LWT - Food Science and Technology 74 (2016) 411–419

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Effect of high pressure carbon dioxide processing on pectin methylesterase activity and other orange juice properties

H. Briongos, A.E. Illera, M.T. Sanz*, R. Melgosa, S. Beltrán, A.G. Solaesa

Department of Biotechnology and Food Science (Chemical Engineering Section), University of Burgos, 09001 Burgos, Spain

ARTICLE INFO

Article history:
Received 22 April 2016
Received in revised form
14 July 2016
Accepted 29 July 2016
Available online 31 July 2016

Keywords:
Orange juice
HPCD
Pectin methylesterase
Cloud stability

ABSTRACT

Inactivation of pectinmethylesterase (PME) and quality parameters of orange juice have been studied after high pressure carbon dioxide (HPCD) treatment. The HPCD treatment conditions covered a wide range of temperature from 2 to 40 °C, far below normal thermal treatment, while operating pressure was varied from 10 to 30 MPa and exposure time from 3 to 60 min. A decrease in PME activity was found, even at the lowest temperature studied in this work, 2 °C. Different inactivation kinetic models were used to correlate the PME residual activity: the two-fraction model, the fractional-conversion model and the Weibull model. The two-fraction model presents the lowest mean relative deviation. Some quality parameters such as colour, pH, °Brix, turbidity, ascorbic acid, total acidity and particle size distribution (PSD) were also determined right after HPCD treatment and along storage at 4 °C up to 12 days. PSD shows that HPCD treatment results in a volume increase of small particles and a volume decrease of large particles regarding the non-treated orange juice. Calcium content was also determined before and after HPCD treatment to check for insoluble calcium carbonate formation but not significant changes were observed in calcium content after HPCD treatment.

1. Introduction

Fruit juice and nectars consumption amounted to 9.7 million litres in 2014 in the EU, of which orange juice is one of the most consumed (European Fruit Juice European Fruit Association, 2015). Cloud loss is a quality defect in orange juice, since cloud particles are involved in the colour, flavour, texture and aroma of orange juice (Klavons, Bennett, & Vannier, 1991). Additionally, consumers associate the cloud loss with spoilage and quality loss. Citrus cloud is a complex mixture of protein, pectin, lipid, hemicellulose, cellulose and other minor components. Cloud particles of citrus juices range from 0.4 to 5 μm, being particles smaller than 2 μm the most stable clouds (Ellerbee & Wicker, 2011). In the literature, one of the most accepted theories of cloud destabilization is based on pectin demethylation by pectinmethylesterase (PME) (EC 3.1.1.11) in a blockwise fashion. The negative charges generated by PME activity allow subsequent formation of insoluble calcium pectate gels with calcium ions present in the juice. These gels can precipitate pulling the cloud with them causing orange juice clarification due to the loss of turbidity (Ellerbee & Wicker, 2011). Thermal treatment of orange juice at 90 °C for 1 min is the method currently used to prevent microbial spoilage as well as the inactivation of the PME (Oulé, Dickman, & Arul, 2013). However thermal treatment causes undesirable changes in several quality parameters such as flavour, colour and texture and can also destroy heat-sensitive nutritional components such as vitamins (Hu, Zhou, Xu, Zhang, & Liao, 2013). Non-thermal technologies have gained interest and acceptance as food processing methods due to the consumer increased demand for fresh-like products. Among them, high pressure carbon dioxide (HPCD) has been proposed as an alternative non-thermal pasteurization technique for foods. HPCD can also cause the inactivation of certain enzymes that affect the quality of some foods such as PME in the orange juice under mild operation conditions (Damar & Balaban, 2006). In HPCD treatments, operating temperatures can range between 5 and 60 °C and pressures usually below 50 MPa. Some other advantages of using HPCD as non-thermal treatment are that carbon dioxide is nontoxic, nonflammable, inexpensive and readily available. It can also be easily removed after treatment by depressurization.

Some previous studies dealing with the effect of HPCD treatment on orange juice quality can be found in the literature. The first work was carried out by Balaban, Arreola, Marshall, Peplow, Wei, and Cornell (1991), who found 100% PME inactivation when using

* Corresponding author.
E-mail address: tersanz@ubu.es (M.T. Sanz).

http://dx.doi.org/10.1016/j.lwt.2016.07.069
0023-6438/

a commercial Milton Roy Supercritical X-10 System while only 86% PME inactivation was achieved when a custom-made supercritical system was used. These authors also found that, when using the custom-made system, cloud significantly increased. Kincal et al. (2006) also reported a high increase in the cloud values (between 446 and 846%) in orange juice, when using a continuous system but a maximum PME inactivation degree of only 46.3%. Recently, Zhou, Bi, Xu, Yang, and Liao (2015) reviewed the effects of HPCD processing on flavour, texture and colour of foods including orange juice. Combined technologies of high power ultrasound assisted SC-CO_2 (HPU-SCCO_2) have been also reported to inactivate PME of orange juice (Ortuño, Balaban, & Benedito, 2014). These authors found a lowest residual activity of 10.65%. Therefore, different inactivation degrees have been reported in the literature when treated freshly squeezed orange juice to HPCD. This regard, in the literature it has been reported an improvement of inactivation of different enzymes by increasing the CO_2 concentration in the enzyme solutions when CO_2 was fed through a cylindrical filter nozzle (Ishikawa, Shimoda, Kawano, & Osajime, 1995). Unfortunately, in most of the previous studies, no information about the way CO_2 is put in contact with the substrate can be found and comparison is difficult to stablish. Additionally, differences in inactivation levels are related to cultivars, original pH of the juice, isoenzyme forms, total solid content and other processing factors.

