

Shelf life of minimally processed potatoes

Part 1. Effects of high oxygen partial pressures in combination with ascorbic and citric acids on enzymatic browning

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Received 7 July 2005; accepted 26 October 2005

Abstract

The shelf life of minimally processed potatoes is limited by enzyme-catalysed browning reactions. Generally, this phenomenon is controlled by the use of chemical reagents such as ascorbic acid, citric acid, or 4-hexyl resorcinol, but it seems that “oxygen shock” treatments are also particularly effective in inhibiting enzymatic browning. The aim of this work was to study the effects of high oxygen partial pressures in combination with ascorbic and citric acid on the development of the enzymatic browning of peeled and cut potatoes (‘Primura’ variety) that were packaged in flexible pouches and stored at 5 °C for 10 days. Different treatments, chosen in according to a central composite design, were applied to the sliced potatoes. The browning development during storage was measured by a tristimulus colorimeter. Second-order polynomial models were computed for three periods of storage (3, 7 and 10 days) to relate the independent variables (oxygen partial pressure, ascorbic and citric acid concentrations) to the colour function attributes.

The effectiveness of the statistical approach offered the possibility to investigate the effects of several processing conditions involved in the enzymatic browning of minimally processed potatoes, while the response surface methodology allowed the identification of the optimum range of the independent variables which prevented browning.

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Keywords: Minimally processed potatoes; Enzymatic browning; High oxygen treatment; Ascorbic acid; Citric acid; Central composite design

1. Introduction

The shelf life of peeled and cut potatoes is strongly limited by enzymatic browning that leads to a decrease in food quality, since it implies spoilage. Enzyme-catalysed browning reactions involve the oxidation of phenolic compounds by the enzyme polyphenoloxidase (PPO) that act as catalyst in two different reactions: the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones. These *o*-quinones are highly reactive compounds that react non-enzymatically to give rise to brown, black or red pigments, called melanins, that are responsible for less attractive appearance and loss in nutritional quality (Tomas-Barberan and Espin, 2001; Cantos et al., 2002). These reactions result

in a deterioration of flavour, colour and nutritional quality, and continue after the food is harvested (Friedman, 1997).

In potato tubers, the phenolic compounds are mainly distributed in the tissues between the cortex and the peel. Indeed, about 50% of the phenolic compounds are located in the potato peel and adjoining tissues, while the remaining 50% decreases in concentration from the outside towards the centre of the tuber (Friedman, 1997). The most important factors determining the rate of enzymatic browning in fruit and vegetables are the concentrations of active PPO and phenolic compounds, pH, temperature, and oxygen availability in the tissues (Martinez and Whitaker, 1995). In the last few years, great interest has been shown in fruit or vegetables presented for sale which have been conveniently peeled, cored or sliced in prepacked containers (Reyes-Moreno et al., 2001). Minimal processing operations cause the disruption of cellular compartments, allowing the substrate and enzymes located

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in the chloroplast to come into contact (Rocha and Morais, 2001). The common way of inhibiting the enzymatic browning of peeled and sliced potatoes is to dip, or immerse, them in anti-browning agents. Among such compounds, ascorbic acid inhibits enzymatic browning very effectively, primarily because of its ability to reduce quinones to phenolic compounds before they undergo further reaction to form pigments (Iyengar and McEvil, 1992). Unfortunately, once the ascorbic acid has been completely oxidised to dehydroascorbic acid, the quinones accumulate and undergo browning. Another anti-browning agent widely used in the food industry is citric acid, which may have a dual inhibitory effect on PPO: it lowers the pH and chelates the copper at the active site of the enzyme. In fact, at pH values below 4, the looser binding of copper at the active enzyme site causes the PPO activity to decrease further, permitting the citric acid to remove the copper (Martinez and Whitaker, 1995).

Since browning is an oxidative reaction, it can be retarded by eliminating oxygen from the cut surface of the vegetables. However, this is not always feasible and browning will occur rapidly when oxygen is reintroduced. In recent years, the application of high oxygen atmospheres for packaging ready to eat vegetables has been evaluated as an alternative preservation technique. High oxygen partial pressures have been found to be particularly effective in inhibiting enzymatic discoloration, preventing anaerobic fermentation reactions and inhibiting microbial growth (Wszelaki and Mitcham, 2000; Jacxsens et al., 2001). It has been hypothesised that high oxygen levels may cause substrate inhibition of the enzyme PPO or, alternatively, that high levels of subsequently formed colourless quinines could lead to PPO feedback inhibition (Kader and Ben-Yehousha, 2000).

In a preliminary part of the work the susceptibility of the 'Primura' variety and the sensitivity of the colorimetric reflectance procedure were studied when two different and constant oxygen partial pressures were applied around the product. The aim of the storage experiment therefore, was to investigate the role of high oxygen partial pressures, combined with ascorbic and citric acid, on the browning of minimally processed potatoes in order to find the best combinations of the three variables to prevent the development of enzymatic browning in this product. Also, mathematical modelling was proposed to determine the optimum levels of oxygen partial pressure, ascorbic and citric acid in order to obtain the minimum colour changes of potatoes slices after packaging.

2. Materials and methods

2.1. Susceptibility of the 'Primura' variety to enzymatic browning

Potato (*Solanum tuberosum* L.) tubers of the cultivar 'Primura' (from Montagnana, Italy) were purchased from a local supplier and kept at 20 °C in darkness prior to process-

ing. Slices of about 6–7 cm diameter were cut and laid down in closed vessels (1 l volume) that were kept at 20 °C and at two constant oxygen partial pressures (20.9 and 100 kPa, respectively) in the presence of a source of humidity (about 100% RH). In this experiment no chemical inhibitors of enzymatic browning were used. Oxygen levels were established by a flow-through system connected to the lid of the vessels. In order to verify the real gas composition, samples of the vessel headspace were periodically extracted through a sampling port in the lid and analysed using a gas chromatograph (Hewlett Packard HP 5890 series II) equipped with a thermoconductibility detector and a steel column (2 m × 6 mm; CTR I Alltech, Milan). Tristimulus colorimetry was performed on the surface of the same slice after short time intervals (5–10–30–60–90–120–150–180 min) using a colorimeter MINOLTA Chroma-Meter mod. CR210. The instrument was standardized against a white tile ($L^* = 97.63$; $a^* = -0.68$; $b^* = 2.77$) before each measurement. The CIE parameters were expressed as ΔL^* , Δa^* and Δb^* with regard to time zero. All values were the average of ten measurements, each carried out on three different slices.

