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Effect of storage conditions on the quality and food safety of hazelnuts and almond pastes

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Abstract

Hazelnuts and almonds are nuts that are widely consumed worldwide. However, there are some problems with their preservation. The dissertation work developed in the present work is part of the ValNuts Project, in which it is intended to valorise dried nuts. The present work intends to study hazelnuts and almonds. In this sense, the effect of temperature (4, 25 and 35 ºC) on hazelnut kernel storage over time (0, 3, 6, 9 and 12 months) was examined in terms of fatty acids, oxidative stability and microbiological parameters. Regarding the almond, it was proposed to prepare a paste, for which it was desired to determine the shelf life using an accelerated test. The temperatures tested were 4, 25, 45, 55 and 65 ºC, over three months. The parameters evaluated were colour, water activity, moisture content, fat content and fatty acids profile, oxidative stability, microbiological parameters and sensory analysis.

Concerning hazelnuts, after 12 months of storage, no significant differences were observed in fatty acid percentages. The induction times decreased after three months, remaining similar until 12 months. Nevertheless, no significative oxidation occurred along storage at 4, 25, and 35 ºC. Thus, the temperatures at 4, 25 and 35 ºC are not critical factors to consider in the storage of hazelnut kernels, considering the measured parameters. Some mycotoxins were determined in hazelnuts, such as beauvericin, bikaverin, 3-nitropropionic acid, citreohybridinol, flavoglaucin, quinolactacin A, abscisic acid, leecanoric acid, asperglaucide, asperphenamate, cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Val), infectopyron and tryptophol; however, they were detected in low quantities.

Regarding almond pastes, the brightness and hue decreased and the a^* increased after three months at 65 ºC, indicative of Maillard reactions. The a_w values were constant over time (0.32 and 0.44). Concerning the storage time, the peroxide values increased after one month; however, applying high temperatures, such as 55 and 65 ºC, did not cause a considerable increase in peroxide value after three months. Furthermore, high temperatures, such as 55 ºC and 65 ºC, did not cause very evident secondary oxidation. Concerning the microbiological counts, these were always <1.7 log CFU/g. On the contrary, the temperature of 65 ºC negatively affected the sensory quality of the almond pastes after three months. As this temperature is high and it is uncommon to find it during storage, it is suggested that the almond pastes present a high shelf-life (> 6 months).

Keywords: Hazelnuts; Almond pastes; Storage; Physicochemical properties; Oxidative stability; Microbiological parameters; Accelerated studies.

Resumo

As avelãs e amêndoas são frutos secos amplamente consumidos em todo o mundo. No entanto, existem alguns problemas na sua conservação. O trabalho de dissertação desenvolvido no presente trabalho insere-se no Projeto ValNuts, no qual se pretende valorizar os frutos secos. No presente trabalho pretendeu-se estudar avelãs e amêndoas. Nesse sentido, o efeito da temperatura (4, 25 e 35 ºC) foi estudado ao nível do armazenamento de avelã ao longo do tempo (0, 3, 6, 9 e 12 meses), em termos de ácidos gordos, estabilidade oxidativa e parâmetros microbiológicos. Relativamente à amêndoa, foi proposta a preparação de uma pasta de amêndoa, para a qual se pretendeu determinar o tempo de prateleira através de um teste acelerado. As temperaturas testadas foram 4, 25, 45, 55 e 65 ºC ao longo de três meses. Os parâmetros avaliados foram a cor, atividade de água, teor de humidade, teor de gordura e perfil de ácidos gordos, estabilidade oxidativa, parâmetros microbiológicos e análise sensorial.

Em relação às avelãs, após 12 meses de armazenamento, não foram observadas diferenças significativas nas percentagens de ácidos gordos. Os tempos de indução diminuíram após três meses, permanecendo semelhantes até 12 meses. No entanto, não ocorreu oxidação significativa ao longo do armazenamento a 4, 25 e 35 ºC. Assim, as temperaturas de 4, 25 e 35 ºC não são fatores críticos a serem considerados no armazenamento de miolo de avelã, considerando os parâmetros medidos. Algumas micotoxinas foram determinadas nas avelãs, como beauvericina, bikaverina, ácido 3-nitropropiónico, citreo-hibridinol, flavoglaucina, quinolactacina A, ácido abscísico, ácido leecanórico, asperglaucida, asperfenamato, ciclo(L-Pro-L-Tyr), ciclo(L- Pro-L-Val), infectopirona e triptofol. No entanto, elas foram detectadas em pequenas quantidades.

Em relação às pastas de amêndoa, o brilho e a tonalidade diminuíram e o a^* aumentou após três meses a 65 ºC, indicativo de reações de Maillard. Os valores de a_w apresentaram uma pequena variabilidade ao longo do tempo (0,32 e 0,44). Os valores de índice de peróxidos aumentaram após um mês. Contudo, a aplicação de altas temperaturas, como 55 e 65 ºC, não causou um aumento considerável nos índices de peróxido após três meses. Este facto sugeriu que a aplicação de altas temperaturas, como 55 ºC e 65 ºC, não causou oxidação secundária muito evidente. Quanto às contagens microbiológicas, estas sempre foram <1,7 log UFC/g. Ao contrário, a temperatura de 65 ºC afetou negativamente a qualidade sensorial das pastas de amêndoas após três meses. Como essa temperatura é

alta e não é comum aplicá-la durante o armazenamento, os resultados sugerem que as pastas de amêndoas apresentam um tempo de prateleira elevado (> 6 meses).

Palavras-chave: Avelãs; Pastas de amêndoa; Armazenamento; Propriedades físico-químicas; Estabilidade oxidativa; Parâmetros microbiológicos; Ensaios acelerados.

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1. FRAMEWORK AND MAIN OBJECTIVES

1. FRAMEWORK AND MAIN OBJECTIVES

The present dissertation work is part of the ValNuts Project, which intends to valorise nuts. This project is currently taking place at the Escola Superior Agrária of IPB, Polytechnic Institute of Viseu and Universidade de Trás-os-Montes e Alto Douro (UTAD). The nuts to be studied in future work will be hazelnuts and almonds, with significant economic relevance in our country.

As these nuts present some problems in terms of preservation, and due to the consumers' more significant demand for new nuts-based products, the work to be carried out in the future aims to obtain knowledge on these two themes. So, the main objectives of the future work to be developed will be:

- To study the effect of storage temperature (4, 25 and 35 ºC) as a function of time (up to 12 months) of hazelnut kernels concerning the fatty acid profile, lipid oxidation through induction times by the Rancimat method and microbiological parameters, to assess the quality and food safety of the products over time;

- To determine the shelf life of almond pastes using an accelerated test carried out for 3 months through the evaluation of physicochemical and microbiological properties.

It should be noted that these specific objectives are included in the general objectives of the project mentioned above. Furthermore, the tasks to be carried out are already a sequence of others previously carried out, such as, for example, studies on the preservation of almond kernels, a topic no longer addressed in the present study.

2. INTRODUCTION

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2.1. World, European and national hazelnut and almond productions

Hazelnuts and almonds are nuts that are widely consumed worldwide. Figures 1A and 1B show the production values and planted area for these two fruits worldwide from 2010 to 2020. In 2020, the two nuts presented an average production of 4.14×10^6 and 1.07×10^6 tonnes, respectively, with almond production about 3.9 times higher than hazelnut production.

As we can see in Figure 1, with regard to the hazelnuts' world production, there were more fluctuations in terms of production than in the case of almonds. However, there was a growing trend in the production for the latter from 2017 onwards. Hazelnuts showed a very significant increase in area in 2016 (FAOSTAT , 2021). With some fluctuations, this increase in the area has increased production, having reached the maximum production in 2019, corresponding to 1 125 178 tonnes. However, from 2015 to 2019, there was an increase in production. There was also a slight increase from 2015 onwards regarding the cultivated area, reaching the maximum value of 1 015 216 ha in 2020 (FAOSTAT , 2021). For the production/planted area ratio, the best year was 2012, with a ratio of 1.5.

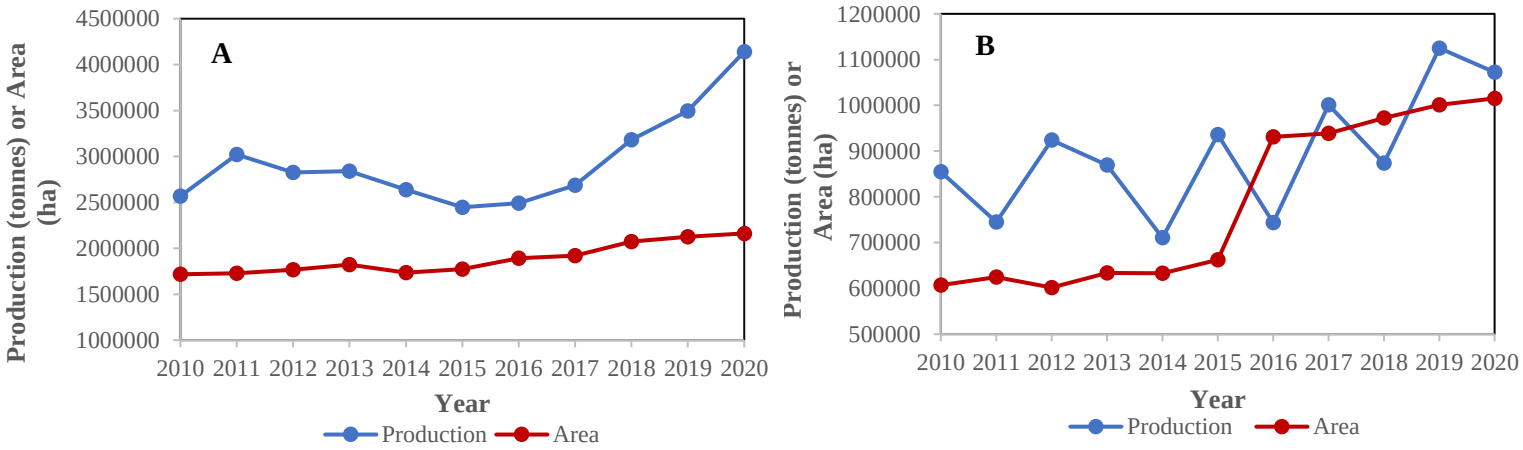

Figure 1 World production (tonnes) and planted area (ha) for the years 2010 to 2020 for almond (A) and hazelnut (B) in shell (Source: FAOSTAT, 2021).

Table 1 shows the ten countries with the world´s largest in-shell almond production of almonds for the year 2020. It was found that the United States of America stood out compared to the rest, with a production of 2 370 021 tonnes (representing 57.2% of world production), followed by Spain with 416 950 tonnes. Australia is also a significant producer of this fruit (221 886 tonnes), as well as Iran and Turkey (164 348 and 159 187 tonnes, respectively). Portugal occupies the fourteenth place.

Table 1 Countries with the highest production of almonds in shell for 2020 (FAOSTAT , 2021).

Countries	**Production** (tonnes)
United States of America	2 370 021
Spain	416 950
Australia	221 886
Iran	164 348
Turkey	159 187
Morocco	134 436
Syrian Arab Republic	123 017
Italy	80 520
Tunisia	62 000
Algeria	60 832

Table 2 shows the top ten hazelnut-producing countries in the world in 2020. The main hazelnut-producing countries are Turkey, Italy, and the United States of America, with 665 000, 140 560 and 64 410 tonnes. Concerning Portugal, it is the twenty-fourth, with 21 tonnes for 2020.

Table 2- Countries with the highest hazelnut production in shell for the year 2020 (FAOSTAT , 2021).

Countries	**Production** (tonnes)
Turkey	665 000
Italy	140 560
United States of America	64 410
Azerbaijan	49 465
Chile	33 939
Georgia	32 700
China	24 263
Iran	13 407
France	9 690
Poland	7 600

Figure 2 shows the European production of almonds and hazelnuts in the shell from 2010 to 2020. In the case of almonds in the shell (Figure 2A), European production behaved differently from world production, except in the last years. There was a progressive increase in production and cultivated area. World production is more dependent on the United States of America than on Europe. Europe only accounts for approximately 14 and 39% of production and cultivated area worldwide, respectively, considering 2020 data. In Europe, Spain and Turkey are the biggest almond producers.

Figure 2B shows the production and cultivated area of hazelnut in the shell. It can be seen that the production of this fruit fluctuated between 2010 and 2014. However, in the years between 2015 and 2018, there was an increase in production, followed by a drop in 2019. European level registered slight fluctuations from 2010 to 2013, showing a slight increase in subsequent years.

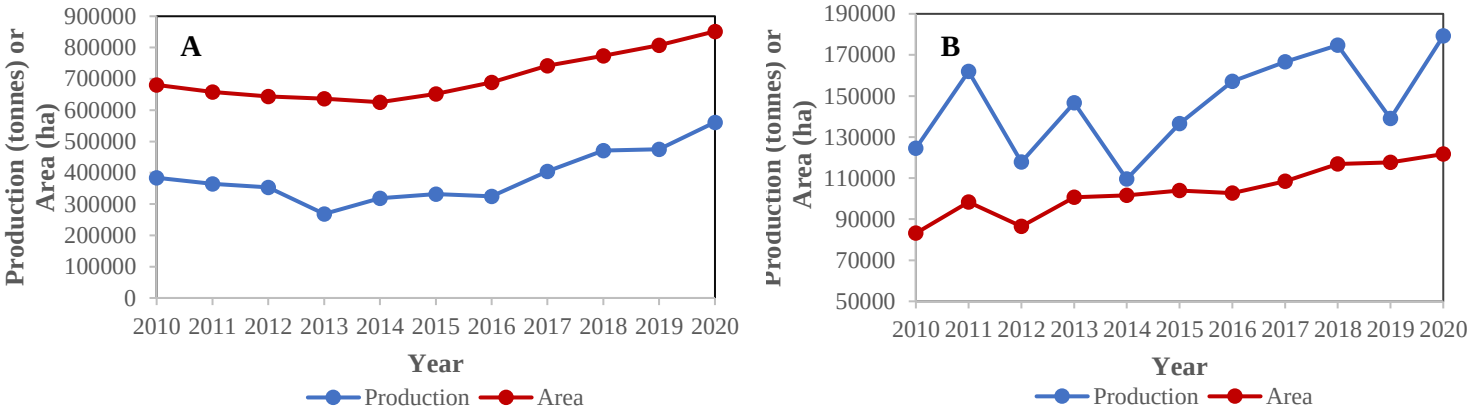

Figure 2 European production (tonnes) and planted area (ha) for the years 2010 to 2020 for almond (A) and hazelnut (B) in the shell (Source:FAOSTAT, 2021).

In 2020, Portugal produced 31 610 tonnes of almonds in the shell, spread over 52 340 ha, corresponding to 5.6 and 6.1% of European production and area, respectively. The production and area planted in the last ten years in Portugal for almonds are shown in Figure 3A. Observing Figure 3B, concerning hazelnuts, it can be concluded that in 2020 the production of hazelnuts in the shell had the lowest value of the last ten years of 210 tonnes, distributed over 320 hectares.

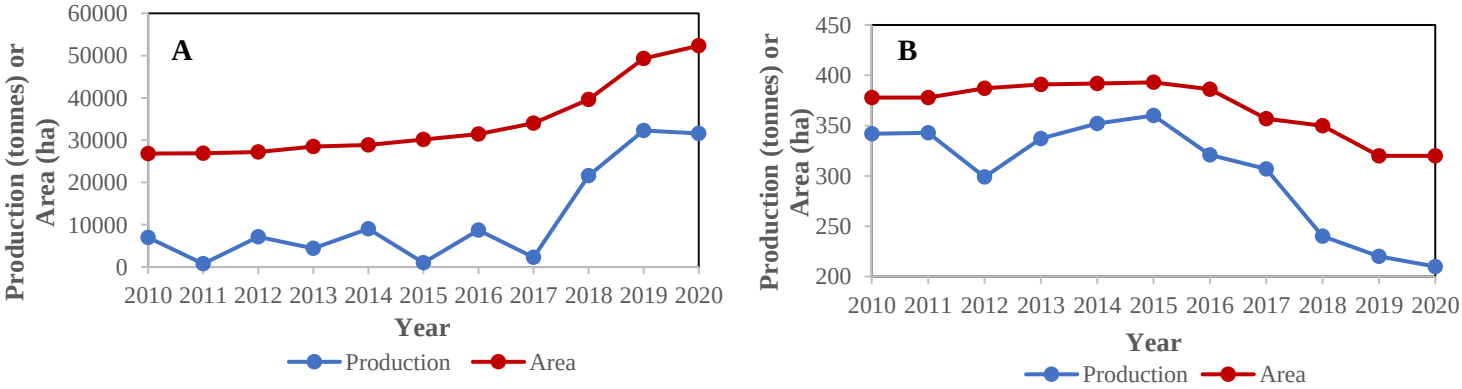

Figure 3 Production in Portugal (tonnes) and planted area (ha) for the years 2010 to 2020 for almond (A) and hazelnut B in the shell (Source:FAOSTAT, 2021).

