Received: 29 June 2022 | Revised: 23 November 2022 | Accepted: 20 December 2022

DOI: 10.1111/1750-3841.16453

Journal of Food Science WILEY

ORIGINAL ARTICLE

Food Microbiology and Safety

Effect of different storage conditions on the stability and safety of almonds

Paula Rodrigues[1] | **Arij Jelassi**[2] | **Elifa Kanoun**[2] | **Michael Sulyok**[3] | **Paula Correia**[4] | **Elsa Ramalhosa**[1] | **Ermelinda Lopes Pereira**[1]

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

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

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

[4]CERNAS Research Centre, Polytechnic Institute of Viseu, Viseu, Portugal

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

Funding information
PDR2020 Partnership Agreement; FEADER, Portugal 2020, Grant/Award Number: PDR2020-101-03,0756; Foundation for Science and Technology (FCT, Portugal)FCT/MCTES; CERNAS, Grant/Award Number: UIDB/00681/2020; European Agricultural Fund for Rural Development; CIMO, Grant/Award Number: UIDB/00690/2020

Abstract: Almond production in Portugal is of great importance for the economy of their main producing areas. However, the contamination of these nut fruits with fungi and mycotoxins poses a significant risk to food safety and security. This work intended to evaluate the influence of storage conditions on the microbial and mycotoxin stability and safety of almonds throughout long-term storage. Two almond varieties—Lauranne and Guara—were submitted to three different storage conditions, namely, 4°C with noncontrolled relative humidity (RH), 60% RH at 25°C, and 70% RH at 25°C, for a storage period of 9 months. Samples were collected after 0, 3, 6, and 9 months of storage and analyzed for microbial loads (aerobic mesophiles, yeasts, and molds), mold incidence and diversity, and mycotoxin contamination. In total, 26 species were identified belonging to 6 genera: *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Paecilomyces*, and *Talaromyces*. For the variety Guara, mycotoxins related to *Aspergillus* sect. *Flavi*, such as aflatoxins, averufin, versicolorin C, and norsolorinic acid, were detected only after 9 months of storage at 70% and 60% RH. *Penicillium* mycotoxins, such as quinolactacin A and roquefortine C, were also detected. For the variety Lauranne, *Penicillium* mycotoxins were detected, such as citrinin, quinolactacins A and B, roquefortines C and D, cyclopenin, cyclopenol, penitrem A, viridicatin, and viridicatol. Mycotoxins related to *Aspergillus*, such as aspulvinone E, flavoglaucin, paspalin, asperglaucide, asperphenamate, cyclo(L-Pro-L-Tyr), and cyclo(L-Pro-L-Val), were also detected.

KEYWORDS
food safety, mycotoxins

to maintain the stability and safety of nuts in terms of microbial and mycotoxin contaminations.

1 | INTRODUCTION

Almond (*Prunus dulcis* [Mill.] D. A. Webb; syn. *Prunus amygdalus*, *Amygdalus communis*, and *Amygdalus dulcis*) is the most important nut in the world in terms of commercial production. Based on FAO (https://www.fao.org/faostat/en/#data/QCL, accessed 21/06/2022), the production of almond in shell is constantly increasing worldwide, and it was, as of 2020, at around 4.1 million tons. Almonds are produced mainly in the United States (47.5%) and Asia (19.5%), followed by Europe (17%), Africa (12.7%), and Oceania (3.4%).

In Portugal, almonds are produced mostly in the northeast region of Trás-os-Montes and the southernmost region of Algarve. The region of Trás-os-Montes integrates one Protected Designation of Origin (PDO), "Amêndoa do Douro," since 1996 (EC, 1996).

Even though Portugal is responsible for only 0.5% of almonds worldwide production, this commodity represents significant cultural and economic incomes for local populations since, under the traditional culturing methods; no major inputs are made other than harvest. Moreover, Portuguese almonds are usually exported as high-value products to other European countries, such as Spain, France, and Germany (Rodrigues et al., 2012a). However, very stringent safety parameters are established between commercial partners.

The almond kernel is valued for its particular physicochemical, nutritional (Massantini & Frangipane, 2022), and sensory characteristics, and it has a high market potential as an ingredient in many snacks and other processed foods as well as in the pharmaceutical and cosmetic industries (Blaak & Staib, 2022; Čolić et al., 2019). Almonds are rich in nutrients. A standard serving of 28 g (as a % of the daily value, DV) is an excellent source (containing >20% DV) of monounsaturated fatty acids (MUFA) (67%), vitamin E (36.4%), and manganese (36.0%), and a good source (10% > DV > 20%) of magnesium (19.5%), copper (16.0%), phosphorus (13.4%), fiber (13.2%), riboflavin (13.5%), and protein (12.1%) (Chen et al., 2006). In addition, almonds are the least allergenic tree nut and contain minute quantities of cyanogenic glycosides (Tomishima et al., 2022). Moreover, almonds also contain phenolic and polyphenolic compounds at 118.5 mg per serving (Chen et al., 2006).

Almonds have anti-inflammatory and antioxidant activities due to their content of α-tocopherol and polyphenols, including flavonoids, not forgetting the anticancer food components and the neuroprotective, hepatoprotective, and cholesterol-lowering properties (Berryman et al., 2011; Seo et al., 2015).

Nevertheless, almonds are known to be a favorable matrix for the growth of various microorganisms, including toxigenic and pathogenic fungal species (Tournas et al., 2015). Because of their hygroscopicity, nuts may be susceptible to undesirable variations during storage, such as mycotoxigenic fungi contamination and mycotoxin accumulation. In addition, postharvest transportation and storage could degrade both the nutritional value and the taste of the nuts (Rodrigues et al., 2012b).

As a result of the general contamination of foods with mycotoxigenic fungi, mycotoxins control in food is of utmost importance to safeguard consumer's health. Maximum Tolerable Levels (MTL) for the major mycotoxins present in food products have been established by the European Community (EC, 2006), with regular revisions and amendments. Nuts, in particular, are only regulated for their MTLs of aflatoxins (AFs). MTLs of aflatoxin B1 (AFB1) and total AFs are set to 12.0 and 15.0 μg/kg in almonds, respectively, when intended for further sorting or other physical treatment, before human consumption or use as an ingredient in foodstuffs. In ready-to-eat almonds, MTLs of AFB1 and total AFs are reduced to 8.0 and 10.0 μg/kg for almonds, respectively (EC, 2010). There were 400 notifications for mycotoxins in the Rapid Alert System for Food and Feed in 2020 (RASFF, 2021). Nuts, nut products, and seeds are involved in most of the notifications.

The general objective of this work was to establish postharvest long-term storage conditions to reduce loss and ensure the quality and safety of almonds, relating to fungal development and mycotoxin contamination. The specific aims of the work were as follows: (i) to study the effect of different storage conditions on microbial loads, mold diversity, and mycotoxin contamination of almonds throughout long-term storage (9 months); (ii) to identify the prevalent molds, and to establish a correlation between fungi and mycotoxins in the studied fruits and conditions; (iii) to establish the best postharvest condition to reduce loss and ensure quality and safety of almonds, mostly in terms of fungal development and mycotoxin presence.

2 | MATERIALS AND METHODS

2.1 | Experimental design

A storage assay was applied to determine the effect of storage conditions on the microbial stability, fungal contamination, and mycotoxin accumulation throughout storage in two different almond varieties—Lauranne and Guara. The objective was to determine not only the effect of storage conditions and time, but also the variety effect on the microbial and mycotoxin stability and safety.

