CHARACTERIZATION OF ACORN MEAL

by

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(Under the Direction of Ruthann Swanson)

ABSTRACT

Acorns have had a role in human diet where ever oak trees have grown. A method was developed to prepare acorn meal for testing physiochemical properties and use as wheat flour replacement in foods. Spice cookies and pumpkin muffins where made from the acorn meal. Total phenolics of acorn meals range from about 39 mg GAE/g in red oak acorns to 6.6 mg GAE/g in white oak acorn. Red oak acorn meal was best suited for replacing all-purpose wheat flour. 50% replacement was performed for the cookies. Acorn meal cookies had some differences in texture, appearance, and flavor, but a consumer sensory panel (n=128) found the cookies to be overall acceptable. 25% replacement of wheat flour in muffins had no effect or a beneficial effect on most instrumental tests associated with organoleptic properties. Panelists expressed interest in cookies that carried an antioxidant claim.

INDEX WORDS: acorn, phenolic, consumer panel, cookies

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1. Introduction

New and exotic super foods commonly appear as functional ingredients. Although acorns have had an important role in the human diet for thousands of years where-ever the oak tree has grown, they are not widely used today as food or food ingredients despite extensive availability. Historically, many Native American cultures used acorns as a main staple. In many European hunter-gatherer societies, acorns were also used as food. Acorns are still used in many modern Asian cultures. As a food source, acorns are calorie dense because of high levels of fat, they are a good source of protein, and are high in phenolic compounds. Differences in composition due to acorn species have been reported (Bainbridge, 1986; Bettinger et al., 1997).

One of the health benefits of consuming acorns is the high levels of phenolic compounds present (Cantos et al., 2003; Kobs, 2008). Phenolics may play a part in reducing risks or symptoms for cardiovascular disease (CVD), cancer, HIV, microbial infection, diabetes, and inflammatory diseases (Gonzalez de Mejia, et al., 1999; Halliwell et al., 2005; Jiang & Dusting, 2003; Kahkonen et al., 1999; Kruk et al., 2005; Lee, et al, 2005; Ullah & Khan, 2008). CVD prevention can be linked to long-term consumption of fruits and vegetables which contain compounds with antioxidant activity and hypolipidimic activity such as dietary phenolics (Jiang & Dusting, 2003). Reduced risk of cancers can be linked to phenolic compounds' influence on several biological mechanisms. Phenolic compounds are important in humans' diet to reduce reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by normal biochemical functions in the body and by environmental factors. Both have been linked to chronic diseases (Halliwell et al., 2005; Kahkonen et al., 1999; Kruk et al., 2005). Phenolics also

can act as pro-oxidants to regenerate vitamins C and E. The phenolic compounds and reduced compounds may activate or limit gene expression of pathways that influence the risk for disease and they may produce natural antioxidant enzymes (Yeh, et al, 2009). Phenolics once were considered anti-nutrients due to the protein binding properties of the compound; however, phenolic protein binding to signaling pathways and to DNA may be the reason for some of the health benefits seen from some phenolic compounds (Chen et al., 2007; Fang, et al, 2007; Faried et al., 2007; He, et al, 2008; Neto, et al, 2008; Wang, et al, 2008; Yeh et al., 2009).

The levels of phenolic content in acorn nut meats range from about 14.3 mg/g GAE in Overcup oak, a white oak species, to 107 mg/g GAE in Laurel oak, a red oak species; generally red oak species tend to be higher in total phenolics than are the white oak species (Kobs, 2008). Typically, the raw acorns are leached and processed to remove tannins and make the meal more palatable. Tannins are associated with bitterness, which many consumers find objectionable. The leaching process reduces the total phenolics as well as the tannins present. Despite this reduction, leached acorns remain a good source of phenols with levels equal to or exceeding those found in hazelnuts, almonds, and peanuts (Kobs, 2008). The total phenolics of leached and processed acorn meals range from about 39 mg GAE/g in black oak acorns (*Quercus velutina)* to 6.6 mg GAE/g in white oak acorn (*Quercus alba*). The food industry has traditionally removed phenols from various foods to increase their palatability to consumers; however, in recent years phenols have been added or not removed from foods to increase the nutritional functionality of foods. Estimated intake of phenolics among American consumers is 1000 mg/ day, with coffee and tea as the major dietary sources (Kobs, 2008; Scalbert, et al, 2005).

When introducing a novel flour, the flour's physical, chemical and sensory properties need to be evaluated. Several novel flours have been investigated in the last decade (Chinma &

Gernah, 2007; Sindhuja, et al, 2005). A proximate analysis is used to determine nutritive value and to predict functionality in a food system. Physical and chemical characteristics such as color, particle size, damaged and undamaged starch content, functional proteins, pH, and phenolic content can be evaluated and used to determine quality and predict functionality in various food systems. The novel flour is then evaluated in a food system like cookies, cakes, or breads to determine its functional performance. The food produced, if successfully made, is evaluated by a sensory panel. The data are then used to generate a characteristic profile of the novel flour for immediate industry use or future evaluation.

Acorn meal could be a nutritionally functional ingredient in foods that use wheat flour. Functional foods are value-added foods that have been shown to have a growing presence in the food industry. Availability of foods that contribute to health benefits and disease prevention is a great tool for nutritionists to employ when trying to improve the eating habits of individual clients and the general population. Food products prepared with acorn meal rather wheat flour could improve the nutritional value of foods such as cookies, muffins, breads, bars, noodles, pastries, breaded foods, and deserts. Extensive tests must be performed to insure acorn meal is a viable substitution for wheat flour, and to identify the types of food products best suited for product reformulation.

Problem Statement

Acorns have been an important part of traditional diet's of peoples throughout the world and are reported to have potential health benefits. However functionality of acorn meals in food systems typical of present day US diets is unknown. Species-specific characteristics that may influence functional performance in food systems, palatability and variability in potential health benefits associated with total phenolic levels are unknown.

Hypothesis

Acorn meal's physicochemical profile will differ from that of wheat flour, but partial replacement of wheat flour with acorn meal in cookies and muffins will produce products with acceptable quality characteristics. Consumer sensory panelists will find the cookies to be acceptable but less acceptable than the 100% wheat flour cookies due to the novelty of the flavors, appearance and texture.

Objectives

1. To create a physicochemical profile that characterizes acorn meal from black oak (*Quercus velutina)* and white oak (*Quercus alba*) acorns for future research and food reformulation of wheat flour-based foods.
2. To successfully reformulate cookie and muffin formulations to incorporate acorn meal with little difference in instrumental quality assessments and an increase in total phenolics.
3. To design a functional food, spice cookies, formulated with acorn meal that is acceptable to consumers in appearance, texture, and flavor as well as overall acceptability.

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2. Review of Literature

Acorns in Culture

Acorns have had a role in the human diet where-ever the oak tree was found. Among Native Americans, acorns were a dietary staple. European cultures also used acorns as food. Today, acorns are still used in many modern Asian cultures. As a food source, acorns are calorie dense with of high levels of fat; acorns are also a good source of protein, and are high in phenolic compounds which have health benefits. Different varietals vary in composition (Bainbridge, 1986; Bettinger et al., 1997).

The use of acorns in Native American culture has been well-documented. Some Native American tribes still use acorns in celebrations and ceremonies. Bettinger and others (1997) describe the gathering patterns and methods used by Native Americans in California with a central place model theory. Acorns were gathered in large baskets about 25 L in volume, and then taken to outlying camps. In the camps, the acorns were dried and shelled and then taken to the central villages for crushing, leaching, grinding, storage, and cooking. Annual harvests by Native Americans of California were reportedly over 60,000 tons and would sometimes constitute 50% of the diet (Bainbridge, 1986).

The acorns found in California, which were often bitter and high in tannins, were typically leached prior to consumption. The leaching reduced tannin levels, thereby reducing associated bitterness. Native American would sometimes “sweeten” the acorns by neutralizing acids with red earth, wood ash, and other ingredients. Thus, both processing and cooking

techniques were used to improve the palatability of the dietary staple. Acorn meal was mostly used in soups and stews by Native Americans. The meal was used as a substitute for corn meal in many recipes. Coffee-like drinks also were made with acorns. Acorns were also pickled or brined like olives. In addition, they were a substitute for many of the tree nuts more commonly consumed today (Bainbridge, 1986).

Acorns were used in many European and Middle Eastern cultures. Both Italy and Spain were previously large consumers of acorns. An acorn coffee-type drink known as Eichel kaffee was consumed in Europe (Bainbridge, 1986). Racahout, a food consumed in the Middle East, is defined by *Larousse Gastronomique* (2001) as containing sweet acorns with salep, cocoa, potato flour, rice, flour, sugar, and vanilla mixed with water or milk to make a drink or soup. Acorns are still used as feed for Iberian pigs (Cantos et al., 2003).

Asia is one of the few places where acorns are widely used as a foodstuff in today's society; although whole acorns are not as widely used, the acorn starch is used in some traditional foods found in Korea. Acorns also are used in traditional Asian medicines to help with digestive problems such as diarrhea (Adams, et al, 2008). In modern America, acorn starch can be found in some Korean grocery stores.

Phenolic Compounds in Acorns

One of the health benefits of acorns is the high levels of phenolic compounds present. Acorn phenolic content varies among varieties. The level of phenolics in acorns harvested in Georgia range from about 14.3 mg/g GAE in Overcup oak (white oak species) to 107 mg/g GAE in Laural oak (red oak species). In general, red oak varieties tend to be higher in total phenolics than are the white oak varieties. The phenolic content of black oak acorns, a red oak species

(*Quercus velutina)* prior to leaching was 39.4±3.6 mg GAE/g and of white oak acorns, a white oak species (*Quercus alba*) 27.4±.3 mg GAE/g. The total phenolics of leached and processed acorn meals range from about 39 mg GAE/g in black oak acorns (*Quercus velutina)* to 6.6 mg GAE/g in white oak acorn (*Quercus alba*). Despite this reduction, leached acorns remain a good source of phenols with levels equal to or exceeding those found in hazelnuts, almonds and peanuts, and acorns are calorically less dense than all of the nuts identified in figure 2.1. (Kobs, 2008).

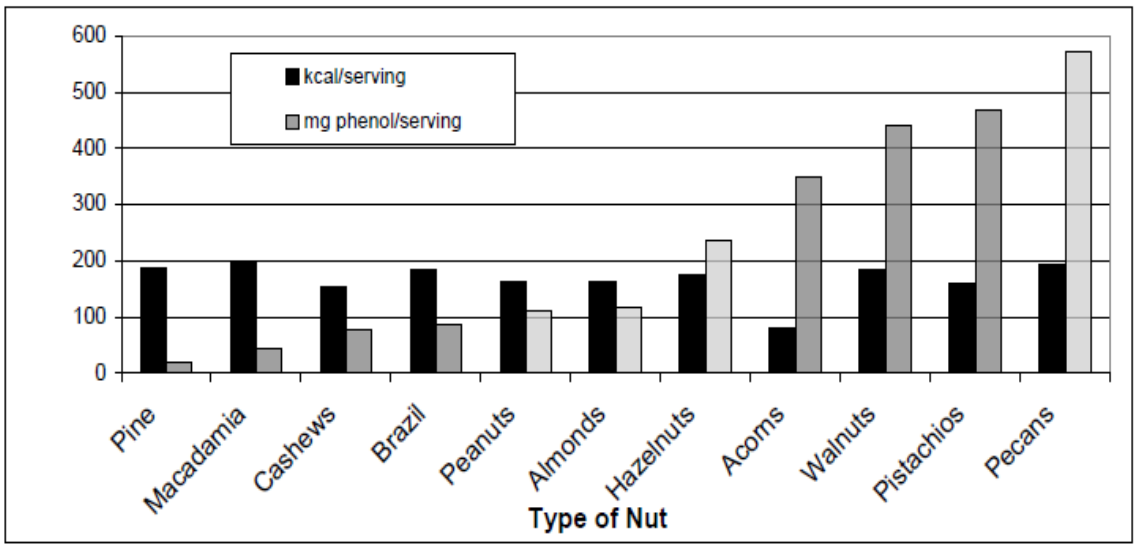

Figure 2.1: Comparison of kcal and total phenolics (mg GAE) per serving of various tree nuts. One serving is 28.35 g (Kobs, 2008).

Phenolic compounds are one of five categories of phytochemicals found in food. Phytochemicals are bioactive plant-derived compounds known to provide health benefits (Liu, 2004). Many types of phenolic compounds exist in foods. Chemically, phenols are defined as having hydroxyl groups on aromatic rings, although the number of phenolic rings and the configuration of the rings differ. Figure 2.2 depicts the different families of phytochemicals differentiated by the chemical structure.

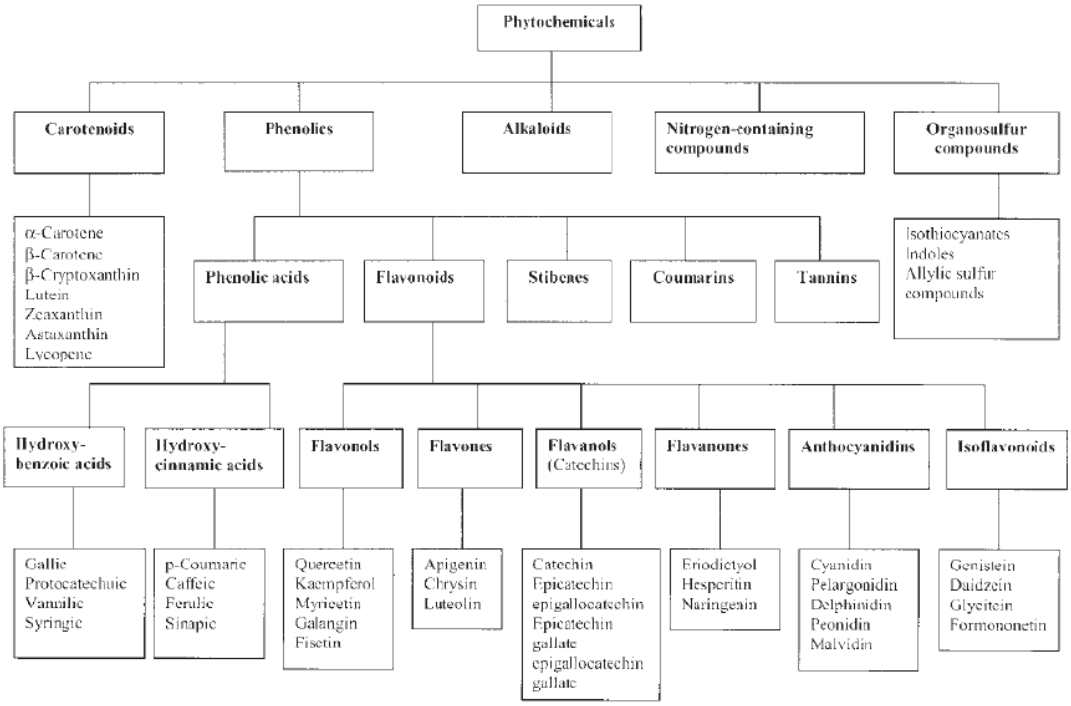

Figure 2.2: Classes of phytochemicals (Liu, 2004)

Of the 32 phenolic compounds that have been found in acorns from *Quercus spp.* most are phenolic acids in the hydroxy-benzoic acids group (Cantos et al., 2003). Figure 2.3 shows some of the chemical structures of the phenolic acids found in *Quercus spp.* The phenolic acids identified are gallic acids and gallic acid derivatives. The gallic acids are further divided into galloyl esters of glucose, the combinations of galloyl and hexahydroxydiphenoyl esters of glucose, tergallagic *O*- or *C*-glucosides, or ellagic acid derivatives (Cantos et al., 2003).

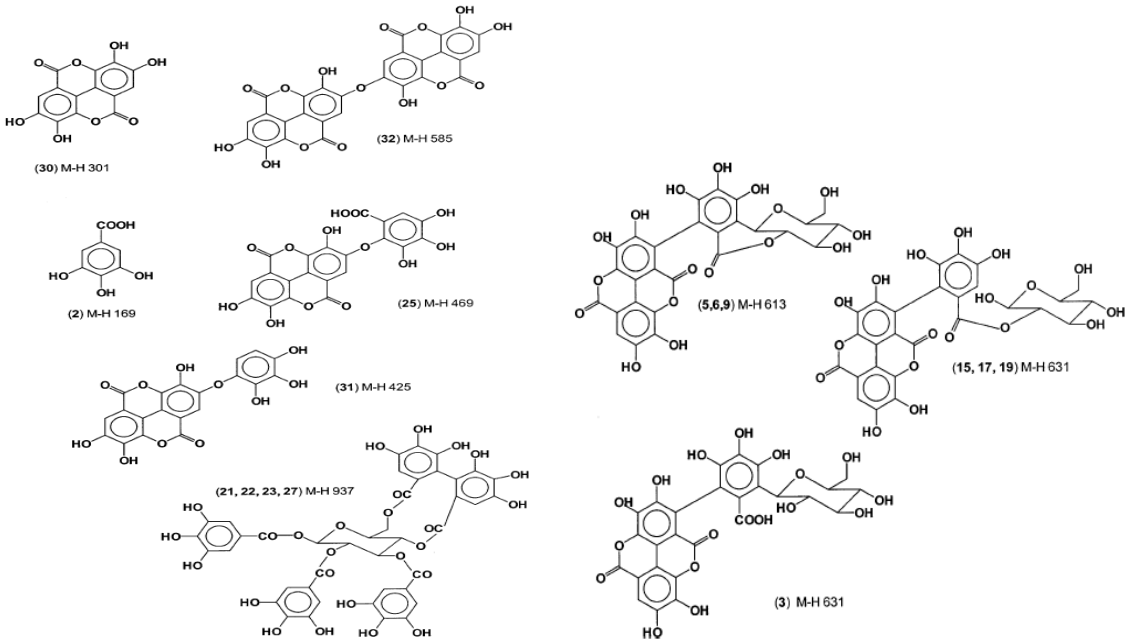

Figure 2.3: Structures of phenolic acids present in acorns (Cantos et al., 2003)

Phenolics contribute to color, flavor, functionality, and nutritive value of foods. Phenolic compounds are known to contribute to bitterness and astringency when present in food. Because the phenols will undergo oxidation before the volatile flavor compounds are oxidized, gallic acids and ellagic acids can contribute to extended shelf life of food flavors (Escalona, et al, 2002). Phenolic acids can cause cross-linking of polysaccharides and proteins and change the texture of foods (Waldron, et al, 1997; Wang, et al, 2002). The cross-linkages can also explain the traditional view of phenols as anti-nutrients. In addition to polysaccharides and proteins, minerals are also bound by phenols reducing their bioavailability. Because phenols were thought to be anti-nutrients and contributed to bitter and astringent flavors, the food industry has commonly removed phenolics from foods to make them more acceptable to the consumer. For example, pomegranate juices are blended with apple juices to reduce astringency and bitterness.

Wheat flours are bleached and the hulls and bran that contain phenols are removed. Some plants are selectively bred to have lower phenol content (Drewnowski & Gomez-Carneros, 2000).

Health Influence of Phenolic Compounds

Ironically, one of the potential health benefits of consuming acorns is the high levels of phenolic compounds contained in the acorn (Cantos et al., 2003; Kobs, 2008). Research shows that phenolics may play a part in reducing risks or symptoms for cardiovascular disease (CVD), cancer, HIV, microbial infection, diabetes, and inflammatory diseases (Gonzalez de Mejia et al., 1999; Halliwell et al., 2005; Jiang & Dusting, 2003; Kahkonen et al., 1999; Kruk et al., 2005; Lee et al., 2005; Ullah & Khan, 2008). Reduction of risk seems to be dose-dependent with daily consumption and a variety of phenols required (Scalbert et al., 2005).

