Autoxidation of Packed Almonds as Affected by Maillard Reaction Volatile Compounds Derived from Roasting

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Shelled almonds of two Italian varieties, *Romana and Pizzuta,* peeled and unpeeled, were roasted and packed under different conditions: air (control), vacuum, and Maillard reaction volatile compounds (MRVc) derived from the roasting process. Samples were stored for ~8 months at room temperature, without light, and, at regular intervals, were collected and analyzed to evaluate the progress of lipid oxidation. Peroxide values, triglyceride oligopolymers, and oxidized triglycerides were evaluated during the storage time. Results showed that, although the MRVc atmosphere did not protect the lipid fraction of almonds as well as the vacuum condition; nevertheless, it was more protective than the control atmosphere, showing an antioxidant effect. The effect of the natural coating was a strong protection against lipid oxidation; in fact, only the unpeeled samples showed peroxide values lower than the threshold of acceptability (25 milliequiv of O_2/kg of oil). Moreover, at the end of the storage period, Pizzuta almonds showed a greater deterioration than those of the Romana variety.

Keywords: *Almond; Maillard reaction volatile compounds; lipid oxidation; packaging*

INTRODUCTION

The stability and shelf life of nuts are highly dependent on packaging and storage methods. Some authors have reported on extending the marketing period of nuts by using packaging materials with a different barrier effect against light or gas or by using different atmospheres inside the package (Senesi et al., 1991, 1996; Sattar et al., 1990).

Severini et al. (1995) found that the shelf life of roasted hazelnuts strongly depends on packaging. In particular, a total protection against loss of moisture and volatile compounds, by using a hermetically sealed package, led to a greater stability of the lipid fraction. The authors formed the hypothesis that volatile compounds, derived from the Maillard reaction during the roasting process, have an antioxidant effect on lipid oxidation. The antioxidant capability of Maillard reaction products (MRP) has been well-known for a long time (Evans et al., 1958; Lingnert and Eriksson, 1980; Eichner, 1981), and recently a great interest has been shown in the application of natural compounds with antioxidant properties because of doubts about the safety of synthetic molecules in preventing rancidity (Hemeda and Klein, 1990). Also, the antioxidant properties of Maillard reaction volatile compounds are studied in model systems (Elizalde et al., 1991, 1992; Severini and Lerici, 1995), whereas no papers have been published on the application of natural volatile compounds with antioxidant activity in prolonging the shelf life of fatty foods. With this study the authors have evaluated the antioxidant capability of volatile compounds derived from the roasting process of almonds in order to assess the possibility of using natural MRVc, rather than air, as the atmosphere around roasted almonds and so prolonging their shelf life. The eventual substitution of vacuum storage with MRVc atmosphere inside packaging was moreover investigated, because the vacuum condition (commonly used in packaging nuts and very effective in protecting oilseeds against oxidation) might reduce the flavor of the product with the total release of the volatile compounds.

MATERIALS AND METHODS

Two Italian varieties of almonds *(Amygdalus communis*), Romana and Pizzuta, have been chosen for experiments. Two lots of samples, each derived from the same harvest in the same area, have been provided, already shelled, from the Caffè Sicilia Co. (Noto-SR, Italy). Half of each lot was peeled. After preliminary trials of roasting time and temperature, 210 °C for 8 min was chosen as the optimal roasting condition by evaluating the sensory characteristics of products. Samples were roasted in a static oven (De Longhi model 862 F, Milan, Italy), and quantities of 100 g were packed in air, after natural cooling (four peeled and four unpeeled samples—as control), under vacuum conditions after natural cooling (four peeled and four unpeeled samples), and under vacuum condition before cooling immediately after roasting (four peeled and four unpeeled samples). The latter samples saturated the atmosphere of the packaging with MRVc by their release during natural cooling.

Samples were packed using a plastic film (OPET/PE/EVOHIPE: 72 mil thickness; 1.21 g/cm^2 density; 4 g/m^2/day water vapor transfer; <2.5 cm^3/m^2/day carbon dioxide transfer) (Ambar Flex, AMB srl, S. Daniele del Friuli-UD, Italy).

A sample of 100 g was analyzed after roasting at time zero of storage, and a sample of both types of almond was analyzed immediately before roasting. The trial was carried out in duplicate, and the total of samples was 104.

