DIETARY POLYPHENOLIC INTAKE FROM ACORNS AND ACORN MEAL

by

LISA KOBS

(Under the Direction of James Hargrove)

ABSTRACT

Antioxidant consumption can increase through strategic food choices. Acorns could contribute to those strategic choices. We surveyed antioxidant content in a variety of acorns from the southeastern United States. The phenolic content of *Quercus velutina* prior to leaching was 39.4±3.6 mg GAE/g and of *Quercus alba* was 27.4±.3 mg GAE/g. After 5 leaching stages, the phenolic content of *Q. velutina* was 12.3±.8 mg GAE/g and of *Q. alba* was 10.8±.7 mg GAE/g. After milling, the phenolic value for Q. *velutina* was 39.9±4.5 mg GAE/g and for Q. *alba* was 6.6±.1 mg GAE/g. A spice cookie made with all-purpose wheat flour contained 2.7±.1 mg GAE/g. When red oak acorn meal was substituted, the cookie contained 9.6±3.3 mg GAE/g, and when substituted with white oak acorn meal contained 4.2±.4 mg GAE/g. Ingredient interactions influenced the phenolic content. Acorns and acorn products can be a strategic method of increasing polyphenolic intake.

INDEX WORDS: Polyphenols, Acorns, DASH Diet

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CHAPTER I

INTRODUCTION

The occurrence of chronic disease in the United States is increasing due to several possible factors. One factor includes changes in eating habits compared to historical norms. The highly refined and processed diets of today are far different from diets of the past (Cordain, 2002). The refining and processing that occurs eliminates possible disease-fighting compounds found in unrefined foods, an example being polyphenols. Polyphenols are antioxidants that may help prevent negative effects of oxidative stress. Oxidative stress has been associated with various degenerative diseases including obesity (Erdeve and others 2007), aging (Kregel and Zhang, 2007), (Beckman and Ames, 1998), cardiovascular disease (CVD) (Ignarro and others 2007), hypertension (Harrison and others 2007) and diabetes (Devangelio and others 2007), (Han and others 2007), (Valko and others 2007), (Ames and others 1993).

Because free radicals are produced during normal metabolism of nutrients and drugs, the oxidation that occurs within biological systems cannot be avoided entirely. However, the extent of the oxidation and associated damage can be lessened by taking certain actions. Because humans are exposed to multiple sources of potential oxidation, the methods to decrease the consequences of oxidation vary. For example, exercise causes oxidative damage; however, exercise has many beneficial effects (Ji, 1999). Therefore decreasing exercise may not be as beneficial as making other changes, such as dietary changes, that protect and support the body during exercise.

Dietary efforts to decrease oxidation include increasing the consumption of nutrient antioxidants such as vitamins A, C and E (Scalbert and others 2005b). Although not considered nutrients, dietary constituents such as phytochemicals can also decrease oxidation. Phytochemicals encompass thousands of compounds varying in structure, and the oxidation prevention potential is based primarily on the structure of the compound. Categories of phytochemicals include carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds. Several thousand compounds containing phenolic structures have been identified, with several hundred existing in edible plants (Manach and others 2004). Specific classes of polyphenols have structures with better antioxidant potential compared to other classes of polyphenols (Bors and Michel, 2002). The structure of polyphenols has been shown to be even more effective in free radical scavenging than vitamins C and E (Rice-Evans and others 1997), (Yan and others 2002). Because of the ability of polyphenols to scavenge free radicals, increasing polyphenol consumption in the diet should have beneficial effects on damage due to oxidation in the tissues and bloodstream.

Phenolics are found in several different types of foods including beverages, fruits, vegetables, grains, and spices. The most concentrated source of polyphenols is possibly from the spices category because most spices have been dried and contain little food energy (kcal). Increasing the consumption of spices could have many beneficial effects without increasing calorie consumption due to the high polyphenol density and low energy content in spices (Wu and others 2004), (Ninfali and others 2005).

Multiple recommendations have been made in terms of making healthy dietary choices, one being the Dietary Reference Intakes (Food and Nutrition Board Institute of Medicine, 2005). Dietary recommendations have also been made for those individuals who need to treat an

existing disease. Two examples are carbohydrate counting and use of the Exchange System for individuals with diabetes and the Dietary Approaches to Stop Hypertension (DASH) diet for individuals with hypertension. These recommendations are comprehensive in terms of dealing with the specific nutrients that are associated with the disease such as fats and carbohydrates in the case of diabetes and sodium and potassium with hypertension. However, these guidelines do not yet include specific recommendations for non-nutritive phytochemicals. The DASH diet encourages fresh fruit and vegetable consumption because of the naturally low sodium content and abundant potassium and magnesium in these foods. Fruits, vegetables, legumes, and nuts are also high in phytochemicals. If the recommendations were to include foods based on the antioxidant capacity, in addition to the nutrient content, the recommendations could provide more benefit in terms of disease prevention. More specifically, foods could be included in the recommendations to increase polyphenol content, such as berries or adding spices such as cinnamon to foods already recommended.

Nuts also can be a good source of polyphenols in the American diet. Several varieties of nuts that are easily accessible in grocery stores are high in polyphenols including pecans, pistachios, and walnuts (Wu et al., 2004). Other nuts not typically thought of in terms of human consumption, and not as widely available, are also high in polyphenols, such as acorns. There is evidence that acorns have served as dietary staples in many parts of the world where oak trees grow, and may provide up to 25%-50% of calories. This suggests that certain traditional diets may have provided much higher levels of polyphenolics than are found in modern diets based largely on refined ingredients (Bainbridge, 1986).

The objectives of this study include:

1. To show the wide range of antioxidant intake that can be obtained by combining different foods and ingredients suggested by the DASH diet plan
2. To determine the total phenolic content of acorns from selected oak species during various stages of processing and in a finished cookie product.
3. To evaluate the effect of baking and the interactions of different ingredients on the total phenolic content of cookies made with acorn meal.

CHAPTER II

LITERATURE REVIEW

DIET RECOMMENDATIONS

Dietary Reference Intakes

The Dietary Reference Intakes (DRIs) are the reference values established for individuals to ensure adequate intake of nutrients, based on age, gender, and life stage. The DRIs consist of the Recommended Dietary Allowance (RDA), Estimated Average Requirement (EAR), Adequate Intake (AI), and Tolerable Upper Intake Level (UL). Values have not been established for all nutrients due to limited information in the published literature about specific effects of the particular nutrient. In some instances if the literature supports a nutrient functioning as an antioxidant, the DRIs will be established at levels where that nutrient can optimally function as an antioxidant (Food and Nutrition Board Institute of Medicine, 2000).

The RDA for vitamin C is 75 mg/d for females and 90 mg/d for males and is set at those amounts to provide antioxidant protection. Because of the oxidative stress that smokers experience, the RDA for vitamin C for smokers is increased by 35 mg/d. The suspected main function of vitamin E is to prevent lipid peroxidation. The RDA for α-tocopherol for males and females is 15 mg/d. Selenium is also described as an antioxidant, and the RDA for males and females is 55 μg/d. The DRIs address carotenoids, but because the functions of carotenoids, other than preventing vitamin A deficiency, have not been identified, no DRI has been established for any of the carotenoids except retinol (Food and Nutrition Board Institute of Medicine, 2000).

A definition for dietary antioxidant is provided as: "a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans" (Food and Nutrition Board Institute of Medicine, 2000). Vitamins C, E, and selenium meet this definition. β-carotene and the other carotenoids, however, do not (Food and Nutrition Board Institute of Medicine, 2000). DRI values for non-nutrients, such as polyphenols, have not currently been established due to a lack of scientific data (Food and Nutrition Board Institute of Medicine, 1998).

Dietary Guidelines for Americans 2005

The Dietary Guidelines for Americans 2005 were developed to promote adequate intake of nutrients to support growth and health. They also emphasize the desirability of consuming those nutrients in adequate amounts from food sources, not supplements. This is because of the additional compounds present in food such as carotenoids, flavonoids, isoflavones, and protease inhibitors, which are not present in nutrient-specific supplements. The compounds found in whole foods, and not always in supplements, may provide the benefit of preventing chronic disease (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2005).

The dietary guidelines encourage consuming a variety of fruits and vegetables within estimated daily calorie needs. Vegetable intake should be from all 5 vegetable subgroups, including dark green kinds, orange types, legumes, starchy varieties, and other vegetables. The consumption of a variety of vegetables ensures adequate intake of all of the nutrients provided in the various groups. Whole grains are also encouraged because of the increased fiber compared to refined grains; however, the bran fraction of whole grains such as wheat, oats, barley and flax also provides a rich source of non-nutrient compounds. Phenolic compounds are only mentioned

in reference to their removal during the refining of grains (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2005).

The dietary guidelines state that the dietary approaches to stop hypertension (DASH) diet and USDA Food Guide are both good examples of eating patterns to adopt to follow the dietary guidelines. They promote the intake of green leafy vegetables, orange vegetables, and fruit at higher levels than current consumption patterns in the US. These guides also recommend a lower intake of refined sugars and refined grain products than current consumption. The DASH Diet Plan specifies the nutrients provided in each food group, however, it does not specify the non-nutrient compounds provided in the food groups. The USDA Food Guide provided in the dietary guidelines does not explain the benefits of choosing foods from the different food groups. Both food plans provide recommendations based on various calorie levels to provide recommendations for the majority of the population. Neither plan describes the non-nutrient compounds present in food that can provide multiple additional benefits besides promoting growth and health while preventing nutrient deficiencies (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2005).

The DASH Diet was developed to help individuals decrease blood pressure. The diet emphasizes controlling sodium intake in order to control blood pressure. The research the plan is based on shows a decrease in blood pressure by decreasing intake of saturated fat, cholesterol, and total fat. The research also shows a diet high in fruits, vegetables, and low-fat or fat-free milk products can positively influence blood pressure(U.S. Department of Health and Human Services and others 2006). The DASH Diet makes recommendations concerning some specific nutrients, such as fat, carbohydrate, sodium, potassium, calcium, and magnesium. It also provides information concerning maintaining a healthy weight, including physical activity into a

healthy lifestyle, and if drinking alcohol, doing so in moderation. The DASH Diet does suggest using cinnamon as a substitution, however this is a strategy suggested to lower the sodium content of the meal. It is not suggested due to the antioxidant properties of cinnamon (U.S. Department of Health and Human Services et al., 2006).

The dietary guidelines discuss the fact that several of the vitamins that act as antioxidants, vitamin A (carotenoids), vitamin C, and vitamin E, are possibly not being consumed in high enough amounts. This recommendation is based on their ability to prevent deficiency symptoms rather than other vital functions the vitamins may provide (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2005).

In conclusion, the recommendations currently do encourage foods containing antioxidants. In addition, the current recommendations have been established taking into account the antioxidant properties that some of the nutrients have. However, recommendations for the non-nutrient antioxidants have not yet been established. Until further data is gathered concerning what amounts provide optimal function of the non-nutrient antioxidants, recommendations cannot be made. If the recommendations are followed concerning fruits and vegetables, non-nutrient antioxidants will be consumed. However, the consumer should know that additional sources of antioxidants exist, and more of an effort can be put in place to consume those foods once the optimal amount for promoting health is determined.

REACTIVE OXYGEN SPECIES

The damage that occurs to cells and cellular components that antioxidants can prevent occurs due to the process of oxidation. The damage arises from the actions of free radicals, where free radicals are compounds with an unpaired electron that can exist independently. Because they contain an unpaired electron, they are highly reactive and have a short half-life.

Free radicals can either donate or accept one electron acting as an oxidant or a reductant (Young and Woodside, 2001).

The most damaging free radicals come from compounds involving oxygen that are called reactive oxygen species (ROS). ROS and free radicals when reacting with other biomolecules are capable of starting a chain reaction where additional free radicals may form. In order for this chain reaction to be stopped, one free radical must react with another free radical or an antioxidant to eliminate the unpaired electron (Nordberg and Arner, 2001).

Some examples of ROS that are formed in living organisms include superoxide and hydroxyl radicals. Superoxide is formed from the leakage of electrons from the electron transport chain, cytochrome p450 oxidase in the liver, and enzymes utilized in the synthesis of adrenal hormones (Young and Woodside, 2001). According to in vitro experiments, an estimated 1-2% of all electrons traveling in the electron transport chain leak out and form ROS (Frei, 1994). Whenever superoxide is formed, hydrogen peroxide is also formed due to a dismutation reaction (Young and Woodside, 2001). Superoxide is not terribly active however, because it cannot cross lipid membranes, and therefore must act within the space in which it was produced (Nordberg and Arner, 2001). Hydrogen peroxide, however, can cross cell membranes. Therefore, hydrogen peroxide can cause damage in multiple cell and tissue types (Young and Woodside, 2001). Another ROS that does not readily react with other biomolecules is nitric oxide (NO). NO does, however, react with other free radicals resulting in the production of less reactive molecules. The role NO plays in a cellular system can vary depending on pH, temperature, and the characteristics of other compounds present (Nordberg and Arner, 2001).

ROS perform both harmful and beneficial functions in living organisms. ROS can damage organisms by altering DNA, lipids, proteins, and other compounds. ROS can cause

cleavage of DNA, cross-link proteins, and oxidize purines. If these alterations are not repaired, mutations in DNA may result. This DNA damage may be related to cancer development and the process of aging. Lipids, especially polyunsaturated fatty acids, make excellent targets for ROS damage because of multiple double bonds. Lipid oxidation can result in the formation of harmful atherosclerotic plaques. ROS can alter proteins resulting in less active or inactive enzymes or denatured proteins. Proteins containing sulfur or selenium residues are most susceptible (Nordberg and Arner, 2001).

A beneficial function in which ROS are involved is intracellular signaling. NO, hydrogen peroxide, and superoxide all are involved in transcription and gene expression. The antioxidant response element, for example, is a sequence of genetic code that is transcriptionally activated in response to the presence of oxidants (Rushmore and others 1991). Other compounds that use ROS in signaling include cytokines, growth factors, hormones, and neurotransmitters. ROS are also utilized in defending organisms against infections. ROS are produced by phagocytic neutrophils (a kind of white blood cell) in high enough amounts to kill invading bacteria (Nordberg and Arner, 2001).

Antioxidants work against ROS via two methods, hydrogen atom transfer (HAT) and single electron transfer (SET) methods. HAT methods involve the transfer of a hydrogen atom. The reactions occur rapidly, but reducing agents such as metals can inaccurately inflate reactivity. SET methods quantify the ability of antioxidants to transfer one electron reducing the other compound. Both types of reactions occur simultaneously within one sample, but the proportion of reactions that occur depends on pH and the structure of the antioxidant (Prior and others 2005).

