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Effects of atmospheric cold plasma and ozone on prebiotic orange juice

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ABSTRACT

In this study, the effect of plasma and ozone treatments on the quality of orange juice was evaluated. The juice was directly and indirectly exposed to a plasma field at 70 kV for different treatment times: 15, 30, 45 and 60 s. For ozone processing, different loads (0.057, 0.128 and 0.230 mg/O_3 mL of juice) were evaluated. After the treatments, the oligosaccharides were quantified by HPLC. The juice pH, color, total phenolic content and total antioxidant activity were also determined. Both processes promoted a partial degradation of the oligosaccharides in the juice. However, the juice maintained an enough amount of oligosaccharides to be classified as a prebiotic food. The phenolic content and antioxidant capacity of the treated samples was also well preserved as the pH and color. Thus, atmospheric cold plasma and ozone are suitable non-thermal alternatives for prebiotic orange juice treatment.

Industrial relevance: Consumers are looking for safe food products with high quality. Thus, the food industry is currently considering non-thermal processes as an alternative to reduce the nutrient loss in processed foods. Despite atmospheric cold plasma and ozone are technologies already evaluated as an efficient non-thermal alternative for pathogens inactivation in orange juice, no previous studies on their effects on the oligosaccharides in functional fruit juice was published. This study is of industrial relevance because it demonstrates that after plasma and ozone treatment the overall quality of prebiotic orange juice was preserved and the product maintained its functional appeal.

1. Introduction

Nowadays there is a great interest in the use of prebiotics as functional food ingredients to improve the human health (Liu et al., 2014). The beneficial health effects attributed to the intake of prebiotics have been reported in several studies (Moreno-Vilet et al., 2014; Tako et al., 2014). Prebiotic oligosaccharides are usually obtained by polysaccharide hydrolysis or enzyme synthesis.

Lactic acid bacteria (LAB) are able to produce a wide variety of glucansucrases (GS) and fructansucrases (FS), which synthesize glucans and fructans, respectively, from sucrose with food and nutritional applications (Bivolarski et al., 2013). *Leuconostoc mesenteroides* NRRL B512F produces the enzyme dextransucrase when cultivated in a medium containing sucrose as substrate, a nitrogen source and minerals (Rodrigues, Lona, & Franco, 2003). Dextransucrase (EC 2.4.1.5) is a bacterial extra cellular enzyme, which uses sucrose as a substrate to promote the synthesis of dextran releasing the fructose moieties form sucrose (Rodrigues, Lona, & Franco, 2005). When another substrate (acceptor) is also present, besides sucrose, dextransucrase produces prebiotic oligosaccharides in a reaction called acceptor reaction due to the addition of glucose units from sucrose to the acceptor molecule (maltose, glucose and fructose) instead of into the dextran chain (Rabelo, Honorato, L.R.B, G.A.S, & S., 2006). Oligosaccharides are well documented as effective prebiotic ingredients that modulate the intestinal microbiota and provide other beneficial health effects such as stool improvement, weight management, allergy alleviation (Johnson, Thavarajah, Combs, & Thavarajah, 2013; Sangwan, Tomar, Ali, Singh, & Singh, 2014) and mineral absorption (Tako et al., 2014).

Orange juice is one of the most consumed juice worldwide due to its high nutritional value and pleasant taste (Agcam, Akyıldız, & Evrendilek, 2014). Commonly, thermal treatments are used in the preservation of fruit juices in order to extend their shelf life. However, oligosaccharides are liable to hydrolysis in the pasteurization temperatures of fruit juices and drinks (Matusek, Merész, Le, & Örsi, 2009).

Nowadays, the consumer's demand and the shortcomings of the existing technologies are stimulating the development of alternative non-thermal approaches in food processing (Misra et al. 2014). In this context, atmosphere cold plasma (ACP) and ozone processing are emerging technologies that offer many potential applications.

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Recently, these technologies emerged as powerful tools for surface decontamination of both foodstuffs and food packaging materials (Pankaj, Bueno-Ferrer, Misra, Milosavljević, et al., 2014; Pankaj, Bueno-Ferrer, Misra, O'Neill, et al., 2014). Several studies have been focused on the application of ozone and plasma such as good alternative processing technologies for decontamination of foods (Misra et al. 2014; Ziuzina, Patil, Cullen, Keener, & Bourke, 2013). However, there are no available data and studies concerning the effects of ozone and cold plasma on the quality characteristics of functional foods.

Our group has successfully studied the oligosaccharides synthesis in fruit juices through the dextransucrase acceptor reaction. The oligosaccharides synthesized by *L. mesenteriodes* B-512F dextransucrase present α-1,6 glycosidic bonds, which are not digested in the stomach (Araújo et al., 2014; Rabelo, Fontes, & Rodrigues, 2009; Silva, Rabelo, & Rodrigues, 2014). In this context, this paper aims to evaluate the effect of the atmospheric cold plasma and ozone processing on the quality of orange juice containing prebiotic oligosaccharides.

2. Materials and methods

2.1. Orange juice

Orange juice (Squeez©, Fruit Juices Ltd., Ireland) was purchased from a local supermarket (Dunnes, Dublin 1, Ireland). The pH of the juice was determined by direct measurement in a 420A potentiometer (Orion research Inc., Beverly, MA. US). The pH meter was calibrated before use with buffer solutions of pH 4.0, 7.0 and 10.0.

