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Short communication

# Microbiological, antioxidant and physicochemical stability of a fruit and vegetable smoothie treated by high pressure processing and stored at room temperature

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ABSTRACT

This study aims to evaluate the physicochemical, antioxidant and microbiological stability of a fruit and vegetable smoothie treated with a previously optimized high pressure processing treatment (HPP: 630 MPa, 6min, 20 °C), stored at 25 °C. The control samples presented a significant increase in microbiological counts during the first days, while treated samples showed counts below the detection limit (< 1.0 logCFU/g) throughout the 26 days of storage. Total soluble solids and pH did not change with treatment or along storage. Initially, HPP-treatment reduced pectinmethylestearase, peroxidase and polyphenoloxidase activities (PME, POD, PPO) by 83.9%, 31.4%, and 9.7%, respectively. During storage, PPO was maintained whereas POD decreased significantly on treated and control samples, while PME decreased on control, the slow value of treated ones was maintained. All the antioxidant indicators presented an initial increase in their values (5–75%) with treatment, presenting similar or better performance than control during storage. All samples presented initially a reddish color (a*: 12.4 ± 0.8) tending towards an orange-brownish color with storage time, probably due to the significant loss of betacyanin, smoothie's main red pigment. In conclusion, although adjustments are necessary to achieve pigments's stability, HPP-treatment is adequate to ensure the microbiological and antioxidant stability of the product at 25 °C.

## 1. Introduction

Fruit and vegetable (F&V) smoothies are tasty, healthy, convenient and ready to drink, fulfilling all the current demands of consumers. This has led to an accelerated increase in their popularity, becoming in recent years one of the food industry sectors with the highest growth worldwide (Morales-de la Peña, Welti-chanes, & Martín-belloso, 2016). However, they have a short shelf life mainly attributed to microbial and enzymatic spoilages. Thermal pasteurization is the traditional method used to obtain safe and stable beverages. However, the high temperatures achieved during processing usually cause detrimental effects on heat-labile nutrients such as certain vitamins and bioactives (Rickman, Bruhn, & Barrett, 2007), and also generates a “cooked taste” (Moshonas & Shaw, 1989). This explains why thermal pasteurization could not be considered as a way of preserving a healthy F&V smoothie. Hence, in recent years the interest in the search for alternative preserving methods for these products has been increased (Barba, Esteve, & Frígola, 2012). Among them, high pressure processing (HPP) has proven to be a highly effective preservation method, achieving microbial and enzymatic inactivation, conserving the sensory and nutritional quality of the fresh product. Indeed, their effect has been proven in several fruit and vegetables juices and nectars (Barba et al., 2012; Cao et al., 2012; Liu, Wang, Li, Bi, & Liao, 2014; Wang et al., 2012). Nevertheless, the impact of preservation treatments on the evolution of quality factors during storage of complex matrices such as mixed fruits and vegetables smoothies remains largely unexplored.

In previous studies, an HPP-treatment was optimized to pasteurize a F&V smoothie (Fernandez, Denoya, Agüero, Jagus, & Vaudagna, 2018).

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Moreover, the product was stable during refrigerated storage at 5 °C (Denoya et al., 2017). If the product could be stable at room temperature, it would open the possibility of marketing it in this way. This would greatly reduce energy, transport and marketing costs. Hence, this study aims to evaluate the overall stability of a HPP-treated F&V smoothie during its storage at 25 °C.

## 2. Materials and methods

### 2.1. Smoothie preparation

Smoothie formulation (orange juice: 59%, apples: 15%, carrots: 15%, beet leaves: 6% and beet stems: 5%) was selected based on previous studies (Denoya et al., 2017) in which a sensory acceptability test was conducted where properties like color, appearance, taste, phase separation, among others, were considered and characteristics such as the intense red color, fresh fruits taste and cloud stability, were positively valued. A single batch of smoothie (4 L) was prepared according to the procedure described in Fernandez et al. (2018). After that, the smoothie was packed into polyethylene terephthalate (PET) bottles (100 mL).

