SHIMADZU

Excellence in Science

The cube of food analysis

In order to comply with the General Food Law Regulations of the European Commission (EC), the European food industry needs a full range of analytical tools, indispensable for research, development and quality control. Whether highthroughput QA/QC laboratories in agriculture, flavor & fragrances, food & beverages or food processing and packaging, Shimadzu provides instruments and software solutions for every analytical challenge.

Serving all food industries
with high-speed analysis of food and drinks ingredients and contaminants

Complete range of analytical instrumentation
including chromatography, mass-spectrometry, spectroscopy and material testing

Total support from farm to fork
providing tools for monitoring food safety from production to packaging

Access to expert knowledge
through download of free of charge application handbook

www.shimadzu.eu/food

Received: 10 June 2022 | Revised: 28 September 2022 | Accepted: 7 October 2022

DOI: 10.1111/1750-3841.16369

Journal of Food Science WILEY

ORIGINAL ARTICLE

Food Microbiology and Safety

Effect of relative humidity on the quality and safety of peeled almond kernels (*Prunus dulcis* Mill.) during simulated maritime transport/storage

Luana Fernandes[1,2,3] | **Francieli Graeff**[1,4] | **Arij Jelassi**[5] | **Michael Sulyok**[6] | **Carolina Garcia**[4] | **Nuno Rodrigues**[1,2] | **José Alberto Pereira**[1,2] | **Albino Bento**[1,2] | **Alifa Kanoun**[5] | **Paula Rodrigues**[1,2] | **Ermelinda Lopes Pereira**[1,2] | **Elsa Ramalhosa**[1,2]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal

[2]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Bragança, Portugal

[3]MORE, Laboratório Colaborativo Montanhas de Investigação - Associação, Bragança, Portugal

[4]School of Food Science, Federal Technological, University of Paraná, UTFPR, Medianeira, Brazil

[5]Ecole Polytechnique, Université Libre de Tunis, Tunis, Tunisia

[6]Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna, Austria

Correspondence
Elsa Ramalhosa and Ermelinda Lopes Pereira, Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal.
Email: elsa@ipb.pt and epereira@ipb.pt

Funding information
PDR2020 Partnership Agreement and co-financed by FEADER, Portugal 2020 (ValNuts Project: PDR2020-101-030756); the Foundation for Science and Technology (FCT, Portugal), National funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021)

Abstract: Long-term transport and storage of peeled almonds under unsuitable conditions may cause the product's rejection. To get knowledge in this topic, peeled almonds were stored at 25°C and 60, 70, and 80% relative humidity (RH). The maintenance of high RH (80%) caused some visual defects after 4 months. Even though the 60, 70, and 80% RH did not clearly affect the production of primary and secondary products formed in the lipid oxidation during the 6 months of storage, sometimes an increase in the values of the specific extinction at the wavelength of 268 nm (K_{268}) was observed at 80% RH, suggesting the occurrence to some extent of secondary oxidation. Concerning microbial counts, the almonds stored at 60 and 70% RH presented a satisfactory microbial quality until 6 months; however, at 80% RH, the mold counts were higher than the reference values after 2 months. Several mycotoxins were detected at low levels, including aflatoxins B1 and G1, although some showed higher amounts at 80% RH. In general, it is recommended that almond producers and industrials should consider the use of low RH (< 80%) for maritime transport and long-term storage of almond kernels.

KEYWORDS
aflatoxins, microbiology, mycotoxins, packaging, peeled almonds, relative humidity, lipid oxidation, microbial counts, mycotoxins

Practical Application: High levels of relative humidity during storage/transport of almond kernels favor fungal growth, mycotoxin production, and secondary oxidation (rancidity). It is recommended to keep the almond kernels under low RH (< 80%) in maritime transport and long storage, especially in tropical countries.

1 | INTRODUCTION

Almond (*Prunus dulcis*) is a nut very appreciated worldwide since it is consumed in raw or processed form (laminated, granulated, flour), and being added to various pastries and chocolates. Consumption of in-shell almonds has grown in the last few years, with a 45% increase in the world production from 2,416,428 tons in 2009 to 3,497,148 tons in 2019 (FAOSTAT, 2019).

Almonds are a rich source of oil. They have lipid contents between 40% and 67% (dry matter, d.m.), the five major fatty acids being the oleic (18:1), linoleic (18:2), palmitic (16:0), stearic (18:0), and palmitoleic (16:1) (Yada et al., 2011). The oleic and linoleic acids generally account for about 90% of the total lipid, while saturated fatty acid levels are low (< 10%) (Yada et al., 2011). These two parameters, high lipid content and lipids with a high degree of unsaturation, affect the lipid oxidation rate and almonds' storage stability. Unsaturated fatty acids can easily suffer oxidation, producing a diversity of off-flavors and off-aromas, which cause a quality decay of nuts.

In general, almonds (in-shell) may be held for up to 9–12 months in storage without a severe loss in quality (Zacheo et al., 2000). Still, under unsuitable storage conditions, they become inedible because of the development of off-flavors and oxidative rancidity.

Until now, several studies have investigated the effect of storage regarding the fruit form (in-shell or peeled), storage time, temperature, relative humidity, and packaging material/atmosphere (Carrasco-Del Amor et al., 2016; Cornacchia et al., 2012; Garciá-Pascual et al., 2003; Guiné et al., 2014, 2015; Kazantzis et al., 2003; Lin et al., 2012; Mexis et al., 2009; Mexis & Kontominas, 2010; Raisi et al., 2015; Ziaolhagh, 2013). However, the main producing countries of almonds export this nut (in-shell or peeled), being of extreme necessity to evaluate the effect of storage conditions during transport on the product's quality and safety. Almonds can be transported by land, air, and water, although maritime transport is mainly used for long-distance tropical countries. Furthermore, maritime transport may be more cost-efficient than air transportation and is an essential mode of transportation for those goods produced far from the market and whose shelf-life exceeds the transportation time. However, there are no studies simulating conditions during almond maritime transport to tropical countries to our best knowledge. On maritime routes between regions of tropical climate (Brazil and Africa) and temperate climate (Europe), high temperatures and relative humidities may be reached during transportation, causing changes in quality and safety parameters. Furthermore, peeled almonds are more perishable than in-shell almonds since shells form a barrier to moisture and oxygen exchange. So, peeled almonds are more susceptible to water changes and lipid oxidation and may deteriorate faster than in-shell almonds (Kazantzis et al., 2003). Even though two outbreaks of salmonellosis were linked to the consumption of almonds (Lambertini et al., 2012; Mohammad et al., 2020), some storage conditions may induce mold growth and the production of mycotoxins in almonds (Diella et al., 2015; Rodrigues et al., 2012a; Varga et al., 2013). The mycotoxins more frequently reported in almonds are the aflatoxins (Ait Mimoune et al., 2018; Liao et al., 2015; Rodrigues et al., 2012a, 2012b), but the advent of multianalyte methods allowed the detection of other less common and less searched compounds (Hidalgo-Ruiz et al., 2019; Narváez et al., 2020; Rodrigues et al., 2012a, 2012b; Spadaro et al., 2020).

However, no studies have been performed on their production along defined and controlled storage conditions. Knowledge of this topic is vital as almond producers and exporters aim to maintain their products' excellent quality and safety until the end of the productive chain.

The present study intends to gain knowledge on this subject. The effect of three relative humidities (60, 70, and 80%), at a constant temperature (25°C; the choice of this temperature is explained later), was studied during peeled almond kernels storage (0, 1, 2, 4, and 6 months), simulating the maritime transport/storage of this nut to tropical countries. Several physicochemical parameters were evaluated, such as color, water activity (a_w), moisture, lipid content, quality indicators, and oxidative stability (spectrophotometric examination in the ultraviolet-specific extinctions at the wavelengths of 232 and 268 nm, and Rancimat method). The microbiological quality and presence of mycotoxins were also evaluated in the kernels.