2.2. Hazelnut

For the consumption of hazelnuts to be beneficial to human health, they must be processed and stored correctly. The following two subsections describe the main problems encountered during hazelnut storage and the storage conditions most applied to this dried fruit.

2.2.1 Main problems observed during hazelnut storage

Hazelnuts are a seasonal fruit generally sold for more than one year after harvest. In addition, the percentage of hazelnuts with defects must be small. As indicated by Silvestri et al. (2021), the portion of defective nuts is influenced by genetic factors (some varieties are more susceptible to defects such as the appearance of twin and moldy seeds), environmental conditions (e.g. prolonged rains during hazelnut harvest) and damage caused by pests and diseases. Defects can be manifested by the appearance of hollow fruits, twin kernels, mouldy or black-tipped seeds, wrinkled/withered fruits, among others (Silvestri et al., 2021). Hazelnuts can be spoiled by the action of different types of insects, such as green beetles (*Nezara viridula*) or *Gonocerus acuangulatus* and *Haliomorpha halys* (Silvestri et al., 2021).

In addition to pests, storage conditions are critical. They allow the preservation of hazelnut's nutritional components and reduce the appearance of abnormal flavours caused by lipid oxidation. Temperature, relative humidity and oxygen availability are the most critical factors affecting hazelnut storage.

During storage of hazelnuts, in addition to lipid rancidity, moulds such as *Aspergillus, Penicillium, Cladosporium, Phomopsis* spp. (Silvestri et al., 2021) can grow. Hadi and Kashefi (2012) referred that *Rhizomucor pusillus* was the most common thermophilic fungi found in hazelnuts. Furthermore, as some of these fungi can be mycotoxin producers, this type of microorganism cannot develop to guarantee the food safety of this product. Among the mycotoxins, aflatoxins should be mentioned. These are considered toxic and dangerous, produced mainly by several fungi that include *Aspergillus* spp. (e.g. *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius*) (Silvestri et al., 2021). In nature, there are several aflatoxins; however, among the most widespread are the aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2)

(Silvestri et al., 2020). Beyond aflatoxins, nuts and dried fruits may be contaminated with ochratoxin A (Hadi & Kashefi, 2012).

2.2.2 Most used conditions in hazelnut storage indicated in preservation studies

The uses of low temperatures and modified gaseous atmospheres (rich in nitrogen and/or carbon dioxide) are generally applied to control enzymatic activity and chemical oxidation and preserve health-promoting constituents (San Martin et al., 2001; Mencarelli et al., 2008). It should be noted that to prolong shelf life and to minimise the occurrence of rancidity reactions, the hazelnuts must be dehydrated immediately after harvesting until reaching a maximum moisture content of 5% and then stored in an environment with a lower relative humidity of less than 70% (Ghirardello et al., 2016). Regarding this drying of the fruits, good results have been reported by applying a drying machine operating at 45 °C (Turan and Karaosmanoğlu, 2019) or 50 °C (Turan, 2019).

Table 3 describes some studies published in recent years related to hazelnut storage, referring to the type of sample, applied conditions and parameters evaluated in these studies. The most studied parameters were temperature, relative humidity and packaging/applied atmosphere. It was found that the most applied temperatures were -25, 4-5, 10 and 20-25 ºC and that the relative humidities varied between 70 and 90%. In general, concerning unshelled (in shell) and kernels, it is possible to store the fruits for up to 12 months without significant lipidic changes when temperatures of 4 and 20 ºC (in shadow) are applied (Monchilova et al., 2017). Turan and Karaosmanoğlu (2019) observed that in-shell hazelnuts (a blend of Turkish varieties) could be stored at 20-25 °C, relative humidity (RH) 70-90%, for 24 months without significant changes in the chemical properties. On the contrary, storing the kernels (shelled almonds) at 4 °C is preferable (Momchilova et al., 2017). Furthermore, in some cases, the absence of oxygen (e.g. vacuum) seemed more relevant than low temperature (Ghirardello et al., 2016). In the case of the unripe hazelnut (fresh hazelnut), a modified atmosphere of 100±1 kPa N_2 maintained the quality of the fresh fruit throughout the 12 days storage period (Moscetti et al., 2012). So, an excellent method to preserve raw hazelnut kernels is to store them under vacuum with or without preliminary nitrogen flushing (Ghirardello et al., 2016).

Table 3 Recently published papers on hazelnut storage.

Hazelnut (Country or Variety)	Fruit Shape	Storage Conditions	Parameters Evaluated	Reference
- Blend: Palaz + Tombul + Kalinkara (Turkey)	- Unshelled (in shell)	– Sun-drying (on concrete ground (CG), grass ground (GG), both for 39 h) or drying machine (45 ºC, 23 h) – Storage, 24 months, 20-25 ºC, HR 70-90%	– Protein – Lipid – Moisture – Water activity – Free fatty acids – Peroxide value – Rancimat value – Aflatoxin	Turan and Karaosmanoğlu (2019)
- Çakildak (Turkey)	- Unshelled (in shell)	– Sun-drying (on concrete ground (CG), 156 h; grass ground (GG), 165 h) or drying machine (50 ºC) – Storage, 24 months, 20-25 ºC, HR 70-90%	– Protein – Lipid – Moisture – Water activity – Fatty acid composition – Sum of fatty acids – Oil oxidation properties	Turan (2019)
- Ata Baba - Ran Trapezundski - Tonda Gentile (Bulgaria)	- Unshelled (in shell) - Shelled (kernels)	– 1, 3, 6, 12 months – 4 and 20 ºC (in shadow)	– Fatty acids – Tocopherols – Oxidative stability (induction period) of the oil	Momchilova et al. (2017)

Hazelnut (Country or Variety)	Fruit Shape	Storage Conditions	Parameters Evaluated	Reference
– Tonda Gentile Trilobata (Italy) – Blend: Tombul +Palaz + Kalinkara (Turkey)	– Unshelled (in shell)	– Roasting (hot air, infrared) at 120 ºC, 40 min (light roast) and 170 ºC, 20 min (dark roast)	– Fatty acids – Oxidative stability – Total phenolic content – Antioxidant capacity – Mechanical and acoustic properties – Sensory perception	Belviso et al. (2017)
– Tonda Gentile Trilobata (Italy) – Delisava (Turkey)	In-shell and shelled (kernels)	– 0, 4, 8, 12 months – Ambient temperature, 5 ºC, -25ºC – Oxygen availability (ambient air, vacuum or modified atmosphere)	– Total phenolic content (TPC) – In vitro antioxidant capacity assays – Phenolic compounds (HPLC-DAD) – Hexanal	Ghirardello et al. (2014)
– Portugal with seed coat (PT-s) – Spain with seed coat (SP-s) - Turkey without seed coat (TR-n)	Without the husk (outer shell)	– Temperature controlled - Unpackaged - With packaging:LDPE[1] and LLDPE[2]	– Moisture content – Water activity – Color – Texture	Guiné et al. (2015a)
Gentile delle Langhe	Fruit without outer shell Unpeeled fruit	- Vacuum - "Rafia" bags	– Humidity – Water activity – Fat oxidative stability	Ghirardello et al. (2014)

Hazelnut (Country or Variety)	Fruit Shape	Storage Conditions	Parameters Evaluated	Reference
Tonda Gentile Romana	Unripe, fresh hazelnut	– 12 days at 4 °C or 10 °C – MAP[3]: 100 ± 1 kPa CO_2, 100 ± 1 kPa N_2 - Air	– Colour – Respiration rate – Firmness – Moisture – Peroxidase activity – Polyphenol oxidase activity – Sensory qualities	Moscetti et al. (2012)

[1]Low-density polyethylene; [2]Linear low-density polyethylene; [3] Modified atmosphere

2.3. Almond

2.3.1 Almond-based products

The food industry is a sector in constant evolution, not only in terms of technology but also in developing new products. This continuous innovation is mainly due to changing consumer tastes and eating habits.

The almond is a very versatile fruit that can be purchased *in natura* (with or without the husk – natural almond kernels) or after being processed. After processing, various products can be obtained, namely: raw almond flour; peeled almond kernels – granulated, toothpick or sliced, peeled almond flour; almonds covered with chocolate or sugar; almonds toasted with salt or sugar, or fried; incorporated into chocolates, *snacks*, *nougat*, turrón and marzipan; typical pastry products, such as almonds covered of Moncorvo, pumpkin jam with almonds, among others; liqueurs; oils; and almond-based vegetable drinks (Ramalhosa et al., 2022).

In particular, alternative products to dairy products have become increasingly important. An example of this is the alternative drinks to milk that emerge as more attractive for the consumer. The almond-based vegetable drink is obtained by grinding the seeds with water and separating the liquid component (filtration). New products involving the fermentation of this vegetable drink have been developed, combining the properties of almonds with probiotics. These new products aim to respond to the growing demand for versatile and health-promoting foods. Among the studies carried out so far in this area, it can be mentioned the application of the Response Surface Methodology to the fermentation of the almond vegetable drink with the probiotics *Lactobacillus reuteri* and *Streptococcus thermophilus*. This study aimed to optimise different parameters, such as glucose, fructose, inulin and *starters*, to ensure high survival of microorganisms in the final product (Bernat et al., 2015). Probiotic bacteria have remarkably survived *in vitro* digestion, which adds value to the product developed, demonstrating the potential health benefits of their consumption. (Bernat et al., 2015).

The market for spreads made from nuts and seeds, such as almond pastes, has also been increasing. Almond pastes are associated with good protein sources and a healthy lifestyle. One of the most common ways to include this product in the diet is through spreads like butter. Pastes made from seeds or nuts are still not very popular in Portugal, despite an increase in their consumption. The best-known and most consumed is peanut butter.

2.3.2 Preservation studies carried out on almond-based products – pastes and pastries

As the future work to be carried out involves the study of almond pastes, a literature review took into account only this type of product and its incorporation into pastry products. Table 2 shows the work carried out so far on this type of product.

In general, lipid oxidation, loss of nutritional components and oil separation during storage are the major problems encountered in nut spreads and nut pastes, affecting their commercial value (Ciftci & Ozilgen, 2019; Capanoglu & Boyacioglu, 2008; Baiano & Del Nobile, 2005). Drying on the product's surface may also occur (Capanoglu & Boyacioglu, 2008). In general, peroxide and free fatty acid values increased as the storage time progressed (Capanoglu & Boyacioglu, 2008). Furthermore, some ingredients have been added to almond pastes to reduce some of these effects, such as black carrot juice (Ciftci & Ozilgen, 2019), sorbic acid and cinnamon (Faid et al., 1995), as well as stabilisers (hydrogenated triglycerides obtained from vegetable oil + mixed tocopherols) (Capanoglu & Boyacioglu, 2008). Good results have been achieved by adding these substances. An increase in the oxidative stability of the almond pastes was observed, the initiation of lipid oxidation was delayed, and the maximum attainable peroxide values were reduced (Ciftci & Ozilgen, 2019). Nevertheless, in some situations, adding a stabiliser may affect the formation of an undesirable dry and cracked surface, lowering the quality of the almond paste (Capanoglu & Boyacioglu, 2008). Oxygen scavengers have also been used (Baiano & Del Nobile, 2005; Baiano et al., 2003). However, in some situations, they can cause the hardening of almond paste because it has been supposed that they consume and absorb water (Baiano et al., 2003). Regarding the application of films, good results have been obtained with both EVOH and nylon, which could prolong the shelf-life of almond paste.

Table 4 Preservation studies on almond-based products – pastes and pastries.

Almond-based product	Ingredients	Storage Conditions	Parameters Evaluated	Reference
- Almond paste - Almond paste + black carrot paste juice	- Almond flour - Water or black carrot paste juice - Sugar	31 days at 4, 20, 30, and 60 °C	– Almond paste oil extraction – Peroxide value – Total phenolic content – Kinetics of the almond oil oxidation – Kinetics of total phenolic compounds	Ciftci & Ozilgen (2019)
Almond-based cookies	Typical product: - Sweet almonds - Egg white - Saccharose - Bitter almond aroma -Modified product: Differences in the weight of bamboo fibre, fructose/ saccharose ratio (F/S) and weight of egg white	- Stored in a climatic chamber (25 °C, RH = 40% for 2 h before analyses	– Hardness – Moisture content – Colour	Farris & Piergiovanni (2009)
Turkish almond paste	Control - Almond - Sugar Seven pastes: - Almond - Sugar - Maltose syrup	- Tightly sealed in boxes - 26 days at 4 and 30 °C	– Moisture and ash – Protein – Fat – Total sugars – Fatty acid composition – Peroxide value **Parameters**	Capanoglu & Boyacioglu (2008)

Almond-based product	Ingredients	Storage Conditions	Evaluated	Reference
	-Stabilisers and antioxidants		– Free fatty acid – Rancimat – Sensory analysis	
Almond paste pastries	-Almonds -Sugar -Eggs -Aromas (cinnamon, cloves, lemon, vanilla)	-Two different multilayer films (PE/EVOH/PE/PET or EVOH and PP/Nylon or Nylon) -Conditions: nylon–nitrogen; nylon–oxygen scavenger; nylon–air (control); EVOH–nitrogen; EVOH–oxygen scavenger; EVOH–air (control) -37 ºC during 6 months	– Fat – Peroxide value – Spectrophotometric indexes (K_{232}, K_{270} and ΔK) – *p*-Anisidine value – Moisture – Firmness – Permeation tests: water vapour and oxygen permeabilities	Baiano & Del Nobile (2005)
Almond paste	-Almonds -Sugar -Whole eggs -Aromas (cloves, vanilla) -150ºC, 8 min	-Two multilayer flexible films: PP/Nylon6,6; PE/EVOH//PE//PET -Air and under nitrogen or in the presence of oxygen scavengers -37 ºC during 2.5 months	– Fat – Peroxide value – p-Anisidine value – Spectrophotometric indexes (K_{232}, K_{270} and ΔK) – Moisture – Firmness	Baiano et al., (2003)

Almond-based product	Ingredients	Storage Conditions	Parameters Evaluated	Reference
Almond paste	Comercial samples	- Ambient temperature - Sorbic acid - Cinnamon	– Dry matter – Water activity – pH value – Lipid content – Acid degree value (ADV) – Alcohol determination – Microbiological determinations: Total plate counts at 30 ºC, Fecal and total coliforms; *Staphylococci*; *Enterococci*; Salmonella; Spore-forming bacteria; Yeasts. – Inhibition assays	Faid et al. (1995)

Almond pastes have also been incorporated into cookies. Regarding the ingredients used, the addition of fibres, such as bamboo fibre, caused an increase in the hardness of cookies (Farris & Piergiovanni, 2009). Also, the sugars used in cookies formulation have an essential role. For example, fructose is more hygroscopic than other sugars, namely, saccharose (Farris & Piergiovanni, 2009). So, this needs to be considered in future packaging to avoid caking or the lumping phenomena (Farris & Piergiovanni, 2009). The sugars also play an essential role in the colour's cookies. The reducing sugars (ex., fructose) are fundamental in defining the colour of baked products because they represent the substrates of the Maillard reaction (Farris & Piergiovanni, 2009). Capanoglu & Boyacioglu (2008) also showed that incorporating maltose syrup might improve the almond paste texture.

2.4. Accelerated tests

It is essential to correctly define the expiration date of a product, being the producer's responsibility to determine it. The shelf life is indicated by the date of minimum durability or by the use-by date (Ramalhosa & Pereira, 2015). The first designation, characterised by the expression "Best before" or "Best before end of", is applied to more stable foodstuffs, which do not deteriorate quickly and is associated with quality. The use-by date, indicated by the expression "Use by", must be applied to microbiologically very perishable foodstuffs and is associated with safety. (Ramalhosa & Pereira, 2015).

To establish shelf life, direct and indirect methods can be used. The former is the most common and generally consists of storing the product under pre-selected conditions longer than the expected shelf life. The product is checked at regular intervals to determine when deterioration begins. Indirect methods involve accelerated storage studies and/or microbiological predictive models (Ramalhosa & Pereira, 2015).

