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ORIGINAL PAPER

Hurdle technology for minimally processed radishes: a response surface methodology approach

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Abstract Response surface methodology was used to determine the optimum processing conditions that yielded maximum vitamin C content, minimum discoloration and minimum polyphenoloxidase activity after 4 days of refrigerated storage of minimally processed radishes. Ascorbic acid (0–2 %), sodium chloride (0–1 %) and immersion time (0–2 min) at 50 °C were the factors investigated related to polyphenoloxidase activity (PPO), vitamin C (VITC) and total color difference (ΔE). Experiments were conducted according to a Box-Behnken Design with three factors at three different levels. For each response, second order polynomial models were developed using multiple linear regression analysis. Analysis of variance (ANOVA) was performed to check the adequacy and accuracy of the fitted models. The response surfaces showing the interaction of process variables were constructed and analyzed. Based on desirability function, optimum operating conditions were found to be 2 % of ascorbic acid, 0 % of NaCl and 1.5 min of immersion time at 50 °C. At this optimum point, relative PPO activity, relative VITC content and ΔE values were 0.218, 3.227 and 4.929, respectively.

Keywords Hurdle technology · Minimally processing · Mathematical optimization · Radish

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Introduction

Due to an increasing health consciousness and growing interest in the role of food for maintaining and improving human well-being and consumer health, vegetables are well recognized for their benefits towards healthiness, thanks to their protective function against diseases. Numerous studies have shown that consumer's needs for convenience are correlated with food choice [1].

Minimally processed radishes (*Raphanus sativus* L.) are important constituents in mixed salads. They are preferred for their strong and unique flavor as well as the content of vitamin C, B-complex vitamins and minerals [2]. However, reports on radish physiologic response during processing and storage are still scarce [2–4]. The marketing of fresh-cut salads is limited by a short shelf-life and a rapid deterioration of their components due to tissue damage by the preparation methods. Obtaining products with fresh-like quality and high nutritional value is a crucial quality step in the processing industry [5, 6]. Several preservation technologies such as physical decontamination methods (irradiation, electrolyzed water and ozone treatment), cold chains, controlled and modified atmospheric storage are used to achieve this requirement. Higher capital and maintenance costs associated with these preservation techniques limit their application. In addition, the use of chemical sanitizers like chlorine, hydrogen peroxide and sulphites is questioned from a safety point of view. In order to cope with these facts and with consumer preference for natural additives, low cost and safer preservation, alternative technologies have been proposed [3].

In a previous work, three hurdles technologies were selected as those capable of maintaining the characteristic fresh-like color of radish slices together with acceptable sensory parameters and enzyme inhibition [7]. These

hurdles were ascorbic acid immersion (AA), mild thermal treatment (TT) and sodium chloride immersion (NaCl). Ascorbic acid is probably the most widely used antibrowning agent on vegetables, and in addition to its reducing properties, it also slightly lowers pH. It reduces quinones to phenolic compounds before they undergo further reaction to form pigments inhibiting the polyphenoloxidase that causes a decrease of nutritional value and consumer's acceptance due to browning [2, 8]. NaCl is also known to inhibit PPO; its inhibition increases as pH decreases. Significant control of browning may be possible if the dipping solutions are acidic. Furthermore, heat inactivation of the enzymes is fundamental due to the importance of preserving the color of the raw material before any transformation [9, 10].

Response surface methodology (RSM), a multivariate statistical technique, has been suggested to optimize the levels of various hurdles taking into account food quality [11]. Therefore, the objective of this study was to determine the optimum levels of ascorbic acid, sodium chloride and mild heat shock time as hurdles technologies to minimize enzymatic browning (ΔE) and polyphenoloxidase activity (PPO) as well as maximize vitamin C content (VITC) of minimally processed radish by means of RSM. The experimental responses called PPO, VITC and ΔE were measured immediately after applying the hurdles (zero time) and after 4 days of storage.

Materials and methods

Plant material and sample preparation

Radishes were purchased from a local market in Mar del Plata, Argentina. They were kept at 5 ± 1 °C in darkness prior to processing. Radish roots were separated from the leaves. The roots were washed with tap water to eliminate any surface contamination and cut with a vegetable cutter (HLC-300, Dynam-h, SYSTEL S.A., Argentine) into slices of 4 mm. Then, they were washed again with tap water using a sliced radish to water ratio of 1:10. The slices were dried by a manual centrifuge, and then the hurdles were applied. Thermal treatment was carried out in a thermostatically controlled water bath with recirculation (Lauda E300, Germany). Thereafter, samples were removed from the bath and cooled immediately in cold water at 0–4 °C for 3 min. Then, immersion in solutions was carried out for 5 min. Finally, the slices were dried by a manual centrifuge. After treatment application, samples of 50 g were packed in polyethylene bags (25 cm × 30 cm) of 25.4 μm thickness (with an O_2 transmission rate of 600 cm^3/m^2 day, CO_2 transmission rate of 4000 cm^3/m^2 day, and water vapor transmission rate of 4 cm^3/m^2 day; P = 101,325 Pa, T = 25 °C) using a manual impulse sealer (HL, FS-300, Argentina). Three bags for each treatment were analyzed at 0 and 4 days of storage at 9 ± 1 °C.

