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Almond Skins as a Natural Antioxidant

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ALMOND SKINS AS A NATURAL ANTIOXIDANT

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ABSTRACT

In this thesis, the potential for almond skins in their native state as a natural antioxidant in foods was explored. A literature review of general information on almonds, research done on almond skins, and general information on antioxidants is presented. The almond skins used in this study required an additional heat treatment step to improve their microbiological quality before further treatments and analysis could be performed. A descriptive analysis sensory panel was utilized to identify potential flavor and aroma descriptors and their intensities. The aroma descriptors identified were toasted, bran, and toasted; nutty. The flavor descriptors identified were toasted, bran, nutty buttery, and toasted; nutty. Proximate analysis, GC-MS headspace analysis, and total phenolic content of the almond skin were determined. Several aromatic compounds, including benzaldehyde and hexanal, were identified in almond skins via GC-MS analysis. Total phenolic content ranged from 5-6.1 quercetin equivalents per gram of almond skin. Soybean oil was treated with varying levels of almond skin and analyzed using the Oxidative Stability Index (OSI) as both an analytical method and oxidation chamber prior to other analyses. Peroxide values and GC-MS headspace analysis was done on the treated soybean oil as well. BHA was also used as a control treatment to serve as a comparison. Measures of oxidative stability of the treatments varied depending on the analysis used. The OSI analysis did not show any differences between treatments ($p>0.05$); however, peroxide values of treated oil samples were lower than those of the control oil samples after 6.5 hours of oxidation ($p>0.05$). GC-MS analysis showed that

hexanal concentrations of almond skin treated oil oxidized for 3.25 hours were greater than those of control and BHA treated oil ($p>0.05$).

Results from this study demonstrate the critical need for a thorough review of current processing and handling procedures to improve the microbiological quality of commercial almond skins. Elimination of the additional heat treatment step that was employed to improve the microbiological quality of the almond skins would likely allow for the identification of new sensory descriptors. Peroxide values remain good indicators of oxidation and should be taken at more intervals to allow for better detection of differences between treatments. Normally, hexanal is a good indicator of oxidation; however, the almond skins in this study contained hexanal before treatment. Analysis of more varieties and samples of almond skins should be done to determine good markers of oxidation. Use of varieties of almond skins known to be high in phenolic content should be further explored before eliminating almond skins as a natural antioxidant source for foods.

DEDICATION

I dedicate this thesis to my mom. She has always inspired me to reach for more and strive for the best.

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REVIEW OF ALMONDS AND ANTIOXIDANTS

Abstract

Consumer demands for "natural" and "organic" foods have resulted in an increase in the use of natural antioxidants. This review explores the properties of almonds, more specifically almonds skins, and their potential use as a natural antioxidant. Basic facts on almonds, known antioxidants in almonds and almond co-products, commonly used antioxidants, methods for determining oxidation and antioxidant effectiveness, and characteristics of almonds that could potentially affect a food product are discussed.

Introduction

Over the past decade, consumers have become more focused on healthier eating habits, which have been paralleled by an increase in the number of "natural" and "organic" foods commercially available (OTA, 2006). As the popularity of natural foods increases, so does the demand for natural antioxidants. One potential natural antioxidant source is almonds and more specifically almond skins. This warrants investigation into the antioxidant properties of almond skins and other characteristics as a food component. Several almond skin properties have been studied; however, there is great variation in the results and source of almonds used among published literature. The antioxidants in almond skins have been studied, but not their use in foods.

Almonds – Basic Facts

Almonds (*Prunus dulcis*) come from the Rosaceae family. The fruit consists of an edible seed or kernel, shell, and outer hull. Once the fruit matures the outer hull splits open, and when dry, separates from the shell (Wickens, 1995). Almonds are available in natural (brown skin still attached) and blanched (skin removed) forms. During the blanching process, the hull (not to be confused with outer hull) or brown skin is removed by placing the almonds in 82ºC water for 3 minutes and skinning by hand or machine. The almonds are then dried to less than 8% moisture and the remaining hulls are used as livestock feed (Wickens, 1995).

California is the world's largest producer of almonds; supplying over 70% of the world's supply and exporting to over 80 countries. Almonds are grown exclusively throughout central California with over half a million acres in production. This region has an ideal climate for almond growth, with hot, dry summers and cool, rainy winters. California almonds are broken into four broad marketing categories: nonpareil, carmel, mission type, and California type. It is important to note that these categories do not incorporate all varieties of almonds. Nonpareil almonds are a single variety and have the widest range of uses. They are easily blanched and cut. Their thin outer shell and smooth kernel allow for easy processing that yields blemish-free almonds. Carmel almonds are actually a California type, but have become popular enough to warrant their own category. They are a soft shell variety that is easily processed and often substituted for nonpareil. Mission type varieties are hard shelled with small, wide kernels. The kernel skin is usually darker than nonpareil and wrinkled. This wrinkling enables better salt and flavor adherence; this variety is rarely blanched. Mission type varieties include Butte,

Padre, and Mission. California type includes the varieties Monterey, Sonora, Fritz, Peerless, and Price. They are blanchable and used primarily in manufactured products. These varieties have a wide range of shell hardness, kernel shapes, skin color, and surface characteristics. These differences make the California type almonds suitable for most any application or use (ABC, 2006).

Almonds have been increasing in popularity over the last several years (Spence & Martens, 2006). Consumers view almonds as healthy and adding interest and appeal to food products. Almonds are commonly used in entrees, salads, desserts, baked goods, and breads.

Antioxidants in Almonds and Almond Co-products

Many plants contain phenolic compounds that are primary antioxidants. They donate hydrogen atoms to free radicals and terminate the free radical chain reactions by forming stable products (Eskin and Robinson, 2000). Catechin, protocatechuic acid, vanillic acid, p-hydroxybenzoic acid, and quercetin containing phenolic compounds were isolated from almond skins by Sang et. al. (2002). A hydroxyl group attached to an aromatic ring is the basic structure of phenolic compounds. Flavonoids are polyphenolic compounds; they contain multiple aromatic rings. Quercetin, isorhamnetin, quercitrin, kaempferol 3-O-rutinoside, isorhamnetin 3-O-glucoside, and morin were determined to be the major flavonoids in almond skins by Wijeratne et. al. (2006). Milbury et. al. (2006) showed that even though the skin only compromises about 4.5% of the total weight of the almond, about 95% of the flavonoids originate in the skin. Phenolic extractions from almond skins have been shown to inhibit copper-induced human LDL oxidation, protect

DNA from peroxyl and hydroxyl radicals, exhibit strong metal chelating capacities, and exhibit strong DPPH radical scavenging activity (Sang et. al. 2002; Wijeratne et. al. 2006). It has been shown that flavonol glycoside content varies amongst varieties; Frison and Sporns (2002) found that Wood Colony, Carmel, and Butte varieties contained the most flavonol glycosides while Le Grand, Padre, and Fritz varieties contained the least. Also, almond brown skins contain more phenolic compounds per gram of extract than almond whole seed or green shell cover (Wijeratne et. al. 2006). The brown skin also exhibited a greater effectiveness in prevention of human LDL oxidation than almond whole seed or green shell cover (Wijeratne et. al. 2006).

Oils

Soybean oil naturally contains tocopherols that help prevent oxidation. This antioxidant is only effective when present at relatively low concentrations, 100 – 300 ppm of oil (w/w). Their effectiveness tends to diminish at levels >500 ppm of oil (w/w). Tocopherols are heat stable and not volatile during normal cooking conditions. They have been shown to have a carry through effect in potato chips, pastry, and cookies (Dougherty, 1988). Two negative aspects of soybean oil are its high content of linolenic fatty acids (6-10%) and that it forms β-crystals when hydrogenated (O'Brien, 2004). Linolenic fatty acids can cause off-flavor development at low levels of oxidation (O'Brien, 2004). Hydrogenation reduces the amount of linolenic acid present, thus extending its shelf life; however, hydrogenation produces trans fat. Citric acid is often added to soybean oil to help scavenge trace metals, which initiate off-flavor formation in

soybean oil (O'Brien, 2004). Citric acid acts synergistically with other antioxidants yielding a greater antioxidant effect together than singly.

Soybean oils with varying levels of linolenic acid are now available, since research began in 1968 at Iowa State University to reduce the amount of linolenic acid in soybeans. This was achieved through conventional breeding methods; three genes were found to independently reduce linolenic acid and when combined, yielded soybean oil with 1% linolenic acid. These modified beans were first commercially grown in 2004. Research has also been conducted to increase the oleic fatty acid content in soybean oil due to the greater oxidative stability and longer shelf life of oleic acid compared to linolenic acid. Conventional soybean oil contains 25% oleic fatty acid. By 2005, a variety of soybean yielding 53.3% oleic acid and 1% linolenic acid was produced. The oxidative stability of this oil is more than twice as long as normal (25%) oleic and 1% linolenic oil (Stockhausen, 2006).

Common Antioxidants

Antioxidants were first synthesized in the 1940's. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary-butylhydroquinone (TBHQ) are commonly used synthesized antioxidants; all of which are phenolic antioxidants (Coulter, 1988). These antioxidants are known as chain-breaking antioxidants, because they interfere with the oxidation chain reaction mechanism by donating hydrogen atoms (Byrd, 2001). Each has its own unique properties that make it suitable for different applications. PG is heat sensitive and decomposes at 148ºC; thus having poor carry through properties. It is also difficult to

dissolve in fats and oils. TBHQ is a powder that is the most effective at inhibiting oxidation of vegetable oils. BHA and BHT both have good carry through properties and are often used in combination (Byrd, 2001). Sources disagree on which antioxidant is better. Byrd states that while BHT is relatively ineffective in vegetable oils, it is better than BHA (2001). Eskin and Robinson stated that BHA is better and particularly effective in protecting vegetable and essential oils, while BHT is effective in protecting animal fats (2000). FDA limits the amount of BHA, BHT, PG, and TBHQ to 0.02% or 200 ppm by weight in the fat portion of the food (Byrd 2001).

Tocopherols, rosemary extract, phenolic acids, and flavonoids are just a few commonly used natural antioxidants (Eskin and Robinson, 2000; Dougherty, 1988; Byrd, 2001). Tocopherols are beneficial in products that are low in natural antioxidants, such as lard and butter. They are heat stable and are not volatile or steam distillable under normal cooking conditions. Natural phenolic compounds, like the commercially available phenolic antioxidants, act as primary antioxidants by donating hydrogen atoms to lipid radicals. Substitution in the *ortho* and *para* positions with alkyl groups (ethyl or n-butyl) enhances the antioxidant ability of tocopherol. Introduction of a second hydroxyl group at the *ortho* or *para* position also increases its antioxidant ability (Eskin and Robinson, 2000). Rice-Evans et. al. (1996) showed that when the Trolox equivalent antioxidant activities of various polyphenols were determined, epicatechin gallate and epigallocatechin gallate were the best, followed by quercetin. Gallic acid, morin, catechin, rutin, and other common polyphenols were all less effective than quercetin (Rice-Evans et. al., 1996). Makris and Rossiter (2001) also showed that quercetin was better than morin at preventing oxidative damage to lipids, proteins, and DNA.

Proximate Analysis

While the USDA National Nutrient Database lists the components of almonds with skin, blanched almonds, and various other almond products; it does not list the components of almond skins alone. According to the data base raw almonds are 5.25% water, 21.26% protein, 50.64% fat, 3.11% ash, and 19.74% carbohydrates (USDA 2006). Blanched almonds are 4.47% water, 21.94% protein, 50.62% fat, 3.02% ash, and 19.94% carbohydrates (USDA 2006).

Total Phenolic Content

The properties of phenolic compounds and the ones present in almond skins are listed in the section "Antioxidants in Almonds and Almond Co-products." A recent study determined the total phenolic content of almond whole seed, brown skin, and green skin and used quercetin as the standard (Wijeratne et. al., 2006). Several researchers have used catechin as the standard. Quercetin, however, has almost twice the Trolox (a water soluble vitamin E analog) equivalent antioxidant activity as catechin (Rice-Evans et. al.,1996). Quercetin is the non-sugar portion of several glycosides present in almonds; including rutin, quercitrin, isoquercetin (Wijeratne and others 2006). Protocatechuic acid, a quercetin degradation product, is also present in almonds (Makris and Rossiter, 2001). Makris and Rossiter (2001) showed that quercetin had more antioxidant capacity than morin and the breakdown products of morin and quercetin. Wijeratne, et. al. (2006) used quercetin as the standard in total phenolic content determination of almond extracts and found that almond whole seed had 8±1 mg of quercetin equivalents/g of ethanolic extract, brown skin had 88±2, and green shell cover had 71±2.