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Table 1. Qualitative Characteristics of Romana and Pizzuta Almonds before and after Roasting

sample	a_w	moisture (%)	L^*	breaking strength (N)	CO_2 (%)	PV (mequiv of O_2/kg of oil)	oxidized triglycerides (%)
Romana (raw)	0.625	3.31	82.02	114.4	0.235	1.35	0.93
Romana (roasted)	0.373	1.92	74.75	84.9	0.435	0.48	0.69
Pizzuta (raw)	0.594	2.56	80.28	84.2	0.296	1.41	0.95
Pizzuta (roasted)	0.385	1.77	72.28	40.4	0.610	0.46	0.71

The following determinations were performed:

Peroxide value (PV) is expressed as milliequivalents (mequiv) of active oxygen per kilogram of oil (*Off. J. Eur. Communities*, 1991); the lipid fraction was previously extracted by immersion of ground almonds in a mixture of chloroform/methanol (2:1 ratio) (RPE, Carlo Erba Reagenti, Milan, Italy) at room temperature for 3 h, with a 1:6 (w/v) ratio. After filtration, with Whatman No. 1 paper, the solvent was evaporated by a Rotavapor model R114 (Buchi, Switzerland).

Polar compounds (PC) were determined according to an IUPAC method (IUPAC, 1987) using silica cartridges for SPE Sep-Pak columns (1 g) supplied by Waters (Milford, MA) as indicated in a previous paper (Márquez-Ruiz et al., 1996). PC were submitted to high-performance size exclusion chromatography (HPSEC) to determine oligopolymers (PTG), oxidized triglycerides (Ox-TG), and diglycerides. The chromatographic system consisted of a Perkin-Elmer pump, series 10, a 7125 S sample injector (Rheodyne), a 50 μL injector loop, and a series of three PL-gel columns (Perkin-Elmer Ltd., Beaconsfield, U.K.) of 0.75 cm i.d. $\times$ 30 cm length. The columns were packed with highly cross-linked styrene divinylbenzene copolymers with a particle diameter of 5 μm and pore diameters of 500, 500, and 100 Å, respectively. A PL-gel guard column (Perkin-Elmer Ltd.) of 7.5 mm i.d. $\times$ 5 cm length was used. The detector was a differential refractometer (Shimadzu refractive index detector, RID-6A, Shimadzu Corp., Osaka, Japan) connected to an integrator. The elution solvent used was CH_2C1_2 for HPLC (purity = 99.9%, supplied by Baker, Deventer, Holland) at a flow rate of 1.0 mL/min. Peaks on the chromatograms were identified by polystyrene standards (Supelco, Milan, Italy) of known molecular masses (MW = 4000 and 2000 g/mol) as well as tristearin, distearin, and monostearin standards (Sigma-Aldrich, Milan, Italy). For each standard, the elution volume was measured under the same conditions as used in our analysis. The log of MW as a function of elution volume was plotted, and the line of best fit was drawn by using the least-squares method. From the elution volume of each separated peak in a chromatogram, the corresponding MW could then be obtained (Gomes, 1992). Known amounts of oligopolymers, oxidized triglycerides, and diglycerides were obtained by preparative gel permeation chromatography of PC derived from a refined peanut oil and then used as standards in the HPSEC method. The amount collected for each standard, corresponding to a given class of compounds, was used to prepare a stock solution in CH_2C1_2 and solutions containing different concentrations after successive dilutions. These solutions were analyzed by HPSEC following the analytical method we developed. The calibration curves were obtained by plotting the amounts of standards (micrograms) that had been injected into the HPSEC system loop against the areas of the corresponding chromatogram peaks (Gomes and Caponio, 1999).

Raw and roasted samples at time zero of storage were analyzed as follows:

Moisture (%) was determined by accurately weighing ground almond samples after heating them in an oven at 105 °C for 12 h (AOAC, 1984).

Water activity (a_w) was determined by using a dew point hygrometer, Aqualab model CX-2 (Decagon Devices Inc., Pullman, WA).

Color was determined with a tristimulus colorimeter Chromameter-2 Reflectance, Minolta (Colorlab, Lugano, Switzerland) as described by Barbanti et al. (1990); an average of five measurements was considered. The coefficient of variation for lightness (L^*) was <5%.