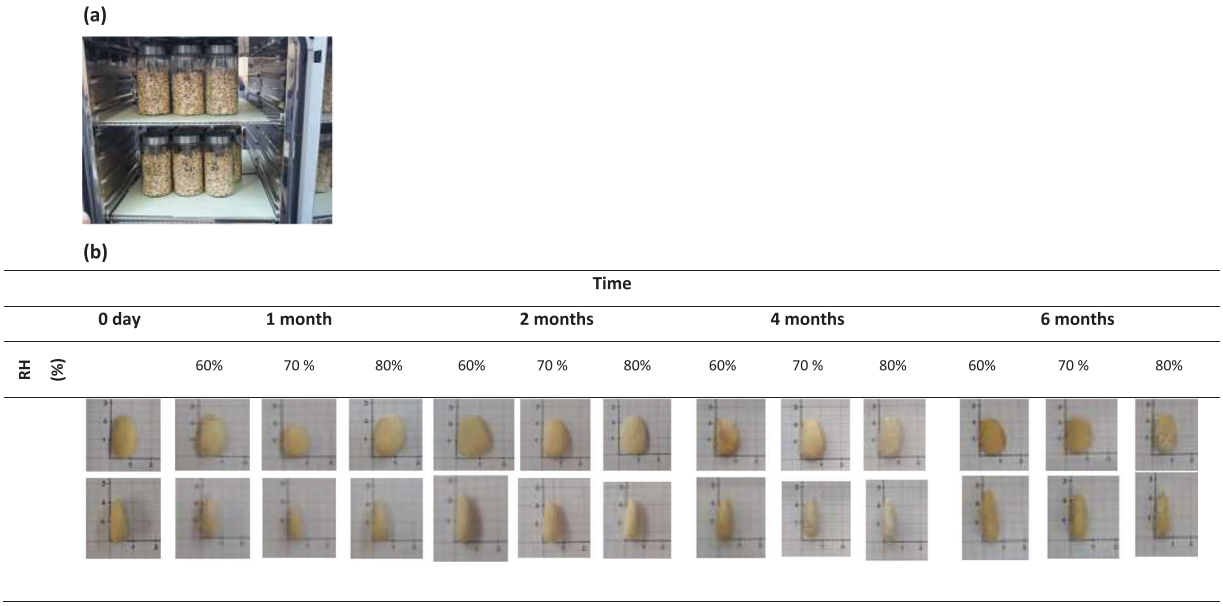

FIGURE 1 Samples at 25°C and three RH (60, 70, and 80%) (a). Visual appearance of almond kernels stored for six months (b)

2 | MATERIALS AND METHODS

2.1 | Samples

A company from Alfândega da Fé (Portugal) supplied raw almond peeled kernels (without internal brown skin) from a commercial blend of softshell varieties, as shown in Figure 1. The almonds peeling was done in a hot water bath for 2–3 min. Afterwards, the fruits were taken to the laboratory and submitted to the next section's storage conditions.

2.2 | Storage conditions

The storage conditions consisted of three relative humidities (RH) (60, 70, and 80%) at 25°C for 0, 1, 2, 4 and 6 months. According to Transport Information Service (TIC, 2021), the favorable travel temperature range for almonds is around 0°C; however, approximately, travel temperatures at 5–25°C are feasible (depending on the journey length). So, almonds do not need to be transported as chilled goods (TIC, 2021).

The choice of the temperature at 25°C was based on the fact that most of the almonds' sea transportation is not done under refrigeration. Moreover, it was intended to evaluate the effect of the maximum recommended temperature and how long the almond kernels can be subjected to this condition to prevent losing quality and guarantee their safety.

Different saturated salt solutions were prepared to obtain the RH of 60, 70, and 80%, namely sodium bromide (NaBr), potassium iodide (KI), and ammonium chloride (Greenspan, 1977), respectively. All solutions were placed in closed bottles. At each time, the samples (150 g of almond kernels) stored in each RH were taken to proceed to its physicochemical and microbiological characterization. All assays were done in triplicate. The samples were previously freeze-dried to perform the lipid analysis (Scanvac, Coolsafe, Lynge, Denmark).

2.3 | Physicochemical analysis

2.3.1 | Visual appearance, water activity, and color

At each storage time (0, 1, 2, 3, and 6 months), pictures of the almond kernels were taken to evaluate visual differences. Water activity (a_w) was determined with a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland). The color of the almond kernels was evaluated with a colorimeter Minolta CR-400 (Osaka, Japan) using the CIELab scale. L^*, a^*, and b^* coordinates, chroma (C^*) and hue angle (h) values were determined. The brightness (L^*) varies between 0 (black) and 100 (white); a^* from $^-$green to $^+$red; and b^* from $^-$blue to $^+$yellow.

2.3.2 | Moisture and lipid contents

The moisture and lipid contents were determined by drying the fresh sample to a constant weight at 105°C and extracting a known weight of freeze-dried sample

with petroleum ether for 16 h, using a Soxhlet apparatus, respectively. After the extraction period, the solvent was evaporated on a rotary evaporator. The flask with the oil was placed in an oven at 30°C and later transferred to a desiccator. The process was repeated until a constant weight was reached. The oil obtained was used to evaluate lipid oxidation (as described in the next section). All measurements were done in triplicate.

2.3.3 | Lipid oxidation—Quality parameters and oxidative stability

The extinction coefficients at 232 nm and 268 nm (K_{232} and K_{268}) were determined according to the official method described in ISO 3656:2011 (2011). Briefly, 0.05 g (for K_{232}) and 0.25 g (for K_{268}) of the oil were weighed into a 25-ml graduated flask. The volume was made up to the mark with iso-octane and homogenized. The extinction values at 232 and 268 nm were measured against iso-octane and should lay within the range of 0.1 to 0.8. When this did not happen, the measurements were repeated using more concentrated or more dilute solutions as appropriate. Then, K_{232} and K_{268} were calculated as follows: $K_{232\text{ or }268} = Extinction$ values $_{\text{at 232 or 268 nm}}/(c \times l)$, where c is the concentration of the oil solution in g/100 ml, and l is the path length of the quartz cell in cm. All analyses were carried out in triplicate.

The Rancimat test was performed in fresh nut (0.5 g) and the oil extracted (3 g). Both samples were weighed into a tube and connected to a Rancimat 743 (Metrohm, Herisau, Switzerland). Air was passed through the samples at 20 L/h while heating at 110 ± 0.2°C. The volatiles released during the oxidation of the samples were carried into a cell containing 60 ml of water. The change in conductivity of the cell was plotted on a graph. The oxidative stability was estimated by measuring the oxidation induction time corresponding to the time taken to reach the conductivity inflexion.

2.4 | Microbial quality

Almond kernels at each storage time were collected to determine the microbial quality. Ten grams of sample were mixed with 90 ml of sterile peptone water solution with 0.05% Tween 80 and homogenized. Decimal dilutions were prepared in the same diluent and plated on appropriate media in duplicate. The growth media and incubation conditions for the studied microorganisms were the following: (i) total mesophilic: Plate Count Agar (PCA, Liofilchem, Italy) for 46–72 h at 30°C (ISO 4833-2:2013) (ISO 4833-2:2013, 2013); (ii) Yeasts and molds: Dichloran Glycerol Agar (DG-18 Agar, Liofilchem, Italy) incubated at 25°C for 5 days (ISO, 21527-2:2008, 2008). All counts were expressed as $\log_{10}$ CFU/g f.w. and calculated according to ISO 7218:2007 (2007).

2.5 | Multimycotoxin analysis

2.5.1 | Sample preparation

A portion of each sample was finely ground (IKA, WERKE M20). Five grams of the ground samples were weighted into 50 ml falcon tubes and sent for analysis.

2.5.2 | Multimycotoxin analysis by LC-MS/MS

A multianalyte method of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was applied to analyze the mycotoxins present in the samples. The analyses were done at the Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna (BOKU).

The detection and quantification were performed with a QTrap 5500 MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray electrospray ionization (ESI) source and a 1290 series UHPLC system (Agilent Technologies, Waldbronn, Germany). The LC-MS/MS protocol was applied as previously described by Sulyok et al. (2020). Confirmation of positive analyte identification was obtained by acquiring two MS/MS transitions per analyte, which yields 4.0 identification points according to Commission Decision, 2002/657/EC (EC, 2002). The method's accuracy is verified for major mycotoxins on a routine basis by participation in interlaboratory testing schemes organized by BIPEA (Bureau Interprofessionnel des Etudes Analytiques, France) and by CODA-CERVA (National Reference Laboratory for mycotoxins, Belgium).

