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Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice
Part II: Impact on specific chemical and biochemical quality parameters

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ABSTRACT

The impact of thermal, high pressure (HP) and pulsed electric field (PEF) processing for mild pasteurization of orange juice was compared on a fair basis, using processing conditions leading to an equivalent degree of microbial inactivation. Examining the effect on specific chemical and biochemical quality parameters directly after treatment and during storage at 4 °C revealed only significant differences in residual enzyme activities. For pectin methylesterase inactivation, none of the treatments was able to cause a complete inactivation, although heat and HP pasteurization were the most effective in limiting the residual activity. Peroxidase was completely inactivated by heat pasteurization and was much less susceptible to HP and PEF. All other quality parameters investigated, including the sugar profile, the organic acid profile, bitter compounds, vitamin C (ascorbic acid and dehydroascorbic acid), the carotenoid profile, furfural and 5-hydroxymethylfurfural, experienced no significantly different impact from the three pasteurization techniques.
Industrial relevance: HP and PEF processing have received important attention during the last years for application as alternatives to traditional thermal pasteurization. For the further implementation of HP and PEF treatment in the food industry, legal approval of such processes is required. Accordingly, an in-depth characterization of products treated by these novel technologies is indispensable. This paper addresses orange juice as a relevant model food product to compare the impact of HP and PEF processing with that of a conventional thermal pasteurization process and to search for significant differences in specific known nutrients, undesired substances and other quality-related aspects of orange juice.

1. Introduction

Because of its unique combination of sensory attributes, such as color, aroma and flavor, and its nutritional value, orange juice is the world's most popular fruit juice, representing approximately 60% of all Western European consumption of juices and juice-based drinks (Fry, Martin, & Lees, 1995). This consumption is estimated at 5 billion liters a year in the European Union (Brown, 2004). Despite its low pH, fresh orange juice stability is rather limited, due to microbial growth and enzyme activities. To prolong this shelf life, thermal pasteurization is the most widely applied technique, successfully inactivating vegetative microorganisms and enzymes (Kimball, 1999). For orange juice shelf-stable at room temperature, conditions of 10–30 s at 95–98 °C are usually applied (Ringblom, 2004). With these conditions, inactivation of pectin methylesterase (PME), responsible for cloud loss, is aimed at; microbial inactivation requires less severe conditions and is already obtained after a few seconds at 70 °C (FDA, 2004; Mazzotta, 2001). Although the intense pasteurization process has proven to be very efficient in microbial and PME inactivation, the great amount of energy that is transferred along with it to the juice may also cause undesirable biochemical and nutritious changes, affecting the overall juice quality (Sandhu & Minhas, 2006). Increased awareness of the relation between health and diet has stimulated a trend towards minimally processed, fresh-like, nutritive and healthy products. Accordingly, there is a growing interest in premium quality juices (not obtained from concentrate), with very mild pasteurization, distributed refrigerated and with a limited shelf life (Esteve, Frigola, Rodrigo, & Rodrigo, 2005). As a consequence, manufacturers seek methods to reduce thermal input during preservation (Mertens & Knorr, 1992; Sloan, 2005). However, despite improvements in heat processing, the application of even minimal thermal treatments can still cause quality losses in foods with heat-sensitive components and/

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or characteristics (Sandhu & Minhas, 2006). Therefore, alternatives to traditional thermal pasteurization, which do not involve direct heat, have been investigated in order to obtain safe juices, but with fresh-like quality attributes (Mertens & Knorr, 1992). Among these, high pressure (HP) and pulsed electric field (PEF) processing have received important attention. These technologies rely on the lethal effect of high hydrostatic pressures and strong electric fields, respectively, for the inactivation of microorganisms, and are claimed to result in better quality retention and longer shelf life (Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003; Min, Evrendilek, & Zhang, 2007; Oey, Lille, Van Loey, & Hendrickx, 2008; Oey, Van der Plancken, Van Loey, & Hendrickx, 2008).

Numerous research papers can be found on the effects of HP and PEF pasteurization on orange juice quality, and some authors have compared their impacts with that of a conventional heat pasteurization process (Bull et al., 2004; Cortés, Esteve, & Frígola, 2008b; Cortés, Torregrosa, Esteve, & Frígola, 2006; Elez-Martínez, Soliva-Fortuny, & Martín-Belloso, 2006; Min, Jin, Min, Yeom, & Zhang, 2003; Plaza et al., 2006; Polydera, Stoforos, & Taoukis, 2005; Sánchez-Moreno et al., 2005; Yeom, Streaker, Zhang, & Min, 2000a). However, in these studies, processing conditions are generally not selected based on principles of equivalence, e.g. equivalent microbial or enzyme inactivation, and therefore, they cannot be considered fair comparisons. Often industrial thermal pasteurization conditions are applied, resulting in complete PME inactivation, designed for production of shelf-stable juices. HP and PEF treatment as such can, however, not sufficiently inactivate PME to result in shelf-stable juice, unless they are combined with elevated temperatures or a high thermal load is created (Goodner, Braddock, & Parish, 1998; Van den Broeck, Ludikhuyze, Van Loey, & Hendrickx, 2000; Van Loey, Verachtert, & Hendrickx, 2002).

The objective of the present work was to compare the impact of thermal, HP and PEF processing for mild pasteurization of orange juice on a fair basis, using processing conditions leading to an equivalent degree of microbial inactivation. Processing was performed in pilot- and industrial-scale equipment, which provided conditions closer to industrial application, compared to previous studies, in which usually only lab-scale equipment is used. In part I (the complementary publication by Timmermans et al., 2011), the effect on overall quality attributes is reported, while this part focuses on specific chemical and biochemical quality parameters, including pectin methylesterase (PME) activity, peroxidase (POD) activity, sugar profile, organic acid profile, bitter compounds, vitamin C (ascorbic acid and dehydroascorbic acid), carotenoid profile, furfural and 5-hydroxymethylfurfural (HMF). All properties were evaluated over a storage period of 58 days at 4 °C.

2. Materials and methods

2.1. Sample preparation and processing

For a detailed description of the orange juice preparation and processing, the reader is referred to part I (Timmermans et al., 2011). In short, thermal pasteurization was conducted at 72 °C for 20 s, high pressure conditions were 1 min at 600 MPa with an initial temperature of orange juice of 5 °C, and PEF treatment was applied in continuous flow using monopolar pulses of 2 μs at 23 kV/cm, 90 Hz and 130 L/h flow rate, with an inlet and outlet temperature of respectively 38 and 58 °C.

2.2. Post-processing sample handling

After treatment and transport, all orange juice bottles were stored at maximum 4 °C, while the temperature was monitored in and out of the bottles, at different locations in the cooling room. At fixed points in time during the shelf life study (day 0, 1, 2, 9, 20, 28 and 58), pooled sampling was performed. For each treatment condition, a number of bottles were taken from the different treatment batches and at different locations in the cooling room, after which the juice from these bottles was mixed jointly and divided uniformly over smaller portions of 30 ml. These volumes were immediately frozen in liquid nitrogen and stored at −80 °C. In this way, a particular analysis could be performed on all samples with different shelf life at once. The stability at −80 °C of the different components examined was verified beforehand. At the time of analysis, one sample tube per condition was thawed in a circulating water bath at 25 °C and homogenized once more.

2.3. Pectin methylesterase (PME) activity measurement

The PME activity in the orange juice samples was determined by monitoring the release of acid during pectin hydrolysis as a function of time at pH 7.0 and 25 °C. The reaction mixture consisted of 1 ml orange juice sample and 30 ml of a 0.35% (w/v) apple pectin solution containing 0.117 M NaCl. During pectin hydrolysis, the pH was maintained constant by addition of 0.01 N NaOH using an automatic pH-stat titrator (Metrohm, Herisau, Switzerland) and the enzyme activity was related directly to the amount of NaOH added per minute. The PME activity of each sample was measured in triplicate.

2.4. Peroxidase (POD) activity measurement

POD was first extracted from the orange juice by mixing 10 ml juice with 10 ml 0.2 M sodium phosphate buffer pH 6.5 containing 1 M NaCl. The mixture was centrifuged for 15 min at 24,000×*g* and 4 °C. The supernatant was filtered through a Whatman no. 1 filter paper and the filtrate was further used for the activity measurement. The extraction of POD was performed in duplicate.

