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Effects of dielectric barrier discharge plasma on the inactivation of *Zygosaccharomyces rouxii* and quality of apple juice

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ABSTRACT

This work aimed to evaluate the effects of dielectric barrier discharge (DBD) plasma on inactivation of spoilage yeast *Zygosaccharomyces rouxii* (*Z. rouxii*), in apple juice. Results showed that DBD plasma treatment at 90 W for 140 s resulted in about 5-log reduction of *Z. rouxii* in apple juice. The levels of extracellular nucleic acids and proteins as well as contents of H_2O_2 and NO_2^- in yeast extract-peptone-dextrose (YPD) medium increased significantly after DBD plasma treatment at 90 W for 40–200 s. The increases in membrane permeability and generation of reactive species would likely contribute to DBD plasma-mediated inactivation of *Z. rouxii*. DBD plasma caused significant changes in pH, titratable acidity, and certain color parameters of apple juice, but had no effect on the contents of total soluble solids, reducing sugar, and total phenolics. This study provides key implications for the application of DBD plasma in fruit juice processing.

1. Introduction

The genus *Zygosaccharomyces*, mainly including *Z. rouxii*, *Z. bailii*, *Z. bisporous*, *Z. bisporus*, and *Z. barker*, belongs to the group of hemiascomycetous yeasts (Pribylova, de Montigny, & Sychrova, 2007). Due to high tolerance to osmotic stress, *Zygosaccharomyces* species can grow in environments with high concentrations of salts and/or sugars. In addition, *Zygosaccharomyces* species also have extreme resistance to organic acids, low levels of oxygen and can grow in excess of legally-permitted concentrations of preservatives (Alonso, Belda, Santos, Navascues, & Marquina, 2015; Stratford, et al., 2013). Therefore, *Zygosaccharomyces* species often causes spoilage of many acidic, high-sugar and canned foods, such as syrups, honey, fruit concentrates, musts, sweet wines, ketchups, wine, mayonnaise, pickles, etc., thereby resulting in significant economic losses in the food industry (Alonso et al., 2015; Stratford, et al., 2013).

In view of serious adverse effects of microbial spoilage, it is extremely important to control or eliminate *Zygosaccharomyces* in foods with low-moisture and high-sugar content. At present, thermal processing technologies and preservatives are the most common methods to inactivate microorganisms and enzymes for extending the shelf life of foods products. While proper thermal processes such as heat pasteurization, ultra-high temperature, and canning are the primary methods for both adding value and ensuring microbial safety of foods, the applied heat may also cause undesirable changes in nutritional and sensory properties of foods (Ortega-Rivas, & Salmerón-Ochoa, 2014). Meanwhile, the adverse health effects of some chemically synthesized preservatives on humans have been widely discussed and food manufacturers are nowadays forced to limit the use of chemical preservatives (Silva & Lidon, 2016). Meanwhile, consumers' demands for minimally-processed fresh-like food products with high sensory and nutritional qualities have been steadily increasing in the last several years. As a result, novel non-thermal technologies for inactivating microorganisms have been well developed in order to address the increasing consumers' interest for safer, healthier, and higher quality foods (Li & Farid, 2016). Some non-thermal food processing techniques have been developed and have shown great promise for their application in food processing, including high hydrostatic pressure (HHP), ultrasound, pulsed electric fields (PEF), cold plasma, pulsed light, oscillating magnetic field, irradiation, electrolyzed water, and so on (Ding et al., 2015; Ekezie, Sun, & Cheng, 2017; Kubo, Augusto, & Cristianini, 2013; Liao, Liu, et al., 2017; Pinela & Ferreira, 2017). These non-thermal pasteurization methods can effectively denature enzymes and eliminate microorganisms involved in foods spoilage with less detrimental effects on sensory and nutritional quality of foods.