ANTIOXIDANTS

Antioxidants are protective to living organisms because they can decrease the negative effects of ROS. Enzymatic antioxidants are intrinsic to an organism and include superoxide dismutase, superoxide reductase, catalases, peroxiredoxins, glutathione peroxidases, and glutathione systems (Nordberg and Arner, 2001). Peroxisomes contain over 50 different enzymes that participate in various cellular functions. Some of the functions of peroxisomes include lipid biosynthesis, β-oxidation of fatty acids, α-oxidation of fatty acids, catabolism of amino acids, polyamines, purines, as well as the metabolism of peroxides and other ROS. The enzymes involved in ROS degradation include catalase, glutathione peroxidase, manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (CuZnSOD), epoxide hydrolase, and peroxiredoxin (Schrader and Fahimi, 2004). Chain breaking antioxidants include lipid phase and aqueous phase antioxidants. Lipid phase antioxidants include tocopherols, ubiquinol, carotenoids, and flavonoids. Aqueous phase antioxidants include ascorbate, urate, and glutathione and other thiols. Chain breaking antioxidants act as electron donors. Transition metal binding protein antioxidants include ferritin, transferrin, and ceruloplasmin. These compounds act on iron and copper and aid in preventing the formation of the hydroxyl radical (Young and Woodside, 2001). Metallothionine, another free radical scavenger, is better at scavenging superoxide radicals compared to other sulfhydryl-containing molecules, such as cysteine (Hussain and others 1996). Due to the interaction of trace metals such as zinc and cadmium with flavonoids, metallothionine levels in intestinal cells may be affected (Le Nest and others 2004).

All aerobic organisms experience oxidative damage. Antioxidants prevent and help fix that damage. ROS only cause damage when they are formed in excess. Specific amounts are

needed for normal physiological function. Maintaining a balance between what is necessary for survival and what is in excess is difficult to determine (Nordberg and Arner, 2001).

Effect of Antioxidants on Disease

An increased intake of fruits and vegetables may decrease risk for certain diseases such as cancer and heart disease (Williams, 1995). It is still unknown what specific compounds in fruits and vegetables are providing the protective effects, and current research is exploring possible mechanism of risk reduction (Duthie and others 2003). Antioxidants from whole foods are suspected of providing protective effects, but when given in supplement form, the expected protective effect is often not seen (Prieme and others 1997). This was demonstrated by the α–tocopherol, β-carotene cancer prevention (ATBC) trial, which showed no decrease in coronary heart disease (CHD) morbidity or mortality during treatment with vitamin E or β-carotene. β-carotene in fact showed a 75% increase in risk of fatal ischemic heart disease in those subjects who had a previous myocardial infarction (MI). The β-carotene and retinol efficacy (CARET) trial involved subjects who were cigarette smokers and exposed to asbestos. No beneficial effect was demonstrated on cardiovascular disease (CVD) mortality, and the mortality risk from lung cancer increased in those subjects taking β-carotene. The Cambridge heart antioxidant (CHAOS) study involving vitamin E supplementation in patients with angiographic evidence of CHD, showed a benefit in CHD morbidity. However, no benefit was demonstrated in terms of CVD mortality (Young and Woodside, 2001). It is possible that no protection occurs unless the antioxidants are provided in a food matrix, or that other compounds in whole foods are needed to observe the protection.

Unlike vitamin E, vitamin C, and β-carotene, polyphenols are non-enzymatic, non-nutrient antioxidants and have also demonstrated some of the protective effects against certain

diseases. Polyphenols have shown several beneficial properties in terms of protecting cellular function. Polyphenols appear to influence cellular signaling cascades and influence gene transcription. For example, polyphenols may down-regulate pro-inflammatory enzymes such as COX 2 and I NOS, influence the activation of mitogen-activated protein kinase, and inhibit apoptosis occurring due to ROS presence (Soobrattee and others 2005), (Han et al., 2007). Perhaps because of these effects, polyphenols may also play a role in cancer prevention. Current consumption may not be adequate to prevent the development of cancer, but increased intakes of polyphenols may have beneficial effects (Mouria and others 2002), (Fresco and others 2006).

Antioxidants have been implicated as having positive effects on preventing the development of cardiovascular disease (CVD) (Huang and others 2005), (Arts and Hollman, 2005), (Tsang and others 2005). Juices containing polyphenols can have very positive effects in vivo. Polyphenol-rich beverages, including red grape juice, tea, and wine have been shown to decrease LDL oxidation (Serafini and others 2000), decrease LDL concentration, and in some cases increase HDL concentration (Castilla and others 2006). The consumption of polyphenol-rich berries has also shown to have beneficial effects on platelet function, HDL cholesterol, and blood pressure (Erlund and others 2008). Olive oil has demonstrated beneficial effects in lipid profiles. It has been shown to increase HDL, decrease triglycerides, and decrease oxidative stress markers (Covas and others 2006). One possible reason for this benefit is the content of hydroxytyrosol and tyrosol, which are among the antioxidants that give virgin olive oil its characteristic taste and color (Salvini and others 2006).

Flavonoids have demonstrated beneficial effects on improving blood vessel health and endothelial cell function (endothelial cells line all blood vessel walls and contribute to relaxation of the vessels). Flavonoids have also been shown to decrease blood pressure and oxidative

stress, both improving CVD risk (Perez-Vizcaino and others 2006). A beverage high in antioxidants, green tea, has demonstrated an inverse relationship between its consumption and all-cause mortality as well as CVD (Kuriyama and others 2006).

In subjects who were HIV+, a condition where oxidative stress may be increased, consuming an increase in fruit juice (blackcurrant, apple, red grape, pear, and green tea-apple) or fruit-vegetable concentrate was shown to increase antioxidant capacity in blood (Arendt and others 2001). A mixture of apple, mango, and orange juices containing additional blueberries and boysenberries showed a statistically significant improvement in antioxidant status and immune function and a reduction in damage to DNA (Bub and others 2003). In rat studies, fruits, such as blueberries and strawberries, have been found to be beneficial in cognitive function. Balance and memory have been shown to improve with specific polyphenol-rich foods (Lau and others 2005). Metabolites of pomegranate have been found to be bioavailable and to exert antioxidant effects in plasma after absorption (Mertens-Talcott and others 2006).

POLYPHENOLS

Polyphenols exist in multiple colors including yellow, orange, red, and blue, and contribute to the taste and odor characteristics of foods (Cheynier, 2005). There are a number of subclasses of polyphenols, indicated in Figure 2.1.

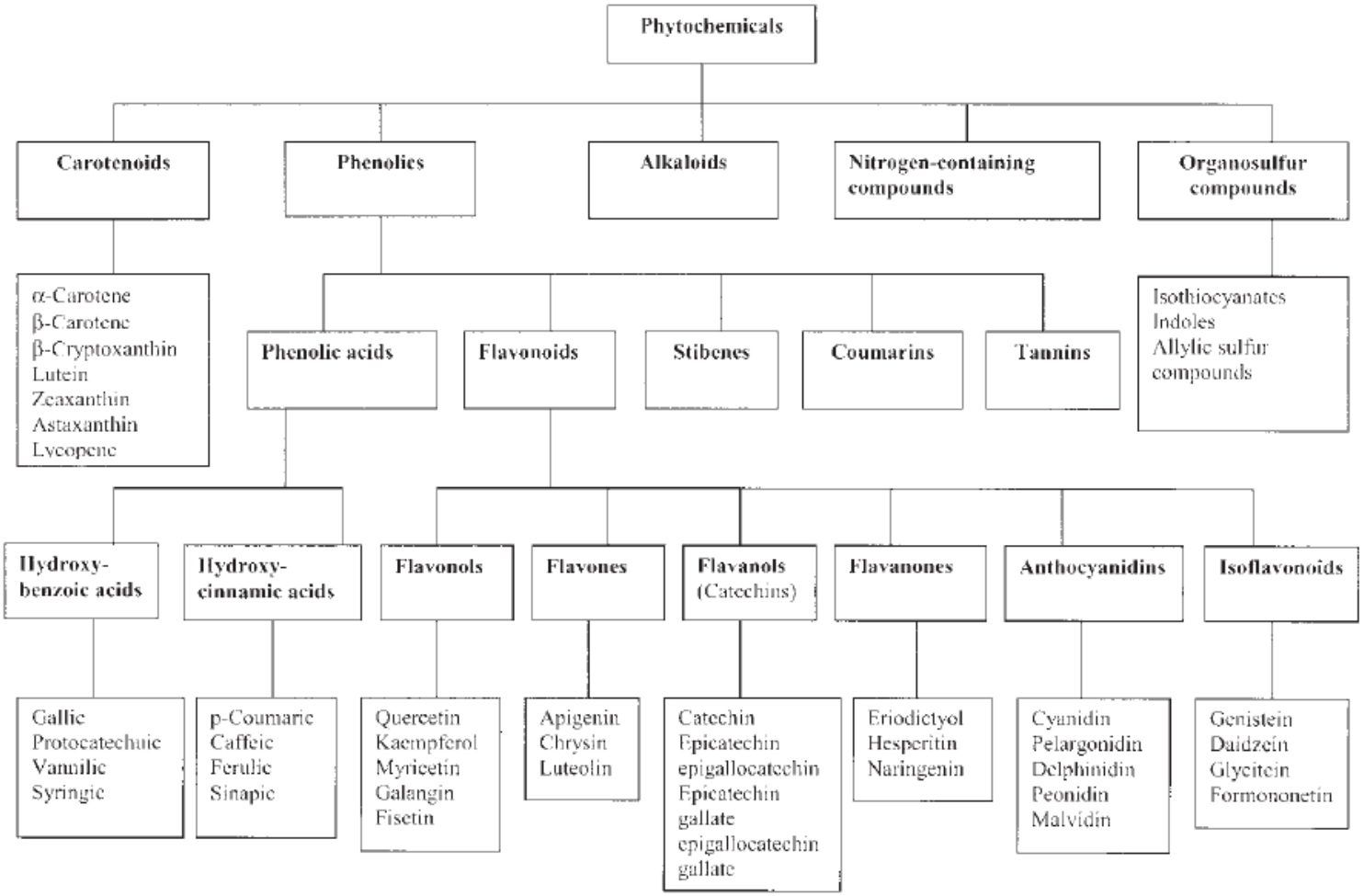

Figure 2.1 Phytochemical classifications including phenolic subgroup classifications (Liu, 2004).

They are divided into groups based on their chemical structure. Polyphenols are defined as having hydroxyl groups on two or more aromatic rings. Thousands of compounds have been identified as fitting this definition. They are divided into different groups based on the number of phenol rings they contain, and on the method in which these rings are bound (Manach et al., 2004). These major groups include phenolic acids, flavonoids, stilbenes, coumarins, and tannins (Liu, 2004).

Phenolic acids consist of two subgroups including derivatives of hyroxybenzoic and hydroxycinnamic acids. Blueberries, kiwis, plums, cherries, and apples contain the most hydroxycinnamic acid derivatives. Caffeic acid is the most abundant phenolic acid, comprising 75 to 100% of the hydroxycinnamic acid present in fruits. Ferulic acid is the most common phenolic acid in grains (Manach et al., 2004).

There are six subclasses of flavonoids, including flavonols, flavones, flavanols, flavanones, anthocyanidins, and isoflavones. Flavonols are the most common in foods that humans consume. Some foods high in flavonols include onions, broccoli, and blueberries. The concentration of flavonols from the fruit on one tree varies from fruit to fruit because the synthesis of flavonols is stimulated by sunlight. Because of this the leaves and skin of plants (or peels of fruit) contain the most flavonols. Flavones are only present in two vegetables that humans consume, parsley and celery. Flavanones are only present in high concentrations in citrus fruits. Flavanols exist in two forms proanthocyanidins, an example of the structure is shown in Figure 2.2, and catechins, of which the richest sources are chocolate and green tea. Proanthocyanidins are common in several fruits, some of which include peaches, berries, and apples. Anthocyanins are the compounds present in flowers and fruit that give them their color, ranging from pink, red, or blue to purple (Manach et al., 2004).

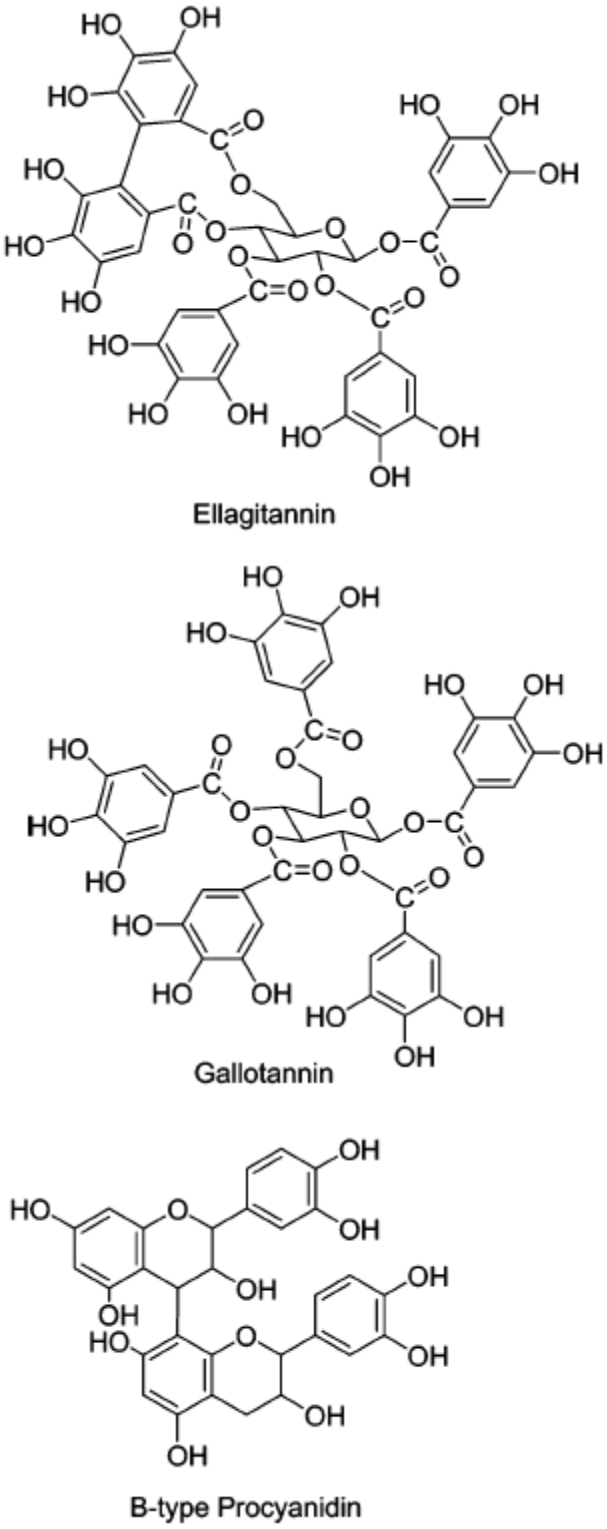

Figure 2.2 Structures of typical tannins and proanthocyanidins (Meyers and others 2006).