Accelerated storage studies are applied to products estimated to have a long shelf life, and it is necessary to accelerate deterioration reactions.

2.4.1 Accelerated tests performed on almonds

Some accelerated storage studies have already been carried out on almonds in fruit and oil. In Table 5, these works are compiled. So far, only an accelerated study has been

carried out on almond oil, with a temperature of 60 °C applied. Differences between sweet and bitter almond cold-pressed oils existed, suggesting that the oil origin may be an essential factor. After four weeks at 60 ºC, sweet almond cold-pressed oil still presented an excellent physicochemical profile, while bitter almond oil presented much less satisfactory properties.

Regarding the fruit, the accelerated studies carried out so far on almonds have been carried out at a single temperature (e.g. 20, 23 and 39 ºC) and relative humidity (15, 27 or 80%). Flavours associated with oxidative rancidity, such as cardboard, painty/solvent, soapy and total oxidised, may increase during the ageing experiment (Franklin et al., 2018), as expected. On the contrary, heptanal, octanal, nonanal, and hexanal detected as headspace volatiles displayed a good correlation with consumer liking (Franklin et al., 2017). Valdés et al. (2015) verified that raw samples were more stable against accelerated oxidation than roasted almonds. Moreover, the roasting degree also affected the peroxide value (Franklin et al., 2017), which measures lipid oxidation, suggesting that roasting affects lipid stability.

Furthermore, storage at low relative humidity did not cause an increase in free fatty acids in roasted almonds subjected to ageing conditions (Franklin et al., 2017). On the contrary, Zacheo et al. (1998) verified that accelerated ageing stimulated lipid peroxidation and inhibited the free radical and peroxide scavenging enzymes, such as superoxide dismutase and peroxidase. These works generally indicate that the choice of conditions to be applied in the accelerated tests is important because they may affect the parameters to be evaluated differently.

Table 5 Accelerated studies carried out on almond products.

Almond product	Storage Conditions	Parameters Evaluated	Reference
Sweet and bitter almond oils	– Transparent glass bottle – Constant temperature of 60 ± 2 ºC	– Fatty acids – Sterols – Tocopherols – Free Fatty Acid content – Extinction coefficients (K_{232} and K_{270}) – Peroxide value – Iodine value – Carotenoid content – Chlorophyll content	El Bernoussi et al. (2020)
Roasted almonds (115 ºC, 60 min; and 152 ºC, 15 min)	– Open brown paper bags – 39 ºC during 12 months, RH=15% – Control (after roasting were maintained at -80 ºC)	– Volatiles – Quantitative descriptive analysis – Consumer hedonic analysis	Franklin et al. (2018)
Sweet almonds	– Different conditions: 115 ± 6 °C for 60 min (light degree of roast) and 152 ± 6 °C for 15 min (dark degree of roast) – Samples were stored at 15 ±1% relative	– Oil – Peroxide Value – Free Fatty Acids – Conjugated Dienes – Headspace Solid-Phase Microextraction (HS-SPME) GC/MS – Tocopherols – Consumer Hedonic Analysis	Franklin et al. (2017)

Almond product	Storage Conditions	Parameters Evaluated	Reference
	humidity (HR) and 39 ± 1 ℃ along 12 months		
Ground almond Almond oil	– 100 ºC (0, 3, 5 and 10 days) – Control: 25±3°C for 4, 8 and 11 months	– Fatty acids – Oxidative indices: Peroxide value and *p*-anisidine – Thermal analysis – Volatiles – ATR-FTIR[1] analysis	Valdés et al. (2015)
Almond Seeds	- Plastic box at 20 °C, HR = 80%, 40 days -	– Lipid extraction – Malondialdehyde (MDA) determination – Enzyme extraction and assays: lipoxygenase, superoxide dismutase and peroxidase – Protein	Zacheo et al. (1998)

[1]ATR-FTIR = Attenuated Total Reflectance Infrared Spectroscopy

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

The following sections describe the experimental work performed on hazelnuts and almond pastes studied in the present work.

3.1 Sampling

The hazelnuts, a commercial sample (mixture of varieties), were supplied by Coopenela - Cooperativa Agrícola de Penela da Beira C.R.L. They were stored at different temperatures (4, 25 and 35 ºC) during 0, 3, 6, 9 and 12 months at the Polytechnic Institute of Viseu (IPV). The samples were stored in a fridge (4º C) and in stovens at 25 and 35 ºC, without control of relative humidity.

Regarding almonds, Amendouro supplied the almond kernels (mixture of varieties). The almond pastes were produced at the laboratory of Polytechnic Institute of Bragança (IPB) by only milling the seeds in an universal mill (IKA, WERKE M20). Then, the almond pastes were stored at five temperatures, namely 4, 25, 45, 55 and 65 ºC, for three months (Accelerated Study) at IPB. Samples were taken at 0 days, 1, 2 and 3 months.

3.2 Physicochemical analyses performed on hazelnut kernels

3.2.1 Fatty acids composition

The oil was extracted in IPV and then transported to IPB under refrigeration in the dark. To determine the fatty acid profile, the Commission Delegated Regulation (EU) 2015/1830 of 8 July was followed. Fatty acids were assessed as methyl esters, after cold alkaline transesterification with methanolic potassium hydroxide solution.

The procedure was the following: 0.1 g of fat was weighed, and 2 ml of *n*-heptane was added. The solution was stirred, and 200 µl of the methanolic KOH solution (2 M) was added. It was stirred for 30 seconds and allowed to stand until the top of the solution was clear. This solution was filtered through a 0.2 µm Nylon syringe filter. The fatty acid profile was established using a Chrompack CP 9001 chromatograph, a split-splitless injector, an FID detector, an autosampler Chrompack CP-9050 and a fused silica Select FAME capillary column (50 m × 0.25 mm i.d.; Varian, Palo Alto, CA, USA). The carrier gas was helium at an internal pressure of 140 kPa. The detector temperature was 270 °C,

and the injector was kept at 250 °C. A 1:50 split ratio was used, being injected 1 μL. The fatty acids contents were quantified in relative percentage, calculated by internal normalization of the chromatographic peak area eluting between myristic and lignoceric methyl esters. A fatty acids methyl esters standard mixture (Supelco 37 FAME Mix) was used for identification and calibration purposes (Sigma, Madrid, Spain).

3.2.2 Oxidative stability by the Rancimat method

Oxidative stability was assessed in hazelnut oil using the Rancimat method (Metrohm, s/d). This is an accelerated ageing test. Air passes through the sample in the reaction vessel at a constant high temperature (e.g. 120 °C). In this process, fatty acids are oxidised. At the end of the test, volatile compounds, side products of the reaction, are formed and are transported to the conductivity measuring vessel by the airflow and absorbed into the measuring solution (deionised water). The electrical conductivity of the measuring solution is continuously recorded, increasing progressively. The time until secondary reaction products are detected is called the induction time. This characterises the oxidation stability of oils and fats.

For the measurement of the oxidative stability of the hazelnut oil, approximately 3 grams were weighed for each tube and placed in the apparatus (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. The induction times were evaluated in triplicate for each sample.

3.2.3 Microbiological Parameters

To determine the microbiological parameters of the hazelnuts, they were processed in a biological safety chamber. Ten grams of hazelnut kernels from each storage condition were measured into sterile bags, and then 90 ml of sterile peptone water with 0.05% Tween 80 were added. After shaking the sample by inversion for 2 minutes, followed by resting at room temperature, three decimal dilutions were performed. The microorganisms analysed were total microorganism count at 30 ºC and moulds and yeasts.

3.2.3.1 Microorganisms at 30ºC

Total microorganism counts at 30°C were performed according to ISO 4833-2 (2013). The Plate Count Agar (PCA, Lyophilchem, Italy) culture medium was used. After seeding 0.2 ml of each dilution in duplicate, the plates were incubated in an incubator at 30 °C for 48h-72h. After the incubation period, colonies were counted. All counts were expressed as $\log_{10}$ CFU/g f.w. and calculated according to ISO 7218:2007 (ISO 7218:2007, 2007).

3.2.3.2 Mould and yeast

Mould and yeast counts were performed according to ISO 21527-2, using Dichloran Glycerol Agar culture medium (DG18, Lyophilchem, Italy). After seeding 0.2 ml of each dilution in duplicate, the plates were incubated for 5 days. All counts were expressed as $\log_{10}$ CFU/g f.w. and calculated according to ISO 7218:2007 (ISO 7218:2007, 2007).

3.2.3.3 Mycotoxins

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 to the Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, University of Natural Resources and Life Sciences, Vienna, Austria.

A multi-analyte method of Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS) was applied to analyze the mycotoxins present in the samples. The detection and quantification were performed with a QTrap 5500 MS/MS system (Applied Biosystems, Foster City, CA) 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).

3.3 Almond paste

3.3.1 Colour

The colour of the almond pastes was evaluated with a colourimeter Minolta CR-400 (Osaka, Japan) using the CIELab scale. *L**, *a** and *b** coordinates, chroma (*C**) and hue angle (h) values were determined. In the beginning, the equipment was always calibrated through a white tile. The brightness (*L**) varies between 0 (black) and 100 (white); a* from –green to +red; and b* from –blue to +yellow.

3.3.2 Water activity

For the measurement of water activity (a_w) in almond pastes, a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland) was used. Prior to carrying out measurements on the samples, the device was calibrated with three solutions of NaBr - 58%, KCl - 84% and LiCl - 11%. After verifying the functioning of the device, the water activity in the samples was determined, as well as the temperature at which the measurement was performed.

3.3.3 Moisture content

To determine the moisture contents of almond pastes, porcelain capsules were numbered and placed in an oven (Memmert) at 105°C for two hours. After the capsules were dried and cooled in the desiccator, two grams of homogeneous sample were weighed. Then, the capsules with the sample were placed in the oven at 105 °C, until the samples reached constant weight. The moisture content (%, f.w.) was determined by the following equation:

$$Moisture\ content(\%, f.w.) = \frac{(Initial\ (sample+capsule) weight - Final\ (sample+capsule) weight)}{Sample\ weight} \times 100 \quad \text{(Eq. 1)}$$

3.3.4 Fat content and fatty acids profile

To determine the fat contents, 30 grams of almond paste was weighed and placed in a cup with 150 ml of petroleum ether with di-*tert*-butyl methyl phenol (BHT) at 0.01% (m/v). The sample was left to stir for 24 hours in the dark. After that, the sample was filtered through analytical filter paper in a Polycarbonate Filter-Holder. The solution was quantitatively transferred to 250 mL round bottom flask. However, this flask was

previously dried in the oven at 105 ºC, cooled in the desiccator and weighed. The round bottom flask with the sample was placed in a rotary evaporator.

The method applied in the quantification of the fatty acids in the almond paste was the same as that used for the hazelnut kernel already explained in Subsection 4.2.1.

3.3.5 Oxidative stability

3.3.5.1 Peroxide value

For the peroxide value determination, 1.2 grams of oil were weighed into a 250 mL Erlenmeyer flask. Then 10 mL of chloroform, 15 mL of glacial acetic acid, and 1 mL of saturated potassium iodide were added. Then the Erlenmeyer flask was capped with parafilm and stirred for 1 minute. Subsequently, the Erlenmeyer was placed for 5 minutes in the dark, and subsequently, 25 mL of distilled water and 5 drops of starch were added. The resulting solution was titrated with sodium thiosulphate (0.1 N), drop by drop, until the solution became transparent.

The peroxide value, expressed in milliequivalents of active oxygen per kg of oil, was calculated from the following formula:

$$Peroxide\ Index\ (mEq.O_2/kg) = \frac{V \times N \times 1000}{m} \qquad \text{(Eq. 2)}$$

Where:

V = number of millilitres of sodium thiosulphate solution spent in the assay, with the correction relative to the blank assay.

N = exact normality of the sodium thiosulphate solution used in the titration.

M = mass, expressed in grams, of the test portion.

3.3.5.2 Ultraviolet extinction coefficients

To determine the ultraviolet extinction coefficients, the method described in Commission Implementing Regulation (EU) 2015/1833 of 12 October was followed. Spectrophotometric analysis in the ultraviolet can provide indications of the quality of the fat, its state of preservation and changes due to the technological processes it has undergone. The absorbances at the wavelengths specified in the method are due to the presence of conjugated diene and triene systems, resulting from oxidation processes

and/or refining practices. The absorbance values are expressed in terms of specific extinction $E_{1cm}^{1\%}$ that is the extinction of a 1% (w/v) solution of the fat in the prescribed solvent in a 10 mm cell, conventionally referred to as *K* and also referred to as "extinction coefficient".

To determine the extinction coefficients at 232 nm and 268 nm, 0.6 g of oil was weighed into a graduated Falcon tube. Then iso-octane (pa) was added to make up 10 mL. From this solution, dilutions of 1:40 were prepared to evaluate the absorbances at 232 nm and of 1:5 to evaluate the absorbance at 268 nm (SHIMADZU UV-1280). The absorbance values were always between 0.1 and 0.8. The formula used to calculate the extinction factors at 232 and 268 nm was as follows:

$$K(\lambda\, nm) = \frac{absorbance(\lambda\, nm)\ \times Dilution\ Factor}{Msample\ \times 10}$$

Where

$absorbance(\lambda\, nm)$ = absorbance measured at the wavelength λ (232 or 268 nm)
Msample = oil weight (g) measured to perform the analysis

3.3.5.3 Rancimat method - Induction times

The method for obtaining the induction times for the almond pastes was the same as for the hazelnut kernel, already explained in Subection 4.2.2.

3.3.6 Microbiological Parameters

3.3.6.1 Microorganisms at 30 °C, mould and yeast

For the quantification of microorganisms at 30°C, moulds and yeasts in the almond paste, the same procedures explained in Subsections 4.2.3.1 and 4.2.3.2, respectively, were applied.

3.3.6.2 Total coliforms and Escherichia coli

For the determination of total coliforms and *E. coli*, 3M Petrifilm™ plates were used (AOAC Method 991.14). These plates contain Red Violet Bile (VRB) medium, a cold water-soluble gelling agent, an indicator of glucuronidase activity and an indicator that

facilitates colony enumeration. For the procedure, 1 mL of the solution was added to the plate and incubated at 35 °C for 24 h. Total coliforms were identified by red and blue colonies with gas and *E. coli* by the appearance of blue colonies with gas around them.

3.3.6.3 Clostridium sulfite-reducing

For the determination of Sulfite-reducing bacteria growing under anaerobic conditions, ISO 15213:2003 - Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions, was followed. Iron Sulphite Agar medium was used. Each plate was seeded 1mL and incubated with two layers of the medium at 37 °C in an anaerobiosis jar for 48 hours.

3.3.6.4. *Staphylococcus aureus*

For testing the presence of coagulase-positive staphylococci (e.g. *Staphylococcus aureus*), ISO 6888-1:1999 was followed. After seeding 0.2 mL of each dilution in triplicate, the Baird-Parker agar plates were incubated in an incubator at 30 °C for 48 h. After the incubation period, if shiny black/grey colonies appear with surrounding clear halos, the presence of *Staphylococcus aureus* must be stated. A positive coagulase test would be required. Nevertheless, in the present work the results were always negative.

3.3.7 Sensory analysis

A descriptive sensory test was conducted with seven semi-trained panelists to track changes in the sensory attributes of almond pastes at different temperatures and over time. The samples were coded using random three-digit numbers. The panelists rinsed their mouths with water, between each tasting. All samples were presented in white cardboard cups, containing 15 g of almond pastes.

The panelists were asked to rate the almond pastes in terms of visual and olfactory, as well as taste properties. Regarding the visual and olfactory properties, the panelists rated the almond pastes in terms of visual appearance, colour, shine, general aroma, intensity (aroma) and rancid aroma at the beginning and along one, two and three months of storage. Simultaneously, the texture, toughness, adhesiveness, chewability, general flavour, bitter flavour, sweetness, rancid flavour and global appreciation were evaluated.

3.4 Statistical Analysis

The statistical analysis of the results was performed using the Minitab software version 14 (Pennsylvania, USA). To analyze the normality and homogeneity of variance, the Shapiro-Wilk and Levene tests were used, respectively. In the case where normality and homogeneity of variance were not observed, non-parametric tests were applied, namely the Kruskal-Wallis Test. When the data followed a normal distribution and homogeneity of variances were observed, ANOVA was applied. Then, the Tukey test was used in case of significant differences between samples ($p<0.05$). When no homogeneity of variances was observed, ANOVA-Welch was applied. In case of significant differences between samples ($p<0.05$), the Games-Howell Post-hoc test was used.

4. RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

In the following sections, the results obtained for the hazelnuts and almond pastes will be presented.

4.1 Hazelnuts

As referred to in the experimental part, the hazelnuts were stored at 4, 25 and 35 °C for 0, 3, 6, 9 and 12 months. In the present work, only the fatty acid profile, the oxidative stability of the oil extracted from the hazelnuts and the microbiological counts were determined. Other parameters were evaluated by another student of the Polytechnic Institute of Viseu.

The following subsections will present the results obtained for the hazelnuts.

4.1.1 Fatty acid composition

The fatty acid composition of hazelnuts during storage is shown in Table 6. During the 12 months of storage, the dominant saturated fatty acid was palmitic acid (C16:0) (6.1-9.2%), followed by arachidic acid (C20:0) (0.110-0.187%). The highest unsaturated fatty acid was identified as oleic acid (C18:1n9) (67.2-84.2%), the major, followed by linoleic acid (C18:2n6) (9.7-17.9%). Similar results were reported in the studies of Oliveira et al. (2008) in Portuguese varieties (C16:0 between 4.95-5.76%; C20:0 between 0.11-0.14%; C18:1n9 between 80.67-82.63%; C18:2n6 between 9.84-11.26%), and Alasalvar et al. (2010) (C16:0 between 5.00-5.95%; C20:0 between 0.087-0.207%; C18:1n9 between 77.77-86.91%; C18:2n6 between 4.42-13.77%), Taş and Gökmen (2015) (C16:0 between 4.59-7.08%; C18:1n9 between 74.0-85.7%; C18:2n6 between 5.49-15.99%), and Kalkisim et al. (2016) (C18:1n9 between 81.90-84.88%; C18:2n6 between 8.69-10.62%) in Turkish varieties.

After 12 months of storage, no significant differences were observed between the three storage conditions (4, 25 and 35 ºC) for all fatty acids, suggesting that the temperatures applied did not affect the fatty acid profile.

Table 6 Fatty acid composition (%) of hazelnut kernels during storage.

Fatty acid	Storage conditions	Storage time (months)				
		0	3	6	9	12
C14:0	4ºC	0.033±0.005^{a}	0.035±0.007^{a,A}	0.038±0.001^{ab,A}	0.058±0.005^{b,B}	0.047±0.013^{ab,A}
	25ºC	0.033±0.005^{a}	0.037±0.006^{a,A}	0.037±0.008^{a,A}	0.040±0.002^{ab,A}	0.058±0.012^{b,A}
	35ºC	0.033±0.005^{a}	0.035±0.002^{a,A}	0.039±0.008^{a,A}	0.047±0.008^{ab,AB}	0.058±0.006^{b,A}
C15:0	4ºC	0.008±0.002^{a}	0.009±0.002^{a,A}	0.009±0.001^{a,A}	0.014±0.001^{a,A}	0.013±0.005^{a,A}
	25ºC	0.008±0.002^{a}	0.007±0.005^{a,A}	0.008±0.003^{a,A}	0.010±0.000^{a,A}	0.013±0.004^{a,A}
	35ºC	0.008±0.002^{a}	0.007±0.000^{a,A}	0.009±0.003^{a,A}	0.012±0.003^{a,A}	0.014±0.003^{a,A}
C16:0	4ºC	5.1±0.3^{a}	5.6±0.2^{a,A}	5.8±0.1^{a,A}	9.2±0.4^{b,B}	7.2±2.3^{ab,A}
	25ºC	5.1±0.3^{a}	5.2±0.4^{a,A}	5.4±0.1^{a,A}	6.1±0.4^{ab,A}	8.5±2.1^{b,A}
	35ºC	5.1±0.3^{a}	5.4±0.5^{a,A}	6.7±2.0^{a,A}	7.4±1.6^{a,AB}	8.5±1.8^{a,A}
C16:1	4ºC	0.197±0006^{a}	0.219±0.015^{a,A}	0.225±0.011^{a,A}	0.339±0.001^{b,B}	0.277±0.078^{ab,A}
	25ºC	0.197±0.006^{a}	0.189±0.023^{a,A}	0.201±0.001^{a,A}	0.244±0.010^{ab,A}	0.331±0.080^{b,A}
	35ºC	0.197±0.006^{a}	0.202±0.032^{a,A}	0.259±0.079^{a,A}	0.291±0.05^{a,AB}	0.317±0.049^{a,A}
C17:1	4ºC	0.068±0.002^{a}	0.070±0.002^{a,A}	0.076±0.004^{a,A}	0.119±0.004^{b,B}	0.097±0.033^{ab,A}
	25ºC	0.068±0.002^{a}	0.071±0.012^{a,A}	0.075±0.006^{a,A}	0.079±0.006^{ab,A}	0.110±0.024^{b,A}
	35ºC	0.068±0.002^{a}	0.067±0.003^{a,A}	0.100±0.040^{a,A}	0.110±0.014^{a,B}	0.101±0.003^{a,A}
C18:1n9c	4ºC	835±1.2^{b}	83.4±0.2^{b,A}	83.1±0.7^{b,A}	70.9±0.5^{a,A}	76.6±8.1^{ab,A}
	25ºC	83.5±1.2^{a}	83.1±0.8^{a,A}	83.7±0.8^{a,A}	82.0±0.8^{a,B}	76.6±8.1^{a,A}
	35ºC	83.5±1.2^{a}	84.2±0.5^{b,A}	80.8±5.8^{b,A}	77.4±4.7^{b,AB}	67.2±0.0^{a,A}

Note: Different capital letters in a column indicate significant differences between samples for a given storage time (p-value <0.05). Different lower-case letters in a row indicate the existence of significant differences between times for a given storage condition (p-value <0.05).

Table 6 (cont.) Fatty acid composition (%) of hazelnut kernels during storage.

Fatty acid	Storage conditions	Storage time (months)				
		0	3	6	9	12
C18:2n6c	4ºC	10.7±0.8^{a}	10.3±0.1^{a,AB}	10.4±0.6^{a,A}	17.9±1.1^{b,B}	13.9±5.4^{ab,A}
	25ºC	10.7±0.8^{a}	11.0±0.6^{ab,B}	10.2±0.7^{a,A}	11.2±0.4^{ab,A}	15.5±3.7^{b,A}
	35ºC	10.7±0.8^{a}	9.7±0.2^{a,A}	11.6±3.6^{a,A}	14.2±3.0^{a,AB}	15.3±3.3^{a,A}
C18:3n6	4ºC	0.015±0.001^{a}	0.012±0.001^{a,A}	0.013±0.003^{a,A}	0.022±0.003^{a,B}	0.016±0.007^{a,A}
	25ºC	0.015±0.001^{a}	0.014±0.002^{a,A}	0.012±0.004^{a,A}	0.013±0.001^{a,A}	0.020±0.007^{a,A}
	35ºC	0.015±0.001^{a}	0.015±0.002^{a,A}	0.016±0.005^{a,A}	0.019±0.004^{a,AB}	0.020±0.004^{a,A}
C18:3n3	4ºC	0.077±0.003^{a}	0.075±0.002^{a,A}	0.072±0.009^{a,A}	0.0126±0.004^{ab,A}	0.099±0.036^{b,A}
	25ºC	0.077±0.003^{a}	0.078±0.010^{a,A}	0.074±0.008^{a,A}	0.093±0.003^{ab,A}	0.122±0.033^{b,A}
	35ºC	0.077±0.003^{a}	0.075±0.002^{a,A}	0.086±0.027^{a,A}	0.103±0.024^{a,A}	0.104±0.020^{a,A}
C20:0	4ºC	0.119±0.021^{a}	0.114±0.001^{a,A}	0.110±0.009^{a,A}	0.187±0.009^{b,A}	0.140±0.038^{ab,A}
	25ºC	0.119±0.021^{a}	0.113±0.007^{a,A}	0.124±0.010^{ab,A}	0.129±0.010^{ab,A}	0.179±0.041^{b,A}
	35ºC	0.119±0.021^{a}	0.118±0.005^{a,A}	0.124±0.048^{a,A}	0.160±0.040^{a,A}	0.174±0.033^{a,A}
C20:1	4ºC	0.138±0.012^{a}	0.127±0.001^{a,A}	0.132±0.018^{a,A}	0.219±0.003^{b,B}	0.168±0.045^{ab,A}
	25ºC	0.138±0.012^{ab}	0.138±0.002^{ab,B}	0.116±0.031^{a,A}	0.143±0.007^{ab,A}	0.197±0.046^{b,A}
	35ºC	0.138±0.012^{a}	0.138±0.004^{a,B}	0.158±0.047^{a,A}	0.187±0.040^{a,AB}	0.199±0.042^{a,A}
C22:0	4ºC	0.035±0.006^{a}	0.030±0.003^{a,A}	0.034±0.004^{a,A}	0.032±0.002^{a,A}	0.033±0.006^{a,A}
	25ºC	0.035±0.006^{a}	0.031±0.004^{a,A}	0.033±0.001^{a,A}	0.036±0.002^{a,A}	0.039±0.003^{a,A}
	35ºC	0.035±0.006^{a}	0.032±0.001^{a,A}	0.031±0.001^{a,A}	0.041±0.007^{a,A}	0.043±0.003^{a,A}

Note: Different capital letters in a column indicate significant differences between samples for a given storage time (p-value <0.05). Different lower-case letters in a row indicate the existence of significant differences between times for a given storage condition (p-value <0.05).

When comparing the beginning (0 days) with 12 months, some significant differences were observed in some fatty acids, mainly at 25 ºC, where an increase in C14:00 (0.033 to 0.058%), C16:0 (5.1 to 8.5%), C16:1 (0.197 to 0.331%), C17:1 (0.068 to 0.110%), C18:2n6 (10.7 to 15.5%), C18:3n3 (0.077 to 0.122%) and C20:0 (0.119 to 0.179%) was observed. Also, at 4 ºC and 35 ºC, an increase was observed in C18:3n3 (0.077 to 0.099%) and C14:00 (0.033 to 0.058%), respectively. However, as no identical behavior was observed at the temperature of 35ºC, the variation obtained at 25ºC may be due to the heterogeneity of the sample and not to an effect of temperature.

The main fatty acid classes, mainly saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and the ratios between unsaturated fatty acids and SFA (USFA/SFA) and MUFA/PUFA, are represented in Table 7.

The MUFA had the highest percentage, between 67.8 and 84.6%, mainly due to the oleic acid (C18:1n9). The ranges obtained by Oliveira et al. (2008) in Portuguese varieties (81.12-83.05%) and Alasalvar et al. (2010) (78.10-87.26%) were similar to the determined in the present work.

Table 7 SFA, MUFA, PUFA, USFA/SFA and MUFA/PUFA of hazelnut kernels during storage.

Parameters	**Storage conditions**	**Storage time (months)**				
		0	**3**	**6**	**9**	**12**
SFA (%)	4ºC	5.3 ± 0.3^{a}	$5.8\pm0.2^{a,A}$	$5.9\pm0.01^{a,A}$	$9.4\pm0.4^{b,B}$	$7.4\pm2.3^{ab,A}$
	25ºC	5.3 ± 0.3^{a}	$5.4\pm0.4^{a,A}$	$5.6\pm0.2^{a,A}$	$6.3\pm0.4^{ab,B}$	$8.7\pm2.1^{b,A}$
	35ºC	5.3 ± 0.3^{a}	$5.6\pm0.5^{a,A}$	$6.9\pm1.9^{a,A}$	$7.7\pm1.6^{a,AB}$	$8.7\pm1.8^{a,A}$
MUFA (%)	4ºC	83.9 ± 1.1^{a}	$83.7\pm0.2^{a,A}$	$83.5\pm0.7^{a,A}$	$71.5\pm0.5^{a,A}$	$77.1\pm8.0^{ab,A}$
	25ºC	83.9 ± 1.1^{a}	$83.5\pm0.8^{a,A}$	$84.1\pm0.9^{a,A}$	$82.4\pm0.8^{a,B}$	$77.2\pm8.0^{a,A}$
	35ºC	83.9 ± 1.1^{b}	$84.6\pm0.5^{b,A}$	$81.3\pm5.6^{b,A}$	$77.9\pm4.6^{b,AB}$	$67.8\pm0.1^{a,A}$
PUFA (%)	4ºC	10.8 ± 0.8^{a}	$10.4\pm0.2^{a,AB}$	$10.5\pm0.6^{a,A}$	$18.1\pm1.1^{b,B}$	$14.0\pm5.5^{ab,A}$
	25ºC	10.8 ± 0.8^{a}	$11.1\pm0.6^{ab,B}$	$10.3\pm0.7^{a,A}$	$11.3\pm0.5^{ab,A}$	$15.6\pm3.7^{b,A}$
	35ºC	10.8 ± 0.8^{a}	$9.8\pm0.2^{a,A}$	$11.7\pm3.6^{a,A}$	$14.3\pm3.0^{a,AB}$	$15.3\pm3.4^{a,A}$
USFA/SFA	4ºC	18.0 ± 1.1^{c}	$16.2\pm0.7^{bc,A}$	$15.8\pm0.1^{bc,A}$	$9.5\pm0.4^{a,A}$	$13.1\pm3.8^{ab,A}$
	25ºC	18.0 ± 1.1^{b}	$17.5\pm1.3^{b,A}$	$16.8\pm0.5^{b,A}$	$15.0\pm0.9^{ab,A}$	$11.0\pm3.2^{a,A}$
	35ºC	18.0 ± 1.1^{b}	$16.9\pm1.6^{b,A}$	$14.1\pm3,6^{ab,A}$	$12.4\pm3.0^{ab,AB}$	$9.7\pm1.8^{a,A}$
MUFA/PUFA	4ºC	7.9 ± 0.7^{b}	$8.0\pm0.1^{b,AB}$	$8.0\pm0.5^{b,A}$	$4.0\pm0.3^{a,A}$	$6.2\pm2.4^{ab,A}$
	25ºC	7.9 ± 0.7^{b}	$7.5\pm0.5^{ab,A}$	$8.2\pm0.7^{b,A}$	$7.3\pm0.4^{ab,B}$	$5.2\pm1.8^{a,A}$
	35ºC	7.9 ± 0.7^{ab}	$8.6\pm0.2^{b,B}$	$7.4\pm2,4^{ab,A}$	$5.6\pm1.5^{ab,AB}$	$4.6\pm1.1^{a,A}$

Note: Different capital letters in a column indicate significant differences between samples for a given storage time (p-value <0.05). Different lower-case letters in a row indicate the existence of significant differences between times for a given storage condition (p-value <0.05).

PUFA was the second main fatty acid class. It varied between 9.8 and 18.1%. The fatty acids that most contributed to this class were linoleic acid (C18:2n6) and α-linolenic acid (C18:3n3). The range for PUFA determined in the present work was similar to the ones determined by Oliveira et al. (2008) in Portuguese varieties (9.99-11.43%) and Alasalvar et al. (2010) (3.92-13.86%).

Considering the storage time, although some minor changes occurred in the fatty acid classes when comparing the beginning with 12 months of storage, the SFA, MUFA and PUFA classes, in general, remained identical, with the exceptions at 25 ºC in SFA and PUFA. In other words, the three storage conditions presented similar magnitudes of fatty acid profiles. These results were identical to those mentioned by Momchilova et al. (2017). They also stated that fatty acids had not been changed significantly during hazelnut storage for up to 12 months irrespective of different conditions such as temperature (4 ºC or 20 ºC) and the presence or absence of shell. It must be referred that Momchilova et al. (2017) studied Tonda Gentile hazelnuts of the specie *Corylus avellana* L., but also Ata Baba (*Corylus pontica* C. Koch) and Ran Trapezundski (*Corylus maxima* Mill.).

MUFA and PUFA, which make up the USFA and are considered heart-healthy fatty acids, accounted for more than 90% of the total fatty acids. In terms of their high proportion in USFA, hazelnut oils are much healthier than other major vegetable oils, such as coconut (MUFA+PUFA equal to 7.6%) and palm (MUFA+PUFA equal to 48.2%) oils (INSA, 2022). Consumption of high amounts of USFA decreases the low-density lipoprotein-cholesterol and increases the high-density lipoprotein-cholesterol concentrations, diminishing the risk of developing heart disease, stroke, and certain cancers, among others (Willet, 1997; Hu et al., 1999).

