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Minimally processed potatoes Part 2. Effects of high oxygen partial pressures in combination with ascorbic and citric acid on loss of some quality traits

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Abstract

Sliced potatoes were stored in flexible packaging under different oxygen partial pressures (10, 55 and 100 kPa) after dipping treatments with ascorbic and citric acid at different concentrations. The levels of oxygen, ascorbic and citric acid were modulated according to a Central Composite Design. The response surface methodology allowed an assessment of the effects of these variables and their interactions on the respiration rate in a closed system, on the carbon dioxide accumulation rate and the volatile metabolites production inside flexible pouches. The results showed that the respiration rate did not increase in direct linear proportion to the oxygen partial pressure and there was no significant difference in respiration between 55 and 100 kPa, even though the respiration rate increased with the increase in ascorbic acid concentration. However, at the highest level of ascorbic acid tested (5%), the respiration rate decreased. During storage in a high barrier plastic pouch, a higher CO_2 accumulation rate was generally observed under 55 kPa than under 10 and 100 kPa. High oxygen partial pressures (55 and 100 kPa) did not stop the production of hexanal but they had an inhibitory effect on the anaerobic volatiles production. © 2006 Elsevier B.V. All rights reserved.

Keywords: Minimally processed potatoes; High oxygen packaging; Ascorbic acid; Citric acid; Central Composite Design; Respiration rate; Volatile compounds; Flexible packaging

1. Introduction

The market of ready-to-use and ready-to-eat fruit and vegetables has increased in the last years as a response to the demand for quality and the modern way of life of consumers (Cantos et al., 2002).

The shelf life of those foods is strongly limited by different factors: browning of peeled and cut surfaces, more intense respiration, microbial proliferation, control of temperature during storage and the right choice of the packaging solution.

Generally, pre-treatments are used to reduce the extent of enzymatic browning. In fact, minimal processing operations cause the disruption of cellular compartments, allowing the substrate and enzymes located in the chloroplast to come into contact (Rocha and Morais, 2001). The most common way of inhibiting enzymatic browning of peeled and sliced potatoes is to dip or immerse them in anti-browning agent solutions containing, for example, ascorbic and citric acid. The first inhibits enzymatic browning very effectively, because of its ability to reduce quinones to phenolic compounds before they undergo further reaction to form pigments (Iyengar and McEvil, 1992), while the second lowers the pH and chelates the copper at the active site of the enzyme (Martinez and Whitaker, 1995).

Modified atmospheres and vacuum packaging are largely used in order to extend the quality storage life of a wide range of cut fruit and vegetables. Specific levels of CO_2 and O_2 may reduce the rates of metabolic reactions and consequently inhibit respiration rate, ripening, microbial growth and ethylene production. Different studies have given indications of recommended O_2 and CO_2 combinations for the

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storage of fruit and vegetables in relation to the packaging material (Cameron et al., 1995; Beaudry, 1999). Usually, O_2 and CO_2 partial pressures under 21 kPa are used but atmospheres necessary for decay control are often close to the tolerance limits. In recent years, the application of high O_2 atmospheres has been suggested as an improvement over the traditional treatments consisting of elevated CO_2 and/or reduced O_2 (Wszelaki and Mitcham, 2000).

High O₂ partial pressures seem to be particularly effective in inhibiting enzymatic discoloration, preventing anaerobic fermentation reactions and inhibiting microbial growth (Wszelaki and Mitcham, 2000; Jacxsens et al., 2001). With regard to the effect on enzymatic browning, it has been hypothesised that high O₂ levels may cause substrate inhibition of the enzyme PPO or, alternatively, that high levels of subsequently formed colourless quinones could lead to PPO feedback inhibition (Jacxsens et al., 2001). Recently, the synergic effects of high O₂ atmospheres and dipping treatments with ascorbic and citric acid on the enzymatic browning of minimally processed potatoes have been investigated. Limbo and Piergiovanni (2006) showed that modified atmospheres containing 55 and 100 kPa of O2 had different effects on enzymatic browning. Colour changes were more evident on slices packaged with 55 kPa of O2, while some positive effects were highlighted at 100 kPa, especially if the composition of the dipping solution was accurately modulated.