### 2.2. High-pressure processing

The treatment was performed in a HPP equipment (Stansted Fluid Power Ltd. High-Pressure Iso-Lab System Model: FPG9400:922, UK) with a vessel of 2-L capacity. The effective capacity of the equipment was 4 bottles at a time, thus 5 repetitions of treatments were carried out, and obtaining 20 treated bottles that then were randomly distributed among sampling days. A pressure level of 630 MPa for 6 min (holding time) and at an initial temperature of 22 ± 2 °C were applied. These conditions were optimized in a previous study (Fernandez et al., 2018) minimizing detrimental factors and maximizing quality indicators immediately after treatment. An equal number of bottles without treatment were prepared and maintained as control.

### 2.3. Storage and sampling

All the samples were stored during 26 days at 25 ± 2 °C. At day 0, 4, 7, 12, 19 and 26, samples were taken (control and HPP-treated) for analysis. Three samples were analyzed each sampling day for each treatment.

#### 2.3.1. Quality parameters evaluated during storage

*2.3.1.1. Microbiological counts*. **Mesophilic aerobic bacteria (MAB), Enterobacteriaceae (EB)** and **molds and yeasts (M&Y)** counts were determined according to the method described by Fernandez et al. (2018). **Lactic acid bacteria (LAB)** were determined in agar Man Rogosa Sharpe (Biokar Diagnostics, France) with a double layer, after 3–5 days at 37 °C. The detection limit of the methods was 1.00 logCFU/g.

*2.3.1.2. Physicochemical parameters*. The **pH**, **total soluble solids (TSS**, °Brix), **firmness** (g) and **consistency** (g s) of samples were determined as described by Fernandez et al. (2018). **Pectinmethylesterase** activity **(PME)** was determined as described by Vicente, Costa, Martínez, Chaves, and Civello (2005) and **poliphenoloxidase (PPO) and peroxidase (POD)** as described by Chen et al. (2015). One unit of enzyme activity (UEA) was defined as the change of 0.001 of absorbance at the corresponding wavelength. Details can be found in the complementary material section (S.2.3.1.2).

*2.3.1.3. Antioxidant indicators*. **The total phenolic content (TPC)** was determined by Folin-Ciocalteau methodology and expressed as mg of Gallic acid equivalent per 100 g of smoothie (mg GAE100 $g^{-1}$). **Antioxidant capacity** was determined using the **DPPH** and **FRAP** assays, according to Fernandez et al. (2018) and expressed as μmol of Trolox equivalents antioxidant capacity (TEAC) per 100 g of smoothie. Details can be found in complementary material section (S.2.3.1.3).

*2.3.1.4. Chromatic parameters and main pigments*. **Chromatic parameters** (L, b* and a*) of smoothies as well as **betaxanthins (Bx)** and **betacyanins (Bc)** content were determined as described by Fernandez et al. (2018).

### 2.4. Statistical analysis

The results were expressed as the mean of all the repetitions together with the standard deviation (SD). The statistical analysis was performed with Origin® 8 software (OriginLab®, USA). For treatments comparison a *t*-test was used, while to analyze time variations on treated samples an ANOVA was conducted. Differences were determined using the Tukey multiple comparison test ($p < 0.05$).

## 3. Results

### 3.1. Microbiological quality

Microbial counts of HPP-treated and control samples during storage at 25 °C are showed on Table 1. Control samples presented a significant increase in their microbial load during the first days of storage. Moreover, the bottles were found to be swollen, with evident signs of fermentation, so it was decided not to continue with their storage, nor with their sampling, from day 4 onwards. Conversely, in HPP-treated samples, MAB, M&Y, EB, and LAB counts remained below detection limit (< 1.0 logCFU/g) during the 26 days of storage.

**Table 1**
Microbiological counts (log CFU/mL) of a fruit and vegetable smoothie treated with high pressure processing treatment (HPP: 630 MPa-5 min) or not (control) during storage at 25 ± 2 °C.

