# ASSESSING THE SHELF STABILITY OF RAW NONPAREIL ALMONDS THROUGH SENSORY, CHEMICAL AND INSTRUMENTAL MEANS

by

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(Under the Direction of Ruthann Swanson)

#### **ABSTRACT**

Suboptimal storage conditions and/or lengthy storage cause degradation of almond quality and results in consumer rejection. Raw Nonpareil almonds were stored in polypropylene bags and unlined cartons under different conditions (15, 25 or 35°C with 50 or 65% relative humidity and 4°C/no RH control). Over 24 months sensory, chemical and instrumental testing was conducted. Consumers rejected all samples stored in unlined cartons and four samples stored in polypropylene bags during the storage period. Regression results indicated that flavor was the strongest sensory determinant and free fatty acid and peroxide levels were the strongest nonsensory determinants of overall acceptability. The sample stored in unlined cartons at 4°C experienced the greatest degradation of quality due to the lack of RH control. Almond quality was best maintained when stored in polypropylene bags at low temperature and low RH; under these conditions, shelf life was determined to be at least two years.

INDEX WORDS: acceptability, consumers, degradation, flavor, free fatty acid, peroxide, quality, rancidity, raw almonds, sensory, shelf life, storage, texture

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#### CHAPTER 1

#### INTRODUCTION

Nut consumption in general has been linked to numerous health benefits including reducing risk of cardiovascular disease, type 2 diabetes and obesity, among others (Albert and others 2002; Berryman and others 2015; Bes-Rastrollo and others 2009; Hull and others 2014; Jaceldo-Siegl and others 2004; Jackson and Hu 2014; Martinez-Gonzalez and Bes-Rastrollo 2010; Wien and others 2003). Almonds are the highest produced tree nut in the world (International Nut and Dried Fruit Foundation 2010) and are the most consumed nut in America, with the consumption of almonds over double the consumption of walnuts, hazelnuts, pecans and pistachios combined (Almond Board of California 2013a; Almond Board of California 2014). Not only are almonds the most popular nut, the consumption has been steadily increasing over the last few decades. The per capita consumption of almonds has more than quadrupled since 1980 and was 2 pounds in 2013 (USDA ERS 2014). Furthermore, over 82% of the world's almond production occurs in California, with approximately two-thirds of the almonds being shipped internationally (Almond Board of California 2013a). Almonds are marketed and consumed in a variety of product forms, including in-shell, shelled kernels and peeled seeds; whole or as pieces; and can be found both raw and roasted (Almond Board of California 2013b; Harris and Ferguson 2013; Shahidi and John 2013).

#### **Nutritional content and health benefits of almonds**

Almonds are a nutrient dense source of numerous micronutrients (Chen and others 2006). Almonds are an excellent source (containing >20% Daily Value) of vitamin E and manganese and a good source (containing 10-20% Daily Value) of magnesium, copper, phosphorus, fiber and riboflavin (Chen and others 2006; USDA ARS 2013). In a standard 28 g (1 oz) serving, raw almonds provide 6 g of protein, being one of the highest protein-containing nuts, and 164 kcal of energy (Ros 2009; USDA ARS 2013). The energy provided by almonds is largely due to their fat content; 49.4% of the weight of raw almonds is from fat, 76% of the fat being monounsaturated fatty acids. Almonds also have good antioxidant activity due to their high vitamin E ( $\alpha$ -tocopherol), phenolic and polyphenol contents (Chen and others 2006; Huang 2014).

Almonds and other nuts have been shown to have anti-inflammatory action; inflammation is known to play a significant role in the risk and progression of cardiovascular disease and diabetes (Chen and others 2006; Jenkins and others 2008a; Ros 2009). Frequent consumption of nuts (including almonds) is believed to have an inverse relationship with the biomarkers of these diseases. There is also evidence that frequent consumption of nuts, grains and cereals is protective against prostate cancer (Chen and others 2006).

Due to their low carbohydrate content, nuts make little contribution to increasing postprandial glycemic responses (Jenkins and others 2008a) and have even been found to reduce the glycemic response in a dose-dependent manner (Jenkins and others 2008a; Josse and other 2007; Kendall and others 2011). As the consumption of almonds increases from 0 to 90 grams as an addition to a high carbohydrate meal, the glycemic index of carbohydrate foods has been seen to decrease linearly with increasing consumption (Jenkins and others 2008a; Josse and others 2007). Josse and others (2007) believe that the reduced glycemic effect can be explained in part

by the high concentration of polyphenols and phytic acid found in the skin of raw almonds; phenolics and phytates have been shown to be associated with a decrease in postprandial glycemia in vivo as well as a decrease in amylolytic digestion in vitro (Josse and others 2007). While the consumption of mixed nuts, including almonds, with bread has been found to have an inverse relationship with glycemic index, the reduction in glycemic response in individuals with type 2 diabetes was about half that seen in nondiabetic individuals (Kendall and others 2011).

Daily consumption of almonds as a snack has been shown to have numerous health benefits and to positively impact body composition of individuals (Albert and others 2002; Berryman and others 2015; Bes-Rastrollo and others 2009; Hull and others 2014; Jaceldo-Siegl and others 2004; Jackson and Hu 2014; Ros 2009; Martinez-Gonzalez and Bes-Rastrollo 2010; Wien and others 2003). The results of the Physicians' Health Study showed an inverse relationship between nut consumption (including almonds) and total coronary heart disease death; Albert and others (2002) believe that this inverse relationship is largely due to a reduction of the risk of sudden cardiac death. In 2003 the FDA decided there was sufficient evidence that the consumption of nuts (including almonds) was inversely associated with the risk of cardiovascular disease (Albert and others 2002) and they created the following qualified B-level health claim: "eating 1.5 ounces per day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease" (US FDA 2003).

Nut consumption has been shown to be inversely associated with the risk of type 2 diabetes (Jackson and Hu 2014; Jenkins and others 2008a), as well as potentially being protective against development of type 2 diabetes (Jackson and Hu 2014). The results of a randomized control trial among 418 subjects at high risk of developing diabetes found that the group who consumed a Mediterranean style diet supplemented with mixed nuts, including

almonds, had a lower incidence of diabetes than the group who did not consume nuts (Jackson and Hu 2014; Salas-Salvadó and others 2011). When the effect of almonds on insulin secretion was investigated by Jenkins and others (2008b) in nondiabetic hyperlipidemic individuals, they found that there was a significant decrease in 24-hour insulin secretion, as indicated by a reduction in urinary C-peptide output, in individuals that consumed 37 g or 73 g of almonds each day when compared to individuals who did not. Although this study investigated the effect over a short period of time, the implication was that there was a reduction in insulin demand and postprandial glucose tolerance (Jenkins and others 2008b).

Hull and others (2014) found a portion-dependent relationship between consumption of almonds as a mid-morning snack and food intake at subsequent meals throughout the day in healthy females. The researchers also determined that self-reported appetite ratings of the individuals during the time between the mid-morning snack and lunch were related to the amount of almonds consumed as the snack in a dose-dependent manner. Additionally, there was no net increase in energy consumed over the day, regardless of the amount of almonds (0, 28 or 42 g) consumed as the mid-morning snack; this indicates that the participants compensated for the excess calories contributed by the almonds and experienced satiety at the same time (Hull and others 2014).

The consumption of nuts has been associated with lower weight gain, increased weight loss, and/or lower incidence of overweight/obesity (Berryman and others 2015; Bes-Rastrollo and others 2009; Jackson and Hu 2014; Martinez-Gonzalez and Bes-Rastrollo 2010; Wien and others 2003). An inverse relationship has been seen between frequency of nut consumption and weight gain over long periods of time (Bes-Rastrollo and others 2009) and typical nut consumption of up to 4 servings per week has not been shown to result in weight gain in the

long-term (Martinez-Gonzalez and Bes-Rastrollo 2010). Nut consumers also tend to have lower BMIs than individuals who do not consume nuts (Martinez-Gonzalez and Bes-Rastrollo 2010). The potential mechanisms that explain the decrease in body weight and prevention of weight gain due to nut consumption are shown in Figure 1.1 (Jackson and Hu 2014).

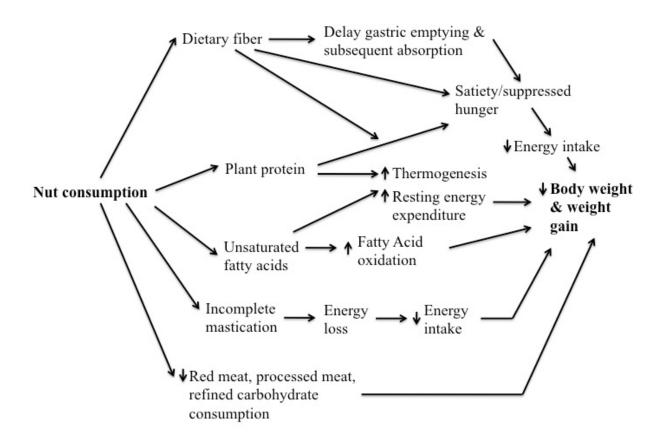


Figure 1.1: The potential link between nut consumption and body weight/weight gain (adapted from Jackson and Hu 2014)

Over a 24-week period, an almond-enriched low-calorie diet (84 g almonds/day) resulted in a significantly greater reduction in weight, BMI, waist circumference, fat mass, total body water and systolic blood pressure when compared to a complex carbohydrate-enriched low calorie diet among 65 overweight and obese adults (Wien and others 2003). Specifically, Wien

and others (2003) found a 62%, 50% and 56% greater reduction in weight/BMI, waist circumference and fat mass, respectively, among the individuals who consumed the almondenriched diet. Additionally, high-density lipoprotein cholesterol levels increased in the group consuming complex carbohydrate enriched diet while the HDL cholesterol levels decreased in the group consuming the almond enriched diet (Wien and others 2003).

A controlled-feeding study in individuals with elevated LDL cholesterol in which a cholesterol lowering diet with 1.5 oz almonds consumed per day versus an isocaloric muffin substitution (no almonds consumed) showed that the diet containing almonds resulted in decreased LDL and VLDL cholesterols. Additionally, almond consumption was found to result in decreased abdominal and leg fat, despite no differences in total body weight (Berryman and others 2015). Jaceldo-Siegl and others (2004) followed approximately 80 individuals for one year and found that daily almond consumption resulted in an overall improvement of nutrient content of the individuals' diets. The diet that included daily supplementation of almonds (average 52 g/day) resulted in an increase in mono and poly unsaturated fatty acids, fiber, vegetable protein and alpha-tocopherol intake as well as a decrease in intake of trans fatty acids, animal protein, cholesterol, sugars and sodium (Jaceldo-Siegl and others 2004).

### Characterization and production of almonds

Almonds (*Amygdalus communis*), which are categorized botanically as a fruit, can be classified as either sweet or bitter (Almond Board of California 2013b; Harris and Ferguson 2013). California is the only state in the United States that grows almonds commercially, which is due to the hot, dry summers and cool, rainy winters common to California. There are approximately thirty varieties of sweet almonds produced in California, which are categorized into broad classifications based on distinctive characteristics such as shape, size and

blanchability. The three major classifications of almonds produced in California are California, Mission and Nonpareil (Almond Board of California 2013b), with Nonpareil almonds being the highest produced variety and accounting for approximately 36-40% of the yearly almond production (Almond Board of California 2013a; Almond Board of California 2014). Nonpareil almonds are easily blanched and have a variety of uses; these almonds are often used when an attractive appearance or a distinctive almond identification is desired (Almond Board of California 2013b).

Almonds grow on trees, which are pollinated by bees and bloom from mid-February through March; almond kernels develop in a shell surrounded by a hull that splits open once the nut is mature. The nuts dry naturally in their shell until they are harvested by mechanical tree shakers between mid-August and October. Once harvested, the kernels go through processing where cleaning and grading occurs (Almond Board of California 2013b). In almonds, the kernel is the edible product for human consumption and the dried hulls are typically used for animal feed (Harris and Ferguson 2013).

Almonds are graded based on standards established by the USDA. USDA grading parameters include dissimilar nuts or double nuts (when two nuts develop in one shell); presence of chips, scratches, foreign material, particles or dust; and being split or broken. Grades used to classify almonds, in decreasing order of quality are "US Fancy," "US Extra No. 1," "US No. 1," "US Select Sheller Run," "US Standard Sheller Run," "US No. 1 Whole and Broken" and "US No. 1 Pieces." Any lot containing more than two varieties is classified as "Mixed varieties." The highest grade of almonds, "US Fancy," is not widely used; in contrast, "US Extra No. 1" is commonly used and is ideal for food applications where the appearance of the almond is very important. "US No.1" almonds are typically used in food applications where further processing

such as blanching or roasting is desired and are sometimes referred to as "Supreme". "US Select Sheller Run" are typically used for applications where the mid-quality almonds can be mixed in with other ingredients and "US Standard Sheller Run" are a good grade for further processing such as chopping, grinding, paste or oil (Almond Board of California 2013b; Harris and Ferguson 2013; USDA 1997).

Once graded, raw almonds are either stored, shipped to consumers or undergo further processing, and the key to maintaining almond quality is that they be properly handled. Almonds are typically held in bins, silos or other bulk containers and it is recommended they be stored under controlled conditions. The Almond Board of California recommends almonds be stored under cool, dry conditions (less than 10°C/50°F and less than 65% relative humidity), in which case they believe whole natural almonds can be stored for two years without a significant decrease in quality (Almond Board of California 2013b; Huang 2014). One reason low temperatures and humidity are recommended for the storage of almonds is because almonds can undergo non-enzymatic browning when stored at high temperatures (>38°C/100°F), and this is especially true at high humidity (Huang 2014). It is also recommended that almonds be protected from oxygen, either through nitrogen flushing and/or vacuum packaging, and that the nuts should avoid exposure to strong odors so that they do not absorb those odors (Almond Board of California 2013b).

Another important processing step that all almonds undergo is pasteurization, which is the final step before the almonds are shipped to consumers. Effective September 1, 2007, the USDA requires that "handlers must subject their almonds to a treatment process or processes that achieve in total a minimum 4-log reduction of *Salmonella* bacteria" (USDA 2007). One common method of pasteurization utilized for natural almonds is polypropylene oxide (PPO), which is an

EPA registered pesticide approved as a fumigant. PPO is not only effective against *Salmonella*, but also against bacteria, yeast, mold and insects. The same protocol is followed for almond kernels and almonds in-shell. The control factors are that the PPO concentration be a minimum of 0.5 oz PPO/ft<sup>3</sup> in vapor and that the almond kernels reach a minimum temperature of 30°C prior to treatment (Prakash 2013).

Almonds are one of the most versatile nuts, being available in numerous forms and for a variety of applications, such as confectionary, baked goods, dairy, prepared foods and snacks. Almonds can be found plain, roasted and sometimes flavored (such as salted) and common forms of almonds include whole, sliced, diced, meal or flour and paste or butter, all of which can be made from natural or blanched almonds and blanched almonds also can be found slivered (Almond Board of California 2013b; Harris and Ferguson 2013). The degree of processing (almond form) also plays a role in the rate of deterioration of quality such as oxidation or rancidity development (Huang 2014).

## **Almond composition**

García-Pascual and others (2003) found that despite Nonpareil almonds having a low fat content (compared to other varieties of almonds), they have high peroxide values ( $5.8 \pm 0.07$  meq/kg before storage, increasing to as much as  $23.8 \pm 0.09$  meq/kg depending on storage conditions). The researchers suggest this could indicate that Nonpareil almonds are more sensitive to rancidity than other varieties of almonds (García-Pascual and others 2003), although this study indicated higher peroxide levels than other studies (Lin and others 2012; Mexis and Kontominas 2010; Senesi and others 1991). The most commonly recommended cutoff for peroxide values of raw almonds in industry is 5 meq/kg (Almond Board of California 2013b; Huang 2014).

Almonds are a low moisture food that have high levels of natural antioxidants, which can help prolong the shelf life if properly handled (Almond Board of California 2013b). In the study by Xiao and others (2014), a moisture level of  $5.05 \pm 0.09\%$  for raw almonds was found, which is consistent with moisture contents reported in other literature (3.5-6%, Almond Board of California 2013b;  $4.3 \pm 0.04\%$ , García-Pascual others 2003). It is generally recommended that almond moisture be maintained below 6%, but almonds can absorb or lose moisture during storage depending on their initial moisture content and the humidity of the storage environment. If almonds absorb moisture during storage, they can lose their crunch, lipid oxidation can increase and their microbial stability can decrease (Huang 2014).

Volatile compounds in nuts are responsible for the characteristic flavor properties (Xiao and others 2014); however, development of volatile compounds over time beyond what is naturally present has been shown to have negative consequences on food quality and consumer acceptability (Mexis and Kontominas 2010). Xiao and others (2014) analyzed raw almonds to determine the volatiles present and identified 41 different volatiles.

#### Significance and innovation of research on the shelf life of almonds

Since almonds are harvested once per year, determining the optimal storage conditions is important to prevent deterioration in quality during storage and shipment (Almond Board of California 2013a; Shahidi and John 2013). During the 2013-2014 crop year, California produced a crop of 2.01 billion pounds of almonds with roughly one-third shipped domestically and the remainder shipped abroad (Almond Board of California 2014). While there have been studies on the optimal storage conditions of almonds, there is currently a lack of information on the shelf life of almonds when held under varying conditions and the best indicator of consumer rejection

is unknown. Additionally, the relationship between consumer acceptability/intent to consume and chemical/instrumental indicators of almond quality is not well established.

The research question for this thesis project was "under which storage conditions (packaging, temperature and relative humidity) is the shelf life quality of whole raw Nonpareil almonds best maintained?" The overall hypothesis was that raw almond quality would be best maintained when the nuts were stored in sealed bags under low humidity and low temperature. The objectives for this research project were:

- 1. Conduct consumer sensory panels at 2 month intervals for almonds stored under a variety of conditions once triggered by a chemical assessment that suggests deterioration of quality.
- 2. Determine if there is a relationship between the sensory attributes as evaluated by the consumer panelists and almond acceptability and/or rejection rate.
- Compare the results from the consumer sensory panels to the results of the chemical and instrumental analyses to identify which chemical/instrumental tests best explain sensory acceptability.
- 4. Define the shelf life for almonds stored under varying conditions.

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#### **CHAPTER 2**

#### LITERATURE REVIEW

This chapter represents a review of literature about how quality and stability of food is monitored, specifically through shelf life testing. The shelf life of nuts is outlined with an emphasis on the primary mechanisms leading to decreased quality of almonds and how a decline in quality may be evaluated, both through sensory evaluation and via chemical and instrumental testing. Finally, factors that are most important when considering the shelf life of almonds are outlined.

### Food stability and quality

When evaluating the stability and quality of food, it is important to consider the formulation, processing and storage of the product. In order to do that, the properties that are important characteristics of safe, high-quality foods and the chemical and biochemical reactions that influence quality and/or wholesomeness of foods must be determined (Fennama and Tannenbaum 1996).

Many changes occur during the processing and storage of food products and these changes can be both desirable and undesirable (Taoukis and Labuza 1996). Quality attributes that most commonly undergo changes due to food handling, processing and/or storage include color, texture, flavor, nutritive value and safety and with the exception of nutritive value and sometimes safety, these changes are typically perceivable by the consumer (Fennama and Tannenbaum 1996). Chemical and biochemical reactions that can lead to changes of food quality or safety

include enzymatic or nonenzymatic browning, oxidation, hydrolysis, metal interactions, lipid isomerization or cyclization, protein denaturation or cross-linking and glycolytic changes. Many of these reactions can act as a primary causative event that will result in a secondary event, which in turn leads to changes in quality attributes (Fennama and Tannenbaum 1996).

Factors that affect food stability and quality

Factors that affect the stability of foods during handling, processing and storage can be classified as either product factors or environmental factors. Product factors include chemical properties of the individual constituents, oxygen content, water content, water activity and pH of the product. Environmental factors include time, temperature, composition of the atmosphere, exposure to light, contamination, physical abuse and chemical, physical or biological treatments the product undergoes (Fennama and Tannenbaum 1996).

Temperature is often the most important variable that impacts the storage and processing of food because it influences many different types of reactions. Time is especially important during storage of a food product because consumers want to know how long the product is expected to retain a specific level of quality. Water activity has been shown to strongly influence the rate of enzyme-catalyzed reactions, lipid oxidation and nonenzymatic browning, among others (Fennema and Tannenbaum 1996), and food stability and safety can be more reliably predicted using water activity rather than water content (Fennema 1996). Other important factors related to the composition of the atmosphere are relative humidity and oxygen content (Fennama and Tannenbaum 1996).

Modeling changes in food quality

Taoukis and Labuza (1996) explain how the loss of quality of a product "can be represented by the loss of a quantifiable desirable quality attribute [...] or by the formation of an

undesirable attribute." The simplest change in quality over time is when the loss of a desirable attribute or the formation of an undesirable attribute is expressed as a linear function of time, which is referred to as a zero-order reaction. Often, the change in quality over time is more complicated and must be expressed as a first- or second-order reaction.

When considering the shelf life of a product, it is important to study a product for a sufficient amount of time. For example, when there is less than 50% loss of a quality attribute, both zero- and first-order reactions can appear to represent the rate of quality deterioration, but when more than 50% of the attribute is lost, different equations may best model the change in quality. Knowing the appropriate model for quality deterioration is important for accurate shelf life estimations. However, it is sometimes not feasible to study a product through the complete deterioration of a quality attribute, in which case caution should be utilized in making shelf life proclamations (Taoukis and Labuza 1996).

### **Shelf life testing**

Shelf life is defined as "the length of time that a commodity may be stored without becoming unfit for use or consumption" (OED Online 2015). However, deterioration of a product occurs over time, which is why it is important to recognize the resulting changes in product quality. Some factors that impact the change in product quality over time include ingredient quality, product composition, processing conditions and water activity of the product, in addition to storage conditions such as temperature, humidity and packaging (Sewald and Devries 1993).

Shelf life testing can be used in order to identify the sensory end-point of a product's life, which is useful for managing business risk and to meet business needs. The end-point can be defined as the point at which the product's overall sensory profile has changed, the point at

which the product's attributes known or suspected to be key to the consumers' perception of the product quality have changed, or the point when consumers no longer consider the product to be acceptable. Additionally, shelf life testing can be used to understand the amount of time a product can be stored before a sensory defect is detectable and to estimate the time at which a consumer product no longer has the intended sensory characteristics and is no longer stable or fit for consumption (ASTM 2011).

There are two main approaches for predicting the shelf life of products. The most common approach is to use accelerated storage or aging to determine the sensory end-point; this is done by subjecting the product to extreme or abusive conditions (such as high temperature or humidity) in order to create changes in the product that are assumed to simulate product aging (ASTM 2011; Sewald and Devries 1993; Taoukis and Labuza 1996). The quality of the product is then evaluated and the results are extrapolated to normal storage conditions (Taoukis and Labuza 1996).

Products can also be stored under a variety of conditions and then tested over time to see the rate of quality deterioration and the dependency of the various quality attributes on the different storage conditions (ASTM 2011; Sewald and Devries 1993; Taoukis and Labuza 1996). Environmental parameters controlled for often include temperature, humidity, light, oxygen, and packaging, which are manipulated and monitored in order to evaluate the effect of these parameters on the shelf life (ASTM 2011; Sewald and Devries 1993). When determining the conditions at which the product will be sampled, it is important to consider the conditions under which the product will be sold and consumed. This alternative method of shelf life testing has higher costs but results in more accurate shelf life predictions (Taoukis and Labuza 1996).

Products tested for shelf life must be from representative production batches and be from the same production date, as well as being subjected to the same processing and packaging, unless the later are the variables of interest. The amount of product required for shelf life testing is dependent on the number of storage conditions, the estimated length of storage, the frequency and types of testing before and during the storage period and the methods of evaluation (ASTM 2011).

#### Shelf life of nuts

The high content of unsaturated fatty acids found in most nuts makes them highly susceptible to oxidation and loss of quality if stored poorly or for too long. Additionally, the content of individual fatty acids in nuts influences the rate of oxidation, flavor deterioration and shelf life. Each of the different unsaturated fatty acids found in fats can also oxidize through different mechanisms and at different rates, yielding various volatile products and flavors (Hudson and Gordon 1999; Shahidi and John 2013). For example, the rates of oxidation for stearic, oleic, linoleic and alpha-linolenic acids are approximately 1:10:100:200, respectively (Shahidi and John 2013).

Depending on variety, almonds are composed of 3.2-6.3% water, 7.9-16.0% dietary fiber, 18.5-24.0% protein and 44.7-54.1% fat. Table 2.1 shows the fatty acid profile of raw almonds as reported in literature (Karatay and others 2014; Mexis and Kontominas 2010; Miraliakbari and Shahidi 2008; Robbins and others 2011; Sathe and others 2008; Senesi and others 1991; Yada and others 2013). All researchers reported that oleic acid is the most abundant fatty acid present in almonds, and its content has been reported to range from approximately 60% to 80%. The second most predominant fatty acid is linoleic acid, which typically ranges from 13% to 33%. Combined, oleic and linoleic acid generally represent over 90% of the fatty acids present in

almonds. Miraliakbari and Shahidi (2008) and Robbins and others (2011) analyzed the fatty acid profile of seven and ten tree nut oils, respectively, and both reported that oleic acid is always the predominant fatty acid, almonds having the second highest level. The differences in reported fatty acid profile by the various researchers are likely due to varying techniques of extracting the almond oil and analyzing the fatty acids, as well as difference in varieties and growing location of the almonds in question. Sathe and others (2008) found that variety, growing location and harvest year all significantly affect the fatty acid profile of almonds.

Table 2.1: Comparison of raw almond fatty acid composition as reported by different researchers

		Karatay and	Mexis and	Miraliakbari	Robbins and	Sathe and	Senesi and	Yada and
		others 2014 <sup>a</sup>	Kontominas	and Shahidi	others 2011 <sup>d</sup>	others 2008e	others 1991 <sup>f</sup>	others 2013g
			$2010^{b}$	$2008^{c}$				
(9)	14:0		0.04	0.00	nd			
(mean %)	16:0	5.34	5.31	7.28-7.43	6.45	5.15-6.65	5.64	
lea]	16:1	0.70	0.77	0.77 - 0.78	0.43	0.31-0.57	0.30	
	18:0	0.85	2.69	1.86-1.89	1.47	0.24-1.66	1.19	
acids	18:1	74.46	71.89	69.89-70.01	67.62	59.52-73.80	79.94	
ac	18:2	17.89	19.04	19.49-19.57	24.03	19.49-33.29	12.94	
tty	18:3	0.75		0.00	nd	0.05-0.09		
Fatty	20:0		0.07	0.00	nd	0.03-0.07		
Saturat	ed fatty acids	6.19	8.15					3.2-4.7
MUFA	_	75.16	72.81					24.9-36.1
<b>PUFA</b>		18.65	19.04					9.4-15.1
Oleic:1	inoleic	4.45				1.79-3.79		
Oleic +	- linoleic	90.48-93.78				91.69-93.95		

<sup>&</sup>lt;sup>a</sup> 32 almond genotypes from Anatolia

<sup>&</sup>lt;sup>b</sup> Raw, shelled, unpeeled almond kernels from Ioannia, Greece

<sup>&</sup>lt;sup>c</sup> Commercially purchased raw, shelled, unsalted almonds; range represents difference in results from hexane extraction versus chloroform/methanol extraction

<sup>&</sup>lt;sup>d</sup> Commercially purchased raw almonds; 'nd' indicates 'not detected'

<sup>&</sup>lt;sup>e</sup> Range in composition for 8 almond varieties from 12 different California counties from two crop years; varieties included Butte, Carmel, Mission, Monterey, Nonpareil, Padre, Price and Sonora

<sup>&</sup>lt;sup>f</sup> Raw, shelled, peeled almonds from Sicily, Italy

<sup>&</sup>lt;sup>g</sup> Range in composition for 7 almond varieties from three growing regions in California from two crop years; varieties included in the included Butte, Carmel, Fritz, Mission, Monterey, Nonpareil and Sonora; reported as g per 100 g

The total fat content of Nonpareil almonds has been reported between 47.2±1.5 g/100 g (García-Pascual and others 2003) and 49.6±1.9 kg/100 kg (Yada and others 2013). The fatty acid profile of Nonpareil almonds, shown in Table 2.2, is representative of almonds as a whole – oleic acid is the most predominant fatty acid, followed by linoleic, and together they account for over 90% of the total fat. The range for fatty acid content shown in Table 2.2 is due to the variability found when analyzing almonds from different counties in California from different crop years, as significant fluctuations in fatty acid profile exist between almonds of different production lots (Sathe and others 2008).