The following treatments were applied: 60% relative humidity (RH) carried out in an oven with controlled RH and temperature (25°C), 70% RH (KI saturated solution, at 25°C), and refrigeration (approx. 4°C), for a storage period of 9 months. The refrigeration condition was used as control, because this is the storage method usually performed by the industry. Refrigeration is a very expensive storage process, mostly if we consider the dimension of the stored almond lots, thus the industry requires alternative, less expensive and more practicable, and storage methods. The most adequate storage temperature for almonds is around 0°C; however, storage and travel temperatures at 5–25°C are permitted (TIC, 2021). In the present study, storage at 25°C was tested based on the fact that most of the almonds' storage is not done under refrigeration. The RH levels were selected based on previous studies (Fernandes et al., 2022).

Samples were taken for analysis at time points zero (T0), 3 months (T3), 6 months (T6), and 9 months (T9). All samples were provided by PABI—Produtos Alimentares da Beira Interior, SA (Pinhel, Portugal), on October 2019. Each variety was analyzed in triplicate for each sampling time and for each storage condition.

2.2 | Microbial analysis

Ten grams of each sample were weighted into sterilized bottles containing 90 ml of sterile peptone water with 0.05% Tween 80 and vigorously homogenized by inversion for 2 min. After performing serial decimal dilutions, 0.2 ml aliquots of each dilution were plated in duplicate in Plate Count Agar (PCA, Liofilchem, Italy) to determine the aerobic mesophiles according to ISO 4833-2:2013 (ISO, 2013) and in Dichloran 18% glycerol (DG18, Liofilchem, Italy) for yeasts and molds according to ISO, 21527-2:2008 (ISO, 2008). The plates with PCA and DG18 were incubated at 30°C for 48–72 h and at 25°C for 5 days, respectively. After incubation, the colonies were counted, and the means and standard deviations of the three replicates were calculated. The results were expressed in decimal logarithm of the number of colony-forming units per gram of food (Log10 CFU/g), according to ISO, 7218:2007 (ISO, 2007).

2.3 | Identification of molds

2.3.1 | Isolation of molds

After counting fungi in DG18 plates, colonies of representative molds were selected and cultivated on Malt Extract Agar (MEA, Liofilchem, Italy) for 5–7 days at 25°C to obtain pure cultures for subsequent identification. At first, isolates (within each sample type) were grouped according to their morphological similarity (morphotypes) based on their cultural characteristics, such as colony diameter, obverse and reverse colony colors, and the production of exudate. Then, one isolate representative of each morphotype was selected for molecular identification.

In total, 117 fungi were isolated from almond samples, and from those, 48 were selected for molecular identification. Isolates were preserved in 30% glycerol with 0.05% of Tween 80 at −20°C (Rodrigues et al., 2013).

2.3.2 | Molecular identification of molds

Genomic DNA was extracted following the procedure described by Rodrigues et al. (2018). The genes Calmodulin (CaM), β-Tubulin (β-tub), and Translation Elongation Factor 1-α (TEF1-α) and the ITS region were amplified by PCR depending on the genus (*CaM* for *Aspergillus* spp.; *β-tub* for *Penicillium* spp.; *TEF1-α* for *Fusarium* spp., and ITS for other genera), using the primer pairs CL1/CL2A (O'Donnell et al., 2000), Tub-F/Tub22 (Hubka & Kolarik, 2012; Scherm et al., 2005), EF1-728/EF-1567R (Rehner & Buckley, 2005), and ITS1-F/ITS4 (White et al., 1990), respectively.

Before sequencing, PCR products were purified with the commercial kit GF-1 PCR CleanUp Kit (Vivantis), following the manufacturer's instructions. Sequence analyses were carried out on an ABI 3730xl DNA Analyzer (Applied Biosystems), by outsourcing. The sequences were manually adjusted by chromatogram analysis using the program BioEdit sequence alignment editor 7.0.5.1 and then aligned with the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/) using the BLAST algorithm.

2.4 | Multi-mycotoxin analysis of samples by LC–MS/MS

A portion of each sample was finely ground with a laboratory mill (IKA, WERKE M20), to obtain particles of approximately 0.5 mm diameter. Five grams of the

ground samples were weighed into 50 ml falcon tubes and sent for mycotoxin analysis at the Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna (BOKU).

Samples were analyzed by the multi-mycotoxin method of liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Detection and quantification were performed with a QTrap 5500 MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray electrospray ionization source and a 1290 series UHPLC system (Agilent Technologies, Waldbronn, Germany). The LC–MS/MS protocol was applied as previously described (Sulyok et al., 2020) and has in the meantime been extended to cover 710 metabolites overall. Confirmation of positive analyte identification was obtained by the acquisition of two MS/MS transitions per analyte (with the exception of moniliformin and 3-nitropropionic acid, that each exhibit only one fragment ion), which yields 4.0 identification points according to the Commission Decision 2002/657/EC.

The method's accuracy is verified for major mycotoxins on a routine basis by participating in interlaboratory testing schemes organized by BIPEA (Bureau Interprofessionnel Des Etudes Analytiques, France) and by CODA-CERVA (National Reference Laboratory for mycotoxins, Belgium).

2.5 | Statistical analysis

Statistical analysis was performed using the Software Statistical Program Social Sciences (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). Shapiro–Wilk test was used for normality testing. The results are presented as mean ± SD (standard deviation) ($n = 3$). Analysis of variance (ANOVA) was used to analyze the statistical difference in microbial load among different treatments, and the means were compared using Tukey's test ($p < 0.05$).

The association between fungi and mycotoxins was determined by the Spearman coefficient. Association values were interpreted as follows: very low association $0.00 \leq \rho < 0.30$, low $0.30 \leq \rho < 0.50$, moderate $0.50 \leq \rho < 0.70$, high $0.70 \leq \rho < 0.90$, and very high $0.90 \leq \rho \leq 1.00$. Principal component analysis (PCA) was also performed using SPSS.

3 | RESULTS AND DISCUSSION

3.1 | Microbial loads

Tables 1 and 2 show the results of microbial counts (aerobic mesophiles and molds), in the two varieties of almonds—Guara and Lauranne, respectively—stored at 4°C, and at 60% RH and 70% RH at 25°C, for up to 9 months. Significant differences ($p < 0.05$) in microbial growth were detected during the storage time, as well as between storage conditions. In both assays, the main differences observed were between storage conditions at 3 and 9 months. For the aerobic mesophiles, comparing the storage periods of the Guara variety (Table 1), the microbial count in T0 was 3.6 log CFU/g at 4°C, 60% and 70% RH, whereas after 6 months, the number was decreasing to 2.9, 2.9, and 3.3 log CFU/g, respectively. However, after 9 months of storage, an increase to 4.8, 3.9, and 3.8 log CFU/g, respectively, was noted.

For the mold counts in the Guara variety (Table 1), in T0 was 3.4 for 4°C, 60% and 70% RH, whereas after 3 months of storage, the total number of molds decreased for 4°C and 60% RH to 3.3 and 2.9 log CFU/g, respectively. Nevertheless, after 9 months of storage, the lowest mold counts were noted at 60% RH at 2.9 log CFU/g, whereas the highest were noted at 4°C.