Singleton and Rossi (1965) examined the many variations of the official method of the Association of Official Agricultural Chemists for determining tannins in wines and spirits. Singleton and Rossi (1965) compared the Folin-Denis procedure to the Folin-Ciocalteu procedure and showed that the latter is preferable for use. They suggest gallic acid as the preferable reference standard; however, other standards are commonly used and vary with the sample being tested.

Methods for Assessing Antioxidant Capability

Oil Stability Index (OSI)

Every fat or oil has a certain resistance to oxidation. This resistance is commonly referred to as the induction period or length of time before the acceleration of oxidation. The OSI is a method of determining this induction period. Basically purified air is bubbled through an oil or fat sample and as the sample oxidizes organic acids, such as formic acid, are produced and carried via air to a deionized water sample. The conductivity of this water sample is constantly monitored. The OSI is defined as the point of maximum change of the rate of oxidation. This can be determined mathematically by calculating the maximum of the second derivative of the conductivity with respect to time. (AOCS, 1993).

De Mario et. al. (2000) found the OSI value of refined soybean oil to be approximately 6 hours. The addition of antioxidants extended the OSI value. Several researchers have suggested the OSI is not a suitable method for evaluating potential antioxidants, because of the possibility of degradation of the antioxidant at the high temperatures of the Oxidative Stability Instrument. However, cooking oils are frequently

exposed to high temperatures and examination of their stability at higher temperatures is necessary.

Peroxide Value

Unlike the OSI, which measures the resistance to oxidative rancidity and aids in knowing the shelf life of oil, the peroxide value determines the presence and concentration of hydroperoxides which are oxidation byproducts. Hydroperoxides readily decompose at elevated temperatures, so it is important that a cold extraction be done to obtain the lipid (Steele, 2000). This is a highly empirical method and any variation in procedure may result in variation in results (AOCS, 1986).

Peroxide values also have a high correlation with organoleptic flavor scores. Peroxide values of 1 or less for soybean oil indicate freshness; 1-5 PV indicates low oxidation; 5-10 moderate oxidation; 10 or more high oxidation; and over 20 indicates poor flavor. It is important to remember that as oxidation progresses peroxides degrade, so while a high peroxide value generally indicates poor flavor, a low value does not necessarily indicate a good flavor (O'Brien, 2004).

Gas Chromatography

Gas chromatography (GC) is becoming a popular method of determining oxidative rancidity in oils and foods. Examining the headspace of a product allows determination of volatile oxidation products present. Aldehydes, particularly hexanal, have been identified as key markers for oxidative degradation. There have also been good correlations between increasing hexanal concentrations and the sensory perception of

rancid odor and flavors (Steele, 2000). However, several problems have been identified with this method though. Primarily, heating at high temperatures is necessary to drive the volatiles into the headspace which risks further oxidation and degradation of current oxidation byproducts (Allen and Hamilton, 1994).

High Pressure Liquid Chromatography (HPLC)

Wijerantne et. al. (2006) discovered 8 phenolic compounds: protocatechuic acid, quercetin 3-O-rhamnoside, kaempferol 3-*O*-glucoside, morin, kaempferol 3- *O* -rutinoside, isorhamnetin 3- *O* -glucoside, quercetin, and isorhamnetin. Amarowicz et. al. (2005) separated and analyzed phenolic acids, flavonoids, proanthocyanidins, and procyanidins from almond seeds. Vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, delphinidin, cyaniding, quercetin, kaempferol, isorhamnetin, procyanidin B_2, and procyanidin B_3 were all found (Amarowicz et. al., 2005). Milbury et. al. blanched almonds and then analyzed the blanched almond kernel, skin, and blanch water. HPLC revealed the presence of 20 flavonoids and phenolic acids; 8 of the flavonoids and 3 phenolic acids were found exclusively in the skin. Kaempferol and quercetin were found only in the skin, while p-hydroxy-benzoic acid, quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, dihydroxykaempferol, and eriodictyol were found only in the skin and blanch water. This observation is consistent with the role of flavonoids as compounds that localize in the skin layer around seeds to protect it from bacteria, fungus, and other environmental stresses (Milbury et. al., 2006).

Descriptive Analysis

Descriptive analysis is defined as a sensory methodology that provides quantitative descriptions of products, based on the assessments from qualified panelists. The evaluation can be all encompassing or focus on only one aspect of the product. Either way, descriptive analysis is based on several fundamentals. They are the subject/panelist selection process, the extent and duration of the training (including development of the descriptive language), quantification of the judgments, and the analysis of the data. Descriptive testing generally involves 10-20 subjects that are screened with a product or products in the same category as the product that will be used in the actual test. Roughly 30% of initial participants will not successfully pass the screening phase. Once the participants are screened they must be trained. Training involves identifying and defining references for attributes. Subjects are encouraged to use any common words they want to describe the product, but the group must be able to come to agreement as to what those words mean. It is useful to have references on hand for subjects to compare to the product. The major cause of incorrect results is a panel leader with insufficient knowledge (Stone and Sidel, 2004).

One method for descriptive analysis is Spectrum™ Descriptive Analysis. It has been described as a "custom design" approach; panelists may be selected and trained to evaluate only one product and selected attributes. The panelists, however, must understand all aspects of the attribute, ex: if determining flavor; panelists must understand olfactory sensations, taste sensations, and oral feeling factors. A panel of only four to six subjects is needed. They are chosen from a larger group and then further trained on basic sensory principles and evaluation of products. This training can be rather

extensive, requiring up to 3-4 hours a week for 14 weeks. During language development, the panel leader must not interfere with creation of the terms, but be able to detect when the subjects are ready to move forward. Quantitative data is also essential and the preferred collection method is the use of a 15 cm line scale. Quantitative data can be collected on aspects such as intensities. Duplication is important to ensure accuracy and reliability; four replicates seem to be optimal, but it varies with each test. The analysis of variance (ANOVA) model is the most common and appropriate statistical method for analysis of responses from descriptive testing (Meilgaard, 1999).

The Almond Board of California commissioned Sensory Spectrum to develop a lexicon of the appearance, aroma, flavor and textural characteristics of almonds. Trained staff members of Sensory Spectrum evaluated fifteen randomly selected samples from a pool of thirty six almond samples representing twenty varieties of almonds. The initial lexicon contained 72 terms; it now contains 86. Lexicon creation is dynamic and may evolve over time as new attributes are revealed and a greater understanding of terms is achieved. The current lexicon contains 15 appearance, 9 aroma, 36 flavor aromatics, 3 basic taste and 4 chemical feeling factor terms; and 19 textural terms and definitions. Aroma terms describe the intensity of odors when they enter the nasal passages, while flavor aromatics describe the portion of flavor perceived by the sense of smell when the sample is in the mouth (Maan, 2006). A second study was done to further investigate the differences amongst varieties. Butte, Carmel, Nonpareil, Fritz, Mission, Monterrey, and Sonora varieties were used, since they comprise 75% of the almonds produced (Maan, 2006). All varieties shared the aromatics of almond nutmeat, benzaldehyde, and woody. Fritz variety did not have any unique aromatic attributes (Maan, 2006).

Almonds and Microbes

Tree nuts are considered low-risk foods, because they are generally consumed dry. A water activity of <0.65 (approximately 6% moisture) is desired for stored tree nuts. This level inhibits growth of bacteria and fungi, including those that produce aflatoxins. They are also rarely associated with foodborne illness outbreaks, but in 2000-2001 a salmonellosis outbreak associated with raw almonds occurred. During that harvest season, heavy rains occurred after the almonds had dropped to the orchard floor and delayed harvesting and drying by 1-2 weeks (Uesugi and Harris, 2006). This outbreak was eventually linked back to the grower and handler, which led the Almond Board of California to further investigate ways of preventing *Salmonella* spp. growth and ensuring safe almonds. In August 2006, the Board recommended that a mandatory treatment that would ensure a 4-log *Salmonella* reduction be implemented by all handlers (Day, 2006); this change will take effect September 1, 2007 and will require all handlers to submit their almonds to an approved treatment of their choice (Pello & Kimmel, 2007). Approved treatments currently include treatment with propylene oxide, oil roasting, blanching, and moist heat processing (Pello & Kimmel, 2007).

Conclusions

Much research has been done on the benefits of incorporating almonds into the diet and on the antioxidants in almonds and almond co-products. Methods for evaluating sensory aspects of almonds and measuring oxidation rates of oils are also well established. Little research, however, has been done on the sensory aspects of almond skins, ability of almond skins to prevent oxidation of oils, and basic components of

almond skin. Since numerous antioxidant compounds have been identified in almond skins, each of these areas warrant further exploration.

Literature Cited

[ABC] The Almond Board of California. 2006 [cited 2007 Feb 1]. California Almonds Technical Information [Internet]. Available from: http://almondboard.files.cms-plus.com/TechTradeKitFINAL2.pdf

Allen, C, Hamilton, RJ, Editors. 1994. Rancidity in Foods. Bishopbriggs, Glasgow: Chapman & Hall. p 26-53.

Amarowicz, R, Troszynska, A, Shahidi, F. 2005. Antioxidant Activity of Almond Seed Extract and Its Fractions. Journal of Food Lipids. 12: 344-358.

[AOCS] American Oil Chemists' Society. 1986. Official and Tentative Methods of the American Oil Chemists' Society. Chicago, IL: AOCS.

[AOCS] American Oil Chemists' Society. 1993. Official and Tentative Methods of the American Oil Chemists' Society. Chicago, IL: AOCS.

Byrd, S. 2001. Using Antioxidants to Increase Shelf Life of Food Products. Cereal Foods World. 46 (2): 48 – 53.

Coulter, R. 1988. Extending Shelf Life By Using Traditional Phenolic Antioxidants. Cereal Foods World. 33 (2): 207-210.

Day, LC. 2006 [cited 2007 Feb 4]. Almonds Grown in California; Outgoing Quality Control Requirements and Request for Approval of New Information Collection [Internet]. 7 CFR, pt.981. Available from: http://www.almondboard.com/files/PDFs/Outgoing%20Quality%20Control%20Rule.pdf.

De Maria, CAB, Santos, MCM. 2000. Stabilization of Soybean Oil with heated quercetin and 5-Caffeoylquinic acid in presence of ferric ion. J. Agric. Food Chem. 48 (9): 3935-3938.

Dougherty, M. 1988. Tocopherols as Food Antioxidants. Cereal Foods World. 33 (2): 222-223.

Eskin, NA, Robinson, DS, editors. 2000. Food Shelf Life Stability: chemical, biochemical, and microbiological changes. Boca Raton, FL: CRC Press LLC. 370 p.

Frison, S, Sporns, P. 2002. Variation in the Flavonol Glycoside Composition of Almond Seedcoats as Determined by MALDI-TOF Mass Spectrometry. J. Agric. Food Chem. 50 (23): 6818 – 6822.

Makris, DP, Rossiter, JT. 2001. Comparison of Quercetin and a Non-Orthohydroxy Flavonol As Antioxidants by Competing In Vitro Oxidation Reactions. J. Agric. Food Chem. 49 (7): 3370 – 3377.

Maan, H (The Almond Board of California, Modesto, CA). RE: almond skins sensory [electronic mail on the Internet]. Message to: Heather Johnson (Clemson University, Clemson, SC). 2006 Nov 17, 4:37 pm [cited 2007 April 29]. [one paragraph and three attachments; attachments used are "Almond Lexicon Poster" and "Almond Variability Poster"].

Meilgaard, M. Descriptive Analysis Techniques. In Sensory Evaluation Techniques, Third Edition. 1999. Civille, GV, Carr, BT, editors. Boca Raton, FL: CRC Press LLC. p 161-180.

Milbury, PE, Chen, C, Dolnikowski, GG, Blumberg, JB. 2006. Determination of Flavonoids and Phenolics and Their Distribution in Almonds. J. Agric. Food Chem. 54 (14): 5027 – 5033.

O' Brien, RD. 2004. Soybean Oil. In: Fats and Oils: formulating and processing for applications, 2nd Ed. Boca Raton, FL: CRC Press LLC. 592 p.

[OTA] The Organic Trade Association. 2006 [cited 2007 June 12]. OTA's 2006 Manufacturer Survey: U.S. Organic Industry Overview [Internet]. Available from: http://www.ota.com/pics/documents/short%20overview%20MMS.pdf

Pello, M, Kimmel, K. 2007 [cited 2007 June 11]. Almonds Grown in California; Outgoing Quality Control Requirements [Internet]. 7 CFR, pt.981. Available from: http://www.almondboard.com/files/Rule.pdf

Rice-Evans, CA, Miller, NJ, Paganga, G. 1996. Structure-Antioxidant Activity Relationships of Flavonoids and Phenolic Acids. Free Radical Biology & Medicine. 20 (7): 933-956.

Sang, S, Lapsley, K, Woo-Sik, J, Lachance, P, Chi-Tang, H, Rosen, RT. 2002. Antioxidative Phenolic Compounds Isolated from Almond Skins (Prunus amygdalus Batsch). J. Agric. Food Chem. 50 (8): 2459 – 2463.

