Innovative Food Science and Emerging Technologies 47 (2018) 170–179

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Optimization of high pressure processing parameters to preserve quality attributes of a mixed fruit and vegetable smoothie

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ABSTRACT

Fruit & Vegetable (F&V) smoothies are rich in nutrients and other health related compounds. However, they have a short shelf-life and the traditional methods applied to preserve them generate losses in their natural flavor and nutrients. The aim of this study was to optimize the pressure level (35–650 MPa) and holding time (1–9 min) of High Pressure Processing (HPP), performed at an initial temperature of 20 °C and only modified by adiabatic heating of a F&V smoothie in order to achieve microbial and enzymatic inactivation while maintaining its natural attributes. Response surface methodology with a Doehlert design and Desirability function were employed to simultaneously optimize these quality attributes. Results showed that HPP enhances microbial quality and does not affect pH, total soluble solids, texture and total phenolic content. Moreover, the optimal HPP treatment (627.5 MPa/6.4 min) leads to reductions of 85%, 45% and 10% on PME, POD and PPO, increases antioxidant capacity by 75% and maintains or slightly improves the color of the smoothie.

Industrial relevance text: 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. However, they have a short shelf life mainly attributed to microbial and enzymatic spoilages. HPP is proposed as a non-thermal method able to prolong shelf-life of the products by means of microbial and enzymatic inactivation, while preserving bioactive compounds and quality characteristics. An optimization assay was carried out in order to find optimal process conditions for the F&V smoothie's preservation. The promising results obtained can help to promote the use of HPP as an alternative technology for the preservation of this kind of products.

1. Introduction

In recent years consumers have become aware of the impact that their diet has on their health. This is why the demand for healthy, nutritious, free of additives products has noticeably increased. Additionally, the current lifestyle has led consumers to look for more convenient, ready-to-eat products (Hendrickx & Knorr, 2002). In this sense, consumption of smoothies is an excellent way to increase the intake of nutrients and bioactive compounds, present in fruits and vegetables (F&V). Their sensory properties, mainly appearance and taste, and the fact that they are ready-to-drink are all decisive factors for the consumption success of smoothies (Andrés, Villanueva, & Tenorio, 2016). Moreover, blending is a good way to incorporate non-traditional and underutilized, yet highly nutritive, vegetables (Jayachandran, Chakraborty, & Rao, 2015) such as beet leaves and stems (Fernandez, Jagus, & Agüero, 2014) into value-added products.

However, untreated F&V beverages have a short shelf-life that generally can be attributed to both microbial and enzymatic spoilage. Although they are usually highly acidic products (pH lower than 4) and this condition inhibits the growth of most of bacterial spores, some acid tolerant microorganisms such as yeast, lactic acid bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*), *Alicyclobacillus acideoterrestris*, *Listeria monocytogenes*, some species of *Salmonella* and some strains of *E. coli*, among others, could survive and

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https://doi.org/10.1016/j.ifset.2018.02.011

Received 24 May 2017; Received in revised form 6 February 2018; Accepted 11 February 2018

Available online 13 February 2018

grow (Gram et al., 2002; Jayachandran et al., 2015). Moreover, the natural presence of enzymes such as peroxidase (POD), polyphenol oxidase (PPO) and pectinmethylesterase (PME), among others, causes loss of phenolic compounds, induces browning and cloud loss in beverages, resulting in a reduced nutritional and sensory quality (Chakraborty, Kaushik, Rao, & Mishra, 2014).

Traditionally, fluid foods have been preserved by thermal treatments such as pasteurization and sterilization. These processes are capable of ensure safety and preventing spoilage; however, they can also result in a loss of heat-labile nutrients such as certain vitamins or bioactive phytochemicals (Rickman, Bruhn, & Barrett, 2007), among other compounds responsible for nutritional and organoleptic attributes, during the preservation/processing treatment (Barba, Esteve, & Frígola, 2012). Retaining the nutritional value and fresh-like quality of F&V beverages is a major challenge for the food industry. Therefore, research in non-thermal preservation processes is rapidly increasing (Duong & Balaban, 2014). In this sense, High Pressure Processing (HPP) is an alternative technology that involves applying very high hydrostatic pressures (100–1000 MPa) at refrigeration or room temperature for a short time (a few seconds to some minutes) on packed food in order to eliminate vegetative microorganisms (pathogens and spoilage microbiota), and to inactivate enzymes, with minimal modifications in nutritional and sensory quality (Oey, Lille, Van Loey, & Hendrickx, 2008). Certainly, one of the main advantages of this technology is its ability to maintain different compounds, such as pigments, volatiles, vitamins and other health-promoting compounds, rather unaffected (Denoya et al., 2016; Patras, Brunton, Da Pieve, & Butler, 2009; Varela-Santos et al., 2012). This has been attributed to the stability of covalent bonds under high pressure (Knorr, 1993). Regarding microbial inactivation, Lado and Yousef (2002) reported that HPP alters cell structure and physiological functions by breaking DNA strands, disrupting cell membrane integrity, inactivating key enzymes, irreversibly denaturing proteins and disabling membrane selectivity. Enzyme inactivation is also associated with conformational changes induced in the protein structure (Ludikhuyze, Van Loey, Indrawati, & Hendrickx, 2003; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). However, depending on the intensity of the treatment applied, HPP can either enhance or inhibit the enzymatic activity (Oey et al., 2008). This also depends on the enzyme type, its origin, its microenvironmental condition and process conditions (Duong & Balaban, 2014).

Therefore, the present study aims to investigate the effect of HPP on quality attributes of a mixed F&V smoothie and to optimize the main processing parameters (pressure level and holding time) in order to achieve microbial and enzymatic inactivation while maintaining nutritional quality attributes, texture and color of the product.

2. Materials and methods

2.1. Smoothie preparation

Smoothie formulation was selected based on previous studies of our group (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. The composition by weight of the selected smoothie was: orange juice (59%), apples (15%), carrots (15%), beet leaves (6%) and beet stems (5%). Oranges (cv. Salustiana, Argentina) were harvested from an experimental orchard in Concordia, Entre Ríos, Argentina, their juice was extracted using a home squeezer (Oster, USA). Apples (cv. Granny Smith, Argentina), carrots (cv. Flakee, Argentina) and beets (cv. Detroyt, Argentina) were obtained from a local retailer. Before processing, all fruits and vegetables were washed and disinfected by dipping in chlorinated water for 5 min and dried. The carrots and apples were then manually peeled and chopped into small pieces. Finally, all the ingredients were blended in a homogenizer (JTC OmniBlend, Guangdong, China) for 60 s. The smoothie was packed into polyethylene terephthalate (PET) bottles (100 mL). Bottles containing fresh control smoothies were immediately stored at 4 ± 1 °C, while the other samples were kept under refrigeration (4 ± 1 °C) until HHP treatments were applied. Four bottles were prepared for each treatment.

Since the developed product aims to meet the needs of consumers who are aware of the importance of food on health and consequently look for nutritious and fresh tasting products, thermal treatment was not considered as an option in this study.

2.2. High-pressure processing

Samples were subjected to different pressure levels and holding times, selected according to the experimental design. HPP was performed in a high hydrostatic pressure equipment with a 2-L capacity (Stansted Fluid Power Ltd. High Pressure Iso-Lab System Model: FPG9400:922, UK) and a maximum working pressure of 900 MPa. A mix of distilled water and propylene glycol (70/30) was used as the compression fluid. The proportion of water and propylene glycol was selected considering the working temperature applied, in order to avoid changes in the physical state of the water. Pressure was increased at 5 MPa s^{-1}. Conditioning temperature of vessel and initial temperature of compression fluid were between 21 °C and 24 °C. After processing, samples were kept at 4 ± 1 °C until further analysis.

