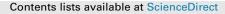
LWT - Food Science and Technology 75 (2017) 85-92



LWT - Food Science and Technology

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

Quality change during high pressure processing and thermal processing of cloudy apple juice



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ARTICLE INFO

Article history: Received 21 March 2016 Received in revised form 18 August 2016 Accepted 19 August 2016 Available online 21 August 2016

Keywords: Color Enzyme Volatile fraction Multivariate data analysis

ABSTRACT

This work evaluates the impact of high pressure processing (HPP; 600 MPa, 3 min) on apple (Pink Lady, Granny Smith and Jonagold) juice quality changes compared to thermal processing (TP; $P_{8.3}^{8.5} C_{-}^{C} = 5$ min). This comparative study was performed by integrating both an untargeted headspace-GC-MS finger-printing and targeted approach to analyze the volatile fraction and *a priori* selected quality attributes (color, sugars, acids and enzymes) respectively. A significant higher total color difference (ΔE^{+} >6.0) was determined for thermally treated juice. Polyphenol oxidase and peroxidase can be inactivated below detection limits by TP, while residual activity (>50%) was detected after HPP. Sugars and acids were almost not affected nor by HPP nor by TP. Using the untargeted fingerprinting approach, aldehydes, alcohols, ketones and organosulfur were detected in higher amounts after TP compared to untreated and HPP treated samples, which could be linked to the Maillard reaction and oxidative reactions. In general, the overall quality of HP treated apple juices is much more comparable to that of the fresh juice, in particular HPP results in lower amount of thermally induced compounds that are related to cooked notes of pasteurized apple juices.

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1. Introduction

Nowadays, consumers demand minimally processed fruit juices with natural appearance, fresh flavor and free from preservatives and other additives. Fruit juices have been traditionally pasteurized by thermal processing (TP), but the fresh quality can be greatly reduced due to the processing intensity. Due to these limitations, food scientists and industries have been investigating emerging food processing technologies, which do not rely on an increased temperature as the most important processing variable. Among these technologies, high pressure processing (Barba, Esteve, & Frígola, 2012; Knorr et al., 2011). In the juice industry, one of the main challenges is to produce juices with a quality close to that of freshly-squeezed ones and to guarantee consumers with a constant original quality.

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Apple juice is one of the most popular fruit juices. For processed apple juice, color and flavor are important guality indicators. The color of apple juice changes during and/or after processing, due to enzymatic and non-enzymatic reactions. Enzymes like polyphenol oxidases (PPO) and peroxidase (POD) have a considerable impact on the color and flavor quality of apple juice (Terefe, Buckow, & Versteeg, 2014). During apple juice processing, non-enzymatic browning reactions occur in apple juice, e.g. Maillard reactions, ascorbic acid destruction and pigment degradation (Ibarz, Pagan, & Garza, 2000). The flavor of apple juice generally depends upon taste mainly related to sweetness (sugars) and sourness (organic acids) and aroma due to the odor-active volatile compounds such as esters, alcohols, aldehydes, ketones and ethers (Dixon & Hewett, 2000). Although over 300 volatile compounds have been detected and identified in the aroma profile of apples by gas chromatography (GC) and mass spectrometry (MS) (Dixon & Hewett, 2000; Yahia, 1994), only 20-40 odor-active volatiles including ethyl-2-methyl butanoate, ethyl acetate, ethyl butanoate, (E)-2-hexenal and 1butanol are considered as being responsible for apple aroma (Cunningham, Acree, Barnard, Butts, & Braell, 1986; Dixon &



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Hewett, 2000; López et al., 2007; Yahia, 1994). Thus, in this context, it is important to evaluate the capacity of HPP to maintain fresh juice color and flavor in comparison to TP.

Although some studies on the impact of HPP and TP on specific quality aspects of apple juices are available (Bayındırlı, Alpas, Bozoğlu, & Hızal, 2006; Juarez-Enriquez, Salmeron-Ochoa, Gutierrez-Mendez, Ramaswamy, & Ortega-Rivas, 2015; Landl, Abadias, Sárraga, Viñas, & Picouet, 2010; Valdramidis et al., 2009), the objective of this work is to develop a holistic multi-quality dimension analysis of cloudy apple juice processed by HPP and TP using targeted, analyzing color, taste related compounds (sugars and organic acids) and enzyme activities and untargeted approaches, fingerprinting volatile fraction. The potential of currently available instrumental and the state-of-the-art data analysis (e.g. multivariate) techniques are also exploited.

2. Materials and methods

2.1. Sample preparation

Apples (Pink lady; Granny smith; Jonagold) were purchased at a local market in Belgium. These three cultivars were chosen in this study because (i) they represent the top three cultivars with highest awareness in Belgium and (ii) a preliminary study including Golden Delicious, Granny Smith, Jonagold, Pink Lady and Red Chief clearly showed that represent three distinctly different apple flavor profiles (data not shown). Apples were juiced by using laboratory-scale equipment (Angel Juicer 8500S, the Netherlands). Ascorbic acid was added to the juice (500 mg/l) in order to slow down enzymatic browning. The whole procedure was performed in a cooling room at 4 °C.