The POD activity was measured spectrophotometrically by adding 1.1 ml 0.2 M sodium phosphate buffer pH 6.5, 0.5 ml enzyme extract, 1 ml o-phenylenediamine solution (10 g/L in 0.2 M sodium phosphate buffer pH 6.5) as substrate (proton donor) and 0.5 ml hydrogen peroxide solution (15 g/L in 0.2 M sodium phosphate buffer pH 6.5) as oxidant to a 1 cm path cuvette. The formation of the colored oxidation product (2,3-diaminophenazine) was measured as the change in absorbance at 485 nm and 25 °C for 10 min. The POD activity of each extract was determined in duplicate.

2.5. HPLC analysis of sugars

Sugars were determined by reversed phase (RP) HPLC with evaporative light scattering detection (ELSD).

10 ml juice was clarified with 0.5 ml Carrez reagent I and II (respectively 15% (w/v) $K_4[Fe(CN)_6]$ and 30% (w/v) $ZnSO_4$) during a period of 30 min rest, after which the samples were centrifuged at 24,000×*g* and 4 °C for 15 min. The supernatant was filtered through a 0.45 μm syringe filter (Chromafil A-45/25, Macherey-Nagel, Düren, Germany) and 5 μl of a 10-fold dilution of the filtrate was injected into the HPLC system.

The apparatus consisted of an Agilent 1200 Series HPLC system (Agilent technologies, Diegem, Belgium) equipped with an external Alltech 3300 ELSD detector (Grace, Deerfield, USA). For all HPLC analyses, the autosampler was cooled to 4 °C. Sugars were separated on a Prevail carbohydrate ES column (250×4.6 mm, 5 μm particle size, Alltech, Grace, Deerfield, USA), protected with a Prevail C_{18} guard cartridge (7.5×4.6, 5 μm particle size, Alltech, Grace, Deerfield, USA), by isocratic elution using 75% (v/v) acetonitrile/water at a flow rate of 1 ml/min and 30 °C. The drift tube temperature for ELSD was set at 38 °C and nitrogen was used as nebulizer gas at a flow rate of 1.5 ml/min. Identification and quantification of the sugars were performed respectively by comparison with retention times and by using calibration curves based on peak area. For this, standard solutions of

different sugars were prepared in milli-Q water. Sugar analyses were performed in triplicate. All solvents used for HPLC analyses were HPLC grade.

2.6. HPLC analysis of organic acids

For RP-HPLC analysis of organic acids, the samples were clarified, centrifuged and filtered analogous to the sugar analysis (2.5). 2 μl of the filtrate was injected in the HPLC system equipped with a Prevail Organic Acid column (250 × 4.6 mm, 5 μm particle size, Alltech, Grace, Deerfield, USA) and Prevail C_{18} guard cartridge. Separation occurred at 25 °C by isocratic elution with 25 mM potassium dihydrogen phosphate buffer pH 2.5 at a flow rate of 1 ml/min. Organic acids were detected at 210 nm using a UV-DAD detector (G1315B, Agilent technologies, Diegem, Belgium). Their identification was obtained by comparing the retention times and UV spectra with those of standard solutions of a wide range of organic acids in milli-Q water, while quantification was performed by external calibration based on peak area. All samples were analyzed in triplicate. Ascorbic acid was quantified by the HPLC method described below (cfr. 2.8).

2.7. HPLC analysis of bitter compounds

The presence of the bitter compounds naringin, neohesperidin and limonin was investigated by RP-HPLC analysis, analogous to the method of Ribeiro and Ribeiro (2008). After extraction of the components as described for sugar analysis, both a 2-fold and a 5-fold dilution in 0.2 M sodium acetate buffer pH 4.0 (20 μl) were injected in the HPLC system. Separations were performed on a Prevail C18 column (250 × 4.6 mm, 5 μm particle size, Alltech, Grace, Deerfield, USA), protected with Prevail C18 guard cartridge, by gradient elution of acetonitrile (A)/water (B) at 1 ml/min and 25 °C: 0–11 min 23% A, 11–21 min 23–65% A, 21–28 min 65–70% A, 28–29 min 70–23% A, 29–35 min 23% A. Limonin was detected at 210 nm, naringin and neohesperidin at 280 nm.

Detection limits (LOD) were determined as the lowest concentration of a standard solution that yields a signal-to-noise ratio (S/N) of 3, with noise level defined as the peak-to-peak noise of the baseline measured over a period of 5 min. Stock solutions of naringin, neohesperidin and limonin were prepared in a 1/1 (v/v) mixture of acetonitrile/sodium acetate buffer 0.02 M pH 4.0 and further diluted in sodium acetate buffer to appropriate concentrations.

The presence of the bitter compounds was verified in three extracts for each sample of orange juice.

2.8. HPLC analysis of vitamin C: ascorbic acid (AA) and dehydroascorbic acid (DHAA)

The analysis of the total vitamin C amount was based on the RP-HPLC method described by Kall and Andersen (1999). A dual detection system was used, after HPLC separation, by which AA was directly detected by UV and DHAA indirectly by fluorometric detection after a post-column on-line derivatization with o-phenylenediamine.

AA and DHAA were extracted from the juice by dilution of 2.5 ml juice in 1% (w/v) metaphosphoric acid with 0.5% (w/v) oxalic acid, adjusted to pH 2.0, to a volume of 25 ml. After homogenization, the samples were flushed with nitrogen and centrifuged for 15 min at 24,000 × *g* and 4 °C. The resulting supernatant was filtered through a 0.45 μm syringe filter and an aliquot (20 μl) was separated by RP-HPLC.

The analytical HPLC column used was a Prevail C18 (250 × 4.6 mm, 5 μm particle size) with Prevail C18 guard cartridge, equilibrated at 20 °C. The mobile phase consisted of 2.3 mM dodecyltrimethylammonium chloride and 2.5 mM sodium EDTA in a 66 mM phosphate–20 mM acetate buffer adjusted to pH 4.5 and was pumped through the system at a flow rate of 1 ml/min. AA was detected by means of a UV-DAD detector set at 247 nm. For derivatization of DHAA, a post-column reagent was prepared containing 28 mM o-phenylenediamine in a 12 mM trisodium citrate–55 mM sodium EDTA buffer, adjusted to pH 3.7. It was delivered by a Dionex AXP pump (Dionex, Sunnyvale, USA) at 0.3 ml/min and mixed with the HPLC mobile phase in a 750 μL knitted reaction coil (Dionex PN 42631), kept at 55 °C. DHAA was converted into the fluorophore 3(1,2-dihydroxy-ethyl)furo[3,4-b] quinooxaline-1-one and detected by a Shimadzu RF-10AXL fluorescence detector set at excitation and emission wavelength 350 and 430 nm respectively.

AA and DHAA were quantified using calibration curves based on peak area of external standards. Standard solutions were prepared in the extraction buffer used during the analysis. The DHAA standard solution was prepared from the AA solution by adding small amounts of an iodine solution (0.1 M) stepwise to 50 ml AA solution (1 mg/ml) until a constant light yellow color was exhibited. To reduce the surplus iodine, two crystals of sodium thiosulfate were added and the volume of the solution was adjusted to 100 ml with extraction buffer. The exact concentration of the standard solutions was determined spectrophotometrically, measuring the absorbance of the AA solution at 245 nm and applying Beer's law. AA and DHAA solutions were combined and diluted to the concentrations required.

The vitamin C content of each sample was analyzed in triplicate.

2.9. HPLC analysis of carotenoids

Analysis of the carotenoid profile was similar to the method described by Meléndez-Martínez, Vicario, and Heredia (2007), yet with some modifications. 15 ml orange juice and 30 ml extraction solvent (50/25/25 (v/v) hexane/ethanol/acetone, containing 0.1% (w/v) butylated hydroxytoluene (BHT)) were homogenized with an Ultra-Turrax mixer (IKA T25, Staufen, Germany) at 11,000 rpm, and the resulting mixture was centrifuged for 10 min at 6200 × *g* and 4 °C. 12 ml of the hexane top layer, containing the carotenoid pigments, was transferred to a separatory funnel and washed four times with 10 ml aqueous NaCl (10%) to remove any trace of acetone.

To obtain all xanthophylls in deesterified form, the extracts were saponified by adding 15 ml of 10% (w/v) ethanolic KOH (containing 0.1% (w/v) BHT) at room temperature. After 1 h, the alkali was removed by washing four times with 10 ml aqueous NaCl (10%). 6 ml of the hexane extract was concentrated to dryness in a rotary evaporator at 30 °C and the carotenoids were redissolved in 250 μl of a 2/1 (v/v) methanol–acetone mixture (containing 0.1% (w/v) BHT). The extract was filtered through a 0.20 μm syringe filter (Chromafil PET-20/25, Macherey-Nagel, Düren, Germany) and 5 μl of the filtrate was injected into the HPLC system.