Vitamin C content

Vitamin C content was determined by the titrimetric assay described by Moreira, Roura and del Valle [12]. Twenty grams of radish slices were homogenized (Hr1362, Philips, Argentina) with 40 mL of oxalic acid solution 2 g/100 (w/w). This mixture was vacuum filtered through glass fiber. Five millilitre aliquots of the filtrate were titrated with 2,6 dichloroindophenol. Vitamin C contents were calculated as mg of reduced ascorbic acid/100 g of sample on a wet basis and relative content $VITC_R$ was expressed as:

$$VITC_R = \frac{VITC}{VITC_0} \quad (1)$$

where VITC is the vitamin C content after the treatment and $VITC_0$ is the value of fresh samples. Determination of vitamin C content in radish was performed in triplicate.

Enzyme activity

Polyphenoloxidase activity was measured by the colorimetric method according to Goyeneche, Di Scala and Roura [13]. Ten gram of radishes were homogenized with a commercial mixer (Hr1362, Philips, Argentina) at 1:2 ratio with 0.5 mol/L phosphate buffer (pH 7.0) in the presence of 50 g/L polyvinylpyrrolidone (ICN Biomedicals, Inc. OH) and centrifuged (high speed centrifuge, Spectrafuge, 7M, Labnet, USA) at 12.700×*g* for 30 min. The supernatant was the PPO crude vegetable extract and was maintained at 0 °C until use. Gallic acid 4 mmol/L on phosphate buffer pH 7 was used as the substrate solution. The reaction cuvette contained 2.9 mL of substrate solution and 0.1 mL of PPO extract, and the reference cuvette contained substrate solution. The enzyme activity was defined as a 0.001 change in absorbance at 350 nm between 0 and 60 s under the assay conditions, according to previous experiments [13]. Enzyme activity was measured (spectrophotometer UV-1601 PC, Shimadzu Kyoto, Japan) and residual activity PPO_R was expressed as:

$$PPO_R = \frac{PPO}{PPO_0} \quad (2)$$

where PPO is the enzyme activity after the treatment and PPO_0 is the initial enzyme activity of fresh samples. Each solution was tested in triplicate.

Color

The color development was measured on the sliced surfaces with a colorimeter (Lovibond, RT Series, England),

which had been standardized against a white tile ($L^* = 97.63$, $a^* = 0.3133$, $b^* = 0.3192$). The measurements were made in triplicate over each surface sample. Color was measured using a CIE—L*a*b* uniform color space (Lab), where L* indicates lightness (whiteness or brightness/darkness), a* indicates chromaticity on a green (−) to red (+) axis, and b* chromaticity on a blue (−) to yellow (+) axis [14]. Numerical values L^*, a^*, b^* were converted into total color difference (ΔE) according to Eq. (3):

$$\Delta E = \sqrt{(a^* - a_o)^2 + (b^* - b_o)^2 + (L^* - L_o)^2} \quad (3)$$

ΔE is generally used to inform the difference between two colors as indicated by the following scale: Trace level difference ΔE* = 0–0.5, Slight difference ΔE* = 0.5–1.5, Noticeable difference ΔE* = 1.5–3.0, Appreciable difference ΔE* = 3.0–6.0, Large difference ΔE* = 6.0–12.0, Very obvious difference ΔE* > 12.0 [15].

Sensory evaluation

Sensory acceptability of radish slices was assessed by a trained sensory panel of five members using a descriptive test, as described by Goyeneche, Roura and Di Scala [7]. Color (two scales, brown scale to observe browning emergence –ColorM- and violet scale to observe pigment diffusion –ColorV-) as well as texture was subjectively evaluated on three samples per treatment and evaluation date. For each index, the scale used rated from 0 (best quality) to 5 (worst) and scores above 3 indicated the rejection of the product. Sensory evaluation was not included in the response surface analysis; however, these results were used to complement and improve the discussion of the experimental results.

Experimental design and statistical analysis

Response surface methodology is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously [11]. A Box-Behnken Design (BBD) with three factors at three levels was used to evaluate the experiments, as shown in Table 1. The ranges of the three independent variables were selected on the basis of preliminary assays [7]. The number of experiments (N) required for the development of BBD is defined as $N = 2k(k-1) + Co$ (where k is number of factors and Co is the number of central point). The design included 15 experiments with 3 central points. Each independent variable was coded at three levels: +1, 0, and −1, whereas ascorbic acid concentration: 0–2 % w/v; sodium chloride concentration: 0–1 % w/v, and immersion time at 50 °C: 0–2 min (Table 1).