For the Lauranne variety (Table 2), the microbial count in T0 was 3.1 at 4°C, 60% and 70% RH, whereas after 9 months under the same conditions, an increase was noted, especially at 4°C at 4.3 log CFU/g. For molds, there was a significant difference in the number of CFUs/g from 3.3 at the beginning to 3.1, 2.1, and 3.2 log CFU/g for 4°C, 60% and 70% RH at 25°C, respectively, after 9 months of storage.

The results showed that the storage condition at 60% RH was the best used for the preservation of almonds for the two varieties, Guara and Lauranne, because it showed the lowest mold growth after 9 months of storage. Regarding varieties, Lauranne presented lower counts of aerobic mesophiles and molds than Guara.

3.2 | Fungal contamination

A total of 117 fungal isolates were obtained from almonds belonging to the varieties Guara and Lauranne, 60 and 57 isolates, respectively. Forty-eight isolates were sequenced for molecular identification by the ITS region and the calmodulin, β-tubulin, or translation elongation factor 1-alpha genes. From these, 26 species were identified belonging to 6 genera: *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Paecilomyces*, and *Talaromyces*. The list of identified species and the corresponding number of isolates is presented in Table S1.

Among the filamentous fungi identified for the variety Guara, six genera were found: *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Paecilomyces*, and *Talaromyces* (Figure 1). *Aspergillus* species were identified at all times of storage from T0 to T9 in all storage parameters, which are presented in Figure 2.

TABLE 1 Aerobic mesophile and mold counts (log CFU/g) in Guara almonds throughout 9 months of storage under 4°C and 60% and 70% RH at 25°C

	Storage conditions	Storage time (months)			
		0	3	6	9
Aerobic mesophiles	Control (4°C)	$3.6 \pm 0.2^{a,B}$	$3.4 \pm 0.2^{a,BC}$	$2.9 \pm 0.2^{a,C}$	$4.8 \pm 0.2^{a,A}$
	60% RH	$3.6 \pm 0.2^{a,A}$	$2.9 \pm 0.1^{b,B}$	$2.9 \pm 0.4^{a,B}$	$3.9 \pm 0.3^{b,A}$
	70% RH	$3.6 \pm 0.2^{a,AB}$	$3.3 \pm 0.1^{ab,B}$	$3.3 \pm 0.1^{a,B}$	$3.8 \pm 0.3^{b,A}$
Molds	Control (4°C)	$3.4 \pm 0.1^{a,A}$	$3.3 \pm 0.1^{b,A}$	$2.9 \pm 0.2^{a,B}$	$3.4 \pm 0.2^{a,A}$
	60% RH	$3.4 \pm 0.1^{a,A}$	$2.9 \pm 0.1^{c,B}$	$3.1 \pm 0.2^{a,B}$	$2.9 \pm 0.1^{b,B}$
	70% RH	$3.4 \pm 0.1^{a,A}$	$3.5 \pm 0.1^{a,A}$	$3.2 \pm 0.0^{a,B}$	$3.0 \pm 0.1^{b,C}$

Note: Values are expressed as Mean ± standard deviation. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) between storage conditions by variety; different capital letters on the same line indicate significant differences ($p < 0.05$) between storage time.

TABLE 2 Aerobic mesophile and mold counts (log CFU/g) in Lauranne almonds throughout 9 months of storage under 4°C and 60% and 70% RH at 25°C

	Storage conditions	Storage time (months)			
		0	3	6	9
Aerobic mesophiles	Control (4°C)	$3.1 \pm 0.2^{a,B}$	$3.3 \pm 0.3^{a,B}$	$3.1 \pm 0.3^{a,B}$	$4.3 \pm 0.5^{a,A}$
	60% RH	$3.1 \pm 0.2^{a,A}$	$3.0 \pm 0.2^{a,A}$	$3.4 \pm 0.5^{a,A}$	$3.5 \pm 0.2^{a,A}$
	70% RH	$3.1 \pm 0.2^{a,A}$	$3.5 \pm 0.2^{a,A}$	$3.4 \pm 0.4^{a,A}$	$3.6 \pm 0.2^{a,A}$
Molds	Control (4°C)	$3.3 \pm 0.1^{a,A}$	$2.9 \pm 0.1^{b,A}$	$3.4 \pm 0.4^{a,A}$	$3.1 \pm 0.4^{a,A}$
	60% RH	$3.3 \pm 0.1^{a,A}$	$2.7 \pm 0.5^{b,AB}$	$3.9 \pm 0.2^{a,A}$	$2.1 \pm 0.4^{b,B}$
	70% RH	$3.3 \pm 0.1^{a,BC}$	$5.2 \pm 0.2^{a,A}$	$3.6 \pm 0.1^{a,B}$	$3.2 \pm 0.2^{a,C}$

Note: Values are expressed as Mean ± standard deviation. Different lowercase letters in the same column indicate significant differences ($p < 0.05$) between storage conditions by variety; different capital letters on the same line indicate significant differences ($p < 0.05$) between storage time.

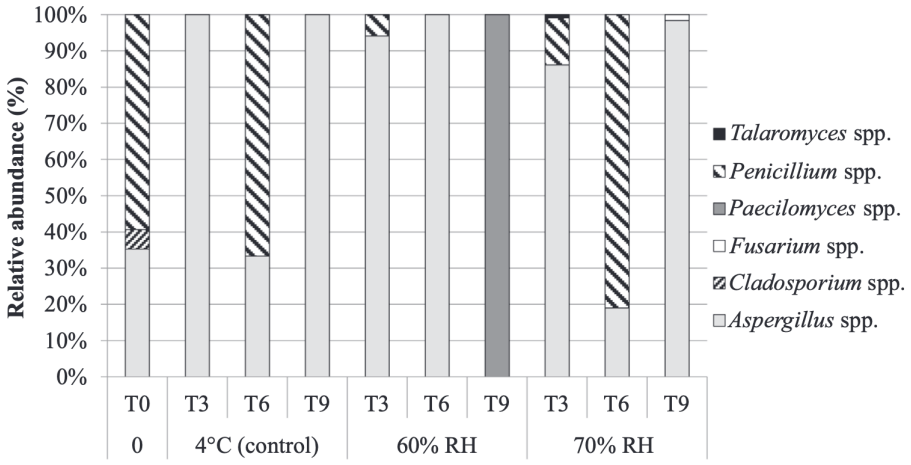

FIGURE 1 Relative abundance of fungi, by genera, in almond samples from Guara variety

Several species of *Aspergillus* were identified belonging to sections *Flavi*, *Nigri*, *Fumigati*, *Circumdati*, *Cremei*, and *Versicolores*. *Aspergillus* section *Flavi* had the highest relative abundance, especially in T3 and T9, where the species *Aspergillus flavus*, *Aspergillus Parasiticus*, and *Aspergillus tamarii* were identified. Second, *Aspergillus* section *Nigri*, where the species *Aspergillus awamori* (syn. *Aspergillus luchuensis*), *Aspergillus Brasiliensis*, and *Aspergillus niger/tubingensis* were also found in high amounts.