The carotenoids were separated on a YMC C30 column (150 × 4.6 mm, 3 μm particle size, Alltech, GRACE, Deerfield, USA), coupled to a corresponding C30 guard cartridge and equilibrated at 25 °C. A gradient elution of methanol containing 0.1% (w/v) BHT (A), methyl-*t*-butyl ether containing 0.1% (w/v) BHT (B) and milli-Q water (C) was used at a flow rate of 1 ml/min: 0 to 5 min 95% A + 5% C, 24 min 95% A + 5% B, 30 min 90% A + 10% B, 40 min 70% A + 30% B, 45 min 50% A + 50% B, 45.01 to 50 min 95% A + 5% C. The effluent was monitored by DAD detection at 350, 430 and 486 nm.

The individual carotenoids were identified by comparison of their retention times and carefully studying UV-VIS spectra with those of standards that were commercially available and with literature data (Britton, Liaaen-Jensen, & Pfander, 2004; Lee, Castle, & Coates, 2001; Meléndez-Martínez, Britton, Vicario, & Heredia, 2005; Meléndez-Martínez, Britton, Vicario, & Heredia, 2008; Mouly, Gaydou, & Corsetti, 1999; Rouseff, Raley, & Hofsommer, 1996)). Antheraxanthin, α-carotene, β-carotene, ξ-carotene, α-cryptoxanthin, β-cryptoxanthin, lutein, mutatoxanthin, neoxanthin, violaxanthin and zeaxanthin were purchased from CaroteNature (Lupsingen, Switzerland), dissolved and further diluted in the 2/1 (v/v) methanol–acetone mixture

(containing 0.1% (w/v) BHT). Quantification was worked out from calibration curves of the available standard solutions. For unidentified peaks, and for peaks for which a standard solution was not available, the amount was determined from the calibration curve of the carotenoid whose spectrum corresponded the most to that of the concerning peak. The total carotenoid content was estimated as the sum of the quantities of the individual carotenoids.

The carotenoid analysis was performed under subdued light to avoid carotenoid degradation during analysis. All samples were analyzed in sextuple.

2.10. HPLC analysis of furfural and 5-hydroxymethylfurfural (HMF)

Extraction and RP-HPLC analysis of furfural and HMF was carried out as described by Lee, Rouseff, and Nagy (1986), with some modifications. First, 10 ml juice was clarified with 0.5 ml Carrez I and II. After 30 min, the mixture was centrifuged at 24,000×*g* and 4 °C for 15 min. The supernatant was filtered through a 0.45 μm syringe filter and 1 ml of the filtrate was applied on a C18 SPE pre-column (Sep-PAK Waters, Milford, USA), preconditioned with 2 ml methanol and 5 ml 0.5% acetic acid. After washing the SPE column with 2 ml of milli-Q water, furfural and HMF were selectively eluted with 4.5 mL of ethyl acetate, and dried with anhydrous sodium sulfate. The eluate was again filtered through a 0.45 pm syringe filter before injection (5 μl).

The chromatographic separation was performed using a Zorbax Eclipse XDB C18 column (150×4.6 mm, 5 μm particle size, Agilent technologies, Diegem, Belgium), coupled to a Prevail C18 guard cartridge, at 25 °C. A mixture of 15/85 (v/v) acetonitrile/water was used as mobile phase at a flow rate of 1 ml/min. The effluent was monitored at 280 nm.

Detection limits were determined as explained for the analysis of bitter compounds. For this, stock solutions of furfural and HMF were prepared and diluted to the appropriate concentrations in 10% methanol. Analyses of furfural and HMF were performed in triplicate.

2.11. Statistical data analysis

Given the magnitude of the overall experiment (Timmermans et al., 2011), it was impossible to perform the treatments in plural. To compare the impact of the treatments on the quality parameters, first changes in the property of interest during storage were modeled with the most straightforward model that provided a good fit. In most cases this was a linear model, since the limited number of data points and frequently the limited change in time often didn't allow a good parameter estimation for description with more complex models. A parameter was considered significant at $P<0.05$. Next, the obtained estimated parameters were compared with each other by means of a two-sided *t*-test, using the standard errors for the estimated parameters. Since multiple pairwise comparisons were conducted on the same dataset, a stricter criterion was applied for deciding whether or not two parameters were significantly different, namely a confidence interval of 99% ($\alpha=0.01$). Model discrimination and parameter estimation were carried out using the linear and nonlinear regression methods of the software package SAS (version 9.2, Cary, USA).

3. Results and discussion

3.1. Pectin methylesterase (PME) activity

Cloud stability has traditionally been considered as an important quality parameter for orange juice, influencing juice grading and market acceptability. It provides turbidity, flavor, aroma and the characteristic color of the juice. The loss of cloud is generally attributed to the action of the endogenous enzyme pectin methylesterase (PME), which demethoxylates soluble pectins, allowing calcium pectates to precipitate and clarifying the juice. Commercial heat pasteurization for the production of shelf-stable orange juice is designed to inactivate PME, which is more thermally resistant than vegetative micro-organisms. In this study, mild pasteurization conditions were chosen, in view of producing high-quality orange juice for (short-time) refrigerated storage (Timmermans et al., 2011). The effect of the three pasteurization processes and cooled storage on the PME activity is shown in Fig. 1. None of the treatments was able to inactivate PME completely. Nevertheless, heat and HP treatment resulted in a substantial activity decrease of respectively 85 and 92% at storage day 1. PEF treatment, on the other hand, was less effective and induced only a decrease of 34%. For heat and HP treatment, it is suggested that the small residual PME activity is due to the persistence of the heat-stable PME fraction. To inactivate this isoform, 15–60 s at 90–95 °C is required at atmospheric pressure (Baker & Cameron, 1999), while in the case of HP treatment, various studies have shown that HP, even when combined with moderate temperatures, is unable to inactivate this form (Basak & Ramaswamy, 1996; Goodner et al., 1998; Nienaber & Shellhammer, 2001b; Ogawa, Fukuhisa, Kubo, & Fukumoto, 1990; Polydera, Galanou, Stoforos, & Taoukis, 2004; Van den Broeck et al., 2000; Van den Broeck, Ludikhuyze, Van Loey, Weemaes, & Hendrickx, 1999). Nonetheless, these low residual activities could still preserve a cloud stability for a relatively long shelf life at refrigerated storage, as shown by Goodner et al. (1998) and Nienaber and Shellhammer (2001a), who proved a stability of more than 50 and 60 days, even though a residual activity of 18 and 4% respectively remained after treatment. As for HP treatment, complete inactivation of orange juice PME is not possible with PEF treatment, also when combined with increased temperatures (Elez-Martínez, Soliva-Fortuny, et al., 2006; Elez-Martínez, Suárez-Recio, & Martín-Belloso, 2007; Espachs-Barroso, Van Loey, Hendrickx, & Martin-Belloso, 2006; Rivas, Rodrigo, Martinez, Barbosa-Canovas, & Rodrigo, 2006; Yeom, Streaker, Zhang, & Min, 2000b; Yeom, Zhang, & Chism, 2002). Recent studies claim that enzyme inactivation by PEF is predominantly caused by thermal effects, rather than the high voltage pulses themselves (Jaeger, Meneses, Moritz, & Knorr, 2010; Van Loey et al., 2002). In the present study, the rather mild temperatures used to supplement PEF treatment resulted in a residual PME activity of still 66%, which inevitably results in cloud loss during storage. For a further discussion on the cloud stability results of the juices, the reader is referred to part I (Timmermans et al., 2011).

For impact comparison of the different treatments on PME activity, the data were best fitted by a linear model (Table 1). Estimated initial activities were significant, confirming the fact that none of the treatments could cause a complete inactivation. In these intercepts, significant differences were found between all processes, except

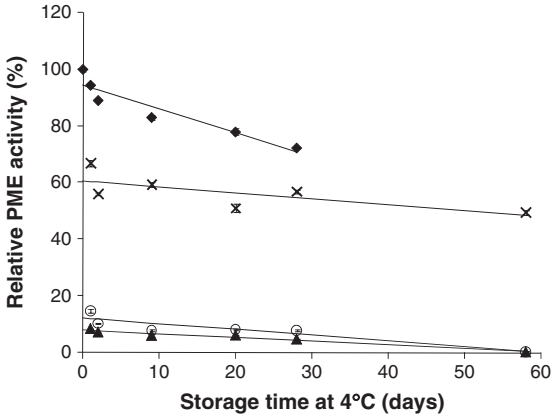

Fig. 1. Residual PME activity of untreated (♦), thermally (○), HP (▲) and PEF (×) pasteurized orange juice during storage at 4 °C. The full lines represent the linear inactivation model.