Table 2.2: Fatty acid profile of raw Nonpareil almonds (Sathe and others 2008)

Fatty acid	Grams per 100 g lipid <sup>a</sup>
14:0	0.00
16:0	5.46-6.64
16:1	0.33-0.57
18:0	0.25-1.01
18:1	61.89-71.90
18:2	21.47-30.73
18:3	0.06-0.09
20:0	0.03-0.06
Oleic:linoleic	2.02-3.33
Oleic+linoleic	92.19-93.55

<sup>&</sup>lt;sup>a</sup> Almonds grown in 12 counties in California between 2004 and 2006 (n=18)

Almonds are a relatively shelf stable nut, due to their low moisture and high antioxidant contents (Almond Board of California 2013b; Huang 2014; Shahidi and John 2013). Since almonds are considered a microbiologically stable food, their shelf life is defined by changes in their sensory attributes (Hough and others 2003). Crispness and chewiness are important textural attributes for almond quality whereas rancid and off flavors and odors are major factors in reduced quality of nuts. Furthermore, because of their high lipid content, nuts easily absorb odors from external sources during storage, resulting in them becoming less acceptable (Kader 2013). The overall development of off flavors and odors and deterioration of texture contributes

significantly to impairing the sensorial quality of the product, until it becomes unacceptable to the consumer (Velasco and others 2010).

### **Rancidity**

While pure food lipids are virtually odorless, lipids play an important role in the overall flavor of foods due to their effect on mouthfeel, with their primary contribution to food flavor being precursors to flavor compounds. Lipids can undergo a variety of reactions during processing and storage of food products and the reactions give rise to intermediates and end products that vary widely in their chemical and physical properties. These intermediates and end products contribute pleasant aromas in addition to offensive odors and flavors (Nawar 1996).

Rancidity, which is often attributable to suboptimal storage conditions and/or lengthy storage, is one of the main causes of reduced quality of nuts and typically results in consumer rejection (Shahidi and John 2010). Rancidity is a general term for the unacceptable off-flavors and off-odors that develop as a result of fats breaking down and can be classified as both hydrolytic and oxidative (Huang 2014; Nawar 1996; Rossell 1999; Sebranek and Neel 2008; Shahidi and John 2010). The flavors and odors resulting from hydrolytic and oxidative rancidity can vary significantly from product to product (Nawar 1996).

### *Hydrolytic rancidity*

Hydrolytic rancidity results from the hydrolysis of triglycerides in the presence of enzymes (lipases) and moisture, releasing free fatty acids from the glycerol molecule (Rossell 1999; Sebranek and Neel 2008; Shahidi and John 2013). Damage to the product, such as crushing or macerating, can begin the process of lipolysis because the lipases are released to act on the lipids. Figure 2.1 shows the mechanism by which lipases and water act on lipids to form

free fatty acids (Hamilton 1999). As little as 0.1% moisture has been shown to result in the liberation of free fatty acids from fat, depending on the fatty acid profile (Rossell 1999).

Figure 2.1: Formation of free fatty acids as a result of hydrolytic rancidity (adapted from Hamilton 1999)

The free fatty acids formed as a result of hydrolytic rancidity are capable of being oxidized by autoxidation or by enzymes such as lipoxygenases. The further breakdown of the fatty acids can result in compounds that have strong off-flavors and off-odors. Hydrolytic reactions can be minimized by inactivating or decreasing the activity of the lipases and reducing exposure to moisture, which can be achieved through sterilization, careful packaging, cold storage and good transportation (Hamilton 1999).

### Oxidative rancidity

Oxidative rancidity is the result of lipids reacting with atmospheric oxygen and is much more complex than hydrolytic rancidity (Rossell 1999; Sebranek and Neel 2008; Shahidi and John 2013). While a degree of lipid oxidation is sometimes desirable for characteristic flavors, as in some fried foods and aged cheeses, lipid oxidation can lead to the development of various off odors and off flavors, which results in a less acceptable product (Nawar 1996). In addition to the development of rancid odors and off-flavors, oxidative rancidity can result in color changes, loss of desirable flavor volatiles and destruction of fat-soluble vitamins (Shahidi and John 2013).

Lipid oxidation can take place via numerous mechanisms, including autoxidation, thermal oxidation, photo-oxidation, hydrolytic oxidation and enzymatic oxidation (Shahidi and

John 2010; Velasco and others 2010). In autoxidation, oxygen reacts with the fatty acids in fats to form primary breakdown products, such as peroxides and conjugated dienes. As oxidation continues to occur, these primary products are further broken down into secondary products such as volatile aldehydes and ketones (Huang 2014; Nawar 1996).

Autoxidation of lipids proceeds via a typical free radical mechanism and consists of three phases: initiation, propagation and termination, as seen in Figure 2.2. The first phase, initiation, involves the removal of hydrogen atoms from fatty acid double bonds in order to get the first few free radicals. The production of these free radicals must be catalyzed and can take place by metal catalysis or by exposure to heat or light (Shahidi and John 2013; Velasco and others 2010). In the second phase, oxygen reacts with the free radical, R·, resulting in the production of a peroxyl radical, ROO·. The peroxyl radical then acts as an initiator and reacts with a new lipid molecule. This results in the chain of reactions beginning again as well as the formation of hydroperoxide, ROOH. Free radicals are unstable so in the termination phase they react with one another to result in more stable non-radical products (Nawar 1996; Velasco and others 2010). Once the initial few radicals are produced, the three phases of oxidation occur simultaneously but at different rates, with the propagation phase being the rate-determining step (Velasco and others 2010).

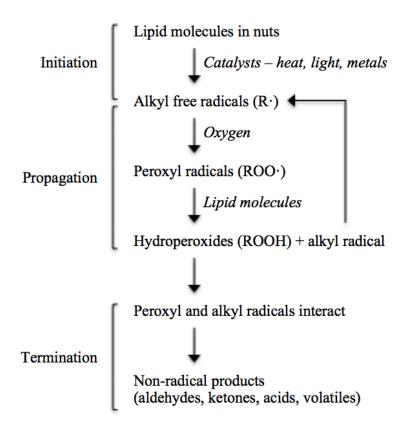


Figure 2.2: Three-step mechanism of lipid autoxidation in nuts (adapted from Shahidi and John 2013)

The intermediate of free radical formation and lipid autoxidation, hydroperoxide, is relatively unstable and can result in the production of various other compounds. The overall result of lipid oxidation is the formation of different chemical compounds of various molecular weights and flavor thresholds. These compounds include aldehydes, ketones, hydrocarbons, volatile organic acids, epoxy compounds and alcohols (Nawar 1996; Shahidi and Zhong 2005). Many of the aldehydes that result in off-flavors and odors are derived from linoleic or linolenic acids, and have been described as rancid, painty, beany, green, metallic and stale (Hudson and Gordon 1999).

Hydroperoxides can also be formed via photo-oxidation in which case photosensitizers become activated by absorption of light. The activated photosensitizers can then act as free radicals and take part in the free radical mechanism discussed above or can produce singlet

oxygen species by energy transfer. The singlet oxygen species formed react directly with unsaturated lipids and are added to the carbons of double bonds (Shahidi and John 2013; Velasco and others 2010).

Factors that influence rate of rancidity

There are many factors that influence the rate of lipid oxidation, which is the primary cause of rancidity, and these factors can be classified as intrinsic or external factors. Intrinsic factors include fatty acid composition, surface area, moisture and the presence of contaminants and/or antioxidants, while external factors include oxygen concentration, temperature and light (Nawar 1996; Sebranek and Neel 2008; Shahidi and John 2013; Velasco and others 2010).

Effect of shelling and product form: Surface area of a food product is directly proportional to the rate of oxidation; as the surface area of a product increases, so does its rate of oxidation (Nawar 1996). In the processing of nuts, any physical damage during shelling will lead to greater exposure to factors such as oxygen, moisture and light, which could trigger the beginning of oxidation thus reducing the shelf life. In-shell almonds can typically be stored twice as long as those without shells. Additionally, intact almond skins help to protect the kernel from minor physical damage and from penetration by atmospheric oxygen (Shahidi and John 2013). Products that have been ground or contain flesh defects are less stable than whole flesh products (Sebranek and Neel 2008). Care must also be taken during transportation to prevent physical damage to products, such as almond kernels, which would provide more opportunities for oxidation (Shahidi and John 2013).

Effect of product composition: Food products typically contain a variety of fatty acids and different fatty acids differ in chemical and physical properties, as well as their susceptibility to oxidation (Nawar 1996; Velasco and others 2010). For example, nonconjugated double bonds

are less reactive than conjugated double bonds and *trans* fatty acids oxidize less readily than their *cis* isomers. Degree of saturation also plays a large role in rate of autoxidation. At room temperature, autoxidation of saturated fatty acids is rather slow while rancidity of unsaturated fatty acids can be detected (Nawar 1996; Berger 1999).

Presence of non-lipid components or contaminants, such as metals, can act as prooxidants and catalyze reactions of the oxidizing lipids or can interact with their oxidation
products (Nawar 1996; Velasco and others 2010). For this reason, metals such as copper, iron,
manganese and chromium should be avoided during processing and storage. Stainless steel or
plastic materials are ideal for preventing oxidation (Sebranek and Neel 2008). Chelating agents
can also be used to control against lipid oxidation (Velasco and others 2010).

Antioxidants naturally present in a product, such as phenolics and tocopherols, can act as protectants, preventing lipid oxidation by reacting with the peroxyl radicals produced during the propagation phase to produce less reactive species (Shahidi and John 2013; Velasco and others 2010). Natural or synthetic antioxidants can be added to food products to achieve the same result (Sekranek and Neel 2008; Shahidi and John 2013; Velasco and others 2010). Almond kernels have a higher tocopherol concentration than other nuts and therefore better storage stability. Specifically, almond skin has high antioxidant content, which is why peeled or blanched almonds are more susceptible to oxidation (Shahidi and John 2013).

Effect of moisture and relative humidity: The moisture present in a product prior to storage plays a major role in the rate of oxidation (Nawar 1996; Shahidi and John 2013). It is believed that there is a protective effect of small amounts of water and that water may quench free radicals and/or interfere with the access of oxygen to the fatty acids. However at high water activities, the rate of oxidation increases (Nawar 1996). For most nuts, it is recommended that

the kernels be dried to moisture levels below 7% (Kader 2013), although it is recommended that raw almonds have a final moisture content between 3 and 6% (Huang 2014). The common industry standard of water activity for almonds is between 0.3 and 0.6, which is optimal for minimal reactions (Huang 2014).

Research shows that during storage, almonds strive to reach equilibrium with their environment by absorbing or losing moisture depending on the storage humidity (Lin and others 2012). Relative humidity above 75% during storage has been shown to stimulate changes in texture and accelerate lipid oxidation. Lower relative humidity, such as 65%, coupled with temperatures exceeding 20°C, can also promote lipid oxidation (Huang 2014). The exchange of moisture and water vapor between nuts and their storage environment results in changes in texture and flavor (Shahidi and John 2013).

Effect of oxygen, temperature and light: At low oxygen concentrations, the rate of oxidation is approximately proportional to the oxygen concentration. However when oxygen is in abundance, the rate of oxidation does not depend on the oxygen concentration (Nawar 1996). Additionally, at high pressure the rate of autoxidation is independent of oxygen concentration but at low pressure the rate of hydroperoxide formation is a function of oxygen partial pressure (Velasco and others 2010). To minimize oxidative rancidity, inert gas such as nitrogen or carbon dioxide can be used to displace the oxygen present in addition to utilizing a packaging material with low oxygen permeability (Sebranek and Neel 2008).

The rate of oxidation also increases as temperature increases (Nawar 1996; Velasco and others 2010) and rancidity has been found to be dependent on temperature with the rate of development increasing at higher temperatures (Shahidi and John 2010). In fact, the rate of reaction between oxygen and lipids roughly doubles for every 10°C increase in temperature

(Berger 1999). To improve the storage stability of a product, lower storage temperatures should be used and temperature fluctuations during storage should be minimized (Sebranek and Neel 2008).

Exposure to light has been shown to significantly accelerate the deterioration of fats and oils (Berger 1999). The presence of light accelerates oxidation by favoring the initiation step and forming free radicals or through a singlet oxygen reaction (Berger 1999; Shahidi and John 2013; Velasco and others 2010). Packaging materials that are impermeable to light should be used to prevent initiation of autoxidation and/or photo-oxidation (Berger 1999; Sebranek and Neel 2008; Velasco and others 2010). However, oxidation via the free radical mechanism can occur in the dark (Hamilton 1999), and therefore other precautions to prevent oxidation should be taken.

Effect of packaging material and storage: Selection of packaging material is important for food products in order to delay product deterioration, preserve desired qualities from processing, maintain overall quality and safety, and to extend shelf life. Ideal packaging provides protection from chemical, biological and physical influences. The most widely used plastics in food packaging are polyethylene and polypropylene due to their strength and flexibility, moisture and chemical resistance, and ease of processability. Polypropylene in particular has good resistance to chemicals and is effective at preventing the penetration of water vapor (Marsh and Bugusu 2007). Almonds for retail sale in North America are typically packaged in sealed plastic bags. However, shipping and storage of almonds post pasteurization are in unlined cartons (Almond Board of California 2012). Paperboard material is moisture sensitive, especially with increasing humidity, and is not recommended for direct food contact (Marsh and Bugusu 2007).

The ideal packaging material for storage of nuts is one that has low permeability to water vapor and gas. Utilizing vacuum sealing is recommended to reduce the available oxygen, which

prevents rancidity and extends shelf life. Successful storage and extension of storage period for nuts is important in part because of the seasonal availability (Shahidi and John 2013)

### **Sensory evaluation**

Sensory evaluation involves the assessment of different food attributes, all of which impact acceptability and preference of foods. Although there is substantial overlap, food attributes are typically perceived in the following order: appearance, which includes color, size, shape, surface texture and clarity; odor, aroma and fragrance; consistency, texture and viscosity; and flavor, which includes the basic tastes, aromatics and chemical feelings such as astringency, cooling or spice (Meilgaard and others 2007).

### *Importance of texture*

Texture, defined as a sensory property that derives from the structure of food and is a combination of characteristics detected by several senses, is one of the most important food attributes to consumers. To consumers, texture is indicative of freshness and excellence in food preparation and texture is especially important for consumer acceptability when it is perceived as undesirable (Szczesniak 2002). A consumer's attitude towards food texture varies based on physiological, social, cultural, economic and psychological factors (Guinard and Mazzucchelli 1996; Szczesniak 2002). The top five liked textural characteristics in America include crisp, crunchy, tender, juicy and firm while the top five disliked textural characteristics include tough, soggy, lumpy, crumbly and slimy. Additionally, consumers appreciate experiencing textural contrast and believe it optimizes the eating experience (Szczesniak 2002).

There are four major mouth behavior groups, each having distinctly different texture preferences: suckers, smooshers, crunchers and chewers, and product choices have been shown to be driven by a person's primary mouth behavior. Suckers and smooshers prefer to manipulate

food between their tongue and the roof of their mouth whereas crunchers and chewers like to use their teeth to break down foods. Chewers, found to be the most predominant group, like foods that can be chewed for longer amounts of time. Crunchers on the other hand are more forceful in their bite than chewers and prefer foods that break up or fracture upon biting; crunchers represent the second most common mouth behavior group (Jeltema and others 2015). Jeltema and others (2015) found that foods that primarily characterized the preferred mouth behavior brought a higher degree of satisfaction to the consumer than foods that did not facilitate that mouth behavior.

# Sensory evaluation methodology

Many factors must be controlled for in order for sensory evaluation to be successful. These factors can be generally classified as controls of the testing booths and controls of sample preparation and presentation. Color, lightening, air circulation, temperature and humidity of the testing booths must all be controlled for. Samples must be served in the same containers and in the same size for all panelists and at the same temperature. Sample should be labeled and their order of presentation should be balanced and random (Meilgaard and others 2007).

The three major classes of sensory evaluation are discrimination, descriptive and affective testing. Discrimination testing is used to determine if two samples are perceptibly different. Examples of discrimination tests include the triangle test, in which three samples are presented and the panelist must identify the sample that is different from the other two identical samples; the duo-trio test, in which two different samples and a reference sample are presented and the panelist must identify which sample matches the reference; and the tetrad test, in which two pairs of samples (four samples total) are presented and the panelist must identify which samples match. Descriptive, or analytical, testing involves the use of trained panelists that are

"calibrated" to be as analytical as laboratory equipment; this method of testing does not investigate acceptability or preference, but rather attempts to quantitatively describe the product (Meilgaard and others 2007).

Affective testing, which assesses preference or acceptance of a product, is used to gain insight into consumers. Consumer sensory evaluation is useful for acquiring knowledge for companies, such as maintaining product quality, product improvement, development of new products, assessing market potential, reviewing product categories, and supporting advertising claims. Affective tests can include preference tests and acceptance tests, as well as the assessment of individual attributes. Assessing individual attributes can be done by asking panelists about personal preference of attributes, responses to attributes or appropriateness of attribute intensities (Meilgaard and others 2007).

Panelist identification of strong and weak points for each sample coupled with acceptability assessments has been used to gain insight into the reasons consumers assign acceptability scores. People can feel positive and negative attitudes toward the different characteristics of a food product; asking them to list strong and weak points allows them to express their positive and negative attitudes to the product in order to see how these attitudes impact their perceptions of the overall acceptability of the product (Rousset and Martin 2001). Rousset and Martin (2001) believe that allowing panelists to give free descriptions of the strong and weak aspects of a food product is more concrete than the hedonic scores, which can be assigned at random by the panelists. The frequency at which consumers list different terms can then be analyzed using correspondence analysis (Rousset and Martin 2001).

Use of consumers can be a good alternative to the classic sensory evaluation methods of using a trained panel. This can be advantageous because consumers do not require training and

therefore testing can be conducted in a shorter time frame, however the sample size needed for consumer panels greatly exceeds that of expert or trained panels. It is generally advised that consumer panels use 80-100 individuals or more while trained panels can be conducted with only 10-12 individuals or less (Worch and others 2010). Although over 100 panelists is typically recommended in the assessment of product acceptability, smaller numbers of panelists have been found to be effective when investigating shelf life (Kilcast 2000).

Many researchers have investigated the sample size required for consumer panels. Gacula and Rutenbeck (2006) compared sample size estimation by computer simulations with the results of various sensory tests in order to determine the optimal sample size for significant results. The researchers found that the agreement between the computer simulations and the sensory tests increased with a larger sample size. They reported that the minimum sample size to see a difference is 40 participants and that a sample size less than 40 results in less repeatability at the significance level. The researchers also reported a statistically significant increase in difference when the sample size is increased from 40 to 100 participants (Gacula and Rutenbeck 2006).

Worch and others (2010) compared results of sensory evaluation from consumers to those from experts and found that both expert and consumer sensory panels can give similar results in terms of discriminatory ability and reproducibility. They found that although there was higher variability related to consumers, the variability was compensated for by a larger sample size (Worch and others 2010). Similar results were published by Ares and others (2011), who found that consumer and trained panelists had similar discriminatory capacity and reproducibility for all of the texture attributes evaluated. These studies indicate that utilizing a consumer panel as opposed to a trained panel to gain insight into product characteristics is justified.

Sensory evaluation as a method of shelf life testing

ASTM International (2011) defines sensory shelf life as "the time period during which the product's sensory characteristics and performance are as intended by the manufacturer. The product is consumable or usable during this period, providing the end-user with the intended sensory characteristics, performance, and benefits." Furthermore, after this period the product has characteristics or attributes that are considered unintended and undesirable (ASTM 2011).

The three most commonly used methods of determining sensory end-point are discrimination, descriptive and affective testing; selection of the testing method depends on the desired knowledge one wishes to obtain and the end-point criteria. Affective testing is the method used when consumer acceptance is chosen as the end-point criterion (ASTM 2011).

Sensory shelf life testing typically utilizes a control product. One type of control product that is frequently used in shelf life testing is a statistical control. A statistical control involves obtaining sensory results (such as acceptance scores) at time zero before the shelf life testing begins. A stable control, in which the product is held under conditions that minimize changes over time, or a fresh control can also be used (ASTM 2011).

Once end point criteria and the type of control have been selected, the evaluation protocol must be determined. The evaluation schedule for products undergoing shelf life testing can be single-point or multi-point. In single-point evaluation, products of different ages are compared in a single evaluation in order to identify the oldest point at which the product still meets the end-point criteria. Multi-point evaluation, shown in Figure 2.3, involves testing samples from a single production lot over time. One advantage to single point evaluation is that the sources of variability often observed in consumer responses can be minimized because the testing is all done at one time. Multi-point evaluation is useful when the objective is to track a single

production lot over the shelf life period and detect early indications of changes in product quality (ASTM 2011).

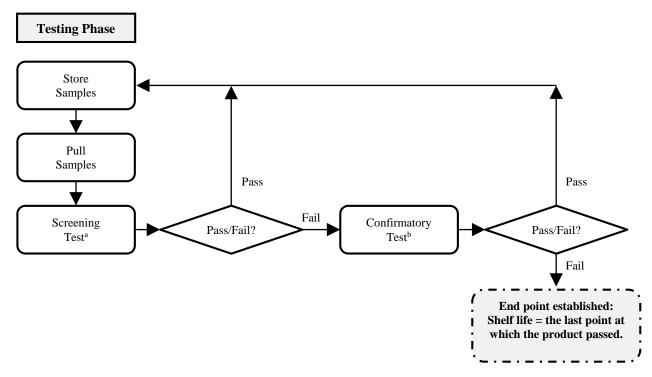


Figure 2.3: Decision making and process flow for multi-point shelf life testing (adapted from ASTM 2011)

Including a pass/fail test in addition to the end-point criteria can be useful for shelf life testing (ASTM 2011) and has been used by many researchers (Araneda and others 2008; Curia and others 2005; Gámbaro and others 2004; Gámbaro and others 2006; Salvador and others 2005). Rejection is typically defined as responding "no" to an intent to consume question and rejection rates of 25% or 50% are commonly used for survival analysis methodology. These rejections rate have been used to study consumer acceptability and shelf life of pears (Salvador and other 2007), sensory shelf life of yogurt (Salvador and others 2005; Curia and others 2005), shelf life of apple-baby food (Gámbaro and others 2006), shelf life of ready-to-eat lettuce

<sup>&</sup>lt;sup>a</sup> Screening tests may include analytical measures, bench top evaluation, or sensory discrimination testing. These should be sufficiently rigorous to avoid passing a product that should have failed.

<sup>&</sup>lt;sup>b</sup> Confirmatory tests may include discrimination testing, descriptive sensory analysis, or consumer acceptance testing, as determined by your pre-established end point criteria.

(Araneda and others 2008), and the shelf life of a chocolate-coated individually wrapped cake (Gámbaro and others 2004), with most researchers concluding that a 25% rejection rate provides the most insight into the shelf life of the various products. A 25% rejection rate has also been found to be more practical and a better reflection of consumer behavior than a 50% rejection rate (Hough and others 2003).

# Non-sensory methods of assessing product quality

Rancidity measurement of food is not only useful as a method of quality control, but it is of growing importance in the study of rancidity development. Research has been aimed at increasing the resistance of a product towards rancidity as well as studying the time for rancidity to develop (i.e. studying the shelf life of products; Rossell 1999). Degree of lipid oxidation can be determined by measuring the presence or accumulation of the primary and/or secondary products (Huang 2014). However, oxidative rancidity is a complex process and a single test alone cannot measure all oxidative events nor be useful at all stages of the oxidative process. For this reason, a combination of different tests is used in order to gain insight into the degree of lipid oxidation (Nawar 1996). Ideally, precise chemical and instrumental tests are used to complement sensory analysis (Shahidi and John 2013).

Peroxides are the first major product from the breakdown of fats and measuring the amount of peroxides, as milliequivalents of peroxide per kg of oil, is useful to detect the early stages of oxidation (Huang 2014; Nawar 1996; Rossell 1999; Shahidi and John 2010). Peroxide values increase as lipid oxidation occurs and higher peroxide values have been correlated with higher rancid flavor (García-Pascual and others 2003), which can result in the product being less acceptable to the consumer. As oxidation progresses, peroxide values peak and then decline as the primary breakdown products are converted to secondary breakdown products; for this reason,

peroxide values should be used with other measurements of quality in order to determine a decline in quality (Huang 2014). In order to determine the stage of oxidation, peroxide concentration can also be monitored over time (Shahidi and Zhong 2005). Peroxide values have been used to monitor almond quality (García-Pascual and others 2003; Lin and others 2012; Mexis and Kontominas 2010; Senesi and others 1991; Zacheo and others 2000), as well as peanuts (Nepote and others 2008) and Brazil nuts (Zajdenwerg and others 2011). The typical industry standard of peroxide values for almonds is less than 5.0 meq. active O<sub>2</sub>/kg oil (Huang 2014).

Conjugated dienes are another primary breakdown product resulting from the rearrangement of double bonds due to the oxidation of fatty acids. Determination of the amount of conjugated dienes is done by measuring absorbance of UV light at 232-234 nm. An increase in absorption indicates the formation of primary oxidation products and this measurement is often correlated with peroxide values (Huang 2014; Shahidi and Zhong 2005). There is no common industry standard for acceptable conjugated diene levels in almonds (Huang 214).

Tests can also be conducted on the secondary products of lipid oxidation in order to assess the extent of rancidity, and these tests include the thiobarbituric acid reactive substances (TBARS) test, *p*-anisidine value test and analysis of volatile carbonyls. In the TBARS test, thiobarbituric acid reagent is reacted with malonaldehyde, a secondary product of lipid oxidation, to produce a pink color. The color can then be measured through spectrophotometry (Nawar 1996; Shahidi and Zhong 2005). The *p*-anisidine test measures the amount of aldehydes, specifically 2-alkenals, produced from the decomposition of hydroperoxides. In this test, the *p*-anisidine reagent is reacted with the aldehydic compounds, which results in a yellow product that is absorbed at 350 nm. Measurement of carbonyl compounds, including aldehydes and ketones,

is important because these compounds contribute significantly to rancid flavors. In addition to an analysis of total carbonyl content, individual carbonyl compounds can also be analyzed, such as hexanal (Nawar 1996; Shahidi and Zhong 2005; Velasco and others 2010). Hexanal, a volatile compound, is one of the major secondary products of lipid oxidation and can be used as an indicator of oxidative rancidity (Huang 2014). Hexanal content has been determined as a quality indicator in the storage of almonds, Brazil nuts and rolled oats (McEwan and others 2005; Mexis and Kontominas 2010; Zajdenwerg and others 2011). There is no common industry standard for acceptable hexanal content in almonds (Huang 2014).

Analysis of volatile oxidation products is also useful in studying deterioration of quality in foods because these products are the first perceived by a consumer as off-odors and off-flavors. Volatile oxidation products are analyzed by gas chromatography (GC) and research has shown that GC analyses of volatile compounds correlate with oxidative flavor scores from sensory analyses (Velasco and others 2010).

