The Stability of Alpha-Tocopherol in Whole-Wheat Flour and Corn Meal During Heating

by

Kelvin Widjaja

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Martin G. Ondrus
Martin G. Ondrus

Committee Members:

Janice Coker
Janice Coker

Carol Seaborn
Carol Seaborn

The Graduate School

University of Wisconsin-Stout

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The Graduate School
University of Wisconsin-Stout
Menomonie, WI

Author: **Widjaja, Kelvin**

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ABSTRACT

Vitamin E (alpha-tocopherol) is a fat-soluble vitamin and a natural antioxidant that has many health benefits according to studies that have been conducted within the past years. In order to meet the recommended consumption level of vitamin E for consumers, it is necessary to know the stability level of the vitamin E in foods. Loss of the vitamin E in cereal grains is frequently caused by heat treatment in food processing. This study, focused on the vitamin E stability related to heating, is necessary in order to determine whether the vitamin E concentration of common types of flour, wheat and corn, decreases significantly after being exposed to the heat processing.

This study focused on the impact of heat (95° C) within three different periods (3, 6, and 9 hours) on the two types of flour (whole-wheat and corn). The vitamin E (alpha-

tocopherol) content of both flour samples was analyzed using High Performance of Liquid Chromatography (HPLC) with fluorescence detection. The data was analyzed statistically on SPSS V. 14 software. The data indicated that the loss rate of alpha-tocopherol in 100 g whole-wheat flour (1.53 mg/hr) and whole-corn meal (0.288 mg/hr) were significantly different ($p \leq .05$). In addition, the results indicated that the effect of type of flour and heating time periods on the alpha-tocopherol loss were significant ($p \leq .05$). The effect of interactions between type of flour and heating time periods on the tocopherol loss was also significant ($p \leq .05$). The difference between alpha-tocopherol loss rate of whole-wheat flour and corn meal may be attributed to fatty acid composition, genetic property, and mineral content of both types of flour.

The Graduate School

University of Wisconsin Stout

Menomonie, WI

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Chapter I: Introduction

Introduction

In big cities, people are so busy with their work that they search for ways to save time. To avoid wasting time, people often skip breakfast and buy fast food (burgers and fries) for meals. In 2005, hamburgers, french fries, and pizza were the most popular and frequently consumed food by adults for take-out (Sloan, 2006). In this modern life, advanced technology has been developed to produce many types of snacks or "junk foods", which have become increasingly popular among teenagers. In 2005, teenagers were the most frequent afternoon customers that purchased grab-and-go and sit down-and-share snacks, such as chips (that contain high amounts of fat/trans fat) (Sloan, 2006). These types of fast food and snacks have a high level of oxidized fat (the result of exposing fat to extremely high temperatures during food processing). The oxidized fat causes loss of functionality and nutritional quality of oil, fat, and vitamin E (tocopherol). Products of lipid oxidation in vivo, cause serious health problems, such as coronary heart disease, atherosclerosis, and cancer (Eitenmiller & Lee, 2004). Because of those problems, many researchers have increasingly conducted studies focused on the role of vitamin E (tocopherol) in the prevention of the chronic diseases and clinical applications of vitamin E therapy (Fuchs & Packer, 1993).

Research on the usefulness of vitamin E in human health benefits has revealed its potential in helping people to prevent and recover from certain illness or diseases, for example:

- Tocopherol has been used widely as a drug in the treatment of various skin diseases (Fuchs & Packer, 1993).

- Vitamin E daily intake has been proven to be useful in treatment of Parkinson disease (Fuchs & Packer, 1993).
- Vitamin E may be used as a protection against various types of cancer (Fuchs & Packer, 1993).
- Tocopherol has an essential role in increasing the normal function of immune system in the human body (Fuchs & Packer, 1993).
- The use of Vitamin E as an antioxidant has become a very important factor in reducing the risk of coronary heart disease and atherosclerosis (Fuchs & Packer, 1993).