CO_2 was used under supercritical conditions in previous reported HPCD treatments of orange juice. The main objective of this work is to assess the effect of HPCD treatment under supercritical and liquid conditions on PME activity. The effect of HPCD processing on other physical and chemical parameters of orange juice will be also studied.

2. Materials and methods

2.1. HPCD equipment and processing

Valencia oranges were purchased from a local supplier. Oranges were squeezed in an orange squeezer. The experimental apparatus used for the HPCD treatment has been designed in our laboratory with a maximum operating pressure and temperature of 30 MPa and 80 °C respectively (Melgosa, Sanz, Solaesa, Bucio, & Beltrán, 2015). It consists of a CO_2 reservoir, a high pressure syringe pump with a pressure controller (ISCO 260 D) and 3 high pressure cells immersed in a thermostatic water bath. In a typical HPCD experiment, orange juice was charged into the high pressure cell, which was then placed in the thermostatic water bath at the preset temperature. Afterwards, the system was pressurized and maintained at constant temperature and pressure for a pre-established treatment time. CO_2 was fed to the high pressure cell through a sintered stainless steel micro-filter with a pore size of 10 μm to increase the concentration of CO_2 dissolved in the sample. The duration of the pressurization and depressurization was less than 2–3 min and it was not included in the treatment holding time. The high pressure cells were magnetically stirred. Experiments were carried out in a temperature (T) range from 2 to 40 °C, pressure (p) from 10 to 30 MPa and exposure time (t) from 3 to 60 min. Different pressure cells were arranged in series to carry out experiments at different operating times. After HPCD treatment, the high pressure cells were depressurized and the treated orange juice was analysed (see section 2.2). During depressurization, a temperature decrease of the orange juice was observed due to Joule-Thomson cooling effect depending on applied pressures (Zhou, Zhang, Leng, Liao, & Hu, 2010).

PME activity, pH and calcium content were determined before and after HPCD treatment at different operating conditions. To evaluate the effect of HPCD treatment on the self-life of orange juice, a sample of orange juice treated at 30 MPa and 40 °C for 40 min was stored in the refrigerator (4 °C). Aliquots were taken after 5 and 12 days of storage, and different quality parameters of orange juice were determined and compared with original freshly squeezed orange juice.

2.2. Physico-chemical analysis

2.2.1. Determination of pectin methylesterase activity

PME activity was determined by using an automatic titrator system (Metrohm® Titrando). A 1% of pectin solution (Alfa Aesar® Pectin Citrus) prepared in NaCl 0.3 M was used as substrate. 50 mL of pectin solution mixed with 5 mL of orange juice were adjusted to pH 7.5 with NaOH 0.02 N. During hydrolysis at room temperature, pH was maintained at 7.5 by adding NaOH 0.02 N. The amount of NaOH added for 30 min was recorded. One PME activity unit (UPE) is defined as the micromoles of carboxylic groups produced per minute and mL of juice at pH 7.5 and room temperature. PME activity was calculated according to the following equation:

$$UPE/mL = \frac{(mL\,NaOH)\cdot(Normality\,of\,NaOH)\cdot(factor\,NaOH)\cdot(1000)}{(mL\,juice)\cdot(minutes)} \quad (1)$$

Results are presented as residual PME activity, defined as the relationship between PME activity after and before HPCD treatment:

$$Residual\ PME\ activity = \frac{PME\ activity\ after\ HPCD}{PME\ activity\ before\ HPCD} = \frac{A}{A_o} \quad (2)$$

2.2.2. Determination of pH, °Brix, total acidity, vitamin C and colour

pH of orange juice was determined with a pH-meter (Crison® pH & Ion-Meter GLP 22). °Brix were measured with a Milton Roy® refractometer (Model 334610) at 25 °C. Temperature and acidity corrections were made (Kimball, 1999).

Total acidity was determined by using an automatic titrator (Metrohm® Titrando). A sample of 2 mL of orange juice was mixed with 50 mL of distilled water. The mixture was titrated with 0.02 N NaOH. Titrable acidity was expressed as citric acid percentage (g citric acid/100 g). Vitamin C was determined with 2,6-dichloroindophenol titrimetric method (Kimball, 1999).

Colour was evaluated by a Konica Minolta® CM-2600d colorimeter. The L*, a* and b* values were obtained representing lightness, red to green colour and yellow to blue colour, respectively. Other conditions are illuminant D65 (daylight source) and a 10° standard observer (perception of a human observer) following the CIE recommendations. Changes in colour were expressed as:

$$\Delta E = \sqrt{\left(L^*_{before} - L^*_{after}\right)^2 + \left(a^*_{before} - a^*_{after}\right)^2 + \left(b^*_{before} - b^*_{after}\right)^2} \quad (3)$$

Differences in perceivable colour can be classified analytically as not noticeable (0–0.5) slightly noticeable (0.5–1.5), noticeable (1.5–3) well visible (3.0–6.0) and great (6.0–12.0) (Yuk, Sampedro, Fan, & Geveke, 2014).

Another parameter that can be used to evaluate alterations in colour of a beverage is the chroma, C, which measures colour intensity:

$$C = \sqrt{(a^*)^2 + (b^*)^2} \quad (4)$$