Considering the filamentous fungi found in the variety Lauranne, four genera were identified: *Penicillium*,

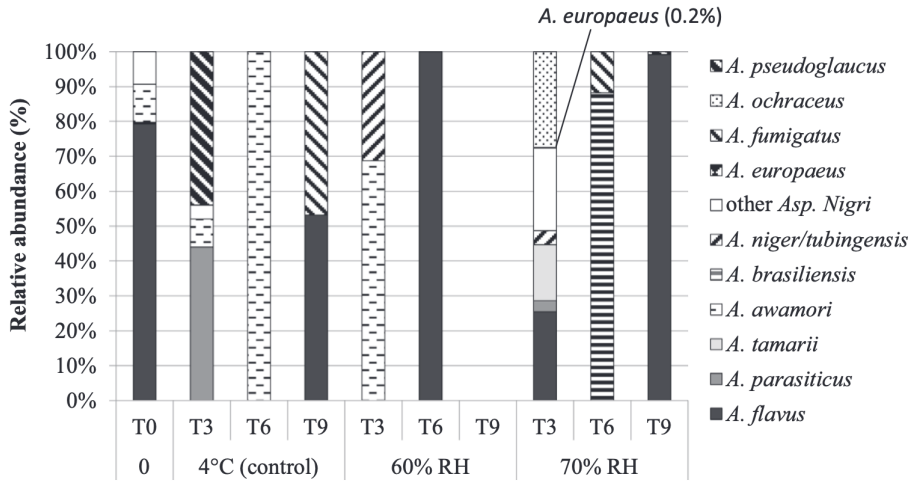

FIGURE 2 Relative abundance of *Aspergillus* species in almond samples from Guara variety

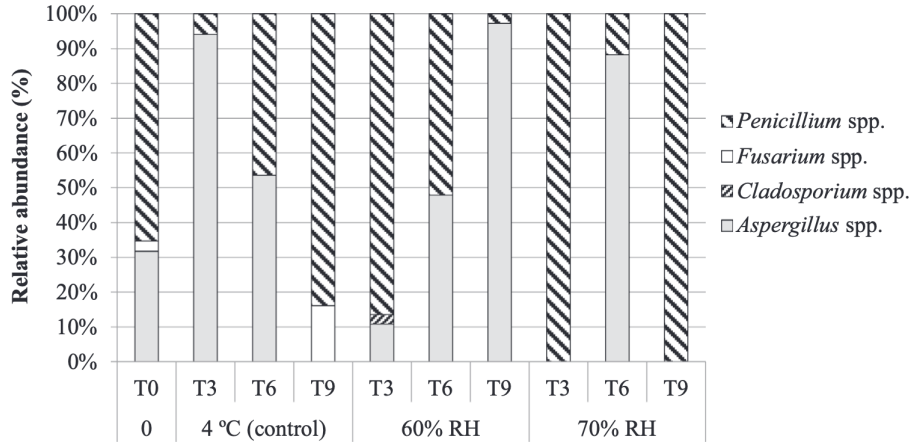

FIGURE 3 Relative abundance of fungi, by genera, in almond samples from Lauranne variety

Aspergillus, *Fusarium*, and *Cladosporium*. *Penicillium* species were identified in all times and parameters of storage (Figure 3). Species of *Aspergillus* were identified, such as *Aspergillus versicolor*, *Aspergillus pseudoglaucus*, *Aspergillus glaucus*, *Aspergillus parasiticus*, and *A. flavus*. *Aspergillus* section *Nigri* were also isolated but not identified to the species level (Figure 4). These fungi are one of the most difficult groups in the *Aspergillus* genus concerning classification and identification, and it is not always possible to discriminate between very closely related species (Samson et al., 2007b, 2014). In this study, "section *Nigri*" was used when it was not possible to identify the fungi to the species level. Several species of *Penicillium* were identified belonging to sections *Canescentia*, *Citrina*, *Fasciculata*, and *Chrysogena* (Figure 5).

3.3 | Mycotoxin contamination

Figure 6 shows the most representative metabolites detected in almonds of Guara variety in T0 (control) and along the storage times T3, T6, and T9 after submission to the treatments 60% RH and 70% RH at 25 and 4°C. Only metabolites present in at least three samples at more than 10 μg/kg are represented, except for aflatoxins, due to its toxicological relevance. All other metabolites are included in the group "others." Table S3 shows a complete list and amounts of metabolites detected. The highest occurrence of cumulative mycotoxins for the Guara variety was detected after 6 months of storage at 60% RH and after 9 months under 70% RH. The lowest occurrence of mycotoxins for Guara was noted under 4°C.

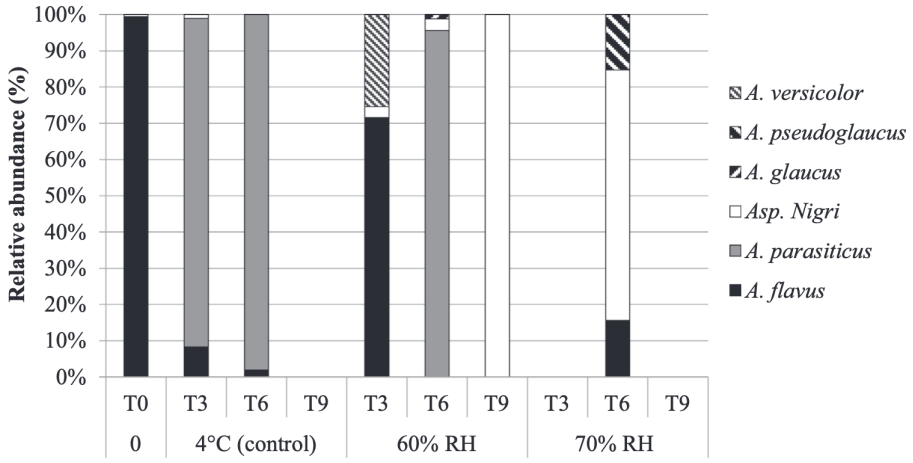

FIGURE 4 Relative abundance of *Aspergillus* species in almond samples from Lauranne variety

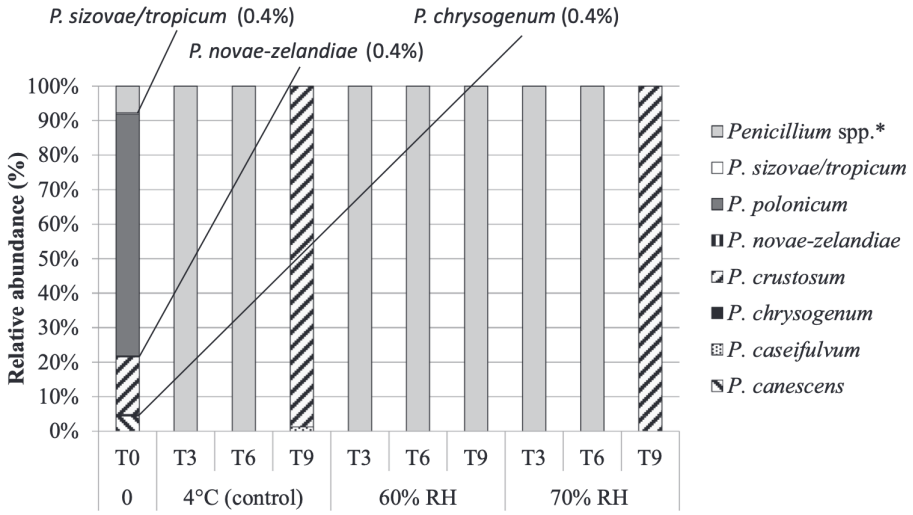

FIGURE 5 Relative abundance of *Penicillium* species in almond samples from Lauranne variety (* non-identified *Penicillium* isolates)

