Influence of Storage Conditions on the Quality of Shelled and Roasted Almonds

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The storage of four varieties of almond, three Spanish and one native to and imported from California (USA), was investigated at two temperatures (8 and 36°C), two packaging atmospheres (air and N₂) and two treatments (raw and roasted) throughout several months, in order to study the storage behaviour. Five chemical parameters were determined during the whole time of the experiment: moisture, fat content, peroxide value, α-tocopherol content and level of aflatoxins. No significant differences were observed between air and nitrogen packaging for the parameters measured. At the end of the study (9 months), almonds stored in their shells at ambient temperature, maintained their high quality. A significant relationship was found between the increase of the peroxide value and the decrease of α-tocopherol content. The aflatoxin contents were always lower than 0.5 µg/kg.

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1. Introduction

The Spanish almond production attains around 250 kt, the Valencia region being the most important producing area (MAPA, 1999). Consumption and consequently production of Spanish almonds has been decreasing in the last 10 yr. In addition, farmers face increased competition from other countries where commercialisation is more advanced and more standardised procedures are employed, for instance the control of storage conditions. Knowing the optimum conditions for processing and storing almonds is an important asset in order to increase competitiveness.

Most of the studies found in the literature on almond storage investigate the influence of different factors (packaging materials, temperature, time, roasting, light or irradiation) on several physical, sensory and chemical parameters of the seeds. Almonds are marketed in different commercial forms: unshelled almonds, shelled kernels and peeled seeds, roasted or not. The only study found in the literature, relating to Spanish varieties of almonds and their storage is the work of Ruiz-Bevià et al. (1999). The work details sorption and diffusion properties of three common Spanish peeled almonds (Marcona, Desmayo Largueta and Comuna), which are of interest in order to establish the influence of temperature and relative humidity during storage.

The classical way of storing almonds nuts is to keep them in the shell after natural drying until their consumption or use in industry. Zacheo et al. (2000) studied the changes associated with postharvest ageing in Italian unshelled almonds stored in the dark at 20°C and 40% relative humidity for 36 months. Some aspects investigated in that study were changes in total lipid content, fatty acid profiles, protein concentration, lipoxygenase activity, hydroperoxide level and tocopherol content. It was found that shelf-life could be extended if peroxidation were to be reduced and the levels of antioxidants, such as tocopherols, were high. The study confirmed previous reports that unshelled almonds maintained at ambient temperature do not show significant chemical and biochemical changes for 1 yr (Senesi et al., 1996). Shelled almonds are another way of storing and consuming the nut, peeled or not. Furthermore, there are other optional ways of treating the almond, such as roasting. Without the shell, the kernel experiences faster deteriorative changes that shorten its shelf-life. Among the different quality parameters considered in the literature throughout the storage time, rancidity is one of the most important.
Consequently, delaying the onset of rancidity could lengthen the available market period of these products. Rancidity is originated by the reaction of unsaturated fatty acids with oxygen, and later the degradation of peroxide fatty acids produces off-flavour compounds. This process is influenced by temperature, the levels of unsaturated fatty acids, the presence of enzymes, trace metals and antioxidants such as tocopherols and light exposure (Sattar et al., 1990a, 1990b; Gou et al., 2000; Zacheo et al., 2000). Researchers have traditionally associated the increase in peroxide values with the onset of rancidity and, since peroxides are easily determined in fats, the peroxide value is frequently used to measure the progress of oxidation (Fourie & Basson, 1989; Senesi et al., 1991, 1996; Zacheo et al., 2000).

In order to prevent the off-flavour of almonds due to rancidity, postharvest techniques can be used. These include the use of different packaging materials, modified atmospheres or refrigeration temperatures. Senesi et al. (1991) studied the effect of different packaging conditions (transparent and metallised films under vacuum or nitrogen) and two storage temperatures (4 and 20°C) on the quality and stability of Italian peeled almonds (cv. Ferraduel). They found that peeled kernels could be stored up to 9 months without a serious loss in quality when packaged in high barrier packaging, regardless of the storage temperature (4°C or ambient). However, in order to maintain the general quality over a longer storage time (more than 9 months), they proposed the use of metallised film under nitrogen and refrigeration (4°C). In another work, Senesi et al. (1996) studied the influence of low barrier packaging material combined with refrigeration (2°C) on the storage conditions of Italian peeled almond (cv. Supernova) quality. They found that it is possible to maintain the quality of peeled almonds up to 12 months in a low barrier material by storing them at refrigeration temperature (2°C).

