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Flavour retention and related enzyme activities during storage of strawberry juices processed by high-intensity pulsed electric fields or heat

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ABSTRACT

The effects of high-intensity pulsed electric fields (HIPEF) processing (35 kV/cm for 1700 μs using pulses of 4 μs at 100 Hz in bipolar mode) and thermal treatments (90 °C for 30 s or 60 s) on lipoxygenase (LOX) and β-glucosidase (β-GLUC) activities as well as on the production of volatile compounds were assessed in strawberry juice for 56 days of storage. HIPEF-treated juice kept higher residual LOX activity than heat-treated juices during the first 28 days of storage. Moreover, β-GLUC increased its initial activity just after HIPEF processing. The concentration of DMHF in HIPEF-processed strawberry juice was above those of untreated and heat-treated juices during the first 14 days of storage. On the other hand, concentrations of ethyl butanoate and 1-butanol obtained after HIPEF processing were better maintained than after thermal processing. However, thermally-treated samples showed an increase in the amount of 1-butanol beyond day 35, causing an unpleasant flavour to the product. Thus, flavour stability in HIPEF-processed strawberry juice was greater than in thermally-treated samples during storage.

1. Introduction

Strawberries are well known for their exquisite flavour and are widely used to prepare juices (Bood & Zabetakis, 2002). Factors such as maturity at the moment of processing or strawberry variety, could substantially affect the content in aroma compounds (Forney, Kalt, & Jordan, 2000). Studies have demonstrated that strawberry aroma is the result of a very complex mixture of about 350 components (Latrasse & Fruits, 1991), a combination of esters, which dominate qualitatively and quantitatively as well as furanones, sulphur compounds, lactones, alcohols and carbonyls (Larsen, Poll, & Olsen, 1992; Zabetakis & Holden, 1997).

Changes in the concentration of compounds related to the typical strawberry aroma have been associated to lipoxygenase (LOX) and β-glucosidase (β-GLUC) activities (Leone et al., 2006; Orruño, Owusu, & Zabetakis, 2001). The formation of volatile C_6- and C_9-aldehydes and –alcohols, which could contribute to the characteristic "fresh green" odour of strawberry fruit, is catalysed by LOX (Leone et al., 2006; Takeoka, 1999). On the other hand, key aroma components such as furanones and esters, present in cells as glucosides, are released from its glycosidic precursor by the action of β-GLUC (Pogorzelski & Wilkowska, 2007; Zabetakis, Gramshaw, & Robinson, 1999). Nevertheless, when the concentration of all of those compounds exceeds the off-flavour limit, the production of disagreeable scents is released (Lunning, Carey, Roozen, & Wichers, 1995; Orruño et al., 2001).

Unfortunately, juice flavour compounds have shown low stability during both storage and processing. To extend the shelf-life of juices, thermal treatments are conventionally used in order to inactivate microorganisms and enzymes. However, heating can adversely affect the sensory and nutritional qualities of juices (Braddock, 1999; Rouseff & Leahy, 1995). High-intensity pulsed electric fields (HIPEF) are being investigated as an alternative to obtain safe products with fresh like sensory and nutritional attributes. It has been demonstrated that HIPEF processing (35 kV/cm for 1700 μs using pulses of 4 μs at 100 Hz in bipolar mode) effectively reduced microbial loads in strawberry juices (Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2008). However, the effects of HIPEF on flavour retention of strawberry juice have not been yet investigated.

Up to now, some studies in other juices indicated that some representative flavour compounds are better retained in HIPEF-processed juices (Jia, Zhang, & Min, 1999; Yeom, Streaker, Howard Zhang, & Min, 2000). Significant flavour compounds of apple were better preserved after HIPEF treatments than after applying heat (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007). Moreover, Min and Zhang (2003) observed an increase in the flavour release of a HIPEF-processed tomato juice (40 kV/cm for 57 μs using pulses of 2 μs at 1000 pulses per second), suggesting that HIPEF could have improved its initial fresh flavour quality.

Therefore, the aim of the present work was to study and compare the retention of volatile compounds contributing to the

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strawberry characteristic aroma during the commercial shelf-life of HIPEF and thermally-processed strawberry juices. In addition, this study evaluated the effect of HIPEF and thermal processing on enzyme activities involved in flavour synthesis.