2.6 | Statistical Analysis

The statistical analysis was conducted using the SPSS version 18.0 program (SPSS Inc., Chicago, IL, USA). Firstly, the one-way analysis of variance (ANOVA) requirements, namely the normal distribution and the homogeneity of variance, were evaluated. As the normality of the data was always verified (except for mycotoxins), the results were then analyzed using ANOVA followed by Tukey's HSD test when the different groups' variances were identical. On the contrary, the Games-Howell test was applied if the

TABLE 1 Colour parameters (L^*, a^*, b^*, C^*, h) of almond kernels stored at 25°C under three relative humidities for 6 months

Colour	RH	Time (months) 0	1	2	4	6
L^*	60%	$67.81 \pm 4.01^{a,b,A}$	$65.96 \pm 2.31^{a,A}$	$71.11 \pm 4.40^{a,b,c,A,B}$	$73.32 \pm 2.32^{b,c,A}$	$76.53 \pm 4.55^{c,A}$
	70%	$67.81 \pm 4.01^{a,A}$	$67.91 \pm 5.72^{a,A}$	$67.48 \pm 4.16^{a,A}$	$73.37 \pm 0.83^{a,A}$	$74.83 \pm 3.21^{a,A}$
	80%	$67.81 \pm 4.01^{b,A}$	$63.03 \pm 1.07^{a,A}$	$74.95 \pm 2.00^{c,B}$	$73.75 \pm 1.84^{c,A}$	$73.72 \pm 2.61^{c,A}$
a^*	60%	$0.21 \pm 0.51^{a,A}$	$0.76 \pm 0.16^{a,B}$	$0.39 \pm 0.93^{a,A}$	$0.61 \pm 0.56^{a,A}$	$0.90 \pm 1.32^{a,A}$
	70%	$0.21 \pm 0.51^{a,A}$	$-0.24 \pm 0.66^{a,A}$	$-0.57 \pm 0.88^{a,A}$	$1.54 \pm 0.57^{b,B}$	$0.26 \pm 0.47^{a,A}$
	80%	$0.21 \pm 0.51^{a,A}$	$0.19 \pm 0.45^{a,A,B}$	$-0.36 \pm 0.34^{a,A}$	$0.64 \pm 0.23^{a,A}$	$0.41 \pm 1.06^{a,A}$
b^*	60%	$20.81 \pm 2.14^{a,A}$	$21.28 \pm 3.36^{a,A}$	$22.19 \pm 1.77^{a,A}$	$21.82 \pm 1.13^{a,A}$	$26.95 \pm 1.47^{b,A}$
	70%	$20.81 \pm 2.14^{a,A}$	$21.98 \pm 2.89^{a,A}$	$21.11 \pm 3.77^{a,A}$	$22.68 \pm 2.02^{a,A}$	$24.76 \pm 2.69^{a,A}$
	80%	$20.81 \pm 2.14^{a,A}$	$21.41 \pm 1.32^{a,A}$	$24.55 \pm 5.33^{a,A}$	$19.60 \pm 2.62^{a,A}$	$22.57 \pm 4.22^{a,A}$
C^*	60%	$20.82 \pm 2.13^{a,A}$	$21.32 \pm 3.40^{a,A}$	$22.21 \pm 1.75^{a,A}$	$21.84 \pm 1.13^{a,A}$	$26.99 \pm 1.46^{b,A}$
	70%	$20.82 \pm 2.13^{a,A}$	$21.59 \pm 2.21^{a,A}$	$21.13 \pm 3.77^{a,A}$	$22.77 \pm 2.04^{a,A}$	$24.77 \pm 2.69^{a,A}$
	80%	$20.82 \pm 2.13^{a,b,A}$	$21.42 \pm 1.33^{a,b,A}$	$26.00 \pm 4.99^{b,A}$	$19.61 \pm 2.61^{a,A}$	$22.59 \pm 4.23^{a,b,A}$
h	60%	$89.32 \pm 1.54^{a,A}$	$87.00 \pm 1.59^{a,A}$	$88.91 \pm 2.55^{a,A}$	$88.41 \pm 1.45^{a,B}$	$88.22 \pm 3.10^{a,A}$
	70%	$89.32 \pm 1.54^{a,b,A}$	$90.52 \pm 1.69^{b,B}$	$91.56 \pm 2.49^{b,A}$	$86.10 \pm 1.50^{a,A}$	$89.43 \pm 1.07^{b,A}$
	80%	$89.32 \pm 1.54^{a,A}$	$89.43 \pm 1.20^{a,A,B}$	$89.82 \pm 5.23^{a,A}$	$88.14 \pm 0.48^{a,A,B}$	$88.84 \pm 2.57^{a,A}$

Note: Values are expressed as: Mean ± Standard deviation. Lowercase letters—Values with different letters in the same row are statistically different ($p < 0.05$). Uppercase letters—Values with different letters in the same column are statistically different ($p < 0.05$).

different groups' variances were not similar. Mycotoxins did not follow a normal distribution, so they were analyzed by the Kruskal-Wallis test. Correlations between parameters were established with the Spearman correlation test (Spearman rho, ρ). In all tests, an α equal to 0.05 was used.

3 | RESULTS AND DISCUSSION

3.1 | Visual appearance and color

The visual appearance and color parameters are shown in Figure 1 and Table 1. Regarding Figure 1, there were no pronounced visible differences between 0 and 6 months of storage for kernels submitted at 60 and 70% RH. On the contrary, in the kernels stored at 80% RH, some visual differences were detected after four storage months. In particular, in some almonds, the white streaks were more perceptible.

Concerning the color parameters, the lightness (L^*) varied along the storage time. However, no specific trend was observed. For the RH of 60 and 80%, a significant increase in the L^* values was detected between 0 month and 6 months. On the contrary, for the RH of 70%, the L^* values remained constant, without significant changes. Guiné et al. (2015) determined a L^* of 78.10 for almonds, without internal skin, from the United States of America, reporting a higher value than those found in this work for 0 days (67.81). Furthermore, they also studied different storage conditions (ambient temperature, stove at 30 and 50°C, chamber at 30 and 50°C and 90% RH, refrigerated and frozen conditions) during 90 days. They found a decrease in the lightness in all treatments, indicative of darkening. However, this effect was more pronounced in the chamber's storage at 50°C and 90% RH and the stove at 50°C. Those results were different from ours, suggesting that the storage at 25°C did not cause such effect, indicating that this temperature is not sufficient to favor oxidation and the occurrence of Maillard reactions. Moreover, except for 2 months of storage, no significant differences were observed between the three RH conditions.

For the other color coordinates (a^*, b^*, C^* and h), with some exceptions, no significant differences were observed along the storage time and the three RH conditions. So, almond kernels stored at 25°C under different RH (60, 70, and 80%) did not suffer pronounced color changes until 6 months of storage, not leading to the formation of compounds with brown coloration.