Flavonoids consist of a nucleus with two phenolic rings and an oxygenated heterocycle. More than 4,000 flavonoids have been identified being differentiated based on the oxidation state of the heterocyclic pyran ring. Flavonols exist as oligomers or polymers, called condensed tannins or proanthocyanidins because they discharge anthocyanidins when heated in acidic environments (Cheynier, 2005).

Isoflavones are flavonoids with structures similar to estrogens, classified as phytoestrogens. They are found mostly in foods containing or made from soybeans. Depending on the method and amount of processing that the food undergoes, the isoflavone content may be altered. The environment in which the soybeans are grown can also influence the isoflavone content. In addition, the Eastern tradition of consuming soy in the form of fermented products such as miso or tempeh alters the isoflavone content compared to Western traditions of consuming soy products (Cassidy and others 2000).

Stilbenes are not very prevalent in the human diet. Resveratrol, found in wine, has been studied the most for its anticarcinogenic effect and for prevention of heart disease. However it is present in such low concentrations, that at normal intake levels, it is unlikely to have much of a protective effect (Manach et al., 2004).

Lignans are found in the highest amounts in linseed (flax seed), however traces are also found in cereals, grains, fruit, certain vegetables, but don't contribute as significantly as linseed. Lignans are metabolized to enterodiol and enterolactone by bacteria in the gut (Manach et al., 2004).

Polyphenols have been identified as superior antioxidants, compared to vitamins C and E in in vitro studies due to the structure of polyphenols. The structure of polyphenols is ideal for hydrogen or electron donating, predicting their free-radical scavenging ability. Polyphenols also

possess the ability to chelate metals acting as preventative antioxidants by preventing the formation of metal-catalyzed free radicals (Rice-Evans et al., 1997).

CURRENT CONSUMPTION

Of all the antioxidants currently being consumed, polyphenols are being consumed in the greatest amounts (Scalbert and Williamson, 2000). Polyphenol intake is estimated to range from 20 mg for individual compounds to about 1000 mg per day for intake of total polyphenolic compounds. This estimated range is higher than the intake of vitamins C and E (Soobrattee et al., 2005), (Scalbert and Williamson, 2000). One reason for the wide range of reported consumption is that, in polyphenol analysis of foods, recovery of polyphenols from foods depends on the method of extraction. In the Spanish diet, polyphenol intake has been estimated at 2590 to 3016 mg per person per day when foods were digested with enzymes before the polyphenols were extracted (Saura-Calixto and others 2007). This method is similar to digestion in the human gastrointestinal tract, and gives a higher recovery of polyphenols from the food matrix than simple blending and chemical extraction. Polyphenols are being consumed from foods such as fruits, vegetables, and various beverages (Scalbert and others 2005a). Data concerning specific polyphenol intake have not been extensive up to now. As of 1976 it was estimated that dietary flavonoid intake was approximately 1 g/day. Since then, flavonols have been researched the most with estimated intakes of 20 to 25 mg/day for specific compounds in the US (Manach et al., 2004), (Sampson and others 2002). Among men and women in the US, quercetin makes up the largest percentage of flavonoid intake (Sampson et al., 2002). The most abundant food sources include onions, tea and apples. Proanthocyanidins are considered to contribute the most significantly to polyphenol intake among Americans due to the high degree in which they are present in food (Cheynier, 2005), (Gu and others 2004). The estimated

average intake per person per day of proanthocyanidins is 57.7 mg (Gu et al., 2004). Because some antioxidant compounds are only found in specific foods, an individual's intake of that specific compound can significantly increase with the specific food consumption, an example being the polyphenol caffeic acid in the cinnamic acid group. When coffee is consumed, caffeic acid consumption increases. Therefore an individual who does not drink coffee would not have as high of an intake of caffeic acid as an individual who does drink coffee (Clifford, 2000). Another example is epicatechins in tea, where consumption of tea greatly increases consumption of epicatechins (Manach et al., 2004).

In addition to specific food choice, human consumption of phytochemicals varies depending on geographic location due to variability in food production around the world. In areas where a large amount of citrus fruits are grown, flavanone consumption is increased because of its high content in citrus fruits. In locations where a large amount of berries are grown, anthocyanin consumption is increased. Isoflavone consumption is higher among Asian cultures due to their high consumption of soya products (Manach et al., 2004).

ANTIOXIDANT EVALUATION METHODS

In terms of quantifying antioxidant capacity there is no single standardized method. Because of this, using certain methods to evaluate some foods may be inappropriate and comparing results using different methods may be inappropriate. Developing a standardized method for analysis would be beneficial in terms of marketing products with nutraceutical benefits in terms of keeping data consistent and reliable (Prior et al., 2005).

Antioxidant assays can be divided into two groups. The first involving hydrogen atom transfer and the second involving single electron transfer. Examples of hydrogen atom transfer (HAT) assays include the total radical trapping antioxidant parameter (TRAP) assay, the oxygen

radical absorbance capacity (ORAC) assay, and the crocin bleaching assay. The single electron transfer (SET) assays include the total phenols by Folin-Ciocalteu reagent, the Trolox equivalent antioxidant capacity (TEAC) assay, the ferric ion reducing antioxidant power (FRAP) assay, the N,N-demethyl-p-phenylenediamine (DMPD) assay, and the Cu(II) reduction capacity assay (Huang et al., 2005).

Before the amount of antioxidants present in a food can be evaluated, the polyphenols must first be extracted. The success of the extraction can depend on a number of factors including the extraction method, storage, interfering compounds, chemical attributes, and particle size. Polyphenols can be chemically very simple monomers or highly complicated polymers. Polyphenols can be attached to other carbohydrate or protein molecules as well as other compounds present in the plant. The solubility of the polyphenol also can widely vary. There is no uniform method of polyphenol extraction. Several variables exist which can affect the level of extracting including the solvent used, the ratio of solvent to the sample, and the time the sample is left in the extraction solvent. The times that have been used range from 1 minute to 24 hours. The solvents that have been used include methanol, water, acidic methanol, ethyl acetate, 70% acetone, and 50% ethanol (Naczk and Shahidi, 2004). The alcohol based methods (ethanol and methanol) have resulted in the highest total polyphenol extraction (Bonoli and others 2004a), (Bonoli and others 2004b). In addition to the method of extraction used, the temperature at which the extraction is made can alter the degree to which polyphenols are extracted (Kalt and others 2000).

Two methods that have been used to determine total phenolics (distinct from antioxidant capacity of these phenolics) without differentiating among specific phenolic compounds are the Folin-Denis assay and the Folin-Ciocalteau assay. A disadvantage of using these 2 reagents is

that they do not take into account reducing substances such as vitamin C which may also be present in the sample being tested. The vanillin HCl method is used to assess proanthocyanidins (condensed tannins). Catechin is often used as a standard in this assay and as a result may lead to the over-estimation of proanthocyanidins. The 4-dimethylamino-cinnamaldehyde (DMCA) assay has also been used to quantify proanthocyanidins with questionable accuracy (Naczk and Shahidi, 2004). The Folin-Ciocalteau method utilizes both HAT and SET mechanisms to eliminate ROS (Prior et al., 2005). The amount of polyphenols present in food can often be seen by the color of the food, with the more colorful the food, the higher the polyphenol content. It has been found that colorless polyphenols also substantially contribute to antioxidant capacity. Therefore color may be one indicator used to evaluate antioxidant capacity, but it should not be the only indicator used (Kalt, 2005).

The FRAP method was originally developed to measure antioxidant potential in blood plasma and tissue extracts, but it also provides a reliable and reproducible method for determining antioxidant capacity of food extracts and dietary antioxidants. The results of multiple methods of determining antioxidant capacity are comparable, including the FRAP, TEAC, and DPPH methods. The values are often not exactly the same but correlate well with one another (Stratil and others 2006), (Pulido and others 2000). In comparing the FRAP and Folin-reagent method specifically, the assays do not produce the exact same values, but do demonstrate a highly significant correlation. Although the assays can not be directly compared, the results can relatively indicate foods that are higher or lower in antioxidants (Agbor and others 2005).

The relationship between the total phenols assay and total antioxidant capacity is not entirely clear. Some research indicates a linear relationship between the phenolic content and the

antioxidant capacity. However other research indicates that the degree of the linear relationship depends on the food group being examined. Fruits and vegetables may be strongly correlated, but not all groups follow that same trend (Wu et al., 2004). The possible lack of association may be due to the lack of an oxygen radical in the total phenols assay (Huang et al., 2005).

In examining specific foods, such as wine and guava fruit extracts, a strong correlation was found between the total phenolic content and the total antioxidant capacity. The total antioxidant capacity of the tested wines could be determined from the total phenolic content expressed as gallic-acid equivalents (GAE). The following equation explains how the total antioxidant capacity can be derived:

$$TAA = 0.0064GAE - 0.2508$$

Total antioxidant activity (TAA) can be derived using the gallic-acid equivalent (GAE) value obtained from the total phenols using Folin-Ciocalteu assay (Lopez-Velez and others 2003).

In the guava fruit extracts the correlation between the total phenolic content and antioxidant capacity was positively high, especially between the total phenols and the FRAP. A high association between total phenols and antioxidant capacity has also been found in other fruits (Thaipong and others 2006).

PRESENCE OF ANTIOXIDANTS IN FOOD

Foods contain antioxidants such as ascorbic acid, vitamin A, and vitamin E which are essential nutrients. However, many foods, fruits and vegetables in particular, contain a wide variety of non-nutrient antioxidants. The antioxidant capacity of certain foods may not be the sum of all the specific antioxidants in that food, however. This could be due to the different

interactions that occur between the nutrients, oxidants, and antioxidants present in the food. From the available literature with testing foods from several categories, it appears that spices have the highest antioxidant capacity (Saxena and others 2007).

The amount and type of polyphenols found in food varies greatly from food to food and within species, an example being that anthocyanins only exist in red grapes and apples. This variation may be due to the environmental conditions in which the food is grown such as sun exposure, rainfall, soil type, field or greenhouse environment, biological culture, and fruit yield per bush or tree. Other factors that may influence polyphenol content include genetics and the growth or maturation stage of the fruit when tested. In addition, flavanols are the major polyphenol in apples, and the amount present differs between different kinds and colors of apples such as dessert and cider varieties. Dessert varieties contain about 1g/kg fresh weight, whereas cider varieties contain about 5 g/kg (Cheynier, 2005) fresh weight and possibly even as high as 10 g/kg (Manach et al., 2004).

The variability in polyphenol content may be attributed to the role polyphenols play in repairing damage. Polyphenols contribute to the lignification process helping cells to heal, and they may possess antimicrobial characteristics. It appears that vegetables grown under conditions of increased stress have an increased concentration of polyphenols, such as vegetables grown organically. More research is needed to state this conclusively (Manach et al., 2004). Sun exposure and the growing environment appear to affect only specific polyphenols in certain types of food (Kalt, 2005). Exposure to disease may also affect polyphenol content. Plants synthesize and accumulate antimicrobial compounds called phytoalexins, which are produced as a result of exposure to infection or stress such as drought or heat. Phytoalexins are considered to be only one of multiple mechanisms in place to protect plants from disease (Kuc, 1995). In

addition, certain types of mold have been shown to increase or decrease the resveratrol content of grapes depending on the degree of exposure to the mold (Kalt, 2005).

PROCESSING OF POLYPHENOL FOODS

Cooking and storage may also significantly alter polyphenolic content depending on the storage conditions and the cooking method (Manach et al., 2004). The presence of polyphenols in processed food products depends on which part of the food was used during processing and the length of time the processing took. An example includes grape processing, where white grape juice does not contain all of the polyphenols present in the grape because the skin is not used in processing. The amount of polyphenols also depends on the amount of time in which foods are processed. Wine produced using longer maceration times, allows increased extraction of polyphenols increasing the amount in the final product (Cheynier, 2005).

Polyphenol loss is quite variable during the heating process depending on the method of heating, the length of heating, and the pH during the heating process. In addition, the specific type of polyphenols lost during heating is quite variable and in some cases depends on the source of the polyphenols (Brenes and others 2002), (Takenaka and others 2006). Vegetables fried in virgin olive oil demonstrate a polyphenolic retention rate of 25% to 70%. Frying in virgin olive oil provides an increase in polyphenols in the form of tyrosol and chlorogenic acid (Kalogeropoulos and others 2007). Heating in water may also affect polyphenol content because polyphenols are water soluble. They can leach out during certain cooking processes. Fruit ripening can also play a role in the type of polyphenol present in fruit. As fruit ripens the anthocyanin content increases as indicated by the change in fruit color. Although the anthocyanin content may change as fruit ripens, anthocyanins only make up one part of total

polyphenol content. The change in total polyphenol content during the ripening process varies between different kinds of fruit (Kalt, 2005).

The storage of fruit can alter polyphenol content depending on the storage conditions and the length of storage (Kalt, 2005). The storage of berries has been shown to have no effect and in some cases be beneficial in terms of increasing anthocyanin content during storage at ambient temperatures (Kalt and others 1999). However, a beneficial effect has not always been found with storage. Rice stored at 4°C lost more polyphenol compounds compared to rice stored at 37°C (Zhou and others 2004). The temperature at the time of extraction can also influence the polyphenol retention during storage even when storage conditions remain constant (Kalt et al., 2000).

When fresh foods are processed and incorporated into new food products, polyphenols can decrease depending on the processing method used. The simple procedure of peeling fruits may decrease polyphenolic content due to the large amount of polyphenols found in the skins of some fruits (Manach et al., 2004). Blueberries exposed to heat processing, such as the baking of blueberry muffins, decrease in polyphenol content compared to products not exposed to heat processing (Schmidt and others 2005). Therefore, using high-polyphenol content foods before exposure to processing, such as raw or unpeeled, would maintain the polyphenol content and allow the high-polyphenol foods to exert the postulated benefits.

