40±6</td>
<td>40±6</td>
<td>30±5</td>
</tr>
</tbody>
</table>

Tab. 1: Mean value (± SD) of the content of some acids and alcohols of wines.

The sensory analysis showed that wine produced with micro-current presented more accentuated typical varietal notes (cherry and iris aromas) of Sangiovese with respect to the control wine (Table 2).

<table>
<thead>
<tr>
<th>Wine sample</th>
<th>Cherry Aroma</th>
<th>Iris aroma</th>
<th>Reduced aroma</th>
<th>Global aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.4a</td>
<td>4.2a</td>
<td>8.3a</td>
<td>3.8a</td>
</tr>
<tr>
<td>M</td>
<td>7.3b</td>
<td>5.8b</td>
<td>3.8b</td>
<td>7.5b</td>
</tr>
</tbody>
</table>

LSD 1.5***  LSD 0.9**  LSD 1.8***  LSD 2.1***

** , *** Significant at p<0.01 and 0.001, respectively

Tab. 2: Intensity of sensory aroma attributes of Sangiovese wine.

3.2 Wine maturation by electrochemical micro-oxygenation

The ORP value of the wine had a decreasing trend from the first day; after ten days was 100 mV. To evaluate the degree of polarization of the titanium surface the potential applied to the net was measured. The electric potential increased in the first 10 days, from 1.9 to 2.5 V. Under these conditions, oxygen developed on the surface of the titanium net (anodic cell). Table 3 shows the concentration of some phenolic parameters and the intensity of bitterness and astringency taste measured after treatments. Only in the case of the wine treated with micro-currents, the content of free anthocyanins and the Hue value were lower, whereas the content of polymeric anthocyanins and the value of colour intensity were higher with respect
to the control. A lower value of bitter and astringency taste was observed in electrochemical treated wines with respect to those micro-oxygenated and barrel aged.

<table>
<thead>
<tr>
<th>Maturation condition</th>
<th>Free anthocyanins(^1) mg/L</th>
<th>Polimeric anthocyanins(^1) mg/L</th>
<th>Color intensity</th>
<th>Hue</th>
<th>Bitterness</th>
<th>Astringency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control in stainless steel tank</td>
<td>180 ±11</td>
<td>190±27</td>
<td>8.84±0.02</td>
<td>0.78±0.01</td>
<td>6.8(^a)</td>
<td>5.8(^a)</td>
</tr>
<tr>
<td>Micro-oxygenation in stainless steel tank</td>
<td>168±14</td>
<td>199±18</td>
<td>9.13±0.02</td>
<td>0.79±0.01</td>
<td>6.4(^a)</td>
<td>5.5(^a)</td>
</tr>
<tr>
<td>Barrique</td>
<td>148±12</td>
<td>207±16</td>
<td>8.85±0.02</td>
<td>0.81±0.01</td>
<td>5.2(^b)</td>
<td>4.9(^b)</td>
</tr>
<tr>
<td>Electrical current</td>
<td>128±15</td>
<td>247±21</td>
<td>10.03±0.02</td>
<td>0.71±0.01</td>
<td>4.8(^b)</td>
<td>4.2(^b)</td>
</tr>
</tbody>
</table>

\(^1\) as malvidin-3-glucoside; *, ** Significant at \(p<0.05\) and 0.01, respectively

Tab. 3: Changes in concentration (± SD) of anthocyanin fractions, colour intensity, Hue, bitterness and astringency taste of wines after 4 months of different maturation conditions.

3.3 Wine maturation by electrochemical modulation of ORP

Titanium tends to polarize itself with the passage of current. To maintain conditions of constant current, it is necessary to raise the electric potential. When the electric potential exceeds 1.23 V, oxygen production occurs. To avoid the oxygen production, a modified titanium electrode with a coating of platinum has been utilised [9].

The ORP value of the wine decreased from 130 to 110 mV in the first two hours and then remained at this value until the end of treatment. The electric potential increased in the 10 hours, from 0.3 to 0.5 V. Under these conditions, no oxygen developed on the surface of the platinated titanium net. Table 4 shows the value of some phenolic parameters measured during the treatment and after 2 months of wine maturation in glass containers. After 1 h of treatment the value of colour intensity increased, whereas the value of Hue decreased to levels that remained the same until the end of treatment (10 h). The content of free anthocyanins showed a slight decrease over the time, whereas the content of polymeric anthocyanins, a slight increase. After two months of storage, the values of colour intensity and Hue remained unchanged in the electrochemical treated wines, whereas the content of free anthocyanins showed a further reduction and consequently the content of polymeric anthocyanins increased.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Wine</th>
<th>Electrical treatment</th>
<th>Control Wine</th>
<th>Treated Wine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hour</td>
<td>hour month</td>
<td>hour</td>
<td>hour month</td>
</tr>
<tr>
<td>Free anthocyanins (mg/L)(^1)</td>
<td>208 ±12</td>
<td>183±11 182±10 172±13</td>
<td>161±10 147±12</td>
<td>179±13 118±14</td>
</tr>
<tr>
<td>Polimeric anthocyanins (mg/L)(^1)</td>
<td>125±16</td>
<td>128±13 139±12 139±15</td>
<td>148±11 170±13</td>
<td>138±14 212±15</td>
</tr>
<tr>
<td>Color intensity</td>
<td>8.2±0.02</td>
<td>9.1±0.01 9.4±0.01 9.4±0.01 9.6±0.01 9.8±0.01</td>
<td>7.4±0.01 9.6±0.01</td>
<td></td>
</tr>
<tr>
<td>Hue</td>
<td>0.72±0.01</td>
<td>0.68±1 0.67±1 0.67±1 0.66±1 0.66±1</td>
<td>0.74±0.01 0.65±0.01</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) as malvidin-3-glucoside

Tab. 4: Changes in concentration (± SD) of anthocyanin fractions, colour intensity and Hue of wines during 10 hours of treatment and after 2 months of maturation.
4 Conclusion

The obtained results suggest that it is possible to substitute oxygenation of the must with the application of micro-current since the consequent modulation of ORP permits the yeast cells to develop and adequately ferment and to highlight the aroma responsible for wine typicity. The application of micro-currents during wine maturation (micro-oxigenation) induces a greater polymerization and stabilization of colour of red wine and a minor bitter and astringency taste than micro-oxigenation and barrel aging of the wines. Micro-current treatment at lower potential under oxygen production induces the formation of stable colour compounds that continues during the wine storage in absence of oxygen.

5. References

ELECTRO-ULTRAFILTRATION OF LIPOSOMAL DISPERSIONS FOR THE REMOVAL OF TRACE MICROPOLLUTANTS

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Abstract:
In our current industrial world, there is an increasing need for improving and updating wastewater treatment methods. An important effort in this field is detection and removal of trace organic materials from the wastewater. Removal of such organic molecules by sorption to vesicles is a promising method which still is not completely characterized and optimized especially regarding the efficiency and energy consumption issues. In this research we are going to study and improve removal efficiency of small organic molecules and lower the energy requirements by a novel system, based on the sorption of these molecules by liposomes, followed by electro-ultrafiltration.

In this document we present the results of the sorption of phenol (as an important trace organic pollutant in industrial wastewater) to Dimethyldistearylammonium Chloride (DODAC) liposomes and afterwards filtration of the resulting dispersion.

According to the results, the sorption of phenol on 0.5% DODAC dispersion at neutral pH and room temperature is 27-39%. This sorption increases to 89% at pH=11.7. Applying electric field improves both the filtrate flow rate and the retention considerably. Applying electric field of 5 V/cm increases the filtrate flux at 200 kPa by a factor of 19.

Key words: ultrafiltration, energy reduction, micropollutant removal, liposomal enhanced ultrafiltration

1 Introduction

Presence of ecotoxic substances such as pesticides, endocrine disrupters, etc. at low levels in wastewater streams has made the wastewater treatment sector to focus on measurement and removal of such micropollutants. In this area phenolic compounds are of great importance because of their toxicity for protoplasm and effect on odour and taste of water and fish [1], [2] and due to their abundance in wastewater streams of different processes such as organic chemicals, plastics, steel and petroleum plants [2], [3]. Different methods have been developed for detection and measurement of organic micropollutants and specifically phenolic compounds. The most widely applied methods
are chromatographic and spectrophotometric methods [2], [4], [5], [6]. The former is more suitable for selective determination and separation of phenolic compounds [4], [7] while the latter is more applied for measurement of total phenolic components [8].

Taking into consideration that the first method is very time-consuming and the second method is not selective, fluorescence spectrophotometry is known to be a fast and moderately selective method and its selectivity can be improved by collecting a two-dimensional fluorescence spectrum [9].

Next step after detection and measurement of the micropollutants is their removal from the wastewater stream. There are many different methods among which biological treatment, adsorption, advanced membrane technologies and reverse osmosis are the most widely used methods. While adsorption and biological processes are not always able to remove these target molecules with satisfactory efficiency, membrane technologies and reverse osmosis are not very efficient concerning the energy consumption aspects. An approach in this regard was adsorption of organic pollutants into micelles of surfactants followed by ultrafiltration of the mixture solution [10], [11], [12], [13], [14]. The sorption of pollutants into the bilayer could be increased when micelles are replaced by liposomes [10]. On the other hand since these vesicles are bigger than micelles in size, the filtration process could be facilitated and energy requirements could be decreased.

The aim of this project is to improve the removal efficiency of low molecular weight pollutants by absorbing them into cationic liposomes and filtering the dispersion afterwards. Since the bottle neck of filtration processes is low filtrate flow rate due to blockage of membrane and fouling, ultrafiltration in previous studies is replaced by electric field enhanced ultrafiltration which can improve the filtration flow rate[15], [16].

In this paper the experimental methodology and the results obtained for the sorption of phenol on DODAC liposomes and the electro-ultrafiltration of the resulting dispersion are explained.

2 Materials and methods

2.1 Preparation of liposomal dispersion

Dimethyldistearylammonium Chloride (DODAC) flakes (>95% purity, TCI, USA), were dissolved in distilled water. 5mM Sodium Chloride was added to assure the electrolytic characteristics of the solution during the electro-filtration process. This batch was then heated up to 60°C with bain-marie method for 1 hour while stirred continuously with a magnetic stirrer.

2.2 Fluorescence spectrophotometry

A Fluorescence spectrophotometer (Cary Eclipse, Varian Analytical Instruments, USA) was used to measure the fluorescence intensity. Samples were measured in a quartz cuvet at excitation and emission wavelengths of 250-280nm and 290-330nm respectively. Calibration curves and all other measurements were based on the integration of the intensity values in the mentioned ranges.

2.3 Ultracentrifugation (UC)

Ultracentrifugation experiments were performed in a L7-55 Beckman ultracentrifuge with a SW55Ti rotor (Beckman, USA) and Polyallomer centrifuge tubes (Beckman,
USA). Samples were centrifuged at a speed of 35000 rpm for ±17 hours at 20°C. After the centrifugation the supernatant layer which contained the liposomes was pierced with spinal needles (Quincke type point, BD Medical, US) and the bottom aqueous layer was collected. The remainder of the liposomes were solubilized by Cetyl trimethylammonium bromide (CTAB, Acros Organics, USA) solution prior to measurement.

2.4 Equilibrium dialysis (ED)
Slide-A-Lyzer dialysis cassettes of 0.5-3 ml (Thermo Fisher Scientific, USA) were used for determination of sorption. Although the equilibrium was reached after 1 hour but all the samples were given 2 hours for equilibrium.

2.5 Filtration setup
Filtration tests were done in a dead-end, double-sided, bench-scale filtration setup (2×38.5 cm² filter area) equipped with inert electrodes. Pall Supor-100 membranes (pore size 0.2 μm) were used in filtration experiments.

3 Results and discussion

3.1 Sorption determination
Sorption of phenol on 0.5% DODAC liposomes was measured by UC and ED methods. The results of the two methods are in agree and express a sorption of 27-39% which is constant disregarding to phenol concentration (fig. 1). Based on the experimental data, statistical tests show that sorption values of phenol with concentrations ≤10 mM into 0.5% DODAC solution are not significantly different. This sorption is a relative value which is equal to absorbed concentration over the total concentration of phenol.

![Bar chart](image)

**Fig. 1**: Sorption of phenol into 0.5% DODAC liposomal solution at 20°C and pH=6.5, measured by ultracentrifugation (UC) and equilibrium dialysis (ED) methods

This can be explained by constant value of partition coefficient (eq. 1). At constant bilayer concentration, increase in total phenol concentration leads to increase in its
absorbed fraction and non-absorbed fraction at the same rate. Once the liposomes are completely saturated with phenol molecules, addition of extra phenol is expected to increase the non-absorbed fraction only. So at that moment the sorption (%) should start to decrease. According to the experimental data this saturation concentration should be above 10mM.

$$k = \frac{C_{bilayer}}{C_{water}} = \frac{M_{bilayer}}{M_{water}} \times \frac{V_{water}}{V_{bilayer}}$$

(1)

k is partition coefficient, $C_{bilayer}$ and $C_{water}$ are the concentrations of phenol in bilayer and water (mol/cm$^3$), $M_{bilayer}$ and $M_{water}$ are moles of phenol in bilayer and water, and $V_{bilayer}$ and $V_{water}$ are volumes of bilayer and water (cm$^3$) respectively.

27-39% sorption which is obtained by experiments is comparable with the value obtained from theory. Considering log $k_{ow}$=1.46 for phenol [17] and density of 0.84 g/cm$^3$ for DODAC and applying eq. (1), one can obtain a roughly estimated value of 17.2% for sorption of phenol on 0.5% DODAC which is in the same order of magnitude as the experimental results.

Since phenol is a weak acid (pK$_a$=9.95), its dissociation rate increases in basic environments. This dissociation seems to be in favour of the sorption to cationic liposomes such as DODAC due to formation of electrostatic interactions. When the spectrum of phenol on fluorescence spectrophotometer was investigated, it was noticed that the spectrum started to change only at pH values above 10. The experiments also show an increased sorption up to 89% at pH=11.7 (fig. 2). The sorption starts to decrease when the liposomes are saturated with phenol.

![Fig. 2: Sorption of phenol into 0.5% DODAC liposomal solution at 20°C and different pH values, measured by ultracentrifugation method](image-url)
3.2 Filtration

To evaluate the kinetics of the filtration process, the filtrate mass was measured and monitored during the filtration process. Results of the filtration without electric field show that the permeate flux at 200 kPa was very low (≈0.16 cm³/cm².h). This is mainly due to the accumulation of the liposomes on the surface of the membrane and obstacle of the membrane pores and consequently increase in the resistance against the filtration. An increase of pressure to 800 kPa caused a slight increase in the permeate flow rate (increase by a factor of ≈2). At very high pressure (1000 kPa) the permeate flux had a considerable increase (fig. 3).

![Graph showing the effect of pressure on permeate mass during filtration of 0.5% (w/v) DODAC dispersion at 20°C.](image)

**Fig. 3:** Effect of pressure on permeate mass during filtration of 0.5% (w/v) DODAC dispersion at 20°C

The effect of applying electric field during filtration is illustrated in fig. 4. In a filtration experiment at a fixed pressure of 200 kPa, applying a low electric field of only 5 V/cm increased the permeate flux very significantly (increase by a factor of ≈19). Filtration at 200 kPa in presence of a 5 V/cm electric field had much higher filtrate flux than filtration at 1000 kPa in the absence of the electric field. This is due to the fact that the cationic DODAC liposomes were repelled by the anode which was positioned at the membrane side and therefore the fouling was retarded very considerably. The same pattern was noticed during the filtration at 800 kPa without and with an electric field of 5 V/cm.

Additional to the improvement of filtration flux, applying electric field during filtration is supposed to have another advantage. This is the increase in retention of both, liposomes and phenol. This supposition is based on the same logical reasoning which was explained for the improvement of permeate flux due to application of electric field (i.e. repulsion of DODAC liposomes by anode).
4 Conclusions

Based on the results and discussions mentioned above it was shown that the sorption of phenol as a model organic micro-pollutants to the bilayer of the cationic liposomes of DODAC is not determined by the concentrations of phenol below the saturation point while the concentration of bilayer is constant. This is due to the constant partition coefficient value at above conditions. Therefore to increase the sorption, the bilayer concentration should be increased or temperature should be raised to above the transition temperature (37.5 °C for DODAC) or pH should be increased to above 10.

Applying electric field during the filtration process can improve both the permeate flux and the retention of phenol as well as DODAC. While increasing the filtration pressure from 200 kPa to 800 kPa improves the permeate flux by a factor of 2, applying only 5 V/cm electric field at the same pressure (200 kPa) increases the flux by a factor of 19. Therefore electro ultra-filtration can be significantly more efficient than ultra-filtration.

References


Abstract:
Process of homogeneous nucleation that occurring spontaneously throughout a supercooled fluid still remains difficult to envisage and control. We present here a novel approach to control ice nucleation in water by application of high electrostatic field. In order to determine the influence of the electrostatic field on ice nucleation, an experimental set-up was designed. A high DC voltage (10 kV) was applied during sample cooling. The temperature was measured using optical fibres calibrated for each experiment. Experimental results indicated a strong effect of the electrostatic field on the ice nucleation process. The increase of the applied voltage tended to shift the nucleation temperature towards higher values. We also explored the influence of the electrostatic field on the nucleation temperature on an aqueous solution of ethanol. Similar trends were measured for concentrations ranging from 5% to 20% (w/w). The electric field strength in the apparatus was calculated using Comsol-Multiphysics®. Numerical results indicated that the electric field was almost homogeneous inside the sample. Moreover, simulations pointed out the difficulty to analyse experimental results from the literature. This study proved that high electrostatic fields could be employed to control ice nucleation and open the way to further investigations on other samples.

Key words: Nucleation, supercooling, freezing, electric field, model

1 Introduction

The control of ice nucleation is of major interest in many processes. The freezing of biological or food products should be conducted at a specific freezing rate to ensure the quality of the products. The nucleation rate governs the number of ice crystals whereas the rate of heat transfer governs the growth of ice crystals [1]. For example, the baking performances of the French frozen dough are altered by high freezing rate [2]. Nevertheless, most of the time high freezing rates are expected to preserve the food quality [1, 3]. As convection and conduction are the main heat transfer modes, the ice crystals firstly appear close to the surface of the product. This apparition strongly depends on the supercooling degree in this region. As in conventional processes, this supercooling degree can not be controlled; innovative processes should be investigated. The pressure shift freezing process is, for example, a process that permits to reach a high supercooling degree in the whole product [4, 5]. Other methods like ultrasound assisted freezing [6], dehydrofreezing [7] and most recently electrofreezing [8, 9] are also studied.

Electrofreezing is based on the ability of a strong electric field to induce the crystallization of supercooled water. In liquid water, clusters of molecules are linked by hydrogen bonds.
Some molecular dynamics simulations confirmed that a DC external electric field can modify the structure of these clusters [11, 12]. In a recent work [13], we underline the thermodynamics aspect of electrofreezing and analyse some previous experimental studies. If some authors did not observe any effects even when the electric field strength was up to $10^6$ V/m [14], other authors reported a decrease of the supercooling degree at lower electric field strength $10^5$ V/m [9]. In the present study, pure water and aqueous ethanol solutions are frozen under a high electrostatic field in an original experimental set-up. Moreover, numerical simulations are performed to analyse the electric field distribution in the set-up and to discuss on the strength of the electrostatic field.

2 Materials and methods

2.1 Experimental set-up

The original experimental set-up consists in a sample container assembled on a cooling-heating system, one pair of plate electrodes (50 x 50 x 2 mm) placed in parallel, a DC voltage generator and a real-time temperature measurement system. The sample container, schematically presented in Figure 1, is made of a dielectric material (Acetal Ertacetal) which dielectric strength is equal to 20 kV/mm.

![Fig. 1: Scheme of the experimental set-up.](image)

The sample is placed in a square hole (20 x 20 x 5 mm) machined in the centre of the container. An aluminum electrode is placed 2 mm above the sample surface; the ground electrode is the metal plate of the MPA100. The sample container is assembled on a Peltier element (Melcor, USA) and the high voltage is supplied by DC voltage generator (Sefelec, France) connected to the upper electrode. Voltage V0 applied in the experiments is 10 kV. Cooling of the sample is obtained by the Peltier element connected to the temperature controller Eurotherm 2408. The temperature of the sample is measured with the Luxtron 790 Fluoroptic Thermometer equipped with optical fiber (Luxtron, model: 00-11511-01) and recorded by the data acquisition system (Datalog – AOIP-France). The temperature sensor is installed inside of the measurement cell in a central location (1 mm lower surfaces of the
sample). Calibration against a reference platinum probe (Comptoir Lyon Allemand – Lyon-France) ensures measurements with an accuracy of ±0.1 °C.

The overall apparatus is placed in a cooling cabinet at 10°C to avoid thermal perturbation from ambient air. Experiments consist in pre-cooling the sample (1.6 ml) at +1°C and then to cool it at -22°C. During the cooling time, a DC voltage \( V_0 \) is applied to the aluminium electrode. Measurements are repeated at least 10 times on a new sample.

The first set of experiments concerns distilled water and the second set concerns aqueous solutions of ethanol at different concentrations (up to 20 % w/w). This choice is justified by the great interest of these solutions in ice slurry generators [15].

2.2 Numerical model

In previous studies [9, 13], authors imposed a potential at one electrode and calculated the electric field strength at the surface of the sample or a mean value (applied potential/interelectrode distance). This latter approach is correct for a homogeneous sample between electrodes but when the sample is not in direct contact with the electrodes, the mean electric field strength is not meaningful. In our experimental set-up, due to the container thickness, the sample is 2 mm above the ground electrode. Air occupies the 2 mm gap between the surface of the sample and the other electrode. To get a better knowledge on the electric field distribution, Maxwell equations are solved in the whole apparatus using Comsol Multiphysic®, a Partial Differential Equations solver based on the Finite Element Method. As it is a pure electrostatic case (no electric current), only Laplace’s equation is solved (Eq. (1)). Lagrange-quadratic elements are chosen as the basis functions with triangular-shaped elements. To limit computational time, a two-dimensional configuration is considered and leads to a mesh with nearly 17000 elements.

\[
- \nabla \cdot (\varepsilon \varepsilon_r \nabla V) = 0
\]

(1)

The dielectric permittivities \( \varepsilon_r \) of the materials are 1, 3.6 and 80 respectively for the air, the container and the sample (water).

3 Results

3.1 Experimental results

Figure 2 presents the typical temperature-time plots during the cooling of 1.6 ml of water with and without the application of the DC electric field. The nucleation temperature TN degree is -8.6°C when 10 kV is applied whereas it is -11.5 °C in the absence of an electric field. Similar results were previously obtained for several applied voltages [13]. The nucleation temperature is shifted towards a higher value by the application of an electric field that modifies the thermodynamical properties of liquid water. There are structural changes in the hydrogen bonded network [9, 11]. Figure 3 presents the temperature evolution during the cooling of an aqueous solution of ethanol at a concentration 10% (w/w). Similar trends are observed whatever the concentration. Table 1 gives the nucleation temperature for these different solutions. At the concentration 20% (w/w), we give no value of nucleation temperature when no electrostatic field was applied because nucleation did not always occur at -22°C. At the other concentrations, the shift of the nucleation temperature towards a higher value is clearly demonstrated for aqueous solutions of ethanol. It can also be noticed that this shift increases with the concentration. At 5% (w/w), the shift is less than 3°C whereas it is close to 5°C at 15% (w/w). These results encourage investigating on the understanding of the electric mechanisms inside the solution. They are also promising to look at other aqueous solutions and even at real food samples.
Fig. 2: Temperature profiles during the cooling of water with and without electric field.

Fig. 3: Temperature profiles during the cooling of an aqueous solution of ethanol 10% (w/w) with and without electric field.
Ethanol % (w/w) | 0  | 5  | 10 | 15 | 20
--- | --- | --- | --- | --- | ---
$T_N$ (°C) | -11.53 ± 0.71 | -14.21 ± 0.17 | -16.11 ± 0.23 | -18.54 ± 0.55 | /
$V_0 = 0$ kV  | (a) |

$T_N$ (°C) | -8.56 ± 1.62 | -10.30 ± 1.71 | -11.65 ± 1.17 | -13.87 ± 1.22 | -18.12 ± 0.68
$V_0 = 10$ kV | (b) |

Shift of $T_N$ (°C) | 2.97 | 3.91 | 4.46 | 4.67 | /
(b-a) |

Tab. 1: Nucleation temperature (average value and standard deviation) for aqueous solutions of ethanol with and without electric field.

### 3.2 Numerical results

Figure 4 presents the electric field strength in the sample and the electric streamlines in the whole apparatus for an applied voltage $V_0=10$ kV. As the materials (sample, air, container) have different electric permittivities, the electric field is deviated and the streamlines are not parallel. Concerning the electric field strength, the mean value calculated from a basic approach (applied voltage/interelectrode distance) would give 1.1x10$^6$ V/m. Our simulation indicates that in the sample, the electric field strength ranges from 4.5x10$^4$ V/m to 1.1x10$^5$ V/m with the highest values located at the corners. The mean electric field is only 5.5x10$^4$ V/m; it is twenty times lower than the value deduced from the basic approach. It clearly means that mean values generally given in the literature should be very carefully considered. For example, we performed another simulation on a device from the literature [9]. Results, not presented in this paper, showed that the electric field strength in their sample was nearly 5.4x10$^3$ V/m whereas they mentioned a mean value of 1x10$^5$ V/m. The electric field strength is in fact mainly reduced in the air gap between the sample and the electrodes.

![Electric field strength: streamlines in the apparatus and distribution in the sample.](image)

### 4 Conclusion

This work confirms that electrofreezing is a promising way to modify and control the nucleation temperature. It demonstrates that the nucleation temperature can be shifted towards higher values by application of an external electric field. In the present experiments on water, the raise of the nucleation temperature is 3°C. On aqueous solutions of ethanol, the shift is higher; it reaches nearly 5°C at 15% (w/w). If reorientation of water molecules by the electric field is the underlying mechanism, a better knowledge of this effect could be obtained by molecular dynamics simulation. This work also demonstrates that the numerical simulation of the electrostatic phenomena is essential to obtain relevant values of the electric field strength inside the sample. This approach can easily be used to design new experimental devices.
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ACCELERATION OF OSMOTIC DEHYDRATION PROCESS THROUGH OHMIC HEATING OF FOODS

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Abstract:

Osmotic dehydration is the process of water removal by immersion of water containing cellular solid in a concentrated aqueous solution during which simultaneous solid gain also takes place. Ohmic heating is a thermal process in which heat is internally generated by the passage of alternating electrical current (AC) through a body such as a food system that serves as an electrical resistance. Ohmic heating is alternatively called resistance heating or direct resistance heating, Joule heating, or Electroheating™.

This research explores osmotic dehydration process of Granny Smith apples using ohmic heating (ODOH) instead of conventional heating. Then their effects were compared with those obtained when performing conventional osmotic dehydration (OD). Both processes were performed at constant temperature of 30°C, a solution of 65°Brix and 10 minutes process time. Then both samples were kept for 30 minutes just applying osmotic dehydration. In the case of ohmic heating electric field intensity (E) of 10 V/cm and 15 V/cm was applied. Two types of samples were utilized: a) a thickness of 9 mm (A) and b) a thickness of 4 mm (B).

The first step was to determine the characteristics of the system necessary to carry out ODOH, considering conductivity and sample size and its location in the system (in relation to the direction of electric field). Firstly, it was determined the conductivity of the tissue block and then defining the composition of the osmotic solution so as to match the conductivity using potassium sorbate and calcium chloride. Parameters used for comparison between both methods were °Brix (soluble solids), % mass loss and Z value (The electrical conductivity disintegration index).

The experimental results showed a significant increase in the % mass loss for samples A and B, in both processes, in addition, a visible decrease in the volume, which is due to loss of water. The water loss can be explained by: a) the electrophoresis in the system and / or b) electroporation of the tissue. The Z value also increases significantly due to a change in the conductivity of the samples, showing alterations in cell structure. In the case of °Brix, there was no significant variation in the results for samples with 9 mm thickness (A), unlike the samples with 4 mm thickness (B), which showed an increase in soluble solids when processing with osmotic dehydration coupled with ohmic heating. This indicates that in sample B the intensity of the electric field was able to generate electroporation increasing the tissue mass transfer, which is reflected in the increased °Brix.
THE EFFECT OF CONVENTIONAL AND OHMIC HEATING ON THE PERMEABILITY OF CELL MEMBRANE IN VEGETABLES TISSUE

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Abstract:
The effect of conventional and ohmic heating on the electrical behaviour and on the degree of permeabilization of cell membrane of vegetable tissue was investigated. Heating curves and electrical conductivity versus temperature profiles of cylindrical potato samples were determined in a static ohmic heating device for different field strength applied (42, 57 and 68 V/cm). The attained degree of membrane permeabilization of vegetable tissue for different final temperature (20, 30, 40, 50, 60, 70, 80 and 90°C) and field strength (0, 42 and 68 V/cm) was also evaluated by measuring complex impedance of the samples at frequency ranging from 100Hz to 10 MHz. Results showed that ohmic heating rate increased with increasing the temperature and the electric field strength applied, as a direct result of the increase of electrical conductivity. Moreover, it was also noted the existence of a transition temperature above which a sharp transition of the electrical conductivity curve was observed. The higher the field strength, the lower the transition temperature. Whatever the thermal treatment, as the final temperature increased, the damage of cell membrane of vegetable tissue also increased leading to a reduction in impedance. However, at all final temperature, the presence of an electric field yielded a higher degree permeabilization of the cell membrane than the one obtained by conventional heating. In particular, with increasing the field strength a higher permeabilization rate was observed.

Key words: Ohmic heating, Conventional heating, Permeabilization, Electrical conductivity, Vegetables tissues

1 Introduction

Ohmic heating is a food processing operation mainly applied for sterilization purpose [7] or enhancing the efficiency of dehydration or extraction processes [5]. During ohmic treatment, food products placed in direct contact with electrodes act as a part of an electric circuit through which alternating electric current flows. As a consequence, heat generation occurs within the whole mass of the food material as a result of its inherent electrical resistance. Thus, electrical properties of foods and their changes during electro-thermal treatment are crucial for the process. In particular, electrical conductivity is the main parameter controlling the ohmic heating rate. Previous studies have demonstrated that the electrical conductivity of food product increases with temperature [1, 2], but the type of dependence is still matter of concern as this parameter is a complex function of biological as well as of physical and chemical characteristics of the product [1]. Further, in addition to the effects occurring in conventional heating, during ohmic processing changes of electrical conductivity take place due to the presence of the electric field [1]. The latter, in fact, may induce electropermeabilization of cell membrane of vegetable tissues which enhances the diffusion of the intracellular fluids through the membrane. This, in turn, increases electrical conductivity and ohmic heating rate as well as the extraction efficiency of valuable compounds [1, 3, 6, 8].
Current literature refers about the effect of final temperature of heating on the electrical properties of vegetable tissues [8], as well as the effect of the applied field strength on the electrical conductivity changes of vegetables during ohmic heating [2, 4]. However, to the best of our knowledge, no detailed work relate the changes of the electrical properties of vegetable tissues due to the simultaneous effect of final temperature of heating and electric field strength applied to the cell membrane permeabilization of vegetable tissues during ohmic heating.

The experimental work presented in this paper aims at investigating the effect of a wide range of final heating temperature and field strength on the electrical behavior and on the degree of permeabilization of cell membrane of vegetable tissue.

2 Materials and methods

Raw potatoes and carrots purchased from a local market were used. Because results obtained for both vegetables were very similar, only those regarding potato samples will be discussed in this paper. Cylindrical samples (2.5 cm long and 2.5 cm diameter) obtained after peeling, were heated by either conventional or ohmic heating.

For ohmic heating treatment, samples were placed coaxially in a cylindrical polycarbonate tube and sandwiched between two plated stainless steel electrodes with slight pressure to ensure good contact without damage of foods. Electrodes were connected to a 240 V, 50 Hz main supply apparatus via a variable transformer to provide up to a maximum a.c. voltage of 180 V at 20 kHz. A teflon coated thermocouple (1 mm in diameter) was inserted to the geometric center of the sample. The applied voltage and current passing through the heater as well as sample temperature were recorded at regular intervals and used to evaluate the conductivity $k$ of the samples (in S/m) by the following formula:

$$k = \frac{L}{A \cdot R}$$

where, L is the length of the sample (m), A the area of cross-section of the sample (m$^2$) and R the resistance of the sample (Ω).

Conventional heating of vegetable was performed by sealing sample in plastic bag and immersing it in a water bath which was kept at 95 °C.

A first set of experiments was carried out to evaluate the effect of temperature rise and electric field changes (42, 57 and 68 V/cm) on the heating rate and electrical conductivity of samples during ohmic heating. In each experiment, a voltage was set and imposed to the system and the current changes were monitored as soon as the temperature increased from room temperature up to about 90 °C.

A second set of experiments was performed to determine the effect of final heating temperature (20, 30, 40, 50, 60, 70, 80 and 90°C) and applied field strength (0, 42 and 68 V/cm) on cell membrane permeabilization. After heating, samples were immediately sealed in a plastic bag and cooled to the center temperature of 25 °C by immersing them in a water-ice bath prior impedance measurements. The complex impedance of intact and treated samples were determined in a wide range of frequency (from 100Hz to 10 MHz) by using an impedance analyser (1260, Solartron, UK) consisting of a generator, an analyser and a sample holder. Results were plotted as both the absolute value of the complex impedance and phase angle as a function of the frequency and for different treatment conditions.
3 Results and discussion

The heating curves and electrical conductivity versus temperature profiles of cylindrical potato samples are shown in Fig. 1 for different field strength applied (42, 57 and 68 V/cm).

Heating rate increases with increasing the temperature and the electric field strength applied as a direct result of the correspondent increase of the electrical conductivity. In fact, the presence of an electric field may induce an enhanced diffusion of intracellular fluids through the cell membrane of biological tissues (electro-osmotic effect) [1] which, in turn, determines the increase of the rate of change of electrical conductivity and ohmic heating rate.

It should be noted that results also show the existence of a transition temperature, above which the electrical conductivity sharply increases and the heating rate is almost independent on the electric field applied. However, the time to reach this temperature is significantly prolonged as the field strength is decreased. Further, the higher the field strength, the lower the transition temperature. In particular, as the field strength increases from 42 to 68 V/cm, the transition temperature decreases from, approximately, 47 to 30 °C and, at the same time, the conductivity curve takes on an increasingly linear aspect.

The observed effect of temperature and applied field strength on the changes of heating rate and electrical conductivity curves (Fig. 1) may be explained in terms of cell membrane permeabilization. To this purpose, measurements of complex impedance of potato tissue before and after conventional or ohmic heating treatment were performed to obtain a measure of the extent of damage cell. Results, reported in Fig. 2 in terms of absolute value of complex impedance |Z| and phase angle theta, show that the impedance of intact vegetable tissue is strongly frequency dependent. This is because in the low frequency field (few Hz up to 50-60 kHz), the cell membrane acts as a capacitor preventing the flow of the electric current in the intracellular medium (ohmic-capacitive behavior). With increasing the frequency, the cell membrane become less and less resistant to the current flow in the intracellular liquid. At very high frequency values (> 500kHz), the membrane are totally shorted out and absolute value of the complex impedance is representative of the contribution of both extra and intracellular medium (pure ohmic behavior). Thus, the tissue permeabilization induced by thermal or electro-thermal treatment is detectable in the low frequencies range while, due to the electrical transparency of the membrane in the high frequency range, even if permeabilization occur, it is not possible to account for the effects of the electrical treatment.
Fig. 2: Absolute value of complex impedance ($|Z|$) and phase angle ($\theta$) changes with frequency for potato sample before and after thermal treatment at different final temperature and field strength applied. (a) and (b) Conventional heating; (c) and (d) ohmic heating at 42 V/cm; (e) and (f) ohmic heating at 68 V/cm.

Whatever the thermal treatment, as the final temperature increases the absolute value of the complex impedance decreases and, correspondently, the phase angle increases from negative values to zero. Therefore, as the temperature increases, the extent of membrane permeabilization also increases and the electrical behavior of the vegetable tissue changes from a ohmic-capacitive behavior towards a pure ohmic one. Interestingly, results reported in Fig. 2 confirm the existence of a transition temperature dependent on the applied field strength, above which damage of the cell membrane occur according to the remarkably lowering of the impedance value. This reduction of impedance results probably in the similar heating rates above the transition temperature whatever the field strength applied.

For the same final heating temperature, the presence of an external field strength yields greater membrane permeabilization than conventional heating. Further, while cell membrane of samples exposed to fields strength of 42 and 68 V/cm are completely permeabilized above
a temperature of 70 and 60 °C, respectively, those heated by conventional treatment still partially preserve membrane integrity also above a final temperature of 90 °C.

As the field strength increases, a higher permeabilization rate is detected. This is confirmed by significant lowering of the impedance value and the correspondent increase of the negative value of the phase angle due to the loss of the capacitive properties of the cell membrane after electropermeabilization.

Therefore the observed increase of the conductivity (and decreased of |Z|) with field strength applied can be correctly attributed to an increase of the concentration of ionic species in the extra cellular space from the permeabilization of the membranes induced by the electrical treatment.

However, in the case of starchy vegetables like potatoes, more work is needed to determine whether the occurrence of gelatinization during heating affects the changing shape of the conductivity curve with increasing applied field strength [3].

4 Conclusions

Present paper, due to the wide experimental work performed, allows to have a deeper insight on some phenomena already observed in previous literature but not fully explained, and to establish a clear correlation between the electrical behavior of vegetable tissues and the degree of membrane permeabilization of their biological cells. Results obtained so far show that electrical conductivity and, consequently, the heating rate of potato tissues sharply increases above a transition temperature depending on the applied field strength. Above this temperature, damage of cell membrane occur according to the remarkable lowering of the impedance value as well as the correspondent increase of the negative value of the phase angle. For the same final temperature, the higher the field strength the lower the transition temperature. Further, the exposure of vegetable tissue to an external electric field strength during ohmic heating yields greater membrane permeabilization than conventional heating. Therefore, the electro-permeabilization of biological tissues may represent a profitable way to increase the permeability of cell membrane of plant tissues at temperature levels lower than those utilized in conventional heating.

References

THE EFFECTS OF PULSED ELECTRIC FIELDS PROCESSING ON TOTAL ANTIOXIDANT CAPACITY IN REFRIGERATED ORANGE JUICE. COMPARISON WITH HHP AND PASTEURIZED TREATED JUICE

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Abstract:
Orange juice is an important source of carotenoids and ascorbic acid, a nutrient that, apart from its vitamin action, is valuable for its antioxidant effect. Knowledge about the concentrations of these substances in foods or their total antioxidant power may contribute to a better diet and a decrease in chronic diseases. All this shows the importance of evaluating citrus juices as sources of antioxidants, estimating the total antioxidant capacity after the application of preservation treatments, either traditional treatments (pasteurization) or emerging technologies (PEF and HHP). The Trolox equivalent antioxidant capacity (TEAC) of the samples after applying the preservation treatments was 4.03 ± 0.04 mmol Trolox/L for untreated orange juice, 3.51 ± 0.04 mmol Trolox/L for PEF treated juice, 3.86 ± 0.13 mmol Trolox/L for HHP treated juice and 2.49 ± 0.20 mmol Trolox/L for pasteurized orange juice. The total antioxidant capacity of the samples during refrigerated storage in the three types of sample analysed were observed, with a greater decrease in the samples stored at 10°C. In comparison with conventional pasteurization, PEF and HHP treatments led to higher total antioxidant activity in orange juice immediately after processing, as well as during storage at 4°C.

Keys words: high hydrostatic pressure, pulsed electric fields, refrigerated storage, antioxidant capacity, orange juice

1 Introduction
Orange juice is an important source of carotenoids and ascorbic acid, a nutrient that, apart from its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported [1,2]. The consumption of foods rich in antioxidant substances may contribute to the prevention of “oxidative stress” situations. Knowledge about the concentrations of these substances in foods or their total antioxidant power may contribute to a better diet and a decrease in chronic diseases.
Although orange juice are an important source of bioactive compounds, the techniques used for their processing and subsequent storage may cause alterations in their contents so they do not provide the benefits expected by the consumer.

In recent years consumers have increasingly sought so-called “fresh” products (like fresh products), stored in refrigeration and demanded for safe processed foods that require minimum preparation time has led the food industry to increase its output of products of this kind and provide means of ensuring that the nutrients and bioactive compounds are maintained or only minimally altered during processing and storage, until they reach the consumer [3-5]. In order to provide a response to the need for greater nutritional and sensory quality in some manufactured foods in which the characteristics of freshness are especially affected by thermal treatments [6,7], and to obtain microbiologically safe foods with physicochemical, nutritional and quality characteristics that are more like those of the fresh product, the food industry The concept of minimal processing is currently becoming a reality with conventional technologies (mild pasteurization) and non-thermal technologies such as pulsed electric fields (PEF) and high hydrostatic pressure (HHP).

All this shows the importance of evaluating citrus juices as sources of antioxidants, estimating the total antioxidant capacity after the application of preservation treatments. The aim of this study was to evaluate the effect on the total antioxidant capacity of an orange juice treated by PEF, HHP and pasteurization, during seven weeks stored in refrigeration at 4 °C and 10 °C in order to determine if the application of non-thermal technologies can lead fruit juices with good retention of bioactive proprieties.

2 Materials and methods

2.1 Sample

Oranges (Citrus sinensis L., ‘Navel’ variety) were purchased in a supermarket in ‘Valencia’. Orange juice was obtained by squeezing (FMC juice extractor with 2-mm perforated plates) and passed through a filter with a pore diameter of 0.23 mm. The filtrate was divided into four aliquots: one to be treated by heat, one by PEF, one by HHP, and one was not treated and that was used to ascertain the value of antioxidant capacity in the fresh juice. Each of the treatments was applied in duplicate.

2.2 PEF treatment system

Sample treatments were carried out in a continuous PEF treatment system designed by the University of Ohio and located in the Instituto de Agroquímica y Tecnología de Alimentos (CSIC) in Valencia. The system consisted of four treatment chambers with a diameter of 0.23 cm and an electrode gap of 0.293 cm connected in series and two cooling coils connected before and after each pair of chambers. The system was immersed in a refrigerated bath in order to keep the temperature within the designated range. The temperature, wave form, voltage and intensity in the treatment chambers were fed into a digital oscilloscope (Tektronix TDS 210, Tektronix, OR U.S.A.). Flow was set at 60 mL/min and controlled by a flow pump (Cole-Parmer 75210-25, Cole-Parmer Instruments, IL). Treatment time was 100 µs and the electric field was set at 30 kV/cm. These treatment conditions were selected on the basis of the results for carotenoid concentration, colour, enzymes and microorganisms obtained when orange juice was treated using different fields (25, 30, 35 and 40 kV/cm) and different times (30–340 µs) [8].
2.3 HHP treatment

Orange juice was placed in a 50-mL PE-LD flask and treated at 4000 bars for 5 min in an high pressure unit (EPSI NV, Belgium). After the treatment, the samples were quickly cooled and then analysed.

2.4 Thermal treatment

To treat the samples, an Armfield FT74P unit with a plate exchanger was used. Juice placed in a feeding tank was impulsed by a pump to the heat exchanger where the treatment conditions (90°C, 20 s) were reached. Treatment conditions were comparable to those used with orange juice in commercial practices; heating 90–99°C for 15–30 s [9]. After treatment, the juice was cooled with cold water from a cooler (Armfield FT61), and it was packed.

2.5 Storage conditions

The juice was packaged in Elopak packages (Pure-pack®), and they were stored in refrigeration and darkness at 4 and 10°C (± 2°C) with controlled humidity. Samples were analysed in duplicate immediately after processing, then after 1, 2, 3, 4, 6 and 7 weeks of storage.

2.6 Total antioxidant capacity

The method, adapted from Rice-Evans and Miller [10], was based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2′-azinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS), which has a characteristic long-wavelength absorption spectrum showing maxima at 734 nm. The ABTS radical cation is formed by the interaction of ABTS (150 µM) with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin (2.5 µM) with H2O2 (75 µM). Antioxidant compounds suppress the absorbance of the ABTS radical cation to an extent and on a time scale dependent on the antioxidant capacity of the substance under investigation.

This inhibition assay used a fixed time point of 3 min. ABTS, myoglobin and a sample were mixed, and the reaction initiated by the addition of hydrogen peroxide. After a fixed time, the absorbance of the solution was read, together with a buffer blank (which has a greater absorbance value). Results were obtained by calculating the difference in absorbance before and after the addition of the oxidant compound (H2O2) and interpolating the value obtained from a calibration curve prepared every day with Trolox standard in the range of 0.5–2 mM.

2.7 Statistical analysis

The results were compared by one-way analysis of variance (ANOVA). To determine differences between during storage of each of the treatments, the LSD test (p < 0.05) was applied (SPSS®, Statistical Package for the Social Sciences, ver. 12.0 for Windows).

3 Results

Table 1 summarize the results obtained for total antioxidant capacity in fresh juice and in juice treated by heat, PEF and HHP and stored at 4 ± 2 and 10 ± 2°C. The Trolox equivalent antioxidant capacity (TEAC) of the samples after applying the preservation treatments was 4.03 ± 0.04 mmol Trolox/L for untreated orange juice, 3.51 ± 0.04 mmol Trolox/L for PEF treated juice, 3.86 ± 0.13 mmol Trolox/L for HHP treated juice and 2.49 ± 0.20 mmol Trolox/L for pasteurized orange juice. TEAC decreased significantly (p < 0.05) after processing the orange juice with both types of treatments, but the decrease was greater in the
Pasteurized juice than in the PEF and HHP treated juices (decrease of 12.9 and 4.2% after PEF and HHP, respectively, and 38.21% after pasteurization).

<table>
<thead>
<tr>
<th>Storage</th>
<th>T weeks</th>
<th>Fresh juice</th>
<th>PEF</th>
<th>HHP</th>
<th>Pasteurized</th>
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<tr>
<td>4 ± 2°C</td>
<td>0</td>
<td>4.03 ± 0.04</td>
<td>3.51 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.86 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.49 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1</td>
<td>*</td>
<td>3.22 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.54 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.93 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>*</td>
<td>2.98 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.26 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.10 ± 0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>3</td>
<td>*</td>
<td>2.94 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.96 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.40 ± 0.16&lt;sup&gt;ac&lt;/sup&gt;</td>
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<td></td>
<td>4</td>
<td>*</td>
<td>2.33 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.46 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.83 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>6</td>
<td>*</td>
<td>2.23 ± 0.02&lt;sup&gt;de&lt;/sup&gt;</td>
<td>2.32 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.80 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>7</td>
<td>*</td>
<td>2.21 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.29 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.80 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>10 ± 2°C</td>
<td>0</td>
<td>4.03 ± 0.04</td>
<td>3.51 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.86 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.49 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1</td>
<td>*</td>
<td>2.37 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.02 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.02 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2</td>
<td>*</td>
<td>2.10 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.79 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.15 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3</td>
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<td>2.37 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.36 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.52 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>4</td>
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<td>1.54 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.88 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.74 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.53 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.50 ± 0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.70 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>7</td>
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T: temperature.
*spoiled samples, not analysed.
Mean ± standard deviation for two samples.
 Differences in letters within a column at same temperature (4 or 10°C) indicate significant (P<0.05) differences (LSD test)

**Tab. 1:** Total antioxidant capacity (mmol Trolox/L) in untreated, PEF and pasteurized orange juice, during refrigerated storage (4 ± 2 and 10 ± 2°C).

Thus, PEF and HHP treated orange juice has an antioxidant capacity more like that of untreated juice. Sánchez-Moreno et al. [11] found that the total antioxidant capacity of orange juice treated by mild pasteurization (70°C, 30s), HHP (400 MPa/40°C/1 min) and PEF (35 kV/cm-1/750 µs) did not undergo significant changes (p < 0.05), whereas pasteurization (90°C, 1 min) produced a decrease. Similarly, Polydera et al. [12] observed a greater decrease in total antioxidant capacity in pasteurized orange juice (80°C, 60 s), whereas when the same juice was processed by HHP (600 MPa, 40°C, 4 min) the decrease in this parameter was slighter. Similarly, Elez-Martínez and Martín-Belloso [13] found that PEF treatment of orange juice does not affect its antioxidant capacity, while pasteurization produces a decrease in this parameter.
Decreases in total antioxidant capacity in the three types of sample analysed were observed (figure 1), with a greater decrease in the samples stored at 10°C. In comparison with conventional pasteurization, PEF and HHP treatments led to higher total antioxidant activity in orange juice immediately after processing, as well as during storage at 4 and 10°C. This is in agreement with results in fresh navel juice and in orange juice reconstituted from frozen concentrate after high pressure treatment [12]. Férnandez-García et al. [14] found that HHP treatment and storage at 4 ± 2°C for 21 days, did not produce significant changes in the total antioxidant capacity of orange juice. Klímčak et al. [15] obtained similar results in orange juice stored for six months at 18, 28 and 38°C, observing that at higher storage temperatures, total antioxidant values decreased to a greater extent (18, 45 and 84% after 6 months at 18, 28 and 38°C, respectively) and more quickly.

4 Conclusions

The results obtained indicate that it is possible to obtain orange juice with a high nutritional value and a content of bioactive compounds similar to that of fresh juice with the emerging technologies studied (PEF and HHP). In comparison with conventional pasteurization, PEF and HHP treatments led to higher total antioxidant activity in orange juice immediately after processing, as well as during storage at 4 ± 2 and 10 ± 2°C.

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ANALYSIS OF THE KINETICS OF PEF-INDUCED CELL DEATH ON THE BASIS OF THE MECHANISM OF CELL ELECTROPORATION

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Abstract:
Here, the kinetics of cell death induced by PEF has been analyzed. Using the set of chosen parameters (the "standard cell"), theoretical relationships between the parameters of the electric pulse which is required to pore cell by square wave, exponential and sine wave ac electric pulses have been obtained and compared with the experimental relationships obtained for several different cell lines. It has been shown that the cell poration time depends on the intensity of the square wave and exponential pulses: the shorter the pulse length $\tau_i$, the higher the field strength should be. This dependence is much more pronounced for short pulses ($\tau_i < 10 \tau_m$). Also, the electric field strength for electroporation, $E_p$, is constant for frequencies of the applied ac field less than 10 kHz but its value depends on the pulse duration and decreases with increasing $\tau_i$. At higher frequencies ($f > 0.5 \pi \tau_m$) $E_p$ is dependent on the frequency of the ac field.

Key words: electropermeabilization, microorganism inactivation, resealing, modeling

1 Introduction

The use of pulsed electric fields (PEF) for killing of microorganisms is a promising new non-thermal food processing technology. To implement this method, the parameters of electric treatment assuring the best yield of microbial inactivation have to be determined. This requires the development of the models that would allow predicting microbial inactivation. Unfortunately, the models currently used for this purpose are, in most part, purely empirical and based on simple approximations of the experimentally obtained dependences by some of the well-known mathematical equations, such as a sigmoid curve, Bigelow, Weibull frequency distribution functions and other ones [1, 2, 3, 4]. Meanwhile, the models describing the kinetics of the microorganism inactivation, should account the actual mechanisms that govern microbial inactivation by PEF [5].

Here, with the purpose of creating the model capable to predict the kinetics of microorganism inactivation, the kinetics of cell death induced by PEF has been analyzed taking into account the following stages of this process: 1) pore formation due to membrane electroporation; 2) increasing of the membrane permeability during the PEF treatment and 3) several processes taking place after PEF treatment (leakage of intracellular compounds, pore shrinkage and disappearance, etc.).
2 Cell electroporation and death/inactivation

The cell electroporation as well as cell killing (inactivation of microorganisms) by PEF treatment should be regarded as an "all-or-nothing events" [6, 5, 7]. Quantitatively, this can be depicted by two curves describing the dependence of the fraction of electroporated and killed (inactivated) cells on the parameters of an electric treatment as shown in Figure 1, where the example of these dependences are shown for Chinese hamster ovary cells. The 'electroporation' curve can be obtained by measuring the loss of intracellular K⁺ [8] and the 'death or inactivation' curve - by the viability tests, e.g. by cells clonogenic [8] or the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays [9]. Because the initial pores created by an electric pulse are very small and leakage of intracellular substances important for cell viability (ATP, proteins, enzymes) is limited, in the majority cases, there will be some difference between the fraction of electroporated and dead cells $F_p - F_d$ as shown in Fig.1 [5].

3 Stages of microorganism inactivation process

The process of microorganism inactivation consists of the following main stages [5]:

(1) Initial stage (with the duration from nanoseconds to milliseconds): creation of pores when an electric pulse is applied ('electroporation' curves in Fig. 1);

(2) Stage of evolution of the pore population: change in the number of pores and their sizes during an electric treatment;

(3) Post-treatment stage: cell death (complete inactivation) ('inactivation' curve in Fig. 1) or returning of the cell to its initial viable state due to pore resealing. In the latter case, the damage of the cells, induced by pulsed electric fields, is sub-lethal. 

3.1 Relationships between the parameters of the electric pulse which is required to porate the cell

The dependence of the fraction of electroporated cells, $F_p$, on the parameters of an electric treatment can be written as [6]:

$$F_p(E_0, \tau_f) = 1 - \exp[-k_f(E_0)\tau_f]$$  

(1)
where \( k_f(E_0) \) is the rate of pore formation in a cell, \( E_0 \) is the electric field strength, and \( \tau_i \) is the duration of the electric treatment. For a spherical cell, \( k_f(E_0) \) can be calculated from [6]:

\[
k_f(E_0) = \frac{2\pi \nu a^2}{a_i} \exp \left[ -\frac{\Delta W_f(0)}{kT} \right] \int_{-1}^{1} \exp \left[ \frac{\pi c_m (\varepsilon_m/\varepsilon_w - 1)}{2kT} r^2 \right] (1.5E_0ay - \Delta \Phi_0)^2 dy
\]

where \( \nu \) is the frequency of lateral fluctuations of lipid molecules, \( a \) is the cell radius, \( a_i \) is the area per lipid molecule, \( k \) is Boltzmann's constant, \( T \) is the absolute temperature, \( \Delta W_f(0) \) is the energy barrier to pore formation at \( \Delta \Phi \equiv 0 \), \( r^* \) is the pore radius corresponding to the top of this barrier, \( \Delta \Phi_0 \) is the resting potential, \( \varepsilon_m \) and \( \varepsilon_w \) are the relative permittivities of the membrane and the water inside the pore, and \( C_m \) is the specific capacity of the membrane.

Using equations (1) and (2), one can obtain theoretical relationships between the parameters of the electric treatment resulting in cell electroporation ('electroporation' curves in Fig. 1) for any type of an electric treatment. The relationships between the external electric field strength required to porate cell, \( E_p \), and the length of the square wave and exponential pulses, \( \tau_i \), calculated by using the set of chosen parameters (the "standard cell") are shown in Fig. 2. It is seen that the shorter the pulse length, the higher the field strength should be. This dependence is much more pronounced for short pulses.

### 3.2 Electric treatment stage: Evolution of pore population

This stage is especially important for achieving microorganism inactivation because often small initial pores do not lead to the cell death. The dynamics of the pore population can be estimated on the basis of Smoluchowski equation [10]:

\[
\frac{\partial n(r,t)}{\partial t} = D_p \frac{\partial^2 n(r,t)}{\partial r^2} + \frac{D_p}{kT} \left( \frac{\partial W}{\partial r} \right) n(r,t) + \frac{D_p}{kT} \left( \frac{\partial^2 W}{\partial r^2} \right) n(r,t)
\]

where \( n(r,t) \) is the pore density function, \( D_p \) – pore diffusion constant, and \( r \) – pore radius.

### 3.3 Post-treatment stage: Cell death, pore resealing

After the end of an electric treatment, the cell or microorganism can either die or regain its viability because of the capability of pores to reseal [11]. The process of pore resealing
consists of a few stages of the quick (microseconds – milliseconds, minutes) reduction of pore size until the value of about 0.5 nm and the stage of the slow (tens of minutes - hours) complete pore closure [11]. Due to this, the membrane barrier function for larger molecules (sucrose, proteins, enzymes) is restored within a few minutes at 37 °C while complete resealing can take a few hours [11]. Pore resealing is important for practical applications of PEF for microorganism inactivation as it effects the number of molecules exchanged between the intracellular fluid and the surrounding medium which influences the cell viability and the likelihood of its death. So, it is necessary to know how long the pores remain open.

Because the last stage is much slower than the first ones, pore disappearance can be considered as a one-step process. This allows describing the dependence of the fraction of completely resealed cells on the post-pulse incubation time, \( F_r(t) \), as [12]:

\[
F_r(t) = (1 - F_{irr}) \sum_{n=1}^{\infty} P_n(0) [1 - \exp(-k_r t)]
\]

where \( k_r \) is the rate of pore resealing, \( F_{irr} \) is the fraction of the cells that have been damaged irreversibly, and \( P_n(0) \) is the probability that there are \( n \) pores in a cell just after the pulse.

Experimental points in Fig. 3 show the dependences of the fraction of Chinese hamster ovary cells, the membrane of which has restored its impermeability to trypan blue, on the time elapsed after the pulse. The data were taken from the paper published by Rols et al. (1990) [13]. Electroporation conditions were 10 square-wave pulses, \( \tau_i = 100 \mu s \), \( \nu = 1 \text{ Hz} \), \( E_0 = 1.5 \) (filled circles), and 1.8 (open triangles) kV/cm, and resealing was monitored at \( T = 21 \) °C. Solid lines in Fig. 3 are the theoretical distribution functions calculated from eq. (4) [12].

Equation (4) shows that increasing the time of incubation at elevated temperature increases the fraction of resealed cells, which is consistent with experimental data [12]. This means that the time necessary for the resealing varies from cell to cell, and thus the process of pore disappearance after electroporation is fundamentally stochastic [12].

The author hopes that the analysis presented here is helpful in the further development of more general models describing the kinetics of microorganism inactivation.

![Experimental points show the dependence of the fraction of Chinese hamster ovary cells, the membrane of which has restored its impermeability to trypan blue, on the time passed after the pulse. Solid curves are the theoretical curves plotted according eq. (4).](image-url)
4 Acknowledgement

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References


Abstract:
The last 20 years have seen the emergence of a novel technology capable of generating gas plasmas in the open air and at temperatures that are only slightly above ambient. These so-called Cold Atmospheric Plasmas (CAP) have characteristics that render them particularly suitable for a variety of applications in the food industry. In this presentation prevalence will be given to microbial decontamination. In particular, understanding the mechanism of microbial inactivation is important as if the most lethal plasma species can be identified, it may prove possible to ‘tune’ the plasmas so as to maximise their production. Preliminary investigations undertaken by our group have gone some way to addressing this issue. One of the most promising applications of CAP is in the treatment of fresh uncooked foods, and results are presented here attesting to the efficacy of plasmas in inactivating a range of micro-organisms at or near the surface of fruits. The possibilities for using plasmas for destruction of allergens and spongiform encephalopathies are also considered.

Key words: Cold gas plasmas, Microbial Inactivation, Foods

1 Introduction

Thermal processing remains an important method for preserving a wide range of foods and it is widely used throughout the world in the food processing industries. Whilst providing an effective method of microbial decontamination of foods there remain a number of types of food that can not be treated by thermal means. This is chiefly because thermal processing can induce unacceptable changes in foods such as destruction of important nutrients, or else can change the appearance or texture of foods so that it is no longer acceptable to consumers.

