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Optimization of high hydrostatic pressure processing for the preservation of minimally processed peach pieces

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ABSTRACT

Consumers demand fresh-cut fruits, free from additives and with fresh appearance. However, the alteration caused by the tissue processing limits their shelf life. The aim of this work was to optimize the pressure level (from 400 to 600 MPa) and the holding time (from 1 to 9 min) of the high pressure processing (HPP) to achieve enzyme inactivation while preserving texture and color of minimally processed peaches. Peach cylinders were processed by combining dipping in organic acid solution, with vacuum packaging and HPP at room temperature. Results showed that higher pressure levels were more effective to inactivate enzymes and to preserve color than longer times. In addition, long treatments affected the microstructure and the texture of the tissues more seriously. Finally, a desirability study and a principal component analysis were performed. These showed that the optimal treatment would be 585 MPa and 1 min and that the best treatment of the ones studied was 600 MPa for 5 min.

Industrial Relevance: There is an increasing demand for minimally processed fruits as a result of their convenience and fresh-like characteristics. Although consumers are familiar with the consumption of canned peaches, the nutritional profile of this product is far from being optimal, and therefore minimal processing offers the unique advantage of maintaining the original quality of the fresh fruit. However, this product is prone to suffer alterations such as browning and softening. High pressure processing (HPP) is proposed as a non-thermal technology able to suitably preserve minimally processed peaches. This study aimed to optimize the conditions of the HPP treatment, to achieve enzyme inactivation while maintaining texture and color. The promising results obtained can help promote the use of HPP as an alternative to preserve the quality and extend the shelf life of minimally processed fruits.

1. Introduction

Modern lifestyle tends to hinder the consumption of fresh fruits and vegetables. In response to this, minimally processed fruits and vegetables represent a convenient way to include these nutritive foods in everyday meals (Oms-Oliu et al., 2010). However, minimally processed products deteriorate at a faster pace than intact commodities, since processes such as peeling and dicing damage the tissues, leading to the release of the cell content, the disruption of the cellular compartmentalization, and the mixture of enzymes and substrates normally separated in intact tissues. In addition, the leakage of juices from the wounded tissues makes these products highly susceptible to microbial spoilage, allowing the growth of bacteria and yeasts, responsible for fermentation and other alterations. Other deteriorative factors such as water loss and osmotic changes cause a decrease in turgor and crispness, which leads to loss of texture. Minimally processed fruits are also prone to suffer browning as a result of the enzymatic oxidation of polyphenols in the presence of oxygen by polyphenoloxidase (PPO) (Toivonen & Brummell, 2008).

High pressure processing (HPP) is a promising non-thermal alternative for the extension of the shelf life of fruit-based products, since it acts as a cold pasteurization process. The main advantage of this technology is its ability to maintain different compounds, such as pigments, volatiles, vitamins, and other health-promoting compounds, rather unaffected (Heinz & Buckow, 2010; Oms-Oliu, Odriozola-Serrano, Soliva-Fortuny, Elez-Martínez, & Martín-Belloso, 2012). However, because of the known baroresistance of PPO, browning reactions cannot be totally avoided, and therefore, there is a need to combine HPP with other technologies such as packaging in films with barrier property to oxygen permeability. By using this combination, the browning can be prevented during storage by the limited access to oxygen exerted by the film and by the partial inhibition of PPO brought about by HPP when the packs are opened. This strategy helps to maintain the color of the product for several hours after the exposure to the normal atmosphere (Perera, Gamage, Wakeling, Gamlath, & Versteeg, 2010). However, the use of vacuum in living tissues is rather limited because the anaerobic environment generated inside the packages could induce a

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fermentative metabolism. Although studies related to the effect of HPP on anaerobiosis are scarce, some authors have suggested that the application of HPP immediately after packing the product under vacuum can prevent this problem. Among them, Trejo Araya et al. (2009) reported that the accumulation of fermentative products such as ethanol and acetic acid was prevented in pressure-treated raw carrots packed under vacuum after 14 days of storage at 4 °C, while these compounds were detected in control samples.

From the textural point of view, HPP causes cell disruption, increases cell membrane permeability, and allows the movement of water and metabolites throughout the cells of plant tissues. As a consequence, the substrates and enzymes that are compartmentalized in the intact cells can be released, leading to their interaction and the consequent development of alterations such as browning. Interestingly, depending on the intensity of the treatment applied, HPP can either enhance or inhibit the activity of enzymes related to cell wall degradation (Oey, Lille, Van Loey, & Hendrickx, 2008). On the other hand, although the texture of firm tissues presenting low amounts of entrapped air (e.g. cauliflower) remains rather unaltered, HPP can cause some alterations such as the induction of a water-soaked appearance (Prestamo & Arroyo, 1998).

Based on the above, the aim of the present study was to optimize the main processing parameters of HPP (pressure level and holding time) in order to preserve minimally processed peaches by achieving enzyme inactivation while maintaining texture and color. Two enzymes, considered as biochemical markers, were included in the study to monitor the effect of treatments: PPO (EC 1.10.3.1—the main enzyme that catalyzes enzymatic browning) and alcoholdehydrogenase (ADH) (EC 1.1.1.1—an enzyme related to induction of fermentation under anaerobic conditions).

2. Materials and methods

2.1. Raw material

Peaches (*Prunus persica* (L.) Batch) cv. Flavorcrest were harvested from an experimental orchard in San Pedro, Buenos Aires, Argentina (33°41_S, 59°41_W) and carefully selected according to uniform weight (mean fruit mass ± 170 g) and ground color. The fruit selected had a mean value of 12°Brix and firmness between 30 and 40 N. Peaches were stored in a cold chamber at 0 °C and 90–95% relative humidity for 1 week before processing.

2.2. Sample preparation

Prior to processing, the peaches were washed in running tap water. Cylinders (15 mm in length, 15 mm in diameter) of parenchyma tissue were obtained using a stainless steel cork borer and knife. To obtain homogenous samples, the cylinders were taken from the middle zone of the mesocarp, parallel to the major axis of the fruit. Subsequently, the cylinders were dipped into tap water containing 20 ppm of HClO for 2 min. The cylinders were then drained and dipped into an aqueous solution containing 1% (*w*/*v*) ascorbic acid (ACS, Biopack, Argentina) and 1% (*w*/*v*) citric acid (USP, Anedra, Austria) for 2 min to prevent surface browning and to wash the remaining HClO. The cylinders were drained again, pooled, and vacuum-packed in Cryovac BB2800 bags (O_2 transmission rate: 6–14 cm^3/m^2/24 h at 23 °C, 1 atm) filled randomly with eight units each, using a double chamber vacuum packaging machine (Rapivac, Model Maximax 800, Argentina). Afterwards, the samples were subjected to HPP treatments with different pressure levels and holding times, selected according to the experimental design (see below). Eight bags were prepared for each treatment. 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. Pressure was increased at 5 MPa s^{-1}. The HPP treatment was carried out at an initial temperature of 21–24 °C and this parameter was modified by adiabatic heating. The maximum temperature of the compression fluid was 35 °C for 400–500 MPa and 38 °C for 550–600 MPa, and upon pressure release reduced to 20 °C.