| | Treatment | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 4 | 7 | 12 | 19 | 26 |
| Mesophilic aerobic bacteria | control | 5.45 ± 0.32 $^{b,A}$ | 7.90 ± 0.37 $^{b,B}$ | – | – | – | – |
| | HPP treated | < 1.00 $^{a,A}$ | < 1.00 $^{a,A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ |
| Enterobacteriae | control | 5.50 ± 0.32 $^{b,A}$ | 5.52 ± 0.10 $^{b,A}$ | – | – | – | – |
| | HPP treated | < 1.00 $^{a,A}$ | < 1.00 $^{a,A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ |
| Molds and yeasts | control | 2.80 ± 0.27 $^{b,A}$ | 6.61 ± 0.14 $^{b,B}$ | – | – | – | – |
| | HPP treated | < 1.00 $^{a,A}$ | < 1.00 $^{a,A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ |
| Lactic acid bacteria | control | 5.14 ± 1.16 $^{b,A}$ | 7.95 ± 1.12 $^{b,B}$ | – | – | – | – |
| | HPP treated | < 1.00 $^{a,A}$ | < 1.00 $^{a,A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ | < 1.00 $^{A}$ |

*Different lowercase letters indicate differences between treatments and different capitals indicate differences over time. Data expressed as means ± standard deviation (n = 3).

**Table 2**
Physicochemical indicators of a fruit and vegetable smoothie treated with high pressure processing (HPP: 630 MPa-5 min) or not (control) during storage at 25 ± 2 °C.

| | Treatment | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 4 | 7 | 12 | 19 | 26 |
| **pH** | control | 3.86 ± 0.01 $^{a,A}$ | 3.89 ± 0.04 $^{a,A}$ | – | – | – | – |
| | HPP treated | 3.87 ± 0.01 $^{a,A}$ | 3.81 ± 0.01 $^{a,A}$ | 3.80 ± 0.02$^{A}$ | 3.85 ± 0.05$^{A}$ | 3.86 ± 0.03$^{A}$ | 3.87 ± 0.03$^{A}$ |
| **TSS** (°Brix) | control | 9.97 ± 0.35 $^{a,A}$ | 9.30 ± 0.31 $^{a,A}$ | – | – | – | – |
| | HPP treated | 9.98 ± 0.38 $^{a,A}$ | 9.88 ± 0.46 $^{a,A}$ | 9.63 ± 0.28$^{A}$ | 9.65 ± 0.07$^{A}$ | 9.93 ± 0.18$^{A}$ | 9.73 ± 0.11$^{A}$ |
| **Firmness** (g) | control | 472.9 ± 48.7 $^{a,B}$ | 210.9 ± 32.6 $^{a,A}$ | – | – | – | – |
| | HPP treated | 390.8 ± 65.6 $^{a,A}$ | 469.0 ± 2.6 $^{a,A}$ | 410.3 ± 43.6$^{A}$ | 504.8 ± 49.3$^{A}$ | 400.9 ± 12.0$^{A}$ | 498.9 ± 31.7$^{A}$ |
| **Consistency** (g s) | control | 13031 ± 67 $^{a,A}$ | 5842 ± 1257 $^{a,A}$ | – | – | – | – |
| | HPP treated | 11383 ± 782 $^{a,A}$ | 13167 ± 935 $^{a,A}$ | 13855 ± 888$^{A}$ | 14861 ± 33$^{A}$ | 14136 ± 2445$^{A}$ | 14757 ± 426$^{A}$ |
| **PPO** (UEA) | control | 16,53 ± 1.01 $^{a,A}$ | 23,33 ± 4.12 $^{a,A}$ | – | – | – | – |
| | HPP treated | 14,93 ± 0.32 $^{a,A}$ | 18,47 ± 1.02 $^{a,A}$ | 19,03 ± 3.15 $^{A}$ | 17,85 ± 1.75 $^{A}$ | 16,07 ± 1.91 $^{A}$ | 14,20 ± 2.80 $^{A}$ |
| **POD** (UEA) | control | 118.23 ± 14.35 $^{b,B}$ | 110.80 ± 14.55 $^{a,A}$ | – | – | – | – |
| | HPP treated | 81.1 ± 6.65 $^{a,C}$ | 72.7 ± 11.66 $^{a,B,C}$ | 59.87 ± 8.10 $^{A,B}$ | 52.40 ± 3.72 $^{A,B}$ | 59.36 ± 1.93 $^{A,B}$ | 50.90 ± 9.68 $^{A}$ |
| **PME** (UEA) | control | 32.30 ± 0.61 $^{b,A}$ | 21.27 ± 1.33 $^{b,B}$ | – | – | – | – |
| | HPP treated | 5.20 ± 0.44 $^{a,A}$ | 4.73 ± 0.51 $^{a,A}$ | 3.63 ± 2.44 $^{A}$ | 3.17 ± 0.57 $^{A}$ | 4.02 ± 0.18 $^{A}$ | 2.93 ± 0.21 $^{A}$ |

