Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments

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ABSTRACT

The effects of high-intensity pulsed electric field (HIPEF) processing (35 kV/cm for 1700 μs applying 4-μs pulses at 100 Hz in bipolar mode) on color, viscosity and PME and PG activities in strawberry juice were studied and compared to those of heat treatments (90 °C for 60 s or 30 s) through 63 days of storage. L* and viscosity values of the HIPEF-processed juices were higher than those found in the thermally treated. In addition, HIPEF-treated juice exhibited lower 5-(hydroxymethyl)-2-furfural (HMF) concentration and browning index than heat-treated juices throughout storage. On the other hand, HIPEF-treated juice maintained low residual pectin methyltransferase (PME) activity (13.1%) for 63 days, whereas in the case of the thermally treated, 22.2 and 48.8% was retained after 60 s and 30 s, respectively. Strawberry juice treated by HIPEF achieved lower residual polygalacturonase (PG) activity (73.3%) than those of heat-processed at 90 °C for 60 s (76.2%) or 30 s (96.8%). Thus, HIPEF could be a feasible alternative to thermal processing to minimize browning and viscosity loss in strawberry juice during storage.

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1. Introduction

Consumers are currently demanding fresh, healthy and flavourful ready-to-eat foods with enhanced shelf life (Hayes, Smith, & Morris, 1998). Thermal processing is the most common method for extending the shelf life of juices, by inactivating microorganisms and enzymes. Nevertheless, concomitant losses in terms of flavor, color, sensory and nutritional qualities occur when foods are heat treated (Goodman, Fawcett, & Barringer, 2002).

The attractive red color of strawberry juice is a commercially valued property that is highly degraded due to heat processing (Rodrigo, Van Loey, & Hendrickx, 2007). The negative effects of thermal treatments include non-enzymatic browning, which mainly cause changes in color and formation of undesirable products such as 5-(hydroxymethyl)-2-furfural (HMF) (Ibarz, Pagán, & Garza, 1999). The formation of HMF is triggered by temperature and time and thus, it is used to evaluate overprocessing in thermal pasteurization (Lee & Nagy, 1988).

Viscosity is an important quality attribute limiting the consumer acceptability of fruit juices that can be determined by factors such as the cultivar of fruits or the maturity of the fruit at the moment of processing (Tiziani & Vodovotz, 2005). The decrease in viscosity can be catalyzed by the action of enzymes such as pectin methyltransferase (PME) and polygalacturonase (PG) (Crelier, Robert, Claude, & Juillerat, 2001). The degradation of pectin substances by both enzymes results in a reduction of the ability of a juice to hold its solid portion in suspension throughout storage (Bemiller, 1986; Chou & Kokini, 1987).

High-intensity pulsed electric field (HIPEF) is a nonthermal food-processing technology which uses short bursts of electricity (microseconds to milliseconds), providing fresh-like and safe foods and reducing quality losses that can be triggered after thermal processing (Dunn, 2001; Morris, Brody, & Wicker, 2007). HIPEF has been demonstrated to be an alternative pasteurization method, even though its effects on enzymes and other parameters can vary depending on the product to be processed. Efficiency of HIPEF treatments to inactivate enzymes commonly present in fruit juices has been compared with that of heat treatments (Min, Jin, & Zhang, 2003; Rivas, Rodrigo, Martínez, Barbosa-Cánovas, & Rodrigo, 2006).

In contrast, extending the shelf life of juices by HIPEF-processing, while keeping natural or appealing color and viscosity is still being a major challenge. Elez-Martínez, Soliva-Fortuny, and Martín-Belloso (2006) reported that HIPEF treatments best maintained the color of orange juice for 56 days of storage compared with thermal processing. Moreover, they reported an increase in the viscosity of the HIPEF-processed juice.

The scarce literature available about HIPEF-treated strawberry juice is only referred to its microbiological commercial shelf life (Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2008). Therefore, the purpose of this study was to evaluate the effects of...
HIPEF and thermal processing on color and viscosity changes as well as on quality-related enzymes during the shelf life of strawberry juice.