2. Materials and methods

2.1. Strawberry juice preparation

Strawberries (cv. Camarosa) were purchased at commercial maturity 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 filtered through a 2 mm diameter steel sieve to obtain the juice. A physicochemical characterisation was carried out to offer detailed information about the strawberry juice used in this study. The electrical conductivity (Testo 240 conductivimeter; Testo GmBh & Co., Lenzkirch, Germany), pH (crison 2001 pH-metre; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), and colour (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) of the strawberry juice were determined (Table 1). The natural pH and sugar concentration of strawberry juice was in the range of the general commercial practice of juices. CIE-Lab values of a^* and b^* were then used to calculate the red–yellow ratio (a^*/b^*), used to indicate the redness of strawberry juice (Min & Zhang, 2003). According to Hayes, Smith, and Morris (1998), an a^*/b^* ratio higher than 1.90 represents a first quality product in terms of colour, meaning that the product may have high colour acceptance.

2.2. HIPEF treatment

HIPEF treatment was carried out using a laboratory scale pulse generator (OSU-4F, The Ohio State University, Columbus) that provides squared-wave pulses within eight cofield flow chambers placed in series. The gap distance between electrodes and treatment chamber volume were 0.29 cm and 0.012 cm^3, 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 spiral (wound), 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 μs 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 in the inactivation of *Salmonella* Enteritidis on strawberry juice.

2.3. Thermal treatments

Strawberry juice samples were pasteurized at 90 °C for 60 s or 30 s. According to Nagy, Chen, and Shaw (1993) 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 loads of the most resistant pathogenic microorganism. Strawberry juice was processed in a tubular stainless steel heat exchange spiral (wound) immersed in a hot water shaking bath using a gear pump to maintain the desired flow rate (University of Lleida, Lleida, Spain). Once processed, the juice was immediately cooled in a heat exchange coil immersed in an ice-water bath.

Table 1
Analytical characteristics of strawberry juice.

Parameters[a]	Strawberry juice
Electric Conductivity (S/m)	0.53 ± 0.02
pH	3.16 ± 0.01
Soluble Solids (°Brix)	7.2 ± 0.1
L^*	22.11 ± 0.02
a^*/b^*	2.75 ± 0.05

[a] Results are the mean ± SD of three measurements.

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 chlorine (100 mL/L) and ethanol (200 mL/L) solutions prior to processing. System was washed with distilled water after passing each solution. The first 200 mL of strawberry juice were discarded to ensure stationary treatment conditions. Polypropylene sterilized bottles of 100 mL were directly filled from the treatment system. After that, the containers were tightly closed, leaving as less amount of headspace as possible, and stored at 4 °C for 56 days. Unprocessed strawberry juice was stored for 14 days due to the rapid microbial growth.

2.5. Determination of enzyme activities

2.5.1. Lipoxygenase (LOX)

LOX activity in strawberry juice was measured using the method described by Anese and Sovrano (2006) and Rodrigo, Jolie, Van Loey, and Hendrickx (2007), with some modifications. LOX was extracted by mixing 5 mL of the HIPEF-treated or -untreated samples with 2 mL of 0.5 mol/L sodium phosphate buffer (pH 6.5) and 2.5 mL of Triton X-100 (5 mL/L) in a centrifuge tube. The homogenate was centrifuged for 15 min at 10,000*g* (Centrifuge AVANTI™ J-25, Beckman Instruments Inc., Fullerton, CA, U.S.A), at 4 °C and the pellet was discarded.

LOX activity was determined by continuously monitoring the formation of conjugated dienes from linoleic acid. The substrate consisted of 10 μL of linoleic acid, 4 mL of H_2O, 1 mL of 0.1 mol/L NaOH and 5 μL of Tween 20. The assay mixture was shaken and diluted up to 25 mL with water. Activity measurements were carried out at 25 °C in a quartz cuvette. Each test contained 2.7 mL of 0.2 mol/L phosphate buffer (pH 6.5), 40 μL of the substrate and 100 μL of enzyme extract. The reaction started by adding the enzyme extract and the absorbance was measured by a spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) at 234 nm for 2 min at 22 °C. The activity was determined from the slope of the linear portion of the curve. One unit of LOX activity was defined as a change of 0.001 units of absorbance per minute and per millilitre of enzyme extract.