ANTIOXIDANT ACTIVITY IN VIVO

It is difficult to ascertain the exact effect that polyphenols have in the body. This is due primarily to the lack of research involving bioavailability and polyphenolic action in the body. In vivo research is difficult to conduct due to the variability of polyphenols in the diet and the variability in polyphenol bioavailability. Plasma concentrations of polyphenols vary greatly

depending on the polyphenol. This could be due to the bioavailability of the intact polyphenol or the bioavailability of metabolites of the polyphenol (Manach et al., 2004). Polyphenols are subject to extensive metabolism at multiple points along the digestive tract. The presence of free polyphenols cannot be detected in blood after the consumption of many foods containing polyphenols. Pharmacologic doses must be consumed for the free forms to be detected. Metabolic processes also alter phenolic structure so as to resemble other compounds which inhibit the use of the metabolites for use as biomarkers (Spencer and others 2007). Polyphenol absorption also depends on the dose of polyphenols consumed (Han et al., 2007). In addition to the dose of polyphenol absorbed, methylation of the compound may also influence its absorption. Methylated flavones have demonstrated increased cellular flux compared to unmethylated flavones(Wen and Walle, 2006), (Walle, 2004).

Although not all ingested polyphenols are absorbed into general blood flow, the polyphenols that are absorbed increase the antioxidant capacity (Ninfali et al., 2005). The original purpose of the FRAP assay was to measure changes in antioxidant activity in the blood. The exact mechanism by which polyphenols are absorbed is unknown (Cao and others 1998). The bioavailability of polyphenols varies among the different varieties of polyphenols and largely depends on the size of the polyphenol. The larger polyphenols such as proanthocyanidins, tea catechins, and anthocyanins are the least absorbed. The best absorbed polyphenols include isoflavones and phenolic acids such as caffeic and gallic acids. In the small intestine, flavonoid glucosides may be absorbed as free aglycones and not as intact glycosides (Han et al., 2007). It has been demonstrated that the consumption of strawberries, spinach, and red wine can increase the antioxidant content of urine in elderly women, suggesting antioxidant absorption (Cao et al., 1998). The consumption of several fruits, including blueberries, plums,

cherries, grapes, and strawberries, has been shown to increase the antioxidant capacity of plasma in healthy human subjects (Prior and others 2007). The consumption of polyphenol-rich berry juice has demonstrated an increase in antioxidant capacity of plasma (Netzel and others 2002). The transporters identified as playing a role in flavonoid absorption include sodium-dependent glucose transporter 1 (SGLT1), monocarboxylate transporter (MCT), and multidrug resistance-associated proteins 2 and 3 (MRP2, MRP3) (Walle, 2004). It has yet to be determined the role salivary proteins play in intestinal absorption of flavonoids (Cai and others 2006). Certain flavonols, specifically quercetin, inhibit the carbohydrate transporter GLUT2, but not other sugar transporters. The flavonol itself is not absorbed via GLUT2 however (Kwon and others 2007).

Select flavonoids have demonstrated the ability to be absorbed via the stomach in rat models. It appears that certain polyphenols can resist gastric acidity and remain intact until reaching the small intestine. Not all polyphenols can be absorbed in the small intestine; some must be absorbed in the large intestine (Manach et al., 2004). The microflora present in the gut metabolize the polyphenols into a wide range of low molecular weight phenolic acids. The benefits of polyphenols can be observed before the compounds are even absorbed, through actions in the lumen of the large intestine (Han et al., 2007). The foods in which the polyphenols are consumed may also influence the bioaccessibility of polyphenols depending on the composition of the food, such as fiber or protein content (Manach et al., 2004). Polyphenol absorption is not affected by the consumption of cocoa with added milk protein (Keogh and others 2007) or black tea with added milk (Reddy and others 2005), (Hollman and others 2001), (Richelle and others 2001). Protein may, however, affect the antioxidant efficacy of flavonoids found in tea when β-casein is added (Arts and others 2002). In addition to the other food consumed with polyphenols, polyphenols may interact with other polyphenols affecting

absorption. In a rat model looking at 3 specific polyphenols, genistein, hesperetin, and ferulic acid, genistein and hesperetin were less available peripherally compared to ferulic acid. This could be due to a high intestinal and biliary secretion of their conjugates. This model also indicates an increased absorption when intestinal secretion of conjugates is saturated (Silberberg and others 2006).

After subjecting food to the digestive conditions experienced in vivo, such as exposure to digestive enzymes, the bioaccessibility can be measured in vitro. However, this measurement is not exactly representative of in vivo conditions due to variations in gut flora. Proanthocyanidins or condensed tannins demonstrate low bioaccessibility in the small intestine. Complete bioaccessibility of polyphenols in the small intestine is estimated at 48%, while in the large intestine it is estimated at 42% (Saura-Calixto et al., 2007).

Biologic absorption of polyphenols also depends on the polyphenolic structure. Differences between individual person's absorption have been found. The majority of ingested polyphenols are not detected in urine. This could be due to a lack of absorption across the intestinal wall, absorption and excretion via bile, or metabolism by gut flora or other tissues. Plasma concentrations of flavonoids rarely go above 1 M after consuming 10 to 100 mg of a single compound. The length of time to reach optimal concentration in plasma is variable between different polyphenols. Whenever the optimal concentration is reached, it is not maintained, meaning in order for optimal concentration to be maintained, consumption would also have to be maintained (Scalbert and Williamson, 2000).

POTENTIAL HEALTH BENEFITS OF PROANTHOCYANIDINS (CONDENSED TANNINS)

Proanthocyanidins have demonstrated many positive effects in several disease processes, including infection, cardiovascular disease (CVD), diabetes, and others. The proanthocyanidins

present in cranberries have demonstrated the ability to prevent the adhesion of bacteria to the urinary tract cell walls, promoting urinary tract health (Prior and Gu, 2005), (Leahy and others 2002). The same anti-adhesion characteristic of bacteria in the urinary tract has been associated with preventing *H. pylori* from attaching to the stomach and preventing peptic ulcers (Leahy et al., 2002), (Prior and Gu, 2005). In addition to the prevention of bacteria adhesion, proanthocyanidins have demonstrated antibacterial properties. Cinnamon stick contains two compounds, cinnamaldehydes and proanthocyanidins, which have demonstrated in vitro antibacterial properties. The antibacterial properties have implications in the use of cinnamon sticks as a natural food preservative (Shan and others 2007).

Proanthocyanidins can positively influence the oxidative state in plasma. Following a meal containing 300 mg grape seed proanthocyanidin extract (GSPE), the antioxidant capacity of plasma increased by decreasing the presence of oxidants and increasing antioxidant levels which increased LDL resistance to oxidation (Prior and Gu, 2005), (Yamakoshi and others 1999). Consuming 110 mg of proanthocyanidins from grapes for 30 days appeared to decrease DNA oxidation and increase vitamin E levels in red blood cell membranes. The consumption of flavanols from a cocoa beverage appeared to decrease F-2-isoprostanes, a marker of lipid peroxidation (Prior and Gu, 2005). Cocoa can decrease inflammatory cytokines while at the same time increase anti-inflammatory cytokines (Kris-Etherton and Keen, 2002), and along with cranberries in in vitro studies has demonstrated the ability to decrease LDL oxidation (Kris-Etherton and Keen, 2002), (Leahy et al., 2002), (Porter and others 2001).

In studies investigating the specific effect of cocoa flavonoids on CVD, plasma concentrations of proanthocyanidins increase, endothelial function and vascular function have been shown to improve, as well as platelet adhesion has been shown to decrease (Prior and Gu,

2005), (Flammer and others 2007). Proanthocyanidins have demonstrated positive effects in terms of cataract formation. They have also demonstrated beneficial effects in lipid profiles and glucose levels in individuals with type 2 diabetes (Prior and Gu, 2005).

GSPE has been shown to be protective against the negative effects that oxidative stress can have on various tissues. Some of the beneficial effects have demonstrated cytotoxicity towards various cancers including breast, lung, and gastric adenocarcinoma. Beneficial effects have also been seen in protection against myocardial infarction in rats. Chronic supplementation of GSPE also has been used to treat pancreatitis in humans (Bagchi and others 2000).

Research indicates that proanthocyanidin absorption across the intestinal cell wall is best for dimers and trimers, and limited for polymers greater than 6. In rat models in instances where only a small percentage of dimers cross to the serosal side of enterocytes, the presence of monomers increase suggesting cleavage of the dimer during transport (Prior and Gu, 2005).

DOSE DEPENDENT BIOACTIVITIES OF POLYPHENOLS

Polyphenols have been shown to have a positive effect on plasma LDL and HDL levels, with the effect varying depending on the amount of polyphenols consumed. Slightly hypercholesterolemic subjects showed a decrease in plasma LDL cholesterol and oxidized LDL, and an increase in plasma HDL compared to baseline after consuming varying levels of cocoa. In the low-cocoa group 13 g was consumed, in the middle group 19.5 g of cocoa was consumed, and in the high cocoa group 26 g was consumed during a 4 week period. The polyphenols present in cocoa thought to provide these beneficial effects include catechin, epicatechin, procyanidin B2, procyanidin C1, and cinnamtannin A2 (Baba and others 2007).

Polyphenols present in red wine have demonstrated a dose-dependent response in terms of angiogenesis triggered by ischemia. A high dose (20 mg/kg/day) of polyphenols present in

red wine showed anti-angiogenic properties in a rat model, while a low dose (.2 mg/kg/day) promoted angiogenesis (Baron-Menguy and others 2007). Consuming juices containing different amounts of polyphenols, such as apple versus pomegranate juice, have demonstrated differences in antioxidant function and reducing damage from oxidative stress. The pomegranate juice, higher in polyphenols, demonstrated a superior ability to provide protective effects compared to the lower phenol juice, apple juice (Guo and others 2008).

The bioavailability of the flavonoids present in cocoa has been shown to be dose responsive. As the amount of consumption of epicatechin increased, the plasma concentration of epicatechin also increased. This also demonstrated a dose response in terms of serum antioxidant capacity (Wang and others 2000).

POTENTIAL FOR POLYPHENOL TOXICITY

The possible benefits of polyphenols in terms of health have been well documented and reported. The ingestion of polyphenols may have some negative side effects however. Polyphenols in tea have demonstrated the ability to precipitate protein, meaning that those proteins are denatured. Tea polyphenols demonstrated an inhibitory ability of α-amylase, pepsin, trypsin, and lipase ranging from 32% to 61% for the 4 enzymes. The inhibition of these enzymes means there may be decreased digestion of protein, carbohydrates, and lipids, indicating possible anti-nutritional effects (He and others 2006). One specific kind of polyphenol has been associated with anti-nutritional effects. Tannins, more specifically condensed tannins or proanthocyanidins have been associated with demonstrating antinutritional effects (Mennen and others 2005). Proanthocyanidins can exist as simple monomers or highly polymerized compounds. The effect that proanthocyanidins have in the body depends on the degree of polymerization due to absorption constraints. It appears that dimers and perhaps

monomers are the only proanthocyanidins that can be absorbed. Therefore the occurrence of any negative effects from large polymers may be limited to the intestinal tract (Beecher, 2004).

The ability of tannins to precipitate protein has been utilized in cultures throughout history. The process of converting animal hide to leather is called tanning. Because tannins were utilized in this process, the compounds were called tannins. The exact effect that the protein binding ability of tannins have in the body remains to be seen (Beecher, 2004).

As with any potential toxicity, the amount of consumption plays a key role in toxicity occurrence. Polyphenol intake at supplemental levels may cause more harm than good. Also important is the specific type of polyphenol consumed. Specific flavonoids have demonstrated the ability to impede thyroid hormone synthesis. The intake of isoflavones at increased intake levels may affect sexual maturation and male and female fertility (Mennen et al., 2005), (Scalbert et al., 2005b). Contrary to much of the published beneficial research regarding polyphenols and CVD, polyphenols may increase homocysteinemia, increasing risk for the development of CVD (Scalbert et al., 2005b). Polyphenols may also influence medication bioavailability, affecting the degree to which intended drug function occurs. It is important to keep in mind the dosing of polyphenol supplements and polyphenol food intake because dietary polyphenol intake is much less than the level of supplemental polyphenol intake. In addition to polyphenol intake amounts, it is important to consider the risk that certain populations may have of consuming increased amounts of polyphenols, such as pregnant women, children, and individuals with specific conditions (Mennen et al., 2005). The testing of one polyphenol supplement called Applephenon, produced from polyphenols present in apples and consumed in Japan, found no adverse effects from consumption at the level of 2000 mg/kg (Shoji and others 2004).

Certain metals including copper and zinc are chelated by polyphenols. The chelation of those metals may be affected by the types of polyphenols present in the foods in which the metals are consumed. Red wine has been found to not affect copper and zinc absorption or tissue levels in rat models (Coudray and others 2000). Due to the possible metal chelation with polyphenols, in certain populations there could be an increase in risk for developing deficiencies. In the Western general population, where individuals have adequate iron stores, tea containing polyphenols has not been shown to be problematic. Only in populations who have marginal iron status does the consumption of tea and the chelation of polyphenols present in tea cause iron deficiency (Temme and Van Hoydonck, 2002). Certain population groups are at risk for developing iron-deficiency anemia due to increased needs. These groups include menstruating women, pregnant women, growing children, and individuals experiencing massive blood loss. If these individuals do not consume adequate amounts of iron, and consume foods high in polyphenols which can chelate the iron, they may not absorb iron in amounts sufficient enough to prevent iron-deficiency anemia (Charlton and Bothwell, 1983). Contrary to not absorbing enough iron, individuals with hemochromatosis, a genetic disease where the body absorbs and stores too much iron, may benefit from the chelation of iron by polyphenols. Polyphenols chelate iron preventing absorption and decreasing the need for other forms of treatment (Kaltwasser and others 1998). Iron chelation may also be beneficial in those individuals developing Alzheimer's and Parkinson's diseases due to the decreased iron available to the neuronal cell tissue (Weinreb and others 2004).

Polyphenol toxicity has only been reported involving animals consuming large amounts of tannin-rich plants. In some animal models, certain polyphenols have demonstrated pro-

oxidant effects, but because of the lack of research regarding the toxicity of polyphenols, the applicability of that research to humans in questionable (Scalbert et al., 2005b).

ACORNS AND POLYPHENOLS

Acorns have been historically consumed all around the world, including North America, Europe, Asia, Africa, and the Middle-East. In California, half of the diet consumed by Native Californians consisted of acorns (Bainbridge, 1986). Among the poorer classes of people in Italy and Spain, acorns contributed up to 25% of calories consumed (Rakic and others 2006). Acorns have a slightly lower protein content compared to cereal grains. The red and black varieties can contain rather high levels of lipid, some as high as 34.3% dry weight. This indicates the benefits acorns may have in terms of providing a calorically dense food option (Ofcarcik and Burns, 1971).

