SAPP XXII

22nd Symposium on Application of Plasma Processes and 11th EU-Japan Joint Symposium on Plasma Processing

Book of Contributed Papers

Štrbské Pleso, Slovakia
18-24 January, 2019

Edited by V. Medvecká, J. Országh, P. Papp, Š. Matejčík

HIDEN ANALYTICAL

E L E V A T E

Book of Contributed Papers: 22nd Symposium on Application of Plasma Processes and 11th EU-Japan Joint Symposium on Plasma Processing, Štrbské Pleso, Slovakia, 18-24 January 2019.

Symposium organised by Department of Experimental Physics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava and Society for Plasma Research and Applications in hotel SOREA TRIGAN***, Štrbské Pleso, Slovakia, 18-24 January 2019.

Editors: V. Medvecká. J. Országh, P. Papp, Š. Matejčík

Publisher: Department of Experimental Physics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava; Society for Plasma Research and Applications in cooperation with Library and Publishing Centre CU, Bratislava, Slovakia

Issued: January 2019, Bratislava, first issue

Number of pages: 386

URL: http://neon.dpp.fmph.uniba.sk/sapp/

NON-THERMAL PASTEURIZATION OF FRESH APPLE JUICE BY COLD AIR PLASMAS

Barbora Tarabová[1], Andrea Žilková[1], Francesco Tampieri[2], Ester Marotta[2], Cristina Paradisi[2], Marco Krewing[3], Julia E. Bandow[3], Zdenko Machala[1]

[1]*Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia*
[2]*Department of Chemical Sciences, University of Padova, Padova, Italy*
[3]*Applied Microbiology, Biology and Biotechnology, Ruhr University, Bochum, Germany*
E-mail: tarabova@fmph.uniba.sk

Cold air plasma (CAP) generated by the transient spark discharge in air was successfully tested for non-thermal pasteurization of fresh apple juice. Inactivation of model pathogens of bacteria and yeasts and extended shelf-life time of the juice were achieved. Minimal effects of CAP treatment on juice chemical and sensory properties (i.e. color, pH, degradation of important juice components, chemical composition) were observed. Successful inactivation of peroxidase enzyme typically responsible for the undesirable juice browning was achieved. By comparing two systems of juice treatment – the static (batch) system was evaluated as more efficient for bacterial inactivation and less affecting the juice properties compared to the electrospray system.

1. Introduction

Conventional methods for inactivation of food borne pathogens are based on thermal treatments, typically referred to as pasteurization. Particularly ultra-high temperature (UHT) processes lead, beside the sterilization, to the loss of food quality (nutrition values, vitamin content, changes in sensory properties, such as taste or color). A growing customers' demand for long-lasting fresh products requires the concept of the "minimal processing". Therefore in recent years, new technologies that can achieve the required level of sterilization and safety without thermal input have been investigated – e.g. pulsed electric field, high pressure, ultrasound, etc [1]. Non-thermal (cold) plasmas known for their bactericidal properties achieved without excessive heat requirement are predetermined for application in sterilization/pasteurization of fresh food and food packaging [2-3].

The objective of this work was to test the use of cold air plasma (CAP) generated by the transient spark discharge in two different systems for the non-thermal pasteurization of freshly squeezed apple juice. We also focused on chemical, nutrient and sensory properties of CAP treated apple juice.

2. Experimental set-up and methods

Transient spark discharge (TS) in positive polarity in direct contact with the juice was generated in ambient air at atmospheric pressure in two different set-ups depicted in Fig. 1. TS discharge is characterized as a self-pulsing repetitive streamer-to-spark transition with very short duration (<100 ns) of spark current pulses (repetitive frequency ~1 kHz) [4]. Both set-ups are based on the point-to-plane geometry using sharp hollow needle as the high voltage (HV) electrode. In the electro-spray system (ES), juice flowed directly through the HV needle with flow rate 1mL/min. Due to the applied HV, the effect of the electrospraying of the juice to the micrometric size droplets occurred. ES set-up enabled the direct contact of the active discharge zone with the sprayed droplets of the juice. Static system (SS) is based on the batch treatment by the discharge generated directly over the juice surface, in which the grounded electrode was submerged with the treatment conditions 1min per 1 mL of the juice.

Freshly squeezed apple juice (cultivar Ontario) was stored in the freezer at -20°C and defrost immediately before plasma treatment. We performed experiments focused on:

- inactivation rate of model pathogens by CAP, where juice was inoculated by bacteria *Escherichia coli* ATCC 25922 or yeasts *Saccharomyces cerevisiae* S228C (initial concentration ~10^6-10^7 CFU/mL);
- effects of CAP treatment on the shelf-life time of juice when containing only its native pathogens.

The pathogen inactivation rate and the spoilage rate were evaluated by the classical thermostatic cultivation and was followed up to 28 days post CAP treatment. During this period, the juice was stored in the fridge at 4°C to simulate the typical behaviour of consumers.

