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Shelf life and sensory attributes of a fruit smoothie-type beverage processed with moderate heat and pulsed electric fields

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ABSTRACT

Smoothie-type beverages are usually preserved by mild pasteurisation (MP) but combining moderate heat (*H*) and pulsed electric fields (PEF) could represent an alternative technique achieving similar, or better, microbiological safety and shelf life, and possibly lowering the thermal impact on physical and sensory product properties. Following *H* (55 °C after 60 s) and PEF (34 kV/cm, 60 μs) the microbiological shelf life of smoothie stored at 4 °C was extended over that of mildly pasteurised (72 °C, 15 s) smoothie (21 vs. 14 days ($P < 0.05$), respectively). Similar trends were obtained for both treatments regarding the shelf life stability of smoothie pH and conductivity ($P \geq 0.05$). H/PEF-treated smoothie achieved better stability of Brix and viscosity than MP ($P < 0.05$) while better colour stability was obtained for the latter than for H/PEF-treated smoothie ($P < 0.05$). Sensory evaluation of untreated, MP-treated and H/PEF-treated smoothie indicated comparable overall acceptability of the products ($P \geq 0.05$). Due to superior microbiological shelf stability and generally comparable attributes of the smoothie-type beverage H/PEF preservation proved to be a feasible processing alternative to fruit smoothie treatment with MP.

1. Introduction

Minimal processing of liquid foods aims at comparable or better microbial product safety and stability than obtained by conventional pasteurisation while better maintaining sensory and nutritional attributes of the product due to the reduced thermal impact. The application of pulsed electric fields (PEF), a non-thermal technology which represents a possible processing alternative to thermal pasteurisation for liquid food preservation, has been studied by various research groups worldwide (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999; Braakman, 2003) for more than a decade. It is generally accepted that PEF achieves food preservation by irreversible poration of the cell membranes of microorganisms (Dimitrov, 1984; Zimmermann, Pilwat, & Riemann, 1974) and that additional heating prior to PEF could enhance the overall germicidal effect. Moreover, it has been reported in some studies that a possible treatment synergism could be obtained when heating and PEF are combined (Amiali, Ngadi, Smith, & Raghavan, 2007; Evrendilek & Zhang, 2003; Heinz, Toepfl, & Knorr, 2003; Li, Zhang, Jin, Turek, & Lau, 2005). Although there has been a particular emphasis on research investigating heat in combination with PEF treatment in the past and PEF shelf life studies have been carried out with milk (Fernández-Molina, Barbosa-Cánovas, & Swanson, 2005; Qin et al., 1995) and single juices (Evrendilek et al., 2000; Hodgins, Mittal, & Griffiths, 2002), to our knowledge, no study on the shelf life of multiple-juice beverages treated with heat and PEF has been reported to date, in spite of the availability of studies on multiple-juice and juice-milk beverages treated with PEF (Rivas, Rodrigo, Martinez, Barbosa-Cánovas, & Rodrigo, 2006; Sharma, Zhang, & Chism, 1998). In addition, few sensory analyses studying the impact of a combined heat and PEF treatment on beverages are available in the literature (Evrendilek, Yeom, Jin, & Zhang, 2004; Min & Zhang, 2003). Smoothie-type beverages, originally consisting purely of fresh fruit and juice, were first introduced in the 1960s and re-emerged in the 2000s (Titus, 2008) as part of a trend towards health-promoting nutrition (McCorquodale, Damian, Richardson, & Gee, 2006; Robinson & Ogawa, 1999). In recent years smoothies have rapidly increased in popularity (i.e. product growth rising 2.39 times from 2002 to 2007 according to food merchandisers) (Lal, 2007) and a loyal consumer base has developed (Lockwood, 2008).

In the present study, which is linked to previous research (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2008), the main objective was to investigate the impact of moderate heat and PEF (H/PEF) on the microbiological shelf life and on the shelf life

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stability of selected quality parameters of a fruit smoothie-type beverage. A second objective was the analysis of sensory characteristics of the fruit smoothie in a consumer acceptability study.

2. Materials and methods

2.1. Fruit smoothie preparation and processing equipment

A smoothie-type beverage was prepared from juiced (Model No. Hinari JEP311, Alba Plc., Elstree, UK) fresh fruits (50 g of pineapples, 28 g of bananas, 12 g of apples and 3 g of oranges per 100 g of smoothie) and coconut milk (7 g per 100 g of smoothie) by blending (Model No. New York Smoothie SB200 Series, Kenwood Ltd., Havant, UK) the ingredients in the same proportions and under the same preparation conditions as described by Walkling-Ribeiro, Noci, Cronin, Lyng, et al. (2008). Product uniformity was ensured by choosing fruit from the same importer and origin.