2.2. Storage experiments: effects of different treatments on enzymatic browning

2.2.1. Raw material and sample preparation

Potato tubers were kept at 5 ± 1 °C in darkness prior to processing. First the potatoes were washed in water to eliminate any surface contamination, then hand-peeled and cut with a manual cutter into slices of about 3–4 mm. The potato slices were immediately dipped into distilled water containing ascorbic acid and citric acid in concentrations chosen according to a central composite design (CCD). Levels of 0–5% (v/w) for ascorbic acid and 0–2.5% (v/w) for citric acid were used. The ratio between the solids (sliced potatoes) and the soaking solution was 1:3. During the immersion, the solution was agitated for 2 min. After each assay, the potato slices were gently dried for 1 min with a manual centrifugal machine.

2.2.2. Storage conditions

After slicing, 150 g of the potato slices were packaged in pouches (15 cm × 20 cm) of PE/PET/EVOH/LDPE (84 μm of layer; $P_{CO_2} < 0.5 \text{ cm}^3 \text{ m}^{-2} \text{ 24 h}^{-1} \text{ bar}^{-1}$, at 5 °C, 0% RH; $P_{CO_2} = 3.4 \text{ cm}^3 \text{ m}^{-2} \text{ 24 h}^{-1} \text{ bar}^{-1}$, at 5 °C, 0% RH) using an automatic packaging machine (mod. S-100 digit, Technovac, Bergamo, Italy), that introduced oxygen according to the central composite design (Table 1). The analyses were performed after 3, 7 and 10 days of storage at 5 ± 1 °C.

2.2.3. Colour measurements

The browning development was measured on the sliced surfaces with a colorimeter MINOLTA Chroma Meter mod. CR210 that, before each measurement, had been standardized against a white tile ($L^* = 97.63$, $a^* = -0.68$, $b^* = 2.77$).

Table 1
Operating conditions of shelf life experiments planned by the CCD

Experiment (run)	Coded units			Natural units		
	X ₁ (O ₂)	X ₂ (AA)	X ₃ (CA)	X ₁ O ₂ (kPa)	X ₂ AA (%)	X ₃ CA (%)
1	-1	0	0	10	2.5	1.25
2	+1	+1	+1	100	5	2.5
3	0	0	+1	55	2.5	2.5
4	0	+1	0	55	5	1.25
5	0	-1	0	55	0	1.25
6	0	0	0	55	2.5	1.25
7	-1	-1	+1	10	0	2.5
8	+1	0	0	100	2.5	1.25
9	-1	+1	+1	10	5	2.5
10	+1	-1	+1	100	0	2.5
11	0	0	-1	55	2.5	0
12	-1	-1	-1	10	0	0
13	-1	+1	-1	10	5	0
14	0	0	0	55	2.5	1.25
15	+1	-1	-1	100	0	0
16	+1	+1	-1	100	5	0
17	0	0	0	55	2.5	1.25
18	0	0	0	55	2.5	1.25

For each CCD run, colorimetric measurements were carried out at time zero on 50 slices (corresponding to 70% of the total weight used). After 3, 7 and 10 days storage, three different pouches were opened and 10 slices from each pouch were analysed. The slices were put into a 12 cm diameter Petri dish and placed on top of a black sheet to avoid stray light. The results were expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness), b^* (yellowness/blueness). From the Hunter L^* , a^* , b^* values, total colour difference (ΔE) (Eq. (1)), chroma (C) (Eq. (2)), hue angle (H , °) (Eq. (3)) were also calculated.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

$$H = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (2)$$

$$C = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

where L_0^* , a_0^* , b_0^* referred to the colour reading of fresh potatoes at time zero.

In the evaluation of H , we used the widely accepted international criterion of assigning the angle of 0° to the semiaxis $+a^*$ (redness), the angle of 90° to the semiaxis $+b^*$ (greenness), the angle of 180° to the semiaxis $-a^*$ and the angle 270° to the semiaxis $-b^*$ (blueness) (Martinez and Whitaker, 1995).

2.2.4. Experimental design and statistical analyses

Oxygen partial pressure (X_1), ascorbic acid concentration (X_2) and citric acid concentration (X_3) were modulated according to a central composite design (CCD) (Box et al., 1978). The three independent variables were studied at three levels coded as -1 (lowest level), 0 (central level), +1 (highest level).

The complete design consisted of 18 experimental trials, which included four replications of the centre point. Table 1 shows the coded and natural levels of each factor. Each of these 18 systems was evaluated in triplicate after 3, 7 and 10 days storage at 5 °C.

The response variables (colour CIE L^* , a^* , b^* parameters and their derived indexes) were estimated using the response surface model described by the following second-order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_3^2 + \beta_7 X_1 X_2 + \beta_8 X_1 X_3 + \beta_9 X_2 X_3 \quad (4)$$

where X_1 , X_2 , X_3 represent the levels of the factors according to Table 1 and β_0 – β_9 represent the coefficient estimates. The variables present in linear terms represent the coordinates of the maximum value predicted, those in quadratic terms represent the surface curvature, and the bi-factorial cross-products represent the directions of the axes of the geometric figure obtained by sectioning the surface area (Zanoni et al., 2000). In order to verify the adequacy of the fitted model, analysis of variance, test of lack of fit and Durbin–Watson statistic test were performed. The response surface methodology was used to analyse the effect of the combination of the three variables on enzymatic browning after packaging. A predictive model (Eq. (4)) was used to graphically represent the systems. The computation was performed with the aid of Statgraphics® Statistical Computer Package (Statgraphics Plus 4.0, Statistical Graphic Corp., USA).

3. Results and discussion

3.1. Susceptibility of ‘Primura’ variety to the enzymatic browning

In this preliminary part of the work, the browning susceptibility of the ‘Primura’ potato variety was verified by making quantitative measurements of the browning rate at 20 °C, for different times after slicing and under two different and constant oxygen partial pressures (20.9 and 100 kPa) applied around the product, without the use of chemical inhibitors. In this way, the oxygen effect was isolated and any discrimination between the two different oxygen partial pressures would have been evident. Moreover, the tristimulus reflectance measurements made on the sliced surface could give an indication of the sensitivity of the method to detect differences in the browning rate of differently treated potatoes.