Singleton, VL, Rossi, JA. 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol. Vitic. 16: 144-158.

Spence, M, Martens, M. 2007 [cited 2007 May 29]. Survey: With a 1-2 Punch of Taste and Health, Almonds are More Popular than ever with Consumers. [Internet]. Available from: http://www.almondsarein.com/almondLovers/nrdetail.cfm?itemnumber=21381&mnItemNumber=3878&snItemNumber=3896&tnItemNumber=3901&qnItemNumber=3944

Steele, R, editors. 2000. Understanding and measuring the shelf-life of food. Boca Raton, FL: CRC Press LLC. 406 p.

Stockhausen, CJ, editor. 2006 [cited 2007 Feb 1]. Soybean Breeding Research for Healthier Oils at Iowa State University [Internet]. Available from: http://www.notrans.iastate.edu/research.html

Stone, H, Sidel, JL. Descriptive Analysis. In: Sensory Evaluation Practices, Third Edition. 2004. Taylor, SL, editor. San Diego, CA: Elsevier Academic Press. p 201-246.

Uesugi, AR, Harris, LJ. 2006. Growth of Salmonella enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. Journal of Food Protection. 69 (4): 712-718.

USDA. 2006 [cited 2007 April 12]. USDA National Nutrient Database for Standard Reference, Release 19, Nuts, almonds [Internet]. Available from: http://riley.nal.usda.gov/NDL/cgi-bin/list_nut_edit.pl

USDA. 2006 [cited 2007 April 12]. USDA National Nutrient Database for Standard Reference, Release 19, Nuts, almonds, blanched [Internet]. Available from: http://riley.nal.usda.gov/NDL/cgi-bin/measure.pl

Wickens, GE. 1995. Major Edible Nuts. In: Edible nuts. Rome, Italy: Food and Agriculture Organization of the United Nations. p 37-39.

Wijeratne, S, Abou-Zaid, M, Shahidi, F. 2006. Antioxidant Polyphenols in Almond and Its Coproducts. J. Agric. Food Chem. 54 (2): 312 – 318.

ALMOND SKINS AS A NATURAL ANTIOXIDANT

Abstract

The use of almond skins as a natural antioxidant and other almond skin characteristics were determined. Using descriptive analysis techniques a sensory panel developed 3 aroma descriptors (toasted, bran, and toasted; nutty) and 4 flavor descriptors (toasted, bran, nutty buttery, and toasted; nutty) and defined intensities for each. Proximate analysis, GC-MS analysis, and total phenolic content of the almond skin were measured. Soybean oil was treated with varying levels of almond skin and BHA then analyzed using the Oxidative Stability Index (OSI), peroxide value determination, and GC-MS analysis. The total phenolic content ranged from 5 - 6.1 quercetin equivalents per gram of almond skin. The OSI did not show any differences between treatments ($p>0.05$). Peroxide values of treated oil samples were lower than those of control oil samples after 6.5 hours of oxidation ($p>0.05$). GC-MS analysis showed that hexanal concentrations of almond skin treated oil oxidized for 3.25 hours were greater than those of control and BHA treated oil ($p>0.05$).

Introduction

The popularity of "natural" and "organic" foods has resulted in an increased demand for natural antioxidants. It is important to have a complete understanding of both the physical and chemical characteristics of almond skins if they are to be used as a food antioxidant. The USDA National Nutrient Database lists the components of almonds with skin, blanched almonds, and various other almond products, but it does not list the

components of almond skins alone. Descriptors for the appearance, aroma, flavor and textural characteristics of almonds have been established through descriptive analysis by Sensory Spectrum, but the characteristics of the skin alone are not documented.

Phenolic compounds are commonly found in plants and are primary antioxidants. Researchers have identified numerous phenolic compounds in almonds and almond skins; including catechin, protocatechuic acid, vanillic acid, p-hydroxybenzoic acid, quercetin, isorhamnetin, morin, and more (Sang et. al., 2002; Wijeratne, 2006). Milbury et. al. (2006) showed that even though the skin only compromises about 4.5% of the total weight of the almond, about 95% of the flavonoids are found in the skin. Also, almond brown skins contain more phenolic compounds per gram of extract than almond whole seed or green shell cover (Wijeratne et. al., 2006). Phenolic extractions from almond skins have been shown to inhibit copper-induced human LDL oxidation, protect DNA from peroxyl and hydroxyl radicals, exhibit strong metal chelating capacities, and exhibit strong DPPH radical scavenging activity (Sang et. al., 2002; Wijeratne et. al., 2006). While the physical and chemical characteristics of almonds have been thoroughly researched, many gaps exist in the data on almond skins as their own entity and need to be further explored.

Material & Methods

Fritz variety almond skins were received from the Almond Board of California (Modesto, CA). Wesson brand vegetable oil with an ingredient statement of "soybean oil" was purchased in gallon bottles from a local grocery store. All chemicals used were reagent grade and purchased from VWR International (Bridgeport, NJ).

Preparation of Almond Skins

Total aerobic plate counts were performed on almond skins upon receipt. Results indicated that the almond skins had a relatively high bacterial population; therefore, they were subjected to a heat treatment to reduce the bacteria to a level safe for human consumption. The ideal treatment methodology was determined by performing microbial analysis on almond skins after heat treatment for 30, 60, and 90 minutes. The results led to the decision to follow the procedures outlined in the next paragraph.

Almond skins were ground in a coffee grinder (Mr. Coffee, Rye, NY) for approximately 20 seconds. Ground almond skins were spread out on a 18 x 13 inch aluminum sheet pan and placed in a Thelco Laboratory Oven (Model 130DM by Precision Scientific Inc., Maharashtra, India) set to 121ºC for 60 minutes; the almond skins were stirred with a sterile metal spoon after 30 minutes of heating. The skins were either used immediately after treatment or vacuum sealed in clear plastic bags and stored at room temperature (25±4ºC) in the dark until use.

Microbial Analysis

Ground almond skins were added to sterile water at dilutions of 1:10 (1 gram of skins to 9 grams of water or 11 grams of skins to 99 grams of water) and 1:100 (1 gram of skins to 99 grams of water). The solution was then hand-shaken for 20 seconds, allowed to settle, and then re-shaken for 20 seconds. Dilutions were made once the almond skins were allowed to settle. Difco™ plate count agar (Becton, Dickinson & Company, Frankin Lakes, NJ) was used to determine the amount of aerobic bacteria

present. Brilliant green agar (Becton, Dickinson & Company, Frankin Lakes, NJ) was used to determine the amount of *Salmonella* spp. populations.

Oxidative Stability Index (OSI)

For the control, 5 grams of oil was placed into the sample tube. For the 2% almond skin, 0.1 grams of ground almond skin and 4.9 grams of oil were placed in the sample tube; the ratio for 6% was 0.3 grams of almond skin to 4.7 grams of oil; the ratio for 10% was 0.5 grams of almond skin to 4.5 grams of oil. The BHA sample was prepared by combining 500 ml of oil with 0.5 grams of a 20% BHA solution (Grindox 105 Kosher by Danisco) and then putting 5 grams of the solution into the sample tube. The AOCS Official Method Cd 12b-92 (1993) was followed. Once prepared, the sample tubes were placed in the Oxidative Stability Instrument (Omion, Inc., Rockland, MA) which was set to 110ºC.

Peroxide Value

Peroxide values were determined on samples taken at 0, 3.25, and 6.5 hours of running time in the Oxidative Stability Instrument. The AOCS Official Method Cd 8-53 (1986) was followed with one exception. Cyclohexane was used in place of chloroform; cyclohexane has been shown by several labs to be a safer and suitable alternative for chloroform, which is a carcinogen.

Total Phenolic Content

Total phenolics were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). The extraction method of Velioglu et. al. (1998) was followed. Two hundred milligrams of almond skin were extracted for 2 hours with 2 ml of 80% methanol containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 minutes and the supernatant decanted into 4 ml vials. The extraction procedure was repeated on the centrifuged pellets. Supernatants were combined and used for total phenolics assay. The determination method of Wijeratne et. al. (2006) was followed. Folin-Ciocalteau reagent (0.5 ml) was added to centrifuge tubes containing 0.5 ml of the supernatant. The contents were mixed and 1 ml of saturated sodium carbonate solution was added to each tube. The volume was adjusted to 10 ml with distilled water and the contents were mixed. Tubes were allowed to stand at ambient temperature for 45 minutes and then centrifuged at 4000g for 5 minutes. Absorbance was read at 725 nm. A blank sample for each extract was used for background subtraction. The content of total phenolics in each extract was determined using a standard curve prepared from quercetin. Total extracted phenolics were expressed as milligrams of quercetin equivalents per gram of extract.

GC-MS

A Hewlett Packard GC System HP 6890 Series with a HP 5973 Mass Selective Detector with a HP 7694 headspace sampler and HP-5MS (5% phenyl methyl siloxane) capillary (30.0 m x 250 μm x 0.25 μm nominal) column was used. For analysis of the almond skin 3.5 grams of ground almond skin were placed in a 20 ml vial and heated at

100ºC for 15 minutes in the headspace sampler. After injection into the column, the volatiles were separated using a temperature profile of -20ºC for 1 minute, followed by a temperature increase of 2º per minute to 60ºC and then 10º per minute to 220ºC for a total of 57 minutes.

Soybean oil with various treatments was heated in the OSI for 3.25 and 6.5 hours. Each OSI sample tube contained a total weight of 5 grams. After oxidation, 9 grams of oil was put in a 20 ml vial and heated at 110ºC for 10 minutes in the headspace sampler. After injection into the column, the volatiles were separated using a temperature profile of -20ºC for 1 minute, followed by a temperature increase of 3º per minute to 100ºC and then 10º per minute to 200ºC for a total of 51 minutes.

Proximate Analysis

The almond skin was analyzed for protein using the kjeldahl method at the Clemson University H.C. Cooper Agricultural Research Laboratory. The percent nitrogen was multiplied by 5.18 to calculate the percent protein ([AOAC] 1995).

Moisture content was determined by placing 2 grams of ground almond skins in disposable aluminum pans. Both heat treated and non-heat (as received) treated samples were subjected to moisture analysis. The samples were then placed in a Thelco Laboratory Oven (Model 130DM by Precision Scientific Inc., Maharashtra, India) set to 100ºC for 5 hours. The samples were then immediately re-weighed. The samples were then held in a desiccator prior to fat analysis.

The heat treated samples from the moisture analysis were put into dried thimbles, stoppered with cotton, and held in a Soxhlet unit for 16 hours. Petroleum ether was used

as the extraction solvent. After extraction, the thimbles were held in a hood overnight. They were then dried in a Thelco Laboratory Oven (Model 130DM by Precision Scientific Inc., Maharashtra, India) for 17.5 hours at 100ºC. The thimbles were then re-weighed and kept in a dessicator before being ash determination.

The samples were placed in porcelain crucibles and put into a Thermolyne Type 6000 programmable furnace to determine ash content. Furnace temperature was started at room temperature and increased 5º per minute until 250ºC is reached. The temperature was held at 250ºC for 2 hours and then increased 10º per minute until 525ºC was reached. The temperature was held at 525ºC for 5 hours. After cooling, samples were re-weighed. Carbohydrates content was determined by difference.

Sensory

Descriptive analysis techniques were used to obtain preliminary sensory data on potential flavor and aroma profiles of the almond skins. Spectrum™ was the general methodology used; however, several modifications were made. Normally, extensive training (3-4 hours a week for 14 weeks) on basic sensory principles and evaluation of products is desired for Spectrum™ analysis. However, due to time constraints, eight panelists were chosen based on their ability to attend sessions and prior experience in sensory analysis. Aroma and flavor were the only aspects of the almond skin explored. Six sessions were held and lasted a total of 5 hours. The first two lasted 30 minutes each while the last four lasted an hour each. During the first two sessions, panelists created descriptors and categorized them. The list of descriptors was narrowed during the third and fourth sessions. Intensities were evaluated twice (once during session 5 and again in

session 6). A survey was also given in the last session (see Table B.1 & B.2 in Appendix B). This was not meant to be a complete and exhaustive descriptive analysis panel, but a basic and introductory panel that would yield results that could be a starting point for further research.

A manual by G. V. Civille and B.G. Lyon (1996) was used to determine reference standards for the descriptors developed and they were prepared according to the manual (detailed preparation directions can be found in Appendix B). Approximately 300 grams of ground almond skins were heat treated on the same day and separated into seven clear plastic bags. Each bag was vacuum sealed and stored at room temperature (25±4ºC) in the dark until use. One bag was opened prior to each session; the almond skins were put into 2 ounce clear plastic cups. Each panelist received a cup containing 5 grams of almond skins to use as their reference.