The oxidation of fats and the rate of rancidity development are highly dependent on the temperature. In that way, the higher the temperature the higher is the rate of rancidity (Harris et al., 1972; Sattar et al., 1990b; Senesi et al., 1991, 1996; Nogala-Kalucka & Gogolewski, 2000). Hence, the influence of temperature must be taken into account when shelf-life studies are undertaken. However, accelerated high-temperature tests produce a different type of change in quality to that found in almonds stored at lower temperatures. Consequently, almonds stored in conditions to accelerate ageing should be maintained below 43°C (Harris et al., 1972).

On the other hand, roasting is a usual way of treating almonds, and this can be done in two ways, either by frying in oil or by a dry process (Young & Cunningham, 1991; Gou et al., 2000). In this last case, the employed methods are characterised by the different ways energy is transferred into the product: convection and radiation. Currently, the most used system is convection. Studies of roasted almonds are scarce in the literature. Roasting promotes different changes in the kernels, for instance in the volatile compounds (Takei et al., 1974; Takei & Yamanishi, 1974; Young & Cunningham, 1991; Gou et al., 2000). Harris et al. (1972) studied rancidity in diced unroasted and roasted almonds for periods up to 6 months at −18 and 38°C. From results obtained from a sensory panel, it was concluded that diced unroasted and roasted almonds became unacceptable after 6 and 3 months, respectively. Gou et al. (2000) studied the influence of oven roasting (two temperatures and six sampling times) on some physico-chemical and sensory properties of Desmayo Largueta variety almonds. Rancidity increased in line with the treatment time up to a maximum value and over-roasting produced antioxidant products during the Maillard reaction, which are absorbed in the oil. Over-roasting also decreased sweetness and increased bitterness and grittiness.

The variety of almond can play an important role in the shelf-life of the product. The content of tocopherols, peroxide values, fats, etc. depends upon the variety, as well as the soils and the climates where almonds are grown. Finally, studies of non-peeled almonds (kernels with tegmen) have not been found in the literature, although this is also a common method of storage.

The aim of this work is to study different packaging and storing methods of Spanish shelled almonds with tegmen, roasted or not. A knowledge of suitable conditions for processing and storing almonds is an important asset in order to increase competitiveness by maintaining product quality during the selling period.

2. Materials and methods

2.1. Raw material

Four almond varieties were selected for this study. Three of the varieties (Marcona, Planeta and Desmayo Largueta) are native to Spain, and are the main varieties as they have the appropriate characteristics for making turrón (a Spanish confectionery product), marzipan and other products. The fourth variety considered was Nonpareil, cultivated mainly in California and imported from the USA. Agronomic and chemical characteristics of the selected varieties are found in the literature (Saura-Calixto et al., 1988).

Three different almond products have been considered in this study: raw with shell, raw, shelled and shelled roasted kernels. The shell was removed using an
industrial sheller. Unlike the previous works (Senesi et al., 1991, 1996; Ruiz-Bevià et al., 1999; Zacheo et al., 2000), the tegmen was not removed, since almonds are often consumed in that way in Spain. Two different atmospheres were considered, oxygen free (nitrogen) and plain air. To simulate both winter and summer conditions, two common storage alternatives were considered, refrigerated or not. Refrigeration lowers the kinetics of many reactions, for that purpose a low temperature of 8°C was chosen. Since the Mediterranean coast is a hot region and in order to accelerate the ageing reaction rates, a high temperature of 36°C was selected according to the findings in the literature (Harris et al., 1972). Differential scanning calorimetry studies of almond have shown that a phase transition occurs around 30°C. Thus, the two selected temperatures are below and above that phase transition. The refrigerated samples were stored for 9 months, whereas the ones submitted to high temperatures were prepared and stored only 4 months after shelling. The shelled seeds were packed and stored under different conditions, as shown in Table 1, but always in the dark. Unshelled almonds were kept at ambient temperature (20 ± 1°C) and analysed at the beginning and at the end of this study to compare them with the almonds stored under the other storage conditions. Before packaging, the roasted almonds were prepared according to a food-processing procedure common to several Spanish companies; the kernels were submerged in a solution that consisted of wheat flour (4%), guar gum (1%) and agar (0.3%) for 10 min. Then, they were drained on a mesh of stainless steel for 5 min and sodium chloride was sprinkled over them. Finally, the nuts were roasted in a convective oven (Hobcart model CS-0611E) at 190°C for 15 min.