TSS: Total soluble solids, PPO: Poliphenoloxidase, POD: Peroxidase, PME: pectinmethylesterase. UEA: unit of enzyme activity.
*Different lowercase letters indicate differences between treatments and different capitals indicate differences over time. Data expressed as means ± standard deviation (n = 3).

### 3.2. Physicochemical parameters

Changes in physicochemical parameters during storage at 25 °C are presented in Table 2. No significant differences in **pH** and **TSS** were observed between treated and control samples, nor during storage. Treated samples showed reductions in the values of **consistency** and **firmness** in relation to the control ones. Nonetheless, these reductions were not significant. Additionally, no significant losses of consistency or firmness were observed during storage. In regard to enzymes activities, HPP-treatment produced a 9.7, 31.4 and 83.9% initial reduction on **PPO, POD** and **PME** activities. During storage, PPO and PME activities were maintained, although PME showed a tendency to reduction (non significant). On the other hand, POD activity decreased significantly, up to 37% on day 26.

### 3.3. Antioxidant indicators

Changes in antioxidant indicators during storage at 25 °C are presented in Table 3. With HPP-treatment, all the evaluated indicators presented an initial increase in their values with respect to control, and they were significant for the antioxidant capacity, determined by **DPPH and FRAP** method (8 and 75%, respectively), but not for **TPC** (5%). During storage, a gradual loss was observed, showing DPPH, FRAP and TPC retentions of 45, 2 and 69%, on day 26.

### 3.4. Main pigments and chromatic parameters

Changes on color indicators for HPP-treated and control samples during storage at 25 °C are showed on Table 4. No significant initial differences between betacyanin and betaxanthin contents on treated and control samples were observed. During storage, a rapid drop on the pigment contents was observed, with losses of 88.1 and 50.8% at day 26. Moreover, while initially all samples presented a similar reddish color (a*:9.60 ± 0.28 and b*:14.75 ± 1.20), during storage color was tending towards orange-brownish tones (a*:3.32 ± 0.03 and b*:23.40 ± 1.18 at day 26).

## 4. Discussion

HPP was highly effective in reducing the native microflora of the product, achieving reductions between 1.8 and 4.5 log cycles in MAB, EB, M&Y and LAB counts. Additionally, its effectiveness was such that no regrowth of any of these microorganisms was observed during the 26 days of storage at 25 °C. According to Houška and da Silva (2017) the main mechanism of action of HPP on microorganism is the alteration of the cell structure and physiological functions, breaking DNA strands, disrupting cell membrane integrity, inactivating key enzymes and irreversibly denaturing proteins and disabling membrane selectivity. It is important to highlight that if the usually accepted microbial limit of 6.0 log CFU/mL for mesophilic microorganisms and molds and yeasts is considered (Varela-Santos et al., 2012), the treated smoothie has a microbial stability of at least 26 days. Similar results to the observed in

**Table 3**
Antioxidant capacity (DPPH and FRAP) and total phenolic content (TPC) of a fruit and vegetable smoothie treated with high pressure processing (HPP: 630 MPa-5 min) or not (control) during storage at 25 ± 1 °C.