The pH of the samples before and after being treated with HPP ranged between 3.4 and 3.5 and presented no significant ($p > 0.05$) differences among the different HPP treatments. After processing, samples were kept at 4 °C until all the determinations were carried out.

2.3. Sample analysis

2.3.1. Chromatic parameters

Chromatic parameters of peach cylinders were measured with a Minolta CR-400 chromameter (Konica Minolta, Japan), using the CIE scale L*a*b*. The instrument was set up for illuminant D_{65} and 2° observer angle. CIE L*a*b* parameters were measured on the surface of cylinders immediately after opening the packages (a^*_0) and 180 min later (a^*_{180min}). The change of a* in time was calculated as the $\Delta a^*/min = [a^*_{180min} - a^*_0]/180$ min ratio.

2.3.2. Texture profile analysis

Instrumental approaching for texture of fresh-cut peaches was performed by running a texture profile analysis. Eight cylinders per treatment were compressed twice to 75% of their original height (1 s interval) simulating mastication. A Texture Analyzer model TA-XT plus (Stable Micro Systems LTD, Surrey, England) was used at room temperature and the following conditions were set according to the instrument manufacturer's recommendations: 3.0 mm/s pre-test speed, 0.8 mm/s test speed, 3.0 mm/s post-test speed, and 25% strain. A 35-mm diameter cylindrical probe (P/35) was used to assure that the surface area of the peach cylinder was completely covered by the probe. Force–distance–time data were recorded for two cycles. Texture parameters: hardness (maximum force attained during the first compression cycle, N), cohesiveness (ratio of positive force area during the second compression cycle to that during the first compression cycle, dimensionless), springiness (sample recovery from deformation, dimensionless), and chewiness (hardness × cohesiveness × springiness, N) were calculated from force, distance, and time data, by using Texture Exponent 32 software (Stable Micro Systems LTD). The distance covered by the probe when subjected to a force of 5 N was measured as an additional parameter named “deformation at 5 N.” This parameter indicates to what extent the material is deformed under the action of a given force.

2.3.3. Light microscopy

Fresh fruit and cylinders from mesocarp of peaches after each HPP treatment were hand-sectioned and mounted with distilled water. The slides were examined using a light microscope (Nikon Eclipse E200, Tokyo, Japan) equipped with a digital camera (Botany Laboratory, Lujan National University, Buenos Aires, Argentina).

2.3.4. Enzyme activities

2.3.4.1. Enzyme extraction. Enzymes were extracted according to the method described by Gonzalez, de Ancos, and Cano (1999), with some modifications. All steps were performed covering samples with ice, to avoid enzyme inactivation. Then, 7 g of peach cylinders was extracted with 20 ml of 0.1 M phosphate buffer pH 7.3 containing 1% *w*/*v* insoluble polyvinylpolypyrrolidone (PVPP, Sigma, USA) as a phenolic scavenger and 0.5 mM phenylmethylsulfonyl fluoride (PMSF, Sigma, Germany) as a protease inhibitor, by using a Homogenizer Power Gen 1000 (Fisher Scientific, Germany). After homogenization, the homogenate was kept in agitation for 30 min. Then, the homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was used as the enzyme source in the following experiment.

2.3.4.2. PPO enzyme assay. The PPO enzyme assay was carried out according to the method described by Jiménez and García-Carmona

(1997) with some modifications. PPO catecholase activity was followed by using a spectrophotometric method based on the measurement of the initial rate of increase in absorbance at 400 nm due to the production of 4-tert-butyl-o-benzoquinone. A volume of 1 ml of 50 mM phosphate buffer solution pH 6.5 with 4.95 mM 4-*tert*-butylcathecol (Aldrich Chemistry, France) as a substrate and 100 μl of the enzyme extract was pipetted into a test tube and mixed thoroughly. Then, the mixture was rapidly transferred to a 1-cm path length cuvette. The absorbance at 400 nm was continuously recorded at 30 °C for 5 min by using a Gilford Response ultraviolet–visible spectrophotometer (Gilford/CIBA-Corning Diagnostics, USA). The activity was expressed as units per gram of fruit. One unit represents the amount of enzyme necessary to change A_{400} nm in 0.01/min at 30 °C. The residual PPO activity was expressed as the percentage of activity in relation to the untreated fruit (%).

2.3.4.3. ADH enzyme assay. The ADH enzyme assay was carried out according to the method described by Walker (1992) with slight modifications. ADH activity was followed by using a spectrophotometric method based on the measurement of the initial rate of increase in absorbance at 340 nm due to NADH (β-Nicotinamide adenine dinucleotide, reduced) accumulation during ethanol oxidation. A volume of 1 ml of 200 mM TRIS (TRIS [hydroxymethyl] aminomethane, USB Corporation, USA) pH 8.8 with 100 mM ethanol (98.5%, Dorwil, Argentina) as substrate and 100 μl of the enzyme extract was added into a test tube and mixed thoroughly. Then, the mixture was rapidly transferred to a 1-cm path length cuvette. The reaction was initiated by the addition of 1 mM of NAD+ (β-Nicotinamide Adenine Dinucleotide, Sigma, USA). The absorbance at 340 nm was continuously recorded at 30 °C for 3 min by using a Gilford Response ultraviolet–visible spectrophotometer (Gilford/CIBA-Corning Diagnostics, USA). The activity was expressed as units per gram of fruit; one unit represents the amount of NADH produced in μM per min at 30 °C. The residual ADH activity was expressed as the percentage of activity in relation to the untreated fruit (%).

2.3.5. Experimental design

The HPP conditions of each treatment were based on a Doehlert uniform shell design with two factors: pressure level (P) and holding time (t). The Doehlert design describes a spherical experimental domain. For two variables, the Doehlert design consists of one central point and six points forming a regular hexagon (Fig. 1). The experimental design required nine trials with the central point in triplicate to determine the experimental error.

Doehlert designs offer some advantages over other designs: they need fewer experiments, and the number of levels is not the same for all variables, which allows flexibility to assign either a small or a large number of levels to the selected variables. Generally, it is preferable to choose the variable with the strongest effect as the variable with maximum levels to obtain maximum information of the system (pressure level in this case) (Ferreira, dos Santos, Quintella, Neto, & Bosque-Sendra, 2004). This method has been successfully applied to optimize different process parameters (Bup, Abi, Tenin, Kapseu, & Tchiegang, 2012; Mayor, Moreira, & Sereno, 2011). Basically, this optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, and predicting the response.