In recent decades, cold atmospheric plasma (CAP) has rapidly

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evolved as one of the newest non-thermal food techniques, which has shown significant potential for applications in food industries (Ekezie, et al., 2017; Pankaj & Keener, 2017; Thirumdas, Sarangapani, & Annapure, 2015). There are many schemes for cold atmospheric plasma production, such as dielectric barrier discharges (DBD), Atmospheric pressure plasma jet (APPJ), gliding arc discharge, corona discharge, and radio frequency plasma (Ekezie, et al., 2017; Thirumdas, et al., 2015). Different gases, such as ambient air, Helium (He), Argon (Ar), Nitrogen (N_2), or a mixture of gases, can be used to produce CAP. Recent research has indicated that CAP can effectively inactivate contaminating microbes (bacteria, yeasts, molds, and viruses) in various areas of food industry including cereal, poultry, meat, dairy, fruits, vegetables, packaging, etc. (Ekezie, et al., 2017; Liao, Xiang, et al., 2017; Misra, Keener, Bourke, & Cullen, 2015). Meanwhile, the underling mechanisms behind CAP induced microbial inactivation have been well investigated. It is generally agreed that reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as atomic oxygen (O), hydroxyl radical ($OH^{\cdot}$), ozone (O_3), and nitric oxide radical ($NO^{\cdot}$), are the key inactivation agents during of cold plasma (Helmke et al., 2011). In addition, CAP can also degrade mycotoxins (ten Bosch et al., 2017), inactivate enzymes in foods (Misra, Pankaj, Segat, & Ishikawa, 2016), and cause modification of foods constituents (Segata, Misra, Cullen, & Innocente, 2015). As an emerging and non-thermal processing technology, CAP has numerous advantages over traditional food processing methods, such as low operating temperature (35–40 °C), higher microbial inactivation efficiency, low energy input, and leaving no toxic residue (Thirumdas, et al., 2015).

In recent years, various CAP technologies have been used for the sterilization of heat-sensitive food products with less influence on the sensory and nutritional qualities (Liao, Liu, et al., 2017; Thirumdas, et al., 2015). However, to the best of authors' knowledge, the research on the bactericidal effect of CAP against *Z. rouxii* in fruit and vegetable juices is very limited. Therefore, the aim of this study was to evaluate bactericidal activity and mechanisms of dielectric barrier discharge (DBD) plasma against *Z. rouxii* in apple juice. The effects of DBD plasma treatment on the main physicochemical properties of apple juice (including color, pH, titratable acidity, total soluble solids, reducing sugar, and total phenolics) were also assessed.

2. Materials and methods

2.1. Chemicals and strain

Yeast extract, peptone, glucose, and agar were acquired from Aobox Biotechnology Co., Ltd. (Beijing, China). Methanol (HPLC grade), 3,3′-bis[*N*,*N*-di(carboxyethyl)aminomethyl]-*o*-cresolsulfonepthalein sodium salt (xylenol orange), and hydrogen peroxide (H_2O_2, 30.0%) were provided by Sigma-Aldrich (Shanghai, China). Ferrous ammonium sulfate, sulfanilamide (SULF), N-(1-Naphthyl)ethylenediamine dihydrochloride (NEDD), 3,5-dinitrosalicylic acid (DNS), $NaNO_2$, isoamyl acetate, and malic acid were purchased from Aladdin Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and were used as received. Double distilled water was used throughout the experiments.

Lyophilized *Z. rouxii* (GIM2.173) was procured from Guangdong Microbiology Culture Center (GIMCC) (Guangzhou, China). The cells were maintained on yeast extract-peptone-dextrose-agar (YPDA) medium (yeast extract, 10 g/L; peptone, 20 g/L; glucose, 20 g/L; and agar, 20 g/L). During preliminary culture it was grown in yeast extract-peptone-dextrose (YPD) liquid medium and incubated at 30 °C, 120 rpm for 18 h.

2.2. Preparation of apple juice

Commercial concentrated apple juice used in the experiments was supplied by Shaanxi Haisheng Fresh Fruit Juice Co., Ltd. (Shaanxi, China) and was diluted in sterile distilled water to the soluble solid content of 14.1°Brix (pH 3.96).