2.2. Packaging

Flexible plastic pouches used in the food industry of low permeability to CO₂, O₂, N₂ and H₂O vapour were used to pack the kernels. The pouches were fitted with a septum in order to check the atmosphere. A partial vacuum was carried out to remove the air before packaging with nitrogen. Immediately after packing the almonds, the gaseous content was checked. The initial O₂ and CO₂ content in the pouches with a plain air atmosphere was 20-6% and 0.04%, respectively, whereas in the pouches with a nitrogen atmosphere the O₂ content was always lower than 0.25%. A periodic measurement of the atmosphere inside the packages was carried out, and if a change in the atmosphere composition due to a lack of airtightness was detected the samples were rejected.

2.3. Moisture determination

The moisture content determination of the raw almond seeds was carried out by freeze-drying. The freeze-drying method was selected because when oven drying is used, which is the most common procedure, the almonds develop a light brown colour. The moisture content measured using the freeze-drying method agreed with values obtained by other authors. The samples of almond were ground, frozen using liquid N₂ and put into a Christ Beta 2-16 freeze-drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). To determine a suitable program for the freeze-drying conditions, freeze-dry moisture measurements were compared with measurements obtained in a vacuum oven until constant weight. The freeze-drying program chosen consisted of a 12 h period of drying at 0.04 mbar followed by a final drying period for 12 more hours at 0.001 mbar. Using liquid N₂ to freeze the samples avoids rancidity of ground kernels, and reduces result variability and subsequently improves the fat extraction.

2.4. Fat content

The oil of the samples was extracted using Tecator Soxtec semiautomatic equipment (FOSS Tecator AB) and quantified according to method 948.22 of the AOAC (1996) applied to freeze-dried samples. Two grams of freeze-dried almonds were placed in the extraction cartridge of the equipment for 45 min. The solvents used were petroleum ether and hexane. The oil extracted was dried in an oven at 102°C for 30 min in order to eliminate the solvent, and then weighed. The oil was frozen at −40°C for tocopherol content analysis.

2.5. Tocopherols

The tocopherol content was measured according to the method described by Slover et al. (1983). A Carlo
2.6. Peroxide value

The peroxide value was determined according to Commission Regulation (EC) No. 2568/91 (1991) methods for the measurement of the characteristics of olive oil and olive-residue oil and the relevant methods of analysis. A sample of around 2–3 g of oil was put into a flask, bubbling pure and dried nitrogen was added to it, with 10 ml of chloroform, 15 ml of glacial acetic acid and 1 ml of water solution saturated with potassium iodide. It was left in darkness for 5 min, after which 75 ml water and several drops of starch were added, then the liberated iodine was titrated with 0.1 N of sodium thiosulphate.

2.7. Aflatoxin

To determine the aflatoxin content (B₁, B₂, G₁ and G₂), the Park et al. (1990) procedure was used. The aflatoxins were extracted from defatted almond powder using methanol and 0.1N HCl (4:1), shaken for 3 min, centrifuged for 10 min, then the extract was filtered through Whatman no. 1 paper, and finally partitioned with methylene chloride. The concentrated extract was passed through a Novapack C18 silica gel column measuring 4.6 mm by 150 mm. Aflatoxins B₁ and G₁ were derivatised with trifluoroacetic acid and the individual aflatoxins were determined by reverse-phase liquid chromatography with a W-470 fluorescence detector.

2.8. Statistical methods

Tests for significant differences were carried out using analysis of variance (ANOVA). When differences were significant, multiple comparisons were made using the least significant differences (LSD) and Tukey’s test.

3. Results and discussion

3.1. Raw material

To characterise the raw material several analyses were carried out (Table 2). The initial water content varied between 4.3 and 5.3 kg [H₂O] per 100 kg. The initial figures for the fat content varied between 47.2 for Nonpareil to 52.3 kg [fat] per 100 kg for Marcona. Desmayo Largueta and Planeta varieties were not significantly different. The peroxide values ranged from 4.2 for the Desmayo Largueta variety to 5.8 mEq [O₂] per kg [fat] for the Nonpareil, and their initial figures were significantly different among the four almond varieties considered. The average content of tocopherol in the almond fat varied between 33.8 and 40.5 g per 100 kg [fat], no significant inter-variety differences were observed. Aflatoxins can appear in foodstuffs contaminated with fungi as Aspergillus flavus and Aspergillus parasiticus under certain environmental conditions, such as temperature between 20 and 30°C, high ambient relative humidity and existence of oxygen. No presence of aflatoxins was initially detected in any sample.