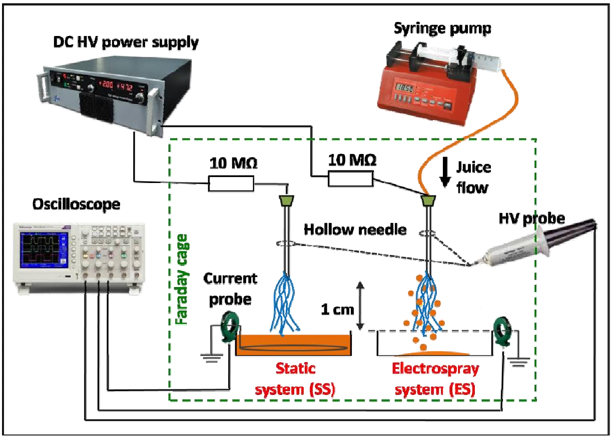

Fig. 1. Experimental set-up of air transient spark discharge in static SS and electrospray ES system for juice treatment.

We also focused on the effects of cold plasma on the chemical, nutrient and sensory properties of the CAP treated juice, such as changes of pH, conductivity; colour changes measured by juice transmittance; concentrations of nitrites/nitrates (NO_2^-/NO_3^-) and hydrogen peroxide (H_2O_2) measured by colorimetric methods; changes of °Brix degree (dissolved sugar content measured by refractive index). The potential chemical changes of juice composition due to the presence of reactive oxygen and nitrogen species (RONS) formed by CAP were evaluated. The most typical juice components including polyphenols, organic acids and sugars, and their CAP induced degradation products were investigated by means of HPLC coupled to UV-VIS, mass spectrometry (MS) and refractive index (RI) detectors. Activity of the peroxidase (POD) enzyme responsible for the natural browning of fruit juices was evaluated.

3. Effects of CAP on pathogen inactivation and spoilage rate of fresh juice

The juice contaminated with model pathogens (*E. coli* or *S. cerevisiae*) was treated in both systems (ES and SS). The efficiency of cold plasma treatment on the inactivation rate (population growth) was followed up to 26-28 days post CAP treatment. In both systems, the initial relatively low (< 1 log) inactivation of *E. coli* bacteria immediately post CAP treatment was followed by a significant decrease of bacterial load within the first 2 days post plasma (~ 3-5 log) that reached further complete inactivation, which remained up to 26 days (Fig. 2). The efficiency of CAP treatment on yeast *S. cerevisiae* remained quite low over the period of 28 days (~1 log), slightly higher for the SS system (not shown). However the high initial yeasts concentration (~10^6 CFU/mL) surpasses by several orders of magnitude the possible natural contamination and therefore much stronger effect can be expected for lower yeast concentration.

Freshly squeezed apple juice may contain its native pathogens (different strains of yeasts and bacteria were detected) responsible for the spoilage of the juice, which primarily affect its shelf-life time. Our preliminary experiments indicated no bacterial or yeast growth up to 26 days post treatment in natural juice treated in both plasma systems and refrigerated at 4°C although both treated and control untreated samples were under detection limit.

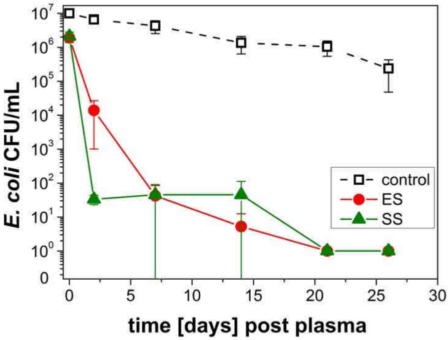

Fig. 2. The time evolution of the inactivation rate of the CAP treated juice contaminated by *E. coli*.

4. Analysis of CAP treated juice chemical, sensory and nutrient properties

Cold air plasmas generated in direct contact with liquids typically induce chemical changes and formation of reactive oxygen and nitrogen species (RONS) in these liquids. Some of the abundant long-lived RONS, especially NO_2^-/NO_3^- or H_2O_2 may be harmful for human health in high doses and their content in food products is regulated. For example the acceptable daily intake (ADI) per kilogram human body weight is 0-3.7 mg NO_3^- and 0-0.06 mg NO_2^- ions. Fig. 3 shows that the concentrations of measured NO_2^- and NO_3^- in CAP treated juice were significantly lower after 24 hours post plasma treatment than the ADI of NO_2^- and NO_3^- per average 60 kg human assuming exaggerated 1L/day juice consumption.

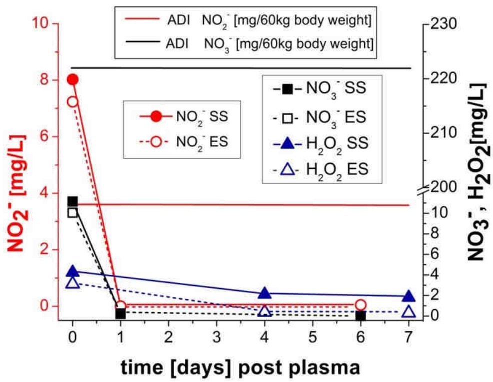

Fig. 3. NO_2^-, NO_3^- and H_2O_2 concentrations measured in CAP treated juice compared to the ADI doses calculated to an average human of 60 kg body weight. The shown measured and ADI values are directly comparable assuming that an average human consumes 1L of the juice daily.

