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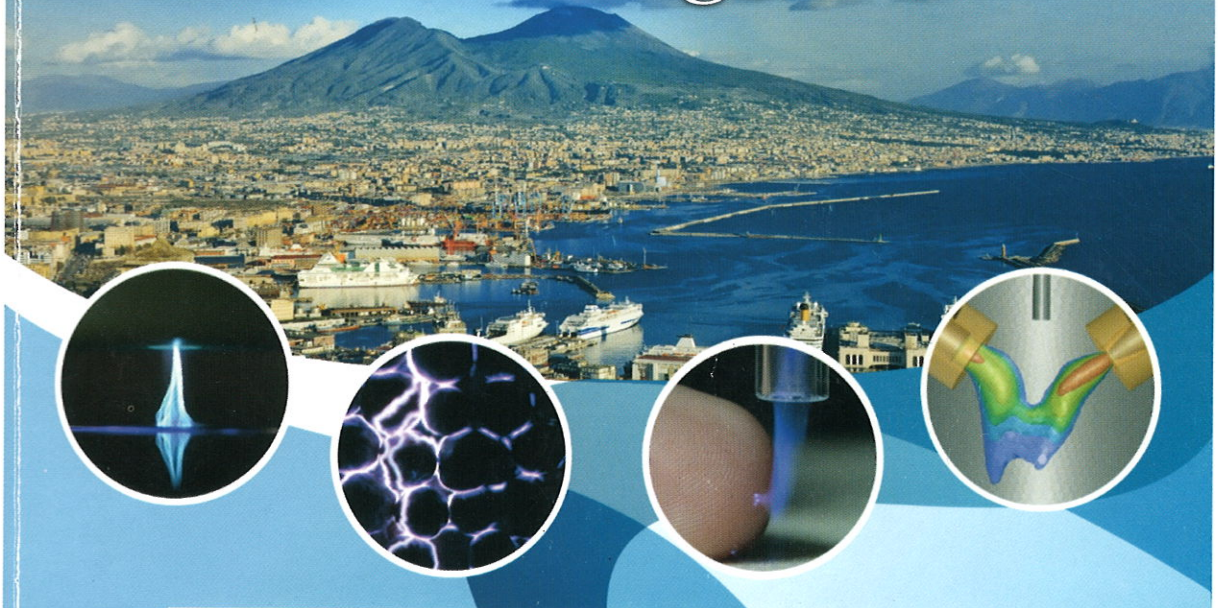

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Treatment of fruit juices inoculated with native microorganisms using two cold air plasma sources

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Abstract: Cold air plasma produced by a transient spark discharge and a plasma synthetic jet actuator were successfully tested for the inactivation of native microorganisms isolated from fresh grape juice. By comparing three different setups based on the use of these sources for the treatment of juice the batch mode employing the transient spark appeared to be the most effective for the inactivation of the native microorganisms in red grape juice.

Keywords: cold air plasma, fruit juices, native microorganisms.

1. Introduction

A growing customer's demand for long-lasting fresh products requires the concept of the "minimal processing". Therefore in recent years, new technologies capable of achieving the required level of sterilization and safety without thermal input are being 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 promising means for application in sterilization/pasteurization of fresh food and food packaging [2-3].

In our work we focused on the inactivation of the native microorganisms isolated from fresh fruit juices. We employed two different cold air plasma sources – a transient spark and a plasma synthetic jet actuator, implemented in two different ways for the treatment of the juice – batch treatment or electrospraying of the juice. We investigated also possible chemical changes of the juice composition due to cold plasma treatment.

2. Experimental set-up and methods

Description of plasma sources and conditions of the plasma treatment

Two different plasma sources, a transient spark discharge and a plasma synthetic jet actuator based on a surface DBD, have been investigated for treatment of fruit juices. Both sources generate cold (non-thermal) air plasma in ambient air and at atmospheric pressure.

Transient spark (TS) discharges in positive polarity were generated in two different set-ups depicted in **Fig. 1 b, c**. TS 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 [4-5]. Both systems are based on a point-to-plane geometry using a sharp hollow needle as the high voltage electrode. In the electrospray system (ES), the juice flowed directly through the hollow anode at a constant flow rate. Due to the applied high voltage, electro-spraying of the juice to micrometric size droplets occurred. This set-up enabled the direct contact of the active discharge with the sprayed droplets of the juice and enhanced the gas-liquid mass transfer of the gaseous reactive species. Batch system (BS) is a static treatment in which the TS discharge was generated directly over the juice surface. A grounded ring wire electrode was submerged in the juice. Ionic wind can play a supportive role in this setup, driving the active species from the discharge towards the juice surface, especially in the streamer phase of the TS discharge which always precedes the spark pulse. The discharge voltage and current were measured by high voltage probe *Tektronix P6015A* and by Rogowski current monitor *Pearson Electronics 2877*. The inter-electrode gap length (from the tip of the anode to the grounded mesh cathode or juice surface) in both systems was 1 cm. Typical electrical parameters were: f ~ 1 kHz, U_{max} ~ 11-12 kV, I_{max} ~ 6 A (batch) or 20-25 A (spray). 10 mL of the juice were treated with a flow rate of 1 mL/min in the spray system or for 10 minutes (in Petri dish with Ø 6 cm) in the batch system.