2. Material and methods

2.1. Strawberry juice preparation

Strawberries (cv. Camarosa) at commercial maturity were purchased from a local supermarket (Lleida, Spain). The fruits were ground and then centrifuged at 23,450 × g for 15 min. The supernatant was collected and then filtered through 2-mm steel sieves to obtain the juice. Strawberry juice samples were immediately treated by HIPEF or heat.

2.2. HIPEF treatment

Pulse treatment was carried out using a laboratory scale pulse generator (OSU-4F, The Ohio State University, Columbus) that provides squared-wave pulses within eight co-field flow chambers placed in series. The gap distance between electrodes and treatment chamber volume was 0.29 cm and 0.012 cm³, respectively. The flow rate of the process was adjusted to 60 mL/min and controlled with a variable speed pump (model 75210-25, Cole Palmer, Vernon Hills, IL, USA). The treatment temperature was kept below 35 °C using a cooling coil, which was connected before and after each pair of chambers and submerged in an ice-water shaking bath. HIPEF treatment was set up at 35 kV/cm for 1700 ms using squared-wave pulses of 4 μs and a pulse frequency of 100 Hz in bipolar mode. These HIPEF conditions were settled according to the results obtained by Mosqueda-Melgar et al. (2008), who studied the effect of HIPEF treatments on the inactivation of Salmonella enteritidis in strawberry juice.

2.3. Thermal treatments

Strawberry juice samples were heat pasteurized at 90 °C for 60 s or 30 s. According to Nagy, Chen, and Shaw (1993) heat pasteurization conditions for fruit juices vary from 95 °C to 90 °C for 15–60 s to assure at least 5 log reductions in the count of the most resistant pathogenic microorganism. Strawberry juice was processed in a tubular stainless steel heat exchange coil immersed in a hot water shaking bath using a gear pump to maintain the desired flow rate (Universidad de Lleida, Lleida, Spain). Once processed, the juice was immediately cooled in a heat exchange coil immersed in an ice-water bath.

2.4. Packaging and storage

The HIPEF fluid handling system was sanitized, first with 250 mL of a solution containing 1.6 g/L of NaOH solution and then with 250 mL of distilled water (100 mL/L) and ethanol (200 mL/L) solutions prior to processing. Between the different solutions, water was passed through the system. The first 200 mL of treated liquid was discarded to ensure stationary treatment conditions. Polypropylene sterilized bottles of 100 mL were directly filled with the strawberry juice from the treatment system. After that, the containers were tightly closed, leaving as less amount of air as possible in the headspace and stored at 4 °C for 63 days.

2.5. Color measurements

The color of the juice was measured using a Macbeth Color-Eye 3,000 colorimeter (Macbeth-Kollmorgen Inst Corp, Newburgh, NY, USA) at room temperature. Equipment was set up for illuminant D65 and 10° observer angle, providing CIE-Lab values of L*, a* and b*.

These values were then used to calculate the red–yellow ratio (a*/b*), used to indicate the redness of the strawberry juice (Min & Zhang, 2003).

2.6. Browning index determination

Browning index (BI) was determined following the method proposed by Meydav, Saguy, and Kopelman (1977). A portion of 10 mL of strawberry juice was mixed with 10 mL of ethyl alcohol (950 g/L) and centrifuged at 2100 g during 20 min at 18 °C. After filtering the supernatant, the absorbance was measured at 420 nm.

2.7. 5-(Hydroxymethyl)-2-furfural determination

A method described by Cohen, Birk, Mannheim, and Saguy (1998) was used for the 5-(hydroxymethyl)-2-furfural (HMF) determination. 5 mL of ethyl alcohol (950 g/L) was added to 5 mL of strawberry juice and centrifuged for 10 min at 7800 g. Then, 2 mL of the supernatant, 2 mL of 3-chloroacetic acid (120 g/L) and 2 mL of thiobarbituric acid (3.60 g/L) solutions were mixed in a 16-mL screw-cap test tube. The tube was placed in a water bath at 40 ± 0.5 °C, heated for 50 min and then cooled immediately to approximately 25 °C. Absorbance was measured at 443 nm. A calibration curve of HMF was used to quantify the HMF concentration.