CAP treatment and RONS formed in CAP treated liquids are known for inducing chemical changes resulting in changes of pH, conductivity or degradation of the organic chemical compounds. Native apple juice contains many organic components including sugars, organic acids and polyphenols known as antioxidants or vitamins. Representative components from each group (i.e. sugars – fructose, glucose and sucrose; organic acids – malic, citric and ascorbic acid; polyphenols – chlorogenic acid, phloridzin and epicatechin) have been tested for possible degradation by CAP treatment [5]. These compounds were CAP treated separately as aqueous solutions and all together in the fresh juice. The results show that in control experiments these compounds undergo reactions when they are CAP treated individually in aqueous solutions at the same concentration as found typically in the juice, leading to their hydroxylated and nitrated products. For instance in Fig. 4 are shown UV-chromatograms of non-treated phloridzine solution and its detected nitrated and hydroxylated degradation products after CAP treatment.

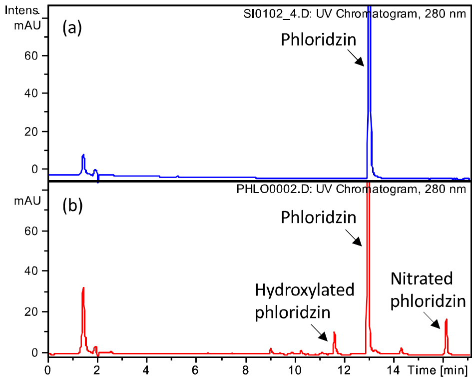

Fig. 4. UV chromatograms (280 nm) of **(a)** non-treated and **(b)** ES treated phloridzin solution.

In contrast, phloridzine seemed to remain unaffected in similarly CAP treated fresh juice as can be seen in Fig. 5. This can be attributed to the fact, that although high concentrations of RONS may be formed in the CAP treated juice, the competition by numerous juice components for the same reactive species limits the effect on each individual substance. We were also able to successfully detect all aforementioned components in the fresh juice and determine the percentage of their decomposition (not shown).

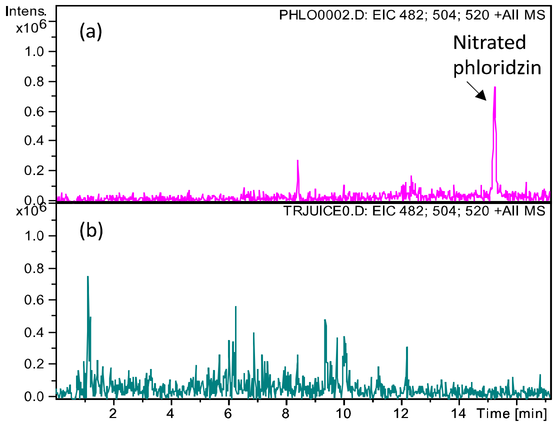

Fig. 5. Extracted ion chromatograms of the signals ($[M+H]^+$ m/z 482, $[M+Na]^+$ m/z 504, $[M+K]^+$ m/z 520) due to nitrated phloridzin **(a)** in ES treated phloridzin solution and **(b)** in ES treated juice.

We also detected no significant changes of pH (3.28→3.19), conductivity and °Brix degree in CAP treated juice. The measured transmittance slightly decreased indicating very mild darkening of the juice due to direct CAP treatment. Peroxidase as one of the enzymes responsible for the undesirable browning (oxidation) and the loss of the juice quality [6] was successfully inactivated. The remaining activity of POD in juice treated in SS system was about 29 % and 47% in ES system.

5. Summary

Cold plasma of the transient spark discharge in air was demonstrated as a promising alternative food processing technique for non-thermal pasteurization of fresh apple juice. Preliminary results showed a significant decontamination rate of pathogens, and shelf lifetime extension up to 26 days. It was shown that the juice quality was not significantly affected by the CAP treatment, i.e. minimal changes of chemical composition (degradation of organic acids, polyphenols, sugars), pH and color were detected. Successful inactivation of peroxidase enzyme known for the undesirable juice browning was achieved.

This work was supported by the Slovak Research and Development Agency APVV-17-0382 and APVV-0134-12, Comenius University in Bratislava, University of Padova (PRAT 2014, Grant CPDA147395/14), and by German Research Foundation (JEB(PAK728)).

6. References

[1] Minimal processing technologies in the food industry 2002 edited by Ohlsson T and Bengtsson N Woodhead Publishing Limited CRC press ISBN 0-8493-154-2.

[2] Niemira B A 2012 *Annual Review of Food Science and Technology* **3**.

[3] Thirumdas R, Sarangapani C and Annapure U S 2014 *Food Biophysics* **10**.

[4] Machala Z, Tarabova B, Sersenova D, Janda M and Hensel K 2019 *Journal of Physics D: Applied Physics* **52**.

[5] Eisele T A and Drake S R 2005 *Journal of Food Composition and Analysis* **18**.

[6] Surowsky B, Fischer A, Schlueter O and Knorr D 2013 *Innovative Food Science and Emerging Technologies* **19**.