Although phenols are known potent antioxidants and are known to bind proteins and minerals, potentially influencing numerous biological pathways (Chen et al., 2007; Fang et al., 2007; Faried et al., 2007; He et al., 2008; Neto et al., 2008; Wang et al., 2008; Yeh et al., 2009) , confirmed mechanisms of action are generally unknown for most of these disease states. To date, most research efforts have been directed toward the effects of phenolics on CVD and cancer. Current theories of action are reviewed in Appendix 1 and are summarized below.

Cardioprotective effects (Kluth, et al, 2007; Yeh et al., 2009 ; Pasten et al., 2007) have been attributed to:

- directly acting as anti-oxidants, although plasma concentrations and bioavailability suggest that free-radical scavenging is not the main mechanism;
- cell signaling through mitogen-activated protein kinases (MAPKs) ;

- gene expression via phase-II detoxification proteins and antioxidant response elements (ARE).

Proposed cancer preventive pathways (Chen et al., 2007; Chen et al., 2009; D'Archivio et al., 2008; Fang et al., 2007; Faried et al., 2007; Gonzalez de Mejia et al., 1999; He et al., 2008; Landis-Piwowar et al., 2007; Lee et al., 2005; Menendez et al., 2008; Neto, 2007; Neto et al., 2008; Pasten et al., 2007; Rahman et al., 2006; Soobrattee, et al, 2005; Ullah & Khan, 2008; Wang et al., 2008; Yang, et al, 1997; Yang, et al, 2009; Yeh et al., 2009; Yi, et al, 2005) include:

- inhibition of growth proliferation in cancer cells
- increase in apoptosis in cancer cells
- stimulation of phase II detoxifying enzyme production
- DNA oxidation reduction in normal cells
- free radical scavenging
- inhibition of DNA methyltransferaces (DNMT)
- regulation of signal transducing systems such as MAPKs
- DNA fragmentation of cancer cells
- inhibition of angiogenesis
- reduction of the migration of cancer cells

At present, most of these cancer preventative pathways are not fully understood, although it is apparent that different types of cancer cells are affected by different phenols and that different phenols act on different or multiple pathways.

Acorns are a good source of a wide array of phenols; most are phenolic acids in the hydroxy-benzoic acids group (Cantos et al., 2003). Gallic acid and other phenolic acids found in

acorns have been widely researched and are associated with reduction of some diseases (Chen et al., 2007; Chen et al., 2009; D'Archivio et al., 2008; Diplock et al., 1998; Fang et al., 2007; Faried et al., 2007; Gonzalez de Mejia et al., 1999; Hsu & Yen, 2008; Jiang & Dusting, 2003; Neto et al., 2008; Scalbert et al., 2005; Yang et al., 2009). However, little data are available on acorn meal or the use of acorn meal in foodstuffs typical of the US diet.

Characterization of Novel Flours

When introducing a novel flour, the flour's physical and chemical composition needs to be evaluated. Several novel flours, including amaranth and soy have been investigated in the last decade (Chinma & Gernah, 2007; Sindhuja, et al, 2005). Proximate analysis is used to determine nutritive value and predict functionality in a food system. Physical and chemical characteristics such as color, particle size, damaged and undamaged starch content, functional proteins, pH, and phenolic content can be evaluated and used to determine quality and functionality in various food systems. The novel flour is then evaluated within a food system like cookies, cakes, or breads. In most cases, flour establishes the crumb structure in these food systems (Pyler, 1988). The final food product, if successful, is evaluated by a sensory panel. The data are then combined to create a profile that characterizes the novel flour and allows its potential for industry use or future evaluation to be assessed.

The proximate analysis uses standardized and industry accepted methods to determine fat, protein, carbohydrate, moisture, and ash content of any food. Fat is usually determined by the gravimetric method (Zhang, et al, 2007). Many novel flours like soy flour and amaranth contain fat (Chinma & Gernah, 2007; Sindhuja, et al, 2005). Standard cake or all-purpose (AP) flour is defatted and contains a minimal amount of fat (Sindhuja et al., 2005). Protein can be

determined by the Kjeldahl method. The sample is digested and N is converted to NH_3 which is distilled and titrated. The collected N is used to determine crude protein (Roccia et al., 2006). The flour moisture percentage is determined by the weight lost when the flour is dried (Qian, et al, 1998). Ash is usually determined by heating the sample to between 550° C and 590° C until a light gray ash is achieved according to AACC method 8-01 (Qian et al., 1998); if a sample cannot be ashed due to high fat content, the carbohydrate + ash is determined. Carbohydrate + ash is determined as the percentage remaining after protein, fat, and moisture are determined (Sayed Razavi et al, 1996). Proximate analysis can be used to predict the possible applications of the novel flour in food products. High-protein flour may be best suited for yeast-breads, assuming adequate protein quality to produce a structural network in the bread system. Flour with a composition similar to soft wheat may be more suitable for cookie applications or quick breads. Fat content can affect staling, so high-fat flours are best suited for use in lower moisture foods such as cookies because moisture can increase lipid oxidation, unless antioxidants are included in the formulation. The proximate analysis also is used to determine nutritive value and calories: protein= 4 kcal/ g, carbohydrate= 4 kcal/ g, fat= 9 kcal/ g. Fatty acid composition is needed to the determine fat content reported on the Nutrition Facts labels as well as to predict possible storage issues associated with fat deterioration.

Physical and chemical characteristics of novel flours determine the way flour acts when heated or combined with other ingredients. Solvent Retention Capacity (SRC) is a method recently accepted by the AACC to determine wheat flour quality by measuring retention of four solvents by the flour sample (Bettge, et al, 2002; Gaines, 2004; Ram & Singh, 2004; Roccia et al., 2006; Zhang et al., 2007). The SRC is the weight of solvent held by flour after centrifugation under specified conditions. As SRC increases, water sequestering increases inhibiting reactions

involving Maillard browning and glass transition. The solvent retention capacity solvents correspond to starch damage (sodium carbonate), glutenin characteristics (lactic acid), and pentosan levels (sucrose), and hydration/ water carrying capacity (water). It is expressed as percentage of flour weight on a 14% moisture basis. Damaged starch influences functionality of a flour by easily absorbing water, reducing the amount of water available for hydration of other flour constituents including the structural proteins. Glutenin, which is always found in combination with the prolamine gliadin, is the glutelin found in wheat. Together these two wheat proteins, once hydrated and manipulated, form the gluten complex which in breadmaking imparts the gas retention property to the dough. Pentosans, which are also referred to as hemicelluloses, are non-starchy polysaccharides. They have the ability to absorb large amounts of water and form viscous solutions or gels. The level of pentosans present will influence flour absorption, mixing requirements, final product moisture content and staling tendencies of the resultant product (Pyler, 1988). In the case of cookies, the primary determinant of the baking potential of cookie flour is the hydration capacity. In cake or muffins, flour with a high moisture carrying capacity is desirable and is determined primarily by fineness of granulation, particle size uniformity and chlorination (Pyler, 1988). Generally high SRC values are associated with decreased cookie spread and decreased volume of cakes (Gaines, 2004; Ram & Singh, 2004; Roccia et al., 2006; Wang et al., 2002), which are the overall indicators of quality for thse products. SRC has also been used to determine the viability of new wheat cultivars for flour production and the potential of novel flours such as triticale (Bettge et al., 2002; Roccia et al., 2006).

pH is an important characteristic that affects the quality (color, flavor and texture) of the final product. Each baked product has an optimal pH range. pH of ingredients used determine

the final pH of each food system, with the flour incorporated one of the contributors. Wheat flour has a pH that typically ranges from 5-8 to 6.1 (Pyler, 1988). However, cake flour is chlorinated to lower pH (4.6-5.1) which influences the functional properties of the flour constituents including lipids, starch and pentosans, although the primary effect appears to be the effect on starch. In cookies, the increased water retention of the chlorinated starch can decrease cookie spread (Donelson & Gaines, 1998; Donelson, et al, 2000).

Color of the flour directly affects the color of the food formulated with the flour. If the color of the flour is darker, the product prepared with the flour will likely be darker. Particle size of a novel flour can affect the distribution in the food system. Larger particles may not be as evenly distributed, and a smaller particle size may indicate an increased amount of starch damage. Also hydration rate is affected, thereby affecting other functional characteristics of the flour.

Use of the flour in food systems such as cookies and cakes further characterizes the novel flour and supports tests performed on the flour. Sugar snap cookies and cake or muffins traditionally are used as a means of determining novel flour quality and viability for flour replacement. Color is measured to determine novel flour's effects on browning and color appearance. Water activity (aW) is the amount of water available for reactions in the food. A lower water activity is associated with better stability and longer shelf life. Cookies have a low aW because of low amounts of water in the formula. Cake and muffins have a higher aW, which is why the shelflife of cakes and muffins is shorter than that of cookies. The loss of water over time due to evaporation in the final baked product changes organoleptic properties and makes the product less acceptable (Baixauli, et al, 2008). Water activity helps predict specific changes that are likely to be issues in food systems.

The texture of the baked product is a very important determinant of quality for novel flours. A three-beam test and probing tests have been used to determine hardness, toughness and fracturability of cookies. The puncture tests (probing) have been determined to be a more appropriate test when sample is limited which is usually the case with novel flours. In addition, the puncture test is better suited to cookies that exhibit a range of textural attributes (Perry, et al 2003; Swanson & Perry, 2007). The cookie is punctured 9 times in a diamond shape pattern avoiding the outer 15% of the cookie. The number of punctures and distribution pattern give an accurate mean hardness of the cookie by sampling the whole area of the cookie when interaction with other puncture points is avoided. Several texture profile techniques exist for muffins and cakes (Baixauli et al., 2008; Bosman, et al, 2000; Meullenet & Gross, 1999; Pong, et al, 1991). The general methods involve compressing the crumb and measuring the force, and time required to compressed a sample a specified percentage. Double compression gives more information, allowing a texture profile of a product to be established (Baixauli et al., 2008; Meullenet & Gross, 1999).

Volume of quick breads and cakes is an indicator of the overall quality of a flour. Standing height is often used to measure volume of these products. Standing height is determined by slicing the baked muffin in half. A photocopy is made of the muffin halves and standing height is determined by measuring height of the middle of the muffin in mm (Bosman, et al, 2000; Pong et al., 1991). The volume is an indication of starch, protein, and fat interactions and reflects overall quality.

Cookie spread is a major component of running quality tests on novel flours (Donelson & Gaines, 1998; Gaines, 2004; Jacob & Leelavathi, 2007; Manohar & Rao, 1999; Miller, et al, 1997; Pareyt et al., 2008; Perry et al., 2003; Sindhuja et al., 2005; Swanson & Perry, 2007).

Many properties of flour influence cookie spread: pH, total fat content, protein, starch, phenolic compounds, moisture, particle size, mixing, and ingredient interactions (Donelson & Gaines, 1998; Gaines, 2004; Jacob & Leelavathi, 2007; Manohar & Rao, 1999; Miller et al., 1997; Pareyt et al., 2008; Perry et al., 2003; Sindhuja et al., 2005; Swanson & Perry, 2007). The intricacy of cookie spread makes the test an excellent measure of overall quality.

Sensory testing of foods prepared with novel flours is essential to determine feasibility of replacement. Two types of sensory profiles are used to test novel flours: descriptive sensory profiling and consumer sensory acceptability profile. Descriptive sensory profiles are usually performed with a trained or semi-trained panel of about 10 to 20 people. A quantitative descriptive analysis method such as the Spectrum Method® is usually used (Meilgaard, et al, 2006). In the Spectrum Method®, the panelists are trained using intensity references regarding a lexicon of flavors, textures, and aromas that are indicative of the product tested. The panelists then rate the product for intensity using a line scale in regards to the lexicon terms in a controlled environment multiple times, thereby acting as a calibrated instrument for organoleptic measurements (Meilgaard et al., 2006). The results form a sensory profile that describes the organoleptic properties of the product. The sensory profile of the novel flour product can then be compared with the instrumental tests to form a complete characterization profile.

The second class of sensory tests is used to ascertain the consumer acceptability of sensory properties of products prepared with the novel flours. Untrained panelists evaluate the product in a controlled environment. The panelists are asked to rate the acceptability of the product in regard to sensory descriptors such as texture, appearance, flavor, as well as overall acceptability. If multiple products are presented, a palate cleanser such as crackers or carrots is provided and water is used to wash the mouth of residual flavors and food particles. The number

of participants is usually 40 or more for reliable results. The results of the sensory acceptability panel reveal whether or not the novel flour product was a successful reformulation in regards to the consumer's perception of an acceptable product (Fennema, 1985; Meilgaard et al., 2006).

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3. Material and Methods

Experimental Design

Acceptability of acorn meal for use as a food ingredient was investigated with factorial design (table 3.1). All tests were replicated 3 times except for the proximate analysis, fatty acid profile analysis, and white oak acorn cookies food system study. Available sample limited the replications to two. Only three flours were used for the particle size test; cake flour was excluded because cake flour was not a variable ingredient. When applicable, formula baking order corresponded to order of post-baking tests and formula baking order was evenly rotated.

Table 3.1: Factorial Design

Test Type	Factors
Flour Tests	
Proximate composition	2 samples x 2-3 replications
Fatty acid profile	2 samples x 2 replications
Color analysis of flours	3 flours x 3 samples x 3 replications
pH of flours	3 flours x 3 replications
Solvent retention capacity	3 flours x 3 replications
Particle size	3 flours x 3 replications

Table 3.1 Cont.

High-moisture Food System:	
Muffin Tests	
Color of muffins	
interior/exterior	3 formulas x 3 samples x 3 replications
pH of muffin batter	3 formulas x 3 samples x 3 replications
Specific gravity muffin batter	3 formulas x 3 samples x 3 replications
aW of muffins	3 formulas x 3 samples x 3 replications
Muffin TPA	3 formulas x 3 samples x 3 replications
Muffin standing height	3 formulas x 3 samples x 3 replications
Total phenolics	3 formulas x 3 samples x 3 replications
Low-moisture Food System:	
Cookie Tests	
Color analysis of cookies	3 formulas x 3 samples x 2-3 replications
pH of cookie dough	3 formulas x 3 samples x 2-3 replications
Specific gravity cookie dough	3 formulas x 3 samples x 2-3 replications
Cookie spread	3 formulas x 3 samples x 2-3 replications
aW of cookies	3 formulas x 3 samples x 2-3 replications
Cookie probing	3 formulas x 3 samples x 9 puncture x 2-3 replications
Consumer panel	2 formulas x 128 panelists
Total phenolics	3 formulas x 3 samples x 2-3 replications

Acorn Selection, Identification and Storage

The acorns were gathered from known red oak (*Quercus velutina*) and white oak (*Quercus alba*) tree species on the campus of the University of Georgia in October 2007. Dr. Tim Smalley of the Horticulture Department at The University of Georgia identified the trees from which the acorns originated. Gathered acorns from each species were sorted to remove

rotted and undesirable acorns. Sorted acorns were dried in a vented rotary oven (National Mfg. Co., Lincoln, NE) at 65.5˚ C for 3.5 hours to prevent germination, stop enzymatic activity, and prevent other micro-organisms from growing. Final moisture content was less than 13% (AACC Method 44-15A, n=2). The dried acorns were then stored at 4˚ C until used in subsequent tests.

Acorn nut yield

Acorn nut meat yield was determined by weighing ten whole acorns, and then the acorn meat after shelling using various tools such as nut crackers, hammers, pliers, and picks. This procedure was repeated three times. The difference in nut meat weight and whole nut weight was reported as a percentage. The yield was about 50% irrespective of acorn size or species. The acorn meat was stored at 4˚ C until used in subsequent tests.

Leaching process

Once shelled, the acorn meats were leached to lower the tannin content. By leaching the acorn meat, the acorn meat became less bitter and astringent and more palatable. The acorn meat was subjected to a total of six 30-minute leaching periods under controlled conditions. The 500 g nut meat were combined with 80° C distilled water in 10:1 water to acorn ratio and stirred with a magnetic stirring plate for 30 minutes. Once 30 minutes elapsed, the nut/water mixture was drained through a sieve. The process was repeated three additional times with fresh 80° C distilled water. The acorns were then coarsely chopped with a Cuisinart food processor (Model #DLC-7, Greenwich, CT) for 24 pulses, where each pulse was one second. The leaching process was then performed two more times.

Drying and grinding

The leached wet acorn meat was evenly spread on half-sheet pans (41.9 x 30.5 cm) lined with parchment paper and dried in a vented rotary oven at 65.5° C. The parchment paper was replaced every 60 minutes throughout the drying process when the acorn meats were stirred. After drying for 6.5-8 hours, the chopped acorn meats were removed from the oven and cooled at room temperature for two hours. The dried acorn meat was then ground in a Porkert model 150 manual grain mill (Skulrov nad Belou, Czech Republic), twice. The acorn meal was stored in a sealed container at 4˚ C until used in subsequent tests.

Proximate composition

An analysis of macronutrients was performed so a nutrient analysis could be performed on the products in which the acorn meal was incorporated, and so that ingredient interactions could be accounted for when explaining results from rheological tests.

Protein analysis of the nut meals was performed by the Kjeldahl method (Zhang, et al, 2007). Samples were sent to Medallion Labs (Minneapolis, MN) where this analysis was performed with 2 replications.

Fat was determined by the gravimetric method on the nut meals (Zhang et al., 2007). Samples were sent to Medallion Labs (Minneapolis, MN) where this analysis was performed with 2 replications.

Moisture of the nut meals and all-purpose wheat flour was determined using AACC method 44-15A (Qian, et al, 1998) in triplicate.

CHO+ ash was determined as the percentage remaining after protein, fat, and moisture were determined (Sayed Razavi, et al, 1996) for the nut meals.

Fatty acid profile

Fatty Acid analysis was performed in duplicate by Dr. Michael Azain of the Animal and Dairy Science Department at the University of Georgia. Acorns meals were analyzed using gas chromatography of fatty acid methyl esters (FAME) prepared by in situ transesterfication (ISTE) (Carrapiso & García, 2000; Park & Goins, 1994).

Total phenolics

The Folin-Ciocalteu reagent method was used to determine total phenolic content (TP) in triplicate. Folin-Ciocalteu reagent and bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, MO).

All acorn meal samples were extracted with 50% ethanol. 9 ml of ethanol was combined with one gram of sample (as-is) and homogenized for 60 seconds. The solution was then shaken for 2 hours at 300 RPM. The samples were then centrifuged at 1100 RPM for 10 minutes at 10˚ C. The samples were then stored at 0˚ C for future tests. The sample absorbance was determined at 765 mμ. The TP was expressed in gallic acid equivalents (GAE) and determined by comparison with a standard curve (Bonoli, et al, 2004; Kobs, 2008; Naczk,et al, 1998; Singleton & Rossi, 1965; Soong & Barlow, 2004; Yang, et al, 1997). Phenolic compounds contribute to flavor, color, aromas, and can have functional effects in a formula and human health.

Color

Color was measured using a Minolta Spectrophotometer (Model CM-508d, Tokyo Japan) calibrated using a white calibration cap (CM-A70) and open air calibration. The spectrophotometer was set at 10-degree observer function, F6 illuminant setting for cool white florescent light source (4150K), and the specular component was excluded. Each reading was an average of the three closest readings out of five taken. Color was recorded in three values, L* a* and b*. L* is a measure of lightness on a 0 to 100 scale, where 0 is black and 100 equals white, and is an indication of saturation. The reading a* measures red-green axis, where positive a* is redness and negative a* is greenness. The reading b* is a measure of the yellow-blue axis, where positive b* is yellowness and negative b* is blueness. a* and b* measure hue. Color data were collected for black and white oak acorn meal, and all-purpose flour. Three samples were analyzed for each of the three repetitions (Baixauli, et al, 2008; Perry, et al, 2003; Pong, et al, 1991; Swanson, et al, 1999). The effects on color of the products due to reformulation were determined.

pH

The pH of black oak, white oak, all-purpose flour, and cake flour was measured using AACC method 02-52 (Seguchi, et al, 1997). The pH of the meals and flour was measured using a pH meter (Model 520A, Orion, Boston MA) calibrated using 4.00 pH and 7.00 pH buffers obtained from Fisher Chemical Labs (Fairlawn, NJ). Three samples were analyzed for each of the three replications.