Table 1 Experimental design

Run	Coded			Uncoded		
	AA	NaCl	TT	AA (% w/v)	NaCl (% w/v)	TT (min)
1	−1	−1	0	0	0	1
2	−1	1	0	0	1	1
3	1	−1	0	2	0	1
4	1	1	0	2	1	1
5	0	−1	−1	1	0	0
6	0	−1	1	1	0	2
7	0	1	−1	1	1	0
8	0	1	1	1	1	2
9	−1	0	−1	0	0.5	0
10	1	0	−1	2	0.5	0
11	−1	0	1	0	0.5	2
12	1	0	1	2	0.5	2
13	0	0	0	1	0.5	1
14	0	0	0	1	0.5	1
15	0	0	0	1	0.5	1

Performance of the process was evaluated by analyzing the responses (Y), which depend on the input factors X_1, X_2,…,X_k. A second-order polynomial equation was used to fit the experimental data to identify the relevant model terms using statistical software (SAS software, Version 9.0, SAS Institute, Cary, NC):

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} X_i X_j \quad (4)$$

where Y is the response; X_i and X_j are variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj}, and β_{ij} are interaction coefficients of linear, quadratic, and the second-order terms, respectively; and k is the number of independent parameters (k = 3 in this study) [11].

Response surfaces graphics were obtained using the fitted model by keeping the least effective independent variable at a constant value, while changing the other two variables. In order to limit the influence of systematic errors, the sequence of the experiments was randomly established. The central point experiments allowed estimating the influence of the experimental error, whereas the other experiments allowed the calculation of the regression coefficients of the model.

The goodness of the fit for the developed regression models was evaluated by means of the following tests: P value, R^2 and mean square error (MSE). Effects with $P > 0.05$ were considered as insignificant at the 95 %

confidence level, and were discarded. The adequacy of the fitted models was verified by calculating the F-lack of fit statistic, the adjusted R-squared, and carrying out the Durbin–Watson statistic test. The Durbin–Watson (DW) statistic tested the residuals to determine if there was any significant correlation based on the order in which they occurred in the data file. A Durbin–Watson value greater than 1.4 indicates probably no serious autocorrelation in the residuals [16].

Simultaneous optimization

During optimization of industrial processes, usually several response variables describing the quality characteristics and measuring performance of the systems, are to be optimized. Some of these variables are to be maximized and some are to be minimized. In many cases, these responses are competing, i.e., improving one response may have an opposite effect on another one. Several approaches have been used to tackle this problem. One approach is to solve the problem of multiple responses through the use of a Desirability function (D) that combines all the responses into one measurement [17]. Once individual response surfaces have been determined for each response, predicted values obtained from each response surface can be transformed to a dimensionless scale d_n. Then, the following modified desired function is applied [18]:

$$d_n = \begin{bmatrix} 0 & if\ y_n \le y_n^{min} \\ \left(\dfrac{y_n - y_n^{min}}{y_n^{max} - y_n^{min}}\right) & if\ y_n^{min} \le y_n \le y_n^{max} \\ 1 & if\ y_n \ge y_n^{max} \end{bmatrix} \quad (5)$$

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; 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.

$$D = (d_1, d_2, \ldots, d_n)^{1/n} \quad (6)$$

where n is number of responses studied in the optimization process. An algorithm is then applied to the D function in order to determine the set of variable values that maximizes it [17].

In the present study, desirability functions were developed for the criteria of maximum vitamin C content and minimum values of ΔE and PPO activity using the SAS program (SAS software, Version 9.0, SAS Institute, Cary, NC).

Validation test

In order to test the reliability of the simultaneous optimization, a new set of experiments using optimal operating conditions obtained with the Desirability function was performed. The experimental and predicted values of PPO, VITC and ΔE were compared in order to determine the goodness of the model. Data were analyzed using the software package SAS (version 9.0, SAS Institute Inc., Cary, USA, 2002).

Results and discussion

Model fitting

The experimental responses or quality indices called relative polyphenoloxidase activity (PPO_R), relative vitamin C content ($VITC_R$) and ΔE immediately after applying the hurdles and after 4 days of storage are presented in Table 2. These experimental data were used to calculate the coefficients of the second order polynomial equations to obtain the significance of the coefficients of the models (Eq. 4). Table 3 shows the regression coefficients of the second order polynomial equations by means of linear, quadratic and interaction terms at different levels of significance as well as the correlation coefficients (R^2), adj R^2 and DW. Based on these results, statistical analysis by means of ANOVA showed that all the models generated were significant ($P < 0.05$) while lack of fit was insignificant ($P > 0.05$). High values of R^2 are also evidenced for the fit goodness of the models. In all cases, values for DW were greater than 1.4, indicating that there were no correlation in the residuals [16].

The visual inspection of the residual graphs can also generate valuable information about the model suitability. Thus, if the mathematical model is well fitted, its graph of residuals presents a behavior that suggests a normal distribution [17]. Normal probability plot (data not shown) showed nearly a linear pattern, which indicated that the normal distribution was a good model for this data set. Moreover, after verifying the normality of the data, the homogeneity of variances was verified by Bartlett's test [19].