3.2 | Water activity and moisture and fat contents

Figure 2a,b shows the water activity (a_w) and moisture contents determined in the almond kernels stored at 25°C, under different RH (60, 70, and 80%), for 6 months, respectively. The water activity is the amount of free water available for chemical and enzymatic reactions and microbial development. So, it is an essential factor to take into account the stability and shelf-life of the food. In the

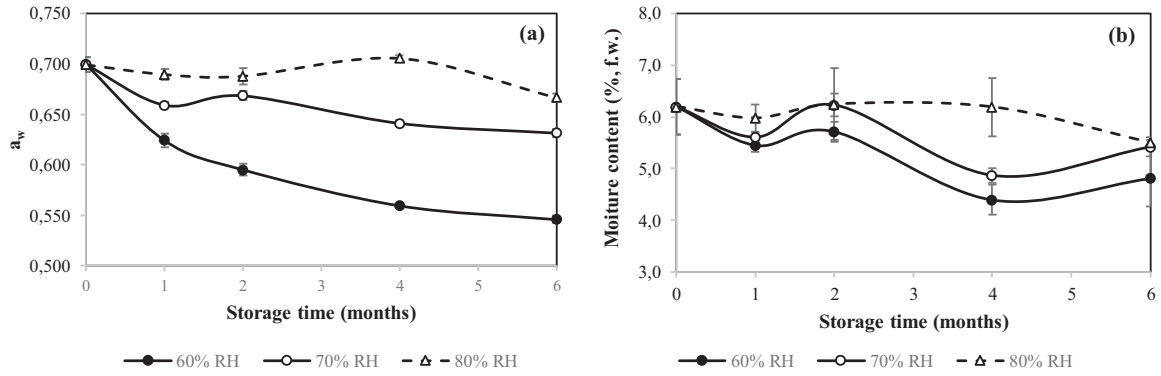

FIGURE 2 Water activity (a) and Moisture content (%, f.w.) (b) of almond kernels stored at three relative humidities (60, 70, and 80%) for 6 months

present study, significant differences in a_w values were always observed between the three RH at each storage time, being $a_{w,80\%\ RH} > a_{w,70\%\ RH} > a_{w,60\%\ RH}$. Concerning the storage period, the a_w remained almost constant at 80% RH. On the other hand, it decreased significantly during storage in 60 and 70% RH, although this decrease was more pronounced in 60% RH (0.699 to 0.546 from 0 months to 6 months). So, the a_w reduced faster when the RH of storage was low. Only almonds stored at 60% RH after 2 months achieved a_w values lower than 0.6. A_w values lower than 0.6 guarantee that the product is safe against microbial deterioration. Regarding the other RH (70 and 80%), the values stood between 0.71 and 0.63, allowing xerophilic molds' growth.

Concerning the moisture content, at day 0, it was 6.20%. Özcan et al. (2011) reported lower values than ours, varying between 3.11% for the Guara cultivar to 3.62% for Ferragnès and Nonpareil, as well as, Guiné et al. (2015) for almonds from the United States of America (≅ 4.5%). This point is critical and must be reported to the almond producers, who must perform a correct drying of the kernel before its storage; otherwise, fungus development may occur. Regarding the storage time, a decrease in moisture values was observed in all storage conditions when comparing the 0 days with 6 months. These reductions were equal to 22.4, 12.6, and 11.3% for 60, 70, and 80% RH, respectively, being more visible in 60 and 70% RH. However, the moisture contents were still 4.8 and 5.4% f.w., respectively, at the storage end. No reduction in the moisture content was observed in 80% RH until 4 months of storage, maintaining a moisture content near 6%. As moisture content and a_w are related to each other, the samples with the highest moisture contents (80% RH) also had the highest a_w values. At the end of storage, a moisture content equal to 5.5% and an a_w of 0.67 were obtained in 80% RH. These values were slightly high and may favor the growth of fungus.

Regarding the fat content, the values varied between 51% d.m and 60% d.m. No significant differences were detected during storage for the three RH and along the time.

3.3 | Lipid oxidation—Quality parameters and oxidative stability

Lipid oxidation is a major cause of quality deterioration in food (Shahidi & Zhong, 2010). The specific extinction coefficients, K_{232} and K_{268}, are shown in Table 2. The absorption at the wavelengths specified is due to the presence of conjugated diene and triene systems resulting from oxidation processes (Commission Regulation (EEC) No, 2568/91, 1991). The K_{232} values decreased significantly after 1 month, remaining almost constant until 4 months of storage for the 60 and 70% RH. On the contrary, for 80% RH, this behavior was only observed until two storage months. Nevertheless, no significant differences were detected when comparing the beginning (0 months) with the end of storage (6 months). Furthermore, when comparing different RH at the same time of storage, no significant differences were identified in almost all situations. The only exception was at 4 months of storage, where higher values were determined in 80% RH. Thus, RH values of 60, 70, and 80% did not significantly affect the production of primary products formed in the lipid oxidation during the 6 months of storage since K_{232} is related to the presence of conjugated dienes and their oxidation products (Salek et al., 2017). At the beginning and after 6 months at 25°C and 60% RH, our values of K_{232} (4.99 and 4.70, respectively) were higher than those reported by Kazantzis et al. (2003) for shelled "Ferragnès" (initial = 3.72–3.41; and 20°C and 60% RH [6 months] = 2.20–2.28), suggesting that the variety and postharvest conditions may influence the oil quality.

TABLE 2 Specific extinction coefficients (K_{232} and K_{268}) of oils extracted from almond kernels stored at 25°C under three relative humidities for 6 months

Specific extinction coefficient	RH	Time (months) 0	1	2	4	6
K_{232}	60%	$4.99 \pm 0.17^{b,A}$	$3.37 \pm 0.11^{a,A}$	$3.72 \pm 0.20^{a,A}$	$3.31 \pm 0.10^{a,A}$	$4.70 \pm 0.42^{b,A}$
	70%	$4.99 \pm 0.17^{b,A}$	$3.55 \pm 0.35^{a,A}$	$3.53 \pm 0.25^{a,A}$	$3.54 \pm 0.23^{a,A}$	$4.75 \pm 0.34^{b,A}$
	80%	$4.99 \pm 0.17^{c,A}$	$3.54 \pm 0.34^{a,A}$	$4.11 \pm 0.43^{a,b,A}$	$5.11 \pm 0.13^{c,B}$	$4.60 \pm 0.40^{b,c,A}$
K_{268}	60%	$0.06 \pm 0.01^{a,A}$	$0.06 \pm 0.01^{a,b,A}$	$0.07 \pm 0.01^{a,b,c,A}$	$0.07 \pm 0.01^{b,c,A}$	$0.08 \pm 0.01^{c,A}$
	70%	$0.06 \pm 0.01^{a,A}$	$0.07 \pm 0.01^{a,b,C}$	$0.06 \pm 0.01^{b,A}$	$0.09 \pm 0.01^{b,c,A}$	$0.10 \pm 0.02^{c,A}$
	80%	$0.06 \pm 0.01^{a,A}$	$0.06 \pm 0.01^{a,A,B}$	$0.08 \pm 0.01^{a,b,A}$	$0.14 \pm 0.01^{c,B}$	$0.09 \pm 0.02^{b,A}$

Note: Values are expressed as: Mean ± Standard deviation. Lowercase letters—Values with different letters in the same row are statistically different ($p < 0.05$). Uppercase letters—Values with different letters in the same column are statistically different ($p < 0.05$).

The specific extinction coefficient of K_{268} corresponds to the absorbance of the conjugated trienes and secondary oxidation products (Salek et al., 2017). The values of K_{268} measured during the storage are indicated in Table 2 and were lower than 0.1, except for 80% RH at 4 months (0.14). After 4 and 6 months, an increase in the values of K_{268} suggested the occurrence to some extent of secondary oxidation. On the contrary, concerning RH's effect, no significant differences were observed between the three conditions at the end of storage.

Kazantzis et al. (2003) also studied the specific extinction coefficients of two cultivars' almond kernel oil (Ferragnès and Texas). However, they reported a different behavior during storage than ours, particularly the K_{232} absorption coefficient decreased along with storage, and the values of K_{270} remained stable. Considering the present work and the UV absorption coefficients, the kernel almonds' oil quality did not deteriorate after 6 months of storage.

The aldehydes produced from hydroperoxide decomposition may be further oxidized to organic acids and other tertiary oxidation products that may be evaluated by monitoring the system conductivity changes (Shahidi & Zhong, 2010). One method that can be used to evaluate these changes is the Rancimat. The induction times obtained by the Rancimat method for the fruit and oil are shown in Figure 3. Regarding almonds (Figure 3a), the oxidative stability varied from 17.3 h (day 0) to 18.0 h after 6 months of storage at 60% RH; 19.4 h at 70% RH, and 18.0 h at 80% RH.

