SOURCING OF SPICES TO MAXIMIZE TOTAL PHENOLIC LEVELS IN FOOD PRODUCT DEVELOPMENT

by

ELIZABETH METHERELL

(Under the direction of Ruthann Swanson)

ABSTRACT

Spices are a significant poylphenolic source. Total phenolics in spice sample suites of cinnamon, cloves, and nutmeg were assayed. Phenolic values for the cinnamon sample suite (n=4) ranged from 44.0 ± 1.1 mg GAE/g to 136.8± 4.5 mg GAE/g; cloves (n=3) ranged from 142.1 ± 16.0 mg GAE/g to 166.7 ± 5.4 mg GAE/g; nutmeg (n=3) from 15.2 ± 0.9 mg GAE/g to 19.3 ± 0.8 mg GAE/g. Sensory panelists (n=134) evaluated pumpkin-spice muffins reformulated using specific spice combinations that minimized, and maximized total phenolics by combining those with the lowest and highest mg GAE/g in each sample suite; all supermarket spices served as the control. On a 5-point just-about-right scale, pumpkin flavor, sweetness and spice intensity were just-about-right and did not differ with formulation. All muffins were equally preferred (P=0.93); 42% of the panelists were willing to pay 20-40% more when an antioxidant claim was present.

INDEX WORDS: Spices, Phytochemicals, Polyphenols

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ELIZABETH METHERELL

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ELIZABETH METHERELL

Major Professor: Ruthann Swanson

Committee: James Hargrove
Barbara Grossman

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2010

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CHAPTER I

INTRODUCTION

Chronic diseases—such as heart disease, cancer, and diabetes—are the leading causes of death and disability in the United States. Chronic diseases account for 70% of all deaths in the U.S., which are estimated at 1.7 million each year (CDC 2008). Of chronic diseases, cardiovascular diseases (CVDs) are the leading cause of deaths globally, claiming 17.1 million lives a year (WHO 2009). The most important causes of heart disease and stroke include unhealthy diet, physical inactivity, tobacco use, and harmful use of alcohol. These causes are termed 'modifiable risk factors' (WHO 2009). Diet and nutrition are increasingly recognized as the most modifiable determinants of chronic disease (WHO 2002; Roberts and Barnard 2005).

There is growing interest in inflammation and its role in a variety of chronic diseases. Several medical conditions are actually inflammatory states, which may be modulated with proper nutrition intervention. An integrated approach to inflammation, with both the medical and nutrition fields involved, offers promise in the abatement of the inflammatory response, and therefore lessens the likelihood of developing a chronic illness (Jensen 2006).

Oxidative stress has been associated with several chronic diseases including CVD and hypertension (Ignarro and others 2007; Harrison and others 2007). Oxygen is required for human metabolism. However, its presence in the body can result in the development of reactive oxygen species (ROS). ROS are very small molecules or ions, such as free radicals, peroxides, and oxygen ions that are highly reactive due to the presence of an unpaired valence shell electron (Chapple 1997). Several diseases have been associated with cellular damage due to the accumulation of these reactive oxygen species, including Alzheimer's, cancer and several forms of cardiovascular dysfunction (Taniyama and Griendling 2003). However, free radicals also serve important physiological functions such as helping the body destroy bacteria, producing vasodilation, stimulating cell proliferation and aiding in immune function. In excess though, free radicals exert oxidative stress contributing to a number of disease states (Nordberg and Arner 2001). As part of cellular functioning, excessive free radical species can have deleterious effects (Patel and others 1999). Negative effects include: damage to DNA, promotion of apoptosis, aging, inflammation, degenerative progression and malignant growth. The key factor is to maintain a balance between excessive ROS and inadequate ROS.

The Institute of Medicine (IOM), Food and Nutrition Board defines a dietary antioxidant 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' (IOM 1998). Dietary antioxidants may be nutritive or non-nutritive. Nutritive antioxidants include vitamins C and E which are found in a variety of food sources. Many plant-based foods, such as vegetables, fruits, spices and herbs are high in natural non-nutritive antioxidants, including flavonoids and polyphenols. Increased antioxidant consumption has a positive role in protecting the body from oxidative stress (Willcox and others 2004). Antioxidants circulate throughout the body, seek-out free radicals, and destroy them before they are able to damage cells.

Over millions of years, plants have developed the capacity to synthesize a diverse array of chemicals. Phytochemicals, literally meaning "plant chemicals", are non-essential nutrients derived from plant compounds. Groupings of phytochemicals include phenolics, carotenoids, alkaloids, nitrogen-containing compounds and organosulfur compounds. These groups include thousands of compounds that vary in structure. Phenolics, a sub-category of phytochemicals, are currently being researched for their potential health-promoting properties and have been linked to a reduced risk of several major chronic diseases. Phenolics exhibit strong antioxidant properties (Ho 1992). These antioxidant effects may positively affect the development of chronic diseases such as cancer and CVD.

Dietary intake of polyphenols is the greatest of all antioxidants (Scalbert and Williamson 2000). Intake is largely due to consumption of foods such as fruits, vegetables, and various beverages (Scalbert and others 2005). Scientific studies show that a high dietary intake of fruits and vegetables is strongly associated with the reduced risk of developing chronic diseases in general (Liu 2004). The estimated range of consumption of polyphenols is higher than that of vitamins C and E (Soobrattee and others 2005; Scalbert and Williamson 2000). However, it is difficult to accurately establish consumption because determination of polyphenol recovery from foods depends on the method of extraction. This leads to the wide range of estimated consumption. As increasing numbers of foods have been added to the phenolic database, spices and herbs have emerged as particularly potent sources (Halvorsen and others 2006). It is suggested that increasing spice and herb consumption is a fairly simple adopted strategy to increase polyphenolic intake.

Throughout history, spices have been used in a variety of fields worldwide. The earliest uses included: magic, medicine, religion and food preservation. Today, spices are primarily used as flavoring agents. The American Spice Trade Association (ASTA) defines spice as “any dried plant product used primarily for seasoning purposes.” This includes: tropical aromatics (pepper, cinnamon, cloves, etc.); leafy herbs found in the temperate zone (sage, basil, oregano, etc.); spice seeds (sesame, mustard, caraway, etc.); and dehydrated vegetables used as spices (onion, chili peppers, garlic, etc.). In food products, they contribute a characteristic flavor and pungency, as well as color and exhibit antioxidant, antimicrobial and nutritional properties (Hirasa and Takemasa 1998).

Recently, research has been aimed at studying various functional compounds within spices and applying these compounds to better the health of the population. Compared with other foods, herbs and spices are typically a more concentrated source of dietary antioxidants with little kilocalories (Tapsell and others 2006). In one study, researchers reported that one teaspoon of cloves had a higher level of antioxidants than a ½ cup of blueberries, and that 13 of the top 50 food products highest in antioxidant concentrations were spices (Halvorsen and others 2006). Because of their high polyphenol density and low energy amount, increasing spice consumption will likely have favorable effects on health. However, due to a lack of scientific data, DRI values for non-nutritive polyphenols have not been established (IOM 1998).

Increased use of herbs and spices as flavorings in foods is a major trend worldwide with sales growth of 20-30% over the past five years in both the UK and the US (Williams 2006). The value of total U.S. spice imports increased from $426 million in 1998 to $597 million in 2007 (USDA 2009). There was an increased demand for a variety of spices including nutmeg, saffron, fennel, turmeric as the interest in diverse flavors also increased. US imports of cloves, cumin, and cardamom also increased over 100% from 1998 to 2007 (USDA 2009). Upward trends in consumption are likely due to food trends that favor spice use in place of excess salt and fat, a growing population and an increased interest in ethnic foods (Buzzanell and others 1995). As spice use in food continues to increase, the reported health benefits of spices are also increasing. Of the top 50 foods highest in antioxidants, spices are the top five. However, it is unknown how the source or origin of a particular spice impacts its total phenolic value.

Hypotheses

Spices identified by growing locale/species and marketed by a single company will differ in total phenolics. Sourcing of spices and selection of those with maximum phenolic levels may allow the marketing of an acceptable food product capable of carrying an antioxidant claim.

The objectives of this study include:

1. To determine the total phenolics in select spices from varied growing areas marketed by a single company and compare to supermarket counterpart.
2. To determine expected total phenolics in a pumpkin muffin and the contribution of all ingredients including alternative spice sources.
3. To determine acceptability of the pumpkin muffin and spice intensity when total phenolics are maximized using consumer sensory techniques.
4. To determine the potential of an antioxidant claim as a marketing/nutrition education tool when applied to a reformulated pumpkin muffin high in total phenolics.

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CHAPTER II

LITERATURE REVIEW

REACTIVE OXYGEN SPECIES

Molecular oxygen is required for all aerobic organisms, but its presence in electron transfer reactions can lead to the development of reactive oxygen species (ROS) (Thannickal and Fanburg 2000). ROS are partially reduced metabolites of O_2 that are highly reactive due to the presence of an unpaired electron. With this lone electron, free radicals are capable of either donating or accepting an electron, acting as an oxidant or a reactant (Young and Woodside 2001). These O_2 metabolites include superoxide anion ($O_2^{-}\cdot$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($OH\cdot$). At high concentrations, ROS are capable of damaging cell structures, nucleic acids, lipids and proteins (Valko and others 2006). Accumulation of these oxidants and their subsequent damage leads to a state of oxidative stress, which has been linked to several human diseases, including cancer, Alzheimer's disease, cardiovascular diseases and aging (Thannickal and Fanburg 2000).

Mitochondria are the energy powerhouses of the cell as they generate the bulk of the cell's supply of adenosine triphosphate (ATP) that is used as a source of chemical energy. Therefore, mitochondrial DNA is increasingly susceptible to oxidative stress. Reactive oxygen species (ROS) and subsequent cellular stress have long been implicated in cancer in a variety of ways. Data suggest ROS may induce cancer and also contribute to the progression of cancer as altered cancer cells use ROS signals to drive proliferation and other events required for tumor progression (Schumacker 2006).

Recent evidence of age-dependent damage and deterioration of respiratory enzyme activities with normal aging may contribute to the delayed onset and age dependence of neurodegenerative diseases (Beal 2004). Alzheimer's disease is an incurable, degenerative disease affecting neurons in the brain. Data suggest that the brain in Alzheimer's disease may be under increased oxidative stress, which may be the cause of neuronal damage and eventually the cause of death (Markesbery 1997).

Increased oxidative stress has been linked to impaired endothelial function in atherosclerosis and may play a role in the pathogenesis of cardiovascular events (Heitzer and others 2001). Specifically, over-production of oxygen-derived free radicals such as the superoxide anion has been linked to damaged endothelial vasomotor function in experimental models of atherosclerosis (Heitzer and others 2001).

ANTIOXIDANTS

Antioxidants are molecules capable of slowing or preventing the damage to the human body caused by oxidation. Antioxidants inhibit the progression of free radical chain reactions by themselves being oxidized. Therefore, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies 1997).

Antioxidants are derived from intrinsic antioxidant defense systems and also from extrinsic sources, such as diet or pharmacotherapy (Gotto 2003). Exogenic antioxidants, those supplied outside the body, are the topic of increased research. These specific lines of defense are essential for counteracting oxidative stress associated with cancers, cardiovascular diseases, and inflammatory disorders. Dietary antioxidants such as alpha-tocopherol, ascorbic acid, and carotenoids represent one possible defense against oxidative stress, which may prevent or delay the development of atherosclerotic disease (Gaziano and Hennekens 1993). Epidemiological studies indicate an association between increased intake of dietary antioxidants and reduced risk of coronary events (Enstrom and others 1992; Kushi and others 1996; Rimm and others 1993; Willcox and others 2008). Most of the exogenic antioxidants have a plant-based origin and are found in the form of phenolic compounds (Lagurre and others 2007).

Increasing intake of dietary antioxidants may help to maintain the normal physiological function of a living system (Kaur and Kapoor 2001). In general, the most universal dietary sources of antioxidants include fruits and vegetables. The most common antioxidants present in these foods are vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds.

However, because reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to get rid of oxidants entirely, but instead to keep them at a balanced level (Rhee 2006). The ability of an antioxidant to protect a cell depends on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts (Vertuani and others 2004). Cells have several antioxidant enzymes that help to maintain the proper balance between oxidants and antioxidants. This includes superoxide dismutase, catalase and glutathione peroxidase (Thannickal and Fanburg 2000).

Mechanisms of Action

Antioxidant mechanisms of action can be divided into three categories: preventive, chain-breaking antioxidants, and proxidant effects (Laguerre and others 2007). Examples of antioxidant compounds and their specific mechanisms of action can be found in Table 2.1 (Ali and others 2008).

As the trend of the future moves toward functional foods with specific health effects, researchers are looking to discover antioxidant compounds in several types of plant materials. This includes vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs (Ramarathnam and others 1997). Researchers continue to examine food samples in order to discover additional dietary ways of promoting health. Halvorsen and others (2006) performed a study in which 1113 food items were analyzed for antioxidant capacity. The top 50 samples are presented in Table 2.2. Of the top 50 foods highest in antioxidants, 13 are spices. Due to the well-known and studied health benefits of antioxidants, a general recommendation to the consumer is to increase the intake of foods rich in antioxidant compounds, such as polyphenols (Katalinic and others 2006).

PHYTOCHEMICALS

Phytochemicals are non-nutritive plant chemicals that exhibit protective or disease preventive properties. It has been estimated that more than 5000 individual phytochemicals have been identified in a variety of fruits, vegetables, and grains (Liu 2004). They display a number of protective actions including: antioxidant activity, hormonal action, stimulation of enzymes, and interference with DNA replication (Lampe 2003). The five groupings of phytochemicals include phenolic acids, flavonoids, tannins, stilbenes, and coumarins (Liu 2004). A detailed division chart can be found in Figure 2.1.

Polyphenols

Phenolic compounds or polyphenols are a widespread group of chemical substances found in plants that are characterized by the presence of hydroxyl groups on one or more aromatic rings. They make-up one of the most numerous groups of substances in the plant kingdom, with more than 8000 phenolic structures known (Harborne 1993). The variability of these plant metabolites ranges from simple molecules to highly polymerized compounds (Bravo, 1998). Phenolics are divided into groups based on the number of phenol rings they contain and the way in which the rings are chemically bound (Manach and others 2004). As products of secondary metabolism, polyphenols provide essential functions in the growth and reproduction of plants. They also act as defense mechanisms against pathogens, predators, and parasites (Lampe 2003). In addition to the protective roles in plants, phytochemicals also provide humans with protective health benefits (Liu 2004). Epidemiological studies suggest associations between the consumption of polyphenol-rich foods or beverages and the prevention of disease (Scalbert and Williamson, 2000).

Polyphenols were originally thought of as anti-nutrients by animal nutritionists, because of the adverse effect of tannins, a type of polyphenol, on protein digestibility (Bravo, 1998). However, during the last decade, researchers and food manufacturers have become increasingly interested in the study of polyphenols due to the recognition of their antioxidant super-power (Manach and others 2004). Some of the most common plant phenolic antioxidants include flavonoid compounds, phenolic acids, coumarins, and tannins. In response to oxidative stress, plant phenolics are multifunctional and are capable of acting as reducing agents (free radical terminators), metal chelators and singlet oxygen quenchers (Mathew and Abraham 2006).

On a cellular level, polyphenols may alter signaling cascades and influence gene transcription. There is increasing interest in the role nutrition may have in the modulation of inflammation. Inflammatory disease, illness or injury results from an overproduction of pro-inflammatory cytokines (Jensen 2006). Research has shown that polyphenols may down-regulate pro-inflammatory enzymes such as COX 2 and I NOS and inhibit apoptosis that occurs because of excess ROS (Soobrattee and others 2005). These effects led to the hypothesis that polyphenols may aid in the prevention of cancer. In the prevention of CVD, polyphenols have been shown to have positive effects on platelet function, HDL cholesterol, and blood pressure (Erlund and others 2008).

Phenolic Acids

Phenolic acids are plant metabolites that are widely spread throughout the plant kingdom. They are one of the main classes of phenolics and are commonly found in both edible and non-edible plants. They can be subdivided into two major groups, hydroxybenzoic acids and hydroxycinnamic acids, which may display various effects in biological systems (Liu 2004). Of the commonly consumed polyphenols, phenolic acids account for about one-third of the total intake (Scalbert and Williamson 2000). The most common phenolic acids include caffeic acid and to a lesser degree, ferulic acid (Scalbert and Williamson 2000). Physically, phenolic compounds are typically white solids, although the complex electronic conjugation of some of the flavonoids results in a yellow color, or red in the case of anthocyanins. Some low-molecular weight phenolics possess a large amount of volatility and have characteristic aromas. Examples include: methyl salicylate, vanillin, and eugenol (Parr and Bolwell 2000). Current interest in determining total phenolics in food stems from their protective role against diseases caused by oxidative damage (Tsang and others 2005).