Whole grains have been included in human diets for centuries. In order to extend the health benefits of whole grain consumption, the variety of grains consumed have grown from common grains (wheat, corn, oats, rye, and barley) to many other grains such as amaranth, brown rice, bulgur, sorghum, and millet. Because of the common health benefits (reducing the risk of diabetes, coronary heart disease, and assisting weight management) offered by whole grain products, the 2005 Dietary Guidelines for Americans, American Heart Association, and Healthy People 2010 recommend the consumption of at least three servings of whole grains (3 oz) per day. In order to increase the average whole grain consumption (typical daily intake = one serving per day), over 650 whole grain products have been created, in 2005 alone, by large food companies (General Mills, Kellogg's, Sara Lee, and Campbell Soup Co.). The popularity of whole grain has encouraged scientists to conduct further clinical research studies on biologically active components of various whole grain products. These research studies can be very helpful in revealing the additional health benefits of food product that are not grain-

based, which include the active components of whole grain into the food products (Marquart & Cohen, 2005).

There are various kinds of flour based products available in food markets nowadays. These products are made of different types of cereal grains such as corn, wheat, rye, oats, barley, and rice (Godon & Willm, 1994). They each have different nutritional compositions that are important for making a variety of food products and to meet consumer needs. The nutritional quality of these flour products depends on the cereals they are made of because of the distinguishing chemical characteristics of the grains; total alpha-tocopherol in whole-wheat is 7-10 mg/100 g, rye is 2.2-5.7 mg/100 g, oats is 1.8-4.9 mg/100 g, rice is 0.4 mg/100 g, and corn is 9.5 mg/100 g (deMan, 1999). Based on the nutritional information for those various grains, whole-wheat and whole-corn have significantly higher alpha-tocopherol content when compared to the others. Therefore, it is very reasonable to conduct research on the vitamin E stability of whole-wheat flour and whole-corn meal.

People around the world have consumed bread as a common bakery product since ancient times; the baking technology of the bread has been refined and improved over the years. In addition, many scientists struggle to discover update methods of bread baking by utilizing the advanced technology (Matz, 1991). As a result, various kinds of bread (northern & southern corn bread, cracked rye bread, canned white bread, cracked wheat bread, whole-wheat bread, and boston brown bread) have become available. During baking, the temperature inside the bread rarely exceeds 95° C. Because the nutritional components are inside the bread, it is very important to conduct a study on the stability of an important heat liable nutrient (alpha-tocopherol) in a high temperature (95° C)

environment (Tressler & Sultan, 1975). In addition, bread is a very crucial part of the diets of almost everyone in the U.S. and other people in the world. Among all the bakery products, bread is inexpensive so that even people with limited incomes can still purchase this starchy food (Matz, 1991). Also, it is simple enough for everyone to make this food by buying some standard ingredients that are easy to find in the market and by following the recipes that can be found on the internet and elsewhere

Hypothesis

The hypothesis of this study is that there is a significant difference in the loss rate of alpha-tocopherol content between the whole-wheat flour and the corn meal after specified periods of heating time in an oven at 95° C. Therefore, there is also a significant difference in the stability level of alpha-tocopherol in both flour samples. The hypothesis was tested by using statistical analysis (T-test and 2-factor ANOVA). The SPSS V.14 software was used for the analysis.

Statement of the Problem

This study was conducted in order to explore the effect of thermal processing on the alpha-tocopherol concentration in whole-wheat flour and corn meal. There were several factors involved in the conventional oven heating process: nutritional (chemical) composition of the whole-wheat flour and corn meal, the amount of time that they were heated, and the temperature at which they were heated.

The stability level data of alpha-tocopherol in both flour samples, obtained from a milling company (Archer Daniels Midland / ADM), was collected by using methods of food analysis (Vitamin E extraction, determination of Vitamin E concentration, and High

Performance Liquid Chromatography). These methods were performed on both flour samples.