Gas chromatographic analysis of carbon dioxide present in the headspace vapor was performed by using a Dani GC 8610 (Monza, Milano, Italy) connected to a personal computer. Data were processed with IQ software v. 2.2 for Windows (Dani). The instrument was equipped with a hot wire detector (HWD) and a 2 m $\times$ 2 mm i.d. glass-packed Supelco column, filled with Porapack Q 80−100 mesh (Supelco, Milano, Italy). The operating conditions were as follows: column temperature, 100 °C; detector temperature, 180 °C; injector temperature, 110 °C; filament temperature, 230 °C; carrier gas flow (He), 40 mL/min; 0.5 mL of headspace volume was injected using a pressure lock A-2 syringe (Dynatec Precision Sampling Co., Baton Rouge, LA).

Breaking strength was determined using a dynamometer Instron Universal Testing Machine, model 4301 (Instron International Ltd., Wycombe, U.K.).

The analyses were performed at least twice.

RESULTS AND DISCUSSION

Pizzuta almonds are the most valuable raw material for the sugared almond industry, whereas Romana almonds are less used despite their good sensory and physical characteristics. In Table 1 data displaying the qualitative characteristics of almonds, before and after roasting, are shown. The effects of the roasting were in terms of moisture loss, water activity decrease, browning, and carbon dioxide percentage increase, an early indicator of Maillard reaction, according to Lerici et al. (1990). The moisture decrease occurred up to the BET monolayer value (Özgül Evranuz, 1993), above and below which the rate of lipid oxidation increases (Labuza, 1970). Therefore, the roasting process, in itself, made the products more stable against lipid oxidation. The peroxide values decreased as did the percentage of oxidized triglycerides; this effect could be due to reactions between peroxide radicals or carbonyl compounds formed from peroxides and Maillard reaction products. Moreover, after roasting, almond samples became more brittle.

The strongest difference between the two varietes of almond was in the resistance against breaking: the Pizzuta variety was less resistant than the Romana variety. Figure 1 shows the behavior of the peroxide value of unpeeled Romana almonds, packed under air, MRVc, and vacuum conditions, as a function of the storage time. As expected, vacuum-packed samples showed the lowest peroxide values. Samples stored under MRVc conditions were less oxidized than the control (air-packed samples) until ~200 days of storage. From the results it seemed that the antioxidant capability was due in particular to the volatile compounds acting in the headspace of the bags rather than the Maillard reaction products with high molecular weight (melanoidins) present in all roasted samples. Studies published in the literature report that other compounds, such as ethanol and hexanal, showed a protective effect, in these cases against the growth of microorganisms, as a gaseous compound of the headspace rather than as a component of the system (Guerzoni et al., 1994; Lanciotti et al., 1999; Corbo et al., 2000).

The mechanism of antioxidant action of the MRVc could be linked to its structure, so these compounds

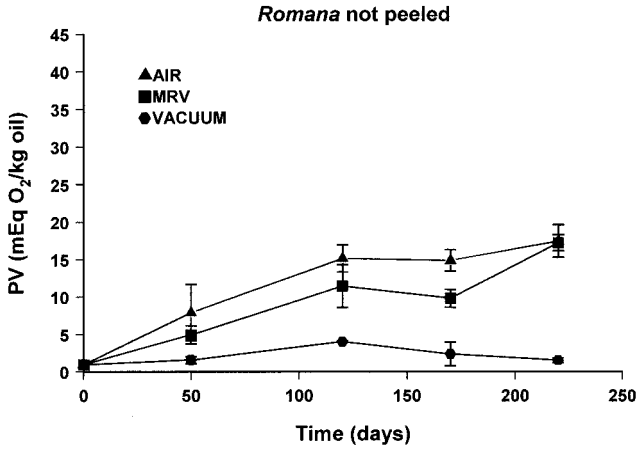

Figure 1. Peroxide values, expressed as milliequivalents of active oxygen per kilogram of oil, of unpeeled Romana almonds, packed under air (control), Maillard reaction volatile compounds (MRVc), and vacuum as a function of storage time (days).

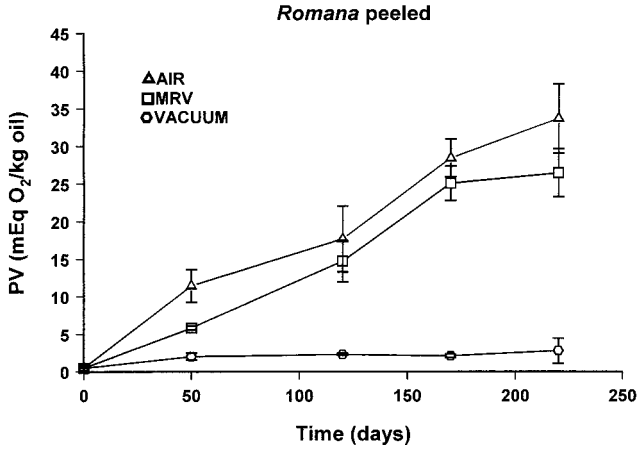

Figure 2. Peroxide values, expressed as milliequivalents of active oxygen per kilogram of oil, of peeled Romana almonds, packed under air (control), Maillard reaction volatile compounds (MRVc), and vacuum as a function of storage time (days).

