Chemical Understanding of Almond Quality and Safety

By

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It takes a village to raise a child. -African proverb

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Trust in the Lord with all your heart and lean not on your own understanding; in all your ways submit to him, and he will make your paths straight. -Proverbs 3:5-6

ABSTRACT

Almonds are one of the highest valued crops in the United States, resulting in an industry that produces more than 2 billion pounds of kernels annually. With an increasing yearly production, it is critical to understand the effects of weather and processing on almond quality and determine chemical safety. This research addresses three aims: determine the effect of postharvest moisture (i.e. rain) exposure during storage on raw and roasted almonds, elucidate the effect of pasteurization on raw almond quality during shelf life, and establish glutathione as an endogenous nucleophile involved in acrylamide scavenging during storage. The first aim of this work determined that postharvest moisture exposure followed by pre-processing drying shortens almond shelf life up to 50%. Shelf life is especially compromised in almonds roasted at high temperatures to achieve darker color roasts. This established that almonds exposed to moisture and/or high humidity after harvest should be prioritized in processing and avoid high temperature processing. The second aim addresses the effect of chemical (propylene oxide) and moist heat pasteurization on lipid oxidation in raw almond during storage. Chemical and sensorial analyses demonstrated that moist heat pasteurization protected raw almonds from lipid oxidation with significantly lower rancidity-associated volatiles and negative sensory attributes starting at 4 months of storage, which suggests longer shelf life. The third aim of this work determined scavenging mechanisms to explain acrylamide (a naturally occurring probable carcinogen) losses in almond products during storage. We observed significant decreases in acrylamide levels (21.8 %) over 12 weeks of storage. Acrylamide undergoes Michael addition reactions with nucleophiles including some amino acids and glutathione. Free amino acids and glutathione concentration were measured for the first time in California almonds before and after roasting. An acrylamide-glutathione conjugate was identified in roasted almonds. This product increases during storage demonstrating that

endogenous nucleophiles are involved in acrylamide scavenging during storage. Overall, this work provides the nut industry with information on how to better manage the crop to reduce food waste in response to climate change. Additionally, these studies support the use of steam pasteurization of almonds for ensuring food safety and promoting extended shelf life for food security. The improved understanding of the acrylamide rate of decline and the reaction between acrylamide and free amino acids and glutathione encourages future improvement in chemical food safety.

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1.1 Introduction

Sweet almonds (*Prunus dulcis*) are the seeds of a drupe in the rose. Almond were first cultivated around 5000 years ago in the Fertile Crescent region of the Middle East. Today, California is the main producer of almonds worldwide and the predominant variety grown in California is Nonpareil[1]. Almonds are nutritionally dense and have been studied extensively for their positive impact on serum lipids[2-4] and cardiovascular health[5-7]. The almond is composed of a seed (kernel; 32 %) surrounded by a hardened endocarp (shell; 13 %) and a thin mesocarp (hull; 55 %)[8]. Although only the almond kernel is consumed, the shells and hulls are increasingly used as materials for value-added ingredients, bioenergy for green energy production, in biosolarization, and as crude materials for manufacturing[9-12].

The major composition of the almond kernel are lipids, ranging from 35 to 54% by weight for California almonds varieties and protein ranging from 10 to 29% by fresh weight[13]. The fatty acids found in almonds are oleic acid (18:1, 50-81%), linoleic acid (18:2, 6-37%), palmitic acid (16:0, 5-16%), and linolenic acid (18:3, 0-11%)[14]. The high amount of unsaturated fatty acids make almonds susceptible to oxidation and formation of aldehydes and acids associated with rancidity. Almonds are recognized as one of the most protein dense tree nuts, where essential amino acids account for 30% of the proteins[13, 15]. The most abundant amino acids found in California sweet almonds are glutamine/glutamic acid, asparagine/aspartic acid, and arginine[15, 16], with lysine and threonine being the top limiting amino acids. However, among free amino acids, asparagine accounts for 20-50% of the free amino acids measured followed by glutamic acid and aspartic acid[17]. Carbohydrates account for 14-28% of almond kernel composition, consisting of sugars (3-8%), starch (0-1%), and dietary fibers (3-16%)[18]. Among the soluble sugars, sucrose compose more than 90% followed by glucose and maltose[18].

However, varieties, harvest year, and growing region can all play a role in the composition profile[19].

1.2 Almond Harvesting and a Changing Global Climate

In California, almonds are harvested between July and October, depending on the variety. Once the hull splits indicating maturity, the almond fruit is shaken off the tree mechanically[20]. The almond fruit is then dry on the orchard floor for 7-10 days and is then swept into windrows between the rows of almond trees for additional drying. Once the moisture content of the kernel reaches < 6%, the windrows are collected and transported to processing facilities. At the processing facilities the almond is dehulled and deshelled. Raw almond kernels can be stored at relative humidity < 65 % and temperature < 50 F (10 °C) up to 48 months with little oxidation. Due to the dramatic increase in production of almonds over the past decade and the limited number of hullers, almonds are increasingly left in the field and in stockpiles for extended periods of time prior to processing[21].

California has been experiencing more extreme weather with increased precipitation during fewer rain events, increased temperatures and severe drought[22]. Postharvest rain and high humidity can lead to decreases in almond quality. Almonds are vulnerable to rain while in windrows or on the orchard floor, and although most stockpiles are covered, moisture exposure occurs through raised relative humidity caused by rain. When almonds are exposed to moisture and heat while in windrows and stockpiles, a defect termed concealed damage can occur. Concealed damage is defined by the almond industry as a brown discoloration of the kernel interior after moderate to high heat treatment (e.g. roasting). The kernel browning has been associated with off flavor and is also observed in macadamia, hazelnut, and pecan[23-25]. There are no visible defects in the raw almond kernel, hence the term “concealed”. In almonds, exposure to

postharvest moisture has been reported to increase reducing sugars, free fatty acids levels, and metabolic activities of certain enzymes[26, 27]. Volatiles related to increased enzymatic activities and lipid oxidation were also measured in almonds exposed to moisture[28, 29]. When almonds are exposed to postharvest moisture, the industry practice is to dry the "wet" almonds to ~ 6% moisture content prior to processing. This practiced helps to decrease mechanical damages to the kernel (e.g. chipping, scratching) during the dehulling and deshelling process. Drying "wet" almonds has also been shown to decrease the discoloration associated with concealed damage after roasting[30]. However, the effect of the moisture exposure and the industry practice of subsequent drying of "wet" almonds on lipid oxidation and quality is not well understood.

1.3 Almond Pasteurization and Roasting

In the 2000s, several *Salmonella* outbreaks were linked to almond consumption. After intensive investigation, the Almond Board of California along with the United States Department of Agriculture instituted a mandatory program for all outgoing California almonds to reduce the potential of *Salmonella* with at least 4-log reduction[31]. The program began in 2007 order to provide safer almonds to the public. Since then, the Almond Board of California has developed certified procedures for pasteurization (e.g. roasting, fumigation, and steam) and the industry has established proprietary equipment to pasteurize almonds to meet a 4-log reduction in *Salmonella*. Among the procedures for pasteurization, steam and fumigation are the only methods of pasteurization approved for the sale of raw almonds. Steam pasteurization involves exposing the almonds to hot moist air for short periods of time to kill the surface *Salmonella*[32, 33]. Moist heat (i.e. steam) is more effective than dry heat at inactivating microorganisms[34]. The higher effectiveness of moist heat pasteurization led to the development of controlled condensation steam which can operate under elevated pressure, at atmospheric pressure or under vacuum.

Vacuum process can reduce the saturation vapor pressure allowing the process to be below 100°C. These derived methods from traditional steam pasteurization utilizes proprietary parameters to pasteurize almonds to reduce the amount of moisture in order to prevent alteration of the moisture content during the process[33]. Propylene oxide is a fumigant approved by the Environmental Protection Agency and is effective for reducing *Salmonella* on almonds. Propylene oxide pasteurization has been used in different food commodities (e.g. spices, nuts) prior to being approved and validated for almond pasteurization since the 1970s[35-37]. The process involved exposing the almonds to vaporized propylene oxide, which can alkylate with protein and achieve the log kill needed. The process also requires an extended post-ventilation period (e.g. 2-5 days, depending on temperature) to minimize fumigant residue. The parameters for propylene oxide pasteurization on almonds have been standardized by the Almond Board of California[38].

Pasteurized raw almonds have sensory attributes described as sweet, slightly astringent, benzaldehyde, and woody[39]. The benzaldehyde/marzipan and woody flavors can be used to differentiate between sweet almond varieties[40]. The predominant aroma compounds in raw almond are 1-octen-3-one (mushroom) > octanal > nonanal > acetic acid and methional, however they all have low odor-activity values after considering their concentration measured and odor detection threshold[41]. The typical shelf life of inshell almond is up to 3 years and raw almond kernels can be stored up to 2 years when stored in bulk bins with a plastic liner at relative humidity < 65 % and temperature < 50 F (10°C). Although pasteurization has been a standardized practice for the almond industry since 2007, the potential effect of pasteurization on storage quality and sensory attributes are unknown. The pasteurization process can expose

almonds to heat, moisture, or fumigants, which can impact the shelf life by contributing to lipid oxidation.

After pasteurization of the raw kernels, almonds can be further processed into a wide variety of products. Almonds are consumed as raw kernels, roasted kernels, flour, almond milk, and as almond butter. Roasting is needed to generate the toasted aroma and flavor. The heating process generates new compounds from several reactions, including the Maillard reaction, sugar pyrolysis, and lipid oxidation. Different roasting processes can generate different volatile compound profiles. Oil roasted almonds have higher levels of furanones and nitrogen-containing aroma compounds and lower levels of aldehydes and sulfur-containing compounds when compared to dry roasted almonds[41]. For roasted almond products, the shelf life varies depending on the roasting levels and packaging. The light to dark roasting levels are defined by the color of the nutmeat after roasting. Typically, the roasting temperature can range from 129 °C to 182 °C combined with different lengths of time to achieve the color desired[42]. Dark roast has been associated with higher degree of rancidity development and can lead to shorter shelf life[43]. The key factor to prolong the shelf life of almonds is to store in packaging that protects against oxygen exposure[44, 45].

1.4 Chemical Reactions in Almonds: Lipid Oxidation and Maillard Reaction

1.4.1 Lipid oxidation

Unsaturated fatty acids are more susceptible to lipid oxidation and decomposes into volatile compounds with low molecular weights that produce off-flavors and off-aromas associated with rancidity. Although polyunsaturated fatty acids (PUFA) are generally considered as healthier for consumers, these fatty acids are more vulnerable to oxidation and subsequently have shorter shelf life. Almonds are composed of mainly unsaturated fatty acids and the quality

of the almonds are mainly affected by lipid oxidation development. However, almonds contain high levels of tocopherols that can protect unsaturated fatty acids against peroxidation and lengthening kernel storage[43, 46]. The tocopherol levels measured in California almonds range from 18.2 to 32.9 mg per 100 g almonds at fresh weight[19].

Lipid oxidation is a radical reaction that can be categorized by three phases: initiation, propagation, and termination. Initiation begins with the abstraction of a hydrogen from the fatty acid generating an alkyl radical (R•). The abstraction occurs at the methylene carbon of PUFA or the carbon next to the double bond of monounsaturated fatty acids. The more double bonds found on the fatty acids, the faster it will oxidize[47]. The initiation of lipid oxidation can be promoted by enzymes, metals, light, and high temperature. After initiation, the PUFA alkyl radical rearranges to form conjugated double bonds to lower the energy. The propagation step occurs after the alkyl radical undergoes the addition of an atmospheric oxygen to form a peroxyl radical (ROO•). The peroxyl radical has sufficient energy to abstract a hydrogen from another unsaturated fatty acid forming a lipid hydroperoxide (ROOH) and another alkyl radical. The propagation phase involves the formation of lipid hydroperoxides and transforming more fatty acids into alkyl radicals. The lipid oxidation process and its measurable markers are shown in **Figure 1.1**. Lipid hydroperoxides undergoes β-scission reaction, which decomposes the lipid hydroperoxides into alkoxyl radicals (RO•). This radical can also attack fatty acids to initiate the lipid oxidation process and increase the oxidation rate. Alkoxyl radicals are high in energy and can break the aliphatic chain of the fatty acids to form low molecular weight volatiles contributing to the rancid smell of oxidized fat.

In almonds, the major fatty acids are oleic acid and linoleic acid, which both can form hydroperoxides during oxidation. Oleate hydroperoxides decompose into carbonyl volatiles

including 2-undecenal, 2-decenal, octanal, nonanal, and decanal[48]. Shorter chain aldehydes (e.g. pentanal, hexanal) can also be formed but in lower concentrations. Other minor volatile products, such as organic acids, methyl ketones, and γ-lactones, have also been observed. Linoleate hydroperoxide mainly decomposes into carbonyl volatiles hexanal and 2,4-decadienal[48]. However, 1-octen-3-ol and 2-heptenal are also the decomposition products of the less favorable linoleate hydroperoxides (10- and 12-hydroperoxides). Lipid oxidation products have been linked to different sensory attributes and can be used to better predict the shelf life. Octanal, nonanal, hexanal, 1-octen-3-ol, and γ-hexalactone have all been reported to be associated with consumer liking[49]. Whereas hexanal and pentanal have been associated with cardboard flavor, total oxidized attributes, and painty/solvent flavor[49].

1.4.2 Maillard reaction

The Maillard reaction (nonenzymatic browning) is another complex reaction that can occur in almonds. The process typically involves a reaction between the nucleophilic amino group on amino acids and the carbonyl group from a reducing sugar. In lipid-rich systems, the oxidation and subsequent degradation of lipids also contribute reactive carbonyl groups to the Maillard reaction[50-53]. Some commonly reactive carbonyls reported from this process are glyoxal, glyceraldehyde, decanal and octanal[54]. Maillard reactions generate a number of flavor precursors, flavors, and polymerized brown pigments (melanoidins)[55, 56]. In addition, acrylamide is formed in the Maillard reaction through reactions between free asparagine and reactive carbonyls (e.g. reducing sugars) at temperatures above 120°C[57] (Figure 1.2). Acrylamide is a compound classified as a probable human carcinogen (IARC, 2002)[57] and identified on the Proposition 65 list in California as a carcinogen and reproductive toxin since the 1990s. In food systems, acrylamide can occur naturally during heat processing[58]. Although the FDA has not set

tolerance levels for acrylamide in food, the recommendation to food manufactures is to reduce levels in food products (generally below 200 ppm). Almonds contain high levels of asparagine (560-4000 mg/kg) and glucose (1693-3154 mg/kg) and are therefore susceptible to acrylamide formation during roasting. Zhang et al. demonstrated that both the variety of almond and the roasting process will influence the levels of acrylamide formation in almonds[42]. Acrylamide can also form via another pathway, in which lipid oxidation contributes precursors (i.e. acrolein and/or acrylic acid) that can react with ammonia, generated through the decomposition of amino acids, to form acrylamide during heating[53] (**Figure 1.2**).

1.5 Methods to Evaluate Almond Quality

Almond quality can be affected by many factors, including the physiological development of the almond kernel, harvesting conditions, hulling/shelling/processing conditions, and storage conditions. There are different methods and approaches to evaluate the quality of almonds, which stem from monitoring the products of lipid oxidation and/or Maillard reaction.

1.5.1 Chemical measurement of lipid oxidation

As mentioned previously, almonds are high in lipids and there are methods that the oil industry uses to monitor the lipid oxidation process. The free fatty acid method is a titration based procedure that monitors for hydrolytic rancidity in oil. The hydrolysis of triglycerides results in the release of free fatty acids from the glycerol backbone, where the fatty acids are now more susceptible to oxidation. Peroxide value is another common oxidative marker based upon a titration method measuring the amount of hydroperoxide present in an oil sample. Hydroperoxides are later decomposed into volatiles that contribute to sensory changes in the product. Conjugated dienes levels can be measured spectrophotometrically in oil and arise from the rearrangement of the double bonds after hydrogen abstraction. In almonds, this level reflects

linoleic acid (6-37%) and linolenic acid (0-11%) levels. The volatile compounds generated from the decomposition of hydroperoxides can also be measured in the headspace of the sample. Solid phase microextraction (SPME) coupled with gas chromatography mass spectrometry (GC-MS) can be used to identify and quantify changes in the headspace volatile profile during storage or in response to various processing treatments (e.g. pasteurization). However, SPME is limited to the selectivity of the solid phase coated on the fiber when used to capture the volatile compounds. Hexanal is another common marker measured to evaluate almond quality due to the high concentration found in oxidized almonds. However, other lipid oxidation related volatile compounds (e.g. 2-heptanal, nonanal) have been shown to be better volatile markers of quality due to their better association with negative sensory attributes[43, 49].

1.5.2 Sensory evaluation

Chemical measurements often need to be associated with sensory measurements to aid in the understanding on how humans perceive the product. Sensory evaluation involves the human response to physio-chemical properties in food, which can include texture, color, aroma, and flavor. Two main sensory evaluations are often used by the food industry, descriptive analysis and hedonic testing. Descriptive analysis is performed by trained panelists to evaluate the intensity of different attributes based on established lexicons and standardized scales. Descriptive analysis has been used to evaluate coated[59], roasted[43, 60], and raw almonds during storage[40, 61]. A team of trained panelists quantifies the perceived intensity of the individual sensory attributes and provides important and useful information about the product characteristics. Some examples of the sensory attributes evaluated in these almond studies are clean roasted aroma and flavor (positive attributes related to fresh roasted almonds), total oxidized aroma and flavor (negative attributes related to lipid oxidation), color, texture, sweetness, and astringency. On the other

hand, hedonic testing is a consumer-based sensory evaluation that measures consumer liking or preferences for the food and requires a large number of participants. The result provides valuable information regarding the product perception of daily consumers, which is less analytical than descriptive analysis. Consumer testing can include preferences, willingness to purchase[62], or the use of a 9-point hedonic scale (1=extremely dislike, 9=extremely like) to express liking[43]. Sensory measurements along with chemical measurements can provide a better picture of how the quality of the product is changing and allows the discovery of chemical markers that can be used to help predict sensory attributes.

1.5.3 Acrylamide measurement

Roasted almonds can contain relatively high levels of acrylamide (> 200 µg kg^{-1}) depending on variety and processing methods[42]. Acrylamide formation is inevitable during almond heat processing, but studies have shown that acrylamide levels can be mitigated by controlling heat, various processing parameters (e.g. time) and through the use of additives pre- or post-processing[42, 63, 64]. Acrylamide has been reported to decrease during storage in almonds, coffee beans, and canned coffee[42, 65, 66]. In roasted Nonpareil almonds, Zhang et al. (2011)[42] demonstrated that the levels of acrylamide decreased by 0 - 17.7 % (average decrease of 6.7 %) at room temperature storage for 1 month. Under accelerated storage condition of 60 °C for 6 days, acrylamide levels in roasted almonds were on average 55.5 % lower than the initial levels[67]. The mechanism of acrylamide reduction during storage is not well understood, but model systems have shown the loss of acrylamide during storage depends on the nucleophilic groups (-SH, $-NH_2$) present to form Michael additions[66, 68-70]. Zamora et al. (2010) demonstrated that acrylamide can form Michael addition reactions with sulfhydryl or amine groups present in amino acids[68]. Yoshioka et al. (2020) was the first to report the conjugate formation (cysteine

and lysine) in real food systems (canned milk coffee)[66]. The conjugate formation is responsible for 69.6% of the acrylamide removal during storage by forming Michael adducts in canned milk coffee (**Figure 1.3**). Zhu et al. (2020) showed the inhibition effect of glutathione on acrylamide formation in cookies and the elimination of acrylamide through the Michael addition[71, 72]. Total amino acid profiles have been reported in almonds, yet free amino acid profiles have not been reported in sweet almond varieties[73, 74]. The levels of free amino acid and glutathione present in a food system can have an impact on the formation and removal of acrylamide. The understanding of the amount of free amino acids and glutathione in almonds will aid in selecting varieties with greater endogenous potential to reduce the amount of acrylamide in roasted products. Acrylamide adducts also have less bioavailable when compared to acrylamide, which can also lower the toxic burden of acrylamide in a food[66, 75]. Ultimately, almonds are a complex matrix and the increased understanding of the levels of different compounds and their interaction can help the industry improve the quality and safety of almonds.

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1.7 Figures

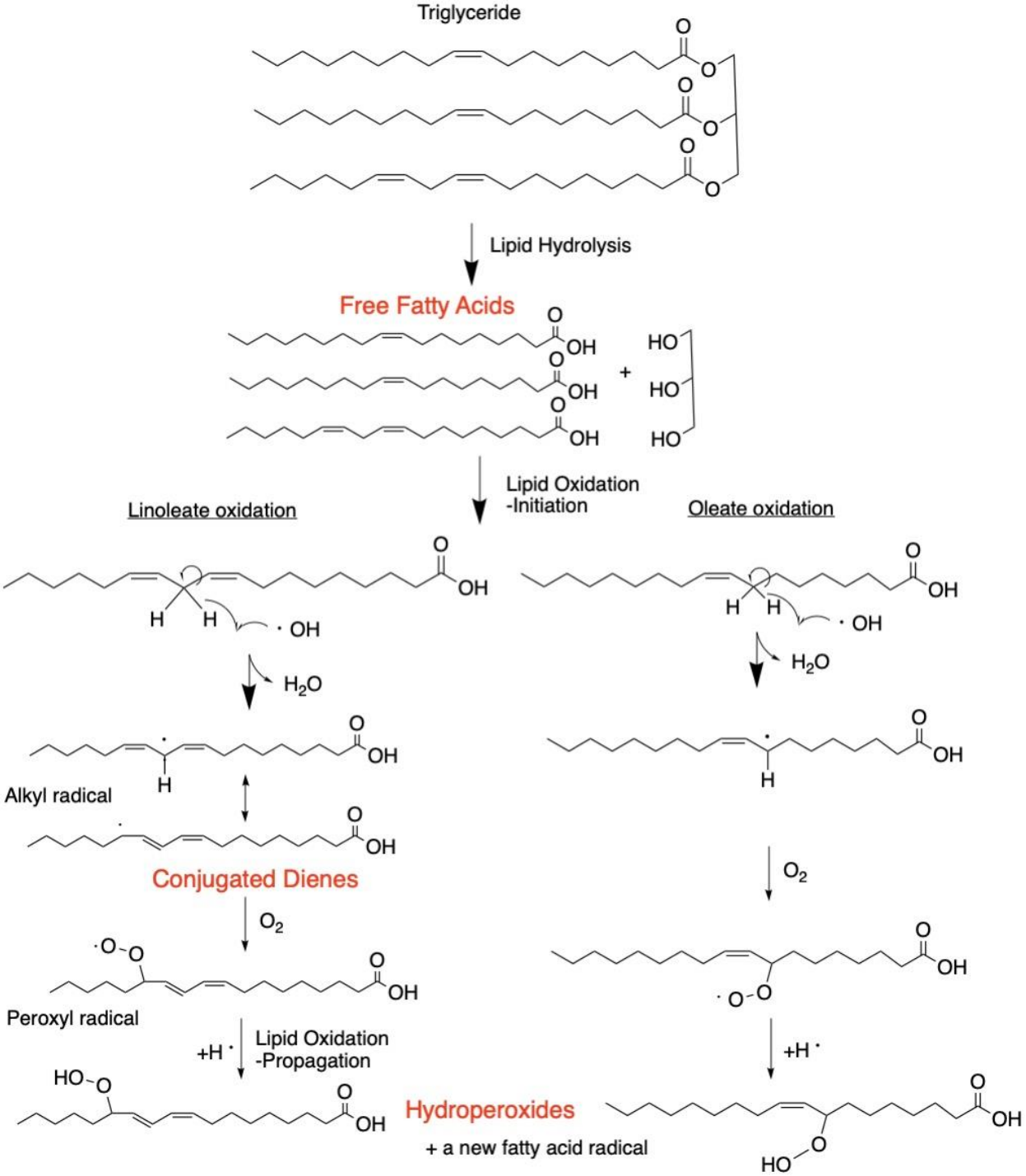

Figure 1.1. Lipid oxidation pathway illustrated with different chemical markers (red) that can be used to monitor lipid oxidation.

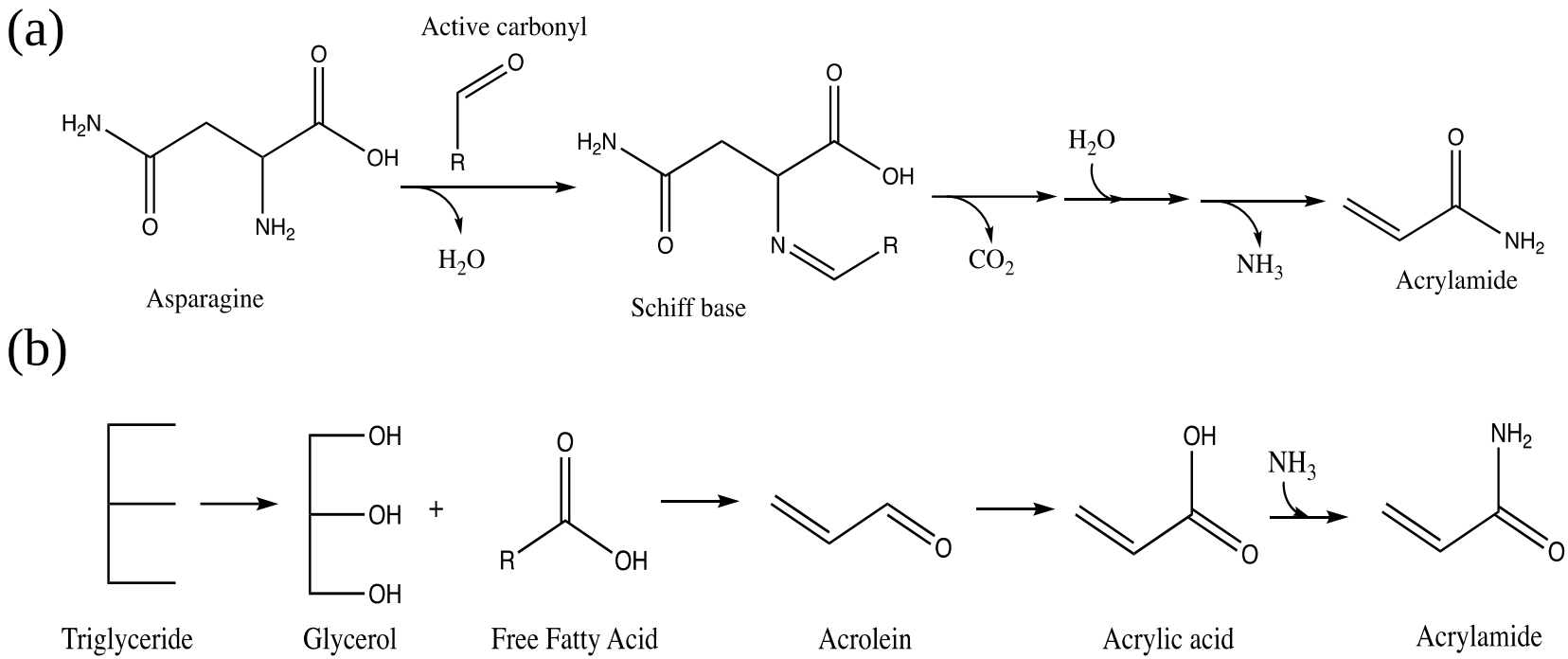

Figure 1.2. Acrylamide formation pathway involving (a) asparagine and carbonyl functional group through Maillard reaction and (b) acrolein and acrylic acid.

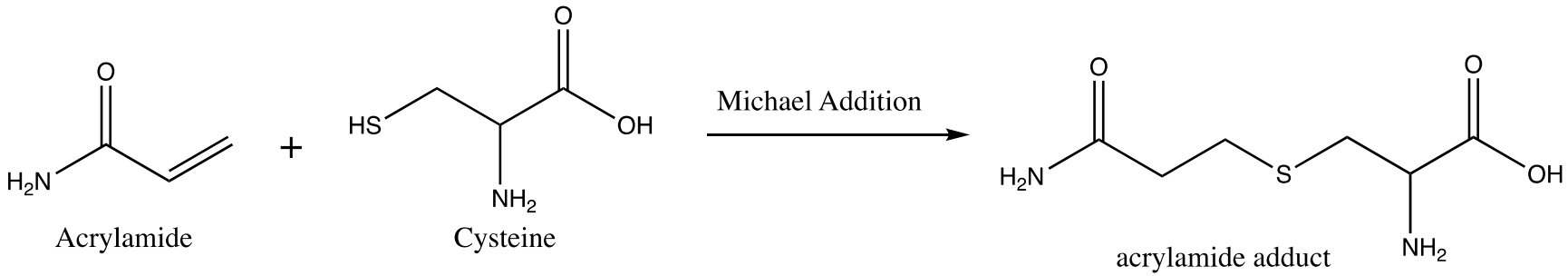

Figure 1.3. Michael addition of acrylamide with cysteine and forming an acrylamide conjugate.

Chapter 2: Influence of Post-Harvest Moisture on Roasted Almond Shelf Life and Consumer Acceptance

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2.1 Abstract

Background: Sweet almonds (*Prunus dulcis*) harvest weights have significantly increased to meet consumer demand and now exceed processing facility capabilities. Crops are stockpiled for longer periods of time increasing the probability of moisture exposure. Wet almonds can be mechanically dried prior to processing; however, it is unclear how this practice influences lipid oxidation, shelf-life and consumer acceptance. To address this, almonds were exposed to 8% moisture and dried with low heat (ME). Almonds were roasted and stored under accelerated conditions for 12 months and markers of lipid oxidation, headspace volatiles, sensory attributes, and consumer liking were evaluated.

Results: At 7 months of storage, light roast ME almonds had higher levels of volatiles related to lipid oxidation as compared to non-moisture exposed almonds (NME) and were significantly higher in oxidized, cardboard and painty/solvent flavors. Although untrained consumers did not show significant preferences between the light roast ME and NME almonds, there were quality losses related to lipid oxidation that trained panelists could detect. Dark roast ME almonds demonstrated significant lipid oxidation by 5 months of storage and indicating they will have a compromised shelf-life. Findings also indicate that octanal, nonanal, 2-octenal, and hexanoic acid are good indicators of consumer acceptability.

Conclusion: Results of this research illustrate that post-harvest moisture exposure with mechanical drying has a significant effect on the storage quality of roasted almonds and is most pronounced in dark roast product.

Keywords: almond, moisture, HS-SPME GC/MS, descriptive analysis, sensory, shelf-life

2.2 Introduction

Sweet almonds (*Prunus dulcis*) are the seeds of a drupe in the rose family[1] and have been consumed since the early bronze age (1000-2000 BCE)[2]. Almonds are an excellent source of α-tocopherol (vitamin E), high value protein, essential minerals and monounsaturated fats[3]. The consumption of almonds is associated with lowering LDL and reducing the risk of heart disease[4]. Almonds are consumed worldwide raw and in roasted snacks, confectionary, bakery products, nut butters, and increasingly as alternative protein in plant-based diets.

California produces more than 80% of the global almonds supply and the crop more than doubled in weight between 2005-2018[5]. Almonds are harvested mechanically using tree shakers. The fallen almonds are swept into windrows and allowed to dry to ~5% moisture. These almonds are collected and stored in stockpiles[6] until they are processed, which involves removing the hulls and shells followed by controlled storage (i.e. indoor storage with controlled temperature). Increased harvests have surpassed processing facility capabilities and crops are stockpiled for longer periods of time increasing the probability of moisture exposure due to rain and humidity.

Raising the moisture content of the hull and kernel after harvest can affect the quality of almonds by increasing the potential of the nutmeat to form a dark brown discoloration upon heating (termed Concealed Damage) and form off-flavors[7-10]. The discoloration and formation of off-flavors results from the hydrolysis of triglycerides and carbohydrates, initiated by moisture exposure. These hydrolysis products serve as precursors for the Maillard browning reaction[7]. Zacheo et al. (1998)[11] was the first to demonstrate a relationship between post-harvest moisture exposure and increased lipid oxidation in almonds.

Lipid oxidation plays a vital role in the sensory attributes of food rich in unsaturated fatty acids[12]. Almonds are susceptible to lipid oxidation as they are 50-60 % lipid by weight and are

composed primarily of oleic acid (60-70 %) and linoleic acid (20-30 %)[13]. Ideally, almond hulls and shells are removed at a kernel moisture content of ≤ 5% as this helps prevent mechanical damage to the nutmeat (e.g. chipping and scratching) during the hulling/shelling process[14]. If the kernel moisture content is > 7% (e.g. from post-harvest moisture exposure), the almonds are mechanically dried to ~5-6% prior to hulling and shelling[14]. Rogel et al. (2017)[7] demonstrated that drying almonds exposed to post-harvest moisture at ≤ 65°C can reduce the degree of brown discoloration upon roasting, whereas drying above 75°C promotes the brown discoloration and the formation of volatiles related to lipid oxidation upon roasting. The preliminary accelerated storage study from Rogel et al. (2017)[7] showed that raw almonds exposed to moisture and drying still showed increase level of lipid oxidation similar to those without drying. Although mechanical drying below 65 °C can reduce the visible browning that occurs after roasting, oxidative damage may still be present and result in a decreased shelf-life of these stored roasted almonds[7]. Roasted almond kernels can be stored for 18-24 months depending upon the roasting conditions and packaging used[15]. Roasting promotes the formation of volatile heterocycles associated with roasted aroma (e.g. pyrazines, furans, pyrans, pyrroles)[16] as well as volatile products arising from oxidation of fatty acids[17]. The decomposition of lipids produces a wide range of aldehydes, alcohols, ketones, and organic acids (e.g. hexenal, pentanol, acetic acid) that contribute to the off-flavors associated with rancidity development in almonds[16, 18].

To date, there are no studies investigating the impact post-harvest moisture exposure and drying have on the development of lipid oxidation in roasted stored almonds although this practice has the potential to significantly affect product shelf life. Moreover, there is no data available evaluating if this practice influences consumer acceptance of these almonds.

2.3 Materials and Methods

Chemicals and Reagents

Stable isotope internal standards: octanal-d_{16}, 2-methylpyrazine-d_6, and n-hexyl-d_{13} alcohol were purchased from C/D/N Isotopes Inc. (Quebec, Canada). All other standards, solvents, and reagents were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Hampton, NH). These include HPLC grade solvents acetic acid, chloroform, and 2,2,4-trimethylpentane; analytical grade sodium hydroxide, ACS grade hydrochloric acid, potassium iodide (99.9%), sodium thiosulfate (99%) and the volatile compounds (95-99%) identified with authentic standards.

Sample Treatment and Storage

A 300 kg sample of newly harvested raw Nonpareil almond kernels (from 2015 harvest year), that were not exposed to post-harvest moisture, was obtained from Blue Diamond Growers (Sacramento, CA). The moisture content of the almonds was determined gravimetrically as 4 %. Almonds were separated into a control group with no moisture exposure (NME), and a moisture exposed (ME) group. The moisture content of the ME group was increased to 8% by incubating almonds in a KMF 240 Constant Climate Chamber (Binder Inc., Bohemia, NY) at 38 °C and 90 ± 1 % relative humidity (% RH) for 36 hours. The ME almonds were dried in a R-4 Harvest Saver Dehydrator (Commercial Dehydrator System Inc., Eugene, OR) at 50 ± 1°C for 12 hours to reduce the moisture content to 4%. Both NME and ME almonds were dry roasted in an E32D5 Turbofan electric convection oven (Moffat Inc., Winston-Salem, NC). Kernels were roasted under two different conditions: 115 ± 3 °C for 60 min (light roast, LR) and 152 ± 3 °C for 15 min (dark roast, DR) to achieve different nutmeat color. Almonds were cooled and divided into

paper bags of 460 g each and placed into the climate control chamber. The chamber was set at 39 ± 1°C and 15 ± 1% RH. Almonds were stored for up to 12 months. The location of each individual bag in the chamber was randomly assigned. Randomized samples were removed from the chamber every month, mixed thoroughly, and repackaged into vacuum sealed polyethylene bags then stored at -80 °C until analyzed. A total of 52 sample type (2 treatments with 2 roasting levels) were analyzed monthly from 0-12 months for lipid oxidation markers.

Analysis of Lipid Oxidation

Whole almonds were ground for three 1-second pulses using a Waring laboratory grinder (Waring Laboratory Equipment, Torrington, CT). The ground almonds were sieved through a size 20 Tyler standard screen (W.S. Tyler Industrial Group, Mentor, OH). The oil was extracted from the ground almonds using a 12-ton Carver manual oil press (Carver, Inc., Wabash, IN). The oil extracted was collected in an amber vial and stored at -20 °C until analyzed. Peroxide values (PV), free fatty acid values (FFA), and conjugated dienes (CD) were measured in the extracted oil. Peroxide values were determined according to the AOCS Official method Cd 8-53, with the results expressed as peroxide milli-equivalents (mEq) per kg[19]. The amount of free fatty acids was determined according to the AOCS Official method Cd 3d-63, with the result reported as % Oleic acid[20]. Conjugated dienes level was measured according to the AOCS Official method Ti 1a-64, with the results expressed in %[21]. The solvents used in these protocols (e.g. chloroform, iso-octane, acetic acid) were flammable and toxic. Proper personal protection was used according to each chemical hazard class, and all work was performed in the chemical fume hood.

Color Measurement

One hundred almond kernels were randomly selected from the LR samples for color analysis to correlate with the appearance attribute in the descriptive analysis. Individual almonds were sliced into 2 identical halves using a razor blade and color of the nutmeat was measured on one half using a ColorFlex colorimeter (HunterLab, Reston, VA), with color values reported in L*, a*, and b* according to the CIE Lab color scale. The port size was 0.5 inch (13 mm) with standard D65 illuminant at 10° observer angle.

Headspace Volatiles Analysis

Headspace volatile analysis was adapted from the method of Franklin et al. (2017)[16]. Twenty grams of almonds were ground with a Waring laboratory grinder and sieved with a size 20 Tyler sieve. An aliquot of 5 ± 0.02 g of the sieved material was weighed into a 20 mL amber headspace vial. Vials were capped and crimped immediately, then equilibrated for at least 4 hours at room temperature (23 ± 2 °C) prior to headspace sampling. An external instrument standard was analyzed in duplicate during each day to account for possible fiber and instrument changes. The external instrument standard was prepared by the same procedure as a sample but using de-volatized almonds instead[9]. After weighing the de-volatized almonds into a 20 mL headspace vial, a 400 µL vial insert containing a 0.5 uL glass capillary filled with methylpyrazine-d_6, hexanol-d_{13}, and octanal-d_{16} in methanol, each at a concentration of 1000 µg mL^{-1}, and placed into the vial. The headspace vial was capped immediately, incubated for 4 hours, and analyzed. A response factor to correct for instrument and fiber variation was calculated according to Franklin et al. (2017)[16].

The volatiles were analyzed using an Agilent 7890A gas chromatograph equipped with a GC injector 80 (Agilent Technologies, Santa Clara, CA). Samples were equilibrated at 35 °C for 45 min with agitation at 400 rpm. The volatiles were extracted with a 1 cm 30/50 µm StableFlex

(Supelco Inc., Bellefonte, PA) divinylbenzene/carboxen/polydimethylsiloxane fiber for 45 min with agitation at 250 rpm. The fiber was desorbed using splitless injection at 250 °C. At 0.9 min the split vent opened at 50:1 ratio for a total injection time of 10 min. The fiber was cleaned in a helium-flushed needle heater for 5 min to prevent carryover. The headspace volatiles were separated using a 30 m x 0.25 mm x 0.25 μm DB-Wax UI column (Agilent Technologies, Santa Clara, CA) at a flowrate of 1.2 mL min^{-1}. The oven program was set at 35 °C for 1 min followed with a ramp of 3 °C min^{-1} to 65 °C, followed by another ramp of 6 °C min^{-1} to 180 °C, and finally 30 °C min^{-1} to 250 °C with a 5 min hold. The mass spectra were collected using an Agilent 5975C MSD with 230 °C source temperature and 150 °C quadrupole temperature. The volatile profiles were collected scanning the range of 30-300 m/z. Tentative volatile identification was performed using the 2017 NIST Mass Spectral Search Program. Identification was confirmed using a retention index calculation or authentic standards when available. Relative concentrations of the headspace volatiles were calculated following the procedure by Franklin et al. (2017)[16].

Quantitative Descriptive Analysis

Ten trained, experienced assessors employed by The National Food Lab, Inc. (Livermore, CA) performed the descriptive analysis. Panelists participated in a 2-hour orientation session to discuss the samples, develop the ballot, and review the references. The final ballot contained one appearance attribute, four aroma attributes, seven flavor attributes and nine texture attributes listed in Table 2.S1[22]. Three evaluations (replicates) were obtained from each panelist per sample with a total of 30 evaluations obtained for each sample. Almonds (2 oz, 57 g) were served in a 3 oz (85 g) opaque soufflé cup with lids coded with a random 3-digit code. Panelists evaluated only LR almonds to minimize bias that can occur from advanced lipid oxidation of DR almonds.

Panelists evaluated 10 test samples per 2-hour evaluation session, each served along with a labeled control sample (NME-LR, 0 month). Panelists used a 15-point degree of difference scale to indicate how different each test sample was from the control sample on an overall basis. In addition, panelists used 15-point intensity scales to indicate the intensity of key sensory attributes for each sample. Samples were assessed in a monadic-sequential order.

Consumer Testing Analysis

One hundred untrained consumers between the ages of 18 and 65, who were not pregnant, were recruited in the city of Davis, California for hedonic testing. Consumers were served 5 pairs of LR almond samples that were evaluated in descriptive analysis, each pair was comprised of one ME sample and one NME sample at the same amount of accelerated storage. Each sample contained 6-7 almond kernels, at room temperature, identified with randomly generated 3-digit codes. Consumers were instructed to taste at least 2 almonds at a time and indicate their liking on a 9-point hedonic scale. After tasting both samples within a pair separately, the consumer was asked to choose a preferred sample within the pair. Consumers tasted the samples in a random and balanced order both among and within the pairs to minimize order effects. Verbal and written instructions were given to the participants, along with a tray of samples, a paper ballot, a bottle of water, an expectoration cup, and unsalted crackers for palate cleansing.

Statistical Analysis

Calculated concentrations are reported as mean ± standard deviation of triplicate measurements. A two-way analysis of variance (ANOVA) with interactions, evaluating moisture exposure (ME) and sample age as main effects was performed ($p < 0.05$). When main effects

were found, post-hoc comparisons using Tukey's HSD test was applied. Binomial testing was performed on the paired preference data. Discriminant analysis and multiple factor analysis were performed as multivariate analysis using XLSTAT statistical and data analysis solution (version 2019.3.1). Data were centered prior to processing. Hierarchical clustering was performed using JMP ® (version 14.3.0. SAS Institute Inc., Cary, NC).