Table 3 also summarizes the results of the variance analysis of minimally processed radish after storage. Afterwards analysis and discussion of each response as affected by the applied hurdle technologies at time zero and after storage based on the ANOVA test are presented.

Effects of hurdle application on quality parameters

Polyphenoloxidase activity

The AA, as an individual factor, had a significant but negative effect on PPO (Table 3) at time zero. This result

Table 2 *E*xperimental responses (PPO_R, $VITC_R$ and ΔE) at zero time and after 4 days of storage

Run	PPO_R [PPO/PPO_0]		VITC [$VITC/VITC_0$]		ΔE	
	Storage time (days)					
	0	4	0	4	0	4
1	0.85 ± 0.10	0.66 ± 0.02	0.92 ± 0.09	0.92 ± 0.03	4.01 ± 1.49	4.86 ± 1.26
2	0.88 ± 0.09	0.81 ± 0.04	0.97 ± 0.16	1.01 ± 0.03	3.59 ± 0.46	4.24 ± 1.93
3	0.35 ± 0.03	0.40 ± 0.07	7.76 ± 1.31	3.01 ± 0.41	6.03 ± 0.79	5.93 ± 1.26
4	0.43 ± 0.02	0.22 ± 0.09	9.12 ± 1.72	5.18 ± 0.70	8.28 ± 0.33	7.75 ± 0.25
5	0.63 ± 0.04	1.39 ± 0.29	3.08 ± 0.59	1.83 ± 0.12	3.20 ± 0.14	9.85 ± 1.87
6	0.68 ± 0.26	0.60 ± 0.04	4.72 ± 0.19	1.70 ± 0.21	3.26 ± 0.24	4.38 ± 0.41
7	0.58 ± 0.08	1.56 ± 0.32	3.87 ± 0.78	1.68 ± 0.06	6.35 ± 0.47	8.02 ± 0.62
8	0.62 ± 0.02	0.73 ± 0.14	4.66 ± 0.10	1.94 ± 0.03	5.79 ± 1.09	9.68 ± 0.89
9	1.03 ± 0.00	1.55 ± 0.24	1.03 ± 0.08	0.95 ± 0.04	2.99 ± 0.49	8.30 ± 1.06
10	0.38 ± 0.09	1.48 ± 0.14	5.50 ± 1.97	2.50 ± 0.09	5.39 ± 0.04	6.70 ± 0.33
11	0.88 ± 0.02	0.66 ± 0.01	0.95 ± 0.08	0.89 ± 0.00	4.22 ± 0.43	4.65 ± 1.55
12	0.36 ± 0.10	0.41 ± 0.14	7.64 ± 1.67	3.78 ± 0.48	7.38 ± 1.87	6.74 ± 0.35
13	0.69 ± 0.07	1.04 ± 0.11	3.94 ± 0.61	1.63 ± 0.01	4.64 ± 0.29	5.00 ± 1.82
14	0.58 ± 0.08	1.10 ± 0.11	3.93 ± 1.01	1.79 ± 0.13	3.86 ± 0.26	3.82 ± 0.16
15	0.53 ± 0.04	1.10 ± 0.05	3.97 ± 0.95	1.69 ± 0.02	4.86 ± 0.07	5.08 ± 1.40

Table 3 Regression coefficients of the second order polynomial models for PPO_R, $VITC_R$ and ΔE at zero time (0 days) and at the end of storage (4 days)

Coefficients	PPO_R (days)		$VITC_R$ (days)		ΔE (days)	
	0	4	0	4	0	4
β_0 (intercept)	0.600***	1.080***	3.947^{NS}	1.703**	4.650*	4.633***
β_1 (X_1)	−0.530***	−0.292*	6.537***	2.675***	2.793*	1.643^{NS}
β_2 (X_2)	0.000^{NS}	0.068^{NS}	0.535^{NS}	0.588^{NS}	1.603^{NS}	1.542^{NS}
β_3 (X_3)	-0.020^{NS}	−0.895***	1.123^{NS}	0.338^{NS}	0.680^{NS}	−1.855*
β_{11} (X_1^2)	0.063^{NS}	−0.602**	0.443^{NS}	2.675^{NS}	1.448^{NS}	-0.698^{NS}
β_{22} (X_2^2)	-0.008^{NS}	−0.512**	1.048^{NS}	0.584^{NS}	0.758^{NS}	2.072^{NS}
β_{33} (X_3^2)	0.063^{NS}	0.492**	-0.777^{NS}	-0.416^{NS}	-0.758^{NS}	4.627**
β_{12} (X_1: X_2)	0.025^{NS}	-0.165^{NS}	0.655^{NS}	1.040^{NS}	1.885^{NS}	0.470^{NS}
β_{13} (X_1: X_3)	0.065^{NS}	-0.090^{NS}	1.110^{NS}	0.670^{NS}	0.380^{NS}	1.845^{NS}
β_{23} (X_2: X_3)	-0.005^{NS}	-0.020^{NS}	-0.425^{NS}	0.195^{NS}	-0.310^{NS}	3.565*
R^2	0.944	0.976	0.980	0.940	0.854	0.920
Adj-R^2	0.844	0.933	0.943	0.833	0.591	0.776
DW[a]	2.309	2.240	1.575	1.977	2.567	2.233