2.5.2. β-glucosidase (β-GLUC)

Determination of β-GLUC activity was carried out following the method described by Orruño et al. (2001). Strawberry juice (10 g) was mixed with 10 mL of 0.1 mol/L citrate–0.2 mol/L phosphate buffer (pH 4.0). The obtained homogenate was centrifuged at 9600*g* for 20 min at 4 °C. The supernatant was filtered through muslin cloth and then kept in an ice bath.

β-GLUC was assayed using *p*-nitrophenyl-β-D-glucopiranoside (Aldrich, UK) as substrate. The assay was performed by adding 100 μL of enzyme crude extract to 500 μL of 0.04 mol/L substrate solution and 400 μL of 0.1 mol/L citrate–0.2 mol/L phosphate buffer (pH 4.0). The reaction mixture was incubated for 30 min at 40 °C and then 2 mL of 3 mol/L sodium carbonate (Aldrich, UK) were added to stop the reaction. The liberated *p*-nitrophenol liberated was measured from absorbance measurements at 405 nm.

2.5.3. Relative residual activity

Residual LOX and β-GLUC activities were calculated throughout storage time and related to those of the untreated juice. The relative residual activities *RA* (%) were defined as indicated by

$$\mathrm{RA}(\%) = \frac{A_t}{A_o} \quad (1)$$

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

2.6. Analysis and identification of flavour compounds

Selected flavour compounds in the headspace of strawberry juice were analysed by a combination of solid-phase microextraction (SPME) and gas chromatography (GC) (Jia, Zhang, & Min, 1998). Samples of 10 mL of strawberry juice were transferred into 30 mL vials. The SPME fibre (Supelco, Bellefonte, PA, USA) coated with 100 μm polydimethylsiloxane was inserted into the headspace of each vial. Afterwards, the vials were heated at 50 °C for 15 min to facilitate the release of volatile compounds to the headspace. During the extraction, the strawberry juice in the vial was stirred with a magnetic stirrer. The SPME fibre was removed from the sample vial, inserted into a GC injection port, and held for 4 min at 250 °C to desorb the flavour compounds absorbed on the SPME coating. The desorbed flavour compounds were separated using an Agilent 5973 Network GC/MS equipment (Agilent Technologies, Palo Alto, CA, USA) equipped with a capillary column of 0.25 mm internal diameter × 30 m length, and coated with 0.25 μm thick diphenylpolysiloxane. The carrying gas was helium at a rate of 1.5 mL/min. The GC oven temperature was programmed from 40 °C to 250 °C at 20 °C/min and held 10 min at the final temperature. At the end of the experimental run, volatiles were tentatively identified by comparing their mass spectra with those contained in the Wiley libraries. The presence of the main volatiles reported to be present in strawberry juice were confirmed by comparing the retention times of gas chromatographic peaks to those of standards. The standard calibration curve of each flavour compound was obtained by plotting the GC peak area against the known concentrations of standard flavour compound in a model solution simulating the strawberry juice matrix. flavour compounds in strawberry juice were quantified using the calibration curve.

2.7. Statistical analysis

Triplicate samples were packaged for analytical determination and triplicate measurements were performed for each sample. Differences amongst 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. 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. Lipoxygenase and β-glucosidase activities

The effects of HIPEF and thermal processing on LOX and β-GLUC activities of strawberry juice for 56 days of storage are illustrated in Figs. 1 and 2, respectively.

Residual LOX activity (RA_{LOX}) after HIPEF processing was significantly ($p < 0.05$) higher (66.7%) than after heat processing at 90 °C for 60 s (37.7%) or 30 s (45.3%) (Fig. 1). LOX resistance to HIPEF treatments has been related to the presence of both heat labile and heat resistant fraction of LOX isoenzymes in fruits (Min, Min & Zhang, 2003). It has been suggested that the heat resistant fraction could also be HIPEF-resistant.

Initial values of LOX activity in the fresh juice decreased around 50% after 14 days of storage. On the other hand, HIPEF-treated juice

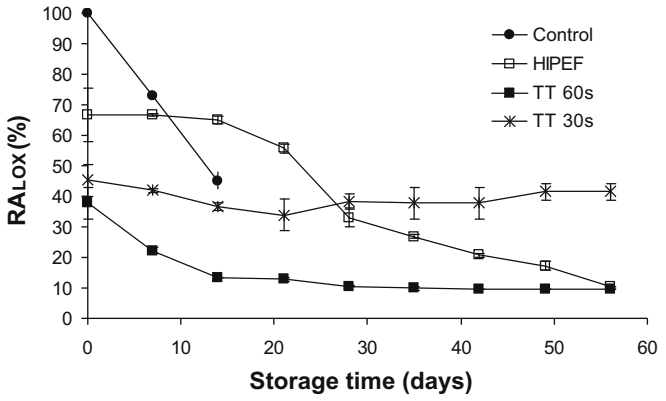

Fig. 1. Effects of HIPEF and thermal treatments, 90 °C for 60 s (TT 60 s) or for 30 s (TT 30 s) on residual lipoxygenase (RA_{LOX}) activity (mean ± SD) of strawberry juice throughout storage at 4 °C.

