Shelf-life extension of multi-vegetables smoothies by high pressure processing compared with thermal treatment. Part II: Retention of selected nutrients and sensory quality.

Shelf-life of multi-vegetables smoothies (part II)

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Abstract

Consumers are increasingly demanding food products based on minimally processed fruit and vegetables (Part I), which are associated with “fresh-like” qualities and convenience. Smoothies may well fit these needs and contribute to increasing fruit and vegetable intake. In this Part II, the objective was to assess the sensory and nutritional quality for up to 28 days at 4ºC in a vegetable smoothie with apple that was stabilized by high-pressure processing (HPP) (350MPa/10ºC/5min) or mild heating (MH) (85ºC/7min). HPP provided smoothies without a cooked fruit odour that maintained their “fresh-like” characteristics for at least 14 days. Furthermore, HPP resulted in a higher retention of vitamin C but not of total phenols and flavonoids, while sucrose rapidly was degraded to glucose and fructose during storage. Thus, mild pressurizing may be used to obtain "fresh-like" vegetable smoothies, although the treatment should be improved to retain their sensory traits and nutrients for longer.

Practical applications

The food industry is adapting its production strategies offer “fresh-like” vegetable smoothies with better sensory and nutritional qualities. This involves substituting conventional thermal treatments for other treatments, such as HPP, that may provide products with an adequate shelf life. Sensory and nutritional aspects are crucial for "fresh-like" smoothies to be developed at industrial scale.

Keywords

High hydrostatic pressure, vegetable smoothie, shelf life, sensory, nutrients.

Abbreviations

HPP: High-pressure processing, MH: Mild heating

1 INTRODUCTION

One of the more attractive features of HPP applied to the stabilisation of vegetable products, in particular smoothies, is the preservation of compounds of nutritional interest and sensory properties. However, depending on the pressurizing conditions, HPP treatments might modify enzymatic and chemical reactions that could result in the formation of compounds associated with undesirable changes in colour, odour and flavour (Oey et al., 2008). The stability of the sensory properties is another very important feature of HPP applied to vegetable-based products, since they are exposed to altering reactions catalysed by enzymes, which are frequently not inactivated by the HPP treatments (Terefe et al., 2014). The effects of HPP on sensory properties have been reported in strawberry purée (Lambert et al. 1999), mandarin juice (Takahashi et al., 1993), orange–lemon–carrot juice and multi-fruit smoothies (Fernández-García et al., 2001; Hurtado et al., 2015, 2017a,b; Picouet et al., 2016). In most of these studies it was seen that the use of 450-600 MPa at room and cold temperatures carries a certain risk of altering odour and flavour, leading to the conclusion that mild pressurization conditions are more adequate for stabilizing ”fresh-like” fruit and vegetable products, such as smoothies. HPP treatments have little effect on fruit and vegetable compounds of nutritional interest, depending on the pressurizing conditions and the nature of fruit or vegetable matrix (Oey et al., 2008; Keenan et al., 2012a,b; García-Parra et al., 2016). However, the remaining enzymatic activities may lead to the greater destruction of nutrients during storage. Vitamin C loss is considered as a handicap for the nutritional quality of fruit derivatives, particularly in products made from fruits rich in vitamin C, such as orange or strawberry. In general, vegetables contain substantial amounts of phenolic antioxidants, such as flavonols, tannins and anthocyanins, which are considered to be good promoters of human health (Nile and Park, 2014), but these, too, may be degraded during storage. Similarly, the inability of HPP treatments to inactivate some enzymes, such as β-fructosidase, might modify the sugar profile in fruit products (Butz et al., 2003). A decrease in the amount of ascorbic acid, phenolic compounds, carotenoids and carbohydrates has been reported after HPP (Fernández-García et al., 2001; Butz et al., 2003), while the content of total phenols increased or were not affected (Patras et al., 2009; Vega-Gálvez et al., 2014, 2016). It is very interesting, even necessary, to know whether HPP treatment permits the better retention of nutrients during cold storage of vegetable smoothies than thermal treatments, which frequently modify sensory and nutritional properties of fruits and vegetables (Deliza et al., 2005) and affect their acceptability (Gao et al., 2016). The enzymes involved in fruit spoilage (Part I) may produce noticeable sensory and nutritional changes, a feature which requires further evaluation.