X_1, ascorbic acid concentration; X_2, sodium chloride concentration; X_3, immersion time at 50 °C

*** Significant at 0.001 level; ** Significant at 0.01 level; * Significant at 0.05 level; ^{NS}: Not significant

[a] Durbin–Watson values

indicated that an increase in AA concentrations resulted into a decrease on PPO activity. Similar results were found by Chow, Louarme, Bonazzi, Nicolas and Billaud [6] on apples and Altunkaya and Gökmen [20] on fresh lettuce. The other hurdles did not show significant differences ($P > 0.05$).

PPO activity of the radish slices depended significantly on the linear terms of AA ($P < 0.05$) and TT ($P < 0.01$), and the quadratic terms of AA ($P < 0.01$), NaCl ($P < 0.01$) and TT ($P < 0.01$) at the end of storage. These results indicated that an increased in AA decreased the PPO activity (Table 3). Polyphenoloxidase inhibition by ascorbic acid has been attributed to the reduction of enzymatically formed *o*-quinones to their precursor diphenols. Similar results were reported for apple polyphenoloxidase [6]. Suttirak and Manurakchinakorn [21] also reported that the antibrowning efficiency of AA in fresh-cut vegetables correlate with the concentrations of the acid employed. Thus, concentrations between 0.2 and 10 g/L of ascorbic acid did not inhibit the enzyme,

although such concentrations would activate PPO of apple. The increase of PPO activity could be due to an insufficient concentration of ascorbic acid, which at low concentrations might act as a prooxidant [22].

Regarding NaCl, high concentrations of this hurdle also reduced PPO activity, as can be seen in the quadratic terms of NaCl (Table 3) at the end of the storage. The inhibitory effect of sodium chloride is attributed to the anion chloride which correspond to a noncompetitive type for purified PPO from apples [22]. Moreover, this effect on PPO activity has been described in grape, banana and sunflower due to enzyme conformational changes, or altered protein association/dissociation due to modified ionic strength [23–25]. Concentrations between 0.5 and 1 % of sodium chloride had an inhibiting effect on the enzymatic browning of apple pieces, but only concentrations about 20 % inactivated PPO isolated from apple [22].

At time zero, TT did not show any effect on PPO activity. However, at the end of the storage, high concentrations of this hurdle increased the PPO activity, as can be seen in the quadratic terms of TT (Table 3). Higher heat treatment increased the cell damage, favoring the activity of the enzyme, while a smaller time promotes its inhibition. Thermal inactivation kinetics of PPO is well reported; however, it is a matter of controversy in published reports [26]. Sunflower PPO required more than 3 min at 100 °C to be fully inactivated, and it still showed a significant activity after exposure to 80 °C for 15 min [23]. The above mentioned data suggest that the inactivation of PPO depends on the source, sub-type, environmental and physicochemical conditions such as pH and temperature [26].

Figure 1 shows the response surfaces graphics of the predicted PPO activity as function of two factors, holding the other constant, after 4 days of storage. It seems that with longer immersion time, a greater inhibition of enzymatic activity is obtained. Also, higher AA concentrations reduced PPO activity. Meanwhile, low levels of NaCl showed more PPO inactivation. After 4 days of storage, the lowest PPO activity was achieved with the combinations of the three inhibitors in runs 4 and 12, or with two inhibitors in run 3 (Table 2). The common factor of these three runs is the high AA concentration (2 % w/v). While in run 4 the highest concentration of NaCl (1 % w/v) with an immersion time at 50 °C of 1 min was required, in run 12 the higher inhibition of PPO was achieved with half NaCl (0.5 % w/v) and maximum heat treatment time (2 min). In run 3, the PPO inhibition was achieved only with AA and TT at 1 min. According to the results of run 3, a combined treatment of heating and AA was more effective in reducing PPO activity. PPO was increasingly inactivated with increasing temperatures, as reported in previous work for apples. It was significantly affected by heating time at 45 and 60 °C and residual activity decreased to 50 % after about 200 and 20 min of residence time at 45 and 60 °C, respectively. According to Chow, Louarme, Bonazzi, Nicolas and Billaud [6], adequate temperature conditions and AA concentration should be carefully chosen to totally inactivate PPO activity from apple and prevent browning in minimally processed apple foodstuffs. These authors also found increases in the PPO activity for apple cubes dipped in ascorbic acid (0.2–10 g/L range) and in NaCl (0.2–1 g/L range) solutions for 5 min. Also, 90–100 % of PPO inhibition was obtained with a 5 min dip in mixtures of ascorbic acid and citric acid (10 + 2 g/L), or ascorbic acid and sodium chloride (10 + 0.5 g/L).