The hypotheses about the reduction of respiration rate at high O_2 levels are different: Barker and Mapson (cited in Kader and Ben-Yehousha, 2000) attributed it to a marked inhibition of CO_2 production as a block in the TCA cycle between citrate and α -ketoglutarate. At super-atmospheric concentrations, O_2 could enhance the production of reactive oxygen species that damage the cytoplasm and inhibit various metabolic activities, leading to deterioration of produce quality. Purvis and Shewfelt (1993) suggested an alternative oxidase pathway that provides a means for oxidation of substrates in stressed plant tissue, when exposed to high O_2 levels, without excessive production of reactive oxygen species. This could result in a reduction of physiological damage and, consequently, in a decreased respiration rate.

The lack of understanding of the biological mechanisms involved in minimally processed vegetables stored at high O_2 levels means that knowledge of the effects of these atmospheres on respiratory activity is necessary, especially when the proper design of packaging and the product storage conditions have to be planned (Jacxsens, L. et al., 2001).

At the same time, it would be of great interest to study the effects of high O_2 atmospheres on production of volatile compounds. After cell disruption, enzymatic and oxidative attack on inherent lipids results in a vast array of unsaturated and saturated aldehydes and alcohols, some of which have extremely low odour thresholds and thus have a possible negative impact on vegetable flavour (Mazza and Pietrzak, 1990; Maga, 1994; Petersen et al., 1998). In fruit and vegetables, it could be that elevated O_2 atmospheres affect the synthesis and accumulation of some volatile compounds associated with fermentative metabolism, such as acetaldehyde, ethanol and ethyl acetate, and could also interfere with some oxidative reactions of the aroma biosynthesis pathway (Whitaker et al., 1998; Perez and Sanz, 2001). A possible explanation is that even under high O_2 the cell is stressed and anaerobic metabolite production may be a stress response (Pesis, 2005).

In the first part of the work, the effects of high O_2 partial pressures, combined with ascorbic and citric acid, on colour changes of sliced potatoes, were studied (Limbo and Piergiovanni, 2006). In the present work, the attention was focused on the changes in respiration rates and in the accumulation of volatile compounds when the same acid solutions and O_2 partial pressures were used.

2. Materials and methods

2.1. Raw materials and sample preparation

Potato (*Solanum tuberosum* L.) tubers of the cultivar Primura (from Montagnana, Italy) were purchased from a local supplier and 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 (AA) and citric acid (CA) 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 treatment solution was 1:3. During immersion, the solution was agitated for 2 min. After each dipping treatment, the potato slices were gently dried for 1 min with a manual centrifugal machine.

2.2. Storage conditions

After preparation, 150 g of the potato slices were packaged in pouches (15 cm × 20 cm) of PE/PET/EVOH/LDPE (84 μ m of thickness, oxygen permeability coefficient <0.5 cm³ m⁻² 24 h⁻¹ bar⁻¹, at 5 °C, 0% RH) using an automatic packaging machine (mod. S-100 digit, Technovac, Bergamo, Italy), that established O₂ partial pressures according to the Central Composite Design (Table 1). The analyses regarding the CO₂ accumulation rate and volatile metabolites production were performed after 3, 7 and 10 days of storage at 5 ± 1 °C.

2.3. Respiration rate

Apparent respiration rate of the sliced potatoes was measured for each combination of the Central Composite Design using the closed system method as reported by Kang and Lee (1997). The measurements were carried out in triplicate at $5 \,^{\circ}$ C.