The aim of the Part II of the present study was to compare the effects of an HPP treatment (350MPa/5min/10°C) and a MH treatment (85°C/7min) on the sensory quality and the level of selected nutrients in a vegetable smoothie (apple, carrot, zucchini, pumpkin and leek), kept in refrigeration for up to 28 days.

2 MATERIALS AND METHODS

2.1 Sample preparation

The smoothie formulation was based on commercial smoothies selected for their sensory properties. Smoothie composition by weight consisted of 20 % blanched carrot (*Daucus carota*), 20 % apples (*Pyrus malus* golden delicious), 20 % *Citrus* pectin solution 1 %, 19.9 % zucchini (*Cucurbita pepo*), 15 % pumpkin (*Cucurbita moschata* butternut), 5 % blanched leek (*Allium ampeloprasum* porrum) and 0.1 % salt. Fruit and vegetables were purchased at a local market.

Juices were obtained using a C40 juicer (Robot Coupé, Montceau-en-Bourgogne, France) and blended in a tank to achieve the above-mentioned composition. During sample preparation room temperature was stabilized at 14 ºC. Smoothies subjected to HPP were packaged in 250 ml polyethylene terephthalate (PET) bottles (Sunbox, Madrid, Spain), while a specific HT300 pouch (Seal Air

Cryovac, Milano, Italy) was used in MH samples. Both packages were selected for high pressure and heat processing, respectively, to avoid the effect of packaging materials on the quality of the smoothie.

2.2 Thermal and high-pressure treatments

For the MH treatment, the samples were introduced into an Ilpraplus autoclave (Ilpra Systems, Mataró, Spain) and heated at 85ºC for 7 min, including the initial ramp of 5.7ºC/min, the total heating lasted 27 min. The conditions of mild heat treatment were selected in previous works with fruit smoothies (Picouet et al., 2016) to find, under mild heat conditions, microbiological safety (destruction of pathogens). HPP stabilisation consisted of the pressurization at 350 MPa for 5 min at an initial temperature of 9 - 10 ºC in a 120 l HPP system Wave 6500/120 (Hyperbaric, Burgos, Spain). The pressure ramp was 200 MPa/min and the total processing time was 7.3 min. The HPP treatment was selected based on the results obtained in a previous study with a fruit smoothie, in which three HPP treatments ranging from 350 to 600 MPa (350MPa/5min/10ºC, 450MPa/5min/10ºC, 600MPa/3min/10ºC) were tested (Hurtado et al., 2015), and in a preliminary HPP treatment of the multi-vegetable smoothie (Part I). After the respective treatments, samples were cooled in water (5ºC) for 1 hour (HPP) or 2 hours (MH), and then stored for up to 28 days at 4±1 ºC in darkness.

2.3 Experimental design

The different parameters studied were measured in untreated products (only at day 1), after MH and HPP treatments (day 1) and throughout refrigerated storage at 4±1 ºC (day 7, 14, 21 and 28) in darkness, representing retailed conditions as mentioned before. Selected nutrient compounds (vitamin C, sugars, flavonoids and total phenols) measurements were taken on three independent samples (3 different 250 ml bottles/pouches) per day of sampling. Two replicates of the experiment were performed.

2.4 Determination of the nutritional quality

2.4.1 Vitamin C

The total vitamin C content, L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA) were analysed according to the method described by Zapata and Dufour (1992) with slight modifications (Gil et al., 1998; González Hidalgo et al., 2019). For AA determination, a purified extract of a sample in methanol:water at 5:95 (V:V), 0.5 g l^{-1} citric acid and 0.5 g l^{-1} ethylendiaminetetracetic acid was used. For DHAA determination, 3 ml of the above extract was reacted with 1 ml of a solution of o-phenylenediamine (OPDA) in methanol:water at 5:95 v:v (333.72 mg OPDA per 100 ml solution), which was kept at 4 ºC in darkness for 40 min before analysis. The reverse-phase high performance liquid chromatography (RP-HPLC) system was made up as follows: L-6200 pump (Merck-Hitachi, Darmstadt, Germany); 2050 plus autosampler (Jasco Inc., Easton, UK); L-7420 UV detector (Merck-Hitachi); and a Gemini C18 column (300 x 4.6 mm, 5 µm) connected to a C18 reverse phase guard column, both from Phenomenex, Torrance, CA, USA. The mobile phase used was methanol/water containing cetrimide and KH_2PO_4. The operating conditions were: 20 µl injection volume, 260 nm (AA) or 340 nm (DHAA) nm detector wavelength and 0.9 ml min^{-1} flow rate. Results were expressed as mg of L-ascorbic acid 100 ml^{-1}, mg of L-dehydroascorbic acid 100 ml^{-1}, and the total vitamin C content (mg 100 ml^{-1}) was calculated as the sum of L-ascorbic acid and L-dehydroascorbic acid.