We could not use the absolute value of the reflectance measurements to compare the browning of the samples because of potato-to-potato variability in natural pigmentation (Sapers and Miller, 1993). Therefore the differences between the final and initial values of L^* , a^* and b^* were considered.

Fig. 1 shows the colour parameters from the two oxygen treatments for ΔL^* , Δa^* and Δb^* , respectively. Tristimulus reflectance measurements made on the cut area of the sliced

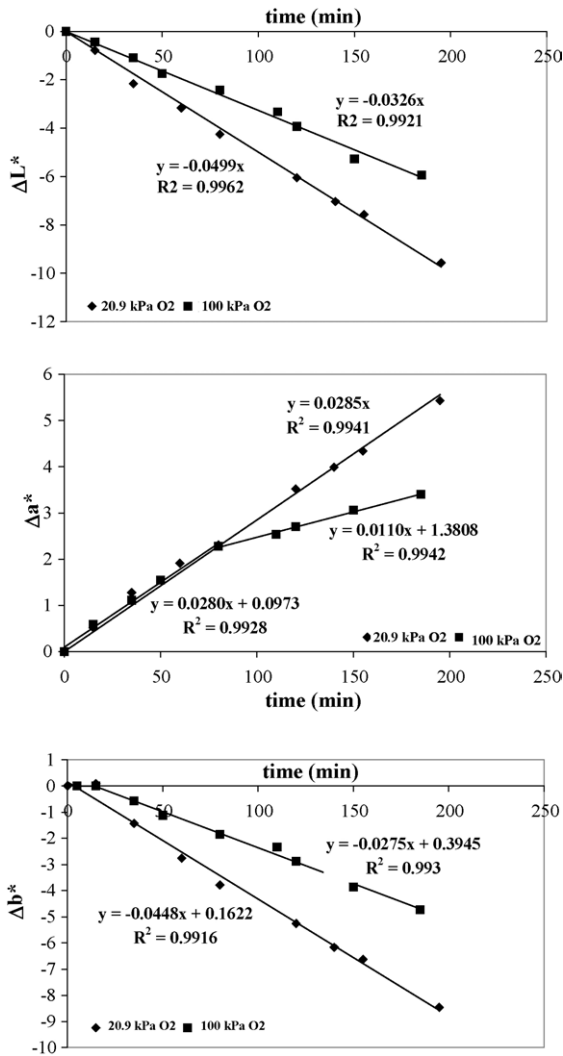


Fig. 1. Reflectance ΔL^* , Δa^* and Δb^* values at the cut surface of potatoes stored under two different and constant oxygen partial pressures.

potatoes yielded linear or bilinear values when plotted against time, and appeared to be related to the extent of browning. The samples treated at 100 and 20.9 kPa of O₂ showed a large decrease in L^* values (the samples darkened) and, at the same time, there was a marked decrease in b^* values, i.e. the samples lost the typical yellow colour (Fig. 1). Even immediately after slicing it is evident from the two parameters (ΔL^* and Δb^*) that the 100 kPa of O₂ treatment was more effective in delaying the browning reactions than the 20.9 kPa one. With regard to the Δa^* coordinate, Fig. 1 clearly shows that the rate of colour change was independent of the oxygen treatment for up to 120 min: after this time, treatment with O₂ at 100 kPa seemed to inhibit the extent of browning, and the absolute value of the slope of the straight line decreased.

The differences among the tristimulus coordinate versus time curves, especially for the highest oxygen pressure, suggest that reflectance measurement can give a profile of the complex reactions involved in the enzymatic browning of peeled potatoes.

Table 2

Comparison of the effect of the two oxygen treatments on enzymatic browning expressed as percent inhibition

	Percent inhibition		
	Calculated from ΔL^*	Calculated from Δa^*	Calculated from Δb^*
35 min	49.5 (1.3 ^a)	13.0 (0.9)	60.2 (1.5)
120 min	45.0 (1.6)	28.0 (1.4)	56.1 (1.7)
190 min	37.9 (1.2)	37.4 (1.2)	51.1 (1.8)

^a Standard deviation of ten measurements carried out on three different samples.

To demonstrate the applicability of the reflectance procedure to cut potato surfaces, and to investigate the effect of oxygen treatment when no chemical inhibitors were used, the degree of inhibition were calculated from changes in the L^* , a^* and b^* values (ΔL^* , Δa^* , Δb^*) as proposed by Sapers (Sapers and Frederic, 1987; Sapers and Miller, 1992). For example, the % inhibition (%I) at time t , expressed on ΔL^* values can be written as follows:

$$\%I = \frac{\Delta L^*_{(20.9 \text{ kPa})} - \Delta L^*_{(100 \text{ kPa})}}{\Delta L^*_{(20.9 \text{ kPa})}}$$

where, $\Delta L^* = (L^*_t - L^*_{\text{initial}})$.

Positive values of the % inhibition between 0 and 100 indicate that oxygen treatment at 100 kPa could really be an effective browning inhibitor, while negative values indicate that the oxygen treatment promoted rather than inhibited the browning.

Table 2 shows the effects on enzymatic browning of the oxygen treatments after 35, 120 and 190 min, expressed as percent inhibition. From the data it is evident that oxygen had different effects on the main components of the reflectance tristimulus colorimetry. In all cases the % inhibition was positive, showing that the high oxygen treatment actually affected the enzymatic browning extent, compared with storage at 20.9 kPa. Note that the degree of inhibition, calculated on the ΔL^* and Δb^* values after 35 min storage, was not very far from the limit of acceptability for potatoes which, in previous work, was estimated to be 50–60% (Sapers and Miller, 1992, 1993). The values recorded after 120 and 190 min tended to decrease progressively, suggesting that the “oxygen shock” effect did not guarantee the complete inhibition of PPO activity. For the % inhibition calculated on the Δa^* values, it is evident from Table 2 that the effect of oxygen on the red potato components was not very evident at the beginning of storage, but over time this effect became more noticeable.

