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Impact of ultra high pressure homogenization on pectin methylesterase activity and microbial characteristics of orange juice: A comparative study against conventional heat pasteurization

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The effect of different ultra high pressure homogenization (UHPH) treatments on pectin methylesterase (PME) activity and on the contaminating microorganisms of orange juice was investigated. The treatments consisted in combinations of two inlet temperatures (10 or 20 °C), three pressures (100, 200 or 300 MPa) and two holding times (≤0.7 or 30 s). Results were compared with two thermal pasteurization treatments (1 and 2 min at 90 °C). Shelf-life of treated juices was evaluated for a period of 50 days at 4 °C. Mean bacterial counts in raw orange juice were: mesophilic aerobic bacteria (MAB) 4.75 ± 0.48 Log CFU/ml; psychrotrophic aerobic bacteria (PAB) 4.58 ± 0.30 Log CFU/ml; lactic-acid bacteria (LAB) 4.69 ± 0.40 Log CFU/ml; yeasts 4.26 ± 0.16 Log CFU/ml. UHPH treatments at 200 and 300 MPa reduced significantly the counts of all the microbial groups up to 4.38 Log CFU/ml for MAB; to 4.43 Log CFU/ml for PAB; to 4.69 Log CFU/ml for LAB and to undetectable from the rest of the group. No significant differences were observed with the thermal pasteurization. These treatments also reduced the PME activity above the 96% of its initial activity. The effect of increasing the inlet temperature from 10 to 20 °C, or the holding time (time during which the sample remains at the maximum temperature achieved) did not increase the efficacy of treatments above 200 MPa. During the later 50 days of storage at 4 °C neither the microbial count nor the PME activity increased their values and no differences were observed with the pasteurized samples during this period.

Industrial relevance: Today, consumer demands are more and more directed toward high-quality, additive free, minimally processed, nutritious, and fresh like products. Thermal pasteurization in fruit juice processing has as primary purpose to destroy pathogenic and deteriorative organisms as well as inactivate undesirable enzymes. Nevertheless, high pasteurization temperatures impact negatively on the nutritional quality and taste of orange juice. On the other hand, Ultra-High Pressure Homogenization (UHPH) is an emergent technology based on the application of high pressures, that allows to process in continuous fluid foods and that has been proposed by its germicidal effect as an alternative to the pasteurization for foods with heat-sensitive properties. Recently, several improvements introduced in the design and materials of UHPH equipments allowed to increase the performances of these equipments in order to bring them closer to the industry requests. We believe the article contains new data and information about the UHPH pectin methylesterase and microbial inactivation effects on orange juice and may be relevant to the scientific and industrial community.

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1. Introduction

Today, one must consider the change in consumer habits, the increasing demand for fresh juices and the desire for them to be free of chemical preservatives. Thus, fruit juice industries have directed their studies to look for alternative processing technologies to produce foods with a minimum of nutritional, physicochemical, or organoleptic changes induced by the technologies themselves. This concept of minimal processing is currently becoming a reality with conventional technologies (mild pasteurization) and non-thermal technologies (Esteve & Frígola, 2007; Tribst, Sant'Ana, & de Massaguer, 2009).

The inactivation of spoilage and pathogenic microorganisms as well as the inactivation of endogenous pectin methylesterase (PME) are prerequisites for the extension of the shelf-life of the juice (Katsaros, Tsevdou, Panagiotou, & Taoukis, 2010). Many organisms, particularly acid-tolerant bacteria and fungi (yeasts and molds), can use fruit as substrate and cause spoilage, producing off-flavors and odors and product discoloration. If the contaminating microorganisms are pathogens, they could also cause human illness. Historically, acid foods such as fruit juices have been considered safe; however, unprocessed orange juice has been recognized as vehicle of foodborne diseases (Burnett & Beuchat, 2001; Parish, 1997, 1998a).

The juice cloud, which is composed of finely divided particles of pectin, cellulose, hemicellulose, proteins and lipids in suspension (Irwe & Olsson, 1994; Klavons, Bennett, & Vannier, 1994), is considered a desirable characteristic of orange juice; however, it shows a loss of cloudiness and concentrates gelation a short time after squeezing, which has been associated to the PME activity. PME is present in all citrus fruits as a cell wall-bound enzyme forming a complex with pectin through electrostatic interactions. During the juice extraction process the enzyme is released into the juice hydrolysing the pectin (methyl esters of homogalacturonan) and transforming it gradually to low methoxy pectin and pectic acids, which may then form insoluble complexes with calcium ions, leading to precipitation of the pectins and cloud loss (Basak & Ramaswamy, 1996). Several PME isoenzymes are present in oranges, with both the heat-labile and heat-stable forms (Versteeg, Rombouts, Spaansen, & Pilnik, 1980). Thermal pasteurization of juice is based on the PME inactivation level of >90% because its thermotolerance is higher than the majority of spoilage microorganisms found naturally in this type of product (Tribess & Tadini, 2006). Severe conditions (90 °C, 1 min or 95 °C, 30 s) are necessary to inactivate orange PME (Cameron, Baker, & Grohmann, 1998; Do Amaral, De Assis, & De Faria Oliveira, 2005). The required heat treatment to inactivate the heat-stable isoenzymes may result in flavour and aroma changes that reduce the 'fresh-like' attributes of the juice (Farnworth, Lagacé, Couture, Yaylayan, & Stewart, 2001), hence there is much interest in the use of non-thermal processing technologies for the inactivation of PME in citrus fruit juices such as high hydrostatic pressures or pulsed electric fields (Bull et al., 2004; Sampedro, Geveke, Fan, & Zhang, 2009).