Table 1
Parameter estimates of the linear inactivation model describing the changes in residual PME activity of (pasteurized) orange juice during storage at 4 °C. *P* values larger than 0.05, denoting an insignificant parameter estimate, are marked in bold. For each parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Treatment	Intercept	*P*	Slope	*P*
Untreated	94.6 ± 2.2^{a}	<0.0001	-0.84 ± 0.15^{a}	0.005
Thermal pasteurization	12.2 ± 1.2^{b}	0.0006	-0.20 ± 0.04^{ab}	0.010
HP pasteurization	8.0 ± 0.4^{b}	<0.0001	-0.13 ± 0.01^{b}	0.001
PEF pasteurization	60.7 ± 2.8^{c}	<0.0001	-0.21 ± 0.10^{ab}	**0.109**

between the heat treated and HP treated juice. During storage at 4 °C, the activity decreased further, although this decrease was not significant for the PEF treated samples. Comparing the slopes revealed only a significant difference between the activity change of the untreated and the HP treated juice.

3.2. Peroxidase (POD) activity

Peroxidase (POD) is traditionally considered responsible for a wide range of oxidative quality and flavor alterations in fruits and vegetables (Vámos-Vigyázò, 1981). Nevertheless, its direct link with quality deterioration has never been proven. Although a negative correlation between POD activity and flavor appreciation has been found, this observation only demonstrates that juice extraction conditions leading to a higher POD activity (high-yield extraction) also lower flavor quality, by liberating flavor-degrading substances (Bruemmer, Roe, Bowen, & Buslig, 1976). In spite of this, its application as an indicator for heat treatment in food processing has been widely investigated, because it is generally considered as the most thermostable enzyme in plants (Clemente, 2002; Hirsch, Forch, Neidhart, Wolf, & Carle, 2008). Thus, a heat treatment sufficient to inactivate POD would also eliminate other undesirable enzymes.

The residual POD activity after orange juice processing and its further evolution during storage is illustrated in Fig. 2. The conditions applied for thermal pasteurization (20 s at 72 °C) caused a complete activity loss. This conflicts with the general conviction of POD having the highest heat stability considering plant enzymes. However, it is important to point out that inactivation studies demonstrating this thermostability are usually performed on crude and purified POD extracts at (nearly) neutral pH values. In the case of orange juice though, the acidic conditions lower the overall thermal stability of POD, as shown by Hirsch et al. (2008). Earlier reports on horseradish POD describing the high impact of the pH value on POD heat stability, confirm this hypothesis (Lu & Whitaker, 1974; Tamura & Morita, 1975).

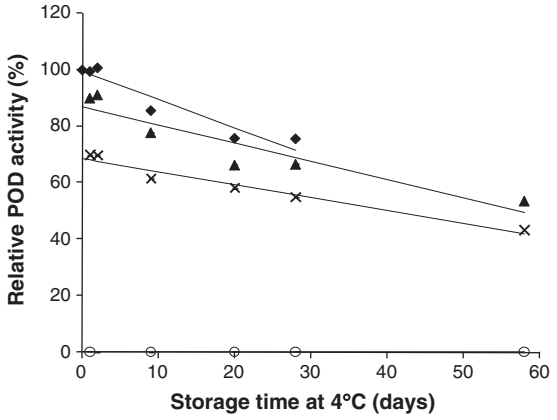

Fig. 2. Residual POD activity of untreated (♦), thermally (○), HP (▲) and PEF (×) pasteurized orange juice during storage at 4 °C. The full lines represent the linear inactivation model.

With an activity decrease of 30% at storage day 1, PEF treatment had a comparable impact on POD as on PME. These results are inconsistent with the high POD inactivations reported by Elez-Martínez, Aguiló-Aguayo, and Martín-Belloso (2006), Elez-Martínez, Soliva-Fortuny, et al. (2006). However, these authors made use of long treatment times and high frequencies, which inevitably entail high thermal loads in the treatment chamber, most likely contributing greatly to the inactivation (Jaeger et al., 2010; Van Loey et al., 2002). Moreover, sufficient evidence is at hand proving the fact that differences in treatment chamber design causes non-uniform electric field and temperature distributions (Barbosa-Cánovas & Altunakar, 2006; van den Bosch, 2007).

In contrast to PEF treatment, orange POD was much less susceptible to HP than PME. Where PME activity was reduced to 8% residual activity after HP treatment (measured at day 1), POD retained 90% of its initial activity. Although several studies have been reported on HP inactivation of POD in extracts of various fruit and vegetable sources, records on HP inactivation in orange juice are limited. Cano, Hernandez, and De Ancos (1997) reported a residual activity of 75% after treatment at 400 MPa and room temperature, although a rather long process time of 15 min was used.

The change in activity during storage at 4 °C, after the four processing conditions, was best fitted by a linear model (Table 2). For the heat treated samples, intercept and slope estimates were not significant, indicating a complete inactivation without regeneration. For the other three conditions, significant parameter estimates were found; in other words, a residual POD activity was retained which decreased further during storage. This activity decrease during refrigerated storage was also noticed by Elez-Martínez, Soliva-Fortuny, et al. (2006) and Hirsch et al. (2008), and may be ascribed to the acidic environment. Comparing the impact of these three treatment conditions, significant differences in initial activity (just after processing) were perceived, except between the untreated and HP treated samples. The negative slopes though were not significantly different.

3.3. Sugar profile

Sugars are among the major components of orange juice, representing about 80% of the total soluble solid content (Ting, 1980). They are inherently responsible for the sweetness of the juice, making its content an important quality attribute. In the present study, the initial total sugar concentration of the fresh orange juice was 86.9 g/L, which can be considered as an average value for orange juice (Lee & Coates, 2000). Sucrose, glucose and fructose were determined as the three sugar compounds of the orange juice, with a respective concentration of 45.7 g/L, 18.7 g/L and 22.5 g/L (Fig. 3), confirming the 2:1:1 ratio usually mentioned in literature (Lee & Coates, 2000; Ting, 1980).

Processing and storage had limited to no effect on sugar composition. Between the different processing conditions, no significant differences were found in initial concentrations, estimated by a

Table 2
Parameter estimates of the linear inactivation model describing the changes in residual POD activity of (pasteurized) orange juice during storage at 4 °C. *P* values larger than 0.05, denoting an insignificant parameter estimate, are marked in bold. For each parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Treatment	Intercept	*P*	Slope	*P*
Untreated	99.5 ± 2.3^{a}	<0.0001	-1.005 ± 0.158^{a}	0.0032
Thermal pasteurization	0.1 ± 0.1^{b}	**0.143**	0.002 ± 0.002^{b}	**0.2873**
HP pasteurization	86.9 ± 3.4^{a}	<0.0001	-0.645 ± 0.122^{a}	0.0061
PEF pasteurization	68.4 ± 1.3^{c}	<0.0001	-0.458 ± 0.046^{a}	0.0006

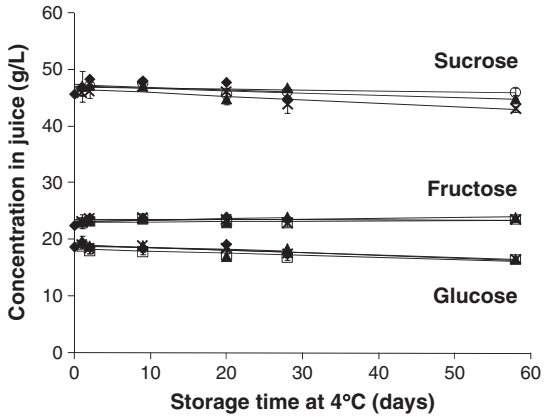

Fig. 3. Sugar concentrations of untreated (♦), thermally (○), HP (▲) and PEF (×) pasteurized orange juice during storage at 4 °C. The full lines represent the linear degradation model.

linear model (Table 3). During storage, some significant changes occurred (a decrease in sucrose concentration in the heat and PEF treated samples, and a decrease in glucose concentration in the heat, HP and PEF treated samples), although this did not result in any significant difference in slope between the four processing conditions. This equivalence between treatment conditions agrees with Brix results reported in part I (Timmermans et al., 2011). The effect of HP processing on the sugar profile of orange juice was also described by Donsi, Ferrari, and Di Matteo (1996) and Fernández García, Butz, Bognàr, and Tauscher (2001), who found no significant changes after HP treatment (respectively 1 min at pressures between 200 and 500 MPa and 5 min at pressures between 500 and 800 MPa) at room temperature. Moreover, according to the latter authors, subsequent storage of 21 days at 4 °C did not alter this picture either.