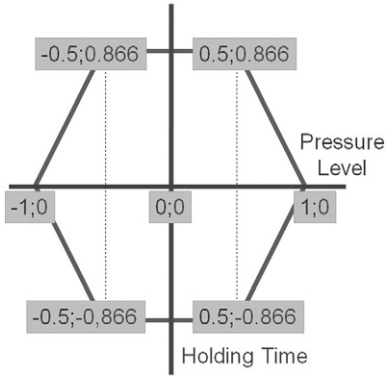

Fig. 1. Experimental Doehlert design for two factors in coded values.

The values of the factors were coded according to Eq. 1:

$$x_i = (X_i - X_0)/\Delta X \quad (1)$$

where x_i is the (dimensionless) coded value of the factor X_i, X_0 is the value of X_i at the central point, and ΔX is the step change.

Table 1 shows the coded and actual values of the factors of the experimental shell design and their levels. The selected ranges of pressure level and holding time were determined according to several preliminary studies (Denoya, Vaudagna, & Polenta, 2015; Denoya et al., 2010).

2.3.6. Statistical analysis

The response surface methodology was used for the analysis of the Doehlert design. For each response variable (textural and chromatic parameters, enzyme activity), 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 $p < 0.05$:

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

where b_0 is the regression coefficient for the mean effect, b_1 for the linear effect of pressure level, b_2 for the linear effect of holding time, b_{11} for the quadratic effect of pressure level, b_{22} for the quadratic effect of holding time, and b_{12} for the simple interaction effect of pressure level and holding time.

Eight cylinders from different bags were analyzed for each treatment to determine texture and chromatic parameters. Three pooled samples were prepared from different cylinders to carry out biochemical determinations (enzyme activities).

To optimize the HPP conditions, the so-called desirability function was applied. This function was used to select the main processing factors (pressure level and holding time) and optimize the application of HPP to minimally processed peaches. This method integrates the regression equations obtained for each quality parameter simultaneously. Thus, the optimization is carried out by using a multi-response method termed desirability (Derringer & Suich, 1980), which consists in transforming each response variable (Y_i) into an individual function (desirability, d_i) (Eqs. 3 and 4) whose values range from 0 to 1. Basically, if the value of the response is outside an acceptable region, it is set to 0, while if the response attains a desired target, it is set to 1.

In the present research, the desirability function was designed to optimize the inactivation of the enzymes related to alterations, while preserving a suitable texture and color. The objectives established included the maximization of hardness and chewiness and concomitantly, the minimization of other parameters of interest such as the displacement at 5 N (rubbery-like texture), the Δa*/min relation (variation of a*, a CIELab chromatic parameter related to the increase in redness, after the exposure of peaches to air), and the activities of PPO and ADH. Therefore:

$$d_i = \frac{Y_i - Y_{min}}{Y_{max} - Y_{min}}, \quad (3)$$

where Y_{min} is the minimum response value, and Y_{max} is the maximum response value. This equation was used for hardness and chewiness. In the case of displacement at 5 N, Δa*/min, % PPO, and % ADH activity,

Table 1
Coded and actual values of independent variables in the experimental shell design. Experimental responses.

Exp. no.	Coded values		Actual values		Experimental responses					
	Y1	Y2	Pressure level (MPa)	Holding time (min)	Vel Δa*/min	Hardness (N)	Chewiness (N)	Deformation at 5 N (mm)	Residual PPO activity (%)	Residual ADH activity (%)
1	0	0	500	5	0.038 ± 0.001	39.6 ± 0.4	16.6 ± 0.2	1.23 ± 0.07	48.6 ± 0.1	55.8 ± 0.7
2	0	0	500	5	0.037 ± 0.002	39.6 ± 0.5	15.1 ± 0.1	1.14 ± 0.01	46.8 ± 0.5	49.8 ± 0.4
3	0	0	500	5	0.034 ± 0.001	33.7 ± 0.2	16.1 ± 0.2	1.17 ± 0.03	45.8 ± 0.1	49.2 ± 0.4
4	−1	0	400	5	0.039 ± 0.002	35.7 ± 0.4	13.9 ± 0.2	1.04 ± 0.02	62.2 ± 0.5	50.6 ± 0.7
5	1	0	600	5	0.024 ± 0.001	33.8 ± 0.4	13.7 ± 0.1	1.36 ± 0.07	41.7 ± 0.4	16.5 ± 0.1
6	−0.5	−0.866	450	1	0.037 ± 0.001	53.5 ± 0.4	20.9 ± 0.2	0.85 ± 0.04	61.9 ± 0.3	56.7 ± 0.9
7	−0.5	0.866	450	9	0.041 ± 0.001	35.7 ± 0.3	14.2 ± 0.1	1.03 ± 0.02	60.1 ± 0.2	51.9 ± 0.8
8	0.5	−0.866	550	1	0.038 ± 0.001	45.4 ± 0.5	17.4 ± 0.2	0.99 ± 0.01	48.6 ± 0.3	49.1 ± 0.6
9	0.5	0.866	550	9	0.034 ± 0.001	33.8 ± 0.5	12.5 ± 0.2	1.07 ± 0.02	38.1 ± 0.1	49.8 ± 0.4

Data for experimental responses are expressed as means ± std. error (n = 8 for textural parameters, "vel Δa*/min" and n = 3 for enzyme activities).

we had to use a redesign of the function, to obtain the minimum values for these responses:

$$d_i = \frac{Y_{max} - Y_i}{Y_{max} - Y_{min}} \quad (4)$$

The d_is from the responses considered were then combined into a single function called the overall desirability (D), which represents the geometric average of the individual desires (Eq. 5).

D was later maximized.

$$D = (d_1, d_2, \ldots, d_k)^{1/k} \quad (5)$$

where k is the number of responses. Thus, since $0 < D < 1$, a high *D* value would mean that all individual functions of desirability are closer to the target value, and therefore the maximum is considered as the optimal solution of the system.

The study was complemented by a PCA, a relational exploratory method, which is a multivariate statistical analysis based on the calculation of linear combinations of the variables. As variables were measured in different units, there were large differences between them with respect to the mean, variance, and standard deviation. To solve this problem, the data were centered and weighted before applying principal component models. Then, data were transformed to a new coordinate system called principal components (PCs), which were summarized in a biplot graphic that also highlighted relationships among different variables. On the other hand, the score plot represented the projection of each sample into the PCs; this was used to define the best treatment among the ones tested.

All procedures were carried out using the statistical software STATISTICA (trial version 12, Stat Soft, OK, USA) except for the PCA which was carried out with SPSS® (version 12 SPSS Inc., Illinois, USA).