2.4 Results and Discussion

Almonds are stored in the field in stockpiles for longer periods of time due to increased harvests and limited processing facilities. In-field storage increases the probability of post-harvest moisture exposure. Pre-processing drying is increasingly used prior to hulling and shelling without an understanding of how this practice can influence product shelf life and quality. However, previous studies demonstrate that post-harvest drying > 65 °C prior to roasting can increase lipid oxidation in raw almonds which has the potential to shorten product shelf life. To address this, PVs, FFAs, CDs, and headspace volatiles were measured in almonds exposed to 8 % moisture and subsequently dried to 4 % moisture then roasted to a commercial light or dark roast (LR and DR, respectively). Roasted almonds were stored under conditions known to promote lipid oxidation and rancidity development over a 12-month period. Chemical data was correlated to sensory data (descriptive analysis and hedonic testing) to better understand the impact this has on consumer liking and acceptance.

<u>Markers of primary oxidation in roasted almonds</u>

The amount of FFAs, reported as % oleic acid, reflects the hydrolytic rancidity due to enzymatic or spontaneous hydrolysis of triglycerides[16, 23]. FFAs are more vulnerable to lipid oxidation, as compared with fatty acids esterified to glycerol[23]. Industry guidelines suggest any product with an FFA value > 1.5 % is at risk for rancidity development[15]. Herein, the FFA levels

did not exceed 1.0 % oleic over the 12 months of storage similar to other studies[16, 24] as roasting destroys enzymes responsible for the hydrolysis of FFAs (Table 2.1). At 12 months of storage, ME-DR almonds (0.61 ± 0.02 % oleic acid) had significantly higher ($p < 0.05$) FFAs than the NME-DR almonds (0.40 ± 0.00 % oleic acid) (Table 2.1). However, there were no significant differences between the FFA values of ME-LR and NME-LR almonds at 12 months of storage (Table 2.1).

Peroxide value (PV) is commonly used as a rancidity indicator in almonds and most processors use a value of PV < 5 mEq kg^{-1} oil to ensure that kernels have not undergone significant oxidation[15]. Herein, PV levels were below the limit of detection at time 0 for all samples (Table 2.1). At one month, the PV levels in the ME-LR almonds (0.96 ± 0.11 mEq kg^{-1}) were significantly higher than the NME-LR (0.34 ± 0.11 mEq kg^{-1}). Starting at two months, PV levels were significantly higher in the ME-DR almonds as compared to the NME-DR almonds. The PV in LR almonds reached a maximum between 6-8 months for both NME (0.80 ± 0.06 mEq kg^{-1}) and ME almonds (1.41 ± 0.10 mEq kg^{-1}) (Table 2.1). This result is similar with the results of Franklin et al. (2017)[9] in accelerated storage studies of Nonpareil almonds. The PV in DR almonds increased throughout storage and were significantly higher at 12 months in the ME-DR samples (44.46 ± 1.12 mEq kg^{-1}) as compared with the NME-DR (24.48 ± 0.27 mEq kg^{-1}) samples. Overall, ME almonds have significantly ($p < 0.05$) higher PV value than NME almonds for both LR and DR almonds. However, Tukey's post-hoc analysis indicated that most values were not significantly different between NME-LR and ME-LR almonds, whereas the ME-DR almonds were significantly higher than NME-DR after 5 months of storage. The PVs in all LR almonds remained below 5 mEq kg^{-1} throughout the 12 months of storage, whereas levels

exceeded 5 mEq kg^{-1} at 7 months in the NME-DR almonds and at 5 months in the ME-DR almonds, indicating that these products will have shorter shelf-life.

Levels of CDs are not currently used as a quality marker in almonds and no industry standards exist; however, levels have been reported to significantly correlate with consumer acceptance of roasted almond products[9]. Over 12 months of storage, CD levels in NME-LR and ME-LR increased by 68 % and 78 % respectively. However, at 12 months of storage, there was no significant difference ($p > 0.05$) between NME-LR and ME-LR almonds. In contrast, the ME-DR almonds showed significantly higher CD values ($p < 0.05$) than NME-DR at 12 months of storage (Table 2.1). The levels of CD in the ME-DR almonds were significantly higher after 4 months of storage.

These results indicate that post-harvest moisture exposure increases fatty acid oxidation and that the high temperature roasting amplifies this effect with respect to low temperature roasting.

Headspace Volatiles

Headspace volatiles are linked to sensory attributes and can be used to evaluate almond quality[18]. A total of 69 volatiles were identified in the headspace of all roasted almonds and 34 were confirmed with authentic standards (Table 2.S2). The remaining 35 volatiles were tentatively identified by comparing the MS spectra with the NIST 17 library and Kovats' retention indices with literature values listed in NIST Chemistry WebBook under comparable conditions[25]. Among the 69 volatiles identified, only 46 were significantly different between NME-LR and ME-LR almonds whereas 49 volatiles were significantly different between NME-DR and ME-DR samples (Table 2.2 and 2.3).

Volatile organic compounds are generated from various pathways, including the Maillard reaction, sugar pyrolysis, and via lipid oxidation. The Maillard reaction is favorable in high heat and low moisture systems[26]. Almonds are a low moisture (less than 10 % moisture w/w), high fat (44−61 % fat by weight) food[27]. Frequently reported Maillard reaction related volatiles found in heat-treated almonds includes Strecker aldehydes, alkylpyrazines, and furans[28]. Strecker degradation product of leucine (2-methylbutanal) and isoleucine (3-methylbutanal) are low odor threshold compounds that contribute to the malty aroma in almonds[29]. 2,5-Dimethyl pyrazine, 2-methylpyrazine, and trimethyl pyrazine are highly correlated with clean nutty flavor/aroma and clean roasted flavor/aroma in roasted almonds[30]. In this study, 2,5-dimethyl pyrazine and 2-methylpyrazine were the only pyrazines detected in the headspace (Table 2.2 and 2.3) and at levels 2-4 times lower than other studies[16, 17] which can be attributed to different roasting conditions.

The major decomposition products of oleic acid alkoxy radicals include decanal, 1-decane, heptanoic acid, octanol, 2-undecenal, nonanal, octanal, heptanol, and heptanal[18]. Heptanal and octanal are proposed as good indicators of rancidity in almonds due to their strong negative correlation with consumer liking and because they exist at concentrations above the aroma threshold for these compounds[16]. Hexanal, the major decomposition product of linoleic acid, is a common rancidity marker in lipid rich foods. Linoleic acid is the second most abundant fatty acids in almonds and its decomposition products (e.g. hexanal, 2-heptenal, and 2-octenal) have been used to assess almond quality[16, 31, 32]. Levels of heptanal and octanal found in this study are comparable to other studies of almonds undergoing accelerated storage[16, 33]. At 7 months of accelerated storage, the ME-LR almonds had significantly greater levels of heptanal (387.30 ± 35.63 μg kg^{-1}) and octanal (341.24 ± 17.77 μg kg^{-1}) as compared with the NME-LR

almonds (331.84 ± 25.43 μg kg^{-1} and 260.48 ± 11.97 μg kg^{-1}) indicating a higher level of oxidation. Similar to PV values, several oxidation products of oleic and linoleic acid (e.g. hexenal and pentanal) peak around 7 months.

Levels of acetic acid, pentanoic acid, and hexanoic acid were significantly higher in all ME almond samples as compared with the NME almonds for both DR and LR almonds (Table 2.2 and 2.3). Organic acids (i.e. acetic, pentanoic, hexanoic, and heptanoic acid) are tertiary lipid oxidation products that increase during almond storage[9, 33]. The concentration of these volatiles increased 55-779 times over the 12 months of storage for both ME and NME almonds (Table 2.2 and 2.3) with levels of hexanoic acid increasing the most significantly. Rogel et al. (2017)[3] demonstrate higher levels of acetic acid in the headspace of ME almonds. Our results indicate that organic acids, and in particular hexanoic acid, may be a useful marker for identifying almonds exposed to post-harvest moisture.

Hierarchical clustering analysis of the volatiles that were significantly different ($p < 0.05$) between ME and NME almonds (Figure 2.S1 and 2.S2) indicates that storage time has a greater effect on the sample clustering than moisture exposure. The shorter storage times (1-5 months) clustered together and the longer storage times (7-12 months) clustered together. Overall, pyrazines and pyrrole concentrations decreased with increased storage time and aldehydes, ketones, and organic acids increased with increased storage time.

Volatiles identified in the headspace of almonds were placed into groups (12) based on their structure and functional group chemistry (Table 2.S2) and analyzed using discriminant analysis (Figure 2.1A and B). Samples separate based on degree of roasting (i.e. LR or DR; Figure 2.1A). Significant overlap was observed between the ME and NME almonds within each quadrant indicating that roasting level has a greater effect on separating the samples than

moisture exposure. Figure 2.1B shows the variables driving the separation observed in Figure 2.1A. The left quadrants, occupied by DR samples, separated based on Maillard reaction products (e.g. pyrazines and low molecular weight aldehydes) and lipid oxidation products (e.g. high molecular weight aldehydes and organic acids). The top quadrants, occupied by the centroids of ME-LR and ME-DR, were driven by organic acids, ketones, and high molecular weight alcohols. Discriminant analysis indicates that there is no distinct class of volatiles that can be used to differentiate between ME and NME samples and that roasting level has a greater effect on discrimination as dark roasting correlates more strongly with lipid oxidation products (e.g. high molecular weight aldehydes and organic acids).

Sensory Analysis of Light Roasted Almonds

Descriptive analysis and consumer hedonic testing were used to study differences between ME and NME almonds in LR almonds. DR almonds were not evaluated as the roasting conditions used to produce DR almonds result in significant lipid oxidation at time points past 4 months and could bias sensory evaluations. NME almonds at 0 months of storage were used as the control for all sensory analyses. The sensory evaluations were limited to 0-7 months to cover a significant part of shelf-life and to allow for the completion of hedonic testing within one sitting.

Twenty-two attributes were evaluated during the descriptive analysis (Table 2.S1). Of these, eleven attributes were statistical different between NME and ME almonds ($p < 0.05$) across storage times (Table 2.4). The ME samples were significantly higher in overall degree of difference (DOD), total oxidized aroma and flavor, cardboard flavor, painty/solvent flavor, and initial hardness, and had significantly darker color as compared to the NME almonds. Additionally, the ME samples were significantly lower in clean nutty aroma and flavor, and

clean roasted aroma and flavor, which are attributes that have positive association with fresh roasted almonds. Attributes such as total aroma and flavor, bitter flavor, and initial and secondary chewing textures were not significantly different between treatments across storage times. These data indicate that exposing almonds to moisture and drying them before roasting does not have a statistically significant effect on the texture attributes measured. When comparing the individual storage times, 5 attributes were significantly different ($p < 0.05$) between treatments at 7 months (Table 2.4). These attributes include DOD, color, total oxidized flavor, cardboard flavor, and painty/solvent flavor. A significant difference between the DOD scores occurred at 7 months of storage and indicates that the trained panelists were able to distinguish the two products from one another. The attributes that were significantly different between treatments at 7 months of storage are characteristics observed in oxidized products[30, 34].

Cardboard flavor is predicted by increased levels of unsaturated aldehydes, such as 2-octenal and 2-heptenal[30]. Herein, 2-octenal, 2-nonenal, and 2-decenal were significantly different between ME and NME almonds (Table 2.2) and correspond to an increase in the description of cardboard flavor in ME samples (Table 2.4). Pentanal and heptanal levels were not significantly different ≥ 7 months of storage, and 1-octen-3-one and dimethyl trisulfide were not detected. Total oxidized flavor and solvent/painty flavor were associated with similar volatiles as total oxidized flavor. Some proposed volatiles markers for monitoring lipid oxidation in almonds are pentanal, hexanal, 2-heptanol, heptanal, octanal, hexanoic acid, 1-pentanol, 2-octenal, nonanal, 2-heptanone, and 2-pentylfuran[16, 31]. Among these markers, only octanal, nonanal, 2-octenal, and hexanoic acid were significantly different between NME and ME almonds at time points when the trained panelists were able to statistically differentiate the products. These compounds have been reported to have low odor thresholds[18]. Our findings

suggest that octanal, nonanal, 2-octenal, and hexanoic acid may be the most sensitive indicators of almond acceptability in roasted almond products.

Almonds that are exposed to moisture after harvesting can develop a dark brown discoloration of the kernel nutmeat when heated (e.g. roasting). This discoloration is termed *concealed damage* as the color appears only after heat treatment[8]. Browning is attributed to the hydrolysis of carbohydrates and lipids and formation of precursors that contribute to Maillard browning. Raw almonds that have *concealed damage* induced by moisture have significantly lower CIE L* color values as compared to controls[8]. Although a previous study indicated that drying almonds below 65 °C prior to roasting can reduce discoloration in roasted almonds[7] we found that not to be the case. Herein, the ME almonds were found to exhibit lower CIE L* color values (i.e. darker in color) than NME almonds after roasting across all time points (Table 2.1). This result was consistent with the descriptive analysis with ME almonds having a higher score in darkness (Table 2.4). The discrepancy between our study and the previous study may be explained by differences in how almond moisture was increased between studies. In the study of Rogel et al (2015), the almonds were sprayed with water and incubated at 45°C for 24 hr to achieve a moisture content of 8-9 %, herein the moisture content of the almonds was increased to 8 % using a climate controlled chamber at 38 °C and 90 ± 1 % RH over 36 hours.

Hedonic testing of the almonds indicated that there are no significant differences in the mean liking scores of between ME and NME almonds over all time points (Table 2.4). Consumer paired preference testing indicated no strong preference between the treatments (Figure 2.S3), with the exception of the 3 month samples where consumers preferred the NME over the ME almonds ($p < 0.05$). The average hedonic testing scores demonstrate that the storage time has a significant influence on the liking score with the highest average score of 6.68 for

NME at 0 month and lowest score of 5.08 for ME at 7 months (Table 2.4). Although no statistically significant differences were found between the liking scores between treatments, the ME samples showed a lower average score than the NME. This suggests that a difference between the ME and NME almonds was detected by the consumers, but it was not significant enough to influence consumer preferences.

A multiple factor analysis (Figure 2.2A and B) was performed to show the relationship between the chemical analysis, volatile analysis, and sensory analysis of LR almonds at 0, 1, 3, 5, and 7 months of storage. The observation plot (Figure 2.2A) demonstrates that samples separate based on the storage time in the first dimension with longer storage times in the right quadrants. Figure 2.2B shows the space generated by the grouped volatiles and the sensory attributes that were significantly different between treatments as variables. The first two dimensions explain 90 % of the variables, with clean roasted flavor/aroma and clean nutty flavor/aroma correlating with low molecular weight aldehydes and alcohols (left quadrants) and total oxidized flavor/aroma correlating with high molecular weight alcohols and aldehydes (right quadrants). PV, FFA, and CD correlate with lipid oxidation volatiles and sensory attributes, whereas hedonic testing correlates only to fresh roasted sensory attributes. This demonstrates that average consumer liking correlates with fresh roasted samples. However, trained panelists are able to determine treatment differences at 7 months of storage with ME sample correlating with lipid oxidation attributes. In addition, at 7 months of storage, both ME and NME samples demonstrated increased levels of volatiles related to lipid oxidation and were rated as having noticeable rancid attributes by descriptive panelists.

2.5 Conclusions

This study demonstrates that post-harvest moisture exposure and subsequent drying has a significant effect on the quality of roasted almonds during storage and this is most pronounced in dark roast product. ME-DR almonds experience significantly higher levels of lipid oxidation than NME-DR almonds at 5 months of storage and will have shorter shelf life. Although the shelf life may be similar in NME-LR and ME-LR almonds, trained panelists can detect sensory attributes related to lipid oxidation at 7 months of storage that correlate with increased levels of volatiles related to lipid oxidation. This result indicates that ME-LR almonds will have a shorter shelf life than NME-LR almonds. This information is critical for providing the industry with tools to help improve product management. For example, lots of almonds arriving at processors that need to be dried prior to hulling and shelling, may be better suited for product streams that undergo light roasting and/or are used in products that are consumed within 12 months.

Abbreviations

ME, moisture exposed; NME, no moisture exposure; LR, light roast; DR, dark roast; PV, peroxide value; FFA, free fatty acid; CD, conjugated dienes; HT, hedonic testing; DA, descriptive analysis; DOD, overall degree of differences.

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Supporting Information Description

Includes clustering of headspace volatiles measured among light roasted and dark roasted samples, graph of consumer paired preference, list of sensory attributes and definitions for descriptive analysis, and a table of the headspace volatiles identified in all samples.

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2.7 Tables and Figures

Table 2.1. Average value of chemical analyses of light roast (LR) and dark roast (DR) almonds during 12 months of accelerated storage of almonds exposed to moisture and subsequently dried (ME) and almonds with no moisture exposure (NME).

		Free Fatty Acids (% Oleic acid)		Peroxide Value (mEq/kg)		Conjugated Dienes (%)		CIE L* value
Storage Month	Treatment	LR	DR	LR	DR	LR	DR	LR
0	NME	0.10 ± 0.02^{ijk}	0.09 ± 0.00^{q}	-	-	0.19 ± 0.00^{hi}	0.18 ± 0.00^{op}	78.63 ± 4.01
	ME	0.09 ± 0.00^{k}	0.10 ± 0.02^{nopq}	-	-	0.18 ± 0.00^{ij}	0.15 ± 0.01^{p}	77.04 ± 5.34
1	NME	0.09 ± 0.00^{jk}	0.09 ± 0.01^{opq}	0.34 ± 0.11^{fg}	0.47 ± 0.19^{m}	0.18 ± 0.00^{ij}	0.17 ± 0.00^{op}	79.23 ± 3.16
	ME	0.09 ± 0.00^{k}	0.10 ± 0.00^{nopq}	0.96 ± 0.11^{b}	0.81 ± 0.05^{m}	0.16 ± 0.00^{j}	0.18 ± 0.00^{o}	77.68 ± 4.43
2	NME	0.09 ± 0.00^{jk}	0.09 ± 0.01^{q}	0.21 ± 0.05^{g}	0.64 ± 0.00^{m}	0.19 ± 0.01^{hi}	0.21 ± 0.01^{n}	80.19 ± 3.05
	ME	0.10 ± 0.00^{hijk}	0.10 ± 0.01^{nopq}	0.46 ± 0.00^{defg}	2.17 ± 0.11^{l}	0.18 ± 0.00^{ij}	0.24 ± 0.01^{mn}	79.58 ± 3.53
3	NME	0.10 ± 0.02^{hijk}	0.12 ± 0.00^{mno}	0.50 ± 0.00^{defg}	2.36 ± 0.12^{l}	0.18 ± 0.01^{hij}	0.27 ± 0.01^{m}	79.52 ± 2.84
	ME	0.12 ± 0.00^{efghij}	0.12 ± 0.01^{mn}	0.71 ± 0.18^{bcd}	4.16 ± 0.11^{ij}	0.19 ± 0.00^{ghi}	0.30 ± 0.01^{l}	78.22 ± 4.35
4	NME	0.11 ± 0.00^{ghijk}	0.09 ± 0.01^{pq}	0.70 ± 0.00^{bcd}	3.08 ± 0.19^{kl}	0.26 ± 0.02^{bc}	0.30 ± 0.03^{kl}	78.44 ± 3.06
	ME	0.11 ± 0.01^{hijk}	0.12 ± 0.01^{mno}	0.53 ± 0.06^{cdef}	4.09 ± 0.00^{ijk}	0.21 ± 0.00^{efg}	0.32 ± 0.00^{jk}	77.77 ± 3.84
5	NME	0.11 ± 0.00^{fghijk}	0.11 ± 0.00^{nop}	0.53 ± 0.06^{cdef}	4.95 ± 0.08^{i}	0.20 ± 0.00^{fgh}	0.35 ± 0.01^{j}	79.74 ± 3.23
	ME	0.14 ± 0.01^{bcdefg}	0.16 ± 0.01^{kl}	0.53 ± 0.06^{cdef}	11.95 ± 0.03^{f}	0.23 ± 0.01^{de}	0.45 ± 0.01^{h}	78.64 ± 4.03
6	NME	0.12 ± 0.00^{defghi}	0.14 ± 0.01^{lm}	0.64 ± 0.08^{cde}	3.68 ± 0.01^{jk}	0.22 ± 0.00^{def}	0.34 ± 0.00^{j}	79.80 ± 2.90
	ME	0.15 ± 0.00^{abcd}	0.16 ± 0.01^{kl}	0.56 ± 0.06^{cdef}	6.08 ± 0.30^{h}	0.21 ± 0.00^{defg}	0.40 ± 0.01^{i}	77.97 ± 3.62
7	NME	0.12 ± 0.00^{efghij}	0.17 ± 0.00^{jk}	0.80 ± 0.06^{bc}	10.09 ± 0.22^{g}	0.26 ± 0.01^{c}	0.47 ± 0.01^{gh}	79.61 ± 3.34
	ME	0.14 ± 0.00^{bcdef}	0.20 ± 0.00^{hi}	0.67 ± 0.00^{bcd}	12.71 ± 0.52^{f}	0.23 ± 0.01^{d}	0.48 ± 0.01^{g}	78.28 ± 4.27
8	NME	0.14 ± 0.00^{bcdef}	0.19 ± 0.01^{ij}	0.74 ± 0.19^{bcd}	12.14 ± 0.09^{f}	0.23 ± 0.01^{de}	0.54 ± 0.00^{ef}	79.33 ± 3.71
	ME	0.15 ± 0.01^{abc}	0.24 ± 0.01^{f}	1.41 ± 0.10^{a}	17.83 ± 0.17^{d}	0.26 ± 0.00^{bc}	0.56 ± 0.00^{e}	78.04 ± 3.91
9	NME	0.15 ± 0.00^{bcde}	0.22 ± 0.01^{gh}	0.68 ± 0.00^{bcd}	12.35 ± 0.07^{f}	0.25 ± 0.01^{c}	0.53 ± 0.00^{f}	78.73 ± 3.56
	ME	0.17 ± 0.01^{ab}	0.28 ± 0.01^{e}	0.62 ± 0.12^{cdef}	16.00 ± 0.34^{e}	0.26 ± 0.01^{c}	0.54 ± 0.01^{ef}	77.01 ± 3.77
10	NME	0.13 ± 0.01^{defgh}	0.23 ± 0.01^{fg}	0.50 ± 0.20^{defg}	12.00 ± 0.19^{f}	0.27 ± 0.00^{bc}	0.54 ± 0.00^{ef}	77.56 ± 3.29

	ME	0.16 ± 0.01^{ab}	0.31 ± 0.00^{d}	0.56 ± 0.11^{cdef}	16.06 ± 0.16^{e}	0.28 ± 0.00^{b}	0.60 ± 0.01^{d}	77.85 ± 3.43
11	NME	0.15 ± 0.00^{abc}	0.23 ± 0.00^{fg}	0.46 ± 0.06^{defg}	9.43 ± 0.01^{g}	0.25 ± 0.00^{c}	0.47 ± 0.01^{gh}	80.02 ± 3.32
	ME	0.18 ± 0.01^{a}	0.46 ± 0.01^{b}	0.58 ± 0.11^{cdef}	27.97 ± 0.82^{b}	0.28 ± 0.00^{b}	0.68 ± 0.00^{c}	78.25 ± 4.00
12	NME	0.16 ± 0.03^{ab}	0.40 ± 0.00^{c}	0.60 ± 0.14^{cdef}	24.48 ± 0.27^{c}	0.32 ± 0.00^{a}	0.72 ± 0.01^{b}	80.41 ± 2.85
	ME	0.16 ± 0.02^{ab}	0.61 ± 0.02^{a}	0.35 ± 0.05^{efg}	44.46 ± 1.12^{a}	0.32 ± 0.00^{a}	0.91 ± 0.00^{a}	78.53 ± 3.25

[1]Letters shared within the same column indicates there is no significant differences ($p < 0.05$) using ANOVA

[2] - indicates a value was not detected

Table 2.2. Average volatile concentration (μg kg^{-1} almond) measured in light roasted (LR) almond headspace at month 0, 1, 3, 5, 7, 9, and 11 months of storage of almonds exposed to moisture and subsequently dried (ME) and almonds with no moisture exposure (NME).

Months	0		1		3		5		7		9		11	
Treatment	NME	ME	NME	ME	NME	ME	NME	ME	NME	ME	NME	ME	NME	ME
3-methyl-butanal	471.19 ± 65.63	495.51 ± 81.71	300.33 ± 35.69	306.86 ± 47.35	141.91 ± 6.66	115.02 ± 10.62	53.03 ± 4.03	61.89 ± 5.93	45.18 ± 6.93	45.81 ± 5.28	35.60 ± 4.42	30.59 ± 3.01	26.84 ± 3.56	30.46 ± 2.62
2-methyl-butanal	508.72 ± 71.23	532.08 ± 91.00	303.02 ± 34.48	311.66 ± 48.76	131.70 ± 9.09	108.33 ± 10.13	50.18 ± 2.55	58.44 ± 7.99	35.65 ± 4.75	38.29 ± 4.83	39.71 ± 14.76	28.21 ± 8.70	35.32 ± 17.31	16.52 ± 2.98
2,2,4,6,6-pentamethyl-heptane	10.81 ± 1.71	2.67 ± 0.34	2.47 ± 0.96	1.17 ± 0.53	6.77 ± 1.52	3.56 ± 1.19	3.37 ± 0.86	3.50 ± 0.68	4.05 ± 0.79	5.18 ± 1.07	13.36 ± 2.22	14.86 ± 3.20	4.93 ± 1.29	4.51 ± 1.34
Pentanal	26.47 ± 4.23	32.52 ± 4.49	76.03 ± 11.72	132.81 ± 38.82	519.75 ± 53.55	444.72 ± 61.55	454.96 ± 41.43	438.68 ± 52.56	552.09 ± 69.15	582.04 ± 69.88	416.43 ± 55.26	423.27 ± 53.03	351.39 ± 41.16	321.46 ± 29.65
Decane	54.13 ± 3.97	24.49 ± 6.68	15.66 ± 4.87	9.96 ± 4.81	37.48 ± 8.69	23.21 ± 8.72	18.60 ± 4.84	20.16 ± 5.55	22.55 ± 4.56	37.04 ± 13.16	87.85 ± 2.95	116.83 ± 36.97	41.99 ± 14.47	31.96 ± 9.42
2-propyl-furan	0.98 ± 0.13	0.59 ± 0.12	0.84 ± 0.09	0.73 ± 0.18	3.08 ± 0.47	2.37 ± 0.46	3.43 ± 0.61	3.35 ± 0.73	4.95 ± 0.70	5.22 ± 0.78	4.23 ± 0.80	4.17 ± 0.76	3.59 ± 0.60	3.47 ± 0.51
Dimethyl disulfide*	1.09 ± 0.12	1.54 ± 0.22	3.73 ± 0.15	3.00 ± 0.80	2.96 ± 0.33	1.96 ± 0.34	1.18 ± 0.11	1.03 ± 0.16	1.08 ± 0.13	0.92 ± 0.15	0.62 ± 0.10	0.57 ± 0.10	0.27 ± 0.06	0.26 ± 0.03
Hexanal	43.59 ± 0.85	35.75 ± 2.67	133.60 ± 8.09	207.70 ± 53.08	993.06 ± 101.04	850.37 ± 136.41	968.76 ± 116.51	965.21 ± 139.20	1,267.91 ± 118.17	1,339.29 ± 139.68	1,037.44 ± 125.16	1,006.33 ± 135.69	811.23 ± 88.51	779.30 ± 79.15
2-methyl-1-propanol	2.25 ± 0.20	2.07 ± 0.23	3.87 ± 0.34	3.20 ± 0.70	2.54 ± 0.18	2.31 ± 0.29	1.18 ± 0.11	1.20 ± 0.14	1.05 ± 0.10	0.83 ± 0.12	0.55 ± 0.08	0.95 ± 0.13	0.31 ± 0.06	0.62 ± 0.09
2-n-butyl furan	0.45 ± 0.04	0.45 ± 0.08	1.16 ± 0.13	1.45 ± 0.34	9.95 ± 1.14	8.27 ± 1.49	11.99 ± 1.54	12.09 ± 1.71	19.36 ± 1.52	21.57 ± 2.61	18.59 ± 2.39	17.95 ± 3.18	15.93 ± 2.21	15.39 ± 1.95
pentyl-oxirane*	0.05 ± 0.02	0.03 ± 0.01	0.37 ± 0.05	0.90 ± 0.23	4.39 ± 0.45	4.78 ± 0.92	6.16 ± 0.82	6.51 ± 0.86	13.12 ± 1.14	14.79 ± 1.63	14.96 ± 1.71	15.09 ± 2.46	15.11 ± 1.75	14.33 ± 1.69
1-butanol	5.16 ± 0.34	4.48 ± 0.71	4.85 ± 0.34	5.26 ± 1.01	8.96 ± 0.77	8.83 ± 0.78	8.35 ± 0.79	8.77 ± 0.61	12.15 ± 1.00	12.70 ± 1.09	10.03 ± 1.01	11.08 ± 0.95	8.66 ± 0.82	9.27 ± 0.68
2-heptanone*	5.85 ± 0.55	6.13 ± 0.87	9.03 ± 1.13	12.38 ± 3.02	69.87 ± 7.04	61.34 ± 10.43	115.51 ± 15.90	118.38 ± 17.23	270.42 ± 24.75	311.44 ± 35.38	323.46 ± 38.01	338.89 ± 58.20	334.02 ± 41.55	323.48 ± 41.37

Heptanal*	6.70 ± 0.73	6.25 ± 0.44	15.22 ± 2.20	21.31 ± 3.42	109.42 ± 3.48	103.38 ± 12.26	163.28 ± 20.37	171.89 ± 21.65	331.84 ± 25.43	387.30 ± 35.63	368.96 ± 34.31	390.52 ± 56.74	337.24 ± 33.56	327.20 ± 36.95
D-limonene	0.35 ± 0.04	0.37 ± 0.04	0.29 ± 0.07	0.22 ± 0.04	0.40 ± 0.03	0.45 ± 0.00	0.22 ± 0.03	0.21 ± 0.01	0.36 ± 0.03	0.33 ± 0.05	0.41 ± 0.06	0.41 ± 0.02	0.50 ± 0.02	0.69 ± 0.06
2-methyl-1-butanol	53.87 ± 4.10	55.74 ± 7.65	118.39 ± 11.17	99.44 ± 21.93	121.55 ± 9.64	99.24 ± 10.31	57.57 ± 4.18	54.06 ± 5.47	60.92 ± 4.06	47.80 ± 4.59	36.68 ± 3.95	42.88 ± 5.22	25.03 ± 1.71	34.64 ± 1.16
3-methyl-1-butanol	49.48 ± 4.52	55.90 ± 8.38	85.58 ± 6.24	76.39 ± 16.25	84.55 ± 6.94	76.01 ± 8.08	41.61 ± 3.25	40.20 ± 4.19	45.32 ± 3.10	36.51 ± 3.12	28.11 ± 2.80	34.33 ± 4.01	19.39 ± 1.56	27.12 ± 0.96
2-pentyl-furan*	3.85 ± 0.23	3.75 ± 0.43	6.87 ± 1.42	8.57 ± 1.23	41.86 ± 2.22	37.96 ± 4.29	50.74 ± 4.95	51.17 ± 4.68	84.85 ± 5.40	95.54 ± 7.14	87.05 ± 5.09	89.81 ± 10.29	84.97 ± 6.93	80.75 ± 6.96
Styrene	0.93 ± 0.07	1.12 ± 0.14	1.21 ± 0.14	1.26 ± 0.16	5.03 ± 0.54	4.00 ± 0.65	4.91 ± 0.65	4.64 ± 0.67	10.76 ± 0.88	11.02 ± 1.34	14.62 ± 1.66	14.51 ± 2.61	19.71 ± 2.37	18.74 ± 2.36
1-pentanol*	28.71 ± 2.84	24.61 ± 3.98	31.30 ± 2.18	44.38 ± 10.75	124.25 ± 12.43	130.45 ± 17.98	142.24 ± 13.53	149.34 ± 16.69	230.87 ± 15.08	244.27 ± 18.01	213.60 ± 20.06	218.67 ± 24.57	194.40 ± 18.21	201.60 ± 14.28
methyl-pyrazine	8.50 ± 0.92	8.77 ± 2.01	4.31 ± 0.39	4.70 ± 0.61	6.19 ± 0.94	5.51 ± 1.19	3.91 ± 0.55	4.83 ± 0.74	4.93 ± 0.51	4.72 ± 0.67	4.11 ± 0.53	4.03 ± 0.71	2.90 ± 0.43	3.13 ± 0.42
Acetoin	74.40 ± 5.32	84.64 ± 8.08	30.17 ± 1.06	27.89 ± 1.55	15.94 ± 0.70	12.65 ± 0.78	9.08 ± 0.30	10.34 ± 0.56	9.55 ± 0.10	8.07 ± 0.15	7.36 ± 0.31	5.75 ± 0.15	4.37 ± 0.18	5.49 ± 0.13
2-octanone*	0.46 ± 0.10	1.24 ± 0.22	0.77 ± 0.20	1.72 ± 0.26	5.83 ± 0.39	6.55 ± 0.56	11.06 ± 1.13	12.54 ± 1.18	30.65 ± 1.15	39.16 ± 2.45	44.69 ± 2.40	52.62 ± 6.44	56.00 ± 4.07	53.79 ± 4.87
Octanal*	1.69 ± 0.27	1.95 ± 0.32	5.95 ± 1.43	11.09 ± 1.46	67.18 ± 3.02	71.72 ± 5.63	120.14 ± 10.43	133.89 ± 9.71	260.48 ± 11.97	341.24 ± 17.77	320.89 ± 12.30	381.44 ± 34.26	332.46 ± 20.39	320.95 ± 26.08
1-hydroxy-2-propanone*	20.35 ± 0.71	28.69 ± 1.69	9.04 ± 0.64	9.43 ± 1.02	4.75 ± 0.13	5.50 ± 0.06	4.98 ± 0.30	5.46 ± 0.25	4.83 ± 0.52	5.39 ± 0.63	4.45 ± 0.62	4.05 ± 0.44	2.38 ± 0.27	2.88 ± 0.22
1-chloro-2-propanol*	577.17 ± 44.82	438.92 ± 53.95	398.18 ± 39.68	236.02 ± 33.83	236.82 ± 15.41	166.77 ± 15.11	113.63 ± 3.73	87.72 ± 3.80	94.46 ± 3.61	85.36 ± 5.17	61.58 ± 2.84	72.48 ± 5.93	39.62 ± 2.67	44.51 ± 0.92
2,5-dimethyl-pyrazine	13.47 ± 1.35	14.49 ± 2.68	8.10 ± 1.14	9.87 ± 1.02	14.96 ± 1.30	12.69 ± 2.05	9.50 ± 1.01	11.59 ± 1.36	13.60 ± 0.91	12.94 ± 1.40	12.19 ± 0.87	11.43 ± 1.70	9.43 ± 0.95	9.48 ± 1.06
Methylthio-2-propanone*	7.76 ± 0.45	9.08 ± 1.36	13.43 ± 1.81	9.33 ± 1.78	16.07 ± 1.88	7.16 ± 1.42	7.10 ± 0.84	4.49 ± 0.60	5.00 ± 0.33	2.54 ± 0.23	2.26 ± 0.20	1.64 ± 0.26	1.32 ± 0.09	1.20 ± 0.03
1-hexanol	83.72 ± 5.72	70.92 ± 10.64	113.05 ± 14.29	126.98 ± 21.94	350.76 ± 25.35	449.04 ± 53.28	379.69 ± 30.11	353.30 ± 27.31	670.45 ± 17.78	560.57 ± 36.94	554.84 ± 30.08	606.64 ± 54.12	486.75 ± 33.62	556.69 ± 31.14
2-chloro-1-propanol*	3.70 ± 0.29	3.33 ± 0.37	2.67 ± 0.22	1.84 ± 0.27	1.65 ± 0.11	1.21 ± 0.14	0.89 ± 0.04	0.73 ± 0.04	0.71 ± 0.04	0.68 ± 0.04	0.52 ± 0.06	0.63 ± 0.02	0.34 ± 0.02	0.42 ± 0.04
2-ethyl-6-methyl-pyrazine*	0.46 ± 0.04	0.53 ± 0.07	0.32 ± 0.02	0.48 ± 0.01	0.63 ± 0.03	0.59 ± 0.05	0.47 ± 0.04	0.58 ± 0.02	0.63 ± 0.03	0.68 ± 0.05	0.66 ± 0.03	0.60 ± 0.07	0.56 ± 0.04	0.53 ± 0.07
2-nonanone*	0.33 ± 0.02	0.44 ± 0.10	0.32 ± 0.10	0.49 ± 0.08	2.29 ± 0.17	2.68 ± 0.24	5.72 ± 0.38	6.81 ± 0.40	18.98 ± 0.62	28.53 ± 1.19	34.08 ± 1.43	48.94 ± 2.73	54.49 ± 1.74	51.61 ± 3.70

Nonanal*	30.24 ± 7.93	29.21 ± 6.47	56.98 ± 6.78	38.36 ± 5.92	124.21 ± 25.88	137.76 ± 29.32	124.98 ± 2.25	148.59 ± 3.77	247.35 ± 8.15	361.85 ± 12.92	311.27 ± 5.03	410.95 ± 14.36	387.07 ± 7.41	375.86 ± 17.39
Trimethyl-pyrazine	1.48 ± 0.06	1.64 ± 0.18	1.02 ± 0.18	1.40 ± 0.07	1.98 ± 0.12	1.69 ± 0.17	1.36 ± 0.11	1.70 ± 0.09	2.01 ± 0.08	2.05 ± 0.13	2.00 ± 0.07	1.87 ± 0.17	1.84 ± 0.12	1.71 ± 0.14
3-octen-2-one*	0.52 ± 0.01	0.26 ± 0.02	1.24 ± 0.38	1.92 ± 0.25	14.19 ± 0.73	16.28 ± 1.47	22.08 ± 1.93	24.56 ± 1.65	46.96 ± 1.91	59.93 ± 3.07	56.94 ± 1.76	65.46 ± 5.28	58.67 ± 2.74	58.04 ± 4.41
3-ethyl-2-methyl-1,3-hexadiene*	1.04 ± 0.01	0.99 ± 0.08	2.10 ± 0.45	3.13 ± 0.41	14.54 ± 0.85	15.06 ± 1.29	19.07 ± 1.38	21.47 ± 1.31	34.61 ± 1.55	47.27 ± 1.67	40.01 ± 1.69	47.71 ± 4.34	44.92 ± 2.64	43.10 ± 3.50
(E)-2-octenal*	0.36 ± 0.01	0.45 ± 0.03	1.52 ± 0.47	2.95 ± 0.30	12.70 ± 0.54	15.60 ± 1.21	12.42 ± 0.91	14.07 ± 1.00	16.06 ± 0.50	29.18 ± 1.09	19.40 ± 0.51	30.84 ± 2.36	22.95 ± 1.06	21.11 ± 2.39
3-ethyl-2,5-dimethyl-pyrazine*	0.12 ± 0.01	0.14 ± 0.01	0.08 ± 0.02	0.12 ± 0.01	0.14 ± 0.00	0.13 ± 0.01	0.10 ± 0.00	0.13 ± 0.00	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.00	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
2,6-diethyl-pyrazine*	1.44 ± 0.06	1.76 ± 0.16	1.10 ± 0.19	1.73 ± 0.07	1.97 ± 0.06	1.77 ± 0.14	1.33 ± 0.06	1.84 ± 0.08	1.97 ± 0.05	2.11 ± 0.10	2.06 ± 0.04	2.04 ± 0.14	1.98 ± 0.05	1.80 ± 0.10
Acetic acid*	14.85 ± 0.94	45.13 ± 2.93	10.81 ± 0.88	19.01 ± 4.43	7.68 ± 0.52	20.56 ± 1.03	17.53 ± 1.92	36.42 ± 2.06	24.95 ± 2.91	48.93 ± 5.54	28.06 ± 2.88	42.89 ± 2.11	20.92 ± 1.55	37.14 ± 1.19
1-octen-3-ol*	4.22 ± 0.06	3.07 ± 0.13	3.11 ± 0.49	3.19 ± 0.33	7.51 ± 0.34	8.16 ± 0.69	8.38 ± 0.54	11.05 ± 0.25	17.77 ± 0.69	28.10 ± 1.59	27.06 ± 0.13	34.50 ± 2.48	37.97 ± 2.32	35.71 ± 3.52
Furfural*	39.23 ± 3.37	36.35 ± 5.01	16.78 ± 0.89	14.00 ± 1.65	12.24 ± 1.31	9.32 ± 1.38	8.56 ± 0.57	8.67 ± 0.46	10.44 ± 0.36	10.52 ± 0.62	9.88 ± 0.25	8.75 ± 0.51	6.33 ± 0.71	6.62 ± 0.84
1-heptanol*	2.22 ± 0.03	2.51 ± 0.24	3.14 ± 0.70	6.33 ± 0.83	16.90 ± 0.70	24.97 ± 2.06	28.89 ± 1.93	37.25 ± 2.02	64.42 ± 2.22	92.21 ± 3.82	83.77 ± 2.06	111.93 ± 7.56	101.59 ± 5.90	106.35 ± 6.67
2-decanone*	0.17 ± 0.02	0.17 ± 0.02	0.15 ± 0.04	0.20 ± 0.06	0.61 ± 0.04	0.76 ± 0.11	1.30 ± 0.06	1.72 ± 0.07	3.88 ± 0.12	7.15 ± 0.39	8.11 ± 0.30	13.90 ± 0.39	14.12 ± 0.20	13.78 ± 0.78
Decanal*	0.58 ± 0.12	0.70 ± 0.19	0.62 ± 0.16	0.79 ± 0.05	2.67 ± 0.22	3.89 ± 0.27	5.00 ± 0.22	6.32 ± 0.10	10.61 ± 0.15	19.47 ± 1.08	16.24 ± 0.81	26.53 ± 0.26	21.46 ± 0.53	22.20 ± 0.93
trans-3-nonen-2-one*	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.03	0.10 ± 0.02	0.53 ± 0.04	0.67 ± 0.12	0.80 ± 0.06	0.94 ± 0.16	2.03 ± 0.11	3.16 ± 0.14	3.16 ± 0.18	4.59 ± 0.19	5.30 ± 0.32	5.17 ± 0.29
Pyrrole	2.99 ± 0.25	3.44 ± 0.56	1.54 ± 0.04	1.60 ± 0.13	1.58 ± 0.08	1.22 ± 0.09	0.88 ± 0.08	1.09 ± 0.11	1.08 ± 0.06	0.99 ± 0.09	0.90 ± 0.08	0.69 ± 0.05	0.89 ± 0.07	0.94 ± 0.04
Benzaldehyde	8.87 ± 0.55	9.92 ± 1.10	8.79 ± 0.73	8.63 ± 0.49	15.60 ± 0.80	13.47 ± 1.39	12.64 ± 0.98	13.85 ± 0.95	20.06 ± 0.70	20.69 ± 1.36	22.19 ± 0.91	20.56 ± 2.04	19.45 ± 1.45	19.40 ± 1.64
(E)-2-Nonenal*	0.49 ± 0.01	0.52 ± 0.07	0.34 ± 0.06	0.38 ± 0.05	0.70 ± 0.06	0.69 ± 0.09	0.82 ± 0.06	1.00 ± 0.05	1.21 ± 0.04	2.34 ± 0.08	1.85 ± 0.10	3.27 ± 0.11	2.52 ± 0.20	2.50 ± 0.34
2-butyltetrahydro-furan*	0.03 ± 0.00	0.05 ± 0.01	0.60 ± 0.13	1.51 ± 0.29	5.05 ± 0.14	9.62 ± 0.52	4.75 ± 0.45	6.19 ± 0.50	4.85 ± 0.09	10.71 ± 0.42	4.80 ± 0.03	9.41 ± 0.73	4.43 ± 0.11	3.72 ± 0.60
1-octanol*	0.60 ± 0.04	0.67 ± 0.02	0.78 ± 0.26	1.32 ± 0.13	3.41 ± 0.05	6.08 ± 0.43	5.73 ± 0.24	8.34 ± 0.42	13.51 ± 0.24	22.21 ± 0.74	19.11 ± 0.31	31.32 ± 1.05	26.16 ± 0.60	28.95 ± 1.40
2-methyl-1H-pyrrole	0.22 ± 0.02	0.26 ± 0.04	0.11 ± 0.01	0.11 ± 0.01	0.09 ± 0.00	0.07 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.08 ± 0.00	0.09 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01
Butyrolactone*	2.25 ± 0.18	3.11 ± 0.41	1.14 ± 0.12	1.30 ± 0.09	1.09 ± 0.05	1.67 ± 0.14	0.91 ± 0.02	1.51 ± 0.02	1.30 ± 0.02	1.67 ± 0.01	1.34 ± 0.04	1.45 ± 0.09	1.09 ± 0.07	1.52 ± 0.06
Benzeneacetaldehyde	62.23 ± 3.03	70.64 ± 6.45	10.33 ± 1.64	8.74 ± 0.20	3.80 ± 0.41	3.72 ± 0.22	3.27 ± 0.07	3.39 ± 0.07	3.46 ± 0.08	3.29 ± 0.15	3.52 ± 0.40	3.15 ± 0.19	2.35 ± 0.17	2.86 ± 0.21