3.4. Organic acid profile

After sugars, the most predominant soluble constituents of orange juice are the organic acids. They represent about 10% of the total soluble solid content (Ting, 1980). Identification and quantification of the organic acid composition can be of considerable importance, since they mark the juice's taste characteristics and organoleptic quality, and provide useful information regarding its authenticity and possible microbiological alterations during storage. In the present juice, three organic acids were detected and identified as citric, malic and ascorbic acid. Because of its limited stability, ascorbic acid was quantified by the method described under 2.8 and is further discussed under 3.6. Citric and malic acid are generally known as the major organic acids found in orange juice and determine its acidity (Karadeníz, 2004; Sinclair, Bartholomew, & Ramsey, 1945). Their respective initial concentrations of 12.2 and 1.44 g/L fall within the range of average values for sweet oranges, reported in literature (Karadeníz, 2004; Saavedra, Garcia, & Barbas, 2000; Sinclair et al., 1945).

The changes in citric and malic acid concentration during storage were best described by a linear and quadratic model respectively, which is depicted in Fig. 4. Tables 4 and 5 contain the corresponding parameter estimates. For citric acid, the increase in the HP treated samples was the only change that could be considered significant. For malic acid, the estimated parameters describing the quadratic course were significant for all processing conditions. Considering this peculiar trend, no records on similar changes could be found in literature and an explanation remains forthcoming. Furthermore, no formation of additional acids was detected during storage. Between the four different treatment conditions, no significant differences in initial citric and malic acid concentration nor in evolution during storage were detected. Analogous conclusions were drawn in part I on pH values (Timmermans et al., 2011).

Table 3
Parameter estimates of the linear model describing the changes in sugar concentration of (pasteurized) orange juice during storage at 4 °C. *P* values larger than 0.05, denoting an insignificant parameter estimate, are marked in bold. For each parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Sugar	Treatment	Intercept	*P*	Slope	*P*
Sucrose	Untreated	47.4 ± 0.8^a	<0.0001	-0.043 ± 0.055^a	**0.478**
	Thermal pasteurization	46.9 ± 0.4^a	<0.0001	-0.017 ± 0.016^a	**0.359**
	HP pasteurization	47.1 ± 0.5^a	<0.0001	-0.040 ± 0.018^a	**0.093**
	PEF pasteurization	46.6 ± 0.5^a	<0.0001	-0.061 ± 0.019^a	0.030
Glucose	Untreated	19.0 ± 0.4^a	<0.0001	-0.037 ± 0.024^a	**0.202**
	Thermal pasteurization	18.3 ± 0.2^a	<0.0001	-0.035 ± 0.009^a	0.017
	HP pasteurization	19.2 ± 0.4^a	<0.0001	-0.047 ± 0.015^a	0.037
	PEF pasteurization	19.1 ± 0.2^a	<0.0001	-0.043 ± 0.008^a	0.005
Fructose	Untreated	23.2 ± 0.3^a	<0.0001	0.025 ± 0.021^a	**0.317**
	Thermal pasteurization	23.2 ± 0.2^a	<0.0001	0.005 ± 0.006^a	**0.468**
	HP pasteurization	23.3 ± 0.2^a	<0.0001	0.012 ± 0.008^a	**0.220**
	PEF pasteurization	23.6 ± 0.2^a	<0.0001	-0.002 ± 0.008^a	**0.791**

3.5. Bitter compounds

Orange juice can be characterized by a certain degree of bitterness, that can be detrimental to the taste if it is too pronounced. This bitterness can be attributed to the components of two chemical classes: the flavonoids and the limonoids. In the class of flavonoids, naringin and neohesperidin represent the two predominant components responsible for a bitter taste in orange juice. They are primarily found in the membranes and albedo of the fruit and are extracted into the juice, giving it an 'immediate' bitterness. Limonin, on the other hand, is the principal limonoid accounting for a bitter taste, but is not present itself in the intact orange fruit. However, its non-bitter precursor, limonoate-A-ring-lactone, occurs endogenously in the segment membranes, and is transformed into limonin after juice extraction, by the acidic pH attained upon physical disrupture of the juice sacs. In this way, limonin is said to be responsible for a 'delayed' bitterness (Puri, Marwaha, Kothari, & Kennedy, 1996; Sandhu & Minhas, 2006).

The HPLC method applied allowed a simultaneous detection of these three bitter compounds. Detection limits of 400, 50 and 500 ppb were established, respectively for naringin, neohesperidin and limonin, which are far below human detection limits (Kelly, Jewell, & O'Brien, 2003; Sandhu & Minhas, 2006; Soares & Hotchkiss, 1998). Nevertheless, none of these three compounds was detected in fresh samples, nor were they formed during processing or storage.

3.6. Vitamin C

The most important contribution of orange juice to human nutrition is perhaps attributed to its high vitamin C content. Although orange juice is not the only fruit product containing large quantities of vitamin C, it is definitely an important source, because of its relatively high consumption by humans. In addition to its vitamin action, vitamin C is valuable for its antioxidant effect, stimulation of the immune system and other health-related benefits. An important issue associated with orange juice quality is vitamin C loss during processing and/or storage. Because of its heat-labile properties and instability during storage, ascorbic acid is often used as an indicator for the overall quality of fruits and vegetables, providing information on the loss of other vitamins as well as organoleptic and/or nutritional components (Cortés et al., 2008b; Lee, Downing, Iredale, & Chapman,

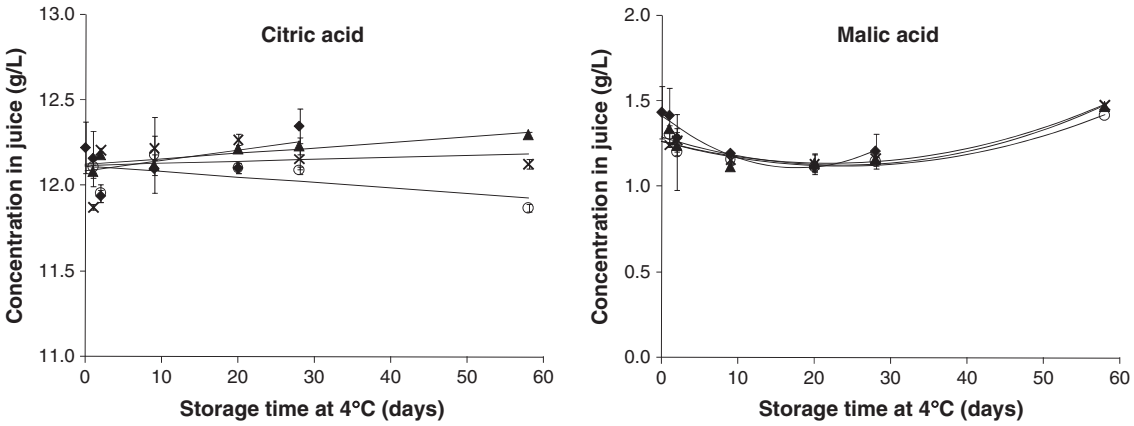

Fig. 4. Organic acid concentrations of untreated (♦), thermally (○), HP (▲) and PEF (×) pasteurized orange juice during storage at 4 °C. The full lines represent the linear and quadratic degradation model respectively.

1976; Polydera, Stoforos, & Taoukis, 2003; Zulueta, Esteve, & Frigola, 2010).