For moderate heating the smoothie was pumped (Model No. SR25 S300, ESSKA Maschinen GmbH, Hamburg, Germany) through a heating coil submerged in a heated water bath (Model No. Lauda E103 Ecoline, Lauda GmbH & Co. KG, Lauda-Königshofen, Germany) and then cooled in a coil submerged in a refrigerated water bath (Model No. Viscotherm VT100, Physica, Stuttgart, Germany), thereby minimising the thermal exposure of the beverage. Subsequent PEF treatment was carried out in a lab scale system as described by Walkling-Ribeiro, Noci, Cronin, Riener, et al. (2008) and as outlined in Fig. 1. The latter consisted of a PEF unit (C-Tech Innovation Ltd., Capenhurst, UK) generating 1 μs-square-wave pulses at frequencies up to 25 Hz in a custom-built PEF treatment chamber (electrode area and gap of 7×10^{-4} m^2 and 2.5 mm, respectively), a high-voltage probe (Model No. P6015A, Tektronix, Beaverton, OR, USA) for pulse monitoring and a digital oscilloscope (Model No. TDS 2012, Tektronix, Beaverton, OR, USA) for evaluation of the pulse data. Mild pasteurisation (MP) was carried out using a pilot scale tubular heat exchanger (Model No. FT74 UHT/HTST Processing System, Armfield Technical Education Co. Ltd., Ringwood, UK). This thermal control treatment was applied in order to determine the feasibility of the H/PEF preservation method as a possible alternative to mild thermal pasteurisation of fruit smoothie.

2.2. Preservation of fruit smoothie by moderate heat and pulsed electric fields (H/PEF)

Smoothie passed through a heating coil over 60 s which raised the product temperature from 15 °C (inlet) to 55 °C (outlet). The product was cooled to 10 °C, and subsequently entered the PEF chamber at 15 °C. PEF treatment was applied at 34 kV/cm for 60 μs inducing a specific energy input of 650 kJ/L, with the product temperature increasing to 55 °C at the chamber exit. The product was then cooled to 10 °C by passing through a further cooling coil (submerged in a refrigerated bath at 0 °C). Samples were placed in iced water prior to analysis.

2.3. Preservation of fruit smoothie by mild thermal pasteurisation

For the thermal control treatment fruit smoothie was pasteurised at 72 °C for a holding time of 15 s using a flow rate of 180 mL/min. At the heat exchanger outlet the product temperature was 15 °C which was decreased further as the samples were placed in iced water before analysis.

2.4. Microbiological shelf life evaluation

The processed smoothie samples were stored in a refrigerated room at 4 ± 0.1 °C for the assessment of its microbiological shelf stability over a period of 28 days. In accordance with the legislation of the European Union for fruit juices (EU, 2005) microbiological shelf life was considered acceptable until 10^3 $\log_{10}$ colony forming units (CFU) per mL were exceeded. Triplicate product samples obtained after H/PEF or mild pasteurisation treatments were stored in pre-sterilised screw-top glass bottles (30 mL). Total bacteria counts (TBC) and total yeasts and moulds counts (TYMC) were analysed for both treatments on day 0, 1, 7, 14, 21 and 28. Triplicate samples of untreated smoothie served as a control and were analysed on day 0. For TBC and TYMC plates tryptone soy agar (TSA) (CM0131, Oxoid Ltd., Basingstoke, Hampshire, UK) and potato dextrose agar (PDA) (CM0139, Oxoid Ltd., Basingstoke, Hampshire, UK) were inoculated, correspondingly, using either 100 μL of sampled juice or its serial dilutions in Ringers solution (BR0052G, Oxoid Ltd., Basingstoke, Hampshire, UK). Following incubation of TSA and PDA at 37 °C for 24 h and at 25 °C for 120 h, respectively, the CFU were counted.

2.5. Shelf life evaluation of selected quality parameters

Selected quality parameters namely conductivity, soluble solids, pH, viscosity and colour were measured before and after processing and throughout the 28 day shelf life period under the sampling and storage conditions as outlined in Section 2.4, using a conductivity meter (Model No. Cyberscan CON 400 Series, Eutech Instruments, Singapore), a hand-held refractometer (Bellingham and Stanley Ltd.,

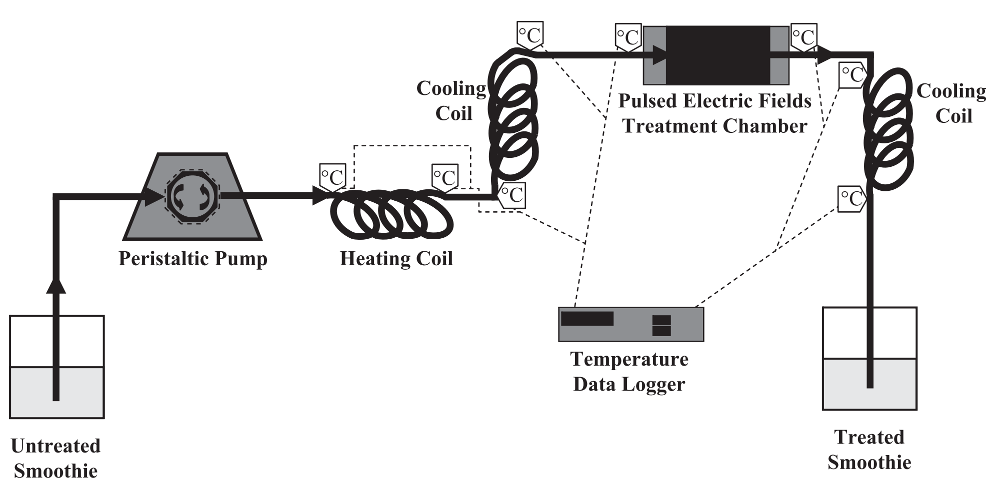

Fig. 1. Schematic layout of a processing system for a smoothie-type beverage by a combination of moderate heat and pulsed electric fields.