The results of this research (the loss rates of alpha-tocopherol content of whole-wheat flour and corn meal) were statistically analyzed by using SPSS V. 14 software provided by University of Wisconsin-Stout.

Purpose of the Study

This study was conducted in order to investigate the stability of alpha-tocopherol, the most effective antioxidant in vitamin E, in whole-wheat flour and corn meal in high temperature environment (95° C) within three different heating time periods.

The objectives of the research were:

1. To obtain the tocopherol concentration in whole-wheat flour and corn meal after heating (at 95° C) for 3, 6, and 9 hours (including the control).
2. To determine the tocopherol loss rate (mg/hr) of whole-wheat flour and corn meal.

Use of Findings

The findings of different alpha-tocopherol (vitamin E) stability between whole-wheat flour and corn meal could be very crucial in making special flour from the breeding technique (genetic engineering) of grains. In addition, the consumption of bread (made of the special flour) could significantly reduce the risk of cancer or heart disease. Also, fortifying flour by synthetic vitamin E would no longer be a consideration and the production cost of bakery products could be maintained. In addition, the synthetic vitamin E forms (commonly used in fortification) have lower biological activity levels when compared to the natural ones (Eitenmiller & Landen, 1999). Therefore, the

consumer demand for greater health benefits may be achieved by the consumption of natural vitamin E preserved in the flour during baking.

Definition of Terms

There are four terms that need to be clarified for better understanding. These terms are:

High Performance Liquid Chromatography (HPLC) – a method of food analysis that is used in a laboratory to separate and identify the chemical components present in a food sample and to determine the concentration of each known chemical substance in the sample. This approach was performed to determine the concentration of alpha-tocopherol in whole-wheat flour and corn meal.

Starchy-Foods – foods that are made of cereal grains. Examples include wheat bread, rye bread, oat bread, and corn bread.

Tocopherol – a major chemical substance of vitamin E. There are four types of tocopherol: alpha-tocopherol, beta-tocopherol, gamma-tocopherol, and delta-tocopherol. Alpha-tocopherol is the chemical form that is most biologically active in the human body.

Assumptions and Limitations

In this study, it is assumed that whole-wheat flour and corn meal produced by the ADM Company are similar to the ones from other milling companies. In addition, it is assumed that the oven used in this research is similar to the ovens used by bakeries and research and development departments of baking companies. There are two limitations to this study. First, many varieties of wheat and corn have been produced through hybridization. The type of wheat and corn flour samples (used in this study) could not be identified. The lack of information related to the samples may cause significant

difference between alpha-tocopherol content of the control (whole-wheat flour and corn meal) reported in other studies and in text books. Secondly, both flour samples may contain contaminants that may affect the stability level of alpha-tocopherol in the samples.

Chapter II: Literature Review

Vitamin E

Due to advances in our understanding of the relationship between oxidative prevention in the human body and control of major chronic diseases like coronary heart disease and cancer, vitamin E (a natural antioxidant) has been widely used to improve the nutritional quality of food products. Unlike other antioxidant components such as vitamin C, carotenoids, selenium and flavonoids, the popularity of vitamin E has recently increased tremendously because of product marketing, its availability for consumers, the variety of health benefits it offers and its antioxidant property at the cellular level (Eitenmiller & Lee, 2004).

In 1936, Evans and other scientists were able to isolate the active compound of vitamin E successfully from wheat germ oil for the first time in research history. They named the compound alpha-tocopherol from the Greek words *tocos* (birth) and *ferein* (bringing), because it was important for rats to bear young. There were three other tocopherol compounds: beta-tocopherol, gamma-tocopherol (isolated from vegetable oil in 1937), and delta-tocopherol (isolated from soybean oil in 1947). Among those four tocopherol compounds of vitamin E, alpha-tocopherol was recognized as the most essential and effective tocopherol in prevention of vitamin E deficiency. Around that time, four other compounds of vitamin E were discovered; they were named alpha, beta, gamma, and delta tocotrienols. It was recognized that tocopherols and tocotrienols naturally existed in foods as vitamin E (Eitenmiller & Lee, 2004).