(Z)-2-decenal*	0.16 ± 0.04	0.11 ± 0.03	0.15 ± 0.04	0.19 ± 0.03	0.44 ± 0.03	0.78 ± 0.08	0.60 ± 0.04	0.91 ± 0.08	0.71 ± 0.04	2.31 ± 0.21	1.10 ± 0.02	3.57 ± 0.16	1.25 ± 0.04	1.35 ± 0.22
2-methyl-, anhydride pentanoic acid*	0.17 ± 0.01	0.26 ± 0.02	0.26 ± 0.08	0.57 ± 0.05	1.96 ± 0.06	3.36 ± 0.36	3.96 ± 0.20	5.44 ± 0.30	6.57 ± 0.03	12.84 ± 0.51	8.90 ± 0.10	13.88 ± 0.66	9.67 ± 0.37	9.51 ± 1.00
2-furanmethanol*	2.01 ± 0.12	3.02 ± 0.26	1.14 ± 0.13	1.40 ± 0.13	0.91 ± 0.02	0.92 ± 0.07	0.69 ± 0.01	1.09 ± 0.05	0.80 ± 0.02	0.98 ± 0.04	0.69 ± 0.02	0.68 ± 0.01	0.41 ± 0.03	0.46 ± 0.02
1-nonanol*	0.26 ± 0.04	0.26 ± 0.03	0.29 ± 0.09	0.28 ± 0.02	0.63 ± 0.02	1.21 ± 0.07	0.87 ± 0.04	0.93 ± 0.10	1.89 ± 0.11	2.30 ± 0.18	2.19 ± 0.25	4.08 ± 0.03	3.12 ± 0.07	4.09 ± 0.15
3-methyl-butanoic acid*	0.47 ± 0.05	1.00 ± 0.12	0.28 ± 0.04	0.52 ± 0.10	0.74 ± 0.07	0.91 ± 0.09	1.15 ± 0.04	1.85 ± 0.04	1.99 ± 0.07	2.30 ± 0.05	2.53 ± 0.04	3.24 ± 0.08	2.84 ± 0.07	2.60 ± 0.08
5-ethyldihydro-2(3H)-furanone*	2.09 ± 0.07	3.85 ± 0.28	2.80 ± 0.68	5.54 ± 0.53	19.53 ± 0.74	27.10 ± 1.80	34.09 ± 2.15	41.92 ± 1.67	72.69 ± 2.04	103.39 ± 3.32	98.00 ± 1.78	123.56 ± 6.88	123.72 ± 4.95	127.44 ± 8.45
Pentanoic acid*	0.12 ± 0.02	0.25 ± 0.03	0.20 ± 0.00	0.50 ± 0.10	2.78 ± 0.43	6.36 ± 0.17	10.39 ± 0.80	16.12 ± 0.84	28.68 ± 1.71	53.81 ± 0.64	50.47 ± 0.45	76.21 ± 4.53	72.75 ± 2.01	79.79 ± 7.37
tetrahydro-6-methyl-2H-pyran-2-one*	0.09 ± 0.00	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.00	0.20 ± 0.01	0.24 ± 0.01	0.27 ± 0.01	0.34 ± 0.02	0.56 ± 0.01	0.89 ± 0.02	0.80 ± 0.00	1.13 ± 0.06	1.07 ± 0.03	1.15 ± 0.07
dihydro-5-propyl-2(3H)-furanone*	0.06 ± 0.01	0.18 ± 0.02	0.07 ± 0.02	0.16 ± 0.01	0.31 ± 0.01	0.50 ± 0.02	0.53 ± 0.02	0.75 ± 0.02	1.30 ± 0.03	2.15 ± 0.06	2.08 ± 0.02	3.24 ± 0.11	3.17 ± 0.05	3.38 ± 0.21
Hexanoic acid*	0.35 ± 0.04	0.64 ± 0.11	0.43 ± 0.07	0.85 ± 0.22	7.56 ± 1.97	26.81 ± 2.30	50.31 ± 4.87	85.16 ± 3.43	127.76 ± 3.51	301.10 ± 6.00	237.26 ± 4.36	420.50 ± 14.90	350.17 ± 13.76	393.04 ± 40.67
Benzyl alcohol*	0.44 ± 0.08	0.57 ± 0.03	0.40 ± 0.10	0.40 ± 0.02	0.43 ± 0.01	0.58 ± 0.03	0.37 ± 0.00	0.45 ± 0.02	0.52 ± 0.02	0.64 ± 0.01	0.58 ± 0.03	0.70 ± 0.02	0.51 ± 0.01	0.58 ± 0.01
5-butyldihydro-2(3H)-furanone*	0.04 ± 0.01	0.08 ± 0.00	0.07 ± 0.02	0.13 ± 0.02	0.44 ± 0.02	0.80 ± 0.04	0.86 ± 0.03	1.33 ± 0.08	1.89 ± 0.02	4.07 ± 0.22	3.50 ± 0.12	6.74 ± 0.16	5.43 ± 0.10	6.39 ± 0.38
Phenylethyl alcohol*	1.58 ± 0.05	2.28 ± 0.18	1.94 ± 0.40	1.73 ± 0.05	1.96 ± 0.07	2.54 ± 0.16	1.75 ± 0.04	2.18 ± 0.07	2.47 ± 0.06	2.40 ± 0.10	2.51 ± 0.11	3.35 ± 0.08	2.40 ± 0.01	2.80 ± 0.04
Heptanoic acid*	0.07 ± 0.01	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.04	0.27 ± 0.07	0.43 ± 0.11	0.58 ± 0.05	1.02 ± 0.11	1.59 ± 0.12	4.85 ± 0.50	3.11 ± 0.05	9.32 ± 0.98	6.45 ± 0.36	7.74 ± 1.02
2-vinyfuran*	0.18 ± 0.02	0.19 ± 0.03	0.25 ± 0.02	0.20 ± 0.02	0.22 ± 0.01	0.31 ± 0.01	0.22 ± 0.03	0.27 ± 0.01	0.29 ± 0.04	0.36 ± 0.02	0.35 ± 0.04	0.38 ± 0.03	0.30 ± 0.02	0.41 ± 0.04

[1]*Significantly different between treatments across all time points ($p < 0.05$) using ANOVA

[2]Bolded: volatiles that have significant difference between treatments at 7 months of accelerated storage.

Table 2.3. Average volatile concentration (μg kg^{-1} almond) measured in dark roasted (DR) almond headspace at month 0, 1, 3, 5, 7, 9, and 11 months of storage of almonds exposed to moisture and subsequently dried (ME) and almonds with no moisture exposure (NME).

Months	0		1		3		5		7		9		11	
Treatment	NME	ME	NME	ME	NME	ME	NME	ME	NME	ME	NME	ME	NME	ME
3-methyl-butanal	530.79 ± 90.12	576.14 ± 103.87	409.54 ± 42.72	530.23 ± 40.39	208.54 ± 19.08	167.02 ± 13.40	54.79 ± 7.64	64.86 ± 15.96	44.22 ± 1.79	45.79 ± 3.30	26.20 ± 2.01	22.42 ± 3.36	24.15 ± 4.46	22.40 ± 2.93
2-methyl-butanal[1]	584.89 ± 107.50	640.85 ± 115.22	433.16 ± 47.71	572.45 ± 45.06	266.91 ± 27.33	214.95 ± 18.26	60.81 ± 10.94	71.15 ± 6.60	63.35 ± 12.08	65.95 ± 6.75	90.63 ± 26.53	78.22 ± 27.09	108.33 ± 21.78	205.12 ± 29.72
2,2,4,6,6-pentamethyl-heptane	5.27 ± 0.67	3.32 ± 0.30	2.99 ± 1.35	6.68 ± 0.86	5.82 ± 0.19	5.29 ± 1.09	3.34 ± 1.03	2.86 ± 0.40	5.65 ± 1.66	4.92 ± 1.25	16.76 ± 3.55	10.46 ± 4.22	5.76 ± 1.95	5.53 ± 1.83
Pentanal[1]	47.05 ± 12.54	29.71 ± 5.59	115.96 ± 16.30	184.28 ± 16.10	599.47 ± 37.16	668.39 ± 38.62	655.11 ± 32.23	576.85 ± 42.34	939.32 ± 43.22	960.93 ± 36.08	720.86 ± 19.14	826.42 ± 53.16	807.71 ± 13.94	983.50 ± 19.11
Decane	34.72 ± 5.35	26.07 ± 3.73	18.47 ± 6.92	41.17 ± 8.06	29.61 ± 4.70	30.66 ± 5.73	14.63 ± 6.10	18.22 ± 4.60	35.31 ± 10.79	31.52 ± 6.43	118.59 ± 30.55	76.67 ± 30.82	38.33 ± 15.21	39.31 ± 9.77
2-propyl-furan	0.39 ± 0.06	0.26 ± 0.04	0.64 ± 0.13	0.89 ± 0.09	2.41 ± 0.37	2.47 ± 0.37	2.21 ± 0.39	2.33 ± 0.16	1.68 ± 0.24	2.28 ± 0.37	2.45 ± 0.42	2.07 ± 0.26	1.90 ± 0.33	1.70 ± 0.25
Dimethyl disulfide	1.82 ± 0.19	2.42 ± 0.29	3.42 ± 0.49	4.80 ± 0.72	2.19 ± 0.33	1.53 ± 0.17	0.55 ± 0.09	0.55 ± 0.06	0.30 ± 0.04	0.34 ± 0.04	0.24 ± 0.03	0.19 ± 0.04	0.09 ± 0.03	0.06 ± 0.00
Hexanal[1]	65.05 ± 4.43	44.50 ± 2.39	180.73 ± 18.12	296.97 ± 16.59	1138.32 ± 86.21	1352.78 ± 82.78	1286.24 ± 59.07	1183.99 ± 90.70	1796.39 ± 61.50	1833.55 ± 27.58	1548.90 ± 26.92	1566.24 ± 50.76	1437.71 ± 18.49	1508.48 ± 8.26
2-methyl-1-propanol	0.52 ± 0.04	0.52 ± 0.08	1.54 ± 0.09	1.82 ± 0.16	1.73 ± 0.34	1.31 ± 0.19	0.64 ± 0.15	0.71 ± 0.11	0.37 ± 0.03	0.34 ± 0.07	0.19 ± 0.04	0.20 ± 0.09	0.11 ± 0.01	0.08 ± 0.05
2-n-butyl furan	0.43 ± 0.06	0.49 ± 0.09	1.35 ± 0.11	1.91 ± 0.08	7.97 ± 0.61	9.50 ± 1.12	9.92 ± 1.20	10.43 ± 1.22	8.99 ± 1.37	12.04 ± 1.37	15.15 ± 1.81	12.73 ± 1.51	13.28 ± 2.27	9.79 ± 1.08
pentyl-oxirane[1]	0.07 ± 0.01	0.07 ± 0.02	0.43 ± 0.07	1.01 ± 0.04	5.15 ± 0.30	8.95 ± 0.97	13.31 ± 1.74	8.58 ± 0.94	38.52 ± 7.55	38.98 ± 2.97	45.00 ± 4.91	37.87 ± 9.89	35.93 ± 6.29	58.97 ± 4.28
1-butanol[1]	4.00 ± 0.41	3.18 ± 0.30	4.33 ± 0.21	5.37 ± 0.06	8.97 ± 0.50	9.59 ± 0.28	9.66 ± 1.12	9.57 ± 0.44	11.42 ± 1.45	12.98 ± 1.42	13.46 ± 1.60	11.26 ± 0.85	10.06 ± 3.09	11.46 ± 3.01
2-heptanone[1]	5.33 ± 0.41	4.62 ± 0.34	10.31 ± 1.22	15.22 ± 0.65	73.92 ± 5.01	110.14 ± 10.94	173.76 ± 21.75	154.89 ± 18.11	402.40 ± 75.28	468.18 ± 39.26	440.24 ± 57.23	545.06 ± 159.29	550.91 ± 92.47	969.09 ± 96.20
Heptanal[1]	8.38 ± 0.59	7.48 ± 1.12	20.39 ± 0.39	25.60 ± 0.98	117.32 ± 5.28	168.12 ± 10.79	234.56 ± 11.57	223.35 ± 18.57	456.64 ± 40.20	508.41 ± 18.84	426.51 ± 32.82	548.67 ± 120.19	566.42 ± 37.85	627.85 ± 3.00

D-limonene[1]	1.97 ± 0.09	3.23 ± 0.15	1.88 ± 0.12	2.87 ± 0.09	2.46 ± 0.04	2.89 ± 0.25	1.72 ± 0.07	1.81 ± 0.10	1.79 ± 0.18	1.92 ± 0.09	1.52 ± 0.04	1.48 ± 0.07	1.14 ± 0.05	0.94 ± 0.07
2-methyl-1-butanol	11.49 ± 1.04	14.58 ± 2.09	44.88 ± 6.25	61.68 ± 3.49	77.43 ± 6.86	63.58 ± 5.42	30.69 ± 3.42	33.36 ± 3.39	25.34 ± 4.72	26.21 ± 2.57	19.79 ± 2.94	15.75 ± 1.51	11.19 ± 1.57	9.80 ± 0.93
3-methyl-1-butanol	11.32 ± 0.97	16.52 ± 2.22	31.73 ± 4.69	45.30 ± 2.52	54.79 ± 4.81	46.42 ± 4.31	23.61 ± 2.71	25.32 ± 2.50	22.30 ± 4.17	23.27 ± 2.87	18.85 ± 2.79	15.77 ± 1.17	12.17 ± 1.83	14.18 ± 1.08
2-pentyl-furan	4.26 ± 0.15	4.13 ± 0.12	7.80 ± 0.60	9.61 ± 0.35	32.85 ± 1.34	43.60 ± 3.62	47.36 ± 3.78	45.40 ± 3.26	52.28 ± 5.87	65.54 ± 5.41	81.98 ± 9.05	78.63 ± 15.26	96.43 ± 13.69	112.96 ± 10.33
Styrene	0.87 ± 0.06	1.17 ± 0.16	1.37 ± 0.19	1.78 ± 0.05	5.22 ± 0.38	5.66 ± 0.22	5.35 ± 0.56	5.37 ± 0.59	10.52 ± 2.05	11.59 ± 1.04	14.76 ± 1.94	18.21 ± 0.51	18.26 ± 3.07	17.27 ± 1.36
1-pentanol[1]	20.89 ± 1.62	16.56 ± 1.97	28.31 ± 2.29	42.57 ± 2.65	134.15 ± 9.48	170.02 ± 13.65	182.77 ± 17.34	166.71 ± 13.13	256.90 ± 36.89	280.05 ± 20.10	253.72 ± 26.89	235.61 ± 26.38	205.86 ± 26.61	215.28 ± 17.26
methyl-pyrazine	15.48 ± 1.50	12.76 ± 2.39	6.97 ± 2.41	15.15 ± 2.64	10.96 ± 1.07	10.40 ± 1.21	4.93 ± 0.50	4.32 ± 0.51	5.89 ± 0.99	5.17 ± 0.52	5.22 ± 0.65	3.46 ± 0.47	3.02 ± 0.63	2.79 ± 0.31
Acetoin[1]	114.75 ± 4.72	86.46 ± 7.91	32.89 ± 0.13	38.15 ± 1.37	18.61 ± 0.61	15.87 ± 0.66	7.79 ± 0.26	6.26 ± 0.46	6.41 ± 0.13	5.40 ± 0.22	4.13 ± 0.31	3.18 ± 0.27	2.64 ± 0.26	1.42 ± 0.32
2-octanone[1]	0.37 ± 0.08	0.32 ± 0.07	1.02 ± 0.13	1.59 ± 0.24	5.77 ± 0.33	10.23 ± 1.07	17.33 ± 1.10	17.24 ± 1.19	53.17 ± 6.32	62.72 ± 2.44	64.73 ± 6.08	95.11 ± 29.80	106.69 ± 13.66	266.01 ± 11.37
Octanal[1]	2.16 ± 0.09	2.10 ± 0.49	8.37 ± 0.07	10.73 ± 0.36	66.27 ± 1.01	118.26 ± 6.55	177.94 ± 6.19	184.49 ± 9.45	397.84 ± 23.79	458.28 ± 12.65	396.54 ± 20.68	569.14 ± 154.43	578.26 ± 34.04	752.73 ± 11.05
1-hydroxy-2-propanone[1]	32.24 ± 0.88	38.82 ± 2.30	10.90 ± 0.22	12.60 ± 1.52	5.37 ± 0.10	8.85 ± 0.09	4.73 ± 0.30	3.33 ± 0.20	5.44 ± 0.59	4.33 ± 0.34	3.37 ± 0.55	2.83 ± 0.49	1.72 ± 0.29	2.04 ± 0.06
1-chloro-2-propanol[1]	660.35 ± 41.18	392.46 ± 47.44	378.03 ± 14.68	274.44 ± 5.31	236.54 ± 10.83	158.88 ± 9.67	84.70 ± 3.67	75.35 ± 3.05	59.31 ± 6.06	55.24 ± 3.84	45.65 ± 4.54	30.58 ± 3.55	21.94 ± 2.68	11.45 ± 0.99
2,5-dimethyl-pyrazine	34.02 ± 2.18	28.41 ± 3.73	17.14 ± 4.82	41.63 ± 7.36	33.04 ± 1.67	30.46 ± 2.81	15.41 ± 0.99	11.58 ± 1.03	19.68 ± 2.50	15.12 ± 0.95	17.50 ± 1.47	11.09 ± 1.95	10.37 ± 1.65	10.86 ± 0.59
Methylthio-2-propanone	4.00 ± 0.30	5.03 ± 0.59	4.52 ± 0.63	5.11 ± 0.15	3.11 ± 0.26	2.98 ± 0.29	1.02 ± 0.07	0.75 ± 0.05	0.25 ± 0.01	0.29 ± 0.02	0.22 ± 0.01	0.16 ± 0.01	0.12 ± 0.01	0.04 ± 0.00
1-hexanol	53.13 ± 3.16	49.70 ± 4.53	55.26 ± 4.93	70.47 ± 4.81	216.47 ± 9.83	210.78 ± 14.08	227.16 ± 14.13	225.70 ± 16.14	367.02 ± 47.41	344.67 ± 17.77	354.59 ± 28.16	313.50 ± 11.39	270.83 ± 22.46	217.75 ± 7.50
2-chloro-1-propanol[1]	3.95 ± 0.23	2.92 ± 0.31	2.35 ± 0.07	2.06 ± 0.05	1.68 ± 0.06	1.24 ± 0.07	0.68 ± 0.03	0.63 ± 0.03	0.51 ± 0.03	0.51 ± 0.03	0.37 ± 0.04	0.28 ± 0.02	0.19 ± 0.01	0.09 ± 0.01
2-ethyl-6-methyl-pyrazine	1.02 ± 0.04	0.92 ± 0.07	0.64 ± 0.16	1.60 ± 0.34	1.26 ± 0.02	1.31 ± 0.12	0.76 ± 0.02	0.56 ± 0.01	1.23 ± 0.10	0.79 ± 0.03	0.93 ± 0.06	0.62 ± 0.12	0.61 ± 0.06	0.63 ± 0.01
2-nonanone[1]	0.28 ± 0.02	0.30 ± 0.05	0.38 ± 0.07	0.42 ± 0.01	2.21 ± 0.07	4.73 ± 0.27	10.72 ± 0.64	10.98 ± 0.58	45.79 ± 3.25	55.06 ± 1.32	55.87 ± 2.52	107.08 ± 36.94	127.11 ± 10.12	370.98 ± 4.77
Nonanal[1]	29.64 ± 5.12	32.27 ± 11.07	61.41 ± 21.79	29.24 ± 1.11	107.95 ± 11.90	166.35 ± 6.87	195.52 ± 14.05	208.50 ± 10.57	439.02 ± 21.93	482.77 ± 11.25	436.56 ± 13.38	639.35 ± 135.19	666.01 ± 14.58	877.19 ± 16.59
Trimethyl-pyrazine	5.32 ± 0.16	4.33 ± 0.20	3.10 ± 0.48	7.17 ± 1.05	5.69 ± 0.15	5.60 ± 0.29	3.32 ± 0.13	2.13 ± 0.18	4.76 ± 0.33	2.96 ± 0.11	3.55 ± 0.16	2.29 ± 0.44	2.24 ± 0.21	2.95 ± 0.05
3-octen-2-one[1]	0.48 ± 0.00	0.17 ± 0.01	1.53 ± 0.09	2.61 ± 0.29	17.76 ± 0.65	31.77 ± 1.79	40.75 ± 2.03	40.23 ± 2.11	114.33 ± 8.74	117.76 ± 1.75	123.00 ± 4.98	116.14 ± 12.21	104.68 ± 8.82	195.73 ± 4.27

3-ethyl-2-methyl-1,3-hexadiene[1]	0.98 ± 0.05	0.69 ± 0.01	2.36 ± 0.09	3.65 ± 0.12	15.39 ± 0.46	25.98 ± 1.45	29.61 ± 1.44	30.93 ± 1.77	69.04 ± 6.42	74.84 ± 2.36	77.70 ± 4.21	90.08 ± 20.51	87.65 ± 8.50	164.13 ± 3.27
(E)-2-octenal[1]	0.47 ± 0.04	0.47 ± 0.06	1.93 ± 0.19	3.62 ± 0.08	13.28 ± 0.26	31.54 ± 1.96	33.16 ± 1.94	26.21 ± 1.35	114.22 ± 10.72	130.24 ± 1.12	128.11 ± 4.82	145.14 ± 34.41	124.97 ± 11.62	293.77 ± 4.05
3-ethyl-2,5-dimethyl-pyrazine	0.31 ± 0.01	0.23 ± 0.01	0.22 ± 0.02	0.45 ± 0.07	0.36 ± 0.01	0.39 ± 0.01	0.26 ± 0.01	0.16 ± 0.01	0.38 ± 0.02	0.23 ± 0.00	0.25 ± 0.01	0.17 ± 0.03	0.16 ± 0.01	0.21 ± 0.01
2,6-diethyl-pyrazine	4.33 ± 0.08	3.27 ± 0.04	3.06 ± 0.24	6.58 ± 0.92	5.40 ± 0.04	5.68 ± 0.24	3.83 ± 0.08	2.26 ± 0.16	5.46 ± 0.09	3.29 ± 0.11	3.79 ± 0.11	2.61 ± 0.58	2.46 ± 0.20	3.29 ± 0.05
Acetic acid[1]	23.09 ± 0.17	31.51 ± 1.18	12.66 ± 2.03	15.73 ± 12.64	12.01 ± 1.55	33.28 ± 2.00	37.06 ± 2.44	30.06 ± 4.02	73.03 ± 8.12	62.79 ± 10.62	52.87 ± 7.51	52.52 ± 11.07	40.84 ± 1.19	89.29 ± 7.48
1-octen-3-ol[1]	2.88 ± 0.07	1.85 ± 0.11	3.08 ± 0.14	4.05 ± 0.26	13.06 ± 0.73	21.55 ± 1.47	26.60 ± 1.15	22.16 ± 2.70	127.22 ± 9.24	126.94 ± 7.97	157.58 ± 4.04	150.04 ± 34.43	144.55 ± 16.00	395.23 ± 4.01
Furfural[1]	41.65 ± 2.68	53.02 ± 4.87	17.47 ± 2.30	21.80 ± 1.69	15.63 ± 0.55	18.05 ± 1.10	12.37 ± 0.35	9.78 ± 0.37	15.89 ± 1.63	13.29 ± 1.32	14.08 ± 0.35	12.24 ± 1.65	10.68 ± 1.04	16.31 ± 0.49
1-heptanol[1]	1.75 ± 0.05	1.83 ± 0.07	2.87 ± 0.11	3.98 ± 0.07	14.84 ± 0.19	35.49 ± 1.96	49.50 ± 2.78	49.20 ± 2.33	123.78 ± 9.35	144.37 ± 5.29	129.40 ± 6.05	181.70 ± 52.25	178.44 ± 16.12	283.18 ± 3.27
2-decanone[1]	0.16 ± 0.02	0.14 ± 0.03	0.23 ± 0.02	0.23 ± 0.07	0.52 ± 0.01	1.36 ± 0.05	2.63 ± 0.12	3.09 ± 0.18	12.74 ± 0.88	15.21 ± 0.54	15.89 ± 0.40	34.16 ± 11.83	40.45 ± 2.06	123.67 ± 1.19
Decanal[1]	0.64 ± 0.20	0.99 ± 0.20	0.90 ± 0.06	0.70 ± 0.09	2.40 ± 0.13	5.30 ± 0.37	7.88 ± 0.63	10.72 ± 0.42	24.28 ± 2.64	28.53 ± 0.39	26.50 ± 1.58	47.60 ± 12.30	48.64 ± 0.55	80.56 ± 1.19
trans-3-nonen-2-one[1]	0.15 ± 0.05	0.24 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.74 ± 0.07	1.38 ± 0.04	1.73 ± 0.19	1.72 ± 0.29	12.69 ± 0.78	11.45 ± 0.41	17.14 ± 0.33	22.53 ± 7.99	26.93 ± 1.81	103.28 ± 2.91
Pyrrole	4.16 ± 0.36	3.47 ± 0.58	1.67 ± 0.31	2.19 ± 0.31	1.13 ± 0.05	0.90 ± 0.01	0.65 ± 0.04	0.65 ± 0.01	0.42 ± 0.04	0.43 ± 0.05	0.49 ± 0.07	0.49 ± 0.04	0.65 ± 0.02	1.56 ± 0.02
Benzaldehyde[1]	7.24 ± 0.24	8.11 ± 0.12	9.03 ± 0.44	12.55 ± 0.65	18.86 ± 0.59	21.66 ± 1.20	16.60 ± 0.57	15.35 ± 0.89	27.25 ± 2.60	28.92 ± 1.03	29.79 ± 1.87	28.80 ± 4.71	26.62 ± 3.01	42.87 ± 1.96
(E)-2-Nonenal[1]	0.52 ± 0.10	0.62 ± 0.13	0.39 ± 0.03	0.44 ± 0.03	0.63 ± 0.05	1.27 ± 0.15	1.36 ± 0.05	1.59 ± 0.15	5.35 ± 0.33	6.14 ± 0.06	6.29 ± 0.12	9.87 ± 3.41	10.10 ± 0.74	24.81 ± 0.56
2-butyltetrahydro-furan[1]	0.03 ± 0.01	0.05 ± 0.01	0.90 ± 0.19	1.72 ± 0.15	7.37 ± 0.08	22.83 ± 0.94	19.41 ± 2.57	16.24 ± 0.74	74.05 ± 5.97	71.61 ± 3.13	61.30 ± 5.62	79.43 ± 22.46	56.37 ± 1.82	135.79 ± 8.51
1-octanol[1]	0.55 ± 0.06	0.59 ± 0.01	0.72 ± 0.04	0.80 ± 0.05	2.69 ± 0.10	8.06 ± 0.43	10.41 ± 0.57	11.73 ± 0.63	32.12 ± 2.02	38.11 ± 0.68	33.79 ± 1.13	53.28 ± 14.71	51.12 ± 3.07	88.67 ± 1.76
2-methyl-1H-pyrrole[1]	0.39 ± 0.03	0.45 ± 0.04	0.14 ± 0.02	0.22 ± 0.02	0.08 ± 0.00	0.06 ± 0.02	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
Butyrolactone[1]	1.45 ± 0.06	2.30 ± 0.15	0.76 ± 0.05	1.36 ± 0.10	0.95 ± 0.02	1.61 ± 0.07	0.77 ± 0.02	0.93 ± 0.01	1.05 ± 0.04	1.24 ± 0.02	1.04 ± 0.03	1.20 ± 0.19	1.07 ± 0.10	1.89 ± 0.07
Benzeneacetaldehyde[1]	101.31 ± 2.39	136.95 ± 4.89	8.43 ± 1.52	8.44 ± 0.35	2.12 ± 0.05	4.63 ± 0.30	2.15 ± 0.23	2.31 ± 0.12	1.84 ± 0.16	2.89 ± 0.32	1.95 ± 0.02	1.95 ± 0.21	1.46 ± 0.04	1.63 ± 0.09
(Z)-2-decenal[1]	0.11 ± 0.07	0.07 ± 0.03	0.22 ± 0.06	0.15 ± 0.03	0.39 ± 0.04	2.04 ± 0.26	1.98 ± 0.20	2.20 ± 0.20	8.70 ± 0.93	9.98 ± 0.22	9.05 ± 0.67	16.38 ± 6.88	13.25 ± 0.62	31.88 ± 0.95
2-methyl-, anhydride pentanoic acid[1]	0.19 ± 0.01	0.24 ± 0.00	0.40 ± 0.08	0.59 ± 0.05	2.09 ± 0.27	9.56 ± 1.28	12.94 ± 0.89	10.40 ± 0.48	64.66 ± 4.80	67.31 ± 1.92	76.68 ± 1.85	86.86 ± 21.73	68.15 ± 6.36	219.57 ± 7.24
2-furanmethanol[1]	2.40 ± 0.14	3.13 ± 0.19	1.40 ± 0.10	2.04 ± 0.28	1.19 ± 0.04	1.58 ± 0.06	0.94 ± 0.10	0.58 ± 0.05	0.98 ± 0.08	0.84 ± 0.03	0.77 ± 0.04	0.77 ± 0.14	0.65 ± 0.09	1.46 ± 0.16

1-nonanol[1]	0.20 ± 0.03	0.24 ± 0.01	0.16 ± 0.00	0.14 ± 0.02	0.30 ± 0.03	0.43 ± 0.03	0.58 ± 0.03	0.69 ± 0.07	1.90 ± 0.46	1.97 ± 0.29	2.05 ± 0.35	3.47 ± 0.72	3.10 ± 0.11	4.84 ± 0.80
3-methyl-butanoic acid[1]	0.49 ± 0.04	0.31 ± 0.05	0.28 ± 0.07	0.70 ± 0.15	1.46 ± 0.13	2.71 ± 0.25	1.94 ± 0.04	1.92 ± 0.01	5.62 ± 0.13	4.69 ± 0.04	6.85 ± 0.08	9.92 ± 4.12	4.30 ± 0.20	7.68 ± 0.04
5-ethyldihydro-2(3H)-furanone[1]	1.76 ± 0.03	3.25 ± 0.06	2.77 ± 0.18	4.09 ± 0.18	16.76 ± 0.35	37.87 ± 1.67	50.39 ± 2.40	55.85 ± 2.67	135.29 ± 8.21	150.71 ± 2.38	144.68 ± 5.71	201.01 ± 48.80	201.97 ± 15.27	480.49 ± 5.64
Pentanoic acid[1]	0.12 ± 0.02	0.18 ± 0.02	0.39 ± 0.19	0.38 ± 0.08	2.75 ± 0.20	12.15 ± 2.14	22.70 ± 2.33	23.03 ± 1.59	99.71 ± 4.74	106.51 ± 4.03	102.62 ± 3.35	183.44 ± 65.25	183.69 ± 14.04	516.29 ± 9.83
tetrahydro-6-methyl-2H-pyran-2-one[1]	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.02	0.06 ± 0.00	0.13 ± 0.01	0.33 ± 0.02	0.39 ± 0.02	0.45 ± 0.03	1.22 ± 0.08	1.39 ± 0.01	1.28 ± 0.02	2.03 ± 0.60	2.09 ± 0.17	5.61 ± 0.01
dihydro-5-propyl-2(3H)-furanone[1]	0.04 ± 0.00	0.15 ± 0.00	0.10 ± 0.02	0.10 ± 0.01	0.25 ± 0.02	0.62 ± 0.03	0.82 ± 0.04	1.04 ± 0.05	2.83 ± 0.20	3.36 ± 0.05	3.25 ± 0.18	5.69 ± 1.39	6.29 ± 0.39	15.79 ± 0.03
Hexanoic acid[1]	0.46 ± 0.10	0.69 ± 0.06	1.59 ± 0.98	0.76 ± 0.22	9.96 ± 1.04	69.31 ± 9.84	130.85 ± 13.78	146.35 ± 15.81	587.04 ± 46.14	619.53 ± 43.59	598.48 ± 27.79	906.51 ± 220.58	928.90 ± 38.14	1423.71 ± 11.55
Benzyl alcohol[1]	0.25 ± 0.03	0.61 ± 0.03	0.17 ± 0.02	0.24 ± 0.01	0.25 ± 0.01	0.46 ± 0.01	0.29 ± 0.01	0.30 ± 0.02	0.46 ± 0.03	0.49 ± 0.02	0.48 ± 0.02	0.50 ± 0.04	0.37 ± 0.01	0.47 ± 0.02
5-butyldihydro-2(3H)-furanone[1]	0.04 ± 0.00	0.07 ± 0.00	0.15 ± 0.07	0.08 ± 0.00	0.39 ± 0.02	1.14 ± 0.07	1.53 ± 0.13	2.27 ± 0.16	5.97 ± 0.63	7.31 ± 0.26	6.74 ± 0.43	12.35 ± 2.69	12.78 ± 0.39	28.64 ± 0.33
Phenylethyl alcohol[1]	0.48 ± 0.01	0.90 ± 0.03	0.48 ± 0.07	0.65 ± 0.03	0.58 ± 0.00	1.17 ± 0.02	0.69 ± 0.05	0.88 ± 0.10	1.08 ± 0.07	1.42 ± 0.11	1.23 ± 0.02	1.42 ± 0.05	1.07 ± 0.02	1.16 ± 0.08
Heptanoic acid[1]	0.06 ± 0.02	0.09 ± 0.02	0.11 ± 0.06	0.09 ± 0.01	0.23 ± 0.08	0.80 ± 0.03	1.36 ± 0.20	1.81 ± 0.14	9.05 ± 1.84	12.67 ± 2.00	10.68 ± 0.27	30.17 ± 13.06	32.88 ± 1.16	123.39 ± 3.37
2-vinyfuran[1]	0.19 ± 0.02	0.22 ± 0.01	0.35 ± 0.07	0.20 ± 0.01	0.24 ± 0.01	0.37 ± 0.05	0.26 ± 0.00	0.21 ± 0.04	0.37 ± 0.06	0.31 ± 0.09	0.39 ± 0.08	0.37 ± 0.10	0.48 ± 0.06	0.43 ± 0.11

[1]Significantly different between treatments across all time points ($p < 0.05$) using ANOVA

Table 2.4. Average value of hedonic testing and descriptive analysis attributes that were significantly different between treatments of light roasted almonds at 0, 1, 3, 5, and 7 months of accelerated storage.

Sensory Analysis	Treatment	Storage Month				
		0	1	3	5	7
Hedonic Testing	NME	6.68^{a}	6.43^{ab}	5.98^{abc}	5.29^{cd}	5.44^{cd}
	ME	6.36^{ab}	6.36^{ab}	5.76^{bcd}	5.44^{cd}	5.08^{d}
Degree of Difference	NME	0.25^{f}	0.73^{de}	1.88^{c}	2.97^{b}	3.2^{b}
	ME	0.39^{ef}	0.94^{d}	2.1^{c}	3.32^{ab}	3.59^{a}
Color	NME	7.43^{f}	7.49^{de}	7.51^{c}	7.64^{b}	7.53^{b}
	ME	7.49^{ef}	7.58^{d}	7.61^{c}	7.7^{ab}	7.74^{a}
Clean Nutty Aroma	NME	4.11^{a}	3.22^{b}	2.32^{c}	1.77^{de}	1.56^{ef}
	ME	4.02^{a}	3.16^{b}	1.99^{cd}	1.52^{ef}	1.43^{f}
Clean Roasted Aroma	NME	3.51^{a}	2.81^{b}	2^{c}	1.59^{de}	1.54^{de}
	ME	3.43^{a}	2.76^{b}	1.78^{cd}	1.47^{e}	1.35^{e}
Clean Nutty Flavor	NME	4.41^{a}	4.1^{ab}	3.27^{c}	2.18^{e}	1.99^{ef}
	ME	4.3^{ab}	3.92^{c}	2.76^{d}	1.94^{ef}	1.71^{f}
Clean Roasted Flavor	NME	2.99^{a}	2.71^{bc}	2.16^{d}	1.67^{e}	1.6^{ef}
	ME	2.92^{ab}	2.57^{c}	1.96^{d}	1.49^{ef}	1.41^{f}
Total Oxidized Aroma	NME	0.02^{f}	0.58^{e}	1.92^{d}	2.65^{bc}	2.98^{ab}
	ME	0.05^{f}	0.67^{e}	2.58^{c}	3.1^{a}	3.2^{a}
Total Oxidized Flavor	NME	0.05^{g}	0.55^{f}	1.67^{e}	2.84^{c}	3.14^{bc}
	ME	0.1^{g}	0.62^{f}	2.1^{d}	3.35^{ab}	3.6^{a}
Cardboard Flavor	NME	0.04^{e}	0.46^{e}	1.01^{d}	1.26^{c}	1.26^{bc}
	ME	0.09^{e}	0.57^{e}	1.29^{d}	1.41^{ab}	1.5^{a}
Painty/Solvent Flavor	NME	0^{e}	0.6^{e}	0.86^{d}	1.92^{c}	2.13^{bc}
	ME	0.01^{e}	0.12^{e}	0.98^{d}	2.34^{ab}	2.52^{a}

[1]Letters shared within the same chemical measurement indicates there is no significant differences ($p < 0.05$) using ANOVA

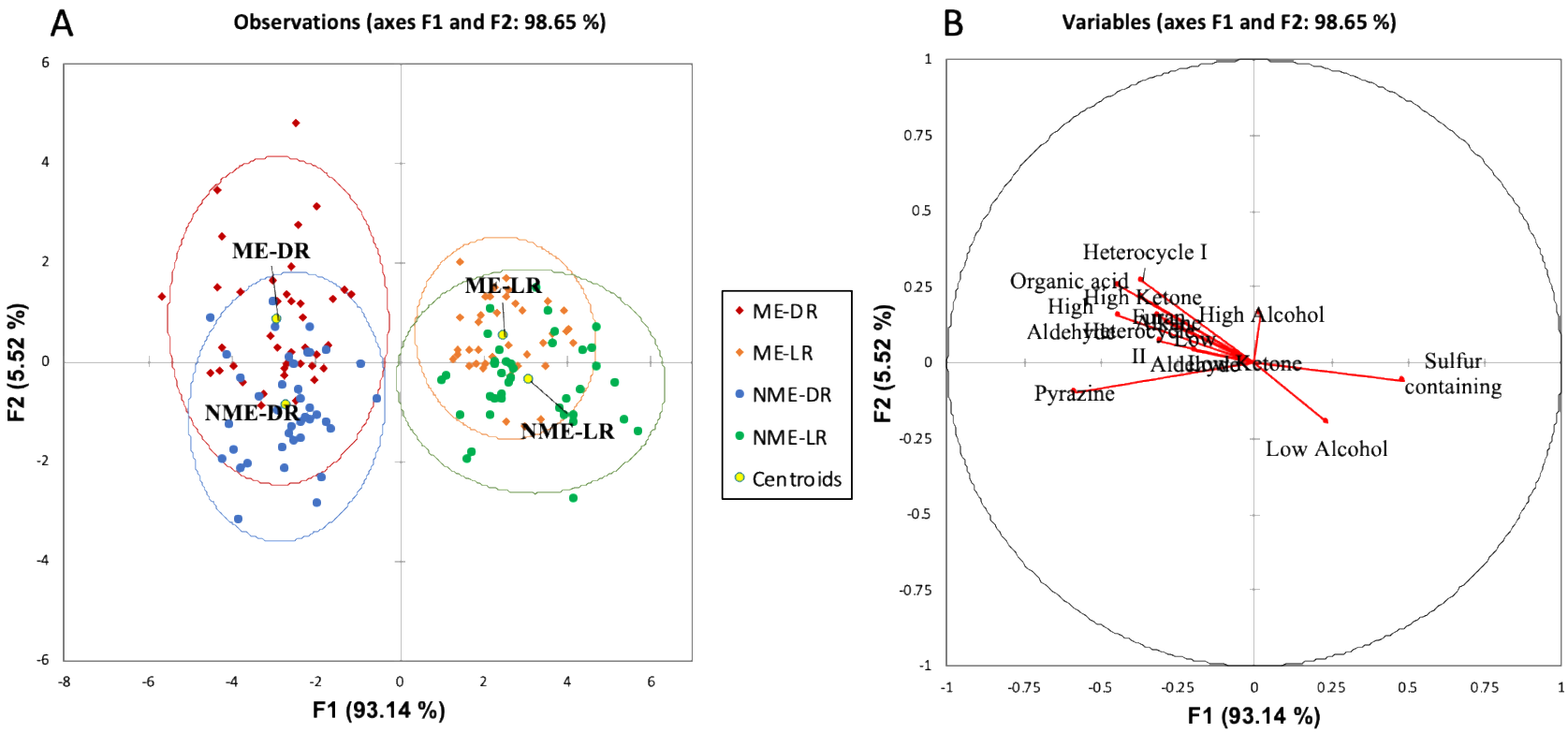

Figure 2.1. Discriminant analysis of volatile compounds (69) identified then grouped by chemical functionality (Shown in Table 2.S2) in almonds either exposed to 8 % moisture and dried to 5 % moisture (ME) or not exposed to moisture (NME) and roasted to achieve either a light roast (LR) or dark roast (DR). (A) the observation plot showing the grouping of each category, and (B) the loading plot showing the variables contributing to both factors (F1 and F2).