2.3. Experimental design

Response surface methodology (RSM) was used to estimate the main effects and interactions of HPP parameters on quality attributes of the smoothie. A total of 9 experiments were conducted according to a Doehlert uniform shell design with two factors: pressure level (P) and holding time at working pressure (t). This method has been successfully applied to optimize different process parameters (Bup, Abi, Tenin, Kapseu, & Tchiegang, 2012; Denoya et al., 2016; Mayor, Moreira, & Sereno, 2011). In this research, pressure was studied at five levels (350, 425, 500, 575, and 650 MPa) and holding time at three levels (1, 5, and 9 min). Furthermore, the central point of the design was triplicated in order to validate the model by means of an estimate of experimental variance. Table 1 shows the coded and actual values of the factors of the experimental shell design and their levels.

For each response variable the linear, quadratic, and simple interaction effects of the factors were compared with each other. Each response variable (Y) was analyzed as a function of the two independent factors (P, t) and the significance of the equation coefficients for each response variable was obtained by multiple regression analysis using the F test with a $p < 0.05$:

$$Y = b_0 + b_1 P + b_2 t + b_{11} P^2 + b_{22} t^2 + b_{12} Pt$$

where b_0 is the regression coefficient for the mean effect; b_1 and b_2 for the linear effect of pressure level and holding time, respectively; b_{11}

Table 1
Coded and actual values of independent variables in the Doehlert design.

Exp. no.	Coded values		Actual values	
	×1	×2	Pressure (MPa)	Holding time (min)
1	0	0	500	5
2	0	0	500	5
3	0	0	500	5
4	−1	0	350	5
5	1	0	650	5
6	−0.5	−0.866	425	1
7	−0.5	0.866	425	9
8	0.5	−0.866	575	1
9	0.5	0.866	575	9

and b_{22} for the quadratic effect of pressure level and holding time, respectively, and b_{12} for the interaction effect of these variables. Four bottles of each treatment were analyzed to determine quality parameters.

It is important to highlight that the initial effects of HPP on the product is determinant for its quality and after treatment, during the storage time, the losses will be due to coexisting chemical reactions, such as oxidation, and biochemical reactions when endogenous enzymes or microorganisms are incompletely inactivated (Oey et al., 2008). That is why numerous researchers optimized the process with the same criterion (Denoya et al., 2016; Duong & Balaban, 2014; Kaushik, Rao, & Mishra, 2016; Swami Hulle, Chakraborty, & Rao, 2017).

2.4. Sample analysis

2.4.1. Microbiological analyses

Smoothie samples (10 g) were taken aseptically from the bottles and homogenized with 90 mL of sterile 0.1% peptone water (Biokar Diagnostics, France) in a stomacher (Interscience Laboratories Inc. BagMixer ® 400P, France) for 60 s. Decimal dilutions were prepared with sterile 0.1% peptone water and plated in the appropriate media for microbial counts. The mesophilic aerobic bacteria (MAB) count was determined in plate count agar (PCA, Biokar Diagnostics, France) incubated at 37 °C during 24–48 h; Enterobacteriaceae (EB) were determined in Mac Conkey agar (Biokar Dignostics, France) incubated at 37 °C during 24–48 h and molds and yeast (M&Y) counts were determined in yeast extract glucose chloramphenicol agar (YGC, Biokar Diagnostics, France) incubated at 28 °C during 48–72 h. The results were expressed as the logarithm of colony forming units per gram of smoothie (log CFU g^{-1}). The detection limit of the methods was 2.00 log CFU g^{-1}.

2.4.2. pH and total soluble solids (TSS)

The pH was measured in smoothies at room temperature (20 ± 1 °C) using a digital pH meter (Hanna, HI99163, Rumania) with a pH electrode (FC232D, Italy). The TSS was determined as °Brix at room temperature (20 ± 1 °C) using a Milwaukee MA871 Refractometer (Milwaukee Instrument, Rocky Mount, USA). Three measurements were performed for each sample and the results were averaged.

2.4.3. Chromatic parameters

Chromatic parameters of smoothies were determined with a Minolta CR-400 chromameter provided with a sample holder CR A505 and a Glass Cell 20 mm CM-A99 for measuring liquids (Konica Minolta, Japan), using the CIE scale L*a*b*. These values were then used to calculate Hue degree (h^0 = arctangent [b*/a*]) and Chroma [Ch = (a*2 + b*2)1/2], which is the color intensity or saturation. The instrument was set up for illuminant D_{65} and 2° observer angle. Three measurements were performed for each sample i.e. glass cell was filled three times, and the results were averaged.

2.4.4. Back extrusion analysis

Back-extrusion (pseudo-compression) test was performed using a Texture Analyzer model XT plus (Stable Micro Systems LTD, Surrey, England) equipped with a 5 kg load cell and a 50-mm diameter back extrusion cell (A/BE Rig). The samples were tested immediately after removal from storage (4 ± 1 °C), using an extrusion disc (Ø = 45 mm) operating at a fixed test speed of 0.5 mm s^{-1} to a depth of 30 mm. The force-time curves were analyzed using Exponent Software version 6.1.10.0 (Stable Micro Systems Ltd., U.K.) and the textural parameters derived were: maximum force in compression (firmness (g)) and positive area of the curve (consistency (g s)), which indicates the thickness of the sample.

2.4.5. Antioxidant capacity and total phenolic content

The extraction phenolic compounds and antioxidants was conducted following the methodology proposed by Viacava, Roura, and Agüero (2015) with some modifications. Briefly, 5 g of smoothie was mixed with 20 mL of extracting solvent (ethanol acidified with 2% citric acid) in a 150 mL Erlenmeyer flask. Extraction was carried out during 1 h, under agitation at 28 °C. Once extraction finished, homogenates were centrifuged (11,000 rpm) for 15 min at 4 °C. The supernatant was the source of phenolic compounds and antioxidants. Antioxidant capacity was determined using the DPPH and FRAP assays, according to Viacava et al. (2015) and Chen et al. (2015), calculated by using 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxilic acid (trolox, Sigma-Aldrich, St. Luis, USA) as standard and expressed as μmol of trolox equivalents antioxidant capacity (TEAC) per 100 g of smoothie. Total phenolic content was determined by Folin-Ciocalteau methodology and calculated by using gallic acid (Merk, Germany) as standard and expressed as mg of Gallic acid equivalent per 100 g of smoothie (GAE100 g^{-1}). These determinations were carried out by duplicated for each sample. The detail of each technique can be found in complementary material section (S.2.4.5.a, S.2.4.5.b, S.2.4.5.c, respectively).

2.4.6. Betaxanthins and betacyanins

Betaxanthins (Bx) and betacyanins (Bc) were determined by an adaptation of the methodology proposed by Moßhammer, Stintzing, and Carle (2006). Briefly, 5 g of smoothie was weighed and diluted in 10 mL of McIlvaine buffer (pH = 6.3). Absorbances at 600, 536 and 476 nm were determined and the contents of Bx and Bc in the extracts were calculated as:

$$Bx \text{ or } Bc\ (mg/L) = \frac{A \cdot DF \cdot MW \cdot 1000}{e \cdot l}$$

where A is the absorbance at 536 or 476 nm for *Bc* or *Bx* corrected by reading at 600 nm (baseline), respectively; DF is the dilution factor; l is the path length (1 cm) of the cuvette; MW is the molecular weight of Bc (550 g mol^{-1}) or Bx (308 g mol^{-1}); and e is the molar extinction coefficient (60,000 or 48,000 L mol^{-1} cm in H_2O for *Bc* and *Bx*, respectively).