Samples preserved at 8°C by refrigeration did not change significantly in their chemical parameters until the sixth month. Hence, in this study only the data corresponding to the last 4 months (sixth to ninth storage months) are shown. Samples for ageing were prepared later from unshelled almonds, and stored at 36°C for 4 months.

For the sake of presentation, the results have been grouped per almond variety. Each almond variety has been separated into three groups: temperature of storage, packaging and treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Marcona</th>
<th>Desmayo Largueta</th>
<th>Planeta</th>
<th>Nonpareil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, kg per 100 kg</td>
<td>4.5 ± 0.10a</td>
<td>5.1 ± 0.05b</td>
<td>5.3 ± 0.20b</td>
<td>4.3 ± 0.04c</td>
</tr>
<tr>
<td>Fat, kg per 100 kg</td>
<td>52.3 ± 2.5a</td>
<td>49.5 ± 1.8b</td>
<td>48.9 ± 2.3b</td>
<td>47.2 ± 1.5c</td>
</tr>
<tr>
<td>Peroxide value, mEq [O₂] per kg [fat]</td>
<td>4.5 ± 0.02a</td>
<td>4.2 ± 0.05b</td>
<td>5.2 ± 0.09c</td>
<td>5.8 ± 0.07d</td>
</tr>
<tr>
<td>Aflatoxin, μg per kg</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>a-tocopherol, g per 100 kg [fat]</td>
<td>40.5 ± 6.8a</td>
<td>38.6 ± 4.5a</td>
<td>33.8 ± 3.7a</td>
<td>36.5 ± 5.8a</td>
</tr>
</tbody>
</table>

N.D.: not detected. Values followed by different letters in the same row are significantly different at the 5% level.
3.2. Moisture

As shown in Table 2 there are significant differences in the raw material moisture, when roasting they attain almost equal moisture content in all cases (2.33 ± 0.05 kg [H₂O] per 100 kg) at 0 months. Throughout the experiments, there is no water loss either in those kept refrigerated at 8°C or in the others kept at 36°C as expected. This is due to the low permeability of the plastic film to CO₂, O₂, N₂ and H₂O.

3.3. Fat

Table 3 shows the average values of fat content of both shelled and roasted almonds, respectively, for the four varieties considered. Significant changes in the fat content as compared to the initial values only occurred for the shelled Marcona, Planeta and Nonpareil almonds. A reduction in total lipid content of unshelled almonds was previously observed by Zacheo et al. (2000) after 18 months of storage. However, throughout the 9 months of this study, the fat content of the unshelled almonds did not vary.

3.4. Peroxide value

Table 4 shows the average peroxide values obtained for the four varieties studied and the different treatments and storage conditions applied to the almonds. The packaging atmosphere did not have effect in the modification of the peroxide content for any almond variety. It seems that there could be an auto-oxidation in the kernels. Several factors can promote this process, such as metal content or water activity (Troller, 1989; Sattar et al., 1990a; deMan, 1999). There were significant differences between shelled and roasted Marcona, Desmayo Largueta and Planeta almonds, but none at all when studying the Nonpareil.

There were significant differences according to the storage temperature in the following cases: in shelled Marcona, in roasted Desmayo Largueta, and in both Planeta and Nonpareil, shelled as well as roasted. The main increase in peroxides occurs in the roasted almonds, since roasting accelerates deterioration (Harris et al., 1972; Young & Cunningham, 1991; Uthman et al., 1998; Gou et al., 2000), with figures ranging between 20 and 46 mEq [O₂] per kg [fat]. Salvo et al. (1986) observed that the peroxide value in almond oil stored at 4°C increases from an initial figure of 9.6 mEq [O₂] per kg [fat] up to 21.3, 29.6 and 129.5 mEq [O₂] per kg [fat] after 1, 2 and 3yr, in that order. These variations are more significant at ambient temperature. In this study, as the raw nuts are stored at a higher temperature of 8°C, it could be an explanation of why they reach higher figures in a shorter time. Although there is no significant relationship between the peroxide value and the results of an untrained sensory panel, it can be observed that a tendency towards the higher peroxide value correlates with higher rancid flavour. More experiments will be needed to determine thresholds and more definite relationships, and an expert sensory panel should be involved to study those relationships (González et al., 2001).