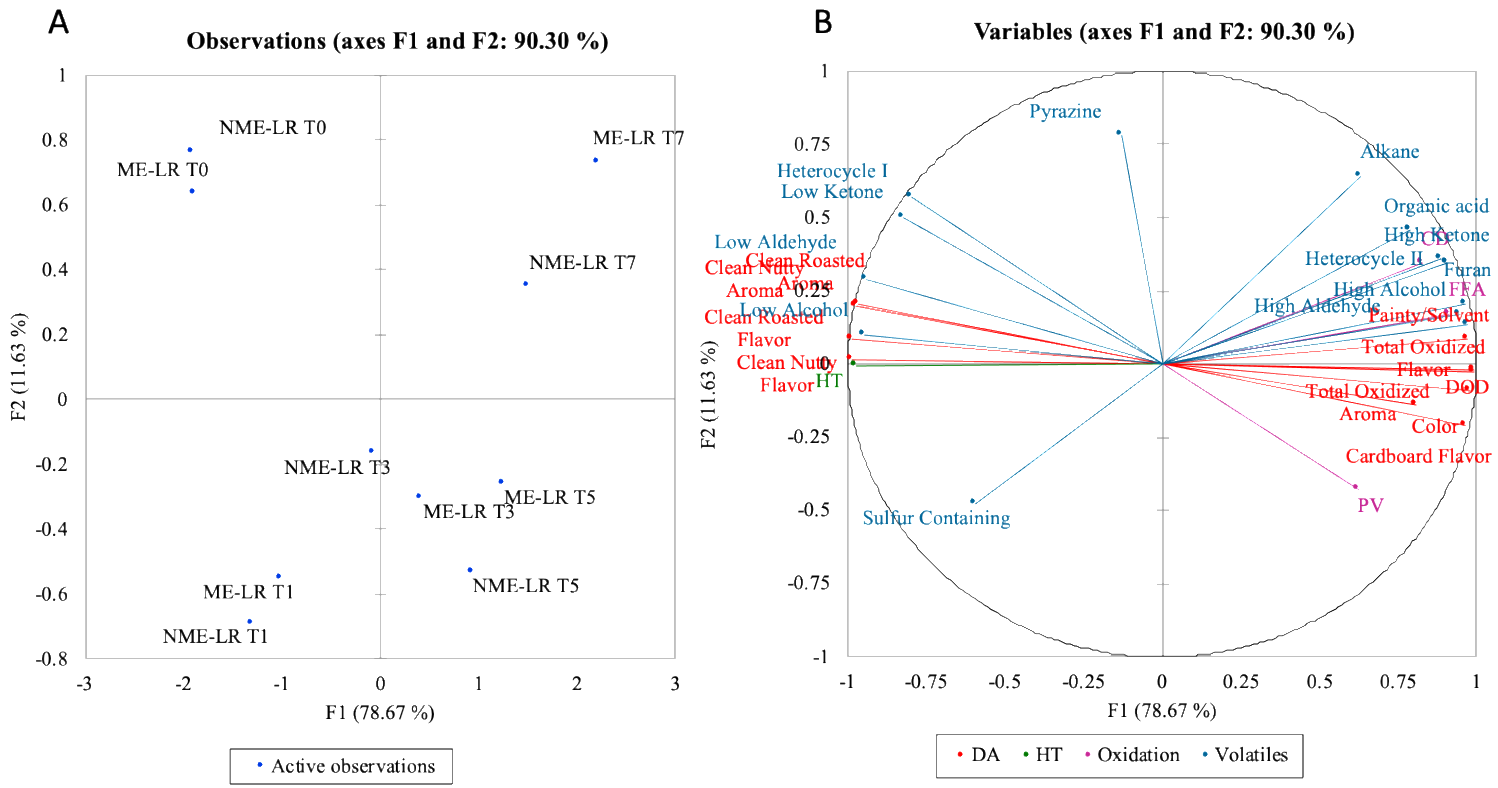

Figure 2.2. Multiple factor analysis of volatile compounds (69) identified then grouped (Shown in Table 2.S2), descriptive analysis attributes (Table 2.4), chemical analyses, and hedonic testing in almonds exposed to 8 % moisture and dried to 5 % moisture (ME) or not exposed to moisture (NME) and roasted to achieve light roast (LR) at 0, 1, 3, 5, and 7 months of storage. (A) the observation plot showing the separation of each sample, and (B) the loading plot showing the variables (DA, descriptive analysis) contributing to both factors (F1 and F2) with oxidation (PV, CD, and FFA) and hedonic testing (HT) projected as supplemental variables into the space.

2.8 Supporting Information

Table 2.S1. List of sensory attributes, references, and definitions used in the descriptive analysis of almonds that were either exposed to moisture and subsequently dried (ME) or no moisture exposure (NME), light roasted then sampled at 0, 1, 3, 5, and 7 months of accelerated storage.

Overall Degree of Difference			
Sensory Attribute	Scale	Reference	Definition
Overall Degree of Difference			The overall impression of how different the sample is from the reference sample.
Appearance			
Sensory Attribute	Scale	Reference	Definition
Average Darkness of Color	0.0	White	The average darkness of the internal side of the almond when cut in half, rated from light to dark.
	10.0	Sepia crayola	
	15.0	Black crayola	
Aroma-Flavor			
Sensory Attribute	Scale	Reference	Definition
Total Aroma/Flavor			The total intensity of all the aromas or flavors in the sample.
Clean Nutty	5.0	Trader Joes Dry Roasted, Unsalted Almonds	The total intensity of clean or fresh nut character in the sample, including woody, marzipan/ benzaldehyde, sweet aromatic and fruity.
	NR	Walnut nut	
Clean Roasted	4.0	Trader Joes Dry Roasted, Unsalted Almonds	The intensity of notes reminiscent of roasted or toasted.
Total Oxidized			The total intensity of notes associated with an old/stale oil character or oil that is oxidized, painty, solvent, rancid or soapy.
Cardboard	NR	Cardboard soaked overnight at room temperature in Alhambra water	The intensity of notes associated with cardboard, stale, musty, dusty or sawdust.
Painty/Solvent	NR	Oil Library	The intensity of notes reminiscent of oil based paint, solvent, spoiled fish or rancid oil.
Bitter	2.0	0.06 g caffeine in 250 mL water	One of the basic tastes, common to caffeine.

	5.0	0.1 g caffeine in 250 mL water	
			Texture
Sensory Attribute	Scale	Reference	Definition
Hardness	5.0	Nabisco Chips Ahoy Cookies	Force required to chew through the sample using the molars, from soft to hard.
	7.0	Nabisco Wheat Thin Crackers	
	8.0	Nabisco Oreo	
	10.0	Old London Melba Toast	
	11.0	Nabisco Ginger Snap	
Fracturability	4.0	Nabisco Regular Chips Ahoy	The force with which the sample breaks, includes brittleness. Generally, an increase in auditory signals results from higher fracturability.
	5.0	Nabisco Graham Cracker	
	7.5	Nabisco Oreo	
	10.0	Old London Melba Toast	
	11.0	Nabisco Ginger Snap	
Crunchiness	4.0	Nabisco Regular Chips Ahoy	The amount of low-pitched noise a heavier, harder product makes during the chewing process.
	6.0	Nabisco Oreo	
	7.0	General Mills Wheat Chex	
Denseness	5.0	Pringles Potato Crisp	The compactness of the cross-section from airy to dense.
	7.0	Nabisco Regular Chips Ahoy	
	11.0	Keebler Pecan Sandie Cookie	
	12.0	Nabisco Fig Newton	
Chewiness	6.0	Snickers Bar	The total amount of "work" or force required to chew the sample once the bolus has broken down prior to swallowing.
Cohesiveness of Mass	1.5	Bush Garbanzo Beans	The degree to which the sample sticks to itself or forms a tight bolus as it is being chewed.
	5.0	Pringles Potato Crisp	
	7.5	Nabisco Graham Cracker	
Moistness of Mass	1.0	Nature Valley Granola Bar	The degree to which the sample mass is moist (tender) or dry (tough).
	4.0	Nabisco Regular Chips Ahoy	

	6.0	Snickers	
Mealy Mouthcoating	7.5	Almond Flour	The amount of mealy, grainy or particulates coating the mouth, perceived particularly in the back of the throat after swallowing.
Awareness of Skins			The awareness of skins in the sample during chewdown, including toughness and skin flakes.

Table 2.S2. Volatile compounds (69) identified in the headspace of both exposed to moisture and subsequently dried (ME) and no moisture exposure (NME) at both roasting level (light roast, LR; dark roast, DR) across 12 months of accelerated storage.

Volatile Compound	External Standard [a]	t_R [b] unkonwn	Standard KI	Unknown KI	Literature KI [c]	Extracted Ion [d]
Low MW Aldehydes						
3-methyl-butanal	Ald	3.44		913	900-937	58.1
2-methyl-butanal[f]	Ald	3.42		911	896-926	57.1
High MW Aldehydes						
Pentanal[f]	Ald	4.53		974	950-979	44.1
Hexanal[f]	Ald	7.1		1077	1041-1108	57.1
Heptanal[ef]	Ald	10.66		1180	1151-1196	70.1
Octanal[ef]	Ald	14.25	1285	1287	1247-1291	84.1
Nonanal[ef]	Ald	17.19		1391	1382-1400	57.1
(E)-2-octenal[ef]	Ald	18.04	1423	1425	1424-1434	83.1
Decanal[ef]	Ald	19.67	1495	1497	1474-1508	57.1
Benzaldehyde[f]	Ald	20.17	1512	1516	1486-1521	105.1
(E)-2-Nonenal[ef]	Ald	20.53		1533	1530-1551	83.1
Benzeneacetaldehyde[f]	Ald	22.7	1629	1633	1592-1651	91.1
(Z)-2-decenal[ef]	Ald	23.19		1642	1599-1644	70.1
Alkanes						
2,2,4,6,6-pentamethyl-heptane	Ald	4.17		955	954-957	112.1
Decane	Ald	4.98		1000	1000	57.1
D-limonene[f]	Ald	10.8	1189	1185	1181-1213	136.1
Styrene	Ald	13.11		1252	1248-1259	104.1
3-ethyl-2-methyl-1,3-hexadiene[ef]	Ald	17.67		1410		67.1
Furans						
2-propyl-furan	Pyr	5.75		1027	1011-1043	81.1
2-n-butyl furan	Pyr	8.84		1129	1119-1140	81.1
2-pentyl-furan[e]	Pyr	12.42		1231	1229-1241	81.1
2-butyltetrahydro-furan[ef]	Pyr	20.56		1536		71.1
2-vinyfuran[ef]	Pyr	29.36	1937	1941	1054-1085	94.1
Sulfur Containing						
Dimethyl disulfide[e]	Pyr	6.78	1060	1063	1044-1081	94.1
Methylthio-2-propanone[e]	Pry	15.45		1326		104.1

Heterocycles I						
Pentyl-oxirane[ef]	Pyr	9.25		1139	1153	71.1
2-methyl-1H-pyrrole[f]	Pyr	21.33		1569	1549-1570	80.1
5-ethyldihydro-2(3H)-furanone[ef]	Ald	23.91	1685	1689	1671-1724	85.1
Tetrahydro-6-methyl-2H-pyran-2-one[ef]	Pyr	25.63		1768	1751-1830	70.1
Dihydro-5-propyl-2(3H)-furanone[ef]	Pyr	25.84		1778	1767-1817	85.1
5-butyldihydro-2(3H)-furanone[ef]	Pyr	27.86		1872	1846-1950	85.1
Heterocycles II						
Furfural[ef]	Ald	18.85	1455	1459	1437-1449	96.1
Pyrrole	Pyr	20.09	1508	1512	1490-1547	67.1
2-furanmethanol[ef]	Alc	23.19	1652	1658	1614-1666	98.1
Low MW Alcohols						
2-methyl-1-propanol	Alc	7.64	1092	1096	1048-1114	74.1
1-butanol	Alc	9.44	1144	1146	1113-1175	56.1
2-methyl-1-butanol	Alc	11.76		1211	1180-1227	57.1
3-methyl-1-butanol	Alc	11.79	1210	1212	1180-1218	55.1
1-chloro-2-propanol[ef]	Alc	15.07	1310	1313		45.1
2-chloro-1-propanol[ef]	Alc	16.52	1363	1361		57.1
High MW Alcohols						
1-pentanol[ef]	Alc	13.27	1254	1257	1213-1271	55.1
1-hexanol	Alc	16.31	1356	1359	1316-1359	56.1
1-octen-3-ol[ef]	Alc	18.74		1455	1437-1462	57.1
1-heptanol[ef]	Alc	18.88	1459	1461	1441-1461	70.1
1-octanol[ef]	Alc	21.16	1561	1563	1519-1570	70.1
1-nonanol[ef]	Alc	23.19	1658	1660	1640-1666	56.1
Benzyl alcohol[ef]	Alc	27.21	1838	1842	1821-1885	108.1
Phenylethyl alcohol[ef]	Alc	27.84	1867	1871	1859-1923	91.1
Pyrazines						
methyl-pyrazine	Pyr	13.47	1259	1262	1254-1274	94.1
2,5-dimethyl-pyrazine	Pyr	15.22	1316	1318	1309-1332	108.1
2-ethyl-6-methyl-pyrazine[e]	Pyr	16.99		1383	1363-1393	121.1
Trimethyl-pyrazine	Pyr	17.48		1401	1391-1413	122.1
3-ethyl-2,5-dimethyl-pyrazine[e]	Pyr	18.51	1443	1445	1408-1477	121.1
2,6-diethyl-pyrazine[e]	Pyr	18.52		1445	1432-1444	135.1
Low MW Ketones						
Acetoin[f]	Ald	14.04	1276	1280	1263-1287	45.1

1-hydroxy-2-propanone[ef]	Ald	14.46		1292	1266-1317	74.1
Butyrolactone[ef]	Pyr	22.39		1618	1595-1645	86.1
High MW Ketones						
2-heptanone[ef]	Ald	10.57	1176	1177	1175-1190	58.1
2-octanone[ef]	Ald	14.06	1282	1283	1281-1304	58.1
2-nonanone[ef]	Ald	17.09	1386	1388	1386-1397	58.1
3-octen-2-one[ef]	Ald	17.52		1404	1363-1440	111.1
2-decanone[ef]	Ald	19.64		1493	1480-1493	58.1
trans-3-nonen-2-one[ef]	Ald	20.13		1511	1523	55.1
Organic Acids						
Acetic acid[ef]	Alc	18.53	1447	1449	1400-1465	60.1
2-methyl pentanoic acid[ef]	Alc	23.19		1654		99.1
3-methyl-butanoic acid[ef]	Alc	23.42	1661	1666	1621-1697	60.1
Pentanoic acid[ef]	Alc	24.76	1723	1727	1686-1749	60
Hexanoic acid[ef]	Alc	26.56	1814	1818	1803-1857	60.1
Heptanoic acid[ef]	Alc	28.63	1902	1905	1916-1967	60.1

[a]External standard used for quantification, Pyr: 2-methylpyrazine-d_6, Ald: octanal-d_{16}, Alc: hexanol-d_{13}.
[b]t_R, retention time.

[c]KI, Kovats Retention Index, the values were obtained from NIST Chemistry WebBook, Standard Reference Database 69.

[d]Extracted ion from the total ion scan used for quantification. [e]Significantly different between treatments for LR almonds. [f]Significantly different between treatments for DR almonds.

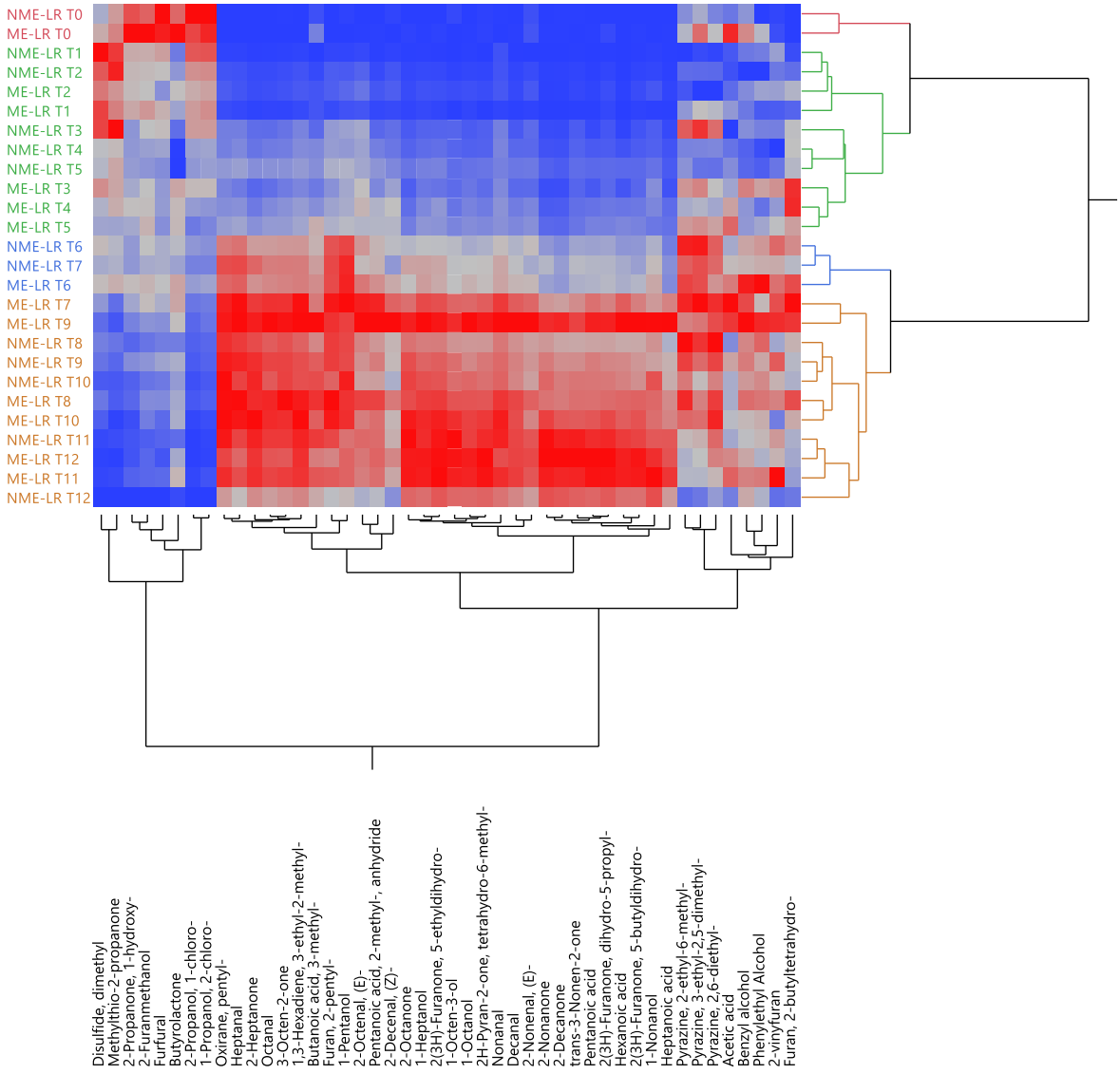

Figure 2.S1. Heatmap indicating the clustering of the clustering of the samples and the clustering of the 46 headspace volatiles that were significantly different among treatments in LR almonds. The relationship between the changes in volatile concentration and samples can be observed. Red indicates high concentration whereas blue is low concentration.

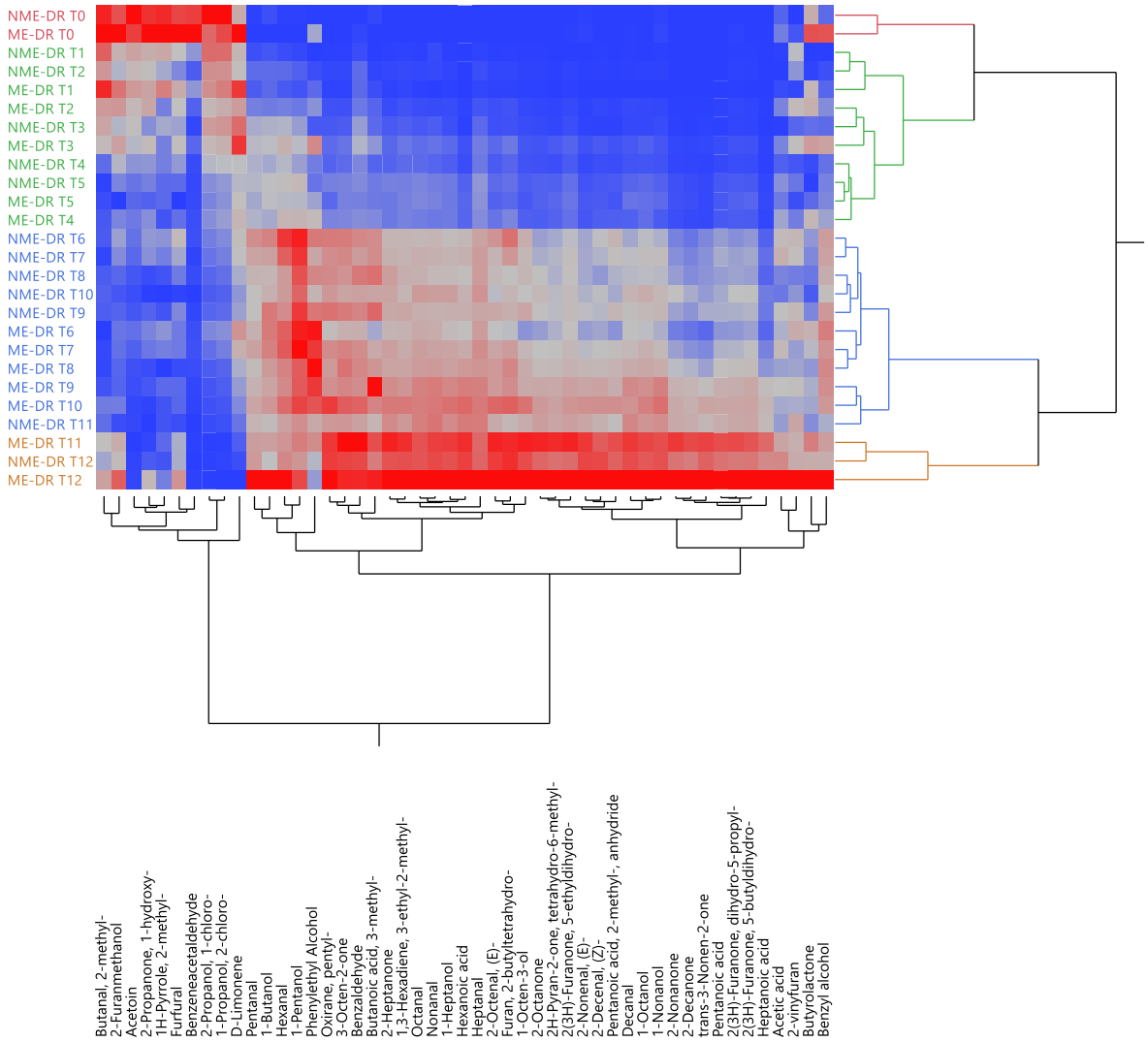

Figure 2.S2. Heatmap indicating the clustering of the samples and the clustering of the 49 headspace volatiles that were significantly different among treatments in the dark roasted almonds. The relationship between the volatile concentration and aged sample can be observed through the color pattern change. Red indicates high concentration whereas blue is low concentration.

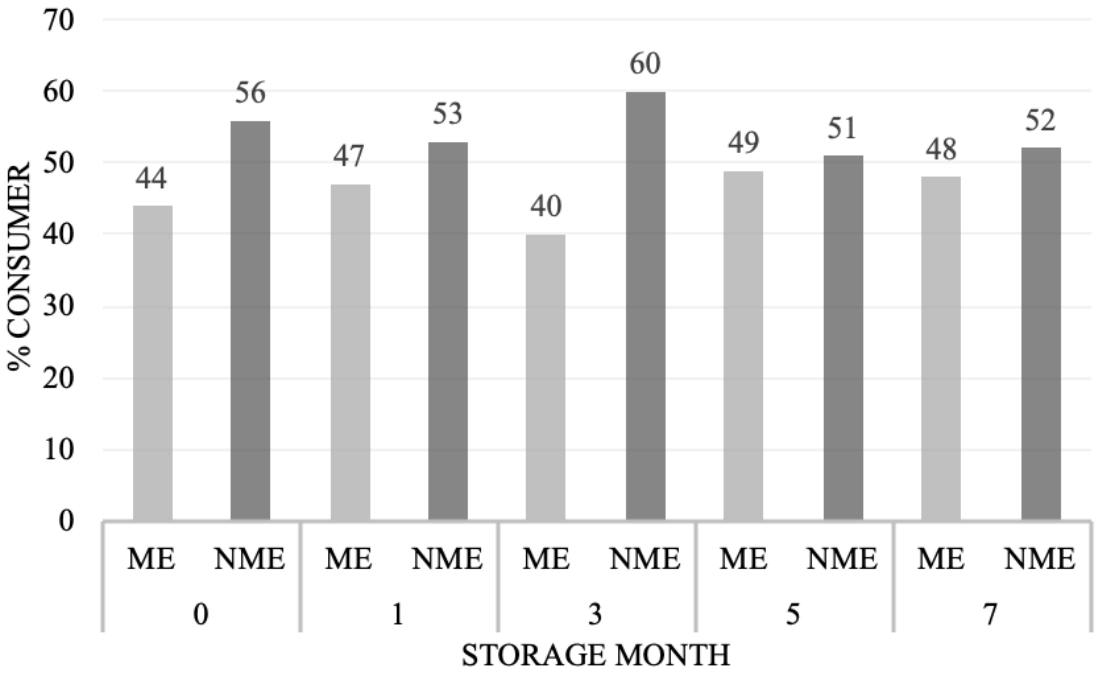

Figure 2.S3. Bar graph of the paired preference test between moisture exposed almonds (ME) and non-moisture exposed (NME) from the consumer analysis for light roast almonds at 0, 1, 3, 5, and 7 months of storage.

Chapter 3: Acceleration of Lipid Oxidation in Raw Stored Almond Kernels in Response to Postharvest Moisture Exposure

Published at Luo, K.K., Huang, G. and Mitchell, A.E. (2021), Acceleration of lipid oxidation in raw stored almond kernels in response to postharvest moisture exposure. J Sci Food Agric. https://doi.org/10.1002/jsfa.11452

3.1 Abstract

Background: Almonds are an important crop in California, and increased yields necessitate that dried in-hull almonds are stored in the field for longer periods increasing the potential for postharvest moisture exposure (e.g. rain, fog, etc.). Processors are increasingly drying these "wet" almonds to a moisture content of < 6% using low heat before the hulling and shelling process in order to reduce mechanical damage to the nutmeat. To date, there is no information on the impact that moisture exposure and drying prior to hulling and shelling, has on lipid oxidation and storage shelf life of raw almonds.

Results: Raw almonds exposed to ≤ 8% moisture and subsequently dried (MEx) and almonds not exposed to moisture exposure (≤ 4% moisture; control) were stored under accelerated shelf life conditions and evaluated monthly over 12 months for free fatty acids value (FFA), peroxide value (PV), and headspace volatiles. At 12 months of accelerated storage, MEx almonds have 1.4 times higher FFA and 3.5 times higher PV than the control indicating significant oxidative damage. MEx almonds also demonstrated higher levels of headspace volatile compounds related to lipid oxidation (i.e. hexanal, octanal, hexanoic acid) throughout storage.

Conclusion: Drying almonds exposed to postharvest moisture prior to storage results in a higher degree of lipid oxidation during storage and a significant reduction in shelf life.

Keywords: Almonds, moisture, oxidation, concealed damage, shelf life, volatiles

3.2 Introduction

Climate change is causing extreme and less predictable weather patterns around the world and is significantly affecting agriculture[1, 2]. California which grows ~80 % of the world's supply of almonds, is experiencing more extreme seasonal drought (summer) and heavy rain events (fall and winter), and is predicted to have higher annual rainfall with larger storm events during the next century[3]. Almonds in California are harvested July through September depending on variety. At hull-split, almonds are shaken from the tree, dried then swept into windrows in the orchard for additional moisture reduction[4]. After drying in windrows, almonds are cleaned of debris and stored in stockpiles prior to processing (i.e. hulling and shelling) and final kernel storage[4]. California almond production has increased by ~50% over the past 10 years fueled by consumer demand as almonds are a good alternative to animal protein, dairy and wheat flour and are considered an excellent source of vitamin E[5-9]. Production now exceeds processing capabilities, and almonds are frequently left in stockpiles for longer periods, where they are more susceptible to changes in the environment. The moisture content of almonds is a critical parameter in determining optimum conditions for their handling, processing and storage, and exposure to postharvest moisture (e.g. rain, fog) can negatively impact the quality and shelf life of almonds.

Unsaturated fatty acids are susceptible to lipid oxidation and the development of rancidity in foods. Almonds contain 44-61 % lipid by weight, the majority of which are the unsaturated fatty acids oleic acid (70-80 %) and linoleic acid (10-20 %)[10]. Lipid oxidation is initiated and accelerated by oxygen, heat, enzyme activity (e.g. lipases), moisture, and UV radiation exposure. Lipid oxidation initiates the degradation of lipids and formation of volatile compounds that result

in the unpleasant “rancid” aroma/flavor that is the primary determinant of shelf life[11]. Common ways to evaluate the shelf life of lipid rich foods include measuring primary lipid oxidation markers, such as peroxide values (PV) and free fatty acid values (FFA) and/or measuring volatile compounds that are secondary or tertiary lipid oxidation products. PV measures the lipid hydroperoxides that are formed early in the oxidation process and represent the amount of active oxygen present in fat and oil[12]. A PV < 0.5 meq/kg (milliequivalents of peroxide per kg oil) is used by the almond industry to establish product acceptability. FFAs reflect the amount of fatty acids hydrolyzed from triglycerides and is a useful marker of hydrolytic rancidity. Free fatty acids are generally considered more susceptible to lipid oxidation than triglyceride bound fatty acids. FFA values of < 1.5% are used by the almond industry to establish product acceptability. Secondary and tertiary lipid oxidation compounds (i.e. volatile organic compounds) are the 7- to 9-carbon aldehydes and alcohols, and/or the 5- to 9-carbon organic acids that are associated with rancidity flavor/aroma and are frequently used to monitor oxidative rancidity in lipid rich foods[11, 13]. All these measurements have been used to monitor the shelf life of almonds and in some cases correlated with the sensory attributes of almonds[13, 14].

Postharvest moisture exposure is reported to cause kernel browning in almonds, macadamia nuts, pecans, and hazelnuts[15-17]. Kernel browning is an undesirable attribute that is frequently associated with off-flavors and consumer rejection of nut products[16]. Rogel et al. (2015) reported that almonds with kernel browning have higher levels of volatile organic compounds related to lipid peroxidation and amino acid degradation[16]. In macadamia nuts, the kernel browning appears at high moisture content and elevated temperature, affecting ~1% of macadamia nuts and costing the Australian macadamia industry around AU$2 million annually[15, 18]. In hazelnuts, kernel browning occurs in the inner layer; which has significantly higher

amounts of oil and sugar, and lower amounts of protein relative to the outer layer[17]. The kernel browning found in nuts is linked to the Maillard reaction and/or enzymatic browning[19]. In hazelnuts, enzymatic hydrolysis provides reducing sugars for the Maillard reaction[17]. In these lipid rich nuts, lipid oxidation byproducts (e.g. carbonyls compound) may also contribute to the Maillard reaction[19, 20].

Postharvest moisture exposure (rewetting) is shown to promote the hydrolysis of proteins, carbohydrates, and lipids and increases levels of lipid oxidation products in almond kernels[21, 22]. Earlier studies have shown that almonds kernels exposed to a moisture content of ≤ 8 % and subsequently heated at high temperatures (e.g. roasting) form dark brown centers[16, 22]. This phenomenon was termed *concealed damage* as the discoloration appears only after heating. The dark discoloration is related to increased products formed via the Maillard reaction[4, 16].

When stockpiled dried in-hull almonds are exposed to postharvest the current industry practice is to dry these almonds to a moisture content ≤ 6 % by applying low heat (40 – 50 °C) as this reduces nutmeat damage during the hulling and shelling process (i.e. chipping and splitting)[4]. This practice has the added benefit of reducing concealed damage in thermally processed almonds[19]. Although this practice reduces cosmetic damage to the nutmeat during processing, it is not understood if the initial moisture exposure increases FFAs and/or induces lipid oxidation in these nuts which could result in decrease product shelf life.

Under controlled conditions and proper packaging (i.e. < 10 °C and < 65% relative humidity and/or vacuum packaging), raw almond kernels can be stored up to two years without experiencing the lipid oxidation that leads to consumer rejection[14]. Nonetheless, various lots of almonds, stored under optimal conditions have a shorten shelf-life and the reason for the shorten-shelf life is not always understood. In this study, we hypothesize that almonds exposed to

postharvest moisture, and dried prior to kernel storage, may have a shorten shelf-life due to the initiation of triglyceride hydrolysis and lipid oxidation during the rewetting phase. Understanding how this increasing practice influences product shelf-life is critical towards improving inventory control and decreasing product loss and importantly food waste.

3.3 Materials and Methods

Almond Samples and Storage

Raw Nonpareil almond kernels (from 2015 harvest year) not exposed to postharvest moisture were obtained from Blue Diamond Growers (Sacramento, CA). Moisture content was measured gravimetrically at ~ 4 % upon receiving. Almonds were then separated into a control group and a moisture-exposed group (MEx). The MEx group was exposed to moisture by incubating kernels in a KMF 240 Constant Climate Control Chamber (Binder Inc., Bohemia, NY) at 38 °C and 90 ± 1 % relative humidity (% RH) for 36 hours. Once the moisture content of the MEx almonds was increased to 8 %, the almonds were subsequently dried in a R-4 Harvest Saver Dehydrator (Commercial Dehydrator System Inc., Eugene, OR) at 50 ± 1 °C for 12 hours to reduce the moisture content back to 4 %. MEx almonds represent crops that have been exposed to moisture (i.e. rained on) and undergo drying prior to processing. The control almonds maintained a moisture content of ~ 4% and did not undergo drying prior to processing. Both the control and MEx group were divided into paper bags containing 460 g each and placed into the climate control chamber at 39 ± 1 °C and 15 ± 1 % RH and stored for up to 12 months. Samples were randomized and analyzed every month. Triplicate sampling was made for each group at each time point.

Chemicals

Acetic acid (HPLC grade), chloroform (HPLC grade), hydrochloric acid (ACS grade), potassium iodide (99.9 %), sodium hydroxide (analytical grade), sodium thiosulfate (99 %), and 2, 2, 4-trimethylpentane (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Hampton, NH). Authentic volatile standards (95 - 99 %) used for identification were purchased from Sigma-Aldrich. Stable isotope internal standards: n-hexyl-d_{13} alcohol, octanal-d_{16}, and 2-methylpyrazine-d_6 were purchased from C/D/N Isotopes Inc. (QC, Canada).

Analysis of Conjugated Dienes, Free Fatty Acids, and Peroxide Value

Whole almond kernels were crushed and ground for three 1-second pulses using a laboratory mill (Waring Laboratory Equipment, Torrington, CT). The oil was extracted from the ground almonds using a 12-ton Carver manual oil press (Carver Inc., Wabash, IN), and collected into an amber vial, and stored at -20 °C until analyzed. Free fatty acids level (FFA) and peroxide values (PV) were measured in the extracted almond oil according to the AOCS official methods Cd 3d-63[23] and Cd 8-53[24] respectively.

Solid Phase Microextraction (SPME) Headspace Volatile Analysis

Almonds were ground with a laboratory mill and sieved with a size 20 Tyler sieve. An aliquot of 5 ± 0.02 g of the sieved almonds was weighed into an amber headspace vial, capped, and equilibrated at room temperature (23 ± 2 °C) for at least 4 hours. The headspace volatiles were measured and analyzed according to Luo et al.[25]. Briefly, the volatiles were extracted with a 1 cm 30/50 μm StableFlex divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco Inc., Bellefonte, PA) attached to an Agilent GC injector 80 (Agilent Technologies, Santa Clara, CA). The volatiles were separated on a 30 m x 0.25 mm x 0.25 μm DB-Wax UI column using an Agilent 7890A gas chromatograph (GC) coupled with an Agilent 5975C Mass Selective Detector

(MSD). An external instrument standard was used to provide a response factor to correct for instrument and fiber variation. The external instrument standard contained a mixture of n-hexyl-d_{13} alcohol, octanal-d_{16}, and 2-methylpyrazine-d_6 in de-volatilized ground almonds capped in a 20 mL amber headspace vial. The headspace volatile profiles were collected in scan mode (*m/z* range 30 – 300). Tentative identifications were made through NIST v.17 Mass Spectral Library Search Program. Identification was further confirmed using retention index calculation or authentic standards when available. Relative concentrations of volatiles with confirmed identification were calculated as described by Franklin et al. (2017)[11].

Statistical Analyses

The statistical analyses were calculated using two-way analysis of variance (ANOVA) including treatment and storage month interaction. Statistically significant differences were considered when $p < 0.05$. Tukey's posthoc test was employed to reveal the grouping for the chemical measurements. Principal component analysis (PCA) was performed on the 48 volatiles that were significantly different ($p < 0.05$) from ANOVA results to visualize the clustering formation among samples and the relationship between volatile compounds with the samples. Agglomerative hierarchical clustering (AHC) was performed after the PCA to cluster the samples based on dissimilarity with data centered and reduced. All statistical analyses were performed using Addinsoft XLSTAT statistical and data analysis solution (version 2020.3).

3.4 Results and Discussion

Lipid oxidation is a dynamic processes and multiple markers are usually used to estimate the extent of oxidation in almonds. FFAs reflect hydrolytic rancidity as free fatty acids are released from triglycerides by lipases in the presence of moisture. Although we expected an increase in FFAs levels in MEx almonds due to hydrolysis, no significant difference was

observed between the MEx and control samples for the first 7 months of accelerated storage (Table 3.1). This suggest that significant hydrolysis of triglycerides does not occur with short moisture exposure (herein it was 36 hr). Almonds typically require 48 hrs of soaking in water to break dormancy and another 3 to 5 months to germinate[26]. Interestingly, FFAs increased significantly in MEx almonds at 8, 9, 11, and 12 months of storage relative to the controls. This may result from the additionally drying step these almonds underwent as compared with controls. Drying (i.e. dehydration with heat) has been shown to change the microstructure of almonds, creating extracellular pores that allow oxygen exposure and increasing lipase contact with oleosomes[27]. The increase of FFAs after 7 months of storage in MEx almonds correlates with an increase in organic acids (i.e. hexanoic acid, heptanoic acid, and pentanoic acid) from secondary lipid oxidation (Table 3.2). Over 12 months of storage, FFAs never exceeded the industry rejection standard of 1.5 %.

PV is a common marker used to monitor oxidative rancidity in the nut and oil industries. The PV values were consistently higher in the MEx almonds relative to the controls beginning at 1 month (Table 3.1). The PVs did not exceed the industry rejection standard of 5 mEq kg^{-1} for either control or MEx samples throughout the 12 months of storage. The PVs reach maximum levels at 5 months of storage for MEx almonds (2.01 ± 0.11 mEq kg^{-1}) and 8 months of storage for the control (1.39 ± 0.09 mEq kg^{-1}). A decrease in PVs results from the decomposition of hydroperoxides into secondary lipid oxidation products (i.e. aldehydes)[28]. MEx almonds have higher PVs and earlier maximum values suggesting an acceleration of lipid oxidation with respect to controls. Although moisture exposure has been shown to increase lipoxygenase activity and lead to an increase of linoleic hydroperoxide formation[29], this effect was not observed herein as the initial PVs were below the limit of detection for both MEx and control

almonds. It is more likely that the increase in PV observed in the MEx almonds is due to low heat induced disruption of the microstructure of almond kernels[27]. The PVs measured were comparable with other studies of raw almonds stored under different temperatures and relative humidity with a PV ranging between 0.5-2 mEq kg^{-1} during 1 year of storage[30, 31].

These results suggest that the drying step, an industry practice after moisture exposure, has the greatest influence on lipid oxidation in almonds. Although the mechanical drying of "wet" almonds can improve processing and decrease concealed damage in roasted almond products[4, 19], the process accelerates lipid oxidation and decreases raw almond shelf-life. Pleasance et al. (2018)[14] proposed a consumer assessment prediction model for raw almonds using lipid oxidation markers. The model reported PVs and FFAs are negatively associated with the overall assessment[14]. The higher level of PVs and FFAs in MEx almonds suggest that these almonds will have shorter shelf life then the control. However, both control and MEx almonds have lipid oxidation measurements below industry thresholds indicating shelf stability up to 12 months in this study.

SPME headspace volatiles

A total of 53 volatile compounds belonging to the chemical classes of organic acids, alcohols, hydrocarbons, aldehydes, furans, oxirane, pyrazine, ketones, and lactones were identified in the headspace (Table 3.2). Two chlorinated alcohols (1-chloro-2-propanol and 2-chloro-1-propanol) were identified in the headspace in both treatments (Table 3.2). These propylene chlorohydrins are often present in foods that have undergone propylene oxide pasteurization such as almonds[32, 33]. These propylene chlorohydrins are not considered genotoxic and have been observed in other studies of almonds; with levels highest at the start of storage and decreasing with time[11, 34, 35]. Hexanol, which has been reported to be a major headspace

volatile detected in Nonpareil almonds[36], had the highest concentration in the headspace. The hexanol concentration found in MEx almonds was higher ($p > 0.05$) than controls when comparing within each month (Table 3.2). Levels of benzaldehyde, a key contributor to raw almond aroma, were higher in the MEx almonds relative to the controls. Benzaldehyde is a hydrolysis product of amygdalin, which ranges from 2.16 to 157.44 mg kg^{-1} in sweet commercial almond varieties[37, 38]. Adding water to ground raw almonds during extraction was shown to increase levels of benzaldehyde measured in the headspace due to the hydrolysis of amygdalin[36, 37]. The postharvest moisture exposure may have contributed to the hydrolysis of amygdalin and the higher concentration of benzaldehyde found in MEx almonds. Hexanal is a common quality indicator of oils as it results from the oxidation of linoleic acid and is associated with off-flavor in almonds[39]. Hexanal levels were significantly higher in MEx almonds than in control almonds at each month (Table 3.2), suggesting a higher degree of linoleic oxidation. The summed concentration of each class of volatile compound (e.g. organic acid, aldehyde, etc.,) was plotted over the storage time (Figure 3.1). The propylene chlorohydrins were not included in the summed data as they are an artifact from the pasteurization process. MEx almonds display higher total volatile concentrations relative to the control samples, with higher levels of aldehydes, alcohols, and organic acids. Higher levels of aldehydes, alcohols, and organic acids are associated with lipid oxidation and are observed in almonds[11, 34].