Acorns are edible with certain varieties requiring processing to become palatable, while other varieties among the genus *Quercus* are considered sweet. The processing required to make acorns palatable includes leaching the bitter-tasting tannins from the nut using hot water. Tannins have been shown to decrease from 9% to .18% after leaching without altering essential amino acid content. In the past to reduce bitterness, Native Americans sweetened acorns with iron-rich red earth, wood ashes, and other ingredients (Bainbridge, 1986). Acorns from the genus *Quercus* contain 300 μg of hydrolysable tannins per gram. Tanoak (*Lithocarpus densiflorus*) acorns have been determined to contain 464 mg condensed tannins per 100 g acorn pericarp (Meyers et al., 2006).

Acorns have been used in a variety of manners in cooking. Acorn meal has been used in place of corn meal in baked products. Acorn pieces and acorn meal can be used in soups and

stews. Acorns can be used as a replacement of other nuts in recipes. Acorns have also been used in various beverages, such as coffee drinks (Bainbridge, 1986).

One benefit of consuming acorns can be attributed to the high polyphenol content, although acorns are also good sources of protein, carbohydrate and fats. The different varieties of acorns contain differing amounts of polyphenols. The polyphenol content of *Q. robur* ranges from 11.76% to 14.93% (Rakic et al., 2006). Total phenols for *Lithocarpus densiflorus* using the Folin-reagent method have been measured at 71 mg/g dry weight (Meyers and others 2007). Using the same method total phenols for two acorn varieties, *Q. robur* and *Q. cerris* were respectively determined to be .223 and .229 mg gallic acid equivalents per mg dry weight extract (Rakic and others 2007). 32 phenolic compounds have been identified in three different species of the genus *Quercus* (Cantos and others 2003).

Acorns are widely consumed by many animals and birds without apparent harm. For example, Iberian pigs have been identified as large consumers of acorns. They have been observed eating amounts as high as 7 to 10 kg per day. Iberian pig meat is also considered to be of superior quality compared to other pig meat. This may be due to the high consumption of acorns. In testing phenolic content related to Iberian pig consumption, of the 3 varieties tested, *Q. ilex* contained the highest amount of polyphenols at greater than 2 g/kg ingested acorn. Because of the large amount of acorns that Iberian pigs can consume per day, it is estimated that they can consume between 14 and 20 g of polyphenols per day, solely from acorns. It is this high amount of polyphenol consumption which is suspected of creating superior meat due to decreased lipid peroxidation (Cantos et al., 2003). However, certain varieties of acorns that have been used to feed livestock in large percentages have shown to be poisonous (Bainbridge, 1986).

Quercetin, part of flavonol group of phytochemicals, is derived from quercitron bark and used as a dye. The term quercitron comes from the inner bark of the Bark Oak, *Quercus discolor*. The prefix 'querc-' comes from the oak genus, *Quercus*, which is the kind of tree that acorns grow on ("quercitrin", 2008).

PHYTOCHEMICAL DATA AVAILABLE

Several databases exist specifying the phytochemical content of certain foods. The USDA Database for the *Proanthocyanidin Content of Selected Foods* contains the proanthocyanidin content of various fruits, vegetables, nuts, spices, and beverages. High-performance liquid chromatography (HPLC) was used to determine the proanthocyanidins present in the food samples. The monomer, dimer, and trimer forms are reported individually. The tetramers, pentamers, and hexamers are reported as one group, as well as the heptamers, octamers, nonamers, and decamers. Polymers greater than 10 were reported as the last group. The complexity of reporting the phytochemical contents of foods is demonstrated by the variation of proanthocyanidins, which is only one class of flavonoids present in food. Table 2.1 contains values for select food items. The following spices contained no detectable proanthocyanidins: dried basil, chili powder, ground cloves, garlic powder, ground ginger, yellow mustard seeds, onion powder, dried oregano, paprika, dried parsley, black pepper, poppy seed, and ground turmeric (Nutrient Data Laboratory and Agriculture, 2004).

Table 2.1 Proanthocyanidin content of various foods

Food		mg/100 g edible portion[a]
Nuts		
Almonds	Monomers	7.8
	Dimers	9.5
	Trimers	8.8
	4-6mers	40.0
	7-10mers	37.7

	Polymers	80.3
Pecans	Monomers	17.2
	Dimers	42.1
	Trimers	26.0
	4-6mers	101.4
	7-10mers	84.2
	Polymers	223.0
Walnuts	Monomers	6.9
	Dimers	5.7
	Trimers	7.2
	4-6mers	22.1
	7-10mers	5.41
	Polymers	20.0
Spices		
Cinnamon, ground	Monomers	23.9
	Dimers	256.3
	Trimers	1252.2
	4-6mers	2608.6
	7-10mers	1458.3
	Polymers	2508.8
Curry powder	Monomers	0.0
	Dimers	9.5
	Trimers	22.9
	4-6mers	41.78
	7-10mers	0.0
	Polymers	0.0

[a] (Nutrient Data Laboratory and Agriculture, 2004)

Data concerning antioxidant content of a large variety of common foods has already been published using FRAP analysis. 1113 food samples were analyzed for antioxidant capacity and ranked according to the resulting values. The 50 foods with the highest antioxidant capacity are displayed in Table 2.2. Also in Table 2.2 is a conversion of the FRAP values to total phenol values in mg GAE/g. This was done using previously calculated FRAP values and multiplying by 1.019 to calculate the total phenolic value. The factor 1.019 was determined from calculating the correlation between the FRAP and total phenol values in previously analyzed food samples (Rice-Evans and others 1997). Also calculated is the amount of phenols per calorie. This was

calculated using the calorie content per g of food product. This may be a more useful value to maximize phenol consumption while controlling caloric intake so as to maintain weight (Halvorsen and others 2006).

Table 2.2 Antioxidant capacity of 50 foods highest in antioxidants – The ferric antioxidant reducing power (FRAP) values, total phenol (TP) values, and mg of phenols per calorie for the 50 foods highest in antioxidant capacity determined from 1113 foods sampled.

Food	FRAP mmol/ 100 g[a]	TP mg GAE/g	mg phenol/kcal[b]
Cloves, ground	125.6	127.9	38.4
Oregano leaf, dried	40.3	41.1	13.7
Ginger, ground	21.6	22.0	6.6
Cinnamon, ground	17.7	18.0	6.9
Turmeric powder	15.7	16.0	16.0
Walnuts	13.1	13.4	2.1
Basil leaf, dried	12.3	12.5	8.8
Mustard seed, yellow, ground	10.5	10.7	2.4
Curry powder	10.0	10.2	2.9
Pecans	9.7	9.9	1.4
Chocolate, baking, unsweetened	8.9	9.1	1.8
Paprika	8.6	8.8	3.1
Chili powder	8.4	8.5	2.8
Parsley, dried	7.4	7.6	2.3
Molasses, dark	4.9	5.0	1.2
Pepper, black	4.4	4.5	1.9
Artichokes, prepared	4.2	4.3	8.6
Chocolate, dark	4.2	4.3	0.2
Blackberries	4.0	4.1	9.4
Whole-grain cereal	3.4	3.5	1.0
Cranberries	3.3	3.4	7.2
Pudding mix, chocolate, cook-and-serve	3.0	3.1	0.3
Bran cereal	2.9	3.0	1.1
Power bar, chocolate flavor	2.8	2.8	0.8
Chocolates, sugar-free	2.6	2.6	0.5
Raspberries	2.3	2.4	4.6
Strawberries	2.2	2.2	6.9
Blueberries	2.2	2.2	5.1
Cabbage, red, cooked	2.2	2.2	7.5
Wine, red	2.1	2.2	2.6
Barley malt syrup, organic	2.1	2.2	2.1
Prunes	2.0	2.1	0.6
Cherries, sour	1.8	1.9	1.2
Peppers, red, cooked	1.6	1.7	1.3
Chocolate cookies w/ vanilla crème filling	1.6	1.6	0.4
Cocoa Krispies cereal	1.6	1.6	0.4

Chocolate chip cookies	1.5	1.6	0.2
Mustard, yellow, prepared	1.5	1.5	14.3
Milk-chocolate candy	1.5	1.5	0.3
Pistachios	1.4	1.5	0.3
Plums	1.3	1.4	3.0
Kiwi Fruit	1.3	1.4	2.2
Corn Flakes	1.3	1.3	0.4
Coffee	1.3	1.3	150.8
Spinach, frozen	1.2	1.3	4.1
Flaxseed, ground or milled	1.1	1.2	0.2
Rice and corn cereals	1.1	1.1	0.4
Toasty peanut crackers	1.1	1.1	0.1
Cupcakes, chocolate	1.1	1.1	0.4
Grape juice	1.0	1.0	0.6

[a] (Halvorsen et al., 2006)
[b] (U.S. Department of Agriculture, 2007)

The total phenolic content and total antioxidant capacity for several kinds of nuts has also been gathered, shown in Table 2.3. The total antioxidant capacity in addition to being expressed as μmol of TE/g, has been expressed as TAC per serving (Wu et al., 2004).

Table 2.3 Antioxidant capacity for select nuts – Total antioxidant capacity (TAC), total phenols (TP), and TAC per serving for select nuts**.**

Nut	serving size (g)[a]	TAC μmol of TE[b]/g[c]	phenol mg/g[b]	mg phenol/serving
Almonds	28.4	44.5	4.1	1262
Brazil nuts	28.4	14.2	3.1	403
Cashews	28.4	20.0	0.2	567
Hazelnuts	28.4	96.5	8.4	2736
Macadamia nuts	28.4	17.0	1.6	482
Peanuts	28.4	31.7	4.0	899
Pecans	28.4	179.4	20.1	5086
Pine nuts	28.4	7.2	0.7	204
Pistachios	28.4	79.8	16.6	2262
Walnuts	28.4	135.4	15.6	3839

[a] (U.S. Department of Agriculture, 2007)
[b] TE = Trolox Equivalents
[c] (Wu et al., 2004)

In addition to water, protein, fat, carbohydrate, vitamins, and minerals, the USDA National Nutrient Database for Standard Reference also contains information concerning non-nutrients. The phytochemicals included in the database are β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin. Data for these phytochemicals although available is not presented here.

GENERAL HYPOTHESIS

Typical U.S. diets that rely on relatively refined foods may provide less than 500-1000 mg of polyphenolic phytochemicals per day.

Specific Hypothesis

The phenolic content of a diet can be increased more than two-fold through the use of acorns and leached acorn products.

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CHAPTER III

DIETARY POLYPHENOLIC INTAKE FROM ACORNS AND ACORN MEAL[1, 2]

[1]Kobs LM, Power J, Swanson RS, Hartle DK and Hargrove JL. To be submitted to *The Journal of Food Science*.

[2]An abstract of this work has been presented at the Institute of Food Technologists annual meeting, New Orleans, LA (June, 2008).

ABSTRACT

Antioxidant consumption can increase through strategic food choices. Acorns could contribute to those strategic choices. We surveyed antioxidant content in a variety of acorns from the southeastern United States. The phenolic content of *Quercus velutina* prior to leaching was 39.4±3.6 mg GAE/g and of *Quercus alba* was 27.4±.3 mg GAE/g. After 5 leaching stages, the phenolic content of *Q. velutina* was 12.3±.8 mg GAE/g and of *Q. alba* was 10.8±.7 mg GAE/g. After milling, the phenolic value for Q. *velutina* was 39.9±4.5 mg GAE/g and for Q. *alba* was 6.6±.1 mg GAE/g. A spice cookie made with all-purpose wheat flour contained 2.7±.1 mg GAE/g. When red oak acorn meal was substituted, the cookie contained 9.6±3.3 mg GAE/g, and when substituted with white oak acorn meal contained 4.2±.4 mg GAE/g. Ingredient interactions influenced the phenolic content. Acorns and acorn products can be a strategic method of increasing polyphenolic intake.

INTRODUCTION

Historically, human diets were probably higher in fruits, vegetables, and unrefined or unprocessed grains and root crops than current diet trends. Populations were hunter/gatherers and consumed diets high in lean protein, omega-3 fatty acids, fiber, and other beneficial phytochemicals. The health of the hunter/gatherer populations was very different from the health of populations today. Hunter/gatherers were generally considered fit and healthy with little incidence of the chronic diseases commonly seen today, such as cardiovascular disease (CVD). Due to the fact that the human genome has basically not changed in the last 10,000 years, some other factor must explain the observed change in disease occurrence. The change in diet patterns could very well explain the change in disease occurrence (O'Keefe and Cordain, 2004). All Paleolithic diets contained animal-based protein, and may have contained amounts of fat the same or even higher than amounts of fat consumed today. Because of other diet characteristics such as low salt, omega-6 to omega-3 fatty acid ratio, and phytochemical intake, historically the occurrence of CVD would not have been as high as it is today (Cordain, 2002).

Polyphenols have been found to have many beneficial properties in terms of improving human health. The consumption of polyphenols is different among different geographical regions and while some consumption trends have changed, some have not. Tea is second to only water in beverage consumption worldwide, and the high tea consumption in Chinese cultures has been associated with reduced hypertension and CVD risk (Yang and others 2004).

Another culture with a possible association between a disease process and dietary intake is the East Indian culture. India practices a wide variety of food habits resulting from religious

and cultural traditions. One of their dietary practices is a high consumption of a variety of spices. The effect that this high consumption of spices has on Indian health is largely unknown; however, the occurrence of cancer in India is less than the occurrence seen in Western countries. Their high consumption of spices is hypothesized to influence cancer occurrences (Sinha and others 2003). Spices have been found to be beneficial in several disease processes including the inflammatory process, cancer, atherosclerosis, diabetes, and digestive stimulation (Srinivasan, 2005).

In addition to tea and spices, historically acorn nuts have been consumed by humans virtually everywhere oak trees existed. In several parts of the world, including North America, Europe, Asia, and Mid-East, acorns were a staple food. Acorns were possibly the most important to natives of California, where up to half of their caloric intake came from acorns. Acorns have many positive attributes including ample content of major nutrients, relative ease of collection, long length of storage, ease of use in food products, and high yields (6,000 pounds/acre). In addition acorns can be grown in hilly land areas, where growth of other crops may cause soil erosion (Bainbridge, 1986). In addition to all of these positive characteristics, acorns may also provide health benefits due to their high polyphenol content.

Whereas the polyphenolic content for fruits (Han and others 2007), beverages (Manach and others 2004), and nuts (Wu and others 2004) has been reported, acorns have not been as extensively researched. Acorns, once properly processed, can be a delicious addition to a diet, and if processed beyond simply a nut, can serve many roles in a diet. This research is concerned with testing the phenolic values of whole acorns, meal made from acorns, and a cookie made using acorn meal.