3. Results and discussion

Table 1 summarizes the mean values for the experimental responses of six variables that were selected because the regression coefficients were statistically significant in at least one of the effects considered in the model (Δa*/min, hardness, chewiness, deformation at 5 N, % residual PPO activity, % residual ADH activity). Table 2 shows the regression coefficients obtained in the analysis of variance, which relate the pressure level and the holding time of the HPP with these response variables. The coefficients of determination (R^2) for each equation are also indicated. These values give an idea of the degree of fit of the model and can be also regarded as the degree of variability in the dependent variables, which is considered by the regression analysis (Sin, Yusof, Sheikh Abdul Hamid, & Rahman, 2006). Although the equations obtained for each response variable explained the variation adequately ($R^2 > 0.8$), only the regression coefficients for the linear effect of the pressure level were significant at 0.05 level for deformation at 5 N and the variables related to enzyme activities (Δa*/min, % residual PPO activity, % residual ADH activity). In the case of the variables related to textural parameters (hardness, chewiness), only the regression coefficients for the linear effect of the holding time were significant at 0.05 level.

3.1. Chromatic parameters

L*, a*, and b* values remained unaffected immediately after the packages were opened, independently of the HPP treatment applied. L*, a*, and b* values ranged from 40 to 45, from −2 to −5, and from 24 to 29, respectively. The fact that these parameters were rather unaffected by the treatments ($p > 0.05$) is in agreement with a previous study with nectarines (Miguel-Pintado et al., 2013), which also showed that pressures between 200 and 600 MPa for 3 min had no effect on the color parameters L* and a*. In HPP-treated carambolas, it has also been found that regardless of the pressure level and holding time of the HPP treatment, the chromatic parameters remained almost unaffected (Boynton, Sims, Sargent, Balaban, & Marshall, 2002). This evidences the stability of the pigments to the high pressure (Oey et al., 2008).

On the other hand, the L* value of fresh peaches was reduced from 60 to 40 after vacuum packaging, which represents the most important decrease in this parameter, as previously reported (Denoya et al., 2015).

Regression coefficients for Δa*/min were estimated by multiple linear regression analysis (Table 2). This analysis showed that the pressure level was the sole parameter which had a significant linear coefficient ($p < 0.05$) with a negative value. Thus, after 180 min of air exposure, the lower the pressure level used to treat the fruit, the faster was the decrease in the a* parameter. This result suggests that treatments with the highest pressure levels would be more effective to inhibit the browning reactions. The a* value is considered as a suitable indicator of browning development, since it reflects the redness (positive values) or greenness (negative values) of a surface. Thus, when a browning reaction takes place, the color of the tissue will turn redder, and therefore this change will be reflected as an increase in the a* value. The positive correlation between this parameter and the intensity of browning has been reported by Rocha and Morais (2003).

Our results are also in agreement with the study of Guerrero-Beltrán, Barbosa-Cánovas, and Swanson (2004), who observed that for a range of pressures between 100 and 500 MPa, peach purees turned less brown at the highest values of pressure.

Although the decrease in L* is also used as an indicator of browning (Gorny, Hess-Pierce, & Kader, 1998; Rocha & Morais, 2003), this criterion was not used in the present study, since the translucency of peach cylinders caused by the vacuum packaging produced a large decrease in L*, which would have interfered with the analysis of the results.

Table 2
Regression coefficients and R2 values for six dependent variables for minimally processed peaches subjected to different HPP treatment conditions.

Regression coefficient	Δa*/min	Hardness (N)	Chewiness (N)	Deformation at 5 N (mm)	R. PPO Act. (%)	R. ADH Act. (%)
Mean effect	0.0365*	37.65*	15.91*	1.182*	47.08*	51.58*
P (Linear)	−0.006*	−2.34	−0.94	0.137*	−12.72*	−13.11*
t (linear)	−0.001	−14.64*	−5.78*	0.131	−6.12	−2.07
P^2 (Quadratic)	−0.002	−1.44	−1.06	0.008	2.44	−9.09
t^2 (Quadratic)	0.004	10.33	1.76	−0.399*	7.72	9.68
P*t	−0.004	3.11	0.88	−0.048	−4.36	2.72
R^2	0.863	0.905	0.895	0.958	0.936	0.891
Reduced equations for process parameters (coded values):						
$\Delta a^*/min = -0.0060\ P + 0.0358$						
Hardness (N) $= -14.64\ t + 38.98$						
Chewiness (N) $= -5.78\ t + 15.59$						
Deformation at 5 N (mm) $= 0.14\ P - 0.40\ t^2 + 1.19$						
Residual PPO activity (%) $= -12.72\ P + 50.42$						
Residual ADH activity (%) $= -13.11\ P + 47.67$						

Abbreviations: R. = Residual, Act. = Activity, P = Pressure level, t = Holding time.
* Significant at 0.05 level.

3.2. Texture profile analysis (TPA)

Cohesiveness, as determined for the different treatments, ranged from 0.50 to 0.59, while springiness ranged from 0.67 to 0.71. These parameters remained almost unaffected by the treatments ($p > 0.05$), similarly to a previous study with pineapples subjected to pressures between 100 and 700 MPa for 10 min (Kingsly, Balasubramaniam, & Rastogi, 2009a).

In turn, both hardness and chewiness had significant ($p < 0.05$) negative correlation coefficients with the holding time (Table 2), which means that longer holding times render softer peaches. This was also modeled by a surface response (lack of fit non-significant at $p = 0.05$) in which hardness was considered as the response variable (Fig. 2). In potatoes subjected to HPP, Park, Balasubramaniam, and Sastry (2013) found a similar behavior for the crunchiness index (another textural parameter obtained by a puncture test) for pressure levels between 400 and 600 MPa and holding times between 1 and 10 min. According to these authors, an increase in the pressure level had no effect on this parameter, while treatments with the longest holding time (10 min) caused a decrease in the index.

Interestingly, in the present study, in the range studied (400–600 MPa), the pressure level had no effect on any texture parameter, which was reflected in the non-significant ($p > 0.05$) regression coefficient. Similar results were found in nectarine halves subjected to pressures between 200 and 600 MPa for 3 min (Miguel-Pintado et al., 2013). In pineapples, Rastogi and Niranjan (1998) found that, in a range between 100 and 700 MPa, texture losses were evidenced up to 300 MPa, but no further effect was detected above this value. Interestingly, Trejo Araya et al. (2007) found a similar threshold for the evidence of textural changes (300 MPa) in carrots in a range between 100 and 700 MPa. These authors suggested the existence of a pressure threshold value which would cause the maximum compression of the water content. Above this value, which would depend on the structure of products, the tissues might not be further deformed.

