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A novel approach to the non-thermal pasteurization of fresh apple juice by cold air plasma

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Abstract: Non-thermal (cold) air plasma generated by the transient spark discharge in air was successfully tested for non-thermal pasteurization of fresh apple juice. Inactivation of model pathogens (*E. coli* and *S. cerevisiae*) and extended shelf-life time of the juice were achieved:higher in the static (batch) system than in the flowing electrospray system. Minimal effects of cold plasma on juice chemical and sensory properties (chemical composition, color, pH) were shown. Successful inactivation of peroxidase enzyme known for the undesirable juice browning was achieved.

Keywords: non-thermal plasma, pasteurization, apple juice.

1. Introduction

Conventional methods for food processing and inactivation of food borne pathogens are based on using thermal treatments, typically referred to as *pasteurisation*. Particularly ultra-high temperature (UHT) processes may lead, beside the sterilization, to the damage of heat-sensitive products and the loss of food quality. A growing customers' trend demanding long-lasting fresh products leads to the concept of the "minimal processing" technologies. Therefore in recent years, food processing technologies that can achieve the required level of sterilization and safety without thermal inputs have been investigated - e.g. high hydrostatic pressure, pulsed electric field, ionizing radiation, etc. [1]. Cold plasmas known for their bactericidal properties achieved without heat effects have been successfully tested for sterilization or processing of various food products including fresh juices [2-3].

The objective of this work was to test the use of cold air plasma generated by the transient spark discharge in two different systems for the non-thermal pasteurization of freshly squeezed apple juice.

2. Experimental set-up and methods

Transient spark discharge in positive polarity and in contact with liquid was generated in ambient air at atmospheric pressure in two different set-ups depicted in Fig. 1. Transient spark discharge is a self-pulsing repetitive streamer-to-spark discharge with very short duration (< 100 ns) of spark current pulse with the repetitive frequency ~ 1 kHz [4]. Both systems are based on the point-to-plane geometry using sharp hollow needle as the high voltage electrode. In the electro-spray system (ES), juice was pushed by the syringe pump directly through the needle. Due to the applied high voltage, the effect of the electro-spraying of the juice to the micrometric size droplets occurred. This 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 static, i. e. batch treatment by the discharge generated directly over the juice surface, in which the grounded electrode was submerged.

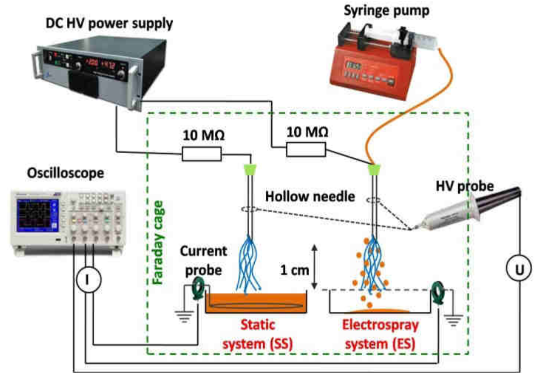

Fig. 1. Set-up of air transient spark discharge in static and electrospray system.

Freshly squeezed apple juice (from apples of variety Ontario) was stored in the freezer at -20°C. We performed experiments focused on:

- inactivation rate of pathogens by cold plasma, where juice was inoculated by model pathogens: bacteria *Escherichia coli* ATCC 25922 and yeasts *Saccharomyces cerevisiae* S228C with the initial concentration ~ 10^6-10^7 CFU/mL;
- effects of cold plasma treatment on the shelf-life time of juice when containing only its native pathogens;
- chemical and sensory analysis of the cold plasma treated juice.

The plasma treatment conditions of the juice were as follows: flow rate 1 mL/min in ES and 1 min treatment per 1 mL of juice in SS. Either the pathogen inactivation rate or the spoilage rate was evaluated by the classical thermostatic cultivation and was followed up to 26 days post plasma treatment. During this period, the juice was stored in the fridge at 4°C to simulate the typical behaviour of consumers.

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

3. Effects of cold plasma on pathogen inactivation rate and shelf-life time of fresh juice

The juice contaminated with model pathogens 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 days post plasma treatment. In both systems, the achieved inactivation of *E. coli* immediately post plasma treatment was quite low (< 1 log) followed by a significant increase within the first 2 days post plasma (~ 5-6 log) that remained up to 26 days (Fig. 2).

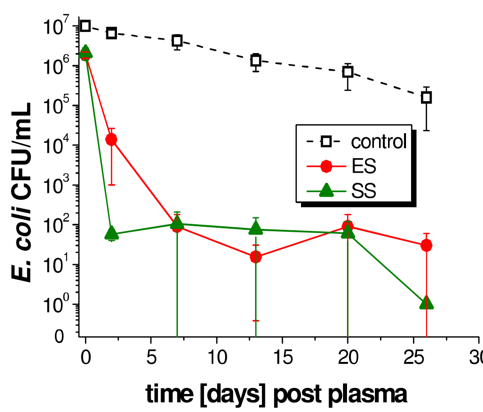

Fig. 2. The time evolution of the inactivation rate of the treated juice contaminated by *E.* coli.

The efficiency of plasma treatment on yeast *S. cerevisiae* remained quite low over the period of 26 days, slightly higher (0.6-0.8 log) in the SS system.

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 affects its shelf-life time. Our preliminary experiments indicated no bacterial or yeast growth up to 7 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. The long-term experiments (up to 28 days post plasma treatment) are to be performed.