MATERIALS AND METHODS

Chemicals and Materials

Bovine serum albumin and the Folin-Ciocalteu reagent were purchased from Sigma Chemical Company, (St. Louis, MO). Acorns were collected, along with appropriate specimens for identification purposes, on the campus of the University of Georgia in October 2007. After collection, the trees were identified by Dr. Tim Smalley, a horticulturist at the University of Georgia. After an initial survey of acorns for polyphenol content and suitability for processing, two species were chosen for our study. These were *Quercus velutina* from a black oak tree in the red oak family and *Quercus alba* from the white oak family. All other food ingredients used in the preparation of the cookies were obtained at a major supermarket in Athens, Georgia.

Acorn Leaching and Processing

After the acorns were collected they were dried in a rotary oven for 3.5 hours at 150°F. The acorns were stored at 4°C after drying. The acorns were shelled using a pecan sheller, pliers, hammers, and nut pick. The shelled acorns were divided into 500 g proportions and stored at 4°C until leaching could take place.

Due to the high tannin content of acorns, leaching was necessary to ensure palatability of the prepared products. The acorns were placed on a magnetic stirring plate with a 10:1 volume of water to acorns. The water was heated initially to 80°C and the acorns and water were stirred for 30 minutes. The acorns were then strained and the process was repeated for a total of 4 times. The acorns were then placed in a Cuisanart® food processor and coarsely chopped. Each batch of acorns was chopped using 24 pulses where each pulse consisted of 1 second of

chopping. The finely chopped acorns were then leached 2 more times with a 10:1 volume of 80°C water with stirring for 30 minutes. The acorns were then dried at 150°F in a National Manufacturing Company rotary oven (Lincoln, Nebraska). Dryness was determined by the feel of the chopped acorns and by the moisture present on the parchment paper the chopped acorns were placed on. The amount of drying time necessary ranged from 6.5 to 8 hours. The dried acorns were then milled into acorn meal using a Porkert model 150 manual grain mill.

Cookie Preparation

The acorn cookies were prepared using the following ingredients:

82.5 g light brown sugar

85 g butter, unsalted

25 g egg

126 g molasses

92.5 g cake flour with 1.9 g water

88.5 g acorn meal with 6.2 g water or all-purpose flour

2.3 g baking soda

1.5 g salt

1.35 g ginger

1.3 g cinnamon

1.1 g nutmeg

.5 g allspice

The control cookies were made using the same ingredients, substituting the acorn meal with all-purpose flour.

The control and acorn cookies were prepared using the following method: The butter and eggs were held at room temperature for 45 minutes prior to mixing. The dry ingredients were blended with a Kitchen Aid (K5SS) mixer at speed 1 for 2 minutes, and then held at room temp until needed. The brown sugar and butter were creamed using the same mixer at speed 2 for 2 minutes. The egg and molasses were incorporated in the sugar/butter mixture at speed 2 for 2 minutes. The previously combined dry ingredients were incorporated over a 3 minute period at speed 2. The cookies were deposited with a # 60 scoop on a 41.9 x 30.5 cm sheet pan lined with parchment paper and lightly sprayed with cooking spray in 6 rows 3 across. The cookies were flattened to .6 cm high using a single back and forth stroke of a rolling pin (AACC, 1999).

Extraction Method

The samples were extracted using 50% ethanol. 1 g of edible portion of the food to be extracted was combined with 9 mL of 50% ethanol and homogenized using a homogenizer for 60 seconds. The 50% ethanol solution was shaken for 2 hours at 300 RPM. The sample was centrifuged at 1100 RPM (430 *g*) for 10 minutes at 10° C. The samples were stored at 0°C until assayed.

Ingredient Interactions

Ingredient interactions were tested in a total volume of 40 mL of distilled water that contained 1 g of the sample being tested and 2 g of the ingredient being tested for interaction (either albumin or sucrose). The 40 mL solution was then shaken for 2 hours at 300 RPM in a rotary shaker, and centrifuged at 1100 RPM (430 *g*) for 10 minutes at 10° C . Supernatants were stored at 0°C until assay.

Total Phenols

Total phenolic content was measured using a modified Folin-Ciocalteu reagent method. The sample absorbance was determined at 765 mμ, and the total phenolic content was expressed in gallic acid equivalents (GAE) and determined by comparison with a standard curve (Singleton and Rossi Jr, 1965).

Calorie Content

The caloric content of the different acorn meals and cookies made with acorn meal was calculated based on the macronutrient profiles of the different samples. The fat and protein content were measured at Medallion Labs in Minneapolis, MN using the Kjeldahl method for protein determination and a gravimetric method for fat determination. The moisture and ash content were determined, and the carbohydrate content was calculated as the difference. The calorie content was then determined using 4 calories/g of carbohydrates, 4 calories/g of protein, and 9 calories/g of fat.

Statistical Analysis

For total phenols analysis, simple linear regression analysis was used to determine the relationship between a standard curve and correlation coefficients (R^2). Microsoft Excel software was used for statistical analysis. The total phenolic means and standard deviations for the cookie ingredients were analyzed using statistical analysis software (SAS). The Student-Newman-Keuls Test was also conducted using SAS.

RESULTS

Acorn Nuts

The total phenolic content of the black oak (*Quercus velutina*) and white oak (*Quercus alba*) acorns were tested at each of the 5 leaching stages. The total phenolic content decreased at practically each stage of leaching process. The dry, unleached acorns contained the most phenols at 39.4 ± 3.6 mg GAE/g for *Q. velutina* and 27.4 ± .3 mg GAE/g for *Q. alba*. The acorn after the fifth stage of leaching contained 12.3 ± .8 mg GAE/g for *Q. velutina* and 10.8 ± .7 mg GAE/g for *Q. alba*. The comparison in decrease of total phenolic content between the 2 species of acorns is shown in Figure 3.1. The caloric content of *Q. velutina* is 81.1 calories/serving, where a serving of acorns is 28.4 g. The total antioxidant capacity expressed as gallic acid equivalents and FRAP values for various species of acorns from the red oak and white oak trees is presented in Table 3.3.

Acorn Meal

After milling the leached acorns into acorn meal, the phenolic content of the *Q velutina* meal was 39.9 ± 4.5 mg GAE/g. The phenolic content of the *Q alba* meal was 6.6 ± 0.1mg GAE/g. The caloric content of the acorn meal made from *Q velutina* is 117.2 calories per serving, where a serving is 31.3 g.

Acorn Cookie

The phenolic content of the ingredients in the acorn meal cookie expressed as mg GAE/g and the standard deviations are in Table 3.1.

The Student-Newman-Keuls Test evaluating the difference in total phenols resulted in the cinnamon and allspice being significantly similar to one another, but not to the other ingredients. The red oak acorn meal was not significantly similar to any of the other ingredients. The nutmeg, ginger, molasses, cake flour, light brown sugar, and salt were all significantly similar to each other but not to the other ingredients. Although nutmeg contains more total phenols than salt, the significant similarity between the nutmeg, ginger, molasses, cake flour, light brown sugar, and salt may be due to the large range of total phenolic content among all of the ingredients.

The ingredients in the acorn cookies were assayed separately to see the influence that each ingredient would have on the total phenolic capacity of the finished cookie. The total phenolic content present in the cookie for each individual ingredient are listed in Table 3.2. The control cookie contained 2.7 ±.1 mg GAE/g. The cookie made with red oak acorn meal contained 9.6 ± 3.3 mg GAE/g, and the cookie made with white oak acorn meal contained 4.2 ± .4 mg GAE/g.

Effect of Baking

An additive effect is not seen in the total phenolic values of the ingredients compared to the finished product. This is demonstrated by the fact that the total phenols present in the sum of the ingredients for 1 serving of cookie dough equals 160 mg GAE, and the acorn meal cookie analyzed equals 128.6 mg GAE/cookie. Because the individual ingredients do not add up to the value in the cookie, other interactions are taking place altering the total phenolic content. An explanation for this occurrence could be the cooking temperature or cooking time. However in testing the phenolic content of the cookie at various stages of baking, the cookie cooked at the time and temperature recommended in the recipe (325°F for 13 minutes) resulted in the cookie

with the highest phenol content, demonstrated in Figure 3.2. This suggests that the heat and baking time do not decrease the total phenolic content in the cookie.

Ingredient Interactions

Another possible explanation for why an additive effect is not seen in the total phenolic content of the cookie is interaction between the ingredients. To test the effect that carbohydrate and protein have on the phenolic content of the acorn meal, the total phenols assay was run with acorn meal and sucrose and another sample with acorn meal and albumin, the results are demonstrated in Figure 3.3. The results at first glance appear to indicate that albumin is high in polyphenols because of the results of albumin and water. However when taking into account that the Folin reagent used in the total phenols assay was first developed to detect protein, it is not surprising that albumin appears to be high in polyphenols. However albumin is not high in polyphenols, the reagent is reacts with the protein making it appear as though polyphenols are present in protein. As comparison to see the effect that carbohydrate and protein has on another high phenolic food, cinnamon was also combined with sucrose and albumin to see the effect, the results are shown in Figure 3.4. The results indicate that sucrose has little effect on the total phenolic content of the sample, whereas added albumin decreases the total phenolic content.

Color

In order to ensure that the color of the sample being tested was not influencing the results of the assay, the assays were run with the samples containing added sucrose and albumin where the reagent was added and where the reagent was not added. The difference in results between the samples with the reagent and without the reagent indicates that color is not influencing the results of the assay (data not presented here).

DISCUSSION

The present study shows that acorns contain relatively high levels of polyphenolic compounds, and that a substantial amount remains after leaching and drying. When substituted for a portion of the wheat flour in a recipe for spice cookies, products containing meal from either white or black oak acorns had substantially elevated polyphenol content. The recipe used was specifically chosen to have high initial content of polyphenolics from blackstrap molasses, cinnamon, and nutmeg, and acorn meal greatly increased the amount of extractable polyphenolics in the batter and finished product. Although acorn polyphenolics do interact with protein in ingredients, approximately 40% of the amount present in starting ingredients is recovered after baking. Recovery of polyphenolics by extraction with 10 parts of 50% ethanol was not affected by baking. Preliminary studies have shown that baked goods prepared with acorn meal are well accepted by a sensory evaluation panel (unpublished data). Furthermore, the present work substantiates the idea that traditional diets that included leached acorn products provided a higher intake of polyphenolics than do modern US diets.

Research pertaining to the antioxidant capacity and total phenolic content of foods is increasing, and numeric values for many food sources have been compiled (Halvorsen and others 2006), (Wu et al., 2004). Research pertaining to the antioxidant contribution that acorns could contribute in the diet has not been as comprehensive. Although today acorns are not always associated with human consumption, acorns have played a substantial role in diets historically (Bainbridge, 1986). The high antioxidant capacity of diets historically may help explain the low occurrence of many of the chronic diseases so common today (Cordain, 2002).

The phenolic content of other varieties of acorns have been determined. *Q. suber, Q. rotundifolia*, and were collected and analyzed. The extraction solvent used was methanol. The phenolic quantification method used was HPLC, and determined 32 different phenolic compounds present, all being gallic acid derivatives. This analysis was performed in relation to Iberian pig consumption of acorns and so did not involve any acorn leaching procedure (Cantos and others 2003).

Tanoak (*Lithocarpus densiflorus*) acorns were widely consumed by the original inhabitants of California, and have also been analyzed for polyphenolic content. The extraction method used included acetone, water, and acetic acid. The total condensed tannin content was determined to be 464 mg/100 g acorn pericarp where monomers through tetramers were identified (Meyers and others 2006).

Acorns can also be processed so that the beneficial phenolic content would be more likely to be consumed without requiring consumption of the intact meat of the nut. Although nuts are a healthy source for multiple nutrients including mono- and polyunsaturated fats, vitamin E, and fiber, only about 1 in 10 consumers ate tree nuts on any given day between 1994 and 1996 (Lin and others 2001). More recent data for 2006 shows that household nut penetration sits at 73%, where penetration consists of using nuts as snacks or in cooking in the past 30 days of when the survey was conducted (Mintel, 2007). About 60% of the tree nuts consumed in the United States are consumed in multi-ingredient foods such as cereals, cakes, and candies (Lin et al., 2001). Therefore, a product containing acorns would be more likely to be consumed than whole acorn nuts alone. Although some of the beneficial properties in acorns are lost in the processing of the nuts, if a product containing acorn meal as an ingredient is more likely to be consumed than the plain nut, the benefits would still be achieved.

In order to make the acorns and acorn meal palatable, the nuts must be leached. Historical methods of leaching involved placing the acorns in a stream and allowing the current to wash over the acorns removing the tannins (Harris, 2000). The leaching process removes the tannins that give the acorns a bitter taste. Tannins are part of the polyphenol classification, so leaching removes some of the beneficial compounds present in the acorns. However, the removal of those compounds is necessary to make consumption of the acorns a more enjoyable experience. Even with the removal of some of the tannins, the acorns remain a good source of polyphenols.

The acorn meal recipe is high in polyphenols without the acorn meal added because of the spices used. Replacing the all-purpose flour with the acorn meal adds more polyphenols to the cookie without drastically changing the taste of the cookie. Therefore if a cookie is going to be consumed, even with the high calorie content usually associated with cookies, choosing a cookie with increased polyphenol content would be the more strategic choice to increasing antioxidant potential in the diet.

We calculated expected values for polyphenolic content of the ingredients used for comparison with the polyphenol content of the finished cookies. The results showed a significant decrease in total phenolic value in the finished cookie. The time and temperature of baking did not reduce the phenolic content of cookies made with acorn meal. This indicates that heat is not having a negative effect on the total phenolic content.

The other ingredients thought to influence the total phenolic content include protein and sucrose. This was tested by combining a food with a known high phenolic value such as cinnamon or acorn meal with albumin or sucrose. The results demonstrate that the acorn meal combined with albumin showed a decrease in phenolic content indicating that the protein binds

with the polyphenols preventing the antioxidant activity of polyphenols. The proteins bind to the hydroxyl groups present in the polyphenols masking the effect of those hydroxyl groups (Hagerman and Butler, 1981), (Siebert, 1999). The binding that occurs between polyphenols and protein is variable between different polyphenols and different proteins. This binding has been demonstrated in vitro, and binding that occurs in vivo can also influence polyphenol antioxidant activity. Bioavailability can be affected by binding with other compounds, and the extent of binding with other compounds is dependent on several factors including pH, temperature, and the concentrations of proteins and other polyphenols present (Arts and others 2002).