When comparing the induction times of almonds at the beginning (day 0) with the end of storage (6 months), no significant differences were detected for all RH studied. However, during storage, significant differences between RH were observed only at 4 months, being the lowest value detected in 60% of RH. Nevertheless, no plausible justification was found for this situation, except for a sporadic case of variability in the sample itself. Some fruit in poor condition may have been collected, causing the observed deviation. This fact demonstrates the importance of sampling significant samples to minimize sporadic contamination and harvesting isolated fruits in bad conditions, which are not representative of the general sample, subjected to the storage condition to be studied. Furthermore, when studying a specific RH along time, no definite trend was observed, indicating that the three RH applied over 6 months of storage did not induce a rancid appearance. According to our knowledge, no studies on the

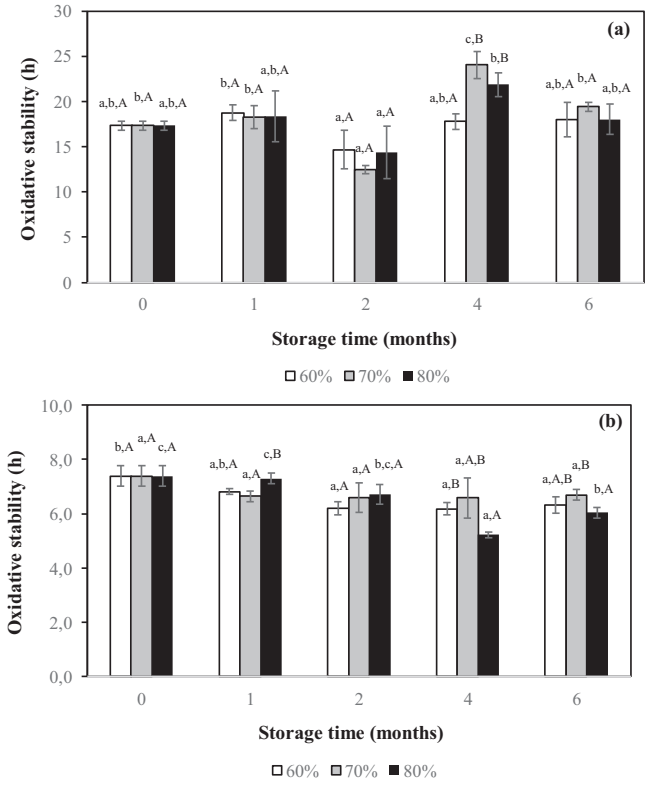

FIGURE 3 Oxidative stability (h) measured by the Rancimat method for the almond kernels (a) and the oils extracted from the almonds (b) that were stored at three RH (60, 70, and 80%). Lowercase letters compare storage times for a specific RH; uppercase letters compare RH kept at the same time

TABLE 3 Microbial quality (log CFU/g) of almond kernels stored at 25°C under three relative humidities for six months

Parameters	RH (%)	Time (months) 0	1	2	4	6
Total aerobic mesophiles (30°C)	60	$3.83 \pm 0.42^{b,A}$	$3.14 \pm 0.28^{b,A}$	$2.82 \pm 0.39^{a,b,A}$	$1.92 \pm 0.24^{a,A}$	$2.02 \pm 0.55^{a,A}$
	70	$3.83 \pm 0.43^{c,A}$	$3.14 \pm 0.04^{b,c,A}$	$2.90 \pm 0.32^{b,A}$	$2.12 \pm 0.22^{a,A}$	$2.03 \pm 0.06^{a,A}$
	80	$3.83 \pm 0.44^{a,A}$	$3.27 \pm 0.19^{a,A}$	$2.72 \pm 0.21^{a,A}$	$3.29 \pm 1.38^{a,A}$	$2.02 \pm 0.55^{a,A}$
Molds	60	$2.06 \pm 0.32^{a,A}$	$1.70 \pm 0.01^{a,A}$	$1.77 \pm 0.13^{a,A}$	$<1.7^{a,A}$	$<1.7^{a,A}$
	70	$2.06 \pm 0.33^{a,A}$	$1.80 \pm 0.17^{a,A}$	$<1.7^{a,A}$	$<1.7^{a,A}$	$<1.7^{a,A}$
	80	$2.06 \pm 0.34^{a,A}$	$1.76 \pm 0.10^{a,A}$	$2.18 \pm 0.52^{a,A}$	$5.45 \pm 0.05^{c,B}$	$4.49 \pm 0.49^{b,B}$
Yeast	60	<1.7	<1.7	<1.7	<1.7	<1.7
	70	<1.7	<1.7	<1.7	<1.7	<1.7
	80	<1.7	<1.7	<1.7	<1.7	<1.7

Note: Values are expressed as: Mean ± Standard deviation. Lowercase letters—Values with different letters in the same row are statistically different ($p < 0.05$). Uppercase letters—Values with different letters in the same column are statistically different ($p < 0.05$).

oxidative stability of almonds (fruit) have been done until now.

Concerning the oil extracted from almonds, some significant changes were detected. However, the mean values only varied between 5.2 h and 7.4 h, being the lowest value obtained after 4 months and 80% RH. However, this decrease was not observed at 6 months. The induction time at day 0 for oil extracted from shelled almonds was 7.4 h. This value was low when compared with other oils extracted from almonds. Arranz et al. (2008) reported a value of 21.8 h, and Houmy et al. (2016) mentioned values between 20.28 h and 27.55 h for Marcona and Ferragnès/Ferraduel mixture, respectively. These significant differences probably may be due to the different temperatures used in the Rancimat method (110°C in the present study and 100°C in the other studies) and different almond varieties. At the end of storage (6 months), 70% RH showed the highest value of induction time (6.7 h), while 80% RH presented the lowest value (6.0 h). Higher relative humidity may cause faster oxidation; however, the present work results did not clearly demonstrate this situation. Nevertheless, high moisture contents may increase the enzymatic activity and facilitate the degradation of oils by lipases, producing free fatty acids, and lipoxygenases that also oxidize polyunsaturated compounds, producing undesirable flavors (Salunkhe & Desai, 1986).

Comparing the fruit with the oil, the former had higher oxidative stability, approximately twice as high as the oil extracted from almonds. This difference may be because the fruit's measurement is more direct, inducing less lipid oxidation than in the oil that has been previously extracted.

3.4 | Microbial quality

The microbial loads of almond kernels stored under different RH for 6 months are described in Table 3. The total aerobic mesophiles (30°C) varied between 1.92 log CFU/g (4 months and 60% RH) and 3.83 (at the beginning), with a decrease along time for 60 and 70% RH but not at 80% RH. No significant differences were observed between mesophiles in the three RH; however, the standard deviations were high in some cases, such as 4 months and 80% RH. Aerobic mesophile loads showed a strong positive correlation with a_w (Spearman $\rho = 0.610$, $p < 0.001$) and a negative correlation with time of storage ($\rho = -0.739$, $p < 0.001$), suggesting that low a_w is effective in the control of bacterial growth, and microorganisms progressively lose viability throughout time under these adverse conditions.

As for molds, values between < 1.7 and 5.45 log CFU/g were determined, being the highest counts observed at 4 and 6 months at 80% RH, where a_w was also the highest. No significant differences were observed for the molds at 60 and 70% RH. Molds showed a moderate correlation with a_w ($\rho = 0.454$, $p = 0.004$) but not with time ($\rho = 0.264$, $p = 0.105$). Molds are more xerophilic than bacteria and more resistant to prolonged drought, and a_w values as low as 0.706 are enough to allow fungal growth. A similar trend was detected in cashew nuts when higher RH increased the molds' growth during storage (12 days) (Onilude et al., 2010). However, these authors noticed an increase at 70% RH, while in the present study, that trend was observed at 80% RH only. Concerning yeasts, the values were always under 1.7 log CFU/g.