2.8. Determination of viscosity

Viscosity was measured from approximately 30 mL of strawberry juice using a rotatory viscometer (model DV-I, Brookfield, Stoughton, Mass., U.S.A) with a precision cylindrical spindle rotating (UL) adapter. Strawberry juice was placed in the UL adapter and viscosity was determined at 60 rpm.

2.9. Enzyme activity measurements

2.9.1. Pectin methylesterase (PME)

PME activity was measured using the method described by Kimball (1991). Pectin, sodium chloride and NaOH were purchased from Acros Organics (NJ, U.S.A), Rectapur (Fontenay, France) and Panreac Quimica (Barcelona, Spain), respectively. A 10 mL aliquot of strawberry juice tempered at 30 °C was mixed with 40 mL of pectin–salt substrate (10 mg/L) (also at 30 °C) and incubated at 30 °C. The solution was adjusted to pH 7.0 with NaOH (80 g/L), and then readjusted to pH 7.7 with NaOH (2 g/L). After the pH reached 7.7, 0.10 mL of NaOH solution (2 g/L) was added. The time required for the solution pH to return to 7.7 was measured. PME activity (A_{PME}) was calculated by Eq. (1):

$$A_{PME} = \frac{[\text{NaOH}] \cdot V_{\text{NaOH}}}{V_{\text{juice}} \cdot t'} \tag{1}$$

where [NaOH] is the NaOH concentration (2 g/L), V_{NaOH} is the volume of 0.10 mL of the NaOH solution (2 g/L), V_{juice} is the volume of juice (10 mL), and t' is the time in minutes required for the solution to return a pH of 7.7 after the addition of NaOH.

2.9.2. Polygalacturonase (PG)

PG activity in strawberry juice was measured using the method described by Aguilo-Aguayo et al. (2008). A sample of 2.5 mL of juice was transferred to a 50 mL centrifuge tube and centrifuged at 7500 g for 10 min. The supernatant was decanted and replaced with cold distilled water. Then, the pH of the mixture was adjusted at pH 3.0 with HCl solution (8.28 mL/L). After that, the sample was centrifuged at 9000 g for 15 min. The supernatant was again decanted, mixed with NaCl solution (70.13 g/L) in a ratio of 1:1. The
mixture was added to the pellet and left for 1 h. After this time, a centrifugation at 18,200g for 10 min was carried out, and the supernatant was assayed for PG activity. All steps were performed at 4 °C.

The polygalacturonase activity assay was based on the release of reducing groups produced by PG and measured by spectrophotometry (Gross, 1982). A portion of 100 μL of the enzyme extract was mixed with 300 μL of polygalacturonic acid (2 g/L) and incubated at 35 °C for 10 min. To stop the reaction, 2 mL of borate buffer (38.14 g/L, pH 9.0) and 400 μL of cyanoacetamide solution (10 g/L) were added to the reaction mixture and boiled for 10 min. After cooling down, the absorbance was measured at 276 nm and 22 °C. A blank was determined in the same way without enzyme addition.

2.9.3. Relative residual activity

Percentage of residual PME and PG activities was calculated through Eq. (2):

$$ RA(\%) = \frac{A_t}{A_o} \cdot 100 $$

(2)

where $A_t$ and $A_o$ are the enzyme activities of treated and untreated samples, respectively.

