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Orange juice processing using a continuous flow ultrasound-assisted supercritical CO_2 system: Microbiota inactivation and product quality

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ABSTRACT

The feasibility of using supercritical CO_2 assisted by ultrasound (SC-CO_2-HPU) in continuous mode (3.06 min residence time) for the non-thermal pasteurization of orange juice was evaluated. The proposed technology was effective for microbial inactivation; complete inactivation was obtained for *E. coli* and total aerobic mesophilic bacteria while 99.7% reduction for *S. cerevisiae*. Results showed that the SC-CO_2-HPU treatment brought about small changes in the pH, °Brix and titratable acidity of the juice. Furthermore, although SC-CO_2-HPU technology produced a higher browning index (211%) and greater changes in color, it was possible to improve the cloud of juice by 173%; what is more, a smaller percentage of phenolic compounds (6.5%) and ascorbic acid (5.5%) was lost compared to the thermally pasteurized juice (10% decrease in both parameters). Moreover, the antioxidant capacity could be increased (12%) with respect to the natural juice. Therefore, SC-CO_2-HPU technology appears to be effective for microbial pasteurization and the mild process conditions used could lead to an increase in the juice quality.

Industrial relevance: The demand for high quality processed foods which preserve their natural and fresh-like characteristics has awakened a growing interest in non-thermal technologies. The ultrasound-assisted SC-CO_2 continuous system is an innovative non-thermal technology that could represent a development in the area of emerging technologies. This technology allows high quality products to be obtained by preserving their natural bioactive compound content while maintaining their fresh-like organoleptic characteristics. In fact, food experts working in academia, industry or governmental agencies worldwide foresee that non-thermal emerging technologies will be among the most impactful novel food processing technologies for the next decade in terms of product commercialization.

1. Introduction

In recent years, while developed countries have witnessed a rise in the consumption of processed fruit juices, that of fresh citrus fruit has been on the wane (Tiwari, O'Donnell, Muthukumarappan, & Cullen, 2009). Worldwide, orange juice is a very popular product due to its high nutritional value, its bioactive components, such as phenolic compounds, vitamin C and carotenoids, and its sensory characteristics (Ortuño, Balaban, & Benedito, 2014).

Despite its low pH, this juice needs to be processed because it is of limited stability due to microbial growth and enzyme activity, which can cause unpleasant organoleptic changes or the degradation of compounds during storage (Fabroni, Amenta, Timpanaro, & Rapisarda, 2010; Ferrentino, Plaza, Ramirez-Rodrigues, Ferrari, & Balaban, 2009; Khandpur & Gogate, 2016; Liu et al., 2010; Zinoviadou et al., 2015).

Although thermal pasteurization remains the most commonly-used method for the preservation of juices, there is growing interest in developing alternative techniques. The new techniques are expected to minimize changes in the nutritional and organoleptic characteristics of food, obtaining fresher and richer juices than traditional thermal technology. Two such techniques are high hydrostatic pressure (HHP) and pulsed electric fields (PEF), which result in better quality retention and adequate shelf life; however, they cannot inactivate enzymes, such as PME, well enough to produce a shelf-stable juice, unless they are combined with elevated temperatures. In addition, these new technologies involve high investment and operational costs, which is an important obstacle to their industrial application (Niu et al., 2010; Ozuna, Paniagua-Martínez, Castaño-Tostado, Ozimek, & Amaya-Llano, 2015;

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Tiwari, Muthukumarappan, O'donnell, & Cullen, 2009; Vervoort et al., 2011). Moreover, at present, HHP processing consists of batch processes, which limits its use because of its low processing capacity (Damar & Balaban, 2006).

For the purposes of processing large volumes of liquid food, such as orange juice, a continuous preservation process is more desirable. This objective can be attained by applying supercritical fluids, a non-thermal preservation technique in which both CO_2 and the product are pumped through the system by high-pressure pumps, mixed and maintained in contact for a period of time (Fabroni et al., 2010; Paniagua-Martínez, Mulet, García-Alvarado, & Benedito, 2016).

Supercritical CO_2 (SC-CO_2) has a density close to that of liquids, as well as gas properties like high diffusivity and low viscosity; therefore, it has excellent transport properties. Furthermore, these properties can be controlled by temperature and pressure changes (Calix, Ferrentino, & Balaban, 2008; Niu et al., 2010; Wimmer & Zarevúcka, 2010). Supercritical CO_2 is considered an excellent alternative to solvents because of its non-toxic and non-flammable nature and its relatively low critical pressure and temperature (73.6 bar, 31.0 °C). Moreover, the SC-CO_2 has a lethal effect on bacteria (Garcia-Gonzalez et al., 2007). This effect is directly proportional to the applied pressure, time and temperature. SC-CO_2 acts on bacteria as follows: first, solubilization occurs in the external liquid phase, causing carbonic acid formation (which dissociates into bicarbonate and hydrogen ions); therefore, it increases cell membrane fluidity and permeability, increasing the diffusion of CO_2 into the cell and causing a decrease in intracellular pH. Thus, the inactivation/inhibition of key cellular metabolic enzymes for microorganisms occurs. As a result, a disorder in the electrolyte balance of intracellular constituents is produced and vital constituents of cells and cell membranes are extracted (Fabroni et al., 2010; Garcia-Gonzalez et al., 2007; Kincal et al., 2005; Ortuño, Martínez-Pastor, Mulet, & Benedito, 2013; Paniagua-Martínez et al., 2016).