Considering the microbiological results, the almonds stored at different RH presented a satisfactory microbial quality until 6 months at 60 and 70% RH; however, at 80% RH, the kernels remained satisfactory only until 2 months. During these periods, our values were consistently lower than the limits established for dried fruits for the microbial counts at 30°C by the FCD (2016) and Gilbert et al. (2000) (< 4 and < 5 log CFU/g, respectively) and for molds and yeasts by Witthuhn et al. (2005) and FCD (2016) (< 3 and < 3.7 log CFU/g, respectively). After 4 months

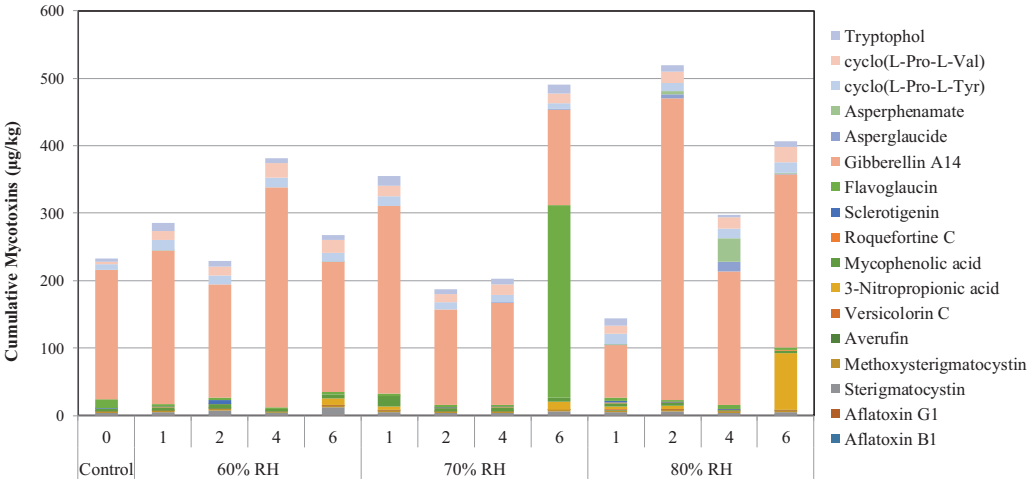

FIGURE 4 Cumulative mycotoxins in almonds before treatment application (control) and after 1, 2, 4, and 6 months of preservation at 60, 70, and 80% RH

of storage at 80% RH, the mold counts surpassed the reference values, probably due to the high a_w values still observed in the kernels (a_w = 0.706 at 4 months) that contributed to the mold's growth.

3.5 | Mycotoxin contamination

Figure 4 shows the different metabolites detected in almonds throughout the 6 months of storage under different RH. In total, 17 fungal metabolites were detected in the analyzed almonds. From those, only Gibberillin A14 is not related to the fungal genera *Aspergillus* and *Penicillium*. To our knowledge, none of the detected metabolites has been reported previously for almonds, except aflatoxins, the only mycotoxins with maximum admissible values set by European legislation (EC, 2006, 2010).

Aspergillus section *Flavi*-related aflatoxins B1 (AFB1) and G1 (AFG1), and other compounds derived from the aflatoxin biosynthetic route—sterigmatocystin (ST), methoxysterigmatocystin (MST), averufin (AV), versicolorin C (VC)—were detected in small amounts in all different treatments, and no significant differences were detected between times of storage or treatments ($p > 0.050$). AFB1 was detected in only five samples (60% RH/2 months; 70% RH/1 and 4 months; 80% RH/2 and 6 months) in very low amounts (0.3-0.8 µg/kg). AFG1 was detected in almost all tested samples with concentrations of 0.2 µg/kg, with higher amounts being determined in only two samples: 70% RH/1 month (1.7 µg/kg) and 80% RH/2 months (4 µg/kg). These amounts are well below the legislated limits of 8 µg/kg for AFB1, and 10 µg/kg for the sum of aflatoxins B1, B2, G1, and G2 (EC, 2006, 2010), but they may be a reflection of the effect of moisture and a_w in the safety of nuts. ST, MST and AV were detected in all samples in average amounts of 4.5 ± 1.3 µg/kg, 2.2 ± 0.8 µg/kg and 0.4 ± 0.2 µg/kg, respectively. This relatively uniform distribution makes us consider that *Aspergillus* section *Flavi* can grow and partially pursue the AF biosynthetic route. Still, AFs are not produced under the tested storage conditions. These toxins showed to be moderately correlated with each other ($0.421 < \rho < 0.699$; $p < 0.008$), but not with a_w or RH. In a study by Rodrigues et al. (2012b), where almonds were always kept at a_w below 0.7, *Aspergillus flavus* and related species could persist or even increase their loads but could not produce AFs. On the other hand, Saleemullah et al. (2006) studied the effect of storage on the AF contamination of almonds and concluded that moisture increase throughout storage significantly affected the level of contamination. In that study, the moisture content of almonds inoculated with aflatoxigenic *A. flavus* increased from 2.7% to 41.3%, after 3 and 18 months of storage, respectively, and the AF contamination increased from nondetected to 12 µg/kg by the end of the storage period.

3-Nitropropionic acid (NPA), cyclo(L-Pro-L-Tyr) (CPT), and cyclo(L-Pro-L-Val) (CPV), which are also associated with *A. flavus* (Frisvad et al., 2019), were generally detected in higher amounts in 6 months and 80 RH% samples. NPA amounts were significantly higher in 6 months than in 2 and 4 months ($p < 0.012$); CPT and CPV were higher in 80% RH than in control ($p < 0.033$); and CPV was higher in four ($p = 0.021$) and six months ($p = 0.015$) than in control. Asperglaucide, produced by the xerophilic fungus *Aspergillus glaucus* (Chen et al., 2017) and asperphenamate (APM), mostly associated with *Penicillium brevicompactum* (Frisvad et al., 2004; Visagie et al., 2014), but also produced by the xerophilic *Aspergillus pseudoglaucus* (Chen et al., 2017), showed a specific trend in terms of RH, being present in significantly higher amounts in

80% RH samples ($p < 0.050$) and were significantly correlated with each other ($\rho = 0.456$; $p < 0.004$). These were the only toxins showing a moderate (but significant) correlation with the applied treatment ($\rho = 0.649$, $p < 0.001$; and $\rho = 0.475$, $p < 0.004$, respectively) and with the mold counts ($\rho = 0.558$, $p < 0.001$; and $\rho = 0.356$, $p < 0.026$, respectively). In fact, samples submitted to 80% RH showed a_w values near to, or even slightly higher than 0.7 (at 4 months of storage, the average a_w was 0.706) and moisture content near 6%, which are the values generally recognized as safe for the adequate preservation of almonds in terms fungal growth and mycotoxin production. This means that, in storage at 80% RH, even small increases in RH that can be caused by the basal metabolism of xerophilic fungi such as *A. glaucus* and *A. pseudoglaucus*, can render the adequate conditions for other fungi to grow (e.g., *A. flavus*) and to produce other highly toxic compounds like AFs.

Penicillium-related mycotoxins—mycophenolic acid (MPA), quinolactacin A (QLA), roquefortine C (roqC) (Frisvad et al., 2004; Visagie et al., 2014)—were found in small amounts, and no correlation was detected with the type of treatment ($\rho < 0.200$, $p > 0.05$).

Gibberellin A14 is the metabolite showing the highest amounts in all samples, with no significant differences among samples. Even though gibberellins are generally known as phytohormones, gibberellin A14 has been reported as a fungal metabolite produced by *Gibberella* (= *Fusarium*) *fujikuroi* (Jones et al., 1968). This compound is not well characterized, and its toxicity has not been established.

4 | CONCLUSION

This study showed that high RH maintenance (80%) during the transport/storage of peeled almonds might cause some visual defects after four months of transport plus storage. Furthermore, moisture contents and a_w values of 5.5% and 0.67 can be obtained, favoring fungus growth. The mold counts increased at 4 and 6 months of storage. Even though the three RH did not clearly affect the production of primary and secondary products formed in the lipid oxidation during the 6 months of storage, sometimes an increase in the values of K_{268} was observed at 80% RH, suggesting the occurrence to some extent of secondary oxidation. The almonds stored for 6 months presented a satisfactory microbial quality only when stored at 60 and 70% RH. Furthermore, some mycotoxins were detected in higher amounts at 80% RH. Thus, keeping the almond kernels under low RH (< 80%) in maritime transport and long storage in tropical countries is recommended.