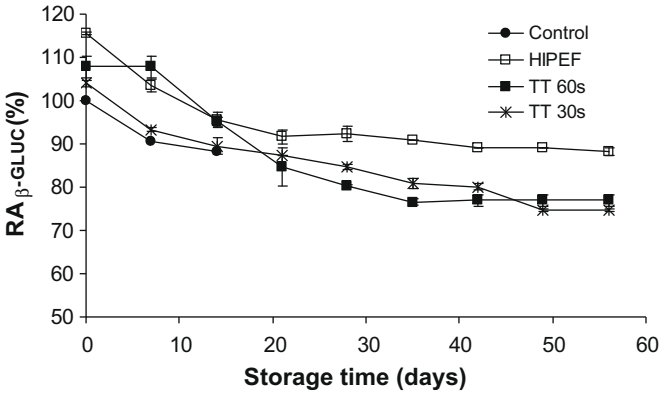

Fig. 2. Effects of HIPEF and thermal treatments, 90 °C for 60 s (TT 60 s) or for 30 s (TT 30 s) on residual β-glucosidase ($RA_{β\text{-}GLUC}$) activity (mean ± SD) of strawberry juice throughout storage at 4 °C.

maintained the initial RA_{LOX} during 21 days. Beyond that period, RA_{LOX} values decreased throughout storage, achieving lower LOX activities than those observed in the juice treated at 90 °C for 30 s. RA_{LOX} obtained after those heat treatment (90 °C for 30 s) was kept constant for 56 days of storage. In contrast, RA_{LOX} of the thermally-processed juice for 60 s decreased up to 13.1% during the first 2 weeks and then, remained unchanged until the end of storage.

It is controversial whether total LOX inactivation should be aimed in minimally processed juices due to the involvement of LOX in juice flavour flavour quality. According to Min, Min, and Zhang (2003), a minimum LOX activity may be desirable in juices stored for a long time.

All the assayed treatments slightly enhanced β-GLUC activity at day 0 in comparison with untreated juice (Fig. 2). HIPEF treatments had little effect on β-GLUC inactivation or even induced a slightly rise in its residual activity ($RA_{β\text{-}GLUC}$). Indeed, the highest β-GLUC activity increase (15.6%) was exhibited after HIPEF processing, while juices treated at 90 °C for 60 s and for 30 s showed 7.9% and 4.1% increases, respectively. This activity increase might be due to the formation of more active sites or the increase of the size of the existing ones (Ho, Mittal, & Cross, 1997). However, these active sites would indicate that the existing native form is not the most active.

During storage, $RA_{β\text{-}GLUC}$ gradually decreased in both treated and untreated juices. Nevertheless, HIPEF-processed strawberry

juice retained higher enzymatic activity than heat-treated juices beyond day 21.

3.2. Flavour analyses

Hexanal, 1-butanol, linalool, DMHF and several esters were identified. These compounds have been reported as important flavour compounds in strawberry juice (Golaszewski, Sims, O'Keefe, Braddock, & Littell, 1998).

3.2.1. Hexanal

Hexanal has been associated to the green and cut grass odour characteristics in fruits (Azodanlou, Darbellay, Luisier, Villettaz, & Amadò, 2003). A low decrease of hexanal content was observed after juice processing. However, no statistical differences between treatments on hexanal concentration were observed at day 0 (Table 2). The hexanal reduction in the treated samples may be related to the inactivation of LOX achieved just after juice processing (Fig. 1). Hexanal could be produced naturally via LOX-lyase oxidation (Hamilton-Kemp, Archbolf, Loughrin, Collins, & Byers, 1996). Therefore, a decrease in LOX could explain a decrease in the production of hexanal. Our results are in the range of those reported by Aguilar-Rosas et al. (2007) who observed a 7% of hexanal reduction after treating an apple juice by HIPEF (1200 pulses per second in bipolar mode of 4 μs at 35 kV/cm). On the other hand, the hexanal retention in the fresh juice seemed to be affected over time, decreasing the initial content from 1.14 mg/100 mL up to 1.00 mg/100 mL at day 14. Microbial proliferation could explain most variations in the aroma release during the storage of the untreated juice (Ayala-Zavala, Wang, Wang, & González-Aguilar, 2004). In contrast, treated juices maintained the initial hexanal content throughout storage.

