High pressure and pulsed electric field pasteurisation of orange juice: evaluation of the substantial equivalence to conventional heat pasteurisation

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ABSTRACT

Nowadays, a new generation of preservation processes, like high pressure (HP) and pulsed electric field (PEF) processing, is presented, which are claimed to result in better quality retention and/or longer shelf life, compared to traditional heat processing. However, a real breakthrough of these novel technologies in the food industry still remains forthcoming. Products treated with HP or PEF are governed by the European “Novel Food legislation” (EC-258/97). This decree states that when adequate evidence can be given that proofs the substantial equivalence of the novel foods to conventional products (regarding their composition, nutritional value, and undesired substances), it is sufficient to inform the European commission about the introduction of these products on the market. The lack of this evidence is currently still a major obstacle for the further implementation of HP and PEF treatment in the food industry.

The objective of the present work was to compare the impact of thermal, HP and PEF processing for mild pasteurisation of orange juice on a fair basis, using processing conditions leading to an equivalent degree of microbial inactivation. The juices were evaluated and compared for significant differences in specific known nutrients (vitamin C, carotenoids, sugars, organic acids,…), undesired substances (products of Maillard reactions and oxidation reactions) and other quality-related aspects of orange juice (pectin methylesterase activity, peroxidase activity, colour,…) directly after treatment and during a storage at 4°C.

Only significant differences in residual enzyme activities were found, all other investigated parameters experienced no significantly different impact from the three pasteurisation techniques. With this research it was proven that there were no significant differences in the analysed components regarding food safety, and therefore no changes in human metabolism after consumption are expected.

Keywords: high pressure processing; pulsed electric field processing; novel food; substantial equivalence; orange juice

INTRODUCTION

Because of its unique combination of sensory attributes, such as colour, aroma and flavour, and its nutritional value, orange juice is world’s most popular fruit juice. Despite its low pH, fresh orange juice stability is rather limited, due to microbial growth and enzyme activities. To prolong this shelf life, thermal pasteurisation is the most widely applied technique, successfully inactivating vegetative microorganisms and enzymes. For orange juice shelf-stable at room temperature, conditions of 10-30 s at 95-98°C are usually applied [2]. 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 [3]. Although the intense pasteurisation 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. 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 pasteurisation, distributed refrigerated and with a limited shelf life. Therefore, manufactures seek for alternatives to traditional thermal pasteurisation, namely novel technologies like high pressure (HP) and pulsed electric field (PEF) processing, which are claimed to result in better quality retention and longer shelf life [4,5,6]. However, a real breakthrough of these new technologies in the food industry still remains forthcoming.

1 A detailed description of the results of this study can be found in Vervoort et al., 2011 [1].
Since HP and PEF processing may induce changes in the composition and/or structure of foods or food ingredients, affecting their nutritional value, metabolism or level of undesirable substances, and because these technologies have not been used in the EU before 15 May 1997, products processed with these technologies are governed by 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 proofs the substantial equivalence of the novel foods to conventional products (regarding their composition, nutritional value, and undesired substances), it is sufficient to inform the European commission of the introduction of these products on the market. The lack of this evidence is currently still a major obstacle for the further implementation of HP and PEF treatment in the food industry.

The objective of the present work was to compare the impact of thermal, HP and PEF processing for mild pasteurisation of orange juice on a fair basis, using processing conditions leading to an equivalent degree of microbial inactivation. The juices were evaluated and compared for significant differences in specific known nutrients (vitamin C, carotenoids, sugars, organic acids, etc.), undesired substances (products of Maillard reactions and oxidation reactions) and other quality-related aspects of orange juice (pectin methylesterase activity, peroxidase activity, colour, etc.) directly after treatment and during a storage period of 58 days at 4°C.

MATERIALS & METHODS

Sample preparation, processing and storage

For a detailed description of the orange juice preparation, processing and storage, the reader is referred to Timmermans et al., 2011 [7] and Vervoort et al., 2011 [1]. For pasteurization, thermal processing was conducted at 72°C for 20 s, HP conditions were 1 min at 600 MPa with an initial temperature 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°C².

Quality analyses

The organic acid profile, the carotenoid profile, ascorbic acid, furfural and 5-hydroxymethylfurfural were analysed by RP-HPLC with UV detection. For the sugar profile, RP-HPLC with ELSD detection was used, and dehydroascorbic acid was derivatized for fluorescence detection after separation by RP-HPLC. Colour measurements (Hunter L*a*b* values) were conducted using a Hunterlab ColorQuest colorimeter (45°/0° geometry, light source D65, Reston, Virginia, USA). Pectin methylesterase activity was measured by titrimetry, monitoring the release of acid during pectin hydrolysis as a function of time. For peroxidase activity measurement, a colorimetric assay was used, in which the formation of the coloured oxidation product of o-phenylenediamine was analysed spectrophotometrically over time. More details on the methods of analysis used in this study can be found in Vervoort et al., 2011 [1].

Statistical data analysis

Given the magnitude of the overall experiment [7], 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 modelled with the most straightforward model that provided a good fit. A parameter was considered significant when the p value was less than 0.05. Next, the obtained estimated parameters were compared with each other by a 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% (α= 0.01).

RESULTS & DISCUSSION

Sugar profile

Sugars are among the major components of orange juice, representing about 80% of the total soluble solids content. They are inherently responsible for the sweetness of the juice, making its content an important

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2 Orange juice preparation and processing were done by Wageningen UR Food and Biobased Research [7].
quality attribute. In the present study, the initial total sugar concentration was 86.9 g/L, which can be considered as an average value for orange juice. 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, confirming the 2:1:1 ratio usually mentioned in literature. 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 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.