2.4.2 Total phenols (TPC)

Total phenols were determined according to Singleton and Rossi (1965) using a UV2 spectrophotometer (Pye Unicam Ltd, Cambridge, UK). The absorbance of a yellow compound formed by the reaction between a sample of the ethanolic extract and Folin-Ciocalteu reagent (containing phosphomolybdate and phosphotungstate) was measured at 765 nm. Results were expressed as mg gallic acid equivalents (GAE) 100 ml^{-1}.

2.4.3 Total flavonoids

Total flavonoids were determined according to the method of Chang et al. (2002) using a UV2 spectrophotometer. The absorbance of a yellow compound formed by reacting a sample of the methanolic extract with aluminium chloride, potassium acetate and water was measured at 415 nm. Results were expressed as mg quercetin equivalents (QE) 100 ml^{-1}.

2.4.4 Sugars

The main sugars (sucrose, glucose and fructose) were determined by HPLC using the method described by Hellín et al. (2001). A water extract sample was directly injected into the HPLC system equipped with an L-7490 Lachrome refractive index detector (Merck-Hitachi) and a Carbosep CHO682 lead column (Transgenomic, Elancourt, France). The mobile phase used was pure water (MilliQ). The operating conditions were: 20 µl injection volume, 0.4 ml min^{-1} flow rate; and 80 °C temperature. Results were expressed as g 100 ml^{-1}.

2.5 Sensory analysis

The descriptors for the Quantitative Descriptive Analysis (QDA) were generated by open discussion in two previous sessions. The retained descriptors and their descriptions are shown in Table 1. Six selected and trained assessors (ISO 8586-1:1993, ISO 8586-146 2:1994 and ISO 8586:2012) undertook the sensory analysis of 50 ml of a multi-vegetable smoothie. A non-structured scoring scale (Amerine et al., 1965) was used, where 0 meant the absence of the descriptor and 10 meant a high intensity of the descriptor. The sensory evaluation was separately performed for each sampling time in two sessions (per sampling time) using one bottle/pouch of 250 ml of each treatment per session. A complete block design was used (Steel and Torrie, 1983), where each taster assessed all of the batches in each session. Eight sensory sessions per taster were performed in total. The samples were coded using three random numbers and presented to the assessors, who balanced the first-order effects and the carry-over effects according to MacFie et al. (1989). The average score of six experts for each sample and session was recorded and used in the data analysis.

2.6 Statistical analysis

Data were analyzed by means of ANOVA using the GLM procedure of SAS 9.01 (SAS Institute Inc, Cary, USA). The model for nutritional data included the treatment (Untreated, MH, HPP), storage time (1, 7, 14, 21 and 28 days) and replicate as fixed effects. For the sensory data, the model included the treatment, storage time, taste session and replicate as fixed effects. Non-significant interactions ($P>0.05$) were removed from the model. Mean differences were tested using the Tukey test ($P<0.05$).

3 RESULTS AND DISCUSSION

3.1 Nutritional quality: Vitamin C, total phenols, flavonoids and sugars

The vitamin C content was very low, 4.3, 3.1 and 2.3 mg 100 ml^{-1} in untreated, HPP and MH smoothies, respectively (Table 2) and it was due mainly to the dehydroascorbic acid. These results were expected because the smoothies were made with vegetables that, in general, are not very rich in vitamin C; for example, zucchini has 15 mg 100 g^{-1}, pumpkin 12 mg 100 g^{-1} or carrot 7 mg 100 g^{-1} of vitamin C (Spanish food composition database [BEDCA]), and also, as reported by González-Tejedor et al. (2017), due to the mincing and blended procedure followed to prepare vegetable smoothies, low ascorbic acid levels were detected, because the ascorbic acid oxidation to dehydroascorbic acid is rapidly catalysed by the enzyme ascorbate oxidase. There were no significant ($p>0.05$) differences in the total content of vitamin C between treated and untreated smoothies at day 1.