Solvent Retention Capacity profile

The solvent retention capacity (SRC) profile analysis was used to determine water absorption, glutenin characteristics, damaged starch, and pentosan characteristics. Distilled water, 50% sucrose solution, 5% sodium carbonate solution, and 5% lactic acid solution were prepared according to the AACC method 56-11. Tests were conducted in triplicate using 1 g of flour in 15-mL tubes with a conical bottom. Material (1 g) was dispersed in 5 mL of solvent and kept for 20 min with intermittent vortexing at 5, 10, 15, and 20 min, followed by 15 min of centrifugation at 1,000 × *g* at room temperature (Ram, 2004). The supernatant was decanted and the tubes were drained at a 90° angle for 10 min. The SRC is the weight of solvent held by flour after centrifugation. It is expressed as percentage of flour weight, on a 14% moisture basis. The results from this analysis were used to compare the acorn meals to wheat flour for hydration ability (water), starch damage (sodium carbonate), glutenin characteristics (lactic acid), and pentosan characteristics (sucrose). Together the values were used to establish flour quality/functionality profile (Ram 2004; Zhang, Qijun 2007; Bettge 2002; Gaines 2004).

Particle size distribution

Particle size of black and white oak acorn meals and AP flour were determined by an adaptation of CFR 137.200 part 21 in triplicate, US # 8, 20, and 40 sieves were stacked on a base. 100g of the flour was placed in the #8 sieve and spread evenly across the sieve. A mechanical shaker (Innova 2000 platform shaker, New Brunswick Scientific Co., Ediston, NJ) was then used to shake the stacked sieves at 150 cycles per minute for 2 minutes. During the 2 minutes the sieves were sharply rapped with the heel of the hand twice every 15 seconds. The flour remaining in each sieve was then removed, weighed, and converted to a percentage of the

total flour. Particle size distribution can affect distribution of the flour and the mixing of ingredients.

High-moisture Food System: Muffins

Black oak acorn meal replaced 50% and 25% of the all-purpose flour (14% moisture-basis) in the pumpkin muffin formula (table 3.2). Muffin tests were used to determine viability of wheat flour replacement with Black Oak acorn meal in a high-moisture food system at various ratios.

Table 3.2: Formula for Pumpkin Muffins[a]

Weight	Ingredient	Source
68 g	Shortening	Crisco, Orrville OH
266 g	Sugar, granulated	Dixie Crystals, Sugarland TX
100 g	Egg	Kroger, Cincinnati OH
245 g	Pumpkin, canned	Stokley, Oconomowoc WI
78 g	H_2O	Athens-Clark County Municipal
201g + 7g H_2O[b]	All-purpose flour	White Lily, Memphis TN
4.6 g	Baking soda	Kroger, Cincinnati OH
4.5 g	Salt	Pocahontas, Richmond VA
1.3 g	Cinnamon, ground	McCormick, Hunt Valley MD
0.53 g	Cloves, ground	McCormick, Hunt Valley MD
0.55 g	Nutmeg, ground	McCormick, Hunt Valley MD
1.15 g	Baking powder	Kroger, Cincinnati OH

[a] 50:50 acorn muffin used all-purpose flour (100.5g +3.5 H_2O) and black oak acorn meal (102.9 g + 1.1 g H_2O) to adjust to 14% moisture, 25:75 muffin used all-purpose flour (150.7 g +5.3 g H_2O) and black oak acorn meal (51.5 g + .5 g H_2O) to adjust to 14% moisture, yields 48

[b] Adjusted to 14% moisture

Muffins were prepared with a Kitchen Aid Mixer (K5SS, St. Joseph, MI) equipped with a paddle beater and baked at 176.7° C in a rotary oven (National Mfg. Co., Lincoln, NE) for 22 minutes (control), 23 minutes (25% acorn meal), or 24 minutes (50% acorn meal).

To prepare the batter, all refrigerated ingredients were held at room temperature for at least 45 minutes before mixing. Wet ingredients (egg, pumpkin, water) were blended for one minute at speed two and then held until incorporation with dry ingredients. In a clean bowl, dry ingredients (sugar, flour, baking soda, salt, cinnamon, cloves, nutmeg, and baking powder) were mixed at speed one for two minutes. Shortening was added to the bowl and mixed for one minute on speed two. The bowl was scraped with a rubber spatula and the wet ingredients were added to the bowl and mixed at speed one for one minute. Paper muffin liners were put in a muffin pan (Wearever No. 2754, Millville NJ). The batter was then scooped into the liners with a leveled # 20 scoop (control= 52.4± 0.18g, 50:50= 48.9± 0.4g, 25:75=50.23± 0.24g). After baking, the muffins were cooled for two hours at room temperature before instrumental tests were conducted. Muffins were baked and tested three times and the order of preparation differed for each replication.

Specific gravity

Specific gravity of muffin batter was measured directly after mixing. Three empty 50 mL beakers were weighed to the nearest 0.1 gram and then filled completely with distilled water and weighed again to the nearest 0.1 gram. The containers were then filled with the sample and weighed to the nearest 0.1 gram, making sure all air pockets were removed and the top was leveled with a straight edge spatula (Pong et al., 1991). The specific gravity was then determined by the following formula:

$$\frac{\text{Wt of filled container – wt of dry container}}{\text{Wt of water-filled container - wt of dry container}}$$

Water activity

The Aqua Lab (Model CX-2, Decagon Devices, Pullman, WA) was used to measure water activity (aW) for muffins. The instrument was calibrated with distilled water. For muffins, three muffins were put into the Cuisinart mini- food processor (model DLC-1, Windsor, NJ) and ground for 15 second on high. Three aliquots were removed from composite sample and tested for aW. aW is an indicator of shelf life and product stability (Perry et al., 2003; Swanson, et al., 1999).

pH

The pH of the muffin batters was measured using a pH meter (Model 520A, Orion, Boston MA) calibrated using 4.00 pH and 7.00 pH buffers obtained from Fisher Chemical Labs (Fairlawn, NJ). The batter pH was measured directly after mixing (Pong et al., 1991). Three samples were analyzed for each of the three replications.

Color

Color of the muffin's interior and exterior was measured using the same methods as used with the meal.

Total phenolics

TP of the muffins was determined using the same methods used to determine the TP of the acorn meal.

Muffin TPA

Compression is an industry accepted, American Institute of Baking, method for determining the texture properties of cake that has been adopted for muffins (Baixauli et al., 2008). A 2-cm wide center slice of 3 muffins per treatment was removed for assessment using TAX.T2 texture analyzer equipped with a 50 kg load cell (Stable Micro Systems, Haselmere, Surrey England) and Texture Expert Exceed software (version 1.20). The 75-mm diameter disc was used to compress the sample height by 50% twice with 5 seconds between compressions. The crossarm speed was 2mm/s pre-test, 5 mm/s test speed, and 5 mm/s post-test. Three samples per treatment were evaluated. Three replications were performed. The treatments were tested in the order of baking; however, baking order was randomized for each replication. Hardness, springiness, and cohesiveness of the muffins were determined (Appendix 2). Hardness was the peak force required to compress the sample by 50% during the first compression. Springiness was the distance the muffin recovered between the first and second compression. Cohesiveness was the ratio of the force area of the second compression to the force area of the first compression (Baixauli et al., 2008).

Muffin standing height

Muffin standing height was determined by slicing the baked muffin in half. A photocopy was made of the muffin halves and standing height was determined by measuring height of the

middle of the muffin in mm (Bosman, et al, 2000; Pong et al., 1991). Three samples per treatment were measured across three replications. Standing height tests are an overall indicator of muffin quality. Presence of tunnels, a common muffin defect, was noted (Appendix 2).

Low-moisture Food System: Cookies

The acorn meal replaced 50% of the total wheat flour (100% of the all-purpose flour) in the spice cookie formula adjusted to 14% moisture (table 3.2). The cookie tests help profile acorn meal and establish viability of wheat flour replacement in a low-moisture food system. After preliminary flour/meal tests, the all-purpose flour, 50% of the total flour in the formula was chosen for replacement with acorn meal because of similarities in pH. Cake flour, the remaining 50% of the flour in the cookie formula, was a constant ingredient.

Cookies were mixed with a Kitchen Aid Mixer (K5SS, St. Joseph, MI) equipped with a paddle beater and baked at 162.8° C in a rotary oven (National Mfg. Co., Lincoln, NE) for 14 minutes. To prepare the dough, all ingredients were weighed the day prior to baking. The day of baking, the eggs and butter were held at room temperature for at least 45 minutes prior to mixing. Dry ingredients (flour, baking soda, salt, ginger, cinnamon, nutmeg, allspice) were blended at speed one for two minutes. Dry ingredients were then held until incorporated with wet ingredients. Brown sugar and butter were creamed at speed two for two minutes in a separate bowl. The bowl was scraped with a rubber spatula; the eggs and molasses were added to the bowl and mixed at speed two for two minutes. The water required to adjust flour moisture to 14% was added in the last five seconds of wet ingredient mixing. The bowl was scraped again and the dry ingredients were added to the bowl and mixed at speed two for three minutes.

Table 3.2: Formula for Spice Cookie (yields 48) [a]

Weight	Ingredient	Source
82.5 g	Light brown sugar	Dixie Crystals, Sugarland TX
85 g	Butter, unsalted	Kroger, Cincinnati OH
25 g	Egg	Kroger, Cincinnati OH
126 g	Molasses	Gradma's, Roseland NJ
92.5 g + 1.9 g H_2O	Cake flour[b]	Swans Down, Reily Foods, New Orleans LA
88.5 g + 6.2 g H_2O	All purpose flour[b]	White Lily, Memphis, TN
2.3 g	Baking soda	Kroger, Cincinnati OH
1.5 g	Salt	Pocahontas, Richmond VA
1.35 g	Ginger	McCormick, Hunt Valley MD
1.3 g	Cinnamon	McCormick, Hunt Valley MD
1.1 g	Nutmeg	McCormick, Hunt Valley MD
0.5 g	Allspice	McCormick, Hunt Valley MD

[a] 100% of the all-purpose flour replaced with black oak or white oak acorn meal adjusted to 14% moisture-basis,
[b] Adjusted to 14% moisture

Cookie dough was deposited with a leveled # 60 scoop (control= 16.12± 0.17g, Acorn= 15.7± 0.29g) on a 41.9 x 30.5 cm sheet pan lined with parchment paper and lightly sprayed with cooking spray (Pam, ConAgra Foods, Inc, Omaha, NE) in three rows of three. After baking, the cookies were allowed to cool on racks for two hours before being individually bagged in plastic zipper sandwich bags (Kroger, Cincinnati, OH) and stored at room temperature until tested. Three replications were obtained for the black oak acorn flour; two replications for the white oak acorn cookies were baked due to limited amounts of white oak acorn meal. The order of baking was different for each replication.

Water activity

The same methods use for the muffins was used for the cookies to determine aW. For the cookies, three cookies were broken up into a Cuisinart mini food processor (model DLC-1, Windsor, NJ) and were ground on low for 15 seconds and then high for 15 seconds. Three aliquots were removed from the ground composite sample and tested for aW.

Specific gravity

Specific Gravity of the cookie dough was determined using the same method used to determine the muffin batter specific gravity.

pH

pH of the cookie dough was measured using the same method as used with the muffin batter.

Color

Color (L*, a* b*) of the cookies' top surface was measured using the same methods as used to determine color of the meal.

Total phenolics

TP of the cookies was determined using the same methods used to determine the TP of the acorn meal.

Cookie probing

Cookies' texture attributes were extracted from a time/force curve (Appendix 2) generated with TAX.T2 texture analyzer equipped with a 50 kg load cell (Stable Micro Systems, Haselmere, Surrey England) and Texture Expert Exceed software (version 1.20). The 3mm probe attachment was used with a crossarm speed of 5 mm per second and readings were taken at 200 PPS. Each cookie was punctured nine times in a diamond shape, excluding the outer 15% of the cookie. Three cookies per treatment per repetition were tested. Three repetitions were performed. The force in grams to puncture the cookie determined hardness and the area under the time (s)/force (g) curve was used to determined toughness. The probing tests were used to determine acorn meal's effect on hardness and toughness (Perry et al., 2003; Swanson & Perry, 2007).

Cookie spread

AACC method 10-50D was used to determine cookie spread. Cookie spread is used as a standard measure of overall cookie quality (Chinma & Gernah, 2007; Gaines, 2004; Goesaert et al., 2005; Miller, et al, 1997).

Consumer sensory panel

A consumer sensory panel was used to test acceptability of the wheat flour control and black oak acorn cookies. The panelists rated the two cookies for acceptability of appearance, flavor, texture, as well as overall acceptability. The panelists used a 9-point hedonic scale where 1 was disliked extremely and 9 liked extremely (Appendix 3). The cookies were coded with random three-digit random numbers and were presented to the panelists one at a time in a

balanced order. The panelists tested the cookies in individual booths lit with cool white florescent light and with positive/negative air pressure. The panelists were given water, unsalted top crackers and carrots to cleanse their palates between samples. Panelists consisted of 128 untrained students, faculty and staff recruited in Dawson Hall at the University of Georgia on the day of testing. Panelists were only told they would evaluate spice cookies and that they would receive a store-bought snack after testing. The panelists also completed a food frequency questionnaire to determine a consumer profile and buying habits. The profile survey asked about age, gender, intent to buy functional foods, and cookie eating and purchasing habits (Appendix 3).

Statistical Analysis

All data were processed using SAS software version 9.1 (SAS institute, Gary NC). Means and standard deviations were determined, and significant differences ($p<0.05$), among samples were determined with the PROC GLM ANOVA procedure and SNK when appropriate. Frequency of response for the questionnaire completed by the sensory panelists was determined with PROC Freq. Total phenolic regression lines were determined with Microsoft Excel 2003 software.

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Chapter 4

CHARATERIZATION OF ACORN MEAL

Sabrin, M. S. To be submitted to *The International Journal of Food Properties*

Abstract

A method was developed to prepare acorns into meal for testing physiochemical properties and use as wheat flour replacement in foods. First, the acorn meal's characteristics were defined using industry accepted methods. Spice cookies and pumpkin muffins were then made from the acorn meal and tests were performed to further characterize the acorn meal. Consumer sensory tests (n=128) were performed on the cookies. Total phenolics of acorn meals range from about 39 mg GAE/g in black oak acorns to 6.6 mg GAE/g in white oak acorn. Major differences between the meal and wheat flour that affected the production of the cookies and muffins were color, solvent retention capacity, fat content, and phenolic content. Black oak acorn meal was best suited for replacing all-purpose wheat flour. 50% replacement was performed for the cookies. Acorn meal cookies had some differences in texture, appearance, and flavor, but a consumer sensory panel found the cookies to be overall acceptable. 25% replacement of wheat flour in muffins had no effect or a beneficial effect on most instrumental tests associated with organoleptic properties. Panelists expressed interest in cookies that carried an antioxidant claim.

Introduction

New and exotic super foods commonly appear as functional ingredients. Although acorns have had an important role in the human diet for thousands of years where-ever the oak tree has grown, they are not widely used today as food or food ingredients despite extensive availability. Historically, many Native American cultures used acorns as a dietary staple. Many hunter-gatherer societies in Europe also used acorns as food. Acorns are still used in many modern Asian cultures. As a food source, acorns are calorie dense because of high levels of fat, they are a good source of protein, and are high in phenolic compounds. Differences in composition due to acorn species have been reported (Bainbridge, 1986; Bettinger, et al, 1997).

One of the health benefits of consuming acorns is the high levels of phenolic compounds present (Cantos et al., 2003; Kobs, 2008). Phenolics may play a part in reducing risks or symptoms for cardiovascular disease (CVD), cancer, HIV, microbial infection, diabetes, and inflammatory diseases (Gonzalez de Mejia, et al, 1999; Halliwell, et al, 2005; Jiang & Dusting, 2003; Kahkonen et al., 1999; Kruk, et al, 2005; Lee, et al, 2005; Ullah & Khan, 2008). CVD and cancer prevention can be linked to long-term consumption of fruits and vegetables which contain compounds with antioxidant and hypolipidimic activity, including dietary phenolics (Jiang & Dusting, 2003). Phenolics once were considered anti-nutrients due to the protein binding properties of the compound. However, recent research suggests that phenolic protein binding to signaling pathways and to DNA may be the reason for some of the health benefits seen from some phenolic compounds (Chen et al., 2007; Fang, et al, 2007; Faried et al., 2007; He, et al, 2008; Neto, et al, 2008; Wang, et al, 2008; Yeh et al., 2009). The total phenolics of

leached and processed acorn meals range from about 39 mg GAE/g in black oak (*Quercus velutina)*, a red oak acorn variety to 6.6 mg GAE/g in white oak (*Quercus alba)*, a white oak variety (Kobs, 2008). The food industry traditionally removed phenols from various foods to make them more appealing to consumers; however, in recent years rather than removing phenols, inherent levels have been retained and in some cases foods have been fortified with phenols to increase their nutritional functionality. Estimated intake of phenolics among American consumers is 1000 mg/ day; with coffee and tea the major dietary sources (Scalbert, et al, 2005).

When introducing a novel flour, the flour's physical, chemical and sensory properties must be evaluated. Several novel flours have been investigated in the last decade (Chinma & Gernah, 2007; Sindhuja, et al, 2005). A proximate analysis is used to determine nutritive value and to predict functionality in a food system. Physical and chemical characteristics such as color, particle size, damaged and undamaged starch content, functional proteins, pH, and phenolic content can be evaluated and used to determine quality and predict functionality in various food systems. Based upon these results, the novel flour is then evaluated in a food system like cookies, cakes, or breads to determine its functional performance. The food produced, if successfully made, is evaluated by a sensory panel. The data are then used to generate a characteristic profile of the novel flour for immediate industry use or future evaluation.

Availability of foods that contribute health benefits and aid in disease prevention are an additional tool that nutritionists can use to help clients as well as the general population improve their dietary choices. Acorn meal, when used as a wheat flour substitute, could improve the nutritional value of foods such as cookies, muffins, breads, bars, noodles, pastries, breaded foods, and deserts. Extensive tests must be performed to insure acorn meal can be successfully

substituted for wheat flour. Acorns have been an important part of traditional diet's of peoples throughout the world and are reported to have potential health benefits. However functionality of acorn meals in food systems typical of present day US diets is unknown. Species-specific characteristics that may influence functional performance in food systems, palatability and variability in potential health benefits associated with total phenolic levels are unknown.

It is hypothesized that acorn meal's physicochemical profile will differ from that of wheat flour, but partial replacement of wheat flour with acorn meal in cookies and muffins will produce products with acceptable quality characteristics despite the novelty of the flavors, appearance and texture imparted by the acorn meal.

The objectives of this study are:

1. To create a physicochemical profile that characterizes acorn meal from black oak (*Quercus velutina)*, a red oak species, and white oak (*Quercus alba*), a white oak species, for potential consideration for future research and food reformulation of wheat flour-based foods;
2. To successfully reformulate cookie and muffin formulations to incorporate acorn meal with little difference in instrumental quality assessments while increasing total phenolics; and
3. To design a functional food, spice cookies, formulated with acorn meal that is acceptable to consumers in appearance, texture, and flavor as well as overall acceptability.