These results clearly show that the ‘Primura’ potato variety was subject to enzymatic browning, therefore it was used in storage experiments proposed in the next section. Moreover the results confirmed the sensitivity of the colorimetric reflectance procedure in recording the extent of browning in pre-peeled potatoes stored under different and constant oxygen partial pressures.

3.2. Storage experiments: effect of different treatments on enzymatic browning

In the second part of the work, colour variation was used as a fast and simple method to describe enzymatic browning on minimally processed potatoes stored in flexible pouches; the combined effects of the initial oxygen partial pressure, the ascorbic and citric acid concentrations were investigated according to the CCD shown in Table 1.

It is obvious that the atmospheric composition changed more or less rapidly inside the pouches because of the natural respiration of the vegetable, its aerobic microflora and also the low permeability of the plastic material. So, in this work, the effects attributed to oxygen were referred to its initial partial pressure.

3.2.1. Effects of the initial oxygen partial pressure on enzymatic browning

Collectively, the results suggested that the Hunter L^* and b^* values tended to decrease, indicating a loss of colour lightness and a loss of yellow hue, while the redness-greenness coordinate (a^*) increased towards red hues, indicating a general browning (data not shown). From the tristimulus values L^* , a^* and b^* , various colour functions were evaluated for each combination of the CCD: total colour difference (ΔE), hue angle (H) and chroma (C), as defined in Section 2. These parameters are characterized by a high correlation with the external visual colour of fruit and vegetables, and can be used in studies on maturation, preservation and storage (Carreno et al., 1995; Dodds et al., 1991; Maskan, 2001). In particular, ΔE is generally used to tell the difference between two colours as indicated by the follow scale:

- $\Delta E < 0.2$: not perceptible difference
- $0.2 < \Delta E < 0.5$: very small difference

- $0.2 < \Delta E < 0.5$: small difference
- $2 < \Delta E < 3$: fairly perceptible difference
- $3 < \Delta E < 6$: perceptible difference
- $6 < \Delta E < 12$: strong difference
- $\Delta E > 12$: different colours

In this study, the absolute values of H and C were not used to compare samples suffering from browning because of potato variability in natural pigmentation at time zero. The differences in the values at each analysis time, with regard to time zero, were considered, and were indicated as ΔH and ΔC , respectively. A negative value of ΔH means that the potato surface tended to go brown, while a negative value of the chroma difference (ΔC) means that the colour at time t was less saturated than that at time zero, i.e. it was paler. For these two indices, the goal was to decrease the differences with respect to time zero, i.e. to obtain values as far as possible close to zero. Table 3 shows the experimental values for ΔE , ΔH and ΔC under the different storage conditions. For simplicity the experiments were assembled on the basis of the different oxygen treatments and, for the sake of brevity, only the results after 3 and 10 days were shown.

Duncan's multiple range test ($p < 0.05$) was conducted on the colour parameters at each analysis time to test the statistic differences among the values. The results showed that, for each colour index, some classes were recognizable among the different oxygen treatments, also during storage. Moreover an initial analysis of the data showed that few significant differences were found among the dipping treatments at low oxygen partial pressure (10 kPa), while these differences were more evident at higher oxygen partial pressures.

In particular, ΔE index increased during storage, while ΔH tended to decrease progressively and statistical differences among the oxygen treatments were evident especially after 3 and 7 days. Also ΔC values became more negative,

Table 3
Experimental values of ΔE , ΔH and ΔC after 3 and 10 days of storage at 5 °C

O ₂ treatment	Run	ΔE		ΔH (°)		ΔC	
		Storage time (days)		Storage time (days)		Storage time (days)	
		3	10	3	10	3	10
10 kPa	1	3.65 ^b	5.97 ^b	-1.29 ^a	-1.08 ^a	-2.56 ^a	-4.22 ^c
	7	1.75 ^a	5.05 ^{ab}	-0.67 ^b	-1.98 ^b	-1.16 ^a	-4.48 ^{bc}
	9	2.74 ^{ab}	3.96 ^a	0.77 ^c	-2.15 ^b	-2.56 ^a	-3.72 ^b
	12	3.12 ^b	4.03 ^a	0.51 ^c	-1.50 ^{ab}	-3.05 ^a	-3.72 ^b
	13	4.30 ^{bcd}	5.06 ^{ab}	0.33 ^{cd}	-3.35 ^c	-4.17 ^c	-2.90 ^a
55 kPa	3	8.53 ^e	8.85 ^d	-6.92 ^e	-5.00 ^d	-4.34 ^c	-7.40 ^f
	4	2.26 ^b	15.41 ^g	-2.23 ^f	-7.54 ^f	-1.63 ^a	-12.83 ^{hi}
	5	2.45 ^a	10.02 ^e	-1.29 ^a	-6.03 ^c	-2.24 ^{ab}	-8.35 ^g
	6–14	6.67 ^{de}	12.02 ^f	-3.21 ^{fg}	-6.41 ^e	-2.98 ^{ab}	-10.78 ^h
	17–18						
100 kPa	11	4.92 ^c	8.98 ^d	-6.11 ^e	-7.94 ^f	-5.08 ^c	-6.66 ^e
	2	5.23 ^c	8.37 ^d	-5.34 ^h	-6.22 ^e	-3.96 ^{bc}	-6.62 ^e
	8	4.60 ^c	5.60 ^b	-4.83 ^h	-5.15 ^d	-2.80 ^a	-4.30 ^c
	10	4.55 ^c	7.17 ^c	-0.70 ^b	-0.50 ^a	-3.23 ^{ab}	-5.99 ^d
	15	9.05 ^f	10.25 ^e	-4.06 ^h	-6.38 ^e	-6.44 ^d	-12.12 ^{hi}
	16	4.46 ^c	8.21 ^{cd}	-4.38 ^h	-5.24 ^d	-2.42 ^a	-7.53 ^f

For each analysis time, different superscripts indicate statistically different groups ($p < 0.05$).

although Duncan's test showed that it was more difficult to find statistical differences, especially between the two experiments carried out at the highest oxygen partial pressures. This should mean that the colour of the slices faded independently of the oxygen pressure used for their storage.