Finally, the initial peroxide content of the unshelled almonds kept at ambient temperature did not change the initial peroxide content through the 9 months of storage. The almond shell itself seems to be an effective package against oxidative deterioration during storage (Zacheo et al., 2000). The mechanism of that protection (atmosphere inside the shell, effective barrier against light or gases exchange) remains, at present, unclear.

![Table 3: Average fat content in the dry matter (dm) ± standard deviation (SD) in kg [fat] per kg [dm]]
3.5. \textit{\alpha-}tocopherol content

Table 5 shows the values of \textit{\alpha-}tocopherol content measured for the four varieties studied. The tocopherol content decreased throughout the storage time in all the four varieties. Neither the storage temperature nor the packaging atmosphere used caused the \textit{\alpha-}tocopherol content to change significantly from variety to variety. The Marcona variety showed the highest figures of \textit{\alpha-}tocopherol content. A statistically significant relationship between the increase in peroxide value and the reduction of \textit{\alpha-}tocopherol content was found. This agrees with the findings of some researchers (Senedi et al., 1991; Zacheo et al., 2000; Sun et al., 2001), in fact tocopherols are effective in quenching lipid peroxide radicals. Moreover, according to the literature, it seems that the oxidation of fatty acids becomes significant after an induction period during which antioxidants are destroyed (Zacheo et al., 2000; Sun et al., 2001).

3.6. Aflatoxin

The infestations of the seeds by fungi that produce aflatoxin can occur mainly during harvesting and early processing steps (Ozilgen & Ozdemir, 2001; Schatzki & Ong, 2001). As aflatoxins are highly toxic and carcinogenic substances, their maximum levels have been regulated. The amount of those substances are the sum of the B₁, B₂, G₁ and G₂ aflatoxins, the highest permitted levels being 4 μg kg⁻¹ for groundnuts, nuts and dried fruit and processed products thereof, which
are intended for direct human consumption and 10 μg kg\textsuperscript{-1} as an ingredient in foodstuffs and for nuts and dried fruit to be subjected to sorting, or other physical treatments, before human consumption or for use as an ingredient in foodstuffs, respectively (Commission Regulation (EC) No. 466/2001, 2001).

In preliminary experiments with almonds stored at 30°C and packed with air, the levels of 14, 3, 12 and 4 μg kg\textsuperscript{-1} of B\textsubscript{1}, B\textsubscript{2}, G\textsubscript{1} and G\textsubscript{2} aflatoxins, respectively, were detected. However, the samples kept in the same conditions but containing N\textsubscript{2} instead of air did not develop aflatoxins. This points to the fact that an inert atmosphere can lessen the risk of growth of Aspergillus flavus in the nuts (Ellis et al., 1994a, 1994b).

Nevertheless, that effect is not observed in the samples of the present work, possibly because the nuts for this study were not contaminated. In this work, aflatoxin contents higher than 0.5 μg kg\textsuperscript{-1} were not observed, regardless of the packaging atmosphere, the roasting procedure, the temperature or the variety considered.

### 4. Conclusions

Significant changes in the fat content as compared to the initial values only occurred for three shelled almond varieties during storage, Marcona, Planeta and Nonpareil. In every case was observed a rise in peroxide value throughout storage time. The roasted almonds presented higher values than the raw packed ones. This increase means a reduction in the global organoleptic quality. No effect of the inert atmosphere (N\textsubscript{2}) on the peroxide value was observed. The Marcona kernels yielded the lowest peroxide value, while the Nonpareil...
gave the highest, the latter indicating a greater sensitivity to rancidity, despite having the lowest fat content. The \( \alpha \)-tocopherol content decreased throughout the experiment in the four varieties considered. There were no significant differences between the samples stored at 8°C for 9 months and the others stored at 36°C for 4 months. The inert atmosphere had no influence on the changes in the \( \alpha \)-tocopherol content. Aflatoxins were not detected in concentrations over 0.5 \( \mu \)g kg\(^{-1}\) in any sample. Finally, the unshelled almonds stored at ambient temperature for 9 months did not undergo any change in their initial fat content, nor the peroxide value, nor the \( \alpha \)-tocopherol content. The explanation for this (atmosphere inside the shell, effective barrier against light or gaseous exchanges) remains, at present, unclear.

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