Chemical Structure Properties

Vitamin E is a fat-soluble, 6-hydroxychroman compound that shows the biological activity of alpha-tocopherol (see Figure 1) indicated by the rat resorption-gestation assay (Eitenmiller & Lee, 2004).

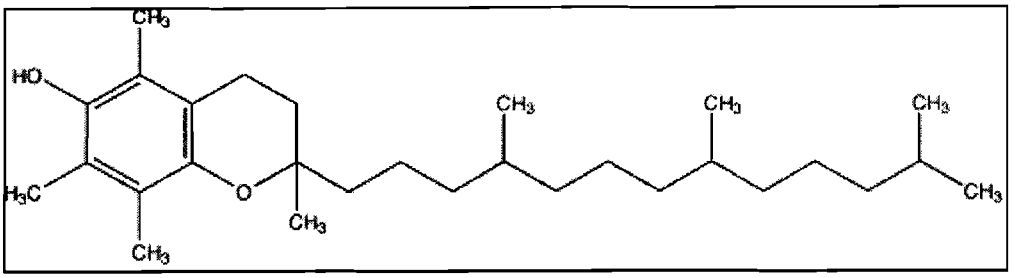

Figure 1. Chemical structure of the alpha-tocopherol

Source: deMan, 1999

The chemical structure of tocopherols and tocotrienols are different; tocopherols do not have double bonds at carbons of the isoprenoid side chain (tail-like structure), but tocotrienols have three double bonds at the carbons of the isoprenoid side chain (see Figure 2). Also, the number and position of the methyl groups on the chroman ring of tocopherols and tocotrienols are different. The differences between the chemical structures of tocopherols and tocotrienols have impacted biological activity levels of the compounds (deMan, 1999). From the stereochemistry perspective, tocopherols have a higher biological activity level than tocotrienols because tocopherols have three asymmetric carbons (chiral centers) at position 2 of the chroman ring and at position 4 and 8 of the isoprenoid side chain (see Figure 2) (Eitenmiller & Lee, 2004).