2.4.7. Enzyme activities analyses

The enzyme activity was determined as described by Vicente, Costa, Martínez, Chaves, and Civello (2005) for pectinmethylesterase (PME) and as described by Augustin, Ghazali, and Hashim (1985) for polyphenoloxidase (PPO) and peroxidase (POD) with some modifications. One unit of enzyme activity was defined as the change of 0.001 of absorbance at the corresponding wavelength. The detail of each technique can be found in complementary material section (S.2.4.8.a and S.2.4.8.b).

2.5. Simultaneous optimization and model validation

A simultaneous optimization was carried out using the Desirability function. This function is commonly used for multi-response simultaneous optimization and was applied in similar studies (Denoya et al., 2016; Duong & Balaban, 2014; Kaushik et al., 2016). For this purpose, predicted values obtained from each model (Yn) were transformed to a dimensionless desirability scale (dn). The desirability scale ranges from 0 to 1, where d = 0 for an unacceptable response value, and d = 1 for a completely desirable one. The individual desirability functions from the considered responses are then combined to obtain the overall desirability D, defined as the geometric average of the individual desirability. An algorithm is then applied to this function in order to determine the set of values of design factors that maximize it (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008).

In order to test the reliability of the simultaneous optimization, a new set of experiments using optimal values of design factors obtained

Table 2
Experimental values for untreated fruit & vegetable smoothies quality attributes.

Microbiological quality	
MAB (log CFU mL^{-1})	3.9 ± 0.1
EB (log CFU mL^{-1})	3.7 ± 0.2
M&Y (log CFU mL^{-1})	2.5 ± 0.3
Physicochemical parameters	
pH	3.79 ± 0.03
Total soluble solids (°Brix)	9.6 ± 0.2
Chromatic parameters	
L*	40.2 ± 0.2
a*	9.4 ± 0.3
b*	14.6 ± 0.1
h°	57 ± 1
Ch	17.3 ± 0.1
Textural parameters	
Firmness (g)	624 ± 13
Consistency (g s)	18,430 ± 2381
Nutritional indicators	
Betacyanin (mg L^{-1})	13.1 ± 0.5
Betaxanthin (mg L^{-1})	4.9 ± 0.1
DPPH (TEAC 100 g^{-1})	350 ± 3
FRAP (TEAC 100 g^{-1})	1040 ± 92
Total phenols content (GAE 100 g^{-1})	62.6 ± 0.6
Enzymatic activity	
PME	37.8 ± 4.0
POD	84.9 ± 2.3
PPO	30.4 ± 2.5

MAB: mesophilic aerobic bacteria, EB: Enterobacteriaceae, M&Y: Mold and yeast, DPPH: radical scavenging capacity, FRAP: Ferric reducing capacity, PME: pectinmethylesterase, POD: Peroxidase, PPO: Poliphenoloxidase.

with the Desirability function were performed. The relative deviation between predicted and experimental value of the response variables (in both cases related to control values) were compared in order to determine the validity of the model.

2.6. Statistical analysis

All the procedures were carried out using the statistical software STATISTICA (trial version 12, Stat Soft, OK, USA). The Lack of Fit test was performed for each model with a 95% confidence level. The significant factors affecting each response variable were selected according to the Student *t*-test establishing a 95% confidence level (Kuehl, 2000).

Moreover, in order to analyze the presence of significant differences between treatments for the responses that do not fit the quadratic model, a one-way ANOVA was performed, also with a 95% confidence level.

3. Results and discussion

Table 2 shows experimental mean values of quality attributes of untreated mixed fruit & vegetable (F&V) smoothie (control sample). Among all the evaluated responses, some were not affected by treatment (pH, TSS, total phenolic, betaxanthin content and some chromatic parameters), others were affected but do not fit the quadratic model (microbiologic and textural parameters) while others were affected by treatment and fit the model (betacyanins content, antioxidant capacity, PME, POD and PPO activities and Chroma value). The mean values for the experimental responses that fit the model can be found in supplementary material section (Table S1), while Table 3 shows the regression coefficients of adjusted models for each response variable. Coefficients of determination (R^2) and lack of fit for each equation are also presented. R^2 in all cases was higher than 0.8, indicating that the equations obtained for each response variable explained the variation adequately. Following, HPP effects on the different parameters evaluated are presented and analyzed.

3.1. Microbial quality

Counts observed on untreated samples (Table 2) were in the range of those usually found in F&V beverages (Andrés et al., 2016; Barba, Esteve, & Frigola, 2013; Chen et al., 2013). As expected, the treatments were effective and samples showed reductions in all microbial counts. For MAB, reductions between 1 and 2 log cycles were observed, without significant differences among HPP treatments. Only the strongest treatments (650 MPa/5 min and 575 MPa/9 min) allowed to achieve reductions of 2 log cycles or more. Both EB and M&Y were more sensitive to HPP than MAB, presenting counts below the detection limit (DL < 2.00 log CFU g^{-1}) in all treated samples.

According to Mújica-Paz, Valdez-Fragoso, Samson, Welti-Chanes, and Torres (2011) differences in the sensitivities of microorganisms to HPP processing arise from their dissimilar cell wall morphology, as well as from the environment in which they are found. Similar results to those presented in this research were obtained by Chen et al. (2015) who worked with papaya beverage with initial counts of 5.54 and 3.73 log CFU g^{-1} of MAB and M&Y. They observed that HPP treatments at 450 MPa or higher reduced M&Y under DL, whereas for MAB, pressure level at least 550 MPa were needed to obtain counts under DL, regardless of treatment time. Moreover, Chen et al. (2013) working with pomegranate juice treated at 400 MPa, found that applying a holding time of 2.5 min, M&Y were eliminated, although 20 min were needed in order to eliminate MAB counts. It is important to mention that the different pressure levels and holding times observed for microorganism inactivation in different studies may be due to the different food matrices (TSS, pH, sugar concentration), type of microorganisms (species and strains) and pressurization and depressurization rates (Chen et al., 2015; Koseki & Yamamoto, 2014).

3.2. Physicochemical characteristics

In the treated samples pH varied between 3.76 and 3.81 and total soluble solids (TSS) between 9.03 and 9.80°Brix. In both cases the observed differences found to be not statistically significant ($p > 0.05$). Moreover, these values were not different than those of the control (Table 2). These results are in agreement with numerous studies on the effect of HPP on different fruits and/or vegetable based beverages (Barba et al., 2013; Chen et al., 2013; Chen et al., 2015; Jayachandran et al., 2015; Swami Hulle et al., 2017; Varela-Santos et al., 2012) that show that pH and TSS are generally unaffected by the treatment.

3.3. Chromatic parameters

The untreated smoothies presented a reddish color with chromatic values as showed in Table 2. The treated smoothies presented the same reddish color. The variations induced by HPP treatment in the chromatic parameters were proven to be statistically insignificant ($p > 0.05$), except for Ch which had a significant ($p < 0.05$) negative correlation coefficient with the quadratic term of the holding time (Table 3). According to the developed second order equation, in low to medium holding times an increase in the value of Ch was observed until reaching a critical point from which the values begin to descend. A high Chroma is associated with high intensity or saturation of color. In this sense, in this study domain, treatments of intermediate pressures and times seem to be the most suitable.

HPP was applied largely to preserve fresh color in many F&V products (Andrés et al., 2016; Oey et al., 2008; Patras, Brunton, Da Pieve, Butler, & Downey, 2009). Nevertheless, like in this study, many authors have informed changes in chromatic parameters. Particularly related with Ch changes, Barba, Esteve, and Frigola (2010) found that HPP treated (100–400 MPa/2–9 min) vegetable beverage had higher color

Table 3
Regression coefficients, R^2 values and lack of fit test results for the response variables of the fruits & vegetable mixed smoothie subjected to HPP.