Statistical analysis

Data were analyzed using SAS (Release 9.1, SAS Inst., Cary, NC, USA) to determine if there was a difference in treatments. The results from the OSI, GC-MS on soybean oil, TPC, and peroxide values were analyzed for least squares differences (LSD) with $p<0.05$. The intensities from the sensory panel were analyzed to determine a confidence interval of 95% ($p<0.05$).

Results & Discussion

Microbial Analysis

Total aerobic plate counts of almond skins received from the supplier averaged above 10^5 cfu/g; therefore, it was necessary to treat the almond skins to ensure safety for human sensory panels. Heat treatment was chosen to treat the skins due to its efficiency and low cost. As shown in Figure 2.1, heating for 60 minutes at 121ºC yielded greater than a 2-log reduction. Heating for an additional 30 minutes yielded less than another 1-log reduction and the almond skins were noticeably darker than those heated for only 60 minutes. Heat treatment for 60 minutes yielded zero growth on the *Salmonella* species specific agar (data not shown). While, the un-heated skins did not have any colonies typical of *Salmonella* species at dilutions above 1:10, the 1:10 plates were too numerous to count, but did not appear to have any colonies typical of *Salmonella* species (data not shown). The bacteria present were most likely lactose or sucrose fermenters (Becton Dickinson and Company, 1999). Therefore, all almond skins used in research were heated at 121ºC for 60 minutes before use.

It is important to note that the almond skins had undergone heat treatment via blanching at a minimum of 190ºF for at least 2.5 minutes and drying at 220ºF for 40-60 minutes before being received; however, the exact processing and handling conditions are unknown. This process is an accepted pasteurization process and should yield greater than a 5 log reduction of pathogens (Huang, 2007). Post-processing handling procedures are likely the reason for the higher microbial content of the almond skins received. Almond skins are currently a by-product and new handling procedures would need to be created to maintain their safety for use in food products.

Total Phenolic Content and GC-MS Analysis of the Almond Skin

Heat treated and non-heat treated almond skins were analyzed via GC-MS and their total phenolic content was determined. As shown in Figures 2.2 and 2.3, heating the almond skins for 60 minutes at 100ºC changed the volatile compounds found via GC-MS. After heating, a large number of compounds with retention times of about 45 minutes were present. Compounds with molecular weights less than 100 were not present after heating and several new compounds with larger molecular weights appear (Table 2.1). One compound present in both heated and unheated almond skins was hexanal. Hexanal concentration was greater in unheated almond skins (peak area of 4.3×10^5 in unheated vs 1.8×10^5 in heated). Heating may have driven off most of the low molecular weight volatiles, degraded hexanal, and formed intermediate fatty acid breakdown products.

It is also worth noting that most of the compounds listed in Table 2.1 have well documented odors associated with them. Methyl butanal exhibits odors of cocoa, almond, and malt. Benzaldehyde is known to smell of almond and burnt sugar and hexanal smells of grass, tallow, and fat. Toluene smells like paint (Acree & Arn, 2004). All of these aromatics, except hexanal, are only present in the non-heat treated skins.

The non-heat treated almond skins had 5.1646 quercetin equivalents per gram, while the heat treated almond skins had 6.1221 querecetin equivalents per gram. These values were not different ($p > 0.05$). Wijeratne et. al. (2006) reported almond skins as having 88 mg quercetin equivalents per gram of ethanolic extract. Ethanolic extraction of almond skin phenolics yielded 8 grams of extract per 100 grams almond skin (Wijeratne et. al., 2006). Based on these values, approximately 7 quercetin equivalents per gram of

Figure 2.1: Effect of Heating in a 121ºC Oven on the Total Aerobic Plate Count of Almond Skins

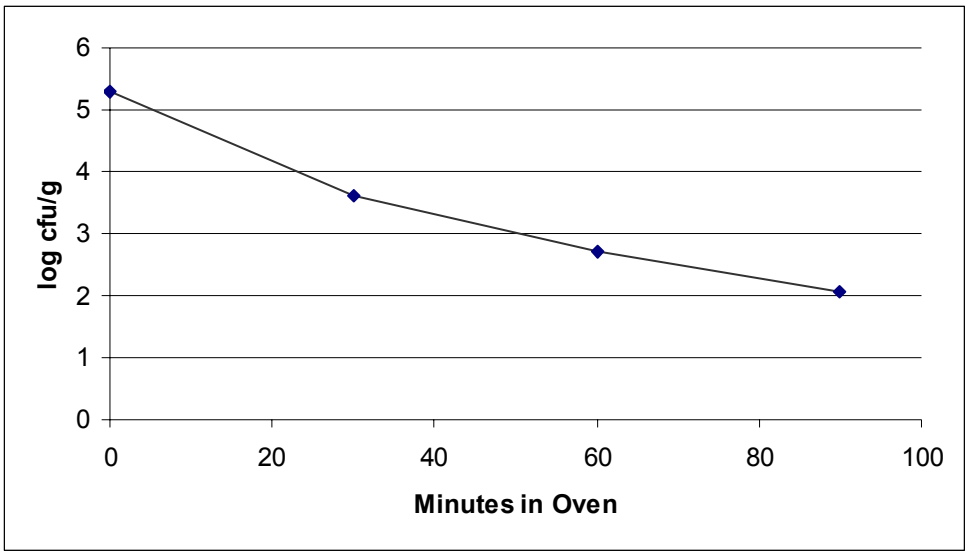


Figure 2.2: GC-MS Chromatogram for Non-heat Treated Almond Skin

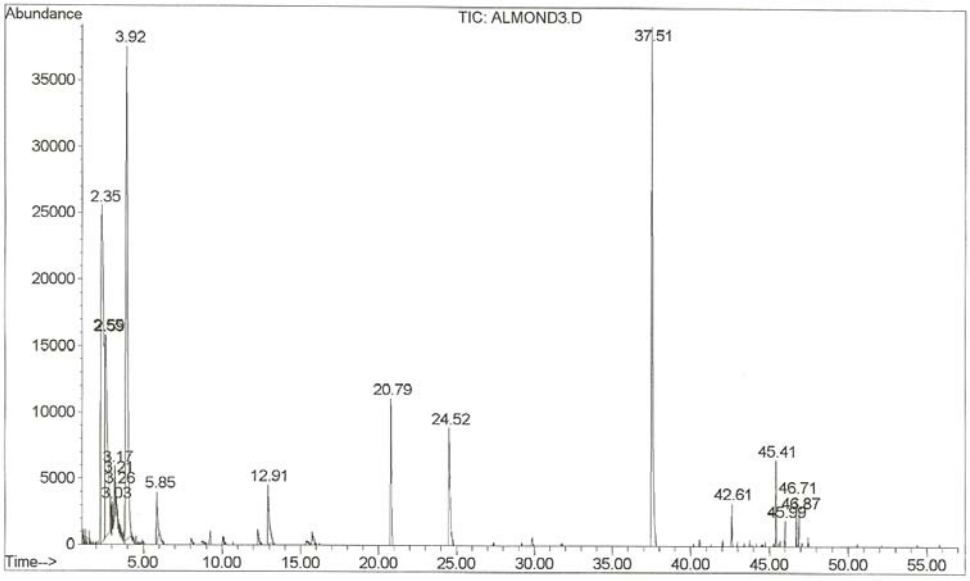


Figure 2.3: GC-MS Chromatogram for Heat Treated Almond Skin

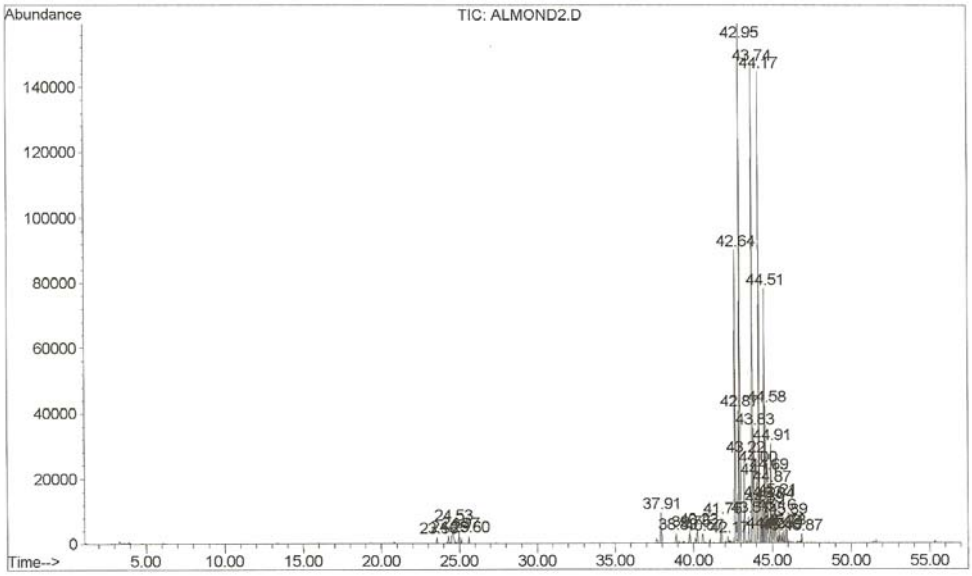


Table 2.1: Compounds Found in Almond Skins via GC-MS Analysis

	Non-heat treated Almond Skins	**Heat treated Almond Skins**
Molecular Weight	**Compound(s) Present**	
85-90	2-methyl butanal (12.91)	
90-95	Toluene (20.79)	
100-105	Hexanal (24.52)	Hexanal (24.53)
105-110	Benzaldehyde (37.51)	
110-115		2-octene (24.97) & cyclooctane (25.6)
170-175		2,2-dimethyl decane (42.64)
180-185		2,7-dimethyl undecane (42.95)

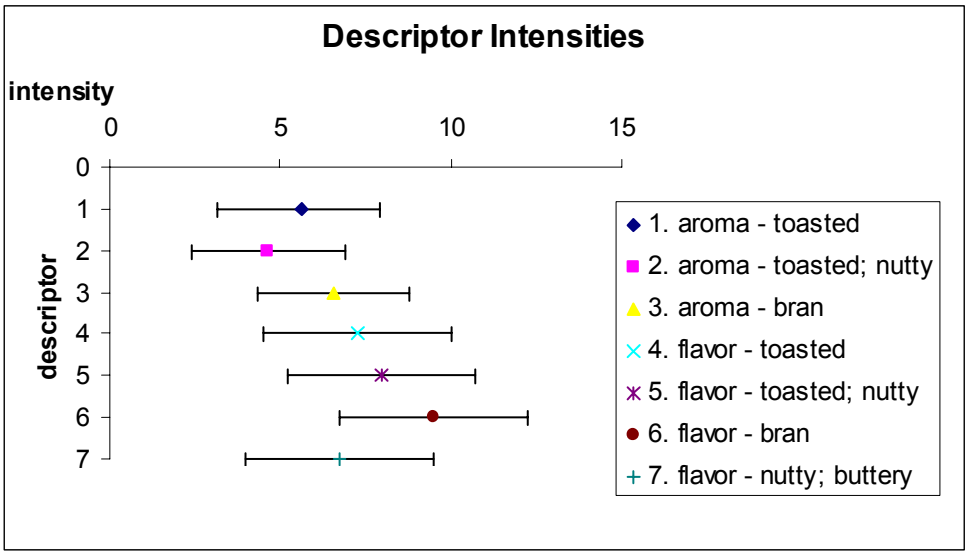
[1]compound name is followed by (retention time) for reference with Figures 2.2 & 2.3

almond skin was reported by Wijeratne et. al. (2006), which is in the range reported in the present study. The variety of almond used was not stated.

Sensory Aspects of Almond Skin

The primary goal of the sensory panels was to identify potential aroma and flavor descriptors for heat treated almond skins. Seven descriptors were developed (3 aroma and 4 flavor) and the intensity of each descriptor was determined (Figure 2.4). Preparation instructions for each of these descriptors can be found in Table 2.2. It is important to note that intensities were given in comparison to the reference standard. Ideally, the intensities of the reference standards would be known, but since they were not, the evaluation form (Figure 2.5) gave the intensity of each reference standard as 7.5 cm on a 15 cm scale. Panelists found "toasted" was the aroma descriptor most worthy of further investigation, while "toasted; nutty" was the least worth pursuing. For flavor descriptors "toasted; nutty" was rated most worthy of further investigation and "nutty; buttery" was the least worth pursuing. The most frequent comment made was that both aroma and flavor were very faint and thus hard to identify, especially in comparison to the strong aromas and flavors of the reference standards. Most panelists stated the almond skins smelled and tasted "woody" and "earthy" but not the same as any of the reference standards to which they were compared. It was suggested that identifying the correct "woody" and "earthy" standards would be beneficial. All comments from the survey can be found in Table B.2 in Appendix B. Complete preparation details and copies of the forms used can be found in Appendix A.

