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Faculty of Food Science

Dissertation

Application of pulsed electric field and high hydrostatic pressure technology for the mild preservation of fruit juices

Prepared by:
Piroska Hartyáni

Supervisor:
Dr. Zsuzsanna Cserhalmi, PhD

Central Environmental and Food Research Institute
Unit of Technology

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Head: Prof. Péter Fodor
Corvinus University of Budapest

Supervisor: Dr. Zsuzsanna Cserhalmi
Head of Unit of Technology
Central Environmental and Food Research Institute
Budapest

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Signature of Head of School Signature of Supervisor

1. Introduction

Investigations into nutrition related diseases are nowadays highly topical. Scientific studies about human nutrition have increasingly demonstrated that a well-balanced diet which is rich in fruits and vegetables serves the health and decreases the risk of formation of many chronic, cardiovascular and cancer diseases. Consequently, the consumption of juices made of fruits and vegetables means effective protection against formation of many illnesses due to the antioxidant capacity of these raw materials, too.

Accordingly, the consumption of fruit juices has emphasized importance. Therefore high attention has to be paid to the application of processing technology with mild character in order to reserve the prosperous nutritional properties of fruit juices after processing. Although the traditional heat processing ensures the microbiological stability of fruit juices and prolongs the shelf life of the product, however, its application may lead to undesirable changes in the nutritional value and sensory properties (colour, flavour, consistency). In order to avoid the possible undesirable changes during the heat preservation, in the last decades the mild, non-heat based food preservation technologies gained increasingly attention and importance. The consumer demands for healthy, fresh-like food products resulted in the research of environmental friendly, mild, so called “novel” technologies. By applying these techniques minimal loss of nutritional value and favourable sensory properties can be observed. Among these novel methods the pulsed electric field (PEF) and the high hydrostatic pressure (HHP) are two international widely researched technologies. The outstanding advantage of these techniques is that beside the inactivation of microorganisms the aroma-, colour-, flavour- and nutritional properties of fruits and vegetables are maintained with high retention.

I proposed in my doctoral work the investigation of application of the PEF and HHP treatment process by examining the nutritional and other quality properties with the application of professional analytical methods. As it is presented in the manuscript, citrus juices were used in the first experimental part, which were subjected to PEF (28 kV/cm electric field strength, n=50 pulse number) and HHP treatment (600 MPa). Beside the investigation of physical-chemical and colour properties, the measurement of organic acid and volatile compounds were accomplished to get an overall feedback from their possible changes as an effect of the treatments. Chemosensor arrays, such as electronic tongue and nose were applied to investigate whether the separation based on the treatment and fruit juice types is possible. The clarification of this statement was of primary interest as far as the comparison of mild technologies (PEF and HHP) had never been done earlier with these equipments.

Because of increasing consumer demands, food technology innovations face continuously changing challenges. One of the most common reasons of consumer rejection is the undesirable flavour and year in year out the food industry gets complainant feedbacks referred to the off-odour which can occurred in the fresh, processed and packed products.

The presence of *Alicyclobacillus acidoterrestris* spp. causes such problems for the beverage industry which have to be solved everyhow, because with its appearance the sensorial properties change inauspicious. It can be observed, that in the presence of this microorganism the cloud of juice gives off a strong odour due to the production of guaiacol. Consequently, it induces food safety and economic damages, too. All these facts underlined the importance of the inactivation studies which can be reached by the combination of mild and traditional preservation technologies.

Therefore 100 % content apple and orange juice were inoculated by the *Alicyclobacillus acidoterrestris* (DSMZ 2498) microorganism to examine the effectiveness of these novel technologies. Combined heat (20, 50, 60 ºC) and high hydrostatic pressure (200, 400, 600 MPa for 10 minutes) treatment, respectively combined heat (20, 50, 60, 80 ºC) and pulsed electric field treatment (3 different levels concerning the energy input) were applied. Afterwards the treated samples were stored at 4 °C temperature up to a period of 4 weeks. Beside the microbiological analysis, physical-chemical and colour parameters had been measured during this 4 weeks storage period (0, 14, 28 days). Furthermore, electronic nose was applied to detect possible changes in the volatile compounds.