Reducing sugars were measured by high-performance liquid chromatography (HPLC) analysis using an Agilent Technologies System. Separation was achieved in a Supelcogel-Ca column at 80 °C. Ultrapure water (MilliQ System, Millipore, Bilberica, MA, USA) at 0.5 mL/min was used as eluent, and the detector temperature was 35 °C. All samples were analyzed in duplicate.

2.2. Dextransucrase production and enzyme activity determination

The dextransucrase production was carried out according to Rodrigues et al. (2003). The enzyme produced was stocked frozen at −20 °C prior to use. The enzymatic activity of the dextransucrase was determined by quantifying the released fructose by the DNS (3.5 dinitrosalicylic acid) method (Miller, 1959) using as substrate a 10% (*w*/*v*) sucrose solution in sodium acetate (20 mM, pH 5.2) at 30 °C (Rabelo et al., 2009; Rodrigues et al., 2005). Dextransucrase activity was expressed in IU/mL. One international unit (IU) is the amount of enzyme that releases 1 μmol of fructose per minute under the assay condition.

2.3. Prebiotic oligosaccharides synthesis in orange juice

Oligosaccharides synthesis was carried out using the partially purified enzyme and the optimum synthesis conditions (30 °C and pH 5.2). Synthesis was carried out in 1000 mL Erlenmeyer flask at 30 °C for 24 h (Rabelo et al., 2006) containing 800 mL of the orange juice and using enzyme with 0.05 UI/mL. The amount of enzyme was modulated to avoid the oligosaccharides overproduction, which can cause intestinal discomfort and or diarrhea.

2.4. Plasma treatment on prebiotic orange juice

The plasma treatment was carried out using a plasma generation (DBD-ACP), model: 6CP120/60–7.5-Phenix Technologies (Fig. 1). The system consisted of a variable high voltage transformer with an input voltage of 230 V at 50 Hz and a maximum high voltage output of 70 kV at 50 Hz. The two 15 cm diameter aluminum disc electrodes were separated by a polypropylene container, which served both as a sample holder and as a dielectric barrier with wall thickness of 1.2 mm. The distance between the two electrodes was 22 mm, equal to the height of the container. Voltage was monitored using an InfiniVision 2000 X-Series Oscilloscope (Agilent Technologies Inc., Santa Clara, CA, USA). All experiments were performed at 70 kV peak to peak at ambient air and atmospheric pressure conditions.

An aliquot of 20 mL of the prebiotic orange juice was transferred to an open Petri dish, which was placed in a polypropylene box, sealed with a polymeric film with 50 μm of thicknesses (Cryovac BB3050). This film served as an additional layer of dielectric barrier (Pankaj, Bueno-Ferrer, Misra, Milosavljević, et al., 2014; Pankaj, Bueno-Ferrer, Misra, O'Neill, et al., 2014). Then, the samples were treated with atmospheric cold plasma (ACP) with processing times of 15, 30, 45 and 60 s and different exposure kinds: direct plasma field (in) and indirect plasma field (out). These treatment times were selected based on previously study carried for pathogens inactivation (Ziuzina et al., 2013), where a complete bacterial inactivation was achieved after 20 s of direct exposure and 45 s of indirect plasma treatment. In the present work, lower and higher exposure times to ACP were evaluated. Treated samples of prebiotic orange juice were stored at room temperature for 24 h, after that, the samples were analyzed. Treatments were done in duplicate and analyzes were performed in triplicate.

2.5. Ozone treatment on prebiotic orange juice

The prebiotic orange juice was also submitted to ozone treatment. Ozone was generated using an ozone generator (Model OL80F, Ozone services, Burton, B.C., Canada; Fig. 2) and produced by a corona discharge generator. Pure oxygen was supplied via an oxygen cylinder (Air Products Ltd., Dublin, Ireland), and the flow rate was controlled using a gas flow regulator. The excess of ozone was destroyed by an

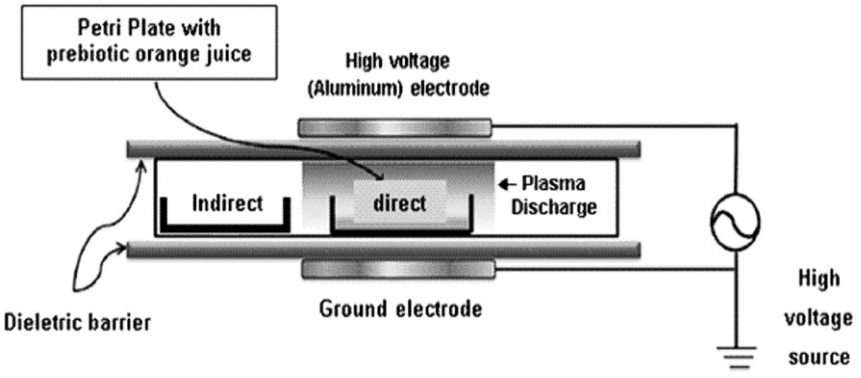

Fig. 1. Experimental setup for plasma treatment. (Adapted from Pankaj, Misra, & Cullen, 2013).