One class of foods that can not be processed thermally by conventional means are fresh cut fruit and salad products. These convenience foods are increasing in popularity in industrialised countries and are seen as a reflection of lifestyle changes that are taking place in such countries. Their consumption has been shown to be associated with increasing incidences of foodborne illnesses [1]. Such foods have conventionally been treated with hypochlorite but this form of treatment is losing popularity partly because it has been shown to be of only limited efficacy but perhaps more significantly because its use is increasingly becoming restricted by health agencies on the basis that chlorine residues could constitute a threat to health.
A method of treating such fresh commodities is therefore urgently required. One possible strategy is to make use of gas plasmas. Plasmas can now be generated under atmospheric conditions under conditions that are compatible with food treatment. Treatment is not strictly restricted to foods; gas plasmas could also be used to treat food processing equipment. This could be carried out in order to decontaminate the equipment of adherent micro-organisms or allergens or even prions. In this article the prospects of treating fresh foods with cold atmospheric gas plasmas is considered.

2 Gas Plasmas

Gas plasmas constitute natural phenomena such as aurora or flames, but they can also be created artificially and have been put to a variety of uses. About 20 years ago methods were developed that enabled gas plasmas to be created in atmospheric conditions and at temperatures that are only slightly above ambient temperatures. Plasmas have been referred to as the ‘fourth state of matter’ after solids, liquids and gases and are actually complex mixtures of ionised, free radical and metastable species all of which are extremely reactive and short-lived.

Apart from chemical species, plasmas result in the generation of a number of important effects that are also useful in inactivating micro-organisms, these include electric fields and UV photons as shown in Figure 1. There are a number of ways of utilizing gas plasmas that could be applicable to food processing applications and these would obviously be related to the specific application. There has yet been no full scale application of cold gas plasmas in the food industry but future developments are likely to revolve around the following possibilities.

The first of these is the barrier glow discharge generated between two parallel electrodes (Fig. 2). In one particular application food could be conveyed through the discharge to achieve microbial decontamination.

A completely different configuration that has considerable potential is the so-called ‘plasma pen’ which is depicted in Figure 3. This results in the production of essentially a stream of gases that can be directed at the object to be treated. Each of these two designs has the capability to be scaled up in a number of different ways. For the plasma pen shown below this could simply be scaled up or else replicated to produce what has been referred to as a ‘plasma brush’ [2].
3 Treatment of foods

As indicated above there are a number of applications of cold gas plasmas for the treatment of foods but there are also different stages at which gas plasmas could be applied to the treatment of foods. For some components of fresh salads the interior is essentially free of micro-organisms and it is the outer layers, or skin, that is contaminated with soil and organisms. The act of cutting is to draw the organisms from the surface into the interior of the flesh and deposit them there. One very good example of this is cantaloupe melons that have a reticulated skin that can trap soil and a number of incidences of Salmonella food poisoning have been attributed to this fruit [3].

Figure 4 shows the inactivation of a number of different organisms on the surface of melon achieved through the use of a plasma pen. Figure 4: Treatment of a variety of micro-organisms on the flesh of melon treated with cold atmospheric plasma pen.
The bacteria are particularly susceptible whilst the yeast species requires longer treatment but high reductions in viable cell numbers were nonetheless obtained. The treatment of the flesh itself is shown below. Treatment was less effective than was achieved during the treatment of the pericarp of the same fruit (Fig. 5). Further investigation revealed that the organisms deposited onto the fresh cut fruit tissue were being internalised. This made it difficult for the lethal plasma species to reach the organisms. Whilst this was to a certain extent a consequence of the experimental protocols employed, it does have consequences for the mode by which treatment must be applied [4]. This relates to the sequence of operations such as cutting and treatment.

![Fig. 5: Treatment of a variety of micro-organisms on the pericarp of melon treated with cold atmospheric plasma pen.](image)

4 Future prospects for cold gas plasmas

Cold gas plasmas have definite potential for the treatment of fresh foods. Studies are being undertaken to identify the plasma species that are most lethal to micro-organisms so that once identified, operating conditions can be selected so as to maximise their production [5]. An often unconsidered aspect of experimental work with gas plasmas is that treatment must be proven not to impact negatively on the organoleptic and nutritional characteristics of the food but to date only limited investigations on this aspect of treatment have been performed. Scale-up remains a challenge to be confronted but there exists a number of different strategies that could be implemented to achieve this. This work has focussed on inactivation of micro-organisms on the surfaces of foods, however, other food-related applications exist. Deng et al. [6] have shown that cold gas plasmas have the potential to inactivate proteins attached to stainless steel. This may have positive implications for the removal of prions – an application that has consequences not only in food processing but also in the healthcare industries. In addition, gas plasmas could also be used to remove allergens from the surface of food processing equipment. Preliminary and as yet unpublished results from our laboratory have confirmed this.
References


OHMIC HEATING APPLICATIONS
ON FRUIT AND VEGETABLE PRODUCTS

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Abstract:

Ohmic heating is an electrical thermal method, which inactivates enzymes and microorganisms without significant adverse effects on flavor and nutrients. It is based on the passage of electrical current through a food product that serves as an electrical resistance. Ohmic heating can be used for different applications in the food industry, such as; preheating in canning process, blanching of vegetables, hygienic production of ready to eat foods, pasteurization of high acidity fruit and vegetable products, production of low acidity meals and pasta, aseptic processing of high consistency particulate food mixtures. Application of Ohmic heating; heat is generated instantly inside of the food and the amount of heat generated is directly related to the current induced by the voltage gradient in the field and the electrical conductivity. The applicability of ohmic heating depends on the electrical conductivity of the product. Depending on the electrical conductivity of food microorganisms and enzymes can be inactivated faster than conventional heating. In addition a high quality product with minimal structural, nutritional or organoleptic changes can be achieved by short operating time. In this study effects of ohmic heating on microorganisms, enzyme activity, color and vitamin C content of fruit and vegetable products were reviewed.

Key words: Ohmic heating, fruit, vegetable

1 Introduction

Ohmic heating is an electrical thermal method that also known as electrical resistance heating, joule heating and electro-heating. In recent years, the world’s food industry has focused increasing attention on ohmic heating of food products. It is a highly attractive technique for food processing. Ohmic heating is based on the passage of an electrical current through a food product, which serves as an electrical resistance [1], [2]. Heat is generated instantly inside of the food. The amount of heat generated is directly related to the current induced by the voltage gradient in the field, and to the electrical conductivity [3]. The applicability of ohmic heating is dependent on the electrical conductivity of the product. Most food preparations contain a moderate percentage of free water with dissolved ionic salts and therefore conduct sufficiently well for the ohmic effect to be applied [4]. Several applications for ohmic heating in the food manufacturing industry include: heating liquid foods such as soups, stews, and fruits in syrup; heat sensitive liquids processing; juices treated to inactivate proteins (such as pineapple or papaya); blanching; thawing; starch gelatinization; sterilization; peeling of fruits; dehydration and extraction [5].

One of the advantages of ohmic heating is that particles can heat faster than the liquid under certain conditions [6], [7], [8]. In addition ohmic heating systems have advantages due to an optimization of investment (increased efficiency), instant shutdown of the system, and reduced maintenance costs because of the lack of moving parts. In order to comply with governmental regulations concerning microbial lethality in food products, heating methods
are applied at the coldest point of a system, which is generally at the center of the largest particle. In conventional heating, the time it takes to increase the temperature at this cold point may overprocess the remaining particles and the surrounding liquid. This overprocessing leads to destruction of nutrients and loss of flavor. Ohmic heating processes the particles and surrounding liquid simultaneously, preventing overcooking [4].

In this study effects of ohmic heating on microorganisms, enzyme activity, color and vitamin C content of fruit and vegetable products were reviewed.

2 The Effects of Ohmic Heating on Microorganisms and Enzyme Activity of Fruit and Vegetable Products

Ohmic heating generally inactivates microorganisms by heating mechanism like thermal processing. Additional non-thermal electroporation type effects have been reported at low-frequency (50–60 Hz), when electrical charges can build up and form pores across microbial cells. However, it is not necessary to claim such effects since heating is the main mechanism [5]. Cho et al. (1996) demonstrated that electrical pretreatment by ohmic heating can reduce the intensity of additional thermal applications for subsequent microbial inactivation [9]. Microbial inactivation curves of ohmic heating are similar to conventional heating curves except for a difference in the slope, which can most likely be explained by the presence of the electric field [10].

An experiment conducted using Bacillus subtilis revealed that a two stage ohmic treatment (ohmic heating, holding period, ohmic heating) resulted in accelerated death rates [11]. Another experiment involving Saccharomyces cerevisae showed that ohmic heating enhanced leakage of intracellular components as compared with the conventional method of boiling in water [12].

Citrus juices are characterized by high acidity conditions, which lead to the growth of yeast and mold in addition to a few types of low acid tolerant bacteria [13]. To avoid microbial spoilage, it is necessary to cause inactivation by applying heat. Both ohmic and conventional thermal treatments reduced microbial counts by at least 2-3 orders of magnitude compared to their number in fresh orange juice, which was \(~10^{25}\) colony-forming units (CFU)/mL. Although there was no detectable difference between ohmic and conventional heating concerning inactivation of microorganisms, a true comparison of this aspect of the techniques should be done by inoculation of samples with a reference microorganism. The results indicate that the significant parameter in the inactivation of microorganisms is the thermal effect, regardless of the kind of thermal treatment. These results are supported by the findings of Palaniappan et al. (1992) determined that suspensions of yeast cells (zygo Saccharomyces bailii) and cells of Escherichia coli were subjected to conventional and ohmic heating. It was found that at thermally lethal conditions, any lethal effect caused by electricity was insignificant when compared to that produced by heat [14]. In another study was conducted on Bacillus subtilis spores treated by conventional or ohmic heating under identical temperatures histories [15]. The study showed that spores heated at 92.3 °C had significantly smaller \(D\) values when heated using an ohmic rather than a conventional method. It was concluded that spore inactivation during ohmic heating was primarily due to the thermal effect. However, there was an additional killing effect caused by the electric current. And both ohmic and conventional heat treatments subjected another research and it was found that heating treatments reduced the initial microbial loads by at least 2-3 log compared to fresh orange juice [16], [17].

There have been limited researches on the effect of ohmic heating on enzymes. Several enzymes are used in the food industry for improving food quality (for example, texture and
flavor), for the recovery of by-products and for achieving higher rates of extraction [18], [19]. On the other hand, enzymes may also have negative effects on food quality such as production of off-odors and tastes and altering textural properties. Therefore, control of enzymatic activity is required in many food processing steps. Castro et al., (2004); determined the inactivation kinetics of several enzymes, most of them used as time-temperature integrators in the food industry. The tested enzymes were polyphenoloxidase (PPO), lipoxygenase (LOX), pectinase (PEC), alkaline phosphatase (ALP), and β-galactosidase (β-GAL), and the inactivation assays were performed under conventional and ohmic heating conditions. The thermal history of the samples (conventional and ohmically processed) was made equal to determine if there was an additional inactivation caused by the presence of an electric field, thus eliminating temperature as a variable. All the enzymes followed 1st order inactivation kinetics for both conventional and ohmic heating treatments. The presence of an electric field does not cause an enhanced inactivation to alkaline phosphatase, pectinase, and β-galactosidase. However, lipoxygenase and polyphenoloxidase kinetics were significantly affected by the electric field, reducing the time needed for inactivation [20].

Castro, Macedo, et al. (2004) compared the deactivation of different enzymes samples heated with ohmic or conventional heating. They showed that the electrical field applied during ohmic heating caused the faster deactivation than the conventional heating [20]. Leizerson and Shimoni (2005) found that ohmic heating reduced pectinmethylesterase activity by 98% [17]. Icier, Yildiz, and Baysal (2006) reported that peroxidase in pea puree was deactivated in a shorter time by ohmic heating as compared to conventional heating. They determined the enzyme activity qualitatively and suggested that ohmic heating caused less browning than conventional heating [21].

PME causes cloud loss in orange juice by de-esterification of pectin; thus, thermal treatment is applied to inactivate the enzyme. The design for thermal pasteurization of orange juice is based on the thermal destruction characteristics of PME, which is more thermally stable than many vegetative microorganisms. The $z$ value of PME ranges from 6.5 to 13 °C in the temperature range of 80-90 °C [22], [23]. PME activity is presented as percentage of PME activity in fresh orange juice. Generally, as the impact of thermal treatments during ohmic heating increases, by applying higher temperatures or times, the residual PME activity decreases. During ohmic heating, PME activity showed a reduction of 90-98% compared to its activity in fresh orange juice where under conventional pasteurization conditions, the residual PME activity was reduced to 5% [16].

Peroxidases are known to be the most heat stable enzymes in vegetables, and their inactivation is usually used to indicate the adequacy of blanching [24]. Browning in juice and purees during manufacture and storage was of vital interest for the industry [25]. Icier et al., (2006) determined the critical peroxidase inactivation time for ohmic blanching applied at different voltage gradients to pea puree and to compare this with water blanching. The pea puree was blanched ohmically by using the voltage gradients of 20, 30, 40 and 50 V/cm and by water blanching at 100 °C. It was observed that the time required for the inactivation of this enzyme changed with the voltage gradients applied. Critical inactivation time decreased as the voltage gradient increased and temperature distribution during ohmic blanching of pea puree was more uniform than water blanching. At high voltage gradients, the electrical current passing through the samples was higher. The electrical effect was also found important on blanching efficiency. Below 30 V/cm, the energy dissipated during ohmic blanching was lower than water blanching, so the longer inactivation time was achieved at 20 V/cm [21]. Mizrahi (1996) has also reported that 2 min blanching time by ohmic heating of whole large vegetables had similar effects with 4 min water blanching [26].
Içier, Yıldız and Baysal (2008), observed that effects of ohmic heating at pasteurization temperature ranges on PPO activity in fresh squeezed grape juice. The enzyme activity was found to be significantly lower at 40 V/cm than at 20 or 30 V/cm at 70 and 80 °C. The ohmic heating rate increases as the voltage gradient increases. The critical deactivation temperature at 40 V/cm was lower than that of at 20 and 30 V/cm, probably because of the faster increase in electrical conductivity at higher voltage gradients causing higher deactivation in PPO. At constant voltage gradient a small increase in the activity with holding time was observed at 60°C until the deactivation started after 15 min. The one step first order kinetic model was found to adequately describe the deactivation kinetics of PPO, for the temperature range of 70–90°C. The activation energy of the PPO deactivation for the temperature range of 70–90°C was found to be 83.5 kJ/mol [27]. The effect of ohmic heating on enzymes structure needs to be further assessed.

3 The Effects of Ohmic Heating on Vitamin C and Color Content of Fruit and Vegetable Products

Ascorbic acid is considered to be one of the most heat sensitive nutrients in food products [28]. Its degradation has been reported to vary with temperature, pH, oxygen, enzymes, metal catalysts, initial concentration and light [29], [30].

The concentration of vitamin C in orange juice is one of the most important attributes for the consumer. Leizerson and Shimoni (2005), followed vitamin C degradation for ohmic heated orange juice during storage. The results showed that no significant difference was observed in the concentration of ascorbic acid between ohmic heating and conventional pasteurization. As it appears, neither electrical heating nor conventional pasteurization had any influence on the degradation rate of ascorbic acid. In addition the shelf life of ohmic-heated orange juice was determined according to vitamin C content and it was found similar to conventionally pasteurized juice which was 79 days. However, the prolonged sensory shelf life of ohmic-heated orange juice may influence the type of thermal treatment applied in the industry. They also observed vitamin C concentration decreased in 7-25% compared to fresh orange juice in conventional heating and 15% reduction determined for ohmic heating treatments [16], [17]. Lima et al. (1999) examined ascorbic acid degradation for orange juice during conventional and ohmic pasteurization. They performed matching time-temperature histories in both conventional and ohmic heating. They found that the type of heating had no significant effect on vitamin C degradation. They measured a decrease of 21-23% in ascorbic acid during both thermal treatments at 90 °C for 30 min [31].

Color deterioration affects the consumer’s perception of the quality of orange juice. Browning occurs in orange juice during storage and can be accelerated due to abusive storage conditions, presence of oxygen or metal ions, or degradation of ascorbic acid. Ascorbic acid provides reactive carbonyl groups that can be precursors to non-enzymatic browning. For vitamin C content, the U.S. Recommended Daily Allowance (U.S. RDA) set forth by the United States Department of Agriculture (USDA) is at least 25 mg per 100 mL at the time of expiration date for 100% vitamin C supply. Ohmic and conventional heat treatments reached this level in 79 days [17]. They also evaluated the effects of ultrahigh-temperature ohmic heating processing on the quality of orange juice and compared to conventionally pasteurize orange juice. The orange juice was treated at temperatures of 90°C for 1.13 s, 120°C for 0.85 s, and 150°C for 0.68 s in an ohmic heating system or 90°C for 50 s in a conventional pasteurizer. Vitamin C degradation of fresh orange juice was occurred due to nonenzymatic reactions, which are accelerated by high temperatures during thermal processing. The vitamin C degradation compared to fresh orange juice and it was found 7-25%, and 16% reduction for
ohmic heated and conventional heated samples respectively. Again, no significant difference could be seen in the two types of heat treatments [16].

Ohmic blanching of pea puree was investigated in another research and above 20 V/cm application of ohmic heating gave better colour values than the water blanched puree samples at critical peroxidase inactivation times. The effect of voltage gradient during ohmic blanching on the colour of puree was found significant. It was described that the colour changes of pea puree follow the first order reaction kinetics [21].

4 Conclusions

Ohmic heating is an excellent alternative food processing technique that shows much promise in the food manufacturing industry. As time goes on, this technology will become even more efficient. The researchs shows that enzyme and microbial inactivations are faster than conventional heating. In addition; color values of ohmic heated products are better than conventional ones. But there were no significant difference evaluated on vitamin C contents of food materials. Further studies must be conducted to explore the effect of this treatment on chemical changes of nutritional facts on the long-term stability and shelf life of the products.

5 References


OPTIMIZATION OF OHMIC COOKING OF GROUND BEEF-FAT 
BLENDS: EXERGY APPROACH

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Abstract:
The main objective of this study was to perform the exergetic optimization of the ohmic cooking process by using the response surface methodology. The independent variables were the initial fat content of ground beef-fat blends and the voltage gradient while the responses were the temperature homogeneity, the cooking yield, Hue angle and the exergetic efficiency. It was obtained that exergetic efficiency increased as the initial fat content decreased and the applied voltage gradient increased. The optimum process condition was found to be the voltage gradient of 36.6 V/cm and the initial fat content of 2 %. The responses for this condition were the temperature homogeneity of 0.96, Hue angle of 70.79, the exergetic efficiency of 89.7 % and the cooking yield of 74.52 % with the desirability of 0.913. It is thought that the exergetic performance evaluation would be a novel concept in the optimization of ohmic processing of food materials.

Key words: Exergy analysis, ohmic, cooking, grounded beef-fat blends.

1. Introduction

Recently, the interest in ohmic process has significantly increased because of its low energy consumption and waste potential [1]. It has proved to be a successful technology to process liquids, the application to meat products have not yet found industrial application. The utilization of alternative systems in meat cooking applications has attracted the attention of many investigators. In recent years, several researches have been conducted on the ohmic processing of meat products. These studies has proved the ohmic cooking as an alternative cooking method for meat products by taking account of its faster-homogeneous heating mechanism and its effects on the some quality attributes. [2] concluded that ohmic cooking provided 82-97 % energy saving while reducing the cooking times 90-95% compared to conventional cooking. They suggested that it could be possible to obtain efficiencies greater than 90% in an industrial process in which these losses were controlled by the wall insulation. However there is no information about the use of the performance evaluation in the optimization of ohmic processes, to the best of the authors’ knowledge.

In recent years, thermodynamic analyses, particularly exergy analyses, have appeared to be an essential tool for the system design, analyses and the optimization of thermal systems. Exergy is a novel approach to evaluate the performance of thermal systems and includes both the first and second law of thermodynamics. The minimization of the exergy losses, or equivalently the entropy generation, has a broader objective than the minimization of energy losses within a plant [3]. Although several studies in which covering the energy and exergy analyses of food products especially during drying process have been undertaken there were no information available about the optimization of ohmic cooking process with an exergy approach, to the best of the authors’ knowledge.
In this study, an exergetic optimization of ohmic cooking has been performed by using response surface methodology. The main objective of this study was; (i) to investigate the main effects of process variables on the energy-exergy considerations during ohmic cooking, (ii) to determine optimum process conditions for cooking of ground beef samples by exergetically.

2. Materials and methods

2.1. Sample preparation
Ground lean beef samples were prepared from lean samples provided by a commercial firm, Izmir-Turkey. The subcutaneous fat was used as the fat source to arrange fat levels of ground beef samples. Lean beef and fat ground through a 3 mm plate grinder. Samples with different fat contents (2, 9 and 15 %) were prepared in a cylindrical shape (a diameter of 0.025 m and a length of 0.05 m).

2.2. Proximate analysis
The moisture content of samples was determined with oven method [4]. The protein content (Nx6.25) [5], the oil content [6] and the ash content [7] of grounded beef samples were determined with appropriate methods.

2.3. Cooking procedure
Ohmic cooking experiments were performed using a specially designed laboratory scale ohmic heating system, which consisted of a power supplying system (an isolating transformer and a variable transformer) and a microprocessor board. The detailed technical information about the system used has been given in [8]. Cooking experiments were carried out at the voltage gradients of 20, 30 and 40 V/cm by using frequency at 50 Hz. The temperature measurements in the sample were presented in Fig.1.

Fig. 1. Schematic illustration of temperature measurement points in the ohmic cooking unit.

A surface thermometer (METEX ME-32, Seoul, South Korea) was used to measure the surface temperatures of the test cell and recorded at every 1 s. The ambient temperature was also measured and recorded with a temperature sensor (Omega Eng. Inc., Stanford, CT).

2.4. Temperature homogeneity
To determine temperature homogeneity (TH) of ground beef samples, the surface and the centre temperatures were taken at each 1 s. Temperature homogeneity were determined by the ratio of the centre temperature to the surface temperature at each time interval (eq. 1);

$$TH = \frac{T_{\text{center}}}{T_{\text{surface}}}$$ (1)
2.5. Exergetic efficiency

The overall exergy balance equations for the ohmic cooking (Fig.2) could be written as follows;

\[
Q_{gen} - \sum \left(1 - \frac{T_0}{T_b}\right) E_{loss} + \sum Ex_1 - \sum Ex_2 = Ex_{dest}
\]  

Fig. 2. Terms used in the exergy analysis of the ohmic cooking process.

The energy generation term \(Q_{gen}\) was represented in eq.(3)

\[
Q_{gen} = \Delta V^2 \sigma / K_c
\]

The exergy \(Ex\) values at the initial and end of the ohmic cooking process was expressed as follows;

\[
Ex_1 = m_p (ex_p)_1 + Q_{gen}
\]

\[
Ex_2 = m_p (ex_p)_2 + (m_{water})_2 (ex_{water})_2 + (m_{oil})_2 (ex_{oil})_2
\]

Since the ohmic cooker was the closed system without the volume change, the specific exergy term \(ex\) for the product, the water removed and the fat removed was written as follows

\[
ex = u - u_0 - T_0(s - s_0)
\]

The specific entropy \(s\) of samples was calculated as;

\[
s - s_0 = \left(\frac{C_p + C_{p,0}}{2}\right) \times \ln\left(\frac{T_p}{T_0}\right)
\]

Since the composition of the sample during cooking was changed, the specific heat \(C_p\) of ground beef samples were calculated using the relations proposed by [9] with the specific heat of pure components given in [10];

\[
C_p = \sum C_{p,i} X_i
\]

The reference-dead state condition was taken as \(T_0= 25 \, ^\circ C, P_0= 101.325 \, kPa\) [11]. The exergetic efficiency of the ohmic cooker \(\eta\) was defined as the percentage of the product exergy to exergy inflow for the cooker [12]:

\[
\eta(\%) = \frac{Ex_2}{Ex_1} \times 100
\]

2.6. Cooking yield
The cooking yield was determined by calculating weight differences for sample before and after cooking [13]:

\[
\text{Cooking yield(\%)} = \left( \frac{\text{Cooked ground beef weight}}{\text{Uncooked ground beef weight}} \right) \times 100
\]  

(10)

2.7. Colour measurement

Colour measurements of the ground beef samples were carried out by using a HunterLab Colorflex (CFLX 45-2 Model Colorimeter, HunterLab, Reston, VA). The combination parameter (Hue angle) was calculated by using tristimulus values measured \((L, a \text{ and } b)\) (eq.12) [14]:

\[
\text{Hue angle} = \tan^{-1}\left( \frac{b}{a} \right)
\]  

(11)

2.8. Experimental design, statistical analysis and optimization

Response surface method (RSM) was used to investigate the main effects of process variables on the temperature homogeneity (\(TH\)), Hue angle, cooking yield and the percentage exergetic efficiency (\(\eta\)) during the cooking of ground beef samples. The voltage gradient \((x_1)\) and the initial fat content \((x_2)\) were selected as independent variables. A Central Composite Design (CCD) including 13 experiments formed by 5 central points and 4 \((\lambda=1.0; \text{Face Centered})\) axial points to \(2^2\) full factorial design was used. Model adequacies were checked by \(R^2\), adj-\(R^2\), pre-\(R^2\), Adeq.Precision, PRESS and C.V. \((\text{lack of fit} > 0.1; \ R^2 > 0.95; \ (\text{Adj}-R^2 - \text{Pre}-R^2) < 0.2; \ \text{max.PRESS}; \ C.V. < 10; \ \text{Pre}-R^2 > 0.7; \ Adeq.Precision > 4)\) (Myers and Montgomery, 2002).

After model fitting, residual analyses including the examination of diagnostic plots and calculation of case statistics were conducted to validate assumptions used in ANOVA. Design Expert Ver. 7.0.0 [15] was used to fit response surfaces and optimize the ohmic process exergetically. Several response variables describing the system characteristics are selected for the exergetic optimization of thermal process. In the present study, desirability function was developed for the criteria including maximum \(TH\), maximum \(\eta\), maximum cooking yield and maximum Hue angle.

3. Results Discussion

The volumetric heat generation via ohmic treatment provided very high temperature homogeneity values (0.83-0.97) for meat product. This advantage of ohmic cooking has a crucial point to ensure the safe cooked products [16]. The temperature homogeneity of the samples was represented in Fig.3a. The effect of initial fat level on the \(TH\) of ground beef samples was statistically significant \((p<0.01)\) whereas the effect of applied voltage gradient and their interaction were not significant \((p>0.01)\). This behaviour could be explained by distribution of more heterogeneous fat structures in higher fat contents.

Exergy efficiency was calculated in the range of 67.3-89.7 \% during ohmic cooking of ground beef samples (Fig.3b). It was obtained that both the voltage gradient applied and the initial fat level had affected exergetic efficiency of the ohmic cooking system significantly \((p<0.05)\). The decrease in the treatment times at higher voltage gradients provided the minimization of energy losses or equivalently entropy generation, which meant the increase in the exergetic efficiency of the system. Furthermore, the interaction effect between the voltage gradient and the initial fat level were not significant for \(\eta\) \((p < 0.01)\).

The cooking yield has been reported as the most important criteria to predict behaviour of the meat products during cooking [17]. Ohmic cooking yield decreased as the initial fat content increased \((p<0.05)\) (Fig.3c). The effect of applied voltage gradient and the interaction effects were not significant statistically \((p>0.01)\).
The colour measurement in cooked ground beefs can provide reliable information about eating quality attributes. In the preliminary experiments, colour measurements of samples were performed both from surface and after mincing the cooked sample. It was determined that the mincing the cooked samples represented both the change in the colour and the pinkness in the internal regions best. Hue angle values were in the range of 67.07-71.47 (Fig.3d). The initial fat content had no statistically significant effect (p>0.05) whereas the applied voltage gradient and their interaction had significant effect on the Hue angle values of cooked samples (p<0.01).

By applying desirability function method, the solutions for the optimum cooking condition covering the criteria were obtained as 36.6 V/cm and lowest (2%) initial fat level with the desirability of 0.913. At this point, $TH$, $\eta$, cooking yield and hue angle values were calculated as 0.96, 89.7%, 74.52 and 70.79, respectively.

4. Conclusions

In this study, exergetic optimization of ohmic process was performed by using the response surface methodology. The temperature homogeneity, the exergetic efficiency, cooking yield and Hue angle values were selected as optimization responses. Exergetic evaluation of ohmic cooking systems presents a novel approach in the performance evaluation of ohmic systems, which could be especially used in the industrial implementation of these systems. This study will give available data about the optimum condition for ohmic cooking of meat products that is obtained by considering the performance criteria of the system.
Acknowledgement
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References
ENHANCEMENT OF POLYPHENOLS EXTRACTION FROM INVOLUCRAL BRACTS OF ARTICHOKEs

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Abstract:
The pulsed electric fields (PEF) technique was applied as a pre-treatment stage of permeabilization of cellular membranes in the extraction process of valuable components, such as polyphenols, from involucral bracts of artichokes. The impact of PEF pre-treatment was evaluated in terms of the determination of the effect of operating parameters on cellular permeabilization, and the evaluation of the kinetics of release of the valuable components by means of the determination of yield of the extract. The vegetable matrix was exposed to PEF treatment at varying intensity and characteristics of the electric pulses. The attained permeabilization was evaluated by measuring electrical impedance of the tissue before and after treatment. Extraction process was performed by liquid solvent extraction. Chemical analysis by standard techniques was used to estimate the extract yield. Results showed that the application of a mild PEF treatment, between 0.8 and 1.6 kV/cm, increased the permeabilization of bracts tissue, as showed by impedance measurement, and accelerated the extraction rate of polyphenolic content. In particular, the treatment appeared particularly suitable for extraction using water as solvent, with a remarkable increase in polyphenols yield with respect to untreated samples.

Key words: Pulsed Electric Fields, Extraction, Artichoke Bracts, Polyphenols

1 Introduction

Pulsed electric fields (PEF) treatment can be applied for non-thermal permeabilization of cell membranes of vegetable tissue without post-processing operations. Food tissue is placed between two electrodes and a electric field of moderate intensity (0.5-5 kV/cm) is applied in form of repetitive very short voltage pulses (from 10 μs up to 1 ms). Due to the low values of both field strength and total specific energy input (1-10 kJ/kg) applied and to the very short total duration (less than 1 s) of the electrical treatment, only a negligible ohmic heating occurs. Irreversible permeabilization of both cell membrane and tonoplast causes the free flowing of the intracellular water and of high-value compounds including sugars, juices and pigments [1, 2]. Thanks to its non-thermal impact on foodstuff, the electric treatment can exert a selective permeabilization of the membranes (tonoplast and plasma membrane), while cell wall remains intact, improving the purity and the yield of the extracts [3]. The use of a PEF treatment is advantageous in the extraction of cellular juice from fruits and vegetables [4, 5],
in extraction of beet sugar [6] and in the recovery of high valuable thermosensitive compounds, such as natural antioxidants [7-9].

To the best of our knowledge, PEF technique has not been applied yet to artichoke bracts, which represent a valuable by-products of artichoke processing, rich in polyphenolic compounds. Hence, this work aimed at investigating the conditions of PEF pre-treatment to enhance the extraction and the recovery of polyphenols from involucral bracts of artichokes.

2 Materials and methods

2.1 Materials

Artichokes of Paestum variety were purchased from a local market and stored in refrigerated conditions at 4°C until used. Bracts were obtained manually and cut in strips of about 2 cm of thickness. Immediately after sampling, the strips were dipped for 1 min in 1% citric acid solution to prevent oxidative phenomena. Initial moisture content of samples ranged between 85 and 88%.

2.2 Experimental apparatus and PEF treatment

PEF treatment was carried out using a high voltage pulse generator (Diversified Technologies, Inc, MA, U.S.A), consisting of a high voltage generator and of a pulse modulator able to provide square wave electric pulses in a wide range of values of voltage (0-25 kV), current (0-100 A), pulse width (1 and 10 µs) and repetition frequency (1-1000 Hz).

Vegetables were treated in a treatment chamber consisting of two stainless steel flat electrodes of 105 cm² surface area (15 cm x 7 cm). The distance between the electrodes can be regulated between 1 and 5 cm in a Teflon enclosure.

In this work, samples of 20 g of materials were placed in the treatment chamber and PEF treatment was conducted at two different intensities of electric field: 0.8 kV/cm and 1.6 kV/cm, for 500 pulses with the duration of 10 µs each. Electrical measurements were conducted on treated and untreated samples, to the purpose placed in a cell that was connected both to the generator and to an impedance analyzer (Solartron, 1260, UK).

2.3 Extraction and phenolic analysis

Extracts from both untreated and PEF-treated samples were prepared using bidistilled water as solvent. After PEF treatment, the samples were suspended in the solvent using 1:10 solid/liquid ratio (i.e. 10 g of bracts in 100 g of water). Extraction was carried out under agitated conditions in a shaker (250 rpm) at controlled temperature (30°C).

Total polyphenolic content in the extracts was evaluated by means of colorimetric analysis of the samples using a modified Folin-Ciocalteau method [10, 11]: 0.5 ml of extracts were added for 2 min at room temperature to 2.5 ml of Folin-Ciocalteau reactant diluted 1/10 in water. Hence, 2.0 ml of a sodium carbonate solution (75 g/l) was added and after mixing the obtained solution was brought at 50°C in a thermostatic bath for 15 min followed by cooling to room temperature in an ice bath for 5 min. Due to the reaction between the reactant and the polyphenols, a blue coloration of the solution appeared. Absorbance was measured at 760 nm in a Jasco V-530 spectrophotometer and the total phenolic concentration was expressed in terms of chlorogenic acid equivalents (mg/l). Refraction index of extracts was measured with an Abbe refractometer (DR-A1, Atago CO).

3 Results

The effect of PEF pretreatments, conducted at 0.8 kV/cm and at 1.6 kV/cm, on the kinetics of extraction in bidistilled water is reported in Fig. 1.
The rate of release of polyphenolic compounds is clearly affected by the pre-treatment: while from the control bracts the extraction is apparently completed in more than 2 h and attains maximum values of 60 mg/l of chlorogenic acid equivalents, PEF-induced permeabilization of the cell membranes significantly accelerates the release of polyphenolic content, which is completed in less than 1 h.

Moreover, it can be observed that the total phenolic content increases with increasing the severity of the treatment: at the highest applied electric field (1.6 kV/cm), after 60 min the content of total polyphenols in the extract is 160 mg/l, while it is only 81 mg/l for PEF treatment at 0.8 kV/cm and 64 mg/l for the control. Significant differences can be noticed already after the first 10 min: the control samples results in the lowest content of extracted polyphenols (17 mg/l), while by PEF pretreatment the attained concentration was 21 mg/l at 0.8 kV/cm and 57 mg/l at 1.6 kV/cm.
In terms of extraction yield, maintaining constant all the extraction process parameters, the application of an electric field of 0.8 kV/cm for 500 pulses, with an energy expenditure of ~1 kJ/kg, caused an increase of the extraction yield of ~30% with respect to control, while the application of an electric field of 1.6 kV/cm for 500 pulses, with an energy consumption of ~5 kJ/kg, causes an increase of ~150% with respect to control and of ~100% with respect to PEF treatment at half the electric field (0.8 kV/cm).

Since the major fraction of the phenolic compounds is present in nature in conjugated form, mainly with sugar residuals, the extraction of phenolic compounds from vegetable tissues implies also the release of monosaccharides, which include sucrose, arabinose, galactose, mannose, rhamnose, among those more probably linked to phenols.

Fig. 2 shows the sugar content of the extract, measured by means of a refractometer. In accordance with expectations, also the rate of sugar release is significantly accelerated when a PEF treatment is applied to the vegetable tissues: even though the same asymptotic value is reached, in the control sugar concentration takes 50 min to reach the steady state, while in the PEF-treated samples only 30 min are required.

![Fig. 3: Changes with frequency of the absolute value (|Z|) and phase angle (θ) of complex impedance for bracts disks before and after PEF treatments of different intensity and duration.](image-url)
The observed enhancement of the release rate and of the yield of total extracted polyphenols can be explained in terms of cell permeabilization induced by the PEF treatment (electroporation). The change of the electrical properties of the tissue, before and after PEF treatments of different intensities, can provide a measure of the extent of electroporation. To this scope, both the impedance and the phase angle of untreated and PEF-treated samples were measured and reported in Fig. 3. It can be observed that the effect of a treatment at 1.6 kV/cm and 50 pulses is equivalent to the effect of a treatment at 0.8 kV/cm for 500 pulses, both in terms absolute value $|Z|$ and phase angle $\theta$ of the complex impedance.

On the other side, a more severe treatment conducted at 1.6 kV/cm for 500 pulses leads to a significant decrease of $|Z|$ and a concurrent increase of $\theta$, suggesting a larger extent of tissue permeabilization. In fact, the increase of the conductivity (and decrease of $|Z|$) is the result of ions release in the tissue as a consequence of the permeabilization of the cell membrane. This is also confirmed by the increase of $\theta$, due to the loss of the capacitive properties of the cell membrane after its electroporation.

4 Conclusions

The present work shows that the extraction of polyphenolic compounds from artichoke bracts can be significantly accelerated by the application of a PEF pretreatment (applied electric field of 1.6 kV/cm for 500 pulses of the duration of 10 µs each), which requires an energy consumption of ~5 kJ/kg in order to increase the extraction yield in water of ~150% and to enhance the rate of release. Milder pretreatment conditions (0.8 kV/cm for 500 pulses of the duration of 10 µs each) requires less energy (~1 kJ/kg) but also a yield increase of only ~30% with respect to untreated samples. Extraction enhancement can be correlated to tissue permeabilization, as shown by the measurable increase of conductivity and loss of capacitive properties of the tissue.

References

INACTIVATION OF *S. CEREVISIAE* AND *E. COLI* BY PULSED ELECTRIC FIELDS: THE EFFECT OF FLOW CONDITIONS ON TREATMENT EFFICIENCY

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Abstract:
In this work, microbial inactivation of *E. coli* and *S. cerevisiae* cells inoculated in a model solution has been studied using a continuous flow PEF system. The influence of flow parameters on the lethal effect and the energy efficiency of PEF treatment has been investigated in a wide range of values. Results showed that, at a fixed field strength, higher degrees of stirring of the microbial suspension achieved by increasing the flow rate with a single pass operation through the PEF chamber, or by re-circulate mode at a constant flow rate, provided a significantly increase of the effectiveness and energy efficiency of the pulse treatment. Finally, results of this study highlight that improving of the PEF sterilization efficiency need of a new approach where not only electric and microbial parameters but also flow parameters should be simultaneously involved.

Key words: Pulsed Electric Fields, Microbial inactivation, Flow parameters

1 Introduction

The recent technological evolutions in both electronic field and pulsed power applications have repurposed the cold pasteurization by means of pulsed electric fields (PEF) as a profitable alternative for the thermal treatment of liquid foods. PEF process involves the application of a high intensity electric field (10-50 kV/cm) as a train of short duration pulses (of the order of microseconds) to a food placed between two electrodes. Membrane damage caused by the external electric field is believed to be the cause of cells inactivation [1].

A number of scientific papers on the matter demonstrated the effectiveness of PEF treatment at low temperature to kill yeasts and many bacteria in liquid foods [3-4]. However, in spite of such achievements, the enhancement of energy efficiency of a PEF process still represents one of the most important points to be investigated in view of a future industrial application of this technique as a food pasteurization method. To this purpose, to obtain the desired pasteurization effect without over processing and to reduce energy consumption, PEF treatment uniformity should be improved to ensure that all cells belonging to the microbial population receive the same energy dose. Previous studies carried out using batch chambers, have demonstrated that even when treatment heterogeneity exists due, e.g., to a non-uniform distribution of the electric field in the treatment zone, or cells cluster formation with high pulse resistance [2,6], the stirring of the microbial suspension can help to enhance the effectiveness of PEF process [6]. As a consequence, although classically, microbial reduction by PEF treatment has been related to electrical and biological parameters [4] also hydraulic parameters could be involved when a continuous flow system is used. However, only few investigations are concerned on the crucial role that flow conditions could play on the microbial inactivation by PEF treatment [5,7].
The present experimental study deals with microbial inactivation of *E. coli* and *S. cerevisiae* cells by high voltage pulsed electric fields in a continuous flow system. The aim of this work was to investigate the influence of different flow conditions on the effectiveness and energy efficiency of the process.

2 Materials and methods

2.1 Microorganisms and growth conditions

PEF experiments were carried out using a strain of *S. cerevisiae* obtained from a commercial bakery yeast (Levital) and a non-pathogenic *E. coli* strain (HB 101) as microorganisms test. *S. cerevisiae* cells were growth in MRS broth (OXOID) at 32 °C in an aerated incubator. Samples taken after 40 h were in the stationary growth phase. *E. coli* cells were growth in LB nutrient broth (DIFCO) incubated at 37°C with continuous shaking at 150rpm for 18 h to obtain cells in the stationary growth phase.

2.2 Experimental apparatus

A laboratory scale continuous PEF unit was designed and constructed at the Chemical and Food Engineering Department of the University of Salerno.

As sketched in Fig. 1, the system mainly consists of the following units: stirrer, pump, heat exchanger, treatment chamber, high voltage pulse generator and controlling and monitoring devices to measure and record the voltage, current and sample temperature during the treatment.

The high voltage pulse generator consists of a repetitive capacitor discharge modulator constructed to provide exponential decay electric pulses in a wide range of values of field strength, pulse repetition frequency and pulse width. Microorganisms were treated in a continuous parallel plate treatment chamber with a distance between electrodes of 0.25 cm and an area of 2.6 cm².

2.3 Experimental design

In this work, similar PEF experiments were carried out for both microbial suspensions of *E. coli* and *S. cerevisiae* cells, respectively.

Before PEF treatments microbial cells were recovered from their mother broth culture by centrifugation and resuspended in Trizma HCl buffer 0.05 M (pH 7.2, electrical conductivity 2 mS/cm at 25°C). The final microbial concentration was about 5·10⁶ CFU/ml for *S. cerevisiae* suspensions and 2·10⁷ CFU/ml for *E. coli* suspensions. Experiments of microbial inactivation were carried out to study the effect of different flow conditions obtained, at fixed field strength (18 kV/cm), either by changing the suspension flow rate (1-6 l/h) in a single pass operation through the PEF chamber, or, at a fixed flow rate (2 l/h), by successive passes through the chamber (up to 5) with intermediate cooling of the processed product (multistep treatment). Treated samples were collected in Eppendorf-cup, placed immediately on ice before microbiological analyses. All the experiments were performed at the inlet temperature of 25°C while the outlet temperature was always lower than 44°C.
2.4 Count of viable cells

The enumeration of the cell viability in the samples collected before and after PEF treatment was determined after a proper dilution of the samples in distilled water by plate count method. The count of microbial colonies, grown on MRS agar slants at 32 °C for 48 h for *S. cerevisiae* and on the surface of LB-agar plates at 37 °C for 24 h for *E. coli*, respectively, was expressed in CFU/ml (colony forming units per ml of sample).

3 Results and discussion

3.1 Effect of flow rate

Microbial inactivation experiments, carried out by changing the flow rate at a fixed electric field strength, highlight that, although the microorganisms tested show a different inactivation kinetics, in both case the suspension flow rate play an important role on the effectiveness of PEF process: the higher the flow rate, the faster the inactivation rate (Fig. 2).

For instance, for a fixed amount of energy received by the product, equal to 60 J/mL, enhancing the flow rate from 1 to 4 L/h the inactivation level increases from 3.8 to 5.2 log-cycles for *S. cerevisiae* and from 2.5 to 5.6 log-cycles for *E. coli*, respectively.

In addition, the energy consumption useful to obtain the same level of microbial inactivation drastically decreases as the flow rate increases.

These results clearly demonstrate the key role that the selected flow rate may play to improve the uniformity and, consequently, the efficiency of the PEF treatment.

![Inactivation curves of S. cerevisiae (a) and E. coli (b) as a function of total specific energy input at different suspension flow rate.](image)

In fact, a greater stirring of the product in the treatment chamber may allow cells to move, during the pulse treatment, from zone with lower field strength to those with higher field strength as well as to support their rotation with respect the electrode between each pulse, or to break cells aggregate with higher pulse-resistance [6].

Further experiments should be performed at higher field strength than the ones used in this investigation, in order to determine the optimal process conditions that maximize the lethal effect of the PEF with the minimum energy consumption.

3.2 Multi-step PEF treatment

Multi-step treatment may represent a viable way to increase the lethality and energy efficiency of a PEF treatment using moderate field strength, as well as to reduce the ohmic heating of the product. Further, it may be also used to simulate the performance of a multiple electrode chambers with intermediate cooling system. In fact, during the multi-step treatment
the same microbial suspension is passed several times through the treatment chamber and the subsequent cooling system in order to gradually increase the total amount of the electric energy received.

Fig. 3 shows the inactivation curves of (a) *S. cerevisiae* and (b) *E. coli* cells obtained at fixed field strength (18 kV/cm) and flow rate (2 L/h) as a function of the number of passes \((N_p)\) through the treatment chamber and for different amount of total energy input per pass \((W_p)\) (22, 42, 61 and 82 J/mL).

Results show that the number of passes through the chamber is an important operative parameters that markedly affect microbial inactivation of both *S. cerevisiae* and *E. coli* cells and is strictly related to the energy delivered during each pass. In fact, total inactivation can be obtained by different combinations of number of passes through the chamber and energy applied per pass: the lower the energy per pass, the higher the number of passes through the chamber.

Interestingly, the curves reported in Fig. 3 highlight that the effect on the inactivation level achieved in each step of the multi-step treatment is not additive, but a reduction in the efficiency at adding steps can be observed. In order to clarify this concept, in Fig. 3 (a) and (b) the linear trends (dashes lines) determined by the first PEF steps are reported: at any energy level per pass the first step is more effective than the following ones. This observed non-additive trend may be mainly attributed to the natural distribution of individual cell resistance to field strength.

Fig. 4 shows, at the same field strength and flow rate, a comparison between the inactivation curves of (a) *S. cerevisiae* and (b) *E. coli* cells obtained in a single pass operation through the chamber and those obtained by multistep treatments carried out using an energy input per pass of 42 and 22 J/mL, respectively. The effectiveness of the multiple PEF treatment increases when the same total specific energy input is delivered with lower energy per pass. In addition, from an energy efficiency point of view, the same inactivation level can be achieved with lower energy consumption when a multiple treatment with lower energy per pass is performed.

These results can be explained considering that, when a multiple treatment with a lower energy per pass is used, the same total specific energy is delivered using a higher number of passes. This in turn, provides a greater stirring of the microbial suspension that, consequently, enhances the efficacy and the energy efficiency of the PEF treatment.
Fig. 4: Inactivation curves of (a) S. cerevisiae and (b) of E. coli cells as a function of the total specific energy input during PEF treatment carried out in single pass operation and during multistep treatment with different energy input per pass. $E=18$ kV/cm; $\tau=3.6$ $\mu$s.

4 Conclusions

Results of this work demonstrated for two different microbial strains the possibility of improving the PEF sterilization efficiency by promoting higher degree of stirring of the microbial suspension by means of a proper selection of the flow conditions. In particular, the findings of this work suggest that the set up of a high flow rate multistage process with lower energy input per stage could significantly increase the overall effectiveness of the pulse treatment, while, at the same time, drastically reduces the energy consumption as well as the heating of the food products.

Finally, conclusions of this work support the view that a new approach in which not only electric and microbial parameters but also hydraulic parameters should be taken into account in order to optimize the process parameters and reduce operational costs.

References

STUDY OF ASCORBIC ACID DEGRADATION IN ORANGE JUICE BEVERAGES TREATED BY PULSED ELECTRIC FIELDS

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Abstract:
Pulsed Electric Field (PEF) is an innovative, non-conventional technology with potential for optimizing intake of nutrient and non-nutrient phytochemicals in human foods. The object of the present study, it is to determine the kinetics of degradation of the ascorbic acid in different beverages with orange juice treated by PEF and to analyze the possible influence of the food characteristics on the change of the ascorbic acid by the treatment. The kinetics of degradation of the ascorbic acid was determined in orange juice, orange-carrot juice and orange juice-milk beverage treated by different electric field intensities (15-40 kV/cm) and different treatment times (40-900 µs). The degradation ascorbic acid follows zero-order degradation kinetics in orange juice and orange-carrot juice while in orange juice-milk beverage the ascorbic acid degradation follows a first-order degradation kinetics. The orange juice is more resistant to the changes of field intensity, while to produce the same degradation of the ascorbic acid the treatment time it must be major in the orange juice-milk beverage than in the orange juice and the orange-carrot juice. These results reveal the need to optimize the conditions of the treatment whenever there changes the food or some factor of the PEF treatment.

Key words: ascorbic acid, pulsed electric fields, orange juice, carrot, milk, degradation kinetics.

1 Introduction

Fruit and vegetables contain compounds that have a protective effect against degenerative diseases, known as phytochemical or bioactive compounds (fiber, phenolic compounds, carotenoids, vitamins A, C, and E, glucosinates, organosulfur compounds, etc.), whose biological activity has been studied in numerous ex vivo, in vitro assays and by tests on humans. Epidemiological studies also show that the consumption of fruit and vegetables has a considerable protective effect against the risk of certain diseases such as cancer, cataract, macular degeneration, and cardiovascular diseases [1]. Not only fruit and vegetables are rich in bioactive compounds; dairy products and milk fractions (milk, whey, casein, and lactoferrin) have a powerful biological and functional activity and contain high concentrations of antioxidant compounds.

Growing consumer demand for safe processed foods requiring minimum preparation time and presenting maximum similarity to the fresh product has led the food industry to increase
production of foods of this kind and seek ways of ensuring that bioactive compounds and nutrients are retained or modified only minimally during processing and storage, until they reach the consumer [2]. Considerable importance is currently being gained by fruit juices not derived from concentrates, enriched or mixed with vegetable juices or milk.

Treatment by high-intensity pulsed electric fields is an non-conventional technology with promising results for the inactivation of microorganisms and enzymes, preserving the organoleptic and nutritional characteristics of the treated product [3-8]. A study by the Institute of Food Technology [9] highlights the research needs for emerging preservation technologies, especially the identification of how they may affect bacterial inactivation, quality, nutritional value, and shelf life of foods, changes in critical processing factors, and the introduction of new factors.

Studies on the loss and/or modification of vitamin contents are necessary to learn how treatment affects the product’s nutritional characteristics that are of particular importance for the consumer. Vitamin C is a thermostable vitamin that is especially affected by heat treatment, and it has been used as a quality indicator after application of a preservation process [10,11].

The aim of the present study was to determine the degradation kinetics of ascorbic acid in various orange juice based beverages treated by high-intensity pulsed electric fields, and to analyze the possible influence of the product’s characteristics on the variation in ascorbic acid after the preservation treatment.

2 Materials and methods

2.1 Samples

**Orange juice**: The orange juice (*Citrus sinensis* L., Navel variety) was obtained by squeezing (FMC juice extractor with 2 mm perforated plates) and passed through a filter with pores having a diameter of 0.23 mm.

**Orange–carrot juice mixture**: The orange juice was prepared as described above. To obtain the carrot juice, the carrots were washed first with a solution of sodium hydroxide and then with drinking water. The juice obtained was sieved and mixed with the orange juice in the following proportion: orange–carrot, 80:20 (v/v).

**Orange juice and milk beverage**: The beverage was prepared by mixing 50% (v/v) of orange juice prepared as described above, 20% (v/v) of UHT skim milk, and 30% (v/v) of distilled water. Then 7.5% of saccharose (w/v), 0.1% of citric acid (w/v), and 0.3% of high methoxyl pectin (w/v) were added as sweetener, preservative, and stabilizer of the samples, respectively.

A sufficient quantity of each product was prepared and divided into three parts: one was assigned as a control, one was processed by PEF, and one was pasteurized thermally.

2.2 Heat treatment

The heat treatment was applied in an Armfield FT74P plate exchanger. The beverage was placed in a feed tank and driven by a pump towards the heat exchanger, where it attained the heat conditions selected (90 ºC, 20 s for the orange juice and the juice–milk beverage; 98 ºC, 21 s for the orange–carrot juice mixture, heat treatments of 90–99 ºC for 15–30 s being customary in the industry [12]. The beverage was cooled after treatment by means of a cooler (Armfield FT61), packaged aseptically, and stored in refrigeration (4±1 ºC) until it was analyzed. The treatment was carried out in duplicate.
2.3 PEF treatment

The treatment was applied in continuous mode in an OSU-4D system designed by the University of Ohio (USA) and installed at the Instituto de Agroquímica y Tecnología de los Alimentos (CSIC) in Valencia. The pulse system consisted of six “co-field” treatment chambers connected in series, having a diameter of 0.23 cm and a distance of 0.293 cm between electrodes, and two cooling coils submerged in a refrigerated bath (Polystat, Cole Parmer, IL, USA) connected before and after each pair of treatment chambers in order to keep the temperature within the designated range. The initial and final treatment temperatures were recorded by thermocouples placed at the entrance to the first treatment chamber and at the exit of the last chamber, respectively. The temperature, waveform, voltage, and treatment intensity were recorded with a digital oscilloscope (Tektronix TDS 210, Tektronix, OR, USA). The flow rate was set at 60 mL/min and controlled by a peristaltic pump. The pulse duration selected was 2.5 µs. The treatment time ranged from 30 to 700 µs, and the electric fields assayed were in the range 15–40 kV/cm. The samples were collected after each treatment and stored in refrigeration (4±1 ºC) until they were analyzed. The treatment was performed in duplicate.

2.4 Determination of physicochemical parameters

The physicochemical parameters were determined as follows: Electric conductivity by a Crison 525 conductivity meter (Crison Instruments S.A., Alella, Barcelona, Spain); pH by a Crison 2001 pH meter (Crison Instruments S.A., Alella, Barcelona, Spain), and soluble solids (ºBrix) by an Atago RX-1000 refractometer (Atago Company Ltd, Tokyo, Japan).

2.5 Polarographic determination of ascorbic acid

Five mL of the product was diluted and made up to 25 mL with the extraction solution: 1% oxalic acid (w/v), 2% trichloroacetic acid (w/v), and 1% sodium sulfate (w/v). After vigorous shaking the solution was filtered. Then 9.5 mL of a solution of 1% oxalic acid (w/v) and 2 mL of acetic acid/sodium acetate 2 M buffer solution (pH = 4.8) was added to 0.5 mL of the filtrate in the polarographic cell [13].

3 Results

Table 1 shows the physicochemical parameters of each of the samples. It can be seen that the orange–carrot juice mixture had the highest conductivity, while the orange juice–milk beverage had the highest ºBrix. The ascorbic acid concentration in the untreated orange juice (47.6±1.4 mg/100 mL) was higher than that of the other two samples (27.1±0.4 mg/100 mL and 25.9±1.4 mg/100 mL for the orange–carrot juice and the orange juice–milk beverage, respectively).

<table>
<thead>
<tr>
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<th>Orange juice</th>
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<th>Orange juice–milk beverage</th>
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</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.35±0.01</td>
<td>3.84±0.04</td>
<td>3.92 ± 0.01</td>
</tr>
<tr>
<td>ºBrix</td>
<td>11.8±0.1</td>
<td>10.3±0.3</td>
<td>14.3 ± 0.1</td>
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<tr>
<td>Conductivity (mS/cm)</td>
<td>3.42±0.04</td>
<td>4.55±0.05</td>
<td>2.99 ± 0.05</td>
</tr>
</tbody>
</table>

Tab. 1: Physicochemical characteristics of the beverages analyzed: orange juice, orange–carrot juice mixture, and orange juice–milk beverage

The ascorbic acid concentration in the orange juice after pasteurization at 90 ºC for 20 s did not vary significantly (p < 0.01). The ascorbic acid retention in the pasteurized orange–carrot juice (98 ºC, 21 s) was 83%, while in the orange juice–milk mixture (90 ºC, 20 s) it was 86%.
The retention of ascorbic acid after PEF treatment in the orange juice ranged between 81.6%, after a treatment of 25 kV/cm for 340 µs, and 97.0% when the electric field strength applied was 40 kV/cm and the time was 30 µs. Similar values were obtained in the orange–carrot juice, 83.1 and 97.1% for treatments of 35 kV/cm (150 µs) and 40 kV/cm (30 µs), respectively. In the orange juice–milk beverage, however, the ascorbic acid retention was slightly higher, 90.7 and 97.3% for treatments of 15 kV/cm (500 µs) and 25 kV/cm (40 µs), respectively.

To evaluate the treatments applied, a study was made of the degradation kinetics of ascorbic acid with treatment time in each of the fields applied. When the ascorbic acid retention in orange juice was plotted against treatment time for each of the fields applied (Figure 1), it could be seen that it follows a zero-order kinetic: $C = C_0 - kt$, where $C$ is the ascorbic acid concentration after treatment (mg/100 mL) and $C_0$ is the initial ascorbic acid concentration (mg/100 mL), $k_E$ indicates the ascorbic acid degradation rate ($\mu$s$^{-1}$), and $t$ is treatment time (µs). The degradation constant obtained from the degradation curve calculated by plotting $\ln(C/C_0)$ against time for each treatment was: $-0.00046 \pm 1 \cdot 10^{-4} \mu s^{-1}$ ($r = 0.857$, standard error (SE) = 0.039), $-0.00051 \pm 5 \cdot 10^{-5} \mu s^{-1}$ ($r = 0.980$, SE = 0.009), $-0.00074 \pm 8 \cdot 10^{-5} \mu s^{-1}$ ($r = 0.969$, SE = 0.012), and $-0.00095 \pm 8 \cdot 10^{-5} \mu s^{-1}$ ($r = 0.967$, SE = 0.012), for fields of 25, 30, 35, and 40 kV/cm, respectively. The fit was significant at the 99 percent confidence level ($p < 0.01$) in all cases except the field of 25 kV/cm, where the fit was significant at the 95 percent level ($p < 0.05$). Similar results were obtained in orange–carrot juice mixture. Least squares fitting of ascorbic acid retention versus treatment time in each of the fields showed that it follows a zero-order kinetic. The ascorbic acid degradation rate ($k_E$) obtained was $-0.00037 \pm 3 \cdot 10^{-5} \mu s^{-1}$ ($r = 0.987$, SE = 0.009); $-0.00042 \pm 5 \cdot 10^{-5} \mu s^{-1}$ ($r = 0.975$, SE = 0.009); $-0.00119 \pm 7 \cdot 10^{-5} \mu s^{-1}$ ($r = 0.993$, SE = 0.008), and $-0.00091 \pm 2 \cdot 10^{-4} \mu s^{-1}$ ($r = 0.917$, SE = 0.018), for fields of 25, 30,
35, and 40 Kv/cm, respectively. The fit was significant at the 95 percent confidence level (p < 0.05) in all cases. In orange juice–milk beverage, when the ascorbic acid retention was plotted against treatment time for each of the fields applied, it was seen that the experimental data for each electrical field fit an exponential model of the type: $C/C_0 = ae^{-bt}$. The ascorbic acid degradation rate ($k_E$) obtained was: $-0.00012 \pm 3 \times 10^{-5}$ µs$^{-1}$ ($r = 0.874$, SE = 0.019); $-0.00022 \pm 5 \times 10^{-5}$ µs$^{-1}$ ($r = 0.910$, SE = 0.013); $-0.00042 \pm 7 \times 10^{-5}$ µs$^{-1}$ ($r = 0.934$, SE = 0.010), and $-0.00061 \pm 6 \times 10^{-5}$ µs$^{-1}$ ($r = 0.979$, SE = 0.006), for fields of 15, 25, 35, and 40 Kv/cm, respectively. The fit was significant at the 95 percent confidence level (p < 0.05) in all cases.