3.2.2. 1-Butanol

Alcohols have been identified as constituents of the aroma profile in strawberries (Pino, Marbot, & Vázquez, 2001). 1-butanol was the major alcohol identified in strawberry juice. No significant differences were observed between the 1-butanol concentration in the untreated juices and after processing the juice by HIPEF or by heat treatments at day 0 (Table 2).

Regarding the storage, the initial 1-butanol content obtained in the HIPEF juice was maintained during the time. On the other hand, initial values achieved in the heated samples increased beyond day 35. After long periods at refrigeration temperature, an increase in 1-butanol concentrations can render an unpleasant flavour to the product, due its strong, pungent smell and taste (Boulton, 1995).

3.2.3. Linalool

Floral essences can be attributed to linalool, a terpene considered to be an important contributor to the fresh aroma of strawberry (Azodanlou et al., 2003).

The obtained results did not show differences between the concentrations of linalool (1.01–1.02 mg/100 mL) in the treated and untreated juices at day 0 ($p > 0.05$) (Table 2). Linalool concentration declined steadily in the thermally-treated juices beyond

Table 2
Effects of HIPEF treatment and thermal treatments, 90 °C for 60 s (TT 60 s) or for 30 s (TT 30 s) on the retention of hexanal, 1-butanol, linalool and DMHF (mean ± SD) ($n = 3$) of strawberry juice throughout storage at 4 °C.

Time of storage	Treatment	Aroma compound (mg/100 mL strawberry juice)			
		Hexanal	1-Butanol	Linalool	DMHF
0	Fresh	1.14^{aA}	1.02^{aA}	1.02^{aA}	0.75^{bA}
	HIPEF	1.01^{bA}	1.01^{aA}	1.02^{aA}	1.41^{aA}
	TT 60 s	1.00^{bA}	1.00^{aE}	1.00^{aA}	0.42^{dA}
	TT 30 s	1.02^{bA}	1.01^{aD}	1.01^{aA}	0.56^{c}
7	Fresh	1.01^{aB}	1.01^{aA}	1.00^{aA}	0.45^{bB}
	HIPEF	1.02^{aA}	1.02^{aA}	1.01^{aA}	1.03^{aB}
	TT 60 s	1.01^{aA}	1.01^{aE}	1.00^{aA}	0.42^{bA}
	TT 30 s	1.02^{aA}	1.00^{aD}	1.00^{aA}	0.41^{bA}
14	Fresh	1.00^{aB}	1.01^{aA}	1.00^{aA}	0.42^{aB}
	HIPEF	1.00^{aA}	1.00^{aA}	1.01^{aA}	0.42^{aC}
	TT 60 s	1.00^{aA}	1.00^{aE}	0.92^{bB}	0.41^{aA}
	TT 30 s	1.02^{aA}	1.01^{aD}	0.94^{bB}	0.41^{aA}
21	HIPEF	1.00^{aA}	1.00^{aA}	1.01^{aA}	0.42^{aC}
	TT 60 s	1.01^{aA}	1.01^{aE}	0.90^{bB}	0.41^{aA}
	TT 30 s	1.02^{aA}	1.02^{aD}	0.91^{bB}	0.41^{aA}
28	HIPEF	1.00^{aA}	1.00^{aA}	1.01^{aA}	0.42^{aC}
	TT 60 s	1.00^{aA}	1.01^{aE}	0.86^{cB}	0.41^{aA}
	TT 30 s	1.01^{aA}	1.00^{aD}	0.90^{bC}	0.41^{aA}
35	HIPEF	1.00^{aA}	1.00^{bA}	1.01^{aA}	0.42^{aC}
	TT 60 s	1.02^{aA}	1.10^{aD}	0.83^{bC}	0.41^{aA}
	TT 30 s	1.03^{aA}	1.07^{aC}	0.83^{bD}	0.41^{aA}
42	HIPEF	1.00^{aA}	1.02^{bA}	1.01^{aA}	0.42^{aC}
	TT 60 s	1.02^{aa}	1.14^{aC}	0.80^{bC}	0.41^{aA}
	TT 30 s	1.00^{aA}	1.11^{aB}	0.81^{bD}	0.41^{aA}
49	HIPEF	1.00^{aA}	1.00^{cA}	1.01^{aA}	0.42^{aC}
	TT 60 s	1.01^{aA}	1.20^{aB}	0.76^{cD}	0.41^{aA}
	TT 30 s	1.01^{aA}	1.13^{bB}	0.81^{bD}	0.41^{aA}
56	HIPEF	1.00^{aA}	1.00^{cA}	1.01^{aA}	0.42^{aC}
	TT 60 s	1.01^{aA}	1.25^{aA}	0.76^{bD}	0.41^{aA}
	TT 30 s	1.01^{aA}	1.18^{bA}	0.78^{bE}	0.41^{aA}