2.10. Statistical analysis

Triplicate samples were packaged for analytical determination and triplicate measurements were performed for each sample. Differences among treatments ($p < 0.05$) throughout the storage time were evaluated using an analysis of covariance (ANCOVA) procedure. The Tukey method was used to determine differences between means. Furthermore, principal component analysis (PCA) was conducted on data to obtain an overview of correlations among variables. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be reduced to a set of new variables called principal components (PCs). The correlation matrix is used to standardize the variables which are not measured on the same scale. The loadings of the PC define the direction of greatest variability and the score values represent the projection of each object onto PC. The statistical procedures were conducted with Statgraphics Plus v 5.1 for Windows (Statistical Graphics Co., Rockville, MD, USA).

3. Results and discussion

3.1. Color

The effect of HIPEF and thermal treatments on $L^*$, $a^*/b^*$ and BI of strawberry juice is shown in Fig. 1. The applied treatment significantly affected color parameters, whereas storage time only had a significant influence on $L^*$ and $a^*/b^*$ (Table 1).

HIPEF-treated strawberry juice had substantially higher initial lightness values ($p < 0.05$) than heat-treated juices and untreated juice (Fig. 1A). In addition, $L^*$ values of the HIPEF-processed juice were maintained during storage above those achieved in thermally processed samples. A depletion in lightness during storage was noticed in juices subjected to heat treatments. This decrease in $L^*$ values can be associated to the formation of dark color compounds in the juice caused by the high temperatures applied (Klim & Nagy, 1988; Yeom, Streaker, Zhang, & Min, 2000). In accordance with our results, HIPEF-treated orange juice (35 kV/cm for 1000 μs applying 4-μs pulses at 200 Hz in bipolar mode) and tomato juice (35 kV/cm for 1500 μs using 4-μs pulses at 100 Hz in bipolar mode) exhibited higher lightness than heat-pasteurized (90 °C for 60 s) juices throughout storage (Aguiló-Aguayo et al., 2008; Elez-Martínez et al., 2006).

The red–yellow ratio ($a^*/b^*$) is commonly used to evaluate the redness of juices (Min & Zhang, 2003). The $a^*/b^*$ values significantly decreased after the processing of strawberry juice ($p < 0.05$), compared with untreated samples. Differences between treatments were only appreciated beyond day 28 (Fig. 1B). HIPEF treatment was more efficient than thermal processing in maintaining $a^*/b^*$ values of strawberry juice during storage.

Browning index (BI) is a measurement commonly used to indicate the browning development in samples (Quijão-Teixeira, Aguiló-Aguayo, Ramos, & Martín-Belloso, 2007). No significant difference between BI of HIPEF-processed and fresh strawberry juices was observed. The thermal treatment at 90 °C for 60 s accelerated browning reactions in comparison with the thermal treatment at 90 °C for 30 s, thus reaching the highest BI values (Table 2). Nevertheless, the initial BI values remained constant in all the samples for 63 days of storage. Color deterioration in strawberry juice may be related to a loss of anthocyanin pigments or formation of Maillard reaction products (Kребbers et al., 2003).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time (covariate)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L^*$</td>
<td>8.94**</td>
<td>96.81**</td>
</tr>
<tr>
<td>$a^<em>/b^</em>$</td>
<td>10.42**</td>
<td>21.04**</td>
</tr>
<tr>
<td>BI</td>
<td>2.74</td>
<td>140.18**</td>
</tr>
<tr>
<td>HMF</td>
<td>0.35</td>
<td>160.77**</td>
</tr>
<tr>
<td>Viscosity</td>
<td>29.90**</td>
<td>0.99*</td>
</tr>
<tr>
<td>$R_{PME}$</td>
<td>48.20**</td>
<td>46.59**</td>
</tr>
<tr>
<td>$R_{PG}$</td>
<td>17.88**</td>
<td>8.82**</td>
</tr>
</tbody>
</table>

Numeric values are the F-ratio of the variance explained by a given factor vs. the unexplained variance. Letters in superscript correspond to the level of significance of the differences. “p < 0.001; p < 0.05. (HMF = 5-(hydroxymethyl)-2-furfural; BI – Browning index; $R_{PME}$ – Residual Pectin methylesterase activity; $R_{PG}$ – Residual Polygalacturonase activity).
HIPEF treatments are unlikely to affect the anthocyanin content, which are associated with the redness of strawberry juice. In cranberry juice, Jin and Zhang (1999) observed that HIPEF treatments at 20 kV/cm and 40 kV/cm for 50 μs or 150 μs using pulse width of 2 μs and frequency of 1000 Hz, did not affect the initial content of anthocyanin pigments. On the other hand, they observed that a thermal treatment of 90°C for 90 s reduced a 4% of the initial amount of anthocyanins in the fresh juice.

