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Combined effect of ultrasound, mild heat shock and citric acid to retain greenness, nutritional and microbiological quality of minimally processed broccoli (*Brassica oleracea* L.): An optimization study

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ABSTRACT

Response surface methodology (RSM) and Box–Behnken design were used to study the combined hurdle effect of mild heat time (1–5 min) at 50 °C, ultrasonic processing time (0–10 min) and citric acid concentration (0–2%) on the quality of refrigerated broccoli after 10 d of storage at 5 °C. Treatment effects were evaluated on weight loss, superficial colour (hue angle (H°) and total colour difference (ΔE)), headspace gas composition (O_2 and CO_2), overall browning potential, chlorophyll content, ascorbic acid content, mesophilic counts and overall visual quality (OVQ) and optimize the process by means of the desirability function. Predicted models were found to be significant with high regression coefficients (91–97%). High regression coefficients indicated that second-order polynomial models could be used to predict and optimize the quality retention in minimally processed broccoli during storage. The mesophilic counts, ascorbic acid content and the overall visual quality were significantly influenced by the three independent variables either independently or interactively. Both thermal and ultrasonic treatments were found to be critical factors influencing changes in chlorophyll content, O_2 concentration inside the package, hue angle and ΔE. On the other hand, thermal treatment and citric acid concentration were found to be significant on overall browning potential. By using the desirability function approach and considering superficial colour parameters, O_2 concentration, mesophilic counts, browning potential, ascorbic acid and chlorophyll content, the optimum processing conditions were 7.5 min of ultrasonic treatment, 3 min of a heat shock treatment and a citric acid concentration of 1.5%. These results were in good agreement with the maximum found from the canonical analysis performed from the response surface when only considering sensorial analysis. Under these optimal processing conditions it is possible to employ citric acid treatment in combination with ultrasonic and thermal treatments as hurdles for retention of green colour, nutritional quality, microbial control and for extending shelf life of refrigerated minimally processed broccoli.

1. Introduction

Fresh-cut vegetables are increasingly demanded by consumers due to their convenience and fresh-like quality (Koukounaras et al., 2008). However, processing after harvest such as cutting or slicing enhances deterioration, senescence and induces a consequent loss of commercial quality (Moreira et al., 2011) reducing the shelf life of minimally processed vegetables. The shelf-life of these products is usually extended by means of a combination of appropriate refrigerated storage throughout the entire cold chain, modified atmosphere packaging and good manufacturing and handling practices.

Colour is one of the most important sensory attributes that determine food quality and is one of the first quality attributes detected and therefore, influences consumer perception of a product. Changes in colour during the processing and storage of vegetables need to be measured and controlled.

In recent years, demand on broccoli has increased greatly, as freshly consumed or in salads ready to eat. However, broccoli is a highly perishable product with a short shelf life after harvest. Although differences among cultivars in terms of shelf life have been reported (Toivonen and Sweeney, 1998) it is

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generally accepted that broccoli quality losses are mostly due to surface dehydration, degreening and yellowing of sepals due to chlorophyll breakdown and the presence of microbial growth (Hajizadeh and Kazemi, 2012). Another important chemical change in minimally processed broccoli is enzymatic browning. The formation of dark-coloured pigments during processing and storage is a very common phenomenon. Browning can also appear during long storage, and is generally dependent upon product characteristics and storage conditions (Toribio and Lozano, 1984). Therefore, maintaining the natural colour and controlling microbial growth in minimally processed and stored broccoli has been a major challenge in food processing.

Some techniques used to delay de-greening and senescence include heat treatments, which effectively reduce yellowing of stored broccoli florets. The beneficial effects of heat treatments on storage on broccoli florets have been reported by several authors (Costa et al., 2006; Lemoine et al., 2007; Ansorena et al., 2011; Moreira et al., 2011). Studies on common techniques such as controlled and modified atmosphere storage (Serrano et al., 2006; Eason et al., 2007), hot air treatment (Costa et al., 2005) and packaging (Jacobsson et al., 2004) show an improvement in postharvest broccoli shelf-life. There are numerous reports on the effects of ultrasonic treatments on food processing and preservation (Knorr et al., 2004; Patist and Bates, 2008; Vilkhu et al., 2008). Most of these studies have been focused on the efficacy on microorganisms and enzymes inactivation and antioxidant compounds extraction (Knorr et al., 2004), however there is insufficient information about the effect of ultrasonic treatments on storage quality of fresh produce. Recently, Cao et al. (2010) and Chen and Zhu (2011) reported that ultrasound was effective in inhibiting decay incidence and preserving quality in strawberries and plum fruit, respectively. More evidence is needed to prove the effects of ultrasound on fruits and vegetables to promote the application of this technique. Chemical treatments are used on lightly processed fresh produce mainly for controlling decay, reducing browning and retaining firmness (Sagong et al., 2011). Low pH organic acid solutions are used as antimicrobials to control bacteria or antioxidants to prevent browning, to reduce pigments discolouration and to protect against loss of flavour, changes in texture and loss of nutritional quality (Roura et al., 2003). Citric acid can be used to prevent browning by chelating copper in polyphenol oxidases (PPO). Treatment baths with citric acid solutions prevent browning in fresh prepared potatoes (Langdon, 1987), fresh-cut spinach (Piagentini et al., 2002) and lettuce (Roura et al., 2003).

In recent years, much attention has been paid to the application of minimal preservation processes based on the hurdle principle. These combined procedures avoid the severe application of only one conservation factor with the consequent improvement in product quality. In order to optimize the levels of various hurdles, multivariate statistical techniques such as response surface methodology (RSM) have been suggested. RSM is a useful and well known statistical tool applied in process optimization. Experimental condition that optimizes a process response as a specific quality characteristic of the final produce can be obtained by RSM. However, this condition may be different for another process response. Desirability function is thus a technique that can be used to formulate and solve this conflict as a constrained optimization problem (Montgomery, 2001). It allows finding an optimal experimental condition that meets all the process responses established as ideal.

So far there have been no available scientific literature about the application of ultrasonic treatment for maintaining quality and extending shelf life of minimally processed broccoli during storage. Moreover, the use of the hurdle principle for greenness nutritional and microbiological quality retention of this vegetable has not been investigated.

The aim of this work therefore, was to investigate the combined effect of ultrasonic processing time, mild heat time and citric acid concentration on quality of minimally processed broccoli after refrigerated storage in order to find the best combinations of the three variables to prevent green colour losses and browning, and to retain the microbiological and nutritional quality of this product. Green colour retention and nutritional quality was evaluated through the determination of superficial colour, headspace gas composition, overall browning potential, total chlorophyll and ascorbic acid content, while microbiological quality was determined through total aerobic mesophilic counts.

2. Materials and methods

2.1. Experimental design and statistical analysis

Response Surface Methodology (RSM) with a Box–Behnken (BB) design (Box and Behnken, 1960) was used to analyse the effect of multiple barrier combination on the colour retention and on nutritional and microbiological quality of broccoli during refrigerated storage. BB is a spherical resolving design that involves a central point and middle points of edges. The design requires an experiment number according to $N = k^2 + k + C_p$, where k is the factor number and C_p is the replicate number of the central point (Aslan and Cebeci, 2007).

Three explanatory factors namely ultrasonic processing time (x_1), mild heat time (x_2) and citric acid concentration (x_3) may affect colour retention, weight loss, headspace gas composition, ascorbic acid content, overall browning potential, chlorophyll content and the mesophilic counts of minimally processed broccoli. For each factor, an experimental range was determined based on the results of preliminary experiments.

For a 3-level–3-factor BB experimental design with three replicates at the central point, a total of 15 experimental runs are needed, in which each variable was tested in three different coded levels: low (−1), middle (0) and high (+1).

In developing the regression equation, factors were coded according to the equation:

$$X_i = \frac{x_i - x_{0i}}{\Delta_{xi}} \quad (1)$$

where X_i is the coded value of the ith independent variable, x_i is the natural value of the ith independent variable, x_{0i} is the natural value of the ith independent variable at the centre point, and Δ_{xi} is the steep change value.

All experiments were performed in a random order to minimize any effects of extraneous errors on the observed responses and were independently repeated three times.