The decimal reduction time (D), is the time required at a particular field strength to reduce the initial ascorbic acid concentration by 10%, was obtained from the destruction curve. It was calculated by using a procedure analogous to the one used in thermal destruction studies, from the expression $D = 2.303/k_E$. Table 2 shows the decimal reduction time values for each of the samples studied. The D values were higher in the orange juice–milk beverage, indicating that to produce the same degradation of ascorbic acid the treatment time would have to be longer than that required for the orange juice and the orange–carrot juice. The $Z_E$ value was calculated as the negative inverse of the destruction curve obtained by plotting Log D against the field applied, giving the following values: 42.9 kV/cm ($r = 0.978$, SE = 0.040, p < 0.05), 30.6 kV/cm ($r = 0.856$, SE = 0.156, p < 0.1), and 35.1 kV/cm ($r = 0.999$, SE = 0.010, p < 0.05), for the orange juice, orange–carrot juice mixture, and the orange juice–milk beverage, respectively. The higher value obtained in the orange juice indicates that it is more resistant to changes in electric field strength in PEF treatment. These results show the need to optimize treatment conditions whenever there is a change in the matrix (food) or some processing factor.

<table>
<thead>
<tr>
<th>E (kV/cm)</th>
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<th>Orange–carrot juice</th>
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<td>2.89</td>
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<td>40</td>
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<td>2.52</td>
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</tbody>
</table>

Tab. 2: Decimal reduction time for each of the treatments applied and samples analyzed

4 Acknowledgements

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5 References


INFLUENCE OF HIGH INTENSITY PULSED ELECTRIC FIELD TREATMENT CONDITIONS ON PEROXIDASE ACTIVITY IN TOMATO JUICE

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Abstract:
The increase in the demand for fresh-like products has developed the interest in new techniques for food processing such as high-intensity-pulsed electric fields (HIPEF). Tomato juice is an important source of mineral salts, vitamins and carotenoids, whose health-promoting properties are widely recognised. One of the enzymes that mostly affect the quality of this product is peroxidase (POD), which contributes to color deterioration, off-flavor formation and loss of nutrients. The objective of this work was to establish the HIPEF treatment parameters that assure the greater POD inactivation in tomato juice. A response surface experimental design was used to study the effect of pulse frequency, pulse width, treatment time and polarity on POD inactivation, keeping constant the electric field strength at 35 kV/cm. A second-order polynomial model was adjusted to the experimental data (p<0.0001), being adequate for predicting the response across the design space. POD was completely inactivated at 35 kV/cm for 2000 µs using 4 µs-pulses in bipolar mode applied at 250 Hz without exceeding 35°C. In addition, bipolar treatments were more effective than monopolar treatments. An interaction between the effects of the pulse frequency and treatment time was observed. In this way, a 1% of residual POD activity was reached with either 2000 µs and 170 Hz or 1000 µs and 250 Hz, when bipolar pulses of 35 kV/cm and 7 µs were applied. Furthermore, a reciprocal influence between pulse width and polarity was revealed. Hence, this study demonstrated the effectiveness of HIPEF treatment to achieve the inactivation of tomato juice POD.

Keywords: peroxidase; high-intensity pulsed electric fields; tomato juice.

1 Introduction

High-intensity pulsed electric field (HIPEF) is a non-thermal technology that is being extensively studied as an alternative to the traditional thermal treatment (Dunn, 2001; Martín-Belloso & Elez-Martínez, 2005). In comparison to the extensive research devoted to the destruction of microorganisms by HIPEF, the information available about the effect on enzymes is more limited.

Reducing peroxidase (POD) activity in tomato juice is an important goal to avoid color deterioration, off-flavor formation and loss of nutrients (Robinson, 1991). Nevertheless, the conventional heat treatment applied to tomato juice to inactivate the enzyme, can damage other valuable properties. A relative high specific energy input, defined through high electric field strength and total treatment time, is required in order to achieve enzymatically stable products (Martín-Belloso and others 2005). In addition, other HIPEF variables such as pulse frequency, pulse width and polarity are also important in defining HIPEF treatment conditions necessary to adequately reduce enzyme activity (Qin and others 1995). The objective of this work was to study the effect of HIPEF parameters on the POD activity of tomato juice as well as to establish the treatment conditions to obtain the greatest POD inactivation in tomato juice.
2 Materials and Methods

Fresh ripened tomato fruits (Lycopersicon esculentum var. Flandia Prince) were washed and chopped. Then, they were crushed and the resulting purée was filtered through a screen of 1.27 mm size to remove peel and seeds.

Pulse treatments were carried out using a laboratory scale pulse generator (OSU-4F, The Ohio State University, Columbus) that provides square-wave pulses within eight cofield flow chambers in series whose treatment volume and gap distance are 0.012 cm$^3$ and 0.29 cm, respectively. The flow rate of the process was adjusted to 60 ml/min and controlled by a variable speed pump (model 75210-25, Cole Palmer, Vernon Hills, IL, USA). The treatment temperature was kept below 35 °C using a cooling coil connected before and after each pair of chambers and submerged in an ice-water shaking bath.

A response surface analysis was used to study the effect of the different HIPEF treatment variables on the inactivation of POD in tomato juice, keeping constant the electric field strength at 35 kV/cm. A face-centred central composite design was used. The independent numerical variables were pulse frequency (from 50 to 250 Hz), pulse width (from 1 to 7 µs) and treatment time (from 1000 to 2000 µs), whereas polarity was a categorical variable. Variable levels were chosen according to previous studies. The design was conducted in duplicate, resulting in two blocks of experiments. The order of assays within each block was randomized. Experimental data were fitted to a polynomial response surface. The second order response function was predicted by Eq. 1:

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j>i}^{3} \beta_{ij} X_i X_j,
\]

where $Y$ is the dependent variable, $\beta_0$ is the center point of the system; $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ are the regression coefficients of the linear, quadratic and interactive effects of the independent variables, respectively; $X_i$, $X_i^2$ and $X_iX_j$ represents the linear, quadratic and interactive effects of the independent variables, respectively. The nonsignificant terms ($P \geq 0.05$) were deleted from the second-order polynomial model after an ANOVA test, and a new ANOVA was performed to obtain the coefficients of the final equation for better accuracy.

POD activity of tomato juice was measured using the method described by Elez-Martínez et al. (2006).

3 Results and Discussion

No residual POD activity was observed after a treatment of 35 kV/cm at 250 Hz for 2000 µs using 4-µs bipolar pulses. In contrast, the initial POD activity was kept when tomato juice was treated with 1-µs monopolar pulses at a rate of 50 Hz for a total treatment time of 1000 µs (Fig.1).

The 2nd order model properly fitted the experimental data ($p = 0.0001$). The determination coefficient (R2) was 0.85 and the lack of fit was not significant, indicating that the model was adequate for predicting the response across the design space. Pulse frequency, pulse width and treatment time affected linearly the POD inactivation and only the quadratic term of pulse frequency was significant. There were also differences between the inactivation levels achieved by applying monopolar and bipolar treatments. Thus, residual POD activity values in HIPEF-treated tomato juice can be modeled through equations 2 or 3, which correspond to monopolar and bipolar pulses, respectively:

\[
Y = 183.496 - 0.693 \cdot f - 12.821 \cdot \tau - 0.018 \cdot t + 1.151 \cdot 10^{-3} \cdot f^2 + 1.229 \cdot 10^{-4} \cdot f \cdot t
\]

\[
Y = 128.376 - 0.709 \cdot f - 8.131 \cdot \tau - 6.824 \cdot 10^{-3} \cdot t + 1.151 \cdot 10^{-3} \cdot f^2 + 1.229 \cdot 10^{-4} \cdot f \cdot t
\]
where $Y$ is the residual POD activity ($\%$), $f$ the pulse frequency (Hz), $\tau$ the pulse width ($\mu$s) and $t$ the treatment time ($\mu$s).

The equations show that bipolar treatments were more effective in inactivating POD activity than monopolar treatments. Hence, when tomato juice was treated using bipolar 4-$\mu$s pulses of 35 kV/cm for 1000 $\mu$s at a rate of 250 Hz, residual POD activity was a 26.7% lower than that observed after applying the same conditions in monopolar mode.

The linear coefficient of the variable treatment time, $t$, was negative, meaning that the higher the HIPEF treatment time, the lower the residual POD activity values. The single effect of $t$ depended on the polarity mode used according to equation 2 and 3. Differences in RA among treatment times and polarity suggest that, at the same level of $t$, bipolar treatments led to lower values of POD activity than monopolar treatments.

POD inactivation also depended on the pulse frequency, $f$. Therefore an increase in its value, keeping constant the rest of variables, induced a decrease in POD activity. When $f$ increased from 50 to 200 Hz, applying bipolar 7-$\mu$s pulses for 2000 $\mu$s at 35 kV/cm, the residual activity decreased from 32.1 to 0 %. These results suggest that at least a pulse frequency of 200 Hz was required to reach the highest POD inactivation rates. The effect of pulse frequency, $f$, on the reduction of POD activity from tomato juice was affected by the treatment time, $t$, which was included in the response model as the interaction $f \cdot t$ (equation 3 and 4). The positive value of the interaction term suggests that greater RA reduction can be achieved by increasing both variables. However, the effect of the mutual influence of $f$ and $t$ on residual POD activity followed a nonlinear curve (Fig. 2).
Fig. 2: Effect of pulse frequency and total treatment time on the residual POD activity in tomato juice treated with 7-μs pulses of 35 kV/cm in bipolar (A) or monopolar (B) mode.
Moreover pulse width, $\tau$, and pulse polarity, $P$, show a reciprocal influence, as revealed by the significance of the interaction term $\tau \cdot P$. In this way, greater POD inactivation values could be feasible when selecting pulse widths higher than 5.5 $\mu$s in bipolar mode. Moreover, complete POD inactivation was reached when pulses of 35 kV/cm for 2000 $\mu$s at 200 Hz were applied, using the abovely mentioned conditions of pulse width and polarity (Fig. 3).

Fig. 3: Effect of the pulse width on the residual POD activity of tomato juice treated at 35 kV/cm for 2000 $\mu$s at rates of 50 (□) or 250 Hz (▲) in bipolar (····) or monopolar (—) mode.

4 Conclusions

Complete POD inactivation in tomato juice was observed when a HIPEF treatment (35 kV/cm for 2000 $\mu$s) was performed applying 7 $\mu$s-bipolar pulses at 200 Hz. Among the studied variables; treatment time, pulse frequency, pulse width and the simultaneous influence of treatment time and pulse frequency could be modeled by a second-order equation. Higher POD inactivation levels were attained when treated in bipolar than in monopolar mode. Moreover, a reciprocal influence of pulse width and polarity was revealed, allowing to maximize POD inactivation when selecting pulse widths higher than 5.5 $\mu$s in bipolar mode. This study demonstrates the effectiveness of HIPEF treatment on tomato juice POD inactivation and describes the influence of processing conditions on inactivation.
Acknowledgements

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References


EFFECT OF OHMIC HEATING IN VEGETABLE ACIDIFICATION

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Abstract:

Diffusion limited conventional acidification process necessitates the development of faster processes. This would reduce product pH below 4.6 as fast as possible preventing the growth of Clostridium botulinum. Conventional and ohmic acidifications were thus compared. Conventional (0 V/m, 100°C) and ohmic (1300V/m at 100°C and 2400 V/m at 70°, 80°, 90°, and 100°C) acid-blanching of carrots was done for a blanching time up to 3 minutes. Additionally, carrots, cucumbers, and asparagus were acid-blanchned under conventional (100°C) and ohmic (2400 V/m, initial temperature of 70°, 80°, 90°, and 100°C) process, holding at 100°C (final temperature) for 3 minutes. Results from both studies validated that ohmic acidification significantly accelerates diffusion over conventional acidification.
ACTIVE ELECTRICAL ANSWER OF MEAT TO NANOSECOND PULSED ELECTRIC FIELDS

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Abstract:
The application of brief but intense electric field to muscle tissue charges the cell membranes. Once a critical transmembrane voltage is reached, electroporation happens. This increases the permeability of membranes for charged compounds by orders of magnitude. The most pronounced effect occurs where the electric field lines cross the membranes perpendicularly, i.e. at the pole caps facing the electrodes. Therefore, one expects that both sides of a cell become electroporated. But, due to the time requirement of electroporation, ultrashort pulses with no sufficient field strength may not cause any pores within the membrane structure. Moreover, since the time course of electroporation is different at the depolarization- and the hyperpolarization side, it is expected that in a small window of pulse duration and field strength electroporation occurs only at one side. Using potential sensing in muscle tissue after such a pulse, the resting potential of the non-electroporated membrane should add, so that information about the electrophysiological state of the cell becomes available without sophisticated equipment like patch-clamp.

We explored single side electroporation for early assessment of meat quality, usually 20 min post mortem. It was found that the voltage after single side electroporation decreases with increasing drip loss.

Key words: nanosecond pulsed electric field (nsPEF), electroporation, active potential

1 Introduction

By definition, electroporation is the concept for the electric formation of transient aqueous pathways across the cell membrane [1,2]. The use and optimization of electroporation procedures requires knowledge about the mechanism of pore formation in cell membranes.

The tissue is usually symmetric with respect to the plasma membranes (except polarized tissue like endothelium) and thus the voltage across an pair of electrodes with a distance on the order of centimeters would be zero. A voltage appears only, if one electrode torches a cell or the membranes are asymmetrical with respect to the transmembrane voltage. If the cell membrane or the membranes of organelles are electroporated, the dc-resistance of the tissue decreases [3,4], but this should not cause any persiting voltage difference between the electrodes.

For basic studies on cell electroporation singe cells are advantageous. Using whole cell clamp, hyper- and depolarization with respect to the resting potential can be applied separately. Moreover, the different kinetic of recovery is assessible as well. For studies in meat, using electrodes with a distance of centimeters, any voltage difference appearing at the electrodes would be interpreted as asymmetric electroporation. In the extreme case, only one side of the membrane is electroporated while the electric field affected the membrane at the other side not at all.

Since the outer electric field increases the transmembrane voltage at the hyperpolarization but decreases it at the other side, one would expect electroporation occurring first at the hyperpolarization side, which was also optically imaged using potential sensitive dyes [5]. If the pulse stops after electrroporating the hyperpolarization side, only this side will be dicharged due to the conducting pathways across the membrane but the potential at the other
side remains at the resting potential. Summing over the transmembrane potential of all cells between the electrodes, would result in a voltage with the opposite polarity of the pulse.

The electrically created pores reseal after electroporation. Immediately after resealing only the Donnan-potential exists due to the fixed negative charges at intracellular macromolecules. The Na⁺/K⁺ATPase re-establishes the K⁺-gradient which in conjunction with K⁺-leakage channels forces the transmembrane potential back the resting potential. Parameters interesting for the evaluation of the specimen are the time and the degree of recovery. Especially the jump in potential due to the high voltage stimulus is practically important, since its measurement is fast and simple.

Since these parameters depend on the level of ATP, the density of ATPase as well as the integrity of the membranes, they reflect the actual condition of the cell. Moreover, with this knowledge it should be possible to predict the time course of post mortem curing processes, which is related to quality of meat.

2 Methods

2.1 Assessment of hyper- and depolarization effects in whole cell clamp

A whole cell clamp has been used to characterize single plasma membranes of chinese hamster ovary (CHO) cells (Fig.1). CHO were chosen because, other than muscle cells, they do not contain voltage sensitive channels. Despite significant differences to muscle cells, CHO-membrane can act as a reasonable model for plasma membranes without the need of extremely toxic agents like TTX (tetradotoxin) or TTA (tetraethanoammonia) for blocking channles [6]. The glass pipette has been pulled from borosilicat glass PG120T-7.5 (Harvard apparatus Ltd.) to a tip diameter of \( d = 1 \pm 0.5 \mu m \). The electrical resistance was \( G = 4 \pm 0.5 \, \Omega \) when filled with 150mM KCl solution.

The Ag/AgCl microelectrodes have been made from silver wires by chloridizing with 1 M HCl electrolyte for 1 h at a current density of about 100 \( \mu A/cm^2 \). The cells were sucked onto the tip of the pipette under microscopic observation using a hydraulic micromanipulator (NARISHIGE, Japan).

For the control voltage (\( U_c \)) an arbitrary waveform is generated in MATLAB and transferred via RS 232 as lookup table to a microprocessor (ADµC 812, Analog Devices, Norwood, MA). The 12-bit DAC of the processor converts the signal into an unipolar output which is amplified from \(-10 \, V\) to \(+10 \, V\). The completely sealed and shielded headstage converts the output into a current within the range of \(-350 \leq I/\mu A \leq +350 \). Because of the presence of the extracellular matrix, the seal resistance did not reach gigohms. Once the cell touches the pipette, a slight suction is applied from a hand-driven syringe pump with picoliter precision. The membrane is precisely broken at the site of the tip to provide tight contact between the pipette and the interior of the cell. In this way, the electrical properties of the entire membrane, except the part at the pipette, are covered.

Fig. 1: Experimental setup: The chamber is filled with culture medium and the cell is attached to the pipette. The current \( I \) is injected and the voltage \( U_{out} \), which is the transmembrane voltage plus junction potentials, is measured. The resting potential \( U_M = U_{out} (I = 0) \) is measured when the switch is open. \( U_c \) is the control voltage from the generator (\( I = U_c/R_m \)). A correction for the junction potential has been made by measurements without cell attached.
2.2 Clamping of the samples and nsPEF-generation

The muscle sample was clamped between two parallel 4x4 mm - plate electrodes made from stainless steel. The electrodes are mounted on rails for distance adjustment (1.5 – 5 cm). For keeping a constant pressure at the interface electrode / sample, a rubberband was used. Since the active potential after the stimulus is on the order of microvolt up to several millivolt reversible electrodes were used as monitor electrodes. Briefly, silverwire was chloridized in 0.1 M HCl with a current density of 0.1 mA / cm² and placed in a capillary, filled with 150 mM KCl. A two stroke syringe pump exchanged the KCl within the capillary after each experiment. The end of the 1 mm capillary with a length of 3 cm was pulled to a tip with a opening of 2-3 µm. The both sensing electrodes had a distance of 1 cm and were mounted on rails as well but perpendicularly to the other rails. They have been inserted into the sample after it was clamped between the outer electrodes. (Fig.2).

![Schematic diagram of the high voltage pulser and the measuring chamber.](image)

**Fig. 2:** (A) Schematic of the high voltage pulser and the measuring chamber. The actual setup is shown in (B). (1) pipettes with the Ag/AgCl – electrodes, (2) outer electrodes.

The high voltage was generated by a transverter reaching up to 35 kV. A single line configuration was used. The coaxial cable was charged until the spark gap triggered the pulse (Fig.2a) at about 10 kV. The length of the pulse was determined by the length of the coaxial cable. Using 3 m cable yield a pulse duration of 30 ns. When a calibration load with 50 Ω was used, the pulse was rectangular. Unfortunately, we were unable to adjust the resistance of the specimen, thus the pulse was reflected and several declining pulses resulted.

2.3 Voltage monitor for active potential after high voltage stimulus

In order to save the sensible electronics of the voltage monitor, it was disconnected during the pulse using a high voltage relay. For a high input impedance we used two FET-input operational amplifiers (AD 514, Analog Devices). The difference voltage after an instrumentation amplifier was optically discoupled and feed into the analog input (12 bit ADC) of a microprocessor (ADµC 831, Analog devices). Using digital control lines and a spark-sensor at the spark gap, the microcontroller controlled the high voltage generator as well. This ensured always a well defined pulse protocol.

2.4 Experimental protocol

After placing a sample of fresh muscle tissue (not yet meat !) into the chamber, the voltage across the inner electrodes was monitored for 10 s. Than, the high voltage relay connected the HV-generator and a pulse was triggered by turning on the HV-generator. After a defined number of pulses (i.e. 20 pulses within 20 s), the relay switched back for 1 min, while the voltage was monitored with a sample rate of 2 kS/s.
3 Results

3.1 Hyper- and depolarization

Positive (depolarization) and negative (hyperpolarization) current ramps are applied for 5 ms, resulting in a final current in the range $-200 \leq I_{5\text{ms}}/\text{nA} \leq +350$. The conductance of the membrane is lower during hyperpolarisation which rises the transmembrane voltage much faster than during depolarizing stimulus. The onset of the nonlinear current/voltage behaviour occurs earlier compared to a depolarising current ramp (Fig.3). The dramatic increase in the conductance (discontinuity of voltage during current ramp) found at $U_{\text{out}} = -1.1V$ ($t = 2 \text{ ms}$, $I = -78 \text{ nA}$) for hyperpolarisation and at $U_{\text{out}} = +0.81 V$ ($t = 4 \text{ ms}$, $I = +265 \text{ nA}$) for depolarisation, respectively, is suggested to arise from electroporation.

Comparing hyper- and depolarization, it is obvious, that electroporation condition is first reached at the hyper-polarization side and later at the depolarization side. This is still true when the rise time of the stimulus becomes very short. From these experiments was suggested, that a fast rising stimulus with ultra short duration can electroporate one side of the cell without changing the electrical behavior of the other one.

3.2 Active response of muscle tissue to ultrashort pulses

Since the behavior of the muscle fibers depends on the orientation of the electrodes with respect to the fiber but also on the direction (dorsal-rectal or rectal-dorsal), all sets of experiments with different orientation of the electrodes were treated separately. Here, we use only results from electrodes perpendicularly to the muscle fibers.

The muscle samples were selected by pH in order to ensure a high variety in quality. Most of the carcasses (66 %) showed normal quality (tested by drip loss within 48 h, $D_{48} < 6$) while about 34 % were of PSE quality (pale soft exudative). It should be noted, that PSE-quality appeared usually much less, here it was selected on purpose and does not reflect the general meat quality.

Despite the tissue should be homogeneous with respect to the resting potential of the membranes throughout all the cells, we still found none zero voltage which was Gaussian distributed at $U_M = 0.6\pm5.7 \text{ mV}$ for the perpendicular direction of the muscle fibers. This voltage disappears with the achievement of the rigor mortis.

Only pulses of negative polarity were applied which would result in a negative voltage if no active behavior of the membrane exist. A test setup with conductive agar-cubes showed a rapid discharge within a second and no persistent voltage between the monitor electrodes.

With the muscle sample, immediately after a high voltage stimulus, a change in the active potential was found (Fig.4A). Especially for meat with low drip loss it opposed the polarity of the pulse which hints to an active process. As in case of the active potential without stimulus, there was a trend to higher difference with lower drip loss (Fig.4B).
Discussion

The resting potential at cell membranes of about –60 mV is due to active processes, mostly due to the activity of the \( \text{Na}^+/\text{K}^- \)-pumps. Potassium leakage channels are responsible for equilibration of the electrochemical potential of Potassium, thereby charging the membrane electrically.

These active processes need energy, supplied by ATP. The content of ATP in the moment of stunning as well as the post mortem metabolism will considerably influence the quality of the meat.

Living or tissue or tissue just removed from blood circulation responds with active potentials after ultrashort high voltage pulses. The critical field strength for muscle tissue perpendicular to the fiber and a pulse length of 30 ns necessary for single-side electroporation is 3 kV/cm. This active response of muscle tissue is evident up to 40 min post mortem.

A trend to higher voltage difference before and after high voltage application is encouraging but the scatter of the experimental data is too high for significant prediction of the drip loss immediately after stunning. Further experiments with a higher level of standardization, like the time post mortem, race and age of the animals, are needed.

References

A NEW HARD AND SOFTWARE CONCEPT FOR IMPEDANCE SPECTROSCOPY ANALYSERS FOR BROADBAND PROCESS ANALYSIS

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Abstract:
Despite the acknowledged advantages of impedance measurements for characterization of biological material, rather little effort has been made to market equipment especially for the needs of process control. Here we present a modular concept for broad bandwidth impedance measurement equipment (IMPSPEC) which is especially designed for integration in control systems, for instance in food industry. It utilizes multi-sine-technique from DC to 10 MHz and the maximum length sequence correlation for an extended frequency range up to 5 GHz. This frequency range makes it suitable for characterizing cells but also molecular composition for example the fractional water content or dry matter. The analog front end as well as the interface to bus systems can be adapted as required. The precision is comparable with commercially available network analyzers. Connected to a PC, it becomes a versatile but still affordable laboratory instrument.

Key words: impedance spectrometer, low frequency, ultra-wideband

1 Introduction
Impedance measurements are a fast and simple way for material characterization. In recent years many activities aimed to extend the frequency range [1]. Modern ultra wideband (UWB) devices range behind the dispersion of water, thereby being able to monitor molecular composition of materials [2]. Today, most of the commercially available devices for impedance measurements are network analyzers, sweeping the frequency over the spectral range of interest. Besides some exceptions, they are very precise and offer a wide range of accessory in order to extend their capabilities. These highly versatile units are constrained to laboratory use due to their size, weight and cost. Another important limitation is the need for well trained personal because of their highly complex usage.

Process instrumentation, however, needs to be specially configured, thereby ensuring robustness and affordability. Moreover, the data processing should be automated and sufficiently robust which eliminates the need for specially trained technicians.

Although, a single unit maybe highly specialized, the system should be flexible with respect to the frequency range, bus interface, and analog front end. The software should be suitable for remote control and batch processing but should allow interactive measurements as well.

1.1 Choice for excitation signals
The sweep of sinusoidal signals over the desired frequency range is time consuming and not suitable for many practical applications. Therefore, the low frequency unit of the
IMPSPEC system utilizes multi-sine-excitation [1]. In the frequency range between some Hz and 10 MHz the user can choose discrete frequencies applied at once which greatly reduces the measurement time. At the receiver side, digital processing is used to recover phase and magnitude of each single frequency. Because of the need for high resolution digital analog converters, multi sine excitation is limited to low frequency applications.

For applications up to several GHz, ultra wideband excitation is required. The simplest wideband signal is a short pulse. The bandwidth is determined by the rise time and the pulse length. Pulses generated electronically, however, deviate from the desired rectangular waveform. This affects mostly the high frequency fraction of the power spectrum. Therefore, in order to ensure sufficient signal power, a high pulse amplitude is desired, which may involve problems by measuring at nonlinear material under test (MUT). Especially if biological matter is tested, low signal amplitude will minimize the influence of the measurement on the object characteristics. The bandwidth reduces, if a regularly spaced pulse train is used. A random distribution of pulses however, keeps the bandwidth of the individual pulse. The high frequency adapter of the IMPSPEC-system uses a maximum length sequence for excitation [3].

1.2 Multi-Sine Excitation

Other than in frequency sweeping, all frequencies are applied at once [4]. The generation of the signal in time domain is controlled by the host computer. By superposing multiple sinusoidal signals, local voltage peaks in time may appear. (Fig. 1a) Despite it is acceptable for most MUT, it may change the characteristics of very voltage sensitive materials such as biological cells or tissue [5]. Therefore, the design of the excitation signal should be optimized for a low crest factor, which is the ratio of the peak voltage to the RMS voltage.

![Fig. 1: Multi-sine signal with arbitrary phase of the individual sine waves (a) and with optimized crest factor (b).](image)

The focus for signal optimization is a low crest factor by adjusting the phase of the superposed sine waves. As shown in Fig. 1b, voltage peaks can be avoided using a properly designed excitation signal. Although theoretically each broad bandwidth signal can be generated, this technique is limited practically by the speed and accuracy of the DAC.

1.3 Maximum Length Sequence

In principle, using a single pulse together with high speed sampling will yield a well suited UWB-system. However, if a single pulse is used, averaging over several pulses for noise reduction is not possible. The use of an equally spaced pulse train reduces the bandwidth. A pseudo random binary sequence can circumvent both problems [1]. It has the bandwidth of the individual pulse and using a periodic sequence, averaging is possible. Moreover, in order to save on the hardware, subsampling can be used. This increases the measurement time, which is acceptable in most cases but eliminates the need for a very fast ADC. The IMPSPEC device generates a maximum length sequence (MLS) which is a special case of the pseudo random binary sequence (Fig. 2).
The spectrum of a MLS extends smoothly to high frequencies. Because of the high power even in the GHz-range, it greatly suppresses the noise. Thus, averaging can be less and the measurement speeds up. The signal itself is easy to generate using a clock generator and a chain of shift registers with feedback. The binary signal has a low crest factor which is especially important for biological materials. Another advantage associated with the low voltage needed is the short transition time at the steps which ensures a high bandwidth and a low jitter.

2 System description

Especially designed for process measurement application, the IMPSPEC is a modular impedance measurement equipment with basic units and special modules (Fig.3). While the data processing unit is generally required, the special modules are interchangeable and, if needed, especially developed to optimally adapt the material under test (MUT). IMPSPEC can be used offline or fully integrated in to an industrial application by omitting the general purpose unit.

The application unit (AU) is available as low frequency application unit (LF-AU) up to 10 MHz and ultra wideband application unit (UWB-AU) up to 5 GHz (see Fig 4 and 5).

![Diagram of IMPSPEC system](image)

Fig.3: Modules of the IMPSPEC-system. The general purpose unit (GPU) consists of the data storage, the signal processing and the post-processing features. For high versatility of the entire system, this unit can control additional sensors as well and capture data in several, user programmable channels. In typical applications, a Notebook-PC is used as GPU. The data processing unit (DPU) is the core of the entire system. The DPU is able to generate an excitation signal and to capture the data synchronously in two channels. Moreover, it can be used as a frequency response analyzer (FRA).

Basically, the DPU (Fig. 3) consists of a two-channel frequency response analyzer with 12 bit resolution. The excitation signal is generated by a 14-bit DAC. Preprogrammed excitation waveforms are sinusoidal, chirp and multi sine waves. Customized programs allow arbitrary waveforms as well. The maximum data throughput per channel is up to 70 MS/s, while the analogue bandwidth is limited to 10 MHz. The DPU features a field programmable...
gate array (FPGA) together with a digital signal processor (DSP). Critical fixed point operations are carried out by the FPGA while the DSP performs floating point operations of increased complexity. The DPU can be upgraded with extended memory and a more powerful FPGA in order to meet higher real time demands.

A configuration consisting of different application units together with the DPU can act as a stand alone device with galvanic decoupling. Controlling and data acquisition is possible via LAN or USB. This allows distributed measurement networks. Additionally, digital I/O ports permit the exchange of time critical signals such as trigger signals or status monitoring.

2.1. Low frequency application unit (LF-AU)

The electronics of the LF-AU monitors the injected current and the voltage drop across the MUT (Fig. 4). The MUT is connected via specialized electrode system, either a tetra- or bipolar interface. Other electrode configurations are possible as well. The LF-AU features digitally controlled gain, input enable and selectable resistors ($R_{\text{meas}}$) for extension of the dynamic range of current measurement. Moreover, using a matched multiplexer, up to 32 MUT can be processed in parallel.

![Fig. 4: Low frequency Unit (LF-AU) with the tetrapolar electrode interface as example.](image)

In case of the tetrapolar interface, the signal is connected to the outer electrodes. The voltage across the inner electrodes ($V_1$) is amplified using a broad bandwidth – high input resistance instrumentation amplifier. In order to avoid phase and amplitude aberrations, an identical amplifier is used to monitor the voltage across the current sampling resistor $R_{\text{meas}}$. Additional digital I/O – lines improve the overall feasibilities of the unit. They can be used for additional sensors or as control lines.

2.2. Ultra wideband application unit (UWB-AU)

A binary pseudo noise generator supplies the periodically repeated excitation signal. The RF-clock is feed into a shift register chain. Together with the proper feedback a periodically repeating maximum length sequence appears at the output. The filter cuts the frequencies above the half clock frequency in order to prevent aliasing. The MUT is connected via the reflectometer bridge separating the incident and the reflected signal.

The binary divider serves as digital delay line and controls the sample and hold stage (S&H). The digitized signal is sent to the data processing unit (DPU, Fig.3) where a DSP takes over further processing. Despite some drawbacks, such as longer measurement time, sub-sampling using the binary divider saves cost by using a much slower ADC. Although the configuration shown in Fig.5 is in principle sufficient, a second ADC with S&H is used in the IMPSPEC system for monitoring the non-ideal excitation signal as well.
Fig. 5. Basic structure of an arrangement using maximum length sequence (MLS) excitation with the UWB-AU. In the DPU and the reflectometer bridge are outside the physical measurement head.

Depending on the excitation signal, different post-processing algorithms are implemented. The result is either the impulse or step response or the frequency characteristics of the sensor. Detailed information can be found in (1) and (2).

DPUs can operate in parallel allowing 2D application with arrays of antennas. The GPU features additional I/O-channels (temperature, position, scanner, interface to other actuators, etc.). UWB-AU can be interfaced with electrode systems for transmission or reflection but also with antennas.

The RF-modules are mounted on carrier-substrates which can be used in versatile combinations. All modules are mutually shielded resulting in excellent cross-talk suppression. The shift register, S&H and the synchronization unit are customer made SiGe-circuits which operate currently up to a clock rate of 15 GHz. In the standard case, all feeding lines are symmetrically driven. The asymmetrical operation in a 50-Ω-system is possible as well.

3. Measurements and results

Microsystems are the key for future fast screening with high throughput. Using a suitable microelectrode interface, viability tests down to the single cell level are feasible. An application to cell suspension in a microfluidic system is shown in Fig. 6a. The cell density of yeast above 100 000 Cells/mL could be followed using the LF-resistance of the suspension extrapolated from the spectrum between 10 kHz and 10 MHz.

High frequency and microwave sensors receive increasing attention for applications in food industry [2]. Because of their non-destructive operation, they are preferentially used in online quality assessment.

A key feature is the precision of the measurement which should be comparable to established methods. Besides a test with equivalent circuits, using a real electrode is a much better way to show the performance of the entire system. We used the direct comparison between the IMPSPEC module and the vector network analyzer Agilent E8361A. Both devices were interfaced with a coaxial Dielectric Probe Kit 85070E. For determination of the material properties we used additional the Agilent software 85071E.

As seen in Fig. 6b the measurement with the UWB-system delivers comparable results to commercial network analyzer.

The cell membranes and also molecular superstructures determine the impedance from a low frequency range up to hundreds of MHz. The most distinguishable parameter influencing the impedance at frequencies between 1 and 20 GHz is the water content. In agricultural products like meat or vegetables the freshness correlates with the water content and can be therefore assessed using microwave spectroscopy.
Fig. 6 (a) Resistance of a microelectrode in a 500 µm flow channel at 25 kHz with varying yeast cell density. (b) Permittivity spectra of methanol and propanol, measured with the UWB-system (solid line) and a standard network analyzer (dotted line).

4. Conclusion

The success of impedance measurement in process monitoring is closely connected to the availability of robust and affordable equipment. The IMPSPEC – system is especially designed to meet the requirements of the integrated process environment. Moreover, connected to a PC, it becomes a precise and affordable laboratory instrument. Its strict modular concept, divided into a basic processing unit and the attached application units allows it to be configured individually for integrated control systems. Moreover, besides standard electrode systems, specially designed electrodes and matching electronics ensure high precision, low noise and robust signals. The time domain approach allows fast measurements.

The broad available bandwidth from DC to several GHz opens up new applications for instance combined measurement of structural changes in biological matter as well as monitoring molecular composition such as fractional water content.

References

EFFECTS OF VACUUM AND OHMIC HEATING ON THE OSMODEHYDRATION KINETICS AND MICROSTRUCTURE OF Pears (VAR. PACKHAM’S TRIUMPH)

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Abstract:
The influence of osmotic dehydration at atmospheric pressure (OD) or vacuum impregnation (VI) and ohmic heating (OH) on the osmodehydration kinetics and microstructure of pears, was studied. Pears cubes (1.0 cm³) immersed in a 65% (w/w) sucrose solution were subjected to shaking at 100 rpm, VI (50 mbar by 5 minutes) and OH (AC, 50/60 Hz and 100 to 200 W) treatments, were carried out at temperatures of 30, 40 and 50°C by 5 h. Changes in water loss, solid gain, color and firmness of pears were measured, and microstructure was analyzed using scanning electronic microscopy SEM. Results showed that the application of VI or OH during osmotic treatments, both presented a significant effect on the kinetics of water loss and sugar gain, as well as microstructure of treated samples. The greatest water loss was observed OD-OH treatment at 50ºC, while the largest amount of solute gain and lowest firmness loss were obtained with VI at 50ºC. SEM observations showed that cell deformation and cell rupture were more important in OD treatments, and moderate in VI samples. SEM also showed an increase of permeability and mechanical rupture of membrane by ohmic heating, effect that explains the acceleration of mass transference.

Key words: Osmotic dehydration, ohmic heating, vacuum impregnation, kinetics, pears.

1 Introduction

Osmotic dehydration (OD) at mild temperatures, which is considered as a minimal processing, preserves fresh-like characteristics of fruits, and may be used to obtain pears products or ingredients for many food products. Osmotic dehydration preserves attributes such as colour, texture and aroma and reduces water activity, providing high moisture products with extended shelf-life.

The use of vacuum impregnation (VI) allows an increase in the rate of water, weight loss, and solid gain, and introduces controlled quantities of a solution into the porous structure of fruits and vegetables (1). Mass transfer during osmotic dehydration occurs through the semipermeable cell membranes of the dominant resistance in mass transfer in biological materials. Cell membrane properties may change from partial to total permeability, leading to significant changes in tissue architecture (2).

Two resistances oppose the mass transfer during osmotic dehydration of fruits, one is internal and the other external. The fluid dynamics of the solid-fluid interface governs the external resistance whereas the internal, much more complex, resistance is influenced by the cell tissue structure, cellular membrane permeability, deformation of vegetable/fruit pieces and the interaction between the different mass fluxes.

The phenomenon of electropermeabilization of the cell membranes has been known for several decades, and has recently received increasing attention for the manipulation of cells and tissues (3). Strictly speaking our knowledge is phenomenological, as it is based on measurements of electrical currents through planar bilayer membranes (BLM) under the
influence of strong electric fields, and on molecular transportation of molecules into (or out of) the cells subjected to electric field pulses.

Ohmic heating (OH) is a thermal process in which heat is internally generated by the passage of alternating electrical current (AC) through a body, such as a food system that serves as an electrical resistance. In ohmic heating processes, the food components are parts of the electric circuit through which the alternating current flows and generates heat in the foods based on their intrinsic properties of electrical resistance.

For a food item consisting of a liquid-particulate mixture, heat could be generated at the same or comparable rates in both, the liquid and particulate phases using ohmic heating, if the electrical conductivities of the two phases are equal the same. Thereby ohmic heating thereby provides a technology for processing particulate foods at the rate of an HTST process without the limitations of heat transfer to particulates found in a conventional HTST processes (4).

The aim of this work was to analyze the effect of vacuum and ohmic heating on the osmodehydration kinetics and microstructure of pears (cv. Packam’s Triumph).

2 Material and methods

2.1 Sample preparation

Fresh pears (cv. Packam’s Triumph) from Chillan (Chile) were obtained from commercial sources. Fruits were selected according to their appearance (ripeness, size and color). The pears were peeled and cut into cubes 1 cm³ pieces. Sucrose solution of 65 °Brix was used as osmotic solution.

2.2 Osmotic treatments

Osmotic dehydration was carried out at atmospheric pressure (OD), vacuum impregnation (VI) and ohmic heating (OD-OH) at 30, 40 and 50°C. Processing time was five hours in the three osmotic treatments. Vacuum pulse was applied for 5 min at 50 mb at the beginning of the process, after that, atmospheric pressure was restored until to complete the process time). For ohmic treatments, samples immersed into osmotic solution were subject to an alternating current in 50/60 Hz, 100 V by 5 h. Agitation was performed on an orbital shaking at 100 rpm shaking speed (Barnstead/Lab-Line MaxQ* 2000, Iowa, USA).

2.3 Analysis of physicochemical properties

Water content (\(X^w\)) and soluble solids (\(X^{ss}\)) were measured in fresh and treated samples in order to determine the compositional changes promoted by osmotic dehydration (OD, VI and OD-OH). Moisture content was determined by technique of Association of Official Analytical Chemists (AOAC, 1997). Soluble solids were determined by a digital refractometer (Leyca Mark II, Buffalo, N.Y., USA). All measurements were made in triplicate and average values were reported.

Mass transfer parameters, changes water and soluble solids (\(\Delta M^w_t\) and \(\Delta M^{ss}_t\), respectively) were calculated using the equations (1) and (2); \(M^w_t\) and \(M^{ss}_t\) being the sample weight at time \(t\) and \(0\), respectively, and \(X^w_t, X^{ss}_t, X^w_0\) and \(X^{ss}_0\) the water (w) and soluble solids (ss) mass fractions in sample at time \(t\) and \(0\), respectively.

\[
\Delta M^w_t = \left[ \frac{M^w_t \cdot X^w_t - M^w_0 \cdot X^w_0}{M^w_0} \right]
\]
\[
\Delta M^{ss}_t = \left[ \frac{M^{ss}_t \cdot X^{ss}_t - M^{ss}_0 \cdot X^{ss}_0}{M^w_0} \right]
\]
Pears color was evaluated using a Minolta Chroma Meter CR-200 (Minolta Corp., Osaka, Japan). CIE Lab coordinates were using D65 illuminant a 2º observer as reference system. The measurements were made in triplicate and in three different places from each sample, and then mean values were reported. A Texture Analyser TA-XT2 (Stable Micro Systems, Haslemere, UK) using a Kramer shear cell with five blades. The press crosshead speed was set at 2 mm/s. Rectangular pieces 4x3x0.3 cm were measured in each mechanical test. The mechanical parameter considered was the maximum peak force (F. max.), reported as N. Ten replicates were performed for each treatment.

2.4 Structural analyses

Structural analysis was carried out using SEM technique, with a Jeol JSM–6380LV, OXFORD Instruments, UK. Cubes pieces 1 cm3 were analyzed from surface to the center zone of the fresh and treated samples.

2.5 Statistical analysis

Statistical analyses of data were performed through an analysis of variance (ANOVA) and a LSD test at 5% using Statgraphics Plus 5.1 Software.

3 Results and Discussion

The variations of water content and soluble solids due to osmotic dehydration at atmospheric pressure (OD) with vacuum impregnation (VI) and ohmic heating (OD-OH) at temperatures of 30ºC, 40ºC and 50ºC were compared (Figure 1).

![Fig. 1: Water content (ΔMw) and soluble solid (ΔMss) changes (mean values) of osmotic treatments. Atmospheric pressure (OD), vacuum (VI) and ohmic heating (OD-OH) treatments were carried out with 65ºBrix sucrose solution. (Superscript J becomes w or ss when it referred to water content or soluble solid, respectively)](image)

It can be observed that the water loss is greater in samples treated with 50ºC, especially in OD-OH treatment; likewise, solids gain is greater in those treatments when VI or OD-OH is applied. This is due to the coupled action of different mass transport mechanisms to a different extent (1). The increases of temperature, vacuum and electroporation effect promote gain of osmotic solution into tissue pores, thus reaching an overall concentration in the sample with less water loss. Treatments at 30ºC suggest lower process driving force and subsequent longer treatment times, which may induce greater number of cell layers affected.
by the osmotic treatment at a determined overall concentration of the sample, coherent with a flatter water concentration profile (5).

Table 1 shows mass fraction of water ($X_w$) and soluble solid ($X_{ss}$) fresh and treated samples at the end of process (5h). Vacuum impregnation (VI) and ohmic heating (OD-OH) treatments at 50°C increase concentration levels compared with samples OD treated, in agreement with the action of hydrodynamic mechanisms coupled with diffusional osmotic phenomena which accelerate mass transfer. On the other hand, although an increase of $X_{ss}$ values was observed, they were not significant ($p<0.05$) between treatments VI and OD-OH at 50°C (Table 1). Previous studies show an increase of diffusion through foods using alternating electric fields, this make favorable the solid gain and water loss (6).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>$X_w$</th>
<th>$X_{ss}$</th>
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<tbody>
<tr>
<td>Fresh</td>
<td>0.878 ± 0.026 $^a$</td>
<td>0.099 ± 0.014 $^a$</td>
</tr>
<tr>
<td>OD 30°C</td>
<td>0.493 ± 0.075 $^b$</td>
<td>0.482 ± 0.029 $^c$</td>
</tr>
<tr>
<td>VI 30°C</td>
<td>0.479 ± 0.059 $^c$</td>
<td>0.454 ± 0.009 $^b$</td>
</tr>
<tr>
<td>OD-OH 30°C</td>
<td>0.500 ± 0.004 $^b$</td>
<td>0.453 ± 0.001 $^b$</td>
</tr>
<tr>
<td>OD 40°C</td>
<td>0.489 ± 0.009 $^{b,c}$</td>
<td>0.465 ± 0.022 $^{b,c}$</td>
</tr>
<tr>
<td>VI 40°C</td>
<td>0.487 ± 0.009 $^{b,c}$</td>
<td>0.478 ± 0.027 $^{b,c}$</td>
</tr>
<tr>
<td>OD-OH 40°C</td>
<td>0.413 ± 0.004 $^d$</td>
<td>0.527 ± 0.012 $^d$</td>
</tr>
<tr>
<td>OD 50°C</td>
<td>0.419 ± 0.005 $^d$</td>
<td>0.533 ± 0.026 $^d$</td>
</tr>
<tr>
<td>VI 50°C</td>
<td>0.405 ± 0.004 $^d$</td>
<td>0.579 ± 0.029 $^e$</td>
</tr>
<tr>
<td>OD-OH 50°C</td>
<td>0.354 ± 0.005 $^e$</td>
<td>0.568 ± 0.001 $^e$</td>
</tr>
</tbody>
</table>

$^{a,b,c,d}$ When there are significant differences at 5.0% homogeneous groups in each variable, according to a LSD test, they are identified by the same superscript letter.

Tab. 1: Composition parameters, water content ($X_w$) and solid soluble $X_{ss}$ reached by fresh and processed samples at 65° Brix sucrose solution (Processing time = 5 h).

Table 2 shows the mean and standard deviation of color CIE parameters $L^*$, $a^*$, $b^*$, $h^{ab}$ (Hue angle) and $C^{ab}$ (Chroma) of fresh and processed pear. A decrease of 40% in lightness ($L^*$) was observed in samples vacuum (VI) treated compared to fresh samples. However, in samples treated with OD and OD-OH 5% increase in $L^*$ values were observed. Vacuum impregnation treatments lead to lower values of $L^*$ associated with transparency gained due to air loss, effect produced for total or partial substitution of the air present in the pores by the impregnation solution (Moreno and other 2004). $a^*$ and $b^*$ values decreased in VI treatments and an increased in OD and OD-OH treatments at 40°C and 50°C compared to fresh pear was observed. When the total change of color is evaluated throughout $\Delta E$ parameter, small changes are associated to OD treatments and the greatest to the vacuum impregnation (VI). Therefore, the main differences are being due to loss of clarity in line with transparency gain.

Maximum force values, obtained from mechanical test indicated that samples under osmotic treatments showed a decrease in firmness (Table 2). The greatest difference was observed in treated samples at atmospheric pressure (OD), especially at 50°C. OD-OH treatments implied lower force decrease at 40°C and 50°C. VI treatments showed nonsignificant difference in all treatments. The application of vacuum induced a greater firmness of fruit by replacing osmotic solution in pores due to air loss then obtaining a more compact and less deformed tissue than at atmospheric pressure (1). Moreover, it has been reported that when a treatment for $a_w$ reduction is applied, changes of texture in vegetal tissue are more dependent of physical and chemical changes due to influence of transformation of protopectin to soluble pectin and sugar diffusion in intercellular spaces, which causes loss of turgor and ions movement from the cell wall to the media (5).
Tab. 2: Color and firmness evaluation in fresh samples and at the end of each treatment.

To better understand the effect of osmotic dehydration and ohmic heating at cell level on texture characteristics, an electron microscopy technique was used. Figure 2a shows the microstructure of fresh pear. A high degree of cell compartmentation and small intracellular spaces were observed.

![Micrographs](image)

**Fig. 2:** SEM micrographs of parenchyma tissue from fresh and treated pears with 65% (w/w) sucrose solution and 50°C. (a) Fresh control, (b) OD, (c) VI and (d) OD-OH.

The effects of OD, VI and OD-OH treatments are shown in Figure 2b, 2c and 2d. The cells changed their shape and reduced their size in both processes, which may be explained by the native liquid loss. VI samples were affected by vacuum pulsed application, causing the intracellular spaces to fill with material resembling ice microcrystals (Fig. 2c). From Fig 2b and 2c it is possible to observe that thickness of the middle lamella between cells is greater in

---

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Firmness(^1) (N)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>h(^{ab})</th>
<th>C(^{ab})</th>
<th>∆E*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>964.5 ± 86.5(^a)</td>
<td>76.1 ± 3.9(^c)</td>
<td>-3.7 ± 0.4(^{bc})</td>
<td>14.2 ± 0.9(^{e})</td>
<td>105.0 ± 2.0(^{d})</td>
<td>14.7 ± 0.9(^{h})</td>
<td>0</td>
</tr>
<tr>
<td>OD 30°C</td>
<td>872.0 ± 70.2(^b)</td>
<td>76.9 ± 2.4(^c)</td>
<td>-3.1 ± 0.3(^{ab})</td>
<td>13.6 ± 0.6(^{b})</td>
<td>103.3 ± 1.4(^a)</td>
<td>14.0 ± 0.6(^{bc})</td>
<td>2.6 ± 1.5(^a)</td>
</tr>
<tr>
<td>VI 30°C</td>
<td>894.1 ± 39.7(^{ab})</td>
<td>46.0 ± 2.9(^{ab})</td>
<td>-2.7 ± 0.4(^d)</td>
<td>10.6 ± 0.6(^{a})</td>
<td>109.5 ± 1.6(^{d})</td>
<td>11.3 ± 0.5(^{ab})</td>
<td>25.5 ± 3.7(^f)</td>
</tr>
<tr>
<td>OD-OH 30°C</td>
<td>883.2 ± 54.6(^{ab})</td>
<td>81.9 ± 1.9(^{ef})</td>
<td>-4.8 ± 0.4(^d)</td>
<td>16.6 ± 1.0(^{de})</td>
<td>106.5 ± 1.0(^{d})</td>
<td>17.3 ± 1.0(^{d})</td>
<td>4.1 ± 1.6(^{bc})</td>
</tr>
<tr>
<td>OD 40°C</td>
<td>844.4 ± 28.4(^{b})</td>
<td>83.8 ± 2.4(^{f})</td>
<td>-4.0 ± 0.6(^{d})</td>
<td>16.5 ± 0.6(^{de})</td>
<td>104.4 ± 0.8(^{ab})</td>
<td>17.1 ± 0.5(^{cd})</td>
<td>6.0 ± 0.6(^{cd})</td>
</tr>
<tr>
<td>VI 40°C</td>
<td>870.4 ± 65.3(^{ab})</td>
<td>48.1 ± 1.4(^{b})</td>
<td>-3.5 ± 0.4(^{ab})</td>
<td>11.9 ± 0.8(^{ab})</td>
<td>110.4 ± 1.9(^{e})</td>
<td>12.5 ± 0.8(^{ab})</td>
<td>30.6 ± 2.0(^{f})</td>
</tr>
<tr>
<td>OD-OH 40°C</td>
<td>854.9 ± 41.6(^{b})</td>
<td>80.5 ± 2.0(^{f})</td>
<td>-4.4 ± 0.1(^{de})</td>
<td>17.3 ± 0.9(^{e})</td>
<td>104.2 ± 0.8(^{ab})</td>
<td>18.1 ± 1.4(^{e})</td>
<td>6.9 ± 0.8(^{f})</td>
</tr>
<tr>
<td>OD 50°C</td>
<td>825.8 ± 46.4(^{c})</td>
<td>78.5 ± 1.4(^{cd})</td>
<td>-3.5 ± 0.3(^{ab})</td>
<td>16.0 ± 1.1(^{c})</td>
<td>102.9 ± 1.0(^{a})</td>
<td>16.4 ± 1.1(^{c})</td>
<td>5.6 ± 1.3(^{c})</td>
</tr>
<tr>
<td>VI 50°C</td>
<td>879.8 ± 68.2(^{ab})</td>
<td>42.1 ± 1.0(^{h})</td>
<td>-2.4 ± 0.2(^c)</td>
<td>9.7 ± 0.6(^{a})</td>
<td>104.2 ± 1.2(^{ab})</td>
<td>10.0 ± 0.7(^{a})</td>
<td>36.8 ± 1.1(^{g})</td>
</tr>
<tr>
<td>OD-OH 50°C</td>
<td>851.1 ± 39.0(^b)</td>
<td>80.7 ± 4.5(^{e})</td>
<td>-4.2 ± 0.3(^{d})</td>
<td>16.6 ± 1.0(^{de})</td>
<td>105.0 ± 1.5(^{bc})</td>
<td>17.2 ± 0.9(^{d})</td>
<td>10.7 ± 1.8(^{c})</td>
</tr>
</tbody>
</table>

\(^1\) Values represent the mean and standard deviation of 10 analyses.

\(^2\) Values represent the mean and standard deviation of 9 analyses.

\(a,b,c,d\) When there are significant differences at 5.0%, homogeneous groups in each variable, according to a LSD test, they are identified by same superscript.
VI than OD treatments and 2d cellular breaking by electro-thermal effect, explaining the differences found in firmness values. The greater firmness observed in VI samples may be explained by the presence of this polymeric material. This material could be a polymeric compound or concentrated sugars, which may be formed by interactions of middle lamella pectin with osmotic solution solutes.

4 Conclusions

The application of vacuum impregnation and ohmic heating during osmotic treatment had a significant effect on the kinetics of water loss and solid gain, color and firmness. Vacuum impregnation and ohmic heating (OD-OH) treatments favored mass transfer with the increase of the work temperature. Color differences induced by osmotic dehydration were related to changes in lightness (L*) values of treated pears. On the other hand, a loss of firmness was obtained throughout osmotic treatments. Microstructure of pear showed that osmotic dehydration caused shape changes and size reduction of pears cells as well as the thickness of the middle lamella and cellular breaking by electro-thermal effect. Under the studied conditions, VI and OD-OH and 50ºC are the best process to dehydrate pear pieces in sucrose solution at 65ºBrix.

5 Acknowledgements

We thank to CONICYT, project FONDECYT 1070946 for its financial support.

References


DIFFUSIVITY MODEL IN APPLES TISSUES THROUGH OHMIC HEATING: EFFECT OF SUGAR CONCENTRATION AND TEMPERATURE

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ABSTRACT:
Two major solid-liquid diffusion processes are utilized in the food industry. Leaching, the transfer of solutes from a solid to an adjacent liquid, is used to extract sugar, vegetables oils, coffee, tea, gelatin, pectin, and other food solutes.

The ohmic heating of a food material occurs when electric current passes through it. Ohmic heating is a promising method for different food processing technologies, including sterilization, blanching, dehydration, and extraction. The moderate electric fields (MEF) with the electric field strength $E$ under 100 V/cm are used, as a rule, for ohmic heating. The MEF treatment allows extraction and expressing processes to be enhanced considerably in different food materials.

During ohmic heating, the electric field may cause changes in the permeability of cell membranes of plant cells below the temperature at which membranes are permeabilized due to thermal effects. Diffusion is enhanced, electrical conductivity changes are more linear during heating, and moisture migrates more easily out of the tissue.

The objective of this study was to investigate the diffusion coefficient of sugar into apple tissue under different levels of sugar concentration (65 and 55 °Brix), temperatures ranging 25 through 60 °C and different currents.

Raw materials such as Granny Smith apple and sucrose were acquired in the local market. The sample prepared as an infinite slab and located (separating) two compartments of the same volume, one filled with sugar solution (donor) and the other with distilled water (receiver). The solutions in both compartments were well-agitated with a concentration of potassium chlorite (0.0008g/ml). The concentration in both sides of the cell was assumed uniform at any given time.

Fick’s Second Law is the governing differential equation. This set of equations was solved using an implicit finite difference scheme. Apparent diffusion coefficient of sugar in apple tissue were determined at different temperatures (30, 40 and 50°C), and for two different sugar concentrations (65 and 55 °Brix). The current used was between 6.3-12V/cm. The increments of sugar in the receiver side can be explained by the movement of sugar through slice apple by the effect osmotic pressure and electroporation in case of ohmics treatment.

The apparent diffusion coefficient was higher with ohmic heating than conventional treatment.
COMBINED EFFECTS OF ELECTRICAL METHODS ON ORANGE JUICE PRODUCTION

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Abstract:
The purpose of this study is to investigate combined effects of electroplosamolysis and microwave heating applications on orange juice production. “Washington navel” variety oranges were used as raw material and samples were processed by using electroplosamolysis as pretreatment and microwave heating at pasteurization step. Electroplosamolysis and microwave applications were optimized by using RSM (Response Surface Methodology), one-factor design and central-composite design respectively. Orange samples were divided into two main parts; 1) control group (without any pretreatment), 2) electroplosamolysis application group. After these applications; the orange juices were extracted by using a fruit juicer. Each group were divided again into two parts and processed as given below;

1-a) control group + conventional thermal treatment (95°C for 60 sec.)
1-b) control group + microwave heating (flow rate: 50 ml/ min., 900 W)
2-a) electroplosamolysis group + conventional thermal treatment (95°C for 60 sec.)
2-b) electroplosamolysis group + microwave heating (flow rate: 50 ml/ min., 900 W)

After production of orange juice; effects of electroplosamolysis treatment on the yield of orange juice were calculated. In addition; pectin methylesterase (PME) activity, pectin, total phenolics, vitamin C contents; and colour (L*, a*, b*, ΔE) values, pH, brix, pulp and peel oil contents of orange juice were investigated. Analyses were performed before and after electroplosamolysis and heating treatments. Results showed that; combination of electroplosamolysis and microwave heating (2b) application is more effectual than conventional processing for improving functional properties and PME inactivation of orange juice.

Keywords: Electroplasmolysis, microwave heating, thermal heating, orange juice

1 Introduction
Orange juice is highly consumed in many countries and non-pasteurized fresh orange juice has a limited shelf life. Conventional thermal processing is the most common method to inactivate microorganisms and enzymes in orange juice production. This conventional treatment causes adverse effects on the final products such as colour alterations, flavor damages, vitamin and nutritional losses. As consumers are highly demanding minimally processed and fresh-like food products, the use of novel technologies is gaining popularity. Food industry is interested in novel electrical thermal method which inactivates enzymes and microorganisms without significant adverse effects on flavor and nutrients. Also these novel techniques such as PEF, Ohmic heating, electroplosamolysis and microwave heating are applied to increased juice yield for different fruits.

Electroplasmolysis is the process which is aimed to improve the yield in fruit juice and pulp production by destroying the cell wall. Electroplasmolysis can be applied at low (11-180 V/cm) or high (1800-2000 V/cm) electric fields. EP causes generally a slight (3-5°C) increase in temperature of product. So it can be grouped in non-thermal electrical applications. EP treatment increases product yield approximately 10% in different fruits. Moreover some
improvements in quality were also reported at literature for materials processed by electroplosmolsysis [1], [2], [3].

Microwave heating is another electrical thermal method which inactivates enzymes and microorganisms without significant adverse effects on flavor and nutrients. Microwave heating is based on the transformation of alternating electromagnetic field energy into thermal energy by affecting the polar molecules of a material. In microwave heating, heat is generated throughout the material, leading to faster heating rates, compared to conventional heating where heat is usually transferred from the surface to the interior [4].

In this study oranges were used as raw material and samples were processed by using electroplosmolsysis as pretreatment to determine effects on juice yield and microwave heating were used for pasteurization. The main purpose of this research was to determine combined effects of electroplosmolsysis and microwave heating applications on orange juice yield and some quality parameters.

2 Material and methods

2.1 Preparation and Processing of Orange Juice

“Washington navel” variety oranges were used as raw material. Electroplosmolsysis application and microwave application conditions were optimized by using RSM (Response Surface Methodology), one-factor design (data were not given). And productions were carried out at chosen optimum conditions for electroplosmolsysis at 27 volt/cm for 10 sec operation time. Microwave applications were achieved with using a continuous microwave system (ARÇELIK MD 595, 2450 Mhz). Then productions were carried out at chosen optimum conditions at 50 ml/min flow rate and 900W. Conventional thermal treatment was realized in a water bath (DKZ Series). 200 ml bottled orange juice were heated until 95°C and keep at same temperature for 60 sec.