Tunbridge Wells, UK), a pH-meter (Model No. 9450, Pye-Unicam, Cambridge, UK), a dynamic stress rheometer (Model No. Rheometric Scientific SR-2000, Rheometrics Inc., Piscataway, NJ, USA) and a tristimulus colorimeter (Model No. CR 300, Minolta Co. Ltd., Osaka, Japan), respectively. For viscosity measurements smoothie samples were placed in a couette (cup diameter of 32 mm, bob diameter and length of 29.5 mm and 44.25 mm, respectively) and stress sweeps were applied in a range from 0.25 to 32.0 Pa at 25 °C (with delay, pre-shearing, steady state and strain limit modes turned off) allowing up to 20 s per data point. The total colour difference (ΔE) was determined using the measured colour attributes (i.e. lightness (L^*), redness (a^*) and yellowness (b^*) in Hunter L^* a^* b^* colour space) with the following equation:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

2.6. Sensory analysis

Consumer acceptability of the fruit smoothie was investigated by a panel of 35 (19 female, 16 male) untrained assessors between 18 and 65 years of age. Following the processing preservation treatment smoothie samples were refrigerated, randomly coded and served at 15 °C in aliquots of 20 mL together with non-salted table biscuits and still water (Ballygowan, Ireland) to panellists placed separately in booths for unbiased evaluation of sensory attributes. A comparison between untreated, H/PEF-treated and thermally pasteurised fruit smoothie was carried out estimating product-specific colour, odour, sweetness, acidity, flavour and overall acceptability on a 9 point hedonic scale. The lowest and highest scores for colour, odour, flavour and overall acceptability were 1 and 9, respectively, while optimum thickness, sweetness and acidity were located at the middle of the scale (5). Moreover, flavour characteristics were assessed by the panellists in terms of possible bland, cooked, artificial, pungent and/or metallic flavour.

2.7. Statistical evaluation

The statistical analysis of the experimental data was accomplished with SigmaStat Version 3.1 (SigmaStat, Systat Software Inc., London, UK). A two-way analysis of variance (ANOVA) was performed with preservation treatment and storage time as factors. Statistical differences between the means were indicated by $P < 0.05$ and each treatment was conducted on triplicate batches ($n = 3$).

3. Results and discussion

3.1. Stability of microbial and selected quality parameters of H/PEF-treated smoothie over the shelf life

3.1.1. Bacteria, yeast and mould stability

Initial TBC in fruit smoothie were 5.3 $\log_{10}$ CFU/mL prior to preservation treatments. Following H/PEF treatment and mild thermal preservation native bacteria were reduced, TBCs being 1.9 and 1.8 $\log_{10}$ CFU/mL, respectively ($P \geq 0.05$) as shown in Table 1. Bacterial numbers in smoothie following conventional pasteurisation increased at a similar rate to those treated with H/PEF up to 21 days of storage. At 21 days, TBCs were 2.8 and 3.3 $\log_{10}$ CFU/mL for the H/PEF and conventional treatments, respectively ($P < 0.05$), the latter exceeding the acceptable limit of $\leq$3.0 $\log_{10}$ CFU/mL (TBC), as defined by EU legislation (EU, 2005). Microbiological shelf life of H/PEF-treated smoothie had expired by 28 days (3.4 $\log_{10}$ CFU/mL).

The TYMC in the fruit smoothie prior to the treatment was 5.1 $\log_{10}$ CFU/mL. After H/PEF and thermal preservation the TYMC's were decreased to 1.1 and 1.4 $\log_{10}$ CFU/mL ($P < 0.05$), respectively (Table 1). Over the duration of the shelf life study the TYMC following either preservation treatment did not exceed the acceptable limit of 3.0 $\log_{10}$ CFU/mL. However, at 28 days of storage lower TYMC were obtained for H/PEF-treated than for mildly pasteurised samples (1.9 vs. 2.5 $\log_{10}$ CFU/mL; ($P < 0.05$)), thus indicating a better microbiological shelf life for H/PEF-treated than for mildly pasteurised smoothies.

Table 1
Total bacteria counts (TBC) and total yeasts and moulds counts (TYMC) obtained in fruit smoothie before and after combined moderate heat (H; 55 °C, 60 s) and pulsed electric fields (PEF; 34 kV/cm for 60 μs) treatment (H/PEF) or mild conventional pasteurisation (MP; 72 °C, 15 s) following refrigerated storage at 4 °C for up to 28 days.