Fig. 5 illustrates the changes in ascorbic acid (AA) and dehydroascorbic acid (DHAA) concentration after processing and during storage. In the untreated orange juice, respective initial concentrations of 529 and 7.01 mg/L were found. According to various references, this orange juice can be considered as a rather rich source of vitamin C (Bull et al., 2004; Klimczak, Malecka, Szlachta, & Gliszczynska-Swiglo, 2007; Meléndez-Martínez et al., 2007a; Ting, 1980; Topuz, Topakci, Canakci, Akinci, & Ozdemir, 2005). The decrease in AA and increase in DHAA concentration during storage was best fitted by a linear and logarithmic model respectively (Tables 6 and 7). The difference in model type and in extent of increase/decrease is not unusual since the reversible oxidation of AA into DHAA is not the only reaction that can occur; probably DHAA is further degraded through the aerobic pathway (Bull et al., 2004; Kennedy, Rivera, Lloyd, Warner, & Jumel, 1992; Nienaber & Shellhammer, 2001a; Polydera et al., 2003; Yeom et al., 2000a). Pairwise comparison of the impact of the four processing conditions yielded only a significant difference between the initial concentration estimates of DHAA in the untreated and thermally pasteurized samples, although not between the three pasteurization processes themselves. The stability of AA during pasteurization (by heat, HP or PEF) was also perceived by Sadler, Parish, and Wicker (1992), Donsi et al. (1996), Yeom et al. (2000a), Fernández García et al. (2001), Min et al. (2003), Bull et al. (2004), Cserhalmi, Sass-Kiss, Toth-Markus, and Lechner (2006) and Cortés et al. (2008b). On the other hand, Sánchez-Moreno, Plaza, De Ancos, and Cano (2003); Sánchez-Moreno et al. (2005), Plaza et al. (2006), Elez-Martínez, Soliva-Fortuny, et al. (2006) and Elez-Martínez and Martín-Belloso (2007) described a significant degradation of AA by some pasteurization processes. These contradictory findings can be explained through the temperatures reached throughout processing. AA is a well-known heat-sensitive compound and therefore treatment temperatures can greatly affect the rate of its degradation (Elez-Martínez, Soliva-Fortuny, et al., 2006; Nagy & Smoot, 1977; Saguy et al., 1978). Higher temperatures are frequently chosen to establish a more excessive PME inactivation, but inevitably entail AA breakdown. Furthermore, through process and equipment design for HP or PEF treatment, often more heat is generated than researchers are aware of (Barbosa-Cánovas & Altunakar, 2006; Otero, Molina-García & Sanz, 2000; van den Bosch, 2007).

In spite of the refrigerated and dark conditions during storage, decrease in AA and increase in DHAA were significant, confirming numerous studies (Bull et al., 2004; Cortés et al., 2008b; Elez-Martínez & Martín-Belloso, 2007; Elez-Martínez, Soliva-Fortuny, et al., 2006; Fernández García et al., 2001; Min et al., 2003; Plaza et al., 2006; Sadler et al., 1992; Yeom et al., 2000a). Nevertheless, between all four processing conditions, no significant changes were detected in the parameters describing these courses. Several authors have compared the impact of the novel technologies HP and PEF with the impact of thermal processing on vitamin C stability in orange juice. Hereby, thermal pasteurization is often found to be more detrimental to vitamin C than HP or PEF pasteurization. However, in all these studies, the remark must be made that a comparison is made with thermal process conditions designed for production of shelf-stable juices, and thus for PME inactivation. This cannot be considered a fair comparison since, unless it is combined with elevated temperatures or a high thermal load is created, PEF treatment alone cannot sufficiently inactivate PME to result in shelf-stable juice. In this way, Yeom et al. (2000a), Min et al. (2003), Elez-Martínez, Soliva-Fortuny, et al. (2006), Elez-Martínez and Martín-Belloso (2007) and Cortés et al. (2008b) concluded that PEF treatment resulted in a better AA retention during processing and/or refrigerated storage, compared to thermal pasteurization, while thermal treatment conditions of 20 to 90 s at 90 to 95 °C were used. Further, Polydera et al. (2003, 2005) found a somewhat slower AA degradation during storage at 5 °C after HP treatment than after thermal treatment of 30 and 60 s at 80 °C. Only Bull et al. (2004) and Plaza et al. (2006) observed a better or similar AA retention during thermal pasteurization, in comparison with HP and/or PEF pasteurization, considering thermal treatment conditions of 60 s at 65 °C, 30 s at 70 °C and 25 s at 85 °C.

Table 4
Parameter estimates of the linear model describing the changes in citric acid concentration of (pasteurized) orange juice during storage at 4 °C. *P* values larger than 0.05, denoting an insignificant parameter estimate, are marked in bold. For each parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Treatment	Intercept	*P*	Slope	*P*
Untreated	12.09 ± 0.07^a	<0.0001	0.0060 ± 0.0051^a	**0.303**
Thermal pasteurization	12.12 ± 0.06^a	<0.0001	-0.0032 ± 0.0022^a	**0.211**
HP pasteurization	12.13 ± 0.02^a	<0.0001	0.0032 ± 0.0008^a	0.020
PEF pasteurization	12.12 ± 0.09^a	<0.0001	0.0011 ± 0.0032^a	**0.744**

3.7. Carotenoid profile

Next to vitamin C, also carotenoids are important quality indicators for orange juice. Apart from being responsible for the color of the juice, a number of them have provitamin A activity (e.g. α-carotene, β-carotene and β-cryptoxanthin) and some are known for their antioxidant capacity (e.g. β-carotene and β-cryptoxanthin, zeaxanthin and lutein) (Rao & Rao, 2007). Oranges are a very complex source of carotenoids, containing the largest number of them among all fruit species (Meléndez-Martínez et al., 2007b, 2008). In Fig. 6, an

Table 5
Parameter estimates of the quadratic model describing the changes in malic acid concentration of (pasteurized) orange juice during storage at 4 °C. *P* values larger than 0.05, denoting an insignificant parameter estimate, are marked in bold. For each parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Treatment	Intercept	*P*	Linear coefficient	*P*	Quadratic coefficient	*P*
Untreated	1.42 ± 0.03^a	<0.0001	-0.035 ± 0.007^a	0.017	0.00098 ± 0.00026^a	0.033
Thermal pasteurization	1.27 ± 0.03^a	<0.0001	-0.012 ± 0.003^a	0.027	0.00025 ± 0.00005^a	0.014
HP pasteurization	1.29 ± 0.04^a	<0.0001	-0.014 ± 0.004^a	0.050	0.00030 ± 0.00007^a	0.026
PEF pasteurization	1.27 ± 0.02^a	<0.0001	-0.012 ± 0.002^a	0.008	0.00026 ± 0.00003^a	0.003

example of a typical chromatogram for the untreated orange juice is given, illustrating the complex carotenoid profile. Of the 20 peaks detected, 16 were successfully identified (Table 8). The total carotenoid content of the untreated orange juice, expressed as the sum of the individual concentrations, was 4.40 mg/L at the start of the experiment, which is comparable to values found by De Ancos, Sgroppo, Plaza, and Cano (2002) and Lee and Coates (2003), although higher contents are frequently reported as well (Dhuique-Mayer et al., 2007; Esteve, Barba, Palop, & Frígola, 2009; Gama & de Sylos, 2007; Gama & Sylos, 2005; Sánchez-Moreno et al., 2005). The major carotenoid detected was β-cryptoxanthin; it accounted for about 22.4% of the total carotenoid content. It is said to be the main contributor to the orange color of the juice since it absorbs light at higher wavelengths, and the main provitamin A carotenoid found in oranges (Dias, Camoes, & Oliveira, 2009; Gama & Sylos, 2005; Mouly et al., 1999). In addition, the other major carotenoids found were β-carotene, lutein and zeaxanthin, with a share of 12.6, 11.4 and 11.3% respectively.

To compare the impact of the different treatments on the carotenoid profile, a selection was made of the quantitatively most important carotenoids together with the total content, of which the changes during storage were all best fitted by a linear model (Table 9). Because of their highly saturated conformation, carotenoids are prone to oxidation and isomerization during processing and storage (Dutta, Chaudhuri, & Chakraborty, 2005; Meléndez-Martínez et al., 2007b; Rodriguez-Amaya, 2001). Nevertheless, processing conditions selected for this study had no significant effect on the carotenoid profile. Likewise, no significant changes were detected after thermal treatment by Sánchez-Moreno et al. (2005), after HP treatment by Donsi et al. (1996), Fernández García et al. (2001) and Esteve et al. (2009), and after PEF treatment by Sánchez-Moreno et al. (2005), Cortés, Torregrosa, et al. (2006) and Esteve et al. (2009). On the other hand, some authors did notice a significant processing impact: Lee and Coates (2003), Cortés, Esteve, Rodrigo, Torregrosa and Frígola (2006), Cortés, Torregrosa, et al. (2006) and Esteve et al. (2009) found changes in various carotenoids due to thermal treatment; De Ancos et al. (2002) and Sánchez-Moreno et al. (2003, 2005) reported a better extractability after HP treatment; and Cortés, Esteve, et al. (2006) observed both increases and decreases in certain carotenoids after PEF treatment. As stated before, these contradictory observations can be attributed to differences in processing conditions and equipment design.