3.2. HMF

HMF is widely used as an indicator of Maillard reactions (Ratnananalerk, Chiewchan, & Srichumpound, 2005). The HMF content in strawberry juice depended on the applied treatment, whereas refrigerated storage did not have any influence on this parameter (Table 1). HMF concentration in HIPEF-processed juice was slightly higher than in the untreated juice (Table 2). However, HMF formation was significantly reduced by HIPEF treatments, compared with thermal processing. On the other hand, the increase in the thermal treatment time from 30 s to 60 s provided a rise of 17% in the amount of HMF of the juice (Table 2). Nevertheless, it is important to emphasize that the concentration was very low in all the treated juices and remained below the maximum values allowed (5 mg/L) by the Association of the Industry of Juices and Nectars from Fruits and Vegetables (AIJN, 1996). Accordance with our results, Min and Zhang (2003) reported lower HMF concentration in tomato juice after HIPEF-processing (40 kV/cm for 57 μs using pulse duration time of 2 μs and pulse repetition rate of 1000 pulse per second) than after a thermal treatment (92°C for 92 s). They suggested that the slow browning rates observed in the HIPEF-processed juice could be related to the high retention of ascorbic acid found in the juice. In fact, when ascorbic acid is not oxidized, it does not provide reactive carbonyl groups, which can be precursors of non-enzymatic browning reactions (Joslyn, 1957).

3.3. Viscosity

The effects of HIPEF and heat treatments on strawberry juice viscosity are shown in Fig. 2. HIPEF-treated strawberry juice exhibited the greatest initial viscosity (19.7 mPa s), followed by the juice treated at 90°C for 60 s (17.6 mPa s). Strawberry juice processed at 90°C for 30 s underwent a slight decrease in viscosity, showing lower initial values (10 mPa s) than those of the untreated juice (12.3 mPa s). Aguilo´-Aguayo et al. (2008) also reported the highest values of tomato juice viscosity by HIPEF-processing (35 kV/cm for 1000 μs using 4-μs pulses at 100 Hz in bipolar mode) and by heating (90°C for 60 s) compared with the untreated juice for 35 days of storage. High values of viscosity after processing may partly be attributed to great PME and PG inactivation or protein-tissue coagulation (Porreta, Birzi, Chizzoni, & Vicini, 1995).

Both HIPEF and heated (90°C for 60 s) juices exhibited the same trend during storage, thus decreasing gradually their initial viscosities to 12.3 mPa s and 10.2 mPa s, respectively, for 63 days of storage. On the other hand, no significant changes were noticed throughout storage with regard to the viscosity of strawberry juice subjected to heat treatment at 90°C for 30 s.

3.4. Pectin methylsterase and polygalacturonase

The reduction of PME and PG activity may explain the better maintenance of viscosity values. Pectins are modified by the combined action of PME and PG, causing changes in the viscosity of juices (Marsh, Buhlert, & Leonard, 1980).