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

Experiment (run)	Coded units			Natural units			
	$\overline{X_1(O_2)}$	X_2 (AA)	<i>X</i> ₃ (CA)	$\overline{X_1 \text{ O}_2 (\text{kPa})}$	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	

The potato tissue and the jars were equilibrated for 1 h at 5 °C. Samples of about 500 g were then placed in air in 1.5 L glass jars which were flushed with gas mixtures of different O_2 partial pressures (10, 55 and 100 kPa) and then were tightly covered with metal caps equipped with silicone sampling ports. The jars, in triplicate, containing the samples were immediately stored at 5 °C. Head space gas was periodically sampled to analyze O_2 and CO_2 partial pressures by a gas chromatograph (Hewlett-Packard HP 5890 series II) equipped with a thermoconductivity detector and a steel column (2 m × 6 mm. CTR I Alltech, Milano), until the CO_2 level inside the jars reached 5%.

Respiration rate was calculated from the linear regression of O_2 partial pressures measured during the time of experiment. The slope of the regression line was multiplied by the free volume and then divided by sample weight to obtain respiration in mg kg⁻¹ h⁻¹. The free volume inside each jar was measured by filling it with water. After each determination, the jar was opened and stored at the required temperature for 2 days and re-used for respiration measurements.

2.4. Measurement of the CO_2 accumulation rate

A small quantity (40 μ L) of the atmosphere inside the pouches was sampled at regular intervals (0, 3, 7 and 10 days), withdrawn using a gas tight syringe through a septum glued onto the package surface, and analysed using a gas chromatograph (Hewlett-Packard HP 5890 series II) equipped with a thermoconductivity detector and a steel column (2 m × 6 mm, CTR I Alltech, Milano). The CO₂ accumulation rate expressed as mg CO₂ pack⁻¹ day⁻¹ was calculated from a linear regression of CO₂ volume increasing during the storage. All the data were the averaged values of triplicate samples.

2.5. Analysis of volatile compounds

For each storage time, 1 g of finely cut potato was put into a 22.3 mL glass vial, closed with a silicon/Teflon septum and aluminium crimp top, and analysed using a static head space analyser (HS40 Perkin-Elmer) connected to a gas chromatograph (GC) (Hewlett & Packard 5890), with the following conditions: thermostatting time 15 min, thermostatting temperature 90 °C, injection time of 0.1 min after 3 min of pressurisation with helium; the GC column was a Innowax 20 M, 25 m × 0.53 mm, 1 μ m film thickness; the GC temperature programme was: 50 °C for 5 min, after that 20 °C/min to 200 °C; the FID detector was held at 280 °C. The carrier gas flow rate (helium) was 2.2 mL/min.

The quantitative determination of some volatile compounds was achieved using the standard addition method, as proposed by Kolb and Ettre (1997), and was carried out on an identical matrix: analysis of the original sample was followed by the analysis of the same sample to which known amounts of analytes had been added. All measurements were carried out in triplicate under identical conditions.

2.6. 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) and +1(highest level).

The complete design consisted of 18 experimental trials, which included 4 replications of the centre point (Table 1). Each of these 18 systems was evaluated in triplicate after 3, 7 and 10 days storage at 5 °C. Only results after 3 and 10 days

have been presented for the sake of brevity. Table 1 shows the coded and natural levels of each factor.

The response variables (respiration rate, CO_2 accumulation rate and volatile metabolites production) were estimated using the response surface model described by the following second order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_3^2 + \beta_7 X_1 X_2 + \beta_8 X_1 X_3 + \beta_9 X_2 X_3$$
(1)

where X_1 – X_3 represent the levels of the factors according to Table 1 and β_0 – β_9 represent the coefficient estimated. The variables in linear terms represent the co-ordinates 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 (Box et al., 1978).

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.

A statistic model (Eq. (1)) was used to graphically represent the systems. Response surfaces and contour plots for each of the response variables were obtained to analyse the effect of the combination of the three variables on physiological changes of minimally processed potatoes. The computation was performed with the aid of Statgraphics[®] Statistical Computer Package (Statgraphics Plus 4.0, Statistical Graphic Corporation, USA).

