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Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice

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ABSTRACT

The effect of conventional and microwave pasteurisation on the main bioactive compounds of grapefruit juice and their stability during 2 months' refrigerated and frozen storage was evaluated. Ascorbic acid (AA), vitamin C and organic acids were analysed by HPLC, whereas total phenols and antioxidant capacity (%DPPH) were measured by spectrophotometry. The results showed that conventional treatment led to a significant decrease in citric acid (from 1538 to 1478 mg/100 g) and AA (from 36 to 34.3 mg/100 g), whilst microwave pasteurisation preserved these compounds. Frozen storage maintained AA and vitamin C, especially in treated samples. Frozen non-treated samples and conventional pasteurised ones preserved about a 75% and 20% of the total phenols and antioxidant capacity, respectively, whilst in frozen microwave pasteurised juices this preservation was of 82% and 33%. From these results, the use of microwave energy may be proposed as an alternative to traditional heat pasteurisation in order to preserve the natural organoleptic characteristics and essential thermolabile nutrients of grapefruit juice.

1. Introduction

Evidence from a large number of epidemiological, *in vitro* and *in vivo* studies has shown that the consumption of citrus fruit is generally good for the health and contributes to the prevention of degenerative processes, particularly lowering the incidence and mortality rate of cancer and cardio and cerebro-vascular diseases (Poulose, Harris, & Patil, 2005). Citrus juice is an important dietary source of bioactive compounds, whose beneficial health effects are ascribed, in part, to its high content of ascorbic acid. Vitamin C is a natural antioxidant that may inhibit the development of major oxidative human reactions. In addition to the well-known vitamin C, citrus juice also contains phenolic compounds which contribute to their antioxidant capacity and that may produce beneficial effects by scavenging free radicals (Xu et al., 2008). Vinson and Bose (1988) emphasised the importance of ascorbic acid as a natural component in citrus juice where other natural compounds present in the juice, such as flavonoids, increase the bioavailability of this acid. On the other hand, organic acids, including citric, tartaric and malic acids in citrus juice are important components which contribute to flavour attributes and are usually used as "fingerprints" to detect the quality of the juice and accomplish its authentication (Cen, Bao, He, & Sun, 2007). High concentrations of organic acids and low pH in most fruits are critical for the preservation of derivative products. They also help to stabilise ascorbic acid and anthocyanins (Wang, Chuang, & Ku, 2007).

Nowadays, consumers demand the maximum preservation of the endogenous sensory, nutritional and health related qualities of fruit products. Traditional heat pasteurisation of citrus juices is necessary in order not only to destroy microorganisms and reduce pectin methylesterase (PME) enzymatic activity, but it also leads to detrimental changes in the quality (Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006). The colour and flavour are different from those of freshly squeezed juice and there is also a decrease in the number of biochemical compounds. PME inactivation is important because this enzyme catalyses pectin degradation and alters the colloid stabilizing power of the pectin, which imparts the favourable appearance and mouth feel to orange juice. As PME is more resistant to heat than microorganisms, thermal treatments are focussed on the inactivation of this enzyme. The search for new technologies that cause minimum damage to the organoleptic and nutritional characteristics may be considered as an alternative to conventional thermal juice pasteurisation. In this sense, the use of microwave energy seems to cause smaller changes in the fruit quality attributes (Nikdel, Chen, Parish, MacKellar, & Friedrich, 1993). Several studies have successfully been carried out into the microwave pasteurisation of fruit juices, as it preserves the natural organoleptic characteristics of the juice and reduces the time of exposure to energy, with the subsequently lower risk of losing

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essential thermolabile nutrients (Cañumir, Celis, Brujin, & Vidal, 2002).

The aim of this work was to characterise the main bioactive compounds (vitamin C, total phenol, organic acids) and their relative contribution to the antioxidant capacity of freshly squeezed grapefruit juice and assess the effect of conventional and microwave pasteurisation on these compounds and their antioxidant capacity. Their stability during 2 months' refrigerated and frozen storage was also evaluated.

2. Materials and methods

2.1. Raw material

For this work, grapefruits (*Citrus paradise* var. Star Ruby) from the city of Murcia were purchased from a local supermarket. Grapefruits were selected on the basis of a similar degree of ripeness (ratio °Brix/acidity ≈ 4) and apparent fruit quality (firmness, size, colour and absence of physical damages). Fruit was processed in the laboratory immediately after being purchased.

2.2. Treatments

Freshly squeezed (FS) grapefruit juice was extracted through a domestic squeezer (Braun Citromatic Pulp Control MPZ6), filtered using a sieve (light of mesh diameter 1 mm, Cisa 029077, 1 series) and immediately processed. To obtain conventional pasteurised juice (CP) samples of 40 ml were heated in glass tubes in a thermostatic water bath (Precisterm, Selecta, Spain) operating at 95 °C. In this way, the juice took 80 s to reach 80 ± 2.5 °C and it remained at this temperature for 11 s. In the case of microwave pasteurised juice (MP), samples of 20 ml were heated in 25 ml glass tubes at 900 W for 30 s using a microwave (Moulinex 5141 AFW2, Spain). Treated samples were immediately cooled in ice-water till juice reached 30 °C. Both processes were previously optimised to reach ≈10% of fresh juice pectimethylesterase (PME) residual activity.