Regression coefficient	Betacyanin	DPPH	FRAP	PME activity	POD activity	PPO activity	Chroma
Constant	**26.113***	**747.389***	**6741.105***	**86.946***	**7.808***	**74.613***	**−0.499***
P (linear)	−0.055	−1.290	**−20.281***	**−0.133***	**0.357***	−0.139	0.066
t (linear)	−0.254	−28.247	**−297.726***	−1.738	**0.257***	−4.357	0.868
P^2 (Quadratic)	**0.00006***	0.0012	**0.020***	0.00004	**−0.0004***	0.00011	−0.00007
t^2 (Quadratic)	**0.060***	**2.461***	**20.587***	0.1656	−0.211	**0.2543***	**−0.102***
P*t	−0.00059	0.0109	0.245	−0.0021	0.0014	0.0036	0.00025
R^2	0.938	0.937	0.968	0.963	0.982	0.879	0.895
Lack of fit	0.2578	0.7634	0.1072	0.1173	0.2606	0.8631	0.6586

Reduced equations for process parameters:
Betacyanin ($mg\,L^{-1}$) = $0.00006\,P^2 + 0.060\,t^2 + 26.113$
DPPH (TEAC $100\,g^{-1}$) = $2.461\,t^2 + 747.389$
FRAP (TEAC $100\,g^{-1}$) = -20.281 P-$297.726\,t + 0.020\,P^2 + 20.587\,t^2 + 6741.105$
PME Activity = $-0.133P + 86.946$
POD Activity = $0.357\,P + 0.257\,t - 0.0004\,P^2 + 7.808$.
PPO Activity = $0.2543\,t^2 + 74.613$
Chroma = $-0.102\,t^2 - 0.499$
DPPH: radical scavenging capacity, FRAP: Ferric reducing capacity, PME: pectinmethylesterase, POD: Peroxidase, PPO: Poliphenoloxidase.
* Significant at 0.05 level.

saturation (Chroma) than the unprocessed beverage. Similar results were found by Patras, Brunton, Da Pieve, Butler, and Downey (2009) in tomato purees. Moreover, González-Cebrino, Durán, Delgado-Adámez, Contador, and Ramírez (2013), working with plum purée found similar or higher values of the parameters Ch or h° in HPP treated samples (400-600 MPa/1–300 s), indicating that HPP maintained or even improved the color of the plum puree. In the case of this smoothie the higher Chroma value, may be related to a greater extractability of the red pigments (betacyanins). According to Oey et al. (2008) the cell disruption caused during HPP can result in the leakage of pigments into the intercellular space, which could yield a more intense bright color.

3.4. Back extrusion analysis

All treated samples showed significant reductions ($p < 0.05$) in the values of consistency and firmness in relation to the untreated samples (Table 2). In the case of firmness, reductions between 23 and 36% were observed, without significant differences among HPP treatments. A similar result was observed for consistency with reductions between 17 and 37%.

Similar results were found by Ahmed, Ramaswamy, and Hiremath (2005) who observed significant reductions in the viscosity of mango pulp after HPP (300–400 MPa/15–30 min). Furthermore, Verlent, Hendrickx, Rovere, Moldenaers, and Loey (2006) who worked with high pressure treated tomato pulp (0.1-500 MPa/15 min/30–70 °C) observed drastic losses in consistency of the pulp pressurized at 300–400 MPa, regardless of the temperature applied. They associated these losses with PME and polygalacturonase (PG) activities which were higher in this range. Indeed, due to cell disruption, HPP facilitates the occurrence of enzymatic and non-enzymatic reactions that cause transformations in the cell wall polymers (Oey et al., 2008). Particularly on F&V puree, smoothie or juice texture, activities of PME and PG are highly relevant, since they act synergistically leading to a decrease in textural integrity (Chakraborty et al., 2014). It is important to mention that PG is considerably more baro-sensitive than PME (Chakraborty et al., 2014), hence, achieving PME inactivation will probably prevent texture losses from being greater during the storage of the product.

3.5. Total phenols content

The total phenols content (TPC) of the high pressure treated samples ranged between 62.07 ± 0.91 and 68.33 ± 1.35 mg GAE $100\,mL^{-1}$. Thus, TPC was preserved or increased around 10% after treatment, however this increase was proven to be not statistically significant ($p > 0.05$) in relation to untreated samples (Table 2).

This result is in agreement with other studies reporting similar behavior of total polyphenol content after high-pressure treatment. For instance, Andrés et al. (2016) obtained increases of 6.6% and 4.2% in the TPC of mixed F&V smoothies high pressure-treated at 450 MPa/3 min and 600 MPa/3 min, respectively. Varela-Santos et al. (2012) also reported TPC increments between 3.3% and 11.9% in pomegranate juices treated at 350 MPa/30–150 s and 550 MPa/30–150 s, respectively.

According to Chen et al. (2013), TPC increase could be related with the fact that during the compression stage, the volume of system tends to be reduced, the extracting solvent comes into cells to interact with bioactive components and the pressurized cells show increased permeability, hence, an increased extractability of some components.

3.6. Betaxanthins (Bx) and betacyanins (Bc)

The betalains family represents the principal pigment in red beet, present in their root, stems and in the leaves veins. In particular, two classes of betalains are well-known: the red violet betacyanins, and the yellow orange betaxanthins (Ninfali & Angelino, 2013).

For betaxanthin, all high pressure-treated samples presented higher values (2–15%) than control (Table 2). Nevertheless these increases induced by the treatment, were proven to be statistically non-significant ($p > 0.05$).

On the other hand, betacyanin content of the treated samples were affected by treatments and ranged between 92.1 and 107.1% in relation to control (Table 2). Differences between the behavior of betalains could be associated to the molecular structure of these compounds which differ by the residues attached to the main structure: betacyanins exhibit a closed structure of cycloDOPA (cyclo-3,4-dihydroxy-phenylalanina) and can be substituted with sugars and acyl groups, whereas betaxanthines are conjugated with amines and amino acids. In line with these results Celli and Brooks (2016), who reviewed the effect of different processing conditions and technologies on stability of betalains, also associated differences between betacyanins and betaxanthines behavior to the molecular structure of pigments.

Betacyanin content in high pressure-treated samples had significant ($p < 0.05$) positive correlation coefficients with the quadratic terms of pressure and holding time (Table 3). As in all cases when the quadratic term is significant, there is a critical value (in this case for both, pressure and holding time) that must be considered. The highest contents of betacyanins were found in the most intense treatments: 575 MPa/9 min, 425 MPa/9 min and 650 MPa/5 min (Table S1).

There are few previous studies in which the effect of HPP on these particular pigments was evaluated. Interestingly, Paciulli, Medina-Meza, Chiavaro, and Barbosa-Cánovas (2016) working with beetroot HPP-treated (650 MPa/3–30 min) found significant increases in betanin (betacyanin) content of treated samples, with a time-dependent behavior. They observed that up to 7 min, > 6-fold increase in betanin contents was achieved, because of a better extraction from the broken cells, whereas holding times of 15 min onwards decreased the yields. According to the authors, due to the high sensitivity of betanin to oxidation, the baro-induced increase of oxygen partial pressure in HPP treated samples could be the reason for the observed reduction at more extended holding times. Even though during smoothies' homogenization significant amounts of oxygen are introduced into the system this effect was not observed in our work, probably because we worked with relatively short holding times (1–9 min).