Material and Methods

Suitability for use as a food ingredient of two acorn meals, prepared from black oak (*Quercus velutina*) and white oak (*Quercus alba*) species, was investigated with a factorial design. Tests included characterization of each acorn meal as well as its use in both high and low-moisture food systems. Protein, fat, and fatty acid analyses were limited to the acorn meals;

the remaining tests also were performed on the all-purpose flour (White Lily, Memphis, TN) control. In a previous study (Kobs, 2008), the Folin-Ciocalteu reagent method was used to determine total phenolic content (TP) of the acorn meals and all-purpose flour control used in this study; the same procedures were used to determine total phenolics of the food products formulated in the baking quality tests in this study. In the high-moisture food system (muffins), black oak, a red oak species, was investigated as a potential substitution for 25% and 50% of the all-purpose wheat flour. In the low-moisture food system (cookies), black oak, a red oak species and white oak, a white oak species, were investigated as a potential substitute for 50% of the all-purpose wheat flour. In both food system studies, acorn meal substitutions for all-purpose wheat flour were made on a 14% moisture basis.

Acorn meal processing and evaluation

The acorns were gathered from known black oak (*Quercus velutina*) and white oak (*Quercus alba*) tree species on the campus of the University of Georgia in October 2007; varietals were black oak and white oak respectively. Once sorted to remove rotted and insect damaged nuts, the acorns were dried in a rotary oven (National Mfg. Co., Lincoln, NE) at 65.5˚ C for 3.5 hours to prevent germination, stop enzymatic activity, and prevent micro-organisms from growing. Moisture content was <13%. (AACC Method 44-13A, n=2). Acorn nut meat yield was determined by weighing ten whole acorns, and then the acorns meat was weighed after shelling. The yield was about 50% irrespective of acorn size or species.

Once shelled, the acorns were leached to lower the tannin content. The acorn meat was subjected to a total of six 30-minute leaching periods under controlled conditions. Five hundred grams of nut meat were combined with 80° C distilled water in 10:1 water to acorn ratio and

stirred with a magnetic stirring plate for 30 minutes. Once 30 minutes elapsed, the nut water mixture was drained through a sieve. The process was repeated three additional times with fresh 80° C distilled water. The acorns were then coarsely chopped with a Cuisinart food processor (Model #DLC-7, Greenwich, CT) for 24 pulses where each pulse was one second. The leaching process was performed two additional times on the coarsely chopped acorn meal.

The wet acorn meal was dried in a rotary oven at 65.5° C on half-sheet pans (41.9 x 30.5 cm) lined with parchment paper. After drying for 6.5-8 hours, the chopped acorn meats were removed from the oven and cooled under ambient conditions for two hours. The acorn meat was ground in a Porkert model 150 manual grain mill (Skulrov and Belou, Czech Republic), twice. The resulting acorn meals were stored at 4˚ C until used in subsequent tests.

Acorn meal proximate analysis. Protein analysis was performed by the Kjeldahl method (Zhang et al, 2007). Fat was determined by the gravimetric method (Zhang et al., 2007). Both analyses were conducted by Medallion Labs (Minneapolis, MN) in duplicate. Moisture of the meal was determined using AACC method 44-15A (AACC, 2008) in triplicate. Carbohydrate + ash was determined as the percentage remaining after protein, fat, and moisture were determined (Sayed Razavi et al, 1996).

Acorn meal fatty acid profile. Fatty acid analysis was performed in duplicate. Acorns meats were analyzed using gas chromatography of fatty acid methyl esters (FAME) prepared by in situ transesterfication (ISTE) (Carrapiso & García, 2000; Park & Goins, 1994).

Particle size distribution. Particle size distribution of the acorn meals and all-purpose wheat flour control was determined by an adaptation of CFR 137.200 part 21 in triplicate. US #8, 20 and 40 sieves and a mechanical shaker (Innova 2000 platform shaker, New Brunswick Scientific Co., Ediston, NJ) were employed.

Color. Color of the acorn meals and the all-purpose wheat flour control was measured with a Minolta Spectrophotometer (Model CM-508d, Tokyo, Japan) calibrated with a white calibration cap (CM-A70) and open air calibration; settings were: 10-degree observer function, and F6 illuminant for cool white florescent light source, with the specular component excluded. Color data were collected for red and white oak acorn meal, and all-purpose flour (Baixauli, et al, 2008; Perry, et al, 2003; Pong, et al, 1991; Swanson, et al, 1999) in triplicate.

pH. A pH meter (Model 520A, Orion, Boston MA), calibrated using 4.00 pH and 7.00 pH buffers from Fisher Chemical Labs (Fairlawn, NJ), was used to determine pH of the black oak meal and white oak meal, as well as the all-purpose flour control. Three replications were obtained and AACC method 02-52 (Seguchi et al, 1997) was used.

Solvent retention capacity profile. The solvent retention capacity (SRC) profile analysis (AACC method 56-11) was used to determine water absorption ability, and glutenin, damaged starch, and pentosan characteristics (Bettge, et al, 2002; Gaines, 2004; Ram & Singh, 2004; Zhang, et al, 2007) of the acorn meals and all-purpose flour control. Each assay was replicated three times.

Food system Formulas and Preparation

Pumpkin muffins were used to assess suitability of wheat flour replacement (0%, 25% and 50%) with red acorn meal in a high-moisture food system on a 14% moisture-basis. Formulas and ingredient sources for the muffins are found in Table 4.1.

Spice cookies were used to assess suitability of black oak and white oak meals in a low-moisture food system. In the control cookie formula, all-purpose wheat flour equaled 50% of the total flour, with cake flour the remaining percentage. Acorn meals replaced 0 or 100% or the all-purpose flour only on a 14% moisture-basis. Formulas and ingredient sources for the cookies are found in Table 4.2.

Table 4.1: High-moisture Food System: Pumpkin Muffins Formula[a]

Weight	Ingredient	Source
68 g	Shortening	Crisco, Orrville OH
266 g	Sugar, granulated	Dixie Crystals, Sugarland TX
100 g	Egg	Kroger, Cincinnati OH
245 g	Pumpkin, canned	Stokley, Oconomowoc WI
78 g	H_2O	Athens-Clark County Municipal
201g + 7g H_2O[b]	All-purpose flour	White Lily, Memphis TN
4.6 g	Baking soda	Kroger, Cincinnati OH
4.5 g	Salt	Pocahontas, Richmond VA
1.3 g	Cinnamon, ground	McCormick, Hunt Valley MD
0.53 g	Cloves, ground	McCormick, Hunt Valley MD
0.55 g	Nutmeg, ground	McCormick, Hunt Valley MD
1.15 g	Baking powder	Kroger, Cincinnati OH

[a] 50:50 acorn muffin: all-purpose flour (100.5g +3.5 H_2O) and black oak acorn meal (102.9 g + 1.1 g H_2O)
25:75 muffin: black oak acorn meal (51.5 g + .5 g H_2O) and all-purpose flour (150.7 g +5.3 g H_2O) yields 48
[b] Adjusted to 14% moisture.

Muffins were prepared with a Kitchen Aid Mixer (K5SS, St. Joseph, MI) equipped with a paddle beater and baked at 176.7° C in a rotary oven (National Mfg. Co., Lincoln, NE) for 22 minutes (control), 23 minutes (25% acorn meal), or 24 minutes (50% acorn meal). To prepare the batter, all refrigerated ingredients were held at room temperature for at least 45 minutes before mixing. Wet ingredients (egg, pumpkin, water) were blended for one minute a speed two and then held until incorporation with dry ingredients. In a clean bowl, dry ingredients (sugar, flour, baking soda, salt, cinnamon, cloves, nutmeg, and baking powder) were mixed at speed one for two minutes. Shortening was added to the bowl and mixed for one minute on speed two. The bowl was scraped with a rubber spatula and the wet ingredients were added to the bowl and mixed a speed one for one minute. Paper muffin liners were put in a muffin pan (Wearever No. 2754, Millville NJ). The batter was then scooped into the liners with a leveled # 20 scoop (control= 52.4± 0.18g, 50:50= 48.9± 0.4g, 25:75=50.23± 0.24g). After baking, the muffins were cooled for two hours at room temperature before instrumental tests were conducted. Muffins were baked and tested three times and the order of preparation differed for each repetition.

Cookies were mixed with a Kitchen Aid Mixer (K5SS, St. Joseph, MI) equipped with a paddle beater and baked at 162.8° C in a rotary oven (National Mfg. Co., Lincoln, NE) for 14 minutes. To prepare the dough, all ingredients were weighed the day prior to baking. The day of baking, the eggs and butter were held at room temperature for at least 45 minutes prior to mixing.

Table 4.2: Low-moisture Food System: Spice Cookies Formula[a]

Weight	Ingredient	Source
82.5 g	Light brown sugar	Dixie Crystals, Sugarland TX
85 g	Butter, unsalted	Kroger, Cincinnati OH
25 g	Egg	Kroger, Cincinnati OH
126 g	Molasses	Gradma's, Roseland NJ
92.5 g + 1.9 g H_2O	Cake flour[b]	Reily Foods Co., New Orleans LA
88.5 g + 6.2 g H_2O	All purpose flour[b]	White Lily, Memphis, TN
2.3 g	Baking soda	Kroger, Cincinnati OH
1.5 g	Salt	Pocahontas, Richmond VA
1.35 g	Ginger	McCormick, Hunt Valley MD
1.3 g	Cinnamon	McCormick, Hunt Valley MD
1.1 g	Nutmeg	McCormick, Hunt Valley MD
.5 g	Allspice	McCormick, Hunt Valley MD

[a] In acorn cookie 100% of the all-purpose flour was replaced with black oak or white oak acorn meal on a equal weight basis adjusted to 14% moisture, yields 48
[b] Adjusted to 14% moisture

Dry ingredients (flour, baking soda, salt, ginger, cinnamon, nutmeg, allspice) were blended at speed one for two minutes. Dry ingredients were then held until incorporated with wet ingredients. Brown sugar and butter were creamed at speed two for two minutes in a separate bowl. The bowl was scraped with a rubber spatula; the eggs and molasses were added to the bowl and mixed at speed two for two minutes. The water required to adjust flour moisture to 14% was added in the last five seconds of wet ingredients mixing. The bowl was scraped again and the dry ingredients were added to the bowl and mixed at speed two for three minutes. Cookie dough was deposited with a leveled # 60 scoop (control= 16.12± 0.17g, Acorn= 15.7± 0.29g) on a 41.9 x 30.5 cm sheet pan lined with parchment paper and lightly sprayed with

cooking spray (Pam, ConAgra Foods, Inc, Omaha, NE) in three rows of three. After baking, the cookies were allowed to cool on racks for two hours before being individually bagged in plastic zipper sandwich bags (Kroger, Cincinnati, OH) and stored at room temperature until tested. Three replications were obtained for the black oak formulations; for the white oak acorn cookies, two replications were baked due to limited amounts of white oak acorn meal. The order of baking was different for each repetition

Quality assessment: Muffin batters and cookie dough

The following quality assessment tests were conducted on muffin batters and cookie dough: specific gravity and pH.

Specific gravity. Specific gravity of the muffin batters and cookie dough was measured in triplicate directly after mixing. Three empty 50 mL beakers were weighed to the nearest 0.1 gram and then filled completely with distilled water and weighed again to the nearest 0.1 gram. The containers were then filled with the sample dough or batter and weighed to the nearest 0.1 gram, making sure all air pockets were removed and the top was leveled with a straight edge spatula (Pong et al., 1991). The specific gravity was then determined by the following formula:

$$\frac{\text{Wt of filled container} - \text{wt of dry container}}{\text{Wt of water-filled container - wt of dry container}}$$

pH. Both muffin batter and cookie dough pH was measured in triplicate directly after mixing by inserting the probe directly into the media (Pong et al., 1991). Instrumentation and calibration procedures were identical to those employed in the acorn meal pH assessment.

Quality assessment of baked muffins and cookies: Physical tests

The following quality assessment tests were conducted on both baked products (muffins and cookies): color and water activity. Texture assessments and overall quality indicators were product specific. For muffins, Texture Profile Analysis and standing height were determined. For cookies, texture was assessed with the probing technique and cookie spread, an indicator of overall cookie quality, was determined. All quality assessments were determined in triplicate.

Color. Color (L*, a*, b*) of the crumb and exterior surface of the muffins and the top surface of the cookie was measured on three samples per replication., with 2 replications for the muffins and 3 replications for the cookies. The same instrument and settings employed in meal characterization studies were used.

Water activity. The Aqua Lab (Model CX-2, Decagon Devices, Pullman, WA) was used to measure water activity (aW) for cookies and muffins. The instrument was calibrated with distilled water. For muffins, three muffins were put into the Cuisinart mini- food processor (model DLC-1, Windsor, NJ) and ground for 15 second on high. For the cookies, three cookies were broken up into a Cuisinart mini food processor (model DLC-1, Windsor, NJ) and were ground on low for 15 seconds and then high for 15 seconds. Three aliquots were removed from each composite sample and tested for aW for each treatment (Perry et al., 2003; Swanson, et al., 1999). Two replications were obtained for the muffins and three replications were conducted for the cookies.

Texture assessment: Muffin Texture Profile Analysis. The American Institute of Baking method for determining the textural properties of cake was adapted for muffins (Baixauli et al., 2008). A 2-cm wide center slice from 3 muffins per treatment was removed for assessment using TAX.T2 texture analyzer equipped with a 50 kg load cell (Stable Micro Systems, Haselmere, Surrey England) and Texture Expert Exceed software (version 1.20). The 75-mm disc was used to compress the crumb in the center of each sample by 50%, twice with 5 seconds between compressions. The cross arm speed was 2mm/s pre-test, 5 mm/s test speed, and 5 mm/s post-test (Baixauli et al., 2008). Data were collected form two replications.

Texture assessment: Cookie probing. Cookies' textural attributes were extracted from a time/force curve generated with a TAX.T2 texture analyzer equipped with a 50 kg load cell (Stable Micro Systems, Haselmere, Surrey England) and Texture Expert Exceed software (version 1.20). The 3mm probe attachment was used with a cross arm speed of 5 mm per second and readings were taken at 200 PPS. Each cookie was punctured nine times in a diamond shape excluding the outer 15% of the cookie (Perry et al., 2003; Swanson & Perry, 2007). Three cookies per treatment with three replications were assessed.

Overall quality assessment: Muffin standing height. To determine muffin standing height, representative muffins were sliced in half. A photocopy was made of the muffin halves and standing height was determined by measuring height of the middle of the muffin in mm (Bosman, et al, 2000; Pong et al., 1991). Three samples were assessed for each treatment. Two replications were obtained.

Overall quality assessment: Cookie spread. AACC method 10-50D was used to determine cookie spread. Three assessments per formulation were conducted. Three replications were obtained.

Consumer sensory panel: Cookies. A consumer sensory panel (n=128) was used to test acceptability of the wheat flour control and black oak acorn cookies. The panelists rated the two cookies for acceptability of appearance, flavor, texture, as well as overall acceptability on a 9-point hedonic scale where 1 was disliked extremely and 9 was liked extremely. Water, unsalted top crackers and carrots were provided as palate cleansers. The cookies were coded with three-digit random numbers and presented one at a time in a balanced order. Panelists evaluated the cookies in individual booths under cool, white florescent light. Panelists also completed a food frequency questionnaire to determine a consumer profile and buying habits.

Total phenolics

The Folin-Ciocalteu reagent method was used to determine total phenolic content (TP) of the cookies and muffins in triplicate. Samples were extracted with 50% ethanol in a ratio of 9 ml ethanol to 1 gram food sample and homogenized for 60 sec. After shaking the homogenized samples for 2 hours at 200 RPM, the samples were centrifuged at 1100 RPM for 10 min at 10ºC. Sample absorbance was determined at 765 mμ The TP was expressed in gallic acid equivalents (GAE) and determined by comparison with a standard curve (Bonoli, et al, 2004; Kobs, 2008; Naczk, et al, 1998; Singleton & Rossi, 1965; Soong & Barlow, 2004; Yang, et al, 1997). Folin-Ciocalteu reagent and bovine serum albumin used to determine total phenolics present were purchased from Sigma Chemical Company (St. Louis, MO).

Statistical analysis

SAS software version 9.1 (SAS Institute, Cary NC) was used for data analysis. Means and standard deviations were calculated, and significant differences ($p \leq 0.05$) were determined using two-way ANOVA with treatment and replication, and treatment x replication in the model statement for all non-sensory evaluations. From the fatty acid data, the total percentage of saturated, monounsaturated and polyunsaturated fatty acids present were calculated. Sensory data were analyzed using one-way ANOVA with the PROC GLM procedure ($p \leq 0.05$); Student-Newman-Kuels (SNK) was used for means separation. Frequency of response to survey questions used to profile the sensory panel was determined with PROC Freq. Simple linear regression was used to determine total phenolics; standard curves were determined with Microsoft Excel 2003 software.

Results and Discussion

Acorn meal is a novel ingredient. Many novel flours have been studied using chemical, physicochemical, and baking quality tests designed for wheat flours with success (Chinma & Gernah, 2007; Correia, et al, 2009; Qian, et al, 1998; Roccia, et al., 2006; Sindhuja, et al, 2005). The tests allow the characterization of acorn meal, and its functional characteristics in comparison to wheat flours; and, the prediction of the feasibility of acorn meal use in various wheat flour-based products. Desirable flour quality characteristics differ with end use. Therefore, while characterization of novel flours aids in selection of potential applications, baking tests are the most reliable method for determining their suitability for use in specific food systems (Pyler, 1988). Replacement of wheat flour with acorn meal in food products has the potential to increase phenolic content and provide functional health benefits. In this study, meals

were prepared from black oak acorns (*Quercus velutina)*, a red oak variety, and white oak acorns (*Quercus alba*), a white oak variety.

Acorn meal characterization

Proximate analysis. The proximate analysis of the acorn meals (table 4.3) shows that the black oak acorn meal is significantly higher ($p \leq 0.05$) than the white oak meal in fat and protein and lower in carbohydrate + ash. The all-purpose (AP) flour (White Lily, Memphis, TN), according to the miller, is 77% carbohydrate, 10% protein, and 0% fat. When compared to the all-purpose wheat flour replaced in the food system studies, the protein content of acorn meal was about 4% (black oak) and 5 % (white oak) lower. Protein quality was not assessed, although the structural proteins characteristic of wheat are not present in tree nuts. The carbohydrate + ash content of the white oak acorn meal approximates that of the all-purpose flour; whereas, the black oak meal was about 25% lower than the wheat flour, primarily reflecting the increase in the fat percentage.

Table 4.3: Proximate Analysis of Black and White Oak Acorn Meal[a]

Acorn Meal	Protein %[b]	Fat %[c]	Moisture %[d]	CHO+ Ash %[e]
White Oak	4.93± 0.04a	6.77± 0.48a	8.35± 2.33	79.95± 2.86a
Black Oak	5.88± 0.06b	30.07± 1.45b	13.10± 0.28	50.96± 1.79b

[a] n=2, Different letters within a column represent significant differences ($p \leq 0.05$) between sample means± SD using ANOVA and SNK (SAS statistical software Version 9.1)
[b] Protein analysis using Kjeldahl method
[c] Fat analysis using the Gravimetric method
[d] Moisture determined using AACC method 44-15A
[e] CHO (carbohydrate) + ash was determined as the percentage remaining after protein, fat, and moisture were determined

Fat content of the acorn meal differs significantly due to species. At 30% in the black oak species and 7% in the white oak species, both are higher than typical of wheat flour due to the defatting and processing involved in the production of all-purpose flour.

Fatty acid profile. The fatty acid profile (table 4.4) is similar to acorn oils analyzed in previous studies of various oak species' acorns (Cantos et al., 2003; Leon-Camacho, et al, 2004; Lopez & Bernardo-Gil, 2005). The dominant fatty acids found in the acorn meals were oleic (C18:1), linoleic (C18:2), and palmitic (C16:0) acids. Species differences were not found. Significant differences in the fatty acid profile due to oak species were found for 14:0, and 18:3, although low levels of both were present in both acorn meals evaluated. Specific fatty acids present in wheat flour have been shown to have little to no effect on the functional performance of flours in various food systems (Jacob & Leelavathi, 2007; Manohar & Rao, 1999; Pareyt et al., 2008).