The minimum changes in ΔE , ΔH and ΔC values were obtained using 10 kPa of oxygen inside the pouches especially in combination with high citric acid concentrations (run 7) while, at these oxygen conditions, the ascorbic acid did not seem to influence browning significantly. Although at this oxygen value the changes were the lowest recorded, a progressive but slight browning was evident during storage. A possible explanation was that the potato tuber is a bulky organ with a relatively large capability for retaining gases including oxygen, which should be capable of contributing to partial browning without external oxygen. Therefore the balance among the respiration, the permeated oxygen and the reservoir oxygen in the tissue could have created an environment of sufficient oxygen for slight PPO activity (Kaaber et al., 2002). As Beaudry has pointed out (Beaudry, 1993, 1999), it is important to recognize that extremely low O_2 levels (as with excessively high CO_2 levels) can induce fermentation and result in the generation of off-flavours or visible tissue damage.

Generally, the highest ΔE , ΔH and ΔC values were recorded in the experiments carried out at 55 kPa of oxygen. Probably, when potatoes were packaged under 55 kPa of oxygen, the respiratory metabolism rapidly consumed the gas, reaching values near to 15–20 kPa at the end of storage. These oxygen levels correspond to the homeostatic zone where the plant material is metabolically active and able to generate sufficient energy to sustain, and perhaps accelerate, most metabolic process such as respiration and phenolic metabolism (Beaudry, 1999).

High changes in colour indices were also found when storing sliced potatoes at 100 kPa of oxygen, 0% ascorbic acid and 0% citric acid (run 15). It is interesting to note that after 7 and 10 days of storage, for ΔE and ΔC indexes, the experi-

ment carried out at 100 kPa of oxygen, 2.5% of ascorbic acid and 1.25% of citric acid (run 8) and at 100 kPa of oxygen, 0% ascorbic acid and 2.5% citric acid (run 10) gave values close to some recorded at 10 kPa. In the same way, the changes in hue of potato slices stored for 10 days at 100 kPa, 0% ascorbic acid and 2.5% citric acid (run 10) were not statistically different from some recorded at 10 kPa.

3.2.2. Synergic effects of the variables on the enzymatic browning

In order to better understand the synergistic effect of the variables and to detect the sensitivity of the different colour indexes (ΔE , ΔH and ΔC) in describing the advancing of the enzymatic browning, a statistical approach based on the response surface methodology was used. The relationships among response variables (ΔE , ΔH and ΔC) and the independent variables were evaluated and Table 4 shows the regression coefficients together with the results of the analyses of variance (ANOVA), at different levels of significance, of the second-order polynomial models. Effects with p -values higher than 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, as reported in Table 5 for both the case of all factors inclusion and that of insignificant factor exclusion. In particular, the lack of fit test was used to determine whether the constructed models were adequate to describe the observed data. The test was performed by comparing the variability of the current model residuals with the variability between observations at replicate settings of the factors. When the estimated p -value for the lack of fit is less than 0.05, there is a statistically significant lack of fit at the chosen confidence level. The adjusted R -squared statistic indicates the percentage of the variability of the optimisation parameter that is explained by the model. The Durbin–Watson statistic tested the residuals to determine if there was any significant correlation based on the order in which they occurred

Table 4
Regression coefficients and analyses of variance of the second-order polynomial models for ΔE , ΔH and ΔC

Factors	Estimated effect of ΔE			Estimated effect of ΔH			Estimated effect of ΔC		
	3 days	7 days	10 days	3 days	7 days	10 days	3 days	7 days	10 days
Intercept	2.319	2.594	1.427	0.710	-0.127	-0.559	-3.359	-2.252	-0.081
X_1	0.093***	0.202***	0.320**	-0.108***	-0.186**	-0.205***	-0.040*	-0.149**	-0.334**
X_2	NS	NS	NS	NS	-1.110**	0.077**	NS	0.318*	NS
X_3	NS	2.683*	NS	NS	NS	1.040**	4.040*	-0.375*	NS
X_1^2	NS	-0.001**	-0.003*	0.001*	0.001**	0.001***	0.001*	0.01**	0.03**
X_1X_2	-0.007**	NS	NS	-0.008*	-0.003*	-0.004*	0.006*	NS	NS
X_1X_3	NS	NS	NS	NS	NS	0.009*	NS	NS	0.032*
X_2^2	-0.421***	0.108**	0.302*	0.427***	0.254*	0.030	NS	NS	NS
X_2X_3	0.203*	0.303*	NS	NS	-0.293**	-0.207*	-0.201*	-0.278*	-0.438*
X_3^2	1.115**	-1.638**	-1.222*	-1.335**	-0.974*	NS	-1.136**	0.602*	NS

NS: not significant.

* $p \leq 0.05$.

** $p \leq 0.01$.

*** $p \leq 0.001$.

Table 5
Lack of fit test, adjusted R^2 and Durbin–Watson statistic after ANOVA

	p -Value for lack of fit test ^a		Adjusted R^2		Durbin–Watson statistic ^b	
	All factors included	Insignificant factors excluded	All factors included	Insignificant factors excluded	All factors included	Insignificant factors excluded
ΔE 3 days	0.03	0.23	29.09	46.72	1.52	1.53
ΔE 7 days	0.27	0.23	81.55	83.94	1.61	2.08
ΔE 10 days	0.06	0.11	65.85	76.00	2.12	2.07
ΔH 3 days	0.20	0.29	68.50	75.51	1.80	1.84
ΔH 7 days	0.28	0.41	70.10	73.09	2.07	2.21
ΔH 10 days	0.10	0.15	70.50	76.13	2.77	2.84
ΔC 3 days	0.39	0.37	66.20	71.10	1.28	1.31
ΔC 7 days	0.13	0.47	71.71	73.23	1.78	1.76
ΔC 10 days	0.13	0.49	61.02	66.52	2.22	2.80

^a If the estimated p -value for the lack of fit is less than 0.05 there is statistically significant lack of fit at the 95% confidence level.

^b If the Durbin–Watson value is greater than 1.4, there is not autocorrelation in the residuals.

in the data file. When the Durbin–Watson value is greater than 1.4, there is probably no serious autocorrelation in the residuals. The statistical analysis indicated that the proposed models were often adequate to describe the development of browning by the chosen colour functions after 3, 7 and 10 days of storage. It is evident from Table 5 that there was an improvement in the adjusted R^2 values after the exclusion of the statistically insignificant factors.