However, this vitamin degraded in both types of smoothie during storage, although the remaining content of this vitamin was higher in the HPP smoothies, with its consequent nutritional benefit. The fact that processing and storage could promote vitamin C degradation lowers the content and, as seen in other studies (Hurtado et al., 2015; Landl et al., 2010; Picouet et al., 2016; González-Tejedor et al., 2017), ascorbic acid is reduced to dehydroascorbic acid. In addition, vitamin C is the most labile antioxidant and so seems to be the most affected and prone to degradation during processing and storage, which could be taken as an indicator of the quality of all the antioxidants present in the product (Esteve and Frígola, 2007; Kalt, 2005). It has been observed that HPP retains the vitamin C content better than MH (Barba et al., 2010), as reported in orange juice treated at 300-500MPa/5min/20-35ºC (Velázquez-Estrada et al., 2013; Polydera et al., 2005a, 2005b), in strawberry purée treated at 300MPa/1,5,15min and 400MPa/15 min/20ºC (Marszalek et al., 2015; Patras et al., 2009) and in pumpkin treated at 450MPa/15min/20ºC (Zhou et al., 2014). Our results were consistent with these studies. HPP treatment resulted in a lower degradation of vitamin C compared with the MH.

The total phenol and flavonoid contents were about 10 mg GAE 100 ml^{-1} and 1-1.5 mg QE 100 ml^{-1}, respectively, for both treatments throughout storage (Table 2). The total level of phenols and flavonoids observed in our study was low compared to those reported in other studies for multi-fruit and red-fruit smoothies with high antioxidant capacity (Picouet et al., 2016; Hurtado et al., 2017a). Unlike vitamin C, the levels of both groups of phenolic antioxidants remained stable in smoothies throughout storage, possibly due to the protective action of the vitamin C against oxidation. Some studies indicate higher retention of phenols in pressurized than in pasteurized fruit or vegetable derivatives; for example, in pumpkin treated at 400MPa/5min/20°C (Contador et al., 2014), fruit smoothies after 450MPa/1,3,5min/20ºC (Keenan et al., 2010), and carrot juice, where the phenol content increased after pressurizing at 350MPa/10min/20ºC and decreased significantly after heat treatment (100°C/4min) (Jabbar et al., 2014). The phenol content decreased to a greater extent in turnip treated at 400MPa/5min/20ºC than when pasteurized at 90ºC/3min (Clariana et al., 2011). Our results agree with those of Suárez-Jacobo et al. (2011), who reported no differences in the total flavonoid content between pressurized (300MPa/4ºC) and pasteurized apple juice. Phenolic losses due to heat treatment may be caused by chemical degradation of phenols, depending on the type of vegetable raw materials and the intensity of the treatment applied (Roy et al., 2007). In our study, heat treatment was mild and did not produce any additional degradation of phenolic antioxidants, with the consequent nutritional benefit for smoothies in question.

The total content of sugars (Table 3) was coherent with the result obtained for total soluble solids (Part I). Sucrose levels were higher in the MH smoothies and remained stable during storage. However, in the HPP smoothies, sucrose was completely hydrolysed to glucose and fructose, falling to below the detection limit from day 7 onwards. This result has already been observed in other studies with fruit smoothies (Hurtado et al., 2015, 2017b; Picouet et al., 2016) and fruit juices (Butz et al., 2003), where, probably, the enzyme β-fructosidase was not inactivated by the treatment, thus hydrolysing the sucrose to below the detection limit (<0.5 mg 100 ml^{-1}). Our results also suggest that this hydrolase could have been inactivated by the heat treatment in the vegetable smoothies MH, which retained their initial sucrose content (Table 3), while the amount of glucose and fructose remain constant once the sucrose has been totally hydrolyzed by active enzymes, what happened in the HPP smoothies after day 7 and following days (Table 3).

3.2 Effects on sensory quality

Freshness is one of the most important factors for consumers' expectations as regard the sensory quality of fruit and vegetable juices. Consequently, major concerns with the pasteurization process of fruits and vegetables have been reported with respect to sensory and nutritional aspects (Sentandreu et al. 2005;

Aamir et al. 2013). Freshness could be explained in relation to attributes of appearance, odour and flavour.