3. Results and discussion

3.1. Respiration rate and CO₂ accumulation rate during storage

Respiration processes take place in the mitochondria and O_2 reaches the mitochondria by passing through the skin,

Table 2 Respiration rates and CO₂ accumulation rates of the experimental runs

intracellular spaces and membranes. Obviously, peeling and cutting operations rise the respiration rate because of removal of skin, reduction in gas diffusion path in tissues and increase permeability of membranes. Moreover, the degradation of the membranes lipids leads to production of free fatty acids and the oxidation of these compounds results in an increase in CO₂ production (Brecht, 1995). During the respiration of a MAP commodity, concentration gradients are established between the gases in the tissues and those in the micro-atmosphere surrounding them, and between the microatmosphere gases and the outside air. The complexity of the gas exchanges suggests that the effects of the applied atmosphere on the respiration rate and, in general, on the metabolism of the vegetable, should be investigated. In this study, the respiration rate of sliced potatoes under the conditions described in the CCD was measured in a closed, not permeable system, in order to better understand the influence of O₂ and dipping solution.

During these experiments, the effect of CO_2 on respiration rate was not considered and the analyses were stopped as soon as 5% CO_2 was found inside the jars. This concentration of CO_2 is expected not to have an inhibitory effect on respiration rate (Jacxsens et al., 2000).

Table 2 shows the respiration rates of the sliced potatoes treated according to the CCD. It is evident that the surrounding atmosphere affected the respiration rate. Increasing O_2 from 10 to 55 kPa resulted in about a two to three-fold increase in respiration rate but few differences were found among the slices packaged with 55 and 100 kPa, suggesting that the increase in respiration rate with O_2 did not follow a simple linear proportionality.

It is interesting to note that at each O_2 partial pressure, the slices treated with 5% ascorbic acid gave the lowest respiration rate values. Probably high concentrations of ascorbic acid not only reduce the activity of PPO, but enzymes of the oxidative phosphorylation pathway are also affected and, consequently, the respiration rate is reduced.

Oxygen treatment	Treatment solutions	$RR_{O_2} \ (mg kg^{-1} h^{-1})$	CO_2 accumulation rate (mg kg ⁻¹ day ⁻¹)
10 kPa	0% AA and 0% CA	14.10 ^a	29.11 ^a
	0% AA and 2.5% CA	14.20 ^a	31.12 ^a
	2.5% AA and 1.25% CA	14.90 ^a	29.18 ^a
	5% AA and 0% CA	10.32 ^b	24.71 ^b
	5% AA and 2.5% CA	10.11 ^b	17.23 ^b
55 kPa	0% AA and 1.25% CA	34.12 ^h	97.42 ^e
	2.5% AA and 0% CA	29.40 ^{gh}	$109.40^{\rm f}$
	2.5% AA and 1.25% CA	46.20 ^d	125.42 ^c
	2.5% AA and 2.5% CA	21.30 ^e	100.46 ^e
	5.0% AA and 1.25% CA	27.80 ^g	82.25 ^d
100 kPa	0% AA and 0% CA	62.60 ^c	58.32 ^g
	0% AA and 2.5% CA	45.20 ⁱ	86.54 ^d
	2.5% AA and 1.25% CA	35.80 ^h	85.64 ^d
	5% AA and 0% CA	26.30 ^g	87.5 ^d
	5% AA and 2.5% CA	19.22 ^e	42.31 ^g

For each analysis time, different superscripted letters (a-i) indicate statistically different groups (p < 0.05) AA, ascorbic acid; CA, citric acid.