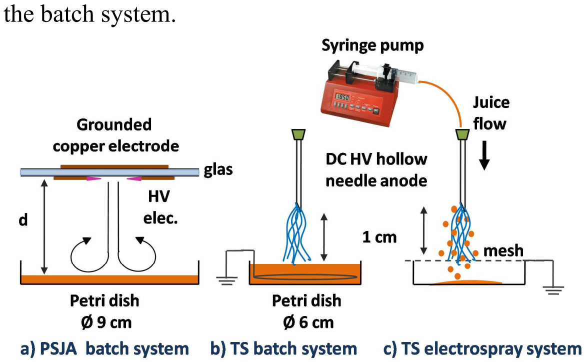

Fig. 1 Scheme of the experimental set-ups: a) PSJA batch system, b) TS batch system, c) TS electrospray system.

Annular plasma synthetic jet actuator (PSJA) producing an ionic wind normal to the surface, where the surface dielectric barrier discharge (DBD) is ignited, was used for batch treatment of the fruit juice [6-7]. The PSJA (**Fig. 1 a**) consists of an annular copper electrode (i.d. 30 mm, o.d. 40 mm), a glass plate (2 mm thick) as dielectric material and a grounded copper circle electrode (d. 35 mm) on the other side. The set-up scheme and the electrical parameters measurement are similar to those published in Neretti *et al.* [6] for a similar actuator. Surface DBD produced ions which were accelerated by the electric field and formed a jet normal to the actuator surface. A sinusoidal power supply system consisting of a push-pull high voltage transformer controlled by *Arduino* was utilized to feed the discharge. Plasma was operated in discontinuous mode with equal on and off times of 1 second. The applied voltage frequency was ~ 28.4 kHz and the peak voltage U_{max} was set to 5.2 kV. PSJA was used for the batch treatment of 10 mL of juice in Petri dish (d. 9 cm) per 10 minutes. The distance *d* from the surface DBD to the juice surface, which can be varied, was set to 3.5 cm for the first set of experiments.

Isolation of native microorganisms from fruit juices and microbiological handling

Native microorganisms were isolated from freshly prepared red grape juice. Briefly, 1 mL of the juice was diluted in 9 mL of Lysogenic Broth (LB) and 200 µL of 10^{-2} or 10^{-3} dilutions were immediately spread on three different agar plates, namely LB (tryptone, yeast extract, sodium chloride, and agar), acidified MRS (casein-peptone, meat extract, yeast extract, glucose, agar, pH 5.0), and OGY (yeast extract, glucose, agar, tetracycline hydrochloride 0.1%). The plates were incubated for 24 hrs at 30°C or 37°C until colonies appeared. Strains were purified by reinoculation. An isolated strain was considered as pure when the microorganism appeared homogeneous.

In a subsequent stage, the isolated microbes were grown for 16 hours under the specific isolation conditions. Sterilized red grape juice was contaminated with 1×10^6 CFU/mL of each strain. 10 mL of microbial suspensions were exposed to the described plasma systems. Immediately after the treatment, microbes were collected, opportunely diluted, and plated on agar culture media. Microbial growth was quantified by colony forming unit enumeration. To assess the long-term antimicrobial effect of plasma treatment, aliquots of juice were stored at room temperature or at 4°C up to 4 weeks.

Chemical analyses of the cold plasma treated fruit juices

We also focused on the potential chemical changes in juice composition due to plasma treatment and due to the presence of RONS. 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. Preliminary results

Characterization of plasma sources

Plasma discharges in direct contact with liquids are known to produce gaseous reactive species which induce formation of reactive species in the bulk liquid. Aqueous RONS induced in the liquid are known as major plasma agents responsible for the bacterial inactivation.

TS discharge generated in ambient air at atmospheric pressure produces cold, non-equilibrium plasma and its chemical activity is comparable with the nanosecond repetitive pulsed discharges. Due to the very short pulse duration, the plasma cannot reach equilibrium conditions and remains at relatively low gas temperature. The identified dominant stable gas phase products in the ambient air without electrospray and in the ambient air humidified by the water electrospray were nitrogen oxides (NO and NO_2), while ozone was negligible (<10 ppm detection limit) [5,8]. Additionally, due to water evaporation, the formation of other abundant species, i.e. $^{\bullet}OH$ and $HO_2^{\bullet}$ radicals, H_2O_2, HNO_2 and HNO_3 occurs. Beside the main stable aqueous RONS (H_2O_2, NO_2^- and NO_3^-) also $^{\bullet}OH$, $^{\bullet}NO$ and $^{\bullet}NO_2$ radicals were identified in water treated by TS discharge. The measured concentrations of the long lived species for the defined conditions of the treatment were:

- electrospray system - H_2O_2 ~ 500 µM, NO_2^- ~ 300 µM and NO_3^- ~ 960 µM;
- batch system - H_2O_2 ~ 270 µM and NO_2^- ~ 340 µM.