Moisture content, which is expressed as a percent or as grams of water per 100 grams of product, is a measure of the total water present in the product (Huang 2014). Moisture content has been used to monitor almond quality by multiple researchers (García-Pascual and others 2003; Harris and others 1972; Sánchez-Bel and other 2008). Water activity (a<sub>w</sub>) is also frequently determined to evaluate changes in quality over time (McEwan and others 2005; Senesi and others 1991) and water activity is believed to better predict food stability than water content (Fennema 1996). Water activity gives an indication of the amount of free water available in a product that can undergo chemical or enzymatic reactions, which would lead to deterioration of quality. Water activity is the ratio of the vapor pressure of water in a product relative to the vapor pressure of distilled water. The water activity of a low moisture product such as almonds is

dependent on the temperature and relative humidity of the storage environment (Fennema 1996; Huang 2014), and water activity levels above 0.4 have been shown to be associated with detrimental effects on almond textural quality (Vickers and others 2014).

Many researchers also conduct fatty acid analyses of a product, both at baseline and/or throughout storage, to get an idea of the free fatty acids present and how they change over time. Amount of free fatty acids is an indicator of hydrolytic rancidity due to the action of lipases. Lipase activity in almonds typically increases when the moisture content exceeds 6% or relative humidity exceeds 65%. The typical industry standard for free fatty acid level in almonds is less than 1.5% (Huang 2014). While free fatty acid analysis is useful as an indicator of rancidity, it cannot be used alone (Harris and others 1972; Lin and others 2012). Fatty acid profiles have been determined in studies of almonds by many different researchers (García-Pascual and others 2003; Harris and others 1972; Lin and others 2012; Mexis and Kontominas 2010; Zacheo and others 2000).

The texture of food is a complex sensory property that is strongly linked to consumer acceptability and the characterization of texture can be a combination of instrumental analyses, such as texture, rheology or microstructural, and sensory analyses (Szczesniak 2002; Varela and others 2008). Since texture and mouthfeel are key factors in a consumer's determination of the acceptability of a food product, instrumental texture analysis is often conducted on food products (Guinard and Mazzucchelli 1996; Szczesniak 2002; Varela and others 2008; Wilkinson and others 2000). Texture analysis is useful in order to gain an understanding of the microstructural changes that occur to a product during processing and storage (Wilkinson and others 2000). Specifically, the degree of jaggedness observed on a force-deformation curve can be used to monitor the textural changes that result from moisture sorption, causing loss of brittleness and

crunchiness (Guinard and Mazzucchelli 1996). For example, a curve with more fracture peaks indicates a crisper, more brittle product (Varela and others 2008). Additionally, it has been found that there is a positive correlation between sensory crispness and the number of fracture peaks and a negative correlation to the force at failure (Varela and others 2006). Maximum force is an assessment of hardness and the extent to which food is deformed (Guinard and Mazzucchelli 1996).

Other chemical and instrumental tests that have been used in shelf life studies include color analysis (Mexis and Kontominas 2010; Sánchez-Bel and others 2008; Senesi and others 1991), tocopherol content (García-Pascual and others 2003; Senesi and others 1991; Zacheo and others 2000; Zajdenwerg and others 2011) and headspace oxygen (Harris and others 1972; McEwan and others 2005).

The use of the combination of chemical and instrumental assessments with sensory evaluation in shelf life testing and assessing product quality is well established. Examples of products that have been investigated using this combination method include peanuts (the relationship between consumer acceptance and descriptive analysis; Nepote and others 2008), Brazil nuts (the correlation between sensory data and chemical markers of oxidation; Zajdenwerg and others 2011), rolled oats (effects of storage on sensory and nutritional quality; McEwan and others 2005), and almonds (Harris and others 1972; Sánchez-Bel and others 2008; Senesi and others 1991; Varela and others 2006; Varela and others 2008).

### Almond shelf life studies

Almonds can have a shelf life of up to 2 years when properly handled, and their shelf life is controlled by three general factors. The first of these factors is the product characteristics, which includes moisture content, unsaturated fatty acid composition, water activity and form

(such as in-shell or out, raw or roasted, size of pieces, etc.). Another factor is the environment during distribution and storage, such as temperature, humidity, oxygen, processing conditions, and presence of insects, pests or microorganisms. The final factor is the packaging, which must provide physical protection as well as be a barrier to moisture and gas (Huang 2014). There have been many studies conducted on the optimal storage conditions for almonds of different forms, looking at both the chemical and the sensory changes that occur (García-Pascual and others 2003; Harris and other 1972; Lin and others 2012; Mexis and Kontominas 2010; Rizzolo and others 1994; Senesi and others 1991).

Effect of product form on shelf life has been incorporated into many shelf life studies. Lin and others (2012) found that blanched almonds had greater increases in peroxide values than the natural samples, indicating that the antioxidant properties of the almond skins played a significant role in preventing lipid deterioration. Rizzolo and others (1994) found that in-shell almonds reached the maximum peroxide value four months later than their shelled counterparts, indicating that the shell acts as a good barrier to oxygen. García-Pascual and others (2003) also found that unshelled almonds stored at ambient temperature for 9 months did not experience any change in fat content, peroxide values or  $\alpha$ -tocopherol content. Different varieties of almonds have also been shown to undergo lipid oxidation at different rates (García-Pascual and others 2003; Rizzolo and others 1994).

Amount of oxygen and ease of accessibility of oxygen to the product affects the rate of deterioration of almonds. Mexis and Kontominas (2010) monitored peroxide value, hexanal content, color, fatty acid composition and volatile compounds to see the effect of different packaging materials on the shelf life of raw, whole, unpeeled almonds. The investigators concluded that using an oxygen absorber in the packaging resulted in an almond shelf life of at

least 12 months, regardless of the packaging material, lighting and storage temperature. Although the researchers stressed that the above conclusion cannot be generalized beyond the storage conditions tested (Mexis and Kontominas 2010), it theoretically shows the importance of nuts being exposed to oxygen as a factor to decrease quality. A similar conclusion was made by Harris and others (1972), who found that a glaze coating on almonds prevented oxygen absorption and therefore rancidity.

It is well established that packaging material plays a significant role in preserving the quality of various products. Senesi and others (1991) studied the effect of packaging and storage conditions for peeled almonds with the hope of extending the shelf life. The packaging materials used had different gas and light permeability, and the storage temperature was also manipulated. Overall, the researchers found that there was no significant quality loss in almonds stored in low or high oxygen permeable packaging up to 9 months at 4 or 20°C; beyond 9 months, however, almond quality was best maintained when stored in low oxygen permeable packaging stored at 4°C. Lin and others (2012) found that raw almonds stored in unlined cartons at high relative humidity absorbed more moisture than raw almonds stored in polyethylene bags.

Studies indicate that the rate of rancidity development in nuts is highly dependent on temperature and that the rate of development increases rapidly at higher temperatures (Shahidi and John 2010). Specifically, García-Pascual and others (2003) found that significant differences in peroxide values existed for raw Nonpareil almonds stored under different temperatures. Looking at acceptability scores, Senesi and others (1991) found that almond samples stored at ambient temperature had greater decreases in acceptability than samples stored at 4°C. Lin and others (2012) also found that free fatty acid levels increased with storage time, temperature and humidity, with the reaction rate increasing faster at higher temperatures.

While there have been studies on the optimal storage conditions of almonds, there is currently a lack of information on how almonds stored under the various conditions are perceived and accepted by consumers. Additionally, the best indicator of consumer rejection is unknown and the relationship between consumer acceptability/intent to consume and chemical/instrumental indicators of almond quality is not well established.

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### **CHAPTER 3**

#### MATERIALS AND METHODS

This study was a collaboration between the Department of Food Science and Technology and the Department of Foods and Nutrition at the University of Georgia in Athens, Georgia. The Department of Food Science and Technology was responsible for conducting chemical and instrumental tests on the almonds at 2-month intervals throughout the 24-month duration of the study and the Department of Foods and Nutrition was responsible for conducting sensory evaluations as well as non-sensory tests on the day of sensory evaluation. All sensory methods and procedures were approved by the University of Georgia Institutional Review Boards on Human Subjects.

### **Characterization of the product**

The nuts investigated in this study were whole, raw, unsalted Nonpareil almonds with skin. The almonds were a composite lot harvested from different orchards in California between September and October of 2012 and were graded Supreme. The almonds were pasteurized by propylene oxide fumigation (PPO) prior to packaging. Before being shipped to Athens, Georgia, the almonds were packaged in 50 lb. unlined cardboard cartons on October 7, 2012 and the initial moisture content was determined to be 4.3%. The shipment of almonds arrived at the University of Georgia on November 14, 2012 via commercial carrier and was placed in refrigerated storage (4 °C) until packaging began on November 15, 2012. Over a 23-day period, the almonds for storage under specified conditions were packaged in the proper packaging.

# Overall study design

The project consisted of an incomplete factorial design in which the almonds were stored under varying conditions over a 24-month period. The storage parameters were packaging, temperature and relative humidity (RH), resulting in 14 different conditions, as seen in Table 3.1. Almonds were stored in unlined cardboard cartons, which served to simulate shipping and bulk storage, and in sealed polypropylene bags to simulate retail packaging in North America. Storage temperatures were 15°C, 25°C and 35°C and the degrees of relative humidity investigated were 50% and 65%. Samples were also held at 4°C without relative humidity control.

Table 3.1: Storage parameters manipulated resulting in 14 storage conditions

<u> </u>	1 0	
Packaging	Temperature (°C)	Relative Humidity (%)
Unlined Cardboard Cartons	4	no RH control
	15	50
		65
	25	50
		65
	35	50
		65
Sealed Polypropylene Bags	4	no RH control
	15	50
		65
	25	50
		65
	35	50
		65

Sample handling and packaging

The raw almonds were removed from the 50 lb. cartons and repackaged for storage in Uline S-17960 (100 $\mu$ m, clean polypropylene) bags or Uline S-15138 unlined cartons (Uline, Waukegon, IL). The polypropylene material had a water vapor transmission rate (WVTR) of 8 g m<sup>-2</sup>d<sup>-1</sup> and oxygen transmission rate (OTR) of 860 cm<sup>3</sup> m<sup>-2</sup>d<sup>-1</sup> and the unlined carton material provided no protection from atmospheric conditions. Thirty polypropylene bags per treatment

were vacuumed using a Henkelman 600 vacuum packaging system (Henkelman bv, Netherlands), flushed with food-grade  $N_2$ , and sealed; the bags were flushed with  $N_2$  such that there was a sufficient pillow. Each polypropylene bag contained  $300\pm 5$  g of raw almonds and the initial oxygen level was below 0.5%. Twelve unlined cartons per treatment were filled with  $900\pm 5$  g of raw almonds.

The almonds requiring relative humidity control were stored in HotPack Environmental Chambers (SP Industries, Warminster, PA); HotPack model 435314 was used to simulate 15°C/50% RH, 15°C/65% RH, 25°C/50% RH, and HotPack model 434304 was used to simulate 25°C/65% RH, 35°C/50% RH and 35°C/65% RH. The almonds without RH control were stored at 4°C in an environmental controlled walk-in unit (Nor-Lake, Inc., Hudson, WI). Temperature and relative humidity of each chamber were monitored throughout the study with an Extech RHT-10 temperature/humidity probe (Extech Instruments Corporation, Nashua, NH).

# Sample evaluation

The sensory evaluation protocol followed in this study was based on ASTM (2011b) methodology. Almonds were evaluated at baseline (0 months of storage) by a consumer sensory panel (n=118); results of this panel served as a basis of comparison over the storage period to determine if significant changes in acceptability occurred. Baseline assessments of all non-sensory tests were also conducted.

Every 2 months over a 24-month time frame, team members in the Department of Food Science and Technology assessed the fourteen almond samples for chemical and instrumental triggers of quality deterioration. When the chemical analysis revealed peroxide values greater than 2.0 meq. active O<sub>2</sub>/kg oil, or there was detection of sensory notes typical of degradation in nuts (ASTM 2011a), consumer sensory evaluation was triggered. Each sample was evaluated for

textural and flavor notes typical of degradation by three experienced sensory analysts. The timing of consumer sensory panels followed the chemical and instrumental triggers to ensure consumer sensory evaluation occurred during the rejection period.

Once triggered by the chemical and instrumental tests, the almonds were evaluated by a consumer screening panel (n=35-40) and the panelists were asked about the acceptability of the sample and their intent to consume the sample. If the sample was not rejected by the panelists (i.e. rejection was less than 25%, meaning at least 75% of the panelists said they would consume the sample), the sample continued to be evaluated at 2 month intervals. If 25% or more of the panelists rejected the sample, a larger confirmatory panel (n=100-110) was triggered. A rejection rate by the confirmatory panel over 25% confirmed rejection of that sample. When the confirmatory panel did not confirm rejection of the sample as indicated by the screening panel, the sample continued to be evaluated at 2 month intervals in screening panels and was only subjected to a subsequent confirmatory panel when the screening panel again indicted a 25% rejection rate. At the conclusion of the 24-month study, all remaining samples were evaluated by both a screening panel and a confirmatory panel. Figure 3.1 shows the flow of procedures for the project, from chemical and instrumental testing through failure of the sample.

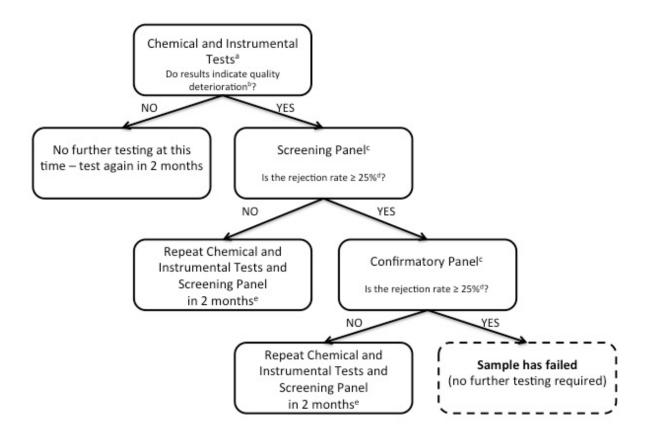


Figure 3.1: Process flow and decision making for chemical, instrumental and sensory testing <sup>a</sup> Tests conducted by the Department of Food Science and Technology included headspace analysis, moisture analysis, water activity, texture analysis, peroxide values, free fatty acids, conjugated dienes, TBARS and Vitamin E

On each day of consumer sensory evaluations, non-sensory tests were conducted in conjunction. The non-sensory tests conducted included instrumental texture analysis (n=36) and water activity (n=6). Baseline assessments were also completed for all non-sensory tests to serve as the control.

<sup>&</sup>lt;sup>b</sup> Peroxide values greater than 2.0 meq. active O<sub>2</sub>/kg oil or detection of off-sensory notes by 3 experienced sensory analysts (ASTM 2011a)

<sup>&</sup>lt;sup>c</sup> Instrumental texture and water activity were assessed on the same day as every sensory panel

<sup>&</sup>lt;sup>d</sup> Response of "No" to "If you had purchased this product would you eat it?" (Hough and others 2003)

<sup>&</sup>lt;sup>e</sup> Once triggered, if the sample was not rejected by a consumer screening or confirmatory panel, the sample continued to be tested at 2 month intervals until rejected

# Participants and demographic information

The participants for the sensory panels were men and women of all ethnic backgrounds over 18 years of age with no peanut or tree-nut allergies. Participants were recruited from UGA faculty, staff, students and visitors via email announcements and through verbal announcements to specific classes and student organizations as well as signage in the hallways. The panelists were told during recruitment that product evaluation would take 6-15 min. An inclusion criterion for consumer sensory panelists was that they consume nuts or nut products at least once per month; this was controlled by asking "If you eat nuts, including peanuts, come evaluate almonds" in the email and verbal announcements and confirmed via questions posed during the sensory evaluation. All participants were required to provide informed consent prior to participation. The panelists were presented with a consent form to read and sign (Appendix A) prior to product evaluation.

# **Consumer panel**

The same protocols were followed for the screening and confirmatory panels, and for the baseline testing and the testing that occurred at the 2 month intervals. Initial protocol dictated that panelists would evaluate no more than 4 samples per session to prevent sensory and mental fatigue. However, after receiving panelist feedback the protocol was changed such that panelists did not evaluate more than 3 samples per session. Each sample was coded with a 3-digit random number code. The almonds were served at room temperature with 3 almonds per sample cup. Almonds to be sampled were selected to be of similar size and shape such that they were similar to "good" quality almonds, as defined by USDA grading standards (USDA 1997); broken pieces, pinched kernels and dissimilar and blemished almonds were discarded.

The panelists were seated in individual sensory booths equipped with white lighting. The samples were presented one at a time in a counterbalanced, randomized order of presentation.

Palate cleansers provided to the panelists were room temperature water and baby carrots. Snacks, such as ice cream and chips, and drinks, such as water bottles and juice boxes, served as participation incentives for the panelists.

The consumer sensory panelists evaluated the almond samples for odor, texture, flavor and overall acceptability by rating the sample(s) on a 9-point hedonic scale for each attribute. The 9-point hedonic scale ranged from "extremely dislike," assigned a score of 1 for data analysis purposes, to "extremely like," assigned a score of 9 for data analysis. Panelists were instructed to evaluate the odor of the sample prior to tasting by holding the cup close to their nose and gently lifting the lid as they take three short sniffs. Once the odor of the sample had been assessed, panelists were then instructed to eat the sample and evaluate the texture, flavor and overall acceptability. The panelists were also asked to indicate the strong and weak points of each sample (Rousset and Martin 2001), by responding to the question "Please indicate WHAT in particular you liked or disliked about this almond sample (use WORDS not SENTENCES)".

The panelists were provided with four lines to give feedback for both "like" and "dislike".

Finally, the panelists were asked to respond to "If you had purchased this product, would you eat it?" (yes or no) (Hough and others 2003) and a negative response to the question resulted in rejection of the sample. The almond sensory scorecard is shown in Appendix B.

A rejection rate of 25% or higher from the screening panel resulted in a confirmatory panel, with the hopes that the larger sample size would validate the rejection. In the event that the confirmatory panel did not confirm rejection of the sample as indicated by the screening panel, the sample continued to be tested at 2 month intervals by screening panels and was only

subjected to a confirmatory panel when the screening panel again indicated rejection. Once the sample rejection was confirmed by the confirmatory panel, the sample was deemed failed and not submitted for further sensory evaluation.

After completing the sample evaluation, panelists completed a general questionnaire (Appendix C). This allowed for characterization of the panel demographics, as well as the typical nut consumption patterns of the panelists.

### **Statistics**

Data were analyzed using SAS (SAS University Edition, SAS Institute Inc., Cary, NC).

Normal distribution was verified through univariate analysis, and outliers were removed as necessary. Descriptive statistics including means and standard deviations were determined for the results of all sensory evaluations. T-tests were conducted to test significant changes over time for sensory tests compared to baseline. Analysis of variance (ANOVA) was conducted on all samples at point of failure to detect significant differences between rejected samples; Student-Newman-Keuls (SNK) was used for means separation when appropriate.

Multiple stepwise regression analyses were conducted in order to explain which sensory attribute (odor, texture, and flavor) best predicted overall acceptability. Stepwise regression was also performed on all sensory results with the means of all chemical and instrumental data in order to identify which tests best predicted acceptability of each of the sensory attributes as well as overall acceptability. Appropriateness of the linear model was verified by plotting residuals vs predicted values for each independent variable.

Frequency statistics were created for the demographic information collected in the questionnaire in order to characterize the panel as a whole. For all statistics, the level of statistical significance was defined at p<0.05.

# **Non-sensory tests**

Texture analysis and water activity were conducted concurrently with the consumer sensory evaluations. All non-sensory tests were conducted on the same day as the sensory testing to ensure the same batch was tested in a similar timeframe after being removed from the environmental chambers.

# *Texture analysis*

Texture analysis (hardness and fracturability) was conducted using TA.XT2 Plus Texture Analyzer (Texture Technology Corp., Scarsdale, NY) equipped with Texture Exponent 32 software (Stable Micro Systems, Haslemere, Surry, England) and a 50 kg load cell following the methodology outlined by Varela and others (2008). Almonds were compressed one at a time by a 40 mm diameter cylinder probe at a test speed of 1 mm/s; the compression distance was set at 5 mm from the baseplate and a trigger force of 10 g was used. For each sample evaluated, 36 replications were performed. All almonds tested were selected for similar shape and size and broken pieces, pinched kernels and dissimilar and blemished almonds were discarded.

### *Water activity*

Water activity of the almonds was determined using an AquaLab Dew Point Water

Activity Meter 4TE (Decagon Devices, Pullman, WA). The almonds for each storage condition
at each testing point were ground in a mini-food processor (Proctor Silex 1.5C Mini Food

Chopper, Hamilton Beach Brand, Inc., Southern Pines, NC) to a final size of 2 mm or smaller for
use in the water activity test. Six aliquots were taken from the composite ground almond sample.

Temperature of the sample was recorded in addition to the water activity.

Chemical and instrumental tests conducted by the Department of Food Science and Technology

Team members in the Department of Food Science and Technology performed chemical and instrumental tests at the beginning of the study and at 2-month intervals throughout the study. The tests included peroxide values (PVs), free-fatty acid values (FFAs), conjugated dienoic acid values (CDs), 2-thiobarbituric acid values (TBARs), headspace-solid phase microextraction gas chromatography with a flame ionization detector (HS-SPME GC/FID), water activity, moisture content and texture analysis. Beginning at 8 months, samples were also evaluated for Vitamin E content. Appendix D details the protocols followed for the chemical and instrumental tests conducted by the Department of Food Science and Technology.

#### **Statistics**

Data were analyzed using SAS (SAS University Edition, SAS Institute Inc., Cary, NC). Descriptive statistics including means and standard deviations were determined for the results of all chemical/instrumental tests. T-tests were conducted to test significant changes over time for specific non-sensory tests compared to baseline (peroxide values, free fatty acids, water activity, force and number of fracture peaks). ANOVA with SNK was conducted to detect significant differences between samples at point of rejection. Means of the chemical/instrumental data were combined with all sensory results and stepwise regression was performed in order to identify which chemical/instrumental tests best predicted acceptability of each of the sensory attributes as well as overall acceptability. Appropriateness of the linear model was verified by plotting residuals vs predicted values for each independent variable. For all statistics, the level of statistical significance was defined at p < 0.05.

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## **CHAPTER 4**

#### RESULTS AND DISCUSSION

In this 24-month multi-point shelf-life study (ASTM 2011b), almond samples were stored at 15, 25 or 35°C and a relative humidity (RH) of 50 or 65%, and at 4°C with no relative humidity control (humidity for the 4°C storage chamber was recorded as 95±3%). Half of the almond samples were stored in clear polypropylene bags (100μm, Uline S-17960, Waukegon, IL), which had a water vapor transmission rate (WVTR) of 8 g m<sup>-2</sup>d<sup>-1</sup>, oxygen transmission rate (OTR) of 860 cm<sup>3</sup> m<sup>-2</sup>d<sup>-1</sup> and initial O<sub>2</sub> level below 0.5%, and the other half of the samples were stored in unlined cartons (Uline S-15138, Waukegon, IL), which provided no protection from atmospheric conditions.

Prior to storage, baseline chemical, instrumental and consumer sensory assessments were conducted. Every two months throughout the study, team members in the Department of Food Science and Technology conducted chemical and instrumental tests in order to monitor changes in almond quality. Peroxide values greater than 2.0 meq. active O<sub>2</sub>/kg oil or detection of sensory notes typical of degradation in nuts (ASTM 2011a) triggered sensory evaluation, as both of these assessments were indices of degrading quality. Once triggered, consumer sensory panels were conducted every two months until the sample was rejected or the study concluded. Rejection of the sample, which signified end of shelf life, was defined as a negative response by over 25% of the consumer panelists (n=91-118) to "If you had purchased this product, would you eat it?" (yes or no) (Hough and others 2003). By the end of the study, all samples stored in unlined cartons

and four samples stored in polypropylene bags (35°C/65% RH at 6 months, 35°C/50% RH at 12 months, 25°C/65% RH at 16 months and 15°C/65% RH at 24 months) were rejected by the consumer panelists. The three almond samples stored in polypropylene bags that did not fail within the timeframe of the 24-month study were stored at 4°C, 15°C/50% RH and 25°C/50% RH. Non-sensory assessments of almond quality were also conducted on the day of each sensory panel.

## Assessment of raw almonds at baseline

Initial assessments of water activity, free fatty acids and peroxide values of the raw almonds at baseline (Table 4.1) fell within the standards typically used in the almond industry, implying that the almonds were fresh and of high quality (Almond Board of California 2013; Huang 2014). Free fatty acid and peroxide levels were within range of raw almonds prior to lipid deterioration as reported in literature (Lin and others 2012; Mexis and Kontominas 2010; Rizzolo and others 1994). In addition, the almonds assessed at baseline fell within the 0.25 to 0.35 water activity range at which lipid oxidation is typically lowest (Huang 2014). Baseline textural assessments (Table 4.1) also suggest that the almonds were hard and crunchy due to the large number of fracture peaks and the maximum force (Varela and others 2006; Varela and others 2008a). The almonds sampled at baseline came from the same supply as the almonds used throughout the study and therefore were representative of the batch of almonds as a whole. These assessments served as the control throughout the storage period.

Table 4.1: Non-sensory test results for raw almonds at baseline

Peroxide value	Free fatty acids	Water	Peaks	Force		
(meq. active O <sub>2</sub> /kg oil) <sup>a</sup>	(acid value) <sup>b</sup>	activity <sup>c</sup>	(number) <sup>d</sup>	$(g)^d$		
	means±SD					
< 0.01	$0.285 \pm 0.028$	$0.289 \pm 0.022$	$6.7 \pm 3.0$	49972±6596		

a n=3

At baseline, 118 individuals participated in a sensory panel to assess the odor, texture, flavor and overall acceptability of the raw almonds. This assessment served as the sensory control throughout the storage period. The sensory panelists were recruited from faculty, staff, students and visitors at the University of Georgia. The panel consisted of 79% females and 21% males, with 76% of the participants being 18 to 27 years old. Approximately 83% of the panelists indicated that they consume nuts, including peanuts, at least several times a month and 65% indicated that they consume almonds at least several times a month.

The results of the sensory evaluation at baseline are shown in Figure 4.1. Mean texture, flavor and overall acceptability were above 7 on the 9-point hedonic scale, where 1 is "extremely dislike" and 9 is "extremely like," whereas mean acceptability of odor was found to be 5.7. It is important to note that consumers were instructed to evaluate the odor of the sample prior to tasting the almonds; therefore fewer aromatic volatile compounds would have been detected than after mastication occurred. It is therefore possible that the lower odor score was due to lack of a prominent aroma as opposed to an undesirable aroma.

The almonds evaluated at baseline were fresh almonds as evidenced by an evaluation date within 2 months of harvesting. On that basis, one may have expected acceptability scores to be close to 9 for all attributes, however this was not the case. It has been shown in sensory science that panelists tend to avoid using the extremes on the rating scales and it is generally

<sup>&</sup>lt;sup>b</sup> n=3; mg KOH necessary to neutralize 1 g of sample

<sup>&</sup>lt;sup>c</sup> n=6 (AguaLab Dew Point Water Activity Meter 4TE)

<sup>&</sup>lt;sup>d</sup> n=36 (TAXT.2 Plus Texture Analyzer equipped with Texture Exponent 32 and a 50 kg load cell as described by Varela and others 2008b)

believed that a 9-point scale is functionally a 7-point scale (Moskowitz and others 2003), essentially ranging from 2 to 8. Therefore, mean texture, flavor and overall acceptability scores at baseline can be considered close to perfect.