The experimental design in the coded (x) and actual (X) levels of variables is shown in Table 1. The measured responses were weight loss, superficial colour (hue angle (H°) and total colour difference (ΔE)), headspace gas composition, total mesophilic counts, ascorbic acid content, overall browning potential, chlorophyll content and sensory quality. These values were related to the coded variables (x_i, i = 1, 2 and 3) by a second degree polynomial. The general form of the second-order polynomial used to fit the measured responses was:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (2)$$

where y is the dependent variable; b_0 is the model constant; b_1, b_2 and b_3 are the linear coefficients; b_{11}, b_{22} and b_{33} are the quadratic coefficients; b_{12}, b_{13} and b_{23} are the coefficients for the interaction effects and X_i is a dimensionless coded value of x_i (independent variable).

Table 1
Initial values of raw broccoli and mean values of all responses of non-treated samples and samples treated under different experimental conditions after 10 d of storage at 5 °C.

Exp no.	Variables			Experimental Responses								
	Ultrasonic treatment time $X_1(x_1)$	Thermal treatment time $X_2(x_2)$	Citric acid $X_3(x_3)$	O_2 (kPa)	CO_2 (kPa)	Hue Angle ($H°$)	Total colour difference (ΔE)	Mesophilic aerobic counts ($\log CFU\,g^{-1}$)	Browning Potential ($Abs_{320\,nm}$)	Ascorbic acid ($mg\,kg^{-1}$)	Chlorophyll content ($mg\,kg^{-1}$)	OVQ
1	0 min (−1)	3 min (0)	0% (−1)	4.51	7.64	113.58	5.76	7.92	0.46	605.7	82.5	2.75
2	5 min (0)	1 min (−1)	0% (−1)	5.33	5.24	115.15	4.44	6.69	0.42	507.1	77.1	2.57
3	5 min (0)	5 min (1)	0% (−1)	5.71	8.37	118.81	6.34	7.14	0.46	447.9	104.0	3.46
4	10 min (1)	3 min (0)	0% (−1)	7.10	5.52	117.47	3.52	6.41	0.37	799.9	91.2	3.04
5	0 min (−1)	1 min (−1)	1% (0)	4.35	7.69	107.74	7.65	6.05	0.43	590.0	82.2	3.74
6	0 min (−1)	5 min (1)	1% (0)	4.48	8.35	113.81	10.61	6.35	0.61	541.0	141.7	4.01
7	5 min (0)	3 min (0)	1% (0)	5.49	4.50	118.81	2.50	4.16	0.33	805.4	156.0	4.65
8	5 min (0)	3 min (0)	1% (0)	5.52	4.14	117.56	2.15	4.08	0.30	854.3	151.6	4.54
9	5 min (0)	3 min (0)	1% (0)	5.66	4.19	117.24	2.17	4.70	0.31	838.7	150.0	4.66
10	10 min (1)	1 min (−1)	1% (0)	6.81	7.22	116.57	5.24	6.81	0.44	864.0	115.0	4.78
11	10 min (1)	5 min (1)	1% (0)	7.67	6.97	120.96	4.98	6.08	0.42	607.1	131.1	4.37
12	0 min (−1)	3 min (0)	2% (1)	4.50	7.40	110.81	5.39	4.08	0.41	773.7	128.1	3.07
13	5 min (0)	1 min (−1)	2% (1)	5.45	6.90	114.50	4.87	5.54	0.37	676.7	137.2	4.57
14	5 min (0)	5 min (1)	2% (1)	5.88	5.71	121.80	3.69	5.23	0.36	457.1	156.7	4.22
15	10 min (1)	3 min (0)	2% (1)	7.33	5.55	123.02	3.56	4.98	0.33	782.6	112.2	4.74
Initial values of fresh broccoli				21	0.03	130.06	0	3.88	0.28	104	20.60	5.00
Non treated samples (control) 10 d				2.06	10.2	102.21	9.42	7.41	0.58	48.6	10.70	2.66

Data were analysed using SAS software (version 9.0, North Carolina, USA). The fit quality of the model was evaluated by R^2 and analysis of variance (ANOVA) using the Response Surface Regression (RSREG) procedure. Statistical testing of the model was done by Fisher's statistical test. The robustness of the model was assessed by the determination coefficient (R^2), correlation coefficient (R), or F-test.

Simultaneous optimization, desirability functional analysis and 3D plots of the responses were performed using Statistica software (version 7.0, Stat Soft Inc., Tulsa, USA).

2.2. Simultaneous optimization

During optimization, several response variables describing the quality characteristics are usually to be optimized. Some of these variables were to be maximized while some were to be minimized. In many cases, these responses were competing, i.e., improving one response may have an opposite effect on another one, which further complicates the situation. In this work, the responses predicted by the models were optimized by means of the "desirability optimization methodology" (Derringer, 1994). The desirability function approach is one of the most widely used methods for the optimization of several responses.

The general approach is to first convert each response (y_n) into an individual desirability function (d_n). The desirability scale ranges from 0 to 1, where, if the response is at its goal or target, then $d_n = 1$, and if the response is outside an acceptable region, then $d_n = 0$. Each response is then standardized in desired functions d_n of the type.

$$d_n = h_n(y_n) \quad (3)$$

where n is the total number of responses in the measure.

Derringer and Suich (1980) used the following modified desired function:

$$d_n = \begin{bmatrix} 0 & \text{if } y_n \le y_n^{\min} \\ \left(\dfrac{y_n - y_n^{\min}}{y_n^{\max} - y_n^{\min}}\right)^r & \text{if } y_n^{\min} \le y_n \le y_n^{\max} \\ 1 & \text{if } y_n \ge y_n^{\max} \end{bmatrix} \quad (4)$$

where $y_n^{\min}$ is the minimum acceptable value of y_n, $y_n^{\max}$ is the maximum value that is considered desirable and r is a positive constant. If $r = 1$, the d_n increases linearly as y_n increases; if $r > 1$, the d_n changes more rapidly towards the $y_n^{\max}$ and if $r < 1$, the d_n changes less rapidly towards the $y_n^{\max}$.

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.

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

where $0 \le D \le 1$, a high value of D indicates the more desirable and best functions of the system, which is considered as the optimal solutions of this system. The optimum values of factors are determined from the value of individual desired functions that maximizes D.

2.3. Plant material

Broccoli heads (*Brassica oleracea* L. var. *italica*) were obtained from a local producer in Mar del Plata, Argentina. Heads were immediately transported in refrigerated bags with polyfreezer (refrigerated gel for cool chain maintenance, Thermics Argentina S.A.) and transported to the laboratory within 1 h of harvesting. Broccoli florets of approximately 5 cm in length were cut from the main stem.

2.4. Sample preparation

Broccoli was subjected to different ultrasonic treatment times at a constant frequency of 40 kHz and ultrasonic power of 180 W by immersion in a water bath (20 °C) dimensions of 330 mm × 180 mm × 310 mm, in an ultrasonic chamber (PS-30A, RoHs, China). The frequency and ultrasonic power level were selected based on previous experiments. Afterwards, broccoli florets were removed from the ultrasonic bath and dipped during different heating times in a well-stirred water bath (M 911 Coleparmer, Germany) at 50 °C. Immediately after the temperature treatment, they were placed in a soaking solution containing different citric acid concentrations for 5 min. Table 1 lists the parameter values for each treatment. After treatment, samples were immediately cooled for 1 min in an ice-water bath and were dried by spreading out on paper towels. The ratio between broccoli and the soaking solution (for the ultrasonic chamber, the thermal bath and the citric acid solution) was 1:10.

After treatments, broccoli florets were packaged in polymeric film bags (multilayered polyolefin PD960, CRYOVAC, Argentine) of 25 μm of thickness (with an O_2 permeability of 0.08 mL m^{-2} s^{-1}, CO_2 permeability of 0.2 mL m^{-2} s^{-1}, and water vapour permeability of 12 μg m^{-2} s^{-1}), placing 3 broccoli florets per bag (approximately 90 g). Bags were sealed (SERVIVAC, Argentina) and stored in a 5 °C refrigerated chamber. According to current Spanish regulations (BOE, 2001), total aerobic mesophilic count should not increase above 7 log CFU g^{-1} for minimally processed produce during their entire storage period. Based on previous works (Ansorena et al., 2011; Moreira et al., 2011) and taking into account total aerobic mesophilic count and sensory quality of minimally processed broccoli, a shelf life of 10 d at 5 °C was determined. Therefore, this was set as the storage time of this experiment. After 10 d of storage and for each applied condition eight bags were used to determine all quality indices. The assays were carried out in duplicate on three independent experimental runs.