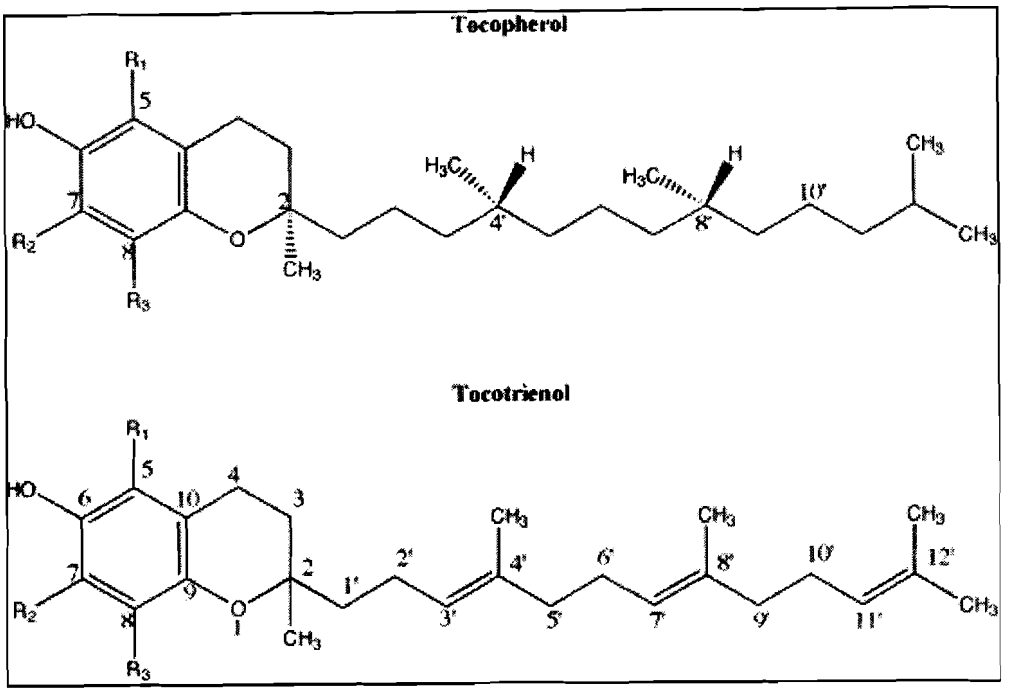

Figure 2. Chemical structure of tocopherols and tocotrienols

Source: deMan, 1999

The chemical structure of tocopherols and tocotrienols give some important characteristics to vitamin E; it is a viscous oil that is fat-soluble, soluble in organic fat solvents, and is colorless to pale yellow (Eitenmiller & Landen, 1999).

Biological Activity

Biological activity of tocopherols and tocotrienols is mostly related to their antioxidant activity (deMan, 1999). Vitamin E compounds have unique biological activity levels that can be measured by using the terms of RRR-alpha-tocopherol (found in nature) equivalents (alpha-TE). One alpha-TE is equal to the activity of 1 mg of RRR-alpha-tocopherol. For foods that only contain natural forms of vitamin E, the biological activity levels can be determined by using the appropriate factors for conversion of tocopherols and tocotrienols to alpha-TE units (see Table 1) (Eitenmiller & Landen, 1999).

Table 1

Conversion Factor of Tocopherols and Tocotrienols to Alpha-TE Units

Vitamin E Forms	Conversion Factor to Alpha-TE Unit
Alpha-tocopherol	mg x 1.0
Beta-tocopherol	mg x 0.5
Gamma-tocopherol	mg x 0.1
Delta-tocopherol	mg x 0.03
Alpha-tocotrienol	mg x 0.3
Beta-tocotrienol	mg x 0.05
Gamma and delta-tocotrienol	unknown

Note: Alpha-TE = RRR-alpha-tocopherol (found in nature) equivalents

Source: Eitenmiller & Landen, 1999

Among all vitamin E compounds, four tocopherols and four tocotrienols, alpha-tocopherol has the greatest biological activity level (antioxidant activity). Therefore, it is very important to measure the vitamin E content of the food products by calculating the amount of alpha-tocopherol in the foods (deMan, 1999). Most biological activity levels of vitamin E compounds are measured by the rat fetal resorption test. These levels are identified using International Units (IU). Synthetic vitamin E form, all-rac-alpha-tocopheryl acetate, is widely used in food fortification. When compared to natural vitamin E, such as alpha-tocopherol, the synthetic form has lower level (see Table 2). Esterification at the C-6 hydroxyl group of chroman ring (vitamin E compounds) plays an important role in stabilizing the vitamin by sacrificing the ability of the vitamin to give a hydrogen atom to free radicals. This ability is required for vitamin E compounds to act as a primary antioxidant. The esterification process, used in making the synthetic vitamin E,

eliminates or greatly reduces the ability of the vitamin to act as an antioxidant (Eitenmiller & Landen, 1999).

Table 2

Comparison of Biological Activity Level (IU) of Natural and Synthetic Vitamin E

Vitamin E Forms	Biological Activity Level (IU/mg)
Alpha-tocopherol (natural)	1.49
All-rac-alpha-tocopherol (synthetic)	1.10
RRR-alpha-tocopheryl acetate (synthetic)	1.36
RRR-alpha-tocopheryl acid succinate (synthetic)	1.21

Source: Eitenmiller & Landen, 1999

Alpha-Tocopherol Content of Foods

Alpha-tocopherol content of food products is usually expressed in milligrams per 100 grams of food. This unit is commonly used in comparisons with the Recommended Dietary Allowances published by the Food and Nutrition Board of the National Academy of Sciences (Fuchs & Packer, 1993). Most alpha-tocopherols can be found in cereal grains, such as wheat and corn. The alpha-tocopherol compounds are not uniformly distributed inside the kernel, and the flour of various degrees of extraction can contain different alpha-tocopherol levels (deMan, 1999). Most of the alpha-tocopherol is deposited and soluble in the germ oil of cereal grains; wheat germ oil contains 2.6 mg/g grain, and corn germ oil contains 0.8-0.9 mg/g grain (Kent & Evers, 1994).