FOODS AND POLYPHENOLS

Foods often contain complex combinations of polyphenols. The main dietary sources of polyphenols are plant foods and beverages, such as vegetables, fruit, tea, red wine, fruit juice, chocolate drinks, coffee, and beer (Manach and others 2004; Scalbert and Williamson 2000). It is estimated that total intake of polyphenols is ~1 g/day (Scalbert and Williamson 2000). However, this estimate is rather uncertain because of the lack of comprehensive data regarding polyphenols in foods. Flavonoids account for roughly two-thirds of the dietary intake of polyphenols (Scalbert and Williamson 2000). Flavonols are found in most fruits, and are typically distinguished by red, blue and purple pigments. Other polyphenols are more specific to certain foods, such as phloridzin found in apples (Manach and others 2004). Even though there are a large number of phytochemical classes, most plants contain only a small number of them. Spices, for example, typically include only a few of these classes. The major classes found in spices are outlined in Table 2.3. Generally, all phenolic compounds and their subsequent characteristics contribute to the sensory quality, consumer acceptability, and marketability of food products.

In food, phenolics may contribute to the bitterness, astringency, color, flavor, odor, and also the oxidative stability of products (Naczk and Shahidi 2004). Tannins are a phenolic compound known for contributing to the bitterness in foods. An important aspect of the defense roles of phenolics lies in making plants unpalatable to herbivores. Tannins have the ability to interact with and precipitate proteins. They exhibit an astringent mouth-feel and an ability to inhibit digestive enzymes. Plants high in tannins are thus rarely consumed by plant eating organisms, although man has acquired a taste for moderately astringent beverages such as black tea and red wine (Parr and Bolwell 2000).

Dietary Intake of Polyphenols

The Dietary Reference Intake (DRI) is a system of nutrition recommendations from the Institute of Medicine (IOM) of the US National Academy of Sciences. The DRI was introduced in 1997 in order to broaden the existing guidelines known as Recommended Dietary Allowances (RDAs). They are the most recent 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) (IOM 1998).

DRI values for non-nutrients, such as polyphenols, have not been established due to a lack of scientific data (IOM 1998). It is neither practical nor feasible to establish DRIs for each polyphenol due to the large number of structurally different polyphenols that exist in nature. However, they have been termed “lifespan essentials” because they are needed to achieve a full lifespan (Williamson and Holst 2008). Even though they do not result in classical deficiencies, a lack of polyphenols can result in the development and progression of chronic diseases (Williamson and Holst 2008). Furthermore, 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 (IOM 2010).

There are however, suggestions and recommendations for the consumption of polyphenols. Polyphenols are pigment-related phytonutrients. Therefore, eating a variety of colorful foods such as fruits and vegetables is advised in order to reap the benefits of these phenolic antioxidants. Eating a variety of colorful and nutrient-rich foods gives the body enhanced nutrition that helps to protect against illness and the development of chronic disease. The Food Pyramid recommends that adults eat 1 ½ to 2 cups of fruit and 2 ½ to 3 cups of vegetables daily (USDA 2010). 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. The Dietary Guidelines for Americans also encourage consuming a variety of fruits and vegetables within estimated daily calorie needs. These guidelines have been published jointly every 5 years since 1980 by the Department of Health and Human Services (HHS) and the Department of Agriculture (USDA) in order to give advice on achieving a healthy diet. The 2010 version is currently in development. The guidelines encourage the intake of nutrients from food sources, rather than supplements. The reasoning for this is that certain compounds present in foods such as carotenoids, flavonoids, and isoflavones are not present in nutrient-specific supplements. Therefore, the most assured way of consuming all of the beneficial compounds and preventing chronic disease is to engage in a whole-food diet (USHHS and USDA 2005).

In addition to the government dietary guidance, there are also several non-government related sources of recommendations. Dr. Andrew Weil, a well known medical doctor from Harvard School of Medicine, is a pioneer in the field of integrative medicine. He has recognized the role of inflammation as a root cause of disease and in doing so, created an anti-inflammatory diet pyramid (Weil 2010). The pyramid is designed to provide a practical eating guide that reduces the risks of inflammatory related diseases and improves overall health for consumers of all ages. The pyramid includes several categories including, herbs and spices, red wine, fish and seafood, healthy fats, and whole soy foods (Weil 2010). Oldways, an internationally known non-profit organization, in conjunction with the Harvard School of Public Health, and the European Office of the World Health Organization developed and introduced the Mediterranean diet pyramid in 1993. It is currently recognized as the "gold standard" eating guide that promotes lifelong good health. The diet is based on the healthy foods and drinks that are traditionally consumed by people living in the countries bordering the Mediterranean Sea (Oldways 2010). At the 15th annual Mediterranean diet pyramid conference in 2008, several updates were made to the pyramid, including the addition of herbs and spices.

Bioavailability

The absorption and bioavailabilty of polyphenols in humans is controversial. To fully understand the implications of dietary phenolic acids in human health, it is essential to determine their bioavailability in the human system. The rate and degree of intestinal absorption is dependent on the chemical structure of the polyphenol (Scalbert and Williamson 2000). Therefore, it is likely that not all polyphenols are absorbed with equal efficacy. They are widely metabolized by intestinal and hepatic enzymes and by the intestinal microflora (Manach and others 2004). For example, phenolic acids found in foods are seldom found in free forms. Therefore, to assess potential in vivo effects, it is necessary to understand phenolic acid absorption from their conjugated forms (Nardini and others 2002). Currently, the types of polyphenols found in various food items and their subsequent bioavailability are the focus of research (Manach and others 2004).

ANTIOXIDANT EVALUATION METHODS

Although antioxidants are recognized as beneficial to health, there is not a "total antioxidant" nutritional index available for food labeling because there is currently not a standard method for quantifying antioxidant capacity (Kaur and Kapoor 2001). There are, however, several methods by which total antioxidant activity or potentials can be measured. The most commonly used assays in recent research include Trolox equivalent antioxidant capacity (TEAC), total radical absorption potentials (TRAP), ferric reducing ability of plasma (FRAP), total phenolics (TP), and oxygen radical absorption capacity (ORAC) (Kaur and Kapoor 2001).

The fundamental basis of these methods relies on either a single electron transfer (SET) or a hydrogen atom transfer (HAT) reaction between an oxidant and a free radical. Both TEAC and FRAP are SET-based methods, where antioxidants are oxidized by oxidants, such as Fe (III);

resulting in a single electron being transferred from the antioxidant molecule to the oxidant. In general, these assays are based on the ability of an antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm (Benzie and Strain 1999). The reducing ability of an antioxidant is measured by an ultraviolet-visible spectrometer, which determines the change of absorbance of either the antioxidant or oxidant. The absorbance value is parallel with the antioxidant's reducing capability with the absorbance decrease proportional to the antioxidant content (Benzie and Strain 1996).

Both the ORAC and TRAP assays utilize the HAT-based method. These employ a radical initiator to generate a peroxyl radical. The radical ideally abstracts a hydrogen atom from the antioxidant, and thus inhibits the subsequent reaction between the radical and target molecule (Kaur and Kapoor 2001).

The FRAP method was originally developed to measure antioxidant potential in blood plasma and tissue extracts. However, using antioxidants as reductants in a redox-linked colorimetric method, it also provides a reliable method for determining antioxidant capacity of food extracts and dietary antioxidants (Benzie and Strain 1999). The principle of FRAP is based on the reduction of ferric tripyridyltriazine (Fe III TPTZ) complex to ferrous form, which has an intense blue color. The reduction is monitored by measuring the change in absorption at 593nm. The change in absorbance is therefore, directly related to "total antioxidant power" present in the reaction mixture (Benzie and Strain 1996).

Because these are all distinct methods based on different chemistry principles, it is necessary to determine whether or not they will provide comparable antioxidant values for the same sample (Kaur and Kapoor 2001). For example, results from a study measuring antioxidants in vegetables demonstrated differences in antioxidant activity, attributing the discrepancies to the principles upon which these methods are built (Ou and others 2002). Researchers concluded that the ORAC method is chemically more relevant to chain-breaking antioxidants activity, while the FRAP has some drawbacks such as interference, reaction kinetics, and quantitation methods (Ou and others 2002).

Measuring Phenolic Content

As antioxidants become better known for providing significant health benefits, current interest is in consuming natural antioxidants, such as polyphenols found in plants (Katalinic and others 2006). Color is one simple way of estimating the amount of polyphenols found in food. Typically, foods that are richer in color are also richer in polyphenol content. However, colorless polyphenols, such as proanthocyanidins, are also significant contributors to overall polyphenol content. Therefore, in order to more accurately quantify polyphenolic compounds, they must first be extracted. There are multiple methods of phenolic extraction. The first step involved in their analysis is the chemical extraction from the plant source. Once extracted, chromatographic and spectrometric analyses are used in the identification of the individual phenolic compounds present (Naczk and Shahidi 2004).

Several factors influence the extraction of phenolic compounds. These issues include the chemical nature of the compound, the extraction method used, storage time and conditions, as well as the existence of interfering substances (Naczk and Shahidi 2004). Particle size is another factor affecting the extraction of phenolics from host plants. Polyphenols range from very simple monomers to complicated polymers. They can be attached to other carbohydrate or protein molecules as well as other compounds present in the plant. Other variables that affect the level of extraction include the time in which the sample is left in the solvent and also the temperature during the extraction (Kalt and others 2000).

Extractability also is influenced by the physical distribution of phenolic compounds in the plant. Specialized cells of plants synthesize phenolics which are located in different plant structures (Beckham 2000). Therefore, the arrangement and concentration varies at the tissue, cellular and sub-cellular levels (Naczk and Shahidi 2004). Phenolic-storing cells have a specialized distribution within the plant in order to take full advantage of the phenolics activity. Insoluble compounds like, lignans and hydroxycinnamic acids, are generally components of the cell wall (Naczk and Shahidi 2004; Beckham 2000; Bengoechea and others 1997). These compounds provide mechanical strength to cell walls and aid in the response to external stress and pathogens (Beckham 2000). Soluble phenolics, on the other hand, are typically compartmentalized within the plant cell vacuoles (Beckham 2000; Bengoechea and others 1997). The outer layers of plants typically contain higher levels of phenolics than those located in their inner parts (Bengoechea and others 1997; Fernandez de Simon and others 1992).

The solvent used in the extraction of the polyphenol also affects the levels retrieved. Solvents that have been used include methanol, water, acidic methanol, ethyl acetate, 70% acetone, and 50% ethanol (Naczk and Shahidi 2004). In a study testing the efficiency of recovering free phenolic compounds from barley by conventional solvent extractions, the alcohol based methods (ethanol and methanol) resulted in the highest total polyphenol extraction. Aqueous acetone and aqueous ethanol solvents extracted the highest amount of catechins and hydrolysable tannins, respectively (Bonoli and others 2004).

Two methods of determining total phenolics include the Folin-Denis assay and the Folin-Ciocalteau assay. The Folin-Ciocalteau method utilizes both HAT and SET mechanisms to eliminate ROS (Prior and others 2005). These assays do not, however, distinguish specific phenolic compounds.

The relationship between total antioxidant activity and total phenolics is not completely understood. Research on several fruits and vegetables show an association between antioxidants and phenolics. However, correlation among other food groups is not clear. The linear relationship most likely depends on the food being studied (Wu and others 2004). In a study evaluating the total phenolic content and the total antioxidant activity (TAA) of five commercial red wines from Spain, results showed that the total antioxidant activity of the wines investigated was related to phenol content (Lopez-Velez and others 2003). The association between the total antioxidant activity and total phenols of wine in this particular study suggests that the antioxidant activity may be obtained directly from gallic acid equivalents (GAE), according to this relationship (Lopez-Velez and others 2003):

$$\mathrm{TAA} = 0.0064\mathrm{GAE} - 0.2508.$$

Total phenolic content and antioxidant capacity of 70 medicinal plant extracts were also investigated. In order to relate the relative antioxidant potential of phenolics, the phenol antioxidant coefficient (PAC) was calculated for each infusion. The PAC is calculated as the ratio between FRAP (µM/L) and total phenolics (µM CE/L). In this study a significant linear correlation was found between total phenolics concentration and FRAP (Katalinic and others 2006).

VARIABLITY IN PHENOLIC CONTENT

Phenolic content can vary considerably in spices as in other plants. The presence of polyphenols in plant foods is largely influenced by genetic factors and environmental conditions. Other factors, such as germination, degree of ripeness, variety, processing, and storage, also influence the content of plant phenolics (Bravo 1998). Data from a study in which the antioxidant capacity of several vegetables was determined showed that the ORAC and FRAP values of vegetable are not only dependent on species, but are also highly dependent on geographical origin and harvest time (Ou and others 2002). Results from this study show nearly 10-fold differences between highest and lowest values of broccoli, almost 6-fold differences in green pepper, and reveals 2-fold differences in spinach (Ou and others 2002). Significant variations in total phenolic content were also found in another study estimating antioxidant phytochemicals and total phenolics in different genotypes of *Brassicaceae* crops (Singh and others 2007). These differences in the antioxidant phytochemicals within the subspecies indicates that the potential health benefits also depend on the genotype. Also, considerable quantitative differences were found in the phenolic composition of apple juices from different varieties (Spanos and others 1990). Overall, the large variability among the same vegetable can be apparently explained by the influences of different variety, growing location, and harvest season, which affect the level of antioxidants present in these vegetables.

Several environmental factors have a major effect on polyphenol content. These factors include soil type, sun exposure and rainfall. Exposure to light has a considerable effect on most flavonoids. For example, the formation of flavone and flavonol glycosides greatly depends on light; therefore, the highest concentrations of these compounds are found generally in leaves and outer parts of plants, with only trace amounts in the subterranean parts of plants (Bravo 1998). The degree of ripeness, particularly of fruits, also considerably affects the concentrations of the various polyphenols. In general, phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations increase (Macheix and others 1990; Manach and others 2004). Similar results were found in a study estimating the phenolics found in leaves of *Quercus robur*

L. trees. The phenolic concentrations of the leaves decreased substantially during leaf maturity and senescence. There was also a greater range of phenolic concentrations in older, aging leaves than in green leaves (Covelo and Gallardo 2001).

Polyphenols are directly involved in the response of plants to stress. They contribute to the plants healing by lignification of damaged areas (Shahidi and Naczk 1995). Lignin is defined as a complex three-dimensional polymer composed prinicipally of oxidatively linked hydroxy-cinnamoyl alcohols. Lignification, therefore, aids in the healing of plants due to a change in character of the cell wall, in which it becomes harder (Parr and Bolwell 2000). Many simple phenolics have antibacterial and antiviral properties (Parr and Bolwell, 2000). Interestingly, phenolic concentrations may increase after stress and infection (Shahidi and Naczk 1995; Parr and Bolwell 2000). This is the reason for the higher polyphenol content found in organic vegetables and other foods produced by sustainable agriculture. Therefore, compared to conventional or hydroponic conditions, plants grown in more natural, stressful environments generally have more beneficial health compounds (Manach and others 2004). This was shown recently in strawberries, blackberries, and corn (Asami and others 2003).

Storage also has a large effect on the polyphenol content of foods. Postharvest storage of fruit crops is complicated because metabolism continues to occur, even though the fruit is removed from the nutrient source (Kalt and others 1999). Maximizing product shelf-life is typically the primary goal of postharvest storage technology. Manipulating fruit and vegetable metabolism is achieved by several methods, including low-temperature storage or storage in a high carbon dioxide atmosphere (Kalt and others 1999). Polyphenols are easily oxidized, which may result in the deterioration of foods (Manach and others 2004). Oxidation is defined as the loss of at least one electron when two or more substances interact. This usually results in changes in the quality of foods, particularly in the browning and other color changes in fruits and vegetables. These types of changes may be harmful to consumer acceptability.

In spices, the drying process leads to a loss or the oxidation of volatile compounds found in the fresh counterpart, resulting in the flavor profile characteristic (Farrell 1999). Drying also reduces or alters flavor and aroma as more volatile components are lost. Typically, as volatile components are lost, non-volatile elements become more concentrated, which results in the creation of bitter tastes (Farrell 1999). Drying may cause the loss of some important dietary components, such as vitamin C and carotenoids (Capecka and others 2005). These changes are induced by respiratory, metabolic and enzymatic effects that occur during the dehydration process. However, drying also typically reduces the antioxidant capacity of plant foods high in phytochemicals, decreasing their potential to positively impact health. For example, fresh garlic is 1.5 times more powerful than dry garlic powder (Palmer 2007). Despite these reductions, dried spices are still a good source of antioxidants.