could present reducing and chelating properties and act as hydrogen donors or electron traps (Elizalde et al., 1991). Furthermore, it could substitute oxygen in the headspace of packed products, thereby hindering lipid oxidation. In Figure 2 it can be observed that the peeled samples were more oxidized than those which were unpeeled when packed in air or MRVc. The different rates of peroxide formation were confirmed by *k* values derived from linear regression of curves (Table 2). In all cases the presence of peel reduced by half the rate of peroxide formation, probably because the product's surface came into contact with oxygen less. If a peroxide value of 25 mequiv of O_2/kg of oil might be taken as a threshold of acceptability for nuts (Narasimhan et al., 1986; Özgül Evranuz, 1993), the retention of external coating could assume a great importance for the shelf life of almonds. In fact, only the unpeeled samples showed peroxide values lower than the threshold.

Although the MRVc atmosphere did not protect the lipid fraction of almonds as well as vacuum conditions, nevertheless, volatile compounds, derived from the Maillard reaction during roasting, resulted in being more protective than the control atmosphere, showing an antioxidant effect.

Table 2. Kinetic Constants (*k*), Coefficients of Correlation (*r*), and Significance (*p*) Calculated from Linear Regression of Curves Shown in Figures 1 and 2 (Romana Samples) and in Figures 3 and 4 (Pizzuta Samples)

sample	*k* (mequiv of O_2/kg of oil × days^{-1})	*r*	*p*
Romana (peeled)			
air	0.148	0.991	<0.001
MRVc	0.127	0.986	<0.001
vacuum	0.008	0.851	<0.05
Romana (unpeeled)			
air	0.072	0.944	<0.01
MRVc	0.067	0.953	<0.01
vacuum	0.004	0.307	ns[a]
Pizzuta (peeled)			
air	0.177	0.998	<0.001
MRVc	0.116	0.982	<0.01
vacuum	0.009	0.914	<0.05
Pizzuta (unpeeled)			
air	0.075	0.952	<0.01
MRVc	0.071	0.992	<0.001
vacuum	0.007	0.769	ns

[a] ns, not significant.

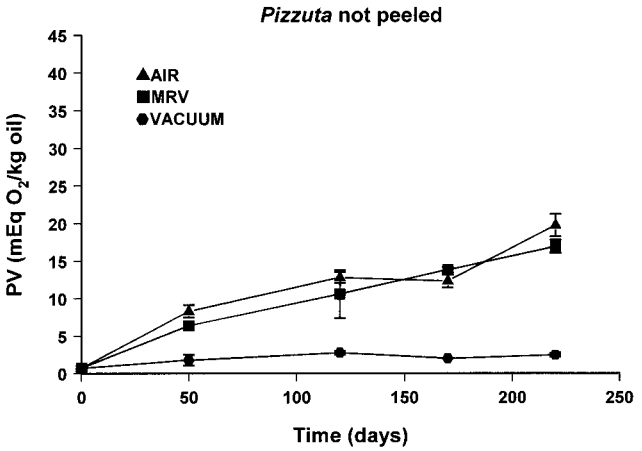

Figure 3. Peroxide values, expressed as milliequivalents of active oxygen per kilogram of oil, of unpeeled Pizzuta almonds, packed under air (control), Maillard reaction volatile compounds (MRVc), and vacuum as a function of storage time (days).

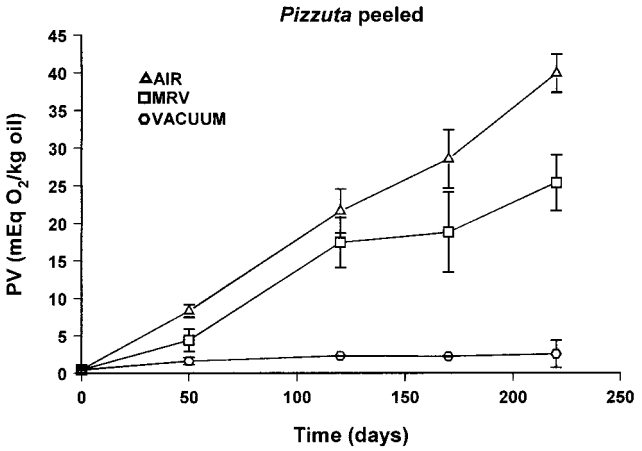

Figure 4. Peroxide values, expressed as milliequivalents of active oxygen per kilogram of oil, of peeled Pizzuta almonds, packed under air (control), Maillard reaction volatile compounds (MRVc), and vacuum as a function of storage time (days).