	TBC [$\log_{10}$ CFU/mL]	TYMC [$\log_{10}$ CFU/mL]
Untreated	5.3^a	5.1^a
H/PEF$_0$	1.9^b	1.1^b
H/PEF$_1$	1.6^b	1.7^b
H/PEF$_7$	2.0^b	1.6^b
H/PEF$_{14}$	2.5^b	2.1^b
H/PEF$_{21}$	2.8^b	2.0^b
H/PEF$_{28}$	3.4^b	1.9^b
MP$_0$	1.8^b	1.4^b
MP$_1$	1.9^b	1.7^b
MP$_7$	1.9^b	1.4^b
MP$_{14}$	2.5^b	1.9^b
MP$_{21}$	3.3^a	1.9^b
MP$_{28}$	3.5^b	2.5^a
SED	0.12	0.09
P	**	*

SED represents the standard error of difference between treatment means. *P* stands for the statistical probability based on confidence intervals applied.
Subscripted numbers specify the storage day on which the samples were analysed.
[a,b] Different superscripted letters in the same column indicate a statistical difference between means of the different treatments on the same storage day.
* and ** refer to a statistical significance of $P < 0.05$ and $P < 0.01$, respectively.

In a study by Evrendilek et al. (2000) a combined PEF (35 kV/cm for 94 μs) and heat (60 °C for 30 s) treatment of fresh apple cider led to shelf life of more than 67 days when stored at 4 °C. For cranberry juice treated with heat (60 °C for 32 s) and PEF (32 kV/cm for 47 μs) a shelf life of 197 days with storage at 4 °C was observed (Evrendilek et al., 2001). Other studies on cranberry juice using more severe PEF treatments of 35 kV/cm for 195 μs (Jin et al., 1998) and 40 kV/cm for 150 μs (Jin & Zhang, 1999) reported TBC and TYMC reductions of more than 4.0 and approximately 5.0 $\log_{10}$ CFU/mL (compared to 3.4 $\log_{10}$ CFU/mL for TBC and 4.0 $\log_{10}$ CFU/mL for TYMC in the present study), respectively. The former study (Jin et al., 1998) allowed a shelf life of 8 months while for the latter study (Jin & Zhang, 1999) a growth inhibition of yeasts and moulds up to 14 days was reported, when juice was stored at 4 °C. Whey-protein fortified orange juice (Sharma et al., 1998), freshly squeezed orange juice (Jia, Zhang, & Min, 1999) and tomato juice (Min, Jin, & Zhang, 2003) exposed to stand-alone PEF treatment (32 kV/cm for 92 μs, 30 kV/cm for 240 μs, and 40 kV/cm for 57 μs, respectively) were stored at 4 °C and were microbiologically stable for 5 months, 6 weeks and approximately 80 days. Although most of the above results reported longer microbiological shelf life compared to the findings of the present study it has to be taken into account that the fruit smoothie used in this study consisted of a multiple blend of fresh juices, rendering it more viscous, thus impeding the preservation process to a greater extent than for single juices (Rodrigo, Barbosa-Cánovas, Martínez, & Rodrigo, 2003). Rivas et al. (2006) reported a shelf stable, fresh orange carrot juice processed with PEF (25 kV/cm, 280 μs) and stored subsequently at 2 °C for a period of 4 weeks which is in agreement with the findings of the present study. Moreover, the present study aimed at achieving minimal processing of the fruit smoothie by choosing the same processing parameters as used in an earlier study (Walkling-Ribeiro, Noci, Cronin, Lyng, et al., 2008) which ensured microbiological safety in line with the US Food and Drug Administration recommendations (FDA, 2001), while the impact of the preservation treatment on the product was minimised (i.e. using a lower overall treatment intensity than reported for most heat and PEF treatments in the literature) in order to retain natural product quality.