The results for the sensory analysis of vegetable smoothies are shown in Tables 4 and 5. In general, the sensory differences observed between both treatments concerned attributes related with smoothie freshness. Odour intensity and pumpkin odour were slightly more intense ($P<0.05$) in the HPP smoothies than in the MH smoothies on day 1 (Table 4). The characteristic cucurbits odour (green melon) was similar in the HPP smoothies (2.3) and the untreated smoothies (2.4) at day 1, and, although this attribute decreased during the lifetime for both treatments, higher scores were achieved for the HPP smoothies (0.8) than for the MH smoothies (0.2) at the end of storage. As more relevant aspect is that the HPP smoothies had intermediate scores (1.3) for the cooked odour at day 1, compared with the untreated (0.8) and MH smoothies (2.1). Whatever the case, flavour alteration by thermal treatment was weak compared with those observed for fruit smoothies (Hurtado et al., 2017b; Picouet et al., 2016). The differences observed for the cooked odour between both treatments were only significant ($P>0.05$) at days 1, 7 and 14 of storage. As described by Hurtado et al. (2017a,b), HPP is an emerging non-thermal technology which has a minimal influence on odour properties.

By contrast, the impact of treatment on colour and appearance attributes was hardly noticeable. Orange and rust-brown colour scored similarly ($P>0.05$) in the HPP and MH smoothies at all storage times; however, there were some differences in colour stability, since MH smoothies suffered a degree of browning (rust colour) from 3.2 at day 1 to 5.5 at day 28. These results were coherent with the instrumental colour data (Part I), which also showed a better colour retention in the HPP smoothies. The lower Browning Index (Part I) of the HPP samples was indicated by a lower rust-brown colour, which was also evident in the sensory analysis. HP processing has a limited effect on the pigments (e.g. chlorophyll, carotenoids, anthocyanins, etc.) responsible for the colour of fruits and vegetables.

HPP is less damaging than thermal processes to low molecular weight food compounds like pigments, since covalent bonds are not affected by pressure (Tauscher, 1995). The colour compounds of HP processed fruits and vegetables change during storage due to incomplete inactivation of the enzymes, which can result in undesired chemical reactions in the food matrix.

The presence of small particles associated with the loss of turbidity was higher initially ($P<0.05$) in the HPP and untreated smoothies than in the MH smoothies throughout storage (Part I). This result was confirmed by the mouth feel score, where there were significant ($P<0.05$) differences in grittiness, which was clearly lower in the MH smoothies than in the HPP smoothies at the end of storage. This could be due to the greater clarification in the HPP and untreated smoothies caused by the residual activities of pectic enzymes during treatment and subsequent chilled storage, which may result in products with a greater quantity of particles and a greater feeling of grittiness.

Another important objective in the processing of fresh vegetables is to maintain their original taste and flavour. Most of the taste and flavour attributes assessed scored similarly ($P>0.05$) in the smoothies for both treatments, except for the carrot and apple flavours (Table 5). Carrot flavour was associated with freshness and was more intense in the untreated and HPP smoothies at the beginning of storage (days 1 and 7), while apple juice flavour was described as cooked flavour and scored higher in the MH smoothies at days 1, 7 and 14, as expected. A similar finding was previously reported in multi-fruit (Picouet et al., 2016) and red fruit (Hurtado et al., 2017a,b) smoothies. Finally, when the overall sensory quality was assessed in the smoothies, there were no significant ($P>0.05$) differences between treatments, although scores decreased ($P<0.05$) in the HPP smoothies during storage, probably due to their gradual loss of freshness associated with the remaining enzymatic activity, which did not occur in the thermally treated product.

It is known that the fresh taste of fruits and vegetables is not greatly altered by HPP, since the structure of small molecules responsible for flavour is not affected by pressure (Oey et al., 2008). Masegosa et al. (2014) showed that the sensory properties of two vegetable products elaborated with pumpkin and broccoli, and with zucchini, eggplant, spinach and chard, were not affected by HPP treatment. In addition, Houska et al. (2006) found no sensory differences in a broccoli and apple juice stored for up to 70 days pressurized at 500 MPa/10min and in a juice that was kept frozen. However, HP treatment could enhance the chemical and enzymatic reactions responsible for the appearance of off-flavours and off-odours. In any case, the resulting sensory properties and further stability of the pressurized smoothies would depend on factors such as the type of vegetables used (matrix), as well as the pressuring and retailing conditions applied (Fernández et al., 2018).