| | Treatment | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 4 | 7 | 12 | 19 | 26 |
| DPPH (TEAC 100 $g^{-1}$) | control | 336.4 ± 12.1 $^{a,B}$ | 233.0 ± 25.7 $^{a,A}$ | – | – | – | – |
| | HPP treated | 362.7 ± 19.7 $^{b,C}$ | 328.4 ± 21.8 $^{bC}$ | 314.7 ± 17.7 $^{C}$ | 218.1 ± 33.1 $^{B}$ | 168.9 ± 1.5 $^{A,B}$ | 162.2 ± 5.4 $^{A}$ |
| FRAP (TEAC 100 $g^{-1}$) | control | 256.9 ± 21.8 $^{a,A}$ | 335.1 ± 35.7 $^{a,A}$ | – | – | – | – |
| | HPP treated | 449.4 ± 37.3 $^{b,C}$ | 252.7 ± 23.9 $^{a,B}$ | 206.5 ± 6.5 $^{B}$ | 45.2 ± 5.1 $^{A}$ | 5.2 ± 0.8 $^{A}$ | 9.42 ± 3.9 $^{A}$ |
| TPC (mgGAE100 $g^{-1}$) | control | 68.0 ± 1.2 $^{a,A}$ | 69.6 ± 2.0 $^{a,A}$ | – | – | – | – |
| | HPP treated | 71.4 ± 1.6 $^{a,C}$ | 71.5 ± 3.3 $^{a,C}$ | 69.2 ± 3.0 $^{C}$ | 59.9 ± 4.5 $^{B}$ | 48.6 ± 1.5 $^{A}$ | 49.5 ± 2.6 $^{A}$ |

DPPH: radical scavenging capacity, FRAP: ferric reducing capacity, TPC: Total phenolic content.
*Different lowercase letters indicate differences between treatments and different capitals indicate differences over time. Data expressed as means ± standard deviation (n = 3).

**Table 4**
Betacyanins and betaxanthines content and chromatic parameters (L[a], a[a], b[a]) of a fruit and vegetable smoothie treated with high pressure processing (HPP: 630 MPa-5 min) or not (control) during storage at 25 ± 1 °C.

| | Treatment | Day | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 4 | 7 | 12 | 19 | 26 |
| Betacyanins (mg/L) | control | 14.25 ± 0.13 $^{a,B}$ | 8.67 ± 2.15 $^{a,A}$ | – | – | – | – |
| | HPP treated | 14.85 ± 0.27 $^{a,D}$ | 7.11 ± 0.36 $^{a,C}$ | 5.51 ± 0.59 $^{B}$ | 2.87 ± 0.12 $^{A}$ | 1.76 ± 0.64 $^{A}$ | 2.70 ± 0.33 $^{A}$ |
| Betaxanthines (mg/L) | control | 6.11 ± 0.29 $^{a,A}$ | 5.95 ± 0.42 $^{a,A}$ | – | – | – | – |
| | HPP treated | 6.53 ± 0.20 $^{a,C}$ | 5.39 ± 0.19 $^{a,B,C}$ | 4.53 ± 0.10 $^{B}$ | 3.27 ± 0.24 $^{A}$ | 2.54 ± 0.40 $^{A}$ | 3.22 ± 0.86 $^{A}$ |
| L[a] | control | 40.04 ± 0.64 $^{a,A}$ | 42.49 ± 1.71 $^{a,A}$ | – | – | – | – |
| | HPP treated | 39.61 ± 0.10 $^{a,A}$ | 42.92 ± 0.27 $^{a,B}$ | 43.59 ± 0.25 $^{B}$ | 45.24 ± 0.17 $^{C}$ | 46.99 ± 0.14 $^{D}$ | 48.05 ± 0.11 $^{E}$ |
| a[a] | control | 9.43 ± 0.61 $^{a,A}$ | 9.63 ± 0.34 $^{a,A}$ | – | – | – | – |
| | HPP treated | 9.81 ± 0.23 $^{a,D}$ | 10.15 ± 0.02 $^{a,D}$ | 8.77 ± 0.20 $^{C}$ | 4.96 ± 0.01 $^{B}$ | 3.88 ± 0.20 $^{A}$ | 3.32 ± 0.03 $^{A}$ |
| b[a] | control | 15.57 ± 0.14 $^{a,A}$ | 22.42 ± 1.68 $^{a,A}$ | – | – | – | – |
| | HPP treated | 13.85 ± 0.10 $^{a,A}$ | 19.39 ± 0.10 $^{a,B}$ | 21.67 ± 0.11 $^{C}$ | 19.45 ± 0.26 $^{B}$ | 24.53 ± 0.22 $^{D}$ | 23.40 ± 1.18 $^{C,D}$ |

[a] Different lowercase letters indicate differences between treatments and different capitals indicate differences over time. Data expressed as means ± standard deviation (n = 3).

the present study were reported by Palou et al. (2000) working with HPP-treated (689MPa/5-20 min) avocado pureé during 30 days of storage at 25 °C. Moreover, Cao et al. (2012) reported similar results on HPP-treated (600 MPa/4 min) strawberry juice, without regrowths during 6 months at 25 °C.