AUTHOR CONTRIBUTIONS

Luana Fernandes: Methodology; Writing – original draft. **Francieli Graeff**: Methodology. **Arij Jelassi**: Methodology. **Michael Sulyok**: Methodology; Validation. **Carolina Garcia**: Supervision. **Nuno Rodrigues**: Methodology. **José Alberto Pereira**: Methodology; Validation. **Albino Bento**: Supervision. **Alifa Kahoun**: Supervision. **Paula Rodrigues**: Methodology; Supervision; Writing – review & editing. **Ermelinda Lopes Pereira**: Methodology; Supervision; Writing – review & editing. **Elsa Ramalhosa**: Conceptualization; Methodology; Supervision; Writing – review & editing.

ACKNOWLEDGMENTS

This work was supported by the PDR2020 Partnership Agreement and co-financed by FEADER, Portugal 2020 (ValNuts Project: PDR2020-101-030756); and the Foundation for Science and Technology (FCT, Portugal), which gave financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021). The authors also thank Amendouro – Comércio e Indústria de Frutos Secos, Lda. (Dr Joana Araújo) for supplying the fruits.

CONFLICT OF INTEREST

None declared.

ORCID

Michael Sulyok https://orcid.org/0000-0002-3302-0732
Paula Rodrigues https://orcid.org/0000-0002-3789-2730
Elsa Ramalhosa https://orcid.org/0000-0003-2503-9705

REFERENCES

Ait Mimoune, N., Arroyo-Manzanares, N., Gámiz-Gracia, L., García-Campaña, A. M., Bouti, K., Sabaou, N., & Riba, A. (2018). *Aspergillus* section *Flavi* and aflatoxins in dried figs and nuts in Algeria. *Food Additives & Contaminants: Part B*, *11*(2), 119–125. https://doi.org/10.1080/19393210.2018.1438524

Arranz, S., Cert, R., Pérez-Jiménez, J., Cert, A., & Saura-Calixto, F. (2008). Comparison between free radical scavenging capacity and oxidative stability of nut oils. *Food Chemistry*, *110*(4), 985–990. https://doi.org/10.1016/j.foodchem.2008.03.021

Carrasco-Del Amor, A. M., Aguayo, E., Collado-González, J., Guy, A., Galano, J.-M., Durand, T., & Gil-Izquierdo, Á. (2016). Impact of packaging atmosphere, storage and processing conditions on the generation of phytoprostanes as quality processing compounds in almond kernels. *Food Chemistry*, *211*, 869–875. https://doi.org/10.1016/j.foodchem.2016.05.132

Chen, A. J., Hubka, V., Frisvad, J. C., Visagie, C. M., Houbraken, J., Meijer, M., Varga, J., Demirel, R., Jurjević, Ž., Kubátová, A., Sklenář, F., Zhou, Y. G., & Samson, R. A. (2017). Polyphasic taxonomy of *Aspergillus* section *Aspergillus* (formerly *Eurotium*), and its occurrence in indoor environments and food. *Studies in Mycology*, *88*, 37–135. https://doi.org/10.1016/j.simyco.2017.07.001

Commission Regulation (EEC) No 2568/91. (1991). Of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. http://data.europa.eu/eli/reg/1991/2568/oj

Cornacchia, R., Amodio, M. L., & Colelli, G. (2012). Effects of storage temperature and duration on quality of unshelled and shelled almonds. *Acta Horticulturae*, *934*, 783–790. https://doi.org/10.17660/ActaHortic.2012.934.103

Diella, G., De Giglio, O., Vallone, L., Iriti, M., Caggiano, G., & Montagna, M. T. (2015). Regulations relating to mycotoxins in almonds in European context. *Annali Di Igiene: Medicina Preventiva E Di Comunita*, *27*(3), 533–538. https://doi.org/10.7416/ai.2015.2044

EC. (2002). Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. https://op.europa.eu/en/publication-detail/-/publication/ed928116-a955-4a84-b10a-cf7a82bad858/language-en

EC. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, *L364*, 5–24.

EC. (2010). Commission Regulation No 165/2010 of 26 February 2010 amending No 1881/2006 as regards aflatoxins. *Official Journal of the European Union*, *L50*, 8–12.

FAOSTAT. (2019). Production—Crops. http://www.fao.org/faostat/en/#data

FCD. (2016). Microbiological criteria applicable from 2015 to private labels, entry-level brands and raw materials in their initial industrial packaging. http://www.fcd.fr/media/filer_public/cb/d0/cbd057e4-e8b3-4ebd-b298-34f92c85267b/1201_fcd_criteres_microbiologiques_2016_produits_ls_mp_28012016.pdf

Frisvad, J. C., Hubka, V., Ezekiel, C. N., Hong, S. - B., Nováková, A., Chen, A. J., Arzanlou, M., Larsen, T. O., Sklenář, F., Mahakarnchanakul, W., Samson, R. A., & Houbraken, J. (2019). Taxonomy of *Aspergillus* section *Flavi* and their production of aflatoxins, ochratoxins and other mycotoxins. *Studies in Mycology*, *93*, 1–63. https://doi.org/10.1016/j.simyco.2018.06.001

Frisvad, J. C., Smedsgaard, J., Larsen, T. O., & Samson, R. A. (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Studies in Mycology*, *49*, 201–241.

Garcıá-Pascual, P., Mateos, M., Carbonell, V., & Salazar, D. M. (2003). Influence of storage conditions on the quality of shelled and roasted almonds. *Biosystems Engineering*, *84*(2), 201–209. https://doi.org/10.1016/S1537-5110(02)00262-3

Gilbert, R. J., de Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C. D., Richards, J., Roberts, D., & Bolton, F. J. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. *Communicable Disease and Public Health*, *3*(3), 163–167.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards Section A: Physics and Chemistry*, *81A*(1), 89. https://doi.org/10.6028/jres.081A.011

Guiné, R. P. F., Almeida, C. F. F., & Correia, P. M. R. (2014). *Efeito da embalagem nas propriedades fisico-químicas de amêndoas durante o armazenamento*. *Jornadas fruteiras tradicionais do algarve* AssociaçãoIn Loco - Equipa do Projecto Cordão Verde, (p. 10). https://core.ac.uk/download/pdf/70645455.pdf

Guiné, R. P. F., Almeida, C. F. F., Correia, P. M. R., & Mendes, M. (2015). Modelling the influence of origin, packing and storage on water activity, colour and texture of almonds, hazelnuts and walnuts using artificial neural networks. *Food and Bioprocess Technology*, *8*(5), 1113–1125. https://doi.org/10.1007/s11947-015-1474-3

Hidalgo-Ruiz, J. L., Romero-González, R., Martínez Vidal, J. L., & Garrido Frenich, A. (2019). Determination of mycotoxins in nuts by ultra high-performance liquid chromatography-tandem mass spectrometry: Looking for a representative matrix. *Journal of Food Composition and Analysis*, *82*, 103228. https://doi.org/10.1016/j.jfca.2019.05.011

Houmy, N., Mansouri, F., Benmoumen, A., Elmouden, S., Boujnah, M., Sindic, M., Fauconnier, M.-L., Serghini-Caid, H., & Elamrani, A. (2016). Characterization of almond kernel oils of five almonds varieties cultivated in Eastern Morocco. In Kodad O., López-Francos A., Rovira M., & Socias i Company R. (Eds.), *XVI GREMPA Meeting on almonds and pistachios*, CIHEAM, (pp. 317–321).

ISO. (2011). ISO 3656:2011—Animal and vegetable fats and oils—Determination of ultraviolet absorbance expressed as specific UV extinction.

ISO 4833-2:2013. (2013). Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 2: Colony count at 30°C by the surface plating technique.

ISO 7218:2007. (2007). Microbiology of food and animal feeding stuffs—General requirements and guidance for microbiological examinations.

ISO 21527-2:2008. (2008). Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of yeasts and moulds—Part 2: Colony count technique in products with water activity less than or equal to 0.95.