4 CONCLUSION

The use of a HPP allows the freshness of vegetable smoothies to be extended. HPP provided “fresh-like” vegetable smoothies with a low intensity of cooked fruit odour at least up to 14 days. Furthermore, HPP resulted in a higher retention of vitamin C but not of total phenols and flavonoids, while sucrose rapidly was degraded to glucose and fructose during storage. The content of the above mentioned antioxidants was low due to the natural composition of the fresh vegetables and the apple juice used. Considering some of the above advantages, HPP may be regarded as an alternative technology to obtain fresh vegetable products, although some sensory degradation occurs more quickly than in the mild-heated product. Notwithstanding, the “fresh-like” sensations are positive for HPP, which provided a superior quality product. Future research on HPP treated vegetable smoothies will be focussed on the effect in enzyme activities related to nutritional compounds.

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TABLE 1 Definition of the sensory attributes included in the sensory profile

Attributes	Definition
Appearance	
Orange colour	Evaluation of the intensity of orange colour.
Rust-brown colour	Evaluation of the intensity of rust-brown colour.
Particles	Evaluation of the loss of turbid cloud.
Odour	
Intensity	Evaluation of the intensity of overall odour.
Pumpkin	Evaluation of the intensity of odour characteristic of pumpkin.
Green melon	Evaluation of the intensity of odour characteristic of cucurbits.
Cooked odour	Evaluation of the intensity of odour characteristic of cooked vegetables.
Taste and flavour	
Intensity	Evaluation of the intensity of flavour.
Sweet taste	Basic taste sensation elicited by sugar.
Bitter taste	Basic taste sensation elicited by caffeine
Carrot flavour	Intensity of flavour characteristic of carrot.
Leek flavour	Intensity of flavour characteristic of leek.
Apple juice flavour	Intensity of flavour characteristic of cooked apple juice.
Aftertaste	Evaluation of the remaining taste in the mouth after swallowing the drink.
Mouth-feel	
Sliminess	Mouth-feel property rated by the degree to which the juice is thick.
Grittiness	Mouth feel sensation related with the perception of particles the size of fine sand.
Overall sensory quality	Scoring of the sensory quality of the sample by reference to the standard of quality for this product.

TABLE 2 Effect of the treatments (HPP *vs* MH) on nutritional quality of vegetable smoothies kept at 4ºC for up to 28 days of storage

Storage day		Ascorbic acid (mg 100 ml^{-1})	Dehydroascorbic acid (mg 100 ml^{-1})	Total vitamin C (mg 100 ml^{-1})	Total phenols (mg GAE 100 ml^{-1})	Flavonoids (mg QE 100 ml^{-1})
Untreated	1	1.0±0.7	3.3±1.1	4.3±1.2	12.1±1.3	1.2±1.2
HPP	1	0.6±0.6^{a}	2.4±0.9^{a}	3.1±1.2^{a}	10.9±1.2	1.3±0.2
MH	1	1.2±0.6^{a}	1.1±0.4^{a}	2.3±0.3^{a}	9.0±1.2	1.6±0.2
HPP	7	<dl	1.5±0.4$^{ab\ x}$	1.5±0.4$^{ab\ x}$	12.8±2.6	1.4±0.3
MH	7	<dl	0.5±0.2$^{b\ y}$	0.5±0.2$^{b\ y}$	11.2±0.9	1.5±0.1
HPP	14	<dl	1.1±0.3$^{b\ x}$	1.1±0.3$^{b\ x}$	10.4±0.9	1.4±0.1
MH	14	<dl	0.5±0.2$^{b\ y}$	0.5±0.2$^{b\ y}$	10.2±1.1	1.5±0.2
HPP	21	<dl	0.7±0.2$^{b\ x}$	0.7±0.2$^{b\ x}$	12.0±2.2	1.3±0.1
MH	21	<dl	0.1±0.1$^{b\ y}$	0.1±0.1$^{b\ y}$	11.0±1.5	1.7±0.3
HPP	28	<dl	0.2±0.0$^{b\ x}$	0.2±0.0$^{b\ x}$	11.4±0.6	1.1±0.1^{y}
MH	28	<dl	<dl	<dl	10.3±0.7	1.8±0.2^{x}

Treatments:
Untreated: Untreated smoothie (raw)
HPP: High Pressure Processing (350MPa/5min/10ºC)
MH: Mild Heating (85ºC/7min)
M±SEM: Mean ± Standard Error of Mean.
GAE: Gallic acid equivalents, QE: Quercetin equivalents

^{xy} treatment effects (within time) for $P \leq 0.05$.