The highest values of residual PME activity (RA_PME) were observed in untreated and thermally processed strawberry juice at 90°C for 30 s. RA_PME was strongly affected by HIPEF treatment (13.1%), whereas residual activities of 22.2 and 48% were achieved after thermal processing at 90°C for 60 s and 30 s, respectively (Fig. 3). Aguilo´-Aguayo et al. (2008) and Elez-Martı´nez et al. (2006) reported a RA_PME of 18% by applying HIPEF to both tomato juice (35 kV/cm for 1500 μs using 4-μs pulses at 100 Hz in bipolar mode) and orange juice (35 kV/cm for 1000 μs using 4-μs pulses at 200 Hz in bipolar mode). Residual PME activity in the untreated juice followed a dramatic decay during the first 2 weeks. Moreover, the low initial RA_PME achieved in HIPEF-treated juice and in the juices processed at 90°C for 60 s decreased to 6.1 and 12.2%, respectively, during the first week of storage. From an industrial point of view, a juice is commercially stable when the residual enzymatic activity remains below 10%. HIPEF-treated strawberry juice was always under these values (Fig. 3).
A total reduction of the residual PG activity (RAPG) was not achieved with the HIPEF treatment. However, RAPG after applying HIPEF (73.3%) was lower than after thermal treatments for 60 s (76.2%) or 30 s (96.8%) (p < 0.05) (Fig. 4). Furthermore, RAPG in fresh and thermally treated juices (for 30 s) decreased gradually to 32% during the first two weeks of storage. Our results agree with those reported by Aguilo-Aguayo et al. (2008), who observed a residual PG activity of 88% in HIPEF-treated tomato juice (35 kV/cm for 1500 μs applying 4-μs pulses at 100 Hz in bipolar mode). These authors reported 78% and 56% of RAPG in tomato juices treated at 90 °C for 30 s and 60 s, respectively. High residual PG activity obtained under all treatment conditions could be attributed to the presence of heat stable PG isomerase (Nogata, Ohta, & Voragen, 1993).

A close relationship between PME and PG of strawberry juice was corroborated in this study. PME de-esterifies the pectin yielding methanol and pectin acid with lower degree of esterification and in turn, PG catalyses the hydrolytic cleavage of the glycosidic a-1–4 bonds in the pectin acid. Thereby, the formation of substrate for PG action could be reduced due to low PME activity.

3.5. Principal component analysis

In order to estimate correlations between strawberry juice variables (L*, a’/b*, BI, HMF, viscosity, PME and PG), principal component analysis (PCA) was carried out. The analysis was performed using the results obtained just after treating the juices. Two principal components (PC1 and PC2) were calculated (Fig. 5).

The factor loadings of the analyzed compounds explain 99.04% of the total variation of the data. The two principal components, PC1 and PC2, explain 54.55% and 44.39% of the total variance, respectively. Fig. 5 shows the loading plot of PC1 vs. PC2. Variables that appear close together in this plot correlate positively. As it can be seen in Fig. 5, there is a close relationship between residual PME and PG enzymes, demonstrating that these enzymes are affected in the same way by the treatment applied. As expected, HMF and BI correlate well, claiming that a major HMF formation induced an increment in the BI of strawberry juice. The negative correlation between the PME and PG enzymes with viscosity suggests that the increase in viscosity of strawberry juice could be related to a decrease in enzymatic activities. In the same way, correlations between L* and a’/b* with the HMF and BI support the fact that high luminosity and redness values observed in the HIPEF-treated juices were related to low HMF formation and consequently low accumulation of brown pigments in the juice.

Fig. 5. Principal compounds plot in untreated, HIPEF-treated and thermally processed strawberry juices (BI = browning index; HMF = 5-(hydroxymethyl)-2-furfural; RAPME = residual pectin methylesterase activity; RAPG = residual polygalacturonase activity).

4. Conclusions

HIPEF can preserve the initial color and reduce browning of strawberry juice throughout storage compared to heat-treated juices. Lightness and a’/b* values of strawberry juice were better maintained for 63 days in HIPEF-treated juices than in heat-processed juices. The application of HIPEF to strawberry juice can assure HMF content and BI levels similar to those found in fresh juices as well as their stability during the product shelf life. In contrast, the higher the heat treatment time, the higher the HMF and browning pigment formation. Thus, greater luminosity and redness in HIPEF-treated juices was associated with low HMF concentrations and accumulation of brown pigments. On the other hand, the greatest PME and PG inactivations achieved by HIPEF-processing allowed to obtain juices with improved viscosity.

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