The results for the parameter termed "deformation at 5 N," which basically measures the displacement of a probe impelled by a force of

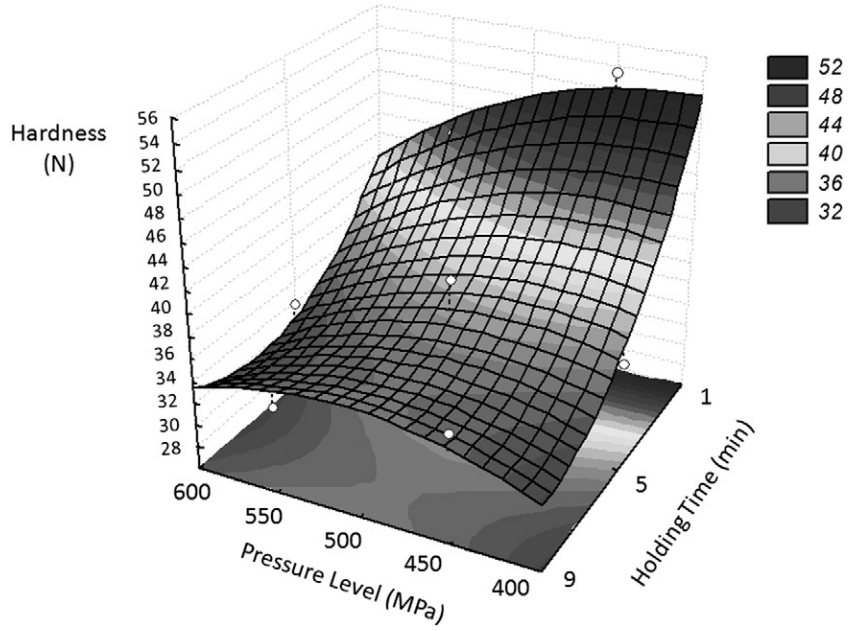

Fig. 2. Effect of pressure level (P: 400–600 MPa) and holding time (t: 1–9 min) on hardness values of minimally processed peach cylinders. Experimental values (–o–).

5 N into a tissue, are also presented in Table 2. This parameter had a positive regression coefficient with the pressure level. In previous studies with the same variety of peaches (cv. Flavorcrest), we found a shorter displacement of the probe for control (untreated) peaches than for HPP-treated peaches. This suggests that HPP imparts a sort of rubbery-like texture to fruit tissue, since a higher value of defomation at 5 N reflects a more deformable material with less cell integrity. An increase in the displacement of the probe due to a force has also been reported in pressurized carrots by Trejo Araya et al. (2007) and in swede by Clariana, Valverde, Wijngaard, Mullen, and Marcos (2011). In another research, Gonzalez, Jernstedt, Slaughter, and Barrett (2010), by analyzing force-deformation curves in onions, considered that the initial behavior of these vegetables treated with different levels of pressure is compatible with a highly viscoelastic liquid.

3.3. Light microscopy

It is known that the instrumental assessment of a complex sensorial characteristic such as texture represents a challenging task. In this regard, research aimed to elucidate the interaction between structure and texture may be helpful to understand and optimize the texture of products (Alzamora, Gerschenson, Vidales, & Nieto, 1997).

Fig. 3 shows the micrographs of the parenchymatous tissues corresponding to the mesocarps of fresh (untreated) peach (a and b) and of

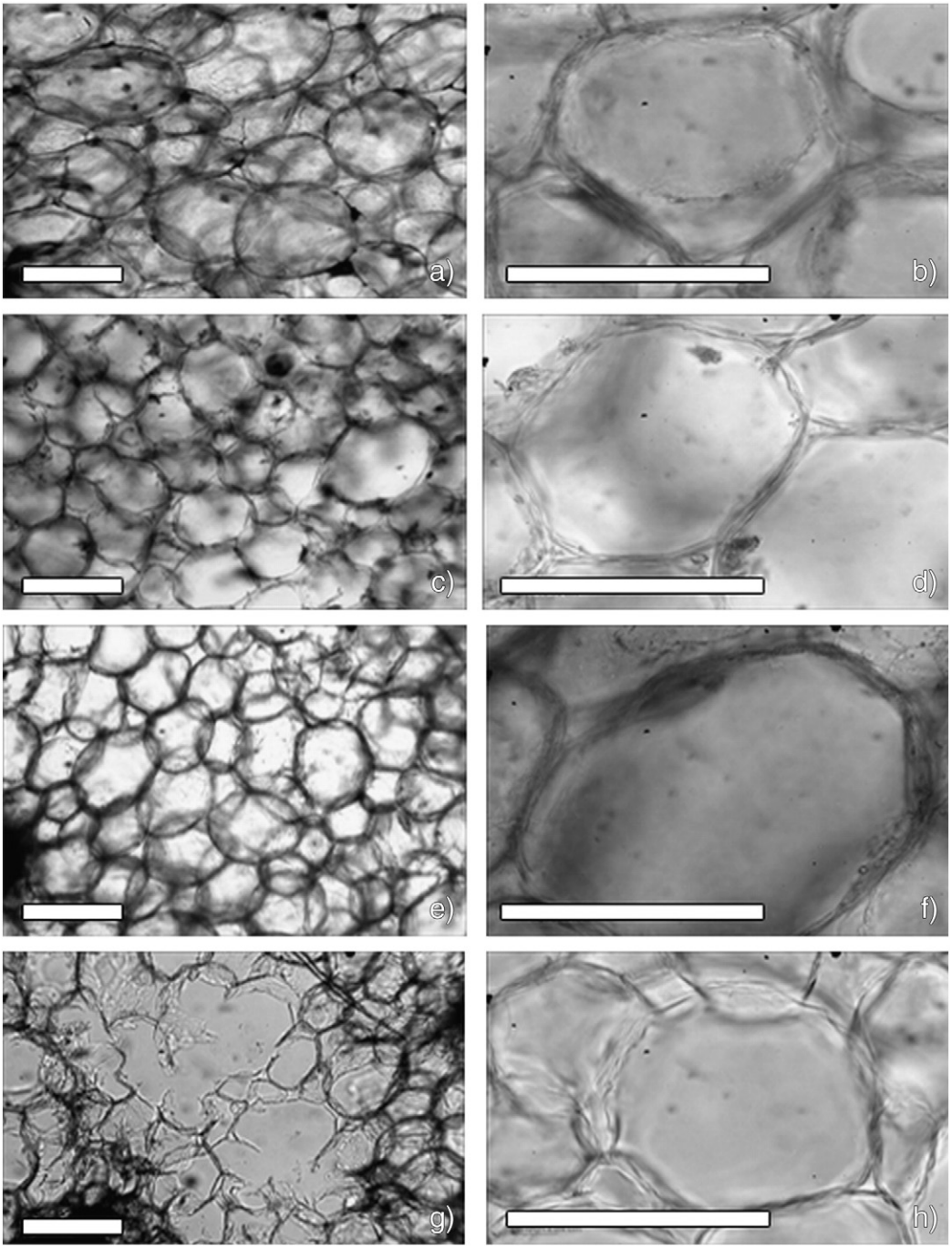

Fig. 3. a, b, Parenchyma from mesocarp of fresh peach (control). c, d, Parenchymatous tissue from peach cylinders treated with a pressure of 500 MPa for 5 min. e, f, Parenchymatous tissue from peach cylinders treated with a pressure of 600 MPa for 5 min. g, h, Parenchymatous tissue from peach cylinders treated with a pressure of 550 MPa for 9 min (longer holding time). Observations using light microscope at 10× and 40×. Bars: 100 μm.

cylinders of the same fruit treated for 5 min with different pressure levels, 500 MPa (c, d) and 600 MPa (e, f), or with a longer holding time, 9 min at 550 MPa (g, h).