Then oranges were divided into two main parts; 1) control group (without any treatment), 2) electroplosmolsysis application group. After peeling process; the orange juices were extracted by using a juice extractor (Moulinex JU5000-800 W). Each group were divided again into two parts and processed as given below with their sample codes;

1) control group (without any treatment); (control)
   1-a) control group + conventional thermal treatment (95°C for 60 sec.); (CH)
   1-b) control group + microwave heating (900 W, flow rate: 50 ml/min.); (MW)
2) electroplosmolsysis application group; (EP)
   2-a) electroplosmolsysis group + conventional thermal treatment (95°C for 60 sec.); (EP+CH)
   2-b) electroplosmolsysis group + microwave heating (900 W, flow rate: 50 ml/min.); (EP+MW)

After production of orange juices, all samples were cooled +4°C in an ice bath and stored at same temperature until analysis.

2.2 Analyses

After production and processing of orange juices; effects of electroplosmolsysis treatment on the yield of orange juice were calculated. In addition; pectin methylesterase (PME) activity [5]; pectin [6]; total phenolics [7]; and vitamin C contents [8]; and colour (L*, a*, b*, ΔE) values (HunterLab Colourflex- Management Company, USA); pH [9]; °brix [10]; pulp [11]; ) and peel oil [11] contents of orange juice were analyzed according to given methods at literature. Analyses were performed before and after heating treatments. Evaluation for significant differences among test media was performed by ANOVA (SPSS-13).
3 Results and Discussion

The juice yield of control group was found 43.56% (±1.2) where the yield found 43.87% (±0.9) for EP group. There were no significant differences found between EP and control samples for juice yield in “Washington navel” variety oranges. But nearly 10% increase were reported by Okilov, (1995) in citrus juices after EP applications [12]. Also the increasing of juice yield in Valencia variety oranges was calculated as 8.74% by Demirdöven and Baysal (2009) [13].

PME causes cloud loss in orange juice by de-esterification of pectin; thus, thermal treatment is applied to inactivate the enzyme. The design for thermal pasteurization of orange juice is based on the thermal destruction characteristics of pectin metilesterase, which is more thermally stable than many vegetative microorganisms [14], [15]. The measured PME activities of orange juices after treatments were presented in table 1 and figure 1a. PME activities of orange juices were determined as 4.063 µmol/min/ml in control and 3.006 µmol/min/ml in EP application group. The inactivation rate of PME in EP group was observed nearly 26%. PME activity reduced 70% for MW heated samples; 83% for EP+MW combination group compared to its activity in fresh orange juice (control). Under conventional pasteurization conditions, PME activity reduced of 66% in CH heated samples; 72% for EP+CH combination group compared to its activity in fresh orange juice (control). The highest reduction on PME activity was found for EP+MW (combined group). All PME activities found significantly different (P ≤ 0.05). Leizerson and Shimoni; (2005) reported that ultrahigh-temperature ohmic heating of orange juices showed a reduction of 90-98% PME activity compared to its activity in fresh orange juice [16]. Also significant differences evaluated for pectin contents of orange juices (P ≤ 0.05). Results of pectin content (table 1-figure1b) were showed good agreement with PME activities; the highest pectin content evaluated for EP group (485 GA-AH, mg/l) followed by EP+MW (477.250 GA-AH, mg/l) group; and the minimum pectin content were determined for control group (413.583 GA-AH, mg/l).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PME activity (µmol/min/ml)</th>
<th>Pectin (GA-AH, mg/l)</th>
<th>Vitamin C (mg/100ml)</th>
<th>Total phenolic (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without heating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.063±0.014</td>
<td>413.583±1.2</td>
<td>47.365±0.6</td>
<td>333.632±1.3</td>
</tr>
<tr>
<td>EP</td>
<td>3.006±0.03</td>
<td>485.000±0.9</td>
<td>57.764±0.41</td>
<td>394.768±1.0</td>
</tr>
<tr>
<td>Microwave heating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW</td>
<td>1.235±0.016</td>
<td>461.166±0.8</td>
<td>44.151±0.6</td>
<td>347.823±1.1</td>
</tr>
<tr>
<td>EP +MW</td>
<td>0.683±0.022</td>
<td>477.250±1.1</td>
<td>57.385±0.33</td>
<td>355.007±1.4</td>
</tr>
<tr>
<td>Conventional heating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>1.397±0.024</td>
<td>454.833±1.3</td>
<td>42.275±0.24</td>
<td>315.695±1.2</td>
</tr>
<tr>
<td>EP+CH</td>
<td>1.137±0.018</td>
<td>433.750±0.75</td>
<td>50.083±0.38</td>
<td>303.139±0.8</td>
</tr>
</tbody>
</table>

Average ± standard deviation for a minimum of three experiments.

Concentration of vitamin C is a significant indicator of orange juice quality, and it may serve as an indicator that all processes, which ensure a high quality of product, have been applied in the production processes [17]. Vitamin C concentrations of control and EP groups were found 47.365 and 57.764 mg/100 ml respectively (table 1). The vitamin C content increased in EP group (22 %) compared to control group of orange juice, because of destroying of the cell wall. At heating applications minimum vitamin C reduction determined for EP+MW combined group (57.385 mg/100 ml) and the highest vitamin C lost was detected CH group (42.275 mg/100 ml). Statistically, there was a significant difference between all treatments for vitamin C contents (P ≤ 0.05). There were no data at literature on combined effects of electrical methods on orange juice production. But in similar researches with different materials highest vitamin C concentrations were also found for electrical treated groups [18], [19].
Total phenolic contents of samples were also found significantly different (P ≤ 0.05) (table 1). Total phenolic content of control group were observed 333.632 mg/l where 394.768 mg/l for EP application group. After heating treatments phenolic contents were evaluated as; 347.832 (MW), 355.007 (EP+MW), 315.695 (CH), 303.139 (EP+CH) mg/l. Gerald and Roberts (2004) was also detected highest total phenolics on apple juices which processed by microwave heating [20].

<table>
<thead>
<tr>
<th>Treatments</th>
<th>°Brix</th>
<th>pH</th>
<th>Pulp content %</th>
<th>Peel oil %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
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<tr>
<td><strong>Without heating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.3±0.1</td>
<td>3.958±0.1</td>
<td>8.27±0.1</td>
<td>0.017±0.02</td>
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<td>EP</td>
<td>12.6±0.0</td>
<td>3.785±0.2</td>
<td>8.30±0.1</td>
<td>0.021±0.01</td>
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<td><strong>Microwave heating</strong></td>
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<td></td>
</tr>
<tr>
<td>MW</td>
<td>12.4±0.1</td>
<td>3.769±0.2</td>
<td>--------------</td>
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</tr>
<tr>
<td>EP +MW</td>
<td>12.6±0.1</td>
<td>3.733±0.2</td>
<td>--------------</td>
<td>------------</td>
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<tr>
<td><strong>Conventional heating</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>12.5±0.1</td>
<td>3.739±0.2</td>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>EP+CH</td>
<td>12.6±0.1</td>
<td>3.683±0.2</td>
<td>--------------</td>
<td>------------</td>
</tr>
</tbody>
</table>

*aAverage ± standard deviation for a minimum of three experiments.

Tab. 2: °Brix, pH, Pulp and peel oil contents of orange juices

Soluble solid (°Brix), pH, pulp and peel oil contents of orange juices were given at table 2. The °brix results of samples were changed between 12.3-12.7 and the highest soluble solid value evaluated for EP application groups (12.6). It means of electro-induced formation and growth of pores in cellular membranes (electroporation) as a result of electric field application. There were no significant differences found between the samples for soluble solid content, pulp and peel oil contents (P >0.05). But significant differences were observed for pH levels (P >0.05) of control samples than others. Orange juice pulp contributes mouth feel, and may or may not be desirable, depending upon the individual consumer and the percentage of pulp present in juice. Suspended pulp, also called sinking or bottom pulp, contributes the opaqueness and smooth mouth feel typical of citrus juices [21]. The United States Department of Agriculture (USDA) standards limit recoverable peel oil content to a maximum of 0.35 ml/l in grade A orange juice. In reality, oil content at this level creates undesirable sensory problems. Florida juice processors attempt to provide an oil level between 0.10 and 0.25 ml/l in juices at the time of purchase by the consumer [22]. Peel oil levels in EP and control groups fell within this range.

Colour values of samples were given at table 3. L* value which indicates the luminosity of the samples were found as; 61.99 (control) and 60.23 (EP) one ; 62.42 (MW); (61.24) EP+MW, and 62.42 (CH), 60.81 (EP+CH). The differences of L* values between the different groups were found statistically significant (P ≤ 0.05). The mean a*, that indicates the...
variation between red and green colour, was found significantly higher ($P \leq 0.05$) for control group orange juice (5.26) than EP group (4.5). The value of this parameter for the MW heated orange juice was 4.3; EP+MW 3.99 and for the pasteurized juices were 4.65 (CH); 4.38 (EP+CH). The $a^*$ values of samples were found significantly different ($P \leq 0.05$) in tested orange juices. Sanchez-Moreno et al., (2005) describe similar results (decrease of the parameter $a^*$) after pasteurization or other thermal treatment of orange juice [23]. Parameter $b^*$, that indicates the variation between yellow and blue colour, is significantly higher ($P \leq 0.05$) for the control samples (60.21) than EP applied sample group (58.00). In heating groups; 60.11(MW); 58.69 (EP+MW); 57.84(CH); 55.51 (EP+CH) $b^*$ values were evaluated. Rodrigo et al. (2003) was also described a significant decrease of the parameter $b^*$ when they analyze different pasteurized orange–carrot juices [24].

<table>
<thead>
<tr>
<th>Treatments</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>$\Delta E$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Without heating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>61.99±0.02</td>
<td>5.26±0.01</td>
<td>60.21±0.05</td>
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</tr>
<tr>
<td>EP</td>
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<td>4.5±0.01</td>
<td>58.00±0.32</td>
<td>2.93</td>
</tr>
<tr>
<td><strong>Microwave heating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW</td>
<td>62.42±0.05</td>
<td>4.31±0.05</td>
<td>60.11±0.01</td>
<td>1.05</td>
</tr>
<tr>
<td>EP+MW</td>
<td>61.24±0.01</td>
<td>3.99±0.03</td>
<td>58.69±0.04</td>
<td>2.12</td>
</tr>
<tr>
<td><strong>Conventional heating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>62.42±0.04</td>
<td>4.65±0.06</td>
<td>57.84±0.015</td>
<td>2.48</td>
</tr>
<tr>
<td>EP+CH</td>
<td>60.81±0.04</td>
<td>4.38±0.03</td>
<td>55.51±0.03</td>
<td>4.93</td>
</tr>
</tbody>
</table>

*Average ± standard deviation for a minimum of three experiments.

Tab. 3: Colour values ($L^*$, $a^*$, $b^*$, $\Delta E$) of orange juices

Total colour differences ($\Delta E$), which indicate the magnitude of the colour difference between orange juices, is shown in table 3 and figure 2. It has been considered that $\Delta E$ of two would be a noticeable visual difference for a number of situations [25], [26]. The minimum $\Delta E$ value determined for MW heated samples (1.05). The highest total colour differences were determined for EP+CH group (4.93).

4 Conclusions

The results showed that there were no significant differences between the processed groups for fruit juice yields. However electroplasmolysis applications increased pectin, total phenolics, and vitamin C contents of orange juice. In heating applications, the highest PME inactivation was found for electroplasmolysis+microwave heating (combined group). Furthermore; minimal vitamin C lost was determined microwave heated samples and the colours of samples were changed significantly according to heating applications. Further studies are being conducted to explore the effect of electroplasmolysis + microwave heating combination treatment on the long-term stability and shelf life of such products.

Acknowledgment:

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References


EFFECT OF PRELIMINARY TREATMENT ON THE RATE OF DRYING AND COLOUR OF TABLE BEETROOT

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Abstract:
The powders prepared from a table beetroot contain many unique biologically active materials and have clear attractive color. They widely used as resistant natural colouring matter, as ingredient of instant food and additives of treatment-and-prophylactic appointment. However, the preparation of a high-quality powder is rather complex technological problem and require the pretreatment of raw materials before the drying. This work was aimed for searching of appropriated regimes of preliminary treatment of the table beet, that allowed realization of high drying rate, effective conservation of natural biologically active components and color substances. The table beet of a "Bordeaux"cultivar was investigated. Both the whole roots and their fragments were pretreated using different modes of preliminary treatment: thermal treatment in water; treatment by hot saturated water vapor; treatment by microwave frequencies; treatment by electric current of a commercial frequency (ohmic heating). The preservation of polyphenolic contents after preliminary treatment and drying was controlled using standard photocolorimetric procedures. The relations between parameters of preliminary treatment, accelerating of drying kinetics and conservation of color substances for different modes of treatment are discussed.

Key words: table beetroot, drying, ohmic heating, microwave treatment.

1 Introduction

Due to unique complex of bioactive substances and specific bright colour of the table beetroot powder, it finds ever increasing application as an ingredient of fast-cooking food products, as a medicinal additive and as a stable natural dyer. The beetroot powder manufacture involves a number of process techniques, including preliminary treatment of the raw stuff before its drying.

The complex chemical composition of the beetroot includes many unstable substances, which eagerly enter into reaction with air oxygen and other compounds on cell integrity damage, thus effecting the depth of chemical and biochemical processes occurring in cells, which modifies the natural properties and process characteristics of the product. Therefore, a comminuted raw material, having larger area of contact with air oxygen, is subjected to more profound changes. Enzymes, jointly with air oxygen, catalyse the polyphenol oxidation processes yielding coloured products [1, 2].

Both enzymatic and non-enzymatic processes can be the cause of colour changes. As a rule, substantial colour changes take place on account of enzymatic processes at temperatures up to 50°C and on account of non-enzymatic processes at higher temperatures. Therefore, creation of conditions for better preservation of the natural beetroot colour due to suppression of enzymatic activity and stabilization of polyphenols is the major task in manufacture of the table beetroot powder. Most commonly, traditional hydrothermal treatment by saturated water steam or by water heated to certain temperature is used in industry in order to stabilise polyphenolic compounds. Recently, other methods of preliminary treatment were also developed. They include electrical treatment of product by fields of different frequency, including microwaves, treatment by hot gases, etc. [3]. One of the basic advantages of electrical treatment is its controlled plasmolytic effect on cell membranes allowing to increase
the rate of product dewatering and to reduce the time of its drying, which is important for
preservation of biologically important components of the raw material and saving of the
power [4 – 7].

This study was aimed at the search of efficient types and modes of preliminary treatment
for table beetroot, which would allow reaching of high rates of beetroot drying with
preservation to the maximum possible extent its natural complex of bioactive substances and
colourants.

2 Methods and Subjects of Study

Four types of thermal treatment were used in this work: hydrothermal treatments by water and
saturated water steam, ohmic heating by industrial frequency current and treatment by
microwave field. Both whole table beetroots of Bordeaux variety and their fragments sized
35х30х15 mm with removed periderm were subjected to treatment.

For increase of the measurement precision and for recording even insignificant changes in
the drying kinetics that can occur at variation of the mode of treatment, dewatering process
was studied using derivatograph Q–1000 (MOM, Hungary) without forced airflow. The
samples of beetroot parenchyma with strictly defined configuration were placed into a
 crucible cup without a lid. Precision of measurement (1 % or higher) was achieved due to
automated computer system of data collection and handling.

The following experimental procedure was applied: After placing a sample into the
measuring unit, the oven was heated at the rate of 20 K/min until reaching the temperature of
sample drying (80°C). Recordings of temperature and sample weight data were started after
due heating of the oven.

The dry matter content in the sample was measured without sample removal from
derivatograph, by raising the temperature in the oven with already dried sample to 104 –
105°C and further continuation of dewatering until constancy of the sample weight.

The effect of preliminary thermal treatment and drying of the sample on preservation of
polyphenolic compounds was estimated from the changes in their content measured by
standard photocolorimetric technique [8]. The table beetroot colorant betanidine is a nitrogen-
containing pigment exhibiting adsorption maximum at 545 – 535 nm.

3 Experimental Results and Discussion

Loss of a portion of beetroot tissue colorants caused by their extraction, oxidation and partial
destruction was observed at hydrothermal treatment of the comminuted tissue. As it was
shown by our study, intensity of colouration of the table beetroot parenchyma after its thermal
treatment by water and of colouration intensity of the powder produced from such tissue
depended on pH of the medium (Fig. 1 and 2). The area of pH values allowing best
preservation and highest stability of betanidine was within 3.6 – 3.8 to 5.2 – 5.4. At pH values
outside this interval, the beetroot pigment was unstable and its structure was undergoing
transformations up to complete destruction.

The time of treatment also played an important role in colour preservation. There existed
an optimum time of treatment providing best colour preservation, practically, for all the types
of thermal treatment of the table beetroot (Fig. 2). The level of tissue colouration below
100%, i.e., lower than colouration intensity of initial raw material, evidenced existence of
processes resulting in pigment decolouration and making inadmissible the relevant type or
mode of treatment. There existed a correlation between stability of colour and preservation of
bioactive components of the table beetroot. The beetroot tissue colouration played a role of a
natural indicator of the product quality.
Thermal treatment of the beetroot fragments by water steam (Fig.2, curve 3) resulted in partial tissue decolouration, including colour loss on account of betanidine extraction from the damaged cells.

The study of beetroot parenchyma of the whole roots after their microwave treatment has shown that such type of preliminary treatment was inadmissible in this case. The beetroots mostly differ by their size and weight, which means that constant correction of the generator power is required. The temperature gradient arising inside large beetroots in the process of their microwave heating was rather high and caused drastic destruction of tissue structure and substantial decolouration (Fig.2, curve 4) and deterioration of the heat and mass transfer during drying. The microwave treatment was more efficient for small beetroot fragments of equivalent size. In such a case, the time of treatment could be considerably reduced, and destructive changes possible in tissues didn’t cause any material deterioration of the drying kinetics.

The powders produced from beetroot tissue after its treatment by microwave field, water and steam, were coloured more intensively than powders obtained from untreated raw stuff. Thus, colour intensity of the powders was 1.65 times higher after microwave treatment and 1.14 times higher after thermal treatment by water. Increase of tissue colouration during its drying may be on account of other anthocyanins or leucoanthocyanins.

Studies of the drying kinetics for different plant materials have shown that not always conditions required for inactivation of enzymes and better preservation of colourants and other bioactive substances coincide with conditions promoting attaining of the highest rate of drying.

The keeping of a vegetable raw material at elevated temperature for quick suppression of enzymatic activity and prevention of reactivation of enzymes results in losses in mechanical
and structural strength of tissue and in worse porosity, which complicates water diffusion to periphery of material and diminishes the rate of drying.

![Graph showing the intensity of beetroot betanidine colour versus time, type and mode of thermal treatment: hydrothermal treatment in acidic (1) and alkaline (2) medium; treatment by steam (3); microwave treatment (4).](image)

**Fig.2:** *Intensity of beetroot betanidine colour versus time, type and mode of thermal treatment: hydrothermal treatment in acidic (1) and alkaline (2) medium; treatment by steam (3); microwave treatment (4).*

Softening of a vegetable tissue at its thermal treatment is accompanied by its microstructure changes resulting from loosening and partial destruction of the cell walls; such tissue softening is determined to a great extent by structural changes in polysaccharides. Softening of the beetroot tissue in the process of its thermal treatment is related to changes in the structure and properties of pectines and hemicelluloses.

The pectines often undergo destruction during thermal treatment and poorly hydrolysing hemicelluloses transform into easily hydrolysing compounds. Thus, beetroot protopectine hydrolyses to hydropectine in the process of thermal treatment. Note that the level of product quality depends not only on the temperature of hydrothermal treatment but also on the time of treatment. The pH of the medium plays an important role in transformations of pectines. Amount of dissolved pectine is minimum at pH 4.5 – 6.5, [9]. It is reasonable to expect that the more protopectine transforms into hydropectine, the more unfavourable will be conditions for internal diffusion during drying. Thus, hydropectine dissolving in the cellular fluid can complicate mass transfer processes [10], which was in fact observed for table beetroot treated by water with pH exceeding the limits corresponding to minimum solubility of hydropectine.

It was found [11] that loosening of cell walls was more intensive when sample was heated in water than when it was treated by steam. Weight losses also depended on the type of thermal treatment. Total weight losses were larger at hydrothermal treatment by steam, while dry matter losses were larger at sample treatment by water. Weight losses during sample treatment by water were mostly due to water evaporation, yet they were on account of extraction of soluble compounds during treatment by water steam.
The process of hydrothermal treatment results in loss of a portion of organic acids and mineral substances. Malic and phytic acids are prevailing in the table beetroot. They make 19 and 50 %, respectively, from the total of acids. Glycolic, oxalic, citric, succinic, ascorbic, galacturonic and other acids are also present, though in small amounts (less then 10%). The total content of acids makes 0.27 % from the beetroot weight. Losses of acids by beetroot in the process of its preliminary thermal treatment make 16% at its treatment by steam and 22% at its treatment by water. A portion of acids passes to water (condensate) and another portion (7 – 10% from the total of acids) destructs irreversibly or enters into reactions with other components of the root. The beetroot composition remains the same on a qualitative level after its hydrothermal treatment by water, but quantitative proportions between beetroot components change.

Those acids, which present in the beetroot in small amounts, undergo destruction to the largest extent (up to 35%). Decomposition of pectines results in accumulation of galacturonic acid.

The beetroot contains great deal of mineral substances (salts of potassium, calcium, manganese, iron). The process of thermal treatment considerably reduces the content of all the mineral substances, including extraction of a part of them by water or by condensate. The losses of mineral salts depend on the type of hydrothermal treatment and degree of beetroot comminution. Thus mineral matter losses by beetroot pieces at their treatment by water were as follows: potassium – 15%, sodium – 33%, calcium – 5%, magnesium – 13%. The losses were materially lower when a whole root was subjected to treatment. The hydrothermal treatment reduced also the content of soluble carbohydrates due to their partial extraction by the medium.

Fig. 3: Comparison of reduced rates of drying of the parenchymal tissue of table beetroot at different types and modes of its preliminary treatment.
The studies that were carried out have shown that kinetics of table beetroot parenchyma drying depends on the type and mode of thermal treatment (Fig. 3). Efficient hydrothermal treatment results in increase of the mass transfer coefficient, enlarges the rate and decreases the time of drying.

As Figure 3 shows, the rate of drying was the highest for samples treated by water with pH 4.25 – 4.45 at 80 – 85°C. Treatment by steam and ohmic heating also increased the rate of drying. At the same time, the time of beetroot tissue treatment by water with pH 7.85 made worse the drying kinetics: the rate of drying remained lower than for parenchyma subjected to no thermal treatment all time during the total continuance of dewatering process. The microwave treatment required a rather strict observance of the process procedure. First of all, the beetroot fragments should be equivalent as far as possible, and the time of their treatment depended on their size and on the power of microwave oscillator. Even insignificant deviation from the chosen process parameters and material inhomogeneity of electromagnetic field could result in spoilage of the product.

4 Conclusion

Conditions for the best preservation of bioactive substances in table beetroot parenchyma concurrently with improvement of kinetic parameters of the drying process were found through selection of the time of preliminary thermal treatment and variation of preliminary treatment parameters.

References

Abstract:

The connection between the properties of the metal oxide nanostructures produced by pulsed electric discharges in water and peculiarities of their interaction with the human blood serum, lysozyme, and albumin solution under various conditions was investigated. By means of dynamic light scattering technique, atomic force and transmissive electronic microscopes it was found that the albumins and lipoproteins of blood serum are aggregated on surface of the nanostructures and forms the supramolecular complexes. Dynamics of the aggregation and concentration dependencies was investigated. In single protein system it was shown that aggregation ability strongly depends on folding state of lysozyme and loading state of albumin. Since the nanostructures have specific effect and various absorptive ability to the biological objects, the possibility to develop new tools for diagnosis of some conformational diseases is appeared. Potential diagnostic significance of new integral characteristics of patient state, characteristic radii of nanostructures-serum components complex is proposed.

Key words: pulsed electric discharges; metal nanoparticles; protein-nanoparticle complexes; conformational diseases diagnostics

1 Introduction

Pulsed electric discharges (PED) in water and other media attract more and more attention [1-5]. There are at least three reasons to that: bactericidal effect on a wide range of pathogenic bacteria, viruses and spores [6], destroying ability to some organic and other compounds [7], high productivity of metal oxide nanostructures manufacture [8,9]. Besides, promising studies of pulsed electric fields effect to human cells and tissues were also carried out [10]. It is known that the PED in water destroy the bacteria by means the shock waves and UV radiation. Shock waves in water are the result of the discharge column widening at early stage [11-16]. The discharge column at the temperature of ~ 10⁴ K, which is typical for these discharges [5], is a source of UV radiation ranging within 200-400 nm [17,18]. UV radiation, being absorbed by water, produces H₂O₂, O, O₃, and OH radicals [19], which destroy the bacteria and oxidize some organic and other compounds. Investigations of physical and chemical properties of water treated by PED and its effect to microorganisms showed that its prolonged microbial resistance is caused by the oxide nanostructures formed by metal electrodes parts [20,21]. Nowadays the interest to the investigations of nanoparticles is increasing essentially. It is caused, in particular, by recently discovered prospects of use of nanomaterials in many...
branches of science and engineering, for example, manufacture of effective and selective catalysts, creation of elements for microelectronic and optical devices, synthesis of new materials [22]. There are many reasons to suppose that the interest to the nanoparticles will have remain for a long time, and the first one is that the nanoparticles have unique properties due to their intermediate state between atom-molecular and condensed matter. Considering it, the current tasks are next: to determine the electronic structure of nanoparticles and their interaction with the medium; to study their surface and its influence on their stability; to investigate the catalytic ability on various chemical reactions.

2 Materials and methods

Experimental setup. Nanostructures are forming in water during the electric discharges between the electrodes supplying from generator of electric pulses [1]. Apparatus for electrodisscharge treatment of water (Fig. 1 a, b) provides continuous manufacturing of water dispersions of nanostructures due to electrodes feeders [3,4], which maintain constant interelectrode gap by supplying the electrode wires into the discharge chamber [2].

![Schematic circuit (a) and photo (b) of electrodisscharge apparatus.](image)

**Fig. 1:** Schematic circuit (a) and photo (b) of electrodisscharge apparatus. 1 - generator of electric pulses; 2 - discharge chamber; 3, 4 - electrodes wire feeders.

PED parameters are the following: duration 0.2-27 μs, energy 0.2-1.5 J/pulse, current rate $10^6-10^9$ A/s, pulses frequency 50-100 Hz [23]. The electrode system of discharge chamber is “wire to wire” or “wire to plate” at the interelectrode gap 5-10 mm and the diameter of the water flow channel $\geq 10$ mm.

**Human blood serum interaction with nanostructures.** The object of the investigation was the interaction of nanostructures water dispersions and biological objects - the standard human blood serum and chicken egg white lysozyme. Before the tests, the samples were centrifuged at 3500 g during 15 minutes. After that, one part of nanostructures dispersion (distilled water at control sample) was added to three parts of serum or lysozyme, and then this mixture was 20 times diluted with physiologic solution. The biological effect of nanostructures dispersion by the changes of subfractional distribution of serum and lysozyme was determined.

**Human blood serum samples preparation.** The samples of serum were exposed for 30 minutes at the room temperature. Blood clots were separated by centrifugation at 3500 g within 15 minutes, after that the serum samples were 20 times diluted with a standard isotonic phosphatic buffer with the ionic force corresponding to the solution of 150 mM NaCl.

**Lysozyme samples preparation.** Chicken egg white lysozyme (CEWL) (Sigma, USA) solution in 25 mM TrisHCl buffer (pH 7.0) at final CEWL concentration 1 mg/ml was used for incubation with nanostructures dispersions (2 times diluted). The protein - nanostructures mixtures were incubated for 1 hour at room temperature with gentle stirring.

**Dynamic light scattering.** Measurements of nanostructures (from here and then monomers – smallest oxide nanoparticles, clusters – nanoparticles aggregations, supramolecular complexes - aggregations of nanostructures, albumins, and lipoproteins) sizes by means of the laser
correlation spectrometer of quasi-elastic light scattering - LCS-03 were carried out. The quasi-elastic light scattering method is based on interaction of monochromatic coherent light with the particles in the liquid. Information about particles size is contained in a spectrum of fluctuations of light scattered on the particles. Nanoparticles sizes are most precisely reset when their lorentzian is determined not less than by five points on a half width and when the range of measurement is not less than ten half widths of lorentzian [24]. The measurement of every sample in each point no less than five times was carried out, and then the data of measurements were averaged.

Transmissive electronic microscopy (TEM). A drop of the nanostructures dispersion was placed to carbon-coated copper grids for 30 sec. The excess of liquid was removed by the filter paper from the grids. The grids at ambient temperature were dried, and then were examined with a JEM-100S (JEOL, Japan) transmission electron microscope at Influenza Institute. For negative staining 1.5% solution (pH 7.4) of sodium phosphomolibdate (SPM) was used.

Atomic force microscopy (AFM). A drop of the nanostructures dispersion was applied to surface of hydrogel-coated slide (Perkin-Elmer, USA) and dried for 1 hour at room temperature. Samples were examined with NT-MDT Solver Bio Scanning Probe Microscope (NT-MDT, Russia) in semi-contact mode, using cantilever NSG01-03 (NT-MDT, Russia).

3 Results and Discussion

Nanoparticles in dispersion. During the investigation, the main attention to Ag, Pt, and Cu nanostructures was paid. AFM examination of smallest Ag nanoparticles shows that some of them are also exists as clusters (Fig. 2).

![Fig. 2: 3D image (a), 2D image (b) and profiles (c) of the silver clusters.](image)

Platinum, as well as silver, forms clusters of various sizes, thus platinum nanoparticles sticking is weaker than silver ones. Fig. 3 shows that platinum “monomers” are almost absent and height of clusters no more than 10 nm.

![Fig. 3: 3D image (a), 2D image (b) and profiles (c) of platinum clusters.](image)

Copper clusters are the largest and steadiest among the investigated ones. Nanoparticles in copper clusters are gathered as a “bunch of grapes”, their size up to 600-1000 nm (Fig. 4).
The distributions of the nanostructures in size are polydispersial and have from three to five characteristic peaks: ~10 nm; 30-100 nm; 100-200 nm; 400-1000 nm; ≥1000 nm (Fig. 5).

It was found also that the nanostructures have a negative surface electric charge of \((0.4-1.6) \times 10^{-2} \text{ C/m}^2\) and volume specific charge of \((0.01-2.6) \times 10^{-2} \text{ C/ml}\) [25].

**Nanoparticles and blood serum.** Many experiments on destroying microbes and spores with the effect of nanoparticles at IEE RAS had been carried out [6,26]. Recently the study of oxide and metal nanostructures and macromolecular complexes of blood serum interaction, due to revealed mechanisms of nanostructures effect to biological objects was performed [27].

Blood serum, as well as many tissue liquids, is a highly concentrated solution of macromolecules, basically, proteins. In such solutions, the forming of macromolecular complexes of various sizes and various degree of stability takes place. These complexes can be formed because of both relatively weak noncovalent interactions, and of strong specific interactions, such as, for example, the antigen - antibody interactions resulting in forming of immune complexes. It can have both normal physiological character, and basis for pathological conditions, as in case of superfluous formation of immune complexes [28] or beta-amyloid oligomers at Alzheimer disease [29]. The registration of forming of such complexes in biological systems can be a model for study of the nanostructures effect on biological systems and can give useful information on many normal and pathological processes in a human organism.
nm. The first peak corresponds, basically, to albumin and antibodies, the second one – to low-density lipoproteins, and the third one – to immune complexes.

After the nanostructures dispersions to standard blood serum adding, two groups of particles with a big $R_h$ have appeared, the first one $\sim 500$ nm, and the second one $\geq 1000$ nm (Fig. 6 b, c, d). The forming of supramolecular complexes of serum components and nanostructures lasts up to 40th minute.

*Aggregation of lysozyme on nanoparticles.* Previously we found the conditions, at which lysozyme in water solution is monomers of single $R_h \sim 2$ nm. Then to determine the peculiarities of the protein - nanostructures interaction, the lysozyme solution in the distilled water as a control sample, and mixtures of lysozyme (see “Lysozyme samples preparation”) and Ag, Cu and Pt nanostructures dispersions were used. Series of experiments were performed for 4-9 times. There were significant difference in hydrodynamic radii spectra of all studied nanoparticles after incubation with lysozyme. The most indicative difference was observed in Ag nanoparticles – lysozyme mixture. Mean $R_h$ values and RMS deviations for this mixture are distributed in Table 1.

<table>
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<tr>
<th>Solution/peak number</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<td>1.97±0.07</td>
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<tr>
<td>Ag</td>
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<td>26.56±1.5</td>
<td>109.17±5.61</td>
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</tr>
</tbody>
</table>

Tab. 1: Difference in hydrodynamic radii distribution peaks of Ag nanoparticles and Ag-lysozyme supramolecular complexes

Fig. 7 shows the distribution of nanoparticles used for this experiment and supramolecular complexes of Ag in mixture.

Fig. 8 shows the TEM-images of Ag, Cu, and Pt nanostructures (Fig. 8 a, d, g) and their supramolecular complexes.
TEM-images (Fig. 8 b, e, h) show blurred outline of oxide nanostructures, which is the protein layer on nanostructures surface. After negative staining by SPM white aureole around nanostructures, which are opaque for electron beam, have appeared (Fig. 8 c, f, i).

4 Conclusion

Nanostructures of all investigated metals in dispersion have five subfractions of sizes: ~10 nm; 30-100 nm; 100-200 nm; 400-1000 nm; and ≥ 1000 nm.

The result of interaction of nanostructures of investigated metals with blood serum is the agglutination of albuminous and lipoprotein structures of blood serum on nanostructures surfaces. Thus, the higher the concentration of nanostructures in blood serum is, the larger supramolecular complexes are formed.

The stages of supramolecular complexes forming are next: small aggregations of albuminous and immunoglobulin, aggregations of lipoproteins, and large aggregations with hydrodynamic radius ≥ 1000 nm.

The interactions of the nanostructure of all metals with blood serum are of the same type and have differed only quantitatively. These distinctions are connected with the sizes of nanostructures aggregates in the dispersions. Known correlation between patient state and blood properties allows us to suppose potential diagnostic significance of new integral characteristics of patient state, characteristic radii of nanoparticles-serum components complex formed (CRNC).

Obtained results, taken together with data available from literature, lead us to possibility of using such nanostructures for diagnosis of some conformational diseases.
Acknowledgements

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References


WEAK PULSED ELECTRIC FIELDS AND BACTERIA RESPIRATION

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Abstract:

A change in the respiratory activity (RA) of the resistant to cyanides bacteria P. fluorescens under the pulsed electric field treatment has been found experimentally. The bacteria reaction to the field treatment (rectangular impulses with the duration of 1 ms, voltage of 20 V, and frequency of 100 Hz) varied from the RA suppression of active culture to restoration and even stimulation of RA in cells with the decreased respiratory function, caused by the cyanide introduction. Theoretical analysis of the experimental data indicates the change of quantity of active respiratory centers in cells under the influence of the pulsed electric field. These changes have reversible character. Depending on the state of bacteria, the processes of inhibition or restoration of the respiratory centers by the pulsed electric field can be observed.

Key words: P. fluorescens, pulsed electric field, respiration

1 Introduction

A convenient object for studying the biogenic influence of fields in a wide range of parameters is microorganisms. The analysis of the data given in the literature testifies to the mostly bactericidal character of the action of fields [1]. The action of pulsed electric fields (PEF) on microorganisms was first described in the 1960s, and then this direction was widely developed. The PEFs used in the technological processes for the inactivation of microorganisms in natural liquid media such as milk and fruit juices have, as a rule, a high strength up to 40-50 kV cm\(^{-1}\). The pulsed electric fields are widely used for the introduction of a genetic material and drugs into eukaryotic and prokaryotic cells (electroporation) and also for the creation of hybrid cells (electrofusion) [2]. Ordinary values of the electric fields applied are \(~15\) kV.

At the same time, there are available the data testifying that the weak electromagnetic and electric fields can stimulate the growth-regulating and physiological characteristics of microorganisms. Mechanisms of the action of fields on living objects are not sufficiently clear; especially this concerns the action of low-intensity fields without any direct physical action.

This work presents the results of investigations of the effect of PEFs on bacteria which are destructors of cyanides. As an objective parameter characterizing the action of a field on a cell, we chose the respiratory activity of bacteria. This choice is due to the fact that cyanides belong to powerful respiratory poisons. Joining a cytochrome \(a_1a_3\) which completes the electron transfer chain from NAD(H) to oxygen, the cyanide-ions block the transfer of electrons to oxygen. An external electric field can directly affect the process of blocking.

The purpose of these studies consists in the verification of the assumption about the significant influence of weak electric fields on the respiratory centers of cells, which would underlie the biotropic effect.
2 Materials, organisms and methods

Organisms and growing conditions. We chose two cyanide-resistant strains of bacteria, *Pseudomonas fluorescens* RCIM B5040 and *Pseudomonas fluorescens* NCIMB11764. The bacteria were grown in 100 ml of the nutrient medium (0.5 g/L peptone, 2.0 g/L KH₂PO₄, 1.0 g/L K₂HPO₄, 0.3 g/L MgSO₄·7 H₂O, 0.5 g/L Na₂CO₃, 0.1 g/L NaCl, 2.0 g/L glucose, 50 mg/L NaCN; pH 7.6) by batch cultivation in a 500-ml conical flask on a shaker at 180 rpm and 28°C. At the end of the exponential growth phase, the biomass was harvested by centrifugation at 3700 g, washed twice, and resuspended in Tris-HCl buffer (pH 7.6).

The treatment with PEFs. The scheme of a setup for the treatment of bacteria with PEFs is given in fig. 1. The setup included a round dielectric cell of 3.0 ml in volume and 1 cm in depth with a lid. On the lid, 7 stainless steel electrodes with needle-like form were fixed. At the ends of needles, there appeared an inhomogeneous field with a maximum strength of 10³ V cm⁻¹. The plane electrode at a voltage of the opposite sign was positioned under the dish bottom. In the lid, we made holes connecting the dish with atmosphere and the inlet for the supply of air from a micro compressor. Field were supplied from a pulse generator and controlled with an oscillograph. Pulses were unipolar and had a rectangular form. Microbial suspension was mixed with the help of a miniature agitator.

Measurement of the respiratory activity (RA). The endogenous respiration was controlled with an oxygen electrode (the Clark-type one, InLab605 “Mettler Toledo”, Switzerland) in an experimental vessel of the open type. The registered parameter was the rate of decrease in the oxygen concentration (θ) in the medium reduced to the biomass unit (specific respiratory activity): $RA = -\frac{d\theta}{dt} \text{ m}⁻¹$, where $d\theta$ – oxygen concentration change in the medium (mmol/L) over a fixed time interval $dt$ (min); $m$ – mass of introduced microbial cells (g/L). The biomass of 20-h cells of the same yield was separated from a nutrient medium by centrifugation at 3700 g, resuspended in 5.0 mM Tris-HCl (pH 7.6) buffer, and deposited under the same conditions. For the PEF processing, we produced a suspension of bacteria from the concentrated suspension and buffer (0.6 – 0.9 g/L of dry cells) and put 2.6 ml of bacteria into the cell for PEF treatment. The treated bacteria was at once transferred into the vessel for respiration tests containing 7.4 ml of Tris-HCl buffer, and the kinetics of the reduction of oxygen with an oxygen sensor was measured. For each series of experiments, we performed the control experiments, in which all the above-mentioned operations were carried out with no field applied.

3 Results and discussion

In fig. 2, we present the data showing both the respiration activity and the oxygen uptake kinetics in a solution due to the vital activity of treated bacteria *P. fluorescens* NCIMB 11764. As the control value, we took the RA of bacteria after 30 min of their stay in the cell for PEF treatment with no field applied (See fig. 2 A, curve 1). The concentration of O₂ decreases sharply for the initial time period and then increases (Shown in fig. 2 A, insert). The
measurements were carried out in the open-type cell; therefore, the subsequent rise of the curve corresponded to the balance of two processes: the reduction of oxygen concentration as a result of its consumption by introduced bacterial cells (the activity proper to bacteria) and the saturation of the solution with oxygen from air. We compared the shallow segments of the curves.

Fig. 2: (A) The RA of P. fluorescens NCIMB11764 culture.

1 Untreated bacteria (control); 2 After the treatment with PEF; 3 After the introduction of NaAg(CN)₂; 4 After the introduction of cyanide and the PEF treatment (U = 20 V, t = 30 min, C_CN = 1.2 ×10⁻⁴ molL⁻¹).

Insert: The concentration of oxygen is presented as a function of the measurement time. Continuous curves are the result of calculations according to the equation (3).

(B) Respiratory reaction of P. fluorescens B5040 to the treatment with the 20-V PEF of various duration.

As seen, the treatment with a 20-V PEF for 30 min caused the clearly manifested decrease of the endogenous respiration of treated cells (See fig. 2A, curve 2). The treatment with silver dicyanide for 30 min without the field treatment led to the analogous result. The consumption of oxygen decreased as a result of a specific binding of cyanides to the cytochrome enzyme. The treatment of bacteria with PEF affected the RA practically in the same way as the treatment with cyanide, i.e., it suppressed the cell respiration. If, simultaneously with the introduction of cyanide, bacteria were treated with a field, then their RA considerably grew (See fig. 2A, curve 4), and cells assimilate oxygen more intensely. This circumstance indicates that the PEF acts as an «anticyanide» factor in the case of the cyanide blocking of respiratory centers. The field rendered the «anticyanide» effect only if cyanide was introduced simultaneously with the switching-on of PEF. The half-hour treatment of bacteria, which were preliminarily incubated with silver cyanide for 1 h, with PEF did not change their RA that remained to be low.

By varying the treatment duration at the constant field strength, one can attain both the suppression and intensification of the RA of bacteria. The results are presented in fig. 2B, where the concentration of oxygen is given in relative units in order to exclude the effect caused by the ordinary stay of bacteria in the cell for PEF treatment. The measured concentration of O₂ was divided by the relevant value obtained in the control experiment (with no PEF applied). In the time interval under study from 5 to 30 min, only the 15-min treatment caused the positive respiratory reaction: the RA increased by more than 60%. In all the rest cases, the field influenced slightly the RA of bacteria (5 and 20 min) or decreased it (30 min).
The respiratory centers (RCs) of bacteria are referred to nanosized objects. Therefore, the motion of electrons in them can be adequately described only within quantum-mechanical methods. The potential energy of a molecular chain, along which electrons move, is modeled by a sequence of potential barriers. Such a system satisfies, as a rule, the conditions for the appearance of resonance tunneling phenomena both in the linear and nonlinear manifestations [3]. The study of the influence of an external electromagnetic field on the process of nonlinear resonance tunneling indicates that the general regularities of such an influence are characteristic of living objects as well [4].

The above-presented arguments allow us to assume that just the electron-transferring protein complexes, which are located in cell membranes and transform the energy of oxidation processes in the chemical energy of ATP, can be a place of the action of external fields in living organisms, in particular in bacteria. A change of the transmembrane potential under the exposure of a cell in an external field can change the conditions for the transport of electrons in RCs. The scheme of the influence of an external field on both respiratory centers and the transmembrane potential is shown in fig. 3.

![Respiratory center diagram](https://via.placeholder.com/150)

**Fig. 3:** Influence of the external electric field on respiratory centers located in membranes of bacteria. (A) Schematic image of electron transport in respiratory center; (B) A bacterial cell in the electric field.

At places where the gradient of an external field coincides with the gradient of the potential on a membrane, the total membrane potential increases by the value of $\ell E/2$, where $E$ is the field strength, and $\ell$ is the size of a cell. On the opposite pole of the cell, the membrane potential decreases by the analogous value. The respiratory centers of bacteria have several sections, in which the transfer of electrons from electronegative redox pairs to electropositive ones is accompanied by a great decrease in the free energy. At these places, the system can be characterized as a structure with double potential barriers [3]. The treatment with weak external fields can cause a significant change of the transmembrane potential and, as a consequence, a blocking or deblocking of the electron transport.

The respiratory activity of living organisms is determined by the number of functioning respiratory centers. Moreover, the rate of consumption of oxygen is directly proportional to the number of operating RCs. In analogy to work [5], the above-discussed scheme can be described on the phenomenological level on the basis of the equations of balance of the number of operating RCs which support a constant value of the transmembrane potential (by means of the forced transport of protons through a membrane) and the amount of consumed oxygen necessary for the support of redox reactions.
Under the condition of low cyanide concentration, two processes take place in bacteria cell simultaneously. The cyanides inhibit the respiratory chains, but at the same time the biochemical destruction of cyanide occurs causing the restoration of respiration. In the framework of experiment, one can consider these processes as mutually compensated. Inasmuch as the total number of RCs in each organism is unknown, it is expedient to introduce the relative variables. Accordingly, the quantity $n$ is defined as the averaged relative number of operating RCs in the solution. It depends directly on the concentration of cyanide in the solution, $C$, according to the equation:

$$n = n_0 - \gamma C$$

(1)

where $n_0$ - the total number of operating RCs in bacteria without cyanide, and $\gamma$ is a certain constant value. When $C > C_{cr}$, all the RCs are blocked, where $C_{cr} = n_0/\gamma$ - the critical concentration of cyanide. The concentration of oxygen in the solution, $\Theta$, can be represented phenomenologically as:

$$\frac{d\Theta}{dt} = -\alpha n + p(\Theta_{sat} - \Theta)$$

(2)

As mentioned above the system under investigation is open. Oxygen can go into the system with a diffusion rate $p$ up to the concentration $\Theta_{sat}$ which corresponds to the equilibrium state with atmospheric oxygen; $\alpha$ - the rate of absorption of oxygen by a single operating respiratory center. The equation (2) has the following stationary solution for the concentration of oxygen $\Theta$:

$$\Theta_s = \Theta_{sat} - \frac{\alpha n}{p}$$

(3)

In the above-described experiments, the solution with bacteria after the treatment with an electric field was diluted by a buffer solution saturated with oxygen, in which we performed the kinetic measurements concerning the respiration of bacteria, by determining a change in the concentration of oxygen. It is obvious that, in this case, the concentration of oxygen in the solution was in a nonstationary state at once after the dilution. The nonstationary solution of the equation (3) takes the form:

$$\Theta(t) = \Theta_0 \exp(-pt) + \Theta_{sat} - \frac{\alpha n}{p}$$

(4)

Formula (4) was compared with the experimental values presented in fig. 2 (see insert). The points correspond to the experimental data, and the continuous curve is the result of calculations. By the conditions of the experiment, $p$ and $\Theta_{sat}$ were constant values: $p = 0.294$ min$^{-1}$ and $\Theta_{sat} = 0.24$ mmol/L.

By comparing the theoretical and experimental values, we can determine only the product $\alpha n_s$, whose measurement was, in fact, the purpose of the studies. On the determination of the temporal dependence of the concentration of oxygen, the parameter $\Theta_0$ varied somewhat at each measurement (because of dilution of the solution, during which the concentration of oxygen in the working vessel was not controlled), which gives rise to certain difficulties for the direct estimate. Therefore, we determined this parameter by means of the comparison of the theory and the experiment. The processing of the experimental data showed that the rate of consumption of oxygen by bacteria after their stay in the cell for PEF treatment with no field applied was $\alpha n_1 = 27.4 \pm 0.33$ μM/min. Under analogous experimental conditions but after the treatment of bacteria with a pulsed field, the rate of consumption of oxygen became $\alpha n_2 = 16.8 \pm 0.20$ μM/min. Thus, as a result of the action of an electric field, the RA of bacteria decreased and was only about 61% of the value for untreated cells. In other words, we observed the suppression of the respiratory activity of cells by an external PEF.
Let’s compare this regularity with that under the action of cyanides, which also inhibit the RA, on cells. After the addition of NaAg(CN)₂ into the experimental cell without treatment with PEF, the rate of absorption of oxygen was
\[ \alpha_{n3} = 15.2 \pm 0.25 \mu\text{M/min.} \]
As would be expected, the RA of cells was decreased due to the blocking of RCs with cyanide. As a result of the joint action of cyanides and PEF on bacteria, we got
\[ \alpha_{n4} = 19.4 \pm 0.12 \mu\text{M/min.} \]
This value is greater than that the sum of the values under the separate action of cyanide or a field on bacteria. In other words, we observed the restoration of the RA under the action of PEF.

4 Conclusions

A specific feature of the interaction of bacteria and PEF consisted in that the RA of active cultures decreased, whereas the cells with decreased respiratory activity, on the contrary, restored or even increased the RA. This can be realized by the mechanism of tunneling of electrons present in the quantum wells formed by separate redox pairs of RCs. The executed estimates indicate the reversibility of the processes of blocking and deblocking of RCs on the application of PEF. The dependence of the respiratory reaction on the treatment duration indicates the cumulative character of this effect. This is related to the fact that the more or less number of centers can be switched-on under the treatment of a cell, by depending on its orientation relative to the field. Since the orientation has stochastic character under mixing, the number of activated RCs will approach a certain value on the long-term treatment. In particular, such an effect can be used for the activation of the bacterial destruction of cyanides in cyanide-containing solutions.

We may assume that the regularities, which are observed in experiments with bacteria of a single genus, have a more general character and are applicable to various biological objects undergoing the action of weak electric (electromagnetic) fields.

5 References


Abstract:

The article discusses the influence of weak pulsed electric fields on cyanide-destructive activity as well as on the surface properties of bacteria Pseudomonas genus. It is shown that during treatment of bacteria by electric impulses with the duration of 1 – 10 ms, voltage of 20 – 70 V, and frequency of 100 – 500 Hz the cyanide destruction increases by 20 - 30%. Simultaneously, the affinity of cells with polar (water) phase rises. The frequency and time interval values of electric field actions at which the hydrophobicity of a culture increased have been detected. The supposition that in the base of the increase in biochemical activity lays the influence of internal electric field on respiratory centers of bacteria associated with the de-blocking phenomenon of electron transport in respiratory centers inhibited by cyanides was made.

Key words: bacteria, pulsed electric field, cyanide

1 Introduction

It is known that in some cases weak electric and electromagnetic fields show stimulating influences on microorganisms. They are: acceleration of growth, adaptation to stress conditions, production of excess metabolites, enzymatic process activation [1,2]. In the majority of cases the mechanisms of such effects are not clear and the field application is of an empiric nature.

Earlier we had shown that in the process of destruction of cyanide complexes of silver, copper, and other transition metals in model solutions and waste water (pulp) of a gold – mining factory the destructive activity of culture P. fluorescens one can increase using DC field in the voltage range of 2 – 5 V/cm or electromagnetic field with the 20 - mW/cm² power in the microwave range of 58 - 60 GHz [3].

Further study on the mechanism of the electric field influence on bacteria displayed respiratory response of culture-destructor on the effect of weak pulsed electric fields (PEFs). The hypothesis that the conditions exist for realization of electron transfer according to the mechanism of the non-linear resonance tunneling was formulated in papers [4,5]. During the exposition of a cell in an external PEF it is possible to block or unblock the chains for electron transfer depending on quantity of working respiratory centers and field parameters that determines sign and value of the respiratory response.

The aim of the given investigation was to study voltage, frequency, duration of treatment, intensity of impulse of weak inhomogeneous PEF influence on the destruction of silver cyanide complex by resistant bacteria belonging to the Pseudomonas genus. Inoculum, before introducing into cyanide-containing solution, was subjected to electrotreatment. The
experiments were carried out in such way in order to exclude decrease of electroactive components at the expense of running of electrochemical reactions.

2 Materials, organisms, and methods

**Microorganisms and the condition of growth.** In the experiments bacteria *Pseudomonas fluorescens* RCIM B5040 and *Pseudomonas fluorescens* NCIMB 11764 were used. The destructive properties of the mentioned cultures were described in papers [6,7]. The cultivation was conducted in the 5M medium of the following composition, g L⁻¹: glucose - 2.0, peptone - 0.5, KH₂PO₄ - 2.0, K₂HPO₄ - 1.0, MgSO₄·7H₂O - 0.3, Na₂CO₃ - 0.5, NaCl - 0.1, NaCN - 0.094; pH 7.8. 25-hour culture (the end of exponential phase of growth) was used in the experiments.

**Procedure of the PEF treatment.** Bacteria of one yield were separated from nutrient media in the centrifuge at 3700 × g, were washed in distilled water and re-suspended in the 5M media containing NaAg(CN)₂. The suspension was stirred in vibration mixer for 1 min; then 2.7 mL were placed in cell and treated by PEF.

**The bacteria treatment by PEF** was conducted in the dielectric cell of 3.0 ml volume. 7 steel electrodes of needle-like form were fixed on cover creating in cell inhomogeneous field. A plane electrode of opposite sign was placed under bottom of the cell. Several vents connecting the cell with atmosphere and also air inlet from microcompressor were in the cover. A content of the cell was stirred with the help of miniature stirring device. Pulsed field was supplied from generator of impulses G5-54 (Russia).

**Microbial destruction and cyanide analysis.** The stable complex NaAg(CN)₂ was used (\(K_{ins} = 8 \times 10^{-22}\)). The [CN⁻] concentration was determined using pyridine – barbituric reagent. Lower threshold of sensitivity was 0.05 mg L⁻¹ of CN⁻. Inoculate of bacteria (70 mL) after the PEF treatment was grown in 250-mL flasks containing of cyanide-bearing nutrient solution by batch cultivation on a shaker at 180 rpm and 28°C. The experiments were conducted by series on the bacteria of one yield. The concentration of seeding was 0.34 - 0.38 mg L⁻¹ (\(D = 0.4 ±0.03\)).

**The degree of total destruction,** \(W_T\), was calculated from kinetic dependencies according to the equation:

\[
W_T = \frac{C_0 - C_T}{C_0} \times 100\%
\]

where \(C_0\) – initial concentration of [CN⁻], \(C_T\) – concentration in the solution after destruction.

**The degree of the biological destruction,** \(W_B\), was calculated according to the equation:

\[
W_B = \frac{C_0 - C_B}{C_0} \times 100\%
\]

where \(C_B\) – concentration of [CN⁻] after destruction with the bacteria not treated by PEF.

**The degree of electrobiotreatment,** \(W_{EB}\), was calculated as a difference between the biological destruction and destruction activated by field according to the equation:

\[
W_{EB} = \frac{(C_0 - C_{EB}) - (C_0 - C_B)}{C_0} \times 100\% = \frac{C_B - C_{EB}}{C_0} \times 100\%
\]

where \(C_{EB}\) – concentration of [CN⁻] after destruction with the bacteria treated by field.

**Surface hydrophobicity,** \(H\), was determined on cell adhesion to \(n\) – octane. The percentage distribution was measured using the relation: \(H = 100(1-D_x/D_0)\), where \(D_0\) and \(D_x\) – optical density of bacteria before and after contact with \(n\) – octane, respectively.
3 Results and discussion

3.1 PEF voltage

Fig. 1 C gives the kinetic curves of the NaAg(CN)₂ degradation depending on the applied PEF voltage (\(C_{CN} = 0.10 \text{ mM}\)). In the given series of the experiments the impulse duration was 1 ms and the repetition frequency was 100 Hz. Spontaneous destruction of the complex does not exceed 1 %. The microbial destruction by the \(P. \text{fluorescens}\) B5040 culture run with the delay about 20 hours, in the following 46 hours the [CN⁻] concentration decreased by 43.4 % up to 0.06 mM. The inoculum treatment with a 10 and 20-V field influenced weakly the degree and destruction kinetics (see curves 4 and 5 in fig. 1). At the same time, the destruction was intensified after the treatment with a 40 and 70-V field, the duration of lag-phase notably shortened (see curves 6 and 7 in fig. 1). The cells at the same time preserved viability that was indicated small growth of biomass in the investigated interval of time (see curves 5, 6 and 7 in fig. 1).

Using the kinetic dependences according to equations (1)-(3), the degree of cyanide destruction depending on the field parameters has been calculated. As seen from fig. 1B the portion of activated by field electrobiodestruction (\(W_{EB}\)) in total index of destruction of cyanide complex by \(P. \text{fluorescens}\) culture increased from 5 % to more than 50 % with the growth of voltage from 10 to 70 V (at fixed frequency of 100 Hz and impulse duration of 1 ms).

While treating by PEF for 75 minutes the bacteria affinity with organic phase was changed. The data are given in fig. 2 C. Bacteria surface acquired more hydrophilic properties. In the field interval from 10 to 70 V the dependence of relative hydrophobicity (\(H_{EB}/H_C\)) changed as a function of voltage \(U\) according to the empirical function:

\[H_{EB}/H_C = 1.05 \exp(-U/21,02).\]
It is possible to suggest that in the result of the treatment by a field, bacteria released exudation containing biomolecules with dominant polar part, for example, polysaccharides, polypeptides and others. The higher applied voltage was the bigger quantity of stress metabolites bacteria released.

3.2 PEF frequency

The study of frequency dependences has shown that PEF in the range of 25-50 Hz at fixed 40-V voltage and the treatment duration 15 min. did not affect distinctly the rate of microbial destruction. As in control experiment without treatment the microbial destruction begins after nearly 20-hour period of lag-phase. The contribution of electrobiodestruction becomes substantial on the frequency more than 100 Hz. The rate of the destruction also increased with growth of frequency. The dependency passed in the plateau in the interval from 250 to 500 Hz.

![Graph](image)

**Fig. 2:** (A) The change of daily rate of the cyanide destruction and (B) relational hydrophobicity ($H_{EB}/H_C$) as a function of PEF frequency.

In the frequency dependence of hydrophobic properties of investigated bacteria given in fig. 2 B conditionally it is possible to separate three regions. The culture after 75-minute of treatment acquired more hydrophilic property as compared with control in the region from 25 to 700 Hz. In the frequency region from 1 000 to 3 000 Hz the dependence changed in extreme manner. The culture hydrophobicity increase essentially, almost by 6 times. At last, the reinforcing tendency to hydrophilization of cell surface corresponded to the third interval of frequencies up to 10 000 Hz.

3.3 Treatment duration and impulse value

It is interesting that the destruction rate was changed depending on impulse value, i. e., the product of field frequency $f$ on impulse duration $\tau$. The data are shown in fig. 3 A. Total statistics of the experimental data obtained at constant value of $(f \times \tau) \approx 0.1$ (increasing frequency from 100 to 10 000 Hz and simultaneously decreasing the impulse duration from 1000 to 10 ms) allows to speak about the downtrend of destruction rate when the impulse duration decrease if the field frequency increased in this case.
Fig. 3: (A) The change in the NaAg(CN)₂ destruction rate \( V_d \) depending on the field impulse value \((f \times \tau)\). Light shading is a total destruction, the deep shading is an electrobiodestruction.

(B) Change in relative hydrophobicity \( (H_{EB}/H_C) \) depending on the duration of bacteria treatment by PEF.

It was noticed that at the 100-Hz field frequency and 1-ms impulse duration bacteria respond to the PEF treatment predominantly by decrease in hydrophobicity. As it seen from fig. 3 B the longer bacterial culture is treated by field, the greater hydrophilic surface becomes. Only in the time interval from 60 to 90 min a small increase of surface hydrophilicity was observed.