2.5. Headspace gas composition and weight loss

Gas composition (O_2 and CO_2) in the headspace of packaged broccoli was measured using an OxyBaby (HTK, Hamburg, Germany) gas analyser. A syringe was inserted into the package through a rubber seal placed on the bag. After 15 s, the analyser stabilized and readings were recorded. The instrument was calibrated to air. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single determination. Results were expressed as kPa of O_2 and CO_2 inside the bags. The reference pressure was 101 kPa.

For weight loss, bags were weighed at the beginning of the experiment (after the application of the treatments) and at day 10 of storage. Results were expressed as percentage of weight loss relative to the initial weight. The assays were carried out in duplicate on three independent experimental runs.

2.6. Superficial colour

Colour determination was carried out using a LoviBond colorimeter RT500 (Neu-Isenburg, Germany) with an 8 mm diameter measuring area. The instrument was calibrated with a standard white plate.

Superficial colour of broccoli florets was determined by measuring L^*, a^* and b^* chromaticity co-ordinates of the CIE-Lab scale (CIE, 1978) where L^* indicates the luminosity (varying from 0 = black to 100 = white), a^* is a measurement varying from green (−60) to red (+60) and b^* varies from blue (−60) to yellow (+60). Five different positions on the surface of each broccoli were measured for each treatment. The parameters $L^*a^*b^*$ were used to describe the hue angle ($H°$) (Eq. (6)) and the total colour difference (ΔE) (Eq. (7)) during storage.

$$H^\circ = \tan^{-1}\frac{b^*}{a^*} \quad (6)$$

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (7)$$

where L_0^*, a_0^* and b_0^* are the readings at time zero and L^*, a^*, and b^* the individual readings at each time during storage.

2.7. Browning potential

Overall browning potential was determined as the absorbance of an aqueous extract of the stem tissue at 320 nm (Pereyra et al., 2005). This method is a modification of the one described by Couture et al. (1993) in which the absorbance is measured at 340 nm. An absorption spectrum of the aqueous extract showed a peak at 320 nm and, therefore, this wavelength was used in the determinations.

Briefly, stem tissue was homogenized with distilled water (1:20) using a tissue homogenizer (Braun, Kronberg, Germany) with a speed of 58.3–117 s^{-1}. The homogenization was performed by immersion on an ice-water bath. The homogenate was filtered through Whatman No. 42 filter paper. The cloudy supernatant was centrifuged at 1400 × g for 15 min. The absorbance of a supernatant aliquot was measured with a spectrophotometer at 320 nm. Browning assays were performed for each treatment combination in duplicate.

2.8. Chlorophyll content

The Chlorophyll content was determined following the methodology described by Moreira et al. (2003). Broccoli florets were homogenized with a tissue homogenizer (Braun, Kronberg, Germany) and two 1-g samples were taken from each homogenate. Each sample was then homogenized with 19 mL of a cold solution 18:1 propanone:ammonium hydroxide (0.1 M). This homogenate was filtered through sintered glass and water was removed from the filtrate with anhydrous sodium sulfate. Absorbance of the filtrate at 660.0 and 642.5 nm was measured with a UV 1601 PC UV–vis spectrophotometer (Shimadzu Corporation, Japan). Chlorophyll content was calculated applying the formula $TC = 7.12A_{660} + 16.8A_{642.5}$; in which TC is the total chlorophyll concentration (mg/L) and A_{660} and $A_{642.5}$ are the absorbance at the corresponding wavelengths. Chlorophyll content is reported as mass of chlorophyll per fresh weight mass of broccoli (mg kg^{-1}).

2.9. Reduced ascorbic acid content

To assay the reduced ascorbic acid content (AA) the titrimetric method described by Ansorena et al. (2011) was followed. Broccoli florets and stems (20 g) were extracted with 100 mL of cold metaphosphoric acid solution (60 g kg^{-1}) for 3 min using a tissue homogenizer (by Braun, Kronberg, Germany) with a homogenizer speed of 58.3–117 s^{-1}. The homogenate was made up to 250 mL with 30 g kg^{-1} metaphosphoric acid and filtered through Whatman No. 42 filter paper. Temperature during ascorbic acid extraction was maintained at 0 °C. Aliquots (5 mL each) of the filtrate were titrated with 2,6-dichloroindophenol. Ascorbic acid content is reported as mass of AA per fresh weight mass of broccoli (mg kg^{-1}).

2.10. Mesophilic aerobic counts

For microbiological analysis, 10 g of broccoli from each treatment bag were macerated in 90 mL of phosphate buffer solution (0.1 mol/L, pH 7.2) and were homogenized with a Stomacher 400

Circulator Homogenizar. Serial dilutions (1:10) of each homogenized sample were made and surface spread in duplicate. The enumeration and differentiation of mesophilic aerobic bacteria was performed according to Alvarez et al. (2013) by using Plate Count Agar (PCA) incubated at 30–32 °C for 48–72 h. Total microbial counts were expressed as log CFU g^{-1}.

2.11. Overall visual quality (OVQ)

For each experimental condition, two individual bags were subjected to a panel of testers to evaluate sensorial quality of samples.

A panel comprised of nine members of the UNMdP Food Engineering Group, aged 30–55 years, and with sensory evaluation experience in vegetable quality, was trained and carried out the evaluation of broccoli quality. During the training, panellists were presented with an array of vegetable products to help the development of terms, which included: superficial colour, browning (overall and of cut edges), brightness, floret opening, odour and texture (Olarte et al., 2009). Then the judges were specifically trained in the discriminative evaluation of broccoli with the same variety and source as those used to prepare experiment samples. The vegetables used in the training sessions had been subjected to various treatments. The products were presented in coded plastic dishes.

Evaluations were performed immediately after removal from storage. The coded (3 digit) samples were presented one at the time in random order to the members who sat at a round table and made independent evaluations.

Sensory quality indices such as colour, brightness, texture, floret opening, smell and browning were evaluated. The intensity of the attributes evaluated was quantified on a scale from 1 to 5 (Ansorena et al., 2011) in the way described by Olarte et al. (2009): colour was rated using 5 = dark green, uniform colour, 3 = light green and 1 = showing yellowish florets. Brightness was rated using 5 = bright, glossy surface, 3 = lighter bright and 1 = opaque surface. Stem texture was rated using 5 = crispy, 3 = rubbery and 1 = very soft. Floret opening was rated using 5 = very tight and firm heads, 3 = slightly loose but acceptable and 1 = very loose and limp. Smell was rated using 5 = no off-odour, 3 = slight but obvious off-odour and 1 = strong off-odour. Browning was rated using 5 = no browning, 3 = moderate browning and 1 = extreme browning. The texture was evaluated by the fracture of broccoli stems with the fingers as described by Rico et al. (2007). The limit of acceptance was three (Ansorena et al., 2011) indicating that a score below 3 for any of the attributes evaluated was deemed to indicate end of shelf life.

3. Results and discussion

3.1. Model fitting

Initial mean values and after 10 d of refrigerated storage for all responses obtained for untreated samples (control) and treated samples, under different experimental conditions, are presented in Table 1. The experimental data were used to calculate the coefficients of the second order polynomial equations to obtain the significance of the coefficients of the models. The regression coefficients for the second order polynomial equations and results for the linear, quadratic and interaction terms as well as the correlation coefficients (R^2) are presented in Table 2. In general, the predicted values were closely correlated with the experimental data (except for weight loss). Correlation coefficients values of 0.9983, 0.9880 and 0.9570 for O_2 concentration inside the package, $H°$ and ΔE, respectively, and 0.9479, 0.9157, 0.9878, 0.9639 and 0.9787 for mesophilic counts, chlorophyll content, ascorbic acid, overall browning potential and OVQ, respectively suggested that the regression models could fit the responses values well.

Table 2
Regression coefficients (from coded data) and R^2 of the response surface models.