Changes in peroxide value of Pizzuta almonds are shown in Figures 3 and 4. The same behavior of Romana samples can be observed; in particular, the effect of the

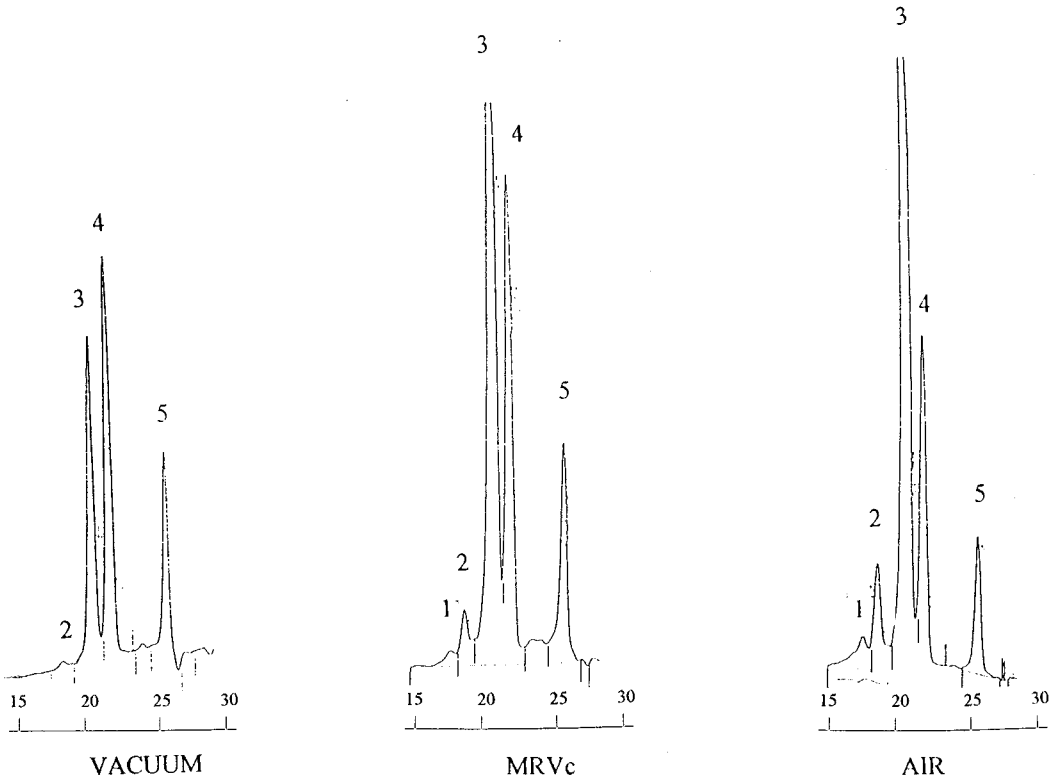

Figure 5. HPSEC analysis of polar compounds derived from oil extracted of Romana almonds, packed under air, vacuum, and MRVc and stored for 8 months: (1) triglyceride trimers; (2) triglyceride dimers; (3) Ox-TG; (4) diglycerides; (5) free sterols and triterpene diols.

Table 3. Polymers (Percent) and Oxidized Triglycerides (Percent) of Peeled and Unpeeled Romana and Pizzuta Almond Samples before and after Roasting at Time Zero and after 8 Months of Storage