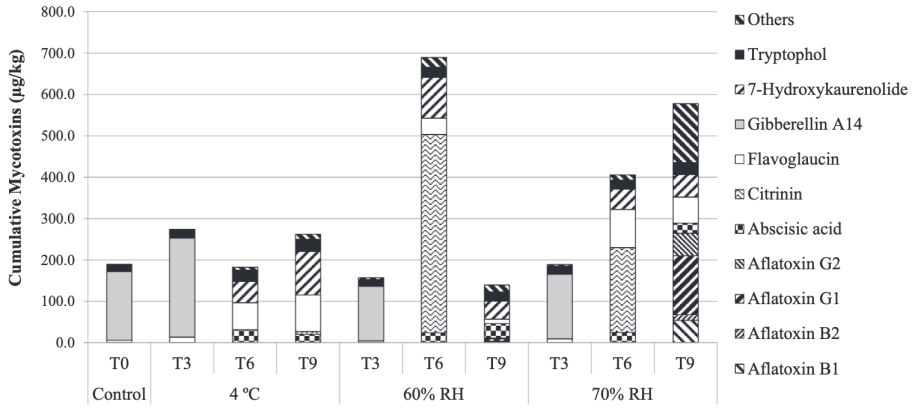

FIGURE 6 Mycotoxins in Guara almonds after 0, 3, 6, and 9 months of storage at 4°C, and 60% and 70% RH at 25°C. (*Notes*: Only metabolites present in at least three samples at more than 10 µg/kg are represented, except for aflatoxins, due to its toxicological relevance. All other metabolites are included in the group "others." See Table S3 for details.)

For T0 and T3 (for all storage conditions), similar metabolite profiles were observed: The *Aspergillus* metabolite flavoglaucin and the unspecific metabolites gibberellin 14 and tryptophol were detected in relatively low concentrations, suggesting that these metabolites are generally present in Guara almonds.

Considering major mycotoxins, only AFs and citrinin were detected in significant amounts in some of the samples. The highly toxigenic aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) were detected in the Guara samples only after 9 months of storage under both 70% and 60% RH. Considering the MTLs established for ready-to-eat almonds for AFB1 (8.0 μg/kg) and for total AFs (10.0 μg/kg) (EC, 2010), the extremely high levels of these toxins (53.9 and 264.1 μg/kg, respectively) observed in the 70% RH samples pose a serious risk to consumers. For 60% RH, these levels are considerably lower (2.7 and 11.6 μg/kg, respectively), suggesting that this storage condition is better at controlling AF-producing fungi and the potential for AF contamination. The AF presence and amounts in the 70% RH samples agree with the fact that all fungi detected in these samples belonged to *Aspergillus* section *Flavi*, responsible for the production of AFs. These fungi are xerophilic and tend to dominate the mycobiota of almonds at low RH (Rodrigues et al., 2013), with the consequent potential for AF production.

In 70% RH samples, other less relevant *Aspergillus* metabolites, like 3-nitropropionic acid (46 μg/kg), averufin (20.9 μg/kg), and averantin (2.4 μg/kg), were also detected. The *Penicillium* and *Aspergillus* metabolite flavoglaucin (63.1 μg/kg), the *Fusarium* metabolite methylequisetin (3.5 μg/kg), and the unspecific metabolites cyclo(L-Pro-L-Tyr) (5.1 μg/kg) and brevianamid F (2.3 μg/kg) were detected at relatively insignificant levels.

After 6 months of storage, the *Penicillium*-related mycotoxin citrinin was detected at extremely high levels in samples submitted to 60% and 70% RH. This result was not expected because, after 9 months, that trend was not observed, suggesting that some nuts in worse preservation conditions may have been collected during sampling, presenting a more significant microbial load, and giving rise to higher levels of mycotoxins. Citrinin is a nephrotoxin compound currently classified as group 3 (IARC, 1993). Although no regulations have been set to this mycotoxin in almonds, a level of no concern has been set at 0.2 μg/kg bw/day by the European Food Safety Authority (EFSA, 2012). The citrinin levels detected after 6 months of storage in the Guara samples submitted to 70% RH and 60% RH are extremely high, suggesting high exposure to this mycotoxin by almond consumers.

After 6 months of storage under 60% RH, other metabolites related to *Penicillium* and *Aspergillus* species were also detected: flavoglaucin (39.6 μg/kg), cyclo(L-Pro-L-Tyr) (7.3 μg/kg), and cyclo(L-Pro-L-Val) (1.4 μg/kg).

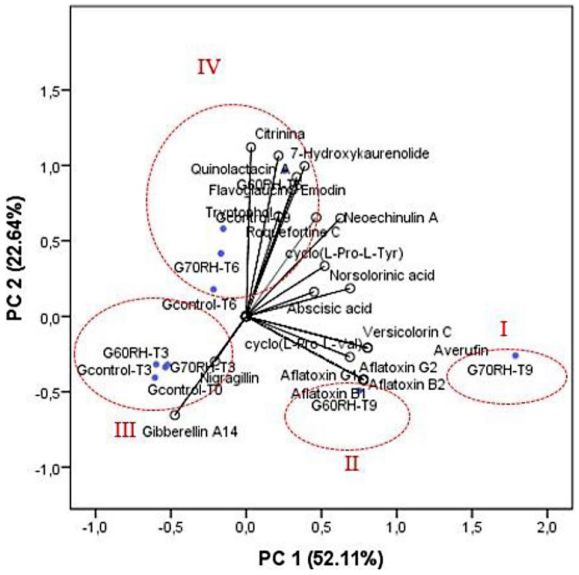

FIGURE 7 Principal component analysis (PCA) applied to Guara almonds stored under different storage conditions, considering the identified metabolites

Spearman correlations were observed between *Asp. Nigri* and gibberellin A14 ($\rho = 0.676$, $p = 0.032$). *Asp.* sect. *Fumigati* and *Paecilomyces formosus* correlated with flavoglaucin ($\rho = 0.683$, $p = 0.029$) and cyclo(L-Pro-L-Val) ($\rho = 0.643$, $p = 0.045$), respectively. Very high correlations were verified among *Asp.* sect. *Cremeii*, *Asp.* sect. *Circumdati*, and *Talaromyces islandicus* with nigragillin ($\rho = 1.00$, $p = 0.00$).

Considering the PCA analysis for the Guara variety, the two main principal components (PCs) accounted for 74.75% of the total data variability (Figure 7). The first component (PC1) explained 52.11% of the variance, and the second component (PC2) 22.64%. The PC1 was mainly related to aflatoxins B1, B2, G1, G2, averufin, versicolorin C, norsolorinic acid, and cyclo(L-Pro-L-Val). The PC2 was mainly related to citrinin, quinolactacin A, roquefortine C, flavoflaucin, 7-hydroxykaurenolide, and emodin.

Four groups were clearly distinguished. The first group (G70RH-T9) presented high values of aflatoxins B1, B2, G1, G2, averufin, versicolorin C, and norsolorinic acid. The second group (G60RH-T9) was distinguished by its high levels of cyclo(L-Pro-L-Val). The third group included the treatments Gcontrol-T0, Gcontrol-T3, G60RH-T3, and G70RH-T3, which presented the highest values of gibberellin A14 and nigragillin. In group 4, Gcontrol-T6 differed from other storage conditions due to high values of citrinin.

Figure 8 shows the most representative metabolites detected in almonds of Lauranne variety in time T0 (control) and along the storage times T3, T6, and T9, under the three treatments (60% RH and70% RH at 25 and 4°C).