At baseline, approximately 6% of the consumer panelists rejected the raw almond sample, as indicated by a negative response to "If you had purchased this product would you eat it?" (Hough and others 2003). Again one may expect that the fresh almonds should not result in rejection by any panelists, however there will have been some people who do not consume almonds frequently and/or do not like to consume almonds and therefore have a bias to reject almonds under any condition. Specifically, three of the seven panelists who rejected the sample at baseline indicated that they consume almonds several times a year or less. It is also possible that the panelists who rejected the sample at baseline are used to eating older almonds (such as those found in the bulk bins at grocery stores) and therefore the sample they evaluated was not acceptable to them. In other words, these panelists may prefer aged or stored almonds as opposed to fresh product, which has been found in some studies (Hough and others 2003).

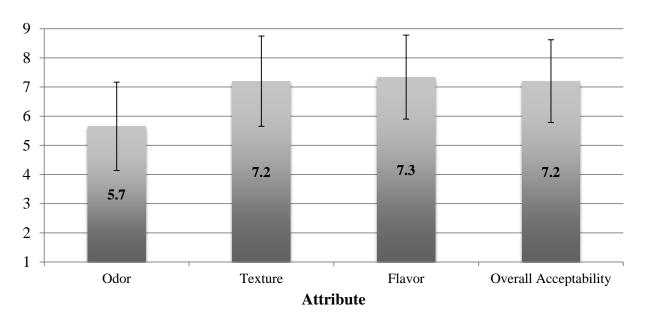


Figure 4.1: Consumer sensory panel responses<sup>a</sup> for raw almonds at baseline (n=118) <sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like" (means±SD)

In addition to rating the individual attributes and responding to the intent to consume question, the consumer sensory panelists were asked to indicate what in particular they liked and disliked about the almond sample (Rousset and Martin 2001). Panelists' responses (Appendices F and G) were used to help explain consumer acceptability and the main reasons the sample was rejected. At baseline, approximately half of the panelists described the almonds as crunchy, indicating that this is something they liked about the sample. Many panelists also said they liked the overall texture and flavor/taste of the sample, with the most commonly used words to describe the flavor being nutty and sweet. Odor was not listed as a quality that was liked by many panelists, however it was listed under dislike. Aside from panelists indicating that they disliked the overall odor, the complaint that the sample had a weak or mild odor was most frequently recorded. The most common complaint about flavor for the sample at baseline was that the sample was bland or mild and the most common textural dislike about the sample was that it was hard and dry. Overall, panelists stated less specific reasons for liking the sample than for disliking and as reported by Rousset and Martin (2001), panelists tend to focus on the most prominent popular and unpopular attributes or characteristics of the sample.

# Sensory evaluation of raw almonds throughout storage

During the 24-month storage period, sixty-nine consumer panels were run; fifty-three of these panels were consumer screening panels (n=35-40) and fifteen were consumer confirmatory panels (n=91-118). The baseline panel is included in the count of confirmatory panels. Although the demographics of each panel differed slightly (Appendix E), the trends were similar. For all panels combined, approximately 78% of the panelists were female (range: 60.5 - 94.7%) and approximately 73% were between the ages of 18 and 27 (range: 42.9 - 94.7%). All participants were consumers of nuts with the percentage consuming nuts, including peanuts, at least several

times per month and the percentage consuming almonds at least several times per month being 88 and 73, respectively.

Consumer sensory results for almonds stored in polypropylene bags

The scores for odor, texture, flavor and overall acceptability as well as the rejection rates for samples stored in polypropylene bags at every testing point are shown in Tables 4.2 – 4.8. Of the 7 samples stored in polypropylene bags, the consumer panelists rejected 4 of the samples during the study. All of the samples stored at 65% relative humidity were rejected (35°C at 6 months, 25°C at 16 months and 15°C at 24 months), and the sample stored at 35°C/50% RH was rejected at 12 months. The two samples stored at 35°C were the first to be rejected, suggesting an influence of temperature on consumer acceptability and rejection. Although all samples were triggered for sensory evaluation within 12 months, three of the samples did not fail within the timeframe of the study (4°C, 15°C/50% RH, 25°C/50% RH).

Results from the screening panel at 16 months indicated that the sample stored at 15°C/65% RH was rejected by the consumer panelists (rejection rate = 30.6%). A confirmatory panel was run and the rejection rate was 22%. Because the confirmatory panel did not result in rejection of the sample, these almonds continued to be tested by a screening panel until triggered again for confirmatory evaluation by the consumer panel at 24 months. The confirmatory panel at 24 months confirmed rejection with a rate of 29.9%. This sample illustrated the necessity to run a confirmatory panel with a larger sample size in order to validate the results of the screening panel and prevent failing a sample that had not yet reached its end of shelf life.

Mean panelist scores for all samples stored in polypropylene bags were above the midpoint for all attributes throughout the entire study except for two samples. The acceptability score for odor for the sample stored at 35°C/50% RH from the screening panel at 12 months was

4.8 (significantly lower than baseline, p<0.05), although it was above the midpoint at all other testing points. The scores for texture, flavor and overall acceptability for the almonds stored at 15°C/65% RH from the screening panel at 24 months were 3.2, 4.2 and 3.9, respectively. However, the scores for texture, flavor and overall acceptability for the almonds stored at 15°C/65% RH were significantly higher in the confirmatory panel than they were in the screening panel at 24 months, illustrating the need to confirm the results of the smaller screening panel with a larger number of consumers.

It also was observed that reducing the temperature from 35°C to 25°C extended the shelf life 10 to 12 months, regardless of storage humidity. This is supported by the results of García-Pascual and others (2003), who found that shelf life was increased with a lower storage temperature. Specifically, they believe there is potentially a protective effect of temperature reduction when samples are exposed to high humidity (García-Pascual and others 2003).

Acceptability scores for all attributes at all testing points were compared to the acceptability scores at baseline to see if statistically significant (*p*<0.05) differences existed, and these results are indicated in Tables 4.2–4.8 by an asterisk. Significant differences in odor were only seen for samples stored at 4°C (24 months confirm), 25°C/50% RH (24 months confirm) and 35°C/50% RH (12 months screen) and these differences were only observed at the end of the study or at the point of failure. Looking at the responses to the open-ended question recorded by panelists for these samples, the most commonly recorded dislikes relating to odor were the overall odor/smell and that the sample had no odor or a mild/weak odor, but similar trends were seen for most other samples at point of failure/end of study. It is important to note that at the confirmatory panel at 24 months, the samples stored at 4°C and at 25°C/50% RH were evaluated in the same panel with the same consumer panelists. A possible explanation for the significant

difference in odor scores for these samples when compared to baseline is that the panelists evaluating these samples were more sensitive to odor than other panelists. Additionally, this significant difference in odor when compared to baseline did not correspond to failure of the sample. Differences in texture, flavor and overall acceptability were seen much more frequently and significant differences in these attributes were observed at point of failure for all rejected samples.

Table 4.2: Consumer sensory panel responses<sup>a</sup> for samples stored in polypropylene bags at 4°C with no relative humidity control and results of t-test when compared to baseline<sup>b</sup>

		Rejection		t-test when comp		Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mear	ns±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	7.2±1.4
12 mo	36	11.1	5.1±1.4	6.6±1.7	7.3±1.1 (35)	7.1±1.2 (35)
14 mo	37	11.1 (36)	5.1±1.6	6.3±1.9*	6.6±1.7*	6.3±1.4*
16 mo	35	5.7	5.7±1.6	6.9±2.0	7.1±1.5	7.0±1.7
18 mo	36	8.8 (34)	5.8±1.9	6.6±2.1	6.8±1.7	6.9±1.7
20 mo	37	13.5	5.6±1.2	6.1±2.1*	6.2±1.9* (36)	6.4±1.8*
22 mo	38	8.1 (37)	5.5±1.5	6.5±1.9*	6.8±1.6	6.6±1.7*
24 mo	38	2.7 (37)	5.6±1.5	6.5±1.8*	7.0±1.5	6.7±1.5
24 mo confirm	102	12.9 (101)	5.3±1.4*	6.1±1.9*	6.4±1.9*	6.4±1.7*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.3: Consumer sensory panel responses<sup>a</sup> for samples stored in polypropylene bags at 15°C and 50% relative humidity and results of t-test when compared to baseline<sup>b</sup>

	n	Rejection Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Overall Acceptability <sup>d</sup>
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	
12 mo	36	11.1	5.4±1.3	6.7±1.5	6.9±1.6	6.8±1.5
14 mo	37	13.9 (36)	5.4±1.5	6.4±1.9*	5.8±1.9*	6.1±1.5* (36)
16 mo	35	8.6	5.9±1.5	6.4±1.9*	6.9±1.7	7.0±1.6
18 mo	36	5.7 (35)	5.3±1.5	6.6±1.8*	6.9±1.6	6.8±1.4
20 mo	37	8.3 (36)	5.9±1.4 (36)	6.3±1.7* (36)	6.9±1.4 (36)	6.7±1.3 (36)
22 mo	38	13.9 (36)	5.2±1.5	6.0±1.8*	6.2±1.9*	6.1±1.7*
24 mo	36	16.7	5.4±1.5	6.1±2.0* (35)	6.2±1.9*	6.2±1.7*
24 mo confirm	100	10.6 (94)	5.8±1.5 (99)	6.7±1.7* (97)	7.1±1.5 (98)	7.0±1.4 (98)

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.4: Consumer sensory panel responses<sup>a</sup> for samples stored in polypropylene bags at 15°C and 65% relative humidity and results of t-test when compared to baseline<sup>b</sup>

	n	Rejection Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Overall Acceptability <sup>d</sup>
				mean	s+SD	
Baseline	118	6.0 (117)	5.7±1.5		7.3±1.4 (115)	7.2±1.4
12 mo	36	8.3	5.3±1.1	6.6±1.8	6.6±1.8*	6.7±1.6
14 mo	35	11.4	5.5±1.4	5.5±2.0*	5.8±2.1*	5.9±1.9*
16 mo	36	30.6	5.6±1.4	5.7±2.0*	5.4±1.9*	5.7±1.6*
16 mo	101	22.0 (100)	5.7±1.7 (100)	6.0±2.0*	6.2±1.8*	6.2±1.7*
18 mo	36	5.7 (35)	5.4±1.4	6.4±1.9*	6.6±1.5*	6.7±1.1 (35)
20 mo	37	21.6	5.6±1.5	6.3±2.0*	5.6±2.0*	6.2±1.9*
22 mo	38	17.7 (34)	5.7±1.5	6.2±1.5*	6.1±1.5*	6.2±1.4*
24 mo	38	60.5	5.1±1.5	3.2±1.5* (36)	4.2±2.0*	3.9±1.8*
24 mo confirm	100	29.9 (97)	5.5±1.8	6.2±1.8*	5.8±2.0*	5.9±1.9*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

<sup>&</sup>lt;sup>e</sup> confirmatory panel resulted in <25% rejection; therefore sample continued to be tested by screening panels at two-month intervals

Table 4.5: Consumer sensory panel responses<sup>a</sup> for samples stored in polypropylene bags at 25°C and 50% relative humidity and results of t-test when compared to baseline<sup>b</sup>

	n	Rejection Rate % c,d	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Overall Acceptability <sup>d</sup>
				mean	s+SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)		7.2±1.4
12 mo	38	16.2 (37)	5.5±1.5	6.4±1.7*	6.4±1.9*	6.5±1.7*
14 mo	36	19.4	5.3±1.6	6.2±2.2*	6.3±2.0*	6.4±1.8*
16 mo	36	17.1 (35)	5.7±1.4	6.2±2.2*	6.7±1.7*	6.5±1.8*
18 mo	37	19.4 (36)	6.2±1.6	6.4±2.1*	6.3±2.0*	6.5±1.9*
20 mo	36	11.1	5.5±1.7	6.6±1.8	6.3±1.8*	6.4±1.6*
22 mo	38	10.8 (37)	5.1±1.8	6.2±2.0*	6.3±2.0*	6.2±1.9*
24 mo	36	31.4 (35)	5.4±1.9	6.0±2.1*	6.1±1.9*	6.2±2.0*
24 mo confirm	102	23 (100)	5.0±1.8*	6.0±1.8*	5.7±2.1*	5.8±2.0*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.6: Consumer sensory panel responses<sup>a</sup> for samples stored in polypropylene bags at 25°C and 65% relative humidity and results of t-test when compared to baseline<sup>b</sup>

		Rejection				Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	7.2±1.4
12 mo	38	17.1 (35)	5.3±1.6	6.1±1.9*	6.4±1.9*	6.3±1.6*
14 mo	36	19.4	5.4±1.9	5.8±1.9*	6.1±1.7*	5.9±1.7*
16 mo	36	28.6 (35)	5.3±1.9	5.6±2.0*	5.3±2.3*	5.4±2.0*
16 mo confirm	101	28.7	5.3±1.8	5.9±2.0*	5.9±2.1*	5.8±1.9*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means $\pm$ SD followed by \* indicate significant difference (p<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels <sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.7: Consumer sensory panel responses<sup>a</sup> for samples stored in polypropylene bags at 35°C and 50% relative humidity and results of t-test when compared to baseline<sup>b</sup>

		Rejection		•		Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	7.2±1.4
6 mo	35	11.4	$6.0\pm2.0$	7.2±1.4	7.5±1.2	7.5±1.3
8 mo	35	14.7 (34)	5.2±1.4	6.9±1.5 (33)	6.7±1.3* (33)	6.3±2.0*
10 mo	37	21.6	5.4±1.8	6.3±2.0*	5.7±2.0*	6.0±1.9*
12 mo	36	31.4 (35)	4.8±1.9*	6.6±1.5* (34)	5.1±2.1*	5.4±2.0*
12 mo confirm	99	28.6 (98)	5.3±1.5	6.3±1.9*	5.9±2.0*	6.1±1.8*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.8: Consumer sensory panel responses<sup>a</sup> for samples stored in polypropylene bags at 35°C and 65% relative humidity and results of t-test when compared to baseline<sup>b</sup>

		Rejection		•		Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	$7.2 \pm 1.4$
2 mo	39	10.5 (38)	5.8±1.4	6.3±1.8*	6.4±1.6*	6.6±1.6*
4 mo	35	14.7 (34)	5.3±1.4	5.5±2.0*	6.0±1.7*	6.1±1.5*
6 mo	36	34.3 (35)	5.3±2.0	5.6±2.4*	5.4±2.4*	5.4±2.1*
6 mo confirm	93	27.2 (92)	5.6±1.5	6.1±1.9*	5.9±2.2*	5.9±2.1*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

Consumer sensory results for almonds stored in unlined cartons

The scores for odor, texture, flavor and overall acceptability as well as the rejection rates for samples stored in unlined cartons at every testing point are shown in Tables 4.9-4.14.

Consumer panelists rejected all of the samples stored in unlined cartons over the storage period (35°C/65% RH at 2 months, 35°C/50% RH at 6 months, 4°C at 6 months, 25°C/65% RH at 12 months, 25°C/50% RH at 16months, 15°C/50% RH at 16 months).

Mean panelists responses for the attributes for all samples tended to be lower than the scores for their counterparts stored in polypropylene bags and acceptability scores were below the midpoint more frequently. It was again observed that reducing the temperature from 35°C to 25°C extended the shelf life 10 to 12 months, regardless of storage humidity. Since the same observation was made for both packaging materials, it can be concluded that the reduction of

b means $\pm$ SD followed by \* indicate significant difference (p<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

temperature has the same benefits for both materials, although packaging in polypropylene bags results in a longer shelf life.

Acceptability scores for all attributes at all testing points were compared to the acceptability scores at baseline to see if significant differences existed, and these results are indicated in Tables 4.9 - 4.14 by an asterisk. For each storage condition, significant differences were seen for more than half of the sensory results, suggesting that storage in unlined cartons results in more significant differences in sensory attributes than storage in polypropylene bags.

The unlined cartons used for storage provided virtually no protection to environmental conditions. Furthermore, packaging in cartons can result in higher product to packaging interaction and in the migration of compounds from the packaging to the product (Guinard and Mazzucchelli 1996). Analysis of the strong and weak points stated by panelists for the samples at point of failure showed that the samples stored in unlined cartons were more frequently described as having a 'cardboard' flavor or odor, especially among individuals who rejected the samples.

Table 4.9: Consumer sensory panel responses<sup>a</sup> for samples stored in unlined cartons at 4°C with no relative humidity control and results of t-test when compared to baseline<sup>b</sup>

		Rejection		•		Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	$7.2\pm1.4$
2 mo	40	20.5 (39)	4.7±1.7*	4.9±2.2*	6.2±1.9*	5.5±1.9*
4 mo	37	13.5	5.0±1.3*	5.6±2.1*	6.8±1.5 (35)	6.6±1.5* (34)
6 mo	36	47.2	4.5±1.8*	3.9±2.5*	5.3±2.2*	4.5±2.3*
6 mo confirm	92	35.2 (91)	4.6±1.6*	4.4±2.3*	5.8±2.1*	5.2±2.2* (91)

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

b means±SD followed by \* indicate significant difference (p<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels c negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.10: Consumer sensory panel responses<sup>a</sup> for samples stored in unlined cartons at 15°C and 50% relative humidity and results of t-test when compared to baseline<sup>b</sup>

		Rejection				Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	$7.2 \pm 1.4$
10 mo	37	16.2	5.9±1.7 (36)	6.5±1.9*	6.6±1.8*	6.7±1.6
12 mo	38	21.6 (37)	5.6±1.3	5.6±1.9*	6.4±1.5*	6.3±1.7*
14 mo	37	17.1 (35)	5.3±1.4	5.9±1.9*	6.1±1.8*	6.1±1.7*
16 mo	36	30.6	5.9±1.7	5.6±2.1*	5.8±1.7*	5.8±1.5*
16 mo confirm	101	40.6	5.2±1.7* (100)	4.9±2.3*	5.6±2.2*	5.4±2.1*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means $\pm$ SD followed by \* indicate significant difference (p<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.11: Consumer sensory panel responses<sup>a</sup> for samples stored in unlined cartons at 25°C and 50% relative humidity and results of t-test when compared to baseline<sup>b</sup>

		Rejection		<u>-</u>		Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	7.2±1.4
12 mo	38	22.9 (35)	5.2±2.2	6.1±2.2*	6.1±1.7*	6.2±1.6*
14 mo	35	8.6	5.2±0.8* (32)	6.4±1.8*	6.6±1.5*	6.6±1.5*
16 mo	36	28.6 (35)	5.6±1.8	6.3±1.8*	5.7±2.0*	5.9±1.6*
16 mo confirm	100	28.9 (97)	5.7±1.6 (99)	5.7±2.0*	5.6±1.8*	5.7±1.8*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

and others 2003)

b means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels c negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.12: Consumer sensory panel responses<sup>a</sup> for samples stored in unlined cartons at 25°C and 65% relative humidity and results of t-test when compared to baseline<sup>b</sup>

		Rejection		•		Overall
	n	Rate % <sup>c,d</sup>	$Odor^d$	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	$7.2\pm1.4$
12 mo	36	61.1	5.6±1.6*	4.5±2.2*	4.0±1.9*	4.3±1.9*
12 mo	100	36.5 (96)	5.5±1.3 (99)	5.4±2.0*	5.3±2.1*	5.6±1.8*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

b means $\pm$ SD followed by \* indicate significant difference (p<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

d sample size reported in parentheses when different from overall panel

Table 4.13: Consumer sensory panel responses<sup>a</sup> for samples stored in unlined cartons at 35°C and 50% relative humidity and results of t-test when compared to baseline<sup>b</sup>

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		Rejection				Overall
	n	Rate % <sup>c,d</sup>	Odor <sup>d</sup>	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5		7.3±1.4 (115)	$7.2 \pm 1.4$
6 mo	36	41.7	5.8±2.1	5.9±2.2*	5.4±2.4*	5.7±2.2*
6 mo confirm	91	29.7	5.5±1.4	5.7±1.9*	5.6±2.0*	5.7±1.9*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

<sup>&</sup>lt;sup>b</sup> means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

Table 4.14: Consumer sensory panel responses<sup>a</sup> for samples stored in unlined cartons at 35°C and 65% relative humidity and results of t-test when compared to baseline<sup>b</sup>

		Rejection				Overall
	n	Rate % <sup>c,d</sup>	$Odor^d$	Texture <sup>d</sup>	Flavor <sup>d</sup>	Acceptability <sup>d</sup>
				mean	s±SD	
Baseline	118	6.0 (117)	5.7±1.5	7.2±1.5 (115)	7.3±1.4 (115)	7.2±1.4
2 mo	40	30.0	5.5±1.7	5.3±2.2*	5.2±1.9*	5.5±2.1*
2 mo confirm	112	32.7 (110)	5.2±1.3*	5.5±2.2*	5.2±2.2*	5.3±2.1*

<sup>&</sup>lt;sup>a</sup> hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"

Comparison of samples stored in polypropylene bags and unlined cartons

The overall timeline of rejection for the fourteen samples is shown in Table 4.15. For each storage condition, samples stored in unlined cartons always failed before samples stored in polypropylene bags when held under similar conditions. Additionally, except for the sample stored in unlined cartons at 4°C, samples failed in the same order for both the bags and the cartons. This could indicate that at ambient humidity (humidity for the 4°C storage chamber was recorded as 95±3%), even with the protection of a low storage temperature, samples stored in unlined cartons are more susceptible to degradation and that storage in polypropylene bags provides sufficient protection to partially compensate for the higher humidity.

It is important to note that although the sample stored in polypropylene bags at 25°C/50% RH did not fail at the confirmatory panel at the conclusion of the study, it did fail at the screening panel at 24 months. It is believed that if the study had continued past 24 months, this sample would have been the next sample to fail, likely at 26 or 28 months. Examining the order

<sup>&</sup>lt;sup>b</sup> means±SD followed by \* indicate significant difference (p<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina); unless indicated as 'confirm,' all panels were screening panels

<sup>&</sup>lt;sup>c</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>d</sup> sample size reported in parentheses when different from overall panel

in which samples failed supports this assumption, as well as the observation that samples stored in polypropylene bags failed 4 to 8 months after their counterparts stored in unlined cartons held under the same storage conditions.

Regardless of packaging material, reducing the relative humidity from 65 to 50% extended the shelf life of the almonds. At 25°C and 35°C, reducing the relative humidity extended the shelf life of almonds at least 4 and 6 months, respectively. Additionally, as mentioned previously, reducing the temperature from 35 to 25°C also extended the shelf life 10 to 12 months, when the relative humidity of the storage environment and the packaging material are held constant.

Table 4.15: Overall failure timeline for samples stored in polypropylene bags and unlined cartons held under the various conditions

	Rejection Rate (%) <sup>a</sup>												
Month	0	2	4	6	8	10	12	14	16	18	20	22	24
Baseline	6.0												
4°C													12.9
15°C /50% RH													10.6
15°C/65% RH													29.9
4 25°C/50% RH 25°C/65% RH													23.0
<sup>m</sup> 25°C/65% RH									28.7				
35°C/50% RH							28.6						
35°C/65% RH				27.2									
4°C				35.2									
Z 15°C/50% RH									40.6				
25°C/50% RH									28.9				
Z 15°C/50% RH 25°C/50% RH Z 25°C/65% RH Z 35°C/50% RH							36.5						
○ 35°C/50% RH				29.7									
35°C/65% RH		32.7											

<sup>&</sup>lt;sup>a</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003); rejection rates in bold font indicate sample failed (>25% of panelists responded 'no' to intent to consume question)

Two-tailed t-tests were performed on the acceptability scores at time of failure/end of study to see if significant differences existed between the samples stored in polypropylene bags and unlined cartons held at the same temperature and relative humidity (Table 4.16). At the

lower storage temperatures (4°C and 15°C), acceptability scores of all attributes differed significantly between the samples stored in bags versus cartons, whereas at high temperature (35°C) only texture differed significantly. This could indicate that storage at high temperature has a greater effect on texture when stored in unlined cartons and that odor, flavor and overall acceptability are equally affected at high temperature for both packaging materials. Specifically, texture acceptability was significantly higher for almonds stored in polypropylene bags at 35°C (6.3±1.9 for 50% RH and 6.1±1.9 for 65% RH) when compared to those stored in unlined cartons at 35°C (5.7±1.9 for 50% RH and 5.5±2.2 for 65% RH).

Table 4.16: Comparison of polypropylene bag versus unlined carton for each storage condition for acceptability of odor, texture, flavor and overall at point of failure/end of study

	Odor	Texture	Flavor	Overall acceptability
4°C	*	*	*	*
15°C / 50% RH	*	*	*	*
25°C / 50% RH	*			
25°C / 65% RH				
35°C / 50% RH		*		
35°C / 65% RH		*		

<sup>\*</sup> indicates significant difference (p<0.05) between samples stored in bags versus cartons held under that storage condition according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina)

## Explaining overall acceptability

Asking a consumer panelist to record what in particular they like and dislike about a particular sample is useful for explaining consumer acceptability and why a specific sample may be rejected (Rousset and Martin 2001). Rousset and Martin (2001) reported that consumers tend to focus on the most prominent popular and unpopular characteristics of a sample. Furthermore, consumers tend to be more descriptive about what they dislike rather than what they like (Greenhoff and MacFie 1994). Additionally, consumers who find a product unacceptable will

tend to be critical on every attribute whereas someone who finds a product acceptable will be less likely to give a negative response to specific attributes (Greenhoff and MacFie 1994).

Panelists' responses for what they liked and disliked about all samples at point of failure/end of study are shown in Appendices F and G, respectively. Overall, panelists stated less specific reasons for liking a sample than for disliking and there were approximately 50% more terms used to describe what was disliked than liked. The most commonly stated reasons for liking a sample were overall flavor/taste, overall texture and crunchiness specifically. These characteristics were among the top five liked traits for all samples at point of failure with the exception of the sample stored in unlined cartons at 4°C, for which crunchiness was not among the top five liked characteristics. The sample stored in unlined cartons at 4°C also had significantly lower texture acceptability scores than all samples. This is not surprising since the samples stored at 4°C were subjected to the highest degree of relative humidity (95±3%) and the unlined carton material provided virtually no protection from atmospheric conditions.

Overall odor, appearance and color were also frequently listed as characteristics that were liked, and appearance characteristics were more commonly stated as attributes liked rather than disliked. In terms of flavor, almond samples were most frequently described as nutty and sweet when panelists were asked what was liked about the sample.

Overall flavor/taste, odor and texture were among the most frequently listed terms under dislike. Soft, chewy and no crunch were the most common specific textural characteristics identified when almonds were disliked and the most common complaint about the flavor of samples at point of failure was that they were bland, not flavorful or only had a mild flavor. The most specific complaint about odor for the samples at point of failure was that they had no odor or a weak odor. Surprisingly, aftertaste and soft were specific terms frequently used to describe

both liked and disliked characteristics of the various almond samples. This suggests that a range of acceptable characteristics exists across a group of consumers.

Contrasting words were also used to describe the texture of the same sample, both for liked and disliked characteristics. For example, some panelists stated that they liked the chewy nature of the sample whereas other panelists stated that they liked the crunchiness of the same sample. In terms of weak points, some panelists indicated that the same sample was hard whereas others indicated it was soft. This further illustrates the range of characteristics consumers used to judge acceptability of a product. Additionally, the use of contrasting words suggests that panelists evaluating the samples were from different mouth behavior groups, which has been shown to drive level of satisfaction of products as well as product choices (Jeltema and others 2015).

It has been shown that products that are valued for crispness or crunchiness, such as almonds, have a more narrow tolerance of texture variation and that products that are 'ideal' in texture have a higher degree of liking (Greenhoff and MacFie 1994). It is not surprising then that when isolating only those panelists who rejected the samples, mushy, soggy and stale textures were common complaints, and a gummy texture was exclusively listed by individuals who rejected the samples. Additionally, the most commonly recorded characteristic panelists listed under 'disliked' was a soft texture. Overall, samples stored in bags were much less frequently described as soft than samples stored in cartons. Of the samples stored in polypropylene bags that were described as soft, the samples stored at the higher relative humidity (65%) were more frequently described as such. The only sample for which a soft texture was never given as a reason for dislike was the sample stored in polyethylene bags at 35°C/50% RH, which failed at 12 months. However, fewer dislikes were listed overall for this sample than for any other.