Table 3 Regression coefficients and analyses of variance of the second order polynomial models for the respiration rate and the CO₂ accumulation rate

Factors or interactions	Estimated effect of RR_{O_2} (mg kg ⁻¹ h ⁻¹)	Estimated effect of CO_2 accumulation rate (mg pack ⁻¹ day ⁻¹)		
Average	5.461	-13.641		
X_1	0.842^{***}	3.390***		
X_2	0.401^{***}	18.269*		
X_3	9.600^{*}	15.382		
X_{1}^{2}	-0.003^{**}	-0.026^{***}		
X_1X_2	-0.060^{***}	NS		
X_1X_3	NS	NS		
X_2^2	-0.127^{**}	-3.24**		
$X_2 X_3$	NS	-3.356^{*}		
X_{3}^{2}	-4.091^{*}	-3.232		
Adj-R ²	75.52	83.22		
<i>p</i> -lack of fit	0.09	0.17		
Durbin-Watson test	2.21	1.44		

*** $p \le 0.001$; ** $p \le 0.01$; $p \le 0.05$; NS, not significant.

The adequacy of the fitted model was verified by calculating the lack of fit test, the adjusted *R*-square, and by carrying out the Durbin–Watson statistic test, as reported in Table 3. The regression model explained 75.52% of the total variation in the values of this response variable, and the lack of fit test indicated that the proposed model was adequate to describe the respiration rate as a function of the selected variables.

The statistical analysis shown in Table 3 highlighted that the respiration rate at 5 °C was strongly influenced by the initial O₂ partial pressure (X_1 , $p \le 0.001$), the ascorbic acid concentration (X_2 , $p \le 0.001$) and by the interaction between O₂ and ascorbic acid (X_1X_2 , $p \le 0.001$).

The negative quadratic effect of O_2 and ascorbic acid terms (X_1^2 and X_2^2 , respectively) indicated that the respiration rate increased with the increase in these parameters, but it decreased as the concentration of the above substances increased at the highest level.

This behaviour is described in Fig. 1 where the interaction between O_2 and ascorbic acid was shown, while keeping citric acid at the intermediate level.

During storage the gas concentration inside a package containing a vegetable product changes more or less rapidly because of its natural respiration and also the permeability



Fig. 1. Response plot of the respiration rate in the closed system as function of O_2 and ascorbic acid.



Fig. 2. CO_2 accumulation rate at 10, 55 and 100 kPa after a dipping treatment with 2.5% ascorbic acid and 1.25% citric acid.

of the plastic material; as a consequence the CO₂ produced could influence the respiration rate of the vegetable. Carbon dioxide levels ranging over about 20% or more can affect respiration, because they could induce anaerobic respiration. Nevertheless the inhibitory effect of CO₂ on respiration is not clear since high concentrations of this gas (larger than 60%) produce, in some vegetable products, a pronounced fall in respiration, and also an increase in other ones (Riquelme et al., 1994; Gorny et al., 2002). For this reason, in this experiment, the change in CO₂ inside the plastic pouches was measured and the CO₂ accumulation rates during storage (10 days at 5 °C) were calculated from a linear regression of CO₂ increasing during storage, and expressed as mg CO₂ kg⁻¹ day⁻¹. As an example, the accumulation of CO₂ in the pouches under the three different O₂ partial pressures is shown in Fig. 2.

It is important to underline that the effects attributed to O₂ referred to its initial partial pressure.

The CO₂ accumulation rates inside the packages recorded during 10 days storage are shown in Table 2. With respect to the data collected in the closed system, some statistical differences (p < 0.05) were found among samples stored at 55 and 100 kPa. When potatoes were packaged under 55 kPa of O₂, the CO₂ accumulation rate was higher than in the other tested conditions, but the inhibitory effects of CO2 were not so evident with regard to colour changes as previously reported (Limbo and Piergiovanni, 2006). Probably, in this condition, the O₂ concentration was sufficient to sustain, and perhaps accelerate, respiratory metabolism. Packaging the potatoes in a modified atmosphere of 100 kPa O₂, resulted in a CO₂ accumulation rate lower than that at 55 kPa initial O₂. In particular, when ascorbic and citric acids were used at their highest levels (5 and 2.5%, respectively), the CO₂ accumulation rate was reduced three fold with respect to what happened at the central point of the experimental design. A similar result was found for respiration rate measurements carried out in the closed system.