3.7. Antioxidant capacity

After HPP, antiradical antioxidant capacity (DPPH) of the treated samples ranged between 96.3 and 114.3% in relation to the untreated smoothie (Table 2). Table 3 shows the regression coefficients of antioxidant capacity as estimated by the application of multiple linear regression analysis. DPPH had a significant ($p < 0.05$) positive correlation coefficient with the quadratic term of holding time. The surface plot of DPPH corresponding to pressure and holding time (Fig. 1a) has a concave shape and provides evidence that holding time had a stronger impact on DPPH values than pressure, meaning that the best results could be obtained working with lower pressures and longer times. Indeed, with longer holding times (9 min) the highest DPPH values were achieved.

Ferric-reducing capacity (FRAP) was increased after HPP with values between 16.4 and 82.9% higher than the control (Table 2). The effects of the pressure level and the holding time were not strictly linear since the equation contains both negative lineal coefficients (first order term) and positive quadratic coefficients for both factors. Therefore, an increase in P and t determines a reduction in FRAP capacity, however, the negative quadratic terms indicate that there are critical values for these parameters from which the tendency is reversed. Furthermore, as it can be observed in Fig. 1b, within the domain, the highest values for FRAP are achieved for the highest pressure level and holding time (650 MPa and 9 min).

Regarding antioxidant capacity, literature data is very variable. While some authors found that is not affected by HPP (Andrés et al., 2016; Chen et al., 2015) others found decreases (Barba et al., 2013; González-Cebrino et al., 2013) and others increases (Swami Hulle et al., 2017; Varela-Santos et al., 2012). Certainly, the effect of HPP on antioxidant activity depends not only on the pressurization conditions but also on the type of food matrix under study and also on the method used for its evaluation. It is therefore fundamental to study the HPP effect for each particular product

Similar results to those obtained in this study were found by Patras, Brunton, Da Pieve, and Butler (2009) who also reported higher antiradical activities at higher level of pressure compared to lower one in strawberry purees (600 vs. 400 MPa). Also, an increase of 37 and 27% in antioxidant potential was reported in carrot and tomato purees, respectively, at 600 MPa/15 min (Patras, Brunton, Da Pieve, Butler, & Downey, 2009). Again, the most accepted reason for this increase is the better extractability of antioxidant components due to changes in the tissue matrix, induced by HPP, resulting in the release of compounds with antioxidant actions into the extracellular environment (Jayachandran et al., 2015).

3.8. Enzyme activities

The PME activity of treated samples ranged between 6.9 and 36.8% in relation to the one of the untreated samples (Table 2). Regardless of the holding time, which did not affected PME activity, the higher the pressure level, the more effective was the inactivation of PME, which was reflected by a significant ($p < 0.05$) negative regression coefficient (Table 3). Thus, for the pressure range studied (350–650 Mpa), an increase in the pressure level will result in a decrease in the PME activity (Fig. 2a). Accordingly, the treatment at 650 MPa and 5 min achieved the highest PME inactivation (81.5%).

Similar results were found by Nienaber and Shellhammer (2001) working with orange juice treated with HPP (400-600 MPa/3 min). They found that PME inactivation increased with the level of pressure, achieving almost complete inactivation at 600 MPa/3 min. Moreover, Timmermans et al. (2011) found a similar level of PME inactivation (92%) in orange juice treated at 600 MPa/1 min. A higher baroresistence was observed by Rao et al. (2014) in HPP-treated (400–600 MPa/5–25 min) peach juice. They observed that PME was inactivated significantly with increasing P and t, although the maximum inactivation achieved was only 50% (600 MPa/25 min).

There have been many studies reporting pressure induced changes in PME, and the extent of changes varies with different commodities, indicating that the source of the enzyme and substrate can also affect the barosensitivity of enzymes (Swami Hulle et al., 2017). Since our smoothie had 60% of orange juice, it was not surprising that our results were closer to those observed in orange juice.

The POD activity of treated samples ranged between 63.1 and 93.1% in relation to the one of the untreated smoothie (Table 2). POD activity was affected by both, pressure level and holding time. The effect of P was not strictly linear since the equation contains both a significant ($p < 0.05$) positive lineal coefficient and a significant

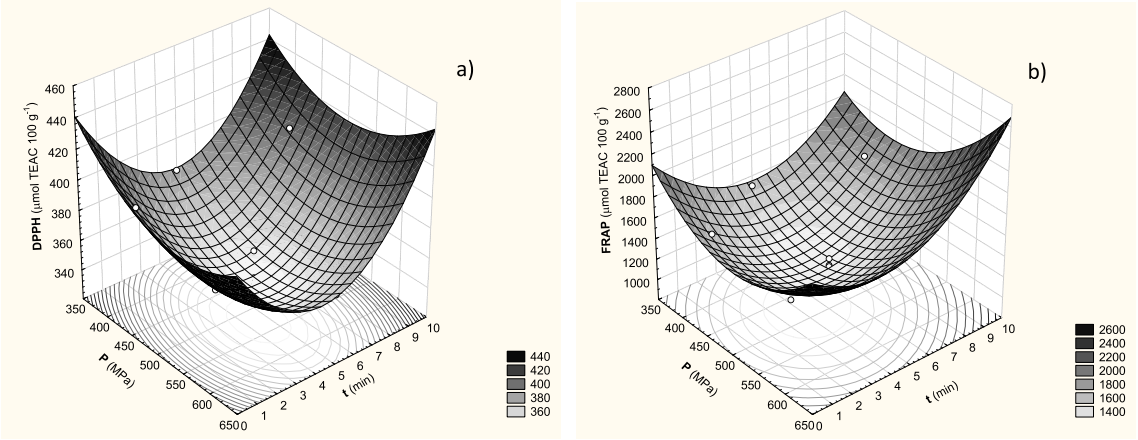

Fig. 1. Effect of Pressure level (P: 350–650 MPa) and holding time (t: 1–9 min) on the antioxidant capacity, a) DPPH and b)FRAP, of fruit & vegetable smoothie.