Pizzocaro, Torreggiani and Gilardi [22] reported that 10 g/L of ascorbic acid with 0.5 g/L of sodium chloride completely inhibited the PPO activity of apple. The explanation of the synergic phenomena between AA and NaCl is that the AA reduced the enzymatically formed quinones and delays browning without altering the enzymatic activity, whereas the anion chloride directly inhibits PPO [2].

Vitamin C content

VITC value of radish slices corresponding to non treated samples (control sample) was 16.5 mg/100 g of fresh weight. This value is comparable with those for Chinese radish (14.16–33.41 mg/100 g) and for red radish (14–27 mg/100 g) [3, 27–30]. Table 3 presents $VITC_R$ values of Eq. 1. At time zero, only the AA, as an individual factor, had a significant and positive effect on VITC. This result can be observed in the positive linear term (Table 3) which corresponded to an increase in VITC when AA is increased. At the end of the storage, increasing concentrations of this hurdle also increased PPO activity, as can be seen in the linear term of AA (Table 3). Moreover, at day four, it was observed that for those treatments including AA and TT (runs 3, 4 and 12) (Table 3), $VITC_R$ content was significantly higher compared to those that do not have these hurdles. Whatever combination of hurdles used, the addition of AA increased the VITC content, which is dependent on the exogenous AA concentration. The external addition of ascorbic acid is the cause of the overestimated value of VITC. This trend was maintained during storage; however, difference between control and samples with AA dropped to lower levels. The samples with lower levels of VITC corresponded to radish slices without AA addition (runs 1, 2, 9 and 11). Therefore, the addition of AA proved to be a key factor for the vitamin C retention of radish slices.

Regarding NaCl, it was observed that higher concentrations of NaCl presented higher $VITC_R$, especially when it was combined with AA. Concerning TT, the lowest

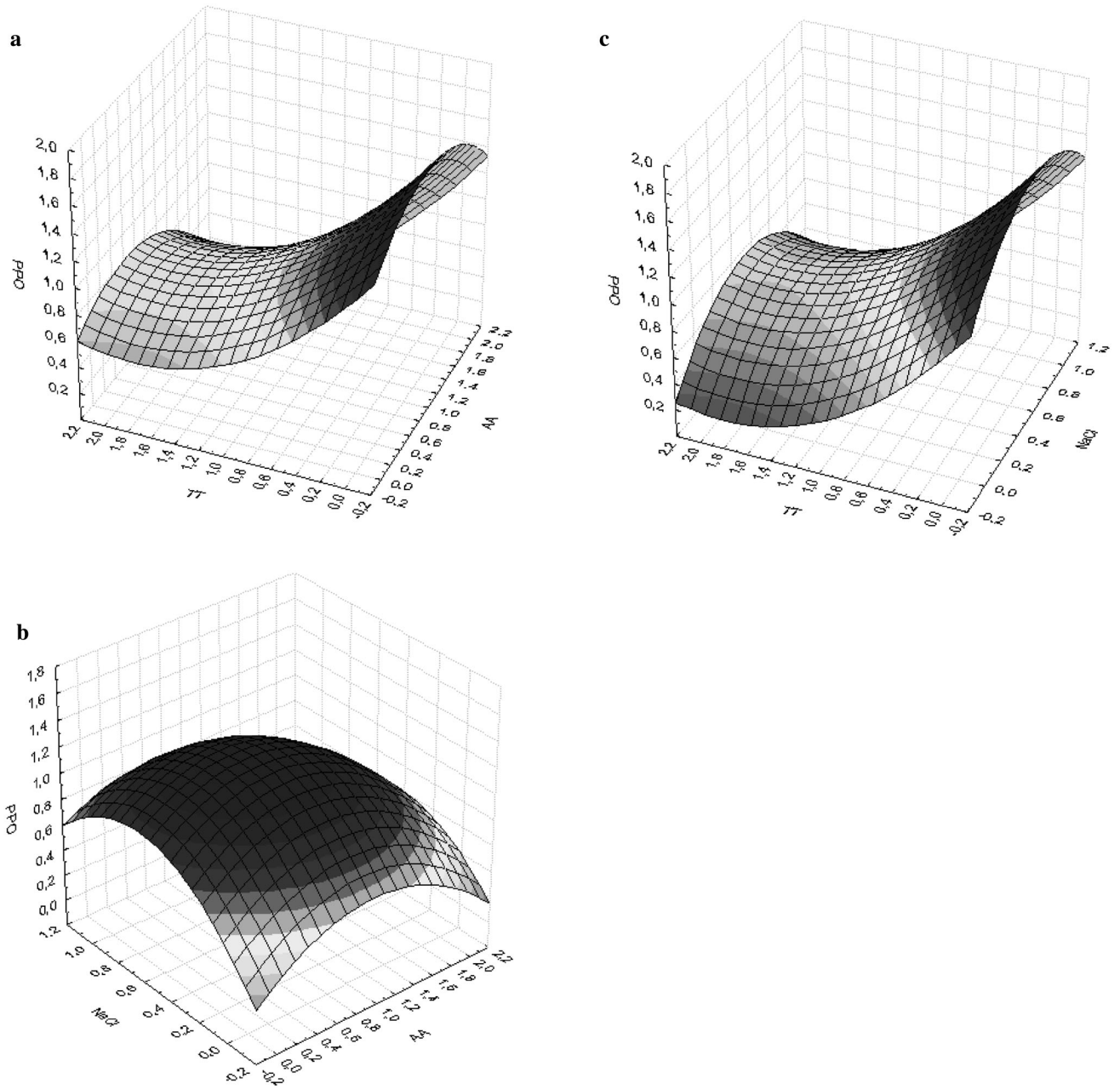

Fig. 1 Response surface plots at the end of storage (day four) for PPO at: **a** NaCl = 0.5 %; **b** TT = 1 min; **c** AA = 1 %