2. Objectives:

Relying upon the statements the following questions were arisen during the doctoral thesis:

1. What type of effect has the application of pulsed electric field and high hydrostatic pressure technique on the physical-chemical properties, included the colour parameters, the organic acids, respectively on the volatile compounds in case of the three freshly squeezed, uninoculated citrus juices (orange, grapefruit, tangerine)?
2. Electronic nose and tongue were applied for the evaluation of volatile compounds in uninoculated juice samples. Due to the lack of reference studies concerning the comparison of the two techniques (PEF and HHP) with the help of electronic nose and tongue additional research questions were included: Are the electronic instruments able to separate and compare the treatment types? Might they even serve as possible detection systems?
3. How could be the PEF and HPP treatment combined with the heat treatment in case of apple and orange juice, which were inoculated with *Alicyclobacillus acidoterrestris (*DSMZ 2498) microorganism? Is the effectiveness of the two treatment types affected by the applied treatment temperatures respectively the 4 weeks long storage time? What types of changes can be observed in the properties of total phenol content and radical scavenging activity beside the determination of the parameters mentioned above?
4. Is the electronic nose able to distinguish between the distinct inoculated fruit juice samples which were treated with different parameters concerning pressure, electric field strength as well as temperature levels and were stored for different periods? Since the electronic nose had been able to differentiate between the HHP and PEF treated juices, afterwards the question was arisen, whether it would be also possible to distinguish between different parameters within one treatment type?

3. Materials and methods

Fresh citrus fruits (orange, grapefruit, and tangerine) were selected from the local fruits and vegetable market. The experiments were carried out from one batch of big amount of fruits in order to use in each case the same initial samples. The preparation for the PEF and HHP treatment started as follows: To the measurement with the help of OSU-4B PEF equipment, the fruits were processed by a Moulinex (Varipulse, Odacio3) citrus juicer. The pulps (100% concentrations) were centrifuged at 3000 rpm 30 minutes long and afterwards filtered through a filter paper (MN 640d/15) to avoid bigger solid components in the juice which can cause problem during the treatment because of the dissimilarity of dielectric properties. All these preparation processes were important because the gap distance between the stainless steel electrodes was 0,29 cm in this type of equipment, thus bigger particle size could cause strangulation. During the treatment 2µs of bipolar pulse duration and square waveform were selected while peak electric field strength of 28 kV/cm with 50 pulses was applied. The total treatment time was 100 µs. The flow rate was adjusted to 84 ml/min and the treatment temperature was 25±1 ºC.

In case of high hydrostatic pressure treatment (Stansted Fluid Power type Food Lab 900, Stansted. Essex, U.K), after the preparation processes the plastic bottles were filled with samples which had to be closed possibly withour air bubble. After filling, the screw capped plastic bottles (4*30 ml) were placed in a high pressure vessel (37 mm internal diameter × 300 mm internal height, Stansted Fluid Power type Food Lab 900, Stansted. Essex, U.K.) which contained a mixture of ethanol and rust inhibitor (castor oil, 15% v/v) and subjected to pressures of 600 MPa. The time to reach the target pressure was approximately 90-100 s and depressurization took 30-40 s. The temperature during the treatment was controlled using the cooling system Haake C40-F6 in order not to rise up to the temperature of 20 °C. After the PEF and HHP treatment the treated samples were placed in sterile glass bottles and stored at 4 °C temperature until further analyses. After the PEF and HHP treatment of 100 % content fruit juices (orange, grapefruit, tangerine) the **physical-chemical properties** were measured in case of control (untreated), HHP-, and PEF treated samples. The pH and the conductivity were determined by Orion-4 Star (Thermo Electron Corporation) conductometer and the Brixº with the help of a refractometer (Zeiss, Jena, Germany).

Since a large amount and microbiologically stable fruit juice basis was needed for the measurements, commercial available 100 % content fruit juices (apple, orange) were selected during the research work at the Technical University of Berlin. The juices were inoculated with the *Alicyclobacillus acidoterrestris* DSMZ 2498 (Deutsche Sammlung von Mikroorganismen und Zellkulturen; Braunschweig, Germany) microorganism. The inoculation happened directly with the Alicyclobacillus suspension in the quantity of 10 ml pro 1000 ml fruit juice. The final microbial concentration of the inoculated juices was 10^6 CFU/ml. The aim was to measure with the electronic nose specifically the changes of metabolite produced by the *A. acidoterrestris.*

The inoculated juices were treated with continuous flow high voltage PEF equipment and U4000 type HHP equipment. The pH and the conductivity were determined by Inolab pH 740, WTW Series, USA and the Brixº with the help of a refractometer (RFM 80, Winopal Forschung, Germany). These properties were measured in case of PEF- and HHP treated samples, furthermore in case of inoculated, but not treated samples which served as control. The changes were also followed up during the storage.