Coefficients	Responses							
	O_2 concentration	Hue angle ($H°$)	Total colour difference (ΔE)	Aerobic mesophilic counts	Browning potential	Ascorbic acid content	Chlorophyll content	Overall visual quality (OVQ)
b_0 (intercept)	5.557***	118.81**	2.273***	4.313***	0.3100***	83.280**	15.253**	4.883**
b_1 (us)	1.383**	4.01***	−1.513**	−0.015**	−0.030	6.790**	0.188**	0.448**
b_2 (tt)	0.225**	2.67***	0.427**	−0.036**	0.023**	−7.308***	1.525*	0.021*
b_3 (ca)	0.063	0.64	−0.318	−1.041**	−0.043**	4.118***	−2.242	0.597***
b_{12} (us × tt)	0.181**	−0.42*	−0.805	−0.2575	−0.0125	−5.197**	−1.085	−0.222
b_{13} (us × ca)	0.058	2.08**	0.1025	0.6025*	0.0025	−4.632**	0.614	0.345*
b_{23} (tt × ca)	0.009	0.91**	−0.77	−0.1900	−0.050**	−4.010*	0.185	−0.310*
b_{11} (us × us)	0.271***	−2.69**	2.284**	0.8533*	0.0050	1.800	−2.515**	−0.455**
b_{22} (tt × tt)	−0.001	−1.35***	2.562**	1.1558*	0.0875**	−20.027***	−0.987	−0.150
b_{33} (ca × ca)	0.032	0.10	−0.0004	0.6808*	0.0775**	−11.032***	−2.391**	−1.027***
R^2	0.9983	0.9880	0.9570	0.9479	0.9639	0.9878	0.9157	0.9787

us, ultrasonic processing time.
tt, thermal treatment time.
ca, citric acid concentration.
* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

Table 3
Results of the ANOVA for regression equation for O_2 concentration, hue angle, total colour difference, mesophilic counts, chlorophyll content, ascorbic acid content, browning potential and OVQ.

Responses	Source	DF	SS	MS	*F* value	Pr > *F*
O_2 concentration	Linear	3	15.737		930.63	<0.0001
	Quadratic	3	0.2749		16.26	0.0052
	Cross-product	3	0.1462		8.64	0.0201
	Total model	9	16.158		318.51	<0.0001
	Lack of fit	3	0.0103	0.0034	0.39	0.7783
	Pure error	2	0.0178	0.0089		
Hue angle ($H°$)	Linear	3	5.971		63.53	0.0080
	Quadratic	3	2.124		22.60	0.0250
	Cross-product	3	1.203		12.80	0.0088
	Total model	9	9.299		32.97	0.0060
	Lack of fit	3	0.156	0.052	4.78	0.0802
	Pure error	2	0.08	0.003		
Total colour difference (ΔE)	Linear	3	20.606		11.51	0.0111
	Quadratic	3	40.871		22.83	0.0024
	Cross-product	3	5.005		2.80	0.1485
	Total model	9	66.483		12.38	0.0064
	Lack of fit	3	2.9066	0.968	25.08	0.0386
	Pure error	2	0.077	0.038		
Aerobic mesophilic counts	Linear	3	8.686		14.06	0.0072
	Quadratic	3	8.181		13.24	0.0082
	Cross-product	3	1.8616		3.01	0.1330
	Total model	9	18.728		10.10	0.0101
	Lack of fit	3	0.8023	0.267	2.35	0.3123
	Pure error	2	0.2274	0.113		
Chlorophyll content	Linear	3	59.075		19.65	0.0034
	Quadratic	3	42.806		14.24	0.0070
	Cross-product	3	6.346		2.11	0.2175
	Total model	9	108.228		12.04	0.0069
	Lack of fit	3	4.809	1.603	36.64	0.1572
	Pure error	2	0.193	0.096		
Ascorbic acid content	Linear	3	931.888		41.04	0.0006
	Quadratic	3	1875.92		82.62	0.0001
	Cross-product	3	258.216		11.37	0.0113
	Total model	9	3066.026		45.01	0.0003
	Lack of fit	3	25.366	8.455	1.36	0.4512
	Pure error	2	12.478	6.239		
Browning potential	Linear	3	0.027		14.19	0.0070
	Quadratic	3	0.047		24.69	0.0020
	Cross-product	3	0.010		5.59	0.0471
	Total model	9	0.085		14.82	0.0042
	Lack of fit	3	0.002	0.0008	2.86	0.2696
	Pure error	2	0.0006	0.0003		
OVQ	Linear	3	4.470		34.43	0.0009
	Quadratic	3	4.434		34.15	0.0009
	Cross-product	3	1.058		8.15	0.0227
	Total model	9	9.963		25.58	0.0012
	Lack of fit	3	0.185	0.061	4.01	0.2061
	Pure error	2	0.030	0.015		

DF, degrees of freedom; SS, sum of squares; MS, mean square.

Regression coefficient for weight loss was poor and lack of fit was significant ($p < 0.01$) showing that the response surface equation did not adequately describe the data (results not shown). The significance of each coefficient was determined by student's *t*-test and *p*-values. A summary of the analysis of variance (ANOVA) for the quadratic models is shown in Table 3. The ANOVA of the regression models indicated that the resultant eight models were highly significant ($p < 0.05$) exhibiting no significant lack of fit. Hence, these models can be used to describe the effects of the selected independent variables (ultrasonic processing time, mild heat time and citric acid concentration) on total quality of minimally processed broccoli.

3.2. Headspace gas composition

The O_2 concentration inside the package was significantly affected by the temperature treatment duration ($p < 0.01$) and the ultrasonic treatment time ($p < 0.01$). Both, linear and quadratic effect of the ultrasonic time were observed on the O_2 content meanwhile only linear effect of temperature treatment duration was observed. The resulting response surface equation described the O_2 concentration inside the package perfectly ($R^2 = 0.9983$). Lack of fit was not significant ($p = 0.7783$) showing that the response surface equation adequately described the data (Table 3).

Canonical analysis show that stationary point was a saddle point suggesting movement away from these points would cause an increased or decreased response, depending upon movement direction. The O_2 concentration inside the polymeric bags was represented in Fig. 1 as a function of ultrasonic and thermal treatment time, holding citric acid concentration at 1%, since this variable presented the least influence on the response. The headspace analysis showed that pretreated samples had significantly reduced metabolic rates in comparison to non-treated samples. Increasing treatment times (ultrasonic and thermal) significantly reduced

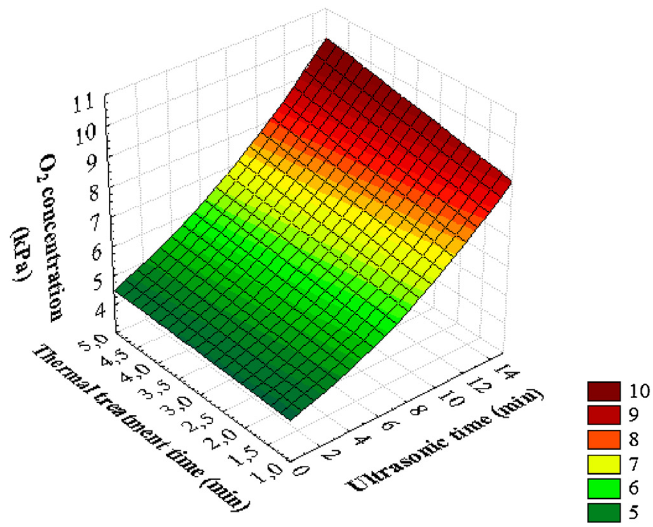

Fig. 1. Response surface plot for O_2 concentration inside the package (kPa) after 10 d of storage: effect of thermal and ultrasonic treatment times on O_2 concentration for a citric acid concentration of 1%.