Because nuts are so calorically dense, eating some types of nuts for their phenol content would substantially increase caloric intake. On a per serving basis, most nuts are relatively similar in terms of their calorie content. Their phenol content, however, on a per serving basis varies greatly between different types of nuts. Walnuts, pistachios, acorns, and pecans provide in some cases more than double the phenolic content per serving, demonstrated in Figure 3.5.

These results indicate that for the typical American diet, which is high in calories, choosing walnuts, pistachios, acorns, or pecans would be more strategic choices because of the high phenolic content per serving in relation to the calorie content per serving. In addition to consuming these nuts whole, incorporating these nuts into other food products would also contribute to polyphenol intake, such as grinding into a flour or meal. Although through processing some polyphenols may be lost, using the nuts high in polyphenols would still provide many of the benefits associated with consuming foods high in antioxidants. Through more strategic food choices, polyphenol intake can be increased, and acorns can be a large contributor to that increased polyphenol intake.

Table 3.1 Total phenols of ingredients used in cookie made with red oak acorn meal – The ingredients used to make acorn meal cookies and the total phenolic content of each ingredient expressed as a mean value with standard deviation (n=3).

Ingredient	Total Phenols mg GAE/g
Red Oak Acorn Meal	60.2 ± 17.2
Allspice	95.2 ± 9.5
Baking Soda	0 ± 0
Butter, unsalted	0 ± 0
Cake Flour	2.45 ± 0.24
Cinnamon	106.6 ± 12.9
Egg	0 ± 0
Ginger	15.7 ± 2.2
Light Brown Sugar	1.35 ± 0.07
Molasses	9.39 ± 0.32
Nutmeg	17.58 ± 0.23
Salt	0 ± 0

Table 3.2 Total phenols in ingredients used to make red oak acorn meal cookie – The ingredients and amounts used to make the acorn meal cookie, as well as the total phenolic content for each ingredient included in the cookie.

Ingredient	Amount (g)	Total Phenols mg GAE/g ingredient
Light brown sugar	165.0	223
Butter, unsalted	170.0	0
Egg	50.0	0
Molasses	252.0	2366
Cake Flour	188	453
Red Oak Acorn Meal	189.4	7062
Baking soda	4.6	0
Salt	3.0	0
Ginger	2.7	42.4
Cinnamon	2.6	277
Nutmeg	2.2	38.7
Allspice	1.0	95.2

Table 3.3. Comparison of Total Phenolics and Antioxidant Capacity (FRAP values) in Red and White Oak Acorns

Oak Family	Common Name	Gallic Acid Equivalents[a] (mg/g)	FRAP value (mmol/100 g)
White	White Oak	32.3 ± 1.62	8.93 ± 0.09
White	Post Oak	17.1 ± 0.49	5.93 ± 0.02
White	Live Oak	56.4 ± 0.15	12.1 ± 0.14
White	Swamp chestnut Oak	32.2 ± 1.20	10.2 ± 0.02
White	Chestnut Oak	26.9 ± 1.33	7.01 ± 0.04
White	Bur Oak	20.3 ± 0.01	6.04 ± 0.06
White	Overcup Oak	14.3 ± 0.01	4.35 ± 0.06
Red	Black Oak	24.3 ± 1.15	5.04 ± 0.08
Red	Northern Red Oak	49.9 ± 0.09	9.94 ± 0.04
Red	Willow Oak	44.0 ± 0.37	9.07 ± 0.09
Red	Shumard Oak	74.5 ± 1.87	16.1 ± 0.08
Red	Water Oak	66.9 ± 3.23	11.4 ± 0.12
Red	Southern Red Oak	70.9 ± 0.51	15.0 ± 0.22
Red	Laurel Oak	106.6 ± 0.37	26.4 ± 0.05
Red	Pin Oak	36.9 ± 1.19	9.09 ± 0.01

[a] Values are means ± standard deviations.

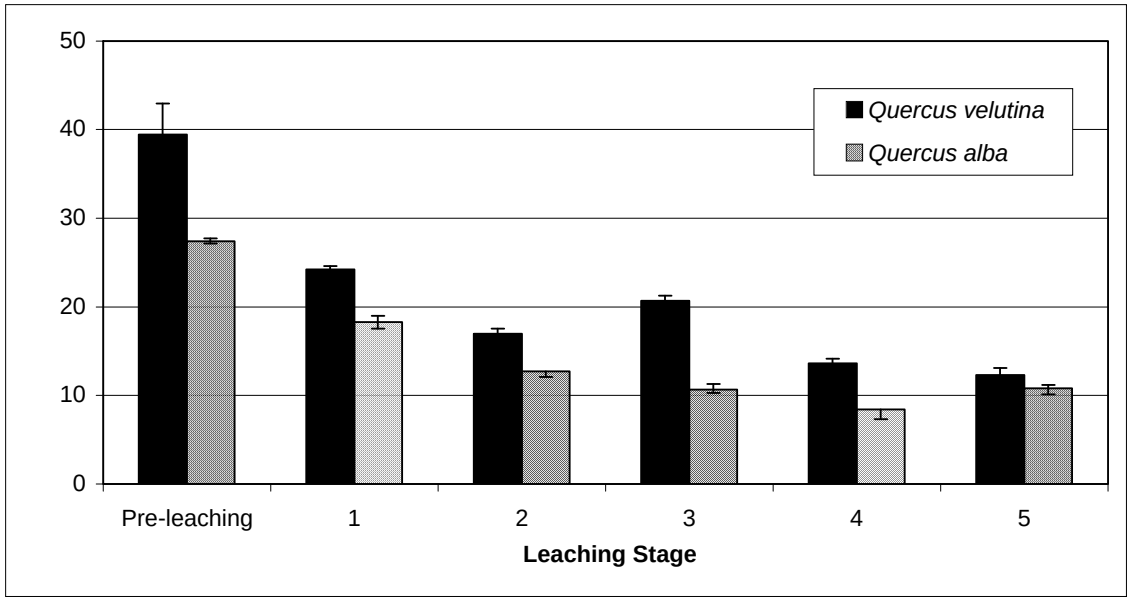

Figure 3.1 The total phenolic content of *Q. velutina* and *Q. alba* at the pre-leaching stage and after the 5 stages of leaching. Data shown are the average of 3 independent samples with standard deviations. (11-30 nuts)

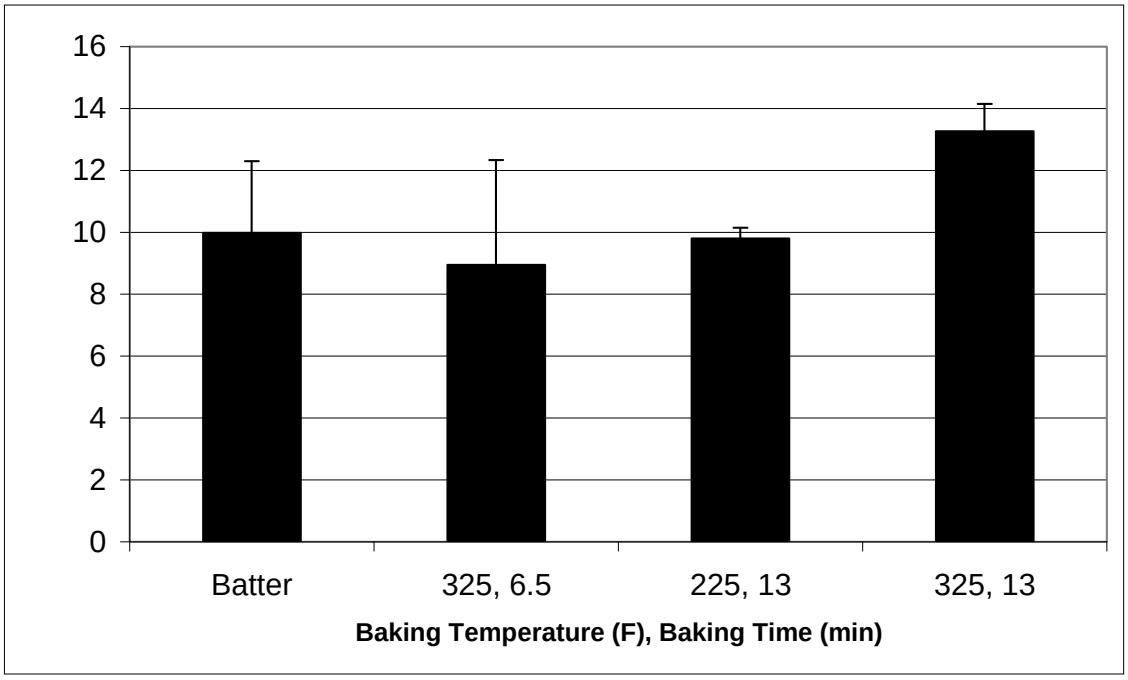

Figure 3.2 The effect of baking time and baking temperature on the phenol content of acorn meal cookies and batter. Baking temperature is expressed in degrees Fahrenheit, and baking time is expressed in minutes. The batter was not exposed to heat for any length of time.

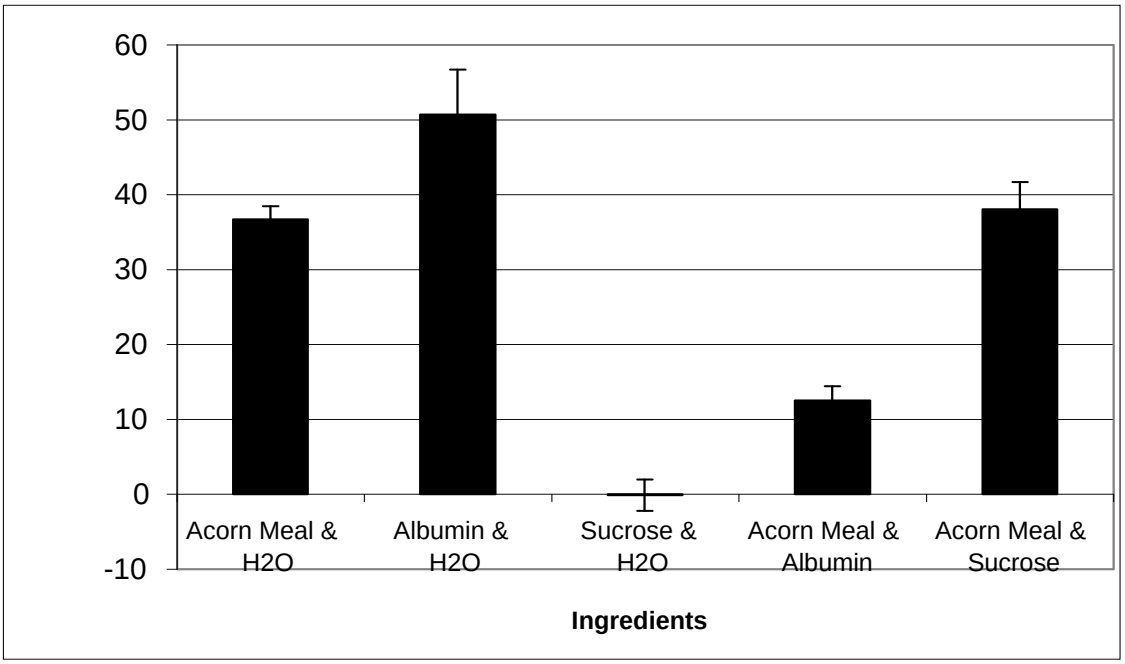

Figure 3.3 The total phenol content of acorn meal, albumin, and sucrose with water, and the effect on total phenol content of acorn meal in solution with albumin and sucrose

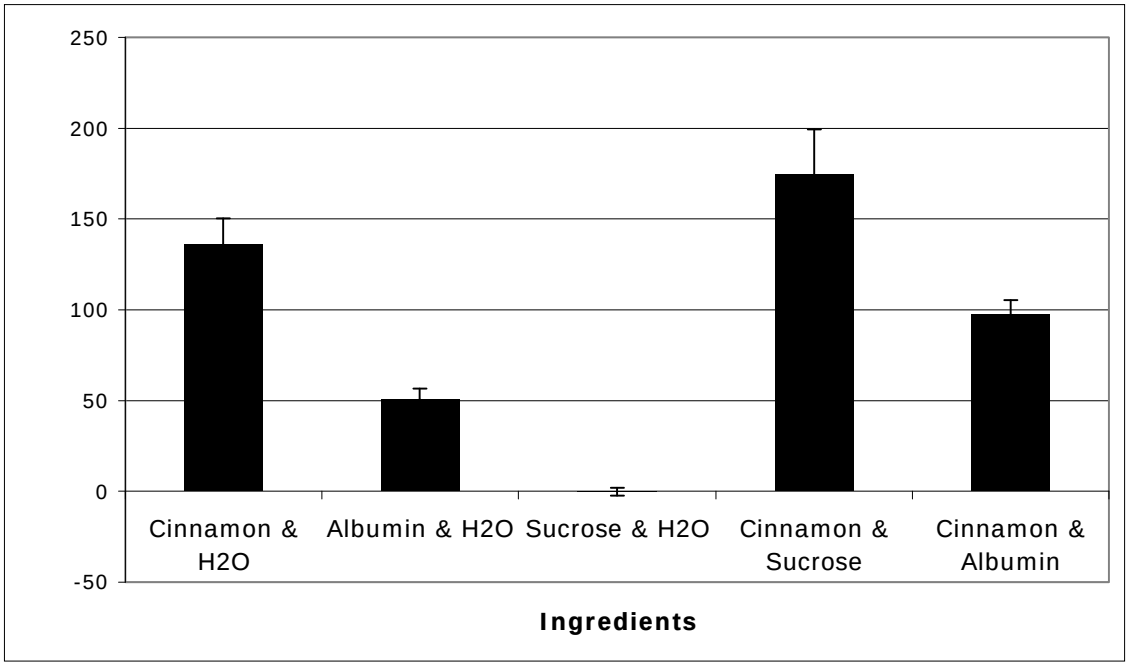

Figure 3.4 The total phenol content of cinnamon, albumin, and sucrose with water, and the effect on total phenol content of cinnamon in solution with albumin and sucrose.