To better understand the possible relationship between the headspace volatiles developed during storage a PCA analysis was performed on 46 of the 53 volatile compounds measured. ANOVA was performed on all the measured volatiles and indicated that 2, 2, 4, 6, 6-heptane, decane, 2-methyl-1-butanol, 3-methyl-1-butanol, and methyl-pyrazine were not significantly different between MEx almonds and controls ($p > 0.05$). Hence, all these volatiles were excluded

from the PCA analysis. 1-chloro-2-propanol and 2-chloro-1-propanol were also excluded from the PCA analysis as they were considered artifacts generated during pasteurization. Two principal components were obtained which explain 84.24 % of the variation (Figure 3.2). Along the PC1 (explaining 74.29 % of the variance), almond samples separate into 2 major groups: all control samples and the 0 – 5 month MEx samples on the left, and 4 – 12 month MEx samples on the right, which are mainly driven by lipid oxidation volatiles including: 2-octanone, 1-heptanol, and 1-octanol. This grouping was supported with the agglomerative hierarchical clustering (Figure 3.3) revealing 2 clusters based on dissimilarity of the headspace volatile profiles: cluster 1 (i.e. control 0 – 12 and MEx 0 – 5) and cluster 2 (i.e. MEx 6 – 12). Within cluster 1, control almonds after 6 months of storage and MEx almonds between 3 – 5 month of storage share similar headspace profiles. The similarity in headspace profile reflects the similarity in lipid oxidation development, suggesting MEx almonds having shorter shelf life than control. The separation of cluster 2 is driven by the majority of the headspace volatiles. Aldehydes found along PC1 (e.g. pentanal, heptanal, octanal, nonanal, decanal, (E)-2-octenal, (Z)-2-decenal) that correlates with cluster 2 have been reported to be products formed from the oxidation of oleic and linoleic acid through ß-scission[39]. Organic acids (i.e. butanoic acid, pentanoic acid, and hexanoic acid) also correlated with cluster 2 along PC1. Most of these volatiles have been reported to be tertiary lipid oxidation products of the major unsaturated acids found in almonds[39]. Control and MEx samples separated along PC2 across storage time (Figure 3.2), which is driven by acetoin (i.e 3-hydroxybutan-2-one) and hexanal. Acetoin is a volatile formed through sugar degradation[40] and is reported as a Maillard reaction product found in roasted almonds[11, 34, 35]. On the other hand, hexanal is a lipid oxidation product of linoleic acid. The correlation between higher levels of acetoin and hexanal found in MEx almonds suggested

that postharvest moisture exposure followed with low heat drying accelerates Maillard reaction and lipid oxidation in almonds.

3.5 Conclusions

Herein we demonstrate that short-term moisture exposure followed by low temperature drying increases markers of lipid oxidation. Although mechanical drying can be used to improve processing and decrease concealed damage in roasted almond products, it accelerates lipid oxidation and significantly decreases raw almond shelf-life (up to 12 months). This information can help processors better control inventories and target these nuts for shorter storage to reduce food waste and product loss.

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3.6 References

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3.7 Tables and Figures

Table 3.1. Average value of free fatty acids and peroxide values in almonds exposed to moisture and subsequently dried (MEx) and almonds with no moisture exposure (control) over 12 months of accelerated storage.

Storage Month	Treatment	Free Fatty Acids (% Oleic acid)	Peroxide Value (mEq kg^{-1})
0	Control	0.09 ± 0.0^{a}	n.d.
	MEx	0.09 ± 0.02^{ab}	n.d.
1	Control	0.09 ± 0.01^{ab}	0.34 ± 0.05^{ab}
	MEx	0.10 ± 0.01^{abc}	0.52 ± 0.21^{a}
2	Control	0.11 ± 0.00^{abcd}	0.46 ± 0.09^{ab}
	MEx	0.09 ± 0.00^{ab}	0.64 ± 0.00^{ab}
3	Control	0.12 ± 0.00^{abcd}	0.55 ± 0.05^{ab}
	MEx	0.12 ± 0.00^{abcd}	1.46 ± 0.15^{fgh}
4	Control	0.09 ± 0.00^{ab}	0.50 ± 0.10^{ab}
	MEx	0.12 ± 0.00^{abcd}	1.58 ± 0.10^{h}
5	Control	0.12 ± 0.00^{abcd}	0.76 ± 0.11^{bcd}
	MEx	0.13 ± 0.01^{bcdef}	2.01 ± 0.11^{i}
6	Control	0.12 ± 0.00^{abcde}	0.50 ± 0.00^{ab}
	MEx	0.15 ± 0.02^{edfg}	1.10 ± 0.00^{de}
7	Control	0.11 ± 0.01^{abcd}	1.35 ± 0.10^{efgh}
	MEx	0.14 ± 0.00^{cdefg}	1.22 ± 0.06^{efg}
8	Control	0.11 ± 0.00^{abcd}	1.39 ± 0.09^{efgh}
	MEx	0.22 ± 0.01^{h}	1.06 ± 0.06^{cde}
9	Control	0.12 ± 0.01^{abcde}	0.76 ± 0.12^{bcd}
	MEx	0.17 ± 0.00^{fg}	1.39 ± 0.27^{efgh}
10	Control	0.13 ± 0.01^{abcdef}	0.73 ± 0.06^{bc}
	MEx	0.15 ± 0.01^{defg}	1.13 ± 0.15^{defg}
11	Control	0.11 ± 0.01^{abcd}	0.56 ± 0.11^{ab}
	MEx	0.18 ± 0.06^{gh}	0.73 ± 0.06^{bc}
12	Control	0.12 ± 0.00^{abcd}	0.43 ± 0.06^{ab}
	MEx	0.17 ± 0.01^{efg}	1.53 ± 0.06^{gh}

Alphabets shared within the same column (treatment) indicates there is no significant differences under Tukey's post-hoc test ($p < 0.05$)
n.d. Not detected

Table 3.2. Average solid phase micro extraction (SPME) headspace volatile concentrations (µg kg^{-1} almond) measured in moisture exposed and dried (MEx) and control raw almonds at 0, 2, 4, 6, 8, 10, and 12 months of accelerated storage

Month		0		2		4		6		8		10		12	
Chemical Class	Treatment	Control	MEx	Control	MEx	Control	MEx	Control	MEx	Control	MEx	Control	MEx	Control	MEx
Organic Acid															
	Acetic acid	0.51 ± 0.06	0.57 ± 0.09	0.99 ± 0.09	2.44 ± 0.57	3.12 ± 0.49	10.42 ± 0.93	9.22 ± 1.65	14.38 ± 1.85	7.90 ± 0.66	11.17 ± 0.50	7.75 ± 0.49	11.06 ± 0.89	7.23 ± 0.50	20.88 ± 1.50
	Butanoic acid	0.09 ± 0.01	0.07 ± 0.00	0.04 ± 0.02	0.07 ± 0.04	0.08 ± 0.02	0.39 ± 0.05	0.24 ± 0.04	0.92 ± 0.05	0.36 ± 0.06	1.19 ± 0.08	0.34 ± 0.04	1.32 ± 0.19	0.29 ± 0.06	1.67 ± 0.11
	2-methyl-Pentanoic acid, anhydride	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.71 ± 0.19	0.09 ± 0.02	3.41 ± 0.49	0.42 ± 0.02	6.41 ± 0.40	0.46 ± 0.35	5.00 ± 0.95	0.39 ± 0.24	2.97 ± 0.96	0.21 ± 0.08	3.67 ± 0.42
	Pentanoic acid	0.03 ± 0.01	0.03 ± 0.00	0.11 ± 0.03	0.80 ± 0.17	0.63 ± 0.16	13.99 ± 1.56	5.57 ± 0.87	32.40 ± 3.01	8.38 ± 2.97	45.01 ± 5.39	9.92 ± 2.59	44.85 ± 10.30	7.19 ± 2.01	46.95 ± 6.07
	Hexanoic acid	0.20 ± 0.04	0.18 ± 0.03	0.19 ± 0.00	3.22 ± 0.17	2.81 ± 1.05	87.63 ± 12.35	18.29 ± 5.10	222.06 ± 30.93	28.11 ± 11.87	269.79 ± 32.94	44.92 ± 14.67	262.42 ± 63.10	25.98 ± 4.75	257.48 ± 37.36
	Heptanoic acid	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.03 ± 0.01	0.01 ± 0.00	1.70 ± 0.61	0.14 ± 0.11	6.36 ± 1.06	0.32 ± 0.09	10.00 ± 0.99	0.63 ± 0.23	14.67 ± 5.93	0.23 ± 0.02	11.35 ± 1.26
Alcohol															
	2-methyl-1-Propanol	0.34 ± 0.03	0.74 ± 0.07	1.96 ± 0.21	1.00 ± 0.15	1.23 ± 0.16	0.71 ± 0.08	1.10 ± 0.19	0.80 ± 0.05	0.74 ± 0.18	0.50 ± 0.05	0.54 ± 0.40	0.65 ± 0.12	0.84 ± 0.12	1.72 ± 0.26
	2-methyl-1-Butanol*	5.65 ± 0.64	15.39 ± 2.14	37.72 ± 5.82	30.87 ± 4.14	30.06 ± 7.86	22.82 ± 4.91	37.30 ± 9.27	31.58 ± 6.14	28.06 ± 6.39	26.15 ± 3.95	30.58 ± 7.59	30.02 ± 5.74	24.78 ± 4.86	35.95 ± 5.06
	3-methyl-1-Butanol*	4.90 ± 0.56	15.82 ± 1.85	34.80 ± 5.13	27.88 ± 3.56	26.96 ± 6.69	21.81 ± 4.51	33.88 ± 8.31	30.44 ± 5.72	24.94 ± 5.97	24.68 ± 3.52	28.50 ± 6.51	28.94 ± 5.11	23.03 ± 5.02	35.28 ± 4.71

1-Butanol	2.74 ± 0.19	2.90 ± 0.25	8.21 ± 0.34	11.23 ± 0.16	12.59 ± 0.22	17.99 ± 1.06	23.17 ± 0.82	26.65 ± 1.51	19.36 ± 1.15	20.86 ± 0.93	18.14 ± 1.37	21.41 ± 1.33	14.82 ± 2.05	41.01 ± 2.10
1-Pentanol	11.15 ± 1.00	12.57 ± 1.33	41.77 ± 2.73	85.16 ± 6.55	86.07 ± 14.30	137.89 ± 19.06	185.14 ± 25.71	276.04 ± 29.13	182.95 ± 31.95	256.44 ± 31.00	212.02 ± 44.90	243.87 ± 42.24	180.39 ± 36.53	307.65 ± 28.46
2-Heptanol	0.20 ± 0.02	0.28 ± 0.06	0.21 ± 0.03	0.19 ± 0.02	0.44 ± 0.13	0.75 ± 0.31	1.38 ± 0.25	2.43 ± 0.20	2.24 ± 0.42	3.43 ± 0.56	2.94 ± 0.89	4.91 ± 0.88	2.94 ± 0.71	6.61 ± 0.31
1-Hexanol	58.61 ± 2.49	68.65 ± 3.37	255.95 ± 20.00	293.91 ± 18.23	531.65 ± 100.30	512.00 ± 86.44	981.35 ± 109.08	1069.00 ± 71.16	911.61 ± 123.18	1038.78 ± 119.15	1014.11 ± 169.49	1040.14 ± 137.34	850.56 ± 143.08	1140.50 ± 51.12
1-Octen-3-ol	0.38 ± 0.01	0.30 ± 0.01	1.72 ± 0.13	3.05 ± 0.30	3.27 ± 0.38	6.65 ± 1.43	9.48 ± 0.96	15.38 ± 1.25	11.05 ± 1.65	19.02 ± 2.45	14.16 ± 2.79	18.67 ± 2.60	13.64 ± 2.45	18.55 ± 0.30
1-Heptanol	1.37 ± 0.03	2.03 ± 0.02	4.30 ± 0.28	14.25 ± 1.25	13.02 ± 2.31	60.03 ± 11.56	35.20 ± 2.27	116.44 ± 2.25	45.48 ± 8.70	140.16 ± 16.37	54.85 ± 11.15	136.84 ± 20.87	46.32 ± 9.93	139.49 ± 6.08
2-Nonanol	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.06 ± 0.00	0.10 ± 0.01	0.18 ± 0.01	0.31 ± 0.02	0.30 ± 0.03	0.61 ± 0.05	0.45 ± 0.09	1.01 ± 0.05	0.52 ± 0.09	1.34 ± 0.13
1-Octanol	0.33 ± 0.01	0.40 ± 0.01	1.10 ± 0.12	3.88 ± 0.29	4.27 ± 0.76	19.15 ± 3.45	13.18 ± 0.53	40.97 ± 0.22	18.40 ± 3.14	53.13 ± 6.55	22.63 ± 4.06	58.54 ± 8.00	19.92 ± 3.99	56.24 ± 1.63
1-acetate-1,2-Propanediol	2.82 ± 0.16	0.28 ± 0.03	0.47 ± 0.04	0.34 ± 0.04	0.73 ± 0.11	0.55 ± 0.13	0.91 ± 0.28	0.76 ± 0.23	0.88 ± 0.02	0.52 ± 0.02	0.57 ± 0.04	0.42 ± 0.02	0.54 ± 0.04	0.85 ± 0.16
1-Nonanol	0.75 ± 0.19	1.74 ± 0.29	1.20 ± 0.49	0.95 ± 0.07	2.20 ± 0.07	4.03 ± 0.55	6.19 ± 0.82	10.39 ± 0.22	7.99 ± 0.87	15.11 ± 2.04	9.50 ± 1.27	18.85 ± 2.15	9.09 ± 1.03	18.69 ± 0.21
Benzyl alcohol	0.18 ± 0.08	0.73 ± 0.04	0.06 ± 0.00	0.12 ± 0.01	0.12 ± 0.01	0.20 ± 0.02	0.27 ± 0.02	0.39 ± 0.04	0.30 ± 0.03	0.35 ± 0.03	0.31 ± 0.02	0.40 ± 0.02	0.27 ± 0.02	0.45 ± 0.01
Phenylethyl Alcohol	0.17 ± 0.02	0.42 ± 0.01	0.17 ± 0.01	0.39 ± 0.01	0.29 ± 0.01	0.55 ± 0.00	0.50 ± 0.03	0.86 ± 0.10	0.65 ± 0.03	1.08 ± 0.08	0.75 ± 0.14	1.36 ± 0.09	0.91 ± 0.08	1.27 ± 0.24
1-chloro-2-Propanol	690.66 ± 52.03	482.26 ± 35.62	205.67 ± 6.34	162.10 ± 1.39	178.62 ± 1.95	135.83 ± 15.14	237.97 ± 4.20	120.59 ± 5.85	139.01 ± 6.74	69.37 ± 4.00	99.69 ± 10.53	62.95 ± 5.59	94.31 ± 8.99	113.72 ± 2.54

	2-chloro-1-Propanol	4.73 ± 0.30	3.61 ± 0.33	1.29 ± 0.06	1.15 ± 0.05	0.99 ± 0.05	0.83 ± 0.02	1.33 ± 0.10	0.72 ± 0.03	0.95 ± 0.07	0.50 ± 0.05	0.70 ± 0.11	0.44 ± 0.09	0.70 ± 0.08	0.83 ± 0.08
Hydrocarbons															
	2,2,4,6,6-pentamethyl-Heptane*	11.39 ± 2.24	12.80 ± 1.83	5.28 ± 1.83	4.40 ± 0.74	5.50 ± 2.32	4.43 ± 1.98	4.25 ± 1.53	4.73 ± 0.74	10.18 ± 5.55	1.42 ± 0.74	7.70 ± 5.74	6.82 ± 3.42	10.03 ± 7.33	0.51 ± 0.07
	Decane*	49.05 ± 2.95	39.64 ± 8.09	13.83 ± 3.40	16.03 ± 6.66	21.65 ± 8.44	20.92 ± 13.95	35.22 ± 9.16	38.44 ± 6.25	50.49 ± 20.81	24.80 ± 12.68	39.84 ± 22.38	44.00 ± 25.85	43.14 ± 27.06	4.75 ± 1.22
	3-ethyl-2-methyl-1,3-Hexadiene	0.37 ± 0.04	0.20 ± 0.02	1.00 ± 0.13	4.45 ± 0.49	2.96 ± 0.77	9.39 ± 1.98	6.34 ± 0.70	18.18 ± 0.57	6.06 ± 1.58	17.16 ± 2.52	6.30 ± 1.74	12.92 ± 2.51	4.15 ± 1.21	12.50 ± 1.03
Aldehyde															
	Pentanal	0.25 ± 0.02	0.31 ± 0.15	1.26 ± 0.24	175.20 ± 25.56	14.48 ± 2.65	272.79 ± 48.87	33.93 ± 6.68	366.56 ± 52.17	23.52 ± 15.72	229.30 ± 18.31	19.62 ± 7.89	153.51 ± 36.41	6.63 ± 7.03	164.09 ± 27.54
	Hexanal	0.61 ± 0.17	0.40 ± 0.14	5.37 ± 0.44	287.21 ± 36.71	35.00 ± 14.64	399.58 ± 73.81	90.81 ± 13.79	573.63 ± 54.63	76.30 ± 40.19	394.40 ± 51.20	59.27 ± 30.84	234.76 ± 57.43	37.18 ± 16.19	192.16 ± 28.38
	Heptanal	0.41 ± 0.08	0.44 ± 0.05	1.29 ± 0.06	30.32 ± 3.80	6.71 ± 2.73	110.72 ± 23.22	22.48 ± 4.27	180.32 ± 9.55	26.54 ± 14.24	179.29 ± 26.67	21.98 ± 10.11	132.00 ± 32.44	15.68 ± 6.02	96.69 ± 11.96
	Octanal	0.13 ± 0.04	0.13 ± 0.03	0.57 ± 0.09	20.92 ± 2.53	5.94 ± 2.50	114.84 ± 26.66	21.30 ± 3.37	187.02 ± 1.97	26.82 ± 12.69	189.88 ± 25.48	24.17 ± 9.29	148.74 ± 31.57	12.93 ± 6.01	98.21 ± 9.40
	Nonanal	11.36 ± 6.31	7.83 ± 2.84	19.47 ± 1.88	56.58 ± 2.53	26.88 ± 1.15	125.69 ± 21.64	84.97 ± 18.83	224.28 ± 15.76	74.83 ± 9.95	233.48 ± 10.93	79.21 ± 5.32	222.77 ± 24.89	93.44 ± 19.51	135.15 ± 3.35
	(E)- 2-Octenal	0.18 ± 0.03	0.20 ± 0.01	0.76 ± 0.09	4.61 ± 0.66	2.16 ± 0.47	9.18 ± 2.45	4.23 ± 0.53	18.12 ± 0.16	4.27 ± 1.16	16.81 ± 2.34	5.05 ± 1.30	10.54 ± 2.01	3.33 ± 0.95	7.68 ± 0.50
	Decanal	0.31 ± 0.11	0.27 ± 0.05	0.29 ± 0.02	0.98 ± 0.17	0.93 ± 0.09	6.02 ± 1.32	2.28 ± 0.24	12.29 ± 0.78	2.43 ± 0.68	14.52 ± 2.46	2.36 ± 0.59	13.03 ± 2.37	1.51 ± 0.40	9.18 ± 0.54

	Benzaldehyde	1.29 ± 0.02	1.57 ± 0.09	1.17 ± 0.03	1.83 ± 0.13	2.10 ± 0.12	3.36 ± 0.28	2.71 ± 0.07	4.11 ± 0.17	2.78 ± 0.35	3.79 ± 0.34	2.36 ± 0.30	3.60 ± 0.34	1.84 ± 0.18	3.70 ± 0.10
	(E)-2-Nonenal	0.20 ± 0.08	0.20 ± 0.02	0.14 ± 0.02	1.28 ± 0.12	0.21 ± 0.04	0.97 ± 0.28	0.52 ± 0.09	2.20 ± 0.03	0.50 ± 0.14	2.86 ± 0.37	0.75 ± 0.28	2.70 ± 0.50	0.70 ± 0.13	1.95 ± 0.13
	(Z)-2-Decenal	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.26 ± 0.06	0.13 ± 0.03	1.64 ± 0.41	0.28 ± 0.03	3.30 ± 0.26	0.32 ± 0.12	3.43 ± 0.45	0.38 ± 0.10	2.55 ± 0.54	0.22 ± 0.07	1.59 ± 0.14
Furan															
	2-n-Butyl furan	0.22 ± 0.04	0.33 ± 0.03	0.75 ± 0.15	1.42 ± 0.33	2.05 ± 0.68	3.03 ± 0.83	4.04 ± 0.57	4.36 ± 0.73	3.51 ± 1.03	4.64 ± 1.08	2.88 ± 0.94	3.90 ± 0.98	2.04 ± 0.69	3.29 ± 0.30
	2-pentyl-Furan	1.86 ± 0.11	1.96 ± 0.23	3.60 ± 0.39	6.23 ± 0.69	8.49 ± 2.15	12.60 ± 2.64	19.50 ± 2.42	21.94 ± 1.98	18.19 ± 4.28	23.86 ± 4.38	17.14 ± 4.53	22.50 ± 4.20	14.22 ± 3.64	20.91 ± 1.20
	2-butyltetrahydro-Furan	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	8.50 ± 1.25	0.46 ± 0.27	12.37 ± 3.74	1.08 ± 0.24	15.02 ± 0.95	0.86 ± 0.48	8.19 ± 0.53	1.31 ± 0.66	3.92 ± 0.53	0.41 ± 0.18	2.13 ± 0.28
Oxirane															
	pentyl-Oxirane	0.10 ± 0.02	0.05 ± 0.01	0.48 ± 0.04	2.54 ± 0.43	1.14 ± 0.38	3.18 ± 0.99	3.53 ± 0.78	7.93 ± 1.18	3.83 ± 1.09	7.54 ± 1.41	4.49 ± 1.52	5.42 ± 1.26	3.32 ± 1.01	5.89 ± 0.63
Pyrazine															
	methyl-Pyrazine*	0.10 ± 0.01	0.12 ± 0.01	0.89 ± 0.06	1.00 ± 0.05	1.22 ± 0.08	1.38 ± 0.11	2.01 ± 0.05	1.97 ± 0.19	1.73 ± 0.21	1.54 ± 0.21	1.35 ± 0.28	1.34 ± 0.22	1.25 ± 0.19	1.70 ± 0.03
Ketone															
	2-Octanone	0.10 ± 0.01	0.65 ± 0.02	0.21 ± 0.04	1.46 ± 0.15	1.08 ± 0.27	8.26 ± 2.27	3.57 ± 0.74	19.84 ± 1.42	5.45 ± 1.97	28.72 ± 5.28	5.31 ± 1.92	25.85 ± 5.43	3.63 ± 1.62	24.60 ± 2.46
	1-hydroxy-2-Propanone	2.88 ± 0.27	2.72 ± 0.08	1.11 ± 0.13	1.75 ± 0.13	0.93 ± 0.21	1.83 ± 0.45	1.57 ± 0.20	1.92 ± 0.37	1.57 ± 0.08	0.97 ± 0.05	0.70 ± 0.09	0.70 ± 0.18	0.57 ± 0.05	1.83 ± 0.41
	1-octen-3-one	0.28 ± 0.09	0.22 ± 0.02	1.10 ± 0.05	5.77 ± 0.48	2.76 ± 0.74	8.06 ± 1.96	6.67 ± 1.07	17.15 ± 1.57	7.64 ± 2.37	17.32 ± 2.77	8.62 ± 2.42	12.45 ± 2.41	6.63 ± 1.82	10.48 ± 1.06
	2-Nonanone	0.11 ± 0.02	0.12 ± 0.06	0.09 ± 0.02	0.54 ± 0.07	0.57 ± 0.18	9.02 ± 2.10	2.71 ± 0.28	22.09 ± 0.37	4.68 ± 1.67	36.50 ± 6.29	5.36 ± 1.66	36.64 ± 7.55	4.33 ± 1.54	30.74 ± 2.42

	3-Octen-2-one	0.37 ± 0.02	0.07 ± 0.01	1.80 ± 0.12	4.35 ± 0.39	4.57 ± 0.93	10.31 ± 1.99	12.34 ± 0.87	24.86 ± 1.13	14.11 ± 2.32	26.44 ± 3.48	16.34 ± 2.78	23.15 ± 3.42	11.98 ± 1.44	21.14 ± 1.00
	2-Decanone	0.05 ± 0.01	0.03 ± 0.01	0.06 ± 0.01	0.18 ± 0.03	0.17 ± 0.06	2.83 ± 0.62	0.83 ± 0.04	7.06 ± 0.17	1.38 ± 0.48	12.36 ± 2.13	1.58 ± 0.50	13.75 ± 2.98	1.45 ± 0.61	11.80 ± 0.98
	Acetoin	0.44 ± 0.05	0.51 ± 0.07	0.51 ± 0.08	3.34 ± 0.32	1.17 ± 0.10	3.33 ± 0.80	1.62 ± 0.69	2.29 ± 1.28	0.86 ± 0.23	1.11 ± 0.07	0.54 ± 0.12	0.96 ± 0.07	0.48 ± 0.02	1.63 ± 0.40
Lactone															
	Butyrolactone	0.57 ± 0.03	0.78 ± 0.03	0.25 ± 0.02	0.49 ± 0.02	0.42 ± 0.05	0.81 ± 0.13	0.63 ± 0.15	1.04 ± 0.23	0.56 ± 0.02	0.83 ± 0.01	0.45 ± 0.07	0.80 ± 0.05	0.47 ± 0.04	1.64 ± 0.24
	5-ethyldihydro-2(3H)-Furanone	0.21 ± 0.01	0.41 ± 0.02	0.55 ± 0.02	1.96 ± 0.19	2.08 ± 0.29	7.03 ± 0.95	5.13 ± 0.02	13.99 ± 0.45	6.36 ± 1.45	15.37 ± 1.89	6.57 ± 1.44	14.49 ± 2.35	5.74 ± 1.32	16.58 ± 0.63
	dihydro-5-propyl-2(3H)-Furanone	0.04 ± 0.00	0.06 ± 0.00	0.04 ± 0.00	0.17 ± 0.01	0.18 ± 0.02	0.99 ± 0.15	0.54 ± 0.01	2.22 ± 0.09	0.74 ± 0.18	3.11 ± 0.47	0.89 ± 0.20	3.81 ± 0.70	0.88 ± 0.18	3.83 ± 0.21
	5-butyldihydro-2(3H)-Furanone	0.02 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.33 ± 0.03	0.28 ± 0.04	2.24 ± 0.42	0.91 ± 0.02	5.01 ± 0.26	1.29 ± 0.38	6.65 ± 1.06	1.64 ± 0.40	8.27 ± 1.62	1.65 ± 0.35	8.16 ± 0.33
	tetrahydro-6-methyl-2H-Pyran-2-one	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.00	0.09 ± 0.01	0.09 ± 0.01	0.32 ± 0.03	0.19 ± 0.00	0.65 ± 0.04	0.25 ± 0.07	0.77 ± 0.10	0.24 ± 0.05	0.76 ± 0.16	0.21 ± 0.06	0.80 ± 0.06

*Not significantly different between treatments using ANOVA ($p < 0.05$)

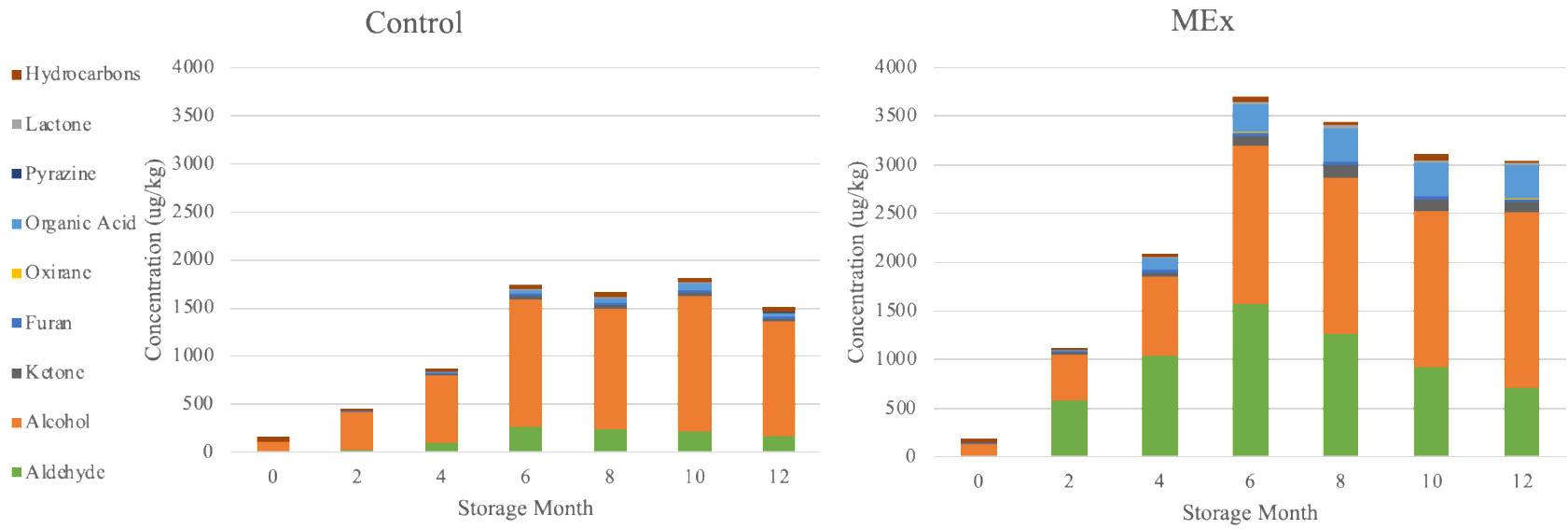

Figure 3.1. The concentration sum of each headspace chemical classes shown in Table 3.2 measured in Control and MEx samples at storage month 0, 2, 4, 6, 8, 10, and 12. 1-chloro-2-propanol and 2-chloro-1-propanol were excluded from the alcohol concentration sum.

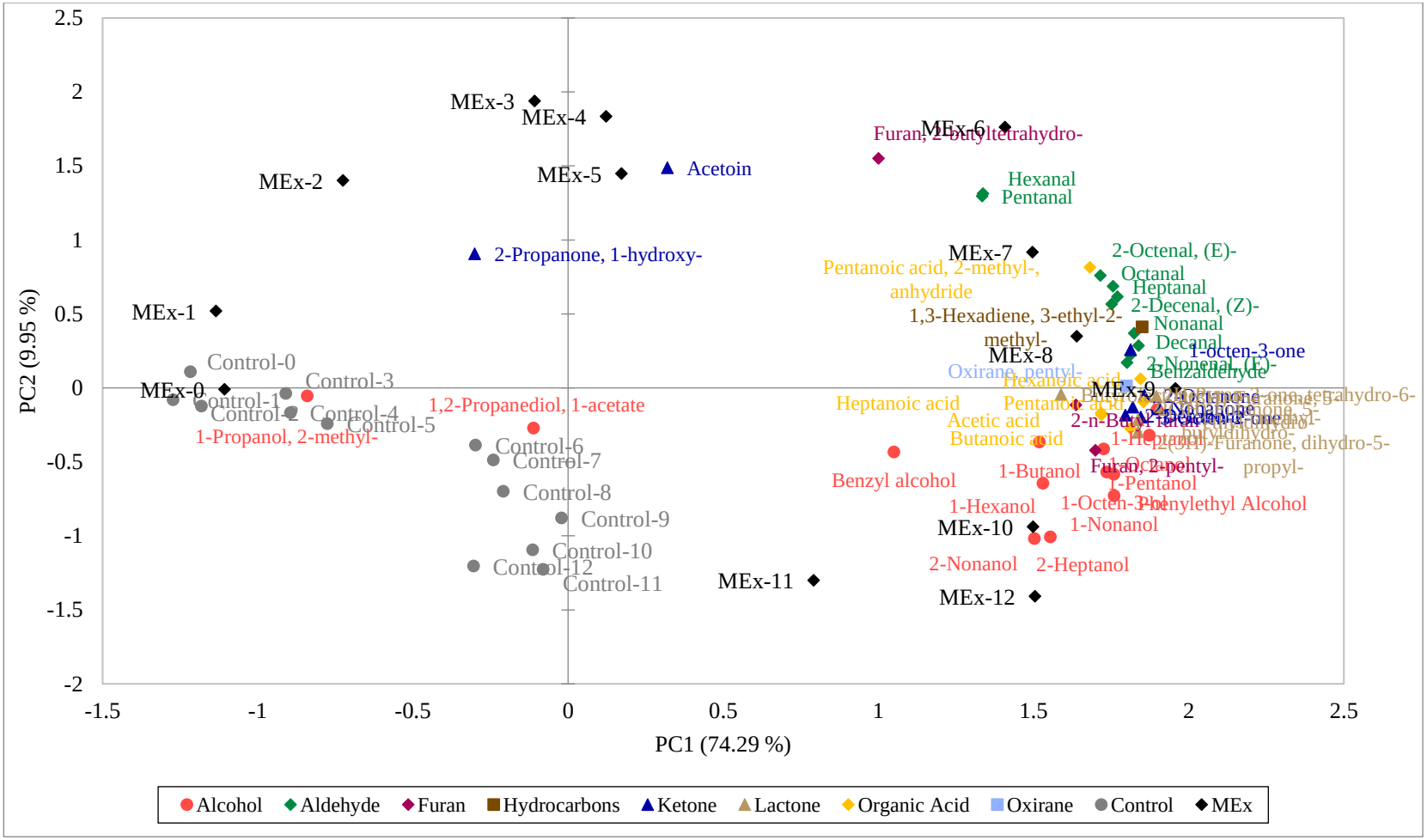

Figure 3.2. The biplot of principal component analysis (PCA) on 46 headspace volatile compounds determined by HS-SPME-GC/MS for almonds that were exposed to moisture and subsequently dried (MEx) and control almonds, stored up to 12 months of accelerated storage. The first two dimension describes 84.24 % of the variables.

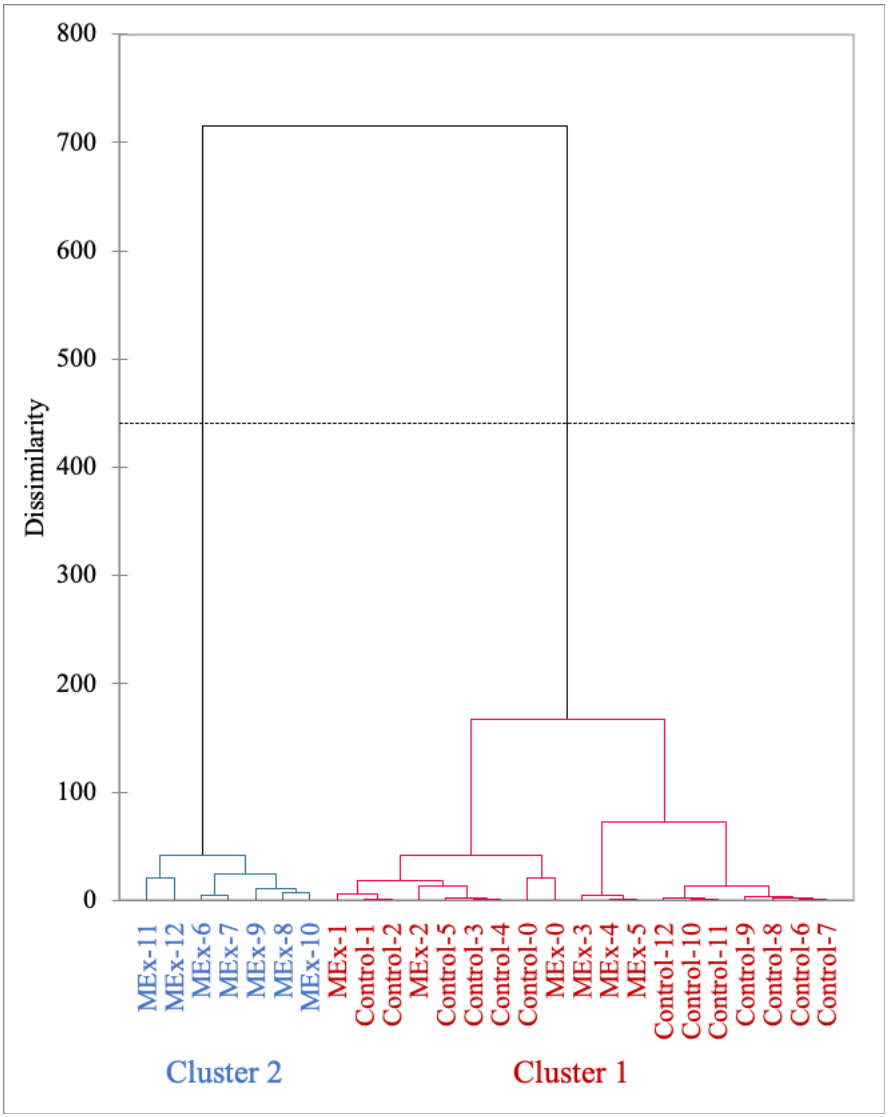

Figure 3.3. Dendrogram obtained from cluster analysis using the 46 headspace volatile compounds in PCA on the moisture exposed sample and control, with the numbers indicating storage month.

Chapter 4: The Effect of Pasteurization on Raw Almond Oxidation During Storage

4.1 Abstract

Since 2007, the majority of commercial almonds grown in California require pasteurized using a validated process such as moist heat exposure (MH) or fumigation with propylene oxide (PO) to reduce the potential of salmonella bacteria contamination. Although these treatments are common, their effect on raw almond storage quality is not well understood. To address this, almonds were treated with either MH or PO or unpasteurized (control) and stored for 12 months under accelerated shelf life conditions to promote rancidity development. Markers of lipid oxidation, headspace volatiles, and descriptive analysis were evaluated monthly. Significant differences were observed between treatments, with control samples expressing significantly higher levels of alcohols and organic acids and highest overall headspace volatile concentrations and MH samples with the lowest. At 8 months of storage, penalties were able to distinguish the MH sample from the control sample with lower scores in rancidity related attributes despite the lower hexanal level. MH samples experience less lipid oxidation during storage than controls and PO treated almonds, and will have a longer shelf life than unpasteurized almonds. However, pasteurization does show to deactivate enzymes and decrease enzymatic activities during storage.

Keywords: Raw almond, propylene oxide, moist heat, pasteurization, shelf life, lipid oxidation

4.2 Introduction

Almonds are a nutritionally dense food that has been studied extensively for their positive impact on serum lipids[1], heart health[2, 3], and weight management[4]. Almonds are also increasingly popular as a dairy alternative (almond milk), in gluten-free diets and as a complimentary protein in plant-based diets[5, 6]. California is the world leader in almond production with 2.55 billion pounds of almond kernels produced in the 2019/2020 crop year and providing 78% of the world's almond supply[7]. Approximately 20% of California almonds are traded as manufactured products whereas 80% are traded as a raw commodity[7]. Currently, it is mandatory for commercial almonds produced in the United States to be pasteurized to reach a minimum 4-log reduction in *Salmonella* bacteria prior to shipment[8]. This decision was in response to *Salmonella* outbreaks in 2001 and 2004 that were linked to the consumption of unpasteurized raw almonds[8]. Pasteurization processes must be validated and can be achieved through roasting (dry and oil), blanching (hot water skin removal), steam processing, moist heat (MH) exposure or through the use of propylene oxide (PO) fumigation[9]. Although it is understood that heat treatments such as oil and dry roasting can lead to accelerated lipid oxidation and shortened shelf life in roasted almond products[10, 11], little is understood about the effects of PO and MH (used for raw almond production) on shelf life.

Steam pasteurization, MH and PO fumigation are common ways to pasteurize almonds and still claim they are a raw commodity. Steam pasteurization involves exposing the almond surface to steam for short periods of time to kill surface *Salmonella*[12, 13]. Alternatively, pasteurization by MH involves exposing almonds to a hot humid environment (similar to steam) for short periods of time. This technique is derived from steam pasteurization but exposes almonds to less moisture to avoid changes in the moisture content during processing[7].

Industrialized steam and MH systems are designed with proprietary parameters to ensure a 4-log reduction of *Salmonella* and are widely used by the almond industry to treat organic and conventional almonds. PO pasteurization has been used since 1970s to treat numerous commodities (e.g. spices) prior to being approved for almond pasteurization by the United States Environmental Protection Agency[14-16]. The PO pasteurization process involves exposing the almonds to vaporized PO, which alkylates proteins to achieve the log kill needed. This process requires an extended post-ventilation period (e.g. 2-5 days, depending on temperature) to minimize fumigant residues. This process is only appropriate for the treatment of conventional almonds.

The shelf life of lipid-rich products such as almonds is largely limited by lipid oxidation and the development of off odors associated with rancidity. Lipid oxidation is a dynamic process involving fatty acid hydrolysis, and the oxidation of unsaturated fatty acids followed by the degradation of oxidized fatty acids into low molecular weight aldehydes, ketones and organic acids[17]. Rancidity is initiated by the hydrothermal and/or enzymatic hydrolysis of triglycerides and the oxidation of free fatty acids[18]. Whole almonds contain 35-66 % lipid, 16-23 % protein, and 2.1-7.4 % sugar (primarily sucrose) by weight[6]. The most abundant fatty acids in almonds are unsaturated and include oleic acid (O-18:1, 50-81% total), linoleic acid (L-18:2, 6-37% total), linolenic acid (Ln-18:3, 0-11% total), and palmitoleic acid (16:1, 0.1-2.5% total) [19]. The most abundant triacylglycerols in almonds are O-L-O (28 %), O-L-Ln (27%), and O-O-O (13%)[19]. Unsaturated lipids are more susceptible to lipid oxidation during storage than saturated lipids. The lipid oxidation process produces primary lipid oxidation products (e.g. lipid hydroperoxides) and secondary lipid oxidation breakdown products (e.g. ketones, aldehydes)[20, 21]. The nut industry commonly relies on lipid oxidation markers, such as peroxide value (PV)

and free fatty acids level (FFA), to estimate primary lipid oxidation in products. FFA measures the hydrolytic rancidity and cleavage of fatty acids from triglycerides. FFA has traditionally been used to monitor oxidative stability in oil by the industry. However, recent studies have shown that FFA may not reflect oxidative stability as bonded fatty acids are the preferred substrate for oxidation[22, 23]. Free fatty acids are associated with off flavors in cheese and dairy, yet free oleic and linoleic acid (long-chain and nonesterfied) has been reported to taste "fatty"[24]. PV reflects the concentration of lipid peroxides and is the most common method used to monitor oxidation in the oil industry. However, PV is a dynamic measurement that changes over time with the breakdown of lipid peroxides into secondary compounds and may not accurately reflect oxidation at later stages of a products shelf-life. Secondary lipid oxidation breakdown products can be measured in the headspace of the samples. These secondary breakdown products originate from the decomposition of lipid peroxides[25]. Lipid peroxide degradation products include alkanes, alcohols, aldehydes, ketones, and organic acids, which often contributes to flavor and aroma changes in food[21]. The most common fatty acid oxidation degradation products in almonds are derived from oleic and linoleic acid due to their overall abundance. Oleate hydroperoxides decompose to form octanal, nonanal, decanal, and 1-heptanol; whereas linoleate hydroperoxides decompose to form hexanal, 2-heptanal, 3-nonenal, and 2-octen-1-ol[25].