During storage at 4 °C, only four carotenoids exhibited significant changes: cis-violaxanthin, antheraxanthin and 9Z-antheraxanthin decreased in concentration, while mutatoxanthin concentration increased. A significant decrease in antheraxanthin, accompanied by a significant increase in mutatoxanthin, during storage was also reported by Cortés, Torregrosa, et al. (2006), Cortes, Esteve & Frigola, 2009), who ascribed this to a conversion of the 5,6-epoxide antheraxanthin to the 5,8-epoxide mutatoxanthin, due to the acidic conditions of the juice. The total carotenoid content slightly decreased, although this decrease was only significant for the thermally treated and PEF treated samples. Nevertheless, no significant differences between the four treatment conditions were found. Some other comparative studies on the impact of heat, HP and PEF treatment on orange juice carotenoid profiles, followed by refrigerated storage, have been carried out by Cortés, Esteve, et al. (2006), Cortés, Torregrosa, et al. (2006), Cortes, Esteve and Frigola (2009) and Esteve et al. (2009), all indicating larger changes after heat treatment. However, in these reports mild HP and/or PEF conditions are compared with a thermal treatment of 20 s at 90 °C, thus the same remark as mentioned above, concerning the fairness of this comparison (cfr. 3.6), can be made.

3.8. Furfural and 5-hydroxymethylfurfural (HMF)

Non-enzymatic browning, accompanied by undesirable off-taste and off-flavor, is considered as one of the major causes of quality loss during processing and storage of orange juice. It is accelerated by temperature and time of processing and storage (Dinsmore & Nagy, 1972; Lee & Nagy, 1988a; Lee & Nagy, 1988b; Nagy & Randall, 1973; Roig, Bello, Rivera, & Kennedy, 1999; Shinoda, Murata, Homma, &

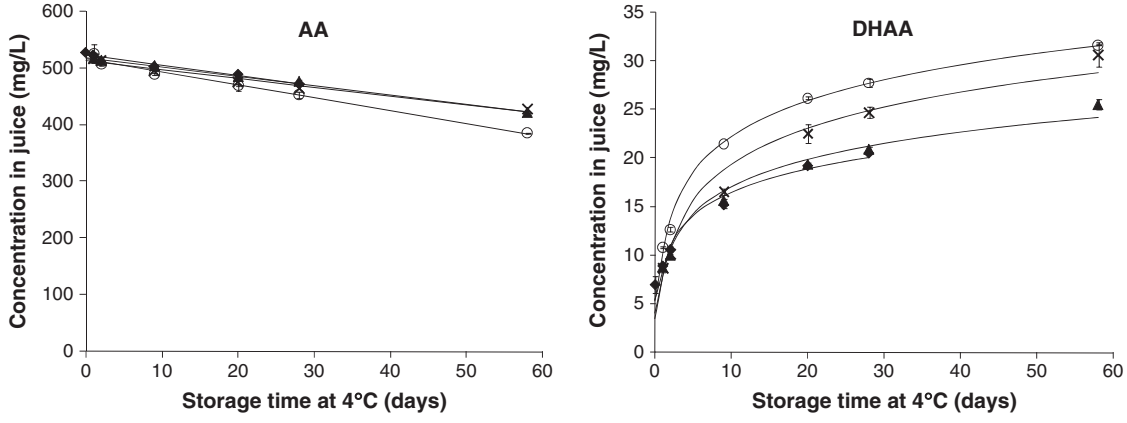

Fig. 5. AA and DHAA concentrations of untreated (♦), thermally (○), HP (▲) and PEF (×) pasteurized orange juice during storage at 4 °C. The full lines represent the linear and logarithmic degradation model respectively.

Table 6
Parameter estimates of the linear degradation model describing the changes in AA concentration of (pasteurized) orange juice during storage at 4 °C. *P* values larger than 0.05, denoting an insignificant parameter estimate, are marked in bold. For each parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Treatment	Intercept	*P*	Slope	*P*
Untreated	523.3 ± 2.7^{a}	<0.0001	-1.80 ± 0.19^{a}	0.0007
Thermal pasteurization	516.7 ± 4.1^{a}	<0.0001	-2.28 ± 0.15^{a}	0.0001
HP pasteurization	518.7 ± 1.3^{a}	<0.0001	-1.64 ± 0.05^{a}	<0.0001
PEF pasteurization	513.8 ± 3.5^{a}	<0.0001	-1.56 ± 0.13^{a}	0.0002

Komura, 2004; Shinoda, Komura, Homma, & Murata, 2005). Furfural and 5-hydroxymethylfurfural (HMF) are indicated as the principal degradation products from ascorbic acid and sugar breakdown, the main sources of this browning. Because of their correlation with browning reactions, furfural and HMF are recognized as useful indicators for temperature abuse during processing and storage, and for quality deterioration in general (Dinsmore & Nagy, 1972; Lee & Nagy, 1988b; Nagy & Randall, 1973; Shinoda et al., 2004, 2005). Furthermore, some concern has been expressed about possible cytotoxic, genotoxic and mutagenic risks of these compounds (Glatt & Sommer, 2006).

The method of analysis enabled detection of both components above a detection limit of 50 ppb. Nevertheless, no measurable quantities of furfural nor HMF were formed during any of the three processing conditions applied, neither were they generated during storage at 4 °C. Likewise, Cserhalmi et al. (2006) and Cortés et al. (2008a) found no increase in HMF levels after heat and/or PEF treatment, although surprisingly, these authors reported small initial quantities of HMF in the untreated product. As remarked by Marcotte, Stewart, & Fustier (1998), furfural and HMF generation can only be detected after more severe treatments than what is usually applied for industrial pasteurization. A similar comment can be made about the storage conditions: within the time frame of the experiment, a storage temperature of 4 °C is not sufficient to significantly increase furfural or HMF concentrations (Lee & Nagy, 1988a,b; Nagy & Randall, 1973; Solomon, Svanberg, & Sahlstrom, 1995). Summarizing, these results suggest that furfural and HMF can not serve as good indicators for quality deterioration of mildly pasteurized orange juice, subsequently stored at refrigerated conditions.

4. Conclusion

A comparison of the impact of thermal, HP and PEF processing for mild pasteurization of orange juice, starting from processing conditions with equivalent microbial inactivation, revealed only significant differences in residual enzyme activities. For PME inactivation, none of the treatments was able to cause a complete inactivation, although heat and HP pasteurization were the most effective in limiting the residual activity. Between these two treatments, no significant differences were found in residual activities at the start of the shelf life, nor in further inactivation during storage. On the other hand, PME inactivation by PEF was limited. POD was completely inactivated by heat pasteurization and was much less susceptible to HP and PEF.

Table 7
Parameter estimates of the logarithmic model describing the changes in DHAA concentration of (pasteurized) orange juice during storage at 4 °C. For each parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Treatment	Intercept	Rate constant
Untreated	3.50 ± 0.24^{a}	8.45 ± 0.53^{a}
Thermal pasteurization	5.33 ± 0.19^{b}	10.02 ± 0.51^{a}
HP pasteurization	4.07 ± 0.30^{ab}	7.71 ± 0.79^{a}
PEF pasteurization	5.38 ± 0.49^{ab}	6.98 ± 1.30^{a}

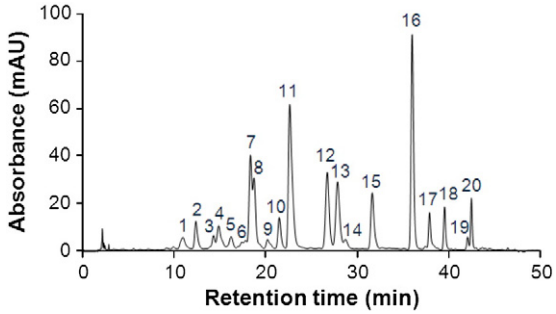

Fig. 6. Example of a carotenoid profile for untreated orange juice at 430 nm. The identification of the different peaks is described in Table 8.

Residual activities after HP and PEF and the following decreases during storage were not significantly different. All other quality parameters investigated (sugars, organic acids, bitter compounds, vitamin C, carotenoids, furfural and HMF) experienced no significantly different impact from the three pasteurization techniques.

The results of this study can be evaluated within the particular context of the European "Novel Food Legislation" (EC-258/97). This regulation imposes the appraisal of substantial equivalence of novel foods to existing foods before introducing them to the European market. Herein, conventional products are used as a reference, since they are considered sufficiently safe because of their safe use for many years. The decree states that when adequate evidence can be given that proves the substantial equivalence of the novel foods to conventional products (regarding their composition, metabolism, nutritional value, and undesired substances), it is sufficient to inform the European Commission of their introduction on the market. The current study provides evidence that HP and PEF pasteurization do not cause any significant differences in the major components regarding public health that were investigated, in comparison to thermal pasteurization, and therefore no changes in the human metabolism after consumption are to be expected. This investigation can be regarded as an important first part of a substantial equivalence study in the Novel Food Legislation framework.