$VITC_R$ were achieved in run 11, where higher immersion time was performed (2 min). Long exposure time resulted in degradation of vitamin C [6, 31, 32].

Total color difference

Based on the tristimulus values obtained (L*, a* and b*), the total color difference (ΔE) was calculated. For fresh radish slices, these parameters were: $L_0 = 74.31 \pm 0.49$, $a_0 = -0.42 \pm 0.01$, $b_0 = 4.68 \pm 0.24$. These values are characterized by a high correlation with the external visual color of fruit and vegetables, and can be used in studies on maturation, preservation and storage [8].

At time zero, the AA, as an individual factor, had a significant and positive effect on ΔE. These results indicated that an increase in AA concentrations resulted in an increase of ΔE. This can be observed in the positive value of the linear term in Table 3 ($P < 0.05$). At day zero, the natural fresh color of the radish was significantly affected by all treatments. Some of them yielded large differences with respect to control sample ($\Delta E > 5.8$), as in runs 3, 4, 7, 8 and 12. The lowest ΔE change was observed in run 9,

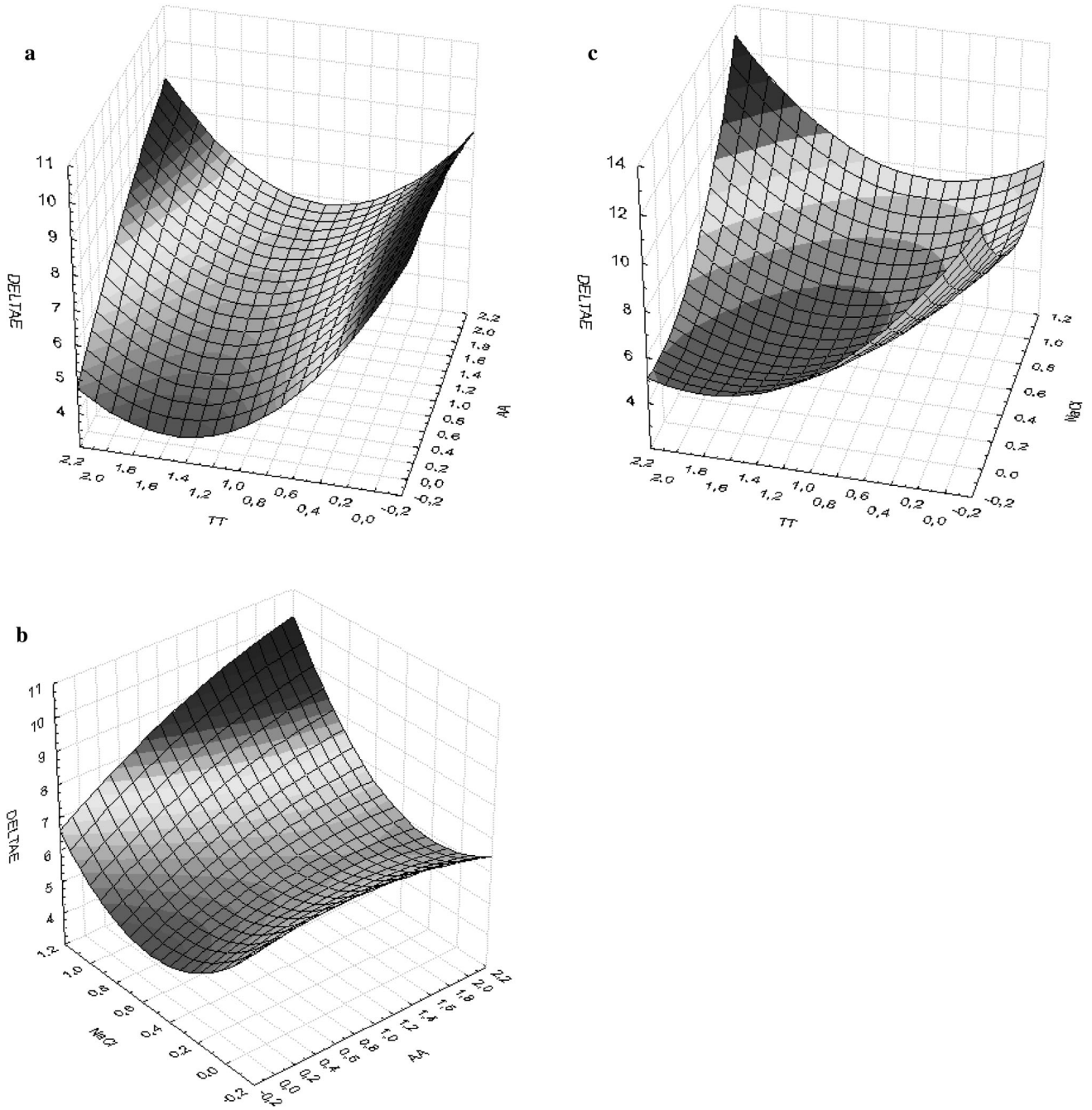

Fig. 2 Response surface plots of ΔE values at the end of storage (day four) for: **a** NaCl = 0.5 %; **b** TT = 1 min; **c** AA = 1 %

where only NaCl was applied (ΔE = 2.99), indicating a noticeable difference according to Chen and Mujumdar [15]. However, only the linear term of ascorbic acid content showed significant and positive differences with ΔE ($P < 0.05$).

At the end of storage, ΔE values of the radish slices depended significantly on the linear term of TT ($P < 0.05$), the quadratic term of TT ($P < 0.01$) and the interaction between TT and NaCl ($P < 0.05$). Based on these results, TT showed a negative effect on ΔE by means of its linear term, but a positive effect on ΔE through quadratic and interaction effect between TT and NaCl. This is in agreement with the results obtained for PPO, since increases in PPO, intensified enzymatic browning and color change, which is reflected in the increased value of ΔE. The lowest ΔE value was achieved in run 14 (ΔE = 3.82), where intermediate levels of the three hurdles were applied.

Figure 2 shows response surfaces graphics of the predicted ΔE values with two independent variables. It seems that higher immersion times presented lower values of ΔE.

Comparable results were reported for other commodities. Siddiq, Roidoung, Sogi and Dolan [10] reported that immersion at 50 °C during 1 min reduced color changes of onion slices stored for 21 days at 4 °C. Siomos, Gerasopoulos, Tsouvaltzis and Koukounaras [33] found that the appearance of a violet coloration on the spear tip was prevented by heat treatment at 55 °C for 3 min.

Sensory evaluation