Conventional cooking methods and other culinary techniques also influence the polyphenol content of foods. Cooking brings about several changes in both the physical characteristics and chemical composition of fruits and vegetables. Significant effects on total phenolics and antioxidant activity were found in a study in which the impact of microwave and conventional cooking methods on several vegetables was examined. After cooking, the total phenolics content of squash, peas, and leeks was significantly reduced, no matter what cooking method was utilized. However, depending on the cooking method, the total phenolic content was significantly increased for bell pepper, broccoli, and green beans (Turkmen and others 2005). Blanching for 1 min in boiling water reduced total phenolics by 12% to 26% in swamp cabbage, kale, shallots and cabbage (Turkmen and others 2005). The total phenolic, ascorbic acid and lycopene contents in tomatoes were also found to be significantly reduced by boiling, baking, and frying methods of cooking (Sahlin and others 2004). Typically, steam cooking of vegetables, which avoids leaching, is preferable. Also, preparation of fruits and vegetables affect the phenolic content. For example, peeling the skin of fruit and vegetables can eliminate a significant portion of polyphenols. This is because phenolic compounds are often present in higher concentrations in the outer parts, such as the skin, than in the inner parts (Manach and others 2004).

Industrial food processing also affects polyphenol content. Bitterness and astringency are found in a variety of foods, including nuts, fruits, chocolate, tea, and wine. The taste of bitterness and the tactile sensation of astringency are elicited primarily by flavanol polymers (proanthocyanidins or condensed tannins) (Lesschaeve and Noble 2005). The process of debittering, in order to make food and beverages more palatable, generally involves the removal of these bitter flavanol phenolics. Flavonoids are also often responsible for discoloration and haze formation in the production of fruit juices. Therefore, industrial processing typically involves clarification or stabilization steps that are aimed specifically at removing these flavonoids (Manach and others 2004). This is why manufactured fruit juices are low in flavonoids. However, not all industrial processing decreases the phenolic content found in foods. Maceration is a process in winemaking where the phenolic materials of the grape, including: tannins, anthocyanins, and flavor compounds, are leached from the grape skins, seeds and stems into the freshly pressed juice. This process of maceration facilitates diffusion of polyphenols in juice, and also occurs during the production of red wine.

SPICES

According to the Merriam-Webster Dictionary, the definition of spice is “any of various aromatic vegetable products used to season or flavor foods”. Typically spices come from the dried seed, fruit, root, or bark of fruits and vegetables. They are distinguished from herbs, which are typically the leafy green part of plants. Herbs are often used fresh, whereas spices are commonly used in their dried form, either ground or whole (Labensky and Hause 2007).

The earliest record of spice use dates back to 50,000 BC. Since these ancient times, spices have been used in food preparations to improve the flavor. Also, in addition to providing flavor, spice uses were numerous, including connections with magic, medicine, religion, tradition and preservation. More recently however, spices are reported to contain bioactive compounds providing food with antioxidant, preservative and antimicrobial properties (Prasad and others 2004). A number of studies concerning the inhibition of microorganisms by spices, herbs, their extracts, essential oils and various constituents have been reported (Zaika 2007). Spices are a concentrated source of several antioxidants beneficial to health. As seen in Table 2.2, spices make up 13 of the top 50 foods highest in antioxidants. The phenolic content of various spices can be found in Table 2.4.

Spice Consumption

The value of total U.S. spice imports increased from $426 million in 1998 to $597 million in 2007. Eight countries including India, Indonesia, China, Brazil, Peru, Madagascar, Mexico, and Vietnam accounted for three-fourths of the spices imported into the United States. India alone, accounted for 24% (USDA 2009).

Consumers’ demand for flavor has driven up spice use per capita about 300% since 1966 (USDA 2005). For the second consecutive year, U.S. annual spice consumption has topped one billion pounds, according to Spice Statistics 2000, a report compiled by the American Spice Trade Association (ASTA) (Food Institute Report 2001). Per capita, the estimated spice consumption increased from 1.2 to 3.3 pounds per year from 1966 to 2005 (USDA 2005). Flavor forecasters predict that spices and herbs, along with fruits and vegetables, will be in the highest flavor growth category over the next 2-3 years (McCormick Science Institute 2009). Rising household use of spices reflects growing Hispanic and Asian populations and also a heightened popularity of ethnic foods. According to the American Spice Trade Association, per capita spice consumption in the United States was ≈4 g/person per day (3.6 lb/person per year) in 1998. Hot spices, such as black and white pepper, red pepper, and mustard seed account for 41% of US spice usage (Lampe 2003). Also, as nutrition and health become increasingly important, a trend toward the use of spices to compensate for less salt and lower fat levels in foods became evident (Buzzanell and others 1995).

Sourcing of Spices

Spice crops are often classified by plant characteristics, fruit size, essential oil content and constituents. This, however, is not adequate as significant differences exist between cultivars from the same region (Weiss 2002). In general, spices contain a complex mixture of aromatic flavoring compounds. The specific flavoring characteristics of a spice are directly related to the comparative measure of its volatile compounds (Ravindran and others 2004). The culinary quality of spices varies according to differences in the separation and milling processes used, with pungency directly related to the concentration of essential oil present (Peter 2001; Hirasa and Takemasa 1998). Essential oils (EOs) (also called volatile oils) are concentrated, aromatic oily liquids obtained from plant material. They can be loosely defined as that part of an extract collected using steam distillation. Essential oils are hydrophobic and comprise a variety of compounds including terpenes, alcohols, aldehydes, ketones, acids, esters, oxides, lactones, acetals (Hedges and Lister 2007). Environmental circumstances influence the quantitative content of the volatile oil, thus, a single spice sourced from different regions will likely differ in flavor profile and pungency (Kustrak 1996). Therefore, it is not surprising that spices are increasingly identified by production region, which is a factor in the marketing of available products. Research was fueled in the 1970's in Japan when Hirahara, Takai and Iwao found an antioxidant variation in the spices depending on the source and type of spice, time of harvest and treatment, and with slight differences in experimental conditions (Kramer 1985). Selective sourcing based upon phenolic levels in available spices may allow increased dietary intake of phenolics without drastic changes in preferred food choices.

Cloves, cinnamon and nutmeg are three of the most familiar spices used in baking. Cloves and cinnamon rank number one and four respectively, on the list of the top 50 foods highest in antioxidants (Table 2.2). These are common household spices and are relatively easy to find at local supermarkets and also from spice retailers such as Penzeys Spices. Penzeys, which is headquartered in Brookfield, WI, has several retail stores, but also makes ordering a wide variety of spices online accessible to consumers.

CINNAMON

Cinnamon (*Cinnamomum verum)* is the sun-dried bark of an evergreen tree of the laurel family (Lauraceae) native to Sri Lanka. The spice is light brown in color and has a fragrant aroma and warm, sweet taste. At one time, cinnamon was regarded as a gift suitable for monarchs and even for the god Apollo (Toussaint-Samat 1994). Cinnamon grows in the tropical highlands of Indonesia, Sri Lanka, China, and Vietnam. According to the Food and Agriculture Organization of the United Nations (FAOSTAT), Indonesia is the largest producer of cinnamon, followed by China, Sri Lanka, and Vietnam (FAOSTAT 2007). Currently the varieties sold by Penzeys are sourced from China, Vietnam and Indonesia. Culinary quality is perceived to vary with source. In the case of cinnamon, not only do growing locations differ, but the plant species may also differ with source origin.

There are two main types of cinnamon, cassia (*Cinnamomum Cassia*, synonym *C. aromaticum*) and ceylon (*Cinnamomum Zeylanicum*, synonym *C. verum*). Cassia cinnamon is native to Southeast Asia, especially southern China, Indonesia (Korintji), and northern Vietnam. Vietnamese cinnamon comes from larger, older trees and has a bold cinnamon flavor similar to that of red-hot candies (Goetze 2010). It has double the amount of volatile oil than is found in Indonesian cinnamon, which equates to greater flavor and aroma intensity (Goetze 2010). Indonesian cinnamon is what most Americans have grown up using in baking and cooking. It has a warm, delicate flavor with a touch of spice (Goetze 2010). Ceylon cinnamon is grown in Sri Lanka and is also known as “true” cinnamon. Ceylon cinnamon, which is milled from the thin inner bark, has a finer and less dense texture, and is considered to be less strong than cassia. It has a more delicate and complex flavor, with citrus, floral and clove notes. Ceylon cinnamon, which is prized in both England and Mexico, has significantly less of the phenolic compound cinnamaldehyde, which imparts the familiar spicy cinnamon flavor and distinct aroma (Jolly 2009). Cassia has a much stronger flavor than Ceylon, is hard and woody in texture, and thicker (2–3 mm thick), as all of the layers of bark are used (Leela 2008). Although both are sold as cinnamon in the United States, cassia dominates in the American marketplace. US labeling laws do not require that the species be identified on packaging.

The chemical constituents of cinnamon include eugenol, eugenol acetate, cinnamaldehyde and benzyl benzoate. Cinnamon bark oil is primarily comprised of cinnamaldehyde (Figure 2.2), which is responsible for the characteristic odor (McCormick and Company Inc. 2010). Extracts of cinnamon have exhibited several beneficial health properties including the improvement of blood glucose, triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol levels in people with type 2 diabetes (Khan and others 2003).

CLOVES

Cloves, *Syzygium aromaticum*, are the aromatic dried, unopened flower buds of an evergreen tree Caryaphyllus aromaticus in the family Myrtaceae. They are native to Indonesia and India, but are used in cuisines all over the world. They are typically used sparingly as they have a very potent flavor. The English name “cloves” is derived from Latin *clavus* 'nail' because of the irregular nail shape of the buds.

The main component of the essential oil of clove is eugenol (Figure 2.3). Additional phenolic constituents include caryophyllin, vanillin, and eugenin (Dearlove and others 2008). Other components include phenylpropanoids such as carvacrol, thymol, and cinnamaldehyde (Chaieb and others 2007). Using thin-layer chromatography (TLC), ultraviolet, infrared, mass spectrometry and high performance liquid chromatography, researchers identified gallic acid and eugenol as the two major antioxidants in clove. Per 100 grams of clove, the amounts of gallic acid and eugenol were determined to be 1.26 g and 3.03 g respectively (Kramer 1985). Eugenol is the primary compound responsible for cloves’ aroma. It is also recognized as having antiseptic, antibacterial, anaesthetic, and antioxidant properties (Chaieb and others 2007). Several species of cloves have been linked with biological means of promoting health. Their antimicrobial properties have also been found to be beneficial against oral bacteria that are commonly associated with dental diseases (Cai and Wu 1996).

NUTMEG

Cultivated for over a thousand years, nutmeg is one of the oldest recorded spices. Nutmeg, *Myristica fragrans*, is an evergreen tree indigenous to the Banda Islands in the Moluccas of Indonesia, or Spice Islands. The nutmeg tree is important for two spices derived from the fruit, nutmeg and mace**.** Nutmeg is the actual seed of the tree, whereas mace is the outer covering of the seed, known as the aril. Currently, nutmeg is grown and selected from both East India and West India. Until recently, Grenadian West Indian nutmeg could not be commercially ground because of its extremely high essential oil content (Penzeys Spices 2009).

Nutmeg contains myristicin (Figure 2.4), a weak monoamine oxidase inhibitor (Truitt and others 1963). Large doses can potentially be dangerous, leading to convulsions, hallucinations, anxiety and nausea (Hallstrom and Thuvander 1998). However, myristicin has been identified as beneficial to health in a number of protective ways including as a potential cancer chemopreventive agent (Zheng and others 1992).

HEALTH BENEFITS OF SPICES

In addition to their antioxidant activity, phenolic compounds found in spices have anti-inflammatory, anti-allergic, anti-microbial and anti-cancer properties (Hedges and Lister 2007). Specifically, spices have a protective effect against inflammation. Inflammation is thought to play a critical role in a number of injury and disease states, which can benefit from enhanced nutrition care (Jensen 2006). It is a protective process by which the body’s vascular tissues respond to harmful stimuli, such as pathogens, damaged cells or irritants (Tracy 2003). In the biological reaction to these stimuli, the inflammation response is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. The local vascular system, the immune system, and various cells within the injured tissue all work together to propagate the inflammatory response (Davies and Hagen 1997). There are several biomarkers of inflammation, including: Interleukin 6, C-Reactive Protein (CRP), Tumor Necrosis Factor (TNF), and Nuclear Factor-κB (NFκB) (Tracy 2003). Herbs and spices have been linked to the suppression or inhibition of inflammation and these biomarkers. Animal and in vitro studies have shown that sage, black cumin, cinnamon, and capsaicin reduce or suppress inflammation biomarkers and thus, inhibit the inflammatory process (Oniga and others 2007; Tekeoglu and others 2007; Kim and others 2007; Huang and others 2006). Curcumin (diferuloylmethane), a component of the spice turmeric, is best known as a modulator of inflammation (Jagetia and Aggarwal, 2007). Through the inactivation of the transcription factor NF-κB, curcumin downregulates the expression of several proinflammatory cytokines including tumor necrosis factor (TNF), interleukin (IL)-1, IL-2, IL-6, IL-8, IL-12 and chemokines (Jagetia and Aggarwal, 2007). More recently, however, curcumin has been studied for its effects on the immune system.

Current evidence suggests a link between cardiovascular disease and free radicals. The oxidation of low density lipoproteins (LDLs) is thought to play a role in the development of atherosclerosis. The compounds found in spices may have the potential to prevent the oxidation of LDL (Tapsell and others 2006). For example, curcumin, derived from turmeric, prevents the oxidation of LDLs and inhibits platelet aggregation, thereby reducing the risk of myocardial infarction (Shishodia and others 2005). Curcumin has also been effective against atherosclerosis (Aggarwal and others 2005). It inhibits the proliferation of peripheral blood mononuclear cells (PBMCs) and vascular smooth muscle cells (VSMCs) which are both characteristic in the development of atherosclerosis (Shishodia and others 2005). The link between culinary herbs and spices and oxidation is an essential area of research and may prove to be critical step in improving overall health and well-being.

The American Institute for Cancer Research notes that herbs and spices should be used as flavor enhancers because of their health-protective phytochemicals, which can help fight cancer and other diseases, much like those found in fruits, vegetables, whole grains, and other plant-based foods (AICR 2003). For example, garlic contains a number of substances now being studied for their anticancer effects, including allicin, allixin, allyl sulfides, quercetin, and organosulfur compounds. Ginger is another spice with apparent anti-inflammatory and anti-cancer effects. Ginger, a mixture of several hundred known constituents, including gingerols, beta-carotene, capsaicin, caffeic acid, curcumin, and salicylate, has been shown to help control inflammation, which can contribute to the development of ovarian cancer cells. Ginger exhibits cancer preventive activity in experimental carcinogenesis (Shukla and Singh 2007). Curcumin, from turmeric, has also displayed several anticancer properties. It has shown the potential of blocking tumor initiation, tumor promotion, invasion, angiogenesis, and metastasis (Shishodia and others 2005). Curcumin has also been shown to inhibit the proliferation of a wide variety of tumor cells, including B-cell and T-cell leukemia, colon carcinoma, and epidermoid carcinoma cells (Chen and others 1999; Kuo and others 1996; Korutla and Kumar 1994). Other spices, including cinnamon, cloves, and nutmeg, are also being examined for beneficial health properties.

Cinnamon has been studied for its antioxidant capacity, antimicrobial effects, and also for its role in insulin activity. Researchers believe that cinnamon’s active ingredients are polyphenol polymers that may imitate the activity of insulin. In three trials involving 164 patients with type 2 diabetes, researchers evaluated the efficacy of cinnamon supplementation. Two of the studies reported modest improvements in lowering blood glucose levels with cinnamon supplementation in small patient samples, while one trial showed no significant difference between the cinnamon and placebo in lowering blood glucose levels. Researchers concluded that cinnamon has a possible modest effect in lowering plasma glucose levels in patients with poorly controlled type 2 diabetes (Pham and others 2007). In another study investigating the effects of cinnamon on insulin sensitivity, cinnamon reduced the mean fasting serum glucose (18–29%), TAG (23–30%), total cholesterol (12–26%) and LDL-cholesterol (7–27%) in subjects with type 2 diabetes after 40 days of daily consumption of 1–6 g cinnamon (Anderson 2008).

Among spices, cloves often exhibit the highest levels of antioxidant activity (Halvorsen and others 2006; Shobana and Naidu, 2000). Therefore, the components of cloves have been extensively studied. Two major constituents of cloves, eugenol and isoeugenol, have a role in blocking tumor necrosis factor (TNF) signaling. As a result, the inhibitory effects of clove components on TNF induced cellular responses may explain its role in suppression of inflammation and carcinogenesis (Aggarwall 2008).

Nutmeg also exhibits phytochemical diversity and thus potential medicinal value (Iyer and others 2009). Recent reports have indicated anti-bacterial, anti-viral, anti-diabetic, anti-leukaemic effects of nutmeg. These reports offer promise in the protective and preventive abilities of nutmeg in human health (Latha and others 2005; Yang and others 2006).