Headspace volatiles play a role in human perception and can be used to monitor flavor changes in food[26]. Descriptive analysis (DA), which utilizes trained panelists to describe and distinguish among product aroma, flavor, and texture characteristics, is frequently used in the food industry to describe sensory changes in food[26]. DA has been used to describe variabilities in California almond varities[27], the effect of different treatments on almonds[28, 29], and evaluating rancidity development in roasted almonds[30]. Common lexicons used to describe rancidity and

lipid oxidation in foods are cardboard, painty and rancid[31]. Among these descriptors, many of the secondary lipid oxidation breakdown volatiles have been shown to be good predictors for rancidity development[30, 32]. Heptanal, 1-pentanol, and octanal have been reported to correlate most strongly with consumer satisfaction in roasted almonds, despite the higher levels of measurable hexanal[32]. For specific sensory attributes, hexanal, pentanal, 1-pentanol, 1-hexanol, 2-butylfuran, 2-pentylfuran, and heptanal were reported to best predict total oxidized aroma in roasted almonds[30]. Having both volatile profile and descriptive analysis can provide a better understanding of the product change from both a chemical and sensory standpoint.

Many studies have evaluated the effectiveness of different pasteurization techniques on removing foodborne pathogens in tree nuts, yet the impact on shelf life is typically not considered or addressed. Although roasted and raw almond shelf life has been studied intensively, no studies address the effect of pasteurization[33-36]. MH and PO are two of the most common treatments for pasteurizing commercial raw almonds. Moisture exposure is linked to increase lipase activity and increase hydrolytic rancidity development in tree nuts and can increase lipid oxidation during storage[37-39]. Mild heat treatments can also increase the enzyme activity of lipases and lipoxygenases[40, 41]. Conversely, elevated temperatures, as in encountered in heated moist air may deactivate lipases and lipoxygenases and decrease the lipid oxidation[40, 41]. Herein, the effect of MH and PO pasteurization on lipid oxidation in raw almonds during twelve months of accelerated storage were studied. Markers of lipid oxidation (i.e. PVs, FFAs, and headspace volatiles) were monitored and compared with sensory descriptive analysis of the product. We hypothesize that MH and potentially PO pasteurization could result in increased hydrolysis of triglycerides and promote lipid oxidation due to the mild heat (MH and PO) and/or

moisture (MH) used. Results can be used to aid in understanding the influence of pasteurization on almond shelf life.

4.3 Materials and Methods

Chemicals and Reagents

Authentic volatile standards (95 – 99 %) and alkane series used for identification were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Chemicals used for measuring the lipid oxidation makers: acetic acid (HPLC grade), chloroform (HPLC grade), hydrochloric acid (ACS grade), potassium iodide (99.9 %), sodium hydroxide 10N solution (analytical grade), sodium thiosulfate (99 %), starch, and sodium chloride (ACS grade were purchased from Sigma-Aldrich or Fisher Scientific (Hampton, NH). The stable isotope internal standards: n-hexyl-d_{13} alcohol, octanal-d_{16}, and 2-methylpyrazine-d_6 were purchased from C/D/N Isotopes Inc. (QC, Canada).

Almond Samples and Storage

Almond kernels (*Prunus dulcis*) Nonpareil variety from 2017/2018 harvest year were obtained from Blue Diamond Growers (Sacramento, CA). All almond kernels were sourced from the same harvest lot. The lot was split into three groups that consisted of (1) a control without pasteurization, (2) MH pasteurization, and (3) PO pasteurization. MH and PO pasteurizations were done using commercial equipment and validated methods to achieve a 4-log reduction of *Salmonella* at Blue Diamond Growers facility (Sacramento, CA). Briefly, the MH process ran for 42 minutes which included preheating, pasteurization, and cooling. The preheat and pasteurization process used heated moist air at 80-90 °C, with the kernel temperature reaching at least 80 °C. The PO process consisted of 4-hrs of pasteurization in a heated chamber (47-51°C) with PO vapor injected at 60-71 °C reaching at least 0.5 oz PO per cubic feet[42]. PO

pasteurization was followed by a post-ventilation at 15 °C for 5 days. All nuts were held under the same ambient conditions post-pasteurization until they were transported to UC Davis. Each pasteurization treatment consisted of 68 Kg.

Almonds from each 68 kg treatment (i.e. MH, PO, control) were randomly divided into paper bags of 400 g with assigned numbers. Control samples were stored in a KMF 240 constant climate chamber (Binder Inc., Bohemia, NY) set at 32 ± 1 °C and 60 ± 1 % relative humidity. Pasteurized samples were stored in an IN 052 incubation chamber (Darwin Chambers, Saint Louis, MO) set at 32 ± 1 °C and the relative humidity was controlled using saturated sodium chloride solution at 60 ± 5 % relative humidity. Randomized samples per treatment were removed from the chamber every month; sensory samples were repacked into vacuum sealed polyethylene bags then stored at -80 °C until sensory analysis could be completed at the end of the study. Three treatments with triplicates were tested monthly from 0-12 months of storage for lipid oxidation markers.

Oxidative Stability Markers

Almond oil was extracted for PV and FFA analysis. Whole almond kernels were ground for three 1-second pulses using a laboratory grinder (Waring Laboratory Equipment, Torrington, CT). The oil was extracted from the ground almonds using a 12-ton Carver manual oil press (Carver Inc., Wabash, IN). The oil was collected into amber vials and stored in -20 °C then warmed to room temperature prior to analysis. All oxidative stability markers were measured according to the American Oil Chemists' Society official methods. PV used official method Cd 8-53, with the result expressed in peroxide milli-equivalents (mEq) per kg oil[43]. FFA was determined according to the official method Cd 3d-63, with the result reported in % oleic acid[44].

Headspace Solid-Phase Microextraction Analysis of Volatile Compounds

The sample preparation, extraction, and analysis methods were adapted from Franklin et al. (2017)[32]. In brief, 20 ± 1 g of almonds were ground and sieved through a size 20 Tyler sieve. An aliquot of 5.00 ± 0.02 g sieved almonds was weighed and incubated in a 20 mL headspace vial at room temperature (23 ± 2 °C) for at least 4 hours prior to headspace analysis. The volatiles were sampled, separated, and detected using Agilent 7890A gas chromatograph coupled with an Agilent injector 80 autosampler and a 5975C MSD (Agilent Technologies, Santa Clara, USA). Headspace vials containing the samples were equilibrated at 35 °C for 45 min with an agitation speed of 500 rpm. The volatiles were sampled using a 1 cm 30/50 μm StableFlex (Supelco Inc., Bellefonte, PA, USA) divinylbenzene/carboxen/polydimethylsiloxane fiber for 45 min. The fiber was desorbed at 250 °C for 10 minutes under splitless injection. The headspace volatiles were separated using a 30 m x 0.25 mm x 0.25 μm DB-Wax Ultra Inert column (Agilent Technologies) at a flowrate of 1.2 mL min^{-1}. The oven program was set to hold for 1 min at 35 °C followed by a ramp of 3 °C min^{-1} to 65 °C, followed by another ramp of 6 °C min^{-1} to 180 °C, and finally a ramp of 30 °C min^{-1} to 240 °C with a 5 min hold. The detector was scanning at a mass-over-charge range of 30 – 300 m/z. Tentative volatile compound identification was performed using the 2017 National Institute of Standards and Technology Mass Spectral Search Program. Further identification was performed either with authentic standards or retention index calculation. Relative concentrations of the headspace volatile compounds were calculated following the procedure described by Franklin et al. (2017)[32].

Descriptive Analysis

Descriptive sensory analysis was conducted by The National Food Lab (Livermore, CA) in July 2020. Due to the COVID-19 pandemic, modifications were made to the typical panel

orientation and data collection protocols to ensure public safety. Ten trained descriptive panelists with 2-30 years of experience participated in the sensory analysis. The panelists were involved in a 2-hour orientation session to review the samples, attribute list, character references, and definition of terms. The session was conducted through virtual meetings, with participants video calling in from their residence. Each panelist was provided with pre-packed reference standards and samples the day of the orientation session.

Descriptive analysis data collection was conducted over three days, with two sessions per day. Panelists were served ~50 g of each sample in 113 g (4 oz) plastic cups coded with random 3-digit numbers. The samples were evaluated in a monadic sequential manner in a balanced William's Latin Square design. Panelists evaluated 1 appearance attribute, 3 aroma attributes, 7 flavor attributes, and 9 texture attributes on a 15-point intensity scale (Table 4.S1). Overall Degree of Differences was evaluated using a 15-point scale against a given reference sample, which is the control sample (without pasteurization) of the matched storage age. The reference sample was served in a 57 g (2 oz) plastic cup containing 6-7 almond kernels. Panelists picked up the pre-packed samples each morning and accessed the ballot online during the designated session times using unique codes for each panelist to ensure the correct sample randomization order. All panelists were asked to conduct the study in an odor and distraction free environment with good natural lighting.

Statistical Analyses

Statistical analyses were performed using XLSTAT statistical and data analysis solution (version 2020.3). Significant differences in concentration among treatments were determined using two-way analysis of variance (ANOVA) followed by Tukey's honestly significantly difference test ($p < 0.05$). Multiple factor analysis was performed on the sensory, headspace

volatiles, and oxidation markers to aid in understanding the relationship between all the measurements and treatments.

4.4 Results and Discussion

FFAs and PVs were used to monitor early stages of oxidation (i.e. hydrolysis and oxidation) in PO pasteurized, MH pasteurized, and unpasteurized (control) raw almonds (Table 4.1, Figure 4.S1). Values were monitored over 12 months to assess impact of MH and PO pasteurization on almond storage quality. Headspace volatiles were measured to monitor secondary lipid oxidation in MH, PO, and control almonds during storage (Table 4.2). A total of 62 volatile compounds were identified and confirmed in the headspace and can be grouped into aldehyde, short alcohol (carbon chain length < 6), long alcohol (carbon chain length ≥ 6), chlorohydrin, ester, furan, hydrocarbons, ketone, lactone, organic acid, and oxirane (Table 4.2). Toluene and 3-pentanol were not significantly different between treatments ($p > 0.05$) (results not shown). Therefore, the concentrations of these two compounds are not reported in Table 4.2.

FFAs were monitored to evaluate hydrolytic rancidity in each treatment. After 5 months of accelerated storage, control samples have significantly higher levels of FFAs than PO samples, and PO samples have significantly higher levels than MH samples (Table 4.1, Figure 4.S1). The rate of FFA increase over 12 months of storage was also highest in controls relative to the PO > MH treatments. Increased levels of FFAs correspond to rancid development, unpleasant sensory attributes (e.g. soapy), and flavor development in cheese[45]. FFAs are precursors to numerous aroma compounds, such as aldehydes, ketones, and lactones[46]. Recent studies have shown that long chain fatty acids (i.e. oleic acid, linoleic acid) may play a role in how human perceive fat, although it has been described as unpleasant[47, 48]. The moisture introduced during the MH pasteurization does not appear to have an effect on the hydrolysis of triglycerides and

release of FFAs. Conversely, the temperature (80-90 °C for 42 mins) used during the MH pasteurization appears to deactivate lipase activity as evidenced by the decrease in FFAs. Lipase enzymes are active between 20-90 °C[49, 50], however activity decreases significantly at temperatures above 60 °C.

PV is a common marker the industry relies on to establish product quality with an acceptable value of < 5 mEq kg^{-1} oil set for almond oil. Herein, no significant differences were observed between treatments (Table 4.1, Figure 4.S1). The reported PV was comparable to a recent study comparing packaging of raw almonds during storage where the PV measured at the time of consumer rejection ranged from 1.4 - 4.3 mEq kg^{-1} oil[36]. Although on average, pasteurized samples showed higher levels of PV (Table 4.1), the PV measured display a comparatively higher level of variance due to the nature of titration measurements. Oxidative stability markers are dynamic and can provide only a snapshot of the complex lipid oxidation process and state of almond quality. Lin et al. (2012) has also reported no correlation between FFA and PV values measured in raw almonds during storage[40]. Franklin et al. (2017) showed that consumers reject roasted almonds despite the PV being below the industry standard of 5 mEq kg^{-1} oil [32].

Almond volatiles can be formed from lipid oxidation, sugar pyrolysis, and the Maillard reaction[51]. Although almonds were exposed to heat during pasteurization, no pyrazines were detected in this study indicating that the heat was not sufficient to generate a significant amount of Maillard reaction related products. After extended storage, control almonds have significantly higher levels of 3-methylbutanol, 2-pentanol, 2-heptanol, 1-hexanol, 2-n-butyl furan, and acetic acid compared to the pasteurized almonds (Table 4.2). 3-methylbutanol was reported as one of the main volatiles observed in sweet almonds extracted with water and is an enzymatic reaction

product[52]. The higher level of 2-methylbutanol found in control suggests that both pasteurization process may deactivate enzymes and reduce enzymatic reactions during storage. On the other hand, acetic acid has been suggested as a sensitive marker to monitor rancidity development in almonds[11]. Acetic acid is a volatile generated during storage through sugar pyrolysis and an aroma active compound in raw almond[53]. Hexanal and 1-pentanol was shown to correlate well with total oxidized attributes and painty/solvent flavor in a roasted almond study[30].

Interestingly, PO and control samples have higher levels of (E)-2-heptenal, hexanal, and 1-pentanol after 4 months of storage (Table 4.2). These volatiles were reported as linoleic acid oxidation products[20]. Linoleic acid represents 18% of the lipid content found in Nonpareil almonds and is more susceptible to oxidation compared to the oleic acid, which is 74% of the lipids reported in Nonpareil almonds[54]. As storage progresses, (E)-2-heptanal and hexanal were significantly higher in PO pasteurized almond relative to controls and MH. While MH showed significantly lower concentration of hexanal, 1-pentanol, 1-hexanol, 2-butylfuran, 2-pentylfuran, and hexanoic acid which associated with total oxidized attributes, cardboard flavor, painty/solvent flavor. This result corresponded with the descriptive analysis (Table 4.S2).

At 8 months of storage, alcohols, furan, ketones, lactones, and organic acids (e.g. 1-heptanol, 2-pentyl furan, 2-octanone, hexanoic acid) were 2-3 times higher in the control and PO samples than the MH samples (Table 4.2). Significantly higher levels of alcohols and organic acids in the control sample suggest higher degree of lipid oxidation during storage. The low concentration of secondary lipid oxidation products measured in headspace indicate that MH samples experience less lipid oxidation during storage than controls and PO treated almonds and will have a longer shelf life than unpasteurized almonds.

A visualization of the changes in headspace volatiles can be found in Figure 4.1 which depicts the summed concentration of each distinct chemical classes (e.g. aldehydes, alcohols, etc.,) for each month of storage. Control samples showed the highest overall headspace volatile concentration, whereas MH has the lowest concentration. The total headspace volatile concentration increases over storage for all treatments, with long chain alcohols being most prevalent. Oliveira et al. (2019) examined different Portuguese, French, and Spanish almond cultivars and reported that the major volatiles found in raw almonds are alcohols[55]. Despite being the most abundant type of volatiles found in raw almond, these alcohols were reported to not be aroma active[53]. Chlorohydrin was found in high concentration in PO samples at the start of the study and decreased over storage (Figure 4.1). Propylene chlorohydrins are found in foods that undergo PO fumigation[39, 40]. The PO level measured in this study was below 1 mg kg^{-1} (< 1 ppm), which is below the acceptable daily intake of 0.03 mg kg^{-1} body weight established by the Food and Agriculture Organization during the Joint Meetings on Pesticide Residues in 2011[56]. The presence of chlorohydrin at high concentration at 0 month of storage only in PO samples support that these volatiles are an artifact during the PO pasteurization process.

Descriptive Analysis

Descriptive analysis involving 20 attributes was performed on three treatments at 0, 2, 4, 6, 8, and 10 months of storage to study the sensory differences between treatments. The descriptive analysis mean data is reported in Supporting Information (Table 4.S2). Fifteen of the 20 sensory attributes evaluated showed significant differences ($p < 0.05$) among the treatments (Table 4.S2). The five attributes that did not show significance were not related to oxidation character (total flavor, benzaldehyde, cohesiveness of mass, moistness of mass, and awareness of skins). Overall degree of differences was evaluated by comparing the pasteurized samples

against the control at the same storage time. MH samples have significantly higher degree of differences after 8 months of storage showing that the panelists were able to distinguish the MH pasteurized samples from the non-pasteurized samples. When compared to the control samples, PO samples have lower mean scores in total oxidized flavor and painty/solvent flavor, whereas MH samples have higher scores in clean nutty aroma and flavor, and lower scores in total oxidized aroma and flavor, cardboard flavor, and painty/solvent flavor.

Radar plots visualizes the mean scores of 6 sensory attributes between treatments at the same storage time (Figure 4.2). Among the 6 attributes, clean nutty flavor and aroma are attributes used to describe fresh almonds that decreases over time, whereas total oxidized flavor and aroma, cardboard flavor, and painty/solvent flavor are attributes used to describe oxidized products which increases over time[30]. At the start of the study (i.e. 0 month), all treatments have similar radar plot profiles with high mean score in clean nutty flavor and aroma. After storage, an increase in total oxidized flavor/aroma can be observed in all samples. At 8 months of storage, MH samples have significantly lower levels of total oxidized flavor and painty/solvent flavor and significantly higher levels of clean nutty flavor. The differences observed contributes to the overall degree of differences, showing MH samples are less rancid than the other samples. The lower mean scores in oxidation related sensory attributes observed in pasteurized samples suggest that pasteurization decreases the undesirable sensory attributes leading to longer shelf life.

Multiple factor analysis was performed on the oxidative stability markers, headspace volatiles, and descriptive analysis to better understand the relationship between all the measurements and the sampling intervals (Figure 4.3(a), (b)). The first 2 factors of the analysis explained 73.33 % of the variables. The observation plot (Figure 4.3(a)) showed the samples

separated by treatments along the first dimension, whereas storage time were separated by the second dimension. The variable plot (Figure 4.3(b)) showed that majority of the volatiles (e.g. long alcohols, lactones, and organic acids) are on the right quadrants correlating with total oxidized flavor/aroma, cardboard flavor, painty/solvent flavor, and free fatty acids value. Sensory attributes associated with the MH samples are clean nutty flavor/aroma. Control samples are associated with oxidized flavor/aroma.

Hexanal (X1), octanal (X2), nonanal (X5), 2-heptanone (X46), and hexanoic acid (X56) were all associated with the later storage time points on the right of the first dimension. These have been previously suggested to be the optimal indicators for rancidity development in almonds[32] and correlate with consumer rejection[30]. Color, overall degree of difference, and hardness were attributes that correlated with the second dimension, indicating that as storage time progressed, samples became darker in color and increased in hardness. This may be due to the accelerated storage condition at 32 ± 1 °C and 60 ± 1 % relative humidity. In this study, no volatiles correlate strongly with the clean nutty aroma/flavor. 2-ethyl-1-hexanol (X26) and pentyl-oxirane (X60) contribute to the upper second dimension separating pasteurized samples from control. 2-ethyl-1-hexanol has been found in raw almonds[57, 58] and in hazelnuts and raisins during shelf life[59, 60]. However, the origin of these volatiles has not been identified but has been reported to be associated with lipid oxidation as levels increase in lipid-rich food during storage. Hexanal (X1), a linoleic peroxide oxidation product commonly used as a lipid oxidation marker, was significantly lower in control samples at the 8 and 12 month time points (Table 4.2). This result supports that hexanal may not be the best marker for rancidity development as the control samples had higher rancid flavor intensity despite a lower level of hexanal measured in the headspace. Among the proposed volatiles that correlate with oxidation sensory attributes, MH

samples showed lower levels of 1-pentanol, octanal, and decanal, which correlates with the significantly lower scores in sensory attributes used to describe rancidity.

These results indicate that MH pasteurization can reduce the rate of rancidity development during storage in almonds. After accelerated storage, MH almonds have significantly lower levels of FFA. Control samples were found to have the highest concentration of lipid oxidation related volatiles (e.g. hexanal, octanal, hexanoic acid) up to 3 times higher than MH samples and the highest mean scores of sensory attributes describing rancidity. Propylene chlorohydrin are characteristic volatiles that are found in PO pasteurized almonds, which decreases significantly after 1 month of storage. MH almonds have significantly lower levels of measurable headspace volatiles after storage and maintained the highest score in clean nutty flavor/aroma sensory attributes. Compared to control, PO samples have a lower mean score in total oxidized flavor and painty/solvent flavor, despite the significantly higher levels of volatiles related to lipid oxidation (e.g. octanal, decanal, (E)-2-heptanal). The overall higher levels of headspace volatiles measured in the control sample may contribute to the sensory differences observed. MH pasteurization has shown to decrease the rate of lipid oxidation in almonds and delay rancidity development. Pasteurization will not only safeguard the food, but can also prolong the shelf life, and has been shown to not cause nutritional loss[61]. These applications may be suitable for other lipid rich tree nuts that are required to undergo similar pasteurization processes.

Abbreviations used

MH, moist heat pasteurization; PO, propylene oxide pasteurization; PV, peroxide value; FFA, free fatty acids level; RH, relative humidity; ANOVA, analysis of variance; MFA, multiple factor analysis.

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Supporting Information Description

Includes the table with the definition and reference scale on the 20 sensory attributes measured, the mean score of each sensory attributes measured on a scale from 0-15, and a figure showing the oxidative stability markers (free fatty acid levels and peroxide values) measured monthly.

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4.6 Tables and Figures

Table 4.1. Average value of free fatty acids level and peroxide value measured in unpasteurized (control), propylene oxide pasteurized (PO), and moist heat pasteurized (MH) almonds over 12 months of accelerated storage.

	Free fatty acids level (% oleic acid)			Peroxide value (mEq kg^{-1} oil)		
Month	Control	PO	MH	Control	PO	MH
0	0.15 ± 0.02^{t}	0.21 ± 0.00^{rst}	0.17 ± 0.00^{st}	0.46 ± 0.05^{b}	1.05 ± 0.14^{ab}	0.55 ± 0.13^{b}
1	0.26 ± 0.01^{qrs}	0.24 ± 0.00^{qrst}	0.21 ± 0.02^{rst}	0.46 ± 0.06^{b}	0.63 ± 0.23^{b}	0.43 ± 0.15^{b}
2	0.41 ± 0.01^{nop}	0.31 ± 0.01^{pqr}	0.23 ± 0.01^{qrst}	1.25 ± 0.15^{ab}	1.25 ± 0.15^{ab}	0.31 ± 0.02^{b}
3	0.43 ± 0.02^{mno}	0.40 ± 0.04^{nop}	0.27 ± 0.02^{qrs}	0.93 ± 0.11^{ab}	1.10 ± 0.10^{ab}	0.36 ± 0.10^{b}
4	0.56 ± 0.03^{ghijk}	0.47 ± 0.02^{jklmn}	0.33 ± 0.02^{opq}	1.09 ± 0.20^{ab}	1.09 ± 0.20^{ab}	0.53 ± 0.15^{b}
5	0.66 ± 0.02^{def}	0.55 ± 0.09^{ghijkl}	0.45 ± 0.03^{lmn}	0.90 ± 0.02^{ab}	0.89 ± 0.00^{ab}	0.79 ± 0.30^{ab}
6	0.65 ± 0.02^{defg}	0.50 ± 0.02^{ijklmn}	0.41 ± 0.02^{nop}	0.72 ± 0.06^{ab}	0.95 ± 0.12^{ab}	0.70 ± 0.22^{b}
7	0.73 ± 0.03^{cd}	0.55 ± 0.06^{ghijkl}	0.45 ± 0.04^{klmn}	1.18 ± 0.11^{ab}	1.15 ± 0.14^{ab}	1.05 ± 0.19^{ab}
8	0.75 ± 0.03^{bcd}	0.62 ± 0.03^{efgh}	0.49 ± 0.02^{jklmn}	1.02 ± 0.05^{ab}	1.50 ± 0.22^{ab}	0.86 ± 0.00^{a}
9	0.85 ± 0.06^{ab}	0.60 ± 0.02^{efghi}	0.53 ± 0.05^{hijklm}	0.99 ± 0.11^{ab}	1.37 ± 0.29^{ab}	0.96 ± 0.26^{ab}
10	0.85 ± 0.02^{ab}	0.61 ± 0.01^{efgh}	0.56 ± 0.01^{fghij}	0.85 ± 0.00^{ab}	1.03 ± 0.19^{ab}	0.75 ± 0.43^{ab}
11	0.81 ± 0.04^{bc}	0.67 ± 0.03^{de}	0.52 ± 0.02^{hijklm}	0.88 ± 0.30^{ab}	1.60 ± 0.65^{ab}	1.06 ± 0.14^{ab}
12	0.92 ± 0.07^{a}	0.76 ± 0.02^{bcd}	0.57 ± 0.01^{efghij}	0.80 ± 0.14^{ab}	1.23 ± 0.72^{ab}	1.02 ± 0.49^{ab}

Tukey's post-hoc test showed that alphabets shared within the same chemical measurement indicates there is no significant differences between treatments ($p < 0.05$)

Table 4.2. The average concentration (µg kg^{-1} almond) of the headspace volatiles measured in unpasteurized (control), propylene oxide pasteurized (PO), and moist heat pasteurized (MH) almonds at 0, 4, 8, and 12 months of accelerated storage. ANOVA was performed at each storage month.

Chemical Class	MFA Code	Volatile	0 Month			4 Month			8 Month			12 Month		
			Control	PO	MH	Control	PO	MH	Control	PO	MH	Control	PO	MH
Aldehydes														
	X1	Hexanal	8.19 ± 1.67	21.16 ± 16.25	6.91 ± 1.24	296.04 ± 89.80^{a}	411.95 ± 107.05^{a}	53.26 ± 53.02^{b}	145.22 ± 40.93^{b}	526.19 ± 73.27^{a}	288.63 ± 210.87^{ab}	89.19 ± 12.51^{b}	306.50 ± 94.15^{a}	173.79 ± 56.04^{ab}
	X2	Octanal	0.42 ± 0.27	0.66 ± 0.39	0.18 ± 0.02	48.68 ± 0.45^{a}	35.09 ± 12.26^{a}	3.27 ± 1.97^{b}	24.10 ± 9.98^{b}	53.39 ± 9.25^{a}	20.24 ± 13.46^{b}	13.44 ± 1.68^{ab}	33.52 ± 14.13^{a}	9.99 ± 4.36^{b}
	X3	(E)-2-Heptenal	0.43 ± 0.20^{ab}	0.63 ± 0.15^{a}	0.23 ± 0.05^{b}	4.69 ± 0.27^{a}	5.07 ± 1.24^{a}	0.73 ± 0.15^{b}	3.69 ± 0.76^{b}	5.61 ± 0.37^{a}	2.68 ± 0.88^{b}	2.11 ± 0.38	2.24 ± 0.78	1.38 ± 0.18
	X4	N,N-dimethyl-formamide	0.35 ± 0.12	0.38 ± 0.14	0.20 ± 0.03	6.74 ± 0.93^{a}	5.49 ± 1.29^{a}	2.00 ± 0.20^{b}	8.91 ± 0.59^{a}	6.92 ± 0.58^{b}	3.22 ± 0.33^{c}	2.82 ± 0.21^{b}	3.96 ± 0.65^{a}	2.18 ± 0.07^{b}
	X5	Nonanal	5.92 ± 3.82	8.89 ± 2.26	8.65 ± 2.35	35.44 ± 2.09^{a}	41.49 ± 7.14^{a}	12.44 ± 4.11^{b}	40.94 ± 2.18	53.17 ± 9.97	28.61 ± 13.65	15.07 ± 1.62	30.36 ± 10.96	14.50 ± 3.53
	X6	Decanal	0.04 ± 0.01^{b}	0.09 ± 0.02^{a}	0.03 ± 0.01^{b}	0.43 ± 0.06^{ab}	0.47 ± 0.14^{a}	0.20 ± 0.10^{b}	0.57 ± 0.13^{b}	0.99 ± 0.13^{a}	0.51 ± 0.18^{b}	0.34 ± 0.06^{b}	0.71 ± 0.19^{a}	0.31 ± 0.04^{b}
	X7	Benzaldehyde	1.09 ± 0.11^{ab}	1.43 ± 0.21^{a}	0.88 ± 0.08^{b}	3.02 ± 0.19^{a}	2.57 ± 0.47^{a}	0.87 ± 0.16^{b}	4.35 ± 0.45^{b}	6.43 ± 0.33^{a}	2.63 ± 0.66^{c}	2.30 ± 0.06^{b}	5.46 ± 1.36^{a}	1.91 ± 0.03^{b}
Short Chain Alcohols														
	X8	Methyl alcohol	7.56 ± 1.83^{b}	20.25 ± 1.31^{a}	8.41 ± 1.65^{b}	21.02 ± 2.80^{a}	2.10 ± 0.81^{b}	2.97 ± 0.81^{b}	19.34 ± 2.51^{a}	1.87 ± 0.59^{b}	2.18 ± 0.26^{b}	2.20 ± 0.07	1.54 ± 0.14	3.66 ± 1.75
	X9	Isopropyl alcohol	59.03 ± 5.16^{a}	23.50 ± 2.17^{c}	34.03 ± 2.01^{b}	10.29 ± 1.14^{a}	3.59 ± 0.84^{b}	10.14 ± 1.80^{a}	9.02 ± 0.46^{a}	4.65 ± 1.28^{b}	9.47 ± 0.28^{a}	3.61 ± 0.26^{b}	3.70 ± 0.44^{b}	7.64 ± 0.82^{a}
	X10	Ethanol	51.07 ± 8.91^{ab}	60.36 ± 8.88^{a}	35.38 ± 3.37^{b}	72.32 ± 8.72^{a}	9.97 ± 1.90^{b}	6.98 ± 1.69^{b}	43.16 ± 2.65^{a}	10.71 ± 1.31^{b}	6.89 ± 0.50^{b}	5.31 ± 0.10	6.54 ± 1.29	4.75 ± 1.41
	X11	2-Butanol	3.25 ± 1.04	2.70 ± 0.21	3.22 ± 0.40	3.52 ± 0.21^{a}	1.75 ± 0.23^{b}	3.82 ± 0.55^{a}	4.80 ± 0.18^{a}	2.00 ± 0.31^{c}	3.32 ± 0.55^{b}	3.15 ± 0.07^{a}	1.93 ± 0.08^{b}	3.05 ± 0.69^{a}
	X12	1-Propanol	2.27 ± 0.32^{ab}	2.81 ± 0.25^{a}	1.74 ± 0.08^{b}	4.22 ± 0.57^{a}	2.10 ± 0.38^{b}	1.36 ± 0.10^{b}	3.58 ± 0.17^{a}	1.98 ± 0.21^{b}	1.40 ± 0.03^{c}	1.16 ± 0.08	1.26 ± 0.26	0.92 ± 0.24
	X13	2-methyl-1-propanol	2.37 ± 0.21^{b}	3.19 ± 0.35^{a}	3.59 ± 0.13^{a}	7.10 ± 1.61^{a}	2.79 ± 0.13^{b}	6.00 ± 1.73^{ab}	6.83 ± 0.44^{a}	3.73 ± 0.67^{b}	5.17 ± 0.73^{b}	3.80 ± 0.31^{ab}	2.91 ± 0.39^{b}	4.80 ± 0.77^{a}
	X14	2-Pentanol	1.59 ± 0.15^{b}	3.90 ± 0.18^{a}	1.62 ± 0.09^{b}	5.64 ± 0.74	4.13 ± 0.71	4.07 ± 1.33	10.01 ± 0.79^{a}	6.64 ± 0.78^{b}	5.91 ± 0.68^{b}	7.52 ± 0.00^{a}	5.99 ± 0.21^{b}	4.12 ± 0.73^{c}
	X15	3-methyl-1-butanol	4.16 ± 0.41^{b}	8.81 ± 2.17^{a}	8.80 ± 0.14^{a}	25.86 ± 0.76^{a}	17.82 ± 2.04^{b}	17.29 ± 3.73^{b}	36.66 ± 2.75^{a}	26.52 ± 2.60^{b}	23.16 ± 2.81^{b}	27.23 ± 0.75^{a}	22.13 ± 3.38^{ab}	18.05 ± 3.50^{b}
	X16	3-methyl-3-buten-1-ol	0.11 ± 0.02^{b}	0.10 ± 0.01^{b}	0.22 ± 0.01^{a}	0.21 ± 0.01	0.13 ± 0.02	0.49 ± 0.31	0.33 ± 0.07^{a}	0.11 ± 0.01^{b}	0.38 ± 0.10^{a}	0.29 ± 0.01^{a}	0.07 ± 0.02^{b}	0.21 ± 0.06^{a}

	X17	1-Pentanol	13.11 ± 2.83^{b}	22.38 ± 3.78^{a}	8.31 ± 1.02^{b}	301.98 ± 30.72^{a}	215.20 ± 22.42^{b}	93.42 ± 29.93^{c}	376.22 ± 24.85^{a}	324.18 ± 17.36^{a}	189.40 ± 57.68^{b}	254.08 ± 9.73^{a}	211.38 ± 32.03^{a}	102.44 ± 15.95^{b}
	X18	2,3-Butanediol	3.76 ± 0.30	4.90 ± 1.09	3.28 ± 2.33	12.75 ± 12.67	4.78 ± 1.97	7.56 ± 4.43	8.27 ± 1.18	6.41 ± 0.66	8.60 ± 0.97	6.40 ± 0.30	4.61 ± 0.66	6.22 ± 2.12
Long Chain Alcohols														
	X19	2-Hexanol	0.15 ± 0.03^{b}	0.47 ± 0.02^{a}	0.14 ± 0.01^{b}	7.00 ± 1.72^{a}	4.97 ± 1.12^{ab}	2.14 ± 0.91^{b}	23.35 ± 1.61^{a}	14.03 ± 0.64^{b}	5.40 ± 1.51^{c}	20.42 ± 0.84^{a}	13.32 ± 1.32^{b}	3.73 ± 0.28^{c}
	X20	3-Heptanol	0.02 ± 0.00^{b}	0.04 ± 0.01^{a}	0.02 ± 0.01^{b}	0.63 ± 0.09	0.56 ± 0.09	0.43 ± 0.14	1.30 ± 0.15^{a}	1.10 ± 0.04^{a}	0.73 ± 0.12^{b}	1.13 ± 0.05^{a}	0.90 ± 0.10^{b}	0.46 ± 0.02^{c}
	X21	2-Heptanol	0.95 ± 0.13^{b}	2.82 ± 0.32^{a}	0.84 ± 0.15^{b}	33.33 ± 8.10^{a}	27.61 ± 8.55^{ab}	12.00 ± 5.75^{b}	140.11 ± 5.50^{a}	79.94 ± 1.39^{b}	30.08 ± 8.76^{c}	125.39 ± 6.14^{a}	75.31 ± 8.75^{b}	21.02 ± 3.16^{c}
	X22	1-Hexanol	59.51 ± 17.52^{b}	104.49 ± 11.83^{a}	39.88 ± 6.43^{b}	1186.95 ± 206.85^{a}	936.31 ± 198.10^{a}	417.85 ± 159.37^{b}	1671.67 ± 91.87^{a}	1232.54 ± 21.60^{b}	667.80 ± 141.64^{c}	1186.62 ± 42.64^{a}	838.75 ± 186.89^{b}	343.03 ± 32.48^{c}
	X23	2-Octanol	0.10 ± 0.02^{b}	0.28 ± 0.01^{a}	0.08 ± 0.03^{b}	3.74 ± 0.47^{a}	3.22 ± 1.16^{a}	1.06 ± 0.44^{b}	16.17 ± 0.54^{a}	7.83 ± 0.73^{b}	2.63 ± 0.36^{c}	14.56 ± 0.84^{a}	7.38 ± 0.79^{b}	1.61 ± 0.06^{c}
	X24	1-Octen-3-ol	0.28 ± 0.08	0.43 ± 0.09	0.34 ± 0.02	3.14 ± 0.27	3.89 ± 1.53	1.86 ± 0.38	4.71 ± 0.68^{b}	6.85 ± 0.47^{a}	5.27 ± 1.18^{ab}	4.21 ± 0.18^{ab}	5.26 ± 0.89^{a}	3.44 ± 0.38^{b}
	X25	1-Heptanol	1.69 ± 0.40^{b}	3.77 ± 0.77^{a}	1.30 ± 0.19^{b}	161.82 ± 5.79^{a}	121.98 ± 31.31^{a}	27.25 ± 8.63^{b}	294.51 ± 21.99^{a}	206.79 ± 8.89^{b}	69.70 ± 22.42^{c}	201.84 ± 5.07^{a}	147.48 ± 23.68^{b}	39.22 ± 2.96^{c}
	X26	2-ethyl-1-hexanol	0.38 ± 0.07^{b}	0.56 ± 0.02^{a}	0.21 ± 0.03^{c}	0.94 ± 0.08^{b}	14.17 ± 1.68^{a}	19.61 ± 5.57^{a}	1.94 ± 0.10^{b}	31.37 ± 2.92^{a}	37.13 ± 3.10^{a}	1.26 ± 0.03^{c}	29.05 ± 3.03^{b}	36.76 ± 3.38^{a}
	X27	2-Nonanol	0.05 ± 0.01b	0.09 ± 0.01^{a}	0.06 ± 0.01^{b}	0.68 ± 0.10^{a}	0.43 ± 0.05^{b}	0.35 ± 0.10^{b}	4.24 ± 0.20^{a}	1.66 ± 0.18^{b}	1.10 ± 0.19^{c}	3.79 ± 0.14^{a}	1.78 ± 0.25^{b}	0.78 ± 0.02^{c}
	X28	1-Octanol	0.85 ± 0.22^{b}	1.72 ± 0.14^{a}	0.58 ± 0.07^{b}	68.24 ± 13.77^{a}	45.08 ± 5.32^{b}	12.56 ± 3.39^{c}	135.93 ± 11.00^{a}	87.29 ± 5.48^{b}	32.50 ± 8.40^{c}	94.59 ± 5.26^{a}	67.34 ± 14.74^{b}	19.68 ± 1.04^{c}
	X29	(E)-2-octen-1-ol	0.06 ± 0.01^{b}	0.17 ± 0.02^{a}	0.07 ± 0.00^{b}	0.48 ± 0.06^{b}	0.66 ± 0.10^{a}	0.39 ± 0.04^{b}	0.66 ± 0.07^{c}	1.07 ± 0.01^{a}	0.89 ± 0.07^{b}	0.40 ± 0.06^{b}	0.66 ± 0.09^{a}	0.67 ± 0.05^{a}
	X30	2-(2-ethoxyethoxy)-ethanol	0.08 ± 0.02	0.09 ± 0.01	0.06 ± 0.00	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.33 ± 0.01^{a}	0.24 ± 0.01^{b}	0.24 ± 0.03^{b}	0.16 ± 0.01^{a}	0.15 ± 0.01^{ab}	0.14 ± 0.00^{b}
	X31	1-Nonanol	0.69 ± 0.18	0.89 ± 0.03	0.77 ± 0.07	9.00 ± 1.51^{a}	5.80 ± 1.07^{b}	3.34 ± 0.46^{b}	21.52 ± 1.64^{a}	12.78 ± 0.87^{b}	7.01 ± 0.94^{c}	14.40 ± 0.32^{a}	10.21 ± 0.17^{b}	4.70 ± 0.13^{c}
	X32	Benzyl alcohol	0.07 ± 0.01^{b}	0.29 ± 0.15^{a}	0.20 ± 0.01^{ab}	1.61 ± 0.16^{a}	1.07 ± 0.17^{ab}	0.76 ± 0.43^{b}	3.10 ± 0.17^{a}	1.60 ± 0.21^{b}	0.95 ± 0.22^{c}	2.43 ± 0.07^{a}	1.30 ± 0.24^{b}	0.79 ± 0.11^{c}
	X33	Phenylethyl Alcohol	0.23 ± 0.02^{b}	0.70 ± 0.13^{a}	0.61 ± 0.03^{a}	2.45 ± 0.17	1.98 ± 0.22	1.90 ± 0.78	5.52 ± 0.21^{a}	3.50 ± 0.16^{b}	3.38 ± 0.59^{b}	4.67 ± 0.52^{a}	2.69 ± 0.67^{b}	2.17 ± 0.25^{b}
Chlorohydrin														
	X34	1-chloro-2-propanol	2.39 ± 0.34^{b}	614.16 ± 64.06^{a}	10.85 ± 17.69^{b}	0.11 ± 0.02^{b}	43.13 ± 23.65^{a}	29.63 ± 6.15^{ab}	0.06 ± 0.01^{b}	8.38 ± 2.52^{a}	8.40 ± 2.24^{a}	0.05 ± 0.01^{b}	4.27 ± 1.92^{a}	3.25 ± 0.36^{a}
Esters														
	X35	Ethyl acetate	0.21 ± 0.23	0.06 ± 0.02	0.04 ± 0.01	0.14 ± 0.02^{a}	0.02 ± 0.01^{b}	0.01 ± 0.00^{b}	0.19 ± 0.03^{a}	0.03 ± 0.01^{b}	0.02 ± 0.01^{b}	0.03 ± 0.00	0.03 ± 0.01	0.05 ± 0.05
Furans														
	X36	2-n-Butyl furan	0.17 ± 0.02^{ab}	0.20 ± 0.05^{a}	0.09 ± 0.01^{b}	3.64 ± 0.63^{a}	1.88 ± 0.33^{b}	0.78 ± 0.31^{b}	6.31 ± 0.75^{a}	2.81 ± 0.54^{b}	1.19 ± 0.19^{c}	4.21 ± 0.57^{a}	1.96 ± 0.49^{b}	0.70 ± 0.12^{c}