We remind that in our experiments the possibility of cyanide destruction due to the electrochemical reactions on electrodes was excluded. Therefore, the mentioned data concerning the destruction testifies in favour of the physiological nature of bacteria response to the PEF treatment. First of all, the change in metabolite composition and surface philicity under the influence of such parameters as applied voltage, frequency, impulse duration, and treatment duration indicates this. The cell hydrophilicity increased while increasing these parameters. Separate narrow windows in value investigated ranges at which the surface became in greater degree hydrophobic as compared with non-treated bacteria were noticed. The reason of non-monotony can be associated with the peculiarities of biochemical mechanisms for cyanide decontamination. In paper [5] it is shown that the cyanide destruction by \( P. \) fluorescens bacteria take place on the oxidation mechanism with participation of membrane – associated cyanide dioxygenase enzyme, NAD(P)H and oxygen. Cyanide refers to respiratory toxin. It inhibits the main (phosphorylating) respiratory chain on the section for transfer of pair electrons from cytochrome \( a_1a_3 \) on an oxygen molecule. The revealed earlier our effect of the PEF influence on the bacteria respiratory activity in a cyanide solution can testifies the interconnection of destructive and respiratory of cell activity. According to our hypothesis, an additional difference of the potentials, \( \pm \Delta \phi_{me} \), appears on the cell membrane as a result of action of the external field. This value increases the transmembrane potential \( \Delta \phi_{m} \), if its sign coincides with the direction of field and, vice versa, decreases if the signs are opposite. Binding of cyanide - ion at cytochrome site raises the value of the energetic barriers between molecules in the respiratory chain that finally blocks transport of electrons along all the chain. In the case, when the respiratory centers of bacteria appear in zone with a reduced...
transmembrane potential, then the value of barriers decreases. At the same time, the electron transfer along the respiratory chain becomes possible [4]. As a result, two electrons of the respiratory chain and two electrons from cyanide - ion pass to an oxygen molecule. The cyanide oxidation with the formation of carbonic dioxide and ammonia take place according to the following equation: \( \text{CN}^- + \text{O}_2 + 4e^- + 4\text{H}^+ = \text{CO}_2 + \text{NH}_4^+ \).

It is clear, that the value of an expected effect cannot be too big, inasmuch as the realization of the proposed mechanism demands optimal combination of parameters of field and certain bacteria orientation relatively field force lines. The last parameter bears spontaneous character set by the stirring conditions.

4 Conclusions

The obtained experimental data concerning the influence of field strength, frequency, and duration of treatment on the rate of the \( \text{NaAg(CN)}_2 \) degradation by the \( P.\text{fluorescens} \) culture indicate the initiation of the destruction function of bacteria by external impulse electric field. One can reach the growth of the destruction rate by 20-30% increasing the voltage of applied field from 20 to 70V in the frequency range of 250 - 500 Hz. Better results were reached while treating by very short impulses 1 - 10 ms.

The obtained our results and made conclusions due to the specificity of cyanide action realizing the inhibiting influence directly on the respiratory centers of cells can be extend on the other cases, at which cells have a decreased respiratory activity or are in the non-active state. Using the PEF treatment with certain field parameters it is possible to increase substantially their physiologic activity returning in active state.

5 References

INFLUENCE OF CONCENTRATION OF SODIUM CHLORIDE ON THE YEAST CELLS AND QUALITY PARAMETERS OF OHMIC HEATED PALM WINE (RAPHIA HOOKERIA) ON A STATIC MEDIUM

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Abstract:

Ohmic heater was designed on a static medium at 110 V rating and used to pasteurize palm wine. Five hundred milliliters of the fresh palm wine samples were treated with 0.0 g, 0.2 g, 0.3 g, 0.4 g and 0.5 g NaCl and then kept to stand for 20 min. Then heated at 110 V for 5, 10, 15 and 20 min using aluminum electrode. The samples were assayed for yeast cells and measured for TTA, Brix level, pH, ionic strength and alcohol content before and after Ohmic heating, then stored for 21 days, during which analysis were conducted at intervals of 7 days and sensorially evaluated on the 21st day with a fresh palm wine as control. The result showed that Ohmic heated samples with NaCl destroyed yeast faster than samples without salt or non Ohmic heated. Sample with 0.5 g NaCl destroyed $0.7528 \times 10^4$ yeast cells/ml per min, while those without NaCl destroyed $0.5432 \times 10^3$ cells/mL per min. Yeast reduction was dependent on concentration of NaCl, heating time and temperature attained. pH decreased with heating and storage time. Titrable acidity (TTA) increased with heating and storage time, while Brix and alcohol content decreased with storage days. However ionic strength showed an initial increase with heating time and subsequently decreased during storage. The test panel ranked the wines differently but preferred the fresh palm wine, while the sample without NaCl was disliked most. Ohmic heating only at 110 V is not good enough to pasteurize palm wine due to low ionic strength.
Abstract:
Pulsed Electric Fields (PEF) can provoke selective electroporation of plasmatic membranes in multicellular plant tissues. The PEF electroporation of tissues provides direct contact between intra- and extracellular solutions that results in promotion of crystallization and reduction of freezing time. This work investigates the effects of PEF pre-treatment on the freezing rate of different vegetables. Three types of vegetable tissues, leaf (spinach), tuber (potato) and pods (green beans) were treated by PEF with electric field strengths between 400 V/cm and 600 V/cm and different pulse durations between 0.2 and 2 s. The degree of tissue damage was estimated from the electrical conductivity disintegration index Z. Samples were frozen using 3 different freezing modes: ultra-rapid freezing in liquid nitrogen vapour, rapid freezing at -80°C and slow freezing at -35°C. The effective freezing time and the rate of freezing were determined and compared to quantify the gain due to PEF pre-treatment in accordance with the freezing mode used. The quality of products was characterized by measuring the tissue deformation (relaxation test).

Keywords: electroporation, pulsed electric fields, freezing, vegetables

1 Introduction

Freezing is an excellent and fairly widespread method for preserving food products, including fruits and vegetables, which provides high stability of health-beneficial micronutrients [1]. However, freezing of vegetables may alter quality characteristics, such as flavour and texture, which, in turn, can affect their marketing potential. Rapid freezing has an advantage of water freezing resulting in a fine crystalline structure, thereby preventing mechanical deterioration of the cell tissue caused by large ice crystals [2]. Crystals produced by recrystallization damage the cell membrane, reduce water content of the product and cause the loss of nutrients [3], oxidation of lipids, colour changes and undesirable flavours [4]. Pulse Electric Field (PEF) pre-treatment (electric field strength: 0.5-1 kV/cm, treatment time: 2-10 s) allows selective electroporation of the cell membranes without causing any serious deterioration of the semi-rigid cell wall [5-9]. The low power consumption of the PEF processing increases its attractiveness in such applications as drying, pressing, extraction and osmotic dehydration [4]. However, PEF effects on freezing of plants have not been investigated previously in details.

The objective of this work is to investigate the effect of PEF pre-treatment on the freezing processes in three types of vegetables (potato, green bean and spinach).
2 Materials and methods

2.1 Raw materials

Potatoes (variety Agata), fresh green beans and fresh spinaches (Bonduelle, France) were purchased in a local supermarket. They were kept in a refrigerator at 4°C until test. The stock was renewed every week. Potato samples (diameter = 26 mm, thickness = 10 mm) were taken from tubers with a punch. Spinach leaves were cut into disks 26 mm diameter and assembled in a 10 mm thick millefeuille. Green beans (8-9 mm diameter) were cut into sticks of 26 mm length. Juice from each of three vegetables was prepared by a food liquidizer (AV6, Moulinex, France).

2.2 PEF treatment

2.2.1 Experimental equipments

All the materials used for the PEF treatment were UTC-made. PEF-treatment cell consisted of a polypropylene cylindrical tube with a 26 mm inner diameter. The electrodes were connected to a PEF generator (400 V-38 A). The generator provided bipolar pulses of a near-rectangular shape (Fig. 1), which allowed avoiding of asymmetric electroporation at the poles of the cells. All the output data: current, voltage, electrical conductivity, and temperature, were collected using a data logger and relevant software. The electrical conductivity was measured between the inter-series period at the frequency of 0.5 kHz. This frequency was selected to minimise the polarizing effects on the electrodes and tissue sample.

2.2.2 PEF treatment protocols

The parameters characterizing the PEF treatment protocols are presented in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Potato</th>
<th>Green Bean</th>
<th>Spinach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage (V/cm)</td>
<td>400</td>
<td>500</td>
<td>600</td>
</tr>
<tr>
<td>Pulse duration, $t_i$ ($\mu$s)</td>
<td>1000</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>Number of trains, $N$</td>
<td>100</td>
<td>1000</td>
<td>600</td>
</tr>
<tr>
<td>Total time of PEF treatment, $t_{PEF}$ (s)</td>
<td>0.2</td>
<td>2</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Tab. 1: Parameters of PEF treatment for three studied vegetables.**

*Number of pulses: 2, pause between trains: 10 s.*

2.2.3 Estimation of tissue damage

The degree of tissue damage was estimated from the electrical conductivity disintegration index, $Z$ [14]:

$$Z = \frac{(\sigma_u - \sigma)}{(\sigma_d - \sigma_u)}$$

where $\sigma$ (S/m) is the measured electrical conductivity and the subscripts $u$ and $d$ refer to the conductivities of untreated (intact) and completely damaged tissues, respectively. The value of $\sigma_d$ was determined from the measurements of electrical conductivity of a raw tissue slowly frozen in a cold store (-25°C) and then thawed.

2.3 Freezing experiments

2.3.1 Methods of freezing

After the PEF treatment, samples were frozen using different methods.

**Ultra-rapid freezing using nitrogen vapour (fig. 2):** The samples were placed in a stainless steel basket and then introduced into a Dewar vessel containing about 5 L of liquid nitrogen. By placing the bottom of the basket at a distance of approximately 5 cm above the surface of the boiling nitrogen, the samples were brought into contact with the vapour nitrogen.
Rapid and low air-blast freezing (fig. 3): An ultra-low temperature freezer MDF-U2086S (Sanyo, Gunma, Japan) was used for air-blast freezing of a sample. The temperature was set at -35°C for slow freezing and at -80°C for rapid freezing. The samples were placed on a perforated plastic plate to minimize the effect of heat conduction through the bottom of the support. The air rate was fixed at 2 m/s and controlled by an electronic device VEAT 2.5 A (Airtechnic, Firminy, France).

2.3.2 Freezing Control

A modular-type temperature controller SR Mini SYSTEM (TC Ltd, Dardilly, France) and the Specview Plus software (SpecView Corporation, Gig Harbor, USA) were used to measure temperature. A type T thermocouple of 0.5 mm diameter with an accuracy of ± 0.1°C (TC Ltd, Dardilly, France) was introduced into the geometrical centre of a sample. Initial temperature before freezing was uniform inside the whole sample and equal to 20°C. The effective freezing time, $t_f$, was used to quantify the freezing rate. It was defined as the time required for temperature decrease by 10°C from the initial freezing temperature (temperature when the crystallization of water starts) at the centre of the sample, i.e. $\Delta T = -10°C$ (International Institute of Refrigeration, 1986). Freezing rate is the ratio of the effective freezing time and the distance between the outer surface and the centre of the product. In our case, this distance was 0.50 cm for potatoes, 0.40 cm for beans and 0.50 cm for spinaches.

2.4 Product characterization

2.4.1 Cell structure

The cell structure was directly checked using a transmission optical microscope (Leitz ophoplan, Germany). Very thin slices (thickness < 0.5 mm) were prepared using a scalpel and then coloured with Lugol and observed at magnification of ×10.

2.4.2 Textural behaviour

Stress–relaxation tests were performed using a Texture Analyser (model TA-XT2, Stable Microsystems Ltd., Surrey, England). The samples were either discs (potato and spinach) with 10 mm thickness and 26 mm diameter or cylinders (green bean) with 8-9 mm diameter and 26 mm length. The data were obtained by subjecting the discs or cylinders to a constant strain at the rate of 0.1 mm/min until achievement of the maximum force of 5 N. Then further deformation was stopped and stress changes were observed during 100 s.

3. Results and discussion

3.1 Evolution of the disintegration index during PEF treatments
As Figure 4 shows, the electrical conductivity disintegration index, $Z$, versus time of PEF treatment strongly depended on the nature of the product. The half time of tissue disintegration ($Z = 0.5$) was shortest for potato ($\approx 10^{-3}$ s) compared to green bean ($\approx 2.10^{-1}$ s) and spinach ($\approx 10^{-1}$ s). Green bean demonstrated slower increase of $Z$ versus time, while spinach exhibited some initial delay of permeabilization development. These results should be related to tissue structure, which is characterised by wide diversity in geometry and size of cells, as Figure 5 shows. The cells of potato and green bean are polyedric, spinach ones are rather oblate. Potato and spinach cells size ($\approx 60 \mu m$) is larger than green bean one ($\approx 30 \mu m$).

![Graph showing electrical conductivity disintegration index, $Z$, versus time of PEF treatment for three different vegetables (3 replicates)](image)

**Fig. 4:** Evolution of electrical conductivity disintegration index, $Z$ during PEF treatment for three different vegetables (3 replicates)

![Typical tissue structures of raw materials (optical microscopy)](image)

**Fig. 5:** Typical tissue structures of raw materials (optical microscopy)

### 3.2 Effect of PEF treatment on textural behaviour of vegetables

The textural relaxation data indicated dependence of tissue structure versus mode of pretreatment. The effect of PEF treatment on stress relaxation of vegetables was also strongly dependant on their nature (Fig. 6).

The PEF-treatment allowed attaining high levels of membrane disintegration and removing of the turgor component of the texture [10]. The texture became notably disintegrated for the freeze–thawed tissues. However, for potato and spinach, the PEF application was not efficient for complete textural strength elimination, where PEF pre-treated tissues exhibited noticeably stronger elasticity than freeze-thawed tissues (Tab. 2).
Fig. 6: Stress relaxation curves for untreated, PEF treated and rapid freeze-thawed tissues

<table>
<thead>
<tr>
<th>Elasticity (%)</th>
<th>Potato</th>
<th>Green beans</th>
<th>Spinach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>62</td>
<td>70</td>
<td>64</td>
</tr>
<tr>
<td>PEF treated</td>
<td>50</td>
<td>34</td>
<td>4</td>
</tr>
<tr>
<td>Freeze-thawed</td>
<td>34</td>
<td>30</td>
<td>2</td>
</tr>
</tbody>
</table>

Tab. 2: The elasticity (%) for untreated, PEF treated and rapid freeze-thawed tissues of three studied vegetables. Elasticity (%) = 100 × (F∞/F0), where F0 is the initial force (5 N) and F∞ is the residual force after 10 s (green beans and spinach) or 100 s (potato)

3.3 Effect of PEF treatment on freezing time and freezing rate

Figures 7 and 8 show that PEF treatment generally decreased the effective freezing time and consequently increased the freezing rate.

Fig. 7: The effective freezing time, t_f, for different vegetables and freezing modes (3 replicates) Fig. 8: The freezing rate for different vegetables and freezing modes (3 replicates)

The gain obtained with PEF treatment was dependant on both the vegetable nature and the freezing mode [11]. PEF treatment was the most efficient for decreasing freezing time and increasing freezing rate in case of spinach. The PEF treatment was advantageous for potato and green bean only when they were frozen in the ultra-rapid mode (nitrogen vapour). The ultra-rapid freezing following a PEF treatment could lead to 50% or larger decrease of the freezing time (potato and spinach).

The best results obtained with spinach could be partly explained by significant leaf thickness reduction during the PEF treatment. However, the tissue structure (cell size and geometry) and the moisture and ionic content also affected the freezing time. The present
theories of freezing suggest that initiation of the ice nucleation takes place in the extracellular space, and further freezing occurs through extracellular ice propagation into the supercooled cytoplasm [1] or is catalyzed by the plasma membrane in the presence of extracellular ice [12, 13]. The PEF effects on these characteristics are not completely clear yet and should be elucidated.

4. Conclusion

Experiments showed significant effect of PEF pre-treatment on the freezing time and freezing rate of potato, green bean and spinach tissues, especially in the ultra-rapid freezing mode. The electroporation of cell membranes was accompanied by a noticeable decrease of textural resistance, which was dependent on the vegetable variety, probably because of differences in the cellular structures, cell shapes and compositions (water, sugar and salt contents...).

References

DIMENSIONLESS ANALYSIS OF JET OHMIC HEATING PROCESSES WITH FOOD MATERIAL

\( \pi \) - SPACE INCLUDING ELECTRICAL CONDUCTIVITY DEPENDENCE WITH TEMPERATURE

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Abstract:
Ohmic-based food processing technologies have recently caught attention of the food industry that wishes to fulfil consumer demands for high-quality and safety of food products. The development of this attention has led to a number of attempts to design proper ohmic heating systems and to model the process. Unfortunately these studies provide little information concerning scale up rules for ohmic heating processes. A problem arises: How can we ascertain that the laboratory ohmic heating system achieves a complete similarity with the industrial plant? According to the theory of similarity, two processes are similar to one another if they take place in a similar geometrical space and, if all the dimensionless numbers necessary to describe the process, have the same numerical value. Another question arises. How can we do to build these dimensionless numbers in order that they take into account process, geometrical and material parameters? Here, different temperature dependence of physical properties (Electrical conductivity, viscosity) cause a problem because physical properties can no longer be considered as constant in the course of the process and constitute a major difficulty to establish the right set of dimensionless numbers. This contribution deals with this obstacle to cross. More accurately, it will be shown how to proceed to construct a complete list of relevant parameters able to consider the variability of physical material parameters and consequently succeed in elaborating a dimensionless frame without pitfalls in the case of ohmic heating. There is only one ohmic heating system which will be addressed and treated in this paper. In the following, the dimensional analysis for food ohmic heating systems is applied as an example to describe how considering the variability of physical material parameters. However this approach can be adapted for any other physical properties and ohmic systems.

Key words: ohmic heating, food material, theory of similarity, scale up.

1 Introduction
Ohmic heating (also referred to as electrical resistance heating, direct resistance heating, and Joule’s heating) is based on the passage of an alternating electric current through a fluid which serves as an electrical resistance in which heat is generated [1]. The direct resistance heating has potential as an alternative to conventional heating techniques for food processing, due to its speed, energy efficiency and capacity of ensuring better temperature uniformity as well as compact design resulting in a minimal equivalent mass system ([2], [3]). Another advantage offered by the ohmic heater over the more traditional steam-oriented heat exchangers is the elimination of the hot surfaces for heat transfer. This fact greatly reduced the burning on and the fouling of such surfaces, offering less maintenance and better product flavour ([4-8]).

However, spreading of these promising technologies is still limited to sporadic industrial application since Jones in 1897 [9], Beattie in 1914 [10], Anglim in 1923[11], Ball in 1937
The major obstacle for such implementation of ohmic food treatment unit is the lack of guideline concerning scale-up rules [13]. Indeed, even if great efforts have been made by the academic research laboratory to provide study at lab-scale which describe how the course of the food processing is affecting by process parameters and food properties in the ohmic heating system [14], these models fail to assist process developers in designing new industrial plants, new processes and better way of process control at large (industrial) scale. The reason is: ohmic processes are scale dependent, i.e., they have different characteristics in small and large applications and most of the models involve dimensional parameters which cannot integrate this fact. Consequently the model frame is not well-presented to ascertain that the industrial plant achieve a complete similarity with the laboratory ohmic heating system and guarantee that the set of process parameters chosen at industrial scale will avoid undesirable results on product quality. Comparing various ohmic heating processes, requires the ability to represent all processes in a set of dimensionless ratios in sufficient numbers, which integrate all the process parameters, geometric factors and material (food product) properties. All these issues can be addressed through a dimensional analysis of the relevant parameters which describe the ohmic heating process.

The dimensional analysis coupled with the similarity theory provides many advantages for understanding and modelling process where chemical or microbiological conversion of material takes place in conjunction with the transfer of mass, heat, electricity and momentum ([13], [15]). Indeed, dimensional analysis is a tool to quickly and reliably elaborate a dimensionless frame (i.e., a set of dimensionless ratios in sufficient numbers regarded as a set of measures defining the state of a system) in which the experiments are favourably presented, because it is condensed as compared with the dimensional one. Indeed, dimensional analysis provides a compression of the “statement”, this means that this approach allows to reduce the variables defining the physical process. Reducing the number of variables in a model minimizes the number of experiments necessary to establish the relationships between these variables and makes easier the identification of relationship parameters, even for complex process equipments ([16][17]). Further advantage lies in the ‘scale invariance’ of the dimensional analysis enabling from the physical theory of similarity reliable scale-up. According to the theory of similarity, two processes are similar to one another if they take place in a similar geometrical space and, if all the dimensionless numbers necessary to describe the process, have the same numerical value. This means that the behaviour of a food product during the treatment at industrial scale can be predicted from the behaviour of its replica provided that all the dimensionless ratios are equivalent.

Ohmic heating technology is a branch of mechanical chemical engineering where the dimensional analysis have been seldom used [13] and consequently few clear view of the set of dimensionless ratios governing the transformation process have been established. The aim of this work is to fill this gap.

When using the dimensional analysis to model system answer, it is generally assumed that physical properties of the material remain unaltered in the course of the process. However, constancy of physical properties can not be assumed in ohmic heating system. A temperature field may well generate a viscosity field and even an electrical conductivity field. Consequently, it would seem to be absolutely essential to present methods for taking into account this fact in our approach. The aim of this paper will be to discuss the following questions:

“Which cares should be taken in dimensional modelling to address the issue that the electrical conductivity of materials evolves in ohmic heater” Which are the consequences in terms of the set of dimensionless ratios which govern food engineering processes in ohmic heating? Therefore this work offers an example of how to proceed, but also how to avoid mistakes to apply rigorous dimensional analysis.
2 Dimensional analysis: usual practice

The procedure necessary to accomplish a dimensional analysis consists of two parts:

a) The first is the construction of a complete list of relevant parameters which describe the process (‘relevance list’). In each case, a target quantity must be considered. The target quantity is the only dependent variable and the influencing parameters should be primarily independent of each other.

b) The second is the transformation of the relevant parameters into dimensionless ratio (called \( \pi \)-ratio) based on the \( \pi \)-theorem. The \( \pi \)-theorem reads: every physical relationship between \( n \) physical quantities can be reduced to a relationship between \( m=n-r \) mutually independent dimensionless ratios, whereby \( r \) stands for the rank of the dimensional matrix. The dimensional matrix is formed with the dimensions of relevant parameters. The columns of this matrix are assigned to the individual physical quantities of the relevance list and the rows to the exponent with which the base dimensions appear in the respective dimensions of these quantities. The \( \pi \)-theorem is often associated with the name of Buckingham, because he introduced this term in 1914 [18]. Generating dimensionless numbers and possibly their transformation represents an extremely easy undertaking compared to the drawing up of a reliable and as accurate as possible relevance list (see next part). This can be made by matrix calculations. The cited literature [19] offers detailed example of how to handle this technique in order to quickly obtain the complete set of dimensionless numbers. This aspect will be not described in details in this paper.

3 Dimensional analysis with non constant physical properties: state of the art

When using the dimensional analysis to model system answer, it is generally assumed that physical properties of the material remain unaltered in the course of the process. However, constancy of physical properties can not be assumed in ohmic heating system. A temperature field may well generate a viscosity field and even an electrical conductivity field. In the case of materials with constant properties, no special precautions should be built to guarantee that a process relationship correlating a set of dimensionless ratios also apply to another material. This is not true for materials with varying properties, as demonstrated by Zlokarnik in 2001 [15]. In this case, we should first ensure a priori that a certain similarity exists for material to extend the range of validity of the relationship of process to other materials.

We note that the similarity theory has changed very little since its foundation and dimensional modeling involving material with variable physical properties are treated as constants in practically all papers based on theory of similarity. The authors considers in their dimensional analysis that the material properties remains constant throughout their history and in all the space defining the flow domain of reactor. This mean, that all authors ignore the fact that the spatio-temporal variability of physico-chemical properties influences the course of the process in the process equipment!

One exception is the modelling of transformation process involving a material with a temperature dependence of viscosity submitted to heat transfer condition. Practically all attempts which have been made to take into account the variability of physical properties of the product in the reactor have consisted to add an additional ratio raised to a certain power to characterize the system response. This ratio is defined as the ratio of viscosity at bulk temperature on the viscosity at wall temperature. However, this kind of enlargement of the set of dimensionless ratios to define the system answer (due to the variability of the physical property of the product) can be regarded as a theoretically validated technique in only few limited cases. It is justified only if the material function (here viscosity versus temperature) satisfy given criteria. In other words this type of method leads to biased predictions and
cannot be systematic apply when handling other fluids. Despite this fact, it is the same ratio which is used, whatever the product investigated in most of the studies since Sieder and Tate in 1936 [20]....

The right way of proceeding has been introduced by Pawlowski in 1971 [21] and remembered by Zlokarnik in 2001 [15] and 2007 [22]. The method consists to introduce additional parameter in the relevance list to take into account the fact that physical property is described by a function $s(p)$.

It can be sum up from [21]:

With variable physical properties $s(p)$ the relevance list should contained $s(p) = s(p_0)$, transformation parameters $\gamma_0$ and reference point $p_0$.

Transformation parameters are defined by:

$$\gamma_0 = \left( \frac{1}{s(p)} \left( \frac{ds(p)}{dt} \right) \right)_{p=p_0}$$

(1)

Reference $p_0$ could be chosen as possible in the range covered by experiments

Adding the reference point is not systematic, it depends on the function $s(p)$.

It has been proven that it is not necessary to add the reference point when $s(p)$ can be described by the following family of curve:

$$s(p) = (A + Bp)^C$$

(2)

Or

$$s(p) = \exp(A + Bp)$$

(3)

With $A$, $B$ and $C$ are 3 independent constants

In the following,

i) First the dimensional analysis will be applied to obtain dimensionless ratios governing exit temperature profile for a given ohmic heating system as an exemple to describe the variability of the electrical conductivity

ii) In the second step, the validity of the set of dimensionless numbers will be ascertained by comparing the dimensionless ratios obtained by this way and those existing in analytical solution.

iii) By means of this example, we will conclude how the variability of physical properties affects discussions of chemical engineering processes

4 Dimensional analysis of a jet ohmic heater

The task is to design a jet ohmic heater which is a new type of ohmic heater for a continuous production process at a volumetric flow rate $Q$ (Figure 1). Its purpose is to heat a given very viscous Newtonian fluid from the inlet temperature $T_i$ to the mean outlet temperature $T_o$ corresponding at the end of the jet. The fluid jet is shot downwards from a nozzle with a radius $R$ and falling freely on to a conical receptacle. The distance between the nozzle exit and the impact surface of the conical receptacle is $L$. The impingement distance of the jet can be modified by adjusting the fluid level $h$ in the receptacle. An electrical potential $V$ is applied to the fluid jet of electrical conductivity $\sigma(T)$. This induced an electrical field parallel to the flow pattern and generates a heat power per unit volume inside the material. The heating power delivered was controlled by measuring electrical current $I$. More details are given about this apparatus are given elsewhere [7][8].

The temperature dependence of electrical conductivity will also be taken into account in this context.

Thus the relevance list can be built as follows:
**Target data**

Mean outlet temperature of process material:  \( T_O \)

**Process data**

Inlet temperature of process material:  \( T_i \)
Electrical current:  \( I \)
Volumetric Flow rate:  \( Q \)
or Exit jet velocity:  \( u_0 = Q / \pi \cdot R^2 \)
Gravitational constant:  \( g \)

**Geometric factor**

Radius of Nozzle:  \( R \)
Length of the jet:  \( L \)

**Material data of the process**

Density:  \( \rho \)
Specific heat:  \( C_p \)

Electrical conductivity is supposed to describe a linear dependence with temperature:

\[
\sigma = \sigma_0 \,(1 + k \cdot T) \quad (4)
\]

As underlined above, a reference temperature should be selected to compute physical properties; we will choose here the mean inlet temperature  \( T_i \).

The presented material function of \( \sigma(T) \) required to introduce transformation parameter defined by equation 1 in the relevance list:

\[
\gamma_0 = k \quad (5)
\]

As it can be observed the presented material function of \( \sigma(T) \) is independent of the reference point. Indeed \( \sigma(T) \) satisfy equation 2 with \( C = 1; B = k \cdot \sigma_0; A = \sigma_0 \)

Thus the relevance list is:

\[
(T_o, T_i, I, u_0, g, R, L, \rho, C_p, k)
\]

It leads to the dimensionless ratios which govern the ohmic process:

\[
\left( \frac{T_o}{T_i}, \frac{I^2}{R \cdot \rho \cdot u_0 \cdot \sigma_0 (1 + kT)}, \frac{u_0^2}{g \cdot R}, \frac{L}{R}, \frac{C_p \cdot T_i}{u_0^2}, \frac{kT_i}{u_0^2}, \frac{kT_i}{R} \right) \quad (7)
\]

Note that for the kind of fluid tested, the pi –set is enlarged by one pi-number, \( \pi_6 \) compared with material with constant properties.

**5 Analytical process relationship of a jet ohmic heater**

Applying the thermal balance over a fraction of fluid jet of length \( dx \) and of section  \( A(x) \), and integrating between the entrance and the exit, Ghnimi et al (2009) have obtained and validated an analytical model between dimensional parameters predicting the temperature at the exit of the fluid jet [8]:

\[
T_o = \left[ 1 + 2k \left( \frac{kT_i^2}{2} + T_i + \left( \frac{I^2 u_0}{3g \pi^2 R^2 \sigma_0 C_p} \right) \times \left( \left( 1 + \frac{2g}{u_0^2} \frac{L}{R} \right)^{3/2} - 1 \right) \right) \right]^{1/2} - 1 \quad (8)
\]
It can be observed that Equation (8) can be rewritten with the dimensionless ratios obtained 
\((\pi_1, \pi_2, \pi_3, \pi_4, \pi_5, \pi_6)\). Indeed,

\[
\frac{T_O}{T_i} = \frac{1}{\pi_6} \left[ 1 + \frac{2}{\pi_6} \left( 1 + \frac{\pi_2 (1 + \pi_6)}{3 \cdot \pi_2 \cdot \pi_5} \right) \times \left( 1 + \frac{2}{\pi_4} \right)^{3/2} - 1 \right]^{1/2}
\]

(9)

This analytical process relationship ascertains the fact that 5 dimensionless ratios 
\((\pi_2, \pi_3, \pi_4, \pi_5, \pi_6)\) govern this ohmic processes and justify the introduction of additional parameter in the relevant list to take into account the electrical conductivity dependence in the with temperature course of the process proposed. It is clear that this should be not forgotten to design ohmic processes.

6 Conclusion

In this work, dimensional modelling of the outlet temperature induced by an ohmic jet heater for food treatment has been developed. In particular, the methods to integrate the variability of physical properties in dimensional analysis are recap. One of the aims of this paper is to develop the interest of chemical engineer and scientist to take into account these aspects in their dimensional modelling. It is shown that the pi-space, in which the process in question is presented, is extended by at least one dimensionless ratio. Such data would be very useful to identify both material and process parameters which govern food transformation in ohmic heater and better integrate the complexity of material.

Acknowledgements

The authors would like to thank the "Agence de l’Environnement et de la Maîtrise de l’Energie" (Angers, France) for providing financial support.

Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>A, B</td>
<td>Independent constant</td>
</tr>
<tr>
<td>A(x)</td>
<td>cross-sectional area of the jet, m²</td>
</tr>
<tr>
<td>C</td>
<td>Independent constant</td>
</tr>
<tr>
<td>C_p</td>
<td>specific heat capacity, J.kg.°C⁻¹</td>
</tr>
<tr>
<td>E</td>
<td>Electric field, V.m⁻¹</td>
</tr>
<tr>
<td>g</td>
<td>standard gravity, m.s⁻²</td>
</tr>
<tr>
<td>h</td>
<td>fluid level, m</td>
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<tr>
<td>I</td>
<td>electrical current, A</td>
</tr>
<tr>
<td>k</td>
<td>temperature factor, °C⁻¹</td>
</tr>
<tr>
<td>L</td>
<td>length of the jet, m</td>
</tr>
<tr>
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<td>radius of the nozzle, m</td>
</tr>
<tr>
<td>p₀</td>
<td>reference point</td>
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<tr>
<td>q</td>
<td>volumetric flowrate, m³.s⁻¹</td>
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<td>R</td>
<td>radius of the nozzle, m</td>
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<td>function</td>
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<tr>
<td>T</td>
<td>temperature, °C</td>
</tr>
<tr>
<td>T_i</td>
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<td>V</td>
<td>electrical potential, V</td>
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<td>u₀</td>
<td>exit jet velocity, m s⁻¹</td>
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Greek letters

<table>
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<tr>
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<th>Description</th>
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<tr>
<td>γ₀</td>
<td>transformation parameter</td>
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<tr>
<td>μ</td>
<td>dynamic viscosity, Pa.s</td>
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<tr>
<td>ρ</td>
<td>mass density, kg.m⁻³</td>
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<tr>
<td>σ</td>
<td>specific electrical conductivity, S.m⁻¹</td>
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References


APPLICATION OF HIGH VOLTAGE ELECTRICAL DISCHARGES AT SEMI-PILOT SCALE: EXTRATION OF POLYPHENOLS FROM GRAPE POMACE

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Abstract:
Grape pomace is winemaking byproducts that still contain valuable compounds like polyphenols. Recently, high voltage electrical discharges (HVED) have been used for extraction of soluble compounds from bioproducts. During the application of HVED, the production of bubbles and pressure waves are responsible for the fragmentation of cells and consequent removal of intracellular compounds. The aim of this study was to intensify polyphenols extraction from grape pomace by HVED at semi-pilot scale. Electrical treatment of grape pomace by HVED was performed in laboratory and semi-pilot treatment chambers of 1L and 35L respectively. A high voltage generator provided 40kV-10kA discharges. Grape pomace was firstly treated by HVED in water and then introduced in a diffusion cell under agitation. Experiments showed the intensification of HVED on polyphenols extraction from grape pomace at laboratory and semi-pilot scales. The scale extrapolation study required to maintain constant the ratio of energy to product mass (0.53 kJ/kg/pulse). A treatment of 53 kJ/kg at the laboratory scale gives similar results as a treatment of 160 kJ/kg at the semi-pilot scale. In these conditions, the content of polyphenols in extracts is approximately 200 mg/L and the antioxidant activity is almost 90 mg TEAC/L.

Key words: polyphenols, intensification, extrapolation, antioxidant activity, energy

1 Introduction

As an alternative method, electrical treatments that provoke electroporation and biological tissue damage [1] have been proposed for compounds extraction. Additionally to their use for
degradation of organic compounds contained in water [2] or for inactivation of microorganisms [3], high voltage electrical discharges (HVED) were also applied for enhanced aqueous extraction of soluble material. This technology was applied to vegetative raw material [4], linseed [5] and green tea [6]. The HVED is a technique that injects energy directly into an aqueous solution through a plasma channel formed by a high current \((10^3-10^4 \text{ A})/\text{high voltage } (10^3-10^4 \text{ V})\) electrical discharge between two submerged electrodes. The discharge phenomenon is accompanied by the formation of gas bubbles [7] and high-amplitude pressure shock waves (up to 1000 MPa) that are responsible for fragmentation of particles. Thus, this technology allows the recovery of intracellular compounds.

Grape pomace is the main by-product generated during winemaking. It is composed of stems, seeds and skins. The valorisation of this food by-product can be useful for diminishing its environmental impact. Grape pomace contains a high level of valuable compounds like polyphenols not extracted during wine processing [8].

The aim of this study was to intensify polyphenols extraction from grape pomace by HVED at semi-pilot scale. Thus, experiments at laboratory and semi-pilot scales were compared for different applied pulse energies.

2 Materials and methods

2.1 Biological material

Industrial grape pomace (skins, seeds and stems) from red grapes (Vitis vinifera L., cultivar “Pinot Meunier”, vintage 2008) from Epernay (France) was obtained as the residue of pressed grapes. The dry matter content in the grape pomace was 30.0 ± 0.1 wt %.

2.2 HVED experiments

For experiments at the laboratory scale, the experimental apparatus of Tomsk Polytechnic University (Russia) was used. It consisted of a pulsed high voltage power supply (200 nF) and a laboratory one-liter treatment chamber. The treatment chamber contained two electrodes: a stainless steel needle 10 mm in diameter and a stainless disk grounded electrode 35 mm in diameter (Fig. 1). A positive pulse voltage was applied to the needle electrode. The high voltage pulse generator provided \(40kV-10kA\) discharges during few microseconds.

For experiments at semi-pilot scale, the high voltage generator (Pau University, France) was composed of a capacitor (4.4 µF) that discharged towards a triggered air spark gap (Fig. 1). The capacitor was charged with a maximum voltage of 40 kV. The 35 L treatment chamber consisted of a needle electrode of 10 mm in diameter and a grounded electrode of 120 mm in diameter.

Fig. 1: HVED treatment apparatus at laboratory scale (left) and semi-pilot scale (right).
In both cases, the electrodes geometry was the same. The inter-electrode space was 5 mm. The pulse repetition rate was fixed at 0.5 Hz. The laboratory equipment provided electrical pulses of 160 J. The semi-pilot generator could supply a discharge pulse of 4000 J.

2.3 Extraction experiments

The treatment chamber was initially filled with grape pomace which was further on mixed with distilled water (the liquid-solid ratio, w/w, was fixed at the level of 5) at 20°C. The total product masses used for laboratory and semi-pilot experiments were respectively 0.3 kg and 7.5 kg. Electrical discharges (up to 1000 pulses with a pulse energy of 160 J or 4000 J) were applied to the mixture of grape pomace and water. Then diffusion was studied in a cylindrical cell under agitation (160 rpm). In order to avoid any evaporation and degradation of polyphenols under the impact of air or light, the diffusion cell was closed and covered by aluminium foil during the extraction process. For control experiments, the extraction was realized without electrical treatment. The concentration of total polyphenols was controlled every 5-10 min during the diffusion process.

2.4 Quantitative and functional analysis

For determination of the concentration of total polyphenols, the Folin-Ciocalteu method described elsewhere [9] was used with some modifications. Briefly, 0.2 mL of diluted samples was mixed with 1 mL of Folin-Ciocalteu reagent diluted ten times in water and 0.8 mL of sodium carbonate (175 g/L). The mixture was then incubated for 2 h at room temperature and the absorbance of the extracts was measured at 750 nm by using a spectrophotometer UV-VIS (Secomam, Shimadzu, France). Gallic acid (Sigma-Aldrich, France) was used as standard for the calibration curve. Results were expressed as milligram of gallic acid equivalent (GAE) per liter of solution.

For antioxidant activity analysis, the TEAC method was used. The assay is based on the decolorisation of the radical cation \(2,2'\)-azino-bis(3-ethylbenzothiazoline-6-sulfonate) \([ABTS^{+}\)] after reduction to ABTS. Spectrophotometric analyses were performed as published elsewhere [10]. Aqueous solutions of trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) in a range of 40-800 µmol/L were used for calibration of the TEAC assay. The values were expressed as mg of Trolox equivalent antioxidant capacity per liter of solution (mg TEAC/L).

3 Results and discussion

3.1 Comparative study of extraction efficiency at the laboratory and semi-pilot scales

The scaling up was performed by maintaining constant the ratio of energy per pulse to product mass. In our case, it is equal to 0.53 kJ/kg/pulse. Thus, the treatment of a total product mass of 7.5 kg by electric discharges with a pulse energy of 4000 J is compared to the treatment of a product of 0.3 kg with a pulse energy of 160 J. The extraction efficiency in terms of total polyphenols content and antioxidant activity at the laboratory (0.3 kg of product) and semi-pilot scales (7.5 kg of product) are described in Fig. 2. The experiments were performed with the same treatment energy for 100 pulses (53 kJ/kg).
The application of electrical discharges on a higher amount of product mass (from 0.3 kg to 7.5 kg) with the same treatment energy seems to be less effective. Concerning the extraction of polyphenols, the content is more than four times smaller at the semi-pilot scale. The antioxidant activity of extracts was also smaller compared to that obtained at the laboratory scale (∼ 30 mg TEAC/L). As a result, maintaining constant the ratio of energy to product mass is not enough to obtain similar results for laboratory and semi-pilot experiments.

When changing the treatment chamber size, a detailed study on the cell dimensions and cell shape is required. For example, the ratio of the cell diameter to the cell height varies in our case. It may have an impact on the distribution of the pressure waves through the cell and the product. The product located at the extreme parts of the cell may not be well treated in the case of too broad cells. The application of HVED results on shock waves production with elevated pressures (up to 1000 MPa). These pressure waves are responsible for the turbulence and agitation of the liquid inside the treatment cell. However, the propagation of these pressure waves and their effects can be decreased in treatment cells with large diameter. Consequently, the required energy for an effective effect of HVED seems to be dependent on the treatment cell geometry. In our case, a higher energy of treatment is required.

3.2 Effect of treatment energy on extraction efficiency at the semi-pilot scale

3.2.1 Impact of HVED on pH and electrical conductivities

The pH and electrical conductivities of extracts treated by electrical discharges were measured (Fig. 3a). The acidic pH of the extracts is due to the extraction of acidic compounds (tartaric acid, malic acid and citric acid) that composed grape pomace. There is no significant variation of the pH after application of HVED at both laboratory and semi-pilot scales (from 4.0 ± 0.1 to 3.8 ± 0.1).

On the other hand, the electrical conductivity of HVED treated samples (Fig. 3b) is increased by a factor of 1.6. This result reflects the release of intracellular components [4]. Note that the electrical conductivity of extracts from a treatment of 533 kJ/kg at the semi-pilot scale is similar to that of extracts from a treatment of 53 kJ/kg at the laboratory scale.

3.2.2 Impact of HVED on polyphenols content and antioxidant activity

The effects of electric discharges with higher treatment energies on polyphenols content and antioxidant activity are described on Fig. 4. The experiments were performed at the semi-pilot scale by increasing the number of pulses.

The values of antioxidant activity and polyphenols content increase linearly with higher pulses number. The highest results are obtained for 1000 pulses (533 kJ/kg); the polyphenols
content is 350 mg GAE/L and the antioxidant activity reaches 150 mg TEAC/L. We can note that the temperature increase after the application of 1000 pulses was only 9°C. The application of a treatment of 160 kJ/kg at the semi-pilot scale gives similar results as a treatment of 53 kJ/kg at the laboratory scale (Fig. 2 and 4a).

Fig. 4: Effects of the treatment energy on the contents of total polyphenols (a) and antioxidant activity (b) at the semi-pilot scale.

The HVED treatment leads to tissue fragmentation and consequent increase of transfer surface. HVED have already been applied on dehydrated vegetable products (tea, soybeans, pea) after their rehydration and also observed an increased in 40-50 times aqueous extraction kinetics [4]. Electrical discharges have also given good results for the extraction of total solutes and total polyphenols from grape skins [11].

The extraction kinetics during diffusion after HVED application were also studied at the semi-pilot scale (Fig. 5). The evolution of polyphenols content is compared for different applied treatment energies.

For smaller treatment energies, the diffusion process improves polyphenols extraction. For a higher energy input (160 kJ/kg), the content of polyphenols remains quite constant during diffusion. Indeed, the application of HVED results on the fragmentation of particles. This phenomenon is more intense when the treatment energy is increased. Consequently, for a treatment of 160 kJ/kg, a large amount of polyphenols are extracted by the treatment and the diffusion process does not improve more the extraction kinetic. The application of HVED treatment of 160 kJ/kg increases the extraction of total polyphenols four times after only 25 min of extraction compared to the control experiment (diffusion process). Consequently, the HVED would be useful for diminishing the treatment time and increasing the extraction rate.

Fig. 5: Extraction kinetics of total polyphenols during diffusion after HVED application (53 kJ/kg or 160 kJ/kg) at the semi-pilot scale.
4 Conclusions

The application of HVED at both laboratory and semi-pilot scales is efficient for the intensification of polyphenols extraction from grape pomace. The electrical treatment allows accelerating the extraction kinetics of polyphenols.

However, maintaining constant the ratio of energy to product mass (in our case, it was equal to 0.53 kJ/kg/pulse) was not enough to obtain similar results for laboratory and semi-pilot experiments. Other parameters should be taken into account (treatment chamber design, pressure wave distribution, ...). A treatment of 53 kJ/kg at the laboratory scale seems to give similar results as a treatment of 160 kJ/kg at the semi-pilot scale.

Acknowledgment

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PULSED ELECTRIC FIELD ASSISTED PRESSING OF APPLE MASH ON A CONTINUOUS PILOT SCALE PLANT: EXTRACTION YIELD AND QUALITATIVE CHARACTERISTICS OF CIDER JUICE

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Abstract:
A PEF pre-treatment system was placed upriver to a belt press on a first attempt to couple electric treatment to a continuous pressing system. In this paper, the effect of PEF pretreatment on juice yield and qualitative attributes of apple cider juice will be presented for a pilot plant scale. Apple cider variety Bedan was crushed into two types of mash (small and coarse sized). Apple mash was pumped in a continuous PEF cell at 300 kg/h and treated at 1000 V/cm. It was then pressed in a continuous belt press. The juice was separated into two types of juices. Juice 1 was collected under the first roller and juice 2 from the three other rollers. Juice yield increased by 5 % when coarse mash was treated by PEF. Electric treatment had different effects depending on the position of the pressing rollers. Phloridzin concentration increased by 37.5% and 22.5% from the recovered juices beneath the last three rollers for small and treated coarse mashes respectively. But PEF may contribute to the increase of polyphenoloxidase activity leading to a decrease of Hydroxycinnamic acid compounds such as 5-CQA and monomers (CAT, ECAT) of Flavan-3-ol family which generates oxidation compounds responsible of the yellow–orange colour is beneficial from the point of view of cider quality.

Key words: Pulsed Electric Field (PEF), Apple, Polyphenoloxidase (PP0), Belt press

1 Introduction

Pulsed Electric Field (PEF) is an innovative technology that has gained increasing interest in the recent years for several applications in food processing. It is considered as a nonthermal food preservation method, to produce shelf-extended life juices with high sensorial quality [1, 2]. PEF pasteurization of apple juice has proved to be efficient in microbial inactivation and it retained most of the phenolic compounds responsible for color and flavor of the apple juice [3, 4]. On a laboratory scale, PEF application was suggested to be a promising alternative for enhancing juice release and solute extraction by pressing[5, 6], maceration [7] or diffusion [8].

A common will in the scientific community is to introduce this technology on a higher scale due to its benefits. In order to extend our knowledge to pilot plant scale processing, several researches were initiated to study the quality attributes of processed products such as flavor, color and shelf life of PEF-processed tomato and orange juice [9]. This technology has proved its efficiency at this scale and has found its way on the American market where PEF treated fruit juices is commercialized by Genesis society. Another research studied the effect of PEF treatment of apple mash on the polyphenolic content of its corresponding juices in a pilot scale batch hydraulic horizontal filter press [10]. Contrarily to previous results on
laboratory scale [11], this study showed that PEF treatment to apple mash improved the extraction of polyphenolic content in apple juice.

The influence of polyphenols on the organoleptic properties of juices and fermented beverages has been widely reported: thus, polyphenols contribute to the color, bitterness and astringency of ciders [12]. French cider is a slightly alcoholic, fizzy, and sweet drink obtained by slow and partial fermentation of the juice of specific apple varieties [12]. Several references allude to the fact that cider colors are mainly due to the oxidation products of polyphenols by polyphenoloxidase (PPO). First, PPO oxidizes o-diphenols to o-quinones that cause a series of coupled redox reactions with other polyphenols, the end products of which are colored pigments (color range from yellow to brown). At this point, it is interesting to note that apple polyphenols are all colorless, except for flavonols that are yellow. Mainly, hydroxycinnamic acids (5-caffeoylquinic acid) and catechins are the compounds involved in enzymatic oxidations by PPO [13].

The aim of this study was to assess the first attempt to combine the electric processing of the mash and a continuous pressing system. In this paper, the effect of PEF pretreatment of two types of apple mash on juice yield and qualitative attributes of apple cider juice will be presented for a pilot plant scale.

2 Material and Methods

2.1 Mechanical expression of juice

The experimental scheme is presented in figure 1. A lot of 300 kg of apple of Bedan variety was washed and pumped into a mill. Two sized mashes (small and coarse) were prepared. Ascorbic acid (1 g/kg of mash) was injected continuously and the mash was collected in an inerted reception tank to prevent oxidation. Mash was pumped at the rate of 300 kg/h in a continuous flow PEF system. The treatment chamber had a collinear design with a height and diameter of 50 mm. Two treatment chambers were placed serially in order to deliver enough treatment. The PEF generator delivered a maximum of 5 kV – 1000 A (Hazemeyer, Saint Quentin, France). A maximum of 1 kV/cm field intensity was applied considering the configuration of the treatment chamber. Monopolar pulses of near rectangular shape were delivered at 200 Hz and pulse duration of 91 µs. The total energy input was 57 kJ/kg. Temperature was controlled after the electrical treatment by a thermocouple. Although the input energy was high, temperature did not increase more than 5°C which was not a significant variation. Pulse protocols (pulse duration and frequency, input voltage) and all the output data (current, voltage, temperature) were collected using a data logger and software written by Service Electronique UTC, France. Apple mash was then pressed in a continuous simple belt press (Voran EBP500, Germany). The pressure applied to the belt was 3 bars. The juice was separated into two categories. Juice 1 was collected under the first roller and juice 2 from the three other rollers. Juices 1 and 2 were then collected in different inerted tanks. Juice yield was calculated according to equation 1.

\[
\text{Juice yield } (\%) = \frac{M_{\text{juice 1}} + M_{\text{juice 2}}}{M_{\text{juice 1}} + M_{\text{juice 2}} + M_{\text{pomace}}} \tag{1}
\]

M is the mass of juice 1, 2 and pomace respectively.

2.2 HPLC analysis of polyphenols

To prevent oxidation, the samples (0.5 mL) of juice 1 and 2 were mixed with sodium fluoride (0.5 mg) and then they were freeze-dried. The pellets were extracted by acidified methanol (1.2 mL) and the raw extract was analyzed by HPLC. Other freeze dried samples of
juice 1 and 2 were directly submitted to the thioacidolysis reaction in methanol in order to quantify procyanidins. The aDP of flavan-3-ols was calculated as the molar ratio of all of the flavan-3-ol units (thioether adducts plus terminal units) to (−)-epicatechin and (±)-catechin, corresponding to terminal units [14].

2.3 Statistical analysis

Three factors were studied. The factor “A” was the size of the mash, the factor “B” was the electric field intensity and the factor “C” was the type of juice. An analysis of the variance (P=0.05; n=2) at three levels was performed for each polyphenolic compound and juice yield. A Least Significance Differences (LSD) test (P = 0.05) was applied for mean discrimination between different modalities.

3 Results and discussion

3.1 Juice yield

Figure 2A present the final yield of juice 1 collected under the first roller and figure 2B for the yield of juice 2 recovered from the three other rollers (see figure 1). The yield was studied for the two sizes of mash. Each one was treated by PEF (1 kV/cm) and compared to its control (0 kV/cm).

The yield of juice 1 and 2 was increased after PEF treatment of coarse size mash leading to a total enhancement of 5 %. PEF treatment of small size mash did not result in a significant increase of juice 1 yield but was more pronounced for juice 2 yield. Total juice yield of coarse size treated mash was similar to the control small size mash.

Fig. 1: Processing scheme of continuous pilot scale apple juice production with PEF treatment.

Fig. 2: Final yield of juices collected beneath the first roller (juice 1, A) and the three next rollers (juice 2, B). Different modalities were presented for mash size (small and coarse) and electric treatment (0 and 1000 V/cm)
3.2 Polyphenol content

“Mash type” (A), “electric treatment” (B) and “juice type” (C) factors were chosen to evaluate their influence on the phenolic composition of cider juice. Polyphenol content of each modality was analyzed by HPLC (Tab. 1). Hydroxycinnamates, flavan-3-ols and dihydrochalcones families were abundantly present. Flavonols, the 4th family, was present at trace.

The analysis of variance at P<0.05 was carried out for the three independent factors (A, B and C) and their interactions (AB, AC, BC and ABC). Insignificant effect was found for the mash factor between small and coarse size where P value ranged between 0.056 and 0.92 (0.01<F ratio<11.35). Electric treatment factor showed a significant effect between treated and untreated mash for (+)-catechin [CAT] (P = 0.032, F = 5.85), (-)-epicatechin [ECAT] (P = 0.026, F = 6.34), Procyanidin B1 [B1] (P = 0.015, F = 7.96), Procyanidin B2 [B2] (P = 0.006, F = 11.07) of the flavan-3-ol family and phloridzin [PLZ] (P = 0.022, F = 6.91) of the dihydrochalcone family.

A significant difference was noticed between juice 1 and 2. Almost all phenolic compounds were concerned except p-Coumaroylquinic acid [PCQ] (P = 0.22, F = 1.66) and PLZ (P = 0.83, F = 0.05).

The only significant interaction occurred between electric treatment and juice type (BC). PCQ, once again, was not concerned with this difference (P = 0.27, F = 1.31). Additionally, CAT (P = 0.099, F = 3.19), B1 (P = 0.12, F = 2.79) and procyanidin [PCD] (P = 0.19, F = 1.91) were not related as well with this difference. Finally, phloretin xyloglucoside [XPLT] concentration (P = 0.054, F = 4.64) did not significantly differ between the different modalities of electric treatment and juice type factors.

This section attempts to show at which level of juice pressing in the belt press, pulsed electric field had a significant effect. PEF reduced significantly the concentration of EC and B1 when small and coarse mashes were pressed on the first roller (juice 1). The same tendency was observed for 5-CQA, CAT and B1 in the case of coarse mash only. Unlike the first observation, most of the phenolic compounds did not show any concentration difference when treated small and coarse mashes were pressed on the next rollers (juice 2) comparing to their respective controls except for one compound. In deed, Phloridzin [PLZ] concentration increased by 37.5% and 22.5% for small and coarse mashes respectively in the case of juice 2.

The decrease in native polyphenol compounds could be related to polyphenoloxidase (PPO) activity increased by the electric treatment of small and coarse mash comparing to their controls. First, PPO oxidises o-diphenols to o-quinones that cause a series of coupled oxidoreduction reactions with other polyphenols. Mainly, hydroxycinnamates (5-CQA) and catechins (CAT, ECAT) are the compounds involved in enzymatic oxidations by PPO [15]. Their o-quinones, very unstable [16], take part in coupled oxidation reaction with flavan-3-ols [17], dihydrochalcones [18]. The p-coumaroylquinic acid is not a substrate of PPO and may be an inhibitor of PPO activity [19]. This could explain the fact that the concentration of this compound was not affected by the electric treatment in juice 1 and 2. It is established that PLZ is a bad substrate for the PPO activity due to its low catalytic power [20]. This fact could lead to an increase of its concentration in juice 2 after PEF treatment of small and coarse mash. The oxidation compounds are mostly responsible of the yellow-- orange color of the products. This type of colors is beneficial from the point of view of cider quality.

4 Conclusion

Pulsed electric field treatment of apple mash of coarse size at 1000 V/cm increased juice yield by 5 % on one hand. On the other hand, it was shown that PEF had a direct effect on the increase of phloridzin concentration in juice 2 when mash passed on the last three rollers. But PEF may contribute to the increase of polyphenoloxidase activity leading to a decrease of hydroxycinnamic acid compounds such as 5-CQA and monomers (CAT, ECAT) of flavan-3-ol family.
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Table 1: Effect of mash size (small and coarse) and pulsed electric field treatment (0 and 1000 V/cm) on the concentration of polyphenolic compounds in apple slices and raw juices collected under the first roller (juice 1) and the three next rollers (juice 2) on the single belt press.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Apple**(&lt;sup&gt;0.5&lt;/sup&gt;) (mg/kg)</th>
<th>Juice 1</th>
<th>Juice 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small size mash</td>
<td>Coarse size mash</td>
<td>Small size mash</td>
</tr>
<tr>
<td></td>
<td>0 V/cm</td>
<td>1000 V/cm</td>
<td>0 V/cm</td>
</tr>
<tr>
<td>5-CQA</td>
<td>918 ± 18</td>
<td>621±59 (dc)</td>
<td>511±59 (ad)</td>
</tr>
<tr>
<td>pCQ</td>
<td>192 ± 3</td>
<td>147±32 (ed)</td>
<td>134±32 (ad)</td>
</tr>
<tr>
<td>Total HCA</td>
<td>1110 ± 21</td>
<td>769±59 (d)</td>
<td>614±32 (e)</td>
</tr>
<tr>
<td>CAT</td>
<td>272 ± 12</td>
<td>88±15 (cb)</td>
<td>69±15 (dc)</td>
</tr>
<tr>
<td>EC</td>
<td>540 ± 5</td>
<td>174±21 (c)</td>
<td>126±21 (d)</td>
</tr>
<tr>
<td>B1</td>
<td>206 ± 9</td>
<td>48±12 (cb)</td>
<td>34±12 (edc)</td>
</tr>
<tr>
<td>B2</td>
<td>397 ± 19</td>
<td>115±16 (b)</td>
<td>80±16 (c)</td>
</tr>
<tr>
<td>PCD</td>
<td>2332 ± 135</td>
<td>369±33 (d)</td>
<td>367±33 (d)</td>
</tr>
<tr>
<td>aDP</td>
<td>2.7 ± 0.1</td>
<td>2.0±0.1 (e)</td>
<td>2.1±0.1 (e)</td>
</tr>
<tr>
<td>Total Flavan-3-ol</td>
<td>3746 ± 108</td>
<td>794±69 (ba)</td>
<td>675±69 (c)</td>
</tr>
<tr>
<td>PLZ</td>
<td>58 ± 5</td>
<td>14±2 (ed)</td>
<td>15±2 (ed)</td>
</tr>
<tr>
<td>XPLT</td>
<td>27 ± 18</td>
<td>13±2 (dc)</td>
<td>12±2 (dc)</td>
</tr>
<tr>
<td>Total DHC</td>
<td>85 ± 19</td>
<td>28±5 (ed)</td>
<td>26±5 (ed)</td>
</tr>
<tr>
<td>Total</td>
<td>4987 ± 103</td>
<td>1590±124 (ba)</td>
<td>1347±124 (cb)</td>
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</tbody>
</table>

5CQA: 5-Caffeoylquinic acid; PCQ: p-Coumaroylquinic acid; HCA: hydroxycinnamic acids; CAT: (+)-catechin; ECAT: (-)-epicatechin; B1: Procyanidin B1; B2: Procyanidin B2; PCD: Procyanidins; aDP: Flavan-3-ol averaged degree of polymerization; PLZ: Phloridzin; XPLT: Phloretin xyloglucoside; DHC: Dihydrochalcone; “a” to “e”: different indices in one row indicate significant differences (LSD, P<0.05), CI: Confidence Intervals at 95%.
PROCESSING OF PEACH NECTAR BY PULSED ELECTRIC FIELDS WITH RESPECT TO PHYSICAL AND CHEMICAL PROPERTIES AND MICROBIAL INACTIVATION

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Abstract:  
Applicability of pulsed electric fields (PEF) to process peach nectar as a function of electric field strength with respect to microbial inactivation and measurement of physical and chemical properties of peach nectar was carried out in this study. No significant difference was detected on pH, °Brix, titration acidity (% TA), conductivity, color (L*, a* and b*), nonenzymatic browning index (NBI), metal ion concentration, ascorbic acid content and beta carotene concentration between control and PEF-treated peach nectar samples (p>0.05). On the other hand inactivation of Escherichia coli O157:H7, Staphylococcus aureus, Listeria monocytogenes, Erwinia carotovora, Pseudomonas syringae subs. syringae, Botrytis cinerea and Penicillum expansum was significant (p≤0.05). It was revealed that PEF can successfully be applied to peach nectar with significant amount of microbial inactivation without significantly affecting physical and chemical properties.

Key words: ascorbic acid retention, beta carotene retention, microbial inactivation, peach nectar, pulsed electric fields (PEF)

1 Introduction

Processing of fruit juices due to their high acidity and low viscosity in order to inactivate spoilage and foodborne bacteria as well as pathogenic fungi and preservation of physical, nutritional and sensory properties by pulsed electric fields (PEF) has increased recently. However, PEF processing of fruit juices mostly includes juices such as apple, orange, cranberry, grape, strawberry and tomato [1-5] and not enough information was reported in the literature regarding of processing of peach nectar like products by PEF. Therefore, this study was designed to process peach nectar by PEF as a function of electric field strength and treatment time for the determination of the changes in pH, °Brix, titration acidity (%TA), conductivity, color (L*, a* and b*), nonenzymatic browning index (NBI), and metal ion, ascorbic acid and total beta carotene concentrations of PEF-processed peach nectar and for the inactivation of Escherichia coli O157:H7, Staphylococcus aureus, Listeria monocytogenes, Erwinia carotovora, Pseudomonas syringae subs. syringae, P. expansum and B. cinerea inoculated into peach nectar.
2 Materials and Methods

2.1 Food samples
Prepared peach nectar concentrates aseptically opened and the °Brix of the nectar was adjusted to 10.0 and processed by PEF immediately.