While these responses are useful for having a better overall picture of acceptability, it is important to remember that consumers have a limited vocabulary when it comes to describing their opinions of a product and they tend to use attribute scales incorrectly (Greenhoff and MacFie 1994). Therefore, these responses were used in conjunction with other tests to gain the most insight.

# Predicting overall acceptability

Stepwise regression was performed in order to identify the contribution of each attribute as a predictor of overall acceptability for each of the sensory panels (Table 4.17). At baseline, all three sensory attributes were found to be predictors of overall acceptability ( $R^2 = 0.80$ ), with percentage contribution of flavor, texture and odor to overall acceptability being 65.7, 13.1 and 1.4, respectively.

At all but four testing points, flavor was the largest predictor of overall acceptability, ranging from 53 to 89% (71 to 85% for samples at point of failure). The four instances at which texture was the largest predictor were all screening panels with a small sample size (polypropylene bags at 4°C at 12 months, unlined cartons at 4°C at 2 and 6 months, and polypropylene bags at 15°C/50% RH at 20 months). Since flavor was the largest predictor for sixty-five out of the sixty-nine panels, the larger contribution of texture to predicting overall acceptability for these samples is likely due to a random sampling of panelists who were more sensitive to texture. This is further supported by looking at the results of the non-sensory tests, which showed that texture was not significantly different from baseline in these samples (discussed later).

Odor was always the smallest contributor to overall acceptability (<5.1%), and it was omitted from the model over half of the time due to lack of significance. The contribution of

texture to predicting overall acceptability ranged from 3 to 20%, excluding the four samples that had higher texture contribution than flavor contribution to overall acceptability.

Table 4.17: Stepwise regression results: contribution of each attribute to overall acceptability <sup>a</sup>

Stora		p wise reg	51055101110		Rejection	Cucii uu	110 410 10 0	veram accep	ruomi
	_	Tagting	noint		rate % <sup>b</sup>	$\mathbb{R}^2$	Odor <sup>c</sup>	Toxturo	Elovor
cond		Testing	g pomi	n 117				Texture <sup>c</sup>	Flavor <sup>c</sup>
Basel		10			6.0	0.80	0.0138	0.1313	0.6574
	4°C	12 mo	screen	36	11.1	0.90	X	0.5764	0.3213
		14 mo	screen	37	11.1	0.89	X	0.1045	0.7816
		16 mo	screen	35	5.7	0.93	X	0.1000	0.8289
		18 mo	screen	34	8.8	0.87	X	0.0658	0.8041
		20 mo	screen	37	13.5	0.90	X	0.1048	0.7904
		22 mo	screen	38	8.1	0.65	X	0.0604	0.5881
		24 mo	screen	38	2.7	0.85	X	0.1309	0.7165
_	1500 /	10	confirm	101	12.9	0.89	X	0.0532	0.8366
	15°C /	12 mo	screen	36	11.1	0.78	X	0.1713	0.6037
	50% RH	14 mo	screen	37	13.9	0.84	0.0436	0.0872	0.7137
		16 mo	screen	35	8.6	0.78	X	0.1044	0.6802
		18 mo	screen	36	5.7	0.79	0.0505	0.0842	0.6576
		20 mo	screen	37	8.3	0.92	0.0123	0.6788	0.2249
		22 mo	screen	38	13.9	0.89	X	0.1339	0.7565
		24 mo	screen	36	16.7	0.89	X	0.1727	0.7217
_			confirm	94	10.6	0.79	X	0.1508	0.6398
	15°C /	12 mo	screen	36	8.3	0.95	X	0.1069	0.8451
	65% RH	14 mo	screen	35	11.4	0.92	X	0.0299	0.8927
		16 mo	screen	36	30.6	0.87	X	0.1642	0.7067
sgı			confirm	100	22.0	0.86	0.0160	0.0959	0.7530
$\mathbf{B}_2$		18 mo	screen	35	5.7	0.74	X	0.1406	0.5956
ne		20 mo	screen	37	21.6	0.86	X	0.0584	0.8012
yle		22 mo	screen	34	17.7	0.88	0.0197	0.0243	0.8330
idc		24 mo	screen	38	60.5	0.84	X	0.1087	0.7337
Polypropylene Bags			confirm	97	29.9	0.90	0.0219	0.1199	0.7552
oly	25°C/	12 mo	screen	37	16.2	0.84	X	X	0.8396
P	50% RH	14 mo	screen	36	19.4	0.92	0.0117	0.0458	0.8622
		16 mo	screen	35	17.1	0.82	X	0.0968	0.7233
		18 mo	screen	36	19.4	0.90	X	0.0697	0.8262
		20 mo	screen	36	11.1	0.88	X	0.1094	0.7673
		22 mo	screen	37	10.8	0.94	0.0091	0.0449	0.8839
		24 mo	screen	35	31.4	0.90	0.0169	0.1111	0.7672
_			confirm	100	23.0	0.91	0.0055	0.0522	0.8505
	25°C/	12 mo	screen	35	17.1	0.83	X	0.0657	0.7681
	65% RH	14 mo	screen	36	19.4	0.83	X	0.1530	0.6787
		16 mo	screen	35	28.6	0.93	X	0.0990	0.8267
_			confirm	101	28.7	0.91	0.0092	0.0541	0.8422
	35°C/	6 mo	screen	35	11.4	0.74	X	0.2040	0.5338
	50% RH	8 mo	screen	34	14.7	0.89	X	0.1172	0.7722
		10 mo	screen	37	21.6	0.90	X	0.0355	0.8634
		12 mo	screen	35	31.4	0.89	X	0.1097	0.7754
_			confirm	98	28.6	0.87	X	0.0505	0.8166
	35°C/	2 mo	screen	38	10.5	0.79	X	0.0303	0.7611
	65% RH	4 mo	screen	34	14.7	0.83	X	0.1252	0.7004
		6 mo	screen	35	34.3	0.81	X	0.1249	0.6850
			confirm	92	27.2	0.91	0.0063	0.0581	0.8466

	4°C	2 mo	screen	39	20.5	0.84	X	0.7601	0.0768
		4 mo	screen	37	13.5	0.74	X	0.1949	0.5406
		6 mo	screen	36	47.2	0.93	X	0.8583	0.0735
			confirm	91	35.2	0.68	0.0159	0.0853	0.7636
	15°C /	10 mo	screen	37	16.2	0.82	X	0.0837	0.7395
	50% RH	12 mo	screen	37	21.6	0.82	X	0.1200	0.6961
S		14 mo	screen	35	17.1	0.83	X	0.1761	0.6516
ton		16 mo	screen	36	30.6	0.91	X	0.1300	0.7848
Cartons			confirm	100	40.6	0.92	X	0.1147	0.8048
Оp	25°C/	12 mo	screen	35	22.9	0.91	X	0.1865	0.7228
ne	50% RH	14 mo	screen	35	8.6	0.90	X	0.1457	0.7523
Unlined		16 mo	screen	35	28.6	0.81	X	0.1035	0.7035
$\Gamma$			confirm	97	28.9	0.88	0.0182	0.1544	0.7068
	25°C/	12 mo	screen	36	61.1	0.83	0.0281	0.1012	0.7023
	65% RH		confirm	96	36.5	0.86	X	0.0780	0.7836
	35°C /	6 mo	screen	36	41.7	0.90	X	0.0219	0.8749
	50% RH		confirm	91	29.7	0.87	X	0.0567	0.8106
	35°C/	2 mo	screen	40	30.0	0.90	X	0.0707	0.8288
	65% RH		confirm	110	32.7	0.89	X	0.1062	0.7883

<sup>&</sup>lt;sup>a</sup> significant contribution (p<0.05) determined using stepwise regression (SAS University Edition, SAS Institute Inc., Cary, North Carolina); values listed are the partial R<sup>2</sup> of each attribute <sup>b</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

Stepwise regression was also performed on all sensory data in order to investigate the contribution of the individual attributes to overall acceptability. Additionally, the data were subdivided into two consumer groups – those who rejected the sample and those who accepted the sample (responses of 'no' and 'yes' to the intent to consume question, respectively) – in order to develop overall acceptability models for each group. Table 4.18 shows the parameter estimates for the intercept as well as each of the individual sensory attributes – odor, texture and flavor; the table also indicates the partial  $R^2$  for each sensory attribute and the cumulative  $R^2$  (the total explainable variability of the model). The overall acceptability (OA) model equation can be determined as follows (where o, t and f are the parameter estimates for odor, texture and flavor, respectively):

OA = intercept + o \* (odor score) + t \* (texture score) + f \* (flavor score)

<sup>&</sup>lt;sup>c</sup> X in column indicates attribute was not a significant predictor and was therefore excluded from the model

The analyses indicated that odor, texture and flavor were all significant factors in predicting overall acceptability. The model created for all sensory data collected explained the most variability (87.3%) and the model created using only data from panelists who had rejected the samples explained the least (75.1%). In all three models, flavor was the largest determinant of overall acceptability and odor was the smallest. The models created using all data and the data for panelists who accepted the samples were similar, although there was a larger contribution of texture and smaller contribution of flavor for the model of panelists who accepted the samples. The model created using only data from panelists who had rejected the samples indicated that odor and texture were larger predictors of overall acceptability and flavor was smaller; although it is important to note that flavor still explained over 56% of the variability in overall acceptability.

These differences in models for panelists who accepted versus rejected the samples indicate that different attributes are more important when consumers make different decisions. Specifically, odor and texture appear to be more important determinants for panelists who reject samples than panelists who do not, indicating that undesirable odors and textures played a larger role than desirable ones in predicting consumer rejection. On the other hand, flavor played a larger role in predicting consumer acceptability.

Table 4.18: Modeling overall acceptability using all sensory data and stepwise regression<sup>a</sup>

		Intercept	Odor	Texture	Flavor	$\mathbb{R}^2$
All data	Parameter estimate Partial R <sup>2</sup>	0.030	0.097 0.0055	0.326 0.0930	0.591 0.7746	0.873
Panelists who accepted the sample <sup>a</sup>	Parameter estimate Partial R <sup>2</sup>	0.553	0.083 0.0062	0.311 0.1197	0.549 0.6986	0.825
Panelists who rejected the sample <sup>b</sup>	Parameter estimate Partial R <sup>2</sup>	-0.076	0.132 0.0183	0.308 0.1684	0.538 0.5644	0.751

<sup>&</sup>lt;sup>a</sup> significant contribution (p<0.05) determined using stepwise regression (SAS University Edition, SAS Institute Inc., Cary, North Carolina)

# Explaining consumer rejection

Additional t-tests were performed in order to see if there were significant differences in attribute scores between panelists who responded negatively towards the intent to consume question compared to those who responded positively. At baseline, panelist scores for texture and overall acceptability differed significantly between panelists who responded "No" to "If you had purchased this product, would you eat it?" (Hough and others 2003) compared to panelists who responded "Yes". Throughout the study, all rejected samples exhibited significant differences between positive and negative intent to consume responses for all attributes. This is as expected, because one would assume that for a panelist to reject a sample, they did not find the attributes acceptable.

Of the three samples that did not fail, the sample stored in polypropylene bags at 25°C/50% RH differed significantly for all attributes whereas the samples stored in polypropylene bags at 4°C and in polypropylene bags at 15°C/50% RH differed significantly in all attributes except for odor. This could indicate that at the lower temperatures, the odor of the sample was preserved

<sup>&</sup>lt;sup>b</sup> panelists who responded 'yes' to "If you had purchased this product would you eat it?" (Hough and others 2003)

<sup>&</sup>lt;sup>b</sup> panelists who responded 'no' to "If you had purchased this product would you eat it?" (Hough and others 2003)

and did not deteriorate as rapidly or noticeably as at the higher temperatures. This is further supported by the results of the stepwise regression, which showed that odor was not a significant predictor of overall acceptability for the samples stored in polypropylene bags at 4°C and at 15°C/50% RH but was for the sample stored in polypropylene bags at 25°C/50% RH.

Additionally, the fact that the sample stored in polypropylene bags at 25°C/50% RH was more similar to the rejected samples than the other non-rejected samples (i.e. differed significantly for all attributes) further supports the previously discussed speculation that this sample would have been the next to fail if the study had continued past 24 months.

When looking at the dislikes of panelists who rejected the samples at point of failure compared to those who responded positively towards the intent to consume question, two characteristics that were more frequently listed were a lingering flavor or aftertaste and a mushy/soggy texture, indicating that these characteristics could be more important to those consumers who rejected the samples. The most commonly recorded terms that were exclusively listed by individuals who rejected the sample were a gummy texture, a sour/tangy flavor/taste and an oxidized taste. Other specific descriptors that were used more frequently by individuals who rejected the samples than those who responded positively to the intent to consume question include stale flavor/taste and texture, rancid flavor/taste, mushy/soggy texture, cardboard flavor/taste and no/weak almond/nutty flavor and odor. The most commonly stated reason for liking a sample that was exclusively used by panelists who responded positively to the intent to consume question was fresh tasting, followed by describing the texture as not too hard.

## Non-sensory assessment of raw almonds throughout storage

Chemical and instrumental tests have been shown to improve the overall picture of changes in product quality and how these changes affect consumer acceptability (Giménez and

others 2012). Every two months throughout the study, team members in the Department of Food Science and Technology conducted free fatty acid and peroxide value tests to monitor changes in the quality of the raw almonds. Additionally, on the day of each sensory panel, water activity and textural assessments were conducted on the same lot of almonds being evaluated.

Non-sensory results for almonds stored in polypropylene bags

Water activity, free fatty acids, peroxide value, maximum force and the number of fracture peaks as well as the rejection rates for samples stored in polypropylene bags at point of failure are shown in Table 4.19. Of the 7 samples stored in polypropylene bags, the consumer panelists rejected 4 of the samples during the study (35°C/65% RH at 6 months, 35°C/50% RH at 12 months, 25°C/65% RH at 16 months and 15°C/65% RH at 24 months).

The only sample that did not exhibit a significant difference at point of failure in the number of fracture peaks or water activity when compared to baseline was the only sample that experienced a significant increase in maximum force (35°C/50% RH at 12 mo). This sample consistently had a significantly higher force than baseline except for its first testing point (at 6 mo) and never differed significantly in the number of fracture peaks. However, the water activity for this sample was significantly higher than baseline at every testing point except for the sample tested on the day of the confirmatory panel at point of failure. It is possible that the almonds tested on this day were not representative of the sample as a whole since it is implausible that there would be a significant change in the water activity between the screening and confirmatory panels. It should be noted that almonds stored under each condition were selected randomly from the chamber for evaluation at each time point.

Table 4.19: Consumer sensory rejection and results from non-sensory tests for raw almonds stored in polypropylene bags at baseline and at point of failure and results of t-test compared to baseline

					Free fatty	Peroxide value		
Storage	Point of		Rejection	Water	acids	(meq. active	Peaks	Force
Condition	Failure	n	Rate % <sup>a</sup>	activity <sup>b</sup>	(acid value) <sup>c</sup>	O <sub>2</sub> /kg oil) <sup>d</sup>	(number) <sup>e</sup>	$(g)^e$
						means±SDf		
Baseline		117	6.0	$0.289 \pm 0.022$	$0.285 \pm 0.028$	< 0.01	$6.7 \pm 3.0$	49972±6596
35°C / 65% RH	6 mo	92	27.2	0.658±0.020*	$0.297 \pm 0.032$	1.41±0.11*	5.2±1.8*	45292±4916*
35°C / 50% RH	12 mo	98	28.6	$0.331 \pm 0.060$	$0.342 \pm 0.026$	4.26±0.61*	$6.5 \pm 1.9$	83064±7436*
25°C / 65% RH	16 mo	101	28.7	0.557±0.009*	0.538±0.012*	4.15±0.31*	4.6±1.8*	46150±4846*
15°C / 65% RH	24 mo	97	29.9	0.445±0.012*	0.632±0.006*	1.87±0.11*	5.1±2.5*	46659±4538*

<sup>&</sup>lt;sup>a</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>b</sup> n=6 (AquaLab Dew Point Water Activity Meter 4TE)

<sup>&</sup>lt;sup>c</sup> n=3; mg KOH necessary to neutralize 1 g of sample

 $<sup>^{\</sup>rm d}$  n=3

<sup>&</sup>lt;sup>e</sup> n=36 (TAXT.2 Plus Texture Analyzer equipped with Texture Exponent 32 and a 50 kg load cell as described by Varela and others 2008b)

f means $\pm$ SD followed by \* indicate significant difference (p<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina)

The change in peroxide levels over time for samples stored in polypropylene bags is shown in Figure 4.2. Since peroxides are the first major product from the oxidative breakdown of fats, peroxide values should increase as oxidation progresses until it peaks and then declines as the primary breakdown products are converted to secondary breakdown products (Shahidi and John 2010; Huang 2014; Nawar 1996; Rossell 1999). It is the secondary breakdown products, such as aldehydes and ketones, that cause the off-flavors associated with oxidative rancidity (Rossell 1999). This trend is perceptible in all samples except those stored at the higher temperatures (25 and 35°C) at 65% RH. The sample stored at 35°C/65% RH was rejected by consumers at 6 months when its peroxide level was only 1.41±0.11 meq. active O<sub>2</sub>/kg oil, but this sample continued to undergo chemical analyses until 14 months. When the change in free fatty acid content over time is considered for this sample (discussed in detail next), it is observed that this sample had a higher level of free fatty acids than all other samples stored in polypropylene bags. It is likely that the reason for consumer rejection of this sample was due to off-flavor development resulting from hydrolytic rancidity.

The sample stored at 25°C/65% RH was rejected at 16 months when its peroxide level was 4.15±0.31 meq. active O<sub>2</sub>/kg oil and did not undergo further chemical testing. Rejection of this sample was likely influenced by oxidative flavor development because of the high peroxide level, although analysis of the strong and weak points stated by panelists does not confirm this. Rather this sample was described as being bland, not flavorful or having a mild flavor. It was also described as being bitter and the aftertaste was listed as a characteristic that was disliked.

The sample stored at 15°C/65% RH was rejected by consumers at 24 months, however chemical analyses suggest that peroxides had already begun to be converted to secondary products as the level had decreased. The most plausible explanation for this is that the lower

temperature preserved the flavor of the sample. This is supported by the results of the stepwise regression as the contribution of flavor to overall acceptability for the sample stored at 15°C/65% RH was lower at 24 months than it had been in the previous few months.

It is important to note that while a significant increase in peroxide values did occur, levels never exceeded the 5 meq. active O<sub>2</sub>/kg oil recommended cutoff typically used by industry to ensure almond quality (Huang 2014; Almond Board of California 2013).

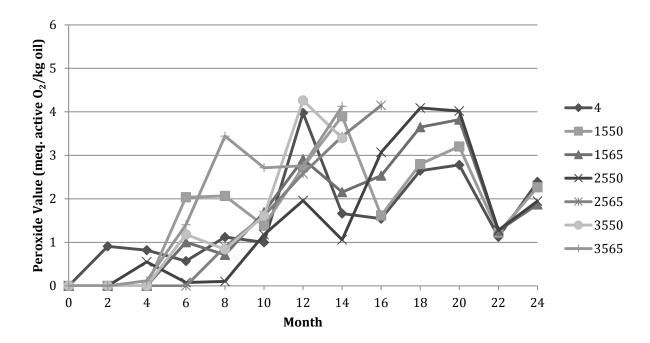


Figure 4.2: Peroxide values for samples stored in polypropylene bags

Free fatty acids are the result of hydrolytic rancidity due to moisture and/or lipase activity. Figure 4.3 shows how the amount of free fatty acids changed over time. At point of failure, only the samples stored at 15°C/65% RH and 25°C/65% RH differed significantly in free fatty acid content when compared to baseline assessments. Research has shown that hydrolytic rancidity, the result of hydrolysis of triglycerides in the presence of moisture, becomes a more prominent issue when free fatty acid levels exceed 0.5 mg/g (Rossell 1999), and this was seen for the two samples that were significantly different from baseline. This indicates that consumer rejection of

these samples could be due to hydrolytic rancidity. Panelists who had rejected both of these samples described them as rancid, stale or oxidized tasting with a rancid or off odor.

Results of this study are similar to results from Senesi and others (1991) who found that at 4°C, free fatty acid levels were relatively constant over the first 7.5 months of storage, and that storage at 20°C resulted in significantly higher free fatty acid levels. Overall, Senesi and others (1991) concluded that storage temperature has a significant effect on lipolytic activity. Figure 4.3 shows that storage at 35°C resulted in significantly higher free fatty acid levels, especially compared to almonds stored at 4°C.

Although the sample stored at 25°C/50% RH was not rejected by consumers at the conclusion of the study, it was postulated that if the study had continued it would have been the next sample to fail. This is supported by examining the free fatty acid level for this sample over time. Over the last eight months of the study, the free fatty acid level for this sample steadily increased from 0.492±0.025 at 18 months to 0.690±0.028 at 24 months. This indicates that hydrolytic rancidity was occurring over time, and it is possible that the increase in hydrolytic rancidity would contribute to consumer rejection.

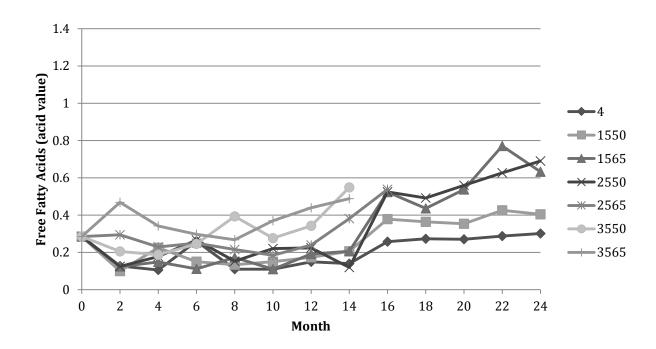


Figure 4.3: Free fatty acids for samples stored in polypropylene bags

Figure 4.4 shows the change in water activity throughout storage for samples stored in polypropylene bags. As expected, water activity increased for all samples during the storage period, especially for the samples stored under higher humidity conditions. All samples stored at 65% RH exhibited significant differences in water activity at all testing points when compared to baseline and all samples stored at 50% RH were not significantly different from baseline for at least one testing point. This indicates that samples stored at a higher relative humidity absorbed moisture from the environment more readily than samples stored at 50% RH. The highest water activities were also seen for the samples stored at the highest temperature, suggesting that temperature affects the rate of increasing water activity. Generally, increases in temperature result in a decrease in the amount of sorbed water, however some researchers are reporting that for some lower moisture foods, such as dried fruits and nuts, water activity actually increases with higher temperatures (Al-Muhtaseb and others 2002).

Water activity levels for the sample stored at 4°C were more similar to the samples stored at 50% RH at 15 and 25°C than the samples stored at the higher humidity levels. This is the opposite of what one would expect because this sample was subjected to the highest humidity (humidity in this chamber was recorded as 95±3%). A probable explanation is that the lower temperature coupled with the low water vapor transmission rate of the polypropylene packaging provided sufficient protection against the high humidity level.

Water activity levels above 0.4 have been shown to have detrimental effects on almond textural quality (Vickers and others 2014), and the water activity for all samples exceeded this level. Additionally, the water activities of all samples at point of rejection were above this level with the exception of the sample stored at 35°C/50% RH (although the water activity of the sample stored at 35°C/50% RH was above 0.4 at all testing points, including on the day of the screening panel at point of rejection). However, water activities exceeded 0.4 over half of the time when samples were not rejected, indicating that any detrimental textural changes occurring were not always sufficient enough to result in rejection of the sample.

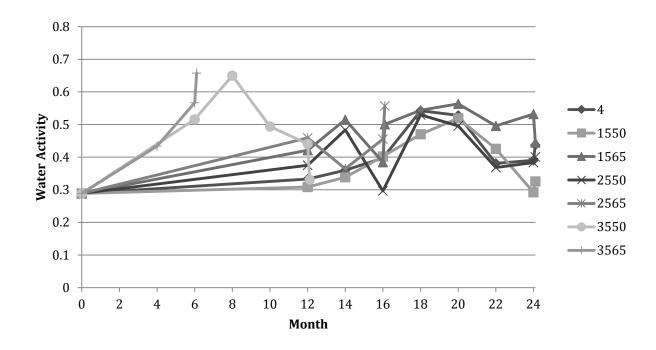


Figure 4.4: Water activity for samples stored in polypropylene bags. Two data points at the same time point represent data collected for screening and confirmatory panels.

Maximum force is an indication of the hardness of a product (Guinard and Mazzucchelli 1996) and the force for almonds stored in polypropylene bags at all testing points are shown in Figure 4.5. Both an increase and a decrease in force was seen when the samples were compared to baseline. Any sample that experienced a significant increase in maximum force saw the increase in the first 14 months of storage and from month 16 on, all remaining samples saw a significant decrease in force.

Although the samples stored at 15°C/65% RH and 25°C/50% RH saw a significant increase in force at 12 months, it is possible that the random almonds sampled were not representative of the conditions as a whole. This is because the sample stored at 15°C/65% RH had a significant decrease in force at every other testing point and the sample stored at 25°C/50% RH had a significant decrease at two testing points and saw no difference at all other testing points. The samples stored at 25°C/65% RH and 35°C/50% RH, on the other hand, more

consistently had significant increases in maximum force. Not only is maximum force an assessment of hardness, it can also be used to study the extent to which food is deformed (Guinard and Mazzucchelli 1996). Those samples that exhibited an increase in force were likely being deformed rather than fracturing, indicating that the samples are tough and chewy rather than crunchy; they require more force to become smashed as opposed to more force to fracture. Analyzing the panelist comments for the samples in question supports this assumption, although it is important to note that since the panels in question were screening panels that did not result in rejection of the sample, fewer panelists listed items that were disliked. These samples were described as chewy and not crisp or crunchy and hard or hard to chew. Other common descriptors recorded under dislike include gummy, sticky and toothpack.

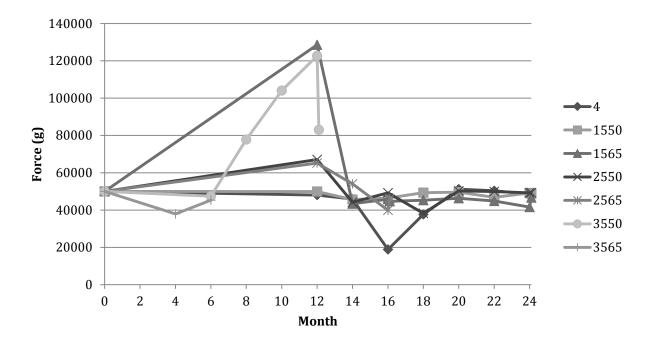


Figure 4.5: Maximum force for samples stored in polypropylene bags. Two data points at the same time point represent data collected for screening and confirmatory panels.

Degree of jaggedness on a force-deformation curve is an indication of the fracturability of a product and a decrease in the number of fracture peaks corresponds to a loss of brittleness

and crunchiness. Figure 4.6 illustrates the number of fracture peaks for almonds stored in polypropylene bags at all testing points. All samples displayed the same trend, with the number of fracture peaks decreasing over storage time. While Figure 4.6 indicates that the number of fracture peaks for the sample stored at 35°C/50% RH increased at 10 months, this increase was not significant. In fact, this sample never experienced a significant change in the number of fracture peaks when compared to baseline.

The number of fracture peaks for the sample stored in polypropylene bags at 15°C/65% RH at the screening panel at 24 months was the lowest of all samples in any packaging material and was significantly different from the results two months prior and the results of the confirmatory panel at 24 months. The almonds tested on the day of the screening panel at 24 months, both instrumentally and evaluated by consumer panelists, were likely not representative of the storage condition as a whole and it is possible that there may have had an improper seal on the bag. The results of the sensory evaluation support this, as this sample had the highest rejection rate by consumers (60.5%) of all sensory panels. This high rejection rate and significant deviation in number of fracture peaks is most likely explained by the almonds being soft and not having any crunch. This sample also had a higher water activity compared to the evaluation two months prior and the evaluation on the day of the confirmatory panel.