Table 8
Chromatographic and spectroscopic features of the orange juice carotenoids detected.

Peak	Retention time (min)	Absorption maxima (nm)[a]	% III/II[b]	Identification
1	11.0	407, 430, 457	56.2	Unidentified
2	12.4	416, 439, 469	91.6	Violaxanthin
3	14.3	411^{sh}, 433, 460	39.6	Unidentified
4	14.9	399, 421, 448	93.4	Luteoxanthin
5	16.2	381, 402, 426, 461	133.6	Auroxanthin A + unidentified
6	17.8	380, 402, 425, 461	142.3	Auroxanthin B + unidentified
7	18.3	411, 435, 464	89.5	cis-violaxanthin
8	18.7	423^{sh}, 445, 473	60.3	Antheraxanthin
9	20.2	395, 417, 443	92.8	Z-isomer luteoxanthin + auroxanthin C
10	21.5	405^{sh}, 427, 452	61.7	Mutatoxanthin
11	22.6	422^{sh}, 444, 472	62.5	Lutein
12	26.7	429^{sh}, 450, 476	30.7	Zeaxanthin
13	27.9	418^{sh}, 440, 467	59.9	9Z-antheraxanthin
14	28.7	423^{sh}, 443, 469	11.4	13Z-β-cryptoxanthin
15	31.6	423^{sh}, 445, 473	62.0	α-cryptoxanthin
16	36.0	430^{sh}, 451, 477	27.0	β-cryptoxanthin
17	37.9	378, 399, 423	70.7	Z-isomer ξ-carotene
18	39.5	424^{sh}, 445, 473	58.8	α-carotene
19	42.1	379, 400, 425	90.8	Z-isomer ξ-carotene
20	42.5	451, 477	22.6	β-carotene

[a] sh indicates a shoulder.
[b] Ratio of the peak height of the longest wavelength absorption band (band III) to that of the middle absorption band (band II).

Table 9
Parameter estimates of the linear model describing the changes in carotenoid concentration of (pasteurized) orange juice during storage at 4 °C. *P* values larger than 0.05, denoting an insignificant parameter estimate, are marked in bold. For each carotenoid and parameter type, estimates which are not significantly different between treatments are indicated with the same letter in superscript.

Carotenoid	Treatment	Intercept	*P*	Slope	*P*
Cis-violaxanthin	Untreated	0.279 ± 0.018^{a}	<0.0001	-0.0045 ± 0.0012^{a}	0.021
	Thermal pasteurization	0.219 ± 0.009^{a}	<0.0001	-0.0029 ± 0.0003^{a}	0.0009
	HP pasteurization	0.268 ± 0.023^{a}	0.0003	-0.0036 ± 0.0008^{a}	0.013
	PEF pasteurization	0.233 ± 0.009^{a}	<0.0001	-0.0029 ± 0.0003^{a}	0.0008
Antheraxanthin	Untreated	0.203 ± 0.014^{a}	0.0001	-0.0019 ± 0.0010^{a}	0.012
	Thermal pasteurization	0.176 ± 0.006^{a}	<0.0001	-0.0014 ± 0.0002^{a}	0.002
	HP pasteurization	0.195 ± 0.015^{a}	0.0002	-0.0016 ± 0.0005^{a}	0.038
	PEF pasteurization	0.186 ± 0.010^{a}	<0.0001	-0.0015 ± 0.0004^{a}	0.018
Mutatoxanthin	Untreated	0.105 ± 0.003^{a}	<0.0001	0.0017 ± 0.0002^{a}	0.002
	Thermal pasteurization	0.115 ± 0.005^{a}	<0.0001	0.0011 ± 0.0002^{a}	0.004
	HP pasteurization	0.113 ± 0.010^{a}	0.0003	0.0011 ± 0.0003^{a}	0.035
	PEF pasteurization	0.114 ± 0.009^{a}	0.0003	0.0011 ± 0.0003^{a}	0.033
Lutein	Untreated	0.459 ± 0.023^{a}	<0.0001	-0.0007 ± 0.0016^{a}	**0.692**
	Thermal pasteurization	0.443 ± 0.011^{a}	<0.0001	-0.0006 ± 0.0004^{a}	**0.199**
	HP pasteurization	0.460 ± 0.027^{a}	<0.0001	-0.0006 ± 0.0010^{a}	**0.568**
	PEF pasteurization	0.450 ± 0.034^{a}	0.0002	-0.0014 ± 0.0012^{a}	**0.325**
Zeaxanthin	Untreated	0.468 ± 0.018^{a}	<0.0001	-0.0010 ± 0.0012^{a}	**0.463**
	Thermal pasteurization	0.451 ± 0.007^{a}	<0.0001	-0.0006 ± 0.0003^{a}	**0.089**
	HP pasteurization	0.462 ± 0.022^{a}	<0.0001	-0.0005 ± 0.0008^{a}	**0.597**
	PEF pasteurization	0.455 ± 0.026^{a}	<0.0001	-0.0014 ± 0.0009^{a}	**0.208**
9Z-antheraxanthin	Untreated	0.176 ± 0.008^{a}	<0.0001	-0.0018 ± 0.0005^{a}	0.026
	Thermal pasteurization	0.155 ± 0.003^{a}	<0.0001	-0.0010 ± 0.0001^{a}	0.0006
	HP pasteurization	0.161 ± 0.010^{a}	<0.0001	-0.0010 ± 0.0003^{a}	0.039
	PEF pasteurization	0.152 ± 0.006^{a}	<0.0001	-0.0011 ± 0.0002^{a}	0.007
α-cryptoxanthin	Untreated	0.240 ± 0.007^{a}	<0.0001	-0.0008 ± 0.0005^{a}	**0.179**
	Thermal pasteurization	0.233 ± 0.004^{a}	<0.0001	-0.0002 ± 0.0001^{a}	**0.259**
	HP pasteurization	0.247 ± 0.007^{a}	<0.0001	-0.0003 ± 0.0002^{a}	**0.243**
	PEF pasteurization	0.246 ± 0.003^{a}	<0.0001	-0.0001 ± 0.0001^{a}	**0.238**
β-cryptoxanthin	Untreated	0.960 ± 0.030^{a}	<0.0001	-0.0040 ± 0.0021^{a}	**0.129**
	Thermal pasteurization	0.927 ± 0.018^{a}	<0.0001	-0.0006 ± 0.0007^{a}	**0.383**
	HP pasteurization	0.963 ± 0.032^{a}	<0.0001	-0.0011 ± 0.0012^{a}	**0.394**
	PEF pasteurization	0.921 ± 0.016^{a}	<0.0001	-0.0004 ± 0.0006^{a}	**0.522**
α-carotene	Untreated	0.271 ± 0.011^{a}	<0.0001	-0.0011 ± 0.0008^{a}	**0.211**
	Thermal pasteurization	0.264 ± 0.006^{a}	<0.0001	-0.0003 ± 0.0002^{a}	**0.279**
	HP pasteurization	0.292 ± 0.010^{a}	<0.0001	-0.0006 ± 0.0004^{a}	**0.206**
	PEF pasteurization	0.278 ± 0.009^{a}	<0.0001	-0.0004 ± 0.0003^{a}	**0.317**
β-carotene	Untreated	0.546 ± 0.014^{a}	<0.0001	-0.0016 ± 0.0009^{a}	**0.163**
	Thermal pasteurization	0.532 ± 0.010^{a}	<0.0001	-0.0004 ± 0.0004^{a}	**0.290**
	HP pasteurization	0.586 ± 0.017^{a}	<0.0001	-0.0007 ± 0.0006^{a}	**0.300**
	PEF pasteurization	0.564 ± 0.017^{a}	<0.0001	-0.0005 ± 0.0006^{a}	**0.426**
Total content	Untreated	4.15 ± 0.15^{a}	<0.0001	-0.017 ± 0.010^{a}	**0.174**
	Thermal pasteurization	3.92 ± 0.06^{a}	<0.0001	-0.008 ± 0.002^{a}	0.016
	HP pasteurization	4.18 ± 0.16^{a}	<0.0001	-0.010 ± 0.006^{a}	**0.168**
	PEF pasteurization	1.03 ± 0.09^{a}	<0.0001	-0.010 ± 0.003^{a}	0.039

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