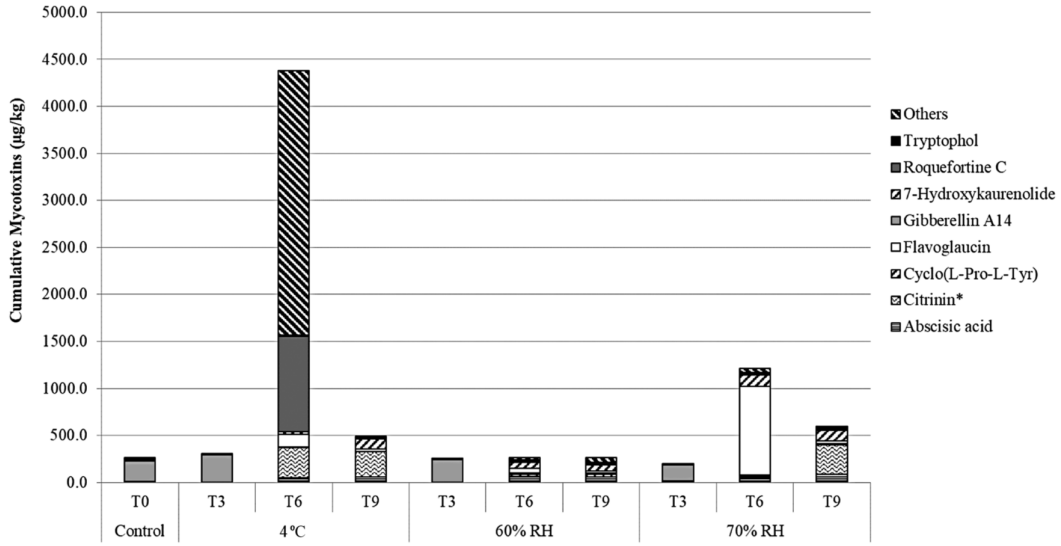

FIGURE 8 Mycotoxins in Lauranne almonds after 0, 3, 6, and 9 months of storage at 4°C, and 60% and 70% RH at 25°C. (*Notes*: The citrinin value in sample T6/4°C is 100× the one presented; only metabolites present in at least three samples at more than 10 µg/kg are represented. All other metabolites are included in the group "others." See Table S4 for details.)

Only metabolites present in at least three samples at more than 10 µg/kg are represented. All other metabolites are included in the group "others." Table S4 shows a complete list and amounts of metabolites detected. Thirty-eight metabolites were detected, from which 35 were detected with the highest amount after 6 months of storage at 4°C. The highest cumulative mycotoxins were *Penicillium*-related toxins (14 toxins), such as citrinin (3247 µg/kg), roquefortine C (1012 µg/kg), viridicatol (764 µg/kg), cyclopenol (324 µg/kg), roquefortine D (296 µg/kg), dihydrocitrinone (130 µg/kg), quinolactacin A (51.1 µg/kg), viridicatin (34.2 µg/kg), cyclopenin (20.3 µg/kg), penitrem (12.7 µg), quinolactacin B (3.6 µg/kg), cyclopeptine (2.5 µg/kg), and asperphenamate (0.5 µg/kg). Metabolites related to *Aspergillus* species (10 toxins) were also detected, such as paspalin (602 µg/kg), aspulvinone E (544 µg/kg), flavoglaucin (137 µg/kg), endocrocin (26.1 µg/kg), cyclo(L-pro-L-val) (6.8 µg/kg), citreorosin (5.1 µg/kg), brevianamid F (3.6 µg/kg), emodin (3.2 µg/kg), and cyclo(L-pro-L-Tyr) (2.4 µg/kg). The second highest frequency of fungal metabolites was noted after 6 months of storage at 70% RH at 25°C, where 14 metabolites were detected: 5 *Aspergillus*-related metabolites, such as aspulvinone E (1289 µg/kg), flavoglaucin (938 µg/kg), cyclo(L-pro-L-Tyr) (13.2 µg/kg), asperglaucide (2.9 µg/kg), and *N*-benzoyl-phenylalanine (0.7 µg/kg). Five toxins related to *Penicillium* sp. were also detected, such as asperphenamate (33.5 µg/kg), citrinin (13.3 µg/kg), roquefortine C (11.3 µg/kg), cyclopenol (2.4 µg/kg), and cyclopenin (0.1 µg/kg). Overall, 60% RH at 25°C was the storage condition with the lowest cumulative mycotoxins. In Lauranne almonds, correlations between mycotoxins

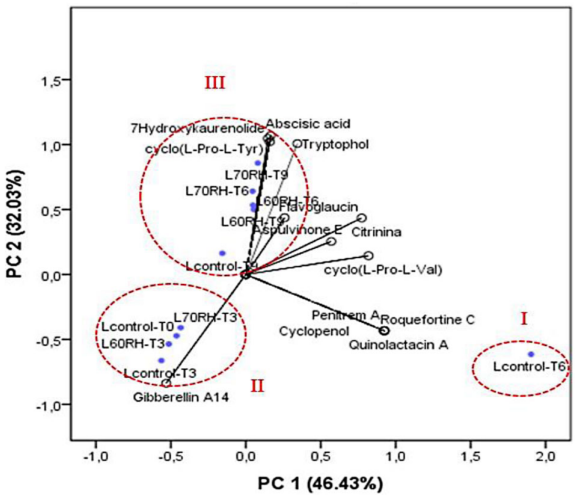

FIGURE 9 Principal component analysis (PCA) applied to Lauranne almonds samples stored under different storage conditions, considering the identified metabolites

and fungi were only significant for *Aspergillus* sect. *Flavi* ($\rho = 0.639$, $p = 0.047$) and *Aspergillus* sect. *Aspergillus* ($\rho = 0.766$, $p = 0.01$) for aspulvinone E.

The results obtained for the PCA analysis of mycotoxin distribution in Lauranne almonds are presented in Figure 9. The first two PCs explained 78.46% of the total variance of the original variables. The first component (PC1) explained 46.43% of the total variance, and the second component (PC2) 32.03%. The PC1 was mainly related to quinolactacin A, roquefortine C, cyclopenol, and penitren A. The PC2 was mainly associated with

abscisic acid, cyclo(L-Pro-L-Tyr), 7-hydroxykauranolide, and tryptophol. Figure 9 shows clearly three groups. The first group included the treatment of 4°C at 6 months (Lcontrol-T6) that showed the highest concentration of quinolactacin A, roquefortine C, cyclopenol, and penitrem A. The second group included the treatments Lcontrol-T0, Lcontrol-T3, L60RH-T3, and L70RH-T3, which showed the highest values of gibberellin A14. The third group included the treatments L60RH-T6, L70RH-T6, Lcontrol-T9, L60RH-T9, and L70RH-T9, which presented the highest values of abscisic acid, cyclo(L-Pro-L-Tyr), 7-hydroxykauranolide, and tryptophol. Within third group, Lcontrol-T9 differs from other storage conditions due to the lower values of tryptophol and cyclo(L-Pro-L-Tyr).

Our results are consistent with previous studies that analyzed the metabolites produced by the various fungi detected in our study, as summarized in Table S2. The metabolites that were found in our study are highlighted.

Effective control of almonds requires good agricultural practices, well-controlled storage conditions, and surveillance at every step, from the harvest to the plate. A particular risk of contamination due to humid and hot climatic conditions may stimulate mold growth, whereas proper control and storage can be difficult to achieve.