The lowest values of the response variable were recorded using 10 kPa of O_2 : in these conditions, the potato metabolism changed from aerobic to anaerobic. When the potato slices were packaged under low O_2 partial pressure (i.e. 10 kPa),



Fig. 3. Response plot of the CO_2 accumulation rate inside flexible pouches as a function of O_2 and ascorbic acid.

 O_2 reached values of 3–4 kPa after a few days of storage. At these O_2 values, off-flavours could accumulate and also fermentation might be induced, which is the only mechanism left to the plant material to generate energy (Zagory and Kader, 1988; Beaudry, 1999; Kaaber et al., 2002).

Analysis of the data showed that the rate of CO₂ accumulation at 5 °C was strongly influenced by the initial O₂ partial pressure (X_1 , $p \le 0.001$) and by ascorbic acid concentration (Table 3). In fact its quadratic term affected the response variable (X_2^2 , $p \le 0.01$) in a negative way, meaning that an high level of ascorbic acid decreased the CO₂ accumulation rate.

The regression model explained 83.22% of the total variation in the values of this response variable, and the lack of fit test indicated that the proposed model was adequate to describe the CO_2 accumulation rate as a function of the selected variables.

Fig. 3, showing the interaction between O_2 and ascorbic acid, confirmed that the rates were maximal when the O_2 par-

tial pressure was about 55 kPa and when ascorbic and citric acid concentrations were taken at their central levels (2.5 and 1.25%, respectively). It is important to note that at the highest O_2 partial pressures, the CO_2 accumulation rate decreased progressively.

At the end of storage the CO_2 concentrations reached high values, but only at 10 kPa O_2 , were undesirable anaerobic conditions ($O_2 < 2$ kPa) created. Nevertheless, between the two super-atmospheric O_2 concentrations tested, only the packaging at 100 kPa O_2 combined with solutions containing ascorbic acid and citric acid at their highest concentrations, could act in a positive way, reducing the respiratory metabolism of the slices.

3.2. Analysis of volatile compounds

In the present study, the effects of high O_2 partial pressures combined with pre-treatments on some constituents of potato aroma were evaluated, quantifying acetaldehyde, ethanol and hexanal as the major indexes of potato off-flavours.

Maga (1994) reported that acetaldehyde is one of the natural aroma components of the volatile fraction of raw and fresh-cut potatoes. In almost every fruit and also in some vegetables it accumulates during ripening, even under aerobic conditions but to a much greater extent under partially or totally anaerobic conditions (Pesis, 2005). Acetaldehyde is formed from pyruvate by the enzyme pyruvate decarboxylase and the two immediate products formed from acetaldehyde are ethanol and acetyl coenzyme A.

Mazza and Pietrzak (1990) and Petersen et al. (1998) found that one of the major components of the headspace concentrates of raw shredded potatoes was hexanal as a