2.2. Processing Conditions

to HPP at 600 MPa/3 min and TP ($P_{8.3 \text{ °C}}^{85 \text{ °C}} = 5 \text{ min}$) (Liu et al., 2014). Fresh apple juice was frozen and kept as a control. To compare thermal and high pressure pasteurization, the processing conditions were selected targeting an equivalent microbial inactivation (a 5-log reduction of *Escherichia coli* O157:H7) (Hiremath & Ramaswamy, 2012). The processing conditions for both HPP and TP are depicted in Fig. 1. Each treatment was repeated six times.

The HPP was carried out using laboratory-scale high pressure equipment (Resato, the Netherlands). The 100% propylene glycol

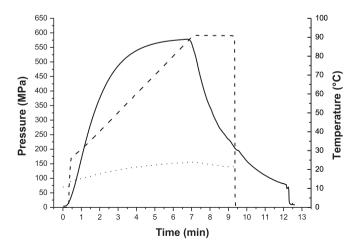


Fig. 1. Profiles of high pressure processing (HPP) with apple juice temperature (dot line) and pressure (dash line) and thermal processing (TP) with juice temperature (solid line).

was used as the pressure medium. Prior to processing, the samples and high pressure vessels were pre-equilibrated at 10 °C. The vessels are jacketed with a heating coil connected to a temperature control unit. The pressure (Fig. 1, dash line) and temperature (Fig. 1, dot line) of the sample were automatically recorded. The temperature of samples never exceeded 21 °C during the whole procedure. The TP was carried out in a temperature-controlled water bath at 90 °C. The samples were kept in the water bath until the aimed pasteurization value was achieved ($P_{8.3 \circ C}^{85 \circ C} = 5 \text{ min}$). Temperature profile was recorded at the geometric centre of the sample in a tube using a type T thermocouple connected to a thermocouple box (TR9216, Ellab, Hilleroed, Denmark) and a CMC-92 data acquisition system (Ellab, Hilleroed, Denmark) (Fig. 1, solid line). After both treatments, the apple juices were immediately cooled in ice water to stop further reactions, then were frozen in liquid nitrogen and stored in a freezer at -40 °C until analysis.

2.3. Color

The color of apple juice was determined using a Hunterlab ColorQuest colorimeter (Hunterlab, Reston, VA) as explained in previous work (Yi et al., 2016). L^* (brightness), a^* (greenness/ redness) and b^* (blueness/yellowness) values were measured. The total color difference (ΔE^*) was determined by the following Eq. (1):

$$\Delta E^* = \sqrt{\left[\left(L^* - L_0^* \right)^2 + \left(a^* - a_0^* \right)^2 + \left(b^* - b_0^* \right)^2 \right]}$$
(1)

In Eq. (1), the subscript '0' indicates initial color of untreated apple juice (reference sample).

2.4. Enzyme activity

2.4.1. Polyphenol oxidase (PPO)

PPO extraction and activity assay were carried out based on the procedure described by Gui et al. (2006) with some modifications. The juice was centrifuged for 10 min at 3000 g at 4 °C (Microfuge 22R, Beckman Coulter, Fullerton, CA, US). Then the obtained supernatant as crude PPO extract was measured using spectrophotometer at 420 nm and 25 °C. The reaction cuvette contained 0.5 ml of PPO extract and 2.5 ml of substrate solution (catechol 0.05 mol/l in 0.2 mol/l sodium phosphate buffer solution, pH 6.5). The activity measurement of each extract was determined in triplicate. The percentage of residual activity was defined as indicated by Eq. (2):

Residual activity =
$$100 \times \frac{A_t}{A_0}$$
 (2)

Where A_t and A_0 were the enzyme activities of treated and untreated samples, respectively.

2.4.2. Peroxidase (POD)

POD extraction was performed using the method of Yi et al. (2016). The juice (1 ml) was mixed with 400 μ l sodium phosphate buffer (0.2 mol/l, pH 6.5) containing 1 mol/l NaCl and 10 g/l polyvinylpyrrolidone (PVPP) in 1.5 ml tube. After a centrifugation step for 30 min at 22,000 g (4 °C), supernatant was used as POD crude extract. The POD activity was analyzed using a spectrophotometer at 485 nm and 25 °C. The reaction mixture including POD extract (200 μ l) and 2.8 ml substrate solution, containing 0.2 mol/l sodium phosphate buffer (pH 6.5), 3 g/l *p*-phenylenediamine and 1 ml/l hydrogen peroxide. POD activity of each extract was determined in triplicate. The percentage of residual POD activity was calculated using Eq. (2).

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