The ratio of USFA/SFA varied between 9.5 and 18.0 in the present work. This range is similar to the one determined by Taş and Gökmen (2015) in Turkish varieties collected in 2013 and 2014 years (8.2 to 14.0). The ratio MUFA/PUFA, which practically corresponds to the ratio of oleic acid to linoleic acid, varied between 4.0 and 8.6. This range is narrower than the one determined by Alasalvar et al. (2010) for Turkish varieties in natural form, 5.6-22.5. As mentioned by these authors, the ratio of oleic to linoleic is considered an important criterion for evaluating kernel quality. The higher the value, the better the oxidative stability.

When comparing the results obtained over time, the USFA/SFA ratio decreased, especially when comparing 0 and 12 months. Nevertheless, for the MUFA/PUFA ratio, a reduction was only observed at 25 ºC.

4.1.2 Oxidative Stability

The oxidative stability of oils is an important indicator of their allowable shelf-life. It depends on the fatty acid composition, the availability of natural antioxidants (tocopherols, etc.), and the presence of synergists (e.g. phytosterols). The oxidative stability was evaluated by the Rancimat method by determining the induction period in hours. The higher the induction time, the better the oxidative stability. In Table 8 are presented the induction times determined for the hazelnuts stored for 12 months at 4, 25 and 35 ºC.

Table 8 Induction times (h) of hazelnut kernels during storage.

Parameter	**Storage conditions**	**Storage time (months)**				
		0	**3**	**6**	**9**	**12**
Rancimat oil	4ºC	24.2 ± 0.1^{c}	$15.7\pm1.5^{b,A}$	$17.0\pm2.3^{b,A}$	$9.0\pm2.0^{a,A}$	$13.9\pm3.2^{ab,A}$
	25ºC	24.2 ± 0.1^{b}	$16.2\pm1.8^{a,A}$	$16.5\pm3.3^{a,A}$	$14.7\pm1.5^{a,B}$	$16.6\pm1.2^{a,A}$
	35ºC	24.2 ± 0.1^{c}	$14.9\pm1.8^{b,A}$	$17.2\pm5.1^{b,A}$	$7.0\pm0.8^{a,A}$	$14.2\pm2.7^{b,A}$

Note: Different capital letters in a column indicate significant differences between samples for a given storage time (p-value <0.05). Different lower-case letters in a row indicate the existence of significant differences between times for a given storage condition (p-value <0.05).

The highest induction time was obtained at the beginning (24.2 h). Then, the induction times decreased after three months, remaining similar until 12 months. Nevertheless, no significant differences were determined between the 3 and 12 months, suggesting that no significative oxidation occurred along storage at 4, 25, and 35 ºC. Furthermore, no significant differences were detected when comparing storage temperatures, except for nine months (25 ºC *versus* 4 and 35 ºC). This situation may be due to sample heterogeneity.

Our induction time at the beginning (24.2 h) was lower than the value mentioned by Momchilova et al. (2017) for Tonda Gentile (59 h). However, these authors used 100 ºC in the Rancimat method, while a higher temperature was used in the present work, explaining the difference. Momchilova et al. (2017) observed that during the storage of

nuts, the induction times gradually decreased, and some effects of the temperature and shell were seen. Momchilova et al. (2017) detected that the decrease in oxidative stability was higher at 20 ºC than at 4 ºC. Furthermore, the kernels showed slightly higher stability than nuts in the shell. A possible explanation of these results might be the moisture held by the shell that induces/favours undesirable processes such as lipid oxidation and degradation (Momchilova et al., 2017).

4.1.3 Microbiological evaluation

Table 9 presents the mean counts for the microorganisms growing at 30 ºC and the moulds and yeasts.

Table 9 Mean count (log CFU/g ± standard deviation) of microorganisms at 30°C and moulds and yeasts in hazelnuts stored under different conditions over 0, 3, 6, 9 and 12 months.

Microorganism	Storage conditions	Storage time (months)				
		0	3	6	9	12
Microorganisms at 30 ºC	4ºC	2.2±0.0 ^{A}	2.3±2.0 ^{a,A}	2.5±03 ^{a,A}	2.5±0.4 ^{ab,A}	1.5±1.4^{a,A}
	25ºC	2.2±0.0 ^{A}	3.4±0.6 ^{a,AB}	<1.7 ^{a,B}	3.6±0.2 ^{a,A}	1.8±1.7^{a,AB}
	35ºC	2.2±0.0 ^{A}	2.5±0.5 ^{a,A}	<1.7 ^{a,AB}	2.1±0.8 ^{a,A}	<1.7 ^{a,B}
Moulds and yeasts	4ºC	1.8±0.1 ^{A}	3.2±1.5^{a,A}	2.0±0.4 ^{a,A}	1.7±0.2 ^{a,A}	<1.7 ^{a,A}
	25ºC	1.8±0.1 ^{A}	<1.7 ^{a,A}	1.8±0.2 ^{a,A}	<1.7 ^{a,A}	<1.7 ^{a,A}
	35ºC	1.8±0.1 ^{A}	<1.7 ^{a,A}	<1.7 ^{,A}	<1.7 ^{a,A}	<1.7 ^{a,A}

Note: Different lower case letters in a column indicate significant differences between samples for a given storage time (p-value <0.05). Different capital letters in a row show significant differences between times for a given sample (p-value <0.05).

When comparing the temperatures, the values were always similar, not being detected significant differences between them. These results indicate that applying higher temperatures did not induce microorganisms' growth.

In terms of temporal evolution, it was observed that the applied conditions caused a decrease or maintained similar levels of aerobic mesophiles, moulds and yeasts to those obtained at time zero.

These results indicated that the conditions applied did not cause significant microbial growth. Thus, the temperatures at 4, 25 and 35 ºC are not critical factors to consider in the storage of hazelnut kernels. However, as previously mentioned, the fulfilment of a

correct initial drying of the hazelnut kernels is extremely important to guarantee the maintenance of their quality.

Mould spoilage can be a food safety problem due to the production of mycotoxins or allergens by these moulds. To our knowledge, no microbiological levels were established for hazelnuts to evaluate their quality and safety. Nevertheless, when considering the limits set for ready-to-eat foods (INSA, 2019), the values to be considered satisfactory (Group 3) for raw fruits and vegetables must be < 6 log CFU/g of total aerobic mesophilic. Concerning moulds, the values should be < 2.7 log CFU/g. Considering these values, the counts obtained in the present work were satisfactory for the microorganisms at 30 ºC and moulds and yeasts, except at 4 ºC, 3 months. This exception may be due to the heterogeneity of the sample.

4.1.4 Mycotoxins

In Table 10 are described the mycotoxins determined in hazelnuts. The mycotoxins detected were: Beauvericin, Bikaverin, 3-Nitropropionic acid, Citreohybridinol, Flavoglaucin, Quinolactacin A, Abscisic acid, Lecanoric acid, Asperglaucide, Asperphenamate, Cyclo(L-Pro-L-Tyr), Cyclo(L-Pro-L-Val), Infectopyron and Tryptophol. Some mycotoxins were detected, but were impossible to quantify.

Beauvericin (BEA) was detected at a very low concentration, showing no toxicological significance. BEA is an emerging mycotoxin produced as a secondary metabolite by several toxigenic fungi, mainly belonging to *Fusarium* genus and other genera such as *Beauveria bassiana*. BEA is primarily identified as a cereal and cereal-based product contaminant, but it is also found in other products such as nuts and coffee (Mallebrera et al., 2018; Caloni et al., 2020).

Bikaverin was detected at low concentrations, showing no toxicological significance. Bikaverin is a red pigment produced by several *Fusarium* species. Its production is stimulated by nitrogen depletion, high sucrose and low pH. It shows antimicrobial and antitumoral activity, but it is also considered a potential mycotoxin, although there are no toxicological studies in humans to support this (Wiemann et al., 2009, Limón et al., 2010).

3-Nitropropionic acid (3-NPA) was detected at low concentrations, showing no toxicological significance. It is a neurotoxin naturally produced by some fungi (such as *Aspergillus* and others) that infest plants and vegetables. It is an inhibitor of succinate

dehydrogenase, a key enzyme in the citric acid cycle and the mitochondrial respiratory chain (revised by Skogvold et al., 2021). It has been detected in hazelnuts with high frequency, at average levels of 40 μg/kg (Varga et al., 2013). In the present work, this mycotoxin was only detected at 25 ºC and 35 ºC at 3 months, in 4,99 and 60,0 μg/kg concentrations. On the contrary, this mycotoxin was not detected in the other storage times.

The abscisic acid (ABA) was detected in all samples, varying between 161 and 202 μg/kg. While ABA is known as a hormone produced by plants through the carotenoid pathway, a small number of phytopathogenic fungi, e.g. *Botrytis cinerea*, are also able to produce this sesquiterpene (Izquierdo-Bueno et al., 2018). Nevertheless, given the consistency of the concentrations detected in all samples, they probably indicate the production of ABA by the plant and not by the fungi.

Tryptophol was detected in all samples, varying between < LOQ and 71.4 μg/kg. It is a metabolite produced by various organisms, from plants to bacteria and fungi, and shows several types of biological activity (revised in Palmieri and Petrini, 2019).

In some situations, infectopyron was detected in some samples, namely 4º C for 3 months, 35 ºC for 6 months, 4 ºC for 9 months, and 4 ºC for 12 months. Infectopyron is a toxin associated with grain and straw (Spanic et al., 2020; Drakopoulus et al, 2021). It is a mycotoxin not very frequent (Escrivá et al., 2017), and there are no toxicological studies for this compound.

Table 10 Mycotoxins detected in hazelnuts stored under different conditions.

Time (Months)	Temperature ºC	Fusarium metabolites		Aspergillus metabolites	Penicillium metabolites			Other fungal genera
		Beauvericin	Bikaverin	3-Nitropropionic acid	Citreohybridinol	Flavoglaucin	Quinolactacin A	Abscisic acid
0		0.61	10.4	< LOD	< LOD	0.70	< LOD	170
3	4ºC	0.96	24.7	< LOD	< LOD	0.53	< LOD	195
	25ºC	detected (< LOQ)	6.60	4.99	< LOD	< LOD	< LOD	193
	35ºC	detected (< LOQ)	8.96	60.0	< LOD	< LOD	< LOD	198
6	4ºC	0.74	23.8	< LOD	< LOD	< LOD	< LOD	197
	25ºC	0.48	14.2	< LOD	< LOD	0.53	< LOD	181
	35ºC	detected (< LOQ)	6.59	< LOD	< LOD	< LOD	< LOD	202
9	4ºC	0.47	15.0	< LOD	< LOD	0.55	< LOD	161
	25ºC	0.47	16.6	< LOD	< LOD	< LOD	< LOD	197
	35ºC	detected (< LOQ)	834	< LOD	< LOD	< LOD	0.04	176
12	4ºC	0.56	17.0	< LOD	4.86	0.54	0.13	172
	25ºC	0.36	15.3	< LOD	< LOD	< LOD	< LOD	170
	35ºC	Detected (< LOQ)	detected (< LOQ	< LOD	< LOD	< LOD	< LOD	162

Time (Months)	Temperature ºC	Aspergillus metabolites	Unspecific metabolites					
		Lecanoric acid	Asperglaucide	Asperphenamate	Cyclo(L-Pro-L-Tyr)	Cyclo(L-Pro-L-Val)	Infectopyron	Tryptophol
0		0.56	< LOD	1.63	1.63	detected (< LOQ)	< LOD	13.8
3	4ºC	< LOD	< LOD	< LOD	< LOD	detected (< LOQ)	detected (< LOQ)	13.8
3	25ºC	0.45	0.88	< LOD	< LOD	7.25	< LOD	29.2
3	35ºC	< LOD	< LOD	< LOD	< LOD	13.0	< LOD	64.8
6	4ºC	< LOD	< LOD	1.39	1.39	< LOD	< LOD	23.7
6	25ºC	< LOD	< LOD	< LOD	< LOD	10.8	< LOD	50.9
6	35ºC	< LOD	< LOD	< LOD	< LOD	16.0	79.6	70.7
9	4ºC	< LOD	< LOD	< LOD	< LOD	2.75	104	detected (<LOQ)
9	25ºC	< LOD	< LOD	< LOD	< LOD	12.9	< LOD	71.4
9	35ºC	< LOD	< LOD	< LOD	< LOD	12.9	< LOD	34.8
12	4ºC	< LOD	< LOD	< LOD	< LOD	detected (< LOQ)	171	detected (<LOQ)
12	25ºC	< LOD	< LOD	< LOD	< LOD	21.0	< LOD	45.5
12	35ºC	< LOD	< LOD	< LOD	< LOD	16.7	< LOD	55.0

4.2 Evaluation of quality and safety of almond pastes

In this section, the results obtained for the almond pastes are presented. The almond pastes were stored at 4, 25, 45, 55 and 65 ºC. The highest temperatures were applied to accelerate the reactions that decrease the quality of the product, such as those related to lipid oxidation.

4.2.1 Colour

The colour parameters (*L**, *a**, *b**, C*, h) determined in almond pastes during storage are presented in Figure 4.

The luminosity at the beginning was 44±4, increasing after one month. This may possibly be due to a slight separation of the oil from the pulp that may have led to an increase in the L* value. The luminosity maintained constant between 1 and 3 months, except for the sample at 65 ºC. For this sample, a darkening of the paste was observed because of the temperature. This may have caused Maillard reactions (non-enzymatic browning reactions). The enzymatic browning reactions caused by polyphenol oxidase seem not to have been significant since if these had occurred, one would expect a slight browning for the lower temperatures, which possibly favour more the activity of this enzyme.

The coordinate *a** increased in all samples from the beginning to 1 month of storage. These results indicate that all samples acquired a redder tone. The sample kept at 65 ºC was the one that suffered an increase in this parameter from 2 to 3 months, again suggesting the occurrence of Maillard reactions, in line with the results obtained for brightness.

As the *b** values were much higher than the *a** values, the predominant colour of the pastes was yellow, as expected. However, after one month of storage, there was an increase in this parameter, with no differences between the samples. However, at 2 months of storage, the sample at 65 ºC showed a decrease in the value of this parameter, but this was not observed at 3 months.

In the beginning, the chroma value was 20.1+0.8. This parameter increased during one month of storage, suggesting a higher colour intensity. However, after this period, the values remained constant.

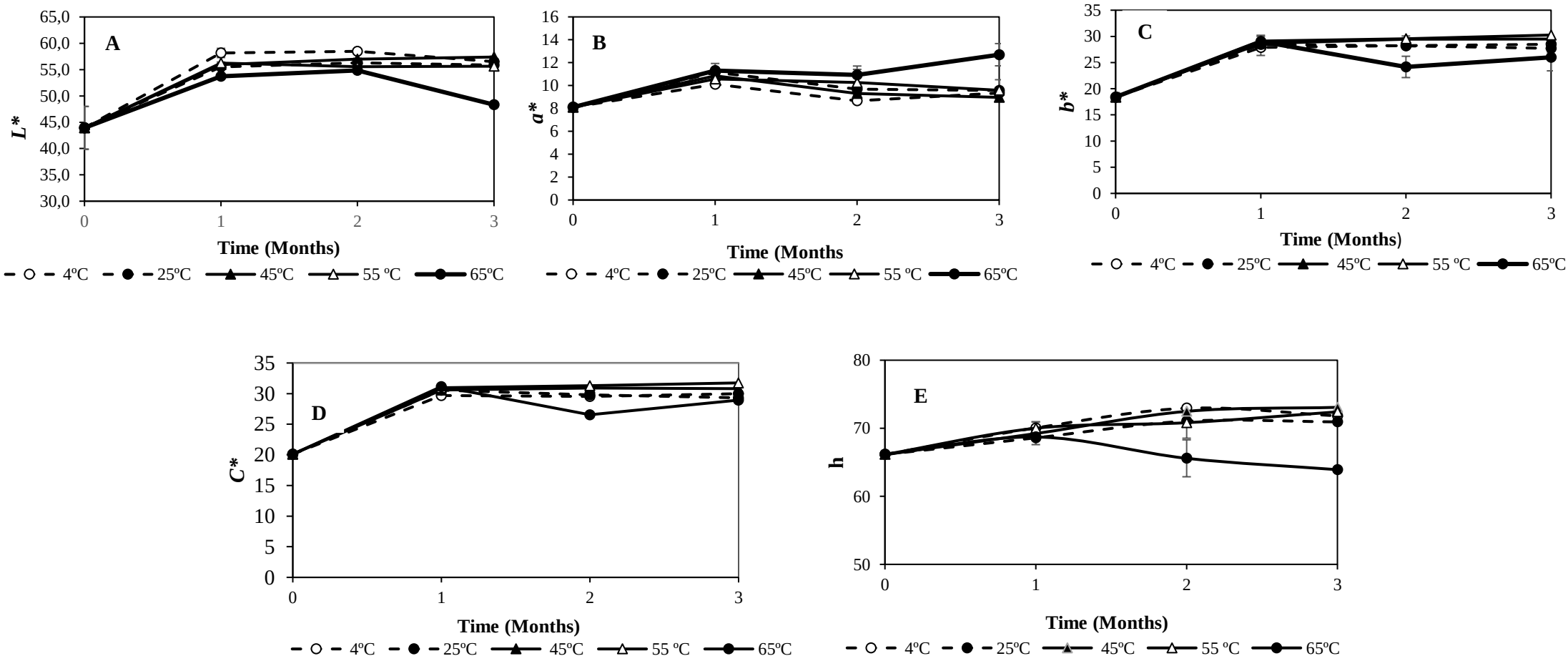

Figure 4. Colour parameters evaluated in the almond paste along storage: (A) Brightness (L*), (B) a* coordinate (red+/green-), (C) b* coordinate (yellow+/blue-), (D) Chroma (C*) and (E) Hue (h).