3.2.2.1. Total colour changes (ΔE). With regard to the ΔE index, the analysis of variance in Table 4 revealed that the statistical importance of the independent variables was not the same during storage and that the oxygen, as an individual factor, had a significant and positive effect on ΔE for all storage times. Generally, this means that increasing the initial partial pressure of oxygen inside the sealed pouch also increased ΔE . The effect on colour changes was not the same of that recorded during the experiment carried out at constant O_2 partial pressure (100 kPa) but during real storage the ΔE changes could be affected not only by the initial oxygen concentration but also by the modification of the atmosphere in the head space. Therefore the oxygen partial pressure was not constant during storage and the ΔE changes could be affected not only by the initial oxygen level but also by the gas composition inside the pouches.

At the beginning of storage (3 days), the total colour variation was also affected by the quadratic terms of ascorbic acid (X_2^2 , $p \leq 0.001$) and citric acid (X_3^2 , $p \leq 0.01$), and by the interaction between ascorbic and citric acid (X_2X_3 , $p \leq 0.05$). In particular, the negative quadratic effect of ascorbic acid indicated that ΔE increased with the increase in this parameter, but it decreased as the concentration of the above substance increased at high level. On the contrary, the positive quadratic effect of citric acid indicated that an increase of its concentration gave an increase in ΔE .

Fig. 2a plots the response surface for the interaction between oxygen and ascorbic acid (X_1X_2) after 3 days. It is evident that the increase in the initial oxygen partial pressure produced an increase in ΔE when intermediate values of ascorbic acid were used, but the highest ascorbic

acid concentrations had a positive effect on the total colour variation.

Though the model chosen explained satisfactorily the effects of variables (Table 5), the adjusted R -squared values obtained at this time were not adequate probably because of the high variability in the experimental data, as can be seen from the standard deviation values shown in Table 3. The analysis of the numerical data of L^* , a^* and b^* (data not presented) had highlighted that only L^* measurements carried out after 3 days gave large confidence intervals. This was probably due to the fact that the translucence that characterized the wet surface of the slices in particular at that storage time could have affected the L^* measurements, introducing a source of error into ΔE calculation. At the end of storage

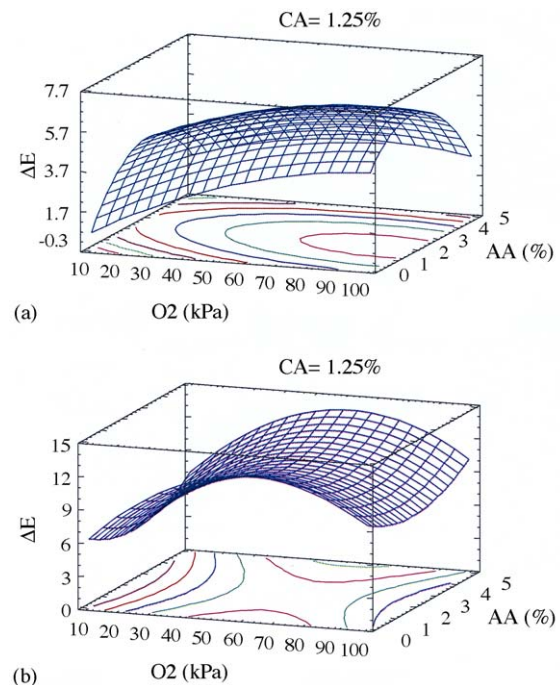


Fig. 2. Response surface for the effect of oxygen partial pressure and ascorbic acid (AA) concentration on ΔE values after 3 days (a) and 10 days (b). CA denotes citric acid.

the ΔE values of the potato slices depended significantly on the linear term of oxygen (X_1 , $p \leq 0.01$), the quadratic term of oxygen (X_1^2 , $p \leq 0.05$), and ascorbic and citric acid (X_2^2 , and X_3^2 , $p \leq 0.05$, respectively). Unlike the situation after 3 days of storage, at this time the highest concentrations of ascorbic acid helped to increase the colour changes (see the positive sign of X_2^2), while very high citric acid concentrations decreased the ΔE values (see the negative sign of X_3^2). A possible explanation is that ascorbic acid acted as antioxidant only for a short period until it was completely oxidised to dehydroascorbic acid and then at a later period it was not able to slow down the enzymatic reactions in a significant way. On the contrary, the beneficial effect of citric acid was more evident over a longer time, probably after a period of adjustment of the vegetables to the new conditions. After 10 days of storage the regression model explains, after exclusion of insignificant factors, 76% of the total variation in the values of this response variable (Table 5). The response surface as a function of oxygen and ascorbic acid, holding the citric acid concentration at the middle level (1.25%), showed a strong surface curvature due to the high significance of the quadratic terms, and presented a saddle shape (Fig. 2b): the highest ΔE values were reached at around 55 kPa of initial partial pressures of oxygen and at the highest ascorbic acid concentration. Since the optimum response for each day of storage did not fall exactly in the same region in the two-dimensional space formed by the process variables X_1 and X_2 , the superimposition of individual contour plots for 3 and 10 days results in the identification of the regions where the ΔE values would be minimum throughout the whole storage period (Fig. 3).

For 1.25% citric acid, the optimum region for the minimum response (ΔE) was very small and reduced to the lowest values of oxygen partial pressures, independently of the ascorbic acid concentration used. This was due to the large differences among the ΔE values at the beginning and at the end of storage. All the same, after 10 days it was possible to find a region corresponding to ΔE values around 6 (which was visually considered an acceptable difference) at high oxygen partial pressures and intermediate values of ascorbic acid.

3.2.2.2. Hue changes (ΔH). The significance of the effects of this response (which was not the same during storage)

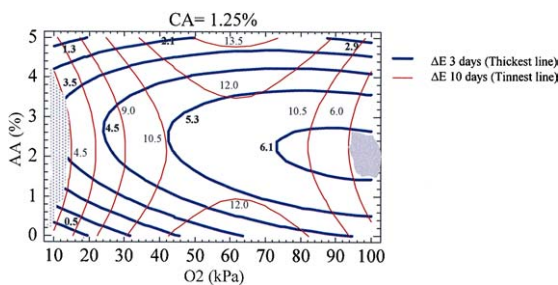


Fig. 3. Overlay plot of ΔE after 3 days (thickest lines) and 10 days (thinnest lines). AA denotes ascorbic acid and CA denotes citric acid.

showed that the ΔH values depended on the initial oxygen partial pressure (X_1 , $p \leq 0.001$) at each storage time (Table 4). During storage, the effect of ascorbic acid as an individual term became significant (X_2 , $p \leq 0.01$), while it was only after 10 days of storage that the ΔH values of the potato slices depended significantly also on citric acid concentration (X_3 , $p \leq 0.01$).