Total mg/ g of fatty acids present differed significantly due to species ($p \leq 0.05$) and reflected the total fat determination (table 4.4) Amount of total fat in a formula contributed by all ingredients has been shown to have effects on texture, cookie spread, flavor, color, starch gel formation, and cake volume in wheat flour-based products (Bosman, et al, 2000; Kaldy, et al, 1993; Manohar & Rao, 1999; Pareyt et al., 2008; Pong, et al, 1991). The high percentage of fat in the black oak species of acorn meal will make it a greater contributor to total fat content than the wheat flour which it replaces in any food system. High levels of unsaturated fats make acorn meal more susceptible to lipid oxidation and spoilage; however, acorns are high in phenolics and tocopherols that act as antioxidants and reduce lipid oxidation reactions (Cantos et al., 2003; Leon-Camacho et al., 2004).

Table 4.4: Fatty Acid Profile[a] of White Oak and Black Oak Acorn Meal[b]

Fatty Acid	White Oak Meal	Black Oak Meal
	----------%----------	
14:0	0.05± 0.005a	0.08± 0.0003b
16:0	12.72± 0.11	10.74± 0.37
16:1	0.01± 0.003	0.34± 0.47
18:0	0.98± 0.02	1.31± 0.10
18:1	58.50± 0.10	57.87± 1.97
18:2	23.8± 0.37	26.50± 1.17
18:3	2.62± 0.06a	1.54± 0.61b
20:0	0.47± 0.06	0.66± 0.20
20:1	0.63± 0.08	0.72± 0.17
20:2	0.02± 0.01	0.01± 0.008
22:0	0.16± 0.05	0.11± 0.006
24:0	0.03± 0.003	0.03± 0.012
Total (mg/ g)	76± 6.16a	240± 6.49b
	----------% fatty acid distribution----------	
Sat	14.4± 0.24	12.9± 0.49
Mono	59.1± 018	58.9± 2.27
Poly	26.4± 0.42	28.2± 1.79

[a] Acorn meals were analyzed using gas chromatography of fatty acid methyl esters (FAME) prepared by in situ transesterfication (ISTE).
[b]n=2; means± SD followed by different letters within a row differ significantly ($p<0.05$), according to ANOVA and SNK (SAS statistical software Version 9.1)

Color. Most phenolic compounds are associated with color in plants. The black and white oak acorn meals (table 4.5) were significantly less light than the AP flour with the black oak meal being significantly less light than the white oak meal ($p \leq 0.05$).

Table 4.5: Color[a] of All-Purpose Flour and Acorn Meals[b]

Flour/Meal Type	L*	a*	b*
All-Purpose Wheat Flour[c]	91.19± 3.70a	0.21± 0.02a	4.18± 0.19a
Black Acorn Meal	29.47± 2.24b	3.24± 0.19b	7.23± 0.47b
White Acorn Meal	52.68± 0.38c	3.72± 0.10c	22.46± 0.40c

[a] Color was measured using a Minolta Spectrophotometer (Model CM-508d, Japan) calibrated using a white calibration cap (CM-A70) and open air calibration, 10-degree observer function, F6 illuminant for cool white florescent light source, with the specular component excluded. L* is a measure of lightness on a 0 to 100 scale, where 0 is black and 100 equals white, and is an indication of saturation. The reading a* measures black-green axis, where positive a* is blackness and negative a* is greenness. The reading b* is a measure of the yellow-blue axis, where positive b* is yellowness and negative b* is blueness.
[b] n=3; different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)
[c]White Lily, Memphis, TN

The higher amount of phenols in the acorn meal likely contribute to the lower L* values recorded by the spectrophotometer. The AP flour has been highly processed to be lighter, closer to white, and to have less saturation. The AP flour approximates neutral on the red/green axis (a*). The acorn meals were significantly redder than the AP flour ($p \leq 0.05$), and the white oak meal was redder than the black oak meal ($p \leq 0.05$). Although the AP flour was low on the yellow-blue axis (b*), the acorn meals were more yellow than the AP flour ($p \leq 0.05$). The white oak meal was significantly higher in yellowness than the black oak meal ($p \leq 0.05$). The higher levels of phenolics in the acorns and the processing and lower natural phenolic content of wheat likely contributed to the major differences in color of the wheat flour and acorn meals. Impacts on color within food systems are expected.

pH. pH can affect many reactions and physical properties in a formulation: starch gelatinization, browning, glass transition, foaming, leavening, protein interaction and denaturation, cookie spread and flavor reactions (Fennema, 1985). The AP flour and the black oak meal have statistically similar pH (table 4.6). Products formulated with black acorn meal rather than white acorn meal will not require adjustment of the pH through manipulation of other ingredients to minimize the impact of pH alteration in food systems.

Table 4.6: pH[a] of All-Purpose Wheat Flour and Acorn Meals[b]

Flour/Meal Type	pH
All-Purpose Wheat [c]	4.68± 0.02a
Black Acorn	4.69± 0.04a
White Acorn	5.14± 0.01c

[a] Measured using AACC method 02-52 and pH meter (Model 520A, Orion, Boston MA) Calibrated using 4.00 pH and 7.00pH buffers from Fisher Chemical labs (Fairlawn, NJ)
[b] n=3, Different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD using ANOVA and SNK (SAS statistical software Version 9.1)
[c] White Lily, Memphis, TN

Solvent retention capacity. Solvent retention capacity (SRC), AACC method 56-11, was developed to evaluate soft wheat flour quality (Ram & Singh, 2004). SRC has been used to evaluate potential quality of new wheat cultivars and novel grain flours as well (Ram & Singh, 2004; Roccia et al., 2006; Sindhuja et al., 2005; Zhang, et al, 2007). Absorption of each solvent is an indication of the characteristics of a flour constituent important in baked product quality, although the relative importance varies with baked food system. Water absorption is an indication of hydration ability. Absorption of the 5% lactic acid solution indicates the strength of the wheat flour structural protein glutenin. The extent of starch damage is indicated by the absorption of the 5% sodium carbonate solution. Pentosan characteristics are indicated by the amount of the 50% sucrose solution absorbed.

Table 4.7: Solvent Retention Capacity[a] of All-purpose Wheat Flour and Acorn Meals[b]

Flour/Meal Type	Water	Sucrose	Lactic acid	Na
	----------(%)----------			
All-purpose wheat flour[c]	63.23± 1.44a	75.85± 1.53a	64.07± 3.1a	73.36± 1.42a
Black Oak Acorn Meal	182.97± 5.09b	189.77± 3.16b	175.77± 1.56b	195.93± 2.02b
White Oak Acorn Meal	183.20± 4.02b	195.17± 2.40b	189.40± 6.08c	202.50± 3.40c

[a] Measured using a modified version of the solvent retention capacity (SRC) profile analysis AACC method 56-11 as described by Ram & Singh, 2004
[b] n=3, different letters within a column represent significant differences ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)
[c] White Lily, Memphis, TN

The solvent levels retained by the acorn meals (table 4.7) were about three times greater and statistically larger ($p \leq 0.05$) than the AP flour for all SRC tests. The black oak and white oak meals were statistically similar to each other for the water SRC and sucrose SRC. Water absorption is an important quality factor in baked products. In high-moisture baked food systems, such as cakes and muffins, high absorption values are associated with increased product yield and extension of shelf life. In cookies, a low moisture system, water absorption affects the viscosity of the dough by impacting the amount of free water available to dissolve the sugar in the formulation (Pyler, 1988). The sucrose SRC results imply that the muffin volume should be decreased, because pentosan content negatively affects cake volume due to water absorption (Kaldy, et al, 1991; Kaldy et al., 1993; Roccia et al., 2006). Similarly, cookie spread should be reduced. The white oak meal lactic acid SRC and Na carbonate SRC were significantly larger ($p \leq 0.05$) than was found for the black oak meal. The implications of these results suggest that whereas both acorn meals should decrease cookie spread due to the increased retention of water by damaged starch and proteins which limits the water available for sugars to form syrups and therefore reduces dough viscosity, the white oak acorn meal will cause an extreme decrease.

However, the effect on cookie spread could be less pronounced than expected due to the low amount of water in the cookie formula and more apparent in the muffin standing height due to the higher water levels in the muffin formula (Gaines, 2004; Roccia et al., 2006).

Particle size distribution. Particle size of flours can affect distribution in a food system. Within the particle size range found in flours, a higher percentage of small particles could mean the flour and other ingredients are more evenly distributed. A higher percentage of smaller particles could also be an indicator of more starch damage which can affect leavening, spread, appearance and texture.

The percentage of flour and meal found in the US #8 and #20 sieves were statistically similar for all samples (table 4.8); however, the standard deviations were high. During the tests seemingly smaller flour and meal particles that should have passed through the larger sieves remained in the top sieves due to static cling. Despite the susceptibility of the AP flour to static cling, a significantly ($p \leq 0.05$) larger percentage of flour passed through all of the sieves when compared to the acorn meals. The smaller percentage of finer particles in the acorn meals should indicate less damaged starch, lower pentosan levels and less meal surface area that would reduce water retention, starch swelling, and gel formation (Sasaki & Matsuki, 1998)

Table 4.8: Particle Size[a] of All-Purpose Wheat Flour and Acorn Meals[b]

Sample	On US #8 sieve	On US #20 sieve	On US #40 sieve	Thru US #40 sieve
	----------(%)----------			
All-Purpose Wheat Flour[c]	7.69± 9.64	82.86± 10.62	4.09± 0.95a	1.44± 0.26a
Black Acorn Meal	15.52± 13.04	81.59± 13.31	1.79± 0.26b	0.44± 0.17b
White Acorn Meal	2.32± 2.99	92.47± 2.83	3.12± 0.71ab	0.6± 0.06b

[a] Determined by an adaptation of CFR 137.200 part 21
[b] n=3, different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD using ANOVA and SNK (SAS statistical software Version 9.1)
[c] White Lily, Memphis, TN

High moisture formula results: Pumpkin muffin

Total phenolics. Phenolic levels in wheat flours range from 126.30-343.5 μg GAE/ g, and storage and processing of the flours dramatically decreases the phenolic content (Klepacka & Fornal, 2006; Sosulski, et al, 1982). Black oak acorn meal total phenolic (TP) content is 12 mg GAE / g and the white oak meal is 6.6 mg GAE/ g as reported by Kobs (2008). A substitution of the AP flour with acorn meal at any level would therefore increase the TP of the final food relative to the amount and type of acorn meal used. Many phenolic-ingredient interactions and reactions can occur during the processing and storage of foods that cause TP of a product to be lower or higher than the sum of the TP of the individual ingredient.

The 50% replacement of the AP flour with black oak acorn meal resulted in significantly higher ($p \leq 0.05$) levels of TP in the muffins by almost 2 mg/g GAE (table 4.9). The 25% replacement of the AP flour was significantly higher ($p \leq 0.05$) then the control, but significantly lower ($p \leq 0.05$) then the 50% replacement. Muffins with higher levels of phenolics could

contribute to a diet that has positive health benefits (Kobs, 2008). Higher TP could also affect taste, appearance, texture, and acceptability.

Table 4.9: Total Phenolics Levels of Muffins Formulated with 25% and 50% Replacement of AP Flour with Black Oak Acorn Meal[abc]

Flour/Meal Type	TP mg GAE /g
100% Wheat Flour	0.70± 0.17a
50% Acorn Meal	2.92± 0.32b
25% Acorn Meal	1.38± 0.15c

[a] n=9, Different letters within a column represent significant difference between sample means± SD using GLM with SNK ($p \leq 0.05$) SAS statistical software Version 9.1
[b] Muffins made with acorn meal replaced 50% and 25% of flour in the control formula at 14% moisture
[c] Total Phenolics were determined using the Folin-Ciocalteu reagent method

Color. Phenolic compounds can contribute to the color of many foods. Phenolics undergo physiochemical reactions and changes when heated and/or when combined with other compounds that can affect color (Klepacka & Fornal, 2006). Phenolics can affect the rates of oxidative and enzymatic browning by acting as an antioxidant or by sequestering water necessary for those color reactions to occurs.

The muffins' exterior and interior lightness (L*) decreased significantly ($p \leq 0.05$) with each treatment as acorn meal increased in the formula (table 4.10). The redness (a*) and yellowness (b*) muffin exterior values significantly decreased as the acorn meal to wheat flour ratio increased ($p \leq 0.05$). Similar trends were seen for the muffin crumb, although redness values were more similar. The interior a* value for the 25% acorn muffin was statistically similar to both the control and the 50% acorn muffin.

Table 4.10: The Color[a] of Pumpkin- Spice Muffins Formulated with 25% and 50% Replacement of Wheat Flour with Black Oak Acorn Meal[bc]

	Exterior			Interior		
Muffin Type	L*	a*	b*	L*	a*	b*
100% Wheat Flour	48.04± 0.87a	8.85± 0.45a	34.32± 2.10a	49.97± 3.33a	7.14± 1.07a	35.41± 1.63a
50% Acorn Meal	31.23± 4.42b	6.62± 0.45b	15.30± 1.69b	28.99± 2.36b	6.34± 0.39b	18.00± 1.42b
25% Acorn Meal	35.89± 1.76c	7.18± 0.31c	19.04± 1.34c	36.15± 2.09c	6.53± 0.48ab	23.75± 1.29c

[a] Color was measured using a Minolta Spectrophotometer (Model CM-508d, Japan) calibrated using a white calibration cap (CM-A70) and open air calibration, 10-degree observer function, F6 illuminant for cool white florescent light source, and the specular component was excluded. L* is a measure of lightness on a 0 to 100 scale, where 0 is black and 100 equals white, and is an indication of saturation. The reading a* measures red-green axis, where positive a* is redness and negative a* is greenness. The reading b* is a measure of the yellow-blue axis, where positive b* is yellowness and negative b* is blueness.
[b] Different letters within a column represent significant difference between sample means± SD using GLM with SNK ($p \leq 0.05$) SAS statistical software Version 9.1
[c] Muffins made with Black Oak acorn meal replaced 50% and 25% of AP flour (White Lily, Memphis, TN) in the control formula at 14% moisture

Maillard browning, oxidative reactions, fat crystal formation, and availability of water for chemical and enzymatic reactions due to starch gelatinization and phenolic interaction could all be affected by flour type and the replacement of the wheat flour with acorn meal which would influence color (Fennema, 1985; Klepacka, 2006). These effects on product color are less intense than might be expected, and likely reflect the presence of the pumpkin in the muffin formulation. Other ingredients and their interactions may also be influential.

pH. The pH of food systems can have profound effects on the final product. A difference in acidity can influence taste and flavors. Changes in pH can alter protein and starch structure, swelling and gelling properties. Enzymatic and non-enzymatic browning reaction rates can be influenced by pH. Cookie spread and leavening reactions are also influenced by the pH of a food system.

The 100% wheat flour muffin was significantly ($p \leq 0.05$) less acidic than the two muffins that used black oak acorn meal (table 4.11); however, despite the statistical significance, the difference in the mean pH is small and would likely have an unnoticeable affect on rheological and organoleptic properties of the muffins (Christensen, et al, 1987).

Table 4.11: Pumpkin-spice Muffin Batters Formulated with 25% and 50% Replacement of Wheat Flour with Black Oak Acorn Meal[a]: pH and Specific Gravity

Flour/Meal Type	Batter pH[b]	Specific Gravity[c]
100% Wheat Flour	6.76± 0.03a	1.16± 0.01
50% Acorn Meal	6.72± 0.03b	1.18± 0.11
25% Acorn Meal	6.71± 0.03b	1.13± 0.02

[a] Muffins made with Black Oak acorn meal replaced 50% and 25% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture
[b] Measured using pH meter (Model 520A, Orion, Boston MA) calibrated using 4.00 pH and 7.00pH buffers from Fisher Chemical labs (Fairlawn, NJ)
[c] Determined by the difference in weight of a 50 ml beaker filled with distilled water and the same beaker filled with batter
[d] n=9, Different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)

Specific gravity. Specific gravity is measured in dough and batters to compare the amount of air incorporated into the food system that will then contribute to leavening, and ultimately volume. The creaming of sugar and butter to form air pockets in the procedure of the formulas is the main determinant of air incorporation and volume pre-baking. Specific gravity was statistically similar across all treatments for the muffins (table 4.11). The replacement of wheat flour with acorn meal did not affect the amount of air incorporated into the batter during the mixing steps of the muffin procedures.

Water activity. Water activity (aW) is a measure of water available for reactions. Muffins have a high aW (Table 4.12)because of the greater amount of water in the formula, Microbial growth can occur at the water activities reported and chemical reactions important in the staling process can occur.. However, no significant differences due to formulation were found. The shelf-life may actually be extended due to the greater amounts of phenolic compounds found in the acorn meal; the phenolics would prevent oxidative reactions that could occur.

Table 4.12: Water Activity (aW)[a] of Pumpkin-Spice Muffins Formulated with 25% and 50% Replacement of Wheat Flour with Black Oak Acorn Meal[bc]

Flour/Meal Type	aW
100% Wheat Flour	0.905± 0.007
50% Acorn black oak	0.909± 0.006
25% Acorn black oak	0.909± 0.006

[a] Aqua Lab (Model CX-2, Decagon Devices, Pullman, WA) was used to measure water activity
[b] n=9, No significant differences were found
[c] Muffins made with acorn meal replaced 50% and 25% of flour (White Lily, Memphis, TN) in the control formula at 14% moisture

Texture Analysis

Texture profile analysis (TPA) was performed to determine hardness, springiness and cohesiveness of the muffins (Table 4.13).

Table 4.13: Texture Analysis[a] of Muffins Formulated with 25% and 50% Replacement of Wheat Flour with Black Oak Acorn Meal[bc]

Flour/Meal Type	Hardness (g)	Springiness	Cohesiveness
100% Wheat Flour	1068.39± 83.77a	755.69± 46.21a	977.60± 55.65a
50% Black Oak Acorn	1237.99± 89.32b	803.12± 56.91b	977.34± 59.47a
25% Black Oak Acorn	865.56± 67.97c	626.64± 46.91c	797.03± 58.46b

[a]2 cm wide center slice from 3 muffins per treatment was tested using the texture analyzer TAX.T2 (Stable Micro Systems, Haselmere, Surrey England) equipped with Texture Expert Exceed software (version 1.20), and a 50 kg load cell. The 75 mm disc was used to compress the sample by 50% twice The crossarm speed was 2mm/s pre-test, 5 mm/s test speed, and 5 mm/s post-test.
[b] n=9, different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)
[c] acorn meal replaced 50% and 25% of the all-purpose wheat flour (White Lily, Memphis, TN) in the control formula on a 14% moisture-basis

The 50:50 ratio of black oak acorn meal to AP flour was the hardest of the three muffin treatments. The control and 25:75 ratio were significantly less hard then the 50:50, and the 100% wheat flour was harder than the 25:75 ($p \leq 0.05$). The 25:75 muffin was the significantly less springy than the 100% wheat flour and 50:50 muffins. The 50:50 muffins were significantly springier than the 100% wheat flour ($p \leq 0.05$). The 100% wheat flour and 50:50 muffins were statistically similar in cohesiveness while the 25:75 was significantly less cohesive ($p \leq 0.05$). The 25:75 muffins had the lowest values for all attributes; therefore, the 25:75 was the least hard and springy.

Standing height.

The standing height (table 4.14), a measurement of volume, was used as an overall measure of quality. The specific gravity, starch gelling, proteins, fat, and sugars can affect muffin standing height.