HIPEF = high-intensity pulsed electric fields treatments at 35 kV/cm for 1700 μs; bipolar 4-μs pulses at 100 Hz. Different lower case letter in the same column for each day indicate significant differences amongst treatments ($p < 0.05$). Different capital letters in the same column for each treatment correspond to significant differences with time ($p < 0.05$).

day 14, whereas in the HIPEF-processed juices the initial values were maintained throughout storage. In black currant juices, Von Sydow and Karlsson (1971) reported a decrease in linalool after heat treatments. This decrease during storage is related to the conversion of linalool to other terpenes via the hydration of double bounds, rearrangements and cyclization. The great retention of linalool in the HIPEF-treated strawberry juice over the time compared to heated sample could be associated to the relatively high levels of β-GLUC activity observed during storage (Fig. 2). Thus, linalool could be released from its glycosidic precursor by enzymatic hydrolysis carried out by β-GLUC (Vasserot, Arnaud, & Galzy, 1993).

3.2.4. DMHF

DMHF is one of the most important furanones that impact on strawberry flavour (Zabetakis & Holden, 1997). The content of DMHF in strawberry juice (0.75 mg/100 mL) increased just after the HIPEF treatment (1.41 mg/100 mL) (Table 2). However, thermal treatments at 90 °C for 30 s and for 60 s reduced DMHF in juices to 0.56 mg/100 mL and 0.42 mg/100 mL, respectively. Interestingly, the high DMHF release in HIPEF-treated samples coincided with an increase of β-GLUC activity after processing (Fig. 2). As suggested for linalool, β-GLUC may also play an important role in the emissions of DMHF in HIPEF-treated juices. According to Orruño et al. (2001), β-GLUC catalyses the hydrolysis of DMHF-glucoside present as the glycosylated form of DMHF. The release of free DMHF from its glucosidic precursor has an important effect on the flavour characteristic of strawberry.

The initial levels of DMHF achieved in the HIPEF-treated juice decreased up to 0.42 mg/100 mL at day 14, and was kept thereafter. However, the initial concentration in the thermally-treated juices did not vary throughout storage. In this way, the decrease in the activity of β-GLUC in HIPEF-processed samples during storage is likely to have an important effect on the reduction of DMHF concentration.

3.2.5. Esters

Esters are one of the most important volatile compounds in fruit flavour. Fruity, green grass and other flavour notes of strawberries are emanated by a complex mixture of esters (Pérez, Sanz, & Olias, 1993). The effects of processing and storage time on the amount of esters in strawberry juices are shown in Table 3. In general, initial ester concentrations found in the fresh juice were significantly reduced by the applied treatments ($p < 0.05$). The substantial losses of butyl acetate and methyl hexanoate compounds were the most remarkable changes observed just after processing (Table 3). Methyl butanoate in HIPEF-treated juice was found in higher concentration (0.28 mg/100 mL) than in samples treated at 90 °C for 60 s (0.16 mg/100 mL). In the same way, ethyl butanoate was released in higher proportion after processing the juice by HIPEF (1.20 mg/100 mL) than after applying heat treatments for 30 s (0.85 mg/100 mL) or 60 s (1.02 mg/100 mL). However, no differences in the content of ethyl hexanoate, butyl butanoate and hexyl acetate ($p > 0.05$) between processed juices were observed at day 0. The literature concerning the effect of HIPEF on strawberry juice flavour is scarce. In orange juice, Jia et al. (1999) observed that

Table 3
Effects of HIPEF treatment and thermal treatments, 90 °C for 60 s (TT 60 s) or for 30 s (TT 30 s) on the retention of esters (mean ± SD) ($n = 3$) of strawberry juice throughout storage at 4 °C.