3.1.2. Stability of pH, °Brix, conductivity, viscosity and colour

Prior to H/PEF or thermal preservation smoothie pH, °Brix, conductivity and viscosities at low and high stress were measured obtaining 4.30, 15.0, 4.62 mS/cm, 0.018 and 1465 Pa s, respectively. A general decrease in pH of H/PEF-treated and pasteurised smoothie was observed over the duration of the shelf life study (Table 2) leading to pH values of 4.16 and 4.21 on day 28, respectively ($P < 0.05$). The soluble solids content remained stable after H/PEF treatment for 28 days ($P \geq 0.05$) while mild conventional pasteurisation led to a rapid decrease in soluble solids between day 1 (15.0 °Brix) and day 28 (11.0 °Brix) of shelf life storage ($P < 0.05$). With regard to conductivity a significant reduction was obtained 7 days after H/PEF treatment (4.49 mS/cm; $P < 0.05$) but stabilised at this level until day 28 of the shelf life study. By contrast, mild smoothie pasteurisation caused an immediate drop of conductivity on day 0 (4.33 mS/cm; $P < 0.05$) which was more pronounced after 28 days of storage when a conductivity of 3.97 mS/cm was obtained. A comparison of both preservation treatments for the product viscosity at low and medium stress levels (0.25 and 2.5 Pa, respectively) generally showed slightly, yet not significant, higher values for H/PEF-treated samples than for mildly pasteurised smoothie over the duration of the shelf life study. When a higher stress (≥24.6 Pa) was induced in smoothie samples H/PEF-treated product retained its structural integrity at the highest stress rate (32.0 Pa) throughout the shelf life study. In contrast, smoothie treated with MP reached the threshold viscosity value (0.015 Pa s, which had been established to prevent possible overloading of the rheometer at higher stress rates) when a lower stress level of 24.6 Pa was applied to thermally pasteurised smoothie, stored for 1 day or more, indicating lower structural resistance of the MP-treated smoothie ($P < 0.05$). Similar trends were observed for colour attributes (Table 3) following H/PEF or pasteurisation. Colorimetric measurements of smoothie prior to both treatments yielded 55.94, 0.20 and 19.55 for L^*, a^* and b^*, respectively. Both treatments generally caused a decrease in L^* and b^* compared to untreated smoothie over the duration of the shelf life study ($P < 0.05$). For H/PEF-treated fruit smoothie after an initial drop in a^* an increase above that of untreated smoothie was noticed after 28 days ($P < 0.05$). Following a decline in a^* MP-treated smoothie showed no difference in a^* to that of untreated smoothie at the end of the shelf life study ($P \geq 0.05$). ΔE values (Table 3) for H/PEF-treated smoothie were similar to the values obtained for the thermally pasteurised product on the same day ($P \geq 0.05$). However, ΔE increased over the storage time for both H/PEF-treated and MP-treated smoothie ($P < 0.05$).

Few studies have examined the shelf life stability of pH, conductivity, viscosity or colour following heat and PEF treatment. In a study by Cortés, Esteve, Frígola, and Torregrosa (2005), which

Table 3
Lightness (L^*), yellowness (a^*), redness (b^*) and total colour difference (ΔE) in fruit smoothie samples before and after moderate heat (H; 55 °C, 60 s) and pulsed electric fields (PEF; 34 kV/cm, 60 μs) treatment (H/PEF) or mild conventional pasteurisation (MP; 72 °C, 15 s) following storage at 4 °C for up to 28 days.

	L^*	a^*	b^*	ΔE
Untreated	55.94^{a}	0.20^{a}	19.55^{a}	N/A
H/PEF$_0$	54.51^{abc}	-0.54^{b}	18.19^{b}	2.1^{a}
H/PEF$_1$	54.43^{abc}	-0.45^{b}	18.39^{ab}	2.0^{a}
H/PEF$_7$	54.00^{b}	-0.16^{ab}	17.86^{b}	2.6^{a}
H/PEF$_{14}$	54.87^{ab}	0.23^{a}	17.31^{b}	2.5^{a}
H/PEF$_{21}$	53.31^{bc}	0.32^{a}	15.21^{c}	5.1^{b}
H/PEF$_{28}$	53.36^{bc}	$+0.68^{b}$	14.04^{d}	6.1^{b}
MP$_0$	55.67^{a}	-0.12^{a}	18.48^{ab}	1.2^{a}
MP$_1$	55.18^{ab}	-0.02^{a}	18.26^{ab}	1.5^{ab}
MP$_7$	55.14^{ab}	0.08^{a}	18.23^{ab}	1.6^{ab}
MP$_{14}$	55.03^{abc}	-0.22^{a}	17.42^{b}	2.4^{ab}
MP$_{21}$	53.41^{bc}	-0.13^{a}	15.61^{bc}	4.7^{b}
MP$_{28}$	54.13^{b}	0.10^{a}	15.08^{bc}	4.8^{b}
SED	0.166	0.059	0.271	0.48
P	**	***	***	***

SED represents the standard error of difference between treatment means. *P* stands for the statistical probability based on confidence intervals applied.
Subscripted numbers indicate the storage day on which the samples have been analysed.
[a, b, c, d] Different superscripted letters in the same column indicate a statistical difference between means of the same treatment over the storage period.
** and *** refer to a statistical significance of $P < 0.01$ and $P < 0.001$, respectively; N/A stands for 'not applicable'.

Table 2
Changes in selected quality parameters such as pH, °Brix, conductivity (σ) and stress dependent viscosity before and after preservation of fruit smoothie with moderate heat (H; 55 °C, 60 s) and pulsed electric fields (PEF; 34 kV/cm, 60 μs) treatment (H/PEF) or mild conventional pasteurisation (MP; 72 °C, 15 s) following storage at 4 °C for up to 28 days.