The possibility of increasing the alpha-tocopherol content in plant foods has been increased dramatically since the success in creating the cloned genes of the responsible enzymes for the tocopherol production. In addition, the advances in understanding of the

biosynthetic steps have improved the method of increasing the alpha-tocopherol levels in plant foods. Therefore, the health benefits of consuming the foods as a source of vitamin E can be increased. There are two groups of alpha-tocopherol biosynthetic enzymes; enzymes that affect the quantitative aspects of creating the homogentisic acid (the origin of alpha-tocopherol), and enzymes that affect the qualitative aspects of the production process (cyclization and methylation enzymes). Although the research for increasing the quantity of alpha-tocopherol in the plant foods has been done successfully, more study is still needed in order to improve the quality and stability of the tocopherol in the plant foods. To solve the quality problem of vitamin E, research involving nutritional biochemistry, food science, plant science and genetics is required. Biochemical and genetic studies are very important in order to help identify genes of plants that affect the metabolism pathways responsible to the stability of vitamin E in plant foods (Eitenmiller & Lee, 2004).

Extraction Method

Many different extraction procedures have been used to extract and quantify fat-soluble vitamins, including vitamin E from food. Since vitamin E is soluble in oil, it can be immediately injected into the HPLC column after the dilution with n-hexane (mobile phase). In order to obtain clear results from the HPLC, the vitamin E must be concentrated and extracted from the food matrix. To obtain the isolated pure vitamin E, saponification of food samples or isolated lipid fraction is required (Eitenmiller & Landen, 1999).

In saponification (alkaline hydrolysis), KOH or NaOH is generally used to initiate the hydrolysis that cuts the ester linkages of glycerides, phospholipids, and plant sterols

to modify the food matrix allowing for vitamin E extraction. The combination of 60% w/v aqueous KOH (5 mL) and ethanol (15 mL) per 1 g of fat can be used for complete digestion of food containing various and complicated nutrition content. Vitamin E is easily destroyed under alkaline conditions during saponification. Therefore, antioxidants, such as pyrogallol or ascorbic acid, should be added to food samples before the digestion process. Sample size, volumes of alkali and ethanol, and time and temperature are important parameters that can be changed to optimize the saponification. After the digestion is completed, it is necessary to dilute the digest with water in order to avoid emulsion formation. Vitamin E is extracted with a solvent mixture containing ether, petroleum ether, and hexane. Vitamin E (unsaponifiable component) is transferred into the solvent mixture during the extraction. Meanwhile, glycerols, fatty acid soaps and other interfering substances stay in the alkaline solution phase. It is difficult to obtain an efficient transfer of vitamin E from the aqueous phase into the organic solvent phase because there are several significant factors that affect the transfer. These factors include concentration of ethanol in digest, composition of solvent used for extracting, and the level of lipids used in the digest. However, the amount of ethanol does not have any effect on the extraction of alpha-tocopherol. During the digestion, fatty acid salts are produced from the lipid. The salts can increase the solubility of vitamin E in aqueous phase and decrease extraction into the organic solvent phase. However, the efficiency of alpha-tocopherol extraction is not affected by the fatty acid salts or lipid levels. The best extracting solvent for vitamin E must be able to penetrate the hard surface of food and break the lipid or protein bonds in the food matrix while minimizing oxidative destruction of the vitamin E. The best solvent for vitamin E extraction includes

chloroform:methanol (2:1), acetone, diethyl ether, and petroleum ether (Eitenmiller & Landen, 1999).