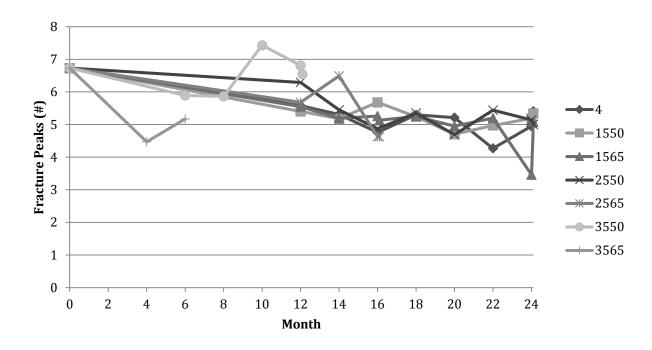


Figure 4.6: Number of fracture peaks for samples stored in polypropylene bags. Two data points at the same time point represent data collected for screening and confirmatory panels.

Non-sensory results for almonds stored in unlined cartons

Water activity, free fatty acids, peroxide value, maximum force and the number of fracture peaks as well as the rejection rates for samples stored in unlined cartons at point of failure are shown in Table 4.20. Consumer panelists rejected all of the samples stored in unlined cartons by 16 months (35°C/65% RH at 2 months, 35°C/50% RH at 6 months, 4°C at 6 months, 25°C/65% RH at 12 months, 25°C/50% RH at 16 months and 15°C/50% RH at 16 months).

Table 4.20: Consumer sensory rejection and results from non-sensory tests for raw almonds stored in unlined cartons at baseline and at point of failure and results of t-test compared to baseline

					Free fatty	Peroxide value		
Storage	Point of		Rejection	Water	acids	(meq. active	Peaks	Force
Condition	Failure	n	Rate % <sup>a</sup>	activity <sup>b</sup>	(acid value) <sup>c</sup>	O <sub>2</sub> /kg oil) <sup>d</sup>	(number) <sup>e</sup>	$(g)^e$
						means±SDf		
Baseline		117	6.0	$0.289 \pm 0.022$	$0.285 \pm 0.028$	< 0.01	$6.7 \pm 3.0$	49972±6596
35°C / 65% RH	2 mo	110	32.7		$0.498 \pm 0.024 *$	$0.56\pm0.14*$		
4°C	6 mo	91	35.2	0.779±0.010*	1.206±0.046*	< 0.01	4.5±1.5*	40890±4285*
35°C / 50% RH	6 mo	91	29.7	$0.668\pm0.029*$	$0.300\pm0.043$	1.10±0.31*	4.7±1.5*	49029±5037
25°C / 65% RH	12 mo	96	36.5	$0.294 \pm 0.009$	$0.356 \pm 0.013$	4.05±0.77*	$6.0\pm2.5$	75523±10233*
15°C / 50% RH	16 mo	101	40.6	0.641±0.002*	$0.276 \pm 0.064$	4.26±0.38*	4.7±1.7*	43815±3775*
25°C / 50% RH	16 mo	97	28.9	0.436±0.015*	0.515±0.015*	3.36±0.29*	4.5±1.9*	50192±5172

<sup>&</sup>lt;sup>a</sup> negative response to "If you had purchased this product would you eat it?" (yes or no) (Hough and others 2003)

<sup>&</sup>lt;sup>b</sup> n=6 (AquaLab Dew Point Water Activity Meter 4TE)

<sup>&</sup>lt;sup>c</sup> n=3; mg KOH necessary to neutralize 1 g of sample

 $<sup>^{</sup>d}$  n=3

<sup>&</sup>lt;sup>e</sup> n=36 (TAXT.2 Plus Texture Analyzer equipped with Texture Exponent 32 and a 50 kg load cell as described by Varela and others 2008b)

f means±SD followed by \* indicate significant difference (*p*<0.05) compared to baseline according to a 2-tailed t-test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina)

The change in peroxide levels over time for samples stored in unlined cartons is shown in Figure 4.7. As discussed earlier, peroxide values are expected to increase as oxidation progresses until it peaks and then declines as secondary breakdown products are formed (Shahidi and John 2010; Huang 2014; Nawar 1996; Rossell 1999). This trend is just beginning to be seen for samples stored in cartons; peroxide values for most samples did not exhibit a decline during the timeframe the samples were analyzed and therefore it is possible the samples were not analyzed to the point of peak peroxide level. Furthermore, consumers rejected most samples while peroxide levels were on the rise. This could indicate that the reasons for rejection by consumers for the majority of samples stored in unlined cartons are not due to oxidative or rancid flavors, but rather textural or other flavor changes (Marsh and Bugusu 2007).

When looking at the peroxide value of all samples at point of failure (Tables 4.19 and 4.20), the sample stored in unlined cartons at 4°C was the only sample that did not exhibit a significant difference in peroxide values when compared to baseline. It is believed that this is a discrepancy in the data as there was a complication during data collection; only one sample was analyzed and less than 0.01 meq. active O<sub>2</sub>/kg oil was detected. However, it is plausible that this sample was in the second phase of lipid oxidation with the production of secondary products because the peroxide level at 4 months was less than at 2 months.

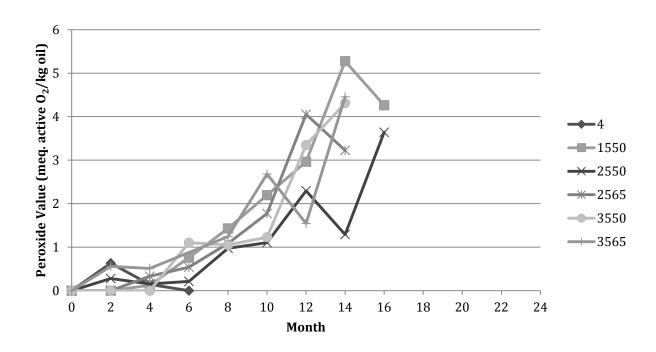


Figure 4.7: Peroxide values for samples stored in unlined cartons

By examining the amount of free fatty acids present in samples at point of failure (Table 4.20), it can be seen that the only samples that were significantly different from baseline were samples that had free fatty acid levels that exceeded 0.5 mg/g, the level at which hydrolytic rancidity is believed to be a problem (Rossell 1999). The changes in free fatty acids over time for the samples stored in unlined cartons are shown in Figure 4.8. Since hydrolytic rancidity is largely due to moisture (Rossell 1999), one would expect the samples stored under higher relative humidity to have higher levels of free fatty acids. This is seen for samples stored in unlined cartons at higher temperatures (25 and 35°C). At the higher temperatures, when 50 and 65% RH are compared for each temperature, samples stored at the higher humidity have higher levels of free fatty acids at the majority of testing points.

In Figure 4.8 it can be seen that the highest level of free fatty acids occurred for the sample stored in unlined cartons at 4°C at 6 months. While it is believed that there is some irregularity in this result (n=1 due to a complication with data collection), it is not surprising that

this sample would exhibit a higher level of free fatty acids than other samples. The relative humidity in the 4°C storage unit was recorded as 95±3%, making this sample subjected to the highest amount of humidity. Additionally, the unlined carton material provided virtually no protection from the high humidity.

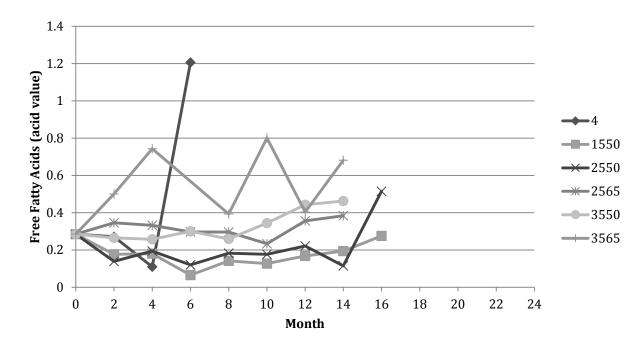


Figure 4.8: Free fatty acids for samples stored in unlined cartons

The change in water activity during storage for samples stored in unlined cartons is shown in Figure 4.9. Water activity was the highest of all samples in both packaging materials for the sample stored at 4°C in unlined cartons (95±3% RH), as expected, because the unlined carton did not provide any protection from the environment. Water activity increased significantly for all samples at all testing points throughout the storage period. The only exception was the sample stored at 25°C/65% RH, for which the water activity on the day of the confirmatory panel at the point of failure (12 mo) was not significantly different from baseline. However, water activity on the day of the screening panel was significantly higher than at baseline. It is unclear which of the water activities is a truer representation of the water activity

of almonds stored under this condition as this sample was only evaluated at 12 months, when it was rejected by the consumer panelists. That being said, all samples stored at 65% RH in polypropylene bags saw a greater increase in water activity than their counterparts stored at 50% RH at the same temperature, so it is therefore likely that the water activity for the sample stored at 25°C/65% RH from the day of the screening panel is more accurate.

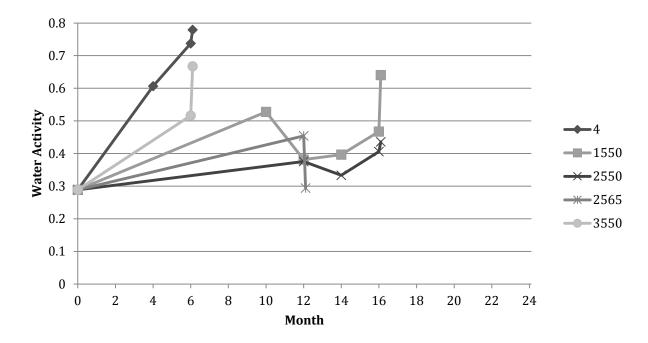


Figure 4.9: Water activity for samples stored in unlined cartons. Two data points at the same time point represent data collected for screening and confirmatory panels.

Maximum force for almonds stored in unlined cartons at all testing points is shown in Figure 4.10. Both samples stored at 15°C (50 and 65% RH) and the sample stored at 25°C/50% RH followed the same trend; initially, force increased significantly and then it decreased until it was significantly lower than the force at baseline. The sample stored at 35°C/50% RH did not differ significantly when compared to baseline. Textural analyses for the samples stored at 4°C and at 25°C/65% again suggest that the random sampling was not representative as high degrees

of variability existed between the testing days. It is important to note that almonds to be sampled on each testing day were randomly selected from the storage chambers.

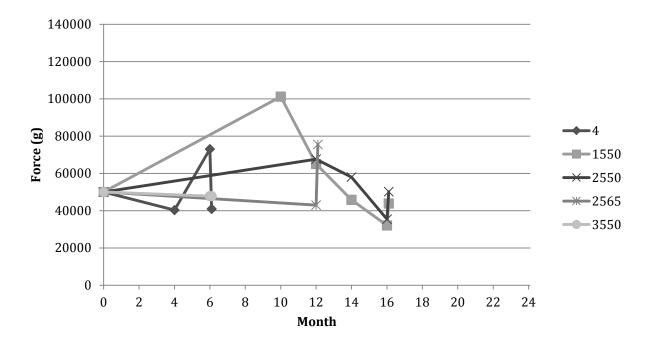


Figure 4.10: Maximum force for samples stored in unlined cartons. Two data points at the same time point represent data collected for screening and confirmatory panels.

Figure 4.11 shows the number of fracture peaks for samples stored in unlined cartons at all testing points. All samples followed the same trend, with the number of fracture peaks decreasing throughout storage, suggesting the almonds were losing their crunch. The only sample that never differed significantly when compared to baseline was the sample stored at 15°C/65% RH. This sample also saw a significant increase in maximum force, and it is possible that this higher force resulted in more fractures.

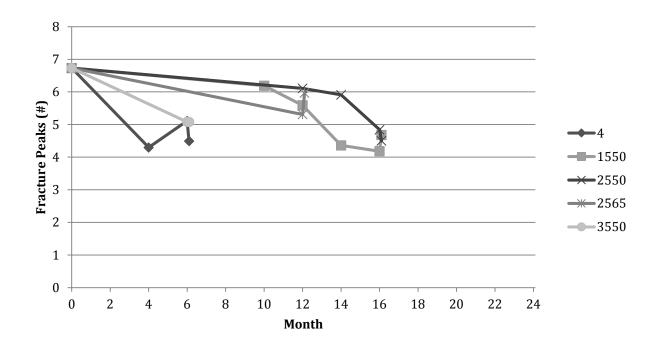


Figure 4.11: Number of fracture peaks for samples stored in unlined cartons. Two data points at the same time point represent data collected for screening and confirmatory panels.

Comparison of samples stored in polypropylene bags and unlined cartons

García-Pascual and others (2003) found that almonds stored at a refrigerated temperature of 8°C did not undergo significant changes in their chemical parameters until the sixth month of storage, which is similar to results of this study. No sample stored at 4 or 15°C failed before 6 months of storage, and except for the sample stored in unlined cartons at 4°C, the other samples stored at 4 or 15°C were not rejected until 16 months, if at all.

Unlined cartons are known to be more sensitive to moisture than plastic packaging, especially with increasing humidity (Marsh and Bugusu 2007) and Mexis and Kontominas (2010) found that packaging material barrier to oxygen had a significant effect on peroxide value and overall shelf life. The same results were found in the present study. Samples stored in unlined cartons were all rejected by consumers before samples stored in polypropylene bags and on average, samples stored in unlined cartons had higher peroxide values than their counterparts

stored in polypropylene bags held under similar storage conditions. While significant increases in peroxide values were seen for samples stored in both polypropylene bags and unlined cartons, all peroxide values were below the 5 meq. active O<sub>2</sub>/kg oil recommended cutoff typically used in industry (Huang 2014; Almond Board of California 2013).

Water activity has been shown to strongly influence the rate of enzyme-catalyzed reactions and lipid oxidation (Fennema and Tannenbaum 1996; Nawar 1996). It is not surprising then that the samples with the highest water activities in each packaging material also had the highest amount of free fatty acids. In particular, the sample stored in unlined cartons at 4°C had a much higher water activity and a significantly higher amount of free fatty acids when compared to all other samples. When the sample stored in polypropylene bags at the same condition (i.e. at 4°C) is compared to the sample stored in unlined cartons, the evidence of lipid oxidation and hydrolytic rancidity is much less. It is therefore concluded that if almonds are to be stored at atmospheric conditions without relative humidity control, storage in polypropylene bags at 4°C is best. Furthermore, sensory evaluation indicates that storage under these conditions results in a shelf life of over two years.

Water activity was generally higher among samples stored in unlined cartons than polypropylene bags. Water activity was also above 0.4 earlier in the storage period for samples stored in unlined cartons and research has shown that water activity above this level can be detrimental to crunchiness of products (Vickers and others 2014), supporting the belief that textural quality deteriorates more rapidly when almonds are stored in unlined cartons.

Additionally, increases in water activity are commonly accompanied by decreases in sensory and instrumental measures of crunchiness (Vickers and others 2014). Texture acceptability ratings have been shown to be positively correlated with crispness, crunchiness and persistence of

crunch and research has shown that a high number of fracture peaks are indicative of a crunchy, brittle product (Vickers and others 2014; Varela and others 2006; Varela and others 2008a).

Results of this study showed that as water activity increased throughout storage the number of fracture peaks decreased for all samples, indicating that the raw almonds lost their crunch.

Varela and others (2008a) found that acceptability parameters were negatively correlated with instrumental hardness/toughness, but this study is not in agreement. Consumer panelists rejected samples when the maximum force was significantly lower, significantly higher and not significantly different when compared to baseline. This suggests that other factors were more important than instrumental textural assessments of hardness in predicting consumer acceptability of raw almonds.

# Comparison of all samples at point of failure

Analysis of variance was performed on the sensory and chemical/instrumental data for all samples at point of failure or at the end of the study for the three samples that were not rejected by consumers. These results confirm the conclusions already discussed in this paper and are shown in Tables 4.21 and 4.22, respectively.

All samples except for the one stored in unlined cartons at 4°C did not differ significantly in odor acceptability when compared to baseline. It has been noted that consumers evaluated the odor of the samples prior to tasting the almonds and therefore the majority of the aromatic compounds had not been released due to mastication. It is highly probable that the volatile compounds formed as a result of hydrolytic rancidity and lipid oxidation were not detectable in the intact almonds.

The sample stored in unlined cartons at 4°C also exhibited the greatest quality deterioration of all samples. It had significantly lower odor, texture and overall acceptability

scores than all other samples. Surprisingly, the flavor score for this sample was not significantly different from most other samples. This suggests that all rejected samples exhibited the same deterioration in flavor; however, whether the decrease in flavor scores of all samples was due to loss of desirable flavors or development of rancid flavors is unknown.

The sample stored in unlined cartons at 4°C also had significantly higher water activity and free fatty acid content than all other samples at point of failure. The apparent relationship between water activity and free fatty acid content is supported by literature (Hamilton 1999; Rossell 1999) and suggests that this sample experienced substantially more hydrolytic rancidity than all other samples. The high degree of water sorption would seem to explain the significant deterioration in texture acceptability of this sample. Additionally, instrumental hardness assessments do not appear to parallel any sensorial attribute, whereas instrumental fracturability assessments decreased for all samples.

It has been speculated that if the study had continued, the sample stored in polypropylene bags at 25°C/50% RH would have been the next to be rejected by consumers. Regarding the difference of flavor acceptability between samples, the samples stored in polypropylene bags at 4°C and at 15°C/50% RH were not significantly different from each other and have the closest flavor scores to baseline of all other samples. The sample stored in polypropylene bags at 25°C/50% RH has the next closest flavor score to baseline but is not significantly different from most other samples when they failed. This indicates that of the three samples that did not fail, the flavor of the sample stored in polypropylene bags at 25°C/50% RH is more similar to the flavor of samples that had failed than samples that had not. Stepwise regression has already indicated that flavor is the largest predictor of overall acceptability, and therefore flavor of this sample being more similar to the other rejected samples further suggests the proximity this sample was

to being rejected by consumers. Finally, when the attribute scores were compared for panelists who rejected the sample compared to those who did not, the sample stored in polypropylene bags at 25°C/50% RH differed significantly for all attributes whereas the other two samples that were not rejected did not differ significantly for odor. This further indicates that this sample was more similar to the samples that had been rejected than those that were not.

The sample that was the most similar to baseline at the end of the study was the sample stored in polypropylene bags at 15°C/50% RH. The scores for odor and overall acceptability for this sample were not significantly different from baseline when the study concluded at 24 months, and although flavor is significantly different when compared to baseline, it had the highest score of all samples. This sample was also the only sample that did not have a significantly different water activity when compared to baseline at the end of the study. This suggests that among the conditions investigated, quality of raw almonds is best maintained when stored in polypropylene bags, which provide high barrier to both oxygen and water barrier, and at low temperature and humidity.

Table 4.21: Sensory ANOVA results<sup>a</sup> for samples at point of failure/end of study

		Point of				Overall
		failure	Odor <sup>b</sup>	Texture <sup>b</sup>	Flavor <sup>b</sup>	Acceptability <sup>b</sup>
				mean	s±SD <sup>c</sup>	
Baseline			5.7±1.5 a	7.2±1.5 a	7.3±1.4 a	7.2±1.4 a
ne bags	4°C		5.3±1.4 a	6.2±1.9 bc	6.6±1.8 b	6.5±1.7 cd
	15°C / 50% RH		5.7±1.5 a	6.6±1.8 b	6.8±1.6 b	6.8±1.5 ab
	15°C / 65% RH	24 mo	5.4±1.7 a	5.4±2.2 def	5.3±2.1 cd	5.4±2.1 de
oyle	25°C / 50% RH		5.1±1.8 a	6.0±1.9 bcd	5.8±2.0 c	5.9±2.0 cd
Polypropylene bags	25°C / 65% RH	16 mo	5.3±1.8 a	5.8±2.0 cde	5.8±2.1 cd	5.7±2.0 d
	35°C / 50% RH	12 mo	5.2±1.6 a	6.4±1.8 bc	5.7±2.1 cd	5.9±1.9 cd
	35°C / 65% RH	6 mo	5.5±1.7 a	5.9±2.1 bcd	5.7±2.3 cd	5.8±2.1 d
Unlined cartons	4°C	6 mo	4.6±1.7 b	4.3±2.3 g	5.6±2.1 cd	5.0±2.3 e
	15°C / 50% RH	16 mo	5.4±1.8 a	5.1±2.3 f	5.6±2.1 cd	5.5±2.0 de
	25°C / 50% RH	16 mo	5.6±1.6 a	5.8±2.0 cde	5.6±1.8 cd	5.8±1.7 d
	25°C / 65% RH	12 mo	5.3±1.5 a	5.2±2.1 ef	5.0±2.1 d	5.2±1.9 de
	35°C / 50% RH	6 mo	5.6±1.6 a	5.8±2.0 cde	5.6±2.1 cd	5.7±2.0 d
	35°C / 65% RH	2 mo	5.3±1.4 a	5.5±2.2 def	5.2±2.1 cd	5.4±2.1 de

a data from screening and confirmatory panels combined (n>115)
b hedonic scale where 1 is "extremely dislike" and 9 is "extremely like"
c means±SD followed by different letters within a column differ significantly (*p*<0.05) according to ANOVA and SNK means separation test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina)

Table 4.22: Chemical and instrumental ANOVA results for samples at point of failure/end of study

Water activity <sup>a</sup>	Force <sup>b</sup>	Fracture peaks <sup>b</sup>	(meq. active O <sub>2</sub> /kg oil)	(acid value) <sup>c</sup>
Water activity <sup>a</sup>	Force <sup>b</sup>	Fracture peaks <sup>b</sup>	O <sub>2</sub> /kg oil)	
			Ozikg OII)	
		means±SD <sup>d</sup>		
0.289±0.022 e	49972±6595 c	6.7±3.0 a	<0.01 e	0.285±0.028 gh
0.419±0.026 d	48779±5324 cd	5.2±2.3 bc	2.39±0.09 b	0.301±0.009 gh
0.310±0.045 e	48927±4838 cd	5.3±2.8 bc	2.27±0.26 b	0.403±0.044 e
0.488±0.055 c	44077±4911 de	4.3±2.2 c	1.87±0.10 bc	0.633±0.005 c
0.393±0.012 d	49242±5242 cd	5.1±2.1 bc	1.95±0.25 bc	0.690±0.025 b
0.506±0.055 c	43013±5834 e	4.6±1.8 bc	4.15±0.28 a	0.534±0.011 d
0.386±0.072 d	103144±22549 a	6.7±2.3 a	4.26±0.54 a	0.342±0.023 fg
0.612±0.050 b	45292±4916 cde	5.2±1.8 bc	1.41±0.10 cd	0.297±0.029 gh
0.758±0.033 a	56972±17507 b	4.8±1.7 bc	<0.01 e	1.206±0.046 a
0.546±0.096 c	37867±7239 f	4.4±1.9 c	4.26±0.34 a	0.276±0.057 h
0.421±0.020 d	42721±9302 e	4.7±2.0 bc	3.64±0.26 a	0.515±0.014 d
0.390±0.099 d	59907±18232 b	5.7±2.3 b	4.06±0.69 a	0.356±0.012 f
0.599±0.081 b	48386±4897 cd	4.9±1.8 bc	1.10±0.28 d	0.300±0.039 gh
	0.310±0.045 e 0.488±0.055 c 0.393±0.012 d 0.506±0.055 c 0.386±0.072 d 0.612±0.050 b 0.758±0.033 a 0.546±0.096 c 0.421±0.020 d 0.390±0.099 d	0.310±0.045 e 48927±4838 cd 0.488±0.055 c 44077±4911 de 0.393±0.012 d 49242±5242 cd 0.506±0.055 c 43013±5834 e 0.386±0.072 d 103144±22549 a 0.612±0.050 b 45292±4916 cde 0.758±0.033 a 56972±17507 b 0.546±0.096 c 37867±7239 f 0.421±0.020 d 42721±9302 e 0.390±0.099 d 59907±18232 b	0.310±0.045 e 48927±4838 cd 5.3±2.8 bc 0.488±0.055 c 44077±4911 de 4.3±2.2 c 0.393±0.012 d 49242±5242 cd 5.1±2.1 bc 0.506±0.055 c 43013±5834 e 4.6±1.8 bc 0.386±0.072 d 103144±22549 a 6.7±2.3 a 0.612±0.050 b 45292±4916 cde 5.2±1.8 bc 0.758±0.033 a 56972±17507 b 4.8±1.7 bc 0.546±0.096 c 37867±7239 f 4.4±1.9 c 0.421±0.020 d 42721±9302 e 4.7±2.0 bc 0.390±0.099 d 59907±18232 b 5.7±2.3 b	0.310±0.045 e 48927±4838 cd 5.3±2.8 bc 2.27±0.26 b 0.488±0.055 c 44077±4911 de 4.3±2.2 c 1.87±0.10 bc 0.393±0.012 d 49242±5242 cd 5.1±2.1 bc 1.95±0.25 bc 0.506±0.055 c 43013±5834 e 4.6±1.8 bc 4.15±0.28 a 0.386±0.072 d 103144±22549 a 6.7±2.3 a 4.26±0.54 a 0.612±0.050 b 45292±4916 cde 5.2±1.8 bc 1.41±0.10 cd 0.758±0.033 a 56972±17507 b 4.8±1.7 bc <0.01 e 0.546±0.096 c 37867±7239 f 4.4±1.9 c 4.26±0.34 a 0.421±0.020 d 42721±9302 e 4.7±2.0 bc 3.64±0.26 a 0.390±0.099 d 59907±18232 b 5.7±2.3 b 4.06±0.69 a

<sup>&</sup>lt;sup>a</sup> AquaLab Dew Point Water Activity Meter 4TE

<sup>&</sup>lt;sup>b</sup> TAXT.2 Plus Texture Analyzer equipped with Texture Exponent 32 and a 50 kg load cell (as described by Varela and others 2008b)

<sup>&</sup>lt;sup>c</sup> mg KOH necessary to neutralize 1 g of sample

<sup>&</sup>lt;sup>d</sup> means±SD followed by different letters within a column differ significantly (*p*<0.05) according to ANOVA and SNK means separation test performed using SAS (SAS University Edition, SAS Institute Inc., Cary, North Carolina)

## Relationship between sensory, chemical and instrumental assessments

Stepwise regression was performed with the aim of identifying if relationships exist between the sensory results as indicated by panelists and chemical and instrumental assessments of almond quality, specifically hardness (force), number of fracture peaks, water activity, free fatty acids and peroxide values. Regression was performed on all sensory data from all consumer panels with the means of the chemical and means of the instrumental assessments matched to storage condition and testing point. The analysis was performed on data across all panels as well as on the two consumer groups – those who rejected the samples and those who accepted the samples (responses of 'no' and 'yes' to the intent to consume question, respectively). Models were created for each of the sensory attributes (overall acceptability, odor, texture and flavor) using the five chemical and instrumental tests – peroxide value (PV), free fatty acid (FFA), water activity (a<sub>w</sub>), maximum force (force) and number of fracture peaks (peaks). Results are shown in Table 4.23. Although the explainable variability was small (R<sup>2</sup> < 0.06), significant relationships were identified for all sensory attributes for at least one chemical or instrumental test and free fatty acid test results were found to be important predictors for all sensory attributes.

The model for all data indicated that free fatty acid level was the most important determinant of overall acceptability, followed by peroxide value and water activity. When the models for overall acceptability by consumer group are compared, free fatty acid is found to be the only test that predicts overall acceptability for panelists who accepted the samples whereas water activity and free fatty acids are important for panelists who rejected the samples. The model for overall acceptability for panelists who rejected the samples has a higher degree of explainable variability than the model for panelists who accepted the samples.