For this index, the oxygen linear term (X_1) displayed a negative effect on ΔH , probably because high partial pressures of this gas reduced the effects of enzymatic browning on the a^* or b^* components of colour. It was possible to note an inverse relationship between oxygen partial pressure and ascorbic acid concentration: in fact, the sign of the interaction factor X_1X_2 was negative. In this case, in order to obtain the same ΔH value the oxygen partial pressures should be increased, and the ascorbic acid concentration decreased. Probably, increasing the oxygen partial pressures leads to a more rapid oxidation of the ascorbic acid to dehydroascorbic acid, and once oxidation is completed the quinones can accumulate and undergo browning. This situation was also confirmed by the positive sign of the X_2 factor quadratic term; in fact, an increase in the concentration of ascorbic acid gave an increase in the absolute values of ΔH .

The response surfaces for ΔH were represented as a function of oxygen partial pressure (X_1) and ascorbic acid concentration (X_2) maintaining the citric acid concentration (X_3) at the maximum level (2.5%), since this variable had the least influence on the hue changes. Fig. 4a and b (in blue on web) illustrates the response surfaces generated after a storage period of 3 and 10 days, respectively. As can be

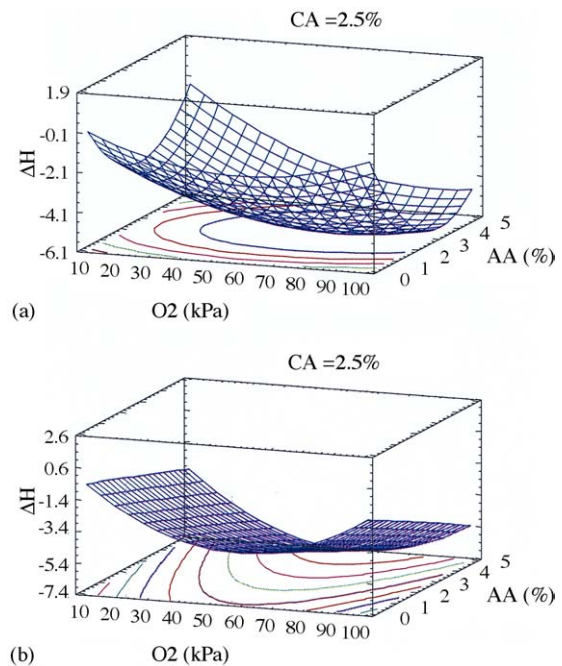


Fig. 4. Response surface for the effect of oxygen partial pressure and ascorbic acid (AA) concentration on ΔH values after 3 days (a) and 10 days (b). CA denotes citric acid.

seen from Fig. 4a, a combination of intermediate values of oxygen (around 55 kPa) and intermediate concentrations of ascorbic acid (2.5%) resulted in the highest changes of hue values (i.e. very negative ΔH), which tended to move towards brown hues, independently of the citric acid concentration. At the highest values of oxygen partial pressure (100 kPa) the hue changes tended to decrease when only citric acid was used, or when the dipping solution contained ascorbic acid at 5% and the citric acid level was not below 1.25%. After 10 days of storage (Fig. 4b) the hue changes were more pronounced and the effect of ascorbic acid on enzymatic browning was not evident as at 3 days of storage; on the contrary oxygen partial pressure played an important role in the enzymatic browning process. Adding the acidulant agent (citric acid) to the dipping solution, which maintained the superficial pH below 4 (data not reported), the browning reactions declined, especially if ascorbic acid was used at low concentrations.

The superimposition of the individual contour plots for 3 and 10 days of storage is shown in Fig. 5. It represents the identification of the region in which the ΔH values would be around close to zero (indicating the minimum hue modification with respect to time zero). These values were achieved at 10 and 90–100 kPa of initial oxygen partial pressure, 0% of ascorbic acid and around 2% of citric acid after both 3 and 10 days of storage. It is well to note that the oxygen inside the package tended to decrease during storage because of the aerobic respiration of the vegetables: in these conditions, the gas chromatographic analyses revealed that at the end of storage the residual oxygen was about 2–4 kPa when the initial partial pressure was equal to 10 kPa. At these oxygen levels the enzymatic browning was more influenced by the residual oxygen than the dipping treatment, while the use of citric acid alone seemed to be enough for a significant inhibitory effect on PPO when very high oxygen partial pressures were used.

3.2.2.3. Chroma changes (ΔC). After 3 days the chroma changes depended significantly on oxygen partial pressure (X_1 , $p \leq 0.05$), citric acid (X_3 , $p \leq 0.05$), quadratic term of oxygen (X_1^2 , $p \leq 0.05$) and citric acid (X_3^2 , $p \leq 0.01$). Interactive effects between oxygen and ascorbic acid

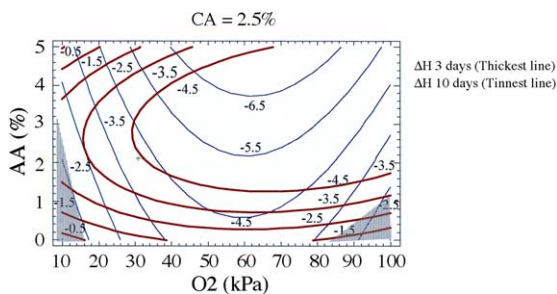


Fig. 5. Overlay plot of ΔH after 3 days (thickest lines) and 10 days (thinnest lines). AA denotes ascorbic acid and CA denotes citric acid.