^{abc} storage time effects (within treatment) for $P \leq 0.05$.

dl: limit of detection

TABLE 3 Effect of the treatments (HPP *vs* MH) on sugar content of vegetable smoothies kept at 4ºC for up to 28 days of storage

	Storage day	Sucrose (g 100 ml^{-1})	Glucose (g 100 ml^{-1})	Fructose (g 100 ml^{-1})
Untreated	1	1.51±0.01	2.11±0.02	3.05±0.02
HPP	1	0.12±0.01^{y}	2.19±0.01^{x}	3.67±0.02^{x}
MH	1	1.75±0.02^{x}	1.40±0.01$^{ab\ y}$	2.74±0.01^{y}
HPP	7	nd	2.55±0.01^{x}	4.53±0.03^{x}
MH	7	1.96±0.01^{x}	1.52±0.01$^{ab\ y}$	3.11±0.02^{y}
HPP	14	nd	2.53±0.02^{x}	4.21±0.02^{x}
MH	14	1.71±0.01^{x}	1.76±0.01$^{ab\ y}$	3.23±0.01^{y}
HPP	21	nd	2.40±0.01^{x}	3.87±0.02^{x}
MH	21	1.47±0.01^{x}	2.81±0.01$^{b\ y}$	2.81±0.01^{y}
HPP	28	nd	2.40±0.01^{x}	3.84±0.05^{x}
MH	28	1.64±0.01^{x}	1.54±0.01$^{ab\ y}$	2.81±0.01^{y}

Treatments:
Untreated: Untreated smoothie (raw)
HPP: High Pressure Processing (350MPa/5min/10ºC)
MH: Mild Heating (85ºC/7min)
M±SEM: Mean ± Standard Error of Mean.
^{xy} treatment effects (within time) for P≤0.05.
^{abc} storage time effects (within treatment) for P≤0.05.
nd: no detected

TABLE 4 Effect of the treatments (HPP *vs* MH) on different sensory attributes related with odour, colour and appearance, and mouth feel of vegetable smoothies kept at 4ºC for up to 28 days of storage.

Storage day		Odour				Colour and Appearance			Mouth-feel	
		Intensity	Pumpkins	Green Melon	Cooked	Orange	Rust-brown	Particles	Sliminess	Grittiness
Untreated	1	5.8±0.4	3.3±0.5	2.4±0.3^{x}	0.8±0.3^{y}	6.0±0.3	2.6±0.5	2.2±0.6^{x}	3.2±0.4	1.2±0.4
HPP	1	5.6±0.3	3.2±0.5	2.3±0.3$^{ab\ xy}$	1.3±0.3^{xy}	5.2±0.4	4.0±0.6	3.0±0.8^{x}	2.8±0.4	1.3±0.4
MH	1	5.3±0.3	2.7±0.4	1.3±0.3$^{a\ y}$	2.1±0.2^{x}	5.8±0.3^{a}	3.2±0.5^{c}	0.4±0.1^{y}	2.8±0.4	0.4±0.2
HPP	7	5.6±0.4	3.5±0.3^{x}	2.6±0.5$^{a\ x}$	1.0±0.3^{y}	5.0±0.4	4.0±0.6	3.9±0.8^{x}	3.3±0.4	2.2±0.6^{x}
MH	7	5.2±0.4	2.1±0.4^{y}	0.7±0.2$^{ab\ y}$	2.9±0.4^{x}	4.8±0.4^{ab}	4.2±0.4^{bc}	1.0±0.4^{y}	2.7±0.4	0.5±0.2^{y}
HPP	14	5.7±0.3	3.5±0.4	1.5±0.2$^{ab\ x}$	1.1±0.3^{y}	4.2±0.3	4.4±0.5	3.7±0.8^{x}	2.5±0.3	2.5±0.5^{x}
MH	14	5.5±0.3	2.7±0.5	0.6±0.2$^{ab\ y}$	2.7±0.4^{x}	3.7±0.4^{bc}	5.3±0.4^{ab}	1.0±0.3^{y}	2.5±0.4	0.7±0.2^{y}
HPP	21	5.3±0.3	2.8±0.5	1.0±0.3^{b}	1.2±0.4	4.1±0.2	5.2±0.5	3.7±0.7	2.5±0.3	2.3±0.5^{x}
MH	21	5.4±0.4	2.1±0.6	0.3±0.1^{b}	2.2±0.3	3.2±0.4^{c}	5.9±0.4^{a}	2.0±0.7	2.4±0.4	0.5±0.3^{y}
HPP	28	4.8±0.6	2.7±0.7	0.8±0.4^{b}	0.8±0.5	3.8±0.4	5.1±0.6	3.7±0.8^{x}	3.1±0.6	3.3±0.8^{x}
MH	28	5.3±0.6	2.0±0.8	0.2±0.1 ^{b}	2.1±0.3	3.4±0.5^{bc}	5.5±0.4^{ab}	0.2±0.2^{y}	2.1±0.5	0.5±0.3^{y}