Time of storage	Treatment	Aroma compound (mg/100 mL strawberry juice)						
		Methyl butanoate	Ethyl butanoate	Ethyl hexanoate	Butyl butanoate	Hexyl acetate	Butyl acetate	Methyl hexanoate
0	Fresh	0.76^{aA}	1.93^{aA}	1.54^{aA}	1.04^{aA}	0.55^{aA}	2.81^{aA}	1.57^{aA}
	HIPEF	0.28^{cA}	1.20^{bA}	1.51^{bA}	1.01^{bA}	0.50^{bA}	1.69^{bA}	1.01^{cB}
	TT 60 s	0.16^{dC}	1.02^{cB}	1.51^{bC}	1.00^{bA}	0.50^{bA}	1.68^{bB}	1.01^{cB}
	TT 30 s	0.37^{bA}	0.85^{dA}	1.51^{bA}	1.01^{bA}	0.50^{bA}	1.70^{bC}	1.08^{bA}
7	Fresh	0.03^{bB}	1.34^{aB}	1.52^{aB}	1.03^{aA}	0.50^{aB}	1.69^{bB}	1.01^{aB}
	HIPEF	0.07^{bB}	1.15^{bA}	1.53^{aA}	1.01^{bA}	0.50^{aA}	1.73^{aA}	1.01^{aB}
	TT 60 s	0.23^{aA}	1.07^{cB}	1.50^{aC}	1.00^{bA}	0.50^{aA}	1.68^{bB}	1.02^{aB}
	TT 30 s	0.26^{aB}	0.57^{dC}	1.50^{aA}	1.01^{bA}	0.50^{aA}	1.67^{bC}	0.64^{bD}
14	Fresh	0.09^{bB}	1.15^{aC}	1.52^{aB}	1.01^{aB}	0.50^{aB}	1.68^{aB}	1.02^{aB}
	HIPEF	0.07^{bB}	1.16^{aA}	1.50^{aA}	1.00^{aA}	0.50^{aA}	1.68^{aA}	1.00^{aB}
	TT 60 s	0.14^{aC}	1.03^{bB}	1.50^{aC}	1.00^{aA}	0.50^{aA}	1.67^{aB}	1.01^{aB}
	TT 30 s	0.15^{aC}	0.79^{cB}	1.51^{aA}	1.01^{aA}	0.50^{aA}	1.69^{aC}	1.01^{aB}
21	HIPEF	0.01^{bC}	1.16^{aA}	1.50^{aA}	1.00^{aA}	0.50^{aA}	1.67^{aA}	1.00^{aB}
	TT 60 s	0.31^{aA}	1.02^{bB}	1.50^{aC}	1.00^{aA}	0.50^{aA}	1.67^{aB}	1.00^{aB}
	TT 30 s	0.09^{bD}	0.74^{cB}	1.51^{aA}	1.01^{aA}	0.50^{aA}	1.70^{aC}	0.98^{aB}
28	HIPEF	0.01^{bC}	1.01^{aB}	1.50^{aA}	1.00^{aA}	0.50^{aA}	1.67^{aA}	1.00^{aB}
	TT 60 s	0.08^{aD}	1.01^{aB}	1.50^{aC}	1.00^{aA}	0.51^{aA}	1.68^{aB}	1.00^{aB}
	TT 30 s	0.09^{aD}	0.60^{bC}	1.50^{aA}	1.00^{aA}	0.50^{aA}	1.67^{aC}	0.68^{bD}
35	HIPEF	0.01^{aC}	1.09^{aB}	1.50^{aA}	1.00^{aA}	0.50^{aA}	1.68^{cA}	1.00^{aB}
	TT 60 s	0.01^{aE}	1.15^{aA}	1.50^{aC}	1.01^{aA}	0.52^{aA}	1.80^{bA}	1.02^{aA}
	TT 30 s	0.02^{aE}	0.64^{bC}	1.51^{aA}	1.01^{aA}	0.50^{aA}	1.87^{aB}	0.78^{bC}
42	HIPEF	0.01^{aC}	1.01^{bB}	1.50^{aA}	1.01^{aA}	0.50^{aA}	1.67^{bA}	1.09^{aA}
	TT 60 s	0.01^{aE}	1.15^{aA}	1.51^{aC}	1.01^{aA}	0.50^{aA}	1.80^{aA}	1.03^{bA}
	TT 30 s	0.02^{aE}	0.64^{cC}	1.51^{aA}	1.01^{aA}	0.50^{aA}	1.69^{bC}	0.79^{cC}
49	HIPEF	0.01^{aC}	1.01^{bB}	1.50^{bA}	1.01^{aA}	0.50^{aA}	1.67^{cA}	1.08^{aA}
	TT 60 s	0.01^{aE}	1.17^{aA}	1.72^{aA}	1.01^{aA}	0.50^{aA}	1.80^{aA}	1.05^{aA}
	TT 30 s	0.02^{aE}	0.61^{cC}	1.51^{bA}	1.01^{aA}	0.50^{aA}	1.72^{bC}	0.58^{bE}
56	HIPEF	0.01^{aC}	1.01^{aB}	1.50^{aA}	1.01^{aA}	0.50^{aA}	1.67^{cA}	1.07^{aA}
	TT 60 s	0.01^{aE}	1.03^{aB}	1.55^{aB}	1.00^{aA}	0.50^{aA}	1.80^{bA}	1.01^{bB}
	TT 30 s	0.02^{aE}	0.61^{bC}	1.52^{aA}	1.02^{aA}	0.50^{aA}	1.95^{aA}	0.77^{cC}