SENSORY TESTING AND MARKETING

The food market is a highly competitive field and manufacturers must meet a constantly changing consumer demand. Sensory testing helps ensure that products enter the market with acceptable sensory qualities that meet the expectations of consumers. Sensory research is necessary to the development and success of new product formulations for packaged goods companies (Lawless and Heymann 1998). That being said, very few new products are successfully formulated. Over the past 15 years, less than 3 percent of new products achieved mega-hit status, or more than $50 million in one-year sales (Toops 2010).

Flavor is often described as the combination of taste and smell (Taylor and Hort 2004). However, appearance, texture, temperature, mouth feel, and past experience also have roles in flavor perception. Therefore, multiple parts of the sensory system are utilized to generate the overall flavor sensation (Goff and Klee 2006). For this reason, phenolics are particularly important to food development companies. In addition to their antioxidant power, phenols are of major interest because of their impact on astringency, bitterness, browning reactions, and color in food products (Singleton and others 1999). These effects are often evaluated using consumer sensory research. To optimize sensory attributes of food products, studies typically use either hedonic scales or just-about-right scales (Epler and others 1998). Hedonic scales generally provide numerical and verbal categories of acceptance (Moskowitz and Sidel 2006). They are formatted with an equal number of positive and negative categories, and a neutral category in the middle; intervals are of identical size. Hedonic scales provide information on products that can be used to compare with other products. Just-about-right scales, however, provide only relative product information (Peryam and Pilgrim 1957).

Just-About-Right Scales

One of the most simple and direct ways of determining consumer approval is asking whether a product is just-about-right (JAR) in regard to a particular attribute (Popper and Kroll 2005). JAR scales are used in product development and marketing research to increase or decrease the intensities of product characteristics to levels that consumers say will be most favorable (Epler and others 1998). A sample format of a just-about-right scale can be found in

Figure 2.5. Other versions of the scale employ three or seven response categories, with the middle category labeled “just-about-right” (Popper and Kroll 2005).

Preference assessment

Consumer taste preferences do not necessarily predict food preferences (Drenowski 1997). Taste preferences are often based on verbal concept of foods. Food preferences on the other hand, are measured using preference checklists or actual taste tests. These are thought to predict food consumption in real life (Conner and others 1988). Food preference is often measured using the standard hedonic preference scale, which consists of a fully anchored 9-point category scale (Peryam and Pilgrim, 1957). Preference can also be measured by ranking of food items using a procedure that has been shown to provide reliable and valid information about children’s food preferences. In this method of ranking, children assign sampled foods to 1 of 3 categories illustrated with cartoon faces depicting “yummy,” “yucky,” and “just okay.” Rank-order scores are then assigned to the food items (Birch 1979).

Health claims as a marketing tool

Research providing scientific evidence linking health and dietary selections is constantly emerging. The marketing issue is how best to communicate this diet and health information to consumers and positively impact sales. One current approach is to include health claims on product labels to differentiate and add value to products in the marketplace. It is beneficial for companies to make antioxidant claims in their products. Nutritive claims, however, are strictly regulated by the FDA.

Food labeling regulations were made mandatory in response to the Nutrition Labeling and Education Act of 1990 (NLEA 1990). This mandated major changes in the development of food labels. Changes included: requiring that nutrition labeling be placed on most foods, requiring terms that characterize the level of nutrients in a food be used according to definitions established by the FDA, and providing for the use of claims about the relationship between nutrients and diseases or health-related conditions. The FDA tightly regulates the use of claims on foods. These FDA regulations on the nutrition labeling of foods are found in Title 21 of the Code of Federal Regulations in section 101.9. Regulations for health claims are found in sections 101.14, 101.70–101.83. Nutrient content claims are found in sections 101.13, 101.54–101.69 and specify the requirements for each type of claim (DHHS 2009).

Claims can be classified into one of three categories: nutrient content claims, structure/function claims, or health claims (Krasny 2004). To include the term “antioxidant” on a label, the product must meet the terms of one or more of these claims. However, even as certain populations become more aware of health and nutrition, previous marketing studies show that taste is the primary influence on consumer food selection (Drenowski and Gomez-Carneros 2001). As we become a more health-conscious society, the impact of health claims is increasingly becoming a major determinant on consumer choice. According to the Food Marketing Institute trends survey, almost all shoppers (96%) said nutrition is a “somewhat” or “very important” factor when they purchase food. The only factor more important than nutrition was taste (97%) (FMI 2000). With 96% of shoppers concerned about nutrition, most have purchased products because of nutrition claims on the label. Nearly 80% have sought out and purchased products because of “low-fat” claims. 59% have done so because of “low-cholesterol” claims and a similar amount of shoppers report purchasing products with “natural” claims (59%) (FMI 2000). Consumers are also more aware of functional foods. In 2007, 90% of Americans were able to relate a specific food or food component with an associated health benefit. This is up from 84% in 2002 (IFIC 2007). 72% of Americans stated that they were aware of the relationship between antioxidants and the protective benefits against free-radical damage that underlies various chronic diseases (IFIC 2007).

Table 2.1: **Mechanisms of action of various antioxidants against different diseases**[a]

Compound	**Pathology**	**Mechanism of action**
Catalase (CAT)	Cancer, diabetic retinopathy	Destroys hydrogen peroxide in high concentration by catalysing its two-electron dismutation into oxygen and water
Superoxide dismutase (SOD)	Neurodegenerative diseases	Catalyse the one-electron dismutation of superoxide into hydrogen peroxide and oxygen
Alkaloids	Cancer, Neurodegenerative diseases, chronic inflammation	Shown a variety of biological activities such as inhibition of topoisomerase I and II; cytotoxicity against different tumor cell lines
Carotenoids	Cancer, diabetic retinopathy, chronic inflammation	Mainly act as physical quenchers of reactive oxygen
Ferulic acid	Diabetes	Decrease lipid peroxidation and enhances the level of glutathione and antioxidant enzymes
Glutathione	Cancer	Glutathione in the nucleus maintains the redox state of critical protein sulphydryls that are necessary for DNArepair and expression
Phenolics	Cancer, diabetic retinopathy, chronic inflammation	Inhibit the oxidation of lipids, fats, and proteins (RH) by donation of a phenolic hydrogen atom to the free radical
Quercetin, Kaempferol, genistein, resveratrol	Colon cancer	Suppresses COX-2 expression by inhibiting tyrosine kinases important for induction of COX-2 gene expression
Tannins	Cardiovascular disorders	Tannins are known to enhance synthesis of nitric oxide and relax vascular segments precontracted with norepinephrine

[a]Table adapted from (Ali and others 2008)

Table 2.2: Antioxidant capacity (FRAP) of 50 foods highest in antioxidants out of 1113 assessed

Food	FRAP mmol/100g[a]
Cloves, ground	125.6
Oregano leaf, dried	40.3
Ginger, ground	21.6
Cinnamon, ground	17.7
Turmeric powder	14.7
Walnuts	13.1
Basil leaf, dried	12.3
Mustard seed, yellow, ground	10.5
Curry powder	10.0
Pecans	9.7
Chocolate, baking, unsweetened	8.9
Paprika	8.6
Chili powder	8.4
Parsley, dried	7.4
Molasses, dark	4.9
Pepper, black	4.4
Artichokes, prepared	4.2
Chocolate, dark	4.2
Blackberries	4.0
Whole-grain cereal	3.4
Cranberries	3.3
Pudding mix, chocolate, cook-and- serve	3.0
Bran cereal	2.9
Power bar, chocolate flavor	2.8
Chocolates, sugar-free	2.6
Raspberries	2.3
Strawberries	2.2
Blueberries	2.2
Cabbage, red, cooked	2.2
Wine, red	2.1
Barley malt syrup, organic	2.1
Prunes	2.0
Cherries, sour	1.8
Peppers, red, cooked	1.6
Chocolate cookies w/ vanilla crème filling	1.6
Cocoa Krispies cereal	1.6
Chocolate chip cookies	1.5
Mustard, yellow, prepared	1.5
Milk-chocolate candy	1.5
Pistachios	1.4
Plums	1.3
Kiwi fruit	1.3
Corn flakes	1.3

Coffee	1.3
Spinach, frozen	1.2
Flaxseed, ground or milled	1.1
Rice and corn cereals	1.1
Toasty peanut crackers	1.1
Cupcakes, chocolate	1.1
Grape juice	1.0

[a] (Halvorsen and others 2006)

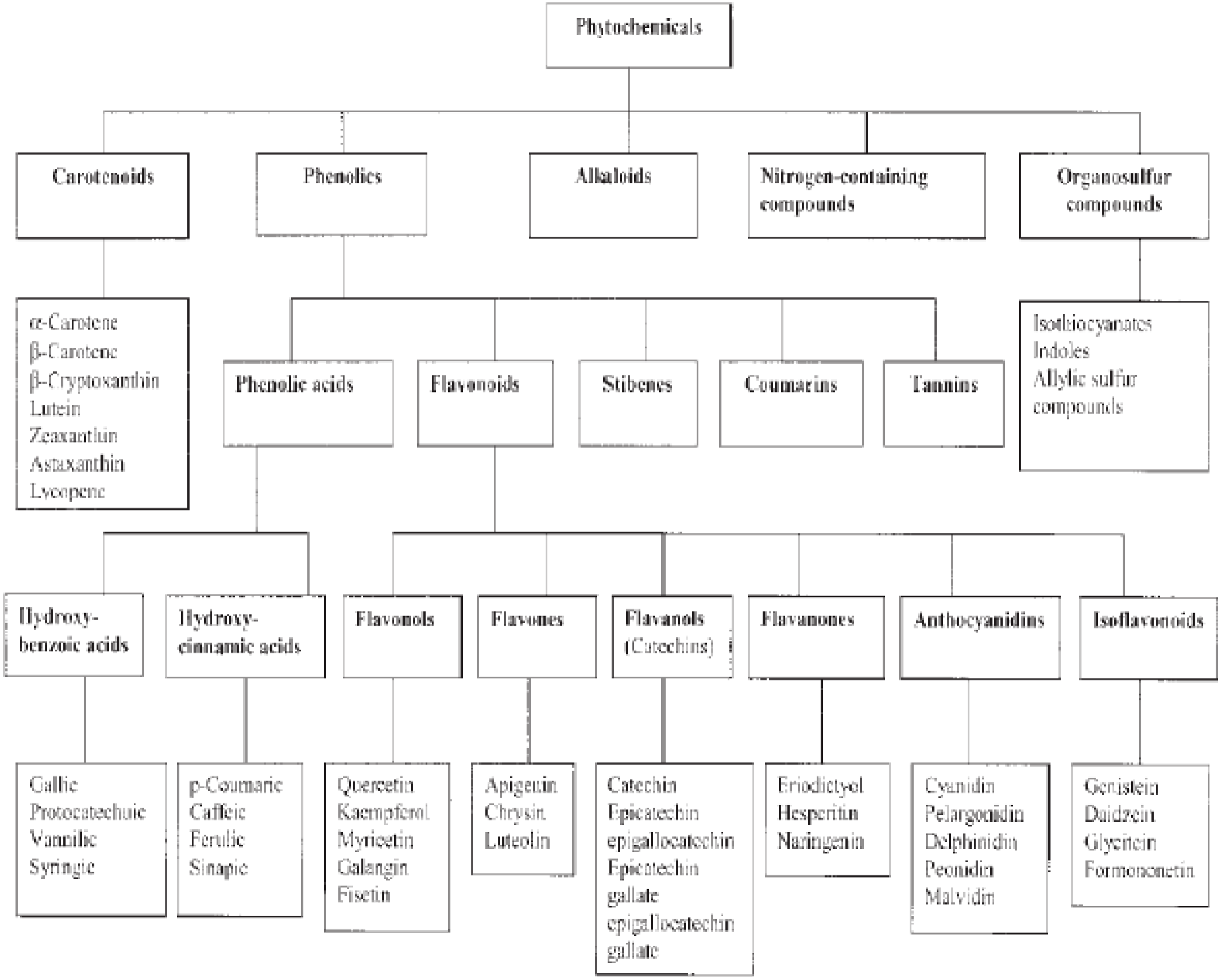

Fig. 2.1: Phytochemical classifications including phenolic subgroup classifications (Liu 2004)

Table 2.3: Major classes of phytochemicals that contribute to the aroma of spices and their sources[a]

Class of Phytochemicals	Source
Terpenes	
Monoterpenes	Apiaceae family (eg, cumin, fennel, caraway)
Tetraterpenes (eg, carotenoids)	Paprika, saffron
Sesquiterpenes	Cinnamon, juniper, ginger, turmeric, galangal
Phenylproponoids	
Cinnamic acid	Cinnamon
Eugenol	Cloves, Nutmeg
Vanillin	Vanilla bean
Methoxysafrole	Nutmeg
Diarylheptanoids	
Curcumin	Tumeric
Isothiocyanates	
Allyl isothiocyanate	Mustard seed, wasabi
Sulfur compounds	
Thiols, sulfides, di- and polysulfides	Garlic, asafetida

[a]Adapted from (Lampe, 2003; USDA ARS, 2003)

Table 2.4: Total phenolic content of select herbs and spices[a]

Herb or Spice	**Phenolic content (mg of GAE/g)**
Cloves	296 ± 3.7
Cinnamon	183 ± 11.5
Allspice	122 ± 7.0
Oregano	82.3 ± 0.9
Sage	59.8 ± 3.0
Thyme	52.0 ± 2.0
Rosemary	48.2 ± 1.0
Bay leaf	36.3 ± 1.1
Turmeric	25.9 ± 1.6
Curry powder	21.6 ± 0.8
Chili powder	19.5 ± 1.2
Basil	18.0 ± 0.5
Nutmeg	17.6 ± 0.9
Ginger	17.7 ± 1.7
Parsley	15.5 ± 0.7
Black pepper	5.1 ± 0.3

[a]Table adapted from Dearlove and others 2008

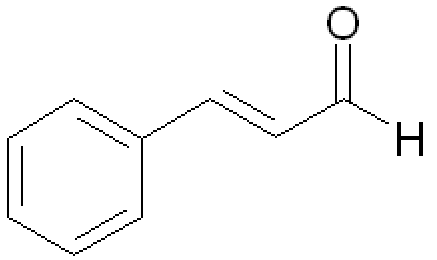

Fig. 2.2 Structural formula of cinnamaldehyde

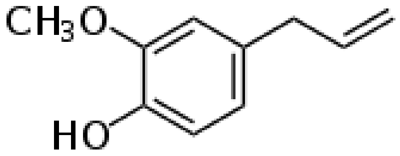

Fig. 2.3 Structural formula of eugenol

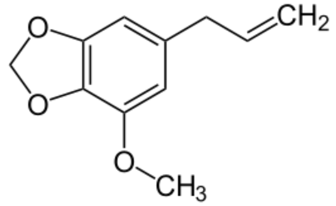

Fig. 2.4 Structural formula of myristicin

Much too strong…………
Somewhat too strong……
Just-about-right………….
Somewhat too weak……..
Much too weak…………..

Fig. 2.5 Five-point Just-about-right scale[a]

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CHAPTER III

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

PHASE 1: SPICE CHARACTERIZATION

Variability in total phenolic levels and color in spices was investigated with a factorial design (Table 3.1). Three spices: cinnamon, cloves, and nutmeg sourced from 3-4 sources, were assayed for total phenolics. The total phenolic assay was replicated three times. Color of individual spices was also determined; color data were collected three times on 3 samples from each source for the three spices.

Spices

A total of 10 samples of three spices were assayed in this study. The three spices tested were: cinnamon (4), cloves (3) and nutmeg (3). McCormick® (Hunt Valley, MD) spices packaged in plastic jars and available in the supermarket served as the control. Origin was unspecified on product labeling. All other samples were purchased online from Penzeys Spices® (http://www.penzeys.com/) (Wauwatosa, WI) September 4, 2009. Origin was specified on the label. These included three types of cinnamon (Chinese Cassia, Vietnamese Cassia, Korintje (Indonesian) Cassia), two types of cloves (Madagascar, Ceylon) and two samples of nutmeg (East Indian, West Indian). All cinnamon and nutmeg spices were purchased in the ground form. The cloves were purchased whole and ground into a fine powder with a Cuisinart Mini-Prep™ (DLC-1) processor (Cuisinart, East Windsor, NJ) before proceeding with analyses.

Total Phenolics Determination

Determination of total phenolics involved three steps: (1) preparation of the sample extracts, (2) removal of the fat via hexane extraction, and (3) determination of the total phenolics using the Folin-Ciocalteu reagent. Details of these steps follow.