 Table 4

 Volatiles compounds production for the experimental runs

O ₂ treatment	Treatment solutions ^a	$\frac{\text{Acetaldehyde } (\mu g g^{-1})^b}{\text{Storage time } (\text{days})}$		Ethanol $(\mu g g^{-1})^b$ Storage time (days)		$\frac{\text{Hexanal } (\mu g g^{-1})^{b}}{\text{Storage time (days)}}$	
		3	10	3	10	3	10
10 kPa	0% AA and 0% CA	0.78h	0.95f	1.25g	2.32g	0.15a	0.15d
	0% AA and 2.5% CA	0.55g	0.75e	1.12g	2.75g	0.18a	0.16d
	2.5% AA and 1.25% CA	0.72h	1.22g	1.34g	2.98g	0.20a	0.26ab
	5% AA and 0% CA	0.44f	0.98f	1.16g	2.49g	0.17a	0.17a
	5% AA and 2.5% CA	0.32e	0.72e	1.10g	2.56g	0.13a	0.13a
55 kPa	0% AA and 1.25% CA	0.12b	0.34b	0.28b	0.52b	1.70f	2.77gh
	2.5% AA and 0% CA	0.13b	0.26a	0.35c	0.76c	0.45b	2.00e
	2.5% AA and 1.25% CA	0.26d	0.63e	0.61de	1.60f	1.33e	3.01h
	2.5% AA and 2.5% CA	0.08ab	0.34b	0.98f	1.56f	0.53b	1.71d
	5.0% AA and 1.25% CA	0.15b	0.44bc	0.55d	0.99d	0.49b	1.97d
100 kPa	0% AA and 0% CA	0.13b	0.20a	0.23b	0.52b	1.89g	2.43g
	0% AA and 2.5% CA	0.25d	0.44bc	0.38c	0.73c	1.78f	2.25f
	2.5% AA and 1.25% CA	0.18bc	0.45bc	0.45d	0.79c	0.98d	2.47g
	5% AA and 0% CA	0.05d	0.35b	0.15a	0.44a	0.56b	1.01c
	5% AA and 2.5% CA	0.03a	0.57d	0.25b	1.03e	0.63c	0.98c

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

^a AA is ascorbic acid and CA is citric acid.

^b All the values are averages of three measurements carried out on three different samples.

product of lipoxygenase-initiated reactions of unsaturated fatty acids that took place soon after disruption of cells. These compounds were quantified using a nonequilibrium static headspace gas chromatographic technique (HS-GC). The vials containing 1 g of ground potato were held at a constant temperature and then the gas phase was analysed before equilibrium was reached. In this way, the analytical method provided for a short thermostatting time (i.e. less than for equilibration) so as not to alter the heat-sensitive sample, as described by Kolb and Ettre (1997). Thus, using a short thermostatting time at moderate temperature, headspace gas chromatography gave valuable information on the relative amounts of volatile compounds present in the matrix (i.e. the potato) as a function of the different dipping solutions and the O₂ partial pressures.

Table 4 shows the concentrations of some volatile compounds. During storage, acetaldehyde and ethanol increased, especially when low O₂ partial pressures were used while high O₂ partial pressures (55 and 100 kPa) had a positive effect on the anaerobic volatiles production: in these conditions, the volatiles concentrations in potatoes were lower than those found at 10 kPa O₂.

Stress induced by high O₂ partial pressures would, most likely, have some effect on the development of oxidative offflavours. With regard to hexanal production, Table 4 shows that the highest concentrations were associated with the central point of the composite design, even if a clear increase was also recorded in potatoes not submitted to the treatment solution and stored at 100 kPa.

Moreover, the combination of the highest O₂ concentrations with the treatment with 5% ascorbic acid helped to reduce the hexanal production after both 3 and 10 days of storage. The statistical analysis of the data showed that the polynomial models were adequate to describe the volatile compounds concentrations at each storage time (Table 5).

0 10 20 30 40 50 60 70 80 90100110 0 O2 (kPa) Fig. 4. Response plots of acetaldehyde and ethanol released from the pota-

toes as function of O2 and ascorbic acid after 3 days of storage.

In particular, it is evident that the O₂ increase had a significant and inhibitory effect on anaerobic volatiles production, as indicated by the negative sign of the linear coefficient (X_1) . Moreover, in the production of these compounds, the acid concentrations were of little importance, just as were the interactions between the variables. Fig. 4 shows, as an example, the response surfaces of acetaldehyde and ethanol after 3 days of storage.