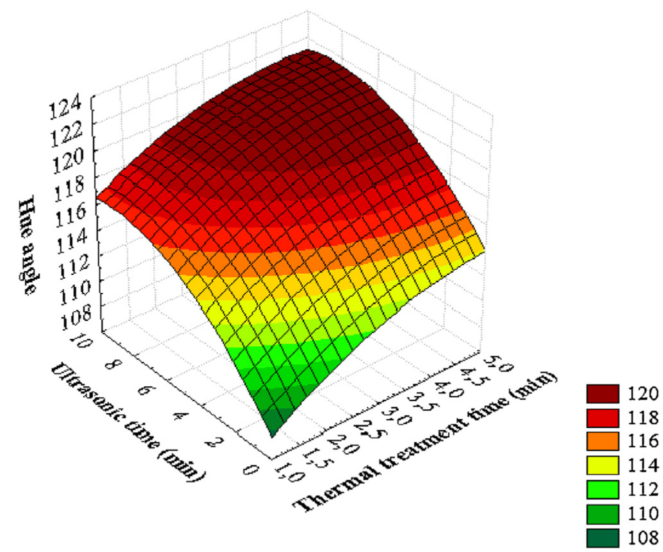

Fig. 2. Response surface plot for Hue angle after 10 d of storage: effect of thermal and ultrasonic treatment times on the Hue angle for a citric acid concentration of 1%.

metabolic rates resulting in a higher O_2 concentration inside the bags at the end of storage.

By applying ultrasound, broccoli always presented the higher O_2 concentration inside the bags at the end of storage. Reduced respiration rates due to ultrasound treatments have already been reported. Zhao et al. (2007) analysing the effect of ultrasound on respiration rates also reported that ultrasound suppressed respiration rates of pears during ripening. Similar results were found by Chen and Zhu (2011) when ultrasound was applied on plum fruit. The application of a thermal treatment, significantly inhibited the respiratory response of the vegetable. Reduced respiration rates due to heat shock pre-treatments have already been reported in several fruits and vegetables (Serrano et al., 2004; Koukounaras et al., 2008). Böttcher et al. (2003) related the total amount of oxygen consumed by the packed produce with the extent of metabolic activity defined as senescence level associated with produce respiration. In this sense, the application of both, a brief heat shock and an ultrasonic treatment, reduced the produce oxygen consumption inhibiting metabolism. Similar trends were obtained for CO_2 concentration inside the packages (results not shown).

3.3. Superficial colour

3.3.1. Hue angle

Broccoli quality losses are mostly related to florets degreening and yellowing of sepals. The hue angle, derived from the Hunter-*L*, *a*, *b* colour scale has been previously used for assessing the quality in broccoli and was used to describe colour change behaviour (Serrano et al., 2006; Olarte et al., 2009; Aiamla-or et al., 2010). The estimated values of the model coefficients describing the effects of the processing parameters on hue angle together with their *p*-values are presented in Table 2. These results indicated that the hue angle was significantly affected ($p < 0.001$) by the mild heat time and the ultrasonic processing time as individual factors.

Thermal and ultrasonic treatments significantly affected ($p < 0.001$) the hue angle in both linear and quadratic manners. Both independent variables showed a positive effect on linear terms but showed a negative effect on its quadratic terms. The interaction effect between ultrasonic and thermal treatments was negative ($p < 0.05$); whereas interactions between citric acid concentration and both, ultrasonic and thermal treatments showed a highly significant ($p < 0.01$) and positive effect on hue angle.

Canonical analysis show that stationary point was a saddle point (mixed signs of all eigenvalues) suggesting movement away from these points would cause an increased or decreased response, depending upon direction of movement. Fig. 2 represents the hue angle retention as a function of ultrasonic and thermal treatment times, holding citric acid concentration at a specified level (1%), since this variable presented the least influence on the response. A strong curvature of the surface is observed due to high significance of pure quadratic terms as shown through the variance analysis. As can be seen in the figure, hue angle values increased with increasing both, ultrasonic and thermal treatment time indicating higher green colour retention of samples. Tian et al. (1997), Dong et al. (2004) and Ansorena et al. (2011) reported that dipping broccoli in hot water at 42–55 °C reduce yellowing during storage.

The application of higher ultrasonic times, significantly increased hue angle retention. Several mechanisms can act concurrently when ultrasound is applied. One factor is the inhibition of the respiratory response of the vegetable. Results showed (Fig. 1) that the application of ultrasound suppressed respiration rates of minimally processed broccoli. Another possible effect could be cavitation, which govern various physical, chemical or biological reactions, such as accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or breakdown of susceptible particles such as enzymes and microorganisms (Tiwari et al., 2008).

3.3.2. Total colour difference

With regard to the ΔE index, the variance analysis in Table 2 revealed that total colour difference was significantly ($p < 0.01$) affected by both, ultrasonic and thermal treatments. Both, linear and quadratic effects of these independent variables were observed on ΔE meanwhile no significant effect of citric acid concentration was observed. Thermal treatment time showed a positive effect in both, linear and quadratic terms while ultrasonic processing time presented negative and positive effects on its linear and quadratic terms, respectively. None of the interactions was significant.

Fig. 3 represents ΔE as a function of ultrasonic and thermal treatment times, holding citric acid concentration at a specified level (1%), since this variable presented the least influence on the response. As can be seen, a strong curvature of the surface is observed due to the high significance of pure quadratic terms as was shown through the variance analysis. At thermal treatment times less than 3 min, ΔE decreased with time, while an increase in

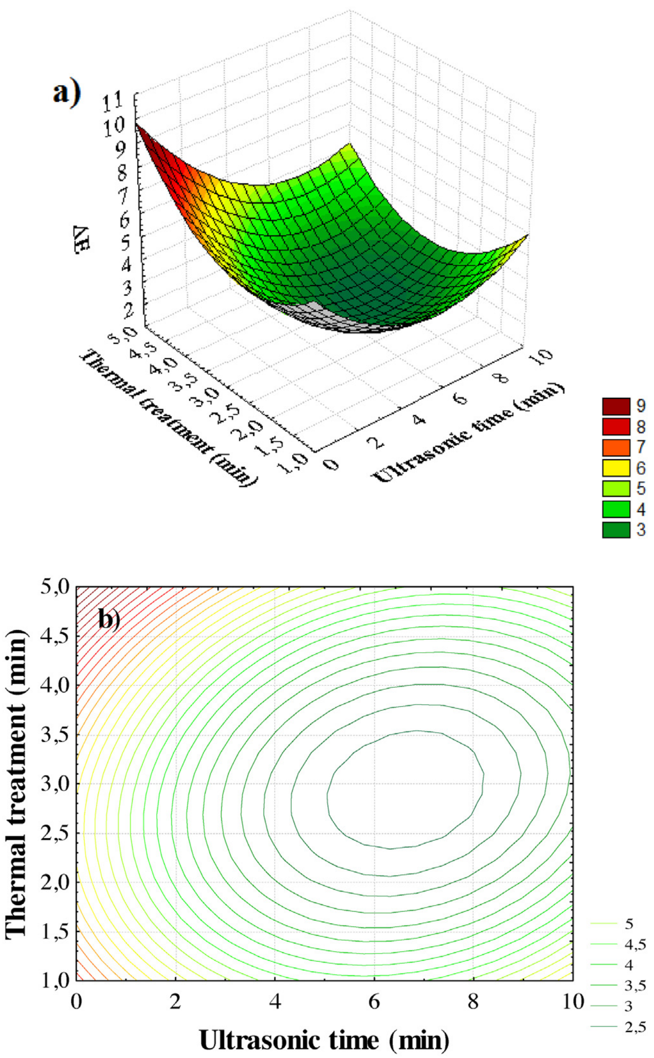

Fig. 3. Response surface plot (a) and contour plot (b) of the combined effects of thermal and ultrasonic time on parameter ΔE of minimally processed broccoli holding citric acid concentration at a specified level (1%).

this index was observed with further increase in thermal treatment time. The same behaviour was observed for ultrasonic processing time. ΔE index tends to decrease with increasing ultrasonic time presenting a minimum at 7 min, time from which ΔE index started to increase with increasing ultrasonic time. It is possible that ultrasound might help citric acid penetrate to inaccessible sites (hydrophobic pockets and folds in leaf surfaces on broccoli) and in this way the effectiveness of aqueous sanitizers could be increased when combined with ultrasound treatment.

3.4. Mesophilic aerobic counts

The regression analysis of the data showed that mesophilic aerobic counts was significantly affected by the ultrasonic processing time, heating time and citric acid concentration. The relationship between mesophilic counts and independent variables is depicted in Fig. 4(a–c). Canonical and stationary point analysis indicated that the stationary point was a minimum response point predicting that the estimated conditions were inside the experimental design region. At the end of storage, results revealed that the application of these combined technologies significantly reduced total microbial counts, respect to untreated broccoli samples.