Figure 2.4: Intensities of Almond Skin Descriptors

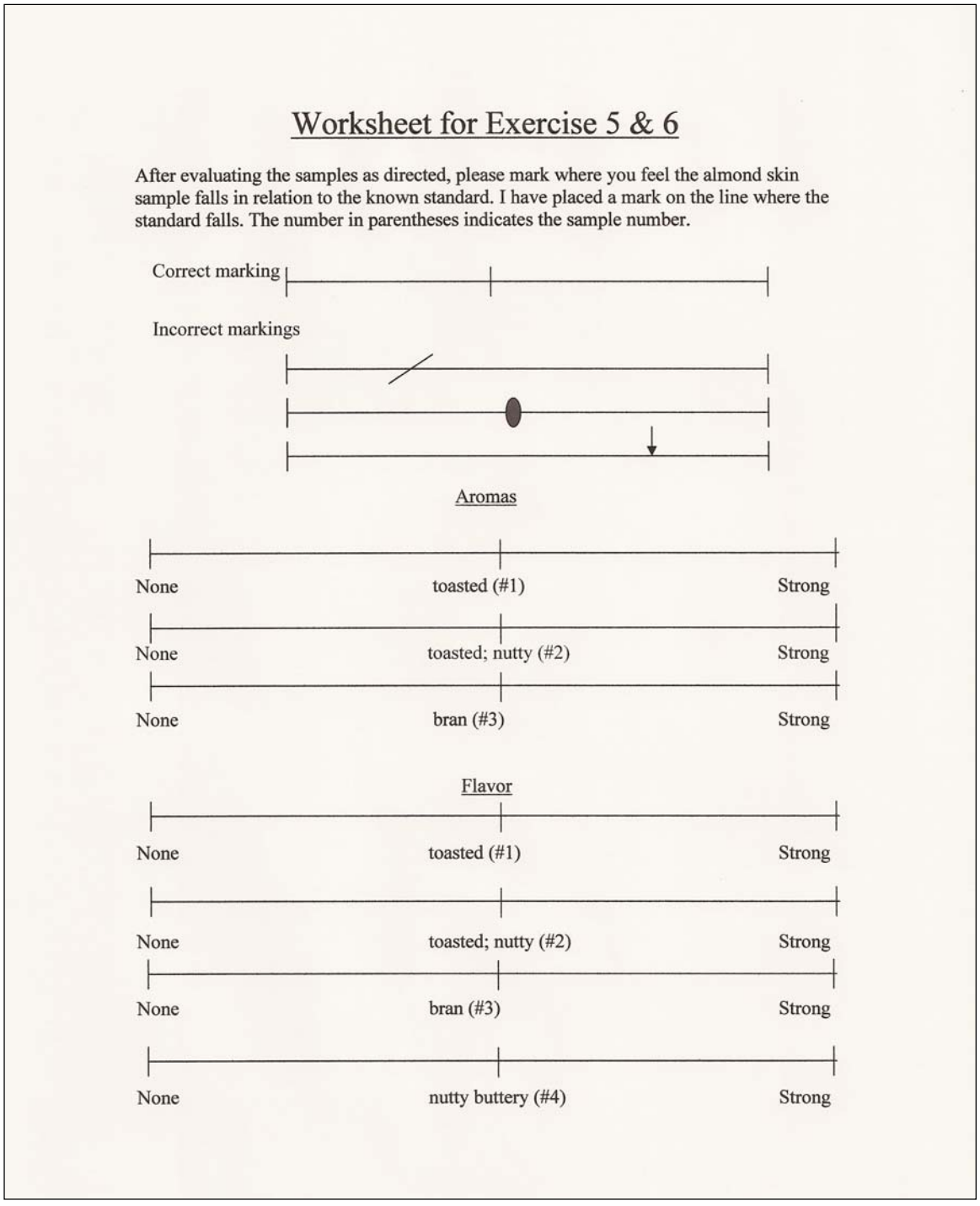


[1] markers show means; error bars mark a 95% confidence interval
[2] references for descriptors from Civille and Lyon (1996)

Table 2.2: Preparation Instructions for Descriptors

Descriptor	How prepared
Toasted	King Arthur All-Natural Traditional Whole Wheat Flour was used. 1 cup was placed in a 9x13 inch Pyrex pan and placed in a 350 ºF oven for 10 minutes; the flour was stirred half-way through. 3 grams was put into a 2 oz clear plastic cup with a lid.
toasted; nutty	5 g of Kretschmer® wheat germ was placed in a 2 oz clear plastic cup with a lid.
Bran	4 g of Bob's Red Mill Wheat Bran was placed in a 2 oz clear plastic cup with a lid.
nutty, buttery	2 tablespoons Land O Lakes unsalted butter and 1 cup Fisher pecan natural pieces were put in a 10 inch skillet and heated over medium-high heat (5-7 minutes or until all butter melted and nuts begin to smell toasted). 10 g was put into a 2 oz clear plastic cup with a lid.

Figure 2.5: Worksheet Used to Evaluate Intensities of Descriptors



Worksheet for Exercise 5 & 6

After evaluating the samples as directed, please mark where you feel the almond skin sample falls in relation to the known standard. I have placed a mark on the line where the standard falls. The number in parentheses indicates the sample number.

Correct marking

Incorrect markings

Aromas

None — toasted (#1) — Strong

None — toasted; nutty (#2) — Strong

None — bran (#3) — Strong

Flavor

None — toasted (#1) — Strong

None — toasted; nutty (#2) — Strong

None — bran (#3) — Strong

None — nutty buttery (#4) — Strong

The results from the sensory panel had great variation – most likely due to the mild flavor and aroma of the almond skin. Therefore, addition of almond skin to food products at low levels would most likely have little effect on the overall flavor and aroma. The effect of almond skin on texture and appearance, however, was not analyzed and would need to be investigated for the food product to which almond skins would be added. Also, this panel was performed using only Fritz variety almond skins. Each variety has its own unique characteristics and more varieties would need to be analyzed before a useful set of lexicon could be established. As with any ingredient, almond skins will most likely behave differently in different environments and therefore need to be tested in multiple mediums to determine in which they are most effective.

Proximate Analysis of Almond Skins

The USDA National Nutrient Database (2006) provides proximate analysis values for almonds with skin and blanched (without skin). Values for the almond skin alone were not found in the literature. Milbury et. al. (2006) stated that the skin compromises about 4.5% of the total weight of the almond, so that information was used to calculate expected values for almond skin proximate composition using the data from the USDA (Table 2.3). When expressed on a dry weight basis, the values for protein and ash content are close to those expected; however, the fat and carbohydrate values are very different. The expected values for fat and carbohydrate were 63.15% and 20% respectively (Table 2.3). The values achieved through proximate analysis in this research were 24.29% fat and 56.9% carbohydrate (Table 2.3). One potential reason for this great disparity is that

Table 2.3: Proximate Analysis of Almond Skins

	Percentage on Dry Weight Basis			
	almond with skin [1]	**blanched almond** [1]	**Expected skin only** [2]	**found skin only** [3]
fat	53.45	52.99	63.15	24.29
protein	22.44	22.97	11.22	12.51
ash	3.28	3.16	5.85	6.3
carbohydrate	20.83	20.87	20	56.9

[1] values for almonds, with skin and blanched, are from the USDA National Nutrient Database

[2] almond skin is 4.5% of the total weight of the almond; expected skin only values were calculated using the following formula: $C = (A - 0.955B)/0.045$ where A = value for almond with skin; B = value for blanched almond and C = value for expected skin only

[3] values are means of triplicates; moisture was 1.01%

the USDA gives few details on the samples it used for analysis. Varying processing conditions and varieties of almonds could also contribute to these differences. The brown skin, also called the seed coat, is meant to protect the almond from oxidation and microbial contamination (Frison and Sporns, 2002). Therefore, you would expect it to be high in carbohydrates not lipids. High lipid content would make the seed coat susceptible to oxidation, while carbohydrates would build a protective structure around the kernel.

Oxidative Stability Index (OSI)

The mean OSI value for the control soybean oil was 8.97 hours. This is 3 hours longer than the OSI value for the refined soybean oil De Mario et. al. (2000) found. This is most likely due to differences in the oils used and recent enhancements in oxidative stability of soybean oil. None of the treatments were significantly different (Table 2.4), which was surprising since there were differences in peroxide values. There are differing opinions on whether BHA is effective in vegetable oils. Also, BHA is volatile and thus not recommended for use in frying application; it is more commonly used in cereal and confectionary products (Eskin, 2000). While 110ºC is below frying temperatures, the BHA may have volatized in the Oxidative Stability Instrument.

The OSI measures the change in water conductivity, which is increased as organic acids are formed during oxidation, and is most likely not the best method of analysis for several reasons. Some of the almond skin settled to the bottom of the tube, potentially altering its antioxidant effect. Also, volatile antioxidant compounds could be lost at the high temperatures. This is likely the reason for the poor effectiveness of BHA in this

Table 2.4: OSI Values of Soybean Oil

Treatment	Mean (h)
Control	8.97 (0.5049)
2% almond skin	8.79 (0.5049)
6% almond skin	8.66 (0.5049)
10% almond skin	9.08 (0.5049)
200 ppm BHA	9.09 (0.5049)

[1] values are expressed as means with (standard error)
[2] OSI temperature was 110ºC
[3] none of the values are different as determined by LSD ($p>0.05$)

experiment. Also, almond skins may contain oxidized lipids before addition to the oil and since the almond skin is not purely oil other compounds not related to oxidation could volatilize as well, yielding a lower OSI value.

Peroxide Value

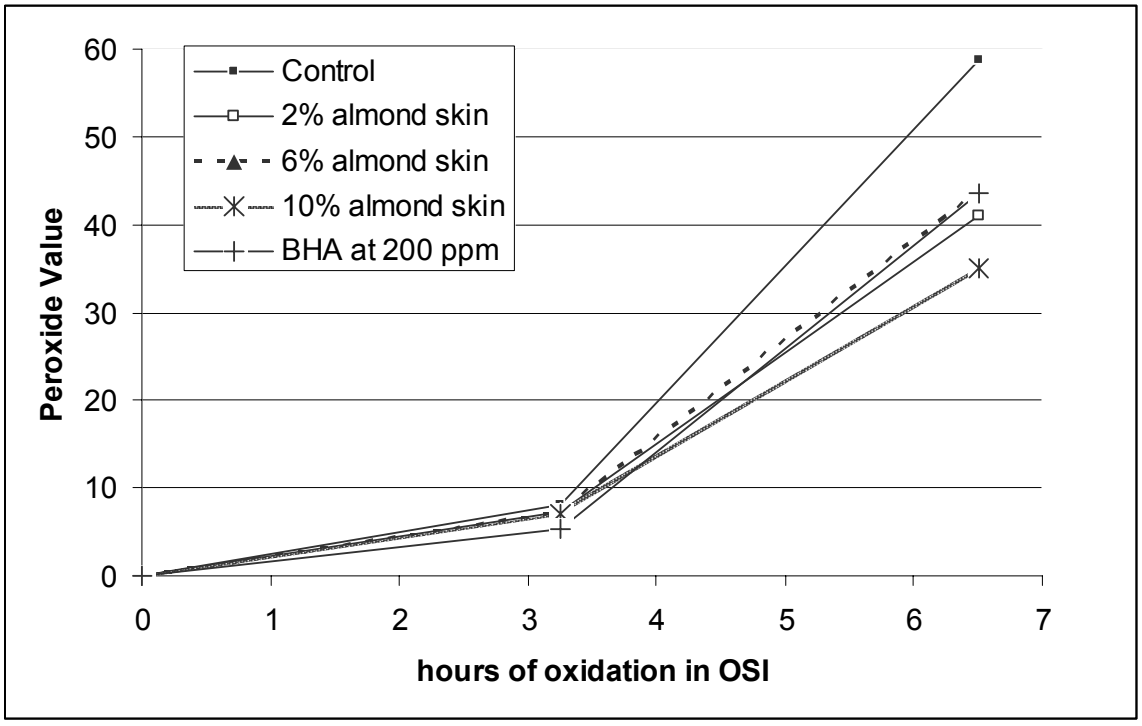
Peroxide values (PV) of 1 or less for soybean oil indicate freshness; 1-5 PV indicates low oxidation; 5-10 moderate oxidation; 10 or more high oxidation; and over 20 indicates poor flavor (O'Brien 2004). Based on peroxide value measures, all soybean oil samples were originally fresh, became moderately oxidized after 3.25 hours of oxidation in the OSI, and extremely oxidized after 6.5 hours of oxidation (Table 2.5 and Figure 2.6). BHA treated oil had lower peroxide values than the control at both times. At 3.25 hours, the 6% almond skin treated oil had a higher ($p > 0.05$) peroxide value than the BHA treated oil, but after 6.5 hours there was no difference between the BHA and almond skin treated oils. All treated samples had lower peroxide values than the control at 6.5 hours.