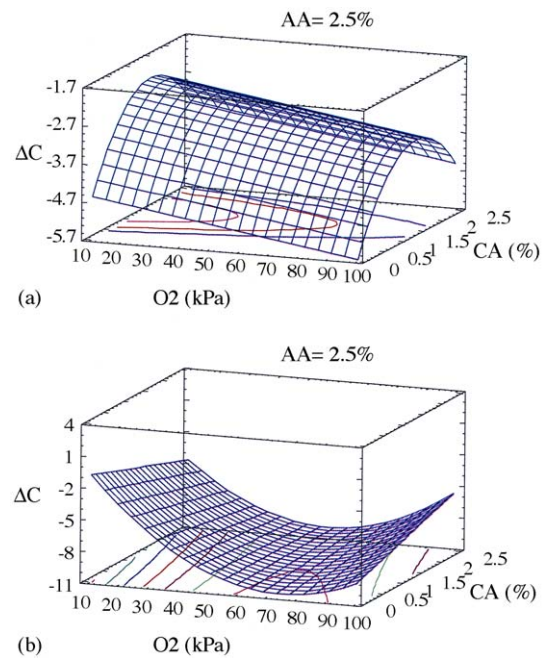


Fig. 6. Response surface for the effect of oxygen partial pressure and citric acid (CA) concentration on ΔC values after 3 days (a) and 10 days (b). AA denotes ascorbic acid.

(X_1X_2 , $p \leq 0.05$) and between ascorbic and citric acid (X_2X_3 , $p \leq 0.05$) also significantly influenced the chroma changes.

The response surfaces after 3 and 10 days of storage were represented as a function of the initial oxygen partial pressure and citric acid, keeping the ascorbic acid concentration at a specified level (2.5%) since this variable had the least influence on the chroma changes (Fig. 6).

At the beginning of storage the regression model explained 71% of the total variation. The lowest ΔC values were recorded when the initial oxygen partial pressure was around 10 kPa, and an intermediate citric acid concentration (around 1.25%) together with ascorbic acid concentration not higher than 2.5% were used (data not shown).

According to Fig. 6a, an increase in the initial oxygen partial pressure produced a decrease in ΔC values especially for all the CA concentrations. It was interesting to note that small chroma index changes were obtained when very high oxygen partial pressures were used and the dipping solution was composed by ascorbic acid at 5% and citric acid at 1.25%.

After 10 days of storage the regression model explained 67% of the total variation (Table 5). The response surface plot was very different from that obtained after 3 days and only few variables were significant. At this time, modifications of the chroma index were more pronounced and, according to Fig. 6b, the ΔC values were maximal when the initial oxygen pressure was about 55 kPa and the citric acid concentration was 1.25% and the ascorbic acid was around 5%.

The overlay plot (Fig. 7) shows that the optimum region for low variations in ΔC values was found both at low oxygen partial pressure with a soaking solution of 1–1.25% of CA

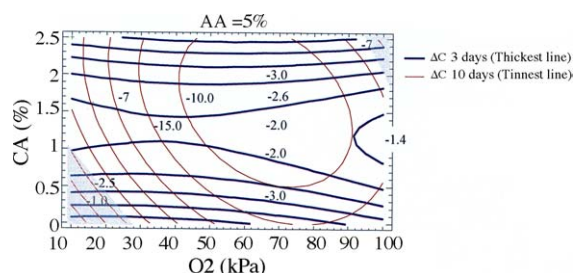


Fig. 7. Overlay plot of ΔC after 3 days (thickest lines) and 10 days (thinnest lines). AA denotes ascorbic acid and CA denotes citric acid.

and at the highest oxygen levels when high concentrations of the dipping agents were used.

4. Conclusions

The results of the preliminary part of the work suggested that enzymatic browning of 'Primura' potatoes was influenced by the oxygen partial pressure. The slices became brown due to the accelerated conditions of storage (20 °C and no chemical inhibitors), although it was evident that browning could be reduced by maintaining a high and constant oxygen partial pressure around the product. Moreover the same experiment confirmed the sensitivity of the colorimetric reflectance procedure in recording the extent of browning in pre-peeled potatoes stored under different and constant oxygen partial pressures.

The storage experiments shown in the second part of the work highlighted that the protection requirements of minimally processed potatoes changed with time, so it was difficult to identify a single optimum combination of the variables that ensured a tolerable browning development. Nevertheless the effectiveness of the statistical approach offered the possibility to investigate the effects of several processing conditions involved in enzymatic browning, while the response surface methodology allowed detection of the optimum range of the independent variables which prevented the same phenomenon. The colours indices chosen (i.e. ΔE , ΔH and ΔC) were able to describe the enzymatic browning development giving useful information with different degrees of sensitivity. The initial oxygen level inside the pouches was the most important factor that affected enzymatic browning. The minimum changes in ΔE , ΔH and ΔC values were obtained using 10 kPa of oxygen inside the pouches especially in combination with high citric acid concentrations while, at these oxygen conditions, ascorbic acid did not seem to influence browning significantly. The other oxygen partial pressures tested in this study (55 and 100 kPa) gave different results in terms of inhibition of browning. The worst results were recorded at 55 and 100 kPa of oxygen when no ascorbic and citric acid were used.

When potatoes were stored in the presence of high oxygen partial pressures, the ascorbic acid concentration was a critical variable and its concentration should be correctly

modulated. In fact, during storage the enzymatic browning was reduced if this agent was not used or it was used at very high concentrations. On the contrary, the use of citric acid alone seemed to be enough for a significant inhibitory effect on PPO when very high oxygen partial pressures were used. It was interesting to note that some experiments carried out at 100 kPa oxygen gave results not statistically different from those at 10 kPa. In particular, after 10 days, low changes in ΔE and ΔC values were recorded in potato slices stored at 100 kPa of O₂, 2.5% ascorbic acid and 1.25% citric acid, while low changes in ΔH values were recorded at 100 kPa of O₂, 0% ascorbic acid and 2.5% citric acid and at 5% ascorbic acid and 1.25% citric acid.

In conclusion, the treatments with high oxygen partial pressures have shown some positive effects on enzymatic browning only if the initial atmosphere was close to 100 kPa and the dipping acid concentrations were chosen with care. As others have shown (Wszelaki and Mitcham, 2000), near 100 kPa O₂ atmospheres could be difficult to maintain either in a package or on a larger scale, as well as being dangerous due to flammability. Nevertheless, because of the limited published information on the effects of elevated O₂ levels of minimally processed vegetables, it could be useful to investigate this further. For this reason, in the second part of this work the results of the same treatments on some quality traits of minimally processed potatoes will be presented.

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