Lipid Oxidation

In plant products (cereal grains), the oils contain unsaturated bonds that can react with oxygen and cause lipid oxidation. The oxidation causes deterioration in flavor (rancidity) of fatty foods, and makes the foods unsuitable for consumption. Several factors that determine the rate of oxidation include temperature, light exposure, packaging material of the products, the amount of antioxidants and prooxidants (copper, heme-containing molecules and lipoxidase), the presence of oxygen, and degree of unsaturation of the lipids. When the lipid oxidation occurs, hydrogen is separated from an olefinic compound (lipids that have double bonds) to produce a free radical (deMan, 1999).

$$RH \rightarrow R\cdot + H\cdot$$

Then, the free radical will combine with oxygen to create a peroxy-free radical, which separates hydrogen from another compound that has degree of unsaturation to make a hydroperoxide (primary oxidation product) and a new free radical; this oxidative reaction part, called propagation, may be repeated up to several thousand times causing a chain reaction (deMan, 1999).

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

$$RO_2\cdot + RH \rightarrow ROOH + R\cdot$$

The reaction (above) can lead to termination process in which free radicals react with themselves to produce inert products (deMan, 1999).

$$R\cdot + R\cdot \rightarrow R{-}R$$

$$R\cdot + RO_2\cdot \rightarrow RO_2R$$

$$nRO_2\cdot \rightarrow (RO_2)_n$$

In the presence of oxygen at high temperatures (90 to 140° C) oleic, linoleic, and linolenic acids are easily oxidized to produce hydroperoxides. Further heating will result in breakdown of hydroperoxides, and formation of aldehydes and formic acids. Hydroperoxides of linolenate break more easily than those of oleate and linoleate because of the presence of active methylene groups (located between a single double bond and a conjugated diene group). Lipids, having more active methylene groups, are more easily oxidized because hydrogen at the active methylene groups can readily be separated to produce dihydroperoxides (deMan, 1999).

Antioxidant Activity in Foods

Alpha-tocopherol compounds are well known for their ability to effectively inhibit lipid oxidation in foods and living organisms. The role of alpha-tocopherol as an antioxidant is mainly due to its ability to donate its phenolic hydrogens to free radicals and stop the activity of oxygen in oxidative reactions. The effectiveness of antioxidant activity of the tocopherol in foods varies from investigation to investigation. This variation may be due to the variety of experimental designs, chemical properties of food matrix, and environmental factors that affect the results of studies applied to oxidation in the foods (Eitenmiller & Lee, 2004).

As a lipid-soluble antioxidant, alpha-tocopherol traps peroxyl radicals in order to slow down or stop the lipid oxidation process and chain reaction (in propagation part of oxidative reaction). Lipid hydroperoxides and relatively stable alpha-tocopheryl radicals

(see Figure 3) (that do not cause propagation and chain reaction) are formed after the tocopherol trap the peroxyl radicals (Fuchs & Packer, 1993).

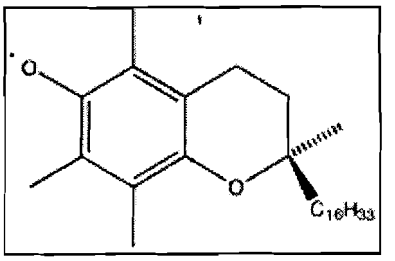

Figure 3. Chemical structure of alpha-tocopheryl radical

Source: Fuchs & Packer, 1993

$$LOO\cdot + \alpha\text{-}TH \rightarrow LOOH + \alpha\text{-}T\cdot$$

Some of the tocopherol compounds may be regenerated from tocopheroxyl radicals by reductants such as ascorbic acid (Fuchs & Packer, 1993).

$$\alpha\text{-}T\cdot + \text{electron donor}_{red} + H^{+} \rightarrow \alpha\text{-}TH + \text{electron donor}_{ox}$$

Tocopheroxyl radicals that do not react with the reductants may react instead with peroxyl radicals to produce inert products (Fuchs & Packer, 1993).