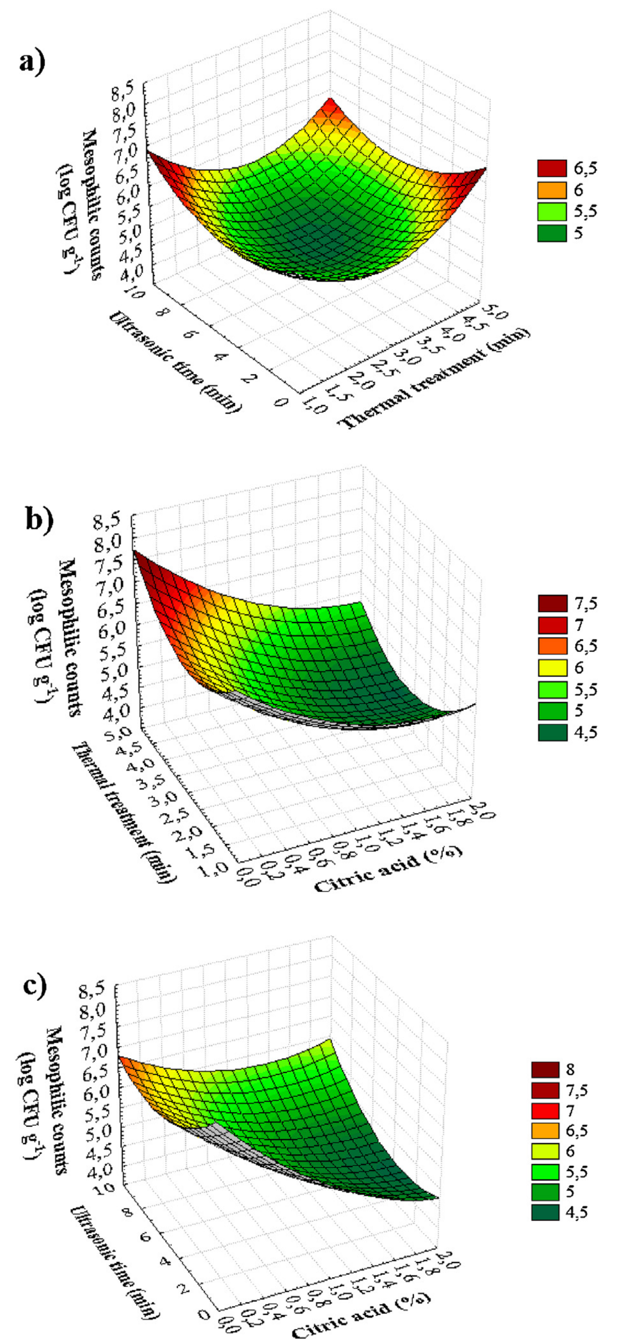

Fig. 4. Response surface curves for microbial load (log CFU g^{-1}); (a) effect of thermal and ultrasonic treatment times on the mesophilic counts for a citric acid concentration of 1%; (b) effect of the mild heat time and citric acid concentration on the mesophilic counts for an ultrasonic processing time of 5 min; (c) effect of the ultrasonic processing time and citric acid concentration on mesophilic counts for a thermal treatment of 3 min.

Heat treatments significantly controlled microbial load. In agreement, several works (Ansorena et al., 2011; Moreira et al., 2011) have already demonstrated that heat shocks are effective as a nonchemical means of improving microbiological quality of minimally processed broccoli. Ultrasonic treatment time significantly ($p < 0.01$) affected the native microflora. Several mechanisms can act concurrently when ultrasound is applied. One possible factor could be cavitation, which govern various physical, chemical or biological reactions, such as accelerating chemical reactions, increasing diffusion rates, dispersing aggregates or breakdown of susceptible particles such as enzymes and microorganisms (Tiwari et al., 2008). Moreover, the bath type ultrasound whose frequencies are in the range of 20–100 kHz is widely used and generates a powerful cavitation phenomenon which can destroy and detach microorganisms from surfaces of fresh produce without affecting quality. Mesophilic counts in minimally processed broccoli declined as the treatment was prolonged, reaching the lowest value at 5 min. Further treatment time resulted in higher populations. Similar results were obtained from Cao et al. (2010) when applying ultrasonic time in strawberry fruit.

Increasing the citric acid concentration to 2% (pH = 4) resulted in a decreased in mesophilic aerobic counts. Once more, the reduction in the microbial load could be explained by a reduction in the pH due to higher citric acid concentrations.

The application of a thermal treatment in combination to citric acid significantly enhanced the inhibition of microbial growth at the end of storage. It is probable that after the thermal treatment the small degree of softening of the broccoli florets improved the penetration of citric acid to the vegetable tissue allowing enhanced access of the organic acid into the irregular texture of the broccoli florets. This fact could be responsible for the higher antimicrobial effect observed at the end of the storage. The same fact took place when ultrasound was combined with the organic acid. In agreement, Sagong et al. (2011) found that the cavitation phenomenon generated by ultrasound detached microbial load from lettuce leaves and simultaneously enhanced the penetration of organic acids to inaccessible sites such as cut surfaces, punctures and cracks in produce surfaces which are hard to penetrate. Results demonstrate that microbial safety of vegetable products could be increased while simultaneously increasing acid concentration and shortening processing time if they use the combination of ultrasound and heat shocks with organic acid solution.

3.5. Overall browning potential

The estimated values of the model coefficients describing the processing parameters effects on overall browning potential of broccoli stems together with their p-values are presented in Table 2. The variance analysis revealed that overall browning potential was significantly ($p < 0.01$) affected by mild heat time and citric acid concentration. Both, linear and quadratic effects of these independent variables were observed meanwhile no significant effect of ultrasonic processing time was observed. Thermal treatment time showed a positive effect in both, linear and quadratic terms while citric acid concentration presented negative and positive effects on its linear and quadratic terms, respectively. The interaction between thermal treatment and citric acid concentration was the only that significantly affected ($p < 0.05$) browning potential.

Canonical and stationary point analysis indicated that the stationary point was a point of minimum response predicting that the estimated conditions were inside the experimental design region. Browning potential was represented in Fig. 5 in a contour plot as a function of thermal treatment times and citric acid concentration, holding ultrasonic time at a specified level (5 min), since this variable presented the least influence on the response. As can be seen, browning decreased with mild heat time up to 3 min and increased thereafter. The same behaviour was observed when considering the citric acid concentration. For concentrations below 1.3% browning decreases with increasing citric acid concentration while an increase in browning was observed with further increase in the concentration.

Thermal treatments are effective in improving postharvest quality of several horticultural products. They disrupt the wound-induced increase in the enzyme phenylalanine ammonia lyase (PAL) activity, the enzyme involved in the first step of the phenylpropanoid pathway that leads to the increasing production of the major phenolic compounds that lead to browning, delaying and

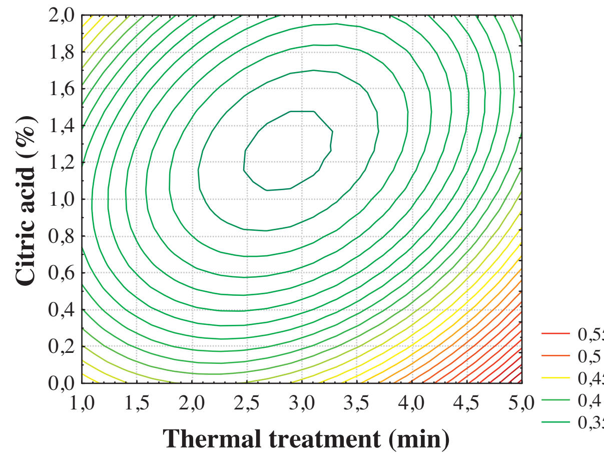

Fig. 5. Contour plot of the combined effects of thermal treatment time and citric acid concentration on browning potential ($Abs_{320\,nm}$) of broccoli stems holding ultrasonic time at 5 min.

diminishing the accumulation of phenolic compounds and tissue browning (Loaiza-Velarde et al., 1997; Pereyra et al., 2005). The application of the thermal treatment at 50 °C probably inhibited the activity of the PAL enzyme located in the superficial tissue of broccoli, delaying the accumulation of phenolic compounds and preventing tissue browning.