Initially, the value of h (hue) was 66.2 + 0.2. After one month of storage, a slight increase in the hue values was observed in all samples. However, after two months, the samples at 65 ºC showed a different hue from the others, being this behaviour again observed at three months. This fact is in line with what was stated before. The sample relative to 65 ºC was the one that presented more distinct values of brightness and *a** than the others, justifying a different tone. These results again indicate the occurrence of darkening reactions, namely Maillard Reactions. These results are identical to the ones mentioned by Padehban et al. (2018) when studying the packaging method, temperature and storage period of wild almond kernel. They also observed a decrease in *L** and an increase in *a** with storage time, leading to a gradual darkening of kernels, especially during air atmosphere packaging. Guiné et al. (2015b) also observed a decrease in lightness when almond kernels were stored in chambers at 50 ºC and 90% relative humidity and in the stove at 50 ºC, suggesting the occurrence of Maillard reactions.

4.2.2 Water activity and moisture content

Figure 5 shows the water activity and the moisture content values determined in the almond paste throughout storage.

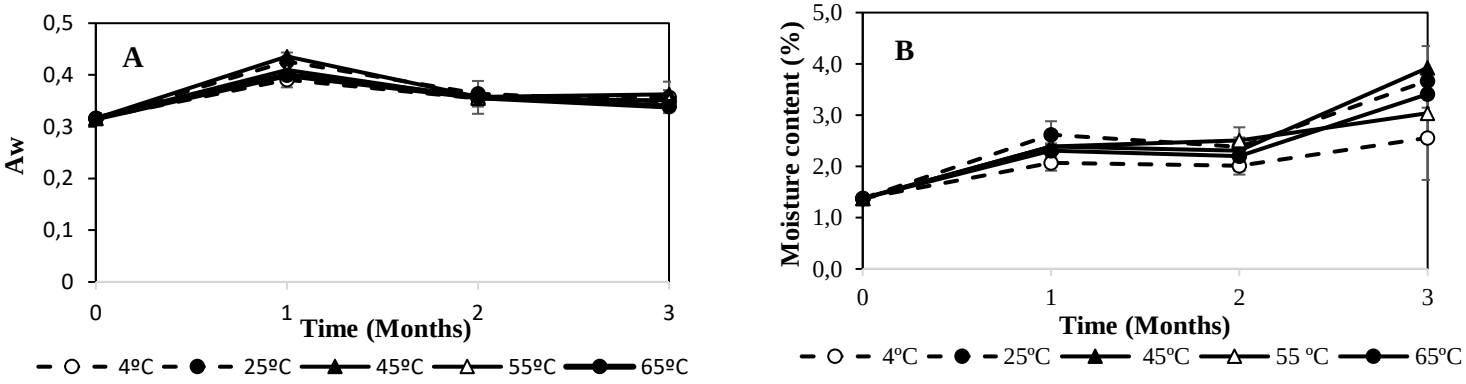

Figure 5 (A) Water activity and (B) moisture content evaluated in the almond paste along storage.

The a_w is an important parameter that assures the quality of the food products because is related to the free water available to chemical and enzymatic reactions and microbial growth. The a_w values were constant over time, varying between 0.32 and 0.44. These a_w values indicated that the samples were microbiologically stable; since below 0.4, no microbial growth is observed (Labcell, s/d). A slight increase was determined after one

month, with no apparent explanation. However, it should be referred that the average measurement temperature at one month was 20.5°C, slightly lower than at 0 months, which was 21.1°C, possibly leading to a somewhat higher a_w value. Regardless of this fact, at a_w values below 0.86, pathogenic microorganisms cannot grow (Barbosa-Cánovas et al., 2003), providing safety to the product.

Furthermore, a product with an a_w of 0.3 will be more stable concerning enzymatic activity (Barbosa-Cánovas et al., 2003). Identical values were obtained in a study where the almond paste was placed at different temperatures (Tazi et al., 2009). The water activity varied between 0.3 and 0.7 (Tazi et al., 2009). When analysing infrared dry-roasted almonds, Yang et al. (2013) also found a_w values between 0.2-0.3.

It would be expected that a decrease in a_w would be observed at higher temperatures. However, this product has a high-fat and low moisture content, which may explain what happened because it is a lipophilic rather than a hydrophilic product.

In the beginning, the moisture content was around 1.38+0.06% (Figure 5B), and an increase was observed after 1 month. The values remained almost constant between 1 and 2 months, increasing for 3 months. The most significant increases were observed for 25, 45 and 65 ºC. On the contrary, the samples at 4 and 55 ºC showed a minor increase. However, in the end, the moisture content of the products was never higher than 4%.

Our moisture contents were lower than those mentioned by Capanoglu & Boyacioglu (2008). These authors evaluated the moisture content of almond pastes stored at different temperatures. It must be referred that the almond pastes prepared by Capanoglu & Boyacioglu (2008) contained maltose, stabilisers, antioxidants and water. In the beginning, the moisture content of the paste was 10.1%. During four weeks of storage, the moisture losses of the paste samples stored at 4°C were in the range of 3.21-12.42%, while at 30°C were slightly higher (4.86-14.67%) (Capanoglu & Boyacioglu, 2008). In our work, moisture losses were not observed.

The moisture contents determined in the almond pastes in the present work were also lower than the moisture contents determined in almonds (kernel) of Spain, Portugal United States of America with skin, and of the United States of America without skin reported by Guiné et al. (2015b), 4.3 to 6.8%. Nevertheless, Yang et al. (2013) indicate that a moisture content of 1.5-2.5% and an a_w of 0.2-0.3 represents the optimal moisture content range for roasted almonds and originates the maximum shelf life.

4.2.3 Fat content and fatty acid profile

Figure 6 represents the fat content throughout almond paste storage.

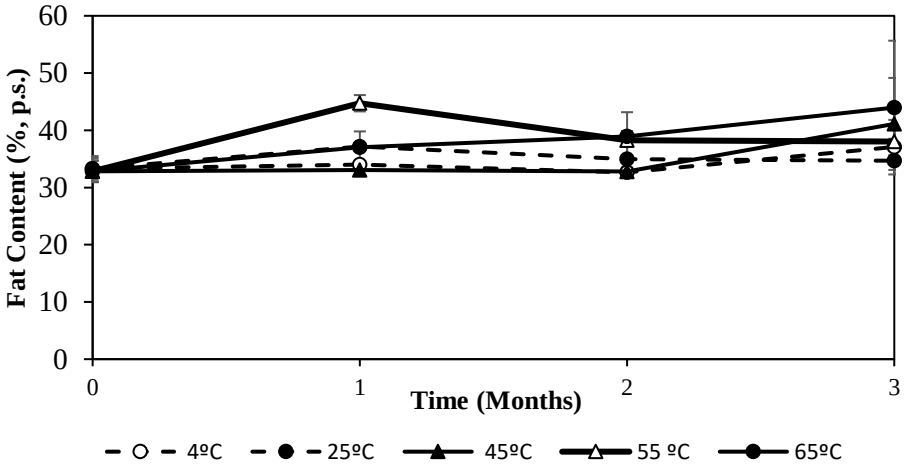

Figure 6 Fat content (%, p.s.) evaluated in the almond paste along storage.

The fat content remained almost constant over time. However, it was observed for the temperature of 55 ºC, a slight increase in the first month concerning the other months, without justification. The percentage of fat in the kernel was between 32.6 and 45.8%, d.w. These values are smaller than the oil content determined by Özcan et al. (2011) in five Turkish almond varieties that varied between 48.84 and 55.69%, d.w.

Table 11 shows the percentage of fatty acids determined in the almond pastes over time. Twelve fatty acids were determined. The predominant was oleic acid (C18:1n9), ranging from 50.8 to 57.0%, followed by linoleic acid (C18:2n6c), ranging from 33.0% to 36.9%, and palmitic acid (C16:0), between 9.5 and 11.2%. All other fatty acids represent less than 1%. Özcan et al. (2011) also reported the presence of oleic, linoleic and palmitic acids in almond kernel oils of five Turkish varieties; however, the percentages were equal to 72.5-79.9%, 13.52-19.77% and 5.87-6.73%. Such differences in the percentages of fatty acids can be explained by variations in variety and location (Holaday and Pearson, 1974; Hamilton and Bhati, 1987).

Concerning the storage time and temperatures evaluated, with minor exceptions, without any defined trend, no significant differences were observed in the percentages of fatty acids between the different conditions studied. Thus, the temperatures tested did not cause significant differences in fatty acid composition.

Table 11 Fatty acid profile (%) evaluated in the almond paste along storage.

Fatty Acid	Storage conditions	Storage time (months)			
		0	1	2	3
C14:0	4ºC	0.03±0,004^{a}	0.032±0.003^{a,A}	0.031±0.002^{a,A}	0.033±0.001^{a,A}
	25ºC	0.03±0,004^{a}	0.031±0.003^{a,A}	0.034±0.002^{a,A}	0.032±0.004^{a,A}
	45ºC	0.03±0,004^{a}	0.030±0.001^{a,A}	0.036±0.006^{a,A}	0.032±0.002^{a,A}
	55ºC	0.03±0,004^{a}	0.032±0.002^{a,A}	0.031±0.001^{a,A}	0.029±0.007^{a,A}
	65ºC	0.03±0,004^{a}	0.034±0.003^{a,A}	0.034±0.005^{a,A}	0.036±0.002^{a,A}
C15:0	4ºC	0.014±0,003^{a}	0.015±0.001^{a,A}	0.014±0.002^{a,A}	0.013±0.001^{a,A}
	25ºC	0.014±0,003^{a}	0.014±0.003^{a,A}	0.014±0.003^{a,A}	0.014±0.001^{a,AB}
	45ºC	0.014±0,003^{a}	0.012±0.005^{a,A}	0.015±0.002^{a,A}	0.014±0.003^{a,AB}
	55ºC	0.014±0,003^{a}	0.012±0.001^{a,A}	0.015±0.001^{a,A}	0.013±0.002^{a,A}
	65ºC	0.014±0,003^{a}	0.014±0.002^{a,A}	0.014±0.002^{a,A}	0.018±0.000^{a,B}
C16:0	4ºC	11.2±1,3^{a}	10.3±0.3^{a,AB}	10.3±0.3^{a,A}	10.3±0.6^{a,A}
	25ºC	11.2±1,3^{a}	10.0±0.6^{a,AB}	10.4±0.7^{a,A}	10.1±0.9^{a,A}
	45ºC	11.2±1,3^{a}	9.7±0.1^{a,A}	10.8±0.9^{a,A}	10.1±0.8^{a,A}
	55ºC	11.2±1,3^{a}	9.9±0.3^{a,AB}	10.1±1.0^{a,A}	9.5±1.0^{a,A}
	65ºC	11.2±1,3^{a}	10.9±0.5^{a,B}	10.6±1.3^{a,A}	10.7±0.7^{a,A}
C16:1	4ºC	0.848±0,113^{a}	0.771±0.007^{a,B}	0.744±0.029^{a,A}	0.702±0.060^{a,A}
	25ºC	0.848±0,113^{b}	0.653±0.044^{a,A}	0.670±0.049^{ab,A}	0.644±0.046^{a,A}
	45ºC	0.848±0,113^{b}	0.622±0.001^{a,A}	0.689±0.061^{ab,A}	0.634±0.050^{a,A}
	55ºC	0.848±0,113^{b}	0.635±0.011^{a,A}	0.655±0.059^{a,A}	0.612±0.053^{a,A}
	65ºC	0.848±0,113^{a}	0.720±0.030^{a,B}	0.695±0.081^{a,A}	0.683±0.051^{a,A}
C17:0	4ºC	0.069±0,012^{a}	0.075±0.005^{a,A}	0.069±0.007^{a,A}	0.077±0.004^{a,A}
	25ºC	0.069±0,012^{a}	0.070±0.008^{a,A}	0.071±0.003^{a,A}	0.078±0.007^{a,A}
	45ºC	0.069±0,012^{a}	0.068±0.010^{a,A}	0.074±0.005^{a,A}	0.073±0.006^{a,A}
	55ºC	0.069±0,012^{a}	0.075±0.002^{a,A}	0.072±0.008^{a,A}	0.071±0.008^{a,A}
	65ºC	0.069±0,012^{a}	0.075±0.004^{a,A}	0.073±0.019^{a,A}	0.077±0.020^{a,A}
C18:1n9	4ºC	50.8±5,1^{a}	55.1±1.0^{a,AB}	55.1±1.3^{a,A}	53.6±1.8^{a,A}
	25ºC	50.8±5,1^{a}	54.5±2.7^{a,AB}	53.7±3.0^{a,A}	54.2±3.4^{a,A}
	45ºC	50.8±5,1^{a}	56.2±0.5^{a,B}	51.9±3.8^{a,A}	55.0±2.8^{a,A}
	55ºC	50.8±5,1^{a}	55.9±0.8^{a,B}	54.3±3.9^{a,A}	57.0±3.0^{a,A}
	65ºC	50.8±5,1^{a}	51.0±2.1^{a,A}	53.0±5.7^{a,A}	52.7±3.4^{a,A}
C18:2n6c	4ºC	36.7±3,6^{a}	33.5±0.8^{a,A}	33.5±1.0^{a,A}	35.1±1.2^{a,A}
	25ºC	36.7±3,6^{a}	34.5±2.0^{a,AB}	34.9±2.2^{a,A}	34.6±2.4^{a,A}
	45ºC	36.7±3,6^{a}	33.0±0.4^{a,A}	36.2±2.7^{a,A}	33.9±2.0^{a,A}
	55ºC	36.7±3,6^{a}	33.1±0.4^{a,A}	34.5±2.7^{a,A}	33.0±2.0^{a,A}
	65ºC	36.7±3,6^{a}	36.9±1.6^{a,B}	35.3±4.2^{a,A}	35.5±2.4^{a,A}
C20:0	4ºC	0.118±0,011^{a}	0.103±0.008^{a,A}	0.112±0.028^{a,A}	0.115±0.003^{a,A}
	25ºC	0.118±0,011^{a}	0.112±0.005^{a,A}	0.117±0.014^{a,A}	0.117±0.012^{a,A}
	45ºC	0.118±0,011^{a}	0.117±0.002^{a,AB}	0.121±0.013^{a,A}	0.113±0.009^{a,A}
	55ºC	0.118±0,011^{a}	0.115±0.004^{a,AB}	0.118±0.016^{a,A}	0.105±0.012^{a,A}
	65ºC	0.118±0,011^{a}	0.130±0.010^{a,B}	0.121±0.010^{a,A}	0.127±0.011^{a,A}
C20:1	4ºC	0.080±0,013^{a}	0.066±0.012^{a,A}	0.069±0.008^{a,A}	0.074±0.005^{a,A}
	25ºC	0.080±0,013^{a}	0.072±0.004^{a,A}	0.075±0.010^{a,A}	0.076±0.008^{a,A}
	45ºC	0.080±0,013^{a}	0.072 ±0.004^{a,A}	0.072±0.007^{a,A}	0.075±0.003^{a,A}
	55ºC	0.080±0,013^{a}	0.068±0.004^{a,A}	0.075±0.008^{a,A}	0.067±0.012^{a,A}
	65ºC	0.080±0,013^{a}	0.078±0.007^{a,A}	0.078±0.011^{a,A}	0.079±0.006^{a,A}

Table 11 (cont.) Fatty acid profile (%) evaluated in the almond paste along storage.