Preparation of sample extracts

All spice samples were extracted using 50% ethanol. 1 g of spice was combined with 9 mL of 50% ethanol and homogenized using a homogenizer for 60 seconds. The 50% ethanol solution was stirred at speed 5 for 1 hour at room temperature using a Thermolyne Nuova II (Dubuque, Iowa) stir plate. The sample was centrifuged at 1100 RPM (430 *g*) for 10 minutes at 10° C to remove unwanted precipitate.

Hexane extraction

Following the centrifuging and removal of unwanted precipitate, a pipette was used to transfer each sample into a separatory funnel. 3 mL hexane was added and the mixture was slowly inverted 20 times to incorporate the hexane into the sample. After sitting under ambient conditions for 15 minutes, the supernatant at the bottom of the funnel was decanted and put into a new tube. This decanted supernatant was used to run the phenol assay.

Determination of total phenolic content

The amount of total phenolics in each sample was determined according to the method described by Singleton and Rossi (1965). Folin-Ciocalteu reagent and bovine serum albumin were purchased from Sigma Chemical Company (St. Louis, MO). Results were quantified using a Beckman (Palo Alto, CA) DU 600 series spectrophotometer at a wavelength of 760 nm with a gallic acid standard. Results were expressed as mg of gallic acid equivalents (GAE)/g of dry sample. All experiments were performed in triplicate. LS-means and standard errors were determined for each sample within each sample suite. Significant differences ($p < 0.05$) were determined with analysis of variance (ANOVA).

Color of spices

Color of each sample in each suite (cinnamon, cloves, nutmeg) was measured using a Minolta Spectrophotometer (Model CM-700d) (Konica Minolta Sensing Inc., Ramsey NJ) calibrated using a white calibration cap (CM-A177) and open air calibration. The spectrophotometer was set at 10-degree observer function, F6 illuminant setting for cool white florescent light source (4150K), and the specular component was excluded. Standard methods of food readings with the spectrophotometer were utilized (Francis and Clydesdale 1975). Each reading was an average of the three closest readings out of five taken. Color was recorded in three values, L* a* and b*. L* is a measure of lightness on a 0 to 100 scale, where 0 is black and 100 equals white, and is an indication of saturation. The reading a* measures red-green axis, where positive a* is redness and negative a* is greenness. The reading b* is a measure of the yellow-blue axis, where positive b* is yellowness and negative b* is blueness. a* and b* measure hue. Two assessments per ground sample were obtained. Data were collected on three samples per spice within in each suite. LS-means and standard errors were determined for each sample within each sample suite. Significant differences ($p < 0.05$) were determined with analysis of variance (ANOVA).

PHASE 2: FOOD SYSTEM STUDY

Phenolics were measured in all of the ingredients in a pumpkin-spice muffin formula to determine if an ingredient provided any contribution to the total phenolic content. Then, in a food system study, pumpkin spice muffins (n=3 formulations) were used to assess the effects of spice source on: pH of muffin batter, interior and exterior muffin color, expected and recoverable phenolics, and a consumer panel. The factorial design can be found in Table 3.2.

pH of the muffin batter and color of the exterior surface and interior crumb of the three muffin formulations were determined on three samples per replication using standard method for pH measurement (McWilliams 2008). Recoverable phenolics were determined in all three muffin samples in triplicate. Three replications were obtained for all chemical and physicochemical tests. A consumer panel was run to determine sensory characteristics and consumer preference for the three muffins. The impact of an antioxidant claim on willingness to purchase, and the amount that consumers were willing to pay when an antioxidant claim was present also were assessed. Factors that affected food choices of the 134 sensory panelists were determined.

Pumpkin-spice muffins

All ingredients except the spices were constant. Amounts of each spice remained constant but sources varied. McCormick® spices were used in the control muffin. Constant ingredients for the pumpkin muffin used in the consumer study were purchased from the local supermarket. The formula for the muffin can be found in Table 3.3. Total phenolic values for each ingredient in the formula were determined in triplicate, using the same procedure used to determine the phenolics in the individual spices. The expected level of total phenolics in each muffin formulation was determined by summing the contribution of each ingredient; the average total phenolic level for each ingredient was used in these calculations.

The three muffins were formulated with specific combinations of spices. The control muffin was prepared using all McCormick® brand spices of unspecified origin. The second muffin was formulated with spices that minimized total phenolics by combining those with the lowest mg GAE/g in each sample suite. The third muffin was formulated with the spice combination that maximized total phenolics by combining those with the highest mg GAE/g in each sample suite. The specific combinations for each muffin can be found in Table 3.4.

Muffins were prepared with a Kitchen Aid Mixer (K5SS, St. Joseph, MI) equipped with a paddle beater and baked at 177° C in a rotary oven (National Mfg. Co., Lincoln, NE) for 22 minutes. To prepare the batter, all refrigerated ingredients were held at room temperature for at least 45 minutes before mixing. Dry ingredients (flour, baking soda, salt, baking powder, cinnamon, nutmeg, and cloves) were measured and sifted together into a large bowl. The shortening and sugar were blended on speed four for one minute. The bowl was scraped using a rubber spatula to incorporate any mixture on the bottom of the mixing bowl. The egg was added and blended on speed four for thirty seconds. The pumpkin and water were added next and blended on speed two for thirty more seconds. The bowl was scraped again to assure proper mixing. The sifted dry ingredients were added to the wet mixture and blended at speed two for fifteen seconds. Stirring was finished with 4 folds using a spatula to make sure all ingredients were properly blended and to avoid over-mixing. The batter was then scooped into the mini muffin pans with a leveled # 40 scoop (46.2 ± 0.24g). After baking, the muffins were left to cool for two hours under ambient conditions before instrumental tests were conducted. Muffins were baked and tested five times and the order of baking was randomized for each replication.

pH of Muffin Batter

The pH of the muffin batters were measured using a pH meter (Model 520A, Orion, Boston MA) calibrated using 4.00 pH and 7.00 pH buffers obtained from Fisher Chemical Labs (Fairlawn, NJ) using standard procedure for pH measurement (McWilliams 2008). The batter pH was measured directly after mixing. Three samples were analyzed for each of the three replications. LS-means and standard errors were determined for each muffin sample. Significant differences ($p < 0.05$) were determined with analysis of variance (ANOVA).

Color

The effects on color of the products due to reformulation were determined by measuring the interior and exterior muffin color, as described for the individual spices. Three muffin samples were analyzed for each of the three replications. LS-means and standard errors were determined for the exterior and interior L*, a*, and b* values for each muffin sample. The L*, a*, and b* values were interpreted (Mabon 1993). Significant differences ($p < 0.05$) were determined with analysis of variance (ANOVA).

Expected vs. recoverable phenolics

Expected phenolic values for each of the muffins were determined by combining the individual phenolic values of each ingredient in the muffin formula. The mean value (n=3) for the total phenolics present in each ingredient was used in this calculation. The ingredients contributing to the expected total phenolic value of the muffin were the spices, cinnamon, cloves, and nutmeg, and also the flour.

Five batches of each muffin formulation were baked. Three muffins from each batch were ground together using a mini food processor (Cuisinart model DLC-1, East Windsor NJ) to generate a composite sample from each muffin formula. A 1-gram aliquot from each composite muffin formula was then used in the determination of total phenolic content using the method described by Singleton and Rossi (1965). Phenolics were determined in triplicate (n=3).
After the phenolic assay was run on the baked muffin, the recovered phenolic values were compared with the expected.

Consumer Sensory Panel – Food Choices Questionnaire

A consumer panel was used to test for acceptability of muffin attributes for each formulation and the relative preference of each muffin formulation. Before evaluating the muffins, the panelists were asked to fill-out a food choices questionnaire to determine a consumer profile and buying habits. The first three questions asked about demographic information, age, gender, and ethnicity. Percentage response was determined.

The remaining questions queried panelists about the relative importance of 38 factors on their choices among available products in the marketplace. The food choices questionnaire was composed of 36 statements developed by Steptoe, Pollard, and Wardle in 1995, which has been validated with urban audiences (Appendix A). Subjects were asked to endorse the statement “It is important to me that the food I eat on a typical day…” for each of the 36 items by choosing between four responses: *not at all important, a little important, moderately important* and *very important*. Two additional items were included that reflected additional impacts that have been reported as emerging influences in the American marketplace. These were: “was grown nearby” and “high in antioxidants” (Sloan 2006).

Frequency of response and means and standard deviations were determined for panelists’ responses to the 38-item food choices questionnaire. Using the SAS statistical program, a frequency procedure (proc freq) was used to calculate the frequency of responses. Survey responses were subjected to an exploratory factor analysis using a common factor model using a factor procedure (proc factor). The Maximum Likelihood Method was used for the initial extraction.

An item was said to load on a given factor if the loading was 0.40 or greater. Factors loading on more than one factor were eliminated as were nonloading factors. After elimination, a new factor solution was derived, following the same procedures. A scree test was done in order to derive factors that were subjected to promax rotation. Scale reliability for each extracted factor was assessed with Cronbach's Coefficient Alpha.

Four factors emerged as significant in the factor analysis. In interpreting the rotated factor pattern, 8 items related to *Nutrition and Health* were found to load on the first factor; Cronbach's Coefficient alpha was 0.85. Seven items loaded on the second factor, which was labeled *Convenience*; Cronbach's coefficient alpha was 0.83. Five items loaded on Factor 3 which was labeled *Mood and Feelings*; Cronbach's coefficient alpha was 0.86. The three factors loading on Factor 4, labeled *Natural*, had a Cronbach's alpha of 0.87.

Estimated factor scores were generated and included in a Pearson Product Moment Correlation analysis ($p<0.1$) between the factors generated from the food choices questionnaire and willingness to pay for the presence of an antioxidant claim on the preferred muffin determined via sensory evaluation.

Consumer Sensory Panel - Sensory Evaluation

The panelists rated each of the three muffins for surface color, interior color, amount of pumpkin flavor, spice intensity, and sweetness (Appendix B). The panelists used a 5- point Just-About-Right (JAR) scale with 1 being much too light/weak/low, 2 being somewhat too light/weak/low, 3 being just-about-right, 4 being somewhat too dark/strong/sweet, and 5 being much too dark/strong/sweet (Popper and Kroll 2005). The muffins were coded with three-digit random numbers and were presented to the panelists one-at-a-time in a balanced order. The panelists tested the muffins in an individual booth lit with cool white florescent light and with positive/negative ventilation to prevent outside aromas. The panelists were given water, unsalted saltine crackers, and carrots to cleanse their palate between samples. Panelists consisted of untrained students and professionals recruited in Dawson Hall at the University of Georgia the day of testing. Panelists were only told they would taste pumpkin muffins and that they would receive a store-bought snack after testing. LS-means and standard errors were determined for each muffin sample for each sensory attribute. Significant differences ($p < 0.05$) were determined with analysis of variance (ANOVA).

Preference Assessment and Marketing Questions

Panelists were asked to rank the muffins in order of preference with 1 as most preferred and 3 as least preferred. Consumer preference data were analyzed using Kruskal-Wallis one-way analysis of variance by ranks. A proc npar1way was used to determine the significance of the ranks of the muffins.

Panelists were also asked a series of marketing questions regarding the pumpkin muffin product and an antioxidant claim. The first question gave an average price for a single muffin ($1.00) and asked if the panelists would be more likely to purchase their preferred muffin (the muffin ranked 1 according to the previous question) if it carried an antioxidant claim. The specific antioxidant claim was provided below the question (Appendix B). The second question asked how much more the panelists who answered “yes” to the first question would be willing to pay for their preferred muffin if it carried the specified antioxidant claim. Lastly, panelists were asked if they would purchase a muffin other than their preferred muffin if it carried an antioxidant claim. All responses to the three questions were analyzed using the frequency procedure.

Statistical Analyses

All statistics were performed using the SAS System program (version 9.1.3, service pack 4) (SAS Institute, Inc., Cary, NC) (Hatcher 1994). Specific analyses used are identified as the methods used for data collection are described. All analysis of variance (ANOVA) tests were completed using General Linear Models (GLM) procedures and pdiff at significance $p < 0.05$ to determine differences among samples. Preference data was analyzed using Kruskal-Wallis one way analysis of ranks. Factor analysis of the food frequency questionnaire was explored using factor procedures followed by correlation procedures. The responses to the marketing questions were analyzed using proc freq.

Table 3.1: Experimental Factorial Design – Phase 1: Spice Characterization

Analysis	Factors
<u>Total phenolics</u>	
Cinnamon	4 sources x 3 replications
Cloves	3 sources x 3 replications
Nutmeg	3 sources x 3 replications
<u>Color</u>	
Cinnamon	4 sources x 3 samples x 3 measurements x 3 replications
Cloves	3 sources x 3 samples x 3 measurements x 3 replications
Nutmeg	3 sources x 3 samples x 3 measurements x 3 replications

Table 3.2: Experimental Factorial Design - Phase 2: Food System Study

Food System Analysis	Factors
Total phenolics – Muffin ingredients	1 formula x 1 sample x 3 replications
pH of muffin batter	3 formulas x 3 samples x 3 replications
Muffin color – exterior	3 formulas x 3 samples x 3 replications
Muffin color – interior	3 formulas x 3 samples x 3 replications
Recovered phenolics	3 formulas x 3 samples x 3 replications
Consumer panel	3 formulas x 134 panelists

Table 3.3: Pumpkin-spice muffin formula[a]

Ingredient	**Amount (g)**	**Source**
Constant		
Shortening	68	Crisco, Orrville OH
Granulated sugar	266	Kroger, Cincinnati OH
Eggs	199	Kroger, Cincinnati OH
Canned pumpkin	245	Libby’s, Solon OH
Water	78	Distilled tap, Athens-Clarke County
All-purpose flour	208	Bakers and Chefs, Bentonville AR
Baking soda	4.6	Arm and Hammer, Princeton, NJ
Salt	4.5	Morton, Chicago IL
Baking powder	1.15	Clabber Girl, Terra Haute IN

Variable		
Cinnamon	4.0	McCormick® (Hunt Valley, MD) or Penzeys® (Wauwatosa, WI) Varied with formulation.
Cloves	0.8	McCormick® (Hunt Valley, MD) or Penzeys® (Wauwatosa, WI) Varied with formulation.
Nutmeg	1.0	McCormick® (Hunt Valley, MD) or Penzeys® (Wauwatosa, WI). Varied with formulation.

[a] Formula yields 32 mini muffins. Average weight of individual muffin is 25.24 ± 0.44g; serving size=2 muffins

Table 3.4: Spice combinations and their sources for the high phenolic, low phenolic, and control muffins along with the expected total phenolic value for each muffin formula

High phenolic (27.62 mg GAE/g)	
Cinnamon	Penzeys Korintje Cassia
Cloves	Penzeys Ceylon Cloves
Nutmeg	Penzeys West Indian Nutmeg
Low phenolic (15.27 mg GAE/g)	
Cinnamon	Penzeys Vietnamese Cassia
Cloves	Penzeys Madagascar Cloves
Nutmeg	McCormick Nutmeg
Control (22.19 mg GAE/g)	
Cinnamon	McCormick Cinnamon
Cloves	McCormick Cloves
Nutmeg	McCormick Nutmeg

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CHAPTER IV

RESULTS AND DISCUSSION

Phenolic Values of Ground Spices

A summary of the measured phenolic levels for each sample can be found in Table 4.1. The phenolic values of cinnamon ranged from a low of 43.96 ± 1.1 mg GAE/g to 136.8 ± 4.5 mg GAE/g. These results demonstrate large variability in phenolic levels depending on the source. Other values for cinnamon phenolics in the literature are comparable to the higher end of the determined range. For example, 50% acetone and 80% methanol extracts of cinnamon in another study resulted in total phenolic contents (TPC) of 186 and 148 mg GAE/g, respectively (Su and others 2007). The value of 183 ± 11.5 mg GAE/g for cinnamon as seen in Table 2.4 (Dearlove and others 2008) is also higher than the determined range of values for cinnamon. The variability may be due to the use of different assays. Often times, phenolic values are calculated from corresponding antioxidant (FRAP) values which may produce varying results and therefore generate dissimilar relative rankings. According to McCormick Spice Institute®, cinnamon has one of the highest antioxidant values of any spice. It is listed, along with ginger, oregano, red pepper, rosemary, thyme, and yellow curry, as one of McCormick's seven super spices for health. One teaspoon of cinnamon has as many antioxidants as a full cup of pomegranate juice or ½ cup of blueberries, both of which are considered high antioxidant foods (McCormick and Co. Inc. 2010a). The primary component of cinnamon responsible for its characteristic aroma is cinnamaldehyde (Figure 2.2) (Ooi and others 2006). Cinnamon is one of the most common household spices in the United States with uses in both baking and cooking. Whether it is utilized in cakes, cookies, stews or curries, the range of uses for this warm, fragrant spice is limitless (Ortiz 1992).