Organic acids have shown high capacity to prevent browning of fresh-cut tissues by the inhibition of polyphenol oxidases (PPO). Several other studies have confirmed the effects of organic acids in controlling browning of plant tissues. In agreement, it was found that citric acid retarded browning on fresh prepared potatoes (Langdon, 1987), lettuce (Castañer et al., 1996), fresh-cut spinach (Piagentini et al., 2002), longan fruit (Whangchai et al., 2006), litchi fruit pericap (Ducamp-Collin et al., 2008), fresh cut artichokes (Amodio et al., 2011) and ash gourd (Sreenivas et al., 2011).

3.6. Reduced ascorbic acid

The regression analysis of the data showed that ascorbic acid (AA) retention was significantly affected by the ultrasonic processing time, heating time and citric acid concentration. The relationship between AA and independent variables is depicted in Fig. 6(a and b). Canonical and stationary point analysis indicated that the stationary point was a saddle point. Although a decrease in ascorbic acid content was observed in both, untreated and treated minimally processed during storage, results revealed that at the end of storage the application of these combined technologies significantly reduced ascorbic loss respect to untreated broccoli samples.

Ultrasonic treatment time significantly controlled AA loss in minimally processed broccoli ($p < 0.01$). Ascorbic acid loss in minimally processed broccoli declined as the treatment was prolonged. Similar results have been obtained from Cao et al. (2010) when applying ultrasonic time in strawberry fruit. In agreement, Chen and Zhu (2011) found that the application of an ultrasonic treatment was effective in preserving titratable acidity and vitamin C content in plum fruit.

Citric acid has shown high capacity to prevent ascorbic acid loss of broccoli at the end of the storage. Organic acids have shown high capacity as an inhibitor of the enzyme polyphenol oxidase (PPO). Activity of PPO results in the production of orthoquinones, which are reduced by ascorbic acid. With the inhibition of PPO by the citric acid, presumably there is lesser demand on ascorbic acid and hence it is better preserved (Pushkala et al., 2012). This could be responsible for the lowest AA loss observed at the end of storage

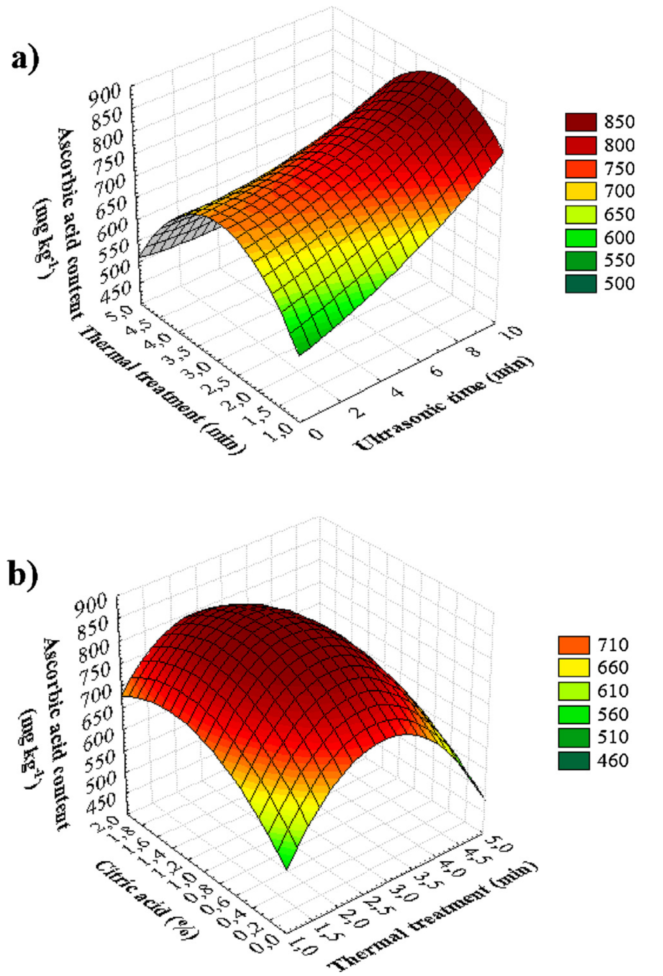

Fig. 6. Response surface curves for reduced ascorbic acid content (mg kg^{-1}); (a) effect of thermal and ultrasonic treatment times on AA content of minimally processed broccoli for a citric acid concentration of 1%; (b) effect of the mild heat time and citric acid concentration on AA content for an ultrasonic processing time of 5 min.

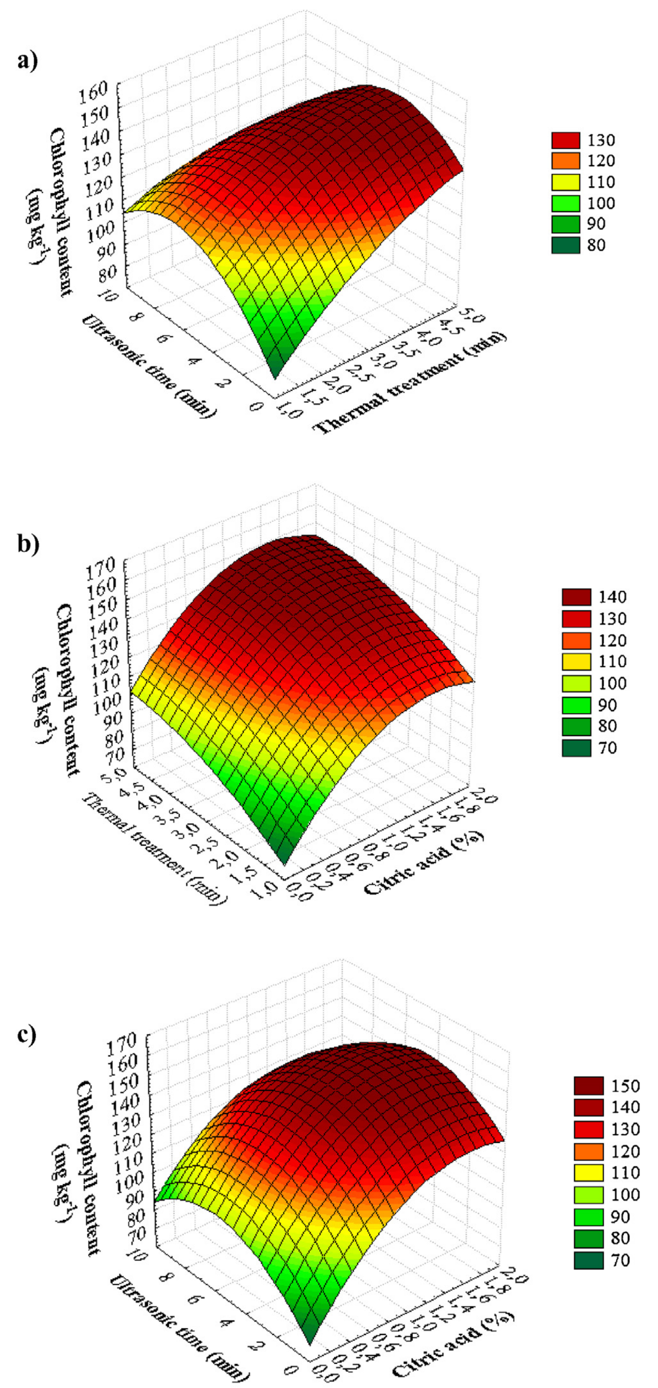

Fig. 7. Response surface curves for total chlorophyll content (mg kg^{-1}); (a) effect of thermal and ultrasonic treatment times on the chlorophyll content of minimally processed broccoli for a citric acid concentration of 1%; (b) effect of the mild heat time and citric acid concentration on chlorophyll content for an ultrasonic processing time of 5 min; (c) effect of the ultrasonic processing time and citric acid concentration on chlorophyll content for a thermal treatment of 3 min.

in broccoli samples treated with citric acid. Similar results were observed by Munyaka et al. (2010) when studying the influence of acidification on vitamin C on broccoli. Moreover, the combined effect of citric acid and ultrasonic treatment significantly enhanced AA retention.