The stability on pH and TSS of HPP-treated juices and purees during storage at room temperature had been observed by many authors (Barba et al., 2012). Additionally, no significant loss of consistency or firmness was observed during storage of treated samples, which could be related to the inactivation of PME, one of the main enzymes associated with the texture loss of this type of product. Indeed, the high barosensitivity of PME had been already noticed in previous studies (Fernandez et al., 2018). Changes on enzymes activities during storage at 25 °C also followed the pattern shown at 5 °C (Denoya et al., 2017), although in that case a slight increase of PPO activity and a smaller decrease in POD activity was observed. Indeed, Guerrero-Beltrán, Swanson, and Barbosa-Cánovas (2005) also observed greater decrease in PPO activity of samples of peach puree stored at temperatures higher than 5 °C (21 and 35 °C). Moreover, the decrease of enzymatic activity during storage is usually attributed to the enzyme being complexed with the available substrates (Keenan, Rößle, Gormley, Butler, & Brunton, 2012). It is important to highlight that HPP-treated samples presented in all cases a lowered enzymatic activity, which is associated with less deterioration and greater stability of the product.

Regarding antioxidant stability, as it is well known, generally the losses during storage are greater at higher temperatures. Nevertheless, the losses observed in HPP-treated samples stored at 25 °C were similar to those observed in refrigerated conditions for FRAP and between 8 and 14% higher for DPPH and TPC (Denoya et al., 2017). Indeed, other authors (Cao et al., 2012; Liu et al., 2014; Wang et al., 2012) have reported small differences on the losses of FRAP, DPPH, and TPC between HPP-treated samples stored at 5 °C and 25 °C. Hence, the storage of the product at room temperature would not be detrimental to its antioxidant attributes. Since this product is aimed to meet the needs of consumers looking for more nutritious and healthy products, their total antioxidant capacity (DPPH + FRAP) can by settled as a biomarker to predict the shelf life based on the antioxidant stability (Cao et al., 2012). If a loss of 50% is considered as limit, a shelf life between 7 and 12 days at 25 °C will be achieved.

The main drawback for the storage of the HPP-treated smoothie at room temperature was related to the instability of the main pigments associated with its reddish color, the betalains. Stability of betalains is favored by low temperatures due to the lower rate in deterioration reactions (Curutchet, Dellacassa, Ringuelet, Chaves, & Viña, 2014). The results observed in this research are consistent with the behaviors reported by González-Sánchez, Séijas-Bernabé, and Séijas-Bernabé (2013). Nevertheless, the stability of betalains at low pH is much lower than at neutral pH (Celli y Brooks, 2016), explaining the high percentage of loss in this smoothie. Accordingly, one of the parameters that was most affected by storage temperature was the color. Similar results were reported by Cao et al., 2012; Guerrero-Beltrán et al., 2005; Wang et al., 2012, among others, comparing color behavior in HPP-treated samples during storage at 5 °C and 25 °C. The color of F&V beverages is an important attribute because it affects consumer acceptability and purchase intention. Since color stability is undoubtedly related to pigments stability, a strategy to manage this situation could be the combination of HPP-treatment with other methods of preservation that allow reducing even more the activities of POD and PPO, main enzymes associated with the deterioration of these pigments.

## 5. Conclusions

The results presented in this study indicates that the treatment of the F&V smoothies with the optimized HPP conditions allowed to obtain a product microbiologically and physicochemically stable and with an antioxidant capacity similar to the fresh product, with the advantage of the possibility of storing it at room temperature. Indeed, a shelf life of at least 26 days can be achieved if microbiological criteria are considered, and of between 7 and 12 days if antioxidant loss criteria are considered. Nevertheless, the instability of the betalains in this condition and the consequent loss of color represent a drawback for a room temperature stored product. Hence, although some adjustments in order to achieve the desired overall stability will be necessary, HPP is a highly promising technology for the development of a room temperature stored F&V smoothie.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2019.02.030.

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