sample	roasted (time 0)	polymers, roasted (after 8 months)	roasted (time 0)	oxidized triglycerides, roasted (after 8 months)
Romana (peeled)				
air	0.05 ± 0	0.55 ± 0.04	0.79 ± 0.13	3.14 ± 0.15
MRVc	0.05 ± 0	0.32 ± 0.01	0.79 ± 0.13	2.45 ± 0.73
vacuum	0.05 ± 0	0.05 ± 0	0.79 ± 0.13	0.85 ± 0.06
Romana (unpeeled)				
air	0.05 ± 0	0.27 ± 0.02	0.72 ± 0.03	2.27 ± 0.21
MRVc	0.05 ± 0	0.26 ± 0.03	0.72 ± 0.03	2.14 ± 0.30
vacuum	0.05 ± 0	0.05 ± 0	0.72 ± 0.03	0.76 ± 0.04
Pizzuta (peeled)				
air	n.d.[a]	0.92 ± 0.05	0.67 ± 0.03	4.33 ± 0.72
MRVc	n.d.	0.21 ± 0.01	0.67 ± 0.03	2.00 ± 0.15
vacuum	n.d.	0.12 ± 0.02	0.67 ± 0.03	1.68 ± 0.11
Pizzuta (unpeeled)				
air	n.d.	0.35 ± 0.03	0.86 ± 0.02	4.32 ± 0.64
MRVc	n.d.	0.26 ± 0.03	0.86 ± 0.02	3.3 ± 0.32
vacuum	n.d.	0.12 ± 0.01	0.86 ± 0.02	1.68 ± 0.21

[a] n.d., not detected.

peel was in this case more evident. The rate of peroxide formation (Table 2) in MRVc-packed samples was lower than in air and higher than under vacuum conditions; the rate of peroxide formation in peeled products was higher than in the unpeeled products.

Regarding the comparison between Romana and Pizzuta, in all cases a greater susceptibility to lipid oxidation was observed in Pizzuta almonds.

The chromatographic analysis of polar compounds allows a more comprehensive evaluation of oxidative and hydrolytic degradation of fats. In fact, as is well-known, hydroperoxide compounds are indicators of the formation of primary oxidation products. These substances are time-unstable and could be transformed in different compounds in relation to the type of packaging, storage conditions or presence of metals; therefore, the only hydroperoxide determination seems not to be completely suitable for evaluating the comprehensive level of fat oxidation. More reliable results are obtained by quantitative determination of triglyceride oligopolymers and oxidized triglycerides. These classes of substances are known to be effective indicators of oxidative degradation by several authors (Perez-Comino et al., 1990; Hopie, 1993). Oxidation and hydrolysis compounds are known to act as pro-oxidants (Yoon et al., 1988; Frankel et al., 1988; Mistry and Min, 1988) and some of these compounds are known to be potentially toxic for human health (Keijbets et al., 1986; Gonzales-Munoz et al., 1996), so their formation should be limited. For example, the chromatograms of Romana samples, vacuum-, air-, and MRVc-packed and stored for 8 months, are shown in Figure 5. Peaks 1, 2, and 3 have

been identified as oligopolymers (dimers and trimers) and oxidized triglycerides, respectively. In Table 3 oligopolymer and oxidized triglyceride data are reported for Romana and Pizzuta samples, with and without peel, packed under air, vacuum, and MRVc conditions. Results confirmed that samples stored without peel, under air conditions, deteriorated the most, whereas samples packed under MRVc conditions showed a midlevel quality between air- and vacuum-packed almonds. In fact, the lower presence of triglyceride oligopolymers and oxidized triglycerides in MRVc-packed almonds, in comparison to air-packed ones, might be related to the antioxidative effect of volatile roasting products.

Also from these data, Pizzuta samples have proved to be less resistant than Romana against lipid degradation, although the two varieties did not show significant differences in terms of fatty acid composition and tocopherol content (Dugo et al., 1979; Salvo et al., 1986). On this subject further investigations could be performed, in particular giving attention to the different morphological characteristics of the two tested varieties. In fact, Pizzuta almonds, with a greater surface/volume ratio, might be less resistant than Romana almonds because of the greater surface area exposed to oxygen.

CONCLUSIONS

The antioxidant effect of Maillard reaction products has been confirmed in a food system (almonds) in addition to what was known in model systems. In particular, the antioxidant capability seemed to be linked to volatile compounds acting as antioxidants in the headspace of bags more than melanoidins present in all roasted samples. Vacuum packing has been the most effective in preventing lipid oxidation of almonds; nevertheless, the possibility of the use of natural MRVc atmosphere, more effective than air conditions, should be further investigated in order to prolong the shelf life of roasted fatty products without reducing their flavor through vacuum packing.

It may be useful to note that the presence of peel led to a strong decrease in the rate of peroxide formation in both Romana and Pizzuta samples under different packaging conditions.

Finally, the results show that in all cases Pizzuta samples had a shorter shelf life than Romana samples. Therefore, a greater use of the Romana variety should be promoted, considering its good quality.

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