In case of freshly squeezed and microorganism inoculated juices the **colour** of control and treated samples was measured at room temperature by using a hand-held tristimulus colour analyser

(Minolta Chromameter-CR200). According to the CIELab system, the total colour difference (ΔE) was determined from the measured L*(lightness), a* (red to green colour), b* (yellow to blue colour) values.

The investigation of **organic acid content** was applied in case of the freshly squeezed untreated, PEF and HHP treated citrus juices (orange, tangerine, grapefruit). The citric, malic, ascorbic acid content of the samples were measured by high-pressure liquid chromatography (HPLC) with the help of Alliance Waters 2690 chromatographic system with PDA detector (Waters 996) on a reverse phase. An YMC ODS_AQ column (ABL and E-Jasco) with the same precolumn was used. The flow rate of the eluent was adjusted to 0,7 ml/min (KH_2PO_4 2,75 g/l). The detection of citric acid was at λ-412 nm and in case of ascorbic acid it was at λ-242 nm. The linearity range was 0,01-1,0 g/l.

For the fast comparison of **aroma profile** of freshly squeezed juices a SPME sampling was developed. A PDMS (polydimethylsiloxane) apolar fiber of 100 μm film thickness (Supelco, Inc.) was applied. In the case of orange juice the method of Jia and co-workers (1999) was adapted. The separation and identification of volatile aroma components was carried out by gas chromatography-mass selective detection. A HP 5890 gas chromatograph coupled with a HP 5971 mass selective detector was used. The column was RH-5ms 30 m x 0.25 mm i.d., 0.25 μm film thickness.

The measurement of changes in the **flavour properties** of citrus juices was carried out by **electronic tongue** (Alpha-MOS α Astree, Toulouse, France) in the Department of Physics and Control, Corvinus University of Budapest. The instrumental measurement of **volatile compounds** in freshly squeezed and inoculated fruit juices was achieved with the help of **electronic nose** (NST 3320 instrument, Applied sensor, Sweden) in the Department of Refrigeration and Livestocks' Products Technology, Corvinus University of Budapest. Multiple statistical analyses were used for the evaluation of the signal responses of electronic tongue and nose.

Microbiological analyses were investigated after the treatment of heat+PEF and heat+HHP of *A. acidoterrestris* inoculated apple and orange juice. The tubes containing the treated samples were mixed carefully with a shaker and a 30 µl quantity of the sample was diluted in ¼ Ringer liquid (TP887925712, Merck KGaA, Darmstadt, Germany) in dilution phase on micro titer plates (Carl Roth GmbH, Karlsruhe, Germany). Afterwards from each dilution unit, 2*50 µl was dropped-plated in petri-dishes on the microorganism appropriate solid medium (DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen- nr. 402; Braunschweig, Germany). The Petri dishes were placed afterwards in incubators at 46 °C for 48 hours before manual enumeration.

The measurement of **total phenol content** of microorganism inoculated juices was carried out according to the method of SINGLETON and ROSSI (1965) in spectrophotometric way (UV-Vis) at 760 nm wavelength with the help of Folin-Ciocalteu reagent. The total phenol content was expressed as µg gallic acid/g raw material. The **radical scavenging activity** of the sample mentioned above was determined according to the method of YAMAGUCHI et al. (1998), with the use of 2.2-diphenyl-1-picrylhydrazyl (DPPH). The radical scavenging activity was expressed as µMol trolox/g raw material quantity.

4. Results and Discussion

Regarding the ***physical-chemical properties*** (pH, Brix°, conductivity) it could be established that no significant differences were observed between the control and treated samples either in case of freshly squeezed uninoculated citrus juices, or in case of inoculated fruit juices. The results obtained by the ***tristimulus colour measurement*** showed that the control and the treated samples were differentiated significantly from each other. It could be established that those citrus juices which were treated by PEF, showed a stronger tendency to the yellow colour tone. In the comparison of control and HHP treated samples of grapefruit juice also visible differences were observed according to the counted value of ΔE^* parameter, as far as the juice was getting dark.