The phenolic values of cloves ranged from a low of 142.1 ± 16 mg GAE/g to 166.7 ± 5.4 mg GAE/g. The lowest value of cloves is still higher than the highest phenolic value established for cinnamon. This finding concurs with the literature. Cloves are generally found to be one of the highest sources of phenolics. Of the 1113 foods evaluated for antioxidant capacity by Halvorsen and others (2006) (Table 2.2) cloves ranked number one. Of the spices in Table 2.4, cloves also rank the highest with a phenolic value of 296 ± 3.7 (Dearlove and others 2008). Cloves are also one of the most potent and flavorful spices, therefore requiring a lesser amount in cooking and baking than other spices. The primary compound responsible for the characteristic aroma of cloves is eugenol. Eugenol makes up 72-90% of the essential oil extracted from cloves, which may be a factor in their strong aroma and flavor (Bensky and others 2004). Cloves have historically been used in Indian cuisine in almost all rich or spicy dishes as an ingredient in a mix of spices making up the blend garam masala. Ethnic cooking, including Indian, Mediterranean, and Mexican, has recently seen a surge in the United States. Americans are more likely now than ever to experiment with spices and exotic flavors (McCormick and Company Inc. 2010a).

The phenolic values for nutmeg ranged from a low of 15.2 ± 0.9 mg GAE/g to 19.3 ± 0.8 mg GAE/g. These values are much lower than those found for both cloves and cinnamon. This result is not surprising as nutmeg is lesser known for being a “super spice” than either cinnamon or cloves. Nutmeg does have one of the highest amounts of volatile oil of all the spices. It may be assumed then that higher essential oil does not necessarily correlate with higher phenolic levels. More research needs to be done in this area to determine whether a relationship exists between oil level and phenolics. Nutmeg is also commonly used in everyday cooking. The annual world production of nutmeg is approximately 13 million pounds, which is relatively small when compared to cinnamon (McCormick and Company Inc. 2010b). However, the US is the largest market for whole nutmegs (Leela 2008).

Color of Individual Spice Samples

Spice imports are sourced throughout the world. Cinnamon, for example, is grown in at least four different countries. Cloves are grown in at least seven countries and nutmeg in at least six (FAOSTAT 2007). Colors of a range of crops vary due to geographical differences in growing region (Malacalza and others 2007). Therefore, it is not surprising that the appearances of the same spices sourced from different regions fluctuate. The color measurements of the individual spices revealed numerous significant differences in the spices of the same suite. The results are found in Table 4.2. The cinnamon suite contained the most wide range of L*, a*, and b* values. The Vietnamese cassia was by far the darkest sample of cinnamon, with an L* value of 20.0. With an L* value of 37.9, Chinese cassia was the lightest sample in the cinnamon suite. The measurements of nutmeg also showed a range of values. West Indian nutmeg was significantly darker and greener than were both the East Indian and McCormick® samples. Cloves, on the other hand, did not differ significantly in lightness, but revealed significant differences in both the red-green and yellow-blue axes. For example, Ceylon cloves revealed more red and yellow color properties.

Phenolic Values of Ingredients in the Pumpkin-Spice Muffins

The ingredients in the pumpkin muffin were assayed separately to see the influence that each ingredient would have on the total phenolic capacity of the baked muffin. All-purpose flour was the only ingredient besides the spices that contributed to phenolic content. The total phenolic contents of individual ingredients present in the muffins are listed in Table 4.3.

pH Measurement of Pumpkin-Spice Muffin Batters

pH is a measure of the acidity or basicity of a solution. Pure water is said to be neutral, with a pH close to 7.0 at 25 °C. Solutions with a pH less than 7 are said to be acidic and solutions with a pH greater than 7 are said to be basic or alkaline. The pH of the batters, ranged from a low of 6.6 to 6.7, which is slightly acidic. No significant differences were found in the values of the pH of the batters (Table 4.4). Therefore, the various spices sourced from different regions had no significant effect on the pH of the muffins. Researchers investigating the antioxidative activity and phenolic content of various yam cultivars grown under varying pH found that phenolic levels were pH dependent. Total phenolic contents from all yam varieties were the highest at pH 5, but began to gradually decrease as pH increased (Chen and others 2008). pH dependence of phenolics is validated by Friedman and Jurgens (2000). In this study, the effects of varying pH on the stability of specific phenolic compounds found in coffee, tea, and fruits and vegetables were investigated. High pH had a damaging effect on phenolic compounds; however, the specific structure of the phenolic compound also proved to be important in the susceptibility to pH. In this study, the lack of difference in the pH of the muffin batter, suggests that the pH effects were uniform for all formulations.

Color Measurement of Pumpkin-Spice Muffins

According to the statistics, significant differences exist in the exterior and interior color of the muffins. Results can be found in Table 4.5. This is not surprising given the significant color differences in the actual spices. The surface color of the high phenolic muffin was significantly darker than both the low and control phenolic muffins. This could be due to the inclusion of Ceylon cloves in the high phenolic muffin, which had the lowest L* value (closest to black) of all the spices. The high phenolic muffin also had the darkest interior, according to the internal L* value.

Spices have long been used as coloring agents throughout history. Certain spices tint food dishes very specific colors, such as curry and saffron. This is relative to consumer and sensory research because sensory attributes, including texture and flavor, have been known to play a significant role in overall perception and acceptance of a food product (Szczesniak 1972). However, the appearance of a product is the first impression a consumer has of a given food item. Color is a major attribute affecting the initial feeling consumers make in regards to the perception of quality. If the color is deemed unacceptable, it is likely that flavor and texture will not even be evaluated (Francis 1995). “We eat with our eyes” meaning that appearance is a key factor in food choice by influencing taste thresholds, sweetness perception, food preference, pleasantness, and acceptability (Clydesdale 1993). In sensory studies, color is capable of replacing sugar in flavored foods while still maintaining the sweetness perception (Clydesdale 1993). Several studies have shown that dark red solutions will be perceived as sweeter than other solutions of the same sucrose concentration in lighter colors or distilled water (Francis 1995). It has been well established that color and appearance effect human perception of quality and subsequent acceptability (Hetherington and MacDougall 1992).

Phenolic Values of Pumpkin-Spice Muffins

The three muffin formulations were reformulated in order to determine sensory and physical differences in the muffins with varying levels of phenolics contributed by the spices. There were expected differences in spice intensity due to volatile oil differences because of the distinction of source origin (Kustrak 1996). The resulting combination of spices was decided upon in order to meet the culinary criteria for spice intensity and balance (Labensky and Hause

2000). It is noteworthy that the spice levels in the pumpkin-spice muffin formulation were increased over those found in the pumpkin–spice muffin formula that served as a basis for this reformulation. The level of cinnamon was increased close to 4 times the original amount, the level of cloves was doubled and the level of nutmeg was increased by 1.5 times. Criteria for appropriate level of spice in product formulations were used to verify the final levels in the muffins. Consumers did not find the levels used to be objectionable. This successful increase in spices incorporated in the muffins suggests that consumers might also increase levels in other recipes. The high phenolic muffin was formulated with the spice combination that maximized phenolic levels, whereas the low phenolic muffin was formulated with the spices containing the lowest phenolic values. The third muffin served as the control and contained supermarket spices of unspecified origin most typically purchased by consumers.. The purpose of this was to determine whether or not consumers could discern differences between the muffins in the categories on the muffin scorecard using the just-about-right scale and to determine if any differences found were reflected in their preference for a particular formulation. It would be beneficial to product developers to demonstrate that a muffin with high phenolics is acceptable to consumers.

The average weight of the baked mini-muffin samples was 25.2 grams. Due to their smaller size and weight a typical serving size would be 2 muffins. Despite the small amounts incorporated in the muffin formulations, spices contributed 74, 62 and 79% of the expected total phenolics in the control, low phenolic and high phenolic muffins, respectively. Therefore, concentrated sources can have a major impact on total phenolics even when used in small quantities.

The recovered phenolics of the muffins were determined after baking and can be found in Table 4.6. Even with the different combinations of spices, there were only slight differences in the recovered phenolics among the three muffin samples. In fact, the muffin formulated with the lowest levels of phenolics from spices generated the most recovered phenolics. A comparison of the expected and recovered phenolic values can be seen in Table 4.7. It is evident that the differing spice combinations did not affect the phenolics recovered from the muffin samples. The expected phenolic values did not match the recovered, which suggests that there may have been binding of the phenolics within the food system.

Consumer Panel - Food Choices Questionnaire

Demographic information provided by the panelists and a food choice questionnaire allowed the consumer sensory panel as a whole to be profiled. Panelists were asked their age, sex, and ethnicity. The greater part of panelists (79.8%) was in the 18-24 age group and were female (85%). In regards to ethnicity, the majority (79%) of panelists were "white", followed by 10% "Asian" and 8% "black".

Food Choices Questionnaire - Factor Analysis

Panelists response to the relative importance of the items queried in the 38-item food choices questionnaire are found in Table 4.8. There was the most agreement in the relative importance of *tastes good*, *is nutritious*, and *keeps me healthy*; at least 60% of the panelists strongly agreed that these influences greatly impact their food selection. *Tastes good* was by far the most important influence with 86% of panelists agreeing that it was 'very important'. The following influence appeared to be of the least importance to these panelists: c*omes from countries I approve of politically* with 62% indicating it is 'not at all important' in the food choice process.

Factor analysis is a type of multivariate statistical analysis whose primary purpose is data reduction and summarization (Hair and others 1979). It is a way of defining the fundamental elements in a large set of variables and assigning a factor loading to each underlying element. Four underlying factors emerged as most relevant in the food frequency questionnaire. Items and their factor loadings for each of the four factors retained are reported in Table 4.9. These four factors together accounted for 38.8% of the variance in food selection; Eigen values ranged from 13.8 to 3.6. That only about 40% of the variance in food selection was explained suggested that other unknown factors are important in the selection among available foods in the marketplace.

The *nutrition and health* factor appears to be more related to general health and nutrition statements rather than specific ones. For example, foods that are high in protein, fiber, or antioxidants are less related to nutrition and health then foods that are nutritious. *Convenience* seems to be more related to ease of preparation then to price and availability. A particular food may load here because how it is prepared is known and consumers are not likely to prepare a food item that will be disliked. According to the *mood and feelings* factor, it appears that consumers use food as an aid to help cope with life and to relax. Most important in the *natural* category is that a food contains no additives or any artificial ingredients. This seems to be more important than containing natural ingredients. These four factors relate to other studies in regards to what is important to consumers. According to the 2007 IFIC Foundation Food and Health Survey, when it comes to making purchase decisions, consumers cite the taste (88%) as the number one influence on their decision to purchase a food product, followed by price (72%), healthfulness of a product (65%), and convenience (55%) (IFIC 2007).

Muffin Scorecard - Sensory Attributes According to Consumer Panel

Surface and Interior Color

According to the statistics, there were significant differences in the surface color of all three muffins ($p < 0.0001$); however values for all muffins deviated less than one unit from just-about-right. The mean values and statistical results of color and the other sensory attributes can be found in Table 4.10. According to the just-about-right scale with a range of 1-5 where 1 is equal to much too light, 3 is equal to just-about-right, and 5 is equal to much too dark, the high phenolic muffin was seen as the darkest with an average response of 3.4. This value is mid-way between just-about-right (3.0) and somewhat too dark (4.0). The surface color of the low phenolic and control muffins were both seen as just-about-right, with the control muffin just slightly under the 3 mark and the low phenolic muffin just over. The goal of product development is to develop products that will viewed by consumers as just-about-right. In a food choice situation, products with the greatest visual appeal will likely be chosen first (Francis and Clydesdale 1975). Color is a particularly important visual cue. Consumer perception of an acceptable color has been shown to be associated with other quality attributes including flavor, nutrition and overall level of satisfaction (Christensen 1983).

The statistics show that significant differences were also found in the interior color of the muffins ($p < 0.0001$). The interior of the high phenolic muffin is significantly darker than both the low phenolic and control muffins, placing between just-about-right and somewhat too dark. This correlates with the surface color results, as the high phenolic muffin was again seen as the darkest. The low and control muffins were both seen as between somewhat too light (2.0) and just-about-right (3.0).

Amount of Pumpkin Flavor, Spice Intensity, and Sweetness

In product formulation, there are typically three criteria used to determine if the spice levels are appropriate in the final product and whether the product is ready for consumer evaluation. These are: the flavor of the primary ingredients should not be masked, flavors imparted should not overwhelm the palate and flavors should be balanced (Labensky and Hause 2000). According to the statistics, panelists found no significant differences in the appropriateness of the intensity of pumpkin flavor in the three muffins (p = 0.66). All three muffins were viewed very similarly as being half-way between somewhat-too-weak in pumpkin flavor and just-about-right. The spice combination in the muffins, therefore, may have masked the pumpkin flavor, violating the culinary criterion that the flavor of the primary ingredients should not be masked (Labensky and Hause 2000). When re-formulating the muffins then, the spices may need to be decreased.

However, there were no significant differences in the appropriateness of the spice intensity of the three muffins (p = 0.31) with values of 2.8, 2.7, and 2.7 on the just-about-right scale for the control, and low and high phenolic muffins, respectively. Therefore, decreasing the spice levels may not be a remedy for the slightly weak pumpkin flavor as the values for spice intensity were also under the just-about-right mark. These results suggest that phenolic levels do not necessarily have a sensory connection with the flavor balance or perceived potency of the spices. Because the panelists did not discern any significant differences in appropriateness of the spice intensity, it would be ideal to utilize the maximum phenolic spice combination in order to better attain the potential health benefits phenolics provide.

All three muffins were viewed as having equally appropriate levels of sweetness (p = 0.55); values were just-about-right. So, like the pumpkin flavor, it can be inferred that the different spices had no effect on whether the sweetness intensity was perceived as appropriate by the panelists. Spices in food products are believed to exhibit properties related to sugar and sweetness. A study examining the similarities of several spices to sugar found that vanilla had the most similar effects to sugar. In this same study, cinnamon, vanilla, spearmint, and anise were generally found to be more like sugar than nutmeg, ginger, cloves, bay, and salt (Blank and Mattes 1990). Due to their sweet characteristics, adding certain spices in place of sugar to food products is a natural and flavorful way of limiting sugar intake. This is important in the field of product development as consumers become more health conscious and more aware of the negative effects of too much sugar. Excess sugar consumption has been hypothesized to contribute to several chronic diseases including obesity and heart disease (Colombani 2004).

Preference Ranking

Panelists were asked to rank the muffins as 1, 2, and 3 – with 1 as the most preferred and 3 as the least preferred. Mean values of the ranks of the three muffin samples are found in Table 4.11. According to the Kruskal-Wallis non-parametric method used for assessing rank data, there were no significant differences in preference (p=0.93) for the muffins. Therefore, panelists' were not influenced by any differences present in the muffins evaluated. This is an important finding because of the typical bitter taste of phytochemicals. Bitterness often predicts toxicity and can be the principal cause of food rejection (Drewnowski and Gomez-Carneros, 2000). Therefore, the presence of high levels of phytochemicals can alter food consumption. The panelists in this study preferred all muffins equally, so it would be reasonable to produce the muffin that maximizes the phenolic levels in order to make an antioxidant claim and appeal to growing health-conscious society. It would also be reasonable to employ the same screening procedure for other products. However, every formulation will be different due to the specific ingredients and the frame of reference held by the consumer, so this process may not work with all products.

Marketing Questions

In response to the question asking if panelists would be more likely to purchase their preferred muffin if it carried an antioxidant claim, 73% of panelists responded “yes” and 27% of panelists replied “no”. Food producers have recognized that consumers are increasingly aware of the term *antioxidant*. As a result, antioxidant claims are found on a wide variety of food products (Sloan, 2007). Of the 73% that answered “yes”, 57% said they would pay at least 20¢ and up to 40¢ more. Panelists were told that the price of a regular size muffin (about 55 g) was $1.00; The base price was determined after surveying muffin sold in bakeries found in local supermarkets (Athens, GA). Consumers are willing to pay more for added health value of a product. However, when asked if they would be more likely to purchase a muffin other than the preferred muffin if it carried the same antioxidant claim, the majority of panelists (57%) responded “no”. This result is not surprising as several consumer and marketing studies show that taste and convenience, rather than nutrition or perceived health value, are the key influences in food choice (Drewnowski and Darmon 2005).

The correlation analysis between the factors generated from the food choices questionnaire (Table 4.9) and willingness to pay for the presence of an antioxidant claim revealed that the only significant correlation ($p<0.006$) exhibited a very weak association ($r=0.14$) between Factor 1—nutrition and health and cost in the presence of an antioxidant claim.