Despite the difficulty to assess the state of membranes by the use of light microscopy, distinctive features can be clearly distinguished in untreated (control) tissues. Among them, it can be mentioned that both the vacuole and the cell content (e.g. chromoplasts) are clearly intact (Fig. 3 b), which evidences the integrity of cells and the lack of degradation, disruption, or dissolution. In contrast, in HPP-treated tissues (Fig. 3 c–f), the process caused the lysis of cell membranes, which is visualized by the disorganization of the cytoplasm and the agglomeration of the organelles and cytoplasm content. When samples were treated with a pressure of 550 MPa for 9 min, cell walls were partially destroyed, and the cellular content was appreciated filling the lysigenous intercellular spaces (Fig. 3 g–h). In onions subjected to a pressure of 300 MPa, Butz, Koller, Tauscher, and Wolf (1994) also found that the vacuoles of epidermis cells were severely damaged and the cellular components lost. In this regards, Boynton et al. (2002) suggested that the texture loss observed in HPP-treated fruits can be attributed to the disruption of cell membranes and to the reduction of the turgidity of the cells. The damage in the membranes caused by HPP probably induces the movement of water through the cell wall, which is evidenced at the macroscopic level by a soaked or drenched appearance of the treated tissues.

Although membranes of the peach tissues were clearly affected by pressure levels in the range of 500–600 MPa, the cell walls were able to withstand a treatment of up to 5 min (Fig. 3 c–f). Similar results were observed by Gonzalez et al. (2010) in onion tissues subjected to pressures between 300 and 600 MPa for 5 min, in which the pressurized tissue presented a loss of vacuole membrane integrity, although the cell walls remained intact. Besides, these authors associated the damage suffered by membranes with a change in the texture of the HPP-treated onions, which became more deformable and less ductile than the untreated tissues.

In the present study, we observed a clear relation between the damage in the microstructure and the behavior of the peach samples in the TPA at all the pressure levels studied. Interestingly, the longer the holding time in the HPP, the more prominent was the texture loss (i.e. lower hardness and chewiness). In contrast, the pressure level had no significant impact on textural parameters.

The most prominent damage in the microstructure was observed in fruit treated with a pressure of 550 MPa for 9 min, which caused alterations in the cell walls of the parenchyma. The negative consequence of cell wall breakdown is the loss of tissue firmness and turgidity (Norton & Sun, 2008). Other alterations such as the folding and deformation of the cell wall that will eventually affect the textural properties of the tissues can also take place (Rastogi, 2010). In fruit subjected to HPP treatment for 5 min, we verify no injury of the cell walls, regardless of the pressure level.

3.4. Residual PPO activity

Table 2 shows the regression coefficients of the residual PPO activity as estimated by the application of multiple linear regression analysis. The residual PPO activity ranged from 38% to 62% for the different treatments (Fig. 4), and regardless of the holding time, the higher the pressure level, the more effective was the inactivation of PPO, which was reflected by a significant ($p < 0.05$) negative regression coefficient. Thus, for the pressure range studied (400–600 MPa), an increase in the pressure level will result in a decrease in the PPO residual activity. This can be also seen in the surface response figure (lack of fit non-significant at $p = 0.05$) obtained for % PPO activity (Fig. 4).

Several authors have estimated the HPP conditions required for PPO inactivation. Among them, Guerrero-Beltrán et al. (2004) reported that for peach puree subjected to pressures in the range of 100–500 MPa, higher pressure levels increase the effectiveness of HPP to inactivate this enzyme. In mango puree, 550 MPa is more effective than lower pressure levels to decrease the PPO activity (Guerrero-Beltrán, Barbosa-Cánovas, Moraga-Ballesteros, Moraga-Ballesteros, & Swanson, 2006). Interestingly, at a specific pressure level, these authors observed that only a limited additional gain in the effectiveness can be achieved by substantially increasing the holding time. For instance, at 517 MPa, these authors reported a decrease of 81.5 and 95.1% in PPO activity for 5 and 25 min of holding time, respectively. For the production of dried peach slices, Kingsly, Balasubramaniam, and Rastogi (2009b) found

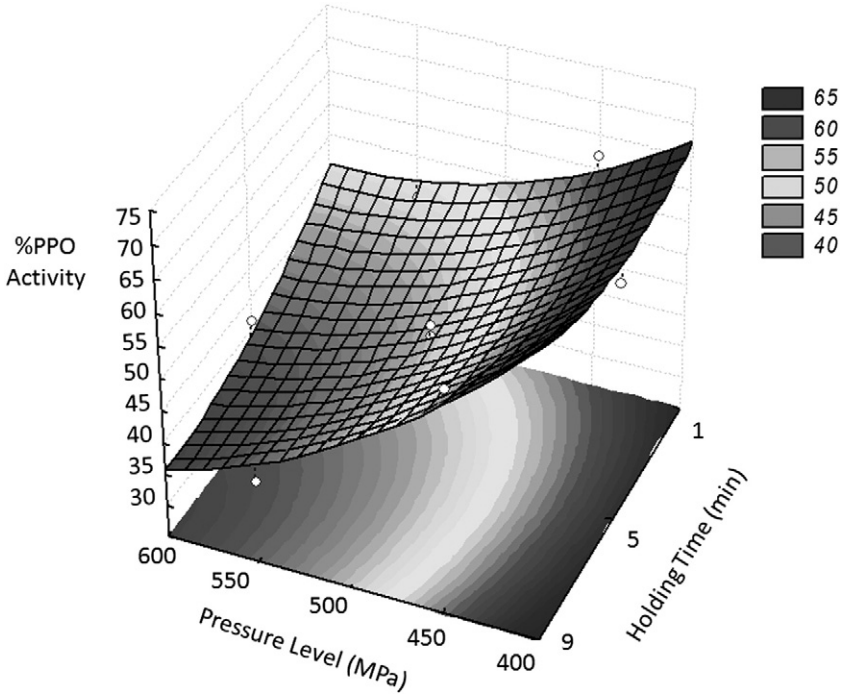

Fig. 4. Effect of pressure level (P: 400–600 MPa) and holding time (t: 1–9 min) on % PPO activity of minimally processed peach cylinders. Experimental values (–o–).

that a combined pretreatment consisting in dipping the fruit in a solution of 1% citric acid followed by HPP at pressures higher than 300 MPa was enough to inactivate PPO.