In the application of combined heat- and PEF, respectively heat- and HHP technique in case of the inoculated juices the followings were established: with the increase of treatment temperature during the HHP treatment the L* lightness value showed increasing tendency when different pressure levels were used. Contrary with the increasing temperature levels the decrease of the L*value could be observed after the PEF treatment. It meant that the PEF treated juice samples showed darker tincture. Taking the passing of storage time into consideration during treating on a given temperature level, the colour results showed the following tendency: those samples became brighter which were treated on higher pressure levels. The increase of temperature in HHP treatments and the decrease of temperature in PEF process resulted in a decrease of the b* value. A decrease of the a* value was reached by increasing the pressure and the temperature level. In contrast, by applying higher electric field strength in PEF process, also a higher value of a* was observed.

Regarding the ***analysis of organic acid and volatile compounds*** it could be established, that the amount of acids decreased in smaller rate in the citrus juices after the PEF treatment in comparison to the HHP treated juice samples. Based on the reference literature and the results of this study it can be concluded that possibly the higher amount of acid content after the treatment can be related to the extended concentration of compounds in some cases. According to the analysis of volatile compounds it can be established that the quantity of ethyl-esters did not decrease significantly after the PEF treatment. Taking the main terpene components into consideration, the quantity of the limonene level seemed to be higher in case of orange juices as a result of PEF treatment. Nevertheless, the limonene level decreased after the HHP treatment whilst the valence content increased. All these results point to the phenomenon of aroma scalping.

Based on the measurements made by ***sensor arrays*** it was clear that both the ***electronic tongue*** and the ***electronic nose*** were able to discriminate those citrus juice samples which were treated by the new techniques (PEF, HHP). Consequently, they could successfully follow up the possible changes of flavour and volatile compounds during the treatment process.

As it can be seen in the results of confusion matrix of CDA cross-validation, only the half of the control samples could be ranked in the correct group, which points to the fact that the applied treatment levels did not cause drastic changes in the volatile compounds of treated samples.

Electronic nose was used for the examination of orange and apple juices which were inoculated with *Alicyclobacillus acidoterrestris* (DSMZ 2498). As it was previously stated, these electronic instruments were able to differentiate samples which were treated by distinct new technologies. The further measurements revealed on the fact that using different parameters (pressure, electric field strength) within one technology type during the process, the treated samples were also distinguishable according to each parameter.

Taking the output values of linear discriminant analysis into consideration which is based on the signal response of electronic nose the following order of the parameters can be set on concerning their ability to separate: In case of HHP treated samples the most dominant factor is the type of the juice, followed by the treatment temperature, the storage time and at last the applied pressure during the treatment. In case of PEF treated juices the same tendency was observed in the ranking, displacing the pressure with energy input values. All these outcomes also demonstrate that electronic nose is an outstanding tool for the examination of the effectiveness of these novel technologies. Additionally it offers an illustrious guideline about the critical parameters of the mild technologies.

Concerning the ***microbiological analysis*** it was established that with the application of combined heat-and high hydrostatic pressure, respectively – pulsed electric field, the inactivation of *Alicyclobacillus acidoterrestris* can be reached in different rates depending on the combination of the selected parameters. After the high hydrostatic pressure treatment at room temperature (20 °C), neither the number of vegetative cells, nor the number of spores decreased significantly in the *Alicyclobacillus acidoterrestris* inoculated apple and orange juice samples.

Regarding the spore numbers, 2-3 log reduction was observed by applying 50 °C treatment temperature whilst the number of vegetative cells was reduced under the detection limit with the increase of pressure level. Even with the rise of energy input levels the inactivation could not be reached after the PEF treatment of inoculated juices at room temperature (20 °C), and still the application of 50 °C and 60 °C treatment temperatures were not enough that this combined process could cause changes in the spore numbers.

With the application of 80 °C treatment temperature and highest energy input values the spore number sunk under the detection limit. The reached inactivation level maintained also during the 4 °C temperature storage, indeed in some cases it intensified with the passing of storage time. These results draw the attention to the importance of chilled storage of mild technology processed food.