	pH	Brix	σ [mS/cm]	Low stress		Intermediate stress		High stress	
				Stress [Pa]	Viscosity [Pa s]	Stress [Pa]	Viscosity [Pa s]	Stress [Pa]	Viscosity [Pa s]
Untreated	4.30^{a}	15.0^{a}	4.62^{a}	0.25^{a}	1465^{a}	2.49^{a}	0.740^{a}	31.9^{a}	0.018^{a}
H/PEF$_0$	4.31^{a}	15.0^{a}	4.62^{a}	0.25^{a}	1472^{a}	2.50^{a}	0.563^{a}	31.9^{a}	0.018^{a}
H/PEF$_1$	4.32^{a}	14.8^{a}	4.61^{a}	0.25^{a}	1495^{a}	2.49^{a}	0.395^{a}	31.9^{a}	0.017^{a}
H/PEF$_7$	4.23^{b}	14.5^{a}	4.49^{b}	0.25^{a}	1829^{a}	2.50^{a}	0.541^{a}	31.9^{a}	0.017^{a}
H/PEF$_{14}$	4.20^{b}	14.8^{a}	4.52^{b}	0.25^{a}	1127^{a}	2.49^{a}	0.938^{a}	32.0^{a}	0.017^{a}
H/PEF$_{21}$	4.22^{b}	15.0^{a}	4.55^{ab}	0.25^{a}	892^{a}	2.49^{a}	1.082^{a}	32.0^{a}	0.017^{a}
H/PEF$_{28}$	4.16^{c}	15.0^{a}	4.48^{b}	0.25^{a}	752^{a}	2.49^{a}	0.852^{a}	31.9^{a}	0.017^{a}
MP$_0$	4.35^{a}	15.0^{a}	4.33^{a}	0.25^{a}	1153^{a}	2.50^{a}	0.243^{a}	32.0^{a}	0.017^{a}
MP$_1$	4.33^{a}	12.7^{ab}	4.29^{a}	0.25^{a}	1381^{a}	2.49^{a}	0.234^{a}	24.6^{b}	0.016^{a}
MP$_7$	4.24^{b}	12.7^{ab}	4.14^{b}	0.25^{a}	1505^{a}	2.50^{a}	0.921^{a}	24.7^{b}	0.016^{a}
MP$_{14}$	4.23^{b}	10.8^{ab}	4.03^{c}	0.25^{a}	453^{a}	2.45^{a}	0.762^{a}	24.6^{b}	0.015^{b}
MP$_{21}$	4.24^{b}	11.0^{ab}	4.10^{bc}	0.25^{a}	208^{a}	2.47^{a}	0.431^{a}	24.8^{b}	0.015^{b}
MP$_{28}$	4.21^{c}	10.5^{b}	3.97^{cd}	0.25^{a}	511^{a}	2.46^{a}	0.190^{a}	24.8^{b}	0.015^{b}
SED	0.009	0.29	0.038	4.307×10^{-5}	120.8	0.068	4.160×10^{-3}	0.559	1.1817×10^{-3}
P	***	***	***	NS	NS	NS	NS	***	**

Threshold value of 0.015 Pa s was applied for viscosity measurements in order to avoid possible overloading of the rheometer. SED represents the standard error of difference between treatment means. *P* stands for the statistical probability based on confidence intervals applied.
Subscripted numbers specify the storage day on which the samples were analysed.
[a, b, c, d] Different superscripted letters in the same column indicate a statistical difference between means of the same treatment over the storage period.
** and *** refer to a statistical significance of $P < 0.01$ and $P < 0.001$, respectively; NS indicates no statistical significance.