In terms of modeling odor acceptability, free fatty acid was found to be the only test that explains the variability in odor scores for all sensory data and for panelists who rejected the samples and no model was created for odor for panelists who accepted the samples. The variability explained by each of these models was only 0.007 and 0.026, respectively, which was not surprising as previous stepwise regression indicated that odor was not a large factor in explaining consumer acceptability. However, odor was found to be more important for panelists who rejected the samples than for those who accepted the samples.

Texture acceptability scores were found to be best explained by free fatty acid and water activity across all sensory data and number of fracture peaks was also important for panelists who accepted the samples. However, number of fracture peaks was not found to be a significant determinant of texture scores for panelists who rejected the samples. Additionally, the contribution of the number of fracture peaks is the only chemical or instrumental test that was found to have a positive effect on any sensory attribute. This suggests that a crunchy texture is more important for increasing texture acceptability among individuals who accepted the samples than a soft/not crunchy texture is for decreasing texture acceptability among individuals who rejected the samples. The order of importance of free fatty acids and water activity in predicting texture acceptability for panelists who rejected versus accepted the samples was also found to be reversed, indicating that the different tests play different roles in predicting how the two consumer groups will rate texture acceptability. Specifically, a higher water activity level was found to have a greater impact on texture acceptability scores for panelists who rejected the samples than panelists who did not.

Flavor acceptability scores for data from all sensory panels were found to be explained by free fatty acids, followed by peroxide value and water activity. This is expected, as free fatty

acceptability by consumer group, free fatty acid level was the only test that predicted flavor scores for panelists who accepted the samples, and chemical and instrumental tests were not found to be significant in predicting flavor acceptability among panelists who rejected the samples. However, stepwise regression results on all sensory data previously discussed (Table 4.18) indicated that flavor was not as important an attribute in predicting overall acceptability among panelists who rejected the samples.

Overall, the difference in models for panelists who accepted versus rejected the samples indicates that different tests best predict consumer rejection versus consumer acceptability. Free fatty acid levels appeared to be the most important chemical test in predicting consumer acceptability of the various sensory attributes as well as consumer rejection. Water activity appeared to be more important in predicting consumer rejection. Instrumental textural assessments, which previously were not considered to reflect sensory texture scores, were found to be important in predicting texture acceptability among panelists who accepted the samples.

Table 4.23: Modeling sensory attributes using chemical and instrumental test results<sup>a</sup>: all sensory results with means of chemical and instrumental data

	Model <sup>b</sup>	$\mathbb{R}^2$
Overall Acceptability		
All data	$OA = 7.622 - 1.146 (FFA) - 0.129 (PV) - 1.552 (a_w)$	0.032
Panelists who accepted the sample	OA = 6.867 - 0.470 (FFA)	0.005
Panelists who rejected the sample	$OA = 4.973 - 1.439 (a_w) - 0.678 (FFA)$	0.043
Odor		
All data	Odor = 5.665 - 0.586 (FFA)	0.007
Panelists who accepted the sample	N/A	
Panelists who rejected the sample	Odor = 5.063 - 1.048 (FFA)	0.026
Texture		
All data	Texture = $7.617 - 1.453$ (FFA) $- 2.342$ (a <sub>w</sub> )	0.057
Panelists who accepted the sample	Texture = $7.525 - 1.022$ (FFA) $- 1.590$ (a <sub>w</sub> ) $+ 0.008$ (peaks)	0.032
Panelists who rejected	Texture = $6.107 - 2.942$ (a <sub>w</sub> ) $- 1.141$ (FFA)	0.085
the sample		
Flavor		
All data	$Flavor = 7.45 - 0.974 (FFA) - 0.163 (PV) - 1.160 (a_w)$	0.021
Panelists who accepted	Flavor = 6.815 - 0.360 (FFA)	0.002
the sample		
Panelists who rejected	N/A	
All data Panelists who accepted the sample	Flavor = $6.815 - 0.360$ (FFA)	

<sup>&</sup>lt;sup>a</sup> significant contribution (p<0.05) determined using stepwise regression (SAS University Edition, SAS Institute Inc., Cary, North Carolina)

<sup>&</sup>lt;sup>b</sup> chemical and instrumental tests are listed in each model in order of importance (the first variable is the largest determinant and the last variable is the smallest); N/A indicates no chemical or instrumental tests were found to be significant

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## **CHAPTER 5**

#### CONCLUSIONS

Of the 7 samples stored in polypropylene bags, 4 of the samples were rejected (35°C/65% RH at 6 months, 35°C/50% RH at 12 months, 25°C/65% RH at 16 months and 15°C/65% RH at 24 months) and all of the samples stored in unlined cartons were rejected by the consumer panelists over the 24-month study (35°C/65% RH at 2 months, 35°C/50% RH at 6 months, 4°C at 6 months, 25°C/65% RH at 12 months, 25°C/50% RH at 16months, 15°C/50% RH at 16 months). For almonds stored in both polypropylene bags and in unlined cartons, the samples stored at 35°C were the first to be rejected. Samples stored in polypropylene bags and unlined cartons failed in the same order, with the exception of the sample stored in unlined cartons at 4°C. It is believed that the reason for this is that the high RH present in the 4°C chamber greatly affected the sample stored in the unlined carton because the cartons provided no protection to atmosphere. Samples stored in unlined cartons always failed before their counterparts stored in polypropylene bags held under similar conditions. Since bulk packaging is primarily in unlined cartons, further studies should be conducted on the best method of preserving almond quality in this packaging, such as utilizing oxygen or moisture absorbers in the packaging.

Mean acceptability scores of almonds stored in unlined cartons tended to be lower than their counterparts stored in polypropylene bags. A statistically significant difference when compared to baseline for odor was much more common in almonds stored in unlined cartons, suggesting that storage in cartons results in more changes in odor. Despite these significant

differences, stepwise regression results revealed that odor only had a small contribution when consumers judged acceptability of raw almonds; flavor was the largest contributor when predicting overall acceptability, followed by texture. Although sensory flavor acceptability decreased for all samples over the storage period, the reasons for the decline in flavor scores are unknown. Use of descriptive sensory evaluation to identify specific changes that occurred during storage would likely help determine if the decrease in flavor acceptability was due to development of undesirable flavors or loss of desirable ones, or both.

When overall acceptability models for the data from all sensory panels and for the two consumer groups (those who rejected the sample and those who accepted the sample, by responding 'no' or 'yes' to the intent to consume question, respectively) were compared, all three sensory attributes (odor, flavor and texture) were found to be significant determinants of overall acceptability. However, the model for panelists who had rejected the samples indicated that odor and texture were more important in predicting overall acceptability than for panelists who accepted the samples. This suggests that undesirable odors and textures may play a larger role than desirable ones in predicting consumer rejection whereas flavor may be more important in predicting consumer acceptability.

While significant increases in peroxide values did occur for all samples throughout the study, levels never exceeded the 5 meq. active O<sub>2</sub>/kg oil threshold used by industry for assessing almond quality (Huang 2014; Almond Board of California 2013). These results suggest this peroxide cutoff may be too high as consumers rejected the almond samples at peroxide levels much lower than the published industry cutoff. It would be worth studying whether rejection is triggered at some lower peroxide level or if other indicators of degraded quality trigger rejection of almonds.

Samples stored in unlined cartons were found to have higher peroxide values than their counterparts stored in polypropylene bags held under similar conditions. This suggests that storage in unlined cartons exposes almonds to an increased risk of lipid oxidation and rancidity. Lower storage temperatures appear to preserve almond flavor as evidenced by lower peroxide values and free fatty acid levels. Additionally, consumers appear to reject samples for reasons other than oxidative or rancid flavor development in some cases.

At higher relative humidity, free fatty acid and water activity levels were found to be higher, although the low water vapor transmission rate of the polypropylene packaging provided sufficient protection against the high humidity when almonds were stored at 4°C (RH 95±3%). Water activity levels above 0.4 have been shown to have detrimental effects on almond textural quality, and although water activities in this study exceeded 0.4, the detrimental textural effects were not seen to the same extent as reported in literature (Vickers and others 2014). Moreover, the high water activities and resulting textural changes were not great enough to cause consumer rejection. Finally, maximum force was seen to both significantly increase and decrease, indicating that almonds became chewy and more tough as well as less crunchy and hard. The number of fracture peaks decreased for all samples, also indicating that the almonds lost some of their crunch during storage.

The sample that exhibited the greatest amount of degradation in quality was the sample stored in unlined cartons at 4°C. This sample had significantly lower odor, texture and overall acceptability scores than all other samples at their respective points of failure and also had the highest free fatty acid and water activity levels of all samples. The sample that was most similar to baseline at the end of the study was the sample stored in polypropylene bags at 15°C/50% RH.

This sample did not exhibit statistically significant differences when compared to baseline for odor and overall acceptability scores or for water activity.

Of the three remaining samples, it is predicted that the next sample to fail would have been the sample stored in polypropylene bags at 25°C/50% RH and that rejection would likely have been at 26 or 28 months. This is supported by an extrapolation of both sensory and nonsensory results. Sensory results indicated that consumer panelists rejected the sample at the screening panel at the conclusion of the study but the sample was not rejected at the confirmatory panel. The flavor acceptability score for this sample at point of failure was not significantly different from most other samples when they failed, whereas the other two samples that were not rejected by consumers (polypropylene bags at 4°C and at 15°C/50% RH) were not significantly different from each other and were the most similar to baseline. This suggests that the flavor of the sample stored in polypropylene bags at 25°C/50% RH was more similar to samples that had failed than samples that had not. Furthermore, the free fatty acid level for this sample had been steadily increasing over the last few months of the study, indicating that more hydrolytic rancidity was occurring.

Models were created to determine the relationship between the chemical and instrumental tests and each of the sensory attributes. Results indicated that free fatty acid level is a significant predictor of acceptability of all of the sensory attributes and peroxide values were found to be significant predictors of overall acceptability and flavor. Instrumental textural assessments, specifically number of fracture peaks, were only found to be significant in predicting texture acceptability among panelists who had accepted the various samples, and maximum force was not found to significantly predict texture acceptability for either consumer group. Water activity level was also found to be important in predicting overall acceptability, texture and flavor, and

appears to be more important in predicting responses for panelists who rejected the samples than panelists who did not.

It was found that reducing the temperature from 35 to 25°C can extend the shelf life of almonds stored in polypropylene bags and unlined cartons by 10 to 12 months regardless of humidity. Although this observation was seen for both packaging materials, packaging in polypropylene bags results in a longer shelf life. Additionally, when considering the higher temperatures (25°C and 35°C), reducing the relative humidity from 65% to 50% was shown to extend the shelf life of almonds at least 4 to 6 months. The ideal condition for storage at atmospheric conditions at 4°C based on this study was in polypropylene bags. Storage in the polypropylene bags provided sufficient protection from moisture and oxygen to retard degradation of the almonds and the lower temperature also contributed to extending the shelf life. Packaging materials with higher oxygen and moisture barriers should be studied to determine if the shelf life could be further extended and how to best retard degradation of raw almond quality at higher temperatures and humidity.

This study showed the importance of utilizing sensory testing in conjunction with chemical and instrumental tests when assessing almond quality over time. Consumer rejection occurred when peroxide value and free fatty acid levels were below those currently recommended as the cutoff point in the almond industry (Huang 2014; Almond Board of California 2013), suggesting that these levels are either too high or other factors played a major role in predicting consumer rejection. Under the study conditions, the longest shelf life was achieved with storage of raw almonds in polypropylene bags at low temperature (≤15°C) and low relative humidity (50%). The polypropylene bag material was less permeable than the unlined carton material and prevented moisture sorption and oxygen permeation. The lower

storage temperature and relative humidity retarded the rate rancidity development. Sensory evaluation indicates that storage under these conditions will result in a shelf life of raw Nonpareil almonds of at least two years.

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## APPENDIX A CONSENT FORM

#### The product today is raw almonds Consent Form

I,, agree to participate in a research study titled Sensory Evaluation of Almonds
conducted by Dr. Ruthann Swanson (706-542-4843) and graduate student Emily Pleasance, Department of Foods
and Nutrition, University of Georgia. I am at least 18 years of age or older. I understand my participation is
voluntary. I can refuse to participate or stop taking part without giving any reason and without penalty or loss of
benefits to which I am otherwise entitled. If I decide to stop or withdraw from the study, or if the investigator
decides to terminate my participation without regard to my consent, the information/data collected from or about me
up to the point of my withdrawal will be kept as part of the study and may continue to be analyzed. The purpose of
this study is to investigate the quality and acceptability of almonds. All products were handled in facilities in which
ServSafe or appropriate GMP procedures were followed. If I volunteer to take part in this study, I will be asked to
do the following things:
Pand and sign the consent form (1.2 minutes)

- Read and sign the consent form (1-2 minutes)
- Complete the demographic and food choices questionnaire (5-8 minutes)
- Evaluate almonds according to directions on the sensory scorecard (4-6 minutes)

Students who have selected participation on this sensory panel as an extra credit option will receive class credit. In classes where extra credit is offered, other options are available, however these options vary with class and are determined by the instructor in the class. Optional commercially prepared snacks will be made available following the participant's involvement in the research study. Food allergies that I have include:

Your participation and the results of this study that are individually-identifiable will be kept confidential. I will be
assigned an identifying number and this number will be used on all questionnaires and evaluation forms that I fill-
out. It will be possible to link specific individuals with specific responses during the evaluation of the product.
However, there is no way to connect specific responses with a specific individual once the test is completed. No
individually identifiable information about me, or provided by me during the research, will be shared with others
without my written permission, except if it is necessary to protect my welfare (for example, if I were injured and
need physician care) or if required by law. An expected benefit is the production of healthier products that are
acceptable to consumers; their availability will empower consumers to improve their dietary choices. My
participation in this hands-on experience may enhance discussions in the classroom, facilitating a better
understanding of the process and limitations associated with development/success of products available to
consumers. There are no expected risks or discomforts associated with participation for any person who does not
have allergies to almonds. In the event I suffer a research-related injury, I will seek treatment at an appropriate

of those involved in the research. If I do seek medical treatment due to research-related injury, I will contact dr. Ruthann Swanson about the injury. As a participant, I do not give up or waive any of my legal rights. If I have further questions about this study, I can call Dr. Ruthann Swanson at (706) 542-4834. I understand the procedures described above and my additional questions have been answered to my satisfaction. I agree to participate in this research study, and I have received a copy of this consent form for my records.

medical facility. However, my medical expenses will be my responsibility or that of my third-party payer, although I am not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part

Ruthann Swanson		
Name of Researcher	Signature	Date
Emily Pleasance Name of Researcher	Signature	Date
Name of Participant	Signature	— — — — — — — — — — — — — — — — — — —

Please sign both copies, keep one and return one to the researcher.

Additional questions or problems regarding your rights as a research participant should be addressed to Chairperson, Institutional Review Board, University of Georgia, 629 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706)542-3100; Email address IRB@uga.edu.

## APPENDIX B ALMOND SENSORY SCORECARD

Product C	Code Nun	Panelist Number:													
	Almond Sensory Scorecard														
You will evaluate XX samples today, each presented in a closed container. Please check the box $(\Box)$ that best reflects your opinion of this sample															
Before tasting, evaluate the odor of the product. Hold the cup close to your nose and gently lift the lid as you take three short sniffs.															
Odor  Extremely Dislike	у						Extremely Like								
	Eat the almonds and evaluate texture, flavor and overall acceptability by marking the box (  ) that best reflects how much you like this product														
Texture	e														
Extremely Dislike	у						Extremely Like								
Flavor															
Extremely Dislike	у У						Extremely Like								
Overall	Accept	ability													
Extremely Dislike	y y						Extremely Like								

Please drink water and eat some crackers and/or carrot to cleanse your mouth

### Turn-over and complete the back

Comments:	Please indicate	WHAT	in particular	you liked	or disliked
about this almo	nd sample (Use	WORD	S not SENT	ENCES)	

LIKED	DISLIKED
If you had purchased this	product, would you eat it?
yes	no
	<del></del>

Please place your tray in the hatch in front of you and close the hatch. You will receive another sample in a moment.

If this was your last sample, you will receive a short questionnaire.

# APPENDIX C ALMOND QUESTIONNAIRE

Panelist _	
Panenst_	

### **Almond Questionnaire**

Now, we would like to know a little more about you. Please check the best response for each item below.

1. Your	gender:	Male or Female
2. Pleas	se check v	your age category:
	18-27	44-51
		52-61
	 _36-43	62 and above
3. How	often do	you eat nuts, including peanuts? (check 1)
		Daily
		Several times a week
		Several times a month
		Once a month
		Several times a year
		Never
4. How	often do	you eat almonds? (check 1)
		Daily
		Several times a week
		Several times a month
		Once a month
		Several times a year
		Never

Please return your completed questionnaire and pull the hatch closed.

Thank you for making our study a success!

### APPENDIX D

### CHEMICAL/INSTRUMENTAL ANALYSES BY THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY AT THE UNIVERSITY OF GEORGIA

The fourteen raw almond samples were subjected to chemical and instrumental testing every 2 months over a 24-month period. For each sampling date, one polypropylene bag of almonds from each storage condition (n=7) and approximately 300 g of almonds in unlined cartons from each storage condition (n=7) were removed from the environmental chambers for analysis. Chemical methods (n = 3) included: peroxide values (PVs), free-fatty acid values (FFAs), conjugated dienoic acid values (CDs), and 2-thiobarbituric acid values (TBARs). Instrumental methods (n = 3) included water activity (a<sub>w</sub>), moisture content (MC) and headspace-solid phase microextration gas chromatography with a flame ionization detector (n = 2) (HS-SPME GC/FID). Texture analysis was also conducted (n = 10). At 8 months, samples were also evaluated for Vitamin E content using values from the USDA National Nutrient Database for Standard Reference as the baseline values for Vitamin E.

Sample preparation for testing of PVs, FFAs, CDs, TBARs and Vitamin E involved extracting oil from the almonds. This was done by cold-pressing the whole almond kernels in a #3912 Carver hydraulic press (Carver, Inc, Wabash, IN, U.S.A.). The cold-pressed oil was held in a 30 mL Fisher Scientific amber screw-cap vial (Fisher Scientific, Suwanee, GA, U.S.A.), flushed with nitrogen and stored in a dark, 4 °C refrigerator until analyses were completed. For HS-SPME GC/FID, analysis a<sub>w</sub> and MC analyses, 45 g of whole almond kernels were ground using a Cuisinart DCG-12BC (Cuisinart, East Windsor, NJ, U.S.A.) coffee grinder for 10 s with vigorous shaking. The ground almond powder was sifted through a 16 mesh Tyler standard screen (W.S. Tyler Industry Group, Mentor, OH, U.S.A.) to create a consistent particle size for analysis.

#### Peroxide Values

Peroxide values were assessed in triplicate using a modified AOAC Method 965.33 (AOAC International, Gaithersburg, MD) and were reported as mean  $\pm$  standard deviation meq. of active O<sub>2</sub> (peroxide)/kg oil. Peroxide values above 2.0 meq. active O<sub>2</sub>/kg oil triggered consumer sensory evaluation.

#### Free Fatty Acids

Free Fatty Acid values were assessed in triplicate using a modified version of AOCS Official Method Ca 5a-40 (AOCS, Urbana, IL) and were reported as mean ± SD FFA value (mg KOH necessary to neutralize 1 g of sample). Cold pressed oil was weighed (1±0.1g) into a 25mL Erlenmeyer flask and 2.5 mL of 95% ethanol that was neutralized with 0.005N NaOH was added to each flask with 0.1mL of 1% Phenolphthalein in 80EtOH:20 H<sub>2</sub>O (v/v). Samples were heated to 70 °C and agitated by a stir bar and titrated with 0.005N NaOH until the solution turned slightly pink color and continued for 30 seconds.

#### Conjugated Dienoic Acid

Conjugated dienoic acid values were assessed in triplicate using IUPAC Official Method 2.505 (IUPAC, Research Triangle Park, NC) and were reported as mean ± SD CD value. Cold pressed oil was weighed (25±5mg) and dissolved in 10mL hexane. Each sample was mixed using a vortex mixer for 10 seconds to ensure proper dilution. The absorptivity was measured at 233 nm in a 10mm quartz cuvette with pure solvent in the reference cuvette.

#### 2-Thiobarbituric Acid Value

2-Thiobarbituric acid values were assessed in triplicate using a modified version of AOCS Official Method Cd 19-90 (AOCS, Urbana, IL) and were reported as mean  $\pm$  SD TBA value (reaction equivalent of 1 mg test sample per 1 mL volume with 2-thiobarbituric acid). Cold

pressed oil was weighed (40mg) in 5mL 1-butanol and 5mL TBA reagent (200 mg 2-thiobarbituric in 100 mL 1-butanol). Samples were then placed in a water bath at 95°C for 120 minutes, then cooled to room temperature under running water. Using distilled water as the reference, sample absorbance was measured at 530nm in a 10mm quartz cuvette.

Water Activity was assessed for each sample in triplicate using a calibrated Aqua Lab CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA). Water activity was reported as a partial pressure of water in the sample compared to distilled water at the same temperature.

Moisture Content

Moisture content was assessed in triplicate by heating weighed ground almond samples in a forced-air convection oven at 105 °C for 24h (AOAC, 2000). Moisture content was reported mean  $\pm$  SD MC (percentage of moisture mass within the total sample mass).

#### Headspace Volatiles

*Water activity* 

Headspace volatiles were assessed in duplicate using an Agilent GC sampler 80 PAL (firmware 4.1.3) system (Agilent Technologies, Inc., Santa Clara, CA, U.S.A.). Ground almond powder was weighed (2.5 g  $\pm$  0.1 g) into a 22.5 x 75.5 mm 20-mL amber glass headspace vial sealed with an aluminum pressure release seal with a 18-mm PTFE/silicone liner (Analytical Sales & Services, Inc., Pompton Plains, NJ, U.S.A.). Samples were allowed to equilibrate at room temperature (24°C  $\pm$  1°C) before being loaded into the agitator (65°C) for 30 min extraction time using a 50/30  $\mu$ m DVB/CAR/PDMS, Stableflex 23 Ga SPME fiber assembly (Supelco, Bellefonte, PA, U.S.A.) before being injected into GC system.

An Agilent 7890A GC-FID (Agilent Technologies, Inc., Santa Clara, CA, U.S.A.) coupled with a FID detector and equipped with an Agilent J&W DB-5ms 60mx0.25 mm non-

polar GC column with a film thickness of 25 μm was used. The gradient run had an initial temperature of 38°C for 1 minute followed by an increase in temperature at a rate of 2.5°C/min until a temperature of 175°C was reached. The temperature was then increased 50°C/min to 220 °C, and held for 2 min. The injector port (0.75mm ID, Supelco) was held constant at 250°C. Other settings included a split ratio of 1:40, split flow of 40.3 mL min<sup>-1</sup>, column head pressure of 132.8 kpa, the flow rate of 1.0 mL min<sup>-1</sup>, and the carrier gas was helium.

#### *Texture analysis*

Almond samples were assessed with 10 replications at each 2-month interval. Almonds were sorted to have similar sizes and appearances. Texture analyses were measured using a Texture Technologies TA-XT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY, U.S.A.) equipped with Texture Expert Exceed 2.64 software (Stable Micro Systems, Haselmere, Surry, England) and loaded with a 25 kg  $\pm$  1g electronic load cell. The load cell was equipped with a TA-94 40mm diameter compression disc, the test speed was set as 1.0 mm/s with a trigger of 0.1N, and compressed to a distance of 50% strain. Area under the curve, force required for compression, number of fracture points, the gradient of the force curve, the average drop-off between peaks, the linear distance of the curve, the average gradient between peeks, the force to the first fracture point, and the distance of compression of the first fracture point were all calculated from the compression test.

#### Vitamin E

At 8 months, the cold pressed oil samples were analyzed in triplicate for vitamin E content by dissolving 1g of oil in mobile phase + 0.01% (v/w) BHT in a 5 mL volumetric flask filled to mark and mixed thoroughly. The sample was then injected into a normal-phase HPLC system via Rheodyne Fixed Loop. The system was equipped with a Shimadzu LC-10AT (Tokyo,

Japan) controller/pump, CBM-20A Prominence communications bus module, DG-14A degasser, RF-10A<sub>XL</sub> fluorescence detector and EZStart chromatography software (Shimadzu Corporation, Columbia, MD, USA). The column was packed with LiChrospher Si 60 (5  $\mu$ m), and an isocratic mobile phase comprising 0.85% (v/v) isopropanol in hexanes with a flow rate of 0.8 mL/min was used. Prior to use, the mobile phase was vacuum filtered through a 0.45- $\mu$ m nylon membrane filter (MSI, Westboro, MA, USA). Total tocopherols were calculated based on standard curves created using the same method.