The application of a thermal treatment significantly reduced ascorbic loss respect to untreated broccoli samples. Results revealed that ascorbic acid retention at the end of storage increased with mild heat time up to 3 min and decreased thereafter. Thermal treatments can delay the loss of ascorbic acid content during postharvest of different products (Shigenaga et al., 2005; Vicente et al., 2006; Lemoine et al., 2010).

3.7. Total chlorophyll content

The effects of independent variables on chlorophyll content are shown in Fig. 7(a and c). The canonical and stationary point analysis indicated that the stationary point was a point of maximum response predicting that the estimated conditions were inside the experimental design region. Total chlorophyll content was significantly ($p < 0.01$) affected by thermal and ultrasonic treatment times and citric acid concentration. It was observed from the statistical analysis conducted on the data that there was a significant ($p < 0.01$) influence of the linear factors of ultrasonic and thermal treatment times. Furthermore, the analysis showed that ultrasonic processing time and citric acid concentration had significant ($p < 0.01$) quadratic effects on the model.

Retention in chlorophyll content during storage significantly ($p < 0.01$) increased with longer exposure of broccoli to the thermal treatment. Previous research has demonstrated that chlorophyll loss during postharvest senescence of broccoli is correlated with an increment in the activities of enzymes involved in their catabolism such as chlorophyllase and Mg-dechelatase (Costa et al., 2006).

Application of thermal treatments during postharvest causes a transitory inhibition of gene expression or decrease of enzymatic activities, which recover when the tissue is returned to non-stressed temperatures (Martínez and Civello, 2008). In the present work, increments of enzymatic activities involved in chlorophyll catabolism may have been delayed by the heat treatment as it was described previously (Funamoto et al., 2002; Costa et al., 2006). In agreement, Dong et al. (2004) reported that broccoli heads treated at 45 °C by immersion with hot water maintained high contents of chlorophyll concentration and Funamoto et al. (2002) indicated that chlorophyll content in broccoli treated with hot air at 50 °C showed almost no change during storage.

The negative quadratic effect of citric acid concentration indicated that chlorophyll content significantly ($p < 0.01$) decreased with the increase in this parameter. These results could be explained by the effect of pH on the rate of chlorophyll degradation, since chlorophyll stability is known to be affected by pH (Aronoff, 1966; Francis, 1985). Similar results have been reported by Gunawan and Barringer (2000) and Koca et al. (2006) in green beans and broccoli, respectively.

3.8. Optimization and validation

Optimum levels of pre-treatments for green colour retention and maximum nutritional and microbiological quality of minimally processed broccoli during refrigerated storage were determined to obtain the criteria; minimum ΔE, overall browning and mesophilic aerobic counts, and maximum O_2 concentration inside the package, AA retention, H° and chlorophyll content. Second order polynomial models obtained in this study were utilized for each response in order to determine the specified optimum pre-treatment condition. The optimization was applied for selected ranges of mild heat time, ultrasonic processing time and citric acid concentration as 1–5 min, 0–10 min, and 0–2%, respectively.

The results of the simultaneous optimization (applying the desirability function) indicated that the levels of the optimized conditions were 3 min of mild heat time, 7.5 min of ultrasonic processing time and a citric acid concentration of 1.5%, with the predicted optimum responses of 6.37 kPa for O_2 concentration, 120.30 for H°, 1.95 for ΔE, 4.32 log CFU g^{-1} for mesophilic aerobic counts, 150.89 mg kg^{-1} for chlorophyll content, 852.68 mg kg^{-1} for ascorbic acid content and 0.2968 for overall browning potential. The general "goodness" score (r) for the simultaneous optimization was 0.8877.

In order to test the reliability of the models in predicting optimum responses and in accordance with the optimization results obtained from RSM with the desirability function, verification experiments were carried out at the optimum levels. The results indicated that the mean experimental values after 10 d of storage were 6.10 kPa for O_2 concentration, 122.27 for H°, 2.23 for ΔE, 4.54 log CFU g^{-1} for mesophilic aerobic counts, 153.5 mg kg^{-1} for chlorophyll content, 848 mg kg^{-1} for ascorbic acid content and 0.312 for overall browning potential at the selected optimum conditions of heat and ultrasonic treatment times, and citric acid concentration. The predicted results matched well with the experimental results obtained using optimum conditions ($p < 0.01$) which validated the RSM models with a good correlation.

3.9. Sensorial quality

The estimated values of the model coefficients describing the postharvest treatments effects on overall visual quality of broccoli together with their p-values are presented in Table 2. The variance analysis revealed that overall visual quality was significantly ($p < 0.05$) affected by the three postharvest treatments. The canonical and stationary point analysis indicated that the stationary point was a maximum (figure not shown). The results indicated that the optimum conditions at day 10 of storage were 8.1 min of ultrasonic processing time, 2.7 min of mild heat time, and a citric acid concentration of 1.2%, with a maximum OVQ value of 4.88. This result was in good agreement with the maximum found from the simultaneous optimization performed when considering to minimize ΔE, overall browning and mesophilic aerobic counts, and maximize O_2 concentration, AA content, H° and chlorophyll content.

3.10. Effect of optimum conditions on quality and shelf life of minimally processed broccoli

In order to verify the effects of optimum conditions on decay incidence, quality maintenance and shelf life of broccoli, a comparison experiment was performed during 20 d of storage at 5 °C between minimally processed broccoli without treatment (control) and treated under the selected optimum conditions. According to these results, combined treatment significantly ($p < 0.05$) inhibited the increase in microbial populations and maintained higher levels of chlorophyll content, ascorbic acid, green colour and sensory quality of broccoli throughout refrigerated storage (results not shown). Interestingly, the main differences between control and treated broccoli were not observed immediately after the treatment, but later during storage probably when broccoli was exposed to secondary stresses. The OVQ and aerobic mesophilic counts of treated broccoli were 43% higher and 38.7% lower, respectively than that in the control on the 10th day. Based on current Spanish regulations for microbial load (BOE, 2001) and on the limit of acceptance for sensory quality, it is possible to extend shelf life of refrigerated minimally processed broccoli up to 18 d (almost twice compared to control) by applying the selected optimum combined treatments.

4. Conclusions

Citric acid treatment in combination with ultrasonic and thermal treatments was employed as hurdles for retention of green colour, nutritional quality, microbial control and for extending shelf life of minimally processed broccoli. Response surface methodology approach was successfully employed to optimize process parameters for minimally processed broccoli with desired microbial, nutritional and sensory qualities.

Analysis of response surfaces indicates that the mesophilic counts, ascorbic acid content and the overall visual quality were significantly influenced by the three independent variables either independently or interactively. Both thermal and ultrasonic treatments were found to be critical factors influencing changes in chlorophyll content, O_2 concentration inside the package, hue angle and ΔE while for overall browning potential only thermal treatment and citric acid concentration were found to be significant. The application of an ultrasound treatment was effective in inhibiting green colour losses, O_2 consumption and for retention of nutritional quality, microbial control and for extending shelf life of minimally processed broccoli. Moreover, the use of the combination of ultrasound with citric acid and a heat treatment could reduce the required concentration of the aqueous sanitizer and thermal treatment times required in processing, resulting in savings to the organic food industry while improving microbial safety and maintaining food quality. Hence, results indicate that ultrasound could be used in combination with other postharvest treatments such as heat and aqueous sanitizers for preserving quality and ensure safety of minimally processed broccoli during refrigerated storage.

By using the desirability function approach and considering superficial colour parameters, mesophilic aerobic counts, browning potential, O_2 concentration inside the package, ascorbic acid and chlorophyll content the optimum processing conditions were

7.5 min of ultrasonic treatment, 3 min of a heat shock treatment and a citric acid concentration of 1.5% for maintenance of quality attributes of broccoli.

Under these optimal processing conditions it is possible to employ citric acid treatment in combination with ultrasonic and thermal treatments as hurdles for retention of green colour, nutritional quality, microbial control and for extending shelf life of refrigerated minimally processed broccoli. These combined treatments can be considered for commercial application during storage and marketing of minimally processed broccoli.

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