4 | CONCLUSION

Based on this evaluation of mycotoxin levels and mold and yeast counts, nuts quality depends on the storage period and the RH and temperature at which they are stored. The RH of the nuts is an important factor in determining the growth of fungal species and their toxin production. At high RH of 70%, there was a different succession of organisms with time. The increase in storage time led to an increase in fungal count. Fungi, such as *Aspergillus* and *Penicillium*, were the genera most commonly identified in the almonds.

Storage under 60% RH was the best condition for the preservation of almonds for the two varieties Guara and Lauranne, which showed the lowest mold growth after 9 months of storage. Moreover, it is necessary to consider that the Lauranne variety presented lower counts of aerobic mesophiles and molds than the Guara variety. A high diversity of species has been detected in almonds. The mycotoxin contamination of the two varieties, Guara and Lauranne, submitted to different relative humidities throughout storage was remarkably important. The highest occurrence of cumulative mycotoxins for the Guara variety was after 9 months under the condition 70% RH. Moreover, the highest occurrence of cumulative mycotoxins for the Lauranne variety was after 6 months under the conditions 4°C and 70% RH at 25°C. On the contrary, the 60% RH condition was the one that caused the lowest cumulative mycotoxins.

Considering the results obtained in the present study, we can conclude that, in terms of microbial and mycotoxin contamination, the preservation of nuts under 60% RH at 25°C is a good storage condition to maintain the stability and safety of nuts.

Studies are being undertaken to evaluate the effect of the same storage conditions on the physical and chemical parameters of nuts. After these two sets of data are analyzed and plotted together, it will be more easy to determine the best overall preservation conditions to apply to almonds.

AUTHOR CONTRIBUTIONS

Paula Rodrigues: Conceptualization; Methodology; Supervision; Writing – review & editing; Writing – original draft; Formal analysis. **Arij Jelassi**: Investigation; Writing – original draft. **Elifa Kanoun**: Supervision. **Michael Sulyok**: Investigation. **Paula Correia**: Investigation. **Elsa Ramalhosa**: Conceptualization; Methodology; Supervision; Writing – review & editing; Funding acquisition. **Ermelinda Lopes Pereira**: Conceptualization; Methodology; Supervision; Writing – review & editing; Writing – original draft; Formal analysis.

ACKNOWLEDGMENTS

This work was supported by the PDR2020 Partnership Agreement and cofinanced by FEADER, Portugal 2020 [ValNuts Project: PDR2020-101-03,0756]; and the Foundation for Science and Technology (FCT, Portugal) under national funds FCT/MCTES for CIMO [UIDB/00690/2020] and CERNAS [UIDB/00681/2020].

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

ORCID

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

REFERENCES

Alwatban, M. A., Hadi, S., & Moslem, M. A. (2014). Mycotoxin production in *Cladosporium* species influenced by temperature regimes. *Journal of Pure and Applied Microbiology*, *8*(5), 4061–4069.

Berryman, C. E., Preston, A. G., Karmally, W., Deckelbaum, R. J., & Kris-Etherton, P. M. (2011). Effects of almond consumption on the reduction of LDL-cholesterol: A discussion of potential mechanisms and future research directions. *Nutrition Reviews*, *69*(4), 171–185. https://doi.org/10.1111/j.1753-4887.2011.00383.x

Blaak, J., & Staib, P. (2022). An updated review on efficacy and benefits of sweet almond, evening primrose and jojoba oils in skin care applications. *International Journal of Cosmetic Science*, *44*(1), 1–9. https://doi.org/10.1111/ics.12758

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

Chen, C. Y., Lapsley, K., & Blumberg, J. (2006). A nutrition and health perspective on almonds. *Journal of the Science of Food and Agriculture*, *86*(14), 2245–2250. https://doi.org/10.1002/jsfa.2659

Čolić, S., Zec, G., Natić, M., & Fotirić-Akšić, M. (2019). Almond (*Prunus dulcis*) oil. In M. Ramadan (Eds.), *Fruit oils: Chemistry and functionality* (pp. 149–180). Springer. https://doi.org/10.1007/978-3-030-12473-1_6

EC. (1996). Commission regulation (EC) no 1107/96 of 12 June 1996 on the registration of geographical indications and designations of origin under the procedure laid down in article 17 of council regulation (EEC) no 2081/92. *Official Journal of the European Union*, *L 148*, 1–10.

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.

EFSA. (2012). Scientific opinion on the risks for public and animal health related to the presence of citrinin in food and feed. *EFSA Journal*, *10*, 2605. https://doi.org/10.2903/j.efsa.2012.2605

Ezekiel, C. N., Chibundu, N., Kraak, B., Sandoval-Denis, M., Sulyok, M., Oyedele, O. A., Ayeni, K. I., Makinde, O. M., Akinyemi, O. M., Krska, R., Crous, P. W., & Houbraken, J. (2020). Diversity and toxigenicity of fungi and description of *Fusarium madaense* sp. nov. from cereals, legumes and soils in north-central nigeria. *MycoKeys*, *67*, 95–124. https://doi.org/10.3897/MYCOKEYS.67.52716

Ezekiel, C. N., Sulyok, M., Frisvad, J. C., Somorin, Y. M., Warth, B., Houbraken, J., Samson, R. A., Krska, R., & Odebode, A. C. (2013). Fungal and mycotoxin assessment of dried edible mushroom in nigeria. *International Journal of Food Microbiology*, *162*(3), 231–236. https://doi.org/10.1016/j.ijfoodmicro.2013.01.025

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

Frisvad, J. C., Hubka, V., Ezekiel, C. N., Hong, S.-B., Nováková, A., Chen, A. J., Arzanlou, M., Larsen, T. O., Sklenár, 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*, *2004*(49), 201–241.

Houbraken, J., & Samson, R. A. (2011). Phylogeny of *Penicillium* and the segregation of *Trichocomaceae* into three families. *Studies in Mycology*, *70*, 1–51. https://doi.org/10.3114/sim.2011.70.01

Hubka, V., & Kolarik, M. (2012). *β*-Tubulin paralogue tubC is frequently misidentified as the benA gene in *Aspergillus* section *Nigri* taxonomy: Primer specificity testing and taxonomic consequences. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *29*, 1–10. https://doi.org/10.3767/003158512x658123

Hubka, V., Nováková, A., Samson, R. A., Houbraken, J., Frisvad, J. C., Sklenář, F., Varga, J., & Kolařík, M. (2016). *Aspergillus europaeus* sp. *nov*., a widely distributed soil-borne species related to *A. wentii* (section *Cremei*). *Plant Systematics and Evolution*, *302*(6), 641–650. https://doi.org/10.1007/s00606-016-1293-7

IARC. (1993). International Agency for Research on Cancer (IARC) monographs on the evaluation of carcinogenic risks of chemicals to humans. vol. 56. In *Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins*. (pp. 397–444). International Agency for Research on Cancer.

Ismail, M. A. (2017). Incidence and significance of black aspergilli in agricultural commodities: A review, with a key to all species accepted to-date. *European Journal of Biological Research*, *7*(3), 207–222. https://doi.org/10.5281/zenodo.834504

ISO 21527-2. (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*. International Organization for Standardization (ISO).

ISO 4833-1. (2013). *Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of microorganisms—Colony-count technique at 30°C*. International Organization for Standardization (ISO).