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 solids content. 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. Results on ascorbic acid analysis are described in a following section. Citric and malic acid are generally known as the major organic acids found in orange juice and determine its acidity. The changes in their concentration during storage were best described by a linear and quadratic model respectively. 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. Between the four different treatment conditions, no significant differences in initial citric and malic acid concentration nor in evolution during storage were detected.

Colour

Food colour affects the perception of orange juice quality and is an important attribute in orienting consumers’ preference. The colour of orange juice is determined by two factors, on the one hand the desired colour of natural pigments, mainly carotenoids, that can fade upon processing, and on the other hand browning that may occur through the formation of pigmented substances due to enzymatic and/or non-enzymatic reactions. Depending on the extent of the reactions involved, the natural colour of orange juice can be lost and/or an unacceptable brownish colour can appear, diminishing the juice acceptance. The untreated samples presented the following initial Hunter values: $L^* = 61.65$, $a^* = 0.42$ and $b^* = 67.45$, which indicate a rather light yellow, less orange, coloured orange juice. During storage, only some minor changes occurred. The PEF treated juice became significantly lighter (significant decrease in $L^*$) and the heat treated juice displayed a significant loss in redness ($a^*$) and yellowness ($b^*$). However, comparison of the three pasteurisation treatments revealed no significant differences in $L^*$, $a^*$ nor $b^*$ values.

Carotenoid profile

Carotenoids are important quality indicators for orange juice. Apart from being responsible for the colour 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). Oranges are a very complex source of carotenoids, containing the largest number of them among all fruit species [9]. The total carotenoids 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. The major carotenoid detected was β-cryptoxanthin; it accounted for about 22.4% of the total carotenoids content. It is said to be the main contributor to the orange colour of the juice since it absorbs light at higher wavelengths, and the main provitamin A carotenoid found in oranges. 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. During storage at 4°C, only four carotenoids exhibited significant changes: cis-violaxanthin, antheraxanthin and 9Z-antheraxanthin decreased in concentration, while mutatoxanthin concentration increased. The total carotenoids 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.

**Vitamin C**

The most important contribution of orange juice to human nutrition is perhaps attributed to its high vitamin C content. 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.

**Figure 1** 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. The decrease in AA and increase in DHAA concentration during storage was best fitted by a linear and logarithmic model respectively. 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 [8]. 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 pasteurised samples, although not between the three pasteurisation processes themselves. In spite of the refrigerated and dark conditions during storage, decrease in AA and increase in DHAA were significant. Nevertheless, between all four processing conditions, no significant changes were detected in the parameters describing these courses.

![Figure 1](image)

**Figure 1** AA and DHAA concentrations of untreated (●), thermally (○), HP (▲) and PEF (x) pasteurised orange juice during storage at 4°C. The full lines represent the linear and logarithmic degradation model respectively.

**Furfural and 5-hydroxymethylfurfural (HMF)**

Furfural and 5-hydroxymethylfurfural (HMF) are indicated as the principal degradation products from ascorbic acid and sugar breakdown, the main sources of non-enzymatic browning, accompanied by undesirable off-taste and off-flavour. Because of this correlation, furfural and HMF are recognized as useful indicators for temperature abuse during processing and storage, and for quality deterioration in general. Furthermore, concern has been expressed about possible cytotoxic, genotoxic and mutagenic risks of these compounds [10].

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. The mild pasteurisation conditions used and the cold storage temperature were not sufficient to generate measurable amounts.

**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, flavour, aroma and the characteristic colour 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 pasteurisation for the production of shelf-stable orange juice is designed to inactivate PME, which is more thermally resistant than vegetative microorganisms. In this study, mild pasteurisation conditions were chosen, in view of producing high-quality orange juice for (short-time) refrigerated storage. The effect of the three pasteurisation processes and cooled storage on the PME activity is shown in figure 2. 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%. The low residual activities of the heat treated and HP treated juice could still preserve a cloud stability for a relatively long shelf life at refrigerated storage [11,12]; the residual activity of 66% in the PEF treated juice, however, will inevitably result in cloud loss during storage.

For impact comparison of the different treatments on PME activity, the data were best fitted by a linear model. 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 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.

**Peroxidase (POD) activity**

Peroxidase (POD) is traditionally considered responsible for a wide range of oxidative quality and flavour alterations in fruits and vegetables. Nevertheless, its direct link with quality deterioration has never been proven. In spite of this, its application as an indicator for heat treatment in food processing has been widely investigated, because of its high thermostability.

The residual POD activity after orange juice processing and its further evolution during storage is illustrated in figure 3. The conditions applied for thermal pasteurisation (20 s at 72°C) caused a complete activity loss. With an activity decrease of 30% at storage day 1, PEF treatment had a comparable impact on POD as on PME. 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. The change in activity during storage at 4°C, after the four processing conditions, was best fitted by a linear model. 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. 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.

![Figure 2](image_url) **Figure 2** 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.

![Figure 3](image_url) **Figure 3** 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.
CONCLUSION

A comparison of the impact of thermal, HP and PEF processing for mild pasteurisation 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 pasteurisation 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 pasteurisation and was much less susceptible to HP and PEF. Residual activities after HP and PEF and the following decreases during storage were not significantly different. All other quality parameters investigated (sugars, organic acids, colour, carotenoids, vitamin C, furfural and HMF) experienced no significantly different impact from the three pasteurisation techniques. With this research it was proven that there were no significant differences in the analysed components regarding food safety, and therefore no changes in human metabolism after consumption are expected.

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