A different result was found for hexanal production, and the situation after 3 days is, as an example, graphically represented (Fig. 5). The hexanal concentration increased only in the packages stored at 55 and 100 kPa. From a statistical point of view, the linear terms of $O_2(X_1)$ and ascorbic acid (X_2) , significantly and positively, influenced this response vari-

Table 5

nd statistical analyses of the second order polynomial models for

Factors	Estimated effect of acetaldehyde Storage time (days)		Estimated effect of ethanol Storage time (days)		Estimated effect of hexanal Storage time (days)		
	Intercept	0.919	1.219	1.389	2.868	-0.143	-0.734
X_1	-0.021^{***}	-0.028^{***}	-0.016^{**}	-0.059^{***}	0.035***	0.087^{***}	
X_2	0.006^{***}	NS	NS	NS	0.156^{***}	0.115^{**}	
X_3	0.081^{*}	NS	NS	0.219^{**}	NS	NS	
X_1^2	0.001***	0.001^{***}	0.001^{**}	0.0003^{**}	-0.0002^{*}	-0.006^{***}	
$X_1 X_2$	0.003**	NS	-0.0006^{*}	NS	-0.003^{**}	-0.003^{*}	
X_1X_3	0.001^{**}	0.002^{**}	0.0001NS	NS	NS	NS	
X_2^2	-0.011^{**}	NS	-0.034^{**}	-0.074^{***}	NS	NS	
$X_{2}X_{3}$	NS	NS	0.018NS	NS	NS	NS	
X_{3}^{2}	-0.063^{**}	-0.1235^{**}	0.119^{**}	NS	-0.266^{**}	-0.407^{**}	
$\operatorname{Adj} - R^2$ (%)	86.43	81.43	71.54	87.41	69.97	89.64	
Lack of fit**	0.06	0.28	0.06	0.06	0.11	0.30	
Durbin–Watson	2.48	1.84	2.19	1.40	1.59	1.52	

 $p \le 0.001; p \le 0.01; p \le 0.01; p \le 0.05; NS, non-significant.$





Fig. 5. Response plot of hexanal released from the potatoes as function of O_2 and ascorbic acid after 3 days of storage.

able for each storage time. Only the highest concentrations of citric acid produced a diminution in hexanal production, as indicated by the negative sign of its quadratic term (X_3^2) .

4. Conclusions

Knowledge of the contribution of each storage variable on quality attributes is essential when the shelf life of minimally processed vegetables has to be predicted. The present study showed that the respiration rate and the volatile compounds production of sliced potatoes were strongly influenced by the initial level of O_2 , but the composition of the treatment solution should be chosen with care, especially if the product was stored at high O_2 modified atmospheres.

In particular, it was more difficult to isolate the contribution of each solution agent than that of the O_2 partial pressure on the quality loss of potatoes. In fact, the concentrations of the solution agents that had a positive effect on the enzymatic browning were not always the same as those that had a positive effect on the other quality attributes.

It was evident that the modified atmosphere of 10 kPaO₂ gave the lowest results in terms of respiration rate and hexanal accumulation. In contrast, the tissue content of anaerobic volatiles was consistently high in the slices from the pouches packaged with 10 kPa O₂. In the closed system, a large increase in respiration rate was found passing from 10 to 55 kPa, while from 55 to 100 kPa the differences were less evident, but at the highest O₂ concentration the value drastically decreased if 5% ascorbic acid and 2.5% citric acid were used in the dipping treatment. The same situation was found during storage, i.e. under dynamic conditions, even if the CO₂ accumulation rate reached its maximum value when potatoes were stored at 55 kPa.

High O_2 partial pressures (55 and 100 kPa) did not stop the production of hexanal but they had an inhibitory effect on the anaerobic volatiles production. In fact, during storage acetaldehyde and ethanol increased less than at 10 kPa, while the highest concentration of hexanal was associated with the central point of the composite design, even if a clear increase was also recorded in potatoes not submitted to the treatment solution and stored at 100 kPa.

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