Table 2.5: Peroxide Values of Soybean Oil Oxidized in OSI

	Number of Hours in OSI at 110ºC		
Treatment	**0**	**3.25**	**6.5**
Control	0	8.0128 (0.7184)[a]	58.6286 (3.5124)[a]
2% almond skin	0	7.3028 (0.7184)[ab]	40.8949 (3.5124) [b]
6% almond skin	0	7.3475 (0.6558)[a]	43.7183 (3.7202) [b]
10% almond skin	0	6.9747 (0.6558)[ab]	35.0067 (3.7202) [b]
BHA at 200 ppm	0	5.3302 (0.6558)[b]	43.4847 (3.5124)[b]

[*] values are expressed as means with (standard error)
[a,b] values that share same lowercase letters within the same column are not different as determined by LSD ($p>0.05$)

Figure 2.6: Peroxide Values of Soybean Oil Oxidized in OSI

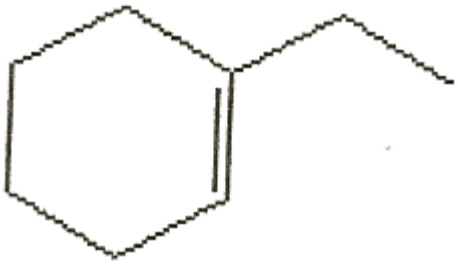


GC-MS Analysis of Soybean Oil

Aldehydes and particularly hexanal have been identified as key markers for oxidative degradation. There have also been good correlations between increasing hexanal concentrations and the sensory perception of rancid odor and flavors (Steele, 2000). Four compounds were identified and used as markers for oxidation in this research: 1-ethylcyclohexene, (E)-2-heptenal, 2-ethylfuran, and hexanal (see Figure 2.7). Retention times for these compounds were slightly different at 3.25 hours and 6.5 hours. As shown in Table 2.6, (E)-2-heptenal and 2-ethylfuran were not present until 6.5 hours of oxidation. Their retention times were 28.14 minutes and 13.02 minutes respectively (see Figure 2.9). The retention time of 1-ethylcyclohexene was 31.02 minutes at 3.25 hours and 31.09 minutes at 6.5 hours; hexanal was 19.26 minutes at 3.25 hours and 19.11 minutes at 6.5 hours (Figures 2.8 and 2.9). The concentrations of 1-ethylcyclohexene and hexanal were significantly greater at 6.5 hours than 3.25 hours. However, as shown in Table 2.6, only concentrations of hexanal at 3.25 hours were significantly different. There was no difference between the control and BHA, but each sample treated with almond skin had a significantly higher concentration of hexanal at the 3.25 hour sample period. This is potentially due to the hexanal present in the almond skins; however, if this was the only reason, hexanal should be present in the samples taken originally (at 0 hours) as well. The hexanal present in the 0 hour samples was probably at extremely low concentrations and therefore not detected. These levels were then supplemented by the formation of hexanal as the lipids in the almond skin oxidized. Differences in fatty acid profiles of the almond skin and soybean oil will lead to differences in the compounds

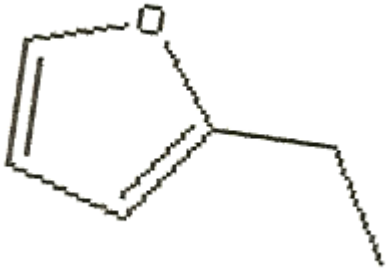
Figure 2.7: Structures of Compounds Identified via GC-MS

A



B.

C.



D.

A. 1-ethylcyclohexene
B. (E)-2-heptenal
C. 2-ethylfuran
D. hexanal

Table 2.6: GC-MS Analysis of Soybean Oil

Time in OSI (h)	Treatment	2-ethylfuran	Hexanal	(E)-2-heptenal	1-ethyl-cyclohexane
		Log of peak area*			
0	Control	0	0	0	0
	2% almond skin	0	0	0	0
	6% almond skin	0	0	0	0
	10% almond skin	0	0	0	0
	BHA at 200 ppm	0	0	0	0
3.25	Control	0	4.1607^{c} (0.0664)	0	4.0366^{b} (0.1479)
	2% almond skin	0	4.3789^{b} (0.0664)	0	4.1838^{b} (0.1479)
	6% almond skin	0	4.3281^{b} (0.0664)	0	4.3032^{b} (0.1479)
	10% almond skin	0	4.5247^{b} (0.0664)	0	4.4426^{b} (0.1479)
	BHA at 200 ppm	0	4.0592^{c} (0.0664)	0	4.3012^{b} (0.1479)
6.5	Control	5.065^{a} (0.2077)	6.1056^{a} (0.0664)	5.7048^{a} (0.0813)	4.5127^{a} (0.1479)
	2% almond skin	4.9999^{a} (0.2077)	6.0302^{a} (0.0664)	5.6522^{a} (0.0813)	4.6440^{a} (0.1479)
	6% almond skin	4.6804^{a} (0.2077)	5.9967^{a} (0.0664)	5.6119^{a} (0.0813)	4.7688^{a} (0.1479)
	10% almond skin	4.9799^{a} (0.2077)	6.0689^{a} (0.0664)	5.6700^{a} (0.0813)	4.7883^{a} (0.1479)
	BHA at 200 ppm	4.9441^{a} (0.2077)	5.9718^{a} (0.0664)	5.5539^{a} (0.0813)	4.8013^{a} (0.1479)

* values are expressed as means with (standard error)

[a-c] values that share same lowercase letters within the same column are not different as determined by LSD ($p>0.05$)

Figure 2.8: GC-MS Chromatogram of Soybean Oil Treated with 2% Almond Skin and Oxidized for 3.25 hours in the OSI

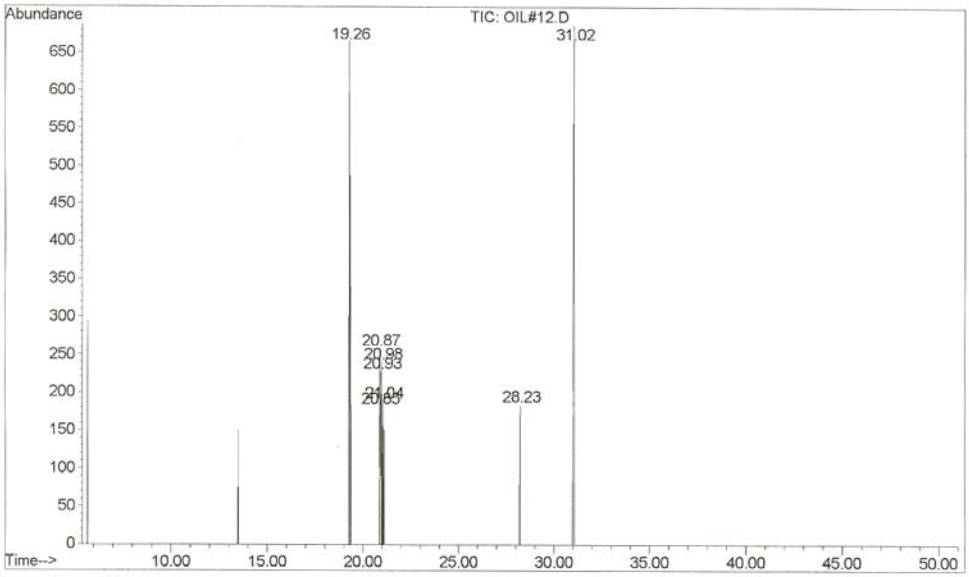
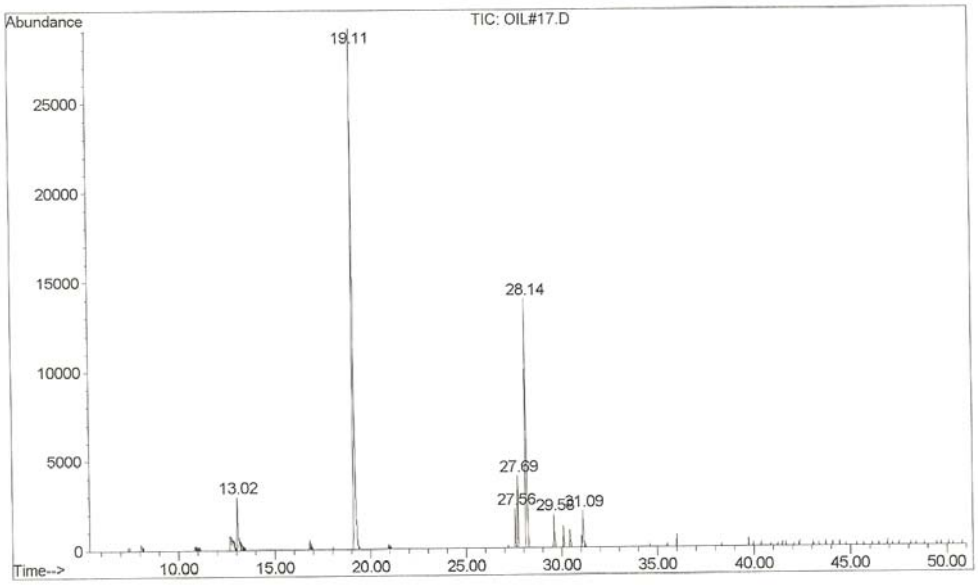


Figure 2.9: GC-MS Chromatogram of Soybean Oil Treated with 2% Almond Skin and Oxidized for 6.5 hours in the OSI



formed during oxidation. The hexanal concentration seems to level out after 6.5 hours of oxidation as smaller molecular weight compounds (ex: 2-ethylfuran) are formed.

Conclusions

Before almond skins can become a viable option for use as an antioxidant in foods, better control of their microbial quality must be developed. They are currently a byproduct and based on microbiological data collected in this study new handling procedures would need to be created. Elimination of the need for additional heat treatment would likely allow for the identification of new sensory descriptors. The aromatic compounds found in the almond skins subjected only to the blanching process contained more aromatics than the almond skins subjected to additional heat treatment. This could also allow for correlation between compounds found via GC-MS headspace analysis and sensory panel results. The primary comment from the sensory panelists was that the flavor and aroma of the skin was very mild, so future sensory work should focus on how the addition of almond skins to a food product affects its texture, aroma, flavor, and appearance. The variety (or varieties) used should always be noted, since it is known that nuances exist within varieties.

While the OSI did not show differences between treated and control oil samples, peroxide value and GC-MS analysis showed potential for use of almond skins as antioxidants in foods. Peroxide values remain good indicators of oxidation and should be taken at more intervals to allow for better detection of differences between treatments. Normally, hexanal is a good indicator of oxidation; however, the almond skins contained hexanal and it is not known if this is because the lipids in the almond skin have already

begun to undergo oxidation or if hexanal is simply inherent to almond skin or both. Analysis of more varieties and samples of almond skins should be done to determine good markers of oxidation. Also, it is known that the variety used (Fritz) is much lower in flavonol glycoside content than most varieties (Frison and Sporns 2002). The total phenolic content of the almond skins was also lower than that discovered by Wijeratne, et. al. (2006). Use of varieties of almond skins known to be high in phenolic content should be further explored before eliminating almond skins as a natural antioxidant source for foods.

Acknowledgements

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Literature Cited

Acree, T, Arn, H. 2004 [cited 2007 June 11]. Flavornet [Internet]. Available from: http://www.flavornet.org/flavornet.html

[AOAC]. 1995. Protein (Crude) in Nuts and Nut Products. 950.48, Sec. 40.1.06. In: Sanders, TH, editor. Official Methods of Analysis of AOAC Int. Gaithersburg, MD: AOAC Int. 3 p.

Becton Dickinson and Company. 1999 [cited 2007 June 12]. BBL™ Prepared Plated Medium for Selective Isolation of *Salmonella* [Technical Information Sheet #88-0622-1]. Sparks, MD: Becton Dickinson Microbiology Systems.

Civille, GV, Lyon, BG, editors. 1996. Aroma & Flavor Lexicon for Sensory Evaluation: Terms, Definitions, References, & Examples. West Conshohocken, PA: ASTM Data Series Publication. 158 p.

De Maria, CAB, Santos, MCM. 2000. Stabilization of Soybean Oil with heated quercetin and 5-Caffeoylquinic acid in presence of ferric ion. J. Agric. Food Chem. 48 (9): 3935-3938.

Eskin, NA, Robinson, DS, editors. 2000. Food Shelf Life Stability: chemical, biochemical, and microbiological changes. Boca Raton, FL: CRC Press LLC. 370 p.

Frison, S, Sporns, P. 2002. Variation in the Flavonol Glycoside Composition of Almond Seedcoats as Determined by MALDI-TOF Mass Spectrometry. J. Agric. Food Chem. 50 (23): 6818 – 6822.