Table 4.14: Standing Height[a] of Pumpkin-spice Muffins Formulated with 25% and 50% Replacement of Wheat Flour with Black Oak Acorn Meal[bc]

Muffin Type	Height (mm)
100% Wheat Flour	38.22± 1.64a
50% Black Oak Meal	35.55± 1.59b
25% Black Oak Meal	38.22± 1.20a

[a] Determined by measuring the height of the center of the Muffin when sliced in half.
[b] n=9, Different letters within a column represent significant difference between sample means± SD using GLM with SNK ($p \leq 0.05$) SAS statistical software Version 9.1
[c] Muffins made with acorn meal replaced 50% and 25% of flour (White Lily, Memphis, TN) in the control formula at 14% moisture

With 50% replacement of all-purpose wheat flour with black oak acorn meal (table 4.14), volume of the 50% acorn meal muffin was significantly reduced; no significant differences in volume were found when substitution levels were limited to 25%. The reduction in height may be due to the specific functional characteristics of the black oak acorn starch and protein, which is congruent with the SRC results (Kaldy et al., 1991; Kaldy et al., 1993; Roccia et al., 2006).

Low moisture product results: Spice cookies

Fifty percent of the flour in all spice cookie formulations was cake flour. In the control, the remaining percentage was all-purpose flour. Only the all-purpose flour was replaced when the spice cookies were reformulated with acorn meal; 100% of the all-purpose flour in the control formulation was replaced regardless of acorn meal species. Spice cookies were reformulated with both black and white acorn meals.

Total phenolics. Incorporation of acorn meal regardless of source increased the total phenolics content of the cookie (table 4.15) with increases reflective of the levels found

in the meals (Kobs, 2008) incorporated in each formulation. At the 50% substitution levels, white oak meal incorporation increased total phenolics nearly 2 times, with black oak meal increasing total phenolics more than 3 times.

Table 4.15: TP Levels of Cookies made with Wheat Flours and Acorn Meal[abc]

Flour/Meal Type	TP mg GAE /g
All-purpose Wheat Flour	2.67± 0.09a
Black Oak Meal	9.57± 3.28b
White Oak Meal	4.21± 0.36a

[a] n=9, different letters within a column represent significant difference ($p \leq 0.05$) between sample means ± SD according to ANOVA and SNK (SAS statistical software Version 9.1)
[b] acorn meal replaced 100% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture
[c] Total phenolics were determined using the Folin-Ciocalteu reagent method

When compared to the cookie dough, baked cookies have been reported to have higher total phenolics levels (Kobs, 2008), likely due to the reduction in the moisture content. However, phenolic interactions with other ingredients are facilitated by the heating process, potentially affecting bioavailability.

Cookie dough tests: Specific gravity and pH. The specific gravity of the wheat cookie dough was similar to the acorn meal cookies (table 4.16), indicating the dough retained similar levels of the air incorporated during the mixing process. pH of the control formulation was adjusted by incorporating cake flour (pH 4.23± 0.05), a non-variable ingredient, in the formulation. The chlorination process to which cake flour is subjected lowers the pH and impacts functional performance of the flour in food system. The pH of the cookie dough was statistically similar for all treatments (table 4.16), despite

significant differences in the pH of the acorn meals (table 4.6), suggesting a lack of pH effects due to acorn meal on cookie quality.

Table 4.16: Specific Gravity[a] and pH[b] of Cookie Dough Formulated with Wheat Flour and Acorn Meals[c]

Flour/Meal	Specific Gravity[d]	pH[d]
All-purpose Wheat Flour	1.13± 0.13	6.85± 0.15
Black Oak Acorn Meal	1.07± 0.02	6.95± 0.02
White Oak Acorn Meal	1.11± 0.04	6.95± 0.10

[a] Determined by the difference in weight of a 50 ml beaker filled with water and the same beaker filled with dough
[b] Measured using pH meter (Model 520A, Orion, Boston MA) calibrated using 4.00 pH and 7.00pH buffers from Fisher Chemical labs (Fairlawn, NJ)
[c] Cookies made with acorn meal replaced 100% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture-basis
[d] n=9, sample means± SD did not differ significantly ($p>0.05$) according to ANOVA (SAS statistical software Version 9.1)

Cookie color. All ingredients in the formula impact color of the baked product directly and indirectly. The cookies' surface measured significantly darker ($p \leq 0.05$) with incorporation of either acorn meal than was found in the control cookie (table 4.17), although species effects were found. The black oak acorn cookie was lighter, than was the white oak despite the black oak meal being darker then the white oak meal (table 4.5)

Despite the higher redness values of the acorn meals, the acorn cookies were significantly less red (a*) than was the control cookie with the white oak acorn cookie exhibiting least detectable redness (a*) axis ($p \leq 0.05$). Yellowness intensity (b*) for the control and the black oak acorn cookie were statistically similar and significantly higher than what was found for the white oak acorn cookies.

Table 4.17: Spice Cookie Color[a] Formulated with Wheat flour and Acorn Meals[bc]

Flour/Meal Type	L*	a*	b*
All-purpose Wheat Flour	44.49± 1.33a	7.9± 0.26a	31.73± 1.04a
Black Oak Acorn	38.86± 1.84b	7.23± 0.64b	29.54± 12.06a
White Oak Acorn	34.62± 1.13c	5.97± 0.33c	20.39± 0.92b

[a] Color was measured using a Minolta Spectrophotometer (Model CM-508d, Japan) calibrated using a white calibration cap (CM-A70) and open air calibration, 10-degree observer function, F6 illuminant for cool white florescent light source, with the specular component was excluded. L* is a measure of lightness on a 0 to 100 scale, where 0 is black and 100 equals white, and is an indication of saturation. The reading a* measures red-green axis, where positive a* is blackness and negative a* is greenness. The reading b* is a measure of the yellow-blue axis, where positive b* is yellowness and negative b* is blueness.
[b] n=9, different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)
[c] Black or white acorn meal replaced 100% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture-basis

In addition to inherent color of the ingredients, protein interactions and anti-oxidative properties of phenolics, a difference in fatty acid levels, and a difference in carbohydrate and starch composition, and the chemical reactions occurring in the batter influence color of the final product (Fennema, 1985; Klepacka et al, 2006).

Cookie Texture Analysis: Probing. The puncture test (table 4.18) was used to determine the force necessary for a probe to break the surface and travel though the cookies. Hardness is a measure of the force to penetrate though the cookie and toughness is the area under the time/force curve (Perry, et al, 2003; Swanson & Perry, 2007).

Table 4.18: Texture Analysis[a] using Nine Point Puncture Test of Spice Cookies Made with Wheat Flours and Acorn Meals[bc]

Flour/meal Type	Hardness (g)	Toughness (g/s)
All-purpose Wheat Flour	2.16± 1.16a	1.73± 0.80a
Black Oak Acorn	1.20± 0.52b	0.97± 0.40b
White Oak Acorn	1.53± 0.88c	0.82± 0.38b

[a] Texture analyzer model TAX.T2 (Stable Micro Systems, Haselmere, Surrey England) equipped with Texture Expert Exceed software (version 1.20), with a 50 kg load cell, a 3 mm probe attachment, crossarm speed of 5 mm per second, and readings taken at 200 PPS
[b] n=9, different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)
[c] acorn meal replaced 100% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture-basis

The wheat cookie was significantly harder and tougher (Table 4.18) than both acorn cookies; acorn species specific differences were found for hardness ($p \leq 0.05$). The acorn cookies are less hard and tough possibly due to the higher fat content of the acorn meal or a difference in starch profile of the meal compared to the wheat flour (Pareyt et al., 2008). Protein strength may have played a role as well.

Cookie spread. Cookie spread is used as an overall measure of flour quality. Based on the SRC assessment (table 4.7) of the acorn meals, the acorn cookie spread should have been smaller than was found for the wheat flour cookies (table 4.19). However, the wheat flour cookie had the lowest spread factor (table 4.19), with the extent to which the acorn cookies spread influenced by acorn species ($p \leq 0.05$). Smaller spread factors are associated with higher protein content, more damaged starch and pentosan levels, lower levels of fat or sucrose, lower pH, and increased protein-phenolic-starch interactions.

Table 4.19: Spread Factor[a] for Spice Cookies Made with Wheat Flours and Acorn Meal[bc]

Flour/Meal Type	Spread Factor
Wheat Flour	93.05± 3.71a
Black Oak Acorn	111.25± 3.87b
White Oak Acorn	170.62± 53.07c

[a] Cookie spread was determined by AACC method 10-50D
[b] n=9, different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)
[b] acorn meal replaced 100% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture-basis

The increase in spread of the acorn cookies could be due to lower levels and types of starches, level of damaged starch granules and heat treatment during the leaching and drying process; higher fat content of the acorn meal could increase spread as well (Chinma & Gernah, 2007; Correia et al., 2009; Donelson & Gaines, 1998; Gaines, 2004; Pareyt et al., 2008; Sindhuja et al., 2005; Singh, et al, 2003). Hydrophobic properties of the meal can also affect cookie spread; the increase in hydrophobicity allows for more available water to form syrup with sugars thereby decreasing viscosity and increasing spread (Donelson & Gaines, 1998; Leon-Camacho et al., 2004). pH and specific gravity could also affect spread, but the pH and specific gravity of the wheat flour and acorn meal cookie dough were not different. The larger spread factor found with acorn meal incorporation means the acorn cookies were thinner and larger in diameter. Although higher spread factors can be associated with higher quality, spread factors that are too large can produce an undesirable appearance and alter texture, potentially impacting acceptability (Roccia et al., 2006).

The contradictory results in flour/meal quality tests and baking system evaluations imply that the SRC is not a good predictor of acorn meal functional performance in these food systems. Previously, presence of other compounds such as natural occurring

emulsifiers like phospholipids in novel flours (Sayed Razavi, et al, 1996) or fat levels that differ from the wheat flour control have been reported to impact the suitability of this flour quality test (Kaldy et al., 1993; Manohar & Rao, 1999; Pareyt et al., 2008).

Water activity. Water activity (aW) (table 4.20) is a measure of water available for chemical reactions or to support microbial growth. Cookies generally have a low aW due to the low water and high sugar levels in formulas. The lower aW makes cookies generally means that cookies have relatively long shelf life.

Table 4.20: Water activity (aW)[a] of Cookies made with Wheat Flour and Acorn Meal[bc]

Flour/Meal Type	aW
Wheat Flour	0.37± 0.04
Black Oak Acorn	0.38± 0.03
White Oak Acorn	0.35± 0.06

[a] Aqua Lab (Model CX-2, Decagon Devices, Pullman, WA) was used to measure water activity
[b] n=9, no significant differences were found
[c] Cookies made with acorn meal replaced 100% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture-basis

The water activity was not significantly different for the cookie treatments SRC results might suggest a greater amount of water would be sequestered by the acorn meals. The results of the aW test suggest that acorn meal would have no effect on shelf-life of the cookies.

Consumer sensory panel tests. The black oak acorn cookie was selected for consumer sensory evaluation. Non-sensory assessments of baking quality suggest that the black oak acorn meal equaled or exceeded the white oak acorn meal in functional performance.

Nut size and the decreased number of nuts lost to insect damage and rotting increased the ease of processing acorn meats into meal. In addition, the higher total phenolics content in the black oak acorn meal positively impacted the total phenolics present in the formulation and has the potential to have a greater impact on dietary intake if these cookies are acceptable to consumers.

The consumer panel consisted of 128 people that were asked to judge a wheat spice cookie control and a black oak acorn spice cookie in regards to acceptability of appearance, texture, flavor, and overall acceptability on a 9-point hedonic scale where 1 was dislike extremely and 9 was liked extremely. 73% of the panelists ate cookies several times a month or more. 82% of the panelists were female and 18% were males. All age groups were represented on the panel, but the majority of panelists were 18-27 years old (85%) and 28-35 (10%).

Overall acceptability of both formulations was rated above mid-point on the hedonic scale, although the control cookie rated higher in all categories of acceptability except for texture (table 4.21).

Table 4.21: Consumer Acceptability[a] of Spice Cookies Made with Wheat Flours and Black Oak Acorn Meal[bc]

Cookie treatment	Appearance	Texture	Flavor	Overall
Control (all-purpose wheat flour)	4.53± 1.57a	5.32± 1.96	6.75± 1.64a	5.86± 1.66a
Black Oak Acorn	4.01± 1.49b	4.94± 1.83	5.78± 1.94b	5.26± 1.72b

[a] panelists rated the two cookies for acceptability of appearance, flavor, texture, and overall acceptability using a 9 point hedonic scale with 1 being disliked extremely and 9 being liked extremely, cookies were coded with random three digit numbers and given to panelists one at a time in a balanced order

[b] n=128, different letters within a column represent significant difference ($p \leq 0.05$) between sample means± SD according to ANOVA and SNK (SAS statistical software Version 9.1)

[c] Black oak acorn meal replaced 100% of AP flour (White Lily, Memphis, TN) in the control formula on a 14% moisture-basis

The panelists found no difference in the acceptability of the texture ($p > 0.05$) even though textural differences were identified in the instrumental tests (table 4.18). Despite significance, the difference in treatment means for all attributes was never greater than one. So, although some statistical differences occurred, the differences exhibited were not vast. The darker color and size of the acorn cookie due to the meal's darker L* value (table 4.5) and greater spread factor (table 4.19) could account for the lower appearance acceptability of the acorn cookie. Panelists' comments made note of the darker appearance. The darker color could have given the perception of over-cooking. The increase in phenolics could have influenced the acceptability of the flavor in the acorn cookie. Most phenolic compounds contribute to flavor. Phenols can contribute to aromatic compounds that are recognized as buttery, citrus, earthy, nutty, herbaceous, and caramel. The flavor acceptability of the acorn cookie was less ($p \leq 0.05$) than the control but was still on the more acceptable end of the scale (5.78± 1.94) with 70% of the panelists ranking the flavor as a 5 or better. Overall acceptability of the acorn cookie, although less then the control ($p \leq 0.05$), was ranked 5 or better by 66% of the panelists and the control cookie was ranked 5 or better by 80% of the panelists for overall acceptability. Scores (Table 4.23) for appearance and flavor acceptability, suggest that the differences in overall acceptability with black acorn meal incorporation are most likely due to the appearance effects rather than flavor effects.

The consumer sensory panelists were asked to complete a survey relating the importance of certain food claims in the selection of foods the panelists purchase (figure 4.1).

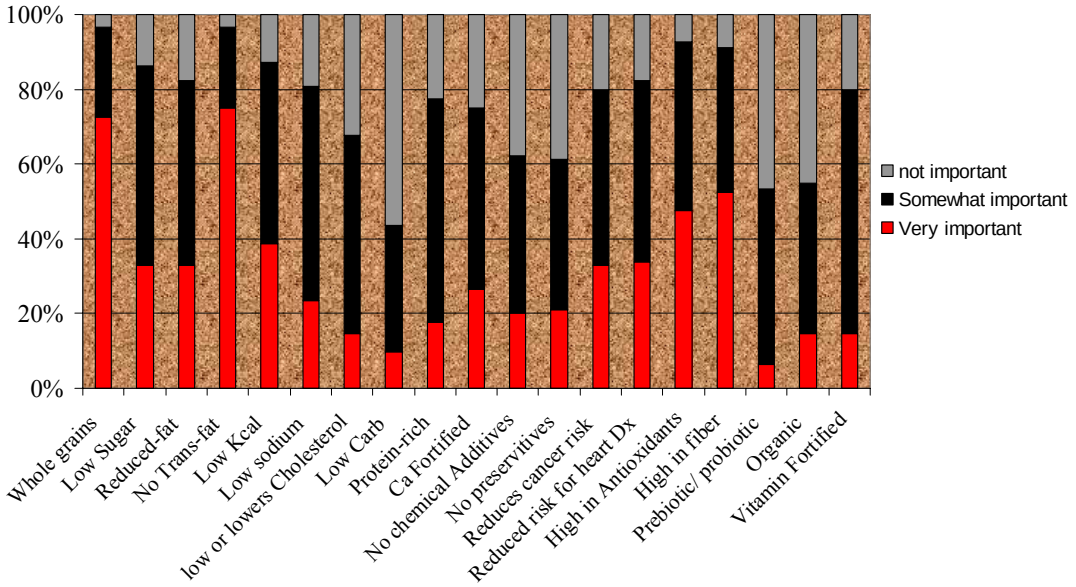

Figure 4.1: Consumer Panel Survey of the Importance of Food Claims in Regards to Selecting Food Items for Purchase[ab]

[a] n=124, panelists were asked how important each claim was when selecting foods to buy

[b] reported as percentage of panelists that responded very important, somewhat important, or not important

The claims that more than 50% of the panelists responded were very important included: whole grains, no trans-fat, and high in fiber (figure 4.1). Low-carb was the only claim that more than 50% of panelists indicated was not important to some extent. A claim of "high in antioxidants" was somewhat to very important in the foods selected for purchase by 115 of 124 (93%) panelists.

The substitution of the black oak acorn meal for all-purpose flour resulted in a cookie that was high in phenolics, an antioxidant; two cookies (one 28 serving) would provide almost 300mg of total phenolics. When queried specifically about the influence of an antioxidant claim on a cookie (figure 4.2), 90 of the 124 respondents indicated that an antioxidant claim on a cookie would positively influence the consumption of the cookie.

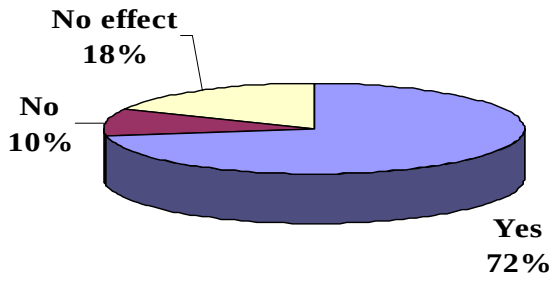

Figure 4.2: Consumer Panel Survey of the Influence of an Antioxidant Claim on Consumption of Cookies[ab]

[a] n=124, Panelists were asked, Would you be more inclined to consume a cookie that carried an antioxidant claim?

[b] Reported as the percentage of panelists who answered Yes, No, or No effect

Conclusions

Potential for the use of meals prepared from the black oak and white oak acorns as a wheat flour substitute were studied. Meals from both acorn sources were characterized; analysis revealed variety differences in proximate compositions, pH, color and Solvent Retention Capacity. Both acorn meals had a fatty acid profile high in monounsaturated fatty acids conducive to a healthy diet. Although fatty acid profiles did not differ with acorn species, fat levels did vary. Acorn species with higher fat percentages have the potential to contribute additional calories to a baked food product, because the meal accounts for a large percentage of the ingredients on a formula-weight-basis. For the two acorn meals studied, those higher in fat were also higher in total phenolics. Acorn meal has a high antioxidant potential due to the high levels of phenolic compounds. From a functional performance perspective, the presence of the phenolics should decrease lipid oxidation, flavor deterioration, and staling in food systems. In addition, the high levels of total phenolics found in the acorn meal may allow foods formulated with acorn meals to be categorized as functional foods. Health benefits have

been attributed to the daily consumption of functional foods high in phenolics (Hasler, 1998; Kobs, 2008). These consumers expressed interest in foods that have higher antioxidants and indicated that they would be more inclined to consume cookies that carried an antioxidant claim, suggesting the importance of labeling. Based on characterizations of the two acorn meals, those milled from the black oak acorn, a red oak species, appears to have the most potential in food systems.

The cookies and muffins formulated with acorn meal exhibited some differences in texture, appearance, and flavor when compared to the cookies and muffins made with 100% wheat flour. However, baking quality tests suggest that overall, black oak acorn meals could be successfully substituted for up to 50% of the total wheat flour present in low- moisture baked products in which soft wheat flours are typically used. In high moisture food systems, a 25% replacement seems to have no effect or a beneficial effect on instrumental tests associated with organoleptic properties in pumpkin-spice muffins, the high-moisture food evaluated. The 50% level of replacement of wheat flour with black acorn meal seems to have a greater effect on quality parameters of the food formulations, although this level was found to be acceptable when incorporated in cookies, a low-moisture food system. Consumer sensory evaluation of high-moisture baked products should be conducted to determine if the effects of wheat flour replacement with acorn meal are deleterious to acceptability and at what replacement level. Further reformulation efforts may also improve acceptability of the spice cookies as well.