investigated the effect of PEF (up to 35 kV/cm for 100 μs) on horchata, a pH reduction (from 6.48 to 5.52) was reported over a storage period of 5 days which is broadly in agreement with the findings of this study. Increased microbial activity was suggested by the researchers (Cortés et al., 2005) to be responsible for the rapid decline in pH but in the present study the microbial growth following either preservation treatment was limited. Rivas et al. (2006) also reported a decrease in pH in the last week of their shelf life examination of blended orange and carrot juice and suggested microbial growth as cause. However, other researchers (Yeom, Streaker, Zhang, & Min, 2000a) reported no changes in pH of orange juice after PEF treatment when stored at 4 °C for up to 112 days and, in most studies (Charles-Rodriguez, Nevarez-Moorillon, Zhang, & Ortega-Rivas, 2007; Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006; Walkling-Ribeiro, Noci, Cronin, Lyng, et al., 2008) no immediate change in pH of fruit juice beverages following exposure to PEF or heat and PEF was obtained. The findings on soluble solids content in the present study coincide with the results of Ayhan, Yeom, Zhang, and Min (2001) and Rivas et al. (2006) indicating no change in °Brix of stored juice beverages (orange juice or blended orange carrot juice) stored at 2 °C after PEF treatment (35 kV/cm for 59 μ or up to 25 kV/cm for 330 μs) for up to 70 or 112 days, respectively. In a study by Cortés, Esteve, and Frígola (2008) no effect of PEF (30 kV/cm for 100 μs) or thermal pasteurisation (90 °C for 20 s) on °Brix of fresh orange juice stored at 2 °C for 7 weeks were obtained. Cserhalmi et al. (2006) obtained significant changes in the conductivity of grapefruit and orange juice after PEF treatment but for lemon and tangerine juice no variations in conductivity were reported which is in line with findings of the present and an earlier study (Walkling-Ribeiro, Noci, Cronin, Lyng, et al., 2008). A study by Aguiló-Aguayo, Soliva-Fortuny, and Martín-Belloso (2008) investigating the viscosity of tomato juice following PEF treatment and thermal treatment indicated a greater decrease in the viscosity of thermally pasteurised than of PEF-treated juice during 30 days of storage at 4 °C. Min, Jin, and Zhang (2003) found no statistical difference between the viscosities of untreated and PEF-treated tomato juice which is also in agreement with the findings obtained in the present study. However, the same researchers (Min, Jin, & Zhang, 2003) obtained different results to those of this study as the viscosities of untreated and thermally pasteurised tomato juice were found to be comparable which the authors associated with effective inactivation of the pectic enzymes. In a study by Hsieh and Ko (2008) on fresh carrot juice treated in a high-voltage electrostatic field and subsequently stored at 4 °C a considerable increase in ΔE (greater than 6.0) after 15 days of storage was detected, which is generally in accordance with the results obtained in this study. However, Evrendilek et al. (2000) reported no impact of combined PEF and heat treatment on L^*, a^* and b^* of fresh apple cider stored at 4 °C in plastic containers over a 14 day period. Similarly, Rivas et al. (2006) reported no changes in colour attributes of PEF-treated orange carrot juice during 8.5 weeks of storage in Elopak packages at 12 °C in contrast to juice samples which were thermally pasteurised (98 °C for 21 s). Moreover, results obtained by Aguiló-Aguayo et al. (2008) showed an increase in colour parameters (L^* and hue angle) of PEF-treated and pasteurised tomato juices stored in polypropylene bottles at 4 °C for up to 77 days, findings not in agreement with the data obtained in the present study. A study by Cortés et al. (2008) conducted on fresh orange juice treated by PEF or pasteurised and packaged in Elopak generally suggested a decrease in L^* and b^* during refrigerated storage (2 °C) in the first 4 weeks of storage which broadly agrees with the results of this study in spite of the initial increase of the colour attributes following the preservation treatments and the fact that the a^* decreased in contrast to the trend observed in the present study.

Possible explanations for these discrepancies in the colour properties of PEF-treated juices could include the effect of different packaging materials (Ayhan et al., 2001), different storage temperatures (Esteve, Frígola, Rodrigo, & Rodrigo, 2005), enzymatic reactions (Van Loey, Verachtert, & Hendrickx, 2002) and non-enzymatic browning (Klim & Nagy, 1988).

3.2. *Smoothie sensory characteristics following preservation treatment with H/PEF*

Panellists evaluated sensory attributes (Table 4) such as colour (4.3 vs. 3.0), odour (5.5 vs. 5.0), acidity (5.2 vs. 5.6), flavour (3.6 vs. 3.0) as better for mildly pasteurised than for H/PEF-treated fruit smoothie, respectively ($P < 0.05$). By contrast, product thickness of H/PEF-treated smoothie was assessed as superior to that of MP-treated fruit smoothie (5.5 vs. 5.9, respectively; $P < 0.05$). Moreover, panellists determined no differences in sweetness (5.0 vs. 4.9, $P \geq 0.05$) and overall acceptability (3.3 vs 3.7, $P \geq 0.05$) of the smoothie beverages as well as in the flavour characteristics ($P \geq 0.05$) examined. This broadly indicates that both preservation methods gave products of comparable organoleptical quality. When untreated and thermally pasteurised and H/PEF-treated smoothie were compared in terms of the overall sensory quality no differences were highlighted ($P \geq 0.05$) A possible explanation for the equally low overall acceptability of all three smoothies could be that the smoothie type did not meet with the general preference of the majority of panellists involved in the sensory evaluation. Furthermore, the main smoothie ingredients were pineapple (50 g per 100 of smoothie) and banana (28 g per 100 g of smoothie) which are both very sensitive fruits, easily affected by quality compromising reactions (e.g. browning, structural degradation) (Montero-Calderón, Rojas-Graü, & Martín-Belloso, 2008; Pan, Shih, McHugh, & Hirschberg, 2008), and this could be the reason that the overall sensory quality of the three products was similar. The addition of antioxidants (López-Nicolás, Pérez-López, Carbonell-Barrachina, & García-Carmona, 2007) or other quality preserving methods (Soliva-Fortuny & Martín-Belloso, 2003) used in commercial smoothie production for the optimisation of organoleptical smoothie quality should be considered in future sensory studies with this specific smoothie-type beverage.

A sensory study comparing heat (88 °C for 120 s) and PEF-treated (40 kV/cm for 57 μs) to thermally pasteurised (92 °C for 90 s) tomato juice was conducted by Min and Zhang (2003) who reported greater

Table 4
Evaluation of colour, odour, thickness, acidity, sweetness, flavour and overall acceptability of untreated, moderately heated (H; 55 °C, 60 s) and pulsed electric fields (PEF; 34 kV/cm, 60 μs) treated (H/PEF), and mildly pasteurised (MP; 72 °C, 15 s) fruit smoothie.