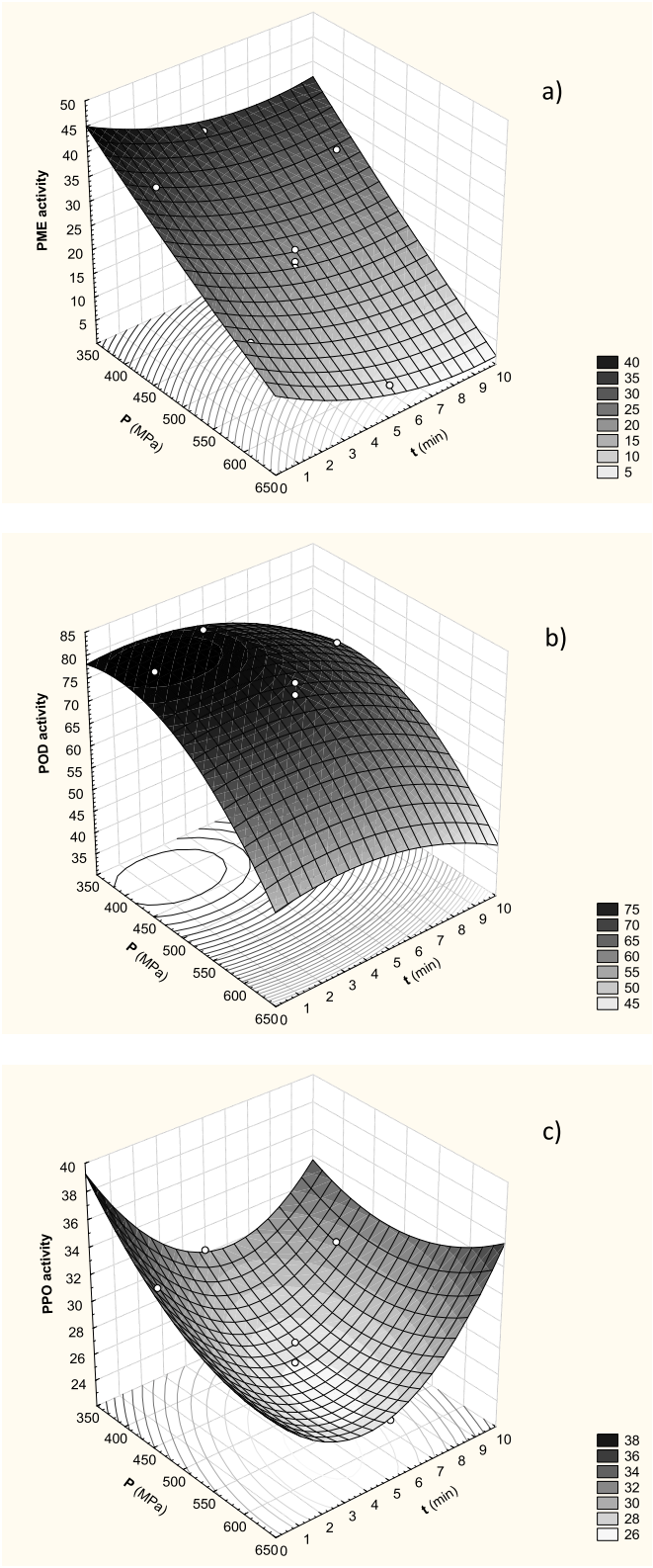

Fig. 2. Effect of Pressure level (P: 350–650 MPa) and holding time (t: 1–9 min) on the enzyme activity, a)PME, b) POD and c)PPO, of fruit & vegetable smoothie.

($p < 0.05$) negative quadratic coefficient. The effect of t in this case was strictly linear, with a significant ($p < 0.05$) positive regression coefficient. Accordingly, an increase in P and t would determine an increase in POD activity. However, the negative quadratic term indicate that there is a critical pressure from which the situation is reversed, thus the maximum inactivation is finally achieved with the highest pressure level and holding time, as shown in Fig. 2b.

Interestingly, Duong and Balaban (2014) reported a similar behavior in feijoa puree, since they observed that at low pressure level (200–400 MPa) and/or with short holding time (1–7 min), POD activities tended to increase. On the other hand, when holding time or pressure level were increased, the enzyme activity decreased. Applying treatments between 400 and 600 MPa, for > 8 min, they achieved a maximum reduction, with a residual activity below 70%. Similarly, Liu, Zhao, Zou, and Hu (2013), studyingthe effect of HPP (200–600 MPa and 5–60 min) in watermelon juice, reported a maximum POD inactivation of about 42% at 600 MPa/60 min. Moreover, they observed a stronger impact of pressure level on the POD activity values.

The PPO activity of treated samples ranged between 78.8 and 106.6% in relation to the one of untreated smoothie (Table 2), resulting in the most baroresistant enzyme among the studied ones. Additionally, under certain conditions, some activation was observed. The PPO activity had a significant ($p < 0.05$) positive correlation coefficient with the quadratic term of holding time (Table 3). In the surface plot of PPO as a function of pressure level and holding time (Fig. 2c) can be observed the concave shape and the stronger impact of holding time on the PPO activity values. In low to medium times a reduction of activity values is observed until a critical point is reached from which the values begin to increase. Within the study domain, the lowest activity values were observed in the treatments with an intermediate (5 min) holding time.

Very variable responses of PPO activity have been observed on different food matrices subjected to HHP treatments. For instance, Duong and Balaban (2014) in feijoa puree observed that at low pressure level (200–400 MPa) and/or with short treatment time (1–7 min), PPO activities tended to increase up to around 120% residual enzyme activity (REA), but as the holding time increased, the enzyme activity tended to decrease. According to González-Cebrino et al. (2013), who worked with plum purée, the higher activity of the PPO after processing could be attributed to the release of membrane-bound enzymes, which could increase the extractability of PPO, counteracting the inactivating effect of the HPP. Another factor which may contribute to this behavior is the activation of latent PPO by the interaction with other constituents in the extract (Terefe, Yang, Knoerzer, Buckow, & Versteeg, 2010). In other studies different degrees of inactivation have been observed. For instance, Keenan, Rößle, Gormley, Butler, and Brunton (2012), working with a mixed fruit smoothie found that a treatment at 450 MPa/5 min resulted in 35% reduction of PPO activity, while a treatment at 600 MPa/10 min resulted in a considerable reduction (83%) of PPO activity.

In this study, low degrees of inactivation of POD and PPO were achieved. Nevertheless, characteristics such as polyphenol content and color remained unchanged or improved. However, future studies are necessary to evaluate how quality evolves with storage time.

3.9. Optimization of HPP conditions and validation

The response variables having at least one coefficient statistically significant in the effects considered in the regression models (betacyanin content, DPPH capacity, FRAP capacity, PME, POD and PPO activities, Chroma) were selected for simultaneous optimization of process condition. As detailed above, HPP affected dissimilarly each response; hence, this tool is fundamental to achieve a compromise solution that allows obtaining good results for all the variables under study. The Fig. 3 shows the predicted profiles at the different levels assayed for each independent variable (pressure level and holding time), while maintaining constant the level of the other independent variable at the estimated optimal value. Fig. 3 also shows each individual desirability function and the global desirability function profiles.