Fatty Acid	Storage conditions	Storage time (months)			
		0	1	2	3
C22:0	4ºC	0.024±0.004^{a}	0.022±0.003^{a,A}	0.022±0.003^{a,A}	0.022±0.001^{a,A}
	25ºC	0.024±0.004^{a}	0.023±0.003^{a,A}	0.023±0.001^{a,A}	0.025±0.003^{a,A}
	45ºC	0.024±0.004^{a}	0.022±0.002^{a,A}	0.026±0.001^{a,A}	0.022±0.001^{a,A}
	55ºC	0.024±0.004^{a}	0.020±0.002^{a,A}	0.025±0.000^{a,A}	0.014±0.012^{a,A}
	65ºC	0.024±0.004^{a}	0.024±0.001^{a,A}	0.024±0.005^{a,A}	0.026±0.002^{a,A}
C23:0	4ºC	0.017±0.001^{a}	0.028±0.011^{a,AB}	0.026±0.008^{a,A}	0.022±0.005^{a,B}
	25ºC	0.017±0.001^{a}	0.033±0.003^{a,B}	0.025±0.010^{a,A}	0.026±0.002^{a,B}
	45ºC	0.017±0.001^{a}	0.021±0.003^{a,AB}	0.024±0.005^{a,A}	0.021±0.003^{a,B}
	55ºC	0.017±0.001^{a}	0.019±0.010^{a,AB}	0.046±0.057^{a,A}	0.011±0.001^{a,A}
	65ºC	0.017±0.001^{ab}	0.014±0.003^{ab,B}	0.012±0.005^{a,A}	0.021±0.002^{b,B}
C24:0	4ºC	0.005±0.001^{a}	0.005±0.001^{a,A}	0.005±0.001^{a,A}	0.004±0.000^{a,A}
	25ºC	0.005±0.001^{a}	0.004±0.001^{a,A}	0.004±0.000^{a,A}	0.003±0.000^{a,A}
	45ºC	0.005±0.001^{a}	0.012±0.017^{a,A}	0.058±0.020^{b,B}	0.008±0.007^{a,A}
	55ºC	0.005±0.001^{a}	0.009±0.003^{a,A}	0.028±0.008^{a,AB}	0.018±0.016^{a,A}
	65ºC	0.005±0.001^{a}	0.024±0.005^{a,A}	0.033±0.019^{a,AB}	0.082±0.083^{a,A}

Note: Different capital letters in a column indicate significant differences between samples for a given storage time (p-value <0.05). Different lower-case letters in a row indicate the existence of significant differences between times for a given storage condition (p-value <0.05).

The fatty acid profile of almonds can affect the product's shelf life since the high degree of unsaturation makes these products very sensitive to oxidation reactions (Severini et al. 2000, 2003). The main fatty acid classes, mainly SFA, MUFA, PUFA and the ratios USFA/SFA and MUFA/PUFA, are represented in Table 12. The MUFA had the highest percentage, between 51.72 and 56.85%, mainly due to the oleic acid (C18:1n9). PUFA was the second main fatty acid class. It varied between 32.96 and 36,89%. The fatty acid that most contributed to this class was linoleic acid (C18:2n6). Considering the storage time, when comparing the beginning with 12 months of storage, the SFA, MUFA and PUFA classes remained identical. In other words, the five storage conditions presented similar magnitudes of fatty acid profiles.

Table 12 SFA, MUFA, PUFA, USFA/SFA and MUFA/PUFA of almond pastes during storage.

Parameters	Storage conditions	Storage time (months)			
		0	1	2	3
SFA	4ºC	11.52±1.35^{a}	10.58±0.26^{a,AB}	10.60±0.37^{a,A}	10.59±0.59^{a,A}
	25ºC	11.52±1.35^{a}	10.29±0.67^{a,AB}	10.65±0.75^{a,A}	10.45±0.91^{a,A}
	45ºC	11.52±1.35^{a}	10.06±0.06^{a,A}	11.15±1.02^{a,A}	10.39±0.76^{a,A}
	55ºC	11.52±1.35^{a}	10.22±0.32^{a,AB}	10.43±1.12^{a,A}	9.76±0.90^{a,A}
	65ºC	11.52±1.35^{a}	11.30±0.48^{a,B}	10.89±1.38^{a,A}	11.04±0.89^{a,A}
MUFA	4ºC	51.72±4.97^{a}	55.96±1.03^{a,AB}	55.89±1.24^{a,A}	54.36±1.77^{a,A}
	25ºC	51.72±4.97^{a}	55.22±2.63^{a,AB}	54.46±2.9^{a,A}	54.96±3.30^{a,A}
	45ºC	51.72±4.97^{a}	56.85±0.48^{a,B}	56.67±3.74^{a,A}	55.65±2.77^{a,A}
	55ºC	51.72±4.97^{a}	56.67±0.74^{a,B}	55.02±3.83^{a,A}	57.27±3.34^{a,A}
	65ºC	51.72±4.97^{a}	51.81±2.07^{a,A}	53.73±5.6^{a,A}	53.46±3.33^{a,A}
PUFA	4ºC	36.74±3.63^{a}	33.45±0.79^{a,A}	33.50±1.00^{a,A}	35.05±1.28^{a,A}
	25ºC	36.74±3.63^{a}	34.48±1.96^{a,AB}	34.87±2.23$^{a.A}$	34.59±2.38^{a,A}
	45ºC	36.74±3.63^{a}	33.09±0.42^{a,A}	36.18±2.72^{a,A}	33.95±2.03^{a,A}
	55ºC	36.74±3.63^{a}	33.10±0.42^{a,A}	34.52±2.72^{a,A}	32.96±2.43^{a,A}
	65ºC	36.74±3.63^{a}	36.89±1.59^{a,B}	35.38±4.24^{a,A}	35.50±2.45^{a,A}
USA/SFA	4ºC	7.75±1.00^{a}	8.45±0.23^{aAB}	8.44±0.33^{a,A}	8.46±0.53^{a,A}
	25ºC	7.75±1.00^{a}	8.74±0.64^{a,AB}	8.41±0.65^{a,A}	8.62±0.88^{a,A}
	45ºC	7.75±1.00^{a}	8.94±0.06^{a,B}	8.03±0.87^{a,A}	8.66±0.71^{a,A}
	55ºC	7.75±1.00^{a}	8.79±0.30^{a,AB}	8.65±0.01^{a,A}	9.32±0.86^{a,A}
	65ºC	7.75±1.00^{a}	7.86±0.38^{a,A}	8.28±1.09^{a,A,}	8.09±0.72^{a,A}
MUFA/PUFA	4ºC	1.42±0.26^{a}	1.67±0.07^{a,AB}	1.67±0.08^{a,A}	1.55±0.11^{a,A}
	25ºC	1.42±0.26^{a}	1.71±0.17^{a,AB}	1.46±0.18^{a,A}	1.64±0.21^{a,A}
	45ºC	1.42±0.26^{a}	1.71±0.04^{a,B}	1.47±0.22^{a,A}	1.64±0.18^{a,A}
	55ºC	1.42±0.26^{a}	1.71±0.04^{a,B}	1.61±0.22^{a,A}	1.74±0.22^{a,A}
	65ºC	1.42±0.26^{a}	1.41±0.12^{a,A}	1.54±0.32^{a,A}	1.51±0.20^{a,A}

Note: Different capital letters in a column indicate significant differences between samples for a given storage time (p-value <0.05). Different lower-case letters in a row indicate the existence of significant differences between times for a given storage condition (p-value <0.05).

MUFA and PUFA accounted for more than 85% of the total fatty acids. Similar to hazelnuts, almond pastes have high quantities of USFA. Again the consumption of high amounts of USFA brings benefits to human health. Thus, the consumption of almond pastes should be promoted. The ratio of USFA/SFA varied between 7.75 and 9.32 in the present work. No significant changes were observed in these ratios over storage time and temperatures, with minor exceptions, with undefined behaviour. Thus, applying high temperatures did not cause changes in these ratios.

4.2.4 Oxidative Stability

The oxidative stability of the almond pastes was evaluated through the determination of peroxide values, spectrophotometric analysis and induction times, measured by the Rancimat method. The results obtained are described in the following subsections.

4.2.4.1 Peroxide values during storage

The peroxide value corresponds to the quantity of substances present in the sample, expressed in milliequivalents of active oxygen per kg of oil, capable of oxidising potassium iodide, as described in Commission Regulation (EEC) No. 2568/91. The peroxide value measures the amount of hydroperoxides formed through oxidation during the storage of an oil. The formation of hydroperoxides (products resulting from primary oxidation) (Salek et al., 2017) correlates with the susceptibility to fatty acid oxidation and the levels of antioxidants present in that same oil.

Figure 7 represents the peroxide values throughout almond paste storage. Initially, the lowest peroxide value was determined (0.812±0.006 mEq. O_2/kg oil). After that, in the first month of storage, all temperatures showed an increase in the peroxide value. However, the temperature at 55 ºC was the one that originated the lowest peroxide values throughout the first two months. There is no justification for this value since it would be expected that the peroxide index value would increase over time at temperatures above 30 ºC as mentioned by Woodroof (1973), Labuza (1982), Hamilton and Bhati (1987), Muego-Gnanasekharan and Resurreccion (1992), Chu and Hsu (1999) and Garcia-Pascual et al. (2003), since the oxidation mechanism slows down at lower temperatures. The highest value was 1.23 mEq O_2/kg of oil at 65 °C at 3 months. However, this value was similar to the one determined at 25 ºC (3 months) (1.21 mEq O_2/kg of oil), suggesting that the application of high temperatures, such as 55 and 65 ºC, did not cause a considerable increase in peroxide value after three months.

In terms of comparison, and since no legal limits were established for almond paste oil, the reference values for olive oil were used. All samples showed peroxide values below 20 mEq O_2/kg, the limit set for extra virgin olive oil, and 5 mEq O_2/kg, the limit established for refined olive oil (Commission Implementing Regulation (EU) 2019/1604 of 27 September 2019).

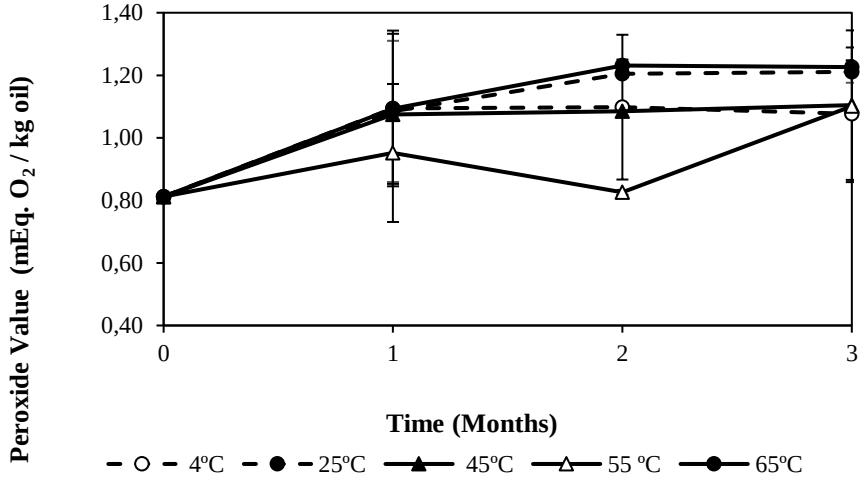

Figure 7 Peroxide values (mEq O_2/kg oil) of almond pastes during storage.

According to industry specifications (Almond Board of California), the peroxide value of "fresh" almond oils must be less than 5.0 mEq. O_2/kg (Yang et al., 2013). The peroxide values determined in the present work were lower than those reported by Yang et al. (2013) after three months of storage for almonds heated at 130, 140 and 150 ºC (1.59, 12.10 and 36.07 mEq. O_2/kg, respectively). On the contrary, our values are similar to those of Raisi et al. (2015) for raw unshelled whole kernels and ground almonds subjected to 4ºC and ambient temperature (23 ºC) for 10 months (0.23-3.41 mEq. O_2/kg). Our work indicates that the method used in almond paste preparation did not promote lipid oxidation.

Furthermore, in the present work, the behaviour of almond pastes was different to that reported by Ciftci and Ozilgen (2019) for almond pastes supplemented with black carrot juice. These authors obtained sigmoidal curves, where the peroxide values stayed almost constant at the early stages of storage and increased exponentially as the storage time progressed (until 18 days) at all storage temperatures (4, 20, 30 and 60 ºC). These results suggest that adding compounds to almond pastes may affect lipid oxidation.

4.2.4.2 Spectrophotometric investigation in the ultraviolet

Spectrophotometric investigation in the ultraviolet can provide indications of the quality of a fat, its state of preservation and modifications due to the technological processes applied. Absorbances at wavelengths 232 and 268 nm are due to the presence of

conjugated diene and triene systems resulting from oxidation processes and/or refining practices (Commission Implementing Regulation (EU) 2015/1833). In more detail, K_{232} is related to the presence of conjugated dienes and their oxidation products. K_{268} corresponds to conjugated trienes and secondary oxidation.

In Figure 8, the K_{232} values are presented. As expected, the lowest value was observed at the beginning (1.50±0.28). Then, a slight increase was observed for all temperatures, except for 4 ºC and 45 ºC after two months. On the contrary, in both situations, a slight decrease was observed; however, there was no explanation for the phenomenon. It was probably due to some natural variability in the initial raw material. In the end, after three months of storage, the highest value was achieved at 45 ºC, closely followed by 55 ºC. These results suggest the occurrence of primary oxidation. Nevertheless, higher values of K_{232} with higher temperatures were not always observed.

For K_{268} (Figure 8B), some variability was observed in the values but without any definite trend. The values were more constant than the K_{232}, varying between 0.10 and 0.30. The lowest value was determined at the beginning and 4°C after one month. In the end, all temperatures gave K_{268} values very similar to each other, 0.15-0.19. This fact suggested that applying high temperatures, such as 55 ºC and 65 ºC, did not cause very evident secondary oxidation.

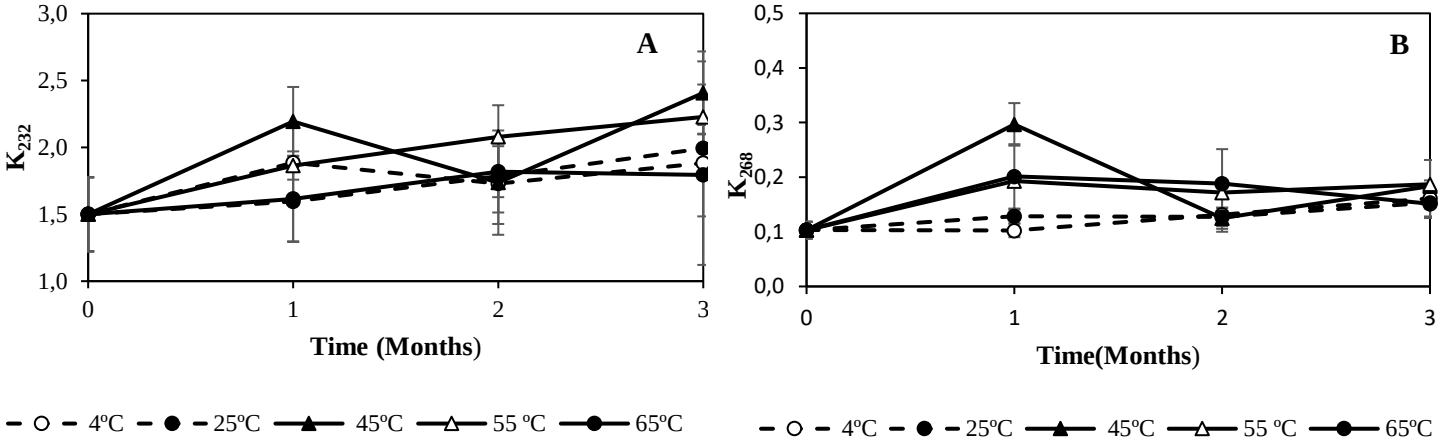

Figure 8 Spectrophotometric investigation in ultraviolet (K_{232} (A) and K_{268} (B)) of almond pastes during storage.