Despite all the aforementioned advantages of SC-CO_2 inactivation, even the continuous systems require long treatment times and high pressures and temperatures (Fabroni et al., 2010; Kincal et al., 2005) to ensure the safety and stability of food, limiting the efficiency of the inactivation process, compromising the food quality and increasing processing costs. In this sense, there is growing interest in process intensification, with the simultaneous application of different non-thermal technologies, in the search for synergistic effects. One of the techniques that synergistically improves the inactivation mechanisms of SC-CO_2 is high power ultrasound (HPU), which accelerates and improves heat and mass transfer processes (Ortuño et al., 2013, 2014; Paniagua-Martínez et al., 2016).

When high power ultrasound propagates in a liquid, cavitation bubbles are generated by pressure changes. These microbubbles collapse violently in the succeeding compression cycles of a propagated sonic wave. This results in localized high temperatures, pressures and significant shearing effects. Consequently, the intense local energy and high pressure bring about a localized pasteurization effect (without causing significant temperature increases, while shortening processing time and cutting energy consumption) (Abid et al., 2013; Tiwari, Mu hukuma appan, O'Donnell, & Cullen, 2008; Tiwari, O'Donnell, et al., 2009). Therefore, with the combination of SC-CO_2 and HPU (SC-CO_2-HPU), an increase is produced both in the solubilization rate of SC-CO_2 in the liquid and in the mass transfer due to the vigorous stirring produced by the ultrasonic field. Thereby, a quick saturation of CO_2 in the medium is achieved, as well as the intensification of the inactivation mechanisms. Furthermore, cavitation and agitation produced by the HPU cause cell wall damage, increasing the SC-CO_2 penetration, the intracellular compound extraction and the death of microbial cells. In addition, thermal, chemical and mechanical effects induced by HPU cavitation contribute to enzyme inactivation (Tiwari, Mu hukuma appan, et al., 2008). The combined use of SC-CO_2 and HPU can be considered as a green processing technique since it can contribute to the reduction of energy and waste, the increase of the product quality and safety and the decrease of the carbon and water footprint (Chemat et al., 2017).

Ortuño, Martínez-Pastor, Mulet, and Benedito (2012) reported that by using a batch-mode SC-CO_2 at 350 bar and 36 °C for 25 min, a reduction of 1 log-cycle in *Escherichia coli* DH1 (*E. coli*) was obtained in orange juice. However, Kincal et al. (2005) reported that a continuous SC-CO_2 treatment (210 bars, 34.5 °C, 10 min residence time) caused at least a 5 log-cycle reduction in pathogens (*E. coli* O157: H7, *Salmonella* Typhimurium and *Listeria monocytogenes*). Consequently, it can be expected that batch-mode equipment requires a much longer inactivation time compared to continuous SC-CO_2 systems. There are a few studies of batch-mode SC-CO_2 intensified using ultrasound (SC-CO_2-HPU); two of them prove the complete inactivation of the *E. coli* and *S. cerevisiae* population in orange juice after 1.5 min (225 bar, 36 °C) and 5 min (350 bar, 36 °C) of treatment, respectively (Ortuño et al., 2012, 2013). In order to improve the efficiency of batch SC-CO_2 treatments, a continuous system was developed by Paniagua-Martínez et al. (2016) who studied the inactivation of *S. cerevisiae* in apple juice, using the continuous flow SC-CO_2-HPU at different juice residence times (3.06–9.2 min), temperatures (31–41 °C) and pressures (100–300 bars). The results demonstrated that the maximum inactivation achieved by the system was 7.8 log-cycles. However, there are no studies covering either the use of this continuous technique (SC-CO_2-HPU) for other types of juices or the effect of the process on the product quality. Therefore, the aim of this study was to determine the effect of SC-CO_2-HPU treatment in a continuous regime on both the inactivation of the microbiota and the quality attributes of orange juice.

2. Materials and methods

2.1. Orange juice

Valencia Navel oranges (*Citrus sinensis*) were purchased from a local market and kept at 4 °C for 2 days until juice extraction. Orange juice was obtained by washing, peeling and extracting the fruit juice (Ultra Juicer, Robot Coupe J80, USA). Juice extraction took place just prior to the treatment application; consequently, an extraction was required for each experiment. Each experiment required about 1.5 L of juice, 1 L was used for processing (SC-CO_2-HPU and thermal pasteurization), and 0.5 L served as control. Juices were not inoculated and only the inactivation of the microbiota was considered.

2.2. SC-CO_2-HPU processing

Laboratory continuous regime equipment was designed and built for supercritical CO_2 assisted by high power ultrasound (SC-CO_2-HPU) (Fig. 1) (Paniagua-Martínez et al., 2016).

The SC-CO_2-HPU process applied to the juice was as follows: first, liquid carbon dioxide was supplied from the tank to the chiller reservoir in which it was compressed to 200 bar by means of the injection of pressurized gaseous N_2. The liquid CO_2 was supplied from the bottom of the chiller reservoir (which stores it at −18 °C) to the pump where it was compressed at the target pressure. The equipment was stabilized at the treatment pressure (P) and temperature (T) by flowing SC-CO_2 at a constant flow rate of 5 mL/min. Thereafter, the ultrasound equipment was connected, and once the process conditions (P, T) were attained, the sample to be treated was pumped to the mixing point (7, Fig.1) where it mixed with the SC-CO_2. The mixture went into the sonication vessel (8, Fig. 1), where the HPU was applied. For the experiments with HPU, the power applied during the whole experiment was 40 W ± 5 W (I = 250 ± 10 mA; U = 220 ± 5 V, measured with a Digital Power Meter, Yokogawa, Model WT210). Pressure and temperature were kept constant during the experiment. The mixture of juice/SC-CO_2 exiting the sonication vessel went into the holding tube (14, Fig. 1) and, finally, into the separation vessel (15, Fig. 1), where it was depressurized and the CO_2 separated from the juice and recirculated to the reservoir (3,