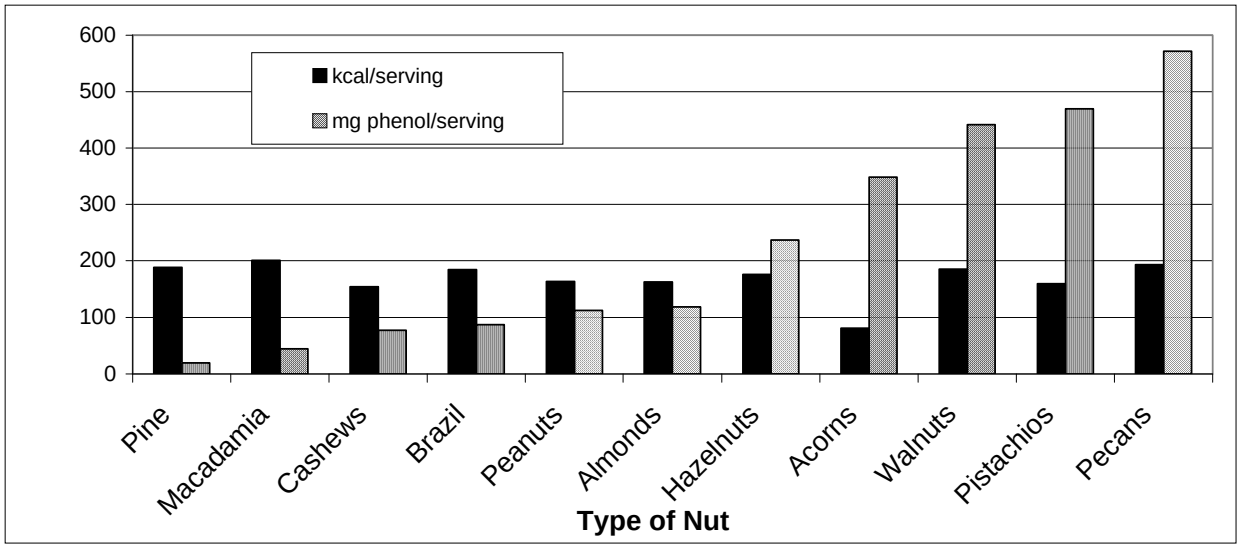

Figure 3.5 A comparison of several different varieties of nuts in calories per serving and polyphenol content per serving. 1 serving of nuts is 28.35 g (Wu et al., 2004).

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CHAPTER IV

IMPROVEMENT TO DASH DIET

Increasing the polyphenolic content of a diet could be easily achieved through the use of strategic food choices. One strategy that could be used to increase polyphenol consumption would be to separate high-phenol foods into different categories, and replace the foods already being consumed in each category with higher containing polyphenol foods. The categories should include fruits, beverages, grains, vegetables, spices, and nuts and seeds. Foods in these categories range in their phenolic content, however strategically choosing a fruit, that is higher in polyphenols would increase overall polyphenol intake. Examples of foods in these categories and their polyphenol values and energy content are shown in Tables 4.1 through 4.6.

The dietary approaches to stop hypertension (DASH) diet is a guide to healthy eating, however improvements in terms of antioxidant capacity can still be made in the diet. Simply by making alternate choices within categories currently provided in the DASH diet, polyphenol intake could be increased. As an example, the lunch menu for Day 6 of the DASH diet meal plan is presented in Table 4.7. With a few simple strategic choices of higher antioxidant containing foods, the antioxidant capacity of that meal can be increased. The changes and additions to the meal are also presented in Table 4.7.

Foods high in antioxidants have also been associated with improved endothelial cell function which has been associated with decreasing blood pressure. Therefore, making adjustments in antioxidant content of the DASH diet may also influence other biological

processes associated with increased blood pressure making the already healthy DASH diet even healthier.

Table 4.1 Beverages

Food	Serving Size[a] (g)	kcal/serving[a]	Phenol mg/g[b]	Phenol mg/kcal	Phenol mg/serving
Wine, red	103	87.0	2.2	2.6	224.1
Juice, Cranberry	180	98.0	1.5	2.7	261.0
Juice, Prune	180	135.0	2.2	2.9	387.0
Juice, Pomegranate & Black Cherry	180	113.0	2.2	3.5	396.0
Juice, Grape	180	128.0	2.3	3.2	409.6
Juice, 100% Prune	240	180.0	2.5	3.3	595.6
Green Tea, Brewed Steeped	240	0.0	3.2	0.0	772.8
Coffee (brewed)	237	2.4	8.2	806.8	1936.3

[a] (U.S. Department of Agriculture, 2007)
[b] adapted from(Halvorsen and others 2006)

Table 4.2 Fruit

Food	Serving Size (g)[a]	kcal/serving[a]	Phenol mg/g[b]	Phenol mg/kcal	Phenol mg/serving
Banana	118.0	105.0	0.2	0.0	0.0
Apple, red delicious w/ peel	138.0	72.0	0.4	0.0	0.0
Orange	140.0	69.0	0.9	0.0	0.0
Raisins	82.0	245.0	0.9	0.3	0.2
Prunes, Pitted	44.0	104.0	1.5	0.6	0.9
Kiwi fruit	76.0	46.0	1.4	2.2	3.0
Plums	66.0	30.0	1.4	3.0	4.0
Cranberries	95.0	44.0	3.4	7.2	24.3
Blackberries	144.0	62.0	4.1	9.4	38.4
Strawberries	152.0	49.0	4.8	14.8	70.9
Blackberries, Frozen	75.5	48.0	10.1	15.8	159.5
Blueberries	74.0	42.0	11.8	20.8	246.2
Raspberries	62.0	32.0	12.4	24.0	297.9
Cherries, Dark	117.0	74.0	4.8	7.6	561.6
Chokeberries	72.0	42.0	25.6	43.9	1123.5

[a] (U.S. Department of Agriculture, 2007)
[b] adapted from(Halvorsen et al., 2006)

Table 4.3 Grains

Food	Serving Size (g)[a]	kcal/serving[a]	Phenol mg/g[b]	Phenol mg/kcal	Phenol mg/serving
Cereal, Shredded Wheat	49.0	167	0.4	0.1	20.1
Toasty peanut crackers	18.0	210	1.1	0.1	20.2
Grits	37.0	140	0.6	0.2	21.1
Bread, White	26.0	70	0.9	0.3	24.2
Oyster crackers	30.0	140	1.0	0.2	30.4
Wafers	17.0	70	1.9	0.5	33.1
Cereals, rice & corn	29.0	82.5	1.1	0.4	33.1
Wheat Bran	15.0	30	2.3	1.1	34.1
Cereal, Corn Flakes	28.0	101	1.3	0.4	35.8
Pancake Mix, Buckwheat	40.0	140	1.2	0.3	48.8
Cereal, Cocoa Krispies	31.0	118	1.6	0.4	49.2
Wasa rye krisp, hearty	25.0	92	2.0	0.5	49.6
Cereal, Multi-grain Hot	40.0	160	1.3	0.3	52.8
Cereal, Oat flakes	33.0	180	2.1	0.6	68.7
Bread, Whole Grains	43.0	110	1.6	0.6	68.8
Wheat Germ	15.0	55	4.9	1.3	73.7
Blue corn chips	28.0	170	2.7	0.5	76.7
Bread, German Dark Wheat	43.0	100	1.9	0.8	80.8
Cereal, Bran	30.0	81	3.0	1.1	89.4
Lentils	32.0	70	3.0	1.4	97.4
Cereal, whole-grain	30.0	100	3.5	1.0	104.3
Cereal, Oat flakes	50.0	110	2.2	0.6	109.3
Cereal, Oat Bran Hot	40.0	120	3.7	1.2	147.2
Cereal, Grape Nuts	58.0	208	2.8	0.8	160.1

[a] (U.S. Department of Agriculture, 2007)
[b] adapted from(Halvorsen et al., 2006)

Table 4.4 Nuts and Seeds

Food	Serving Size (g)[a]	kcal/serving[a]	Phenol mg/g[b]	Phenol mg/kcal	Phenol mg/Serving
Poppy seeds	2.8	15.0	0.0	0.0	0.1
Brazil nuts	28.4	184.0	0.3	0.1	9.2
Peanuts	28.4	164.0	0.4	0.1	10.1
Macadamia nuts	28.4	201.0	0.6	0.1	17.0
Almonds	28.4	163.0	0.5	0.1	15.5
Pine nuts	28.4	188.0	0.7	0.1	20.7
Cashews	28.4	155.0	0.7	0.1	18.5
Hazelnuts	28.4	176.0	1.0	0.2	27.0
Flaxseed, whole brown	28.4	150.0	0.8	0.2	23.2
Flaxseed, ground or milled	28.4	150.0	1.1	0.2	32.5
Pistachios	28.4	162.0	1.6	0.3	44.1
Pecans	28.4	196.0	9.9	1.4	279.3
Walnuts	28.4	185.0	13.3	2.0	378.4
Pumpkin seeds	28.4	153.0	12.8	2.4	362.9

[a] (U.S. Department of Agriculture, 2007)
[b] (Wu and others 2004)

Table 4.5 Spices

Food	Serving Size (g)[a]	kcal/serving[a]	Phenol mg/g[b]	Phenol mg/kcal	Phenol mg/serving
Pepper, black	2.1	5.0	4.5	1.9	9.5
Parsley, dried	0.3	1.0	7.6	2.3	2.3
Mustard Seed, yellow, ground	3.3	15.0	10.7	2.4	35.4
Chili Powder	2.6	8.0	8.5	2.8	22.2
Curry Powder	2.0	7.0	10.2	2.9	20.3
Paprika	2.1	6.0	8.8	3.1	18.4
Ginger, ground	1.8	6.0	22.0	6.6	39.6
Cinnamon, ground	2.3	6.0	18.0	6.9	41.4
Basil leaf, dried	0.7	1.0	12.5	8.8	8.8
Oregano leaf	1.0	3.0	41.1	13.7	41.1
Tumeric powder	2.2	8.0	16.0	16.0	35.1
Cloves, ground	2.1	7.0	127.9	38.4	268.7

[a] (U.S. Department of Agriculture, 2007)
[b] adapted from(Halvorsen et al., 2006)

Table 4.6 Vegetables

Food	Serving Size (g)[a]	kcal/serving[a]	Phenol mg/g[b]	Phenol mg/kcal	Phenol mg/serving
Carrot	61.0	24.0	0.0	0.1	2.1
Cucumber, with peel	52.0	8.0	0.0	0.2	2.0
Celery	60.0	10.0	0.1	0.4	3.8
Potatoes, white, cooked	213.0	182.0	0.4	0.5	93.5
Broccoli	44.0	15.0	0.3	0.7	11.1
Tomatoes	123.0	22.0	0.2	0.9	19.4
Garlic clove	3.0	4.0	1.4	1.1	4.2
Spinach, frozen	78.0	16.0	1.2	6.1	97.4
Broccoli	44.0	15.0	2.3	6.7	101.2
Red Bell Pepper	92.0	24.0	1.9	7.3	174.8
Cabbage, red, cooked	75.0	22.0	2.2	7.5	164.5
Artichokes, prepared	84.0	42.0	4.3	8.6	362.7

[a] (U.S. Department of Agriculture, 2007)
[b] adapted from(Halvorsen et al., 2006)

Table 4.7 DASH Diet Day 6 lunch menu and replacement foods to increase polyphenol intake

Low[a]	TP mg GAE/serving	High	TP mg GAE/serving
Turkey Sandwich		Turkey Sandwich	
3 oz turkey	0.0	3 oz turkey	0
2 slices whole wheat bread	0.3	2 slices whole wheat bread	0.3
1 large leaf romaine lettuce	0.1	1 large leaf romaine lettuce	0.1
2 slices tomato	0.1	1/2 c cooked artichoke hearts	726
2 tsp low-fat mayonnaise	0.0	1/2 c sliced red bell pepper	88
1 Tbsp Dijon mustard	0.2	1 c red cabbage slaw	330
1 c steamed broccoli	1.6	1 tsp mustard seed	0.4
1 med orange	1.3	1 tsp tarragon	83.6
		1 tsp olive oil	0.0
		1c berries	40
		1 tsp cinnamon	476
Total	3.7	Total	1743.9

[a] (U.S. Department of Health and Human Services and others 2006)

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CHAPTER V

CONCLUSIONS

LIMITATIONS

The information obtained concerning the polyphenolic content of acorn nuts, acorn meal, and a cookie made with acorn meal is valuable for comparison with foods that high in polyphenols which can provide beneficial properties in treating and preventing disease. The information is limited in that the values obtained pertain only to the food products tested, and do not reflect the activity of the polyphenolics after being absorbed in the body. The results contain information regarding different ingredient interactions among the ingredients in the acorn meal cookie, however many more interactions may take place between the food products and compounds present in the body. The data gathered here does not reflect the effect acorns or acorn products can have in vivo.

The total phenols assay provides information about all of the polyphenols present in the food sample being tested. However the total phenols assay does not provide information about the total antioxidant capacity of the food sample. Polyphenols may be a significant contributor to the antioxidant properties provided in the sample being tested, but other antioxidants may be present in the food sample and contribute to the antioxidant capacity. The total phenols assay cannot detect those other antioxidants.

IMPLICATIONS

Acorns are a traditional, relatively unrefined food. This research demonstrates that a particular traditional food does contain a higher level of polyphenolics than most contemporary

staples. Per gram of dried product, acorn meal places among the highest 50 sources of dietary antioxidants. It appears to be true that some traditional diets included more polyphenols than modern diets, and acorn meal could be used to increase antioxidant intake, and specifically polyphenol intake. Not only can eating whole acorns contribute to polyphenol consumption, but consuming products made from acorns, such as baked goods prepared from acorn meal, can contribute to polyphenol intake. It is a reasonable hypothesis that increased polyphenol intake may improve the oxidative state of individuals consuming a higher polyphenol diet, ultimately providing a beneficial effect in terms of reduced risk for diseases associated with oxidative processes.

FUTURE RESEARCH

In order for the postulated benefits of consuming a diet high in polyphenols and more broadly, antioxidants, to be proven, more must be learned about polyphenols once they enter the body. A large body of evidence exists regarding the polyphenols and antioxidants present in foods, but more information must be gathered regarding the mechanism of absorption and the fate of the antioxidant compounds once absorbed. Foods high in antioxidants may be very beneficial in treating and preventing diseases, but the exact mechanisms and specific antioxidant compounds providing that beneficial effect is still unknown. Thousands of antioxidant compounds exist in foods, and it is still unclear the specific effect each of those compounds has in the body, in which disease processes those compounds can play the largest beneficial role, and whether those compounds provide their beneficial effects individually or work together.

If polyphenols and antioxidants actually do provide the beneficial effects research thus far is indicating, more information needs to be gathered as to how polyphenol and antioxidant intake can be significantly increased in the typical American diet. Diets that utilize various

strategies to improve food choices, such as the DASH diet with lower salt intake, should be developed to strategize and optimize polyphenol and more broadly antioxidant intake. Even with strategic food choices, supplements could provide high levels of polyphenols and antioxidants and could provide health benefits without adding calories. More research must be done to determine whether different polyphenols contribute different health benefits, whether dietary supplements could provide the most benefit, and what serving size and frequency of consumption should be used to provide the most benefit without causing harm.