Huang, G (The Almond Board of California, Modesto, CA). RE: a few questions [electronic mail on the Internet]. Message to: Heather Johnson (Clemson University, Clemson, SC). 2007 Jan 29, 11:45 am [cited 2007 April 29]. [two paragraphs].

Milbury, PE, Chen, C, Dolnikowski, GG, Blumberg, JB. 2006. Determination of Flavonoids and Phenolics and Their Distribution in Almonds. J. Agric. Food Chem. 54 (14): 5027 – 5033.

O' Brien, RD. 2004. Soybean Oil. In: Fats and Oils: formulating and processing for applications, 2nd Ed. Boca Raton, FL: CRC Press LLC. 592 p.

Sang, S, Lapsley, K, Woo-Sik, J, Lachance, P, Chi-Tang, H, Rosen, RT. 2002. Antioxidative Phenolic Compounds Isolated from Almond Skins (Prunus amygdalus Batsch). J. Agric. Food Chem. 50 (8): 2459 – 2463.

Steele, R, editors. 2000. Understanding and measuring the shelf-life of food. Boca Raton, FL: CRC Press LLC. 406 p.

USDA. 2006 [cited 2007 April 12]. USDA National Nutrient Database for Standard Reference, Release 19, Nuts, almonds [Internet]. Available from: http://riley.nal.usda.gov/NDL/cgi-bin/list_nut_edit.pl

USDA. 2006 [cited 2007 April 12]. USDA National Nutrient Database for Standard Reference, Release 19, Nuts, almonds, blanched [Internet]. Available from: http://riley.nal.usda.gov/NDL/cgi-bin/measure.pl

Velioglu, YS, Mazza, G, Gao, L, Oomah, BD. 1998. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. J. Agric. Food Chem 46 (10): 4113 -411 7.

Wijeratne, S, Abou-Zaid, M, Shahidi, F. 2006. Antioxidant Polyphenols in Almond and Its Coproducts. J. Agric. Food Chem. 54 (2): 312 – 318.

CONCLUSIONS

Before almond skins can become a viable option for use as an antioxidant in foods, better control of their microbial quality must be developed. They are currently a byproduct and based on microbiological data collected in this study new handling procedures would need to be created. Elimination of the need for additional heat treatment would likely allow for the identification of new sensory descriptors. The aromatic compounds found in the almond skins subjected only to the blanching process contained more aromatics than the almond skins subjected to additional heat treatment. This could also allow for correlation between compounds found via GC-MS headspace analysis and sensory panel results. The primary comment from the sensory panelists was that the flavor and aroma of the skin was very mild, so future sensory work should focus on how the addition of almond skins to a food product affects its texture, aroma, flavor, and appearance. The variety (or varieties) used should always be noted, since it is known that nuances exist within varieties.

While the OSI did not show differences between treated and control oil samples, peroxide value and GC-MS analysis showed potential for use of almond skins as antioxidants in foods. Peroxide values remain good indicators of oxidation and should be taken at more intervals to allow for better detection of differences between treatments. Normally, hexanal is a good indicator of oxidation; however, the almond skins contained hexanal and it is not known if this is because the lipids in the almond skin have already begun to undergo oxidation or if hexanal is simply inherent to almond skin or both. Analysis of more varieties and samples of almond skins should be done to determine

good markers of oxidation. Also, it is known that the variety used (Fritz) is much lower in flavonol glycoside content than most varieties (Frison and Sporns 2002). The total phenolic content of the almond skins was also lower than that discovered by Wijeratne, et. al. (2006). Use of varieties of almond skins known to be high in phenolic content should be further explored before eliminating almond skins as a natural antioxidant source for foods.

APPENDICES

Appendix A

Sensory Panel Forms and Instructions

Information Concerning Participation in a Research Study
Clemson University

Developing a Sensory Lexicon for Almond Skins

You are invited to participate in a research study conducted by Dr. Paul Dawson and Heather Johnson. The purpose of this research is to begin developing a sensory lexicon (descriptors) for almond skins.

Your participation will involve tasting, smelling, and evaluating almond skins. You will also smell other products (ex: vanilla, oil, nuts) to aid in your evaluation of the almond skin.

The amount of time required for your participation will be approximately 2 hours a week for 3 weeks (a total of 6 hours). One hour sessions will be held twice weekly. Participants that complete all 6 hours will be awarded a $30 gift card (to the location of your choice) as a token of our appreciation.

Risks and discomforts
People with nut allergies should not participate in this research. There are no known risks to people without nut allergies.

Potential benefits
There are no known benefits to you that would result from your participation in this research. This research may help us to better understand the characteristics of almond skins.

Protection of confidentiality
We will do everything we can to protect your privacy. You will not be asked to identify yourself on any forms other than the allergen form. Your identity will not be revealed in any publication that might result from this study.

Voluntary participation
Your participation in this research study is voluntary. You may choose not to participate and you may withdraw your consent to participate at any time. You will not be penalized in any way should you decide not to participate or to withdraw from this study.

Contact information
If you have any questions or concerns about this study or if any problems arise, please contact Dr. Paul Dawson at Clemson University at 864-656-1138. If you have any questions or concerns about your rights as a research participant, please contact the Clemson University Office of Research Compliance at 864.656.6460.

Developing a Sensory Lexicon for Almond Skins
Session 1 – Agenda

1. Introduction
    a. Thanks for agreeing to assist with this research project. I'm Heather Johnson and I'll be the moderator for today's session. Today's exercise will involve smelling and tasting almond skins. You will be presented with one sample. We will first discuss how to evaluate the sample. Then you will be given blank sheets of paper to record descriptors on. Aroma will be evaluated first, followed by flavor. When evaluating flavor, you may find that the sample is very dry; water is provided.
2. Sample – how to evaluate
    a. Please smell the samples before tasting them. Shake the container and then half-way open the lid to sniff. Repeat if necessary. Please record aroma descriptors below. Once finished recording all aroma descriptors, please taste the sample. Use the spoon provided to place a small amount in your mouth. Please record flavor descriptors below. The sample will be dry, so water is provided; however, do not record flavors you get when combining water and sample.
    b. It is important that everyone follow these procedures each and every time to ensure consistency in results.
3. Sample again – actual evaluation
    a. Panelist record descriptors on paper. When they appear to be done or after 25 minutes the moderator will instruct them to stop.
4. Discussion of descriptors
    a. The moderator will lead a discussion of the descriptors everyone wrote down.
5. Conclusion
    a. Thanks so much for your time today. I hope to see you at our next session. Please give me your sheets before you leave.

Developing a Sensory Lexicon for Almond Skins
Session 2 – Agenda

1. Introduction
    a. Thanks for coming today. I'm Heather Johnson and I'll be the moderator for today's session. Today's exercise will involve smelling and tasting almond skins as we did last session. We will also categorize all the descriptors you come up with. You will be presented with one sample. Please remember to evaluate them the same way as before. You will be given blank sheets of paper to record descriptors on. Aroma will be evaluated first, followed by flavor. When evaluating flavor, you may find that the sample is very dry; water is provided.
2. Sample– actual evaluation
    a. Panelist record descriptors on paper. When they appear to be done or after 25 minutes the moderator will instruct them to stop.
3. Discussion of descriptors
    a. The moderator will lead a discussion of the descriptors everyone wrote down.
4. Categorization
    a. Moderator will lead categorization of descriptors.
5. Conclusion
    a. Thanks so much for your time today. I hope to see you at our next session. Please give me your sheets before you leave.

Developing a Sensory Lexicon for Almond Skins
Session 3 – Agenda

1. Introduction
    a. Thanks for coming today. I'm Heather Johnson and I'll be the moderator for today's session. Today we will compare the sample to known standards. The known standards presented today are based on your responses from the last exercise. As before, please analyze the standards in the exact same manner as you analyze the sample. I will bring out one standard at a time. You have a sheet in front of you that lists each standard; please check if the same flavor is present in the sample. We will be evaluating aroma and flavor descriptors today. Aroma will be done first, followed by flavor. Water is provided. Please use them to cleanse your palette before evaluating the flavor of each sample.
2. Comparison of Known Standards to Sample
    a. Moderator will bring out one standard at a time as needed.
3. Conclusion
    a. Thanks so much for your time today. I hope to see you at our next session.

Worksheet for Session 3 – Flavor

Please check "yes" if the known descriptor is in the almond skin or "no" if the known descriptor is not in the almond skin.

	Same flavor present in almond skin?	
Known Descriptor	**Yes**	**No**
1. toasted #1		
2. toasted #2; nutty #1		
3. bran		
4. cardboard #2		
5. buttery		
6. oil		
7. oxidized/rancid	Do not taste	Do not taste
8. woody #1	Do not taste	Do not taste
9. woody #2	Do not taste	Do not taste
10. nutty buttery		
11. earthy #1		
12. earthy #2	Do not taste	Do not taste
13. grainy		
14. grain, processed		
15. bran		
16. almond		

Comments:

Worksheet for Session 3 – Aroma

Please check "yes" if the known descriptor is in the almond skin or "no" if the known descriptor is not in the almond skin.

	Same aroma present in almond skin?	
Known Descriptor	**Yes**	**No**
1. toasted #1		
2. toasted #2; nutty #1		
3. bran		
4. cardboard #2		
5. buttery		
6. oil		
7. oxidized/rancid		
8. woody #1		
9. woody #2		
10. nutty buttery		
11. earthy #1		
12. earthy #2		
13. grainy		
14. grain, processed		
15. bran		
16. almond		

Comments:

Developing a Sensory Lexicon for Almond Skins
Session 4 – Agenda

4. Introduction
    a. Thanks for coming today. I'm Heather Johnson and I'll be the moderator for today's session. Today we will compare the sample to known standards. The known standards presented today are based on your responses from the last exercise. As before, please analyze the standards in the exact same manner as you analyze the sample. I will bring out one standard at a time. You have a sheet in front of you that lists each standard; please check if the same flavor is present in the sample. We will be evaluating aroma and flavor descriptors today. Aroma will be done first, followed by flavor. Water is provided. Please use them to cleanse your palette before evaluating the flavor of each sample.
5. Comparison of Known Standards to Sample
    a. Moderator will bring out one standard at a time as needed.
6. Conclusion
    a. Thanks so much for your time today. I hope to see you at our next session.

Worksheet for Session 4 – Flavor

Please check "yes" if the known descriptor is in the almond skin or "no" if the known descriptor is not in the almond skin.

	Same flavor present in almond skin?	
Known Descriptor	**Yes**	**No**
1. toasted		
2. toasted; nutty		
3. bran		
4. bran #2		
5. oil	Do not taste	Do not taste
6. oxidized/rancid	Do not taste	Do not taste
7. oxidized/rancid		
8. woody	Do not taste	Do not taste
9. earthy		
10. bran #3		
11. almond		
12. cardboard	Do not taste	Do not taste
13. grain, processed		
14. bitter		

Comments:

Worksheet for Session 4 – Aroma

Please check "yes" if the known descriptor is in the almond skin or "no" if the known descriptor is not in the almond skin.

	Same aroma present in almond skin?	
Known Descriptor	**Yes**	**No**
1. toasted		
2. toasted; nutty		
3. bran		
4. bran #2		
5. oil		
6. oxidized/rancid		
7. oxidized/rancid		
8. woody		
9. earthy		
10. bran #3		
11. almond		
12. cardboard		
13. grain, processed		

Comments:

Developing a Sensory Lexicon for Almond Skins
Session 5 – Agenda

1. Introduction
    a. Thanks for coming today. I'm Heather Johnson and I'll be the moderator for today's session. Today we will compare the sample to known standards and assign intensities to them. As before, please analyze the standards in the exact same manner as you analyze the sample. I will bring out one standard at a time. You have a sheet in front of you that lists each standard and its intensity; please make a mark on the line where you feel the almond skin falls in comparison with the standard. We will be evaluating aroma and flavor descriptors today. Aroma will be done first, followed by flavor. Water is provided. Please use it to cleanse your palette before evaluating the flavor of each sample.
2. Comparison of Known Standards to Sample
    a. Moderator will bring out one standard at a time as needed.
3. Conclusion
    a. Thanks so much for your time today. This was our last session. I truly appreciate your help.

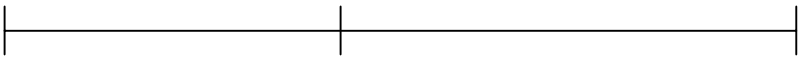
Developing a Sensory Lexicon for Almond Skins
Session 6 – Agenda

1. Introduction
    a. Thanks for coming today. I'm Heather Johnson and I'll be the moderator for today's session. Today we will compare the sample to known standards and assign intensities to them. As before, please analyze the standards in the exact same manner as you analyze the sample. I will bring out one standard at a time. You have a sheet in front of you that lists each standard and its intensity; please make a mark on the line where you feel the almond skin falls in comparison with the standard. We will be evaluating aroma and flavor descriptors today. Aroma will be done first, followed by flavor. Water is provided. Please use it to cleanse your palette before evaluating the flavor of each sample. After this is completed I have a brief survey for you to complete.
2. Comparison of Known Standards to Sample
    a. Moderator will bring out one standard at a time as needed.
3. Conclusion
    a. Thanks so much for your time today. This was our last session. I truly appreciate your help.