PSJA produces an ionic wind due to the electrohydrodynamic interaction of ions with gas molecules. Long-lived charged particles and reactive species generated within the plasma region of the surface DBD are carried on by the jet flow outside the plasma towards the target to be treated. PSJA propagates for several centimetres at a velocity of several m/s. Although the induced tubular jet core has a diameter of about 1 cm, by hitting the target, it spreads homogenously over the whole surface of juice in the Petri dish and the transport of gaseous reactive species is enabled. The analysis of water treated by PSJA showed formation of important aqueous RONS, e.g. H_2O_2, O_3, but also NO_3^- and $^{\bullet}OH$ radicals.The PSJA treatment of water under the same conditions as for the juice treatment showed formation of $^{\bullet}OH$ radicals with a rate ~ 0.25 µM/s, H_2O_2 ~ 15µM and O_3 ~ 18µM, but also a decrease of the solution pH that is due to formation of nitric acid (~200-400µM).

Additionally, we present three different set-ups, which provide different ways for the juice treatment, which seem to have a significant effect on the microorganisms inactivation.

Effect of cold plasma on survival rate of native microorganism in red grape juice

Seven different native microbial strains (J1-J7) were so far isolated from red grape juice. Characterization of microbial colonies is in progress.

Fig. 2 shows the comparison of the survival rates of 5 different native microorganisms directly after plasma treatment of the red grape juice expressed in % of the control samples. After the treatment, all the tested plasma systems decreased the survival of the J1 microbial strain. In particular, PSJA batch completely inactivated J1 whereas TS discharge reduced its survival rate to 25%. The decontamination effect in case of J1 was observed even one week later in samples stored at room temperature and 4°C (data not shown). The other strains (J4, J5, J6 and J7) showed low responsiveness (from ~ 40 to 80% survival rate) toward the different plasma treatments. In overall, TS batch appeared to be the most effective type of plasma treatment for microbial decontamination of grape juice even if the different microbial strains reported diverse responsiveness to plasma systems. The results obtained by the TS spray system were comparable with the PSJA batch system (for given conditions). The evaluation of the long lasting antimicrobial effect (up to 4 weeks post plasma treatment) is in the progress.

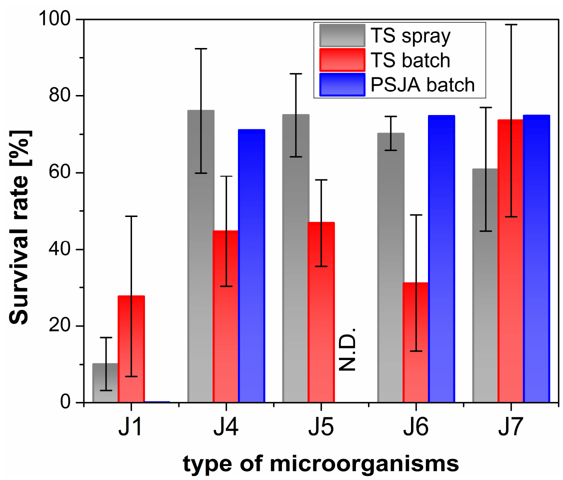

Fig. 2 Comparison of the survival rates of different strains of native microorganisms in the red grape juice (treatment conditions: PSJA batch – 3.5 cm distance, 10mL/10 min, TS batch –1 cm distance,10 mL/10 min, TS spray – 1 mL/min flow rate, treated volume 10 mL).

In the TS electrospray system the juice passes through the HV electrode and through the active discharge zone. In addition, due to the effect of electrospray, we can expect an enhanced transfer of gaseous reactive species into the liquid. However, due to the set flow rate of 1 mL/min, the juice droplets are in direct contact with the discharge only for a very short time (a few millisecond). After crossing the inter-electrode distance, the juice droplets are collected below the grounded mesh electrode, where they can be further affected only by the long-lived neutral species. In the TS batch system, the transport of the plasma RONS to the juice is not enhanced by the high surface-to-volume ratio like in the electrospray, on the other hand, the ionic wind can contribute, similar to PSJA. In addition, the juice surface layer is exposed to the direct plasma, i.e. both short- and long-lived species for the whole time of treatment. Some liquid mixing within the dish is also possible due to the thermal gradients and ionic wind, which may enhance the antimicrobial effect. Interestingly, PSJA represents and indirect plasma treatment (it is not in direct contact with the juice during treatment), in which the flow of charged and reactive species formed in the plasma region can cover the whole juice surface. The antimicrobial effect is certainly due to the strong ionic wind driving the plasma RONS towards the juice surface. Though the PSJA treatment gives comparable results for some strains under the given conditions, a new set of experiments in which the distance between the treated sample and the discharge is decreased, and the electrical parameters are changed (e.g. increase of voltage) is in progress.

Once the optimal conditions will be identified, we will proceed to verify that the chemical components of the juice are not modified by the plasma treatment.

4. Acknowledgement

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