2.2 Test microorganisms and enumeration
Escherichia coli O157:H7 (EDL 931 04054), Staphylococcus aureus (95047), Listeria monocytogenes (Type I 04077), Erwinia carotovora, Pseudomonas syringae subs. syringae, Penicillium expansum and Botrytis cinerea were inoculated into peach nectar at the level of $10^6-10^7$ cfu/mL separately. Both control and PEF-treated peach nectar samples were diluted with 0.1% peptone (Fluka, Germany) water and 100 µL of appropriate dilutions were plated.

2.3 Pulsed electric field processing
OSU-4A bench scale continuous PEF system (The Ohio State University, Columbus, OH, USA) equipped with six treatment chambers having 0.29 cm diameter and 0.23 cm gap distance was used. OSU-4A bench scale PEF generator provided square wave bipolar pulses. For processing of peach nectar 0 (control), 17, 20, 23, 27, and 30 kV/cm electric fields strengths were applied with 50 mL/min of flow rate, 3 µs of pulse duration, 500 pps of frequency, and 131 µs of treatment time.

2.4 Measurement of physical properties
pH, titratable acidity (%TA), °Brix, conductivity color (L*, a* and b*) and nonenzymatic browning index (NBI) of peach nectar were measured. Ascorbic acid content [6], beta carotene analysis [7], and metal ion concentration of Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sb, Se, Sr, and Zn [8] were measured.

2.5 Data Analyses
Data were analyzed by Minitab (13.2 version, Minitab Inc., State College PA, USA) by using one-way or two-way ANOVA at 95% confidence interval. Differences between electric field strength and treatment time were determined by Tukey’s multiple comparison test. Every experiment was repeated at least three times.

3 Results and Discussion
Results revealed that pH, °Brix, TA, conductivity, NBI, color values of L*, a* and b*, ascorbic acid and beta carotene content of the control samples did not significantly affected by the application of electric field strength in the magnitude of 17, 20, 23, 27 and 30 kV/cm ($P>0.05$) (Table 1). The measured ion contents also did not significantly changed with increased electric field strength ($P>0.05$) (Table 2). Initial number of E. coli O157:H7, S. aureus, L. monocytogenes, P. syringae subs. syringae, E. carotovara, P. expansum and B. cinerea inoculated into peach nectar by PEF as a function of electric field strength ($n$:12).
was listed in following order; *B. cinerea* > *P. expansum* > *P. syringae* subs. syringae > *E. carotovara* > *E. coli* O157:H7 > *L. monocytogenes* > *S. aureus.*

<table>
<thead>
<tr>
<th>Measured attributes</th>
<th>Electric field strength (kV/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.90±0.00 a</td>
</tr>
<tr>
<td>°Brix</td>
<td>10.0±0.00 a</td>
</tr>
<tr>
<td>TA (%)</td>
<td>0.19±0.01 a</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>3.86±0.32 a</td>
</tr>
<tr>
<td>NBI*</td>
<td>0.53±0.10 a</td>
</tr>
<tr>
<td>Color (L*)</td>
<td>41.30±2.20 a</td>
</tr>
<tr>
<td>Color (a*)</td>
<td>4.94±0.19 a</td>
</tr>
<tr>
<td>Color (b*)</td>
<td>29.91±1.41 a</td>
</tr>
<tr>
<td>Ascorbic acid (mg/L)</td>
<td>4.46±0.87 a</td>
</tr>
<tr>
<td>Beta carotene (mg/L)</td>
<td>47.26±6.61 a</td>
</tr>
</tbody>
</table>

*Data in the same row with different superscript letter are significantly different (P≤0.05)*

Tab. 1: *Physical and Chemical Properties of Peach Nectar Processed by PEF as a Function of Electric Field Strength*

<table>
<thead>
<tr>
<th>Measured ions</th>
<th>Electric field strength (kV/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Al</td>
<td>1.39±0.1 a</td>
</tr>
<tr>
<td>Ca</td>
<td>64.56±3.6 a</td>
</tr>
<tr>
<td>Cd</td>
<td>0.00±0.0 a</td>
</tr>
<tr>
<td>Co</td>
<td>0.00±0.0 a</td>
</tr>
<tr>
<td>Cr</td>
<td>0.00±0.0 a</td>
</tr>
<tr>
<td>Cu</td>
<td>0.73±0.1 a</td>
</tr>
<tr>
<td>Fe</td>
<td>5.44±0.4 a</td>
</tr>
<tr>
<td>K</td>
<td>985.83±42.9 a</td>
</tr>
<tr>
<td>Mg</td>
<td>84.67±4.4 a</td>
</tr>
<tr>
<td>Mn</td>
<td>0.54±0.1 a</td>
</tr>
<tr>
<td>Na</td>
<td>24.47±5.4 a</td>
</tr>
<tr>
<td>Ni</td>
<td>0.01±0.0 a</td>
</tr>
<tr>
<td>Pb</td>
<td>0.00±0.0 a</td>
</tr>
<tr>
<td>Sb</td>
<td>0.00±0.0 a</td>
</tr>
<tr>
<td>Se</td>
<td>0.00±0.0 a</td>
</tr>
<tr>
<td>Sr</td>
<td>0.24±0.1 a</td>
</tr>
<tr>
<td>Zn</td>
<td>0.79±0.2 a</td>
</tr>
</tbody>
</table>

*Data in the same row with different superscript letter are significantly different (P≤0.05)*

Tab. 2: *Measurement of metal ion concentration of peach nectar processed by pulsed electric fields as a function of electric field strength*
Apparently, inactivation obtained in fungi was higher than that of bacteria, and inactivation of gram negative bacteria was higher than that of gram positive bacteria (Fig. 1). Results obtained in the study were similar to that of previous studies that PEF treatment did not cause significant change in the physical properties of peach nectar with significant inactivation of inoculated microorganisms [9-11].

Peach nectar although considered in fruit juice category has different physical properties than that of juices. Peach nectar, since it is made from the puree, has pulp particles which make it very thick. Therefore, the viscosity of the peach nectar is higher than that of fruit juices which makes it harder to process by PEF. However, in this study, peach nectar was successfully processed by PEF up to 30 kV/cm electric field strength with significant amount of microbial inactivation without significantly affecting important physical and chemical characteristics. In order to understand the effect of PEF on sensory characteristics and shelf life of PEF-processed peach nectar further studies need to be designed.

Acknowledgements

We would like to thank The Scientific and Technological Research Council of Turkey (TUBİTAK) for supporting TUBITAK KARIYER (project number 104O585) project and Dimes Gıda San ve Tic Ltd Şti (Tokat, Turkey) for providing fresh peach nectar.

References

INFLUENCE OF PULSED ELECTRIC FIELD, HEATING AND THEIR COMBINATION ON CELL DISRUPTION AND SOLUBLE MATTER DIFFUSION FROM CHICORY TISSUE

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Abstract:
Nowadays inulin is becoming the important ingredient in the food industry that allow producing the high quality and well-tasting products. Thanks to its gelling properties, it is able to replace up to 100% of fat in dairy products, meat products, sauces and...
Inulin can be also used as a dietary fiber or sugar replacer in tablets [4], [10], [5].

The thermally accelerated extraction of soluble solids is an important step in industrial processing of chicory roots and this process is very similar to that used for sugar production from sugar beet [1]. Recently it was demonstrated that pulsed electric field (PEF) assisted processing could be rather effective tool for acceleration of extraction from sugar beet [3], [6], [11], [8], [12].

This work was aimed at investigation of thermal and PEF treatments on efficiency of soluble solids extraction from chicory roots. The effects of temperature, PEF electric field strength and time of treatments on tissue damage degree and acceleration of kinetics of soluble matter extraction is discussed.

2 Materials and methods

2.1 PEF and thermal treatment experiments

A cell used in PEF and thermal treatment experiments consisted of a polypropylene cylindrical glass with the inner diameter of 29 mm and an electrode at its bottom. The treatment cell, filled with fresh chicory juice, was placed in the water thermostat kept at the desired temperature. The sample was placed inside the cell, then the second electrode was installed on the top of the sample and the electric field was applied straightforwardly to the tissue sample.

The PEF treatment was applied to the sample at different electric field strengths $E$ and different fixed temperatures $T$. Electric field treatment was applied using the PEF generator. The PEF generator provided the bipolar pulses of near-rectangular shape.

All the output data (current, voltage, electrical conductivity, and temperature) were collected using a data logger and special software, adapted by Service Electronique UTC, Compiegne, France. All the experiments were repeated at least three times. The Table Curve 2D (Jandel Scientific, San Rafael, CA) software was used to smooth data and to determine their standard deviations.

The tissue damage degree was estimated from the electrical conductivity disintegration index $Z$ [12]:

$$Z = \frac{\sigma - \sigma_i}{\sigma_d - \sigma_i}$$

where $\sigma$ is the measured electrical conductivity value and the subscripts $i$ and $d$ refer to the conductivities of untreated (initial) and completely damaged tissue, respectively.

The maximally damaged chicory tissues ($Z = 1$) were obtained by their PEF treatment at the electric field strength $E = 400$ V/cm and the total time of the PEF treatment $t_t = 0.3$ s.

2.2 Diffusion experiments

Diffusion cell was a cylindrical glass beaker supplied with magnetic stirrer and digital thermoregulation. The liquid/solid ratio always was 3/1. The untreated and PEF-pretreated samples with maximal disintegration index ($Z = 1$) were used.

Extraction kinetics of chicory water-soluble matter was monitored by solute concentration in the solution ($C \equiv ^\circ$Brix). Extraction kinetics was characterized by the normalized solute concentration (normalized $^\circ$Brix) in the chicory juice defined as

$$B = \frac{C - C_i}{C_f - C_i}$$

where $C_i$ and $C_f$ are the initial and the final soluble matter content, respectively.
To estimate effective diffusivities \( D \) in a chicory slices, an analytical solution of the diffusion equation for the well-stirred limited volume was used [2]:

\[
B = 1 - \sum_{n=1}^{\infty} \frac{2\alpha (1 + \alpha)}{1 + \alpha + \alpha^2 q_n^2} \exp \left( -\frac{Dq_n^2 t}{l^2} \right)
\]

where \( \alpha = V_w/V_s \) is a ratio of volumes of water and slices, \( 2l (\approx 2 \text{ mm}) \) is a thickness slices, \( t \) is a time, and \( q_n \)'s, are the non-zero positive roots of equation \( \tan q_n = -\alpha q_n \).

3 Results and Discussion

3.1 Damage of chicory tissue

The evolution of electrical disintegration index \( Z \) (estimated from eq. (1)) during the thermal treatment alone (at \( E = 0 \text{ V/cm} \)) at different temperatures was studied. The experimental data evidenced that thermal treatment was inefficient at temperatures below 40–45 °C, but it resulted in noticeable tissue damage at elevated temperatures (above 50 °C).

![Graph](image)

Fig. 1: The Arrhenius plots of thermal \( \tau_T \) and electrical \( \tau_E \) characteristic damage times

The time of thermal treatment required reaching high disintegration index \( Z \) noticeably decreases with temperature increasing. However, even at 60 °C and long time of treatment (\( t_T \approx 1 \text{ hour} \)) desintegration index \( Z \) is still smaller than maximum level and is of order 0.8–0.9.

PEF treatment also initiates damage of membranes, but it is ineffective for disruption of cell walls. The efficiency of release of the ionic components at electrically-assisted extraction depends, mainly, on electric field strength \( E \), time of PEF treatment \( t_{PEF} \), and thermal-electric synergism. The treatments show, that damage degree \( Z \) increased during the PEF treatment, and effect was more pronounced at higher electric field strengths.
For qualitative characterization of this effect the thermal ($\tau_T$) and electrical ($\tau_E$) characteristic damage times, were estimated as the treatment time required for $Z$ to attain one-half of its maximal value, i.e., $Z = 0.5$ (Lebovka et al., 2002). Dependencies of $\tau_E$ and $\tau_T$ versus the inverse temperature $1/T_a$ are presented in figure 1 in the Arrhenius form.

It is interesting that the activation energy $W_\tau$ was a decreasing function of applied electric field $E$ (insert in figure 1). Field dependence of activation energy reflects selectivity of PEF-induced changes accounting for the possible distribution of damage resistances and thermal activation energies of different cells.

Figure 2 presents the electric energy input $U$ versus electrical characteristic damage time $\tau_E$ at different electric field strengths $E$ and temperatures $T$.

![Fig. 2: Electrical energy input $U$ versus electrical characteristic damage time $\tau_E$ at different electric field strengths $E$ and temperatures $T$.](image)

Here the energy consumptions $U$ were estimated at the 50% level of tissue damage, $Z \approx 0.5$

The sub-linear $U$ versus $\tau_E$ reflects non-thermal character of PEF induced damage, because the usual ohmical heating should result in linear proportionality between energy consumptions $U$ and time of treatment $\tau_E$.

The electrical energy consumptions $U$ noticeably decreased with increasing of temperature, especially for small fields ($E \leq 200$ V/cm). Such behavior also justifies the presence of specific synergetic effects of simultaneous thermal-PEF treatments at small fields.

### 3.2 Diffusion kinetics

Diffusion curves in the form of the normalized solute concentration $B$ (normalized °Brix) versus time of extraction $t$ were investigated for untreated and PEF-pretreated chicory slices at different temperatures. Both temperature increase and the PEF pretreatment accelerated the extraction kinetics. For untreated slices the diffusion processes were very restricted for temperatures below 50 °C and noticeably accelerates at $T \geq 60$ °C. PEF pretreatment
considerably facilitates diffusion processes even below 50 °C, for example, at 40 °C the satisfactory extraction with $B \approx 1$ required about 1.5 hours.

The Arrhenius plots of the effective diffusion coefficient $D$ for untreated and PEF-pretreated chicory slices are presented in figure 3.

![Fig. 3: Disintegration index Z versus thermal treatment time $t_T$ for chicory tissue](image)

At high temperatures between 60 and 80 °C approximately the high values of $D$ were observed for both untreated and PEF-pretreated slices. At smaller temperatures the diffusion was noticeably higher for PEF-pretreated than for untreated slices. The non-Arrhenius behaviour with evident changes in slopes at $T_c \approx 60$ °C were observed for untreated chicory slices. The diffusion coefficient activation energy $W_D$ for low temperature branch (at $T < T_c$) was approximately estimated as $\approx 210$ kJ/mol. For high temperature branch (at $T \geq T_c$) the value of $W_D$ was estimated as $\approx 19 \pm 3$ kJ/mol. PEF pretreated slices displayed the activation behaviour with the same activation energy, $W_D \approx 19 \pm 3$ kJ/mol, in the whole investigated temperature interval between 20 °C and 80 °C. Slope breakdown at $T_c$ observed for untreated slices evidently reflects crossover between these two mechanisms of diffusion. Note that similar differences in activation behaviour for untreated and PEF-damaged tissues were previously observed for sugarbeet slices [8], and this data are presented, also, in figure 3.

4 Conclusions

The results of this work clearly evidence benefits of the PEF application enhancement of soluble matter extraction from chicory. PEF treatment of chicory tissue with field strength between 100 and 600 V/cm enhances the membrane permeabilisation and allows reaching high level of tissue disintegration even at room temperature. The contribution of temperature factor to the damage efficiency becomes rather unimportant at high fields. However, at smaller fields the contribution temperature factor to electroporation efficiency is important, that reflects synergetic effect of simultaneous thermal-PEF treatments. The moderate electric
energy consumptions \( (U < 10 \text{ kJ/kg}) \) at room temperatures demand application of relatively high electric field strengths \( (400–600 \text{ V/cm}) \), however, value of \( U \) noticeably decreases with increasing of temperature. The non-Arrhenius behaviour of effective diffusion coefficient with evident changes in slops at \( T_c \approx 60 \degree \text{C} \) was observed for untreated chicory. PEF pretreatment removed cell membrane barriers and noticeably accelerated the diffusion even at low temperatures between 20–40 \degree \text{C}. Proposed methodology appears to be promising for future industrial applications of “cold” soluble matter extraction from chicory roots.

References


EFFECT OF CELL DISRUPTION METHODS ON THE BEHAVIOR OF DEAD-END MICROFILTRATION OF YEAST SUSPENSION

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Abstract:

The interior of yeast cells is a rich source of bio-products (proteins, enzymes, etc.). The disruption of cell walls is a key step in the isolation and separation of intracellular bio-products. In this study, for disruption of Saccharomyces cerevisiae suspensions, high voltage electrical discharges (HVED) and High Pressure Homogenization (HPH) were compared. Releasing of intracellular components was characterized by conductivity disintegration index Z (Z=0-1), spectroscopy and turbidity. It was shown that the releasing efficiency of the proteins and other bio-products dramatically depends on the applied disruption technique. For the same value of Z, HPH allows a higher extraction of proteins and other bio-products and better filtration than HVED. The filtration behavior of intact and disrupted yeast suspension in a dead end stirred cell was investigated. It was shown that filtration of intact yeast suspension was governed by cake formation. While filtration behavior of HVED-processed suspensions and homogenized suspensions with different Z revealed suspension filterability deterioration after disruption. The flux decreased and the cake resistance increased with increase of Z.

Key words: Saccharomyces cerevisiae suspension, disruption, high voltage electrical discharges, high pressure homogenization, dead-end filtration.

1 Introduction

The interior cytoplasm of the yeast cells (Saccharomyces cerevisiae) is a rich source of bio-products (proteins, polysaccharides, etc.). Extraction of bio-products from cells usually involves disruption of cell walls and separation of extract from cells, cell debris and other insoluble particles. Therefore, the disruption of cell walls is a key step in the isolation and separation of intracellular bio-products.

Numerous techniques have been proposed for cell disruption [1]. High Pressure Homogenization (HPH) is most appropriate for industrial-scale disruption. This method results in considerable breakage of cells and high recovery of bio-products. However, it is restricted by temperature elevation and requires multiple passes with supplementary cooling.

Nowadays, applications of the electrically induced extraction technologies for protein extraction are very promising. High voltage electrical discharges (HVED) has found application for mechanical damage of cells and disintegration of cell and HVED application seems to have no undesirable effect on the quality of protein extracts [2].

Membrane filtration has been used as an alternative for the separation of soluble intracellular protein from cell lysates. Microfiltration technique allows filtrate recovery with higher efficiency both in crossflow and dead end systems [3]. However, application of
filtration for treatment of bio-suspensions is hindered by membrane fouling which substantially decreases filtration flux rate and transmission of bio-molecules through the membrane. Various mechanisms of filtration, including cake formation and models of standard, complete and intermediate pore blocking, were proposed for description of the membrane fouling [4].

In this work the dead-end filtration of intact and disrupted S. cerevisiae yeast suspensions was investigated. Two methods were used for yeast cells disruption and bio-molecules recovery: HPH and HVED. Influence of the disintegration index of yeast Z on proteins extraction and filterability of the yeast cell suspension was investigated.

2 Materials and methods

Suspensions of 0.5% w/w were prepared by mixing the dry yeast cells and distilled water by magnetic stirring. The yeasts were swelled in an aqueous suspension at room temperature and gentle agitation for 0.5 h was provided before HVED or HPH treatment.

HVED apparatus (Tomsk Polytechnic University, Tomsk, Russia) consists of a pulsed high voltage power supply and a laboratory treatment chamber with electrodes of a needle-plate geometry (Fig 1b). The high voltage pulse generator provided 40 kV - 10 kA discharges in a one-liter chamber. The electrical discharges were generated by electrical breakdown in the aqueous yeast suspension. The treatment chamber was initially filled with 500 mL of yeast suspension (0.5% w/w). HVED treatment lies in application of NHVED successive pulses (NHVED = 1-500). Suspension characteristics were measured between successive applications of the HVED pulses.

HPH was performed in a two-stage high pressure homogenizer at fixed homogenizing pressure of 800 bar (Fig 1a). The flow rate was 10.0 L/h. The temperatures of suspensions were measured by thermocouple just before and immediately after homogenization. Then suspensions were cooled to room temperature for their further characterization.

Dead-end microfiltration was conducted in a stirred cell (inner diameter 6.35 cm and total volume 180 mL). Filtration membrane was nylon membrane (nominal pore size was 0.2 μm), polypropylene filter cloth 25302 AN (pore size 25 μm, SEFAR FYLTIS, France) was used as a filter support. The filtration was conducted under pressure of 1 bar and agitation velocity of 200 rpm. New membrane was used for each filtration. The permeate was collected in a vessel placed on an electronic balance and the permeate weight was recorded by computer software.
3 Results and discussions

3.1 Properties of disrupted yeast suspensions

In order to estimate efficiency of cell disruption, electrical conductivity of suspension was measured and the conductivity disintegration index of yeasts $Z$ was determined from the following expression [5]:

$$ Z = \frac{\sigma - \sigma_{i}}{\sigma_{\text{max}} - \sigma_{i}} $$

where $\sigma$ is the electrical conductivity of suspension after treatment, $\sigma_{i}$ is the initial electrical conductivity of suspension before treatment and $\sigma_{\text{max}}$ is the electrical conductivity of suspension with maximum of disrupted cells. The above equation gives $Z = 0$ for an intact tissue and $Z = 1$ for a maximally disintegrated material.

Evolution of conductivity disintegration index $Z$ and temperature of suspension $T$ during HPH and HVED treatments are presented in Fig 3. $Z$ and $T$ increase gradually with the number of treatment cycles both for HPH and HVED methods. Increase of $Z$ during the treatment is related to extraction of ionic intracellular components from damaged cells and it is proportional to the disruption degree. For HVED treatment, the high level of disintegration ($Z=0.75$) required about 500 pulses which corresponded to the effective treatment time of
1000 μs, while for HPH treatment (Z=0.99) it requires about 14400 s for 20 passages under pressure of 800 bar which is much longer than that of HVED treatment. So the temperature elevation resulting from HPH treatment was higher than that of the HVED treatment. The final temperature of suspension treated by HPH exceeds 40 °C, which is critical for preservation of the intra-cellular bio-products (proteins and cytoplasmic enzymes). So supplementary cooling is used during the multiple passes.

In order to estimate efficiency of extraction of the high molecular weight compounds, absorbance spectra of the supernatant solutions of treated yeast suspensions were measured. In accordance with literature data, the peak observed at the wavelength $\lambda \approx 210$ nm corresponds to absorption by bonds of peptides and proteins, which are the main intracellular constituents of the $S. cerevisiae$, and peak at $\lambda \approx 260$ nm corresponds to nucleic acids [6]. Data show that absorbance of supernatant is much higher for HPH-treated suspensions than for HVED-treated suspensions after maximal disintegration (Fig 4a). Hence, disruption of yeast cells by HPH method seems to be more effective than HVED-treatment and results in higher release of proteins. This conclusion agrees with data on turbidity of supernatants of the treated suspensions (Fig 4b).

![Absorbance spectra and turbidity vs. Z](image)

**Fig. 4:** a) Absorbance spectra for untreated, HVED (Z=0.75) and HPH (Z = 0.99) treated yeast suspension; b) turbidity vs. Z for yeast suspensions after HPH or HVED

Increase of turbidity with Z increase reflects release of proteins and fine cell debris during the treatment. However, turbidity is much higher after homogenization of a yeast suspension than after its HVED treatment at the same level of Z.

### 3.2 Filtration behavior of disrupted yeast suspensions

Increase in content of the cell debris and decrease in the average particle diameter after HVED and HPH disruption results in complication of filtration behavior. Evolution of the filtration curves after HVED treatments of yeast suspensions is shown in Fig. 5. During HVED treatment, the filtrate flux gradually decreases with increase of Z. The variation of filtration flux can be divided into two stages, a quickly decay and a pseudo-steady stage. At the early period of filtration, the flux attenuates very quickly due to the quicker membrane blocking and particle deposition. Filtrate flux data was replotted in conventional filtration coordinates $t/V$ versus $V$ [7]. This coordinates allow to fit the experimental filtration curves by linear equation.

$$t/V = k_1 V + k_2$$

$$k_1 = \frac{\mu \omega \alpha}{2 \Delta P A^*}, \quad k_2 = \frac{\mu R_m}{2 \Delta P} \quad (2)$$

where $k_1$ and $k_2$ are the slope and intercept of the curve $t/V$ versus $V$, respectively. According to Ruth–Carman’s classical cake formation model, $k_1$ is directly proportional to specific cake
resistance. Fig 5b evidences that filtration of intact yeast suspension was governed by cake formation. The gradual increase of the slope with Z indicates increase of specific cake resistance after treatment which is associated with aforementioned disaggregation and damage of cells and formation of cell debris. It results in more dense cake structure with narrow pores and higher compressibility.

Fig. 5: Filtration curves of HVED-treated yeast suspensions as a function of Z: a) filtrate flux J vs. time; b) t/V vs. V

In HPH-treated suspensions, increase of Z also results in decrease of the flux (Fig 6a). It is obvious that initial segments of filtration curves for HPH suspensions are linear for the curve t/V versus V (Fig 6b). It evidences that the cake formation prevails in the beginning of filtration. Slopes of the segments increase with increase of Z. Hence disaggregated and disrupted particles build up a cake with higher specific resistance. Increase of specific cake resistance may be explained by both decrease of the particle diameter and over-clogging effect lying in fouling of the deposited cake of fine-sized cell debris.

Fig. 6: Filtration curves of HPH-treated yeast suspensions as a function of Z: a) filtrate flux J vs. time; b) t/V vs. V

4 Conclusions

The HPH treatment was found to cause a more effective extraction of intracellular compounds than HVED at the same value of disintegration index Z. The filtration results showed that the filtration mechanism of intact yeast suspensions was governed by cake formation. For HVED-processed and high pressure homogenized suspensions, filterability of the disrupted suspensions become worse with increase of Z. Further work will be required for the improvement of the filterability.
References


EFFECT OF MODERATE ELECTRIC FIELD IN THE PHYSICAL AND TRANSPORT PROPERTIES OF CHITOSAN COATINGS

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Abstract:
Edible films and coatings can provide additional protection for food, while being a fully biodegradable, environmentally friendly packaging system. Preliminary works have shown that the presence of a moderate electric field during the preparation of chitosan coating solutions may influence e.g. their transport properties. The aim of this work was to determine the effect of field strength on functional properties of chitosan coatings (obtained from lobster of the Cuban coasts). Four different field strengths (50, 100, 150, 200 V cm⁻¹) were applied during the preparation of the film forming solution, films were cast and, for each electric treatment, the water vapor, O₂ and CO₂ permeabilities of the films were determined, together with their solubility in water. The films were also analysed using scanning electron microscopy (SEM) and X-ray diffractometry (XRD). The results showed that the electric field has statistically significant effects on films’ transport properties (which e.g. for water vapour permeability, varied from 0.3228 to 0.2667(g.(m.day.atm)⁻¹)) and structure, a positive correlation having been found between the water vapor, O₂ and CO₂ permeability coefficients and the applied field strength. XRD indicated that when electrically treated, chitosan films exhibited a more ordered structure and a clearly higher crystallinity when compared with non-treated films. SEM micrographs evidenced that the surface morphologies of chitosan films were influenced by the electric field. In fact, the electric field treatment led to a structure with more regular layers as can be seen in the cross-sections of the films. These results clearly indicate that, when applied to the film-forming solution, the electrical field treatment may be a good tool to finely adjust the transport properties e.g. in tailor-made film formulations.

Key words: edible coatings, electric fields, transport properties.

1 Introduction

Edible coatings can provide an alternative to extend the post-harvest life of fresh fruits and other vegetables and can also result in a similar effect as modified atmosphere storage in modifying the internal gas composition (1). Indeed, this protective barrier can be formulated to prevent the transfer of moisture, gases, flavor or lipids, and thus to maintain or improve food quality and to increase food product shelf life (2). Chitosan has been widely used for the production of edible coatings and edible films (3,4,5). Chitosan films are excellent oxygen and
carbon dioxide barriers and have interesting antimicrobial properties (3,6). Ohmic heating is based on the passage of electrical current through a sample that has electrical resistance. The electrical energy is directly converted to heat and instant heating occurs, at a rate which depends on the intensity of the current passing through the material.

García et al., (2009) analyzed the effect of applying an electrical field during drying on the microstructure of films formulated with different concentrations of chitosan and methyl-cellulose; those authors have shown that the electrical field treatment could be a good alternative to improve film flexibility and to increase water vapor barrier properties.

In this context, the objectives of the present work were to analyze the effect of applying a moderate electric field to film-forming solutions of chitosan, to evaluate the films’ microstructure by SEM and X-ray diffraction and determine the effect of field strength on transport properties of chitosan coatings.

2 Materials and Methods

2.1 Coating Materials
The materials used to prepare the edible coating solutions were: chitosan (obtained in the Pharmaceutical Laboratories Mario Muñoz, Cuba) with a degree of deacetylation of 90% approximately, Tween 80 (Acros Organics, Belgium) as surfactant and lactic acid (90%, Merck, Germany).

2.2 Film Formation
The coating solutions were prepared dissolving the chitosan (1.5% w/v) in a 1% (v/v) lactic acid solution with agitation using a magnetic stirrer during 2 hours at room temperature (20 °C); subsequently, Tween 80 was added as a surfactant at a concentration of 0.1% (w/w) (4).

2.3 Device description
A set of experiments was conducted to determine the effect of the application of a moderate electric field to chitosan solutions. The chitosan solution samples were treated in an ohmic heater using four different field strengths (from 50 to 200 Vem⁻¹) (8).

2.4 Characterization of chitosan films
2.4.1 Conditioning and Thickness
All chitosan films used for permeability tests were conditioned in desiccators, at 20 °C and 25 % RH. Film thickness was measured with a hand-held digital micrometer (Mitutoyo, Japan) having a sensitivity of 0.001 mm. Ten thickness measurements were taken on each testing sample in different randomly chosen points and the mean values were used in permeability calculations.

2.4.2 Gases Permeability
Oxygen permeability (O₂P) and carbon dioxide permeability (CO₂P) were determined based on the ASTM (2002) method.

2.4.3 Water vapor permeability measurement and Film Solubility
The water vapor permeability (WVP) of the films was determined gravimetrically based on the ASTM E96-92 method (9, 10).

The film solubility in water was determined according to the method reported by Gontard et al. 1994 (11).
2.4.4 Scanning electron microscopy (SEM)
Scanning electron microscopy (SEM) analyses were performed with a scanning electron microscope (Nova NanoSEM 200, The Netherlands) with an accelerating voltage varying from 10 to 15 kV.

2.4.5 X-ray diffraction and Crystallinity
X-ray diffraction patterns of the films were analyzed between 2θ = 4º and 2θ = 60º with a step size 2θ = 0.02º in an X-ray diffraction instrument (Bruker D8 Discover, USA). The crystallinity index (CI) was defined using the equation 
\[ CI = \frac{I_{110} - I_{am}}{I_{110}} \] (12), where \( I_{110} \) is the maximum intensity (2θ, 20º) of the (110) lattice diffraction and \( I_{am} \) is the intensity of the amorphous diffraction (2θ, 16º).

3 Results and Discussion

3.1 Oxygen permeability (O2P); Carbon dioxide permeability (CO2P); Solubility in water and Water Vapor Permeability
Table 1 shows O2P and CO2P as measured for chitosan films formed from solutions subjected to electric fields of different intensities. The samples with treatments made at 100 V·cm\(^{-1}\) or higher have lower values (\( p < 0.05 \)) of O2P and CO2P.

<table>
<thead>
<tr>
<th>Electric field strength</th>
<th>Solubility (%)</th>
<th>WVP (g·(m.day.atm)(^{-1}))</th>
<th>CO2 Permeability (10^{14}) (g·(Pa·s·m(^{-2}))(^{-1}))</th>
<th>O2 Permeability (10^{16}) (g·(Pa·s·m(^{-2}))(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 V·cm(^{-1})</td>
<td>39.48±0.01(^a)</td>
<td>0.3228±0.027(^a)</td>
<td>6.98±0.030(^a)</td>
<td>10.60±0.420(^a)</td>
</tr>
<tr>
<td>50 V·cm(^{-1})</td>
<td>39.42±0.03(^a)</td>
<td>0.3219±0.022(^a)</td>
<td>6.97±0.029(^a)</td>
<td>10.60±0.450(^a)</td>
</tr>
<tr>
<td>100 V·cm(^{-1})</td>
<td>39.30±0.01(^a)</td>
<td>0.2740±0.027(^b)</td>
<td>6.74±0.037(^b)</td>
<td>9.54±0.400(^b)</td>
</tr>
<tr>
<td>150 V·cm(^{-1})</td>
<td>39.28±0.01(^a)</td>
<td>0.2728±0.030(^b)</td>
<td>6.72±0.041(^b)</td>
<td>9.42±0.540(^b)</td>
</tr>
<tr>
<td>200 V·cm(^{-1})</td>
<td>39.27±0.02(^a)</td>
<td>0.2667±0.025(^b)</td>
<td>6.72±0.040(^b)</td>
<td>9.62±0.600(^b)</td>
</tr>
</tbody>
</table>

*Different letters in the same column correspond to statistically different samples (\( p < 0.05 \)).

Tab 1: Values obtained from water vapor permeability (WVP), CO\(_2\) permeability and O\(_2\) permeability for the films obtained with film-forming solutions subjected to different field strengths.

Wan, et al 2003 (13) observed that the crystallinity of the chitosan membranes increased gradually with increasing degree of deacetylation ranging from 70 to 90 %. This can be attributed to the fact that chains of chitosan with higher degree of deacetylation are more compact thus facilitating hydrogen-bonding formation and consequently favoring crystallinity formation in the film. Furthermore, chitosan with a higher degree of deacetylation contains more glucosamine groups, which also facilitate the hydrogen-bonding formation; on the contrary, chitosan with a lower degree of deacetylation has more acetyl groups, which hinder the chitosan chain packing due to their rigidity and steric effect (14).

The results obtained in this work show that WVP of chitosan films decrease with the increase of the field strengths for values of 100 V·cm\(^{-1}\) or higher (Table 1).

Anker, et al 2000 (15) concluded that the reason for the increased WVP is probably the larger pores formed at high polymer concentration, compared to the smaller pores formed at low polymer concentration. The work of Miller and Krochta 1997 (16) also points at the fact that the permeability is highly affected by how closely packed the polymer chains are, thus establishing a direct relationship between the crystallinity of the structure and permeability. In the present work the solubility of the chitosan films was evaluated, and it is shown that the
The solubility of chitosan films decreases with the increase of the field strengths for values of 100 V·cm⁻¹ or higher (Table 1). Balau et al. (2004) showed that the electric field plays an important role in the crystallization process, which may also interfere in the water solubility of the films.

3.2 X-ray diffraction

Figure 2 shows that the crystallinity of the chitosan films increases gradually with the increase of the electric field strength. This indicates that, during the moderate electric field treatment, a structure with a different X-ray diffraction pattern was developed. This may be attributed to the fact that the chitosan chains with higher degree of deacetylation are more flexible. Flexible chains will facilitate the hydrogen bond formation and consequently crystallinity formation in the film. In addition, there was one other diffraction peak at around 8°-10° (2θ).

![X-ray diffraction patterns of chitosan films](image)

Fig. 1: X-ray diffraction patterns of chitosan films to (-) Control (-) Conventional heating (-) Electric fields 100 V cm⁻¹ and (-) Electric fields 200 V cm⁻¹

Results indicated that the application of a moderate electric field to the film-forming solutions had significant effects on the crystallinity index (CI), which was higher for films treated with electrical fields.

3.3 Scanning electron microscopy (SEM)

When their film-forming solutions were submitted to an electric field, chitosan films have shown crystals in their structure, evidencing that there must have been morphological influences from that treatment. In fact, the electric field treatment lead to a structure with more regular layers as can be observed in the cross-section images of Figure 2.

The development of films with a uniform and compact layer can be an important achievement towards the improvement of various film properties, such as their permeability to gases.
Fig 2: SEM micrographs of chitosan films and cross-section to (a) control, (b) conventional heating, (c) electric fields 100 V cm⁻¹, (d) electric fields 200 V cm⁻¹.

The search for homogeneous structures thus becomes a target of the research involving edible films. In this perspective, the application of electric fields may provide an interesting solution for that problem and has gained importance in this area of research. García et al. (2009) showed that the surface morphologies of films were influenced by the preparation method, indicating that the application of an electric field during drying was an influencing factor. SEM analyses indicated that this treatment led to a more regular structure.
References


COMPARISON BETWEEN PULSED ELECTRIC FIELD AND THERMAL – EFFECT IN LACTOPEROXIDASE AND ALKALINE PHOSPHATASE INACTIVATION

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Abstract:

Pulsed electric field (PEF) treatment of liquid foodstuff is considered as a non-thermal alternative for inactivation of microorganisms based on cell electroporation. Current flow occurs in the food, which leads to ohmic heating since electrical energy is dissipated. Hence, temperature increase is a PEF side effect that has to be considered when treatments of heat sensitive products are investigated. In order to differentiate between thermal effects occurring during PEF treatment and the remaining electric field effects it is necessary to analyse the total thermal load to which the product is exposed. Based on available heat inactivation data for the target component, thermal inactivation resulting from the PEF treatment can be calculated separately. Activity of lactoperoxidase (LPO) and alkaline phosphatase (ALP) in milk were considered as the indicators for the differentiation of thermal and electric field effects. A co-linear treatment chamber and stainless steel cooling coils were used for PEF treatment. Rectangular pulses of 3µs at a field strength between 20-40 kV/cm were applied at different frequency to allow a total specific energy input between 40-160 kJ/kg with treatment times between 10-34 µs. Heat transfer in the spiral heat exchanger was calculated using finite volume method (FVM). Heat inactivation kinetics of LPO and ALP were obtained in glass capillaries and enzyme activity were determined after PEF treatment. Differentiation of total inactivation effect into thermal and remaining pulsed electric field effect could be performed (an up to 50% contribution of thermal effects to the total inactivation could be detected). An optimisation of the PEF system (including heat exchangers) and a mathematical model were performed based on the calculated thermal inactivation occurring in the different parts of the apparatus in order to minimize thermal effects with regard to the preservation of heat sensitive food constituents.
ON DAMAGE KINETICS OF CELLULAR BIOMATERIAL TREATED BY PULSED ELECTRIC FIELD

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Abstract:
Nowadays, the pulsed electric fields (PEFs) are widely used for enhancement of permeabilization of the cell membranes and partial or complete disintegration of cellular biomaterial. Such processes are related to electroporation phenomenon and are not accompanied by undesirable thermal heating. The PEF-induced changes facilitate drying, diffusion and extraction of useful components. The damage kinetics of different cellular biomaterials and various treatment protocols were recently investigated. The PEF treatment may be followed by a transient behaviour, related to partial resealing of the bio-membrane structure or further increase of the degree of damage. However, the mechanisms of many observed effects are unclear and still under discussion. This work discusses relations between the damage kinetics in a cellular biomaterial and PEF treatment protocol. The damage kinetics of cells was approximated by a first order equation. The effective medium theory was used for estimation of the damage degree P dependence on conductivity disintegration index Z. The mixed linear-power law \( Z = \alpha P + (1-\alpha)P^m \) was shown to be satisfactory for approximation of \( Z(P) \) dependency. Here, value of \( \alpha \) characterises linear contribution to relation between Z and P, and \( m \) is a parameter. The linear contribution \( \alpha \) was rather small (\( \alpha < 0.12 \)) and the power Archie’s equation of type \( Z = P^m \) was a good approximation. Archie’s exponent \( m \) was an increasing function of both \( P_c \) and \( \sigma_i/\sigma_d \), so it reflected location of the percolation threshold and electric contrast of the medium. The model accounted for dependence of the induced transmembrane potential \( u_m \) on Z. Changes in the medium conductivity accelerated the damage process. The simulated \( P(t) \) dependences were exhibitative of a complex damage kinetics comprising fast and slow rate regimes. Acceleration of the process resulted in a noticeable shortening of the characteristics damage time. This effect was more pronounced at small values of the field strength.

Key words: pulsed electric fields, electroporation, biomaterials, damage kinetics

1 Introduction

Many useful examples of the pulsed electric field (PEF) application for enhancing the pressing, drying, extraction and diffusion processes in materials of biological origin were already demonstrated [1]. This minimally invasive method allows avoidance of undesirable changes in a biological material, which are typical for other techniques, such as thermal, chemical and enzymatic ones. Moreover, the PEF treatment is also promising for microbial
killing [2]. For purposes of vegetable tissue damage, this methodology requires a moderately small electric field within \( E = 500–1000 \text{ V/cm} \) and treatment time within \( 10^{-4}–10^{-2} \text{ s} \), microbial killing is efficient only at larger fields \( (E = 10–50 \text{ kV/cm}) \). The damage of cells in a tissue is governed by electroporation of biomembranes without any faults in the cell wall structure, significant temperature increase, and small power consumption. Recent research efforts were aimed on optimisation of the PEF protocol accounting for the structure and state of the treated material, the presence of additives and possible electro-thermal synergy [1, 3].

Barrier functions of biomembranes are rather strong and probability of their damage due to thermal fluctuation at ambient conditions is insignificant. However, external fields increase probability of the fluctuation damage of membranes. Due to huge difference between electrical conductivities of membrane and surrounding medium, the external electric field \( E \) concentrates presumably on membranes, and when the transmembrane potential \( u_m \) exceeds some threshold value (typically, about 0.2-1.0 V), the electroporpermeabilization or damage may occur. Owing to the cell lysis, electrical conductivity of biomaterial is an increasing function of time. This results in more favourable condition of cells permeabilisation and in acceleration of the damage process. However, the exact mechanism of tissue damage is not precisely understood yet and it may depend on the applied pulse protocol, i.e. electric field strength, shape of pulses, pulse duration and intervals between pulses.

This work discusses relations between kinetics of the cellular biomaterial damage and PEF treatment protocol.

2 Description of the model

In biomaterials electroporation is governed by a noticeable amplification of the external electric field \( E \) on the membranes. For spherical cells, field amplification factor \( k \) can be estimated as \( k \approx R/h \sim 2 \times 10^4 \), where \( h \approx 5 \text{ nm} \) is the membrane thickness and \( R \) is the cell radius \((\approx 100 \mu\text{m})\) [1]. In our model, the damage degree of biomaterial \( P \) was defined as the ratio of the damaged cells and the total number of cells. The time evolution of \( P \) under the PEF treatment was approximated by the simplest first-order equation

\[
dP/dt = P/\theta(E, P) \tag{1}
\]

where the cell lifetime \( \theta \) was dependent upon external electric field strength \( E \) and damage degree as [3, 4]:

\[
\theta = \theta_{\infty} \exp W/[1 + (f(P)E^*)^d] \tag{2}
\]

where \( \theta_{\infty} \) is the limiting lifetime (formally, at \( T \to \infty \)), \( W \) is the reduced activation energy, \( E^* = E/E_o \), \( E_o \) characterizes the electroporation response and \( d \) is a parameter. The estimated values for spherical cell \((R=50 \mu\text{m})\) with lipid membrane are \( d \approx 1.3 \), \( W \approx 109 \), \( \theta_{\infty} = 7.4 \times 10^{-7} \text{ s} \), \( E_o = 20 \text{ V/cm} \) at 298 K [3,4].

The coefficient \( f(P) \) is the function of \( h, R \) and electrical conductivities of membrane \( \sigma_m \), extracellular medium \( \sigma(P) \), and cytoplasm \( \sigma_d \) [5]. For estimation purposes, the value of \( \sigma_d \) was identified in our model with electrical conductivity of the totally destroyed cellular biomaterial. In the case of \( \lambda = R \sigma_m/h \sigma_d \ll 1 \) (it is usually true under physiological conditions) it can be simplified to:

\[
f(P) = 1-(1+\sigma_d/\sigma(P))\lambda \tag{3}
\]
In an intact biomaterial (at \( P = 0 \)), which is usually a low-conductivity medium, \( \sigma(P=0) = \sigma_i \), the inequality \( \sigma < \sigma_d \) is satisfied, and \( f = f_i \) can be noticeably smaller than 1. The electrical conductivity \( \sigma_i \) was estimated as \( \sigma_i \approx \sigma_d/(1+1/(2\lambda)) < \sigma_d \) [4]. The value of \( \sigma \) increases with increase of the PEF-induced damage. Finally, it reaches \( \sigma (P=1) \approx \sigma_d \) in the limit of high tissue disintegration and \( f(P=1) \approx 1 \).

Fig. 1: Conductivity disintegration index \( Z \) versus damage degree \( P \) calculated from (4) and (5) at different values of percolation threshold \( P_c \). Insert shows \( x \) and \( m \) versus \( P_c \) calculated from the mean square fitting of \( Z(P) \) by \( Z \approx xP + (1-x)P^m \).

The \( f = f(P) \) was approximated using (3) and general effective medium (GEM) equation for \( \sigma \) versus \( P \) dependence

\[
(1-P)(\sigma_i^{1/s} - \sigma_d^{1/s})/(\sigma_i^{1/s} + A\sigma_d^{1/s}) + P(\sigma_d^{1/t} - \sigma_i^{1/t})/(\sigma_d^{1/t} + A\sigma_i^{1/t}) = 0
\]

where \( s \) and \( t \) are the exponents of random percolation theory, \( s = 0.73, \ t = 2.0 \) for 3d materials, \( A = (1-P_c)/P_c \), \( P_c \) is the percolation threshold corresponding to transition from the low (\( \sigma = \sigma_i \)) to high (\( \sigma = \sigma_d \)) conductivity [6,7].

The conductivity disintegration index \( Z \) was calculated as [1]:

\[
Z = (\sigma - \sigma_i)/(\sigma_d - \sigma_i), \tag{5}
\]

This equation gives \( Z = 0 \) for the intact tissue and \( Z = 1 \) for the totally disintegrated material.

The kinetics of biomaterial damage was calculated using (1)–(3) accounting for dependence \( f(P) \) estimated from (4). For estimation purposes, we put the following values of parameters in all the calculations: \( W = 58, \ d = 1.33 \) (as was estimated in [4] for spherical cells), \( \sigma_i = 0.03 \) S/m, \( \sigma_d = 0.3 \) S/m, \( R = 50 \mu m, h = 5 \) nm.

2 Results and discussion

Figure 1 presents examples of the calculated \( Z \) versus \( P \) dependences at different values of percolation threshold \( P_c \) and two values of \( \sigma_i/\sigma_d \). These calculations were done using (4)–(5).
at $\sigma_i/\sigma_d$ between 0.1 and $\sigma_i/\sigma_d$ that is typical for the cellular tissues. Data show that $Z(P)$ curves are highly non-linear and deviation from linearity increases with increase of $P_c$. So, application of the conductivity disintegration concept may result in underestimation of the real damage degree $P$.

![Figure 2: Conductivity disintegration index Z and damage degree P versus reduced time $t/\tau_\infty$ at different values of $E^* = E/E_o$ and $\sigma_i/\sigma_d = 0.1$. The dashed lines correspond to unaccelerated process ($f(P=0) = f_i$ and $\theta = \theta_i = \text{const}$).](image)

Fig. 2: Conductivity disintegration index Z and damage degree P versus reduced time $t/\tau_\infty$ at different values of $E^* = E/E_o$ and $\sigma_i/\sigma_d = 0.1$. The dashed lines correspond to unaccelerated process ($f(P=0) = f_i$ and $\theta = \theta_i = \text{const}$).

![Figure 3: Examples of log(1–P) versus $t/\theta_\infty$ presentation for kinetics of accelerated (solid line, $t^p$) and unaccelerated (dashed line, $\tau^p_i$) processes, $E^* = 5$. Here, $t^p$, $\tau^p_i$ are characteristic damage times required for attaining $P = 1/2$. $\sigma_i/\sigma_d = 0.1$.](image)

Fig. 3: Examples of log(1–P) versus $t/\theta_\infty$ presentation for kinetics of accelerated (solid line, $t^p$) and unaccelerated (dashed line, $\tau^p_i$) processes, $E^* = 5$. Here, $t^p$, $\tau^p_i$ are characteristic damage times required for attaining $P = 1/2$. $\sigma_i/\sigma_d = 0.1$. 

At small values of $P_c$ (it is expected from predictions of random percolation theory that $P_c \approx 10^{-40}$) the mixed linear-power law $Z \approx xP^m + (1-x)P^m$ was fulfilled. Here, $x$ characterises linear contribution and $m$ is a parameter. Parameter $x$ was rather small ($x \approx 0.12$) and the power Archie’s equation of $Z \approx P^m$ type was a good approximation. Note that Archie’s exponent $m$ was an increasing function of both $P_c$ and $\sigma_i/\sigma_d$ (insert to Fig. 1), so it reflected location of the percolation threshold and electric contrast of the medium.

Examples of the calculated kinetics of $Z$ and $P$ at different values of $E^* = E/E_0$ are shown in Figure 2. Accounting for the $f(P)$ dependence results in more rapid damage kinetics (solid lines) as compared with unaccelerated process (dashed lines, $\theta = \theta_i = \text{const}$). The resulting curves of accelerated process kinetics, which account for $f(P)$, cannot be described by the 1st order equation with the constant lifetime. This conclusion can be easily justified by log$(1-P)$ versus $t/\theta_e$ dependence (Fig. 3).

The curve for accelerated process kinetics has more complex shape and deviates from the linear dependence between log$(1-P)$ and $t/\theta_e$. The most non-linear kinetics was observed at the initial period during the lag-time (at $t < t_{lag}$), however, at long time of treatment the accelerated process kinetics can be approximated by the linear dependence log$(1-P) = c - t/\theta_e$, where $\theta_e$ is the effective lifetime. The lag-time $t_{lag}$ was a decreasing function of the electric field strength $E$ (insert to Fig. 3) and it could reach rather large value at small fields ($\approx 10^3 \theta_e$ at $E^* = 5, E \approx 100 \text{ V/cm}$).

For characterisation of the complex damage kinetics, it is useful to introduce the characteristic damage time $\tau'\theta_e$ (or $\tau Z$), which corresponds to the time required for attaining one half of the maximum damage degree, i.e. $P = 1/2$ (or $Z = 1/2$) (Fig. 3).

![Fig. 4: Relative characteristic damage time $\tau'\theta_e$ versus relative electric field strength $E^*$. Insert show the ratios $\tau^P/\tau^P_i$ and $\tau^E/\tau^P$ versus $E^*.$ $\sigma/\sigma_d = 0.1.$](image-url)
Figure 4 shows field dependence of $\tau_P(E^*)$. At large fields, $E^*>50$ (which corresponds to $E>1000$ V/cm), the value of $\tau_P(E^*)$ approaches the limiting value $\theta\infty$. Insert to Fig. 4 show the ratios $\tau_P/\tau_i$ and $\tau_P/\tau^*$ versus $E^*$. The ratio $\tau_P/\tau_i$ characterises the difference between kinetics of accelerated and unaccelerated processes.

At $E^* = 5$ ($E \approx 100$ V/cm), $\tau_P/\tau_i=0.5$, however the difference between $\tau_P$ and $\tau_i$ reduces at higher field strength. The non-linear relation between $Z$ and $P$ results in larger characteristic damage time obtained from measurements of the conductivity disintegration index $\tau^E$ as compare with the real damage time $\tau_P$ and difference between them grows with increase of $E^*$.

3 Conclusions

Damage kinetics of PEF-treated cellular biomaterial for the model accounted for dependence of the induced transmembrane potential $u_m$ on damage degree $P$ was investigated. The simulated $P(t)$ dependences were exhibitive of a complex damage kinetics comprising different slow and fast rate regimes. The lag-time $t_{lag}$ of fast rate regime was the decreasing function of electric field strength $E$ and reached large values at small fields ($\approx 10^3 \theta_e$ at $E^* = 5$, $E \approx 100$ V/cm).

The effective media theory predicts the mixed linear-power law $Z \approx xP+(1-x)P^m$ in the reasonable interval of percolation threshold value ($P_c \approx 10–40\%$). The Archie’s exponent $m$ was found to be an increasing function of both $P_c$ and $\sigma/\sigma_d$, so, it reflected location of the percolation threshold and electric contrast of medium.

References


Abstract:
The demand for minimally processed food has increased in recent years, owing to its greater retention of flavor, color, and nutritive value. Foods can be non-thermally processed by electrical methods such as electroplasmolysis. Electroplasmolysis provides increased cell wall permeability, electrical conductivity, diffusion and compressibility of plant tissues. Carrot juice holds an important place in vegetable juices with the increased consumption of fruit and vegetable juices. This study focuses on effects of electroplasmolysis applications in carrot juice processing. Carrots were processed by using electroplasmolysis as pretreatment at stationary mode by applying voltage gradients in the range of 20-60 V/cm for 30-90 seconds. Electroplasmolysis conditions were optimized by using RSM (response surface methodology). After production of carrot juice; effects of electroplasmolysis treatment on the yield of carrot juice was calculated. In addition, some quality characteristics of carrot juices were investigated at optimum conditions. The results showed that in control samples yield of juice was calculated 53.7% and the samples applied electroplasmolysis (40 V – 60 sec) the yield increased 4.07%. In addition, electroplasmolysis application increased phenolic, pectin content and soluble solid contents of carrot juice. Color values of samples are comparable with the control samples. As a result; electroplasmolysis application is effectual as a pretreatment in carrot juice production for improving quality and yield of carrot juice.

Keywords: Electroplasmolysis, yield, carrot juice

1 Introduction

Increased consumer demand for fresh-like products with minimal vitamin and flavor losses stimulated the search for new mild preservation techniques. The use of nonthermal methods which are among these new techniques are gained popularity. Food industry is highly interested in electrical methods as a non-thermal technology [1]. One of these electrical methods is electroplasmolysis.

Electroplasmolysis of fruits and vegetables has been reported since the late forties. It is the increased permeability of biological tissue cells after electric field application. Recently, this phenomenon was also called electropereomeabilization. The microstructure of plant and animal tissues changes considerably during electrical treatment as a result of contraction and gaping of cell vacuoles. Since electrical conductivity of intercellular juice is significantly higher than
the conductivity for plasmatic membranes that covers the cells, up to 95% of the voltage applied across tissue samples drop at the cellular membranes [2].

Carrot juice became more popular with the increased consumption of vegetable juices which can prevent certain diseases such as cancer and cardiovascular diseases. But there is no many studies about vegetable juices with electrical techniques. There are limited published works that study the effect of pulsed electroplasmolysis in vegetable tissues [2],[3] and PEF in blended orange and carrot juice [4],[5]. Therefore in this study electroplasmolysis is used as a pretreatment in carrot juice production. Effects of electroplasmolysis treatment on the yield and some quality characteristics of carrot juices were investigated.

2. Materials and Methods

2.1 Material

Carrots (Daucus Carota L) of Nantaise variety used as raw material in this study. Carrots were purchased from local producer (OR-NA Agriculture) and were stored in a refrigerator at 4°C and 92.4 RH) until juice production.

2.2 Processing Methods

2.2.1 Electroplasmolysis application

Electroplasmolysis applied by a continous drum type electroplasmolizator at stationary mode, having two cylinders with stainless steel pins. The electrode gap between drums was chosen 1.8 cm. In this system different voltage gradients and times are studied. Carrots were put between pins and voltage adjusted by using voltage control unit.

Carrots were washed and peeled before processed. Then samples were divided into two groups; 1) control group (without any treatment), 2) electroplasmolysis application group as a pretreatment to determine yield and quality effects. Control group was directly processed to carrot juice by using a juice extractor (Moulinex JU5000-800W). Carrots in electrolasplasmolysis group were processed by using electroplasmolysis as pretreatment by applying voltage gradients in the range of 20-60 V for 30-90 seconds and conditions were optimized by using Response Surface Methodology(RSM) (data were not shown). Then carrot juice was processed at optimized conditions.

2.3 Methods of analysis

After processing of carrot juices effects of electroplasmolysis treatment on the yield of orange juice were calculated. In addititon other quality characteristics were investigated by physical and chemical analyses. The juice samples were analysed to determine the following: pH [6], titrable acidity (% of citric asit) [7], total soluble solids [7], pulp content [5], turbidity [5], total phenolics [8], pectin content [6] and the colour (L*, a*, b*, ΔE) values (HunterLab Colourflex- Management Company, USA) of carrot juice were analyzed according to given methods at literature. Analysis were made in selected optimum point and control samples from the freshly extracted juices for quality characteristics.

3 Results and Discussion

Yield was calculated as 55.91% in optimum point (40 voltage-60 seconds electroplasmolysis application) and 53.7% in control samples. Thus, yield was increased % 4.07 by electroplasmolysis. This can be explained by increasing in cell permeability. Electroplasmolysis has been reported to enhance extraction and dehydration processes in vegetable tissues [2].
Although there is no many studies in vegetable juice it was reported that electroplosmolsysis application was effective in increasing yield of citrus fruits 10% compared to conventional methods, and in apple juice production before pressing electroplosmolsysis was applied as a pretreatment and was increased yield 10-15% [9]. Pulsed electric field can be used for destruction of plant cells and provides increase in yield. Bazhal et al. (2003) indicated that PEF treatment provides significant improvements in juice yield [10].

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Titrable Acidity (%)</th>
<th>Brix</th>
<th>Pulp ratio (%)</th>
<th>Turbidity</th>
<th>Total phenolic (mg/l)</th>
<th>Pectin (GA-AN, mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.24±0.03</td>
<td>0.224±0.02</td>
<td>8.75±0.03</td>
<td>8.75±0.00</td>
<td>3.378±0.00</td>
<td>150.24±0.13</td>
<td>258.67±0.06</td>
</tr>
<tr>
<td>40 V – 60 s EP</td>
<td>6.67±0.04</td>
<td>0.272±0.02</td>
<td>9.28±0.04</td>
<td>10.00±0.00</td>
<td>4.42±0.00</td>
<td>152.74±0.95</td>
<td>296.915±2.24</td>
</tr>
</tbody>
</table>

Average ± standard deviation for a minimum of three experiments.

Table 1: Chemical analyses results of carrot juice produced by conventionally and EP treatment

Table 1 shows the results of chemical analyses of selected optimum point and control groups.

pH value and total titrable acidity increased by EP compared to control samples. Organic acids appear in foods as a result of biochemical processes and Rivas et al. (2006), reported that PEF treatment shows the same effect on total acidity. As shown above water soluble solid contents are higher in electroplosmolsysis groups which can be explained by increasing in cell permeability and soluble solids can be transferred to vegetable juice easily. In citrus juices, brix is used to indicate the percentage of soluble solids and is one of the most important factors for grading the quality of a citrus juice [5]. There are some studies which confirming results that electroplosmolsysis is effective on soluble solid content in fruit juices [11].

Gulyi et al. (1994) reported that juice purity (percent ratio of sugar in the total dry solids of juice) was increased by 1-5% after electrical treatment in comparison with traditional thermal plasmolysis. Bazhal & Gulyi (1983) explained this difference as due to polarization of the tissue during electroplosmolsysis, electrical coagulation of proteins and colloids that increased retention of nonsugar substances in the treated tissue [2].

Pulp content and turbidity results of electroplosmolsysis group were comparable with the control samples. Results of total phenolic contents are also shown in Table 1. Total phenolic content increased by 1.21% in the samples which were applied electroplosmolsysis with respect to the control sample. Schilling et al. (2006), were investigated the effects of PEF treatment of apple mash and found that phenolic contents in apple juice were increased with electrical treatment.

Pectin contents of control samples as shown in Table 1 was found less than the electroplosmolsysis group. Electroplosmolsysis application increased pectin content 14.78% comparing to control group. Pectin is important for the cloud stability in pulp juices. The loss of juice cloudiness will easily indicate to the consumers that the carrot juice is of poor quality [10]. Schilling et all.(2007), found that pectin content of juices were not diminished by PEF treatment in apple juices when EP is applied to apple mash.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE</th>
<th>Hue angle</th>
<th>ΔC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.18±0.13</td>
<td>31.02±0.12</td>
<td>45.62±0.03</td>
<td>-</td>
<td>55.66</td>
<td>-</td>
</tr>
<tr>
<td>40 V – 60 s EP</td>
<td>41.89±0.13</td>
<td>29.81±0.13</td>
<td>44.95±0.06</td>
<td>4.18</td>
<td>56.55</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Tab. 2: Color values of carrot juice produced by conventionaly and EP treatment
The color values (L*, a* and b*), the total color difference (ΔE), Chroma and Hue angle of samples in CIELab system are summarized in Table 2. There is no significant difference between L* values of samples. By electroplasmolysis treatment a* and b* values decreased compared to control group, a/b value also decreased. This can be explained by color of samples became lighter after electroplasmolysis application. The color difference between control and EP samples is found slightly noticeable. Same effects were observed by electroplasmolysis application in other studies [11],[12].

4 Conclusion

Results showed that electroplasmolysis has significant effect on the yield of carrot juice. Electroplasmolysis application increases total phenolic and pectin content of carrot juice compared to control samples. The use of electroplasmolysis as an alternative to pretreatment of carot juice production may be considered a strategically important action to obtain a sensory impaired product. In this study it was determined that electroplasmolysis application has positive effect on carrot juices. There will be further studies about electroplasmolysis effects during storage conditions and other vegetable juices.

References

DESTRUCTION KINETICS OF ESCHERICHIA COLI CELLS
AFFECTED BY MODERATE PULSED ELECTRIC FIELDS

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Abstract:
Among different non-thermal processing methods used in food technologies, pulsed electric field (PEF) treatment is one of the most promising. A number of new PEF applications were demonstrated for microorganism inactivation of liquid food. In PEF, the cellular damage is achieved during high voltage stimulations and its moderate processing conditions are the keys to produce high quality products with sufficient nutritional contents as well as desirable colors and taste. The influence of the initial cell concentration is not yet fully understood and contradictory results are published. This work discusses PEF-induced effects in treatments of Gram negative Escherichia coli cells suspensions. Treatment was done in a static 2 mm electrode gap electroporation cuvette (Eppendorf Laboratories). The PEF generator 1500V-20A (Service Electronique UTC, France) provided monopolar pulses of near-rectangular shape, electric field strength E was within 2.5-7.5 kV/cm, pulse duration t was 300 μs, pulse repetition time Δt was 100 ms, effective PEF treatment time t_{PEF} was within 0.075-0.75 s, the medium temperature was maintained at 25°C. The cells viability was assayed by counting colony forming units (CFU). The initial cells concentration in distilled water was in the range of 0.5 · 10^8 and 5.9 · 10^8 CFU. Results show that the cells inactivation efficiency of MPEF increases with field strength, effective time of treatment and initial cells concentration. In order to obtain a deep knowledge of the damage kinetics of PEF, the establishment of an appropriate model is necessary; Simplified Hulsheger model and Peleg model were tested. With a regression coefficient R² equal to 0.9935, the critical intensity E_c obtained was equal to 1.9 kV/cm which is approximately five times lower than the critical intensity found by Hulsheger (10 kV/cm). Concerning Peleg model E_d was found to be equal to 3.309 kV/cm which is lower than E_d found by Peleg, 1995. A new model that may help in obtaining more reasonable simulation results and more agreements with experimental data is established.
ENHANCING THE AQUEOUS EXTRACTION OF STEVIA GLYCOSIDES FROM STEVIA REBAUDIANA LEAVES UNDER THE ACTION OF ELECTRIC DISCHARGE PRETREATMENT

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Abstract:  
The Extraction of the natural sweetening glycosides from Stevia rebaudiana leaves (Steviosides) can be processed by several methods such as hot water, alcohols (methanol or ethanol) or supercritical fluid extraction. Water is an environmentally friendly and economically preferable alternative to harmful organic solvents in spite of its inferior efficacy. Therefore, different high voltage electric discharge (HVED) treatments (Intensity 40 KV cm\textsuperscript{-1} and 50, 125, and 200 pulses) were used to enhance the water extraction of Stevioside. The extraction kinetics was studied at two temperatures (20 and 55°C) with agitation during 60 minutes. Experimental data were fitted by simplified empirical model. The extract quality was determined by spectrum scanning at wavelength range 190-700 nm for all treatments. The results indicated generally that the HVED treatments accelerated the extraction process of glycosides with a better quality comparing with a control at the end of extraction time.

Key words: High voltage electric discharge; stevia; stevioside; sweetener; extraction.

1 Introduction  
Stevia rebaudiana is a plant native of South America and its active constituents are considered, by Food Science researchers, as the “sweeteners of the future”. Stevia sweetener is a low calorie, heat stable, intensely sweet (300 times sweeter than sucrose at 0.4% concentration), proven safe by many years of continual use in Japan since its introduction to the market in 1970. The consumption of steviosides in Japan has accounted for more than 20 percent of low calorie sweeteners. Interest in this low calorie sweetener was renewed in North America during the 1990s because of export opportunities and positive nutritional properties [1].  
Stevia leaf has several sweetener glycosides such as stevioside, rebaudioside A, B, C, D, E and dulcosides A and B. Stevioside represents the highest proportion (5-10%) followed by rebaudioside A (2-4%), rebaudioside C (1-2%); while other constituents are present in smaller concentrations [2].  
Several processes of extraction of the sweetening substances of Stevia presented in literature follow approximately the same methodology. First, extraction from the leaves of Stevia is made with water or alcohols (ethanol or methanol); the obtained extract is in the form of a solution loaded with colloidal particles of dark brown colour, containing all the active principles, pigments of the leaf, soluble polysaccharides and other impurities. Some primary processes remove the greases, essential oils, chlorophyll and other non polar principles from the leaves with solvents such as chloroform or hexane [3].
Supercritical fluid extraction (SFE) employing CO₂ as a medium for extraction is faster than the previous methods. However, pure CO₂ does not have sufficient solvation power for polar stevioside and therefore it requires the addition of polar cosolvent. Investigated cosolvents have included methanol, water and/or ethanol, a mixture of methanol and water [4]. Methanol showed better extraction ability for isolation of stevioside from Stevia rebaudiana leaves than water within the range 110–160 °C.

Water represents the green alternative to methanol [5], but thermal degradation of stevioside was equally observed in both used solvents within a temperature range of 70–160 °C [5]. Different ways (mechanical, biological, thermal, and electrical) may be applied as pretreatments before and/or during extraction [6]. High-pulsed electric field as a new nonthermal method of foods preservation has been intensively studied [7]. Successful application of pulsed electric fields encouraged the studies focusing on electrical extraction technologies [8].

The treatment by high voltage electrical discharges (HVED) was also proposed for different physical effects as shock waves, cavitation and particles breakage [9]. In paper [10] the HVED were used for acceleration of aqueous extraction from the dry crop products (peat, tea, soya beans). Also, the extraction kinetics was 40 to 50 times faster than the traditional infusion and the obtained yield was about 95%.

The improvement of the aqueous extraction by HVED has been shown for the linseeds oil after pressing. Three successive treatments were done (40 KV, 300 impulses and 0,5 Hz) and almost all the oil quantity was extracted in less than 40 minutes, whereas in absence of HVED, the kinetics were rather slow. More than 300 minutes were required to extract only half of oil extracted with HVED water extraction [11, 12].

The electric discharge treatment with 40 KV (HVED) was found to accelerate the extraction of aqueous solutions of two dry products (dried leaves of black tea and mint). Solution Brix was raised and the extraction kinetics were accelerated by HVED treatment [13].

The objective of this investigation was to study the effect of HVED treatment (0, 50, 125, 200 pulses) on the kinetics of solute extraction from stevia leaves and on the extract quality. Extraction experiments were performed at two different temperatures: 20 and 55 °C.

### 2 Materials and methods

#### 2.1 Raw materials

_Stevia rebaudiana_ leaves, planted under organic conditions, were supplied from Kato Aromatic Company (Giza, Egypt).

The humidity of raw material and total carbohydrate were ~7%, 57% respectively. The composition of leaves is presented in table (1) [14].

#### 2.2 Methods

##### 2.2.1. Electric discharge pretreatment

The generator (Polytechnic University of Tomsk, Russia) provided 40 kV discharges during a few microseconds in a treatment cell with 1 L capacity (fig. 1). These HVED were applied directly to the dried stevia leaves immersed in water with a ratio (r) 1:16 respectively at two temperatures (20 °C and 55 °C). Electrical discharges were applied with a 0.5 Hz frequency, which was imposed by the generator.

<table>
<thead>
<tr>
<th>Nutrient composition</th>
<th>per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate</strong></td>
<td></td>
</tr>
<tr>
<td>Moisture (g)</td>
<td>10</td>
</tr>
<tr>
<td>Energy (K cal)</td>
<td>270</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>10</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>57</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>10</td>
</tr>
<tr>
<td>Crud fibre (g)</td>
<td>14</td>
</tr>
</tbody>
</table>

Tab. 1: Composition of dried leaves

(Stevia rebaudiana)
2.2.2 Aqueous extraction of glycosides

The HVED-pretreated mixture was transferred to the extraction unit. Extraction was effected under gentle rotation at 170 rpm, and two temperatures: ambient (20°C) and 55°C for 60 min. The extraction unit was closed to avoid evaporation. All extracts were filtered with filter paper Rotilabo 601P, Ø 8 µm (Carl Roth, Germany).

![Scheme of the High voltage electric discharge generator](image)

2.2.3 Quantitative and qualitative analyses of extract

The soluble solids (°Brix), as an indication of glycosides extraction, were determined by a refractometer Atago-PR-101 (Brix 0-53% ± 0.10) (Sciencelab.com, Houston, USA). The yield of extraction (Y%) versus time was calculated as

\[ Y = \left( \frac{\text{°Brix}}{\text{°Brix}_0} \right) \times 100 \]

where °Brix∞ was calculated as

\[ \text{°Brix}_\infty = \left( \frac{\text{°Brix}_0}{r + 1} \right) \]

and °Brix0 is the concentration of solute in fresh leaves tissue in g solute per 100 g juice [15].

The crude extract of stevia contains impurities (protein, pectin, pigments and flavonoids) which are practically soluble in water. So, the wavelength ranged between 190 and 900 nm used to determine the extract quality (with the precision of ±1 nm) and recorded by Secomam Anthelie Advanced spectrophotometer (Domont, France).

2.2.4 Empirical model for data fitting

The following empirical equation fits rather well both pressure and solute extraction data [16]:

\[ m = \frac{t}{1/v_0 + at} \quad (1) \]

where \( m \) is the mass of solute (kg) extracted in time \( t \) (s), \( v_0 \) is the initial velocity of solute extraction (kg/s); \( a = 1/m_\infty \), where \( m_\infty \) is the mass of solute (kg) extracted after the infinite time. The coefficients \( v_0 \) and \( a \) of Eq. (1) can be found after the data linearization:

\[ \frac{t}{m} = \frac{1}{v_0} + at \quad (2) \]

2.2.5 Statistical analysis

The results reported in this work are the average of at least three measurements. The data presented in tables and figures represents mean values ± standard deviation (n=3). Significance levels (p<0.05) were evaluated using statistical package of Microsoft Office software.

3 Results and discussions

3.1 Effect of HVED pretreatments on the extraction kinetics

The extraction kinetics at ambient temperature (20°C) presented in fig. (3a), shows changes in the yield of solute after HVED pretreatments followed by the time of maceration (60 min) at ambient temperature. There were noticeable changes between the control (without HVED) and the treated samples in the extraction kinetics. Also, the yield was increased by increasing
the pulses number. After 30 min, the extraction yield reached 78% for the control sample against 85, 88, and 90% for the HVED with 50, 125, and 200 pulses respectively, i.e., the HVED pretreatments enhanced the extraction yield in 1.09, 1.13, and 1.15 times. It may be noticed that the time factor has maximized the extraction yield either for control or pretreated samples. For control, the extraction extent increased by 14 times after 1hr, while for the HVED treatments (50, 125, 200 pulses) it increased only by 1.9, 1.5, and 1.3 times respectively. It can also be observed that the HVED can decrease the time required for high extraction yield. It rests to compare the constitution of the obtained extracts in each case to determine the impact of this pretreatment.

![Graph](http://example.com/graph1.png)

**Fig. 3:** Yield of extraction during 60 min at 20 °C (a) and 55 °C (b) from stevia leaves treated with HVED at different number of pulses (50, 125, and 200).

The data introduced in fig. (3b), show the effect of thermal extraction (55°C) in addition to HVED treatments on the yield kinetics. The heat treatment (55°C) increased just slightly the extraction yield. On the other hand, the extraction yield of HVED-pretreated samples increased only on 6, 5.5 and 4.7% when subjected to 55°C after 30 min, compare to corresponding treatments at 20°C. It can also be observed that the extraction yield has reached its maximum (92.5%) after 60 min tof HVED treatment with 125 pulses at 20°C. The HVED treatment with 200 pulses permitted to attain a higher extraction yield (99.7%).

Applying the thermal treatment accelerated the extraction kinetics due to the increased solute diffusivity [15, 18].

### 3.2 The empirical model

Eq. (1) represents a straight line in coordinates \( t/m \) versus \( t \) which gives the values of coefficients \( 1/v_0 \) and \( a \). The curve \( m(t) \) at \( t \to \infty \) approaches to the constant value of \( m_\infty = 1/a \).

This value characterizes a maximal mass of liquid, which can be extracted from stevia leaves at given conditions. As can be seen from Fig. 4 there is an increase of \( v_0 \) values for higher pulses number and also for higher temperature. This can be explained by increased number of destroyed cells.

![Graph](http://example.com/graph2.png)

**Fig. 4:** Curve of \( v_0 \) as a function of pulses number
3.3 Extracted solutes

The crude water extracts were scanned by UV/Visible light absorption to reveal the interference of juice impurities released during extraction. The results in fig (5) show that samples contained different solutes at the wave length of 250-400 nm. Heat treatment has increased the extraction of impurities. The HVED pretreatment (200 pulses) has relatively reduced the content of impurities especially when the extraction was conducted at room temperature.

The HVED with different number of pulses (50-200) seems to reduce the total content of impurities when the extraction was conducted at 20°C. However, the HVED treatment at 55°C did not confirm this tendency.

The juice absorbance at the wavelength $A_{\text{max}}=340$ nm is presented in figure (6). Extracts treated at ambient temperature had a lower absorbance than ones treated at 55°C. The effect of HVED at different pulses number was noticeable at ambient temperature.

In thermal extraction, the effect of heat resulted in a very small difference between the treatments with different number of pulses; these results of the crude extract quality may help for further purification procedures to obtain a clear extract.

4. Conclusion

HVED noticeably enhanced the yield of extraction from *stevia glycosides*. This treatment can contribute to reduce the duration of maceration. The clarity of extracts pretreated by HVED was higher for the treatment at ambient temperature.

Fig. 5: Spectroscopic scanning of the water extract from *Stevia rebaudiana* leaves pretreated with HVED (200 pulses) and extracted at 20 °C and 55 °C.

Fig. 6: Absorbance at $A_{\text{max}}=340$ nm of the stevioside water extract at two temperatures (20, 55 °C) with different pretreatments by HVED.
References


INCREASE OF THE ROUGHNESS OF THE ELECTRODE SURFACE DUE TO THE EXPOSURE TO HIGH-VOLTAGE ELECTRIC PULSES AS REVEALED BY ATOMIC FORCE MICROSCOPY

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Abstract:
The changes of the surface topography of stainless-steel and aluminium electrodes occurring due to the action of electric pulses which are commonly utilized in cell electroporation procedures, have been studied by using atomic force microscopy. The surfaces of the polished stainless-steel electrodes were smooth - the average roughness was 13-17 nm and the total roughness 140-180 nm. The total roughness of the aluminium electrodes was about 320 nm. After the treatment of the chambers filled with 154 mM NaCl solution by a series of short (20–40 µs), high-voltage (4 kV) pulses with the total dissolution charge of 0.20–0.26 As/cm², the roughness of the surface of the electrodes has increased, depending on the total amount of the electric charge that has passed through the unit area of the electrode. Up to a two- and three-fold increase of the surface roughness of the stainless-steel and aluminium anodes respectively was observed due to the dissolution of the anode material. Therefore, the use of high-voltage electric pulses leads to the increase of the inhomogeneity of the electric field at the electrode, which leads to the non-equal treatment of each cell and facilitates the occurrence of the electric breakdown of the liquid samples-duration pulse.

Key words: electroporation, aluminium, stainless-steel, electrochemical reactions

1 Introduction

Cell electroporation – a temporal increase of cell membrane permeability due to the action of pulses of strong electric field (up to 300 kV/cm) – is widely used in cell biology, biotechnology and medicine [1]. However, when a high voltage is applied to the electrolyte solution, a cell membrane permeabilization is not the only consequence - a variety of electrolysis reactions occur at the electrode-solution interfaces [2]. These reactions cause changes of the chemical composition [3, 4] or pH [5] of the experimental medium.

For many applications of cell electroporation, it is necessary to apply the pulses of as homogeneous electric field as possible [6]. Meanwhile, the rough electrode surface creates local enhancements of the electric field, which lead to the inhomogeneity of an electric treatment of each cell and can facilitate the occurrence of the electrical breakdown of the liquid samples [7].

By using AFM, we have recently demonstrated that the roughness of the stainless-steel anode surface increased due to its dissolution occurring under the action of high-voltage electric pulses commonly utilized for cell electroporation [7]. Here, the changes of the
topography of the aluminium and stainless-steel anode surface, occurring under similar conditions, were compared using the same approach.

2 Materials and methods

Commercially available cuvettes with aluminium electrodes (inter-electrode distance - 2 mm, chamber volume - 1ml, CUV-02, Cyto Pulse Sciences, Inc., Columbia, MD, USA) and a home made cuvette with stainless steel electrodes (inter-electrode distance - 1 cm, chamber volume - 2 ml) were studied. The dissolution of the electrode was obtained by discharging several times a high voltage 1 μF capacitor (charged to 4 kV) through the chamber filled with a 154 mM NaCl solution. Three-dimensional surface topography of the electrodes was studied with atomic force microscope Quesant Qscope-250 (Ambios Technology Company, Santa Cruz, CA, USA). The data were acquired in the contact mode of AFM with silicon cantilevers. The lever parameters of the microfabricated V-shaped silicon cantilevers were: length 200 μm, width 40 μm, thickness 1 μm, resonant frequency 32 kHz, force constant 0.35 N/m, tip curvature radius 10 nm. A set of frames of the same size (30×30 μm) was taken from different areas of the sample surface. All measurements were done in ambient environment at the room temperature. AFM images were processed with software SPIP v. 2.3206 (Image Metrology A/S, Denmark) [7].

Fig. 1: AFM three dimensional images of the stainless-steel anode surface: (A) prior to the exposure by high-voltage (4 kV) electric pulses and after the exposure to (B) 100 exponential pulses with the duration of about 20 μs and dissolution charge $Q_{diss} = 0.20 \text{As/cm}^2$. Scanning area 30 x 30 μm²; z range: (A) 154.02 nm, (B) 301.2 nm.

3 Results and discussion

We have studied the changes of the topography of the of stainless-steel and aluminium electrodes, occurring under the action of high-voltage electric pulses commonly utilized for cell electroporation. At first, the surfaces of the polished stainless-steel and intact aluminium electrodes prior to the electric treatment were investigated by using atomic force microscopy. Fig. 1A shows a typical three-dimensional image of the surface of the freshly polished stainless-steel anode. The scanning area was 30x30 μm².
The height profile of the electrode surface along the white line is shown in Fig. 2A. It can be seen from this figure, that the surface is rather smooth - the average roughness, $R_a$, calculated for the anode surface three-dimensional image shown in Fig. 1A was 13-17 nm and the total roughness (the vertical distance from the deepest valley to the highest peak), $R_t$, was 154 nm. Long straight narrow (~1 µm) not deep (10-50 nm) scratches seen on the electrode surface are most likely a result of the mechanical polishing process.

Although the surface of the untreated aluminium electrode was not smooth - the total roughness of the surface, $R_t$, was about 350 nm and the average roughness was 25-35 nm (Fig. 3A) – no sharp peaks were observed on the surface. The height profile through the electrode surface is shown in Fig. 4A. Then, the chambers filled with 154 mM NaCl solution were exposed to a series of short (20-40 µs), high-voltage (4 kV) pulses. After the treatment, the roughness of the surfaces of the electrodes has increased due to the dissolution of the anode material (mainly Fe$^{2+}$/Fe$^{3+}$ and Al$^{3+}$). The character and extent of the changes were dependent on the anode material and the total amount of the electric charge that has passed through the unit area of the electrode.
Fig. 4: Cross-sections of the stainless steel anode surface (height profiles) along white lines A in Fig. 1: (A) prior to the exposure by high-voltage (4 kV) electric pulses and (B) after the exposure to 100 exponential pulses with the duration of about 20 µs (dissolution charge $Q_{\text{dis}} = 0.20 \text{ As/cm}^2$).

A three dimensional image of Fig. 1B provides quantitative information on the stainless-steel anode surface topography after the exposure to a series of 100 exponential electric pulses giving the total dissolution charge $Q_{\text{dis}} = 0.20 \text{ As/cm}^2$. The height profile of the electrode along the white line shown in Fig. 1B is presented in Fig. 2B. As in the previous case, the scanning area was 30x30 µm. It can be seen that the surface of the stainless-steel anode has become much rougher. The total roughness, $R_s$, exceeded 300 nm (about two times).

After the treatment by 90 short exponential (time constant of about 40 µs) high – voltage (4 kV) electric pulses, surface topography of the aluminium anode has changed remarkably (Fig. 3 B). The total roughness exceeded 0.94 µm for the dissolution charge of 0.26 As/cm$^2$. So, the total roughness of the anode has increased about 3 times (Fig. 4 A). In addition, the topography of aluminium anode surface was altered too – it consisted of small 2-3 µm-size grains (Fig. 3 B).

The AFM images showed the changes of the topography of stainless-steel and aluminium electrodes due to release of metal ions (mainly Fe$^{2+}$/Fe$^{3+}$ and Al$^{3+}$) from them as a result of the oxidation of the metal. The authors hope, that the approach for studying the changes of the surface topography by using AFM, utilized here and the results obtained can be helpful in finding the solutions for increasing the longevity of the electrodes as well as avoiding the problems arising due to the anodic half- reactions and contamination of the solution by the release of metal ions from the electrodes.or presented at the end of the article after the references.

4 Conclusions

It can be concluded that the changes of the surface roughness of stainless-steel and aluminium anodes occur due to the action of electric pulses which are commonly utilized in cell electroporation procedures. The character and extent of these changes depend on the anode material and the total amount of the electric charge that has passed through the unit area of the electrode. Up to a two– and three–fold increase of the surface roughness of the stainless-steel and aluminium anodes respectively has been observed. Increased roughness creates local enhancements of the electric field at the interface between the solution and the electrode surface and can facilitate the occurrence of the electric breakdown of the liquid samples.
5 Acknowledgement

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References


PULSES OF MILLISECOND-DURATION CREATE LARGER PORES THAN PULSES OF MICROSECOND-DURATION

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Abstract:

Modeling studies have revealed that the pulse of nanosecond-duration should create smaller pores than the pulse of micro-millisecond duration. It can be expected, that similar differences may also exist between the pore populations created by the pulses of micro- and millisecond-durations. Here, we compared the size of the pores created by square-wave electric pulses with the duration of 100 $\mu$s and 2 ms. For the cells studied (mouse hepatoma MH-22A and Chinese hamster ovary cells), short 100 $\mu$s-duration pulse created smaller pores than longer 2 ms-duration pulse.

Key words: electropermeabilization, mouse hepatoma, rat glioma, CHO cells

1 Introduction

For many applications of cell electroporation, it is desirable to know the size of the pores created in the cell membrane. Although, the size of the pores created in the cell membrane by electric pulses has been estimated numerous times [1, 2, 3, 4], there is a lack in the studies in which the pore sizes would be estimated for different pulse durations. Meanwhile, modeling studies have revealed that the pulse of nanosecond-duration should create smaller pores than the pulse of micro-millisecond duration [5]. At the same time, the number of pores created by nanosecond-duration pulses should be by 2–3 orders of magnitude higher than in the case of longer pulse [5]. It can be expected, that similar differences may also exist between the pore populations created by the pulses of micro- and millisecond-durations.

Here, we compared the size of the pores created by square-wave electric pulses with the duration of 100 $\mu$s and 2 ms. This has been done by determining the fraction of cells the membranes of which have become permeable to potassium ions (molecular weight $M_r = 39$ Da, $r \approx 0.16-0.22$ nm) and bleomycin ($M_r \approx 1500$ Da, $r \approx 0.8$ nm) as well as the fraction of the cells that were killed by the electric treatment. Experiments were done with mouse hepatoma MH-22A and Chinese hamster ovary (CHO) cells.

2 Materials and methods

The culture medium consisted of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% L-glutamine, 100 U/ml penicillin, and 125 mg/ml streptomycin (all from Sigma-Aldrich Chemie, Steinheim, Germany). As an electroporation medium, either the culture medium (for the determination of cell electroporation) or minimum essential medium Eagle (Sigma-Aldrich Chemie) was used. Bleomycin hydrochloride (Bleocin, Nippon Kayaku, Tokyo, Japan) was obtained as a crystalline powder and dissolved in sterile 0.9% NaCl solution (Balkanpharma-Troyan, Troyan, Bulgaria). Calibration solutions containing 100 nM-100 mM KCl were prepared by diluting a stock solution of 100 mM KCl and adding 150 mM sodium chloride and 8 mM sodium benzoate [6, 7].

Experiments were performed with mouse hepatoma MH-22A, Chinese hamster ovary (CHO), and rat glioma C6 cells. Cells were grown in monolayer cultures in 25-cm$^2$ (60-70-ml) or 75-cm$^2$ (200-ml) flasks at 37 °C and 5% CO$_2$ in water-jacketed incubator IR AutoFlow NU-2500E (NuAire, Plymouth, MN, USA). When cells reached confluence they were trypsinized for 2 to 10 min with 2 ml of 0.25% trypsin-0.02% EDTA solution (Sigma-Aldrich Chemie). When cells detached from the flask bottom, cell suspension was
supplemented with 2 ml culture medium. After centrifugation of the suspension for 5 min at 1000 rpm, cells were resuspended in the culture medium at approximately 2-5×10^7 cells/ml (for the determination of the fraction of electroporated cells) or in a minimum essential medium Eagle at a concentration of approximately 1×10^6 cells/ml (for the determination of the fraction of cells permeable to bleomycin and killed cells). When cell electroporation was determined, the cells were kept for 60–70 min at room temperature (20–21 °C) before electroporation [7].

A 50-µl droplet of cell suspension was placed between a pair of flat stainless-steel electrodes and subjected to a single square-wave electric pulse. The distance between the electrodes was 2 mm. After the electric treatment, the cells were immediately transferred to a chilled Eppendorf tube, kept on ice for 5-10 min, and then kept for 30-40 min at 10-11 °C to prevent pores from closing and to allow equilibration between intracellular and extracellular K⁺ concentrations. Then, the extracellular potassium concentration was measured by means of a mini K⁺-selective electrode and the fraction of electroporated cells was determined [6, 7].

The fraction of the dead cells and the cells whose membrane has become permeable to bleomycin were determined from the reduction of the cell viability [8, 7]. Cell viability was determined by means of a colony-forming assay [9].

3 Results and discussion

The fraction of electroporated cells can be estimated by determining the extent of the release of intracellular K⁺ ions from the cells exposed to an electric pulse [6, 7]. In this study, this approach was used to obtain the dependences of the fraction of electroporated cells on the pulse intensity. The cells were exposed to a single square-wave electric pulse with the duration of 100 μs or 2 ms the amplitude of which was varied from 0.2 to 2.4 kV/cm.

![Graph](image1.png)

**Fig. 1:** The dependences of electroporated, permeabilized to bleomycin, and dead cells on the amplitude of a square-wave electric pulse with the duration of 100 μs obtained for (A) mouse hepatoma MH-22A and (B) Chinese hamster ovary cells.

At first, the dependences of the fraction of electroporated and dead cells as well as the cells permeable to bleomycin on the pulse intensity were obtained for the cells exposed to a single square-wave electric pulse with the duration of 100 μs. The results for mouse hepatoma MH-22A and Chinese hamster ovary cells are shown in Fig. 1. It can be seen, that in the large portion of electroporated cells (permeable to K⁺ ions) the pores created by an electric pulse with the duration of 100 μs are smaller than the molecule of bleomycin. For example, exposure of cells by an electric pulse with the amplitude of 0.8 kV/cm leads to electroporation of 68-75 % of cells. However, the pores permeable to the molecules of bleomycin were created only in 24-38 % of electroporated cells. This means that the pores created in these cells were large enough to allow potassium ions (r ≈ 0.16-0.22 nm) to leave the cells but small enough to prevent bleomycin (r ≈ 0.8 nm) from reaching the cell cytosol.
In the case of 2 ms-duration pulse, the curves showing the dependence of the fraction of the cells that have become permeable to bleomycin are close to the ones showing the release of intracellular potassium ions. That is, irrespective of the amplitude of an electric pulse, in all cells that were electroporated the pores created were larger than the size of the molecules of bleomycin. This is strong evidence that the pores created by the pulse with the duration of 2 ms are larger than the pores created with a 100 µs-duration pulse.

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 2:** The dependences of electroporated, permeabilized to bleomycin, and dead cells on the amplitude of a square-wave electric pulse with the duration of 2 ms obtained for (A) mouse hepatoma MH-22A and (B) Chinese hamster ovary cells.

4 Conclusions

For the cell lines studied (mouse hepatoma MH-22A and Chinese hamster ovary cells), the size of the pores created by a square-wave electric pulse depended on the pulse duration. Short 100 µs-duration pulse created smaller pores than longer 2 ms-duration pulse.

5 Acknowledgements

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References


COMPARISON OF ELECTROPORATION THRESHOLD OF DIFFERENT CELL LINES

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Abstract:
The electroporation threshold was compared at various electric pulse durations for two non-tumor cell lines (human erythrocytes and Chinese hamster ovary cells) and one tumor cell line (rat glioma C6 cells). First, the dependences of the fraction of electroporated cells on the pulse intensity were obtained for the cells exposed to single square-wave electric pulses with the durations of 0.02–2 ms. Then, the average cell radii were measured for each cell line and the transmembrane potential induced by the external electric field was calculated. The obtained values of the transmembrane potential were in the range of 470–910 mV and decreased with increasing pulse duration. The obtained dependences of the transmembrane potential required to electroporate 50% of cells on the pulse duration were close to each other for all cell lines studied.

Key words: electroporation, human erythrocytes, rat glioma, CHO cells

1 Introduction

The permeability of the cell membrane can be modified by exposure of cells to high-voltage electric pulses, which leads to the creation of pores in the cell membrane (electroporation) [1]. When using cell electroporation in clinics, e.g., for tumor and gene therapy, it is important to know in advance whether and how many of cells will become electroporated as a result of a particular electric treatment. This could be achieved by using theoretical models of electroporation [2], optimized by using real experimental data on cell electroporation [3; 4].

There are theoretical models allowing to obtain theoretical relationships between the parameters of the electric treatment resulting in cell electroporation for any type of an electric treatment [4; 5]. However, it is difficult to predict individual responses of different cells to electric treatment [6; 7], because there are no studies in which the electroporation threshold would be compared for different cell lines. Either the electric field parameters needed for the increase of the cell membrane permeability to a particular substance (electropermeabilization) [6] or cell viability [7] have been determined for several cell lines. As a result, the fraction of electroporated cells still needs to be determined empirically for each cell line [7].

The aim of this study was to compare the electroporation threshold for three different cell lines at various electric pulse durations (20 µs – 2 ms) in vitro. Experiments were performed with one tumor (rat glioma C6 cells) and two non-tumor (human erythrocytes and Chinese hamster ovary cells) cell lines.

2 Materials and methods

The culture medium consisted of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% L-glutamine, 100 U/ml penicillin, and 125 mg/ml streptomycin (all from Sigma-Aldrich Chemie, Steinheim, Germany). Electroporation media: 24.5 mM sucrose and 124.7 mM NaCl (for human erythrocytes) and the culture medium (for Chinese hamster ovary and rat glioma C6 cells). Calibration solutions containing 100 nM - 100 mM KCl were prepared by diluting a stock solution of 100 mM KCl and adding 150 mM sodium chloride and 8 mM sodium benzoate [8; 9].
Experiments were performed with human red blood cells, Chinese hamster ovary (CHO), and rat glioma C6 cells. Blood was collected on sodium citrate and the erythrocytes were isolated by centrifugation. The erythrocytes were then washed three times with isotonic sodium chloride solution and suspended in the electroporation medium at a volume concentration of 2–2.5 % [8]. CHO and rat glioma C6 cells were grown in monolayer cultures in 75-cm² (200-ml) flasks at 37 °C and 5% CO₂ in water-jacketed incubator IR AutoFlow NU-2500E (NuAire, Plymouth, MN, USA). When cells reached confluence they were trypsinized for 2 to 10 min with 2 ml of 0.25 % trypsin-0.02% EDTA solution (Sigma-Aldrich Chemie). When cells detached from the flask bottom, cell suspension was supplemented with 2 ml culture medium. After centrifugation of the suspension for 5 min at 1000 rpm, cells were resuspended in the culture medium at approximately 2-5×10⁷ cells/ml and kept for 60–70 min at room temperature (20–21 °C) [9].

A 50-μl droplet of cell suspension was placed between a pair of flat stainless-steel electrodes and subjected to a single square-wave electric pulse. The distance between the electrodes was 2 mm. After the electric treatment, the cells were immediately transferred to a chilled Eppendorf tube, kept on ice for 5-10 min, and then kept for 30-40 min at 10-11 °C to prevent pores from closing and to allow equilibration between intracellular and extracellular K⁺ concentrations. Then, the extracellular potassium concentration was measured by means of a mini K⁺-selective electrode and the fraction of electroporated cells was determined [8; 9].

The average cell diameters were determined by measuring the cell diameters with an improved Neubauer haemocytometer (Heinz Herenz Medizinalbedarf, Hamburg, Germany) by using digital video camera MOTIKAM 2001 connected to optical microscope MOTIK BA400 (both from MOTIK China Group, Ltd., Xiamen, China).

3 Results and discussion

When the cell is electroporated, K⁺ ions leak out of the cell down their concentration gradient until the equilibration between intracellular and extracellular K⁺ concentrations occurs. So, the fraction of electroporated cells can be determined from the extracellular concentration of K⁺ ions [8; 9]. In this study, this approach was used to obtain the dependences of the fraction of electroporated cells on the pulse intensity. The cells were exposed to a single square-wave electric pulse with the duration of 0.02–2 ms the amplitude of which was varied from 0.6 to 2 kV/cm. These dependences obtained for CHO cells are shown in Fig. 1.

![Fig. 1: Dependences of the fraction of electroporated Chinese hamster ovary cells on the amplitude of an electric field pulse for various pulse durations. Cells were exposed to a square-wave pulse with a duration of 20 μs – 2 ms and the fraction of electroporated cells was determined from the amount of potassium ions released from the cells.](image-url)
It can be seen that increasing the intensity or the duration of the electric field pulse increased the fraction of electroporated cells (Fig. 1). The dependences of the fraction of electroporated cells on the pulse intensity were obtained for human erythrocytes and rat glioma C6 cells too (data not shown).

From the relationships of the fraction of electroporated cells on the pulse amplitude obtained at different pulse durations, the pulse amplitude inducing electroporation of 50% of cells, $\Delta E_{50\%}$, can be estimated for each pulse length. The dependence of $\Delta E_{50\%}$ on the pulse duration obtained for human erythrocytes, Chinese hamster ovary, and rat glioma C6 cells are shown in Fig. 2. It can be seen that electroporation of CHO and rat glioma C6 cells require the electric pulses of similar amplitude. But, to electroporate human erythrocytes the pulse of much higher amplitude has to be used.

However, the transmembrane potential generated by the external electric field depends on the cell radius [10]. Because human erythrocytes, CHO, and rat glioma C6 cells differ in their sizes, the electric field strength necessary to electroporate the cells of different sizes cannot be compared. Only the maximal transmembrane potential (at the polar regions of the cell) induced by the external electric field can be compared. So, the transmembrane potential inducing electroporation of 50% of cells, $\Delta \Phi_{m50\%}$, was calculated from equation [10]:

$$\Delta \Phi_m = 1.5E_{50\%}a,$$

where $E_{50\%}$ is the electric field strength inducing electroporation of 50% of cells and $a$ is the cell radius which was 3.0 $\mu$m for human erythrocytes, 7.0 $\mu$m for chinese hamster ovary cells, and 6.5 $\mu$m for rat glioma C6 cells.

The obtained dependences of $\Delta \Phi_{m50\%}$ on the pulse duration are presented in Fig. 3. From Eq. 1 we get the values of the transmembrane potential in the range of 470-910 mV (Fig. 3), which are similar to the transmembrane potentials that have been reported as leading to membrane permeabilization when using the pulses of similar duration [6; 11]. It can be seen from Fig. 3 that the dependences of the transmembrane potential required to electroporate 50% of cells are very close for all three cell lines studied here.

**Fig. 2:** The dependences of (A) the amplitude of a square-wave electric pulse and (B) the maximal transmembrane potential (at the poles of the cell) required to electroporate 50% of cells on the pulse duration, obtained for human erythrocytes, CHO, and rat glioma C6 cells.

**Fig. 3:** The dependences of the maximal transmembrane potential (at the poles of the cell) required to electroporate 50% of cells on the pulse duration, obtained for human erythrocytes, CHO, and rat glioma C6 cells.
4 Conclusions

It can be concluded that the electric field strength necessary to electroporate cells depends on the cell size and decreases with increasing the amplitude or the duration of the pulse. But the transmembrane potential required to electroporate the cells and its dependences on the pulse duration are very close for human erythrocytes, Chinese hamster ovary (CHO), and rat glioma C6 cells.

5 Acknowledgements

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References

EFFECT OF PULSED ELECTRIC FIELDS ON AROMA COMPOUNDS AND SENSORY PROPERTIES OF APRICOT NECTAR

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Abstract:
Effects of pulsed electric field (PEF) processing as a function of electric field strength on aroma active compounds and sensory properties of apricot nectar was prompted in this study. Apricot nectar samples were processed by PEF as a function of electric field strength for 0(control), 17, 23 and 30 kV/cm with 3 µs pulse duration and 500 pps frequency. In order to perform sensory evaluation of apricot nectar, both control and PEF-treated samples were evaluated by at least 30 trained panelists for flavor-aroma, taste, consistency, aftertaste, sourness, sweetness and overall acceptability. For determination of aroma active compounds, relative amounts of each components presented in both control and PEF-treated samples were determined by GC-MS. Results revealed that measured sensory attributes of the control and PEF-treated samples were not significantly changed (P>0.05). Total of 28 aroma active compounds were determined by GC-MS. There was a significant difference in the amounts of hexanoic acid, butyl ester, linalool oxide trans, propanoic acid, propanedioic acid diethyl ester, triacetin, 2H-pyran-2-one, tetrahydro-6-pentyl-, gamma dodecalactone and ethyl citrate (P≤0.05). No significant difference was detected for other components (P>0.05). Although, there was a significant difference in some compounds after PEF treatment, this difference did not affect the sensory properties of the apricot nectar. It was concluded apricot nectar can successfully be processed by PEF without significantly affecting the sensory properties.

Key words: pulsed electric fields, apricot nectar, aroma active compounds, sensory evaluation.

1 Introduction

Pulsed electric field (PEF) processing of fruit juices such as apple [1], orange [2], tomato[3], orange-carrot mixture [4], other juices [5], milk products such as skim milk [6], yogurt [7], yogurt drink [8] and rice pudding [9] were successfully realized. Most of the studies involving PEF treatment usually focused on measurement of physical properties, sensory evaluation, shelf life extension and microbial inactivation. To our knowledge no studies were performed to determine the changes in the aroma active compounds of PEF-treated food products. Moreover, limited studies were performed determination of aroma active compounds of fruit and fruit juices [10]. Therefore, the objectives of the study were determination of aroma active compounds and sensory attributes of apricot nectar before and after PEF treatment and correlation of changes in sensory properties with changes in aroma active compounds after PEF treatment.
2 Materials and Method

2.1 Food samples

Freshly squeezed apricot nectar concentrates were obtained from Dimes Gida Sanayii ve Tic. A.S. (Tokat, Turkey) in aseptic pouches. The pouches were aseptically opened. The Brix of the nectar was adjusted to 11.2 and processed by PEF immediately.

2.2 Pulsed electric field processing

OSU-4A bench scale continuous PEF system (The Ohio State University, Columbus, OH, USA) equipped with six treatment chambers having 0.29 cm diameter and 0.23 cm gap distance was used. OSU-4A bench scale PEF generator provided square wave bipolar pulses. The post- and pre-treatment temperatures (t₂–t₁, t₄–t₃, and t₆–t₅) at outlet and inlet of each pair of treatment chambers were monitored by K type dual channel digital thermocouples (Fisher Scientific, Pittsburgh, PA, USA) attached to the outer surface of the thin wall stainless steel tubing. Specifications of the OSU-4A bench scale PEF units were 12,000 V max of output voltage, 60 A max of output current, 10,000 pulse per second (pps) max of repetition rate, 200–1200 Ω of load resistance, and 16 J of energy storage in the pulse generator when fully charged. For processing of apricot nectar 0 (control), 17, 23 and 30 kV/cm electric fields strengths were applied with 50 mL/min of flow rate, 3 µs of pulse duration, 500 pps of frequency and 131 µs of treatment time.

2.3 Sensory analyses

In order to perform sensory evaluation of apricot nectar, both control and PEF-treated samples were evaluated by at least 30 trained panelists for flavor-aroma, taste, consistency, aftertaste, sourness, sweetness and overall acceptability based on 9-point hedonic scale. The samples, after the PEF treatment, were immediately cooled down and served to panelists.

2.4 Determination of aroma active compounds

Direct solvent extraction of the samples was performed according to MILO and REINECCIUS [11]. After the extraction process, the samples were injected to gas chromatography/mass spectroscopy (GC/MS) and the amount of each component was calculated by methodology indicated in AVSAR et al. [12].

2.5 Data analyses

Data were analyzed by Minitab (13.2 version, Minitab Inc., State College PA, USA) by using one-way or two-way ANOVA at 95% confidence interval. Differences between electric field strength and treatment time were determined by Tukey’s multiple comparison test. Every experiment was repeated at least three times.
3 Results and Discussion

Total of 28 aroma active compounds were determined by GC-MS analysis. 2(3H)-furanone, 5-hexylidihydro-, 1,6-octadien-3-ol, 3,7-dimethyl-, propanoic acid, 2-methyl-, gamma dodecalactone, gamma dodecalactone and n-hexadecanoic acid had the higher concentration among all components. After data analysis it was revealed that there was no significant change for most of the components (P>0.05). However, there was a significant difference in the amounts of hexanoic acid, butyl ester, linalool oxide trans, propanoic acid, propanedioic acid diethyl ester, triacetin, 2H-pyran-2-one, tetrahydro-6-pentyl-, gamma dodecalactone and ethyl citrate (P≤0.05) (Table 1).

Sensory analysis of apricot nectar for flavor-aroma, color, taste, consistency, aftertaste, sourness, sweetness and overall acceptability is shown in table 2. It was revealed that there was no significant difference in the measured sensory properties of the apricot nectar (P>0.05).

Although there was a significant difference in some aroma active compounds this difference did not effect the sensory evaluation of the apricot nectar samples. It is possible that the measured significant changes in some compounds do not have a significant impact on measures sensory properties of the apricot nectar. It is also possible that sensory panel could not identify the small differences in the measured attributes. Thus, further studies need to be designed to explain impact of PEF on aroma compounds as well as sensory analysis of PEF-treated products.

Acknowledgements

We would like to thank The Scientific and Technological Research Council of Turkey (TUBİTAK) for supporting TUBİTAK KARIYER (project number 104O585) project and Dimes Gıda San ve Tic Ltd Şti (Tokat, Turkey) for providing fresh apricot nectar.
References


<table>
<thead>
<tr>
<th>Aroma active compounds</th>
<th>0</th>
<th>17</th>
<th>23</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Limonene</td>
<td>494.7±1.25a</td>
<td>483.2±28.31a</td>
<td>718.0±116.90b</td>
<td>647.5±116.90b</td>
</tr>
<tr>
<td>Butanoic acid, 3-methylbutyl ester</td>
<td>4693.5±128.87a</td>
<td>4962.9±41.80b</td>
<td>5405.0±423.91c</td>
<td>5747.6±423.91c</td>
</tr>
<tr>
<td>2-Butanone, 3-hydroxy-</td>
<td>441.8±9.72a</td>
<td>366.5±8.29b</td>
<td>462.7±26.30c</td>
<td>512.3±26.30c</td>
</tr>
<tr>
<td>2-Hexen-1-ol, acetate, (E)-</td>
<td>3191.4±168.24a</td>
<td>3158.9±15.29a</td>
<td>3768.7±110.43b</td>
<td>3797.8±110.43b</td>
</tr>
<tr>
<td>Hexanoic acid, butyl ester</td>
<td>4192.2±142.12a</td>
<td>4492.3±93.74b</td>
<td>4922.3±348.21b</td>
<td>5275.1±348.21b</td>
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<tr>
<td>Acetic acid</td>
<td>9941.9±659.02a</td>
<td>9772.6±99.59a</td>
<td>10252.8±224.21a</td>
<td>11342.7±224.21a</td>
</tr>
<tr>
<td>Linalool oxide trans</td>
<td>2440.1±104.60a</td>
<td>2536.4±27.18a</td>
<td>2702.1±188.23a</td>
<td>3003.1±188.23b</td>
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<tr>
<td>Propanoic acid</td>
<td>1830.5±71.42a</td>
<td>1405.0±17.85a</td>
<td>1481.1±30.09a</td>
<td>1622.2±30.09b</td>
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<td>1,6-Octadien-3-ol, 3,7-dimethyl-</td>
<td>28746.6±455.78a</td>
<td>27249.2±804.63ab</td>
<td>21756.6±2658.28b</td>
<td>32530.9±2658.28c</td>
</tr>
<tr>
<td>Propanoic acid, 2-methyl-</td>
<td>14559.6±543.73a</td>
<td>8415.5±6870.06a</td>
<td>15763.8±7026.71a</td>
<td>8557.6±7026.71a</td>
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<td>Propanedioic acid, diethyl ester</td>
<td>6716.9±124.00a</td>
<td>6495.6±242.74a</td>
<td>7196.8±150.35a</td>
<td>7713.3±150.35a</td>
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<tr>
<td>Acetic acid, phenylmethyl ester</td>
<td>2839.9±248.50a</td>
<td>2907.5±281.73a</td>
<td>3182.6±477.52a</td>
<td>3415.7±477.52a</td>
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<td>2,6-Octadien-1-ol, 3,7-dimethyl-</td>
<td>5219.6±73.75a</td>
<td>5491.1±138.47a</td>
<td>6113.9±368.44b</td>
<td>6281.3±368.44b</td>
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<td>Acetate</td>
<td>4416.6±189.65a</td>
<td>4492.9±114.05a</td>
<td>5130.2±204.21b</td>
<td>5310.2±204.21b</td>
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<td>Hexanoic acid, ethyl ester</td>
<td>994.0±89.99a</td>
<td>1025.3±83.68a</td>
<td>1109.4±17.46a</td>
<td>1229.1±17.46b</td>
</tr>
<tr>
<td>2,6-Octadien-1-ol, 3,7-dimethyl-, (E)-</td>
<td>1862.9±126.92a</td>
<td>2788.9±1257.42b</td>
<td>2121.4±258.96a</td>
<td>2366.3±258.96a</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td>820.7±95.40a</td>
<td>838.9±45.66a</td>
<td>809.9±106.13a</td>
<td>838.1±106.13a</td>
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<tr>
<td>Triacetin</td>
<td>1542.6±70.48ab</td>
<td>1571.2±73.14ab</td>
<td>1656.0±118.91ab</td>
<td>1811.2±118.91b</td>
</tr>
<tr>
<td>2(3H)-Furanone, 5-hexylidihydro-</td>
<td>32310.6±836.97a</td>
<td>33123.0±1653.87a</td>
<td>35799.6±2229.31a</td>
<td>37082.9±2229.31a</td>
</tr>
<tr>
<td>2H-Pyran-2-one, tetrahydro-6-pentyl-</td>
<td>3742.1±74.47a</td>
<td>3649.3±156.6a</td>
<td>3797.1±142.42a</td>
<td>4095.6±142.42a</td>
</tr>
<tr>
<td>Hexadecanoic acid, ethyl ester</td>
<td>602.8±88.46a</td>
<td>652.8±113.36a</td>
<td>715.1±145.58a</td>
<td>723.3±145.58a</td>
</tr>
<tr>
<td>2H-Pyran-2-one, 6-hexyltetrahydro-</td>
<td>7627.1±193.26a</td>
<td>8265.9±156.04a</td>
<td>8637.6±519.01a</td>
<td>8987.3±519.01a</td>
</tr>
<tr>
<td>Gamma dodecalactone</td>
<td>14451.1±471.15a</td>
<td>17509.7±1685.78a</td>
<td>16222.8±1127.89a</td>
<td>17039.4±1127.89a</td>
</tr>
<tr>
<td>Ethyl citrate</td>
<td>1529.8±07.28a</td>
<td>1822.2±449.22a</td>
<td>1914.5±467.10a</td>
<td>1963.6±467.10a</td>
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<tr>
<td>Linoleic acid ethyl ester</td>
<td>826.3±258.70a</td>
<td>864.9±208.79a</td>
<td>886.5±325.47a</td>
<td>917.9±325.47a</td>
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<td>9,12,15-Octadecatrienoic acid, ethyl ester</td>
<td>13139.30±1280.83a</td>
<td>12907.2±1252.28a</td>
<td>12732.5±1400.41a</td>
<td>14439.0±1400.41a</td>
</tr>
<tr>
<td>(Z,Z,Z)-n-Hexadecanoic acid</td>
<td>11772.6±9722.82a</td>
<td>10685.0±8656.80a</td>
<td>11039.0±10087.41a</td>
<td>11886.8±10087.41a</td>
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<tr>
<td>Squalene</td>
<td>2692.5±123.36a</td>
<td>2676.8±69.97a</td>
<td>2698.8±144.41a</td>
<td>2975.8±144.41a</td>
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<tr>
<td>Octadecanoic acid</td>
<td>1440.5±427.61a</td>
<td>2013.6±82.28a</td>
<td>2215.2±153.53a</td>
<td>2315.5±153.53a</td>
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Tab. 1: Aroma active components of apricot nectar
<table>
<thead>
<tr>
<th>Sensory properties</th>
<th>Electric field strength (kV/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Flavor-aroma</td>
<td>5.50±2.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Color</td>
<td>6.19±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Taste</td>
<td>5.84±1.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Consistency</td>
<td>5.80±1.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>5.84±2.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sourness</td>
<td>5.30±2.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweetness</td>
<td>5.23±2.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>6.19±1.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Tab. 2: Sensory analysis of apricot nectar
PULSED ELECTRIC FIELD PROCESSING OF POMEGRANATE JUICE

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Abstract:
Due to recent finding about positive health effect of pomegranate juice, it is one of the most consumed juices worldwide. However, heat processing of pomegranate juice causes deterioration of nutritional properties. Thus, alternatives such as pulsed electric field processing for pomegranate juice processing are in search. In this study, pomegranate juice was processed by 0 (control), 17, 23, 27 and 30 kV/cm electric field strength with 3 µs pulse duration and 500 pps frequency, and some important properties (pH, °Brix, conductivity, titratable acidity and color) were measured. In addition, inactivation kinetics of Escherichia coli O157:H7 and Staphylococcus aureus under the same PEF processing condition and different processing temperatures (5, 15, 25 and 35°C) were also studied. It was revealed that physical properties of pomegranate juice were not affected by PEF processing, however, inactivation and activation energy of E. coli O157:H7 and S. aureus was increased by both increased electric field strength and treatment temperature. As a result, pomegranate juice was successfully processed by PEF without significantly affecting the physical properties with significant amount of microbial inactivation.

Key words: pulsed electric fields, PEF, pomegranate juice, microbial inactivation

1 Introduction

Recent studies revealed that pomegranate juice may effectively slow the progression of prostate cancer, reduce cholesterol, reduced blood pressure (particularly systolic pressure) and slowed down LDL cholesterol (the bad cholesterol) oxidation due to the contained polyphenols, tannins and anthocyanins and interestingly high levels of antioxidants [1]. Heat processing provides safer pomegranate juice having longer shelf life; however, it causes degradation of abovementioned compounds and reduction of nutritive value. Therefore, studies are needed to determine the applicability of alternative technologies for pomegranate juice processing.

Pulsed electric field (PEF) processing, known as nonthermal preservation method, can be successfully applied to low viscosity high acidity food products such as pomegranate juice. Although different food products were processed by PEF [2-6], literature lacks information about PEF processing of pomegranate juice. Therefore this study was performed to determine the effect of PEF processing on some physical properties of pomegranate juice as well as microbial inactivation.
2 Materials and Methods

2.1 Food samples
Freshly squeezed pomegranate juice was obtained from Dimes Gida Sanayii ve Tic. A.S. (Tokat, Turkey) and processed by PEF immediately.

2.2 Pulsed electric field processing
OSU-4A bench scale continuous PEF system (The Ohio State University, Columbus, OH, USA) equipped with six treatment chambers having 0.29 cm diameter and 0.23 cm gap distance was used. OSU-4A bench scale PEF generator provided square wave bipolar pulses. For processing of peach nectar 0 (control), 17, 23 and 30 kV/cm electric fields strengths were applied with 50 mL/min of flow rate, 3 µs of pulse duration, 500 pps of frequency, and 131 µs of treatment time.

2.3 Test microorganisms and inactivation
*Escherichia coli* O157:H7 (EDL 931 04054) and *Staphylococcus aureus* (95047) after activation, were inoculated into pomegranate juice at the level of $10^6$-$10^7$ cfu/mL separately. For inactivation kinetics studies PEF processing were performed at 5, 15, 25 and 35°C. Both control and PEF-treated pomegranate juice samples were diluted with 0.1% peptone (Fluka, Germany) water and 100 µL of appropriate dilutions were plated.

2.4 Measurement of physical properties
pH, titratable acidity (%TA), °Brix, conductivity and color (L*, a* and b*) of pomegranate juice were measured.

3 Results and Discussion
There was no significant difference between control and PEF-treated pomegranate juice samples for pH, TA, °Brix, conductivity and color values of L*, a* and b* ($P>0.05$). Inactivation of both *E. coli* O157:H7 and *S. aureus* were increase with increased electric field strength and treatment temperature (Figs. 1-2). Under the same PEF processing and treatment temperature conditions inactivation energy of *E. coli* O157:H7 was higher than that of *S. aureus* (Tables 1-2).

![Fig.1. Inactivation of E. coli O157:H7 by PEF and different processing temperatures](image1)

![Fig. 2: Inactivation of S. aureus by PEF and different processing temperatures](image2)
In terms of inactivation kinetic and activation energy of *E. coli* O157:H7, under different processing temperature was calculated as 0.2235 cal/mol K (Table 1). Similarly activation energy calculated for *S. aureus* was 0.4563 cal/mol K (Table 2)

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Inactivation equation</th>
<th>$R^2$</th>
<th>(k)</th>
<th>Ea</th>
</tr>
</thead>
<tbody>
<tr>
<td>278</td>
<td>$y = 0.3572x - 0.201$</td>
<td>0.9851</td>
<td>0.3572</td>
<td>0.70976</td>
</tr>
<tr>
<td>288</td>
<td>$y = 0.3077x + 0.0773$</td>
<td>0.9933</td>
<td>0.3077</td>
<td>0.61139</td>
</tr>
<tr>
<td>298</td>
<td>$y = 0.2998x + 0.3656$</td>
<td>0.9887</td>
<td>0.2998</td>
<td>0.59570</td>
</tr>
<tr>
<td>308</td>
<td>$y = 0.2472x + 0.9392$</td>
<td>0.9972</td>
<td>0.2472</td>
<td>0.49119</td>
</tr>
</tbody>
</table>

Tab. 1: *Inactivation kinetics of E. coli O157:H7.*

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Inactivation equation</th>
<th>$R^2$</th>
<th>(k)</th>
<th>Ea</th>
</tr>
</thead>
<tbody>
<tr>
<td>278</td>
<td>$y = 0.3365x - 0.2494$</td>
<td>0.9929</td>
<td>0.3365</td>
<td>0.66862</td>
</tr>
<tr>
<td>288</td>
<td>$y = 0.2653x + 0.1082$</td>
<td>0.9965</td>
<td>0.2653</td>
<td>0.52715</td>
</tr>
<tr>
<td>298</td>
<td>$y = 0.1965x + 0.4325$</td>
<td>0.98</td>
<td>0.1965</td>
<td>0.39045</td>
</tr>
<tr>
<td>308</td>
<td>$y = 0.1724x + 0.6507$</td>
<td>0.9689</td>
<td>0.1724</td>
<td>0.34256</td>
</tr>
</tbody>
</table>

Tab. 2: *Inactivation kinetics of S. aureus.*

Findings in this study were similar to data reported in the literature. Previous studies revealed that PEF processing does cause significant changes in some physical properties of the food products [7-8]. Moreover, it was also indicated that under the same processing conditions, inactivation of gram negative bacteria are more than that of gram positive due to differences in the cell structure [9-10].

Temperature increase causes increase on the inactivation of both bacteria. Once temperature increased to 35°C, almost all inoculated cells of *E. coli* O157:H7 were inactivated. Similarly inactivation of *S. aureus* was greatly increased especially at 25 and 35°C. The combination of PEF by heat treatment provided increase in microbial inactivation and activation energy. It was also indicated in the literature that temperature increase causes increase in activation energy for a specified reaction [11].

For future studies, total antioxidant capacity, total phenolic content of pomegranate juice after PEF processing as well as sensory analyses need to be conducted to determine whether PEF processing causes any changes in these properties of pomegranate juice.

**Acknowledgements**

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References


OPTIMIZATION OF ELECTROPLASMOLYSIS USED IN SOUR CHERRY JUICE PROCESSING

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Abstract:
In this study, electroplasmolysis applied to crushed sour cherry products was optimized based on the juice yield by using response surface methodology. The voltage gradient and temperature were chosen as optimization factors while the juice yield obtained after pressing was the optimization response. Box-Bencken statistical design was used in experimental study. After the stone of sour cherries was removed, the sour cherry bulk was crushed by using a laboratory type blender. Then, the sour cherry mash was subjected to electroplasmolysis by applying the voltage gradient in the range of 40-110 V/cm. The final temperature reached during electroplasmolysis was in the range of 40-60°C. Finally, the heated sour cherry mash was pressed at 20-30 kN for 2 minutes by hydraulic press. The optimum electroplasmolysis parameters giving maximum juice yield were obtained as 75 V/cm voltage gradient and heating to 60 °C, by the desirability of 0.896. The juice yield obtained at optimum condition was 84.10% while it was 80.34% in the juice heated to same temperature in the agitated vessel by using steam at 1.5 bar. Electroplasmolysis parameters did not affect the colour of sour cherry juice statistically (p<0.01). It was concluded that it could be applied as an alternative method to obtain higher yield in juice.

Key words: electroplasmolysis, sour cherry juice, yield, RMS

1 Introduction

Sour cherry production of Turkey has been increased last 15 years. Although it was 92.000 tones in 1995, it reached to 10.000 tones in 2000, 121.500 tones in 2006, and 170.000 tones in 2007. Most of sour cherry produced is processed to fruit juice in Turkey [1, 2].

Electroplasmolysis is the process which is aimed to improve the yield in fruit juice and pulp production by destroying the cell wall. It can be explained by two main factors: (i) Electroporation; i.e. electro-induced formation and growth of pores in biomembranes as a result of their polarization, (ii) denaturation of cell membranes as a result of their ohmic heating caused by the electrical resistance of membranes, which is much higher than that of cell sap [3]. The main parameters influencing the electroplasmolysis treatment are electric
field strength and treatment time [4, 5]. Depending on the voltage gradient, treatment time, and temperature, electroplasmolysis results to increase in cell wall permeability, electrical conductivity, diffusion, heat and mass transfer coefficients and compressibility of plant tissues [6]. Moreover, it may also affect the some quality characteristics of product. Many researchers reported that the electroplasmolysis increases the yield in juice and pulp production [3].

Juice yield of sour cherry is 70-75% [1]. To increase the juice extraction yield some treatments may be applied, e.g. enzyme addition to mash. McLellan et al. [7] reported that the application of electroplasmolysis increases the yield in apple juice as compared to that of enzyme application and mechanical extraction. They also found that the application of electroplasmolysis improve the color of apple juice as compared to that of enzyme application and mechanical extraction. Since there were limited published data found on the effects of electroplasmolysis treatment to sour cherry, the objective of this study is to evaluate the effects of electroplasmolysis on extraction yield and color of sour cherry juice.

2 Material and Methods

Sour cherries were obtained from a local market in Izmir, Turkey. The sour cherries were washed, de-stoned, and then crushed by using a laboratory type blender (Waring, USA). The sour cherry mash was subjected to electroplasmolysis by applying the voltage gradient in the range of 50-100 V/cm and heating to temperature in the range of 40-60°C (Table 1). Electroplasmolysis application conditions were optimized by using RSM (Response Surface Methodology), Central Composite design. The experimental design is presented in table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Voltage gradients (V/cm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>75</td>
<td>64</td>
</tr>
<tr>
<td>R2</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>R3</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>R4</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>R5</td>
<td>110</td>
<td>50</td>
</tr>
<tr>
<td>R6</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>R7</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>R8</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>R9</td>
<td>75</td>
<td>36</td>
</tr>
<tr>
<td>R10</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>R11</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>R12</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>R13</td>
<td>75</td>
<td>50</td>
</tr>
</tbody>
</table>

Tab. 1: Electroplasmolysis application parameters.

In steam heating methodology, the sour cherry mash was heated in a pan with steam jacket by using 1.5 bar steam. The heated sour cherry mash was pressed at 20 kN for 50 s by using a laboratory type hydraulic press (Karl Kolb (West-Germany)), and then the pressure was increased up to 30 kN and remained at this pressure for 50 s. Total pressing time was 2 min. for each sample. The juice yield was determined gravimetrically. Color of extracted sour cherry juice was immediately measured by HunterLab Colorflex model color-meter (Management Company, USA). L*, a*, b*, total color difference, chroma difference, and hue angle were determined.
3 Statistical analysis

Response surface methodology was used to find out optimal conditions of electroplasmolysis treatment. The experiments were carried out according to the Box Bencken design with 2 factors and 3 levels. Table 2 shows independent variables selected for these two treatments. For each factor, an experimental range was based on our results of a preliminary study (unpublished data). Extraction yield and colour were the dependent variables. The complete design consisted of 13 experimental points. The software Design Expert version 7.0 was used to generate the experimental planning and to process data. Mathematical models were evaluated for each response by means of the multiple regression analysis. Significant terms in the model for each response were found by ANOVA. All experiments were performed in triplicate. The experimental results obtained were expressed as means ± SD. Mean values were considered significantly different when P < 0.05.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Factor level</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage gradients (V/cm)</td>
<td>-1</td>
<td>50</td>
<td>75</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>40</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>

Tab. 2: Independent variables and their levels in the RSM.

4 Results and Discussion

The juice yield extraction and color changes during electroplasmolysis of sour cherry samples were determined. The color values of sour cherry samples were given in table 3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE</th>
<th>ΔC</th>
<th>Hue</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>1.42</td>
<td>5.11</td>
<td>1.34</td>
<td>2.65</td>
<td>2.63</td>
<td>14.69</td>
</tr>
<tr>
<td>R2</td>
<td>0.52</td>
<td>1.67</td>
<td>0.49</td>
<td>6.29</td>
<td>6.17</td>
<td>16.35</td>
</tr>
<tr>
<td>R3</td>
<td>0.65</td>
<td>1.76</td>
<td>0.42</td>
<td>6.19</td>
<td>6.10</td>
<td>13.42</td>
</tr>
<tr>
<td>R4</td>
<td>0.90</td>
<td>2.82</td>
<td>0.82</td>
<td>5.04</td>
<td>4.98</td>
<td>16.21</td>
</tr>
<tr>
<td>R5</td>
<td>0.75</td>
<td>1.46</td>
<td>0.34</td>
<td>6.48</td>
<td>6.41</td>
<td>13.11</td>
</tr>
<tr>
<td>R6</td>
<td>1.68</td>
<td>4.65</td>
<td>0.66</td>
<td>3.26</td>
<td>3.26</td>
<td>8.08</td>
</tr>
<tr>
<td>R7</td>
<td>1.69</td>
<td>6.62</td>
<td>1.87</td>
<td>1.07</td>
<td>1.07</td>
<td>15.77</td>
</tr>
<tr>
<td>R8</td>
<td>1.79</td>
<td>4.28</td>
<td>0.70</td>
<td>3.60</td>
<td>3.60</td>
<td>9.29</td>
</tr>
<tr>
<td>R9</td>
<td>1.42</td>
<td>2.91</td>
<td>0.81</td>
<td>4.90</td>
<td>4.89</td>
<td>15.55</td>
</tr>
<tr>
<td>R10</td>
<td>1.09</td>
<td>1.38</td>
<td>0.43</td>
<td>6.50</td>
<td>6.47</td>
<td>17.31</td>
</tr>
<tr>
<td>R11</td>
<td>1.03</td>
<td>1.48</td>
<td>0.37</td>
<td>6.42</td>
<td>6.38</td>
<td>14.04</td>
</tr>
<tr>
<td>R12</td>
<td>1.38</td>
<td>2.24</td>
<td>0.75</td>
<td>5.57</td>
<td>5.56</td>
<td>18.51</td>
</tr>
<tr>
<td>R13</td>
<td>1.02</td>
<td>1.48</td>
<td>0.30</td>
<td>6.44</td>
<td>6.40</td>
<td>11.46</td>
</tr>
</tbody>
</table>

Tab. 3: Color values of electro-treated sour cherry samples.

Color change has been selected as a quality criterion for optimization. However, statistical analysis shows that process parameters applied were not effective on color of sour cherry juice. Thus, it was not taken as an optimization response. The model adequacies to the juice yield response have been determined. Quadratic model described well the yield changes as a function of voltage gradient and temperature. The results of ANOVA indicated that the model is significant at the considered confidence level since a satisfactory correlation coefficient was obtained and the F-value was more then the listed F-value (table 4).
**Table 4: ANOVA results for quadratic model used in optimization.**

Estimated effects of each variable as well as their interactions on the yield of electroplasmolysis treatment were presented in table 5. The temperature and voltage gradient had significantly positive effects on yield of electroplasmolysis treatment process while their obvious quadratic effects were also observed.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>p-value</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>18.04379</td>
<td>5</td>
<td>3.608758</td>
<td>10.99432</td>
<td>0.0033</td>
<td>significant</td>
</tr>
<tr>
<td>A-Voltage gradients</td>
<td>0.328671</td>
<td>1</td>
<td>0.328671</td>
<td>1.00132</td>
<td>0.3503</td>
<td></td>
</tr>
<tr>
<td>B-Temperature</td>
<td>4.914276</td>
<td>1</td>
<td>4.914276</td>
<td>14.97167</td>
<td>0.0061</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>1.678439</td>
<td>1</td>
<td>1.678439</td>
<td>5.113478</td>
<td>0.0582</td>
<td></td>
</tr>
<tr>
<td>A^2</td>
<td>7.26356</td>
<td>1</td>
<td>7.26356</td>
<td>22.12892</td>
<td>0.0022</td>
<td></td>
</tr>
<tr>
<td>B^2</td>
<td>2.547461</td>
<td>1</td>
<td>2.547461</td>
<td>7.76101</td>
<td>0.0271</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>2.297668</td>
<td>7</td>
<td>0.328238</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>0.432877</td>
<td>3</td>
<td>0.144292</td>
<td>0.309509</td>
<td>0.8187</td>
<td></td>
</tr>
<tr>
<td>Pure Error</td>
<td>1.864791</td>
<td>4</td>
<td>0.466198</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor Total</td>
<td>20.34146</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5: Effects of variables and their interactions on the juice yield.**

Juice yield increased as the temperature and voltage gradient increased. Above 55°C desirability increased. Furthermore, for all temperature and voltage gradients studied, juice yield obtained by electroplasmolysis was higher than that of the conventional treatment (figure 1). The optimum electroplasmolysis parameters giving maximum juice yield were obtained as 75 V/cm voltage gradients and heating up to 60°C by the desirability of 0.896. The juice yield obtained at optimum condition was 84.10% while it was 80.34% in the juice heated to same temperature in the agitated vessel by using steam at 1.5 bar. The treatment at the optimum conditions for the electroplasmolysis increased the extraction yield of sour cherry juice approximately 5.45% compared to that of untreated ones while conventional heating provided only 4.68 % increase.

**5 Conclusion**

Several studies concluded that electroplasmolysis treatment provides increase in juice extraction yield [3, 7, 8]. The present study presents that electroplasmolysis could be applied to the sour cherry mash without undesirable color changes. The voltage gradient and temperature were effective process parameters. Although the optimum electroplasmolysis condition was found on the base of juice yield only, quality attributes rather than color (phenolics and vitamin contents etc.) may be affected by this treatment and must be taken into account in the optimization in further studies.
Design-Expert® Software

Yield
- Design Points
- 84.9505
- 80.6895

X1 = A: Voltage gradients
X2 = B: Temperature

Fig. 1: Contour plot for effect of temperature and voltage gradient on juice yield.

References


A COMPARATIVE STUDY ON PULSED ELECTRIC FIELD-INDUCED PERMEABILIZATION OF DIFFERENT PLANT TISSUES

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Abstract:

In processes where intact plant tissue are handled for obtainment of a specific product, a stage is usually devoted to tissue disintegration. In this stage, an effective permeabilization of cell membranes is of critical importance mainly because the specific products (functional biomolecules or liquids) are localized inside of cell and, intact cell membranes and tissue structure are the main barriers in transferring them out of the tissue.

Compared to classical disintegration methods with several drawbacks, the application of PEF to plant is a promising way offering possibility to permeabilize the entire tissue targeting for each of the cell, thereby not leading to significant temperature and energy increment.

In the past years, effectiveness of PEF for permeabilization of plant tissue, and optimization of the process parameters have been demonstrated in a number of research. However, most of the research focused on parenchyma type of tissue. Additionally, the intracellular specific products to be extracted exist in other tissue types that differs in cell size and tissue structure. Aim of this study is to present data on PEF-induced permeabilization characterizations of other plant tissues in a model system consisting of PEF-generator and LCR meter, and also to include a comparative study for such tissues based on Cell Disintegration Index previously developed.

Key words: Pulsed electric field, plant tissue, cell permeabilization, tissue disintegration, solid-liquid extraction
ELECTROPHORETIC SEPARATION OF THE MILK PROTEIN

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Abstract:
Electrophoresis of the total casein in different system (disc-electrophoresis, disc-electrophoresis with SDS in homogeneous and gradient gel, electrophoresis in homogeneous gel with urea) was carried out. The effective methods of electrophoresis have been suggested for identification of casein fractions and milk serum protein according to the modern classification.

Key words: casein protein, milk serum protein, electrophoresis, identification of caseins

1. Introduction

Cow milk includes two big types of proteins which are combined according to their ability to dissolve at subacid pH value. According to the International Commission of protein, the phosphoprotids precipitated from the skim milk at pH 4.6 and temperature 20°C are called caseins. Proteins, remained in the solution, belong to the proteins of milk whey. Caseins in milk appear to be in four fractions that differ in their primary structure: αS1-, αS2-, β- and κ-casein. Furthermore, αS1-casein can form minor components depending on the number of phosphoserines residues.

Cow milk proteins are used in many medical and food biotechnological processes as well as for increasing biological value of foods and producing some special types of nutrition [1]. Products of casein and some proteins of milk whey proteolysis have different physiological activities [1].

Wide use and great importance of milk proteins in human nutrition and medical biotechnology cause the need in existence of the method to separate and identify proteins of casein type and those of milk whey. Moreover the present data concerning their fractional composition and properties must be taken into account.

General methods of qualitative and quantitative analysis of milk proteins are treated as ion-exchange chromatography on DEAE-cellulose, fast liquid chromatography (FPLC) and electrophoresis on the starch and polyacrylamide gel [2]. Chromatographic methods need much time or complex equipment. As to the milk proteins, even up-to-date fast liquid chromatography of proteins doesn’t separate αS-caseins fractions. Problems of electrophoretic analysis deal with the unsuccessful simultaneous separation of all milk proteins, duration of the process or low efficiency of minor caseins separation [3].

The objective of the paper is to select the structure of electrophoretic systems and conditions of electrophoresis for fast separation and identification of cow milk protein fractions.
2. Material and methods

Total casein of cow milk was extracted by means of reprecipitation of fresh skim milk at the isoelectric point (pH=4.6). Total casein extraction provides inactivation of natural proteolytic milk enzymes by means of extraction in acetic acid at pH 4.0. Caseins being precipitated and low-molecular components being separated, milk whey proteins were extracted [3].

Homogeneous fractions α5- and β-caseins, extracted in our laboratory by means of ion-exchange chromatography on DEAE-cellulose (DEAE-52, Serva) were used for proteins identification on electrophoregrams. All the compounds of milk proteins were dried lyophilically and stored at the temperature of +4°C.

Proteins concentration was measured by Kjeldahl method or with spectrophotometric assays of absorbance at 280 nm [2].

Electrophoresis of protein was conducted on the polyacrylamid gel in the Stadier apparatus. The apparatus itself, electrophoretic chamber and formers were made in our laboratory. The apparatus of “Reanal” (Hungary) was used for the electrophoresis in the tubes of polyacrylamid gel. The reagents produced by the same company were used to prepare electrophoretic system components.

3. Results and discussion

A number of electrophoresis such as: disc-electrophoresis, electrophoresis at the presence of sodium dodecylsulphate, disc-electrophoresis in gradient gel and electrophoresis in alkaline system of homogeneous gel at the presence of urea were used for the comparative analysis [4-7].

In order to determine the composition of total casein we took advantage of variant of disc-electrophoresis, suggested earlier for neutral and acid proteins separation [5]. Typical electrophoregram of separation total casein of cow milk by means of this method is shown in Figure 1.

Main fractions αS1-CN and β-CN were identified while using homogeneous caseins. Identification of αS2-caseins and κ-caseins fractions is complicated and is treated differently.

Milk whey proteins were also used after caseins precipitation, dialysis and lyophilisation. Unlike caseins, milk whey proteins can be separated efficiently in the system of native disc-electrophoresis. The results of this separation are shown in Figure 1.

Electrophoretic system with sodium dodecylsulphate (SDS) and β-mercaptoethanol were used successfully for the analysis of separate casein fractions [1]. SDS at concentration of 2% being a part of buffer and engaged into water-repellent interaction should cause formation of casein monomeric solution. This state is maintained by the detergent in PAAG. Binding SDS by means of caseins depends on the temperature [8]. In addition to β-casein the amount of bound detergent increases when temperature increases from 20 to 80°C. Detergent binding decreases after 40°C in β-casein. Accordingly, total casein treatment by means of buffer was conducted at the room temperature. However, even in this case molecular weight of β-casein being determined electrophoretically reaches the value found on the basis of aminoacid composition. The value of αS1-CN and κ-CN is 8000 and 10000 Dalton bigger than theoretical value respectively [8]. Due to small molecular weight of caseins, and temperature changes during electrophoresis, such variations make their objective identification according to the molecular weight in the electrophoretic with SDS impossible. Typical electrophoregram of total casein is shown in Figure 1.
This abnormal casein behavior can be caused by SDS binding properties. Most proteins are known to react with SDS in a ratio of 1,4 mg of detergent and 1 mg of protein [5]. Caseins are likely to bind more detergent due to their high rendering water-repellent. Our caseins separation cannot be considered efficient, though, the system can be used for the analysis of separate casein fractions homogeneity. Mostly it deals with α\textsubscript{S1}-CN and β-CN fractions.

We also used disc-electrophoresis in the gradient field with SDS for the caseins analysis. The use of gradient separating gel together with the advantages of step electrophoretic system make this electrophoresis one of the most efficient methods for proteins separation [4]. It allows to separate and simultaneously to determine molecular weight in wide rage of values from 10000 Dalton in protein mixture with high heterogeneity. The results of total acidic casein analysis in this system are shown in Figure 1. Calculations show too high molecular weight values for the main fractions (29000-33000 Dalton). Thus, despite using gradient gel and disc-electrophoretic system, abnormal behavior of caseins towards SDS and close values of molecular weight of casein fractions do not allow to separate them efficiently in the given system.

D. Devis and A. Low’s electrophoretic system was assumed as a basis in order to conduct casein electrophoresis in homogeneous PAAG system [7]. As their paper did not contain detailed description of the method and electrophoresis conditions, we made some supplements concerning gel composition and buffers. The first efforts of caseins electrophoresis in this system allowed to determine at least 4 main fractions: α\textsubscript{S1}-CN, β-CN, κ-CN and α\textsubscript{S2}-CN. Electrophoregram is shown on Figure 2. α\textsubscript{S1}- and β-caseins location on the electrophoregram was confirmed while using homogeneous fractions α\textsubscript{S1}-CN and β-CN. Casein location was determined after short term treatment (15 minutes) of total casein solution by means of milk-clotting enzyme of high specificity to κ-CN (Figure 2). This fraction has the lowest electrophoretic mobility and almost disappears under the influence of milk-clotting enzyme. Thus, it can be stated that casein fractions on the given electrophoregram are placed in electrophoretical mobility decreasing as follows: α\textsubscript{S1} > α\textsubscript{S2} > β > κ.
Disadvantage of the applied electrophoretic system is the absence of separate $\alpha_{S2}$-casein fractions. It concerns to $\alpha_{S2}$-CN-13P, $\alpha_{S2}$-CN-12P, $\alpha_{S2}$-CN-11P and $\alpha_{S2}$-CN-10P. All of them are shown on the electrophoregram as one blue band. The same result is with $\kappa$-caseins. In addition minor fraction $\alpha_{S1}$-CN-9P of $\alpha_{S1}$-casein is not separated. Electrophoresis lasted 4-5 hours.

In order to get rid of these disadvantages some changes in PAAG composition, electrode buffer and buffer for the gel and samples were made. First of all, the changes took place in PAAG concentration lowering (4.5 % PAAG was used in the past) [4]. It allows to accelerate the process of electrophoresis due to decreasing the gel heating and prevention widening of protein areas and possible association of caseins. The latter is available even while using disaggregating factors. 2-mercaptoethanol was added to the gel and buffer composition too. $\kappa$-CN and $\alpha_{S2}$-CN efficient separation cannot be obtained without such reagents. Since urea does not migrate during electrophoresis, it was excluded from electrode buffers composition. It simplifies buffer preparing and the electrophoresis itself to some extend, and lowers expenses of high treatment reagent. In addition, the lowering of buffer concentration for the samples (5 times) is very important for the qualitative electrophoretic separation. It allows to make an area with high tension to the gel and to achieve the effect of sample proteins concentration at the start, similar to the process of disc-electrophoresis.

The results of total casein separation in our system are shown in Figure 2. Electrophoregram shows special separation of proteins of casein complex of cow milk. All fractions are placed according to the modern international classification of caseins [9]. The method is comparatively simple, available and it allows to obtain efficient casein separation.
4. Conclusions

The methods of electrophoresis based on protein separation with the use of sodium dodecylsulphate in the homogeneous and gradient gel are slightly suitable for the identification of casein that have close molecular weight and bind sodium dodecylsulphate non-uniformly. Native disc-electrophoresis allows to identify the main fractions of casein (αS1-, β-caseins) as well as whey proteins. It can be used for the preliminary analysis of the proteins mixture. The suggested type of anode electrophoretic system with continuous low concentration PAAG in the presence of urea allows to separate completely casein fractions according to the modern casein classification and it can be used for casein identification.

References

ANALYSIS OF THE ELECTRIC FIELD AND CONVECTIVE HEATING DURING MICROWAVE DRYING OF FOODS

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Abstract:  
The aim of this work is to analyse the changes that appear in the electric field (E), and in the convective heat coefficient (h), during the dehydration of fruits.  
The electric field is affected with the temperature and during the process of drying. This parameter increases when the product reduces its water content.  
The most important influence in the convective heating for a fixed pressure is the drying rate. Through a general analysis of the convective heat coefficient as a function of the drying rate, it is possible to observe a typical behaviour closely related to drying rate.  
Finally, relationships depending of moisture or water content, for E, and of drying rate, for h, were obtained to describe their variation during drying of cut up apples pieces.

Key words: microwave drying, electric field, convective coefficient, apples.

1 Introduction

Microwave heating is a technology that is being used increasingly in the food industry. There are different types of microwave heating, known as radiofrequency or dielectric heating. These are more generic than plain microwave heating, and they refer to the possibility of modifying frequency to improve efficiency in certain applications: e.g. frequency of 900 MHz results in a greater penetration of waves [1]. Nevertheless, the most widely used frequency is 2.45 GHz, because of its lower production cost and applicability [2].  
Recently, the knowledge of the effects derived from electric field application on materials with special dielectric makes microwave an interesting technology used in so many applications like: treatment of food for microbial preservation [3] or improving diffusion in mass transfer [4].

Nevertheless, in the application to the dehydration of food, despite being a more well known due to the considerable amount of water, a deeper knowledge on the effects of removing water is required. To conduct the process adequately a microwave drying process, it is necessary to know the effect of operating conditions such as pressure and temperature control on process kinetics, through the characteristic heating parameters: convective heating coefficient (h) and electric field (E). Both parameters governs the temperature of material and heat assimilated, being directly responsible of the drying rate and quality of final dehydrated product [5].

From previous analysis on food drying, it has been found that both E and h, in a greater or lesser extent, are affected by variables such as product temperature, drying rate and, in more degree, material moisture. One objective of this work is to find which of the former variables affects more strongly on E and h.
In this work, we start with an analysis of microwave drying to relate a given combination of \( E \) and \( h \) to the conditions of pressure, type of product, equipment and control temperature in the product. However, changes in moisture caused by drying suggest that variations in \( E \) and \( h \) along the drying cycle can be quite significant. A general purpose of this study, therefore, is to study the variations of \( E \) and \( h \) along the drying process under different operating conditions. A proper interpretation of the progression of \( E \) and \( h \) during microwave drying is essential to design more and more efficient vacuum dryers with better control on quality of final dehydrated food.

2 Materials and Method

2.1 Equipment

The installation consists of a magnetron that emits microwaves at a frequency of 2.45 GHz, with a maximum power of 300 W. Microwaves is emitted through a guide to the multimode cavity, which is inside the camera that is capable of operating at pressures below atmospheric [6]. The entire system is attached to a computer for data acquisition, which are then used to determine the parameters that are to be calculated. To measure water loss, samples, placed on a Teflon plate were connected to a balance (Sartorius, model PT-1200). Fiber optic probes connected to temperature measurement equipment (NORTECH NOEMI TS-Series) were used to determine the temperature inside the samples.

2.2 Material

Experiments have been done with small parallelepipeds of dimension 20x10x10 mm of apple (Golden Delicious) that have been introduced inside the multimode cavity. It consists of about 85 % of water by weight. Their chemical composition are taken from [7].

The dielectric properties of apple at ambient temperature are 60 for the dielectric constant, and 15 for the loss factor [8]. These and thermophysical properties like specific heat of the product were build on the properties of food components, fundamentally water according Table 1.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \rho_s )</td>
<td>774.08 kg/m(^3)</td>
<td>Singer &amp; Harris, 1987</td>
</tr>
<tr>
<td>( \rho_w )</td>
<td>1000 kg/m(^3)</td>
<td>Lide, 2001</td>
</tr>
<tr>
<td>( C_{ps} )</td>
<td>378,14</td>
<td>Singer &amp; Harris, 1987</td>
</tr>
<tr>
<td>( C_{pw} )</td>
<td>4211</td>
<td>Lide, 2001</td>
</tr>
<tr>
<td>( \varepsilon_s )</td>
<td>3,3</td>
<td>Mudgett, 1986</td>
</tr>
<tr>
<td>( \varepsilon_w )</td>
<td>( \varepsilon_w=87.75-0.39\cdot T+7.3\cdot 10^{-4}\cdot T^2 )</td>
<td>Lide, 2001</td>
</tr>
<tr>
<td>Delta</td>
<td>( \Delta=0.297-0.004\cdot T+3.64\cdot 10^{-5}\cdot T^2 )</td>
<td>Singer &amp; Harris, 1987</td>
</tr>
</tbody>
</table>

Tab. 1: Thermo-physical and dielectric properties of apple (Golden Delicious)

2.3 Mathematical description of MW drying.

In this case, the measured experimental data are the temperature (inside the product and on its surface) and the mass of the samples, which are directly related to the amount of water they have at every moment. From these experimental data, we can calculate both \( E \) and \( h \), using the balance of matter and energy taken from a previous work [9].
3 Experimental Results

To estimate the electric field ($E$) and convective coefficient ($h$), the mass and energy balances were solved. A calculus program in Matlab was designed to estimate $E$ according to an iterative algorithm for a certain combination of $E$ and $h$. The program compares the temperature and water content calculated at each time with the experimental ones. The basis of calculus consists in searching and adequate power factor $mwp$ which normally varies all along the drying to match the experimental values of temperature and moisture.

The term $mwp$ has been defined as the fraction of the maximum power of the microwave generator. Thus, a $mwp$ value of 0.5 during a time interval means the power input is half the maximum that is equivalent to power-off the generator half the time.

Therefore, $mwp$ is regulated according to a determined control strategy. Generator power must be changed throughout the experiment in order to fit a certain control variable. This is usually the product temperature that can change significantly from inside out, being in most cases temperature at the surface lower that the surface; i.e. a contrary temperature gradient to that observed in conventional heating from an external source.

![Fig. 1: Calculated values of $mwp$ for different values of $E$ and the same $h$.](image)

The control system operates increasing or lowering microwave power input to maintain product temperature at surface or inside within the limits of the prefixed set point.

The consequence of the combination $E$, $h$ and $mwp$ leads to a temperature level at the surface or inside the product, depending on the control choose which is responsible of the drying rate at each moment. Figure 1 shows the $mwp$ profiles calculated for different values of $E$ and $h=15$ W/m$^2$K corresponding to the drying of 20x10x10 mm apple pieces at atmospheric pressure, being 55 °C the temperature control at the surface of the pieces. The results show that the electric field of 3000 fits well de $mwp$ variation in the middle of process but present serious deviations at the beginning and the end. Therefore, it seems that a complete fitting of $mwp$ all along the process requires a continuous variation of parameters $E$ and $h$.

A consequence of the above study is that $E$ and $h$ vary significantly throughout the process. Therefore, a new calculus algorithm was proposed, based on the variation of these parameters ($E_t$ and $h_t$) throughout the experiment to get an adjustment to the experimental $mwp$. In this case, the calculus program operates in reverse with respect to the above procedure, taking 200 seconds time intervals. The experimental $mwp$ is introduced and the program searches values of $E_t$ and $h_t$ satisfying the temperature and mass loss observed experimentally. In a first approximation, it was proposed a second order polynomial function ($n = 2$) of the type:
\[ P = \sum_{i+j=0}^{n} a_{ij} x^i y^j \]  
(1)

to correlate \( E_t \) and \( h_t \) as a function of independent variables \( x \) and \( y \).

A total of 10 experiments (Tables 2 and 3) were carried out in which was varied the control temperature in the product (inside or at surface) and pressure (atmospheric or vacuum 70, 30 and 6 mm Hg). With the data of temperature and mass loss, parameters \( E_t \) and \( h_t \) were estimated during drying according to the calculation procedure explained before. A preliminary analysis of the dependence of these variables revealed that electric field was mainly influenced by water content (variable \( x \)) and temperature (variable \( y \)), while the convection coefficient depends on the drying rate (variable \( x \)) by the influence of the thermal boundary layer, and temperature (variable \( y \)). At the same time, it was observed that a second order polynomial was good enough to describe the variation of these parameters.

After a study of significance of the different terms of polynomial function, it was found that only the terms corresponding to the variable \( x \): water content (\( M \)) in the case of \( E_t \) and normalized drying rate (\( R_N \)) for \( h_t \); i.e. terms corresponding to coefficients; \( a_{00} \) or \( a_0 \), \( a_{10} \) or \( a_1 \) and \( a_{20} \) or \( a_2 \), following nomenclature of polynomial in equation (1). It is noted that the \( R_N \) values are normalized, between 0 and 1. Normalization of this variable was performed respect to reference value of \( R \) (g water / g d.s. min) at the end of drying. Finally, to achieve a better fit to the experimental data with a single variable, the term of second order \( (i = 2) \) is substituted by an adjustment parameter \( m \), non necessarily integer and positive, as follows:

\[ E_t = a_0 + a_1 \cdot M + a_m \cdot M^m \]  
(2)

\[ h_t = a_0 + a_1 \cdot R_N + a_m \cdot R_N^m \]  
(3)

<table>
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<th>Drying Exp.</th>
<th>( a_0 )</th>
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<th>( a_2 )</th>
<th>( m )</th>
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Tab. 2: Coefficients for \( E_t(M_N) \) relationship according equation (2).

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Tab. 3: Coefficients for \( h_t(R_N) \) relationship according equation (3).
In Tables 2 and 3, the values of the coefficients corresponding to expressions (2) and (3) are shown. Such equations express the variation of $E_t$ and $h_t$ in the process through the variables $M$ and $R_N$, which decreases with time during the process. Standard deviations found in Table 2 and 3 represent standard errors below 10%, from which an acceptable fitting of equations to experimental data can be deduced in all the experiments. Figure 2 shows some of analysed cases of $E_t(M)$ and $h_t(R_N)$ modelled relationships and experimental values.

![Graphs showing $E_t$ and $h_t$ as functions of $M$ and $R_N$.](image)

**Fig. 2:** Modelled profiles of $E_t(M)$ and $h_t(R_N)$ together with experimental values (dots) in some of the studied drying cases.

### 4 Conclusions

For low values of product moisture, $M$, below 1 g/g d.s. (end of drying), electric field sharply tends to a maximum value of 3500 V/m, independently of pressure and temperature drying conditions. On the contrary, at product moisture above 1 g/g d.s., it has been appreciated a linear dependence of the electric field ($E$). For high values of product moisture, values of $E_t$ range in a narrow interval between 1400 and 1900 V/m, for experiments carried out at atmospheric pressure. For the rest of experiments under vacuum, values of $E$ decrease according to the product temperature lowering, reaching values as low as 500 V/m in the beginning of drying at 6 mm Hg pressure and 5 ºC surface control temperature.

Convective heat coefficient ($h$) was found to be strongly dependent of drying rate, $R$. Coefficient $h$ varies from 0 to 35 (W/m² K) depending on the value of drying rate. The $h$ vs $R$ dependence is so significant at atmospheric pressure drying but decreases in the vacuum drying presenting values of $h$ lower than 7 W/m² K. Coefficient $h$ decreases sharply at low drying rates so that below $R=0.042$ g water / (g d.s. min), convective is practically insignificant.
References


TECHNO-ECONOMIC ANALYSIS OF A PILOT SCALE MICROWAVE DRYING UNIT FOR GARLIC CLOVES

Department of Processing and Food Engineering, College of Technology and Engineering, MPUA&T, Udaipur 313001, India, Email: gpsharma@mailcity.com

Abstract:
A batch type microwave dryer was designed and developed for use by small scale processing industries in India, to dehydrate garlic cloves. The dryer had a capacity to dehydrate about 5 ton of garlic cloves per year. The quality of dried cloves was evaluated in respect of colour and the flavour strength. The techno-economic analysis of the drying process was made, using economic indicators such as net present worth (NPW), benefit cost ratio (BCR) and pay back period (PBP). The initial cost for installation of a drying unit capable of producing 10 ton dehydrated garlic was found to be about 3.42 million INR (1 Euro = 64.65 INR). The net present worth of the total investment made on the industry in next 20 years worked out to be approximately 19.67 million INR. The benefit cost ratio for garlic dehydration unit was estimated as 2.08 with a pay back period of 6 years. The microwave assisted dehydration unit for the garlic appeared as a viable project from techno-economic analysis.

Key words: microwave drying, flavor strength, net present worth, pay back period, benefit cost ratio

1 Introduction
India is the second largest producer of vegetables, contributing about 15% of the world’s production of vegetables; and ranking next to China. Garlic is an important cash crop in India. The production of garlic, worldwide, has been to a tune of about 10.21 MT in the year 2005 and India contributes about 0.6 MT from an area of 92,000 ha under cultivation of this crop. It is used for seasoning of foods because of its typical pungent flavour and is usually used without any pre-processing. More recently, it has found uses in its dried form, as an ingredient of pre-cooked foods and instant convenience foods including sauces, gravies and soup. This has led to a sharp increase in its demand in dehydrated form.

Garlic is a semi-perishable commodity, having moisture content as high as 67% (wb). Lack of suitable storage and transportation facility, about 30% of the fresh produce is wasted due to respiration and microbial spoilage. Above all, garlic bulb has a tendency to lose moisture during storage which causes weight loss and also shriveling of cloves to an extent of 40-50% [1].

The dehydration is an oldest method practiced by the mankind, to preserve the food in a stable and safe condition. Dehydration reduces the water activity and enhances the shelf life much longer than that of the fresh fruits and vegetables, therefore is a means for preserving the foods in a stable and safe condition. The thermal dehydration methods used in garlic dehydration such as hot air drying, vacuum drying, freeze drying result into lower drying rates in falling drying rate period i.e. time consuming processes; and exposure of the food to relatively high temperature for longer times brings about undesirable thermal degradation in the finished product. Moreover, these technologies are energy intensive too [1-3].
Several units are functional in and around the state of Gujarat and Rajasthan in India, dehydrating onion and garlic as primary products for export purpose, using hot air drying technique. Most of these units have been operating somewhat successfully but on low returns due to the fact that their cost of production is much higher. The Indian industries for dehydration of vegetables and fruits can survive in the present scenario only on the strength of product quality and cost competitiveness, which can possibly be had by going into technology up-gradation in the manufacturing process, apart from getting raw materials of superior quality through contact farming.

Microwave (MW) drying, which is considered as fourth generation drying technology, can be a promising solution as it offers opportunities to shorten the drying time and thus improves the final quality of the dried product. The two narrow bands of microwave allocated for use in industrial food processing applications are 915 MHz and 2450 MHz; and the most widely and commercially used frequency is 2450 MHz [1]. A complete MW drying process consists of three drying periods (i) a heating-up period in which MW energy is converted into thermal energy within the moist food, and the temperature of the product increases with time. Once the moisture vapour pressure in the food is above that of the environment, the material starts to loose moisture, but at relatively smaller rates. (ii) rapid drying period, during which a stable temperature profile is established, and thermal energy converted from MW energy is used for vapourization of moisture. (iii) reduced drying rates period, during which the local moisture is reduced to a point when the energy needed for moisture vapourisation is less than thermal energy converted from MW. The loss factor of the food materials decrease with moisture reduction and the conversion of MW energy into heat is reduced at lower moisture content [4].

Most of the reported technologies of MW-related combination drying are based on laboratory scale system, using 2450 MHz domestic microwave ovens as main MW source [5-13]. This leads to difficulties in scaling up the pilot scale results to industrial scale applications. There is a need for further studies to bridge the gap between laboratory research and industrial applications. Adoption of this technology at industrial level relies on positive economic returns, considering the start up and maintenance costs, need to use electricity, the complexity of operations and added value to the final product. The present work was, therefore, aimed at developing a batch type microwave drier usable by the small scale food processing units involved in food dehydration; and to study quality and economic aspects of the microwave drying process, specially for garlic - a commodity that still has tremendous potential for export.

2 Constructional Details of the Microwave Dryer

A batch type microwave dryer, having capacity to dehydrate about 5 tonnes per year (fresh peeled garlic cloves) was designed and developed. The capacity was so chosen that it can be readily used by small scale food processing units. However, the dryer could be used to dehydrate any other agricultural commodity as well. The developed dryer had the following functional details:

It had a cavity (drying chamber) of 700x700x550 mm, made from 16 stainless steel gauge (SS 304). Two magnetrons (2M121A Hitachi make), each of 750W and 2450 MHz frequency were used in the dryer, conveying the microwaves into the cavity through two different waveguides. An air blower was provided to cool the magnetrons continuously during operation period of the dryer. The mode of operation of the dryer was continuous which could be intermittent with a step up of 0.15W of microwave power i.e having duty cycle of 10-100%. The main body of the dryer was made of mild steel.

A circular product holder, made from Teflon, was provided in the microwave cavity. The product holder had a diameter of 600 mm and a rim height of about 120 mm. The bottom of
the product holder had small round perforations (about 2.0 mm size) so that water vapours evolved from the drying material can find a passage, through bottom also, to escape. The arrangements were made to felicitate the movement of product holder by 3600, like a turntable in a domestic microwave oven moves for uniform heating of the product. An exhaust fan was also installed on one side of the dryer to evacuate the moisture laden air, continuously, from inside of the cavity.

![Microwave dryer developed for small scale food process industries](image)

The dryer had an electronic console, comprising of chips, relays, contractors to preset the duty cycle of microwave power and its period of application. The pictorial view of the microwave dryer developed is presented in Fig.1.

3 Materials and Methods

Fresh garlic (Allium sativum) bulbs were used in the present investigation and were procured from the local market of the Udaipur in the state of Rajasthan; and were stored in a cold space (deep freezer) maintained approximately at a temperature of 1±10C until experiments were completed. The garlic cloves had initial moisture content of about 2.03 g H2O/g dry solid.

3.1 Moisture content

The vacuum oven method was used to determine the moisture content of the garlic cloves. Garlic samples of approximately 15 g were placed in a pre-dried aluminium dishes in a vacuum oven, with sulfuric acid as a dessicant. The operating temperature was 700C with a guage pressure of 85 kPa and the sample was kept for 24 h [14]. The samples were then taken out of the oven, cooled in a dessicator and weighed using a top pan digital balance having sensitivity of 0.01 g. The fresh and bone dried weights were used to calculate the moisture content in garlic cloves.

3.2 Experimental procedure

Garlic bulbs were taken out of the cold chamber and allowed to equilibrate with ambient conditions for about 4-5 h, followed by peeling, which was done manually. A batch of 7.5 kg of peeled garlic cloves was charged into the microwave dryer and drying was accomplished by applying the microwaves continuously. The product was weighed intermittently at later stages of drying, which was terminated when weight reached a level corresponding to moisture content of about 0.06 g H2O/g dry solid. An exhaust fan mounted on the outside...
surface (on one side) of the cavity gave a fairly constant air flow of about 0.30 m/s through the cavity. A water load comprising of about 800 ml water in borosil glass beaker was kept in the cavity, during finish drying of product, to prevent overheating of the magnetrons.

3.3 Quality of garlic powder

The quality of dehydrated garlic cloves samples was evaluated with respect of colour and flavor strength, after powdering the garlic to 100 mesh size. The chromacity of the powder was measured by L value on L,a,b scale using a Hunter Lab colorimeter (model: colorflex). The colorimeter was first calibrated against the standard calibration plates, provided with instrument. The measurement of colour was replicated five times on the same samples, shaking the sample each time; and average values were reported.

The volatile oil, comprising of sulfur compounds which are responsible for pungency in the garlic, was determined by Chloramine-T method [15]. The quality attributes of the microwave dried garlic cloves were compared, in respect of flavor strength and colour, with commercially available dehydrated garlic cloves, obtained directly from a manufacturer. This air dried commercial sample served as a control in the present study.

4 Economics of the Garlic Dehydration Process

An agricultural project is expected to return benefits in each of subsequent years of functioning; and therefore it becomes imperative to know the present worth of that future income stream to know how much was it justified investing today to receive that income stream. The capital investment for setting up the garlic processing and dehydration unit including land, building, plant and machinery was evaluated. The operating costs for running the industry was also determined which included cost of raw material, wages and contingencies. The selling of processed product will give cash inflow to the unit. The net benefit expected from the industry was determined after deducting capital investment from gross cash inflow. Following considerations were made to carry out the economic analysis of this project.

The processing unit was envisaged to work for at least 300 days per annum on a double shift basis.

The unit could achieve its full capacity utilization in the first year itself.

The wages for the skill labour were taken as per prevailing rates in a food processing unit typical to present garlic process unit.

Interest rate for total capital investment was assumed as 8 % per annum.

The processing unit had its own building conforming to provisions of FPO.

The cost of machineries and equipments were assumed on the basis of the prices enquired from the machinery manufacturers.

The production capacity of the plant was assumed to be 10 tonnes dehydrated garlic products per annum.

The motive power and water requirement were assumed as 10 kW and 1kL per day respectively.

The life of the microwave dryer and packaging machine was assumed as 20 and 10 years respectively.

The cost of the processed dehydrated garlic product was assumed as Rs 350 per kg.

A piece of land measuring 30 x 20 m was deemed sufficient for establishing a small scale garlic processing unit, which could be close to road as well as production area so that control over the cost of raw material could be had.

4.1 Net Present Worth(NPW)
The discount cash flow measure of project worth is its net present worth (NPW). To obtain the incremental net benefit gross cost is subtracted from gross benefit of the investment cost from the net benefit [16]. The NPW was computed by subtracting the total discounted present worth of the cost stream from that of benefit stream, using eq. 1:

\[ NPW = \sum_{t=1}^{n} \frac{(B_t - C_t)}{(1 + i)^t} \]  

where, \( B_t \) = benefit in \( t \)th year, \( C_t \) = cost in \( t \)th year, \( t = 1, 2, \ldots, n \) year, \( i \) = discount rate (8 per cent)

4.2 Benefit Cost Ratio (BCR)

The benefit–cost ratio was obtained after dividing the present worth of the benefit stream by its present worth. The formal selection criterion for the benefit-cost ratio for measure of the project worth was to accept projects for benefit-cost ratio of 1 or greater. In practice, it more common not to compute the benefit-cost ratio using gross cost and gross benefit, but rather to compare the present worth of the investment cost plus the operation and maintenance cost. The ratio is computed by taking the present worth of the gross benefit less associated cost and then comparing it with the present worth of the project cost. The associated cost is the worth of the goods and services over and above those included in project costs needed to make the immediate products or services of the project available for use or sell. The project economic cost, i.e. sum of installation costs, operation, maintenance costs and replacement costs was obtained from eq. 2 [16].

\[ BCR = \frac{\sum_{t=1}^{n} B_t}{\left(1 + i\right)^t} / \frac{\sum_{t=1}^{n} C_t}{\left(1 + i\right)^t} \]  

where \( BCR \) = benefit cost ratio

4.3 Pay Back Period (PBP)

The payback period is the length of time from the beginning of the project until the net value of the incremental production stream reaches the total amount of the capital investment; and shows the length of the time between cumulative net cash outflow recovered in the form of yearly net cash inflows. The Pay Back Period (PBP) was estimated as the duration in which \( \sum_{i=1}^{n} B_i \geq P_w \) where \( B_i \) is the present worth of cash inflow and \( P_w \) is the present worth of the total cash out flow for setting the processing unit [16].

5 Results and Discussion

5.1 Drying behavior and quality

In the present investigation, drying time required to dry the garlic cloves from initial moisture of about 2.03 kg water/kg solids to the final moisture content of about 0.06 kg water/kg dry solids was about 6.03 h, under continuous application of microwaves. This drying time was much less than that reported for hot air drying process of garlic cloves i.e. 13.5h [13].
The flavour strength and color in terms of L-value for the microwave dried garlic as well as commercial sample are given in Fig.2 and Fig.3 respectively. The flavour strength of microwave sample was 4.86 mg/g dry matter and that of commercial sample was 4.24 mg/g dry matter. The L-value (indicating lightness of colour) of the microwave sample was 73.42 and that of commercial sample was 68.78.

This implied to that garlic cloves dehydrated by microwaves were superior as compared to commercial sample of garlic in respect of their quality attributes. This is in line with earlier findings of Sharma and Prasad [1].

5.2 Economics of the Microwave drying process

The economics feasibility with regard to establishment of a microwave based small scale processing unit having capability of producing approximately 10 tons of dehydrated garlic products annually was assessed, considering the costs involved and the profits received. The total fixed cost of small scale unit for garlic dehydration including land, building, plant and machinery with installation of the three microwave assisted dryers was found to be Rs. 1.92 million INR. The total recurring expenses for successful running of the plant including raw material, wages and other contingencies such as electricity, water and insurance were determined as Rs. 1.50 million INR. Therefore the total initial cost for starting of the unit was summed as Rs. 3.42 million INR (Table 1).

The proposed microwave assisted garlic dehydration plant would comprise of 3 microwave dryers each with a handling capacity of 15 kg of raw material (fresh garlic cloves) in one batch of 6 hours. It is expected that plant can run in 2 batches per day. Therefore the plant can process approximately 100 kg garlic daily and can produce approximately 35 kg of dehydrated product. The raw material (fresh garlic) can easily available at rate of 15 INR per kg and the processing cost per kg inclusive of the man power, electricity and other contingencies can be estimated as 36 INR per kg (Table 1). The dehydrated cloves can easily be sold and can fetch the price up to 350 INR per kg. Therefore, net profit can be found as the 300 INR per kg.

The financial aspects concerning cash outflow and inflow along with net present worth in each year was estimated using the eq.1. In the very first year of the commencement of the unit the total investment required is 3.424 million INR (table 1.) which includes the cost of infrastructures and the raw material for the year. As the unit will start function in the same period and can start processing the material therefore the yearly cash inflow from the selling of the product will be 3.00 million INR. This way the plant will start giving the profit in the first year of its inception. The total profit from the plant is expected as Rs. 0.291 million INR per month (Rs. 3.50 million INR per year).
In each successive year 1.504 million INR will be required for operation and maintenance of the unit, the present worth of this investment was found to vary from 1.39 million INR to 0.322 million INR from first to twentieth year at discount rate of 8 per cent. Similarly the present worth of cash in flow from selling of the dehydrated product in each successive year was also determined and found to vary from 3.240 million INR to 0.750 million INR for the same duration. The net present worth of the total investment made on the industry in next 20 years was found as 19.673 million INR (Table 2).

The payback period of microwave assisted garlic dehydration unit was computed by determining the present worth of the total cash out flow and inflow and also presented in Table 3. It can be seen from the Table that after 6th year of operation the cumulative cash inflow will exceed the present worth of the cash out flow giving the pay back period as 6 years. The economic indicators, such as Net Present Worth, Benefit Cost Ratio and pay back period are presented in Table 4. The benefit cost ratio of the project was estimated with the help of present worth of the total cash inflow and out flow and found to be 2.308.

5 Conclusions

The researchers have conducted several studies on drying of various types of the fruits and vegetables in the microwave assisted drying units and found the system technically feasible. In the present study, microwave assisted drying unit for garlic dehydration was designed and developed and found that the process is technically feasible and economically viable. The dehydrated product was superior in retention of colour and the volatile content, compared to the commercially available samples. The Net Present Worth of the total investment on complete dehydration unit having capacity of 10 tons of dehydrated product per annum was 19.673 million INR. The benefit cost ratio of the project was estimated to be 2.308; with a pay back period being less than 6 years. The economic indicators suggested that microwave assisted small scale industry for garlic dehydration was an economically viable project.

Acknowledgements

The authors are thankful to Khadi and Village Industries Commission, Mumbai (India) for providing the financial assistance to develop the microwave dryer as well as process technology for drying garlic cloves under microwave field. Authors also thank the Dean, College of Technology and Engineering, MPUAT, Udaipur for providing required facilities for carrying out the present investigation.
References

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<td>Garlic</td>
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<td>Packaging material</td>
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<td>Utilities</td>
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<td>Power</td>
<td>10 kW for 10 hours per day</td>
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<td></td>
<td>Water</td>
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<td><strong>Total recurring expenditure</strong></td>
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<td>7.</td>
<td>Total capital investment</td>
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(1Euro= 64.65 INR)

Tab. 1: *Infrastructure requirement and their costs for microwave dehydration unit*
### Tab. 2: Financial aspects for the microwave dehydration unit for garlic

<table>
<thead>
<tr>
<th>Year</th>
<th>Present Worth of total cash out flow in 20 years (Rs.)</th>
<th>Cash inflow (Rs.)</th>
<th>Present Worth of cash inflow (Rs.)</th>
<th>Cumulative cash inflow (Rs.)</th>
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<td>Total</td>
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(1 Rs. = 1 INR)

### Tab. 3: Computation of the payback period of microwave assisted garlic dehydration unit

<table>
<thead>
<tr>
<th>Economic indicators</th>
<th></th>
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<tr>
<td>Net Present Worth of the Project of 20 Years (Rs)</td>
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<tr>
<td>B/C Ratio</td>
<td>2.08</td>
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<td>Pay Back Period</td>
<td>Less than 6 years</td>
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</table>

(1 Rs. = 1 INR)

### Tab. 4: Economic indicators of microwave assisted garlic dehydration unit
Abstract:

Electrical treatment causes the damage of biological membranes through the electroporation mechanism. The influence of pulsed electric fields (PEF) on juice expression from red grapes has been investigated. Effects on juice yield and qualitative juice characteristics of different PEF treatments are discussed. The PEF pre-treatment enhanced expression kinetics from red grapes and modified juice characteristics. Significant effects of PEF were observed for grapes pressed under the lowest pressure (0.2 bar) when the juice yield was multiplied by 3 comparatively to the juice yield of untreated grapes. The PEF treatment reduced juice turbidity by 33% and enhanced polyphenols extraction. The amounts of L-malic and tartaric acids in final juice were decreased by 0.1 g/l and 0.86 g/l respectively after the PEF pre-treatment.

Key words: red grapes, pulsed electric fields, expression kinetics, turbidity, phenolic compounds

1 Introduction

The treatment using Pulsed electric fields (PEF) is a non-thermal processing method for cell tissue damage and enhancement of juice extraction from fruits and vegetables. It can be a good alternative to the conventional thermal, chemical or enzymatic treatments. Under the action of PEF, cell membranes become reversibly or irreversibly damaged [1]. The advantages of PEF-treatment are selective extraction of cell components [2], lower energy consumption comparatively to thermal treatments and process intensification. Recently, it was shown that PEF use might be promising for the production of higher quality juices in white wine making [3]. The grape composition and structure are very important wine technology influencing factors [4,5]. Polyphenols are unevenly distributed in the berry of the grape.
major constituents of grape pulp and skin are phenolic acids of the hydroxycinnamic acids type [6]. Flavonoids comprise mostly flavanols, under monomeric and polymeric forms. In red grape varieties, another flavonoid family, the anthocyanins, is responsible for the red colour of the skin [7]. High expression pressure leads to the extraction of a more turbid juice with higher suspended solids content. This is why industrial practice supports the gradually increasing pressure expression method [8]. High level of polyphenols in juice contributes to bitterness, astringency, and browning and is not desirable in white wine processing. However, the polyphenolic content may be important for red wine production and grape marc valorization.

The aims of this study were to determine the effects of PEF treatment and expression method on juice properties and to optimize the expression parameters from the red grape cultivar treated by PEF.

2 Materials and methods

2.1 Materials

Good quality Pinot Noir grapes (red grapes) were harvested in 2008 from an experimental vineyard of France and were chosen as object of the study. For each experiment \( m = 1.4 \) kg of grapes (untreated or PEF treated) were introduced inside the compression chamber. The moisture content of the fresh grapes was 80 ± 0.2 wt.%.

2.2 Pulsed electric field treatment

A pulse generator, 5000 V–1000 A (Hazemeyer, France) was used for electric field treatment (Fig. 1.a). A sample of grapes was placed in the PEF-treatment cell between two plate parallel electrodes (Fig. 1.b). The PEF generator provided pulses of near-rectangular shape, and series of pulses were used for PEF treatment. Each separate series consisted of \( n \) pulses with pulse duration \( t_i \), time delay between pulses was \( \Delta t \) and a pause \( \Delta t_t \) was allowed after each waves train (Fig. 1.c). The total time of the PEF treatment was set by varying the number of series \( N \) and was calculated as \( t_{PEF} = nNt_i \). The PEF protocol with the following parameters was used in pressing experiments: \( E = 700 \) V/cm, \( n = 100 \), \( t_i = 100\pm 1 \) µs, \( \Delta t = 110\pm 1 \) ms, \( \Delta t_t = 10 \) s, and the number of wave trains \( N \) chosen among 5, 10 and 20, which correspond respectively to 50, 100 and 200 ms of PEF treatment. This protocol allowed fine control of the grape tissue permeabilisation without any noticeable temperature rise (\( \Delta T \leq 5^\circ C \)) during the PEF treatment.

The experiments of expression were carried out using a laboratory compression chamber usually used in the wine industry to estimate the potential expression yield of grapes. The compression chamber consists in a spherical compartment with internal radius of 115 mm with a fitted elastic diaphragm (Fig. 1.d). Pressure was applied to the layer of grapes through this elastic diaphragm by means of an air compressor. Pressing was progressively and step-by-step increased with time (0.2 bar increase each 3 minutes) until the desired pressure was reached (fig 1.d). This protocol was chosen for the scale-down modelling of industrial grape pressing process for white wine production. A filter cloth was placed at the bottom of the pressing chamber in order to reduce the turbidity of the juice flow.
2.3 Quantitative and qualitative analysis

2.3.1 Juice yield

The mass of juice was weighed using a PT 610 (Sartorius AG, Germany) electronic scale. The juice yield $(Y \%)$ was calculated using the following formula:

\[ Y = \frac{m}{m_i} \times 100 \]  \hspace{1cm} (1)

Here, $m$ is the mass of the juice after pressing and $m_i$ is the initial mass of grapes before pressing.

2.3.2 Analytical measurements

After pressing and cloth filter filtering, different analysis of the grape juice (must) were realized. Malic and tartaric acids contents (OIV, 1990) were measured. For determination of total polyphenols, the absorbance of the juice was measured at 280 nm [9] using a spectrophotometer UV-VIS (Libra S32, Biochrom, France) with a 1 mm quartz cell. For optimization purposes, direct reading of absorbance at 280 nm rather than Folin-Ciocalteu method may be preferable for total polyphenols evaluation [10]. Turbidity expressed in nephelometric turbidity units (NTU) was used as a measurement of juice clarity and concentration of suspended particles and colloids in the juice. A ratio XR turbidimeter (HACH Company, Loveland, USA) was used for turbidity measurements. The final turbidity was determined taking into account the dilution.

2.4 Statistical analysis

Each experiment was repeated at least six times, means and standard deviations of data were calculated. Mean test between untreated and PEF treated samples data was done. For each analysis, a significant level of 5% was considered.
3 Results and discussion

Figure 2 shows the evolution of juice yield during expression with gradually increasing pressure for the untreated and PEF treated samples versus pressing time. PEF treatment results in the enhancement of juice extraction. For example, the juice yields after 15 minutes of pressing for untreated and PEF treated samples were respectively 42% and 50%. This difference can be explained by the action of the PEF treatment. It has been demonstrated that PEF treatment provokes membrane permeabilisation and pore formation (electroporation). It can facilitate transfer phenomena, increase expression rate, and decrease the final cake humidity. These effects of PEF were observed for different fruits and vegetables [11].

![Fig. 2: Final juice yield for untreated and PEF treated grapes versus pressing time](image1)

![Fig. 3: Juice yield after 3 min of pressing at 0.2 bar versus PEF duration](image2)

Figure 3 plots the juice yield after 3 min of pressing at 0.2 bar versus PEF pre-treatment duration. In the absence of PEF treatment, the juice yield is equal to 6%. A noticeable effect of PEF treatment can be observed at the initial stage of pressing for a pressure P=0.2 bar. The increase of PEF treatment duration enhances electroporation of cell membranes. In wine making, it is important to extract more juice at lower pressure and during a short time. Higher expression pressure provokes extraction of undesirable impurities, which influence juice quality (extract with a higher turbidity). Longer pressing time induces the oxidation of polyphenols and antioxidant components. The reduction of pressing time and decrease of pressure can contribute to the preservation of juice characteristics and to decrease energy consumption (related to juice clarification and process time).

Figure 4 shows required pressing time to obtain the same value of juice yield (Y=50%) versus different PEF treatment durations. The increase of PEF treatment duration was accompanied with the decrease of pressing time. For example, a 50% yield of juice was obtained from untreated grapes after 24 min of expression, while from PEF treated grapes (200 ms) the same quantity of juice was obtained after only 10 min of expression.

The effect of PEF treatment on the total polyphenols content is shown in figure 5. The same quantities of juice (Y=50%) were obtained with and without PEF application. Statistical analysis shows a significant difference between treated and untreated samples. PEF treatment seems to be favourable to polyphenols extraction from red grapes. Similar results were observed for Chardonnay grapes (a white grape variety) [12].
The measurement of the electric energy consumption for different PEF treatment durations shows a quasi-linear dependency. The energy consumption equals to 4 kWh/kg, 7 kWh/kg and 15 kWh/kg for 50 ms, 100 ms and 200 ms of treatment time respectively.

The analysis measurements of turbidity, tartaric and malic acids for untreated and PEF treated samples for different portions of juice are given in table 1.

<table>
<thead>
<tr>
<th></th>
<th>Juice after 3 min of pressing</th>
<th>Final juice yield Y = 50%</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>PEF treated (100 ms)</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>336</td>
<td>227</td>
</tr>
<tr>
<td>Tartric acid (g/l)</td>
<td>9.33</td>
<td>8.13</td>
</tr>
<tr>
<td>Malic acid (g/l)</td>
<td>10.47</td>
<td>7.80</td>
</tr>
</tbody>
</table>

* No significant difference between both values.

Table 1. Characteristics of juice obtained from untreated and PEF-pre-treated grape

The PEF treatment always resulted to the increase of final juice yield (Fig. 2 and 3). Moreover, turbidity of juices, tartaric and malic acids contents of PEF-treated samples showed lower figures. The value of turbidity depends on the pressing time. It was higher for the first fraction of juice and lower for the final juice yield. This result can be explained by the extraction of higher quantities of pectin during the first minutes of pressing. For the final juice (juice yield = 50 %), equilibrium between clear and unclear juice was established. The quantities of L-malic and tartaric acids were decreased by 0.1 g/l and 0.86 g/l respectively after the PEF pre-treatment. The decrease of the acidity after PEF application was not adapted for white winemaking. These results should be investigated in future studies.

4 Conclusion

Pulsed electric fields application allows acceleration of juice and polyphenols extraction by pressure from red grapes using an increasing pressure protocol. Pressing time can be reduced with PEF pre-treatment use. The obtained juice was less turbid after the PEF pre-treatment (227 NTU for treated samples and 336 NTU for untreated samples). This study presents evidences of the interest of PEF pre-treatment for some uses in red wine making and rejected grape marc.
Acknowledgements

The authors would like to thank the “Pôle Régional Génie des Procédés” (Picardie, France) for providing financial support. Authors also thank the “Comité Interprofessionel des Vins de Champagne” (CIVC, France) for providing grapes and for their help with the realisation of this work.

References