	Untreated	H/PEF	MP	SED[e]	*P*[f]
Colour[c]	4.6[a]	3.0[b]	4.3[a]	0.17	***
Odour[c]	5.4[a]	5.0[b]	5.5[a]	0.17	*
Thickness[d]	5.5[a]	5.5[a]	5.9[b]	0.13	*
Sweetness[d]	4.9[a]	5.0[a]	4.9[a]	0.15	NS
Acidity[d]	5.5[b]	5.6[b]	5.2[a]	0.14	*
Flavour[c]	3.5[a]	3.0[b]	3.6[a]	0.19	*
Overall acceptability[c]	3.4[a]	3.3[a]	3.7[a]	0.20	NS

[a, b] Different superscripted letters in the same row indicate a statistical difference between treatment means.
[c] Sensory attribute score was evaluated by panellists on a 9 point hedonic scale with lowest and highest scores at 1 and 9 points, respectively.
[d] Sensory attribute score was evaluated by panellists on a 9 point hedonic scale with an optimum score at the middle of the scale (5 points).
[e] SED represents the standard error of difference between treatment means.
[f] P stands for the statistical probability based on confidence intervals applied.
* and *** refer to a statistical significance of $P < 0.05$ and $P < 0.001$, respectively; NS indicates no statistical difference.

flavour intensity and overall acceptability for the heat and PEF-treated juice. In addition, several studies on the effect of stand-alone PEF on the sensory properties of fruit juices have been carried out. Qin, Zhang, Barbosa-Cánovas, Swanson, and Pedrow (1994) reported no significant differences in sensory attributes between freshly squeezed and PEF-treated (50 °C for 20 μs) apple juice and in another study by Evrendilek et al. (2000) no impact on the acceptability of fresh apple juice exposed to PEF (35 kV/cm for 94 μs) was determined by sensory panellists. A sensory study by Min, Jin, & Zhang (2003) investigated colour, appearance, texture, flavour and overall acceptability of untreated, thermally treated (90 °C for 90 s) and PEF-treated (40 kV/cm for 97 μs) fresh orange juice and the 30 panellists found that untreated freshly squeezed was better than PEF-treated juice which, in turn, showed superior texture, flavour and overall acceptability than thermally treated juice. Moreover, some studies (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martín-Belloso, & Ortega-Rivas, 2007; Cserhalmi et al., 2006; Jin, & Zhang, 1999) reported small changes in flavour or similar flavour profiles for untreated fresh juices and PEF-processed juices while other researchers (Jia et al., 1999; Qiu, Sharma, Tuhela, Jia, & Zhang, 1998; Yeom, Streaker, Zhang, & Min, 2000b) suggested that orange juice flavour is retained better following PEF than thermal treatment.

The divergence between the results obtained in the present study (i.e. showing no advantage in sensory properties of H/PEF-treated over mildly pasteurised smoothie) and findings in the literature cited above (i.e. all suggesting sensory advantages of H/PEF-treated or stand-alone PEF-treated over thermally pasteurised juices) could be explained by residual enzyme activity which affected both H/PEF-treated and mildly pasteurised smoothie-type beverage. Moreover, MP-treated and H/PEF-treated smoothies were rated with a similar overall acceptability to that of untreated smoothie and consequently, no difference between MP-treated and H/PEF-treated products was found. The changes in pH, conductivity and colour for smoothie exposed to either H/PEF or treatment during the storage time described earlier in the shelf life study are indicators for possible enzymatic product modifications as well as the sensory evaluation of untreated fruit smoothie. In addition to the application of antioxidants, blanching or other quality preserving measures the use of a controlled oxygen-free atmosphere during stages of smoothie processing (i.e. preparation, H/PEF treatment and packaging) could lead to a reduction in quality deteriorating reactions during production and storage. Longer exposure of smoothie to moderate heating prior to PEF processing and more severe PEF treatment conditions could also contribute to achieving a greater overall acceptability and quality of the product.

4. Conclusion

An improved microbiological shelf life was achieved in a fruit juice smoothie-type beverage using a combination of moderate heat and pulsed electric fields compared to mild thermal pasteurisation with shelf life of the products expiring after 28 and 21 days, respectively. With regards to the quality parameters investigated in this study a similar decrease in pH and conductivity over the storage time was observed for both preservation treatments, but the soluble solids content of the H/PEF-treated fruit smoothie was more constant over the duration of the shelf life study. The assessment of viscosity showed less structural degradation for smoothie exposed to H/PEF compared to thermally pasteurised smoothie. However, colour attributes were found to be slightly better for mildly pasteurised smoothie than H/PEF-treated smoothie, a feature which was also confirmed in a consumer acceptability study. Similar overall acceptability was indicated by panellists in the sensory study for fruit smoothie which was preserved with H/PEF, conventionally pasteurised or untreated. For the minimisation of quality losses during the processing stages further optimisation of the smoothie preparation and processing by means of quality retaining substances and techniques is suggested for future studies.

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