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

Kakinuma, N., Iwai, H., Takahashi, S., Hamano, K., Yanagisawa, T., Nagai, K., Tanaka, K., Suzuki, K., Kirikae, F., Kirikae, T., & Nakagawa, A. (2000). Quinolactacins a, b and c. Novel quinolone compounds from *Penicillium* sp. EPF-6. I. Taxonomy, production, isolation and biological properties. *The Journal of Antibiotics*, *53*(11), 1247–1251. https://doi.org/10.7164/antibiotics.53.1247

Massantini, R., & Frangipane, M. T. (2022). Progress in almond quality and sensory assessment: An overview. *Agriculture*, *12*(5), 710. https://doi.org/10.3390/agriculture12050710

Munkvold, G. P. (2017). *Fusarium* species and their associated mycotoxins. *Methods in Molecular Biology*, *1542*, 51–106. https://doi.org/10.1007/978-1-4939-6707-0_4

Nielsen, K. F., Mogensen, J. M., Johansen, M., Larsen, T. O., & Frisvad, J. C. (2009). Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. *Analytical and Bioanalytical Chemistry*, *395*(5), 1225–1242. https://doi.org/10.1007/s00216-009-3081-5

O'Donnell, K., Nirenberg, H. I., Aoki, T., & Cigelnik, E. (2000). A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. *Mycoscience*, *41*(1), 61–78. https://doi.org/10.1007/BF02464387

Ostry, V. (2008). *Alternaria* mycotoxins: An overview of chemical characterization, producers, toxicity, analysis and occurrence in

foodstuffs. *World Mycotoxin Journal*, *1*(2), 175–188. https://doi.org/10.3920/wmj2008.x013

Perrone, G., Stea, G., Epifani, F., Varga, J., Frisvad, J. C., & Samson, R. A. (2011). *Aspergillus* niger contains the cryptic phylogenetic species *A. awamori*. *Fungal Biology*, *115*(11), 1138–1150. https://doi.org/10.1016/j.funbio.2011.07.008

RASFF. (2021). The Rapid Alert System for Food and Feed 2020 Annual Report. Luxembourg: Publications Office of the European Union. ISBN 978-92-76-34377-6, https://doi.org/10.2875/259374. https://food.ec.europa.eu/system/files/2021-08/rasff_pub_annual-report_2020.pdf

Rehner, S. A., & Buckley, E. (2005). A beauveria phylogeny inferred from nuclear ITS and EF1- α sequences: Evidence for cryptic diversification and links to cordyceps teleomorphs. *Mycologia*, *97*(1), 84–98. https://doi.org/10.3852/mycologia.97.1.84. PMID: 16389960.

Rodrigues, P., Venâncio, A., & Lima, N. (2012a). Aflatoxigenic fungi and aflatoxins in portuguese almonds. *The Scientific World Journal*, *2012*, 471926. 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

Rodrigues, P., Venâncio, A., & Lima, N. (2013). Incidence and diversity of the fungal genera *Aspergillus* and *Penicillium* in Portuguese almonds and chestnuts. *European Journal of Plant Pathology*, *137*(1), 197–209. https://doi.org/10.1007/s10658-013-0233-4

Rodrigues, P., Venâncio, A., & Lima, N. (2018). Toxic reagents and expensive equipment: Are they really necessary for the extraction of good quality fungal DNA? *Letters in Applied Microbiology*, *66*, 32–37. https://doi.org/10.1111/lam.12822

Samson, R. A., Hong, S., Peterson, S. W., Frisvad, J. C., & Varga, J. (2007a). Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*. *Studies in Mycology*, *59*, 147–203. https://doi.org/10.3114/sim.2007.59.14

Samson, R. A., Houbraken, J., Varga, J., & Frisvad, J. C. (2009). Polyphasic taxonomy of the heat resistant ascomycete genus *Byssochlamys* and its *Paecilomyces* anamorphs. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *22*, 14–27. https://doi.org/10.3767/003158509x418925

Samson, R. A., Noonim, P., Meijer, M., Houbraken, J., Frisvad, J. C., & Varga, J. (2007b). Diagnostic tools to identify black Aspergilli. *Studies in Mycology*, *59*, 129–145. https://doi.org/10.3114/sim.2007.59.13

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H., Perrone, G., Seifert, K. A., Susca, A., Tanney, J. B., Varga, J., Kocsubé, S., Szigeti, G., Yaguchi, T., & Frisvad, J. C. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in Mycology*, *78*, 141–173. https://doi.org/10.1016/j.simyco.2014.07.004

Scherm, B., Palomba, M., Serra, D., Marcello, A., & Migheli, Q. (2005). Detection of transcripts of the aflatoxin genes aflD, aflO, and aflP by reverse transcription-polymerase chain reaction allows differentiation of aflatoxin-producing and non-producing isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. *International Journal of Food Microbiology*, *98*(2), 201–210. https://doi.org/10.1016/j.ijfoodmicro.2004.06.004

Seo, K. H., Lee, D. Y., Lee, D. S., Kang, H. C., Kim, H. S., Kim, Y. C., & Baek, N. I. (2015). Hepatoprotective and neuroprotective tocopherol analogues isolated from the peels of *Citrus unshiu* marcovich. *Natural Product Research*, *29*(6), 571–573. https://doi.org/10.1080/14786419.2014.952235

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 (Basel)*, *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*, 2607–2620.

TIC. (2021). Transport Information Service—Almonds. https://www.tis-gdv.de/tis_e/ware/nuesse/mandeln/mandeln-htm/#temperatur (accessed 20 september 2021)

Tomishima, H., Luo, K., & Mitchell, A. E. (2022). The almond (*Prunus dulcis*): Chemical properties, utilization, and valorization of coproducts. *Annual Review of Food Science and Technology*, *13*, 145–166. https://doi.org/10.1146/annurev-food-052720-111942

Tournas, V. H., Niazi, N. S., & Kohn, J. S. (2015). Fungal presence in selected tree nuts and dried fruits. *Microbiology Insights*, *8*, MBI.S24308. https://doi.org/10.4137/mbi.s24308

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. http://doi.org/10.1016/j.simyco.2014.09.001

Visagie, C. M., Varga, J., Houbraken, J., Meijer, M., Kocsubé, S., Yilmaz, N., Fotedar, R., Seifert, K. A., Frisvad, J. C., & Samson, R. A. (2014). Ochratoxin production and taxonomy of the yellow *Aspergilli* (*Aspergillus* section *Circumdati*). *Studies in Mycology*, *78*(1), 1–61. https://doi.org/10.1016/j.simyco.2014.07.001

White, T. J., Burns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A.,Innis, D. H., Gelfald, J. J., Sninsky, & T. J., White (Eds.). *PCR protocol: A guide to methods and applications*. (pp. 315–322). Academic press.

Yilmaz, N., Visagie, C. M., Houbraken, J., Frisvad, J. C., & Samson, R. A. (2014). Polyphasic taxonomy of the genus *Talaromyces*. *Studies in Mycology*, *78*(1), 175–341. https://doi.org/10.1016/j.simyco.2014.08.001

SUPPORTING INFORMATION

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How to cite this article: Rodrigues, P., Jelassi, A., Kanoun, E., Sulyok, M., Correia, P., Ramalhosa, E., & Pereira, E. L. (2023). Effect of different storage conditions on the stability and safety of almonds. *Journal of Food Science*, *88*, 848–859. https://doi.org/10.1111/1750-3841.16453