Interestingly, in the present study, the rate of color change caused by the enzymatic browning after 180 min of air exposure (expressed as Δa*/min) had a similar behavior to PPO inactivation. Both parameters showed a statistically significant ($p < 0.05$) negative coefficient of regression for pressure level, which evidences the close relation between the PPO residual activity and the degree of browning.

3.5. Residual ADH activity

The residual ADH activity after the application of HPP was measured to assess the effect of pressure level and holding time on this key reaction of the fermentative metabolism.

The highest percentages of ADH inhibition attained were 43% and 84% for the 450 MPa–1 min and the 600 MPa–5 min treatments, respectively (data not shown). Regression coefficients of the percentage of decrease of ADH activity in relation to the activity measured in raw material were estimated by multiple linear regression analysis (Table 2). Results showed that the higher the pressure level, the more effective the treatment to inactivate ADH, regardless of the holding time. This was reflected by the negative regression coefficient obtained for residual ADH activity vs. pressure level, which was statistically significant ($p < 0.05$) for the range of pressures evaluated (400–600 MPa). Therefore, it was evidenced that an increase in the pressure level will result in a decrease in the percentage of residual ADH activity.

Few authors have evaluated the effect of HPP on ADH. Among them, Dallet and Legoy (1996) found that this enzyme of yeast origin is less baroresistant than other types of enzymes, achieving 28% and 62% of inactivation after only 5 min of exposure to 50 and 150 MPa, respectively. Apparently, for pressures lower than 200 MPa, the inactivation is the consequence of the rearrangement of the monomers of the enzyme to render an inactive polymeric form. To cause the denaturalization of the enzyme, pressures higher than 200 MPa and longer times would be necessary. Eisenmenger and Reyes-De-Corcuera (2009) explained that for dehydrogenases in general, even low levels of pressure (<100 MPa) can result in the activation of the enzyme. This phenomenon is probably caused by the pressure that counteracts the increased volume associated with the denaturation of the enzyme.

3.6. Optimization of HPP conditions

Fig. 5 shows the predicted profiles of the response variables (chewiness, hardness, % residual ADH activity, % residual PPO activity, deformation at 5 N—rubbery-like texture—and Δa*/min) having at

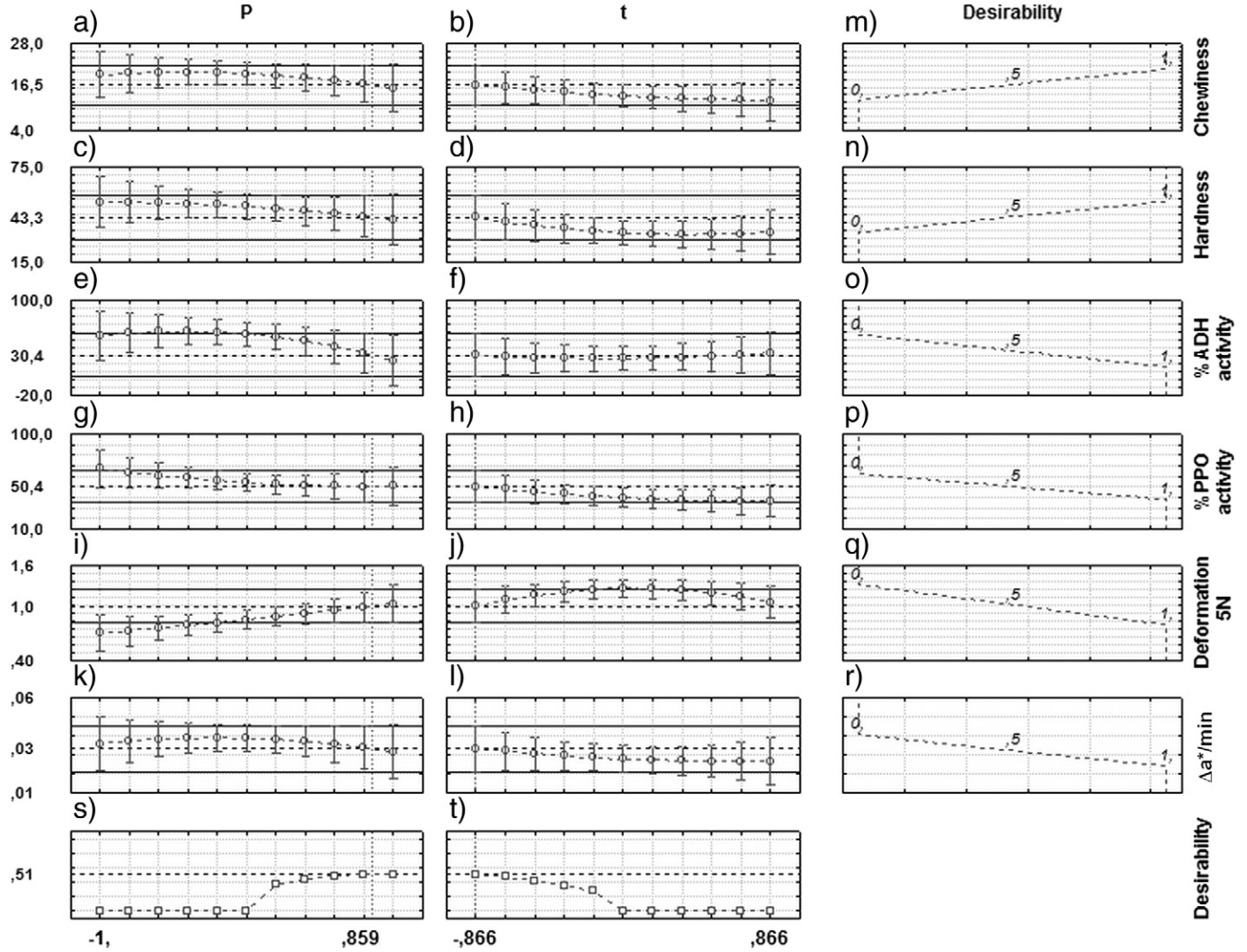

Fig. 5. Simultaneous optimization of process conditions for HPP of minimally processed peaches. Predicted profiles for the response variables at different levels of each independent variable, holding the levels of the other independent variable constant at the estimated optimal value [a) to l)]; for each individual desirability function profile [n) to r)], and for the global desirability function profile [s), t)].

least one coefficient statistically significant in the effects considered in the regression models, at the different levels assayed for each independent variable (pressure level and holding time), while maintaining constant levels of the other independent variables at the estimated optimal value. Fig. 5 also shows each individual desirability function profile and the global desirability function profile.