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# APPENDIX E PANELIST QUESTIONNAIRE RESPONSES

Table E.1: Demographic profile for consumer panelists assessing raw almonds stored in unlined cartons

	<u> </u>			*			Several	Several		Several	
Storage				18-27			times a	times a	Once a	times a	
condition	Panel	n	Female	years old	Consumption	Daily	week	month	month	year	Never
						Fred	quency %				
Baseline		115	79.1	75.7	Nuts	19.1	35.7	27.8	10.4	6.1	0.9
					Almonds	7.0	25.2	33.0	12.2	19.1	3.5
4°C	2 mo	40	75.0	70.0	Nuts	27.5	40.0	15.0	10.0	5.0	2.5
					Almonds	7.5	32.5	25.0	10.0	17.5	7.5
	4 mo	37	86.5	54.1	Nuts	27.0	40.5	24.3	5.4	2.7	0.0
					Almonds	13.5	27.0	32.4	10.8	16.2	0.0
	6 mo	36	72.2	69.4	Nuts	11.1	41.7	30.6	5.6	8.3	2.8
					Almonds	8.3	22.2	33.3	19.4	13.9	2.8
	6 mo	92	72.5	60.9	Nuts	14.1	48.9	26.1	4.4	6.5	0.0
	confirm				Almonds	4.4	30.4	37.0	10.9	14.1	3.3
15°C /	10 mo	36	91.7	61.1	Nuts	25.0	30.6	27.8	5.6	11.1	0.0
50%RH					Almonds	8.3	33.3	30.6	5.6	19.4	2.8
	12 mo	38	60.5	89.5	Nuts	21.1	44.7	18.4	7.9	5.3	2.6
					Almonds	2.6	31.6	36.8	5.3	18.4	5.3
	14 mo	36	77.8	80.6	Nuts	16.7	47.2	25.0	2.8	8.3	0.0
					Almonds	2.9	28.6	45.7	11.4	8.6	2.9
	16 mo	34	82.4	91.2	Nuts	14.7	47.1	32.4	2.9	2.9	0.0
					Almonds	5.9	35.3	44.1	5.9	5.9	2.9
	16 mo	100	78.0	78.0	Nuts	16.0	43.0	32.0	3.0	6.0	0.0
	confirm				Almonds	5.0	38.0	39.0	8.0	7.0	3.0
15°C /	8 mo	35	82.9	62.9	Nuts	11.4	54.3	34.3	0.0	0.0	0.0
65%RH					Almonds	8.6	28.6	51.4	2.9	8.6	0.0
	10 mo	37	75.7	70.3	Nuts	10.8	40.5	37.8	2.7	5.4	2.7
					Almonds	2.7	24.3	45.9	16.2	8.1	2.7
	12 mo	37	86.5	67.6	Nuts	21.6	21.6	43.2	5.4	8.1	0.0
-					Almonds	5.4	27.0	43.2	13.5	10.8	0.0
25°C /	12 mo	38	60.5	89.5	Nuts	21.1	44.7	18.4	7.9	5.3	2.6
50%RH					Almonds	2.6	31.6	36.8	5.3	18.4	5.3

	14 mo	36	72.2	77.8	Nuts	11.1	41.7	30.6	8.3	8.3	0.0
					Almonds	2.8	16.7	41.7	16.7	19.4	2.8
	16 mo	34	82.4	91.2	Nuts	14.7	47.1	32.4	2.9	2.9	0.0
					Almonds	5.9	35.3	44.1	5.9	5.9	2.9
	16 mo	101	83.2	74.0	Nuts	13.9	41.6	33.7	5.0	5.0	1.0
	confirm				Almonds	5.9	30.7	43.6	7.9	9.9	2.0
25°C /	12 mo	35	77.1	88.6	Nuts	11.4	51.4	31.4	2.9	2.9	0.0
65% RH					Almonds	5.7	34.3	37.1	14.3	5.7	2.9
	12 mo	99	80.8	75.8	Nuts	18.2	36.4	36.4	5.1	3.0	1.0
	confirm				Almonds	6.1	32.3	32.3	12.1	11.1	6.1
35°C /	6 mo	36	80.6	47.2	Nuts	19.4	30.6	33.3	13.9	2.8	0.0
50% RH					Almonds	5.6	22.2	27.8	22.2	22.2	0.0
	6 mo	89	64.8	51.7	Nuts	23.6	34.8	24.7	10.1	5.6	1.1
	confirm				Almonds	9.1	27.3	27.3	20.5	13.6	2.3
35°C /	2 mo	40	87.5	77.5	Nuts	22.5	37.5	35.0	0.0	5.0	0.0
65% RH					Almonds	7.5	22.5	55.0	5.0	10.0	0.0
	2 mo	109	81.7	65.1	Nuts	27.5	33.0	26.6	2.8	9.2	0.9
	confirm				Almonds	11.0	28.4	29.4	13.8	11.9	5.5

Table E.2: Demographic profile for consumer panelists assessing raw almonds stored in polypropylene bags

	<u> </u>	-		<u> </u>			Several	Several Several		Several	
Storage				18-27			times a	times a	Once a	times a	
condition	Panel	n	Female	years old	Consumption	Daily	week	month	month	year	Never
						Fred	quency %				
Baseline		115	79.1	75.7	Nuts	19.1	35.7	27.8	10.4	6.1	0.9
					Almonds	7.0	25.2	33.0	12.2	19.1	3.5
4°C	12 mo	35	77.1	88.6	Nuts	11.4	51.4	31.4	2.9	2.9	0.0
					Almonds	5.7	34.3	37.1	14.3	5.7	2.9
	14 mo	36	77.9	80.6	Nuts	16.7	47.2	25.0	2.8	8.3	0.0
					Almonds	2.9	28.6	45.7	11.4	8.6	2.9
	16 mo	35	74.3	85.7	Nuts	22.9	40.0	25.7	8.6	2.9	0.0
					Almonds	2.9	37.1	34.3	17.1	5.7	2.9
	18 mo	35	71.4	42.9	Nuts	26.5	41.2	26.5	2.9	2.9	0.0
					Almonds	11.4	28.6	42.9	5.7	8.6	2.9
	20 mo	37	83.8	83.8	Nuts	10.8	51.4	21.6	10.8	5.4	0.0
					Almonds	35.1	37.8	10.8	10.8	5.4	0.0
	22 mo	38	94.7	76.3	Nuts	18.4	39.5	26.3	5.3	7.9	2.6
					Almonds	5.3	21.1	52.6	5.3	10.5	5.3
	24 mo	37	81.1	73.0	Nuts	21.6	37.8	32.4	0.0	8.1	0.0
					Almonds	5.4	27.0	37.8	13.5	16.2	0.0
	24 mo	102	79.2	80.4	Nuts	18.6	39.2	26.5	6.9	6.9	2.0
	confirm				Almonds	5.9	24.5	40.2	13.7	10.8	4.9
15°C /	12 mo	35	77.1	88.6	Nuts	11.4	51.4	31.4	2.9	2.9	0.0
50%RH					Almonds	5.7	34.3	37.1	14.3	5.7	2.9
	14 mo	36	77.8	80.6	Nuts	16.7	47.2	25.0	2.8	2.9	8.3
					Almonds	2.9	28.6	45.7	11.4	8.6	2.9
	16 mo	35	74.3	85.7	Nuts	22.9	40.0	25.7	8.6	2.9	0.0
					Almonds	2.9	37.1	34.3	17.1	5.7	2.9
	18 mo	36	63.9	58.3	Nuts	11.1	33.3	38.9	11.1	5.6	0.0
					Almonds	2.9	16.7	52.8	13.9	11.1	2.8
	20 mo	34	82.4	82.4		23.5	32.4	32.4	8.8	2.9	0.0
					Almonds	8.8	17.7	47.1	2.9	20.6	2.9

	22 mo	38	92.1	94.7	Nuts	10.5	34.2	34.2	5.3	13.2	2.6
	22 IIIO	30	72.1	74.1	Almonds	5.3	18.4	36.8	13.2	23.7	2.6
	24 mo	36	83.3	72.2	Nuts	25.0	44.4	19.4	8.3	2.8	0.0
	2 <del>4</del> 1110	30	05.5	12.2	Almonds	2.8	38.9	27.8	11.1	19.4	0.0
	24 mo	99	80.6	80.8	Nuts	15.2	41.4	31.3	7.1	5.1	0.0
	confirm		00.0	00.0	Almonds	7.1	23.2	41.4	12.1	15.2	1.0
15°C /	12 mo	36	75.0	47.2	Nuts	25.0	41.7	25.0	2.8	5.6	0.0
65%RH	12 1110	30	73.0	17.2	Almonds	2.8	30.6	36.1	22.2	8.3	0.0
03/01411	14 mo	35	77.1	77.1	Nuts	14.3	45.7	25.7	5.7	8.6	0.0
	1 1 1110	33	7 7 • 1	, , , , 1	Almonds	5.7	28.6	42.9	11.4	8.6	2.9
	16 mo	36	77.8	77.2	Nuts	16.7	33.3	41.7	5.6	2.8	0.0
	10 1110		, , , , ,	, , , _	Almonds	5.6	25.0	52.8	8.3	8.3	0.0
	16 mo	101	83.2	74.0	Nuts	13.9	41.6	33.7	5.0	5.0	1.0
	confirm			,	Almonds	5.9	30.7	43.6	7.9	9.9	2.0
	18 mo	36	63.9	58.3	Nuts	11.1	33.3	38.9	11.1	5.6	0.0
					Almonds	2.8	16.7	52.8	13.9	11.1	2.8
	20 mo	37	83.8	83.8	Nuts	10.8	51.4	21.6	10.8	5.4	0.0
					Almonds	0.0	35.1	37.8	10.8	10.8	5.4
	22 mo	38	92.1	94.7	Nuts	10.5	34.2	34.2	5.3	13.2	2.6
					Almonds	5.3	18.4	36.8	13.2	23.7	2.6
	24 mo	37	81.1	73.0	Nuts	21.6	37.8	32.4	0.0	8.11	0.0
					Almonds	5.4	27.0	37.8	13.5	16.2	0.0
	24 mo	99	80.6	80.8	Nuts	15.2	41.4	31.3	7.1	5.1	0.0
	confirm				Almonds	7.1	23.2	41.4	12.1	15.2	1.0
25°C/	12 mo	38	60.5	89.5	Nuts	21.1	44.7	18.4	7.9	5.3	2.6
50%RH					Almonds	2.6	31.6	36.8	5.3	18.4	5.3
	14 mo	35	77.1	77.1	Nuts	14.3	45.7	25.7	5.7	8.6	0.0
					Almonds	5.7	28.6	42.9	11.4	8.6	2.9
	16 mo	36	77.8	72.2	Nuts	16.7	33.3	41.7	5.6	2.8	0.0
					Almonds	5.6	25.0	52.8	8.3	8.3	0.0
	18 mo	35	71.4	42.9	Nuts	26.5	42.2	26.5	2.9	2.9	0.0
					Almonds	11.4	28.6	42.9	5.7	8.6	2.9
	20 mo	34	82.4	82.4	Nuts	23.5	32.4	32.4	8.8	2.9	0.0

					Almonds	8.8	17.7	47.1	2.9	20.6	2.9
	22 mo	36	94.7	76.3	Nuts	18.4	39.5	26.3	5.3	7.9	2.6
			,,	,	Almonds	5.3	21.1	52.6	5.3	10.5	5.3
	24 mo	36	83.3	72.2	Nuts	25.0	44.4	19.4	8.3	2.8	0.0
					Almonds	2.8	38.9	27.8	11.1	19.4	0.0
	24 mo	102	79.2	80.4	Nuts	18.6	39.2	26.5	6.9	6.9	2.0
	confirm				Almonds	5.9	24.5	40.2	13.7	10.8	4.9
25°C /	12 mo	38	60.5	89.5	Nuts	21.1	44.7	18.4	7.9	5.3	2.6
65% RH					Almonds	2.6	31.6	36.8	5.3	18.4	5.3
	14 mo	36	72.2	77.8	Nuts	11.1	41.7	30.6	8.3	8.3	0.0
					Almonds	2.8	16.7	41.7	16.7	19.4	2.8
	16 mo	34	82.4	91.2	Nuts	14.7	47.1	32.4	2.9	2.9	0.0
					Almonds	5.9	35.3	44.1	5.9	5.9	2.9
	16 mo	100	78.0	78.0	Nuts	16.0	43.0	32.0	3.0	6.0	0.0
	confirm				Almonds	5.0	38.0	39.0	8.0	7.0	3.0
35°C /	6 mo	36	80.6	47.2	Nuts	19.4	30.6	33.3	13.9	2.8	0.0
50% RH					Almonds	5.6	22.2	27.8	22.2	22.2	0.0
	8 mo	35	77.1	47.1	Nuts	20.0	42.9	22.9	8.6	5.7	0.0
					Almonds	8.6	28.6	34.3	14.3	14.3	0.0
	10 mo	37	75.7	70.3	Nuts	10.8	40.5	37.8	2.7	5.4	2.7
					Almonds	2.7	24.3	46.0	16.2	8.1	2.7
	12 mo	36	75.0	47.2	Nuts	25.0	41.7	25.0	2.8	5.6	0.0
					Almonds	2.8	30.6	36.1	22.2	8.3	0.0
	12 mo	99	80.8	75.8	Nuts	18.2	36.4	36.4	5.1	3.0	1.0
	confirm				Almonds	6.1	32.3	32.3	12.1	0.0	0.0
35°C /	2 mo	39	84.6	71.8	Nuts	18.0	35.9	28.2	7.7	10.3	0.0
65% RH					Almonds	7.7	12.8	46.2	10.3	23.1	0.0
	4 mo	35	76.5	62.9	Nuts	20.0	37.1	34.3	2.9	5.7	0.0
					Almonds	2.9	22.9	51.4	2.9	14.3	5.7
	6 mo	36	80.6	47.2	Nuts	19.4	30.6	33.3	13.9	2.8	0.0
					Almonds	5.6	22.2	27.8	22.2	22.2	0.0
	6 mo	90	64.4	51.1	Nuts	24.4	34.4	24.4	10.0	5.6	1.1
-	confirm				Almonds	8.9	28.9	26.7	20.0	13.3	2.2

### APPENDIX F

## PANELIST STRONG POINT RESPONSES FROM CONSUMER PANELS AT POINTS OF FAILURE/END OF STUDY

Table F.1: Panelist responses to the open-ended question "Please indicate WHAT in particular you liked or disliked about this almond sample (use WORDS not SENTENCES)" - Likes presented for baseline and samples stored in unlined cartons at point of failure (bold

font indicates responses from panelists who rejected the sample)

	T P MATERIAL WATER TO	35°C /	35°C /		25°C /	25°C /	15°C /
		65 RH	50 RH	4°C	65 RH	50 RH	50 RH
	Baseline	(2 mo)	(6 mo)	(6 mo)	(12 mo)	(16 mo)	(16 mo)
Appearance							
Appearance	6	3, <b>3</b>	3,1	1, <b>1</b>	1, <b>1</b>	<b>4,1</b>	2
Color	6,1	4,3	2,3	2,3	5,1	1 <b>,2</b>	3 <b>,1</b>
Skin	1				1		
Mouthfeel/texture							
Chewy	1	2, <b>1</b> 2		3	2,1	1	
Consistency		2					
Creamy			1				
Crispness	1	1	1				1
Crispy	1						
Crunchiness	53 <b>,2</b>	14 <b>,4</b>	10,4	2,1	9, <b>2</b>	10, <b>2</b>	8
Crumbly			1				
Easy to chew	1	3	1	1, <b>1</b>	2	2	1
Firm	1	3,2	2		1		1
Grainy	1		1				
Hard	3,1						1
Mouthfeel	5	1	1	1	1		
Not dry					1		4
Not oily							
Not too hard		2	2	4	1		
Not too crunchy					2		1
Not too soft		1 <b>,2</b>	1				
No toothpack				1			
Oily/fatty	1					1	1
Smooth	4	2		1		1	3, <b>2</b>
Soft		2,1	3	6,1	7,2	1	1

Tender							
Texture	24,2	14,4	10,2	9	19, <b>4</b>	15,3	8,1
Flavor							
Aftertaste	7	2,1	4	1	4	3	2, <b>2</b>
Almond flavor	2	1		1	1	3	2,1
Buttery	1	1				1	
Coconut			1				
Earthy				1	2	1	
Flavor/taste	50,2	24,1	23 <b>,2</b>	32,7	19,5	18,3	22,4
Fresh	7	3	1		4		2
Moderate/mild/bland	5	5		3	4	3	4,1
Moist				1			
Natural flavor		1	1		1		
No aftertaste					1		
No off flavor				1			
Not too sweet				3			
Not bitter		2,1		1			1
Not sour					1		
Nutty	10,1	4	3, <b>2</b>	3	3	5	3,1
Pleasant		1					
Raw flavor	3						
Roasted			1		2		
Salty	2						2
Savory							
Sweet	9,2	5,1	3	2,2	3	3	2, <b>2</b>
Unsalted/not too salty	5	1, <b>1</b>	1	1			
Woody			1				1
Odor							
Earthy	1						
Odor/smell	3,1	3 <b>,2</b>	6,1	8	8,4	3,1	6,3
No odor/off odor	3	2,1	3,1	3	2	1	1
Nutty			1		1		

Other							
Filling/satisfying	1						
Fresh		1					
Healthy	2	3, <b>3</b>	1	2		1, <b>1</b>	1
Other <sup>a</sup>	3,1		1	2	1	2, <b>2</b>	1
Natural	3					1	
Plain		1					
Quality							
Simple		2					

<sup>&</sup>lt;sup>a</sup> examples of responses listed under "other": "standard", "almonds in general", "snacks were given", things pertaining to testing procedure (i.e. "lighting", "privacy", "quick", "organized), words that could describe any/all attributes (i.e. "milky", "light", "neutral"), illegible words or unsure of meaning

Table F.2: Panelist responses to the open-ended question "Please indicate WHAT in particular you liked or disliked about this almond sample (use WORDS not SENTENCES)" – Likes presented for samples stored in polypropylene bags at point of failure/end of study (bold font indicates responses from panelists who rejected the sample)

	35°C /	35°C /	25°C /	25°C /	15°C /	15°C /	
	65 RH	50 RH	65 RH	50 RH	65 RH	50 RH	4°C
	(6 mo)	(12 mo)	(16 mo)	(24 mo)	(24 mo)	(24 mo)	(24 mo)
Appearance							
Appearance	2,3	1,1	4	2	1	4	2
Color	1,2	<b>5,1</b>	2,1		2	2,1	
Skin					1		
Mouthfeel/texture							
Chewy	2	2	3	1	3	3	1
Consistency	2				1		
Creamy							
Crispness		1			1		
Crispy							
Crunchiness	23,4	28,4	17,3	25, <b>7</b>	16, <b>1</b>	17,3	25, <b>7</b>
Crumbly							
Easy to chew	1, <b>1</b>	2,1		1	1, <b>1</b>		1
Firm		1	1	1		1	1
Grainy							
Hard	1	1	3	1		3	1
Mouthfeel				1			1
Not dry	1		1			1	
Not oily	1						
Not too hard		2	1			1	
Not too crunchy					1		
Not too soft							
No toothpack							
Oily/fatty			1			1	
Smooth	2,1	3	3		3,1	3	
Soft	1	2	1, <b>1</b>	3	2	1, <b>1</b>	3

Tender		1					
Texture	15, <b>5</b>	19 <b>,8</b>	17,3	23,9	13 <b>,5</b>	17,3	23,9
Flavor							
Aftertaste	1	2	1	5,1	2,1	1	5,1
Almond flavor	2	3,1	1,1	1	3,1	1, <b>1</b>	1
Buttery							
Coconut							
Earthy	3,1	1		1			1
Flavor/taste	34,3	28,1	25,4	34 <b>,2</b>	19,3	25,4	34 <b>,2</b>
Fresh		1	1	1		1	1
Moderate/mild/bland	2	3	1	3	4,1	1	3
Moist	1	1					
Natural flavor		1			1		
No aftertaste							
No off flavor							
Not too sweet		1					
Not bitter			1		1	1	
Not sour		1					
Nutty	5,1	6	5	4,1	4	5	4,1
Pleasant							
Raw flavor					1		
Roasted		1					
Salty			1			1	
Savory		1					
Sweet	2	2,1	5,2	7	3	5,2	7
Unsalted/not too salty	1				1		
Woody							
Odor							
Earthy			1			1	
Odor/smell	6,1	2,3	7,2	8	8,4	7,2	8
No odor/off odor	2	1	3	1		3	1
Nutty	1			2			2

Other							
Filling/satisfying							
Fresh							
Healthy	2	2	2		2	2	
Other <sup>a</sup>	1	2	1	1	3	1	1
Natural							
Plain							
Quality	1		1			1	
Simple		1		1			1

<sup>&</sup>lt;sup>a</sup> examples of responses listed under "other": "standard", "almonds in general", "snacks were given", things pertaining to testing procedure (i.e. "lighting", "privacy", "quick", "organized), words that could describe any/all attributes (i.e. "milky", "light", "neutral"), illegible words or unsure of meaning

### APPENDIX G

# PANELIST WEAK POINT RESPONSES FROM CONSUMER PANELS AT POINTS OF FAILURE/END OF STUDY

Table G.1: Panelist responses to the open-ended question "*Please indicate WHAT in particular you liked or disliked about this almond sample (use WORDS not SENTENCES)*" – Dislikes presented for baseline and samples stored in unlined cartons at point of failure (bold font indicates responses from panelists who rejected the sample)

(controller materials response	1	35°C /	35°C /		25°C /	25°C /	15°C /
		65 RH	50 RH	4°C	65 RH	50 RH	50 RH
	Baseline	(2 mo)	(6 mo)	(6 mo)	(12 mo)	(16 mo)	(16 mo)
Appearance							
Appearance	3						
Color				1			
Skin/outside	2				1	1	
Wrinkly/ridges	2	1, <b>1</b>					
Mouthfeel/texture							
Chalky	1		1				2
Chewy	2	6,1	5 <b>,4</b>	8,7	4,2	7,4	7,10
Creamy		1					
Crumbly							
Crunchy							
Dense	1						
Dry	6	6,2	3,1	1	4	1	1
Grainy/dusty		2	1	2			1
Gritty		2			1		
Gummy			1	2	2		
Hard	8,1		1	1		2	1
Hard to chew/swallow	3						1
Inconsistent texture	1			1			
Mealy					1	1	1
Meaty	1						
Moist		1, <b>1</b>	1	1	1, <b>1</b>		1
Mouthfeel	1	1	1		1	1	1
Mushy/soggy	2	1,3	2,1	2,9	2, <b>2</b>	1,3	1
No crunch	4	7,3	6,2	12,5	5,3	3, <b>2</b>	6,5
Not crisp	1	2, <b>2</b>		1,1	1	1	

Oily						1	
Off		2			1		
Old		_			1		
Outside texture				1			
Raw			1				
Rough	1	1	1			1	
Rubbery		2					
Smooth					1		
Soft	2,1	12,5	6,5	15,7	6,4	7, <b>4</b>	13,9
Spongy	1		,	,	,	2,1	,
Stale	1,2	2, <b>2</b>	1,2	1	2	1	1,3
Texture	2,1	2,5	6,4	4,3	4,2	3 <b>,3</b>	4,7
Too crisp/crunchy	3						
Toothpack	5	4	3	1	1,1	1, <b>1</b>	3 <b>,2</b>
Tough	2	1	1			1	
Waxy	1			1			
Wood chips		1					
Flavor							
Aftertaste/lingering flavor	5	5 <b>,2</b>	3,1	1	2,3	1,3	1, <b>1</b>
Bitter	2	2	1	1	2	1	1
Bland	9	9,1	1	4,2	2,1	5, <b>5</b>	4,3
Burnt/overcooked	1					1	1
Cardboard		1	1,2		2	1	
Coconut flavor		1					
Crayon		1					
Dirt			1				
Earthy	1				1		
Flavor/taste	2, <b>2</b>	6,11	5 <b>,6</b>	2	6,2	2,6	2,4
Fresh/fruity	1	1	1		1		
Greasy/oily/fatty		1	1				
Musty							
No/weak almond/nut flavor	1	1				1	1

Not flavorful/mild flavor	6,1	3	5, <b>2</b>	2,1	2,5	9,3	9,4
Not fresh	•	1	1	·	1		
Not roasted (raw)	3	2		1	1, <b>1</b>	1	
Not salted	4	2		2	2		1
Not sweet					1		
Nutty							
Off	1	2, <b>2</b>	3	2, <b>2</b>	4		1,1
Old		2	1,2	1	1	2,1	1
Oxidized			1		1		
Painty			1				
Plain/flat	1	1,1	1	1	1,1	2	2
Rancid		3	1,3		1		
Soapy		1					
Sour/tangy				1	1	1	
Stale		1,3	4	1,2	1,2	4	
Strong							
Sweet		1					
Tannic							
Unnatural/artificial/metallic		2		1	2		
Unpleasant/unsatisfying	1	1			1		
Woody		1	1		1		
Odor							
Cardboard		1	1				1
Dirt-like		1			1		1
Dough/crayon					1		
Earthy		1				1	1,1
Industrial/chemical/				1,3	1		1
plastic/medical				1,3	1		1
Musty		1	1	1			
No almond/nutty odor	1, <b>1</b>		1	1	1	1	1
No/weak/mild odor	4	3,1	4,3	1	4,4	12, <b>2</b>	3, <b>2</b>
Not fresh		1 <b>,1</b>					

Odor/smell	8	5,1	2	12, <b>2</b>	5,1	2,4	2,5
Off odor				2	1	1	1
Old		1					
Oxidized					1		1
Rancid		1		1			
Stale		1	1				
Strong/pungent/unpleasant	1			1			1
Sweet				1			
Woody	1	1					
Other							
Inconsistent quality							
Other <sup>a</sup>	1, <b>1</b>	1		1	1		1

<sup>&</sup>lt;sup>a</sup> examples of responses listed under "other": "not satisfying", "almonds in general", words that could describe any/all attributes (i.e. "milky", "light", "neutral"), illegible words or unsure of meaning

Table G.2: Panelist responses to the open-ended question "*Please indicate WHAT in particular you liked or disliked about this almond sample (use WORDS not SENTENCES)*" – Dislikes presented for samples stored in polypropylene bags at point of failure/end of study (bold font indicates responses from panelists who rejected the sample)

(bold folk indicates responses	35°C/	35°C/	25°C/	25°C/	15°C /	15°C /	
	65 RH	50 RH	65 RH	50 RH	65 RH	50 RH	4°C
	(6 mo)	(12 mo)	(16 mo)	(24 mo)	(24 mo)	(24 mo)	(24 mo)
Appearance							
Appearance	1	1				1	
Color			1	1			
Skin/outside	1					1	1
Wrinkly/ridges						1	
Mouthfeel/texture							
Chalky		1	1				
Chewy		2,1	5, <b>4</b>	2,1	7,2	3 <b>,2</b>	4
Creamy							
Crumbly	1			1	1	1	
Crunchy		1		1		1	
Dense							
Dry	5,3	3, <b>3</b>	2	5 <b>,2</b>	2,3	6	2, <b>1</b> 2
Grainy/dusty	1, <b>1</b>	1	1		1		2
Gritty	1			1			1
Gummy	1	1					
Hard	3 <b>,2</b>	1	3	5 <b>,2</b>	2,3	2,1	2
Hard to chew/swallow		1				2	2
Inconsistent texture	1						
Mealy	1		1	1			
Meaty							
Moist	1	1	1			1	
Mouthfeel		1					
Mushy/soggy	1,2	1 <b>,1</b>					
No crunch	2, <b>2</b>	1	3,4	3	2,1	2,1	1
Not crisp		2	1				

Oily		1					
Off		1	1				
Old			1				
Outside texture	1		1				1
Raw				1		1	1
Rough	1			1		1	1
Rubbery							
Smooth							
Soft	3 <b>,2</b>		7,3	1	8,2		6,1
Spongy							
Stale			1	1,1		1	2
Texture	3,6	4,2	3,1	4,3	4,5	2,4	5,3
Too crisp/crunchy				1		1	
Toothpack	2		4	1	2	2,1	2
Tough	1			1		1	
Waxy							
Wood chips			1				
Flavor							
Aftertaste/lingering flavor	2,4	4,1	2,5	1,2	2,1	2,1	3
Bitter	2, <b>2</b>	1	2, <b>2</b>	1,2	2		1, <b>1</b>
Bland	1	1,2	6,3	2,1	5,3	3,1	6,1
Burnt/overcooked		1	1				
Cardboard		1			1		
Coconut flavor							
Crayon							
Dirt	1	1,1					
Earthy				1	3,1		
Flavor/taste	8 <b>,2</b>	2,8	1,5	9 <b>,6</b>	2,8	2	2,3
Fresh/fruity						1	
Greasy/oily/fatty							
Musty		1					1
No/weak almond/nut flavor		2	1				

Not flavorful/mild flavor	1	2,3	4,3	3	5,2	3,1	8,1
Not fresh	1	1			1 <b>,1</b>		
Not roasted (raw)	2	1				1	1
Not salted	1		1	1 <b>,1</b>	4	3	3
Not sweet		1	1,1		1		1
Nutty					1		
Off	1, <b>1</b>	1,2	2		2		1
Old	1	1		1	1,1		
Oxidized		1			1		
Painty		1					
Plain/flat	1			1	1		
Rancid	1	1	1 <b>,1</b>	2	3	1	1
Soapy							
Sour/tangy	1		1	1			1
Stale	1			1	2, <b>2</b>		
Strong	2						
Sweet	1		1				1
Tannic				1			
Unnatural/artificial/metallic		1		1			
Unpleasant/unsatisfying					1		
Woody	2, <b>2</b>			1 <b>,1</b>			
Odor							
Cardboard		1		1			
Dirt-like							
Dough/crayon		1 <b>,1</b>			1		1
Earthy			1	1			
Industrial/chemical/		2		1, <b>1</b>			
plastic/medical				·			
Musty		1,1		1			
No almond/nutty odor		1					
No/weak/mild odor	3,1	4,1	2	2	1,1	4,1	6
Not fresh							

Odor/smell	4, <b>4</b>	6,3	2, <b>2</b>	5,8	3,2	5	8
Off odor	1	1	1,1		1		1
Old		1			1		
Oxidized							
Rancid	1, <b>1</b>		1,2	1	1 <b>,1</b>		
Stale		1	1	1		1	
Strong/pungent		2	1, <b>1</b>		1		
Sweet							
Woody		1		2,1	2		
Other							
Inconsistent quality	1				1		
Other <sup>a</sup>	2		1, <b>1</b>		1	1	1, <b>1</b>

<sup>&</sup>lt;sup>a</sup> examples of responses listed under "other": "not satisfying", "almonds in general", words that could describe any/all attributes (i.e. "milky", "light", "neutral"), illegible words or unsure of meaning