Sensory evaluation of radish slices immediately after hurdles application and after 4 days of storage was done as complementary analysis. Values greater than three indicated that the parameter is unacceptable to the evaluator. Panelists also evaluated radish slices without any treatment (control samples). At the end of storage, control sample presented values for ColorM above three; this is directly related to the browning of slices, and consequently rejection by the consumer. This is confirmed by the high value of PPO_R (1.53) and ΔE (16.08) and low value of $VITC_R$ (0.87) obtained for those samples. Therefore, some of the hurdle technologies applied reverted the changes, and maintained the quality of the radish slices during storage. It can be seen that, at the end of storage and for all hurdle combinations, the attributes ColorM, ColorV and texture remained below 3, indicating that from a sensorial point of view all samples were acceptable (data not shown).

Optimization and validation of results

AA and NaCl concentration as well as immersion time, as hurdles, were optimized to obtain radish slices with minimum PPO_R and ΔE together with maximum value of $VITC_R$. For each response, second order polynomials models were used to optimize the combined objective functions by means of the desirability function. Optimum conditions were: AA = 2.0 %; NaCl = 0.0 % and TT = 1.5 min. In order to validate the model, a new set of experiments was performed based on the optimum levels. After 4 days of storage, the responses were determined. Table 4 presents the predicted values and the experimental responses of this new test. As can be observed, there is a very good agreement between the values predicted by the models and the new set of experimental values.

Table 4 Optimization for the hurdles evaluated and validation values

Response	Prediction	Range	Experimental value
PPO_R	0.218	(0.067–0.504)	0.22 ± 0.00
$VITC_R$	3.227	(2.023–4.432)	2.13 ± 0.01
ΔE	4.929	(2.438–7.419)	3.43 ± 0.06

Conclusions

Quality parameters that affect the shelf-life of minimally processed radish treated with hurdles technologies changed with storage time and levels of hurdles. Initially or immediately after the application of the hurdles, only AA affected the measured parameters (PPO_R activity, ΔE and $VITC_R$ content), but at the end of storage the other factors became significant. Therefore, the optimization was done at the end of storage by means of RSM. Based on the evaluation of the mathematical models and the application of desirability function, optimum conditions of hurdles were obtained. From this optimization assay, 2 % of ascorbic acid, 0 % of sodium chloride and 1.5 min of immersion time at 50 °C led to maximum $VITC_R$ content and to minimum PPO_R and ΔE of radishes. Moreover, the validation with a new set of experiments achieved very good results. Therefore, optimal combination of hurdles is a very good alternative to preserve radish slices as a minimally processed food, which is environmentally friendly.

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Compliance with ethical standards

Conflict of interest The authors of this work declare that there is no conflict of interest with any individuals or organizations.

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