Several areas of acorn meal characterization need to be studied further. The processed acorn meal's individual phenolic compounds should be identified. Sterol

content of the acorn meal should be determined. Further testing to verify the suitability of the SRC quality assessment should be performed. In this study, the SRC results did not accurately reflect the functional performance of the acorn meal in either low or high moisture food systems. Acorn meal replacement for soft wheat flours should be investigated in other food systems. The tests performed in this experiment should be repeated in following years to show if any characteristics are variable from year to year.

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6. Conclusions

Although acorns have had an important role in the human diet for thousands of years where-ever the oak tree has grown, they are not widely used today as food or food ingredients despite extensive availability. Acorn meal has a high antioxidant potential due to the high levels of phenolic compounds and tocopherols present. These antioxidants function in food products to decrease lipid oxidation, flavor deterioration, and staling. The high total phenolics present in the acorn meal also have added health benefits, and levels present would potentially allow foods formulated with acorn meal to be categorized as functional foods; dietary incorporation would increase intake of phenolics (Hasler, 1998; Kobs, 2008). Incorporation in a wide variety of popular food products would facilitate increased consumption on a daily basis.

In this study, meals were prepared from black oak acorns (*Quercus velutina)*, a red oak variety, and white oak acorns (*Quercus alba*), a white oak variety. Total phenolic levels in leached and processed acorn meals were 39 mg GAE/g in black oak meal and 6.6 mg GAE/g in white oak meal (Kobs, 2008). In Phase 1 of this study, the meals from both acorn sources were characterized; proximate composition, fatty acid profile, particle size distribution, pH, color and solvent retention capacity were determined. The tests suggested that acorn meals were most suited as a substitute for soft wheat flour. Therefore in phase 2, baking tests were conducted on muffins and cookies. In these baked products, soft wheat flour is preferred; formulations differ in moisture levels. Cookies are a low-moisture system, whereas, muffins are a high-moisture system.

Specific cookie and muffin formulations selected for the baking tests were based on the contribution of individual ingredients to the total phenolics present as well as the presence of flavors that would aid in masking any bitterness or astringency associated with the acorn meal incorporation. Impacts on color were also considered. A spice cookie and pumpkin spice muffin were evaluated. Quality assessments included determination of the specific gravity and pH of the cookie dough and muffin batters. After baking, color and water activity were determined. Finally, an instrumental assessment of texture and overall quality appropriate for each product was conducted. Level of total phenolics present in each baked product was determined. In the muffin system, 25 and 50% replacement of the flour with black oak acorn flour was investigated. In the cookie system, 50% of the total flour was replaced with either white oak or black oak meals.

The cookies and muffins made with acorn meal exhibited some differences in texture, appearance, and flavor when compared to the cookies and muffins made with 100% wheat flour, but overall the acorn meal-based foods met quality standards and successful reformulation was achieved. The higher level of replacement with acorn meal seems to have more effect on quality in food formulations that contain high levels of water (muffins); however, a 25% replacement seems to have no effect or a beneficial effect on instrumental tests associated with organoleptic properties. The 50% level of replacement of wheat flour with acorn meal appears to have a greater effect on quality parameters of the food formulations, although this level was found to be acceptable when incorporated in cookies, a low-moisture food system.

In this study, the black oak acorn cookie was selected for consumer sensory evaluation. Non-sensory assessments of baking quality suggested that the black oak acorn meal equaled or exceeded the white oak acorn meal in functional performance in the spice cookie. Further, nut size of the black oak acorn variety and the decreased number of nuts lost to insect damage and rotting increased the ease of processing acorn meats into meal. In addition, the higher total phenolics content in the black oak acorn meal positively impacted the total phenolics present in the formulation and had the potential to have a greater impact on dietary phenolics intake if the cookies were acceptable to consumers. Consumer sensory panelists (n=128) rated the overall acceptability of the spice cookie in which black oak acorn meal replaced 50% of wheat flour in the acceptable range of the hedonic scale. Of the acceptability attributes (appearance, flavor, texture and overall) evaluated, appearance was rated lowest. Texture acceptability did not differ from the wheat flour control. Ratings for the acorn cookie for all acceptability attributes were within one unit of the control cookie. Additional reformulation efforts may further improve acceptability of the spice cookies. Replacement of the wheat flour with the acorn meal increased the total phenolic content of the spice cookies more than 3 times. Consumer panelists expressed an interest in foods that have higher antioxidant levels and indicated that they would be more inclined to consume cookies that carried an antioxidant claim.

This study was limited to two acorn varieties, one a white oak species, and the other a black oak species. Meals prepared from other acorn varieties may equal or exceed the functional performance of the varieties investigated in this study. In addition, further characterization of the acorn meals investigated in this study should be done. The

processed acorn meal's individual phenolic compounds should be identified and variability due to species and variety should be determined. Further testing of the suitability of the solvent retention capacity assay for assessing quality of this novel flour should be performed. Consumer sensory evaluation of high-moisture baked products should be conducted to determine if the effects of wheat flour replacement with acorn meal are deleterious to acceptability and at what replacement level. Non-sensory results suggest black oak acorn meal can successfully replace at least 25% of the wheat flour in the formulation. Other soft wheat flour-based foods should also be evaluated for potential acorn meal replacement. Year-to year differences in acorn meal functionality should also be investigated. Finally, even though acorn meal has a fatty acid profile high in monounsaturated fatty acids and other lipid related compounds that are conducive to a healthy diet, acorn varieties with higher fat percentages have the potential to contribute additional calories to a baked food product because the meal accounts for a large percentage of the ingredients on a formula-weight-basis. For the two acorn meals studied, those higher in fat were also higher in total phenolics. Whether this relationship exists across all potential acorn varieties should be explored.

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7. Appendices

Appendix A: Proposed Mechanisms for the Beneficial Health Effects of Phenolics

The phenolic compounds and reduced compounds may activate or limit gene expression of pathways that influence risk for disease and produce natural antioxidant enzymes (Yeh, et al, 2009). Phenolics can act as pro-oxidants to regenerate vitamin C and E Although phenolics were once thought as anti-nutrients due to the protein binding properties of the compound, phenolic protein binding to signaling pathways and to DNA may be the reason for some of the health benefits seen from some phenolic compounds (Chen, et al., 2007; Fang, et al., 2007; Faried et al., 2007; He, et al., 2008; Neto, et al., 2008; Wang, et al., 2008; Yeh et al., 2009)

Phenolic Effect on ROS

Phenolic compounds are important in humans' diet to reduce reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced by normal biochemical functions in the body and by environmental factors (Halliwell, et al., 2005; Kahkonen et al., 1999; Kruk, et al., 2005). The structure of phenolic compounds is the reason for the increased ability to scavenge free radicals. Phenolics' ability to perform hydrogen atom transfer (HAT) or electron transfer (ET) to free radicals with greater propensity than tocopherols and ascorbic acid is what makes phenolics such potent antioxidants. Free radicals are compounds with unpaired electrons that exist autonomously. The unpaired electrons make the compound highly reactive with a relatively brief half-life. Reactive oxygen species (ROS), superoxide anion radical ($O_2^{\bullet}$), hydrogen peroxide(H_2O_2),

hydroxyl radical ($HO^{\bullet}$), and singlet oxygen (O), and reactive nitrogen species (RNS) are highly reactive free radicals produced during natural functions in cells and by exogenous pathways such a pollutants radiation. ROS, although important for stimulation of some protective cellular pathways, can cause damage to membranes, DNA fragmentations, lipid oxidation, cellular mutations, and inflammation. Phenolic compounds contain –OH groups that participate in redox reactions scavenging ROS and RNS. The phenolic then becomes a free radical, but is more stable than the ROS or RNS so the phenol is less likely to cause damaging oxidation of other molecules (Bayr, 2005; Diplock et al., 1998).

Cardio-protective Effects of Phenolics

CVD prevention can be linked to increased consumption of fruits and vegetables which contain compounds with antioxidant activity and hypolipidimic activity such as dietary phenolics (Jiang & Dusting, 2003). The exact mechanism by which increased fruit and vegetable consumption reduces risk for CVD is still being debated and studied (Blomhoff, 2005). Antioxidants were once thought to be directly related to the reduction of oxidative damage that caused elevated risk for CVD, but recent intervention studies show limited or no relation to the increase of individual antioxidants by supplementation or diet and increase in plasma antioxidant potential (Blomhoff, 2005). A direct reduction in oxidative stress by antioxidants has been the proposed mechanism by which these compounds work. The antioxidant would directly undergo HAT or ET to reduce the ROS or RNS and prevent lipid oxidation, LDL oxidation, and lower oxidative stress (Bayr, 2005). While this does occur and is an important mechanism in maintaining homoeostasis, the plasma concentrations and bioavailability suggest that free-radical

scavenging is not the main mechanism behind the cardio-protective nature of phenolic compounds.

New mechanisms have been proposed by which phenolics work after studying the effects of phenolic compounds on gene expression and cell signaling in plants and animals. Alterations of cell signaling and gene expression are thought to be ways phenolic compounds can influence plasma antioxidant potential as well as many other biochemical reactions and pathways in the body (Kluth, et al., 2007; Yeh et al., 2009). Several phenols affect redox signaling that can produce phase-II detoxification proteins and contribute to the plasma antioxidant potential. Nuclear redox factor (Nrf2) is a transcription factor that binds to nuclear factor erythroid derived 2(NF-E2) binding sites (Rahman, et al., 2006; Yeh et al., 2009). NF-E2 is part of the antioxidant response elements (ARE) that are regulatory sequences part of phase II detoxification genes. ARE induces production of antioxidant enzymes under oxidative stress. Electrophilic compounds such as certain phenolic compound can elicit ARE activation too (Rahman et al., 2006). Several phenolic compounds have been reported to affect the production of heme oxygenase-1 (HO-1). HO-1 offers defense against oxidative stresses and degrades heme, and is a redox sensitive protein made by means of the ARE (Rahman et al., 2006; Yeh et al., 2009).

In study done by Yeh et. al. (2009), the effects of different phenolic acids on the production on cardio-protective antioxidant enzymes: CuZn Superoxide dismutase (CuZnSOD), Glutathione Peroxidase (GPx), catalase (CAT), and HO-1 were examined. Male Spraque-Dawley rats were given daily oral amounts (100mg/ kg body weight) of four different phenolic acids (gallic acid, ferulic acid, p-coumaric acid, and gentisic acid)

for fourteen consecutive days. The animals were then sacrificed, the hearts removed, and processed to assay enzyme activity, glutathione oxidation, oxygen radical absorbance capacity (ORAC), and RT-PCR to show level of antioxidant enzyme gene expression. Gallic acid, ferulic acid, and p-coumaric acid were found to increase levels of all enzymes' activity in the heart tissue ($p<0.05$). The mRNA expression of the enzymes was increased relative to the increase in enzyme activity, but gentisic acid also increased mRNA expression ($p<0.05$). The ORAC value of the heart homogenate was significantly increased by gallic acid, ferulic acid, and p-coumaric acid ($p<0.05$). Gallic acid increased the ORAC value by 54.3%. Level of Nrf2 protein gene expression was shone to increase with phenolic acid treatment. The results of this study show a marked effect of phenolic acids on antioxidant enzymes in heart tissue. The tissue could handle more oxidative stress and reduce damage to the heart. The dose is quite large, but this illustrates a possible pathway through which phenolics' cardio-protective properties is viable. The study shows that phenolic acids positively effect antioxidant enzyme mRNA expression and phase II cardiac enzyme activity (Yeh et al., 2009).

Another mechanism of the cardio-protective nature of phenolic has been suggested by Pasten and others (2007). In the study, the down regulation of plasminogen activator inhibitor type-1 (PAI-1) gene expression in cultured human coronary artery endothelial cells (EC) by polyphenols is shone. PAI-1 is involved in clot formation and is positively associated with onset and advance of CVD. In a study done previously, the same end-points in Spraque-Dawley rats were investigated (Grenett et al., 2007). Mitogen-activated protein kinases (MAPKs) are serine/threonine protein kinases that are suggested by these researchers to take part in intercellular signaling cascades activated by

polyphenols. MAPKs affect signaling of ARE, transcription of phase II detoxifying enzymes, anti-angiogenic properties, and angiotensin II inhibition. Catechen and quercetin activate the MAPKs p38, ERK1/2, and JNK (Pasten et al., 2007). ERK1/2 and JNK suppressed EC PAI-1 gene expression. The activation of the MAPKs and suppressions of the EC PAI-1 gene expression is limited due to the relatively short half life of the polyphenols; unless the polyphenols are consumed on a daily basis the polyphenols may not be as beneficial (Pasten et al., 2007). Although a suggested mechanism for the cardio-protective nature of polyphenols has been suggested, a binding site for the activation of the MAPKs is still elusive (Pasten et al., 2007).

Phenolics and Cancer

Consumption of foods high in phenolics has been associated with reduced risk for cancer through several biochemical pathways. The pathways implicated in the anti-carcinogenic and anti-mutagenic characteristics of phenolics very with each type of phenolic and cancer cell type. A phenolic compound can work through multiple pathways or single pathways. The pathways and mechanisms are still being widely researched and are not fully understood. Some of the pathways include inhibition of growth proliferation in cancer cells, increase in apoptosis in cancer cells, stimulation of phase II detoxifying enzyme production, DNA oxidation reduction in normal cells, free radical scavenging, inhibition of DNA methyltransferaces (DNMT), regulation of signal transducing systems such as MAPKs, DNA fragmentation of cancer cells, inhibition of angiogenesis, and reducing migration of cancer cells (Chen et al., 2007; Chen et al., 2009; D'Archivio et al., 2008; Fang et al., 2007; Faried et al., 2007; Gonzalez de Mejia,

et al., 1999; He et al., 2008; Landis-Piwowar et al., 2007; Lee, et al., 2005; Menendez et al., 2008; Neto, 2007; Neto et al., 2008; Pasten et al., 2007; Rahman et al., 2006; Soobrattee, et al., 2005; Ullah & Khan, 2008; Wang et al., 2008; Yang, et al., 1997; Yang, et al., 2009a; Yeh et al., 2009; Yi, et. al., 2005). The anti- carcinogenicity is usually not manifested by just one phenolic compound but in combination with other compounds or whole foods containing the compounds.

Inhibition of growth proliferation in cancer cells has been shown in several cancer cell lines with different phenolics. An in-vitro study done by Yi, et. al. (2005) found blueberry extracts and anthocyanins to have anti-proliferation effects on colon cancer cells. Resveratrol and the methylated forms 3,5,49-trimethoxy-trans-stilbens, compounds found in red wine and berries, have been shone to reduce proliferation of lung adenocarcinoma cells by regulating NF-κB and activator protein-1 activity (Yang, et al., 2009b). Lignans and secoiridoids, complex polyphenolic compounds in extra virgin olive oil, have high anti-prolific effects on breast cancer (Menendez et al., 2008). Catechin/epicatechin, monomeric catechins, and quercetin glycosides found in cranberries and polyphenolic extracts from cranberry powder have anti-prolific effects multiple human cancer cell lines: two oral (CAL27 and KB), four colon (HT-29, HCT-116, SW480, and SW620), and three prostate (RWPE-1, RWPE-2, and 22Rv1). The extracted polyphenolics from cranberry powder was more effective than the individual phenolic compounds (Neto et al., 2008). Epigallocatechin gallate **(**EGCG), from green tea, shows anti-prolific properties in breast cancer cells. Phenolic compounds contribute to anti-proliferation of cancer and contribute to the prevention of cancer when consumed regularly (Landis-Piwowar et al., 2007).

Apoptosis, programmed cell death, is a normal function in the life of a healthy cell. Cancer cells are formed when DNA mutates, cannot be repaired, and the apoptosis pathways are inhibited or is involved in the mutation. Initiation of apoptosis in mutated cells and tumor cells is beneficial when trying to treat tumors and prevent growth of cancer (D'Archivio et al., 2008). Several phenolic compounds and foods high in phenols up regulate production of pro-apoptotic proteins (Bax, Bad, Bak, Bid, Bcl-Xs), down regulate anti-apoptotic proteins (Bcl-2, Bcl-XL, Bag-1,Bcl-W), activate apoptosis in cancers cells by disrupting mitochondrial membrane integrity, and/or activate death receptor pathways (Figure 2.4) (D'Archivio et al., 2008). Blueberry extract and the polyphenolic compounds in the extract increased apoptosis in colon cancer cells (Yi et al., 2005). Green tea extracts, EGCG, and derivatives of EGCG have shown to induce apoptosis in breast and prostate cancer cells and tumors. The EGCG inhibits proteasome chyromotrypsin-like activity which increases accumulation of ubiquitinated proteins p27, bax, and IκB-α protein. The anti-apoptotic nuclear factor κB is not activated because of the accumulation of IκB-α protein so the cell goes into apoptosis (Landis-Piwowar et al., 2007). Apoptosis is induced by tannic acids (TA) that can be found in many foods such as tea, wine, acorns and coffee. TA has shone apoptotic activity in squamous cell carcinoma, salivary gland tumor cell lines, and human leukemia HL-60 cells. The TA induced apoptosis in the HL-60 cells by down regulating anti-apoptotic Bcl-2 and increasing expression of bax a pro-apoptosis protein (Cantos et al., 2003, Chen et al., 2009). As_2O_3, a pro-apoptotic treatment of leukemia, sensitivity in HL-60 cells was selectively increased when coupled with TA. A lower dose (2μg/ml) was used with TA (5μM) to achieve the same level of apoptosis as the high dose (5μg/ml) of As_2O_3 without

enhanced cytotoxic effects in normal human cells (Chen et al., 2009). Resveratrol has been shone to induce apoptosis through several pathways involving up regulating caspase activity, decreasing Bcl-2 and Bcl-XL proteins, increasing bax, and activating p53 (D'Archivio et al., 2008). Many phenolic compounds are still being investigated regarding impact on the apoptosis activity of cancer cells and tumors.

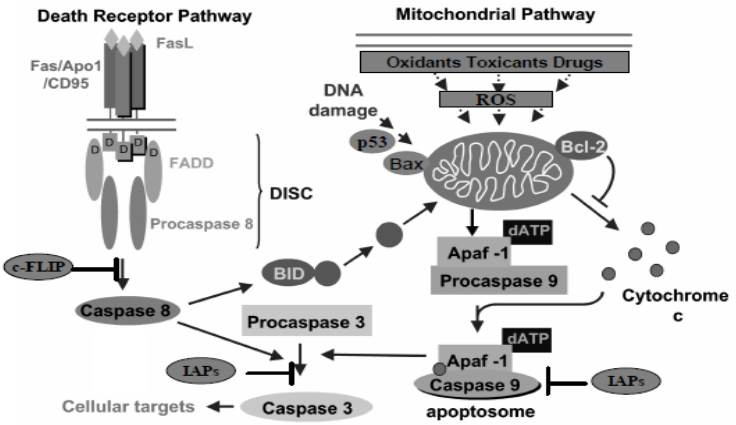

Figure 7.1: Pathways of Apoptosis (D'Archivio et al., 2008)

Oxidative stress can cause mutation of DNA and formation of cancer. The body's ability to quench free radicals and reduce oxidative damage is enhanced by phenolic compounds. Phenolic compounds have high anti-oxidant potential by directly participating in HAT or ET reactions with free radicals (Neto et al., 2008; Scalbert, et al., 2005; Soobrattee et al., 2005; Soong & Barlow, 2004; Yang et al., 1997; Yang et al., 2009a). Phenolics can up regulate signaling pathway that control redox reactions. The same MAPKs, ARE, Nrf2, and phase-II detoxifying enzyme pathways that help lower CVD risk contribute to lowering risks for most cancers by decreasing DNA oxidation damage, increasing repair of oxidized DNA, increasing plasma antioxidation potential, and decreasing the levels of free radicals produced by cancer cells(Chen et al., 2007;

Diplock et al., 1998; He et al., 2008; Kruk et al., 2005; Neto et al., 2008; Rahman et al., 2006; Scalbert et al., 2005; Yang et al., 1997; Yang et al., 2009b).