2.3. Enzymatic determinations

2.3.1. Pectin methylesterase (PME) activity measurement

PME activity in grapefruit juice was measured using the Kimball (1999) method. Briefly 10 ml of grapefruit juice and 40 ml of 1% peel citrus pectin dissolution (60% degree of esterification, Fluka Biochemika, Switzerland) containing 0.02 M NaCl, previously tempered to 30 °C in a thermostat bath, were mixed and kept in continuous agitation. NaOH was used to adjust the resulting solution to pH 7.7 (Consort C830 pH meter, Belgium) and then 100 µl of 0.05 N NaOH were immediately added. The exact time needed to lower the pH back to 7.7 by enzyme's action was then measured. As it is a first order reaction, the enzyme activity (A) can be calculated according to the concentration of acid produced using Eq. (1).

$$A = \frac{(V_{NaOH}) \times (N_{NaOH})}{(t_R) \times (W_{sample})} \quad (1)$$

where V_{NaOH} is the NaOH volume used in the titration (ml), N_{NaOH} is the normality of the NaOH solution used (mEq ml^{-1}), t_R is the reaction time (min) and W_{sample} is the weight of the sample (g).

The percentage of residual enzyme activity (RA) was defined as indicated by Eq. (2):

$$RA = 100 \times \frac{A_t}{A_0} \quad (2)$$

where A_t and A_0 were the enzyme activities of treated and untreated samples, respectively. A_t and A_0 were determined immediately after processing to avoid the effects of storage time.

2.3.2. Polyphenoloxidase (PPO) activity measurement

PPO activity was measured by spectrophotometry. The enzyme was extracted from grapefruit juice using the method of Valero, Varón, and García-Carmona (1988) modified by Rapeanu, Van Loey, Smout, and Hendrickx (2006). Briefly 100 µl of clarified juice were added to 1 ml substrate (0.1 M cathecol in McIlvaine buffer, pH 5) and the increase in absorbance at 400 nm at 25 °C was recorded automatically for 30 min (Thermo Electron Corporation, USA). One unit of PPO activity was defined as a change in absorbance at 400 nm min^{-1} ml^{-1} of enzymatic extract. Enzyme activity was calculated from the linear part of the curve. The percentage of residual enzyme activity was calculated using Eq. (2).

2.3.3. Peroxidase (POD) activity measurement

POD activity in grapefruit juice was measured using the method described by Cano, Hernández, and De Ancos (1997) with some modifications made by Elez-Martínez et al. (2006). Briefly 10 ml of sample were homogenised with 20 ml 0.2 M sodium phosphate buffer (pH 6.5) and centrifuged (15,000 rpm, 20 min) at 4 °C (P-Selecta Medifrigar BL-S, Spain) to obtain the enzymatic extract. POD activity was assayed spectrophotometrically by placing 2.7 ml 0.2 M sodium phosphate buffer (pH 6.5), 0.2 ml *p*-phenylenediamine (10 g kg^{-1}), 0.1 ml hydrogen peroxide (15 g kg^{-1}) and 0.1 ml of enzymatic extract in a 1 cm oath cuvette. The oxidation of *p*-phenylenediamine was measured at 485 nm and 25 °C using a Thermo Electron Corporation spectrophotometer (USA). POD activity was determined by measuring the initial rate of the reaction, which was computed from the linear portion of the plotted curve. One unit of POD activity was defined as a change in absorbance at 485 nm min^{-1} ml^{-1} of enzymatic extract. The percentage of residual enzyme activity was calculated using Eq. (2).

2.4. Analytical determinations

2.4.1. Soluble solids

Total soluble solids were estimated as °Brix with a refractometer (Abbe Atago 89553 by Zeiss, Japan) at 20 °C.

2.4.2. pH

To determine the pH, a Consort C830 pH meter (Belgium) with a penetration electrode was used.

2.4.3. Organic acids

HPLC (Jasco, Italy) was applied to the quantitative determination of citric (CA), malic (MA) and tartaric acid (TA) according to Cen et al. (2007). Samples were centrifuged at 10,000 rpm for 15 min and filtered by 0.22 µm membrane. HPLC method and instrumentation was: Ultrabase-C18, 5 µm (4.6 × 250 mm) column (Spain); mobile phase 0.01 mol/l potassium dihydrogen phosphate solution, volume injection 20 µl, flow rate 1 ml/min, detection at 215 nm at 25 °C. Standard curves of each reference acid (Panreac, Spain) were used to quantify the acids.

2.4.4. Ascorbic acid and total vitamin C

Ascorbic acid (AA) and total vitamin C (ascorbic acid + dehydroascorbic acid) were determined by HPLC (Jasco, Italy). To determine the ascorbic acid (Xu et al., 2008), 1 ml sample was extracted with 9 ml 0.1% oxalic acid for 3 min and immediately filtered before injection. The procedure employed to determine total vitamin C was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol as the reductant reagent (Sanchez-Mata, Cámara-Hurtado, Diez-Marques, & Torija-Isasa, 2000; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006). A 0.5 ml aliquot sample was taken to react with 2 ml of a 20 g/l dithiothreitol solution for 2 h at room temperature and in darkness. Afterwards, the same procedure as that used for the ascorbic acid method was performed. The HPLC