Table 4.1: Scientific names, place of origin, and total phenolic content[a] of cinnamon (n=4), nutmeg (n=3) and cloves (n=3)

Spice common name	Family and scientific name	Country/place of origin	Total phenolic content (mg of GAE/g) -----LSMeans ± SE[b]------
Cinnamon			
Penzeys Chinese cassia (cinnamon)	Lauraceae *Cinnamomum aromaticum*	China	61.4 ± 1.12
Penzeys Vietnamese cassia (cinnamon)	Lauraceae *Cinnamomum loureiroi*	Vietnam	44.0 ± 1.12
Penzeys Korintji cassia (cinnamon)	Lauraceae *Cinnamomum burmannii*	Indonesia	136.8 ± 4.51
McCormick cinnamon	Lauraceae *Cinnamomum cassia*	Unspecified	95.4 ± 9.19
Nutmeg			
Penzeys East Indian nutmeg	Myristicaceae *Myristica fragrans*	East India	17.7 ± 3.33a
Penzeys West Indian nutmeg	Myristicaceae *Myristica fragrans*	West India	19.3 ± 0.83a
McCormick nutmeg	Myristicaceae *Myristica fragrans*	Unspecified	17.6 ± 3.61a
Cloves			
Penzeys Madagascar cloves	Myrtaceae *Syzygium aromaticum*	Madagascar	142.1 ± 15.96a
Penzeys Ceylon cloves	Myrtaceae *Syzygium aromaticum*	Ceylon	166.7 ± 5.43a
McCormick cloves	Myrtaceae *Syzygium aromaticum*	Unspecified	162.7 ± 3.14a

[a] phenolic content was determined according to the method described by Singleton and Rossi (1965)

[b] LSMeans ± SE followed by the same letter in a column are not significantly different ($p>0.05$) according to proc GLM and pdiff; SAS version 9.1.

Table 4.2: Color measurements of spices (n=3) assessed using the Minolta Spectrophotometer[a]

Spice	L*	a*	b*
		LSMeans ± SE [b]	
Cinnamon			
Chinese cinnamon	37.9 ± 1.04a	9.4 ± 0.19a	34.5 ± 0.68a
Vietnamese cinnamon	20.0 ± 1.04b	6.6 ± 0.19b	20.8 ± 0.68b
Korintji cinnamon	37.0 ± 1.04a	9.8 ± 0.19a	32.9 ± 0.68a
McCormick cinnamon	28.3 ± 1.04c	7.1 ± 0.19b	24.1 ± 0.68c
Nutmeg			
East Indian nutmeg	27.8 ± 1.04a	6.9 ± 0.19a	23.4 ± 0.68a
West Indian nutmeg	31.4 ± 1.04b	6.2 ± 0.19b	23.0 ± 0.68a
McCormick nutmeg	26.1 ± 1.04a	7.6 ± 0.19c	23.1 ± 0.68a
Cloves			
Madagascar cloves	12.3 ± 1.04a	5.6 ± 0.19ac	11.5 ± 0.68a
Ceylon cloves	9.7 ± 1.04a	5.2 ± 0.19ab	8.8 ± 0.68b
McCormick cloves	11.9 ± 1.04a	5.9 ± 0.19ac	12.1 ± 0.68a

[a] Minolta Spectrophotometer (model CM-700d, Minolta Corp., Ramsey, NJ, calibration cap number CM-A177, 10 degree observer function, F6 illuminant)

[*]L= lightness: 0-100, where 0 is black and 100 is white

[*]a= red-green axis. –a=green, +a=red, 0=neutral

[*]b= yellow-blue axis. –b=blue, +b=yellow, 0=neutral

[b] LSMeans ± SE followed by the same letter in a column for a specific spice (cinnamon, nutmeg, cloves) are not significantly different (p>0.05) according to proc GLM and pdiff; SAS version 9.1.

Table 4.3: Total phenolic content of ingredients used to make pumpkin-spice muffin –The ingredients and amounts used to make pumpkin muffins, as well as the total phenolic content of each ingredient expressed as a mean value with standard deviation (n=3).

Ingredient	Amount (g)	Total Phenols[a] mg GAE/g ingredient
Shortening	68.0	0 ± 0
Sugar	266.0	0 ± 0
Egg	100.0	0 ± 0
Pumpkin Puree	245.0	0 ± 0
Water	78.0	0 ± 0
All-Purpose Flour	208.0	0.884
Baking soda	4.6	0 ± 0
Salt	3.0	0 ± 0
Baking Powder	1.15	0 ± 0
Cinnamon	4.0	Variable upon source[b]
Nutmeg	1.0	Variable upon source[c]
Cloves	0.8	Variable upon source[d]

[a] Phenolic content was determined according to the method described by Singleton and Rossi (1965)

[b] Four samples of cinnamon were tested: Penzeys Chinese cassia, Penzeys Vietnamese cassia, Penzeys Korintje cassia, and McCormick Chinese cassia.

[c] Three samples of nutmeg were tested: Penzeys East Indian nutmeg, Penzeys West Indian nutmeg, and McCormick nutmeg.

[d] Three samples of cloves were tested: Penzeys Ceylon cloves, Penzeys Madagascar cloves, and McCormick cloves.

Table 4.4: pH[a] of pumpkin-spice muffin[b] batters

Muffin	pH ----- LSMeans ± SE [c]-----
Control	6.6 ± 0.2
High phenolic	6.7 ± 0.2
Low phenolic	6.6 ± 0.1

[a] each bakes' pH result is a mean of 3 measurements. Temp (°C) is held at 25.0

[b]Control muffin was prepared with McCormick brand spices purchased from local grocery store. High muffin was prepared with the spice combination that yielded the highest phenolic content: Penzeys Korintje cassia, Penzeys Ceylon cloves, and Penzeys West Indian nutmeg. Low muffin was prepared with the spice combination that yielded the lowest phenolic content: Penzeys Vietnamese cassia, Penzeys Madagascar cloves, and McCormick nutmeg.

[c] LSMeans ± SE followed by the same letter in a column are not significantly different (p>0.05) according to proc GLM and pdiff; SAS version 9.1.

Table 4.5: Color measurements of pumpkin-spice muffins[a] (n=3) prepared with different sources of spices (cinnamon, nutmeg, cloves) assessed using the Minolta Spectrophotometer[b]

Muffin	Internal Color			External Color		
	L*	a*	b*	L*	a*	b*
	----------------LSMeans ± SE [c]-------------------					
Control	52.0 ± 0.59a	8.5 ± 0.11	41.8 ± 0.78a	54.6 ± 0.61b	6.8 ± 0.12a	46.0 ± 1.01a
High phenolic	50.2 ± 0.59b	9.2 ± 0.11	39.1 ± 0.78b	50.4 ± 0.61a	8.6 ± 0.12b	41.6 ± 1.01b
Low phenolic	52.0 ± 0.59a	8.7 ± 0.11	42.0 ± 0.78a	55.6 ± 0.61b	7.5 ± 0.12a	48.5 ± 1.01a

[a]Control muffin was prepared with McCormick spices purchased from local grocery store. High muffin was prepared with the spice combination that yielded the highest phenolic content: Penzeys Korintje cassia, Penzeys Ceylon cloves, and Penzeys West Indian nutmeg. Low muffin was prepared with the spice combination that yielded the lowest phenolic content: Penzeys Vietnamese cassia, Penzeys Madagascar cloves, and McCormick nutmeg.

[b] Minolta Spectrophotometer (model CM-700d, Minolta Corp., Ramsey, NJ, calibration cap number CM-A177, 10 degree observer function, F6 illuminant)

[c] LSMeans ± SE followed by the same letter in a column are not significantly different (p>0.05) according to proc GLM and pdiff; SAS version 9.1.

[*]L= lightness: 0-100, where 0 is black and 100 is white

[*]a= red-green axis. –a=green, +a=red, 0=neutral

[*]b=yellow-blue axis. –b=blue, +b=yellow, 0=neutral

Table 4.6: Recovered phenolic values of the baked pumpkin-spice muffin samples

Muffin[a]	**Recovered phenolics (mg GAE/g)**	**Total phenolics**[c] **(mg GAE/g) per muffin (25.24 g)**
	---LSMeans ± SE [b] **---**	
High phenolic	0.97 ± 0.40	24.4 ± 0.95
Low phenolic	1.01 ± 0.16	25.6 ± 0.95
Control	0.96 ± 0.09	24.3 ± 0.95

[a] The control muffin was formulated with McCormick brand cinnamon, cloves, and nutmeg. The low muffin was formulated to minimize the total phenolics from spices; consisting of Penzeys Vietnamese cassia, Penzeys Madagascar cloves, and McCormick nutmeg. The high muffin was formulated to maximize the total phenolic content from spice; consisting of Penzeys Korintje cassia, Penzeys Ceylon cloves, and Penzeys West Indian nutmeg.

[b] LSMeans ± SE followed by the same letter in a column are not significantly different ($p>0.05$) according to proc GLM and pdiff; SAS version 9.1.

[c] Total phenolics were determined according to the method described by Singleton and Rossi (1965).

Table 4.7: Expected vs. recovered phenolic values of pumpkin-spice muffins[a] **(n=3)**

Muffin[b]	Expected [c] phenolics mg GAE/g	Recovered [d] phenolics mg GAE/g
High phenolic	27.6	24.4 ± 0.95
Low phenolic	15.3	25.6 ± 0.95
Control	22.2	24.3 ± 0.95

[a] Control muffin was prepared with McCormick spices purchased from local grocery store. High muffin was prepared with the spice combination that yielded the highest phenolic content: Penzeys Korintje cassia, Penzeys Ceylon cloves, and Penzeys West Indian nutmeg. Low muffin was prepared with the spice combination that yielded the lowest phenolic content: Penzeys Vietnamese cassia, Penzeys Madagascar cloves, and McCormick nutmeg.

[b] each batch yielded 32 mini-muffins. Average weight of muffins was 25.24g.

[c] expected phenolics were calculated by adding the phenolic contribution of each spice and the all-purpose flour in each muffin formula and dividing by 32.

[d] recovered phenolics were determined by running a phenolic assay according to the method described by Singleton and Rossi (1965) on each muffin in order to determine mg GAE/g. This value was then multiplied by the average weight of the muffins, 25.24g.

Table 4.8: LSMeans ± SE and frequency of panelists' (n=134) responses (%) on the food frequency questionnaire

		Relative Importance			
It is important to me that the food I eat on a typical day…	LSMeans ± SE	Frequency of Response (%)			
		Not at all important	A little important	Moderately important	Very important
1. is easy to prepare	3.2 ± 0.80	3.0	14.9	41.0	41.0
2. contains no additives	2.0 ± 0.79	26.9	40.3	32.1	0.8
3. is low in calories	2.8 ± 0.83	9.0	23.1	52.2	15.7
4. tastes good	3.9 ± 0.35	0	0	14.2	85.8
5. contains natural ingredients	2.6 ± 0.79	9.0	32.1	49.3	9.7
6. is not expensive	3.2 ± 0.72	0.8	14.2	45.5	39.6
7. is low in fat	3.0 ± 0.75	3.0	21.6	53.0	22.4
8. is familiar	2.3 ± 0.87	19.4	42.5	29.9	8.2
9. is high in fiber and roughage	2.6 ± 0.79	6.7	38.8	42.5	11.9
10. is nutritious	3.6 ± 0.60	0	5.2	34.3	60.5
11. is easily available in shops and supermarkets	3.3 ± 0.69	1.5	9.0	49.3	40.3
12. is good value for money	3.5 ± 0.68	1.5	6.0	37.3	55.2
13. cheers me up	2.8 ± 0.87	7.5	26.9	44.0	21.6
14. smells nice	3.1 ± 0.78	4.5	14.2	52.2	29.1
15. can be cooked very easily	3.2 ± 0.78	3.7	12.7	48.5	35.1
16. helps me cope with stress	2.1 ± 0.88	26.1	44.8	21.6	7.5
17. helps me control my weight	2.9 ± 0.88	6.0	29.1	38.8	26.1
18. has a pleasant texture	3.0 ± 0.80	3.0	23.9	44.8	28.4
19. is packaged in an environmentally way	2.2 ± 0.77	17.9	47.8	30.6	3.7
20. comes from countries I approve of politically	1.6 ± 0.83	61.9	23.9	10.5	3.7
21. is like the food I ate when I was a child	1.9 ± 0.79	34.3	47.8	14.2	3.7
22. contains a lot of vitamins and minerals	3.0 ± 0.78	3.0	18.7	48.5	29.9
23. contains no artificial ingredients	2.3 ± 0.80	18.7	41.8	35.8	3.7
24. keeps me awake or alert	2.2 ± 0.9	23.1	44.0	23.1	9.7
25. looks nice	2.7 ± 0.78	6.0	35.1	45.5	13.4
26. helps me relax	1.9 ± 0.8	32.1	48.5	14.9	4.5
27. is high in protein	2.5 ± 0.86	11.9	42.5	33.6	11.9
28. takes no time to prepare	2.5 ± 0.88	12.7	39.6	34.3	13.4

29. keeps me healthy	3.6 ± 0.61	0	6.0	32.8	61.2
30. is good for my skin/hair/teeth/nails etc	2.9 ± 0.88	6.7	24.6	42.5	26.1
31. makes me feel good	2.9 ± 0.87	6.0	26.9	41.8	25.4
32. has the country of origin clearly marked	1.7 ± 0.82	52.2	35.1	8.2	4.5
33. is what I usually eat	2.2 ± 0.93	23.9	40.3	25.4	10.5
34. helps me to cope with life	1.8 ± 0.82	44.0	39.6	12.7	3.7
35. can be bought in shops close to where I live or work	3.0 ± 0.86	5.2	19.4	41.8	33.6
36. is cheap	3.0 ± 0.79	1.5	27.6	42.5	28.4
37. is high in antioxidants	2.8 ± 0.86	6.7	29.9	41.8	21.6
38. was grown nearby	2.0 ± 0.78	29.9	45.5	22.4	2.2

Table 4.9: Factor analysis of the food frequency questionnaire

Factor (% variance)	Items loading	Factor loading
1. Nutrition and Health (10.1%)	Is high in fiber and roughage	0.55
	Is nutritious	0.74
	Is packaged in an environmentally friendly way	0.43
	Contains a lot of vitamins and minerals	0.72
	Is high in protein	0.56
	Keeps me healthy	0.82
	Is good for my skin/hair/teeth/nails etc.	0.53
	Is high in antioxidants	0.57
2. Convenience (10.2%)	Is easy to prepare	0.81
	Is easily available in shops and supermarkets	0.49
	Can be cooked very easily	0.88
	Takes no time to prepare	0.78
	Is what I usually eat	0.44
	Can be bought in shops close to where I live or work	0.49
	Is cheap	0.49
3. Mood and feelings (9.6%)	Cheers me up	0.66
	Helps me cope with stress	0.76
	Helps me relax	0.79
	Makes me feel good	0.50
	Helps me to cope with life	0.86
4. Natural (8.9%)	Contains no additives	0.87
	Contains natural ingredients	0.71
	Contains no artificial ingredients	0.81

Table 4.10: Mean values[a] of perceived sensory[b] attributes of three muffin varieties[c] prepared with different sources of spices, according to a consumer panel (n=134)

	Formulations (LS-Means)			
Sensory Attributes	**Control**	**Low phenolic**	**High phenolic**	**Standard Error**
Surface color	2.9a	3.2b	3.4c	0.04
Interior color	2.7b	2.8b	3.2a	0.04
Pumpkin flavor	2.5	2.5	2.5	0.06
Spice intensity	2.8	2.7	2.7	0.08
Sweetness	2.9	2.9	2.8	0.06

[a] LS means followed by different letters within a row differ significantly ($p<0.05$) according to proc GLM and pdiff; SAS version 9.1

[b] consumer panelists filled out a just-about-right sensory form with anchors 1 and 5 where 1 = much too low/light/weak and 5 = much too dark/strong/sweet.

[c] The control muffin was formulated with McCormick brand cinnamon, cloves, and nutmeg. The low muffin was formulated to minimize the total phenolics from spices; consisting of Penzeys Vietnamese cassia, Penzeys Madagascar cloves, and McCormick nutmeg. The high muffin was formulated to maximize the total phenolic content from spice; consisting of Penzeys Korintje cassia, Penzeys Ceylon cloves, and Penzeys West Indian nutmeg.

Table 4.11: Mean values of consumer preference responses (ranked 1, 2, or 3) for pumpkin-spice muffins with differing spice combinations

Muffin	Mean
High phenolic	1.98
Low phenolic	2.01
Control	2.01

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CHAPTER V

CONCLUSIONS

IMPLICATIONS

The present study illustrates the fact that spices are a concentrated source of phenolic compounds. When sourced from specific regions of the world, spices are capable of increasing and thus contributing to the overall phenolic content of food products. The muffin formulas used were specifically designed to either maximize or minimize total phenolic levels. From a food procurement standpoint, total phenolics may be a desirable addition to the specification for the spices. To increase total phenolics of common foods, increased spice use is an easily adopted practice that consumers or food and culinary professionals as well as food product developers could employ.