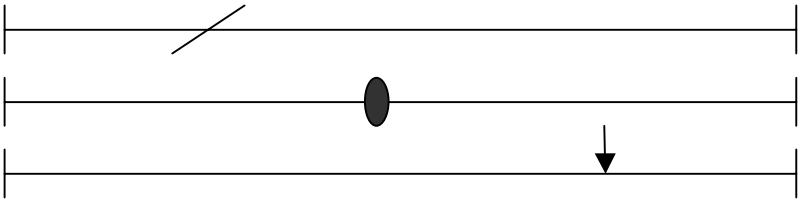
Worksheet for Exercise 5 & 6

After evaluating the samples as directed, please mark where you feel the almond skin sample falls in relation to the known standard. I have placed a mark on the line where the standard falls. The number in parentheses indicates the sample number.

Correct marking



Incorrect markings



Aromas

None — toasted (#1) — Strong

None — toasted; nutty (#2) — Strong

None — bran (#3) — Strong

Flavor

None — toasted (#1) — Strong

None — toasted; nutty (#2) — Strong

None — bran (#3) — Strong

None — nutty buttery (#4) — Strong

Survey for Exercise 6

The following survey is to collect your thoughts on a few topics. The information you provide will help future researchers determine areas that are worth pursuing further.

1. Of all the aroma descriptors developed in this panel, which do you feel (if any) are **most** worth further research and investigation?

2. Of all the aroma descriptors developed in this panel, which do you feel (if any) are **least** worth further research and investigation?

3. Of all the flavor descriptors developed in this panel, which do you feel (if any) are **most** worth further research and investigation?

4. Of all the flavor descriptors developed in this panel, which do you feel (if any) are **least** worth further research and investigation?

5. If this same project were to be repeated, which improvements would you suggest?

Session 3

Descriptor	How prepared
1. toasted #1	King Arthur All-Natural Traditional Whole Wheat Flour was used. 1 cup was placed in a 9x13 inch Pyrex pan and placed in a 350ºF oven for 10 minutes; the flour was stirred half-way through. 5 grams was put into a 2 oz clear plastic cup with a lid.
2. toasted #2; nutty #1	10 g of Kretschmer® wheat germ was placed in a 2 oz clear plastic cup with a lid.
3. bran	7 g of Bob's Red Mill Wheat Bran was placed in a 2 oz clear plastic cup with a lid.
4. Cardboard	5 g of Carnation instant nonfat dry milk was placed in a 2 oz clear plastic cup with a lid.
5. buttery	1 tablespoon of Land O Lakes unsalted butter was placed in a 2 oz clear plastic cup with a lid
6. oil	10 g of Wesson brand soybean oil was placed in a 2 oz clear plastic cup with a lid
7. woody #1	Forster brand mini jumbo craft sticks were broken to fit in a 2 oz clear plastic cup with a lid. Each cup contained one craft stick.
8. woody #2	Hines roasted jumbo Virginia peanuts in shell were put in a 2 oz clear plastic cup with lid. Each cup contained 2 nuts.
9. nutty, buttery	2 tablespoons Land O Lakes unsalted butter and 1 cup Fisher pecan natural pieces were put in a 10 inch skillet and heated over medium-high heat (5-7 minutes or until all butter melted and nuts begin to smell toasted). 10 g was put into a 2 oz clear plastic cup with a lid.
10. earthy #1	ToJo brand button mushrooms were sliced and 3 slices were put into a 2 oz clear plastic cup with a lid.
11. earthy #2	2 oz plastic cups were filled half way full with generic potting soil and lids were put on.
12. grainy	Frito Lay "Fritos" corn chips were crushed and 10 g were put into a 2 oz clear plastic cup with a lid.
13. grain, processed	Equal parts of Cheerios, Post Shredded Wheat Spoon size, and Kellogg's Corn Flakes were crushed. 10 g were put into a 2 oz clear plastic cup with a lid.
14. bran	10 g of Hodgson Mill rye flour was put into a 2 oz clear plastic cup with a lid.
15. almond	1 cup of Fisher natural almond slices were put in a 10 inch skillet and heated over medium-high heat for about 5-7 minutes or until they were lightly browned and had a toasted, nutty aroma. 6 g were put into a 2 oz clear plastic cup with a lid.

Session 4

Descriptor	How prepared
1. toasted	King Arthur All-Natural Traditional Whole Wheat Flour was used. 1 cup was placed in a 9x13 inch Pyrex pan and placed in a 350 ºF oven for 10 minutes; the flour was stirred half-way through. 3 grams was put into a 2 oz clear plastic cup with a lid.
2. toasted; nutty	5 g of Kretschmer® wheat germ was placed in a 2 oz clear plastic cup with a lid.
3. bran	4 g of Bob's Red Mill Wheat Bran was placed in a 2 oz clear plastic cup with a lid.
4. bran #2	4 g of Kellogg's All-Bran Original cereal was placed in a 2 oz clear plastic cup with a lid.
5. oil	10 g of Wesson brand soybean oil was placed in a 2 oz clear plastic cup with a lid
6. oxidized/rancid	Wesson canola oil that had been opened on 10/10/05 and had a best by date of 1/20/07 was used. It clearly exhibited an oxidized odor. 10 g was placed into a 2 oz clear plastic cup with a lid.
7. oxidized/rancid	Great Value Brand ripple cut potato chips were crunched up and allowed to sit in their open bag on the counter for 5 days. An oxidized aroma developed. 2.5 g of crushed chips were put into 2 oz clear plastic cups with lids.
8. woody	3 g of Kingsford hickory wood chips were put in a 2 oz clear plastic cup with a lid.
9. earthy	ToJo brand button mushrooms were used. 1.5 g or about ¼ of a baby button mushroom was put into a 2 oz clear plastic cup with a lid.
10. bran #3	7 g of Hodgson Mill rye flour was put into a 2 oz clear plastic cup with a lid
11. almond	1 cup of Fisher natural almond slices were put in a 10 inch skillet and heated over medium-high heat for about 5-7 minutes or until they were lightly browned and had a toasted, nutty aroma. 3.5 g were put into a 2 oz clear plastic cup with a lid.
12. cardboard	1 g of clean cardboard and 3 g of water were combined in a 2 oz clear plastic cup with a lid.
13. grain, processed	Equal parts of Cheerios, Post Shredded Wheat Spoon size, and Kellogg's Corn Flakes were crushed. 2 g were put into a 2 oz clear plastic cup with a lid.
14. bitter	Approximately 28 g of Polar brand tonic water was put into a 2 oz clear plastic cup with a lid.

Sessions 5 & 6

Descriptor	How prepared
toasted	King Arthur All-Natural Traditional Whole Wheat Flour was used. 1 cup was placed in a 9x13 inch Pyrex pan and placed in a 350 ºF oven for 10 minutes; the flour was stirred half-way through. 3 grams was put into a 2 oz clear plastic cup with a lid.
toasted; nutty	5 g of Kretschmer® wheat germ was placed in a 2 oz clear plastic cup with a lid.
bran	4 g of Bob's Red Mill Wheat Bran was placed in a 2 oz clear plastic cup with a lid.
nutty, buttery	2 tablespoons Land O Lakes unsalted butter and 1 cup Fisher pecan natural pieces were put in a 10 inch skillet and heated over medium-high heat (5-7 minutes or until all butter melted and nuts begin to smell toasted). 10 g was put into a 2 oz clear plastic cup with a lid.

Appendix B

Tables and Figures

Table B.1: Sensory Survey Responses to Questions 1-4

	Question Number			
Answer	**1**	**2**	**3**	**4**
Toasted	62.5% (5)	12.5% (1)	25.0% (2)	25.0% (2)
Bran	25.0% (2)	12.5% (1)	12.5% (1)	0
Toasted; nutty	12.5% (1)	37.5% (3)	37.5% (3)	12.5% (1)
Nutty; buttery	0	0	25.0% (2)	37.5% (3)
Earthy (mushroom)	0	0	0	12.5% (1)

* Question 1: Of all the aroma descriptors developed in this panel, which do you feel (if any) are most worth further research and investigation?
Question 2: Of all the aroma descriptors developed in this panel, which do you feel (if any) are least worth further research and investigation?
Question 3: Of all the flavor descriptors developed in this panel, which do you feel (if any) are most worth further research and investigation?
Question 4: Of all the flavor descriptors developed in this panel, which do you feel (if any) are least worth further research and investigation?
** values are expressed as percentage of panelists that gave answer and (actual number of panelists that gave answer); 8 total panelists

Table B.2: Sensory Survey Responses to Question 5

Responses to Question 5: If this same project were to be repeated, which improvements would you suggest?
1. Either use more almond skins and have them in different containers so we could evaluate aroma better.
2. Would it be possible to extract oil from the skins? That might have more (stronger) aroma and/or flavor.
3. First, I would like to say that the flavor descriptors were the closest to matching the almond skins. I liked how it was done, I thought we really narrowed it down on our descriptors
4. More almond skin samples to smell because after a while the aroma is hard to smell because you have to compare it to so many other samples.
5. It went very well - I still wish you could get tree bark.

Figure B.1: Quercetin Standard Curve for Determination of Total Phenolic Content

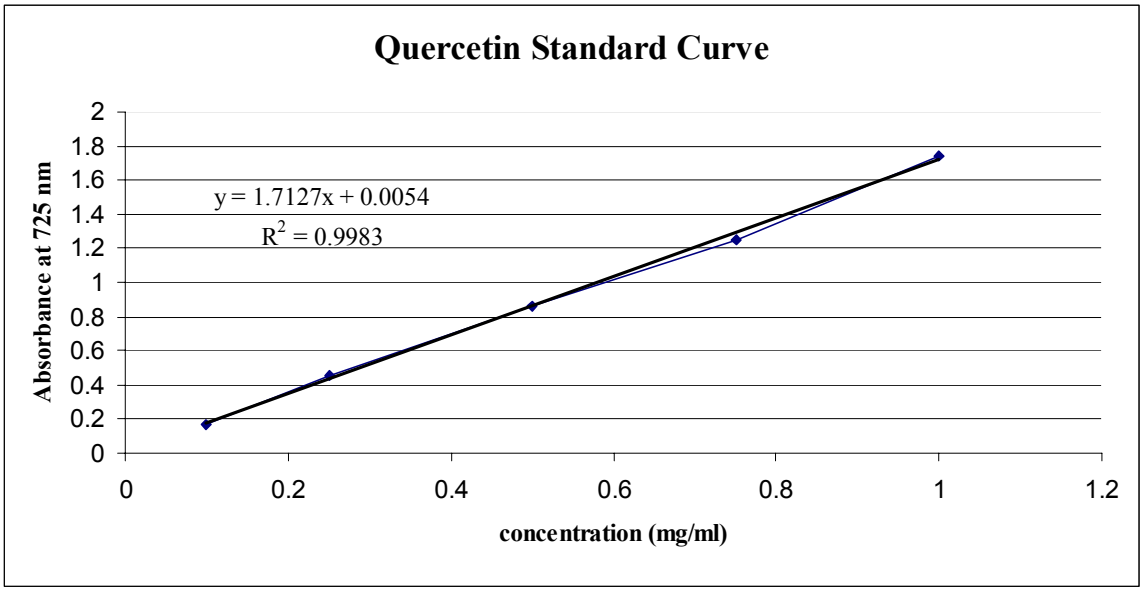


Table B.3: Determination of Total Phenolic Content of Almond Skin

	absorbance at 725 nm				quercetin equiv (mg/ml)	quercetin equiv/mg skin	quercetin equiv/g skin
sample	abs 1	abs 2	abs 3	Mean abs			
heated 1	0.530	0.571	0.561	0.554	0.320	0.006	6.406
heated 2	0.491	0.513	0.512	0.505	0.292	0.006	5.838
original 1	0.400	0.419	0.417	0.412	0.237	0.005	4.748
original 2	0.461	0.494	0.495	0.483	0.279	0.006	5.581

Table B.4: Microbial Analysis Results of Almond Skin Using Brilliant Green Agar

Time at 121ºC	Dilution	Total cfu[1]
0 min	10^{-1}	Tntc[2]
	10^{-2}	0[3]
	10^{-3}	No growth
60 minutes	10^{-1}	0
	10^{-2}	No growth

[1] only colonies indicative of *Salmonella* species
[2] plates had too many colonies to count; however, most did not appear to be indicative of *Salmonella* species
[3] colonies were present, but none indicative of *Salmonella* species