2.3. DBD plasma equipment and treatment conditions

The DBD plasma source used in this study was described in detail by Li et al. (2017), which consisted of a reaction cell (DBD-50), a high voltage alternating current power source (CTP-2000K), a voltage-regulator (Nanjing Suman Electronics Co., Ltd., China), and a quartz dish (with a outer diameter of 70 mm, inner diameter of 50 mm, a wall thickness of 9 mm, and a depth of 4 mm). The quartz dish was placed between the two quartz plates. Three milliliters of YPD medium or diluted apple juice artificially inoculated with *Z. rouxii* cells (about 10^6–10^7 CFU/mL) were placed in the quartz dish and then submitted to inactivation treatments for different time periods (0, 20, 40, 60, 80, 100, 120, and 140 s). The input power of DBD plasma treatment was 90 W and air was used as the gas source. After treatment with DBD plasma for the indicated time point, serial dilutions (10-fold) of the treated samples were made in sterile saline solution (0.85%, w/v), and then 100 μL of each dilution was plated on YPDA medium. After being incubated at 30 °C for 48 h, the number of colonies on agar plates was counted and the results were expressed as $\log_{10}$ CFU/mL.

2.4. Scanning electron microscopy (SEM)

Changes in morphology and structure of *Z. rouxii* cells before and after DBD plasma treatment were observed using a scanning electron microscopy (SEM) (Xu et al., 2017). Briefly, *Z. rouxii* cells were incubated in apple juice and then treated with DBD plasma at 90 W for 0, 120, and 300 s, respectively. After the DBD plasma treatment, the cells were collected with 6000 rpm centrifugation at 4 °C for 10 min, and the obtained precipitates were washed twice using 0.85% NaCl solution. Then, the harvested cells were fixed in a mixture of 3% paraformaldehyde and 1% glutaraldehyde in 0.1 mol/L phosphate buffered saline (PBS, pH 7.2) for 4 h at 4 °C. The cells were then rinsed three times with 0.1 mol/L PBS (pH 7.2), resuspended in 1% o(v/v) osmium tetroxide solution, and held for 2 h at 4 °C. After being washed with PBS for three times, fixed *Z. rouxii* cells were firstly dehydrated a gradient ranging ethanol (30%, 50%, 70%, 80%, 90%, 95%, 100% (v/v)) for 10 min each. Then the ethanol was replaced with isoamyl acetate twice for 15 min each. Afterwards, the samples were dried with a CO_2 critical point dryer (Autosamdri-815, Series A, Tousimis Research Corp., Rockville, MD, USA). The dehydrated cells were then mounted an aluminum stubs and coated with gold for 90 s using a vacuum evaporator HUS-5GB (Hitachi, Tokyo, Japan). Finally, *Z. rouxii* cells were examined with a scanning electron microscope (JSM-6490LV, JEOL Ltd., Tokyo, Japan) at 20 kV accelerating voltage.

2.5. Measurement of nucleic acids and proteins leakage

The leakage of nucleic acids and proteins form DBD plasma-treated *Z. rouxii* cells were measured by UV absorption as previously described (Pillet, Formosa-Dague, Baaziz, Dague, & Rols, 2016). *Z. rouxii* cells were incubated in sterile 0.85% NaCl solution and then treated with DBD plasma for 0, 40, 80, 120, 160, and 200 s, respectively. The yeast suspensions were centrifuged for 10 min at 12,000 rpm (4 °C) and the supernatants were then collected. The total nucleic acids and proteins contents in the supernatant were quantified by measuring the absorbance at 260 nm and 280 nm with a Nanodrop 2000 UV–Vis Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), respectively.

2.6. Measurement of H_2O_2 and nitrite anions

Three milliliters of YPD medium without *Z. rouxii* cells was placed in a quartz dish and treated by DBD plasma at 90 W for 0, 40, 80, 120,