Jones, K. C., West, C. A., & Phinney, B. O. (1968). Isolation, identification and biological properties of gibberellin A14 from *Gibberella fujikuroi*. *Phytochemistry*, *7*(2), 283–IN2. https://doi.org/10.1016/S0031-9422(00)86326-8

Kazantzis, I., Nanos, G. D., & Stavroulakis, G. G. (2003). Effect of harvest time and storage conditions on almond kernel oil and sugar composition. *Journal of the Science of Food and Agriculture*, *83*(4), 354–359. https://doi.org/10.1002/jsfa.1312

Lambertini, E., Danyluk, M. D., Schaffner, D. W., Winter, C. K., & Harris, L. J. (2012). Risk of salmonellosis from consumption of almonds in the North American market. *Food Research International*, *45*(2), 1166–1174. https://doi.org/10.1016/j.foodres.2011.05.039

Liao, C.-D., Wong, J. W., Zhang, K., Yang, P., Wittenberg, J. B., Trucksess, M. W., Hayward, D. G., Lee, N. S., & Chang, J. S. (2015). Multi-mycotoxin analysis of finished grain and nut products using ultrahigh-performance liquid chromatography and positive electrospray ionization–quadrupole orbital ion trap high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry*, *63*(37), 8314–8332. https://doi.org/10.1021/jf505049a

Lin, X., Wu, J., Zhu, R., Chen, P., Huang, G., Li, Y., Ye, N., Huang, B., Lai, Y., Zhang, H., Lin, W., Lin, J., Wang, Z., Zhang, H., & Ruan, R. (2012). California almond shelf life: Lipid deterioration during storage. *Journal of Food Science*, *77*(6), C583–C593. https://doi.org/10.1111/j.1750-3841.2012.02706.x

Mexis, S. F., Badeka, A. V., & Kontominas, M. G. (2009). Quality evaluation of raw ground almond kernels (*Prunus dulcis*): Effect of active and modified atmosphere packaging, container

oxygen barrier and storage conditions. *Innovative Food Science & Emerging Technologies*, *10*(4), 580–589. https://doi.org/10.1016/j.ifset.2009.05.002

Mexis, S. F., & Kontominas, M. G. (2010). Effect of oxygen absorber, nitrogen flushing, packaging material oxygen transmission rate and storage conditions on quality retention of raw whole unpeeled almond kernels (*Prunus dulcis*). *LWT – Food Science and Technology*, *43*(1), 1–11. https://doi.org/10.1016/j.lwt.2009.06.024

Mohammad, Z. H., Murano, E. A., Moreira, R. G., & Castillo, A. (2020). Effect of post inoculation drying procedures on the reduction of *Salmonella* on almonds by thermal treatments. *Food Research International*, *130*, 108857. https://doi.org/10.1016/j.foodres.2019.108857

Narváez, A., Rodríguez-Carrasco, Y., Castaldo, L., Izzo, L., Graziani, G., & Ritieni, A. (2020). Occurrence and exposure assessment of mycotoxins in ready-to-eat tree nut products through ultra-high performance liquid chromatography coupled with high resolution Q-Orbitrap mass spectrometry. *Metabolites*, *10*(9), 344. https://doi.org/10.3390/metabo10090344

Onilude, A. A., Igbinadolor, R. O., & Wakil, S. M. (2010). Effect of time and relative humidity on the microbial load and physical quality of cashew nuts (*Anacardium occidentale* L) under storage. *African Journal of Microbiology Research*, *4*(19), 1939–1944.

Özcan, M. M., Ünver, A., Erkan, E., & Arslan, D. (2011). Characteristics of some almond kernel and oils. *Scientia Horticulturae*, *127*(3), 330–333. https://doi.org/10.1016/j.scienta.2010.10.027

Raisi, M., Ghorbani, M., Sadeghi Mahoonak, A., Kashaninejad, M., & Hosseini, H. (2015). Effect of storage atmosphere and temperature on the oxidative stability of almond kernels during long term storage. *Journal of Stored Products Research*, *62*, 16–21. https://doi.org/10.1016/j.jspr.2015.03.004

Rodrigues, P., Venâncio, A., & Lima, N. (2012a). Aflatoxigenic fungi and aflatoxins in Portuguese almonds. *The Scientific World Journal*, *2012*, 1–9. https://doi.org/10.1100/2012/471926

Rodrigues, P., Venâncio, A., & Lima, N. (2012b). Mycobiota and mycotoxins of almonds and chestnuts with special reference to aflatoxins. *Food Research International*, *48*(1), 76–90. https://doi.org/10.1016/j.foodres.2012.02.007

Saleemullah, I. A. K., Khalil, I. A., & Shah, H. (2006). Aflatoxin contents of stored and artificially inoculated cereals and nuts. *Food Chemistry*, *98*(4), 699–703. https://doi.org/10.1016/j.foodchem.2005.06.034

Salek, R. N., Burešová, I., Kráčmar, S., Lorencová, E., Zálešáková, L., & Dabash, V. (2017). Evaluation of selected physicochemical parameters of extra virgin olive oil commercialized in the Czech market and stored during a period of 5 months. *Potravinarstvo*, *11*(1), 664–672. https://doi.org/10.5219/823

Salunkhe, D. K., & Desai, B. B. (1986). *Postharvest biotechnology of oilseeds*. CRC Press.

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, *39*(11), 4067. https://doi.org/10.1039/b922183m

Spadaro, D., Meloni, G. R., Siciliano, I., Prencipe, S., & Gullino, M. L. (2020). HPLC-MS/MS method for the detection of selected toxic metabolites produced by Penicillium spp. in nuts. *Toxins*, *12*(5), 307. https://doi.org/10.3390/toxins12050307

Sulyok, M., Stadler, D., Steiner, D., & Krska, R. (2020). Validation of an LC-MS/MS-based dilute-and-shoot approach for the quantification of >500 mycotoxins and other secondary metabolites in food crops: Challenges and solutions. *Analytical and Bioanalytical Chemistry*, *412*(11), 2607–2620. https://doi.org/10.1007/s00216-020-02489-9

TIC. (2021). Transport Information Service—Almonds. https://www.tis-gdv.de/tis_e/ware/nuesse/mandeln/mandeln-htm/#temperatur

Varga, E., Glauner, T., Berthiller, F., Krska, R., Schuhmacher, R., & Sulyok, M. (2013). Development and validation of a (semi-)quantitative UHPLC-MS/MS method for the determination of 191 mycotoxins and other fungal metabolites in almonds, hazelnuts, peanuts and pistachios. *Analytical and Bioanalytical Chemistry*, *405*(15), 5087–5104. https://doi.org/10.1007/s00216-013-6831-3

Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S.-B., Klaassen, C. H. W., Perrone, G., Seifert, K. A., Varga, J., Yaguchi, T., & Samson, R. A. (2014). Identification and nomenclature of the genus *Penicillium*. *Studies in Mycology*, *78*, 343–371. https://doi.org/10.1016/j.simyco.2014.09.001

Witthuhn, R. C., Engelbrecht, S., Joubert, E., & Britz, T. J. (2005). Microbial content of commercial South African high-moisture dried fruits. *Journal of Applied Microbiology*, *98*(3), 722–726. https://doi.org/10.1111/j.1365-2672.2004.02500.x

Yada, S., Lapsley, K., & Huang, G. (2011). A review of composition studies of cultivated almonds: Macronutrients and micronutrients. *Journal of Food Composition and Analysis*, *24*(4–5), 469–480. https://doi.org/10.1016/j.jfca.2011.01.007

Zacheo, G., Cappello, M. S., Gallo, A., Santino, A., & Cappello, A. R. (2000). Changes associated with post-harvest ageing in almond seeds. *LWT – Food Science and Technology*, *33*(6), 415–423. https://doi.org/10.1006/fstl.2000.0679

Ziaolhagh, H. (2013). Effect of packaging on shelf life of almond kernels. *International Journal of Agriculture and Crop Sciences*, *5*(1), 15–20.

How to cite this article: Fernandes, L., Graeff, F., Jelassi, A., Sulyok, M., Garcia, C., Rodrigues, N., Pereira, J. A., Bento, A., Kanoun, A., Rodrigues, P., Pereira, E. L., & Ramalhosa, E. (2022). Effect of relative humidity on the quality and safety of peeled almond kernels (*Prunus dulcis* Mill.) during simulated maritime transport/storage. *Journal of Food Science*, *87,* 5363–5374. https://doi.org/10.1111/1750-3841.16369