Ultra high pressure homogenization (UHPH) is an emergent technology based on the application of high pressures, but that allows to process in continuous fluid foods and that has been proposed as an alternative to the pasteurization for foods with heat-sensitive properties by its germicidal effect. Principles of UHPH are similar to conventional homogenization processes, where a liquid is forced through a valve with a very narrow and adjustable gap achieving high pressure and high velocity and bringing physical changes about in the treated product. The potential of UHPH to inactivate pathogenic and spoilage microorganisms has been previously demonstrated in milk (Briñez, Roig-Sagués, Hernández, & López, 2006a; Briñez, Roig-Sagués, Hernández, & López, 2006c; Briñez, Roig-Sagués, Hernández, & López, 2007; Kheadr, Vachon, Paquin, & Fliss, 2002; Vachon, Kheadr, Giasson, Paquin, & Fliss, 2002), in whole egg (Velázquez-Estrada, Hernandez-Herrero, López-Pedemonte, Guamis-López, & Roig-Sagués, 2008) and in orange juice (Briñez, Roig-Sagues, Hernández, & López, 2006b; Briñez et al., 2006a, 2007; Campos & Cristianini, 2007; Tahiri, Makhlouf, Paquin, & Fliss, 2006; Velázquez-Estrada et al., 2011).This technology has also been tested against enzymes of milk (Hayes & Kelly, 2003) and orange juice (Lacroix, Fliss, & Makhlouf, 2005; Welti-Chanes, Ochoa-Velasco, & Guerrero-Beltrán, 2009), but in the last case the UHPH treatments used were not able to inactivate the PME permanently. In order to achieve better results, it has been suggested the possibility of passing the samples two or more times through the valve, which increases the efficacy of the treatments, but this implies to lose the advantage of the continuity of the process. Recently, several improvements introduced in the design and materials of UHPH equipments allowed to increase the performances of these equipments in order to bring them closer to the industry requests. Among these improvements, is worth to mention the possibility to process higher volumes of product at pressures up to 400 MPa where it can be controlled the processing temperatures and extended the effect of the pressure. Nevertheless the real effectiveness of these improvements on the product properties has not been extensively evaluated to date.

In this study we evaluated the potential of newly designed UHPH equipment for orange juice processing as an alternative to pasteurization. To investigate whether the combination of different conditions could be able to inactivate PME or spoilage microorganisms of orange juice, we assessed two inlet temperatures (10 and 20 °C), three levels of pressure (100, 200 and 300 MPa) and two holding times (≤0.7 and 30 s). We compared the results of these treatments with those of two pasteurization treatments 90 °C for 1 and 2 min commonly used in the juice industry. Shelf-life was also compared during storage at 4 °C for fifty days.

2. Material and methods

2.1. Orange juice processing

Raw orange juice from *Citrus sinensis* var. Valencia was obtained from a local juice manufacturer. Most of the pulp was removed using a 2-mm steel sieve before processing.

Two heat pasteurization treatments (90 °C for 1 and 2 min) were applied to raw juice using pilot scale pasteurizer with a tubular heat exchanger (ATI, Granollers, Barcelona, Spain).

UHPH treatments were applied to raw orange juice using a Stansted ultra high pressure homogenizer (FPG 11300:400 Hygenic Homogenizer Unit, Stansted Fluid Power Ltd., Essex, UK), with a flow rate of 120 L h^{-1}. Previously, orange juice was tempered at the required inlet temperatures (10 or 20 °C), and then was pressurized at 100, 200 and 300 MPa, with ≤0.7 or 30 s holding time. Processed orange juice was cooled passing it through a heat exchanger fed with cold water. The inlet temperatures (Ti) as well as the temperature before the homogenization valve (T1) and the temperature after the homogenization valve (T2), and the final temperature (TF) of the orange juice after passing through the heat exchanger were monitored throughout the experiment. UHPH processed orange juice samples were collected in sterile bottles and stored at 4 °C until being analyzed.

2.2. PME activity measurement

Residual PME activity was evaluated using the method described by Rouse and Atkins (1955) using an automatic titrator (Titrando model 842, Metrohm AG, Herisau, Switzerland). PME activity was evaluated by titration of free carboxyl groups at pH 7.5. Briefly, 5 ml of orange sample was added to 50 ml of 1% citrus pectin (Sigma-Aldrich) solution