HIPEF = high-intensity pulsed electric fields treatments at 35 kV/cm for 1700 μs; bipolar 4-μs pulses at 100 Hz. Different lower case letter in the same column for each day indicate significant differences amongst treatments ($p < 0.05$). Different capital letters in the same column for each treatment correspond to significant differences with time ($p < 0.05$).

ethyl butanoate was completely lost after HIPEF treatment (30 kV/cm for 240 or 480 μs, 1 KHz of frequency and 2 μs of pulse duration). However, Cserhalmi, Sass-Kiss, Tóth-Markus, and Lechner (2006) reported that this compound was not significantly affected when orange juice was HIPEF-treated using 50 bipolar pulses of 2 μs at 28 kV/cm. The amounts of volatile compounds typically decrease with an increase in processing temperature. Thus, low processing temperatures during HIPEF treatment (below 35 °C) may result in a higher retention of flavour compounds compared with the dramatic changes occurring in the thermally-treated juices (Yeom et al., 2000).

In general, ester concentrations achieved after treating the juices were maintained during storage, except for methyl butanoate and butyl acetate. Treated juices underwent a substantial loss of methyl butanoate during 21 days of storage. flavour deterioration may be associated with a number of reactions during storage, such as ascorbic acid degradation or nonezymatic browning, which could promote flavour changes in pasteurized juices (Kaanane, Kane, & Labuza, 1988).

On the other hand, thermally-treated juices showed a significant increase in the amount of butyl acetate beyond day 35. Increase in butyl acetate is associated with the availability of 1-butanol, which is readily accepted to butyryl-CoA, catalysing the production of this ester (Azodanlou et al., 2003; Bood & Zabetakis, 2002). Thus, the accumulation of 1-butanol in the heated samples observed at the end of storage (Table 2) could have caused the release of butyl acetate in those juices (Table 3).

4. Conclusions

HIPEF-treated strawberry juice retained higher aroma-related enzyme activities compared to thermally-treated juices during storage. HIPEF (35 kV/cm for 1700 μs using pulses of 4 μs at 100 Hz in bipolar mode) and thermal treatments (90 °C for 30 s and 60 s) led to a significant reduction of the major volatile compounds in relation to the untreated juice. However, HIPEF processing seemed to reduce the loss of methyl butanoate and ethyl butanoate, compared to the thermal treatments. In addition, HIPEF-processed juice enhanced the concentration of DMHF after the treatment and up to the day 14, which is the most important flavour compound of strawberry juice. In contrast to the HIPEF-treated juice, a decrease in the linalool concentration and an increase in the production of 1-butanol was observed in thermally-treated juices, which could render an unpleasant flavour to the product. Hence, HIPEF could help to obtain strawberry juices with improved flavour quality for at least 14 days. However, it is advisable to carry out further research on the chemistry of flavour components in order to deepen the understanding the mechanisms involved in their synthesis.

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