According to these data, the predicted optimal process condition leading to the maximum global desirability value (D = 0.51) for the process under study was a combination of a pressure level of 585 MPa and a holding time of 1 min. This would render the following predicted values of the response variables: 16.55 N for chewiness, 43.32 N for hardness, 30.43% for residual ADH activity, 50.42% for residual PPO activity, 1.08 mm for deformation at 5 N (associated with a rubbery-like texture), and 0.03 Δa*/min (associated with the browning rate). This method has been previously used by other authors to successfully establish the optimal process conditions for the production of films from banana, and for a drying process (Pelissari, Andrade-Mahecha, Sobral, & Menegalli, 2013; Rouissi et al., 2013).

3.7. Principal components analysis (PCA)

Principal components analysis (PCA) was performed to identify the relations among the variables and to establish the most suitable treatment based on this information. This analysis explained 86.34% of the total variability, and among the individual components, the first component (PC_1) explained 59.78% of the variance of the model with a positive loading for hardness, chewiness, residual ADH, and PPO activity. In turn, the second component (PC_2) explained 26.56% of the variance with a positive loading for the change in a* after 180 min of exposure of the product (Δa*/min) (Table 3).

Table 4 shows the correlation matrix of the variables considered and Fig. 6 is a biplot graphic involving treatments and dependent variables. Hardness shows high correlation with chewiness and enzyme activities show correlation with the change in a*. The contribution of the parameters to the first component (PC_1) is based on these correlations.

PC_1 indicates that the treatments on the right side of the biplot rendered higher values of enzyme activities and hardness and chewiness on the peaches, while the second component (PC_2) indicates that the treatments in the quadrant below had a lower rate of browning (lower value of a*/min) and higher hardness and chewiness values. Although PC_2 only explained 26.56% of the variance of the model, it is important to highlight the negative loadings for textural parameters and the positive loadings for residual enzyme activities and Δa*/min. Therefore, the optimal treatment should be selected among those situated in the lower quadrant of the biplot, since they had lower enzyme activities and higher values of hardness and chewiness. On the other hand, PC_1 explained the higher percentage of the variance of the model (59.78%), having positive loadings for all the variables. Thus, the treatments on the right side of the biplot will have suitable values of hardness and chewiness (the highest values) but high activities of PPO, and therefore a faster browning rate (Δa*/min).

Based on the above, it can be stated that the optimal treatment should be located at the bottom of the biplot, since PC_2 had a better description of the characteristics associated with a suitable product quality (good texture, adequate color preservation, and effective enzyme inactivation). Therefore, according to the PCA, the process parameters corresponding to the optimal treatment are a pressure of 600 MPa and a holding time of 5 min (represented with a star in Fig. 6). This is in full agreement with our preview analysis of the surface responses and the regression equations obtained for each variable, which led us to conclude that the treatments with the highest pressure levels will also render the highest percentages of enzyme inactivation, with no significant ($p > 0.05$) effect on textural parameters. In contrast, treatments with the longest holding times will have significant ($p < 0.05$) detrimental effects on hardness and chewiness, with no improvement in the effectiveness to inactivate enzymes linked to alteration.

Results from the desirability function in the present research, which predicts that the optimal treatment would be that with a high pressure level and a low holding time (585 MPa − 1 min), are comparable to those obtained from PCA, which indicates that the best combination was the highest pressure level assayed (600 MPa), applied for a short time (5 min).

Table 3
Eigenvalue analysis of the correlation matrix loading of the significant principal components (PC).

Variables	Components	
	1	2
Δa*/min	.675	.707
Hardness (N)	.818	−.550
Chewiness (N)	.829	−.538
Residual PPO activity (%)	.783	.071
Residual ADH activity (%)	.752	.482

Table 4
Correlation matrix between variables.

		Hardness	Chewiness	% ADH Activity	% PPO Activity	Δa*/min
Correlation	Hardness	1.000	0.978	0.402	0.514	0.163
	Chewiness	0.978	1.000	0.391	0.544	0.190
	% ADH Activity	0.402	0.391	1.000	0.392	0.794
	% PPO Activity	0.514	0.544	0.392	1.000	0.580
	Δa*/min	0.163	0.190	0.794	0.580	1.000

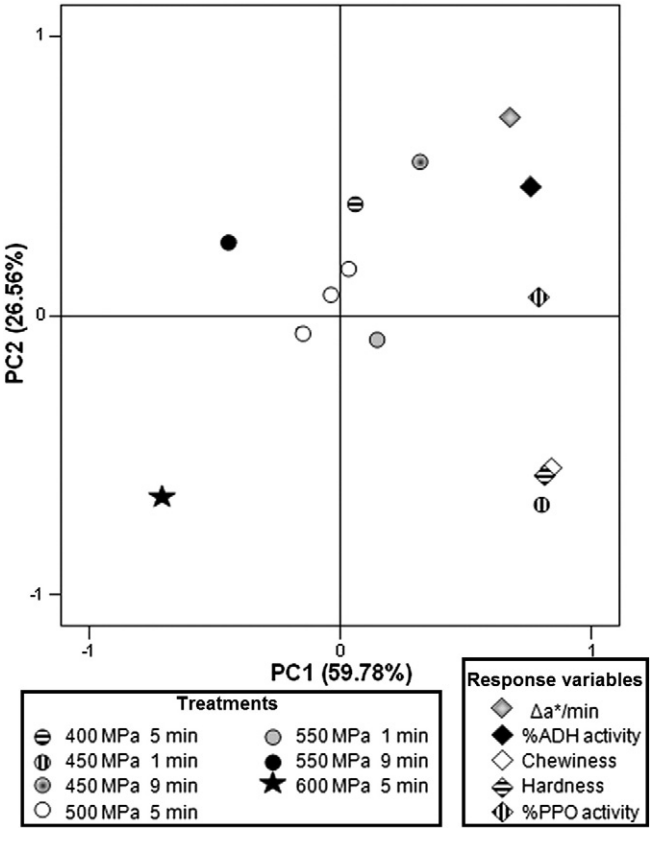

Fig. 6. Biplot of two principal components. Treatments are identified with circles and response variables with diamonds. The treatment suggested as optimal by the analysis is identified with a star.

4. Conclusions

The results of the present study show that HPP is a promising technology for the preservation of minimally processed peaches.

The optimization analysis suggests that HPP applied at pressure levels higher than those evaluated in the present study, in the range of 600–900 MPa, but applied for shorter holding times (0.5–5 min) would lead to a better preservation of minimally processed peaches. The effectiveness of these proposed conditions, as well as the assessment of the theoretical optimal condition predicted for the range used in the present study (585 MPa for 1 min), remains to be evaluated. This will probably result in further improvement, allowing the extension of the shelf life of minimally processed peaches with high quality and nutritional standard.

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