These results differed from those of Padehban et al. (2018) for wild almond kernels stored at 4, 25 and 35 ºC and atmospheres (vacuum, CO_2 and normal air). These authors stated that as storage time increased, the conjugated dienes and trienes of all samples enhanced significantly at all temperature conditions. These results indicate that evaluating these

parameters for each sample is important because different behaviours can be observed and probably due to their different composition in antioxidants.

Raisi et al. (2015) also stated that at low temperatures (4 ºC), all samples of the whole kernels (packed and unpacked) did not show significant differences in the conjugated trienes (K_{268}) during 10 months of storage, in line with our results. Furthermore, Kazantzis et al. (2003), when studying the effect of storage for 6 months at 5 ºC (80% Relative Humidity) versus 20 ºC (60% Relative Humidity) of shelled versus in-shell almonds from Greece, verified that K_{232} decreased overall with storage, while K_{270} remained stable.

4.2.4.3 Rancimat method - Induction times

When comparing the effect of storage temperature over time, it was noticed that the induction times remained almost constant throughout storage, except at 55 ºC, for one month. However, at this condition, a high standard deviation was determined (8.16±1.33 h). These results show good oxidative stability by the almond pastes even when submitted to high temperatures. The condition at 4 ºC gave rise to values with low variation over time, suggesting that it is suitable for storing the kernel paste as expected and already referenced by Capanoglu & Boyacioglu (2008). Nevertheless, the temperatures of 25 and 45°C also proved quite suitable.

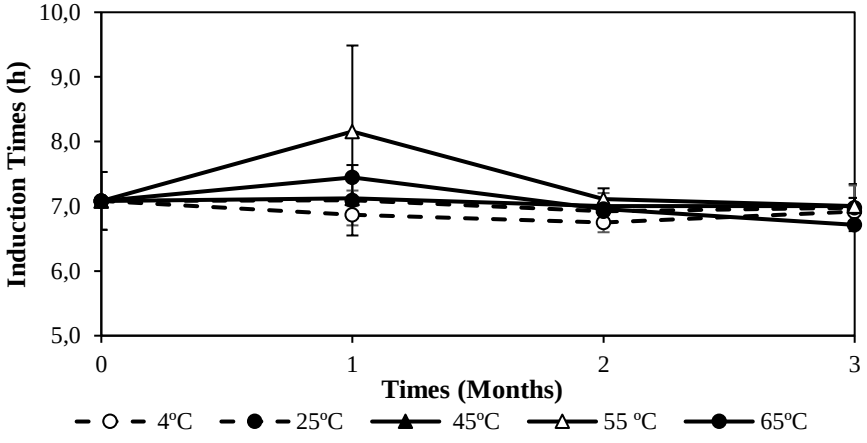

Figure 9 Induction times (h) of almond pastes during storage.

4.2.5 Microbiological evaluation

Total microbiological counts at 30 ºC and of moulds and yeasts were performed at 4, 25 and 45 ºC after three months of storage. This analysis was not performed at 55 and 65 ºC because both temperatures were relatively high and, therefore, not favourable for the growth of microorganisms. Tests were also performed for Coliforms, *Clostridium perfringens*, *E. coli*, and *Staphylococcus aureus* at 0 and 3 months of storage.

The results were always <1.7 log CFU/g, so the applied conditions did not favour microbiological growth. An important factor that contributed to the absence of growth was that the a_w was between 0.3 and 0.48. Such low a_w values are not favourable for the growth of microorganisms. Furthermore, the growth of pathogenic microorganisms is also unfavourable. This is also confirmed by the low moisture content, being the highest value equal to 4% and a_w. It has already been mentioned by Faid et al. (1995) that almond paste has a low microbial load. These authors refer that the relatively low a_w and the low initial microbiota of the raw material might have some effect on the microorganisms. Thus, it is very important always guarantee the hygienic conditions of the product.

4.2.6 Sensory analysis

4.2.6.1 Visual and olfactory properties

Figure 10 depicts the spider diagrams of visual and olfactory evaluations.

Visual appearance, colour, shine, general aroma, intensity (aroma) and rancid aroma were evaluated at the beginning and along one, two and three months of storage. Similar behaviours were observed at one and two months, which were similar to the beginning of the experiment. Thus, the storage temperature did not affect the visual appearance of the almond pastes, their color, shine and general aroma. No rancid aroma was detected at one and two months of storage. On the contrary, after three months, the sample stored at 65 ºC showed a slight rancid aroma and the lowest scores for the visual appearance, colour, shine and general aroma. This situation showed that the temperature of 65 ºC negatively affected the quality of the almond pastes.

4.2.6.2 Taste properties

The taste properties are represented in Figure 11. Texture, toughness, adhesiveness, chewability, general flavour, bitter flavour, sweetness, rancid flavour and global appreciation were evaluated. Again, there were no differences in the taste properties evaluations in the first two months of storage compared to the beginning. However, after 3 months at 65 ºC, the tasters detected that this sample had a very pleasant hardness, but on the contrary, there was an increase in the rancid flavour. This behaviour has also been reported by Capanoglu and Boyacioglu (2008) that although their pastes have other compounds, they showed similar behaviour as the storage time increases. The temperature that originated the highest overall appreciation was 25 ºC, thus being the best temperature to store the almond paste.

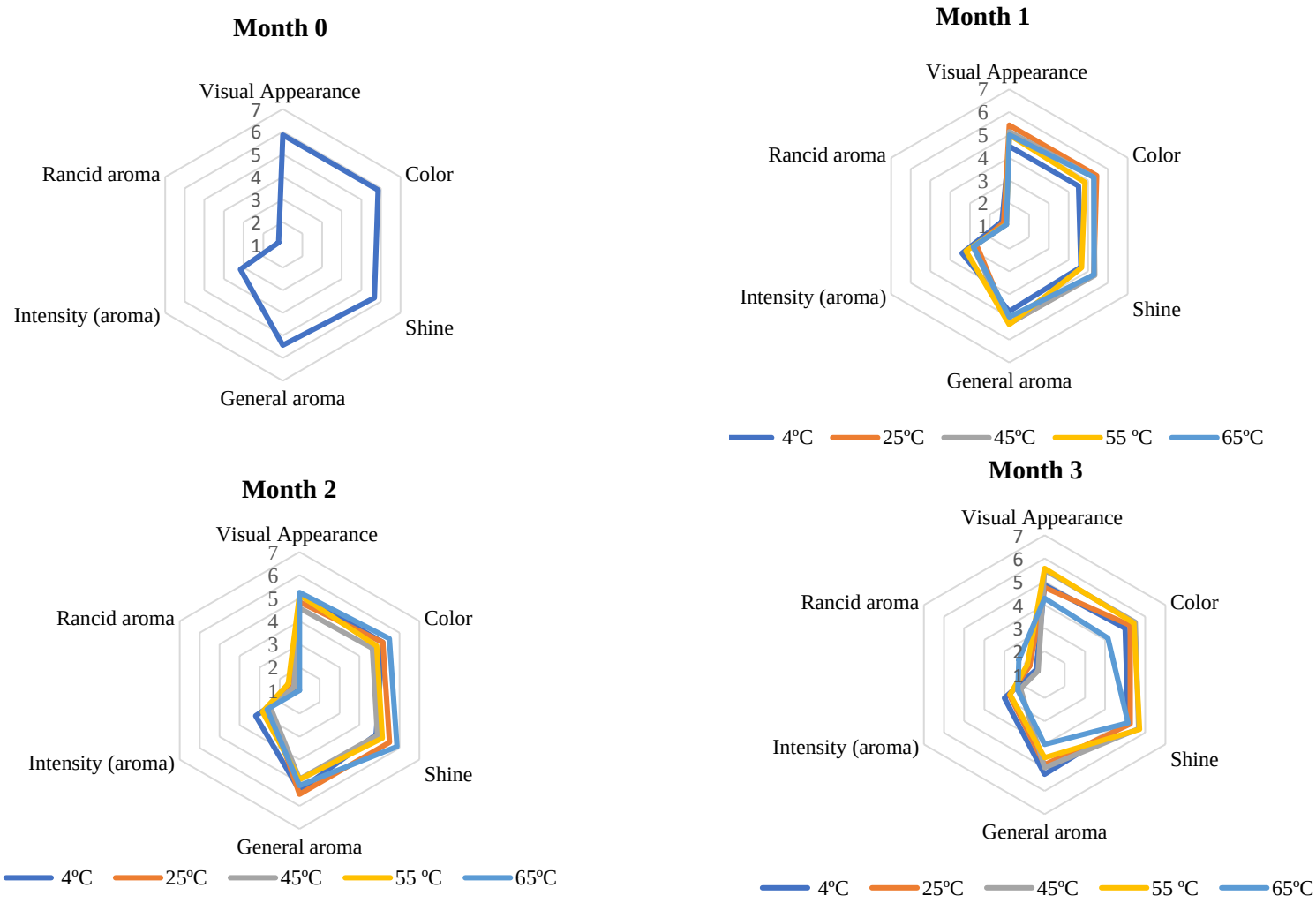

Figure 10 Visual and olfactory properties of almond pastes during storage.

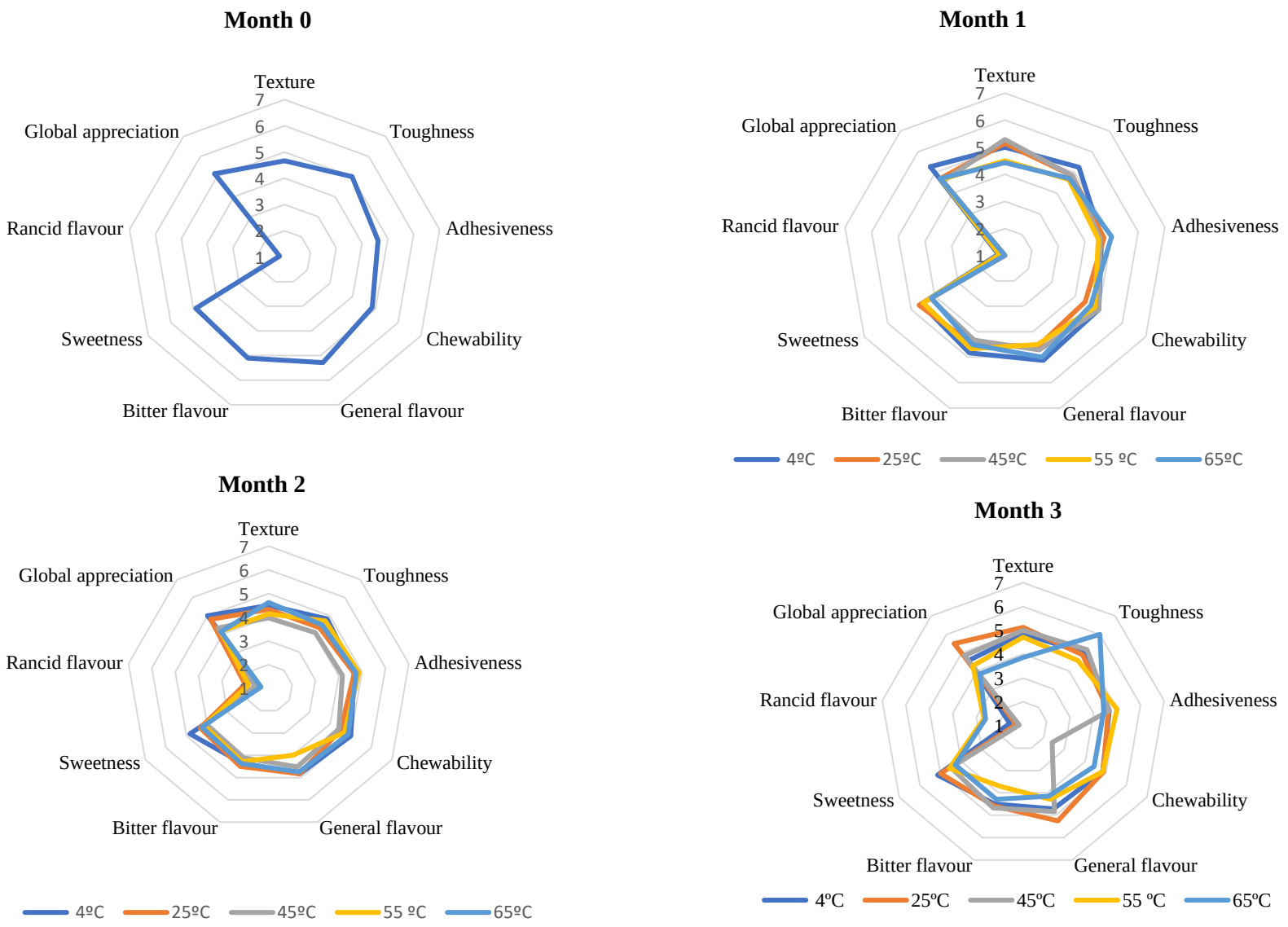

Figure 11 Spider diagram of taste properties of almond pastes during storage.

Our results are in line with those of Padehban et al. (2018), who, when analyzing wild almond kernel, verified that the overall acceptability of all packaged samples at three temperatures significantly decreased during storage time. Furthermore, when increasing the temperature, the sensory scores for the overall acceptability of all samples decreased. Baiano and Del Nobile (2005), when studying almond paste pastries, verified that the loss of moisture and the consequent hardening of samples were the main phenomena affecting almond paste's quality and shelf life. In our work, these phenomena were not observed due to the use of adequate packaging (glass jars) that prevent moisture loss.

Due to the fact that the negative changes were detected at 65 ºC and this temperature is high and it is not common to find this value during the storage of this product, the results indicate that the shelf life of almond pastes will be high (> 6 months). Generally, the rate of reactions doubles or triples for each 10 °C increase. As no negative changes have been observed from 25 to 55 ºC, the shelf life should be significant. However, accelerated studies involving higher temperatures that cause significant changes should be carried out in the future.

5. CONCLUSIONS

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Concerning hazelnuts, the highest unsaturated fatty acid was identified as oleic acid (C18:1n9) (67.2-84.2%), followed by linoleic acid (C18:2n6) (9.7-17.9%). After 12 months of storage, no significant differences were observed in fatty acid percentages between the three storage conditions, suggesting that the temperatures applied did not affect the fatty acid profile. Through the application of Rancimat method, the highest induction time was obtained at the beginning (24.2 h). Then, the induction times decreased after three months, remaining similar until 12 months. Nevertheless, no significant differences were determined between the 3 and 12 months, suggesting that no significative oxidation occurred along storage at 4, 25, and 35 ºC. When comparing the temperatures, the values were always similar, not being detected significant differences between them. Furtermore, the conditions applied did not cause significant microbial growth. Thus, the temperatures at 4, 25 and 35 ºC are not critical factors to consider in the storage of hazelnut kernels, considering the measured parameters. Some mycotoxins were determined in the stored hazelnuts, such as beauvericin, bikaverin, 3-nitropropionic acid, citreohybridinol, flavoglaucin, quinolactacin A, abscisic acid, leecanoric acid, asperglaucide, asperphenamate, cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Val), infectopyron and tryptophol; however, they were detected in low quantities.

Regarding almond pastes, the brightness and hue decreased and the a^* increased after three months at 65 ºC, indicative of Maillard reactions. The a_w values were constant over time, varying between 0.32 and 0.44. These a_w values indicated that the samples were microbiologically stable. The fat content varied between 32.6 and 45.8%, d.w., being twelve fatty acids determined. The oleic acid (C18:1n9) was the major (50.8-57.0%), followed by linoleic acid (C18;2n6c). Concerning the storage time and temperatures evaluated, with minor exceptions, without any defined trend, no significant differences were observed in the percentages of fatty acids between the different conditions studied. The peroxide values increased after one month; however, applying high temperatures, such as 55 and 65 ºC, did not cause a considerable increase in peroxide value after three months. The spectrophotometric investigation in the ultraviolet showed that higher values of K_{232} with higher temperatures were not always observed. After three months, all temperatures gave K_{268} values very similar to each other, 0.15-0.19. This fact suggested that applying high temperatures, such as 55 ºC and 65 ºC, did not cause very evident

secondary oxidation. Concerning the microbiological counts, these were always <1.7 log CFU/g. Thus, the applied conditions did not favour microbial growth. On the contrary, the temperature of 65 ºC negatively affected the sensory quality of the almond pastes after three months. As this temperature is high and it is uncommon to find it during storage, it is suggested that the almond pastes present a high shelf-life (> 6 months). Nevertheless, more assays need to be done to precisely establish this product's shelf-life, such as by using higher temperatures.

6. REFERENCES

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