The criteria selected for the optimization of process parameters

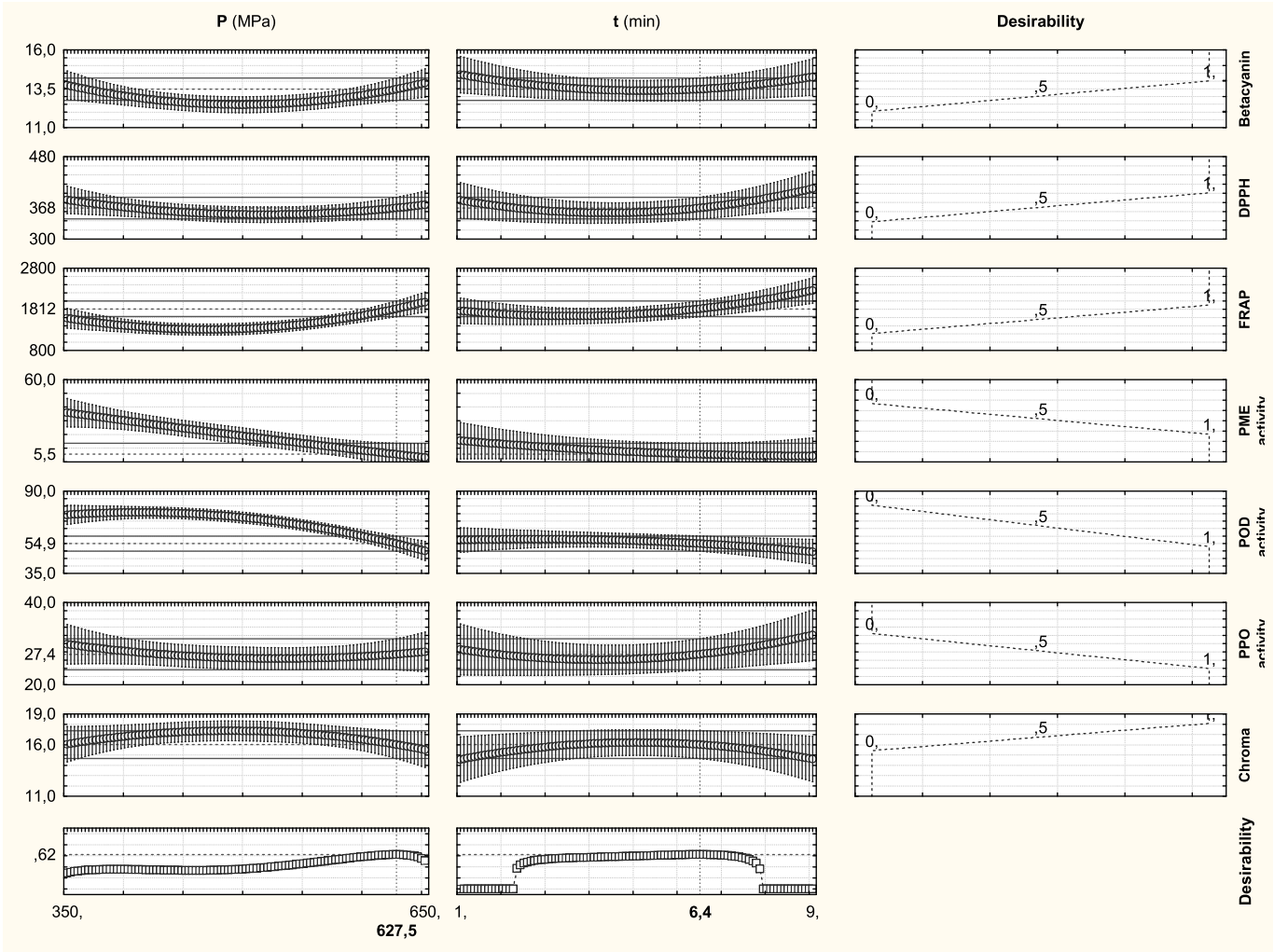

Fig. 3. Simultaneous optimization of process conditions for HPP of fruits & vegetable smoothie. Predicted profiles for the response variables at differents levels of each independient variable, holding the levels of the other independient variable constant at the estimated optimal value; for each individual desirability function pro file and for the global desirability function pro file.

Table 4
Relative error between predicted and actual values for fruits & vegetable smoothie processed under optimized HPP conditions.

Values	Optimized process parameters		Response variables						
	Pressure level (MPa)	Holding time (min)	PME ACTIVITY	POD ACTIVITY	PPO ACTIVITY	Bc (mg L^{-1})	DPPH (μmol TEAC/ 100 g)	FRAP (μmol TEAC/ 100 g)	Chroma
Predicted	627.5	6.4	5.5	54.9	27.4	13.5	368	1812	16.0
Actual	630.0	6.0	4.8	81.2	15.0	14.9	363	1797	17.5
% error**	0.33	−4.76	3.1	−3.1	0.5	1.2	3.0	0.4	0.2

PME: pectinmethylesterase, POD: Peroxidase. PPO: Poliphenoloxidase, Bc: Betacyanin, DPPH: radical scavenging capacity, FRAP: Ferric reducing capacity.
**For the calculation of the error, the values of the response variables were relativized to the value of the corresponding control, since in each new elaboration there may be differences associated to the variability of the raw material. The low %error means that the response was similar regardless of the initial values of the product. The %error was calculated as follows:

$$\%\text{error} = \frac{\%\text{actual from control} - \%\text{predicted from control}}{\%\text{predicted from control}}$$

were: maximization of bioactive component concentration and antioxidant capacity (betacyanins, DPPH and FRAP); maximization of color saturation (Chroma) and minimization of enzyme activity (PME, POD, PPO). Based on the above criteria, the predicted optimal process condition leading to the maximum value of global desirability function for the process under study was a combination of a pressure level at 627.5 MPa and a holding time at 6.4 min (which would correspond to practical operating values at 630 MPa and 6 min).

Further, the smoothie was processed under these conditions and its quality was compared with the predicted response values. The relative deviation was found to be < 5% (Table 4), verifying that with the optimized HPP parameters a high quality smoothie is obtained.

4. Conclusions

The results of the present study show that HPP is a promising technology for the preservation of the mixed fruit & vegetable smoothie, reducing spoilage microorganisms counts and enzymes activity. Moreover, HPP does not affect pH, total soluble solids, texture and total phenolic content, increases antioxidant capacity largely and maintains or slightly improves color of the smoothie. The optimization analysis suggests that HPP applied at 627.5 MPa and 6.4 min would lead to a product with high quality and maximum reduction of spoilage causing factors.

Moreover, the developed quadratic models might be useful to predict the quality characteristics of smoothie during the HPP within the studied domain of process conditions. Future studies will be oriented to evaluate the stability of the different quality attributes during storage, moreover, shelf-life estimation and scale-up studies may be explored in order to transfer HPP to the smoothies industry.

Acknowledgements

This research was financially supported by Universidad de Buenos Aires (20020130100176BA; 20020120300008BA), Instituto Nacional de Tecnología Agropecuaria, INTA (PNAIyAV 1130033) and Agencia Nacional de Promoción Científica y Tecnológica (PICT 2013-0636) projects. The authors also gratefully acknowledge the Peruilh Foundation (FIUBA) for Fernandez MV scholarship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2018.02.011.

References

Ahmed, J., Ramaswamy, H. S., & Hiremath, N. (2005). The effect of high pressure treatment on rheological characteristics and colour of mango pulp. *International Journal of Food Science and Technology, 40*(8), 885–895.

Andrés, V., Villanueva, M. J., & Tenorio, M. D. (2016). The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry, 192*, 328–335.

Augustin, M. A., Ghazali, H. M., & Hashim, H. (1985). Polyphenoloxidase from guava (Psidium guajava L.). *Journal of the Science of Food and Agriculture, 36*(12), 1259–1265.

Barba, F. J., Esteve, M. J., & Frigola, A. (2010). Ascorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. *Journal of Agricultural and Food Chemistry, 58*(18), 10070–10075.

Barba, F. J., Esteve, M. J., & Frígola, A. (2012). High pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: A review. *Comprehensive Reviews in Food Science and Food Safety, 11*(3), 307–322.

Barba, F. J., Esteve, M. J., & Frigola, A. (2013). Physicochemical and nutritional characteristics of blueberry juice after high pressure processing. *Food Research International, 50*(2), 545–549.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta, 76*(5), 965–977.

Bup, D. N., Abi, C. F., Tenin, D., Kapseu, C., & Tchiegang, C. (2012). Optimisation of the cooking process of sheanut kernels (Vitellaria paradoxa Gaertn.) using the Doehlert experimental design. *Food and Bioprocess Technology, 5*(1), 108–117.

Celli, G. B., & Brooks, M. S. (2016). Impact of extraction and processing conditions on betalains and comparison of properties with anthocyanins—A current review. *Food Research International, 100*, 501–509.

Chakraborty, S., Kaushik, N., Rao, P. S., & Mishra, H. N. (2014). High-pressure inactivation of enzymes: A review on its recent applications on fruit purees and juices. *Comprehensive Reviews in Food Science and Food Safety, 13*(4), 578–596.

Chen, D., Pang, X., Zhao, J., Gao, L., Liao, X., Wu, J., & Li, Q. (2015). Comparing the effects of high hydrostatic pressure and high temperature short time on papaya beverage. *Innovative Food Science & Emerging Technologies, 32*, 16–28.

Chen, D., Xi, H., Guo, X., Qin, Z., Pang, X., Hu, X., ... Wu, J. (2013). Comparative study of quality of cloudy pomegranate juice treated by high hydrostatic pressure and high temperature short time. *Innovative Food Science & Emerging Technologies, 19*, 85–94.

Denoya, G. I., Fernandez, M. V., Sanow, L. C., Bello, F., Jagus, R. J., Vaudagna, S. R., & Agüero, M. V. (2017). Revalorización de subproductos hortícolas: desarrollo de un batido mezcla de frutas y verduras estabilizado con altas presiones hidrostáticas. *I Congreso Argentino de Biología y Tecnología Poscosecha. Concordia, Argentina (October 25–27)*.