Research concerning antioxidants and total phenolic content of foods continues to increase as health becomes more important to a growing population of humans who are living longer. Heart disease and cancer remain among America's top health concerns: 58% are *extremely concerned* about heart disease, up 3% over the past two years, and 57% have that level of concern about cancer, up 4% (HealthFocus 2007). As their name implies, antioxidants are instrumental in combating oxidative stress associated with chronic diseases. With the majority (69%) of Americans pursuing a *preventive* lifestyle and 27% taking a *treatment* approach, the growing interest in protective antioxidants is not surprising (Sloan 2008). Quality of life is directly related to physical condition and overall well-being. Therefore, finding simple ways to incorporate health benefits into daily life are increasingly popular and adding spices to flavor foods is a straightforward, uncomplicated way of acquiring these benefits. Because of this, research specific to the use of spices as protective dietary components is growing.

This rise in health consciousness of today's more cosmopolitan society explains the rising consumption of functional foods. A functional food is any healthy food claiming to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients (Agriculture and Agri-Food Canada n.d.). These are typically foods fortified with health-promoting additives, like "vitamin-enriched" products. Examples of functional components added to foods to provide health benefits can be found in Table 5.1.

The functional food industry is experiencing rapid growth and is expected to continue developing. It is estimated by BCC Research that the global market of functional food industry will reach 176.7 billion in 2013, up from 109 billion in 2010 and 75 billion in 2007 (GBA 2007). Young consumers (ages 18-24) are the key target audience for functional foods and beverages. Improving mental performance is one of the most sought-after benefits of functional foods for this age group (Sloan 2008). The current attention given to phytochemicals has made consumers more aware of the potential health benefits. According to data from Health Focus, 75% of shoppers positively link antioxidants with immunity, 65% connect omega-3s and heart health, and 61% associate green tea with disease risk reduction (HealthFocus 2007). It is not surprising then that food companies benefit from the use of the terms “antioxidants” and “polyphenols” in their marketing campaigns. Phytochemicals began to appear in the mass market in 2007 according to Sloan Trends TrendSense™ model (Sloan 2007). Of the phytochemicals, flavonoids and polyphenols are the most familiar. Rather than obtaining vitamins and other bioactive compounds from non-dietary sources, consumers are moving towards the trend of whole food nutrition (Sloan 2008). The IFIC Functional Foods Report indicates that 19% of American consumers put antioxidants in the top 3 when asked what potentially beneficial components in foods and beverages are you looking for. Also, consumers ranked spices and in the top “functional foods” when asked to list foods or food components that come to mind as having health benefits beyond basic nutrition (IFIC 2007).

Spices are a source of many phytochemicals, as well as some core nutrients. Because of their relative pungency, they are typically consumed only in small quantities, resulting in a relatively small dietary contribution. However, if eaten regularly, spices could provide useful amounts of beneficial bioactive compounds, including phenolic antioxidants, (Hedges and Lister 2007). Therefore, embracing a cuisine rich in spice may boost the protective capacity of one’s diet.

LIMITATIONS

The data collected and the information obtained regarding the total phenolic content of individual spices and of the pumpkin spice muffins is reflective of other spice phenolic databases. These data, however, do not demonstrate the activity of phenolic compounds in the human body. Throughout research, spices display protective/preventive effects against harmful substances in both animals and in vitro. In fact, they are amongst the most powerful of all the dietary antioxidants in vitro. However, these types of studies generally use extremely high doses of pure compounds extracted from spices which are not typical of human consumption. In the future, phenolic and antioxidant activity should be measured in humans, by determining the amounts absorbed in the blood and tissues.

This study was also limited in the fact that only total phenolics were measured and used as the indicator of overall antioxidant potential. Though phenolic compounds are typically closely associated with antioxidant activity, antioxidant activity does not necessarily correlate with high amounts of phenolics. For this reason, both phenolic content and antioxidant activity should be discussed when determining overall antioxidant potential (Kähkönen 1999).

FUTURE RESEARCH

Phenolic binding and subsequent availability are unclear. The levels recovered from the muffins suggest that the phenolics may be complexed to a carbohydrate or protein matrix. This may have implications for the type of food systems that should be chosen for phenolic enhancement. Future research should examine the binding of phenolic to potential food components and determine if this impacts bioavailability in humans.

Foods high in antioxidants may be extremely beneficial to the health and well-being of a growing world population. Numerous studies have been performed on the antioxidant power both in animals and in vitro. However, future research should be geared toward learning more about the activity of the antioxidants once they enter the human body. Specific antioxidant mechanisms of absorption are still unknown. It is necessary to fully understand the bioavailability and breakdown of the different polyphenols in order to assess their impending impact on target tissues. Therefore, more randomized, placebo controlled human studies of polyphenols are needed.

The effects of temporal variability on spice crops should also be established. When making conclusions regarding phenolic levels in spices and making health claims, it should first be determined whether or not there are significant differences in the phenolic levels of spices from different growing seasons. Studies have shown definite seasonal variation for the antioxidant content of herbs (Dragland and others 2003).

Further research should also be conducted on determining the recoverable phenolics from food systems. In the analysis of phenolics, the extraction from their food source is the first step

(Naczk and Shahidi 2004). This, however, can be difficult due to the complex chemical nature of phenolic compounds. They may exist as complexes with proteins or carbohydrates, which may result in their insolubility (Naczk and Shahidi 2004). The potential for binding with other plant components also exists, which should be recognized when quantifying phenolic compounds. Research should be done in order to fully understand the binding and determine whether or not it affects the bioavailability.

Considering the established health benefits of phytochemicals, further information should be collected as to how American consumers can increase their consumption of these phenolic compounds. However, their acceptability for use in food products should be determined, as higher phenolics may be associated with objectionable flavor notes—especially bitterness, which may actually reduce their level of use and their potential to contribute to phenolic intake. Polyphenolic supplements could be a strategy for attaining the health benefits, but leaving out the bitterness and calories from food sources. However, research should be conducted to determine whether supplements provide the same benefits as whole foods.

Table 5.1: Examples of functional food components and their potential health benefits

Functional component	Source	Potential benefits
	Carotenoids	
Alpha carotene/Beta carotene	Carrots, fruits, vegetables	Neutralize free radicals, which may cause damage to cells
Lutein	Green vegetables	Reduce the risk of macular degeneration
Lycopene	Tomato products	Reduce the risk of prostate cancer
	Dietary Fiber	
Insoluble fiber	Wheat bran	Reduce the risk of breast or colon cancer
Soluble fiber	Psyllium	Reduce risk of cardiovascular disease. Protect against heart disease and some cancers; lower LDL and total cholesterol
	Fatty acids	
Long chain omega-3 Fatty Acids-DHA/EPA	Salmon and other fish oils	Reduce risk of cardiovascular disease. Improve mental, visual functions
	Phenolics	
Anthocyanidins	Fruits	Neutralize free radicals; reduce risk of cancer
Catechins	Tea	Neutralize free radicals; reduce risk of cancer
Flavonones	Citrus	Neutralize free radicals; reduce risk of cancer
Flavones	Fruits/vegetables	Neutralize free radicals; reduce risk of cancer
Lignans	Flax/rye/vegetables	Prevention of cancer, renal failure
Tannins	Cranberries, cocoa, chocolate	Improve urinary tract health. Reduce risk of cardiovascular disease
	Plant sterols	
Stanol ester	Corn, soy, wheat, wood oils	Lower blood cholesterol levels by inhibiting cholesterol absorption
	Probiotics/Prebiotics	
Lactobacillus	Yogurt, other dairy	Improve quality of intestinal microflora; gastrointestinal health

	Soy Phytoestrogens	
Isoflavones: Daidzein, Genistein	Soybeans and soy based foods	Menopause symptoms, such as hot flashes Protect against heart disease and some cancers; lower LDL and total cholesterol

[a] Table adapted from Agriculture and Agri-Food Canada (www.agr.gc.ca) and International Food Information Council

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Panelist________

APPENDIX A
FOOD CHOICES QUESTIONNAIRE

It is important to our study that you complete both sides of the Food Choices Questionnaire and evaluate all 3 muffins.

Food Choices Questionnaire

Your responses to these questions will allow us to profile the panel as a whole. All information will remain confidential. Please check the best response for each question.

1. **Your Age:** ___ 18-24 ___25-34 ___35-44 ___45-54 ___55-64 ___65+

2. **Your Gender:** ___ Male ___ Female

3. **Ethnic Background:** Do you consider your ethnic/racial background to be…
___ White ___ Black ___ Other: __________
___ Hispanic ___ Asian (please specify)

4. Next, we would like to know how important each of the following factors is when you make choices among available foods. Please indicate the relative importance of each factor in the following list by checking the **most** appropriate box to the right of **each** statement.

It is important to me that the food I eat on a typical day…	**Relative Importance**			
	Not at all important	A little important	Moderately important	Very important
1. is easy to prepare				
2. contains no additives				
3. is low in calories				
4. tastes good				
5. contains natural ingredients				
6. is not expensive				
7. is low in fat				
8. is familiar				
9. is high in fiber and roughage				
10. is nutritious				
11. is easily available in shops and supermarkets				
12. is good value for money				
13. cheers me up				

It is important to me that the food I eat on a typical day…	**Relative Importance**			
	Not at all important	A little important	Moderately important	Very important
14. smells nice				
15. can be cooked very easily				
16. helps me cope with stress				
17. helps me control my weight				
18. has a pleasant texture				
19. is packaged in an environmentally friendly way				
20. comes from countries I approve of politically				
21. is like the food I ate as a child				
22. contains a lot of vitamins and minerals				
23. contains no artificial ingredients				
24. keeps me awake or alert				
25. looks nice				
26. helps me relax				
27. is high in protein				
28. takes no time to prepare				
29. keeps me healthy				
30. is good for my skin/hair/teeth/nails/etc.				
31. makes me feel good				
32. has the country of origin clearly marked				
33. is what I usually eat				
34. helps me to cope with life				
35. can be bought in shops close to where I work or live				
36. is cheap				
37. is high in antioxidants				
38. was grown nearby				

Please return the completed Food Choices Questionnaire through the hatch. You will then receive 3 Pumpkin-spice Muffins to evaluate.

Panelist ______

APPENDIX B

Muffin Scorecard

Please mark the box (□) on the scale below that best indicates how you feel about the following characteristics of each pumpkin spice muffin. Please evaluate the muffins in the order the codes are presented on the scorecard. You will receive 3 muffin samples to evaluate.

Please drink some water and eat a bite of cracker before evaluating the first muffin sample.

Muffin code <u>432</u>

Surface color	☐ Much too light	☐ Somewhat too light	☐ Just-about-right	☐ Somewhat too dark	☐ Much too dark
Interior color	☐ Much too light	☐ Somewhat too light	☐ Just-about-right	☐ Somewhat too dark	☐ Much too dark
Amount of pumpkin flavor	☐ Much too weak	☐ Somewhat too weak	☐ Just-about-right	☐ Somewhat too strong	☐ Much too strong
Spice Intensity	☐ Much too weak	☐ Somewhat too weak	☐ Just-about-right	☐ Somewhat too strong	☐ Much too strong
Sweetness	☐ Much too low	☐ Somewhat too low	☐ Just-about-right	☐ Somewhat too sweet	☐ Much too sweet

---

Please drink some water and eat a bite of cracker before evaluating the next muffin sample.

Muffin code <u>786</u>

Surface color	☐ Much too light	☐ Somewhat too light	☐ Just-about-right	☐ Somewhat too dark	☐ Much too dark
Interior color	☐ Much too light	☐ Somewhat too light	☐ Just-about-right	☐ Somewhat too dark	☐ Much too dark
Amount of pumpkin flavor	☐ Much too weak	☐ Somewhat too weak	☐ Just-about-right	☐ Somewhat too strong	☐ Much too strong
Spice Intensity	☐ Much too weak	☐ Somewhat too weak	☐ Just-about-right	☐ Somewhat too strong	☐ Much too strong
Sweetness	☐ Much too low	☐ Somewhat too low	☐ Just-about-right	☐ Somewhat too sweet	☐ Much too sweet

Turn over to evaluate your next sample.

Panelist _______

Please drink some water and eat a bite of cracker before evaluating the next muffin sample.

Muffin code <u>698</u>

Surface color	☐ Much too light	☐ Somewhat too light	☐ Just-about-right	☐ Somewhat too dark	☐ Much too dark
Interior color	☐ Much too light	☐ Somewhat too light	☐ Just-about-right	☐ Somewhat too dark	☐ Much too dark
Amount of pumpkin flavor	☐ Much too weak	☐ Somewhat too weak	☐ Just-about-right	☐ Somewhat too strong	☐ Much too strong
Spice Intensity	☐ Much too weak	☐ Somewhat too weak	☐ Just-about-right	☐ Somewhat too strong	☐ Much too strong
Sweetness	☐ Much too low	☐ Somewhat too low	☐ Just-about-right	☐ Somewhat too sweet	☐ Much too sweet

Preference: Now that you have sampled all 3 muffins, please number them from most (1) to least (3) preferred.

Muffin code	Preference order: **1 = most preferred** and **3 = least preferred**
XXX (3-digit identifying code)	
YYY (3-digit identifying code)	
ZZZ (3-digit identifying code)	

- **If the cost of your preferred muffin was $1.00:** Would you be more likely to purchase your preferred muffin (#1 above) if it carried the **antioxidant claim** in the box?

> Provides antioxidants called polyphenols. Polyphenols fight free radicals and help prevent cell damage. Foods with polyphenols can help support a healthy heart, mind and immune system.

☐ YES ☐ NO

- If yes, how much more would you be willing to pay if the **antioxidant claim** was present?

_____ 0¢ _____ 0.10¢ _____ 0.20¢ _____0.30¢ _____0.40¢

- If a muffin other than your preferred muffin carried the **antioxidant claim** in the box above, would you be more likely to purchase it rather than your preferred muffin?

☐ YES ☐ NO

Thank you for making our study a success!!!

APPENDIX C

Consent Form

I, __________________________, agree to participate in a research study titled Sensory Evaluation of Pumpkin-Spice Muffins conducted by Drs. Ruthann Swanson (706-542-4834) and James Hargrove (706-542-4678), and graduate student Elizabeth Metherell (706-542-4834), Department of Foods and Nutrition, University of Georgia. I am at least 18 years of age or older. I understand my participation is voluntary. I can refuse to participate or stop taking part at any time without giving any reason and without penalty or loss of benefits to which I am otherwise entitled. I can ask to have all of the information about me returned to me, removed from the research records, or destroyed immediately after my participation as a sensory panelist.

The purpose of this study is to investigate the effects of product formulation on the acceptability of a pumpkin-spice muffin. All ingredients are currently available commercially in the United States and are included in these products at levels at or below those found to be safe by FDA. Further, all products are produced in facilities in which ServSafe procedures are followed. If I volunteer to take part in this study, I will be asked to do the following things:

- Read and sign the consent form (1-2 minutes)
- Complete a demographic and food choices questionnaire (5-8 minutes)
- Evaluate muffins according to directions on the sensory scorecard (8-12 minutes)

Following my participation, I will be offered commercial snacks and beverages upon leaving the study testing site. Students who have selected participation on this sensory panel as an extra credit option will receive class credit. In classes where extra credit is offered, other options are available. No additional compensation will be offered.

Food allergies that I have include
____
____

This study is anonymous. I will be assigned an identifying number and this number will be used on all questionnaires and evaluation forms that I fill-out. However, there is no way to connect responses with a specific individual once the test is completed. No individually identifiable information about me, or provided by me during the research will be shared with others, except as necessary by law. An expected benefit is the production of healthier products that are acceptable to consumers; their availability will empower consumers to improve their dietary choices. My participation in this hands-on experience may enhance discussions in the classroom, facilitating a better understanding of the process and the limitations associated with development/success of products formulated to meet specific dietary needs.

There are no expected risks or discomforts associated with participation for any person who does not have allergies to ingredients in the products. However, in the event that my participation in this study results in a medical problem, treatment will be made available. However, my insurance company or I will be billed for the costs of any such treatment. No provision has been made for payment of these costs or to provide me with other financial compensation. As a participant, I do not give up or waive any of my legal rights.

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

I understand the procedures described above and my additional questions have been answered to my satisfaction. I agree to participate in this research study, and I have received a copy of this consent form for my records.

Ruthann Swanson	____________________________	______
Name of Researcher	Signature	Date
James Hargrove	____________________________	______
Name of Researcher	Signature	Date
Elizabeth Metherell	____________________________	______
Name of Researcher	Signature	Date
________________	____________________________	______
Name of Participant	Signature	Date

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

Additional questions or problems regarding your rights as a research participant should be addressed to Chairperson, institutional Review Board, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706-542-3100; E-mail address IRB@uga.edu.