DNA fragmentation is indicative of apoptosis; however, another pathway of DNA fragmentation has been investigated in several studies. Production of ROS through a polyphenol-Cu^{+2} redox path can cause DNA fragmentation and death of cancer cells (Chen et al., 2007; Hadi et al., 2007; Wang et al., 2008). Cancer cells have higher levels of Cu, so normal cells are not as affected by this pathway (Ebara et al., 2000; Hadi et al., 2007; Wang et al., 2008). Cu levels are greater in the nucleus area surrounding the DNA and in chromosomes where polyphenols, acting as pro-oxidants, bind to the Cu^{+2} to form polyphenol-Cu^{+2} complexes, or the polyphenol can bind to the DNA and Cu^{+2} to form a ternary polyphenol-DNA-Cu^{+2} complex (Hadi et al., 2007; Wang et al., 2008). The Cu^{+2} in the complex is reduced to Cu^{+}. The Cu^{+} is then oxidized, and peroxide and oxygen radicals are formed as byproducts (see Figure 2.5) (Hadi et al., 2007; Wang et al., 2008).

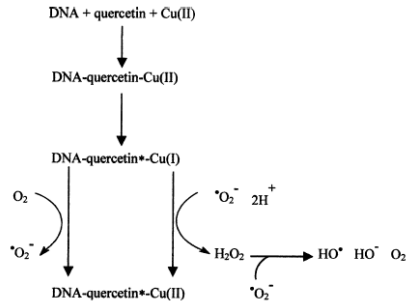

Figure 7.2: Ternary Polyphenol-DNA-Cu^{+2} Redox and Formation of ROS (Hadi et al., 2007)

The ROS immediately causes damage to the adjacent DNA section before the ROS can be scavenged (Wang et al., 2008). The cell then dies or is repaired, but genenomic

expression for DNA repair is usually inhibited in cancer cells so the cancer cell usually dies.

Many cancer cell lines have sections of DNA that have become inhibited by hypermethylation by increased levels of DNA methyltransferaces (DNMT) affecting gene expression of processes such as differentiation, genomic imprinting, DNA mutation, and DNA repair. The hypermethylation and inactivation of the genes were found to occur in early and advanced lesions of cancer cells (Fang et al., 2007). The hypermethylation of genes is theorized to be on of the causes of some cancers. DNMT inhibitor drugs are part of some cancer treatment and can reverse and inhibit hypermethylation (Fang et al., 2007). The DNMT inhibitor drugs could be used for cancer prevention, but the drugs are toxic. Methylation of DNA can be affected by polyphenols found in tea and soybeans. DNMT was inhibited *in vitro* by (-)-epigallocatechin 3-gallate (EGCG), found in green tea, and genistein found in soy beans (Fang et al., 2007). Myricetin, quercetin, hesperetin, naringenin, apigenin, luteolin, garcinol, curcumin, and hydroxycinnamic acid were also found to inhibit DNMT. S-adenosylmethionine (SAM) methylates the polyphenols and therefore cannot participate in DNMT activity (Fang et al., 2007). Demethylization of the DNA and restoration of gene expression activity occurs in the presence of EGCG and other polyphenolic compounds (Fang et al., 2007).

A diet that has many concentrated sources of phenolics is important to reduce cancer risks. Some naturally occurring phenols are being modified for use as anticancer drugs or are being used to enhance the efficacy of current drugs (Chen et al., 2009; Landis-Piwowar et al., 2007; Yang et al., 2009b). The studies of phenolic compounds in

relation to cancer need extensive further study despite the quick and extensive advance in knowledge of the area in the last decade. Many of the tests use high levels of phenolics and are not preformed in humans. The pathways phenols use to decrease risk and fight cancer are very complex. Multiple pathways are stimulated for different phenol types.

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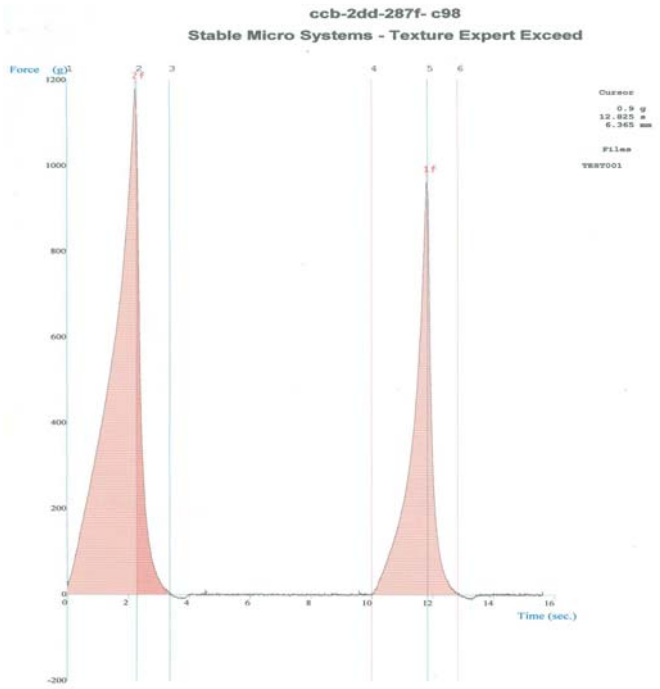
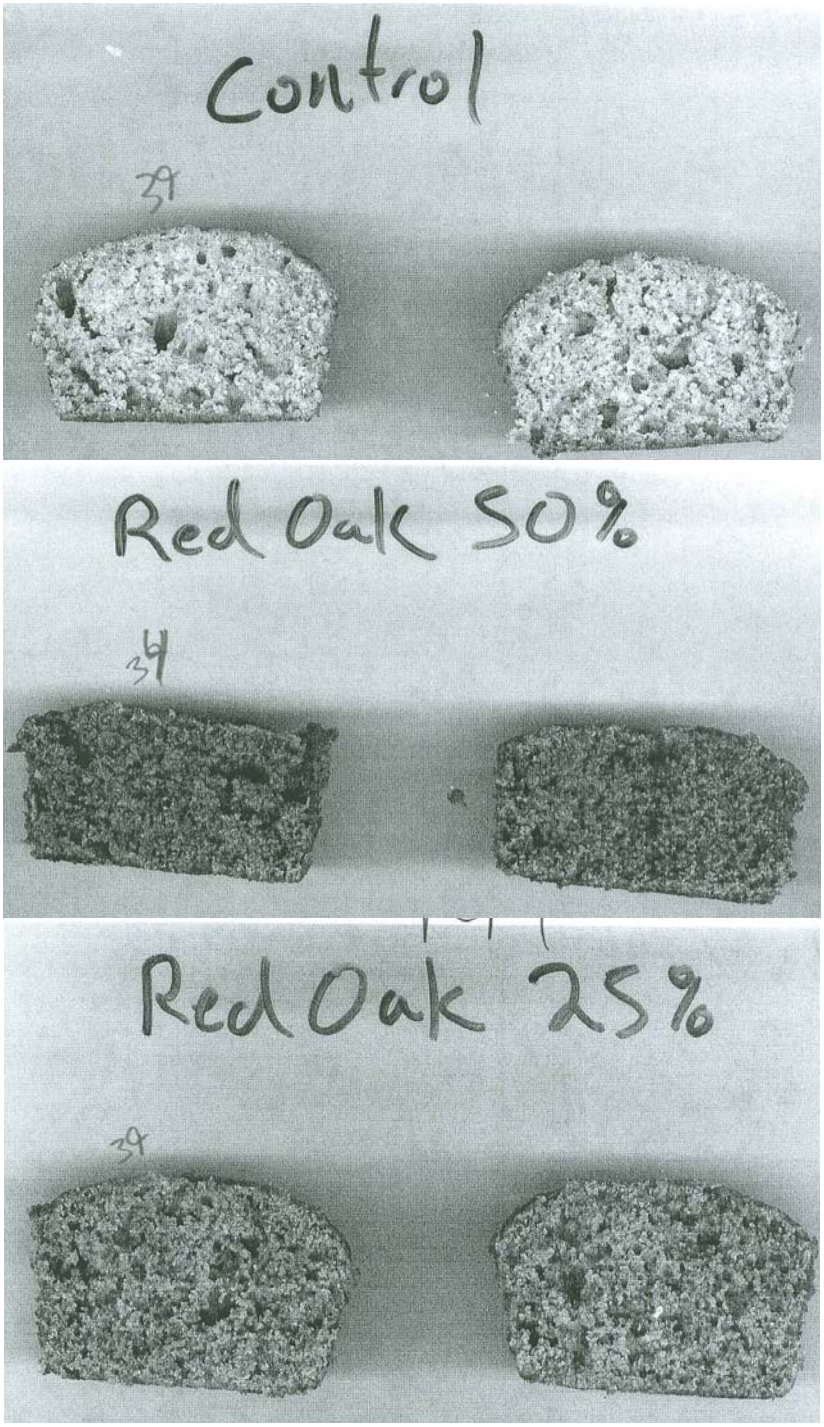
Appendix B. Physical Tests—Data Extraction

Representative graph depicting from where the data were extracted from the Time-Force Curve for texture profile analysis of the muffins.



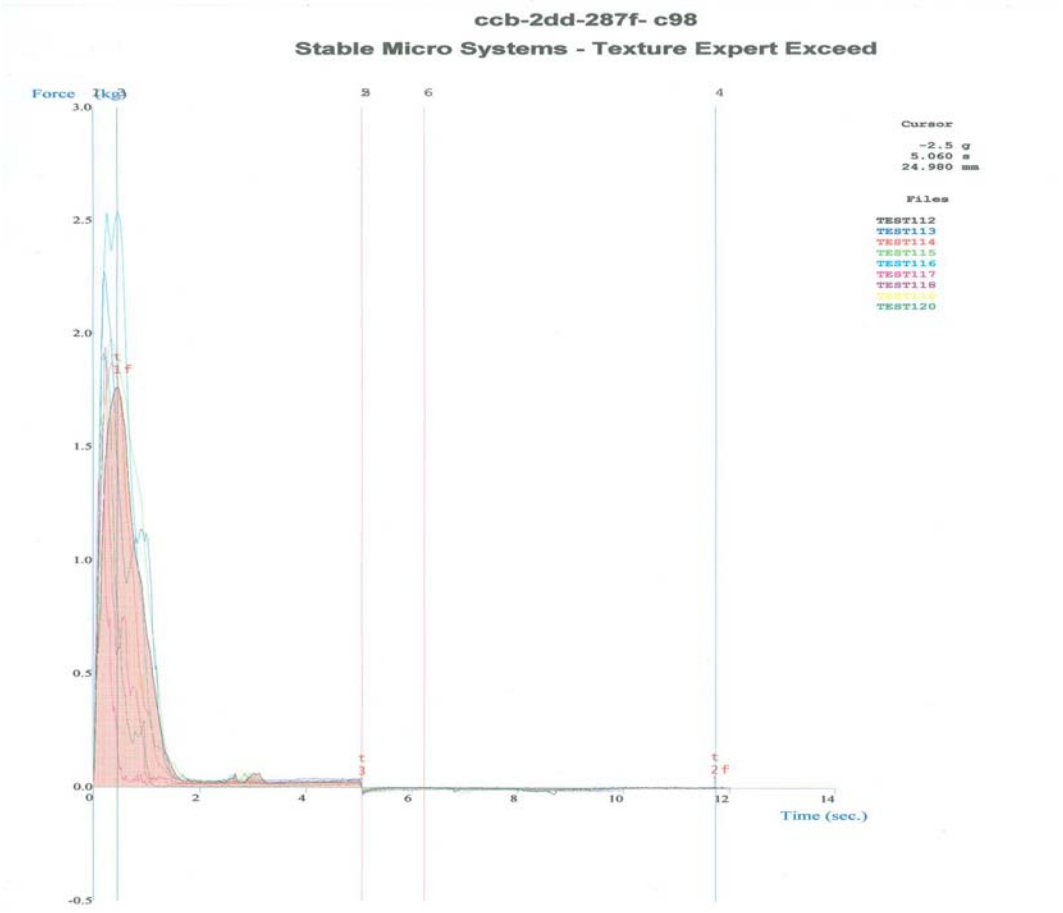
Hardness was taken from the top of the first peak. Springiness was derived by dividing the compression time of compression two to peak by the compression time of compression one to max peak. Cohesiveness is derived by dividing the total area under the second curve by the total area under the first curve (Meullenet & Gross, 1999).

Representative Xerographs for the Pumpkin- spice Muffins [0%-control, 25% replacement with black acorn meal, 50% replacement with black acorn meal)



Standing height determined by measuring the height in mm at the center of each muffin (Pong et al, 1991).

Representative graph depicting from where the data were extracted from the Time-Force Curve for probing analysis of the cookies.



Hardness was derived by the max peak of the curve. Toughness was found by measuring the area under the curve (Perry et al. 2003).

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Appendix C. Consumer Sensory Panel: Consent Form, Scorecard and Survey

Consent Form

I, _____, agree to participate in a research study titled "Consumer Acceptability of Muffins and Cookies" conducted by Dr. Ruthann Swanson, Dr. James Hargrove and Michael Sabrin from the Department of Foods and Nutrition, University of Georgia (706-542-4834). I understand my participation is voluntary. I can refuse to participate or stop taking part without giving any reason and without penalty.

The reason for this study is to investigate factors that affect acceptability of baked products formulated with novel ingredients. To prevent any possible bias to the results, the specific kind of ingredients used will not be disclosed until after the completion of the study. However, the FDA's Consumer Safety Office has verified that this product is generally regarded as safe.

If I volunteer to take part in this study, I will be asked the following things:
- Read the consent form, declare allergies to any food or food ingredient or dietary restrictions due to medical reasons where indicated, and
- Evaluate products using sensory scorecards. (7-10 minutes)
- Complete a demographic and food choice questionnaire. (3 – 5 minutes)

Food allergies or other dietary restrictions due to medical conditions that I have include
_____
_____
_____(please list).

Following my participation, I will be offered commercial snacks and beverages upon leaving the study testing site. Students in FDNS 3100, FDNS 4550/6550 or FDNS 4650/6650, who have selected participation on this sensory panel as an extra credit option will receive class credit. No additional compensation will be offered.

I will be assigned an identifying number, and this number will be used on all questionnaires and evaluation forms I fill out. However, there is no way to connect specific responses with a specific individual once the test is completed. This study is anonymous. An expected benefit is the production of healthier products that are acceptable to consumers; their availability will empower consumers to improve their dietary choices.

Risk is comparable to that found in everyday life. The only potential risk associated with participation is unknown or undeclared food allergies. However, in the event that my participation in this study results in a medical problem, treatment will be made available. However, my insurance company or I will be billed for the costs of any such treatment. No provision has been made for payment of these costs or to provide me with other financial compensation.

If I have further questions about the study, I can call Dr. Ruthann Swanson at (706) 542-4834 or Dr. James Hargrove at (706) 542- 4678.

I understand the procedure 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.

Ruthann Swanson Name of Researcher	R Swanson Signature	3/26/08 Date
James Hargrove Name of Researcher	James Hargrove Signature	3/26/08 Date
Michael Sabrin Name of Researcher	M Sabrin Signature	3/27/08 Date
_____ Name of Participant	_____ Signature	_____ Date

Please sign both copies, keep one and return one to the researcher.
Additional questions and problems regarding your rights as a research participant should be addressed to The Chairperson, Institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706)542-3199; E-mail address IRB@uga.edu

Cookie code _____ Panelist _____

Cookie Scorecard

Please mark the box (☑) on each scale below that best indicates how much you like each characteristic of this cookie.

Please drink some water and eat a bite of cracker before sampling the next product. You will receive 3 cookie samples to evaluate, followed by a short questionnaire. It is important to our study that you complete all of the forms.

Appearance

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Extremely Dislike — Extremely Like

Texture

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Extremely Dislike — Extremely Like

Flavor

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Extremely Dislike — Extremely Like

Overall Acceptability

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Extremely Dislike — Extremely Like

Comments:

Cookie 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. Please check your age category:
   _____18-27 _____44-51
   _____28-35 _____52-61
   _____36-43 _____62 and above

3. How often do you eat cookies?
   _____Daily
   _____Several times a week
   _____Several times a month
   _____Once a month
   _____Several times a year
   _____Never

4. Are you or someone else in your household currently on a diet?
   _____yes _____no

Please answer the questions on the back.

5. How important is each of the following claims when you select foods to buy?

	very important	somewhat important	not important
whole-grain			
low sugar			
reduced-fat			
no trans-fat			
low calorie			
low sodium			
low or lowers cholesterol			
low carb			
protein-rich			
calcium-fortified			
no chemical additives			
no preservatives			
reduces risk of cancer			
reduces risk of heart disease			
high in antioxidants			
high fiber			
low glycemic index			
prebiotic/probiotic			
organic			
vitamin fortified			

6. Would you be more inclined to consume a cookie that carried an antioxidant claim?

_____yes
_____no
_____no effect

Thank you!

Appendix D. Consumer Survey Responses (n=124)

Depicted in Chapter 4, Figures 4.1 and 4.2

Consumer survey subject heading	frequency	Percentage
gender		
Male	22	17.74
Female	102	82.26
Age		
18-27	106	85.48
28-35	12	9.68
36-43	1	0.81
44-51	2	1.61
52-61	2	1.61
62+	1	0.81
How often eat cookie		
daily	7	5.65
several/ week	24	19.35
several/ month	60	48.39
once/ month	21	16.94
several/ year	10	8.06
never	2	1.61
Are you Dieting?		
yes	79	63.71
no	45	36.29
How important is each of these claims when buying food?		
Whole grains		
Very important	90	72.58
Somewhat important	30	24.19
not important	4	3.23
Low Sugar		
Very important	41	33.06
Somewhat important	66	53.23
not important	17	13.71
Reduced-fat		
Very important	41	33.06
Somewhat important	61	49.19
not important	22	17.74
No Trans-fat		
Very important	93	75

Somewhat important	27	21.77
not important	4	3.23
Low Kcal		
Very important	48	38.71
Somewhat important	60	48.39
not important	16	12.9
Low sodium		
Very important	29	23.39
Somewhat important	71	57.26
not important	24	19.35
Low or lowers Cholesterol		
Very important	18	14.52
Somewhat important	66	53.23
not important	40	32.26
Low Carb		
Very important	12	9.68
Somewhat important	42	33.87
not important	70	9.68
Protein-rich		
Very important	22	17.74
Somewhat important	74	59.68
not important	28	22.58
Ca Fortified		
Very important	33	26.61
Somewhat important	60	48.39
not important	31	25
No chemical Additives		
Very important	25	20.16
Somewhat important	52	41.94
not important	47	37.9
No preservatives		
Very important	26	20.97
Somewhat important	50	40.32
not important	48	38.71
Reduces cancer risk		
Very important	41	33.06
Somewhat important	58	46.77
not important	25	20.16
Reduced risk for heart Dx		
Very important	42	33.87

Somewhat important	60	48.39
not important	22	17.74
High in Antioxidants		
Very important	59	47.58
Somewhat important	56	45.16
not important	9	7.26
High in fiber		
Very important	65	52.42
Somewhat important	48	38.71
not important	11	8.87
Low glycemic index		
Very important	12	9.68
Somewhat important	51	41.13
not important	61	49.19
Prebiotic/ probiotic		
Very important	8	6.45
Somewhat important	58	46.77
not important	58	46.77
Organic		
Very important	18	14.52
Somewhat important	50	40.32
not important	56	45.16
Vitamin Fortified		
Very important	18	14.52
Somewhat important	81	65.32
not important	25	20.16
Would you be more inclined to consume a cookie that carried an antioxidant claim?		
Yes	90	72.58
No	12	9.68
No effect	22	72.58