Treatments:
Untreated: Untreated smoothie (raw)
HPP: High Pressure Processing (350MPa/5min/10ºC)
MH: Mild Heating (85ºC/7min)
M±SEM: Mean ± Standard Error of Mean.
^{xy} treatment effects (within time) for P≤0.05.
^{abc} storage time effects (within treatment) for P≤0.05.

TABLE 5 Effect of processing treatment on different sensory attributes related to taste and flavour in mouth and the overall sensory quality of vegetables smoothies kept at 4ºC for up to 28 days of storage

	Storage day	Taste and Flavour							O.S.Q.
		Intensity	Sweet	Bitter	Carrot	Leek	Apple juice	Aftertaste	
Untreated	1	6.1±0.2	3.3±0.3	1.9±0.4	2.3±0.3^{x}	2.4±0.3	0.6±0.2^{y}	1.9±0.4	5.7±0.4
HPP	1	6.1±0.3	3.2±0.3	1.6±0.4	1.9±0.3^{xy}	2.2±0.3	0.8±0.3^{y}	1.7±0.4	6.0±0.2^{a}
MH	1	6.0±0.2	2.9±0.4	1.6±0.2	1.0±0.2^{y}	1.7±0.2	2.0±0.2^{x}	1.6±0.3	5.3±0.3
HPP	7	5.9±0.3	3.0±0.4	1.6±0.5	2.2±0.3^{x}	2.8±0.4	0.6±0.3^{y}	2.0±0.5	5.1±0.4^{ab}
MH	7	6.0±0.3	3.1±0.3	1.2±0.3	0.9±0.2^{y}	2.2±0.3	1.7±0.3^{x}	1.6±0.4	5.0±0.3
HPP	14	5.4±0.3	2.7±0.3	2.0±0.4	1.5±0.2	2.2±0.4	0.6±0.2^{y}	2.0±0.5	5.0±0.3^{ab}
MH	14	6.0±0.2	2.9±0.2	1.4±0.3	1.1±0.3	1.9±0.3	1.4±0.2^{x}	1.6±0.4	4.9±0.3
HPP	21	5.5±0.3	2.4±0.4	1.9±0.4	1.4±0.2	2.3±0.3	0.5±0.2	1.9±0.5	4.5±0.3^{b}
MH	21	5.6±0.2	2.9±0.3	1.3±0.3	0.8±0.3	1.9±0.3	1.0±0.3	1.4±0.3	4.7±0.4
HPP	28	5.3±0.4	2.5±0.6	2.2±0.7	1.6±0.3	2.4±0.5	0.5±0.2	2.4±0.7	4.4±0.5^{b}
MH	28	5.8±0.2	2.8±0.5	1.3±0.5	0.5±0.3	1.6±0.6	0.9±0.4	2.2±0.4	4.6±0.5

Treatments:
Untreated: Untreated smoothie (raw)
HPP: High Pressure Processing (350MPa/5min/10ºC)
MH: Mild Heating (85ºC/7min)
M±SEM: Mean ± Standard Error of Mean.
O.S.Q.: Overall sensory quality

[xy] treatment effects (within time) for $P \leq 0.05$.
[abc] storage time effects (within treatment) for $P \leq 0.05$.