Denoya, G. I., Polenta, G. A., Apóstolo, N. M., Budde, C. O., Sancho, A. M., & Vaudagna, S. R. (2016). Optimization of high hydrostatic pressure processing for the preservation of minimally processed peach pieces. *Innovative Food Science & Emerging Technologies, 33*, 84–93.

Duong, T., & Balaban, M. (2014). Optimisation of the process parameters of combined high hydrostatic pressure and dense phase carbon dioxide on enzyme inactivation in feijoa (Acca sellowiana) puree using response surface methodology. *Innovative Food Science & Emerging Technologies, 26*, 93–101.

Fernandez, M. V., Jagus, R. J., Agüero, M. V., Fito, P., & Gerard, J. (2014). Revalorización de hortalizas subutilizadas: caracterización y evaluación de hojas de remolacha y su potencial como fuente de compuestos beneficiosos para la salud. *Book of abstracts "international conference on food innovation". Concordia, Argentina* (pp. 64–68). (ISBN OC 978-950-698-376-5. ISBN 978-950-698-380-2).

González-Cebrino, F., Durán, R., Delgado-Adámez, J., Contador, R., & Ramírez, R. (2013). Changes after high-pressure processing on physicochemical parameters, bioactive compounds, and polyphenol oxidase activity of red flesh and peel plum purée. *Innovative Food Science & Emerging Technologies, 20*, 34–41.

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., & Givskov, M. (2002). Food spoilage–interactions between food spoilage bacteria. *International Journal of Food Microbiology, 78*(1–2), 79–97.

Hendrickx, M. E. G., & Knorr, D. (2002). Ultra high pressure treatments of foods. *Food engineering series*US: Springer (0306472783, 9780306472787).

Jayachandran, L. E., Chakraborty, S., & Rao, P. S. (2015). Effect of high pressure processing on physicochemical properties and bioactive compounds in litchi based mixed fruit beverage. *Innovative Food Science & Emerging Technologies, 28*, 1–9.

Kaushik, N., Rao, P. S., & Mishra, H. N. (2016). Process optimization for thermal-assisted high pressure processing of mango (Mangifera indica L.) pulp using response surface methodology. *LWT - Food Science and Technology, 69*, 372–381.

Keenan, D. F., Rößle, C., Gormley, R., Butler, F., & Brunton, N. P. (2012). Effect of high hydrostatic pressure and thermal processing on the nutritional quality and enzyme activity of fruit smoothies. *LWT- Food Science and Technology, 45*(1), 50–57.

Knorr, D. (1993). Effects of high-hydrostatic-pressure processes on food safety and quality. *Food Technology (USA), 47*(6), 156–161.

Koseki, S., & Yamamoto, K. (2014). High pressure microbial inactivation: modelling and its features. *Novel food preservation and microbial assessment techniques* (pp. 3–24). Taylor & Francis Group.

Kuehl, R. O. O. (2000). *Designs of experiments: statistical principles of research design and analysis.* Duxbury Press.

Lado, B. H., & Yousef, A. E. (2002). Alternative food-preservation technologies: Efficacy and mechanisms. *Microbes and Infection, 4*(4), 433–440.

Liu, Y., Zhao, X. Y., Zou, L., & Hu, X. S. (2013). Effect of high hydrostatic pressure on overall quality parameters of watermelon juice. *Revista de Agaroquimica y Tecnologia de Alimentos, 19*(3), 197–207.

Ludikhuyze, L., Van Loey, A., Indrawati, S. C., & Hendrickx, M. (2003). Effects of combined pressure and temperature on enzymes related to quality of fruits and vegetables: From kinetic information to process engineering aspects. *Critical Reviews in Food Science and Nutrition, 43*(5), 527–586.

Mayor, L., Moreira, R., & Sereno, A. (2011). Shrinkage, density, porosity and shape changes during dehydration of pumpkin (Cucurbita pepo L.) fruits. *Journal of Food Engineering, 103*(1), 29–37.

Moßhammer, M. R., Stintzing, F. C., & Carle, R. (2006). Cactus pear fruits (Opuntia spp.): A review of processing technologies and current uses. *Journal of the Professional Association for Cactus Development, 8*, 1–25.

Mújica-Paz, H., Valdez-Fragoso, A., Samson, C. T., Welti-Chanes, J., & Torres, J. A. (2011). High-pressure processing technologies for the pasteurization and sterilization of foods. *Food and Bioprocess Technology, 4*(6), 969–985.

Nienaber, U., & Shellhammer, T. H. (2001). High-pressure processing of orange juice: Kinetics of pectinmethylesterase inactivation. *Journal of Food Science, 66*(2), 328–331.

Ninfali, P., & Angelino, D. (2013). Nutritional and functional potential of Beta Vulgaris Cicla and rubra. *Fitoterapia, 89*, 188–199.

Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008). Effect of high-pressure processing on colour, texture and flavour of fruit-and vegetable-based food products: A review. *Trends in Food Science & Technology, 19*(6), 320–328.

Paciulli, M., Medina-Meza, I. G., Chiavaro, E., & Barbosa-Cánovas, G. V. (2016). Impact of thermal and high pressure processing on quality parameters of beetroot (Beta vulgaris L.). *LWT- Food Science and Technology, 68*, 98–104.

Patras, A., Brunton, N., Da Pieve, S., Butler, F., & Downey, G. (2009). Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. *Innovative Food Science & Emerging Technologies, 10*(1), 16–22.

Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies, 10*(3), 308–313.

Rao, L., Guo, X., Pang, X., Tan, X., Liao, X., & Wu, J. (2014). Enzyme activity and nutritional quality of peach (Prunus persica) juice: Effect of high hydrostatic pressure. *International Journal of Food Properties, 17*(6), 1406–1417.

Rastogi, N. K., Raghavarao, K. S. M. S., Balasubramaniam, V. M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition, 47*(1), 69–112.

Rickman, J. C., Bruhn, C. M., & Barrett, D. M. (2007). Review: Nutritional comparison of fresh, frozen, and canned fruits and vegetables. Part I. Vitamins C and B and phenolic compounds. *Journal of the Science of Food and Agriculture, 87*, 930–944.

Swami Hulle, N. R., Chakraborty, S., & Rao, P. S. (2017). Effect of high pressure thermal processing on the quality attributes of Aloe vera-litchi mixed beverage. *Innovative*

Food Science & Emerging Technologies, 40, 68–77.
Terefe, N. S., Yang, Y. H., Knoerzer, K., Buckow, R., & Versteeg, C. (2010). High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. *Innovative Food Science & Emerging Technologies, 11*(1), 52–60.
Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L., Van der Plancken, I., ... Matser, A. M. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: Impact on overall quality attributes. *Innovative Food Science & Emerging Technologies, 12*(3), 235–243.
Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J. E., Pérez-Won, M., Briones-Labarca, V., & Morales-Castro, J. (2012). Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science & Emerging Technologies, 13*, 13–22.
Verlent, I., Hendrickx, M., Rovere, P., Moldenaers, P., & Loey, A. V. (2006). Rheological properties of tomato-based products after thermal and high-pressure treatment. *Journal of Food Science, 71*(3), 243–248.
Viacava, G. E., Roura, S. I., & Agüero, M. V. (2015). Optimization of critical parameters during antioxidants extraction from butterhead lettuce to simultaneously enhance polyphenols and antioxidant activity. *Chemometrics and Intelligent Laboratory Systems, 146*, 47–54.
Vicente, A. R., Costa, M. L., Martínez, G. A., Chaves, A. R., & Civello, P. M. (2005). Effect of heat treatments on cell wall degradation and softening in strawberry fruit. *Postharvest Biology and Technology, 38*(3), 213–222.