$$LOO\cdot + \alpha\text{-}T\cdot \rightarrow \text{nonradical products}$$

In order to inhibit lipid oxidations in foods, antioxidants scavenge hydroxyl, alkoxyl, peroxyl, and active oxygen radicals that can initiate the chain reaction of oxidations. How fast the antioxidants scavenge peroxyl radicals determines the efficiency and effectiveness of the antioxidants in breaking the chain propagation of the lipid oxidations (Fuchs & Packer, 1993).

$$LO_2\cdot + IH \quad k_{inh} \rightarrow \quad LOOH + I\cdot$$

Where k_{inh} is the rate constant for the reaction, $LO_2^{\bullet}$ the lipid peroxyl radical, IH the antioxidant, and $I^{\bullet}$ the radical derived from the antioxidant. Among the tocopherol and phenolic compounds, alpha-tocopherol is the strongest antioxidant in scavenging the

peroxyl radicals (see Table 3) and destroying the chain propagation of the oxidations (Fuchs & Packer, 1993).

Table 3

Rate Constant of Antioxidants in Scavenging Peroxyl Radicals at 30° C

Antioxidant	$10^5 \times k_{inh}$ ($M^{-1} s^{-1}$)
Alpha-Tocopherol	32.0
Beta-Tocopherol	13.0
Gamma-Tocopherol	14.0
Delta-Tocopherol	4.4
2,6-Dimethyl-4-methoxyphenol	9.4
2,6-Di-*tert*-butyl-4-methoxyphenol	1.1
2,6-Di-*tert*-butyl-4-methylphenol	0.14

Source: Fuchs & Packer, 1993

The high antioxidant activity of alpha-tocopherol toward the peroxyl radical is due to its high reactivity of hydroxyl group of the tocopherol compound, which is the result from a high resonance stabilization energy of the alpha-tocopheroxyl radical. In addition, the temperature of environment significantly affects the rate constant of reaction of the alpha-tocopherol with the peroxyl radical. Experimental findings have indicated that alpha-tocopherol can scavenge the peroxyl radical about 10^4 times faster than polyunsaturated fatty acid (PUFA) can react with the peroxyl radical at 37° C in homogeneous solution. Also, the alpha-tocopherol is able to scavenge about 90% of peroxyl radicals before they initiate the oxidative attack on the PUFA (Fuchs & Packer, 1993).

Whole Grain

Whole Grains Council (WGC, 2004) and AACC International (2000) stated that foods can be categorized as whole grains if the foods still contain almost the same relative proportions of bran, germ, and endosperm as the original grain kernel (Marquart & Cohen, 2005).

Grains have three important parts: the innermost germ, which includes plant embryo or seed; the endosperm, which plays an important role in providing food for the growing seed; and the outer hull, which mostly contains the bran and helps protecting the grain from bacteria, mold, insects, and bad weather. To be labeled as whole-grain food, the grain (used as raw material in the food production) has to contain all the three main parts (Marquart & Cohen, 2005).

The bran and germ are the parts where the most biologically active components can be found in the grain: amino acids, vitamin B (thiamin, riboflavin, niacin, and pantothenic acid), vitamin E (tocopherols), and minerals (calcium, magnesium, phosphorus, potassium, sodium, and iron). An abundance of starch can be found in endosperm. Grains also supply phytonutrients (plant components that contribute to several health benefits). One of the most important phytonutrients is vitamin E which acts as antioxidant. Vitamin E can provide protection to nutrients in the plant from oxidation during food processing and storage (Marquart & Cohen, 2005).

Whole-grain foods can be considered as functional foods because of providing health benefits and lowering the risk of chronic diseases. In order to effectively act as functional foods and be accepted well by the consumers, food scientists may want to focus their research on various techniques, such as hybridization, genetic modification,

milling, and processing to maintain the satisfying sensory properties (taste, texture, color) and control the caloric impact of the grain-based foods (Marquart & Cohen, 2005).

Environmental (irrigation, fertilization, pest control) and genetic (selective breeding) methods are the most essential aspects in agriculture. The use of both methods can increase grain