4. Effects of cold plasma on juice properties

Cold air plasmas in direct contact with liquids induce formation of reactive oxygen and nitrogen species (RONS), especially hydrogen peroxide, nitrites/nitrates or $OH^{\cdot}$, $NO^{\cdot}$ and NO_2 radicals. Due to the fact that certain doses of RONS may be harmful for human health, their content in food products is regulated. For example the acceptable daily intake (ADI) per human kilogram body weight is 0-3.7 mg nitrate and 0-0.06 mg nitrite ions. Fig. 3 shows that the concentrations of measured nitrites and nitrates in cold plasma treated juice were significantly lower than the ADI of nitrites and nitrates per average 60 kg human.

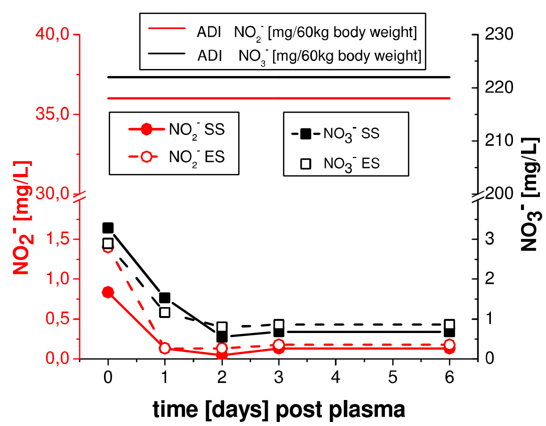

Fig. 3. Nitrites and nitrates concentrations measured in cold plasma treated juice compared with the ADI doses calculated to an average human of 60 kg body weight. The shown measured and ADI values are directly comparable assuming that an average human consumes 1L of the juice daily.

RONS formed in plasma 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. Representative components from each family (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 RONS [5]. Preliminary results show that in control experiments the polyphenols undergo reactions when they are plasma treated individually in aqueous solutions at the same concentration as found inthe juice, leading to their hydroxylated and nitrated products (Fig. 4).

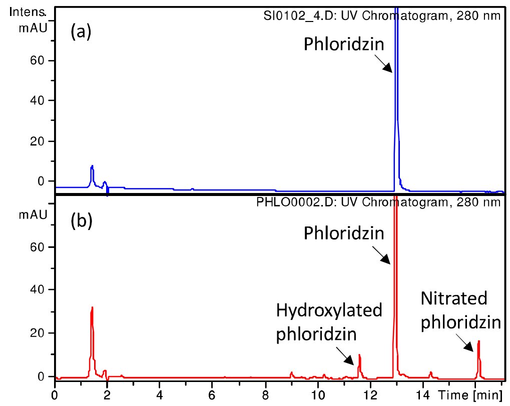

Fig.4. UV chromatograms (280 nm) of (a) untreated and (b) electrospray treated phloridzin solution.

In contrast, the same polyphenols seemed to remain unaffected when similarly treated within the juice (Fig. 5). This can be attributed to the fact, that although high concentrations of RONS may be formed, the competition by many juice components for the same reactive species limits the effect on each individual substance.

We also detected no significant changes of pH (3.28→3.19), conductivity and °Brix degree in plasma treated juice. The measured transmittance slightly decreased in plasma treated juice, indicating very mild darkening of the juice. Peroxidase (POD) is one of the enzymes known for the undesirable browning and the loss of the juice quality [6]. We showed that the transient spark treatment successfully inactivated the peroxidase, the remaining activity of POD in juice treated in SS system was only about 6 %.

5. Summary

Cold air plasma seems to be a promising alternative food processing technique for non-thermal pasteurization of fresh apple juice. Preliminary results showed a significant decontamination rate of pathogenic bacteria and shelf life time extension. In addition, enzyme inactivation and no significant chemical and sensory modifications give a very optimistic potential of this novel method of food product preservation.

6. Acknowledgement

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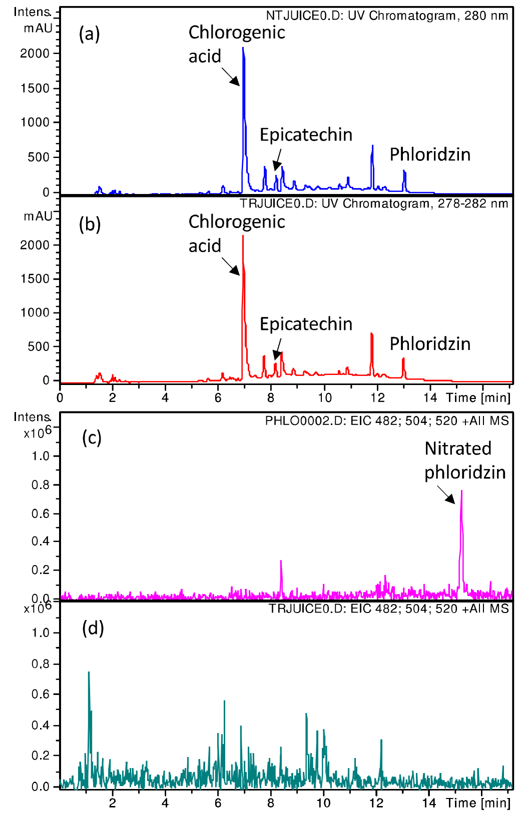

Fig.5. UV chromatograms (280 nm) of (a) untreated and (b) electrospray treated juice. Extracted ion chromatograms of the signals ($[M+H]^+$ m/z 482, $[M+Na]^+$ m/z 504, $[M+K]^+$ m/z 520) due to nitrated phloridzin (c) in electrospray treated phloridzin solution and (d) in electrospray treated juice.

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