	X37	2-pentyl-furan	1.53 ± 0.23^{b}	2.68 ± 0.18^{a}	1.05 ± 0.06^{c}	17.58 ± 2.13^{a}	12.80 ± 1.62^{b}	7.75 ± 1.85^{c}	32.93 ± 2.42^{a}	19.78 ± 3.04^{b}	13.20 ± 0.75^{c}	20.23 ± 2.27^{a}	14.52 ± 2.83^{b}	10.12 ± 1.50^{b}
	X38	2-hexyl-furan	0.02 ± 0.00^{b}	0.03 ± 0.00^{a}	0.01 ± 0.00^{b}	0.55 ± 0.14^{a}	0.31 ± 0.03^{b}	0.17 ± 0.03^{b}	1.32 ± 0.03^{a}	0.55 ± 0.10^{b}	0.34 ± 0.03^{c}	0.76 ± 0.05^{a}	0.41 ± 0.03^{b}	0.23 ± 0.00^{c}
	X39	2-Vinylfuran	0.11 ± 0.02^{b}	0.18 ± 0.01^{a}	0.09 ± 0.02^{b}	0.15 ± 0.01	0.16 ± 0.02	0.17 ± 0.01	0.29 ± 0.00	0.26 ± 0.01	0.29 ± 0.04	0.13 ± 0.01^{c}	0.16 ± 0.00^{b}	0.18 ± 0.01^{a}
Hydrocarbons														
	X40	1-Octene	0.41 ± 0.27	0.57 ± 0.02	0.53 ± 0.18	0.79 ± 0.11^{a}	0.41 ± 0.05^{b}	0.40 ± 0.20^{b}	0.56 ± 0.11^{a}	0.54 ± 0.10^{a}	0.29 ± 0.04^{b}	0.49 ± 0.02^{a}	0.45 ± 0.06^{a}	0.31 ± 0.08^{b}
	X41	Benzene	2.37 ± 0.23^{b}	3.15 ± 0.19^{a}	1.70 ± 0.19^{c}	2.32 ± 0.23^{a}	1.33 ± 0.49^{b}	1.52 ± 0.19^{ab}	3.27 ± 0.35	3.08 ± 0.46	3.06 ± 0.34	1.63 ± 0.05	1.66 ± 0.02	1.59 ± 0.15
	X42	2,2,4,6,6-pentamethyl-heptane	4.52 ± 0.12^{a}	3.62 ± 0.59^{ab}	3.11 ± 0.28^{b}	5.57 ± 1.04^{a}	3.61 ± 0.84^{ab}	2.27 ± 0.56^{b}	4.60 ± 0.66	3.47 ± 1.19	2.76 ± 0.44	2.55 ± 0.50	2.79 ± 0.89	1.58 ± 0.66
	X43	Ethylbenzene	2.06 ± 0.32^{a}	1.24 ± 0.08^{b}	1.05 ± 0.16^{b}	1.55 ± 0.23^{a}	0.91 ± 0.06^{b}	0.97 ± 0.18^{b}	2.45 ± 0.34^{a}	1.17 ± 0.06^{b}	1.36 ± 0.07^{b}	2.13 ± 0.05^{a}	0.79 ± 0.07^{b}	0.78 ± 0.03^{b}
							Ketone							
	X44	Acetone	9.37 ± 1.07^{a}	4.90 ± 0.76^{b}	4.46 ± 0.23^{b}	11.00 ± 0.62^{a}	3.71 ± 0.80^{b}	4.52 ± 0.71^{b}	5.27 ± 0.72^{a}	4.98 ± 0.76^{a}	2.41 ± 0.53^{b}	1.72 ± 0.10	3.00 ± 1.02	1.51 ± 0.12
	X45	2-Butanone	0.37 ± 0.03	0.33 ± 0.09	0.29 ± 0.02	1.82 ± 0.18^{a}	0.69 ± 0.19^{b}	0.32 ± 0.07^{b}	1.17 ± 0.13^{a}	1.04 ± 0.04^{a}	0.42 ± 0.08^{b}	0.41 ± 0.04^{ab}	0.62 ± 0.19^{a}	0.32 ± 0.06^{b}
	X46	2-Heptanone	0.39 ± 0.03^{ab}	0.75 ± 0.27^{a}	0.22 ± 0.04^{b}	37.80 ± 1.46^{a}	21.06 ± 4.68^{b}	2.36 ± 1.16^{c}	55.94 ± 7.31^{a}	40.22 ± 3.40^{b}	7.31 ± 4.00^{c}	31.45 ± 2.11^{a}	24.51 ± 9.94^{a}	3.26 ± 0.64^{b}
	X47	2-Octanone	0.05 ± 0.01^{b}	0.15 ± 0.05^{a}	0.02 ± 0.00^{b}	6.47 ± 1.34^{a}	2.33 ± 0.49^{b}	0.23 ± 0.07^{c}	8.74 ± 1.31^{a}	4.48 ± 0.33^{b}	0.90 ± 0.48^{c}	4.69 ± 0.26^{a}	2.88 ± 0.92^{b}	0.39 ± 0.10^{c}
Lactones														
	X48	Dihydro-5-methyl-2(3H)-furanone	0.26 ± 0.03^{b}	0.48 ± 0.07^{a}	0.16 ± 0.02^{b}	2.19 ± 0.22^{a}	1.46 ± 0.15^{b}	0.79 ± 0.18^{c}	4.96 ± 0.36^{a}	3.16 ± 0.04^{b}	1.49 ± 0.26^{c}	3.96 ± 0.11^{a}	3.13 ± 0.96^{a}	1.15 ± 0.01^{b}
	X49	Tetrahydro-2H-pyran-2-one	0.10 ± 0.02b	0.24 ± 0.04^{a}	0.11 ± 0.01^{b}	0.21 ± 0.03	0.16 ± 0.03	0.22 ± 0.05	0.33 ± 0.03^{a}	0.25 ± 0.04^{b}	0.30 ± 0.03^{ab}	0.22 ± 0.02	0.19 ± 0.05	0.19 ± 0.03
	X50	Butyrolactone	0.66 ± 0.19^{b}	1.33 ± 0.26^{a}	0.61 ± 0.07^{b}	3.70 ± 0.39^{a}	2.60 ± 0.34^{b}	1.38 ± 0.21^{c}	9.10 ± 0.54^{a}	4.21 ± 0.40^{b}	2.14 ± 0.06^{c}	6.14 ± 0.38^{a}	3.81 ± 0.77^{b}	1.59 ± 0.21^{c}
	X51	5-Ethyldihydro-2(3H)-furanone	0.38 ± 0.08^{b}	0.67 ± 0.08^{a}	0.24 ± 0.06^{b}	19.40 ± 4.09^{a}	13.79 ± 2.93^{a}	3.23 ± 0.47^{b}	40.77 ± 3.15^{a}	29.43 ± 0.96^{b}	10.34 ± 4.05^{c}	33.40 ± 0.69^{a}	24.62 ± 4.34^{b}	8.06 ± 0.29^{c}
	X52	Dihydro-5-propyl-2(3H)-furanone	0.08 ± 0.02^{ab}	0.15 ± 0.07^{a}	0.04 ± 0.01^{b}	3.24 ± 0.26^{a}	2.43 ± 0.41^{b}	0.38 ± 0.10^{c}	9.63 ± 1.04^{a}	6.65 ± 0.14^{b}	1.53 ± 0.60^{c}	8.33 ± 0.07^{a}	6.07 ± 1.09^{b}	1.32 ± 0.13^{c}
Organic Acids														
	X53	Methyl ester acetic acid	0.42 ± 0.19	0.61 ± 0.15	0.40 ± 0.01	4.25 ± 0.59^{a}	0.26 ± 0.04^{b}	0.19 ± 0.01^{b}	7.64 ± 1.12^{a}	0.44 ± 0.04^{b}	0.32 ± 0.05^{b}	0.81 ± 0.02^{a}	0.40 ± 0.06^{b}	0.35 ± 0.04^{b}

	X54	Acetic acid	6.31 ± 4.30	6.58 ± 1.97	1.70 ± 0.12	45.97 ± 8.94	39.77 ± 12.62	20.52 ± 12.08	175.91 ± 10.70^{a}	105.97 ± 9.47^{b}	63.13 ± 2.44^{c}	115.26 ± 0.70^{a}	91.67 ± 11.80^{b}	62.02 ± 5.42^{c}
	X55	Pentanoic acid	0.40 ± 0.15	0.26 ± 0.02	0.28 ± 0.05	1.66 ± 0.35^{a}	1.41 ± 0.55^{a}	0.28 ± 0.07^{b}	7.48 ± 1.19^{a}	6.80 ± 0.68^{a}	2.82 ± 1.74^{b}	6.16 ± 0.57^{ab}	10.00 ± 2.80^{a}	3.94 ± 0.58^{b}
	X56	Hexanoic acid	3.59 ± 1.96	2.34 ± 0.37	1.47 ± 0.35	38.20 ± 1.79^{a}	42.58 ± 17.92^{a}	6.62 ± 2.79^{b}	147.63 ± 20.43^{a}	146.76 ± 6.39^{a}	48.71 ± 25.48^{b}	107.04 ± 8.49^{a}	128.49 ± 32.94^{a}	45.50 ± 7.28^{b}
	X57	Heptanoic acid	0.30 ± 0.16	0.10 ± 0.01	0.13 ± 0.03	4.83 ± 0.60^{a}	4.20 ± 1.98^{a}	0.44 ± 0.07^{b}	15.14 ± 2.41^{a}	11.54 ± 0.98^{a}	2.88 ± 1.46^{b}	10.44 ± 1.29^{a}	12.10 ± 2.75^{a}	2.24 ± 0.62^{b}
	X58	Octanoic acid	0.21 ± 0.09	0.16 ± 0.05	0.09 ± 0.02	7.94 ± 0.44^{a}	4.60 ± 2.10^{b}	0.46 ± 0.16^{c}	16.99 ± 3.10^{a}	12.06 ± 2.83^{a}	4.15 ± 2.19^{b}	14.31 ± 1.63^{a}	14.07 ± 0.97^{a}	3.05 ± 0.95^{b}
	X59	Nonanoic acid	0.12 ± 0.03^{ab}	0.20 ± 0.06^{a}	0.06 ± 0.02^{b}	1.73 ± 0.28^{a}	1.19 ± 0.59^{a}	0.20 ± 0.05^{b}	5.06 ± 0.21^{a}	2.63 ± 0.52^{b}	3.08 ± 1.30^{ab}	2.60 ± 0.38^{a}	3.07 ± 0.43^{a}	0.86 ± 0.24^{b}
Oxirane														
	X60	Pentyl-oxirane	0.20 ± 0.06^{ab}	0.32 ± 0.05^{a}	0.11 ± 0.02^{b}	0.14 ± 0.02^{c}	0.39 ± 0.06^{b}	0.65 ± 0.13^{a}	0.08 ± 0.01^{b}	0.38 ± 0.05^{b}	0.91 ± 0.27^{a}	0.05 ± 0.00^{b}	0.16 ± 0.05^{ab}	0.32 ± 0.14^{a}

Alphabets shared within the same month within the same row indicates there is no significant differences under Tukey's post-hoc test ($p < 0.05$)

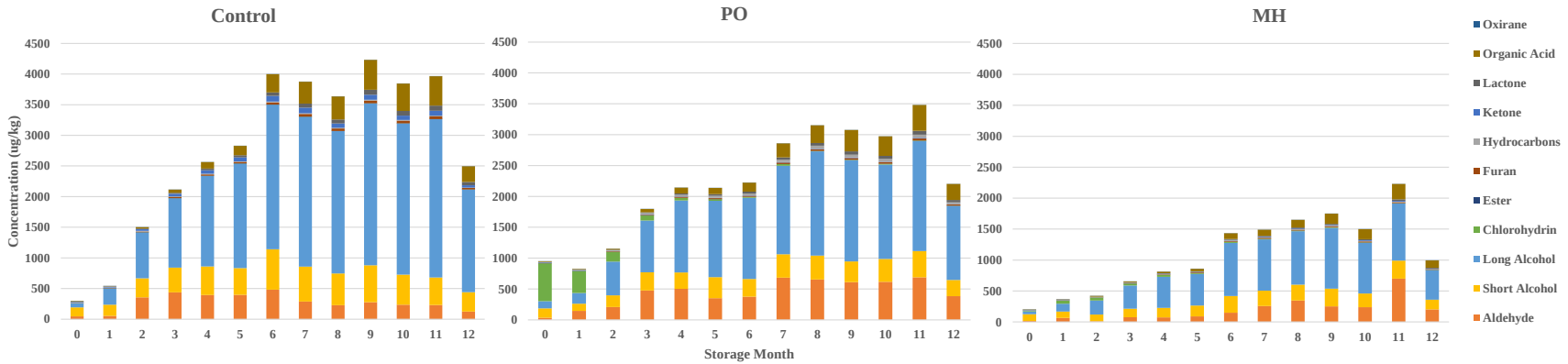

Figure 4.1. The concentration sum of each headspace chemical classes shown in Table 4.2 measured in unpasteurized (control), propylene oxide pasteurized (PO), and moist heat pasteurized (MH) almonds during 12 months accelerated storage sampled once a month.

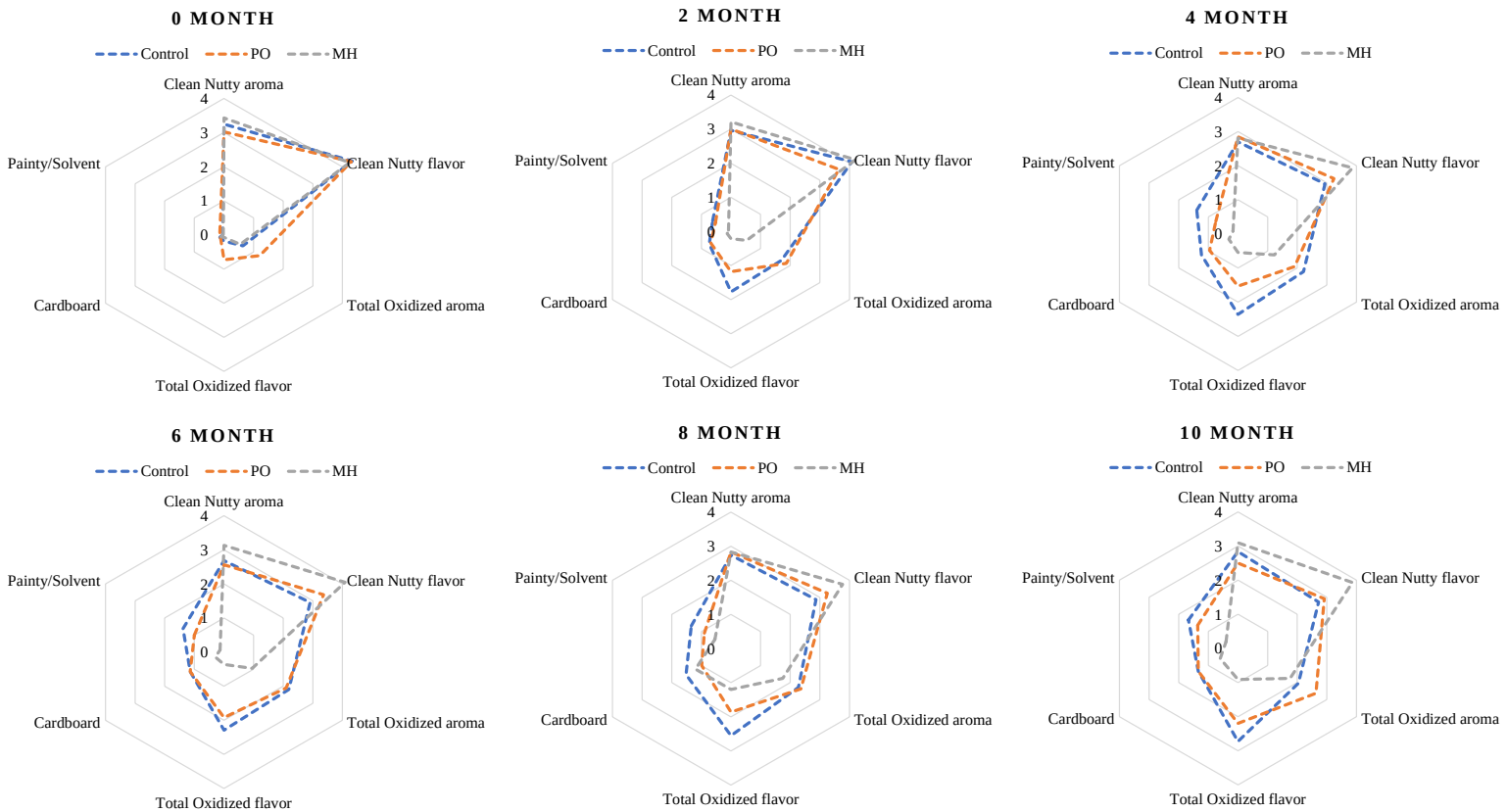

Figure 4.2. Rader plots with 2 positive sensory attributes (clean nutty aroma/flavor) and 4 negative sensory attributes (total oxidized aroma/flavor, painty/solvent, and carboard) measured in unpasteurized (control), propylene oxide pasteurized (PO), and moist heat pasteurized (MH) almonds at accelerated storage month 0, 2, 4, 6, 8, and 10 months.

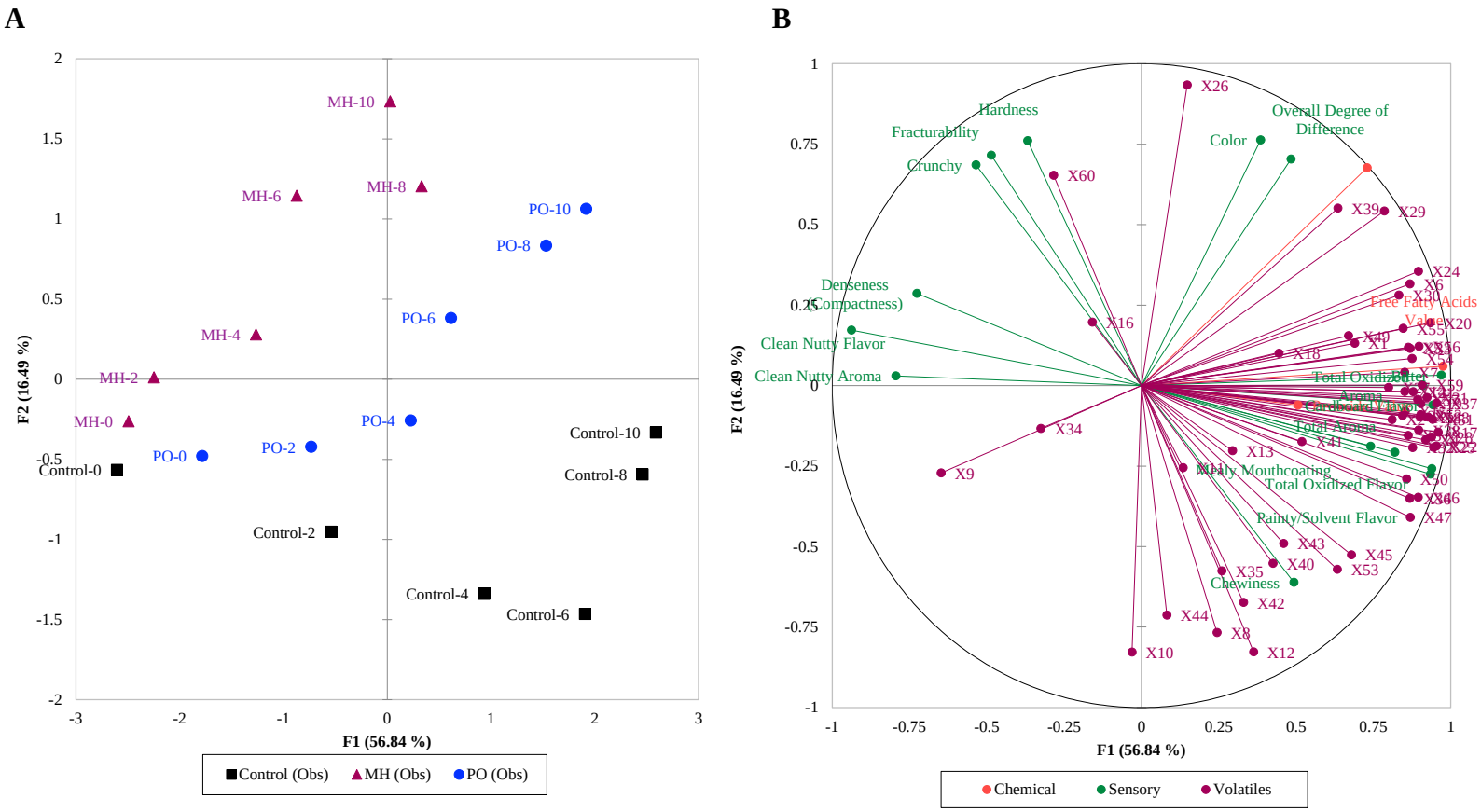

Figure 4.3. Multiple factor analysis (MFA) observation plot (A) and variables plot (B) on the headspace volatiles (volatiles corresponding to Table 4.2), oxidative stability markers (chemical), and sensory descriptive attributes (sensory). First 2 dimensions explained 73.33 % of the variables.

4.7 Supporting Information

Table 4.S1. Definition and reference scale on the 20 sensory attributes measured on the pasteurized and unpasteurized raw almonds stored under 12-month accelerated storage.

Overall Degree of Difference			
Sensory Attribute	Scale	Reference	Definition
Overall Degree of Difference			The overall impression of how different the sample is from the reference sample.
Appearance			
Sensory Attribute	Scale	Reference	Definition
Average Darkness of Color	0.0	White	The average darkness of the internal side of the almond when cut in half, rated from light to dark.
	10.0	Sepia crayola	
	15.0	Black crayola	
Aroma-Flavor			
Sensory Attribute	Scale	Reference	Definition
Total Aroma/Flavor			The total intensity of all the aromas or flavors in the sample.
Clean Nutty	5.0	Trader Joes Dry Roasted, Unsalted Almonds	The total intensity of clean or fresh nut character in the sample, including woody, marzipan/ benzaldehyde, sweet aromatic and fruity.
	NR	Walnut nut	
Benzaldehyde	NR	Maraschino Cherries in Juice	The aroma/flavor intensity associated with Marzipan and/or benzaldehyde; reminiscent to maraschino cherries or almond extract.
Total Oxidized			The total intensity of notes associated with an old/stale oil character or oil that is oxidized, painty, solvent, rancid or soapy.
Cardboard	NR	Cardboard soaked overnight at room temperature in Alhambra water	The intensity of notes associated with cardboard, stale, musty, dusty or sawdust.
Painty/Solvent	NR	Oil Library	The intensity of notes reminiscent of oil based paint, solvent, spoiled fish or rancid oil.

Bitter	2.0	0.06 g caffeine in 250 mL water	One of the basic tastes, common to caffeine.
	5.0	0.1 g caffeine in 250 mL water	

Texture			
Sensory Attribute	Scale	Reference	Definition
Hardness	5.0	Nabisco Chips Ahoy Cookies	Force required to chew through the sample using the molars, from soft to hard.
	7.0	Nabisco Wheat Thin Crackers	
	8.0	Nabisco Oreo	
	10.0	Old London Melba Toast	
	11.0	Nabisco Ginger Snap	
Fracturability	4.0	Nabisco Regular Chips Ahoy	The force with which the sample breaks, includes brittleness. Generally, an increase in auditory signals results from higher fracturability.
	5.0	Nabisco Graham Cracker	
	7.5	Nabisco Oreo	
	10.0	Old London Melba Toast	
	11.0	Nabisco Ginger Snap	
Crunchiness	4.0	Nabisco Regular Chips Ahoy	The amount of low-pitched noise a heavier, harder product makes during the chewing process.
	6.0	Nabisco Oreo	
	7.0	General Mills Wheat Chex	
Denseness	5.0	Pringles Potato Crisp	The compactness of the cross-section from airy to dense.
	7.0	Nabisco Regular Chips Ahoy	
	11.0	Keebler Pecan Sandie Cookie	

	12.0	Nabisco Fig Newton	
Chewiness	6.0	Snickers Bar	The total amount of “work” or force required to chew the sample once the bolus has broken down prior to swallowing.
Cohesiveness of Mass	1.5	Bush Garbanzo Beans	The degree to which the sample sticks to itself or forms a tight bolus as it is being chewed.
	5.0	Pringles Potato Crisp	
	7.5	Nabisco Graham Cracker	
Moistness of Mass	1.0	Nature Valley Granola Bar	The degree to which the sample mass is moist (tender) or dry (tough).
	4.0	Nabisco Regular Chips Ahoy	
	6.0	Snickers	
Mealy Mouthcoating	7.5	Almond Flour	The amount of mealy, grainy or particulates coating the mouth, perceived particularly in the back of the throat after swallowing.
Awareness of Skins			The awareness of skins in the sample during chewdown, including toughness and skin flakes.

Table 4.S2. Mean scores of each sensory attribute measured on a scale from 0 - 15. Almonds that were unpasteurized (control), pasteurized with propylene oxide (PO), and pasteurized by thermal moist air (MH) were measured at 0, 2, 4, 6, 8, and 10 months of accelerated storage. For each row, values with different letters indicates significantly different ($p < 0.05$).

Month	0			2			4			6			8			10		
Treatment	Control	PO	MH	Control	PO	MH	Control	PO	MH	Control	PO	MH	Control	PO	MH	Control	PO	MH
OVERALL DEGREE OF DIFFERENCE:																		
Overall Degree of Difference	0.47^{K}	0.83^{JK}	0.94^{IJK}	1.15^{HIJ}	$1.26^{GH\ IJ}$	$1.27^{FG\ HIJ}$	$1.32^{EFG\ HI}$	$1.30^{FG\ HIJ}$	$1.75^{CDE\ F}$	$1.63^{CD\ EFG}$	1.78^{CDE}	$2.07^{AB\ C}$	$1.59^{DE\ FGH}$	$1.59^{DE\ FGH}$	2.54^{A}	$1.57^{DE\ FGH}$	$1.95^{BC\ D}$	$2.42^{A\ B}$
APPEARANCE:																		
Color	7.18^{H}	$7.49^{EF\ G}$	7.39^{GH}	7.49^{EFG}	$7.59^{CD\ EFG}$	$7.65^{BC\ DEF}$	7.47^{FG}	$7.70^{BC\ DE}$	7.81^{B}	$7.67^{BC\ DEF}$	7.71^{BCD}	7.85^{B}	$7.64^{BC\ DEF}$	7.80^{BC}	7.82^{B}	$7.52^{DE\ FG}$	8.19^{A}	8.20^{A}
AROMA:																		
Total Aroma	$4.71^{C\ DE}$	$4.76^{BC\ DE}$	$4.68^{CD\ E}$	5.04^{ABC}	$4.97^{AB\ C}$	4.50^{DE}	4.98^{ABC}	5.15^{AB}	4.38^{E}	5.24^{A}	4.95^{ABC}	4.42^{E}	5.17^{AB}	5.22^{A}	$4.93^{AB\ CD}$	$4.92^{AB\ CD}$	5.19^{AB}	$4.93^{A\ BCD}$
Clean Nutty	$3.25^{A\ B}$	$3.02^{AB\ CDE}$	3.43^{A}	$2.96^{ABC\ DEF}$	$3.00^{AB\ CDE}$	$3.21^{AB\ C}$	2.70^{DEF}	$2.85^{BC\ DEF}$	$2.81^{BCD\ EF}$	$2.68^{DE\ F}$	2.56^{EF}	$3.13^{AB\ CD}$	$2.74^{CD\ EF}$	$2.83^{BC\ DEF}$	$2.83^{BC\ DEF}$	$2.84^{BC\ DEF}$	2.50^{F}	$3.10^{A\ BCD}$
Total Oxidized	0.64^{EF}	$1.21^{DE\ F}$	0.55^{F}	1.70^{BCD}	$1.87^{BC\ D}$	0.53^{F}	2.21^{AB}	1.91^{BC}	1.23^{CDE}	2.19^{AB}	2.10^{AB}	0.95^{EF}	2.2^{AB}	2.36^{AB}	$1.75^{BC\ D}$	2.04^{AB}	2.63^{A}	$1.76^{B\ CD}$
FLAVOR:																		
Total Flavor	5.25	5.41	5.26	5.38	5.19	5.24	5.35	5.29	5.24	5.43	5.36	5.25	5.3	5.32	5.46	5.38	5.33	5.28
Clean Nutty	4.33^{A}	4.30^{AB}	4.24^{AB}	$4.08^{ABC\ D}$	$3.64^{DE\ F}$	$4.20^{AB\ C}$	2.94^{GHI}	$3.24^{FG\ H}$	3.87^{BCD}	2.91^{HI}	3.36^{EFG}	$4.09^{AB\ C}$	2.87^{HI}	$3.25^{FG\ H}$	$3.77^{CD\ E}$	2.72^{I}	2.91^{HI}	$3.86^{B\ CD}$
Benzaldehyde	0.59	0.64	0.51	0.71	0.63	0.42	0.6	0.4	0.61	0.6	0.51	0.46	0.48	0.5	0.45	0.53	0.41	0.51
Total Oxidized	0.18^{K}	$0.73^{HIJ\ K}$	0.07^{K}	$1.77^{CDE\ F}$	$1.18^{FG\ HI}$	0.21^{K}	2.36^{ABC}	$1.53^{DE\ FG}$	0.54^{IJK}	$2.29^{AB\ C}$	1.92^{BCD}	0.36^{JK}	2.55^{AB}	$1.86^{CD\ E}$	$1.20^{EF\ GH}$	2.73^{A}	$2.20^{AB\ C}$	$0.91^{G\ HIJ}$
Cardboard	0.14^{F}	0.12^{F}	0.10^{F}	0.73^{CD}	0.68^{DE}	0.13^{F}	1.23^{AB}	$0.96^{BC\ D}$	0.31^{EF}	$1.14^{AB\ C}$	1.14^{ABC}	0.29^{EF}	1.52^{A}	$0.99^{BC\ D}$	1.20^{AB}	1.34^{AB}	1.34^{AB}	$0.60^{D\ E}$
Painty/Solvent	0.04^{G}	0.14^{G}	0.02^{G}	0.62^{DE}	0.52^{EF}	0.08^{G}	1.39^{A}	$0.75^{CD\ E}$	0.17^{FG}	1.38^{AB}	1.00^{BC}	0.13^{G}	1.34^{AB}	0.90^{CD}	$0.54^{DE\ F}$	1.67^{A}	1.36^{AB}	$0.40^{EF\ G}$
Bitter	0.36^{H}	$0.55^{FG\ H}$	0.41^{GH}	0.75^{DEF}	$0.80^{CD\ EF}$	0.38^{H}	1.07^{ABC}	$0.88^{CD\ E}$	0.70^{EFG}	$1.05^{AB\ C}$	$0.93^{BCD\ E}$	$0.69^{EF\ G}$	$1.06^{AB\ C}$	$0.99^{AB\ CD}$	$0.89^{BC\ DE}$	1.22^{A}	1.17^{AB}	$0.86^{C\ DE}$
TEXTURE:																		
Hardness	$6.50^{A\ BC}$	$6.40^{BC\ DE}$	$6.43^{BC\ D}$	6.08^{EF}	$6.25^{CD\ EF}$	$6.31^{BC\ DEF}$	6.16^{DEF}	$6.28^{BC\ DEF}$	$6.36^{BCD\ E}$	6.02^{F}	$6.46^{ABC\ D}$	6.77^{A}	$6.18^{DE\ F}$	$6.39^{BC\ DE}$	$6.44^{BC\ D}$	$6.32^{BC\ DEF}$	$6.36^{BC\ DE}$	$6.57^{A\ B}$

Fracturability	8.24^{A} B	8.09^{AB} CD	8.01^{AB} CDE	7.75^{EFG}	7.83^{DE} F	7.99^{AB} CDE	7.43^{G}	7.82^{DE} F	7.87^{CDE}	7.52^{FG}	7.93^{BCD} E	8.18^{AB} C	7.71^{EF} G	8.13^{AB} CD	7.93^{BC} DE	7.73^{EF} G	8.02^{AB} CDE	8.28^{A}
Crunchy	5.34^{A} BCD	5.33^{AB} CD	5.43^{AB}	5.27^{ABC} DE	5.19^{BC} DEF	5.28^{AB} CDE	4.88^{FG}	5.06^{CD} EFG	5.18^{BCD} EFG	4.85^{G}	5.16^{BCD} EFG	5.31^{AB} CDE	5.02^{DE} FG	5.43^{AB}	5.40^{AB} C	4.98^{EF} G	5.23^{AB} CDE	5.55^{A}
Denseness	9.59^{A}	9.41^{AB} CD	9.43^{AB} CD	9.40^{ABC} D	9.32^{AB} CDE	9.46^{AB} C	9.15^{DEF}	9.20^{CD} EF	9.37^{ABC} D	8.98^{F}	9.28^{BCD} EF	9.48^{AB} C	9.21^{CD} EF	9.52^{AB}	9.14^{DE} F	8.98^{F}	9.05^{EF}	9.53^{A} B
Chewiness	2.23^{C} DEF	2.20^{DE} F	2.11 F	2.23^{BCD} EF	2.35^{AB} CDE	2.20^{DE} F	2.30^{ABC} DEF	2.37^{AB} CD	2.30^{ABC} DEF	2.47^{A}	2.29^{ABC} DEF	2.18^{DE} F	2.44^{AB} C	2.16^{DE} F	2.18^{DE} F	2.44^{AB}	2.12^{F}	2.15^{EF}
Cohesiveness of Mass	2.92	2.87	2.91	2.81	3.06	2.8	2.77	2.83	2.75	2.7	2.81	2.86	2.83	2.82	2.81	2.76	2.8	2.75
Moistness of Mass	2	1.95	1.96	1.99	1.97	1.97	1.93	2.09	2	1.87	1.94	2.01	1.99	1.92	1.92	1.96	1.95	1.9
Mealy Mouthcoating	3.97^{C} DE	3.94^{DE}	3.95^{DE}	3.99^{CDE}	4.10^{BC} DE	3.99^{CD} E	4.14^{BCD} E	4.09^{BC} DE	4.02^{CDE}	4.29^{B}	4.21^{BCD}	3.90^{E}	4.57^{A}	4.16^{BC} DE	4.07^{BC} DE	4.23^{BC}	4.16^{BC} DE	4.09^{B} CDE
Awareness of Skins	2.13	2.05	2.07	2.08	2.1	2.07	2.07	2.07	2.14	2.2	2.14	2.1	2.18	2.08	2.12	2.08	2.17	2.04

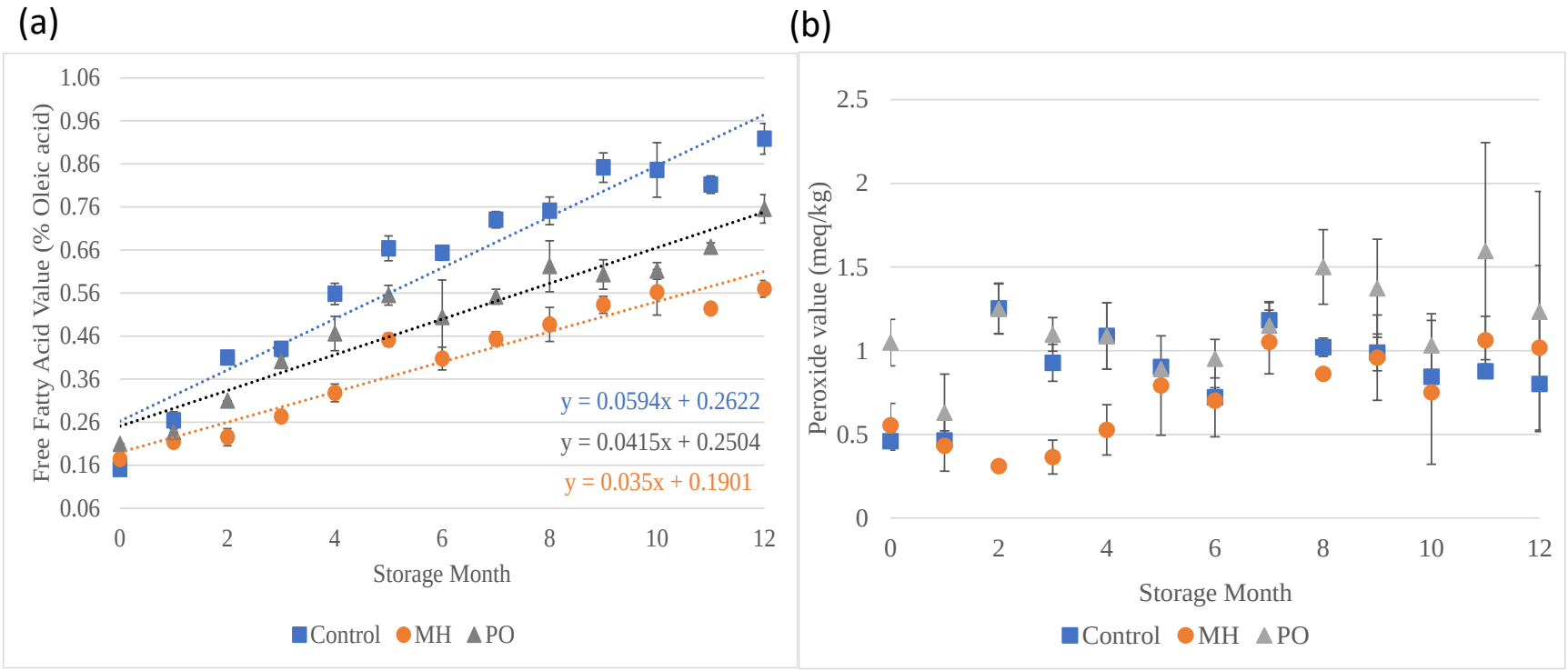

Figure 4.S1. Oxidative stability markers (a) free fatty acid levels and (b) peroxide values measured monthly for unpasteurized almond (control), pasteurized with propylene oxide (PO), and pasteurized by thermal moist air (MH).

Chapter 5: Acrylamide Mitigation in Almonds during Storage

5.1 Abstract

Acrylamide is a probable human carcinogen (Group 2A) classified by the International Agency of Research on Cancer that occurs naturally after food is exposed to high temperature (e.g. roasting). This occurs in food that is rich in asparagine and reducing sugars. However, the level of acrylamide has shown to decrease during food storage. Model systems have demonstrated that acrylamide can undergo Michael addition with nucleophilic groups (-SH, -NH_2) found within amino acids. Free amino acid concentration can contribute to the formation of acrylamide and potentially the reduction during storage. Glutathione, a tripeptide, has also shown to undergo Michael addition with acrylamide in model systems. In this work, the free amino acid, glutathione as well as acrylamide were quantified in three varieties of California almonds and a store-bought almond butter. These levels were measured during storage at room temperature for up to 12 weeks. Raw almonds had showed high concentration of glutathione and free asparagine at 201.34 ± 8.73 mg kg^{-1} and 1755.87 ± 328.38 mg kg^{-1} respectively. After roasting, free amino acids and glutathione concentrations decreased 10 - 90 % and the acrylamide-glutathione conjugate was observed in roasted almonds and found in all almond butter samples. This is the first demonstration of acrylamide-glutathione conjugate detection in almonds. Acrylamide decreased 10 – 33% after 12 weeks of storage and acrylamide-glutathione conjugate showed a negative correlation with acrylamide level. Therefore, glutathione may play a role in the reduction of acrylamide in roasted almonds.

Keywords: Acrylamide, almond, almond butter, free amino acid, glutathione

5.2 Introduction

Acrylamide is classified as a probable human carcinogen (group 2A) by the International Agency for Research on Cancer in 1994[1]. It is a naturally occurring chemical that is formed in foods after heat treatment (e.g. roasting, baking) and has been on the California Proposition 65 list of compounds known to cause cancer, birth defects or reproductive harm since the 1990s[2]. Many studies have investigated the formation of acrylamide, risk assessment, and ways to mitigate acrylamide in food and beverages since the first study published in 2002 by the Swedish National Food Administration and the University of Stockholm3. The mechanism of acrylamide formation involves the reaction between reducing sugars and asparagine while heated under low-moisture conditions as part of the Maillard reaction[3-5]. Almonds contain carbohydrates (2-12 %), proteins (10-35 %), and are low in moisture content[6], making almonds susceptible to acrylamide formation after roasting, especially when asparagine is one of the major free amino acid present[7]. Roasted almonds have been reported to contain ~200 μg kg^{-1} of acrylamide, with a weak correlation between the amount of free asparagine and the amount of acrylamide formed in almonds among different varieties[8]. Acrylamide has been reported to decrease during the storage of almonds, coffee beans, and canned coffees[8-10]. In roasted Nonpareil almonds, Zhang et al. (2011) reported that the acrylamide levels decreased by an average 6.7 % at room temperature storage after one month[8]. Another study reported that the acrylamide level decreased an average of 50.2 % after three days of accelerated storage at 60 °C[11]. However, the mechanism of the acrylamide level decrease during storage is still unknown in almonds.

While the reduction of acrylamide in foods during storage is not well understood, model systems have shown the loss of acrylamide during storage depends on the nucleophilic groups (-SH, $-NH_2$) present to undergo Michael addition[10, 12-14]. Zamora et al. (2010) have shown that

acrylamide can form Michael addition with sulfhydryl groups or amine groups found in amino acids[12]. In some model systems, the amount of acrylamide formed is also related to the relative abundance of different free amino acids present[15]. Koutsidis et al. (2009) showed that the presence of glycine and cysteine lowered the amount of acrylamide formed after roasting, while valine, glutamine, and leucine increased the amount of acrylamide[15]. Recently, Yoshioka et al. (2020) showed that cysteine and lysine from milk formed Michael addition conjugates with acrylamide in black coffee[10]. The conjugate formation was responsible for 69.6% of the acrylamide removal during storage in canned milk coffee. This is the first study identifying and confirming that addition of lysine and cysteine can reduce acrylamide levels in real food systems during storage.

Previous studies indicate that cysteine, lysine, and glycine can react with acrylamide at elevated temperatures to form Michael adducts, yet these are not the most abundant amino acids found in almonds[12, 13, 16, 17]. Total amino acid profiles of almonds showed the cysteine level is 0.2 - 0.3 g/100 g protein, lysine is 2.0 - 2.4 g/100 g protein, and glycine is 5.6 - 5.8 g/100 g protein18. Glutamic acid, asparagine, and aspartic acid have been reported to be the most abundant amino acids in almonds, but no information is available for Michael addition reactions with acrylamide for these amino acids[18, 19]. Besides focusing on the total amino acid profile in a food system, the amount of free amino acids present can also have an impact on the formation and removal of acrylamide. The amount of free amino acids has been reported in different almond varieties but only selected amino acids (asparagine and glutamine) has been studied for the commercially available California varieties[8, 20, 21]. Different varieties have varied amounts of free amino acids which can be considered for breeding to improve almond quality[8, 21].

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) is a tripeptide that is highly reactive and is involved in many cellular processes including cellular detoxification and redox signaling[22]. Glutathione has shown to have an anti-carcinogenic property and can be found in many foods, such as pork, potato, asparagus, and avocado[23, 24]. Although this peptide is present in our diet, the amount of glutathione in tree nuts has not been reported. Glutathione can impact the level of acrylamide through the sulfhydryl group on the tripeptide acting as a nucleophilic group to form a Michael addition with acrylamide (Figure 5.1)[25]. Zhu et al. (2020) demonstrated success in using glutathione to inhibit acrylamide in a model system and in cookies along with the inhibition and elimination pathway[26, 27].