It can be stated that during the combined heat+PEF and heat+HHP treatments even the increasing treatment temperature did not cause significant changes in the ***biological active compounds***, neither in the total phenol content, nor in the radical scavenging activity. It means that the treatments did not have impairing effects to these properties. Moreover in case of heat+ PEF treatment an increase was observed in the total phenol content, which might be the result of the cell membrane permeabilization with a higher rate, due to the effect of the application of higher electric field strength intensity. All these statements strengthen the efficiency and mild effect of combined heat and PEF, respectively heat and HHP treatments concerning the total phenol content and radical scavenging activity.

5. New scientific results, theses

1. It was established that in the case of freshly squeezed citrus juices (orange, grapefruit, tangerine) the pulsed electric field (28 kV/cm , 50 pulse number, 2 µs pulse width) and the high hydrostatic pressure technique (600 MPa, 10 min) did not cause significant changes, as an effect they did not affect the physico-chemical properties (pH, Brix°, conductivity) and the organic acid content of juices.
2. The application of electronic nose and electronic tongue for the determination of volatile compounds of citrus juices (orange, grapefruit, tangerine) was successful. During their adaptation it was stated that the PEF treatments (with an electric field strength of 28 kV/cm and 100 µs treatment time) respectively the HHP treatments (with 600 MPa applied pressure and 10 minutes treatment time) could be distinguished by this electronic instruments.
3. With the investigation of combined heat and PEF respectively heat and HHP treatments of *Alicyclobacillus acidoterrestris* DSMZ 2498 inoculated apple and orange juices the followings were established: regarding the inactivation of spores and vegetative cells the most dominant factor was the temperature. An increase of temperature resulted in a declining tendency in the number of microorganism. Furthermore the spores and vegetative cells of *Alicyclobacillus acidoterrestris* were inactivated more successfully by combined heat and HHP treatment.
4. It was stated that the application of pulsed electric field technique in itself or with its combination with temperature showed an upward tendency in total phenol content of *Alicyclobacillus acidoterrestris* DSMZ 2498 microorganism inoculated juice which can reach also a 20 % rise at 95 % confidential level.
5. With the help of canonical discriminant analysis it was presented that the electronic nose could differentiate the distinct treatment types (HHP and PEF) in case of *Alicyclobacillus acidoterrestris* microorganism inoculated apple-and orange juice. In addition, within the PEF technique the e-nose was able to differentiate the samples which were treated at low, middle and high electric field strength, respectively within the HHP technique the samples which were treated at 200, 400, 600 MPa pressure levels.
6. Based on the sensor responses of the electronic nose it was demonstrated with the application of multiple statistical analysis methods that the conditions of combined heat and HHP respectively heat and PEF treatments affect distinctly to the volatile compounds of fruit juices (apple, orange). Taking the influence of the examined factors into consideration the following ranking can be set up: The treatment time (20, 50, 60, 80 °C) had the highest influence, followed by storage time (0, 14, 28 days) and the technological parameters (electric field strength, applied pressure).

Publications related to the subject of dissertation

<u>Reviewed articles published in international journals (IF):</u>

Piroska Hartyáni, István Dalmadi, Zsuzsanna Cserhalmi, Dávid-Balázs Kántor, Marianna Tóth-Markus, Ágnes Sass-Kiss. (2011). Physical-chemical and sensory properties of pulsed electric field and high hydrostatic pressure treated citrus juices. *Innovative Food Science and Emerging Technologies*. Vol 12(3), pp. 255-260

Piroska Hartyáni, István Dalmadi, Dietrich Knorr. (2012). Electronic nose investigation of *Alicyclobacillus acidoterrestris* inoculated apple and orange juice treated by high hydrostatic pressure. *Food Control (accepted),* DOI: 10.1016/j.foodcont.2012.10.035

<u>Hungarian articles published in non IF journals</u>

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Proceeding in Hungarian language:

Hartyáni Piroska, Cserhalmi Zsuzsanna (2008); Pulzáló elektromos térerővel kezelt gyümölcslevek vizsgálata, FVM Fiatal Kutatók az élhető Földért. pp.62. ISBN 963993502-6

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Hartyáni P., Cserhalmi Zs., Kántor D. B., Dalmadi I., Tóth-Márkus M., Sass-Kiss Á., (2008): Study of pulsed electric field treated fruit juices. International Conference on Science and Technique in the Agri-food business (ICoSTAF), Abstract book, 202-203, pp. Conference book ISBN: (978-963-482-908-9).299-305 pp. 5-7. November

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<u>Citations (without self-citation)</u>

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