California supplies ~80 % of the almonds consumed throughout the world and the almond industry generates 104,000 jobs in California and contributes $11 billion to the state's economy[28]. Being the largest grower of almonds in the world, it is important to ensure the industry is providing healthy and safe products to the consumers. Acrylamide formation is inevitable during almond heat processing, but the acrylamide levels can be mitigated by controlling heat, various processing parameters (e.g. time) and through the use of additives pre- or post- porcessing[8, 29, 30]. Acrylamide conjugates formed through Michael addition have been reported to have less bioavailability compared to acrylamide, potentially making conjugates safer than acrlyamide[10, 31]. This study will first determine the free amino acid and glutathione concentrations in almonds, monitor the acrylamide concentration during storage and determine potential Michael addition conjugates formed that can contribute to the decrease in acrylamide level.

5.3 Methods and Materials

Chemical and Reagents

LC-MS grade acetonitrile, formic acid, ammonium formate and analytical grade hydrochloric acid (HCl) were obtained from Fisher Chemical (Fisher Scientific, Hampton, NH, USA). Water was purified using a Milli-Q purification system (Millipore, Bedford, MA, USA). Analytical standards, acrylamide (> 99%), acrylamide-d3 (98% D), amino acid standards and glutathione (analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The extraction was performed with QuEChERS pouches containing 4 g MgSO4 and 0.5 g NaCl, which were purchased from Agilent Technology (Agilent Technology, Santa Clara, CA, USA).

Experimental Design

Three varieties of almonds (Aldrich, Fritz, and Nonpareil) of 2020 harvest year were obtained from Blue Diamond Growers (Sacramento, CA). Five pounds of kernels of each variety were received. These varieties represent different types and shapes of almonds grown in California and commonly found on the market. Three bottles from three different production lots of the same type of store-bought almond butter containing roasted almonds were used as the almond butter sample. To induce acrylamide formation in the almonds, each almond variety was separated into three portions and dry-roasted in three separate batches at 176 ± 3 °C for 10 minutes using an E32D5 Turbofan electric convection oven (Moffat Inc., Winston-Salem, NC). Samples were then stored in a temperature-controlled incubator (Thermo Scientific, Waltham, MA, USA) kept at 30 ± 2 °C. Every week for up to 12 weeks, an aliquot of the almonds (30 g) and almond butter (5 g) were taken and analyzed for free amino acid, glutathione, acrylamide, and its conjugates.

Almond and Nut Butter Sample Extraction

One composite containing 15 g each was sampled from each batch for the almond samples. The nut meat was crushed with a wooden mallet, ground by a spice grinder (Waring

Laboratory Equipment, Torrington, CT, USA) and sieved through a 20-mesh standard screen (W.S. Tyler Industrial Group, Mentor, OH, USA). Nut butters were homogenized by stirring vigorously prior to sampling and 2 g of each almond butter was sampled each week. The extraction method for acrylamide in almonds were modified from De Paola et al. (2017)[32]. To extract acrylamide, an aliquot of 1.00 ± 0.02 g sample was transferred into a 50mL Falcon tube, 10 µL of 10 µg mL^{-1} internal standard acrylamide-d_3 internal standard was spiked into the sample. The extraction was performed by adding 10 mL of water and 10 mL of acetonitrile to the sample and vortexed for 30 sec after each addition. The salt packet (4 g MgSO4 and 0.5 g NaCl) from the Acrylamide QuEChERS Extraction Kit (5982-5850, Agilent Technology Inc., Santa Clara, CA) was then added and vortexed for 1 min. The sample was then centrifuged at 4000 g for 15 min to separate the water and acetonitrile layer. A 100 µL aliquot of the upper acetonitrile layer was then diluted into 900 µL of water for the acrylamide analysis. All samples were stored at -20 °C prior to analysis.

The extraction method for free amino acids in almonds were modified from Zhang et al. (2011) to maximize the amount of free amino acid measured[8]. To extract for free amino acids, glutathione, and acrylamide conjugates, an aliquot of 0.20 ± 0.02 g ground sieved almond sample was transferred into a 15 mL tube and extracted with 5 mL of 0.1 N hydrochloric acid solution under a shaker at 350 rpm for 1 hour. The extract was centrifuged at 4000 g for 15 min and a 100 µL aliquot of the solution was transferred to a microcentrifuge along with 900 µL of acetonitrile to remove the protein. The mixture was then centrifuged at 15000 rpm for 15 min at 8 °C. All samples were stored at -20 °C prior to analysis.

Detection of Acrylamide

The determination of acrylamide was performed on an Agilent 1290 UHPLC system interfaced with a 6460 triple quadrupole mass spectrometer (QqQ MS/MS, Agilent Technology, Santa Clara, CA, USA). The spectrometer was equipped with an electrospray ionization source via Jet Stream Technology. The extract was separated on a Hypercarb column (2.1 x 100 mm, 3 µm, Thermo Scientific, USA), and the column temperature was controlled at 30 °C. The mobile phase consists of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B) starting at 5 % B. Samples were injected at 5 µL injection volume and was analyzed under isocratic condition at 2.5 min per sample. After 12 injections, a cleanup method was applied by holding at 90 % B for 11 minutes and re-equilibrated to 5 % B for 10 minutes. The QqQ MS/MS source was optimized at 300 °C gas temperature and flow rate at 5 L min^{-1}. The sheath gas temperature and flow rate were at 400 °C and 11 L min^{-1}, respectively. The nebulizer gas pressure, capillary voltage and nozzle voltage was set at 45 psi, 3000 V and 500 V, respectively. The transition settings and the linear concentration ranges are reported in supplemental information (Table 5.S1). Quantification was achieved using an internal calibration curve. The recovery was performed in roasted almonds and seasoned almonds with a 20, 200, and 2000 ng mL^{-1} spike. The recovery was 98.2 ± 3.6 % among different matrix and concentration tested.

Determination of Free Amino Acids and Glutathione

The chromatography method was adapted from Huang et al. (2018) to measure amino acids and glutathione[33]. Amino acids and glutathione were separated using a Poroshell 120 HILIC-Z column (2.1 x 100 mm, Agilent Technology, USA) with temperature controlled at 30 °C and injection volume of 2 µL. A stock solution of 200 mM of ammonium formate was made and adjusted to pH =3 using formic acid for the mobile phase. Mobile phase A consist of 20 mM of ammonium formate in water adjusted to pH = 3. Mobile phase B consist of 10 % of the 200

mM ammonium formate stock solution and 90 % of acetonitrile (volume/volume %). The starting condition was set as 100 % mobile phase B and a slow gradient to 70 % mobile phase B at 10 min with a constant flow rate of 0.3 mL min^{-1}. The column was flushed at 50 % mobile phase B for 3 minutes and equilibrated at the starting condition of 100 % mobile phase B for 10 minutes. The amino acids were measured in the MS/MS under positive mode with gas temperature set at 300 °C flow at 7 L min^{-1}. The sheath gas was set at 400 °C and 11 L min^{-1}. The condition was optimized to 45 psi for the nebulizer and 2000V for the capillary. The transitions and optimized conditions for each amino acid along with the linear concentration ranges are reported in supplemental information (Table 5.S1). Quantification was achieved by external calibration curve.

Determination of Acrylamide Conjugates

The acrylamide conjugates were obtained by reacting acrylamide (1 mg mL^{-1}) with perspective amino acids (cysteine, lysine, glycine, glutamic acid, asparagine, and aspartic acid) or glutathione in water incubated in 40 °C water bath at two concentrations (1 mg mL^{-1} and 10 mg mL^{-1}). All standards were prepared in 0.1 N HCl solution. The acrylamide conjugates were separated on a Hypercarb column (2.1 x 100 mm, 3µm, Thermo Scientific, USA) with the column temperature controlled at 30 °C. Samples were injected at 5 µL. The mobile phase consists of 1 % formic acid in water (A) and 1 % formic acid in acetonitrile (B) delivered at 0.3 ml min^{-1}. The starting condition was a 2 minute hold at 5 % mobile phase B followed with a ramp to 90 % mobile phase B in 8 minutes. The column was cleaned at 90 % mobile phase B for 4 minutes and equilibrated to the starting condition for 4 minutes. The drying gas was set at 250 °C and 8 L min^{-1} and the sheath gas set at 350 °C and 11 L min^{-1}. The nebulizer was set at 35 psi

with the capillary set at 3000 V and nozzle voltage set at 500 V. The transition parameters are reported in supplemental information (Table 5.S1).

5.4 Results and Discussion

The levels of acrylamide, glutathione, free amino acids, and potential acrylamide Michael addition conjugates were monitored in the three varieties of roasted almonds and one type of store-bought almond butter for up to 12 weeks. Among the chosen amino acids (cysteine, lysine, glycine, glutamic acid, asparagine, and aspartic acid) and glutathione reacting with acrylamide in this experiment, only the acrylamide-glutathione conjugate was formed that can be used as a standard. Hence, only the acrylamide-glutathione conjugate was reported in this study.

Free amino acids in almonds

A chromatography method that does not require amino acid derivatization for 18 amino acids and glutathione was used to measure free amino acids and glutathione in almond and almond butter (Table 5.1, Figure 5.S1). The most abundant free amino acid found in raw almond is asparagine (1755.87 ± 328.38 mg kg^{-1} almond at fresh weight) among all varieties. Asparagine is reported to be the most abundant free amino acid found in almonds, with some reporting glutamine or arginine being the second most abundant[20]. The asparagine levels (Table 5.1) were similar to previously reported levels on Nonpareil and Fritz variety, with Fritz having higher level of free asparagine than Nonpareil[8, 19]. Free amino acid levels were last reported by Carratala et al. (2002) in Spanish almonds and showed similar relative distribution of free amino acids found in California almonds[21]. Glutathione levels are reported in raw almonds for the first time with an average of 201.34 ± 8.73 mg kg^{-1} almond at fresh weight.

All free amino acid concentrations decreased after roasting and glutathione was below the limit of detection (< 156.5 mg kg^{-1}) after roasting (Table 5.1). Almond butter was first measured

when purchased, and it showed similar levels of free amino acids as the roasted almonds, but with much higher amount of asparagine (Table 5.1). Similar trend of free amino acid decreasing after roasting was reported by Zhang et al. (2011) who monitored asparagine and glutamine level in almonds before and after roasting[8]. After 12 weeks of storage, most amino acid concentrations remained constant or increased over time (Table 5.1). The high temperature during roasting can denature the protein and release more free amino acids that can be extracted. Only glycine, serine, and histidine have a significant decrease in concentration. These amino acids may contribute to the decrease in acrylamide level.

Acrylamide levels in roasted almonds

Acrylamide was measured in the roasted almonds and almond butter and reported at week 0, 4, 8, and 12 (Figure 5.2). Acrylamide concentrations in roasted almonds (176 °C for 10 min) averaged 1420 ± 269 μg kg^{-1} with no significant differences between the three varieties at the start of the storage study. This level was comparable to Zhang et al. (2011) who reported an average acrylamide concentration of 1469 ± 121 μg kg^{-1} when roasted at 168 °C for 8 min[8]. Another study reported acrylamide concentration to be below 2 μg kg^{-1} in store-bought roasted almonds utilizing a similar extraction method[32]. The roasting parameter used in our study was a dark roast setting, which creates a dark brown color and significantly higher levels of acrylamide[8]. To date, the concentration of acrylamide in almond butters have not been reported in the literature. However, a 2019 survey conducted by the Center of Food Safety and Applied Nutrition under the Food and Drug Administration that surveyed acrylamide in food a reported acrylamide concentration in almond butter found to be between < 10 – 570 μg kg^{-1} depending on the brand[34]. Almond butter contained an average of 493 ± 66 μg kg^{-1} (Figure 5.2), which falls within the range reported by the survey study and within the range of roasted almonds (132 –

1469 µg kg^{-1}) reported by Zhang et al. (2011)[8]. Most almond butters are made by roasted almonds, unless specified as raw almond butter. Currently, there is no regulation on the acrylamide level found in food. However, a recommendation has been placed by the regulatory agencies for the industry to monitor the acrylamide level in their product and suggested ways (e.g. lower heat processing) to mitigate the acrylamide level.

In this work, the acrylamide level decreased 10 – 33 % during 12 weeks of storage at 30 °C (Figure 5.2). A stability study on almonds reported that acrylamide decreased 0 – 17.7 % after 1 month storage at room temperature, a 12.9 – 68.5 % decrease after 3 days of storage at 60 °C, yet the acrylamide level increased after storage at 80 °C due to acrylamide formation at higher temperature[8]. The increase in acrylamide reduction rate when the storage temperature is elevated was also observed in cocoa powder, instant coffee, and baby foods that contained milk powder[35]. Almond butter has an acrylamide decreasing rate of reduction between the 3 varieties of almonds measured in this study. This suggested that the homogenous form of almond butter does not impact the acrylamide reduction rate during storage. However, the composition of the almond affected by the varieties may play a bigger role in the acrylamide reduction rate during storage.

Acrylamide conjugates in almond

Acrylamide Michael addition conjugates have been investigated and discovered in other model systems[10, 12, 26]. After reacting selected amino acids (cysteine, lysine, glycine, glutamic acid, asparagine, and aspartic acid) and glutathione with acrylamide for 3 weeks, only glutathione formed a conjugate. The system yielded a conjugate product that shared the same mass spectrum reported by Zhu et al. (2020) in their model system[27]. The chromatography method used for acrylamide measurement was optimized for the detection of the acrylamide-glutathione conjugates. As a proof of concept that the acrylamide-glutathione can be formed and

found in real food system, the acrylamide-glutathione conjugate extraction nor the limit of the detection were investigated in this study. The extract that contains the free amino acids from roasted almonds and almond butter were measured for acrylamide-glutathione conjugates. Relative quantification was performed to observe the quantity of conjugates present during storage. The peak area of the quantifier mass transition of the acrylamide-glutathione conjugate was divided by the sample weight for comparison (Figure 5.S2). This step corrects for the differences in sample size. Some conjugate levels increased over storage time, but there were no significant differences across storage time (Figure 5.3). The acrylamide-glutathione conjugate was not found in raw almonds prior to roasting but was observed after the roasting at week 0. Almond butter has the highest amount of acrylamide-glutathione conjugate, which may be due to the longer storage that has occurred (the time elapsed between roasting, and measurement is longer than the freshly roasted almonds) and the sample being a homogenous mixture leading to better extraction. Glutathione contributed to binding with acrylamide after roasting by forming Michael addition conjugates. However, our study showed that glutathione may contribute to the reduction of acrylamide during storage with a slight negative correlation between acrylamide and acrylamide-glutathione conjugate during storage ($r(46)= -0.56$, $p < 0.05$).

The free amino acid and glutathione concentration indicated that these compounds may play a role in acrylamide mitigation during storage. Further study will be needed to understand the role of the decreasing amino acids (glycine, serine, and histidine) in acrylamide reduction during storage. Elevated storage temperature may also change the rate of acrylamide reduction and the rate of free amino acid reduction during storage.

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Supporting Information Description

Includes the table for mass transitions and optimized parameters along with linear concentration range for acrylamide, amino acids, glutathione, and acrylamide-glutathione conjugate, two figures containing the sample chromatograms of Aldrich variety, one showing the free amino acids and glutathione before and after roasting and one showing the acrylamide-glutathione conjugate at 0 and 12 weeks of storage.

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5.6 Tables and Figures

Table 5.1. Free amino acid levels and glutathione levels (mg kg^{-1} almond, fresh weight) measured in three almond varieties and an almond butter. Levels were measured in raw almonds, roasted almonds at 0 week of storage, and roasted almonds at 12 weeks of storage.

	Raw			Roasted, 0 week				Roasted, 12 weeks			
	Nonpareil	Fritz	Aldrich	Nonpareil	Fritz	Aldrich	Almond butter	Nonpareil	Fritz	Aldrich	Almond butter
Phenylalanine	154.31 ± 26.29	125.69 ± 5.17	136.75 ± 1.80	31.05 ± 4.63	64.28 ± 9.06	40.32 ± 7.05	73.38 ± 5.46	33.27 ± 6.22	85.51 ± 44.74	39.05 ± 4.10	57.31 ± 5.05
Tryptophan	59.48 ± 4.92	63.39 ± 5.35	53.04 ± 3.50	14.67 ± 2.98	28.66 ± 2.01	13.58 ± 0.52	33.53 ± 2.06	27.85 ± 3.06	70.17 ± 33.97	25.80 ± 1.24	46.91 ± 3.10
Leucine	139.56 ± 4.89	73.95 ± 2.91	103.69 ± 10.95	34.42 ± 5.59	41.39 ± 5.13	28.31 ± 0.93	47.07 ± 1.61	25.47 ± 3.49	30.51 ± 6.60	18.48 ± 1.15	36.57 ± 4.84
Isoleucine	115.92 ± 12.90	84.55 ± 8.26	102.79 ± 8.82	47.90 ± 6.52	59.29 ± 3.16	41.63 ± 1.21	63.78 ± 3.93	51.39 ± 6.79	87.97 ± 44.92	41.19 ± 2.01	54.63 ± 4.93
Methionine	31.17 ± 6.10	35.92 ± 2.95	26.20 ± 3.13	6.71 ± 1.47	16.45 ± 2.01	6.40 ± 0.92	14.10 ± 2.29	5.65 ± 1.46	16.34 ± 9.16	4.85 ± 0.51	10.54 ± 2.10
Valine	136.36 ± 26.62	92.96 ± 7.71	118.45 ± 4.04	45.22 ± 9.56	63.81 ± 5.52	41.93 ± 3.10	76.10 ± 5.79	50.18 ± 8.37	80.41 ± 33.96	42.73 ± 1.17	69.90 ± 9.12
Proline	357.05 ± 66.21	441.68* ± 89.93	530.77* ± 42.09	153.44 ± 16.27	254.03 ± 13.55	219.91 ± 4.58	299.91 ± 44.62	185.39 ± 18.07	348.75 ± 163.83	236.52 ± 2.16	288.12 ± 34.45
Alanine	240.37* ± 33.19	145.43 ± 4.88	161.41 ± 11.96	127.39 ± 15.05	140.60 ± 6.63	112.39 ± 7.72	165.66 ± 13.58	198.40 ± 11.35	226.32 ± 88.48	150.47 ± 13.49	204.98 ± 18.37
Threonine	91.00 ± 13.70	68.43 ± 8.88	72.15 ± 7.98	41.23 ± 12.43	54.26 ± 1.29	33.98 ± 7.28	59.83 ± 5.83	41.96 ± 7.61	61.03 ± 25.49	37.15 ± 2.72	53.13 ± 9.86
Glycine	166.51 ± 7.95	158.88 ± 13.92	171.80 ± 14.61	123.38 ± 4.99	139.78 ± 33.53	122.33 ± 8.20	144.43 ± 42.97	50.46 ± 18.92	101.62 ± 38.00	58.00 ± 7.39	86.93 ± 34.35
Glutamine	1091.86* ± 135.96	1683.34* ± 120.00	1552.98* ± 358.33	40.19 ± 12.93	65.33 ± 8.99	55.58 ± 7.37	60.75 ± 7.16	33.51 ± 7.85	96.02 ± 57.09	42.31 ± 3.55	57.42 ± 13.35
Serine	221.16* ± 7.54	179.30 ± 12.72	193.43 ± 14.37	171.68 ± 3.86	157.94 ± 12.91	127.51 ± 10.52	159.92 ± 10.01	89.55 ± 19.10	124.12 ± 41.75	60.57 ± 14.97	93.97 ± 29.65
Asparagine	1711.87* ± 11.48	2083.02* ± 367.06	1472.27 ± 111.25	496.39 ± 113.78	1177.20 ± 132.71	547.13 ± 77.96	1894.15* ± 143.05	781.93 ± 139.50	1816.52 ± 878.77	762.40 ± 219.64	1977.34 ± 177.15
Glutamic acid	279.90* ± 29.29	237.83 ± 8.06	175.73 ± 25.90	55.18 ± 10.46	85.11 ± 11.01	42.77 ± 2.87	84.20 ± 12.62	86.84 ± 11.38	146.00 ± 77.71	62.48 ± 3.95	101.45 ± 15.94
Aspartic acid	266.47 ± 23.95	284.17 ± 20.36	277.76 ± 11.06	168.80 ± 17.24	253.30 ± 11.80	187.19 ± 15.13	268.50 ± 22.92	212.69 ± 22.64	359.89 ± 89.24	224.34 ± 15.69	276.88 ± 18.58

Histidine	197.15 ± 22.82	205.69 ± 22.39	215.55 ± 10.17	115.58 ± 6.83	149.02 ± 1.07	112.57 ± 9.19	170.32 ± 7.17	56.51 ± 4.59	102.07 ± 36.19	58.09 ± 1.01	83.39 ± 8.21
Arginine	549.16 ± 111.71	1483.22* ± 253.12	757.34 ± 85.52	389.06 ± 161.11	1204.38 ± 178.14	477.93 ± 54.29	1466.42* ± 32.83	285.26 ± 59.81	1152.96 ± 542.80	348.48 ± 46.17	987.54 ± 57.49
Lysine	152.14 ± 26.75	183.23 ± 15.89	162.87 ± 12.51	86.68 ± 6.66	141.75 ± 4.50	94.68 ± 9.45	129.33 ± 11.34	103.23 ± 9.40	340.22 ± 315.07	102.20 ± 3.90	128.75 ± 13.03
Glutathione	207.53 ± 11.08	201.79 ± 7.00	194.71 ± 3.11	< 156.5							

*Concentration above the highest concentration of the linear response range

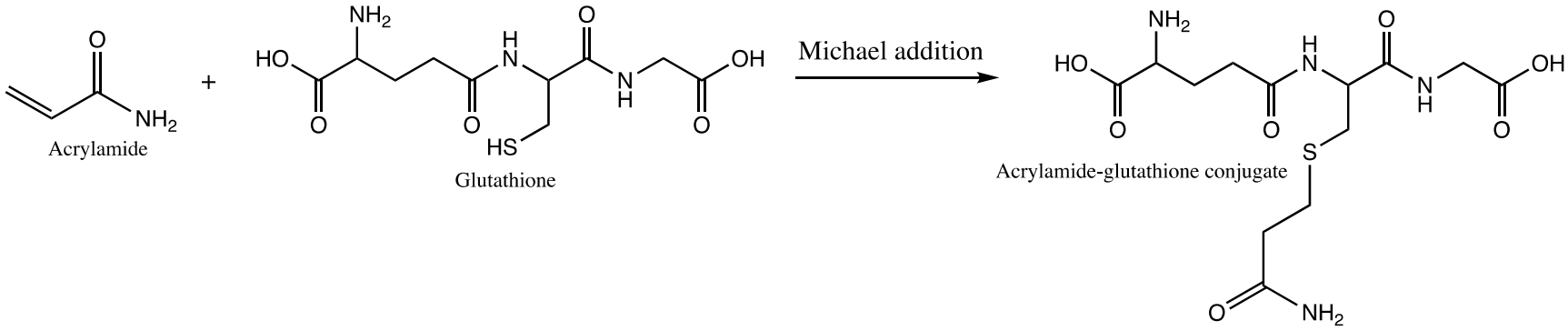
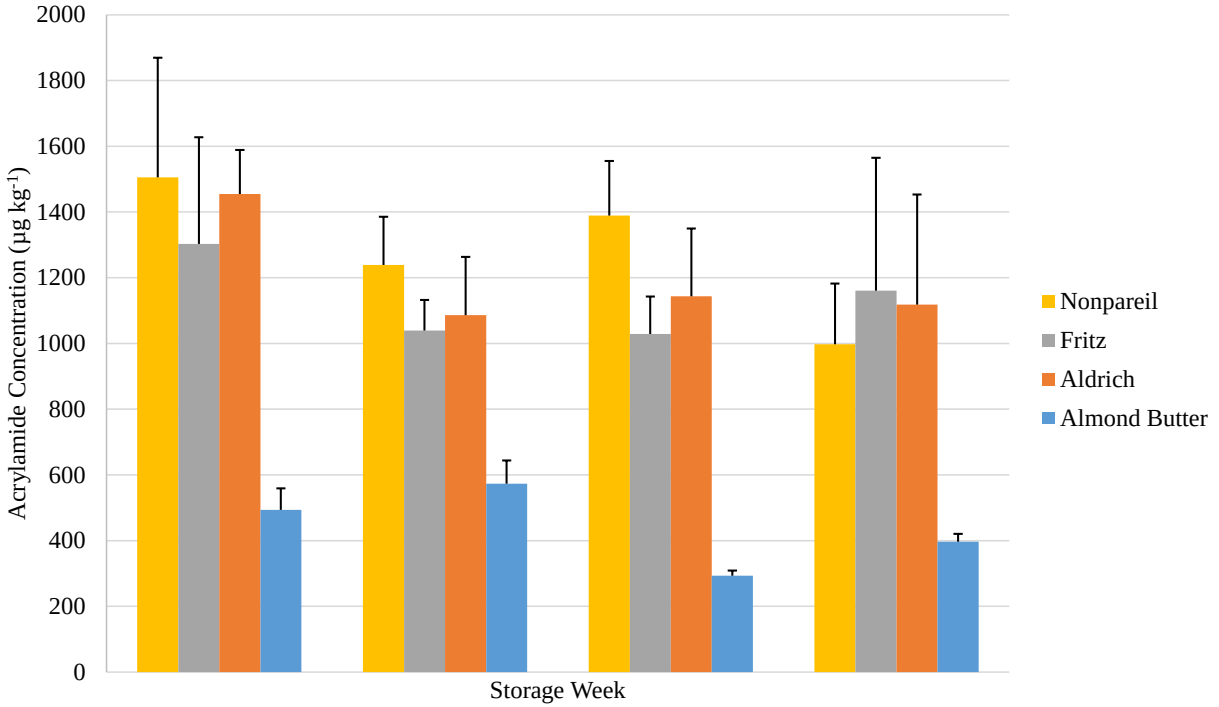


Figure 5.1. Acrylamide-glutathione conjugate possible formation pathway through Michael addition.



	0	4	8	12	Overall decrease (%)
Nonpareil	1505.36 ± 364.07	1238/47 ± 147/32	1389.08 ± 1166.16	997.65 ± 184.38	33.7
Fritz	1302.91 ± 324.42	1039.31 ± 92.95	1028.76 ± 113.83	1161.00 ± 404.18	10.9
Aldrich	1454.46 ± 134.45	1086.01 ± 177.66	1143.18 ± 206.42	1118.51 ± 334.65	23.1
Almond Butter	493.35 ± 66.04	573.14 ± 70.56	293.41 ± 15.13	397.17 ± 23.73	19.5

Figure 5.2. Acrylamide concentration (µg kg^{-1} almond, fresh weight) measured in three almond varieties and commercially available almond butter over 12 weeks of storage at 30 °C with overall % decrease.

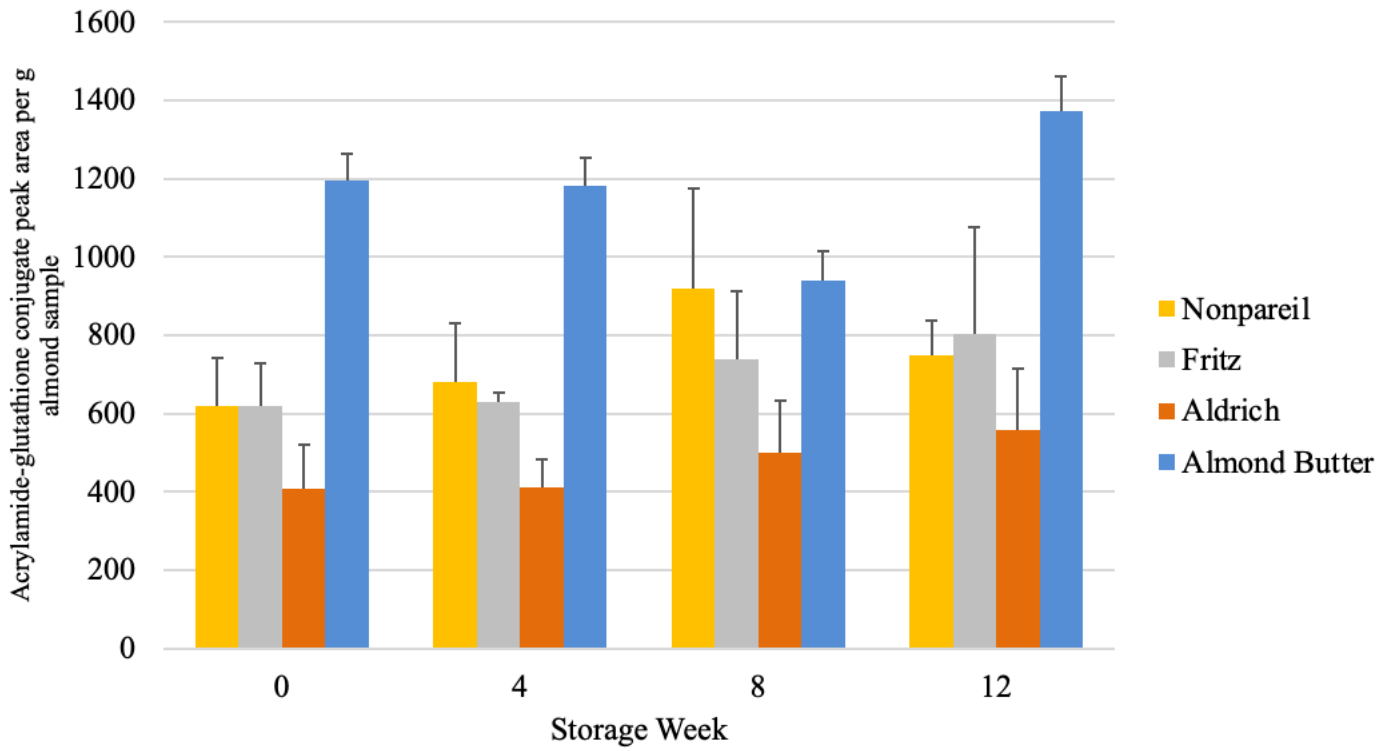


Figure 5.3. Acrylamide-glutathione conjugate levels measured in peak area per gram of almond sample during 12 weeks of storage.

5.7 Supporting Information

Table 5.S1. Mass spectrometer transition condition of acrylamide, acrylamide-d_3, amino acids, glutathione, and acrylamide-glutathione conjugate under positive mode.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Fragmentor (V)	Collision energy (V)	Cell accelerator (V)	Linear range (ng mL^{-1})
Acrylamide (Quantifier)	72.0	55.0	85	8	7	0.1-250
Acrylamide (Qualifier)	72.0	54.0	85	8	7	
Acrylamide-d_3	75.0	58.0	85	8	7	
Alanine	90.1	44.2	70	6	7	215-1612.5
Arginine	175.1	70.1	80	24	7	680-5100
Asparagine	133.1	74.0	65	12	7	418-6270
Aspartic acid	134.0	74.0	60	9	7	171-2565
Glutamic acid	148.1	84.1	65	13	7	134-2010
Glutamine	147.1	84.1	55	13	7	64-2432
Glutathione (Quantifier)	308.0	179.2	70	10	7	626-15650
Glutathione (Qualifier)	308.0	162.1	70	15	7	
Glycine	76.0	30.3	65	4	7	82.25-1645
Histidine	156.1	110.1	60	10	7	123.5-938.6
Isoleucine	132.1	86.1	75	6	7	38.9-972.5
Leucine	132.1	86.1	75	6	7	24.9-249
Lysine	147.1	84.1	55	13	7	162.5-1235
Methionine	150.1	104.0	65	6	7	17.6-352
Phenylalanine	166.1	120.1	70	9	7	30.7-767.5
Proline	116.1	70.1	75	13	7	265-1987.5

Serine	106.1	60.0	57	5	7	108.5-1627.5
Threonine	120.1	74.1	65	6	7	55.25-839.8
Tryptophan	205.1	188	70	4	7	10.7-537.5
Valine	118.1	72.1	70	6	7	28.7-717.5
Acrylamide-glutathione (Quantifier)	379.0	250.0	100	8	4	
Acrylamide-glutathione (Qualifier)	379.0	104.0	100	25	4	

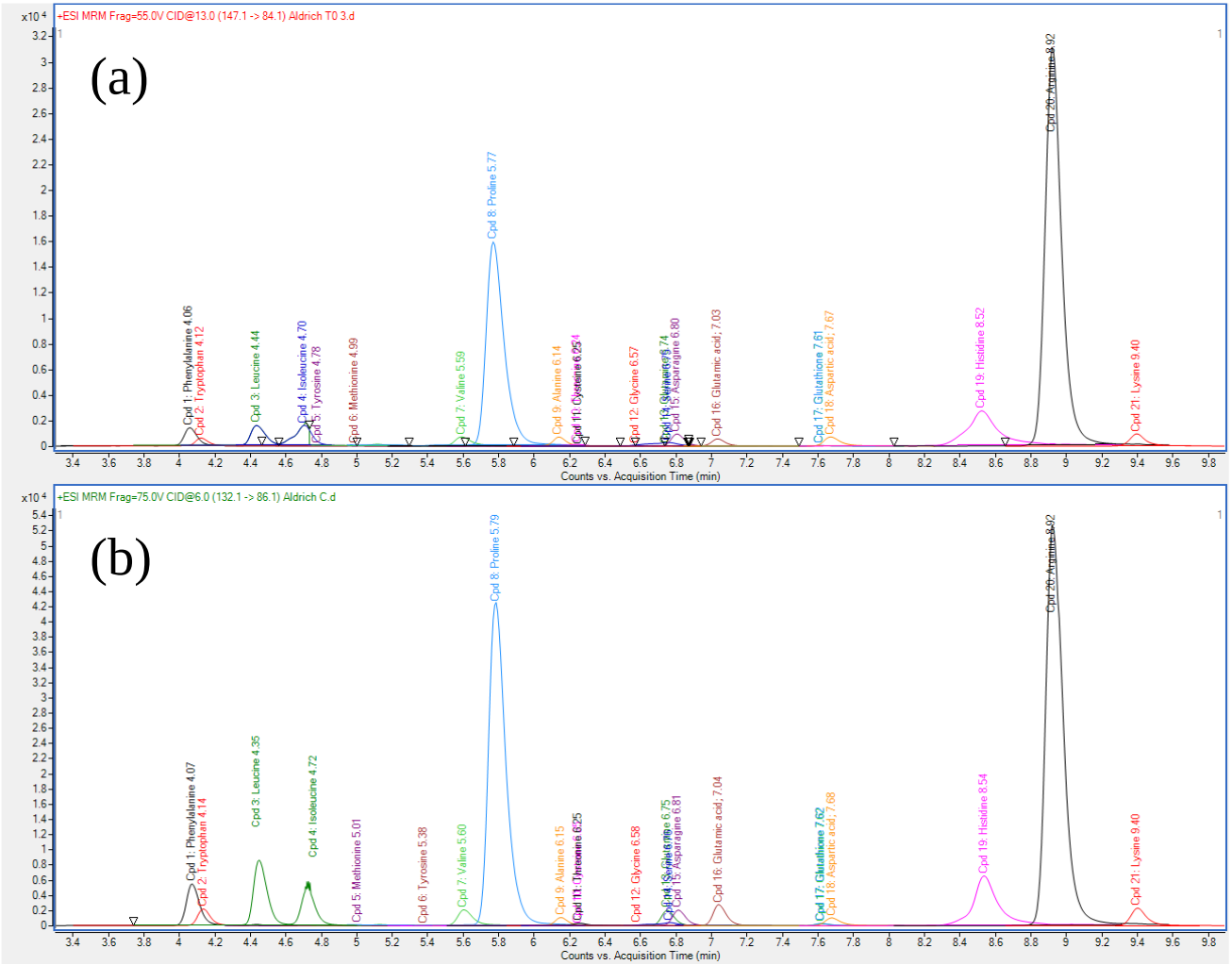


Figure 5.S1. The chromatograms of free amino acids and glutathione showing Aldrich variety almond (a)before roasting and (b)after roasting before storage.

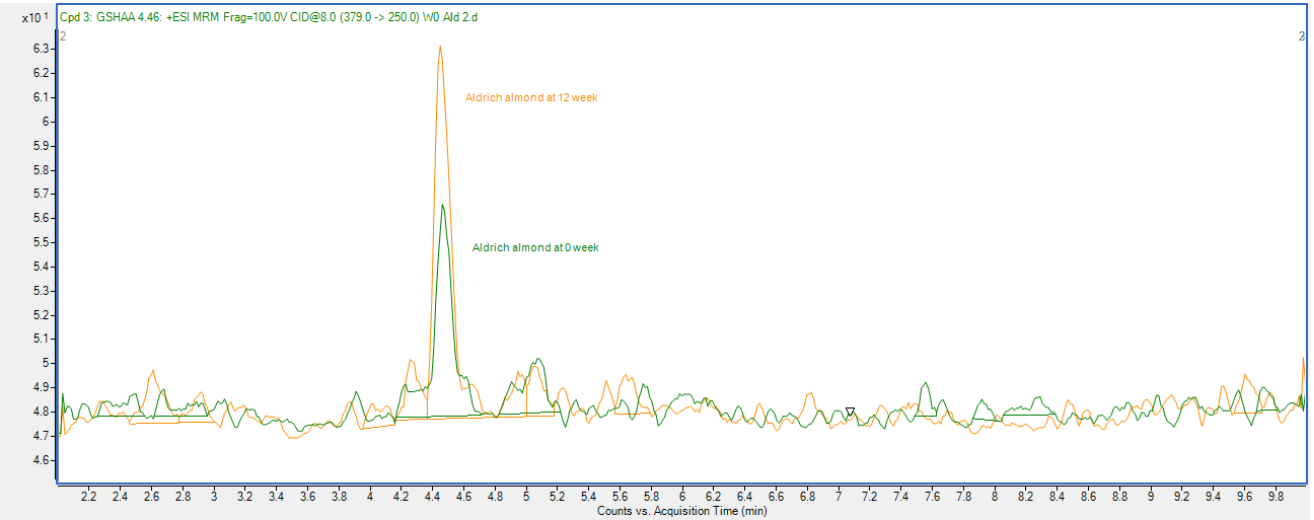


Figure 5.S2. The chromatograms of acrylamide-glutathione conjugate found in roasted Aldrich variety almond at 0 week and 12 week of storage.

Chapter 6: Conclusion

Due to the expansion of tree nut cultivation and an increase in severe weather events, there is a need to better understand the potential impact of rain and processing on almond quality. This research sought to understand the quality and chemical safety of almonds during storage by assessing postharvest treatments and processing methods. In this work, quality is evaluated by measuring lipid oxidation markers, headspace volatiles, and sensory analysis with samples stored in accelerated storage for up to 12 months. To assess chemical safety, acrylamide concentration was measured, and potential mitigation strategies were explored in almonds and almond butter. By understanding the effect postharvest moisture exposure and pasteurization have on almond quality, we can better understand how to improve crop management and how lipid oxidation and rancidity develop in almonds. Studying methods to naturally mitigate the acrylamide level during storage and the natural rate of decline can provide clarity for future regulation for acrylamide levels in almonds and its products.

Due to the increase production in almonds, almonds are left out in the field for longer durations. Chapter 2 and 3 of this work describe the effect of postharvest moisture exposure (MEx) on roasted and raw almonds through the evaluation of peroxide values, free fatty acids, conjugated dienes, headspace volatiles, and sensory analysis during 12 months of accelerated storage. At 5 months of accelerated storage, MEx dark roasted (i.e. roasted at higher temperature) almonds have significantly higher levels of lipid oxidation compared to those without moisture exposure (control). For light roasted (i.e. roasted at lower temperature) almonds, consumers cannot tell the difference between the MEx almond and control. However, trained panelists can detect sensory attributes related to lipid oxidation at 7 months of storage, which correlate with increased levels of volatiles related to lipid oxidation. Although raw almonds have a shelf life of up to 2 years, MEx raw almonds may have only up to 1 year of shelf life due to the increased level of lipid oxidation.

Climate change is causing more extreme weather patterns and unpredictable rain events. Almonds are exposed to postharvest moisture while waiting to be processed. The results from both chapters can help nut processors better control inventories and target these MEx nuts for products with shorter shelf life. By doing so, tree nut processors can improve the quality of their products to consumers and reduce food waste due to product rancidity.

Chapter 4 of this work applies methods developed in Chapter 2 and 3 to measure lipid oxidation in almonds that have undergone pasteurization using either moist heat (MH) or propylene oxide (PO). At the end of the accelerated storage, unpasteurized samples have the highest concentration of lipid oxidation related volatiles (e.g. hexanal, octanal, hexanoic acid) up to 3 times higher than MH samples and the highest mean scores of rancidity related sensory attributes. At 8 months of accelerated storage, MH samples were significantly different from the control with high fresh almond attributes and low rancidity attributes. Pasteurization has shown to decrease the rate of lipid oxidation in almonds and delay rancidity development. This finding indicates that moist heat pasteurization provides protection against biological hazard (e.g. *Salmonella*) and lipid oxidation in tree nuts. Moist heat pasteurization provides safer food to the public and extends the shelf life of tree nuts, which reduces food waste and helps battle food insecurity.

Chapter 5 of this work describes the development of methods for the measurement of free amino acids, glutathione, and acrylamide in roasted almonds and in almond butter over 12 weeks of storage. Nucleophiles can scavenge acrylamide through Michael addition reactions post thermal treatments. We investigated the sulfhydryl-scavenging and amine-scavenging ability of free amino acids and glutathione by monitoring conjugate formation and acrylamide loss. Free amino acid profile before and after thermal processing was established in three California almond varieties

with asparagine being the most abundant. Free glutathione is first reported in raw California almonds at 201.34 ± 8.73 mg kg^{-1} fresh weight. Levels of acrylamide decreased 10 – 33 % during 12 weeks of storage at 30 °C. Acrylamide-glutathione conjugates were detected in roasted almonds and almond butter. Among the free amino acids measured, only glycine, serine, and histidine decreased during storage. Further studies are needed to better understand the role of the amino acids in acrylamide decline during storage. The rate of acrylamide decreasing naturally in almond products provides information for future regulation on acrylamide level in food. The enhanced understanding of the role of free amino acids and glutathione in acrylamide mitigation can help improve the chemical safety of thermal processed almonds.

The shelf life of almonds has been better understood through the utilization of chemical and sensorial measurements. However, further studies are required to understand the complex matrix effects on the relationship between lipid oxidation volatiles and sensory attributes describing rancidity. This information would assist in the determination of how different fatty acid profiles in food contribute to rancidity related sensory attributes. This study has helped gain insight into the free amino acid profiles of almonds and how they change after roasting and storage. Future studies should focus on the competition between free amino acids to form Michael addition conjugates with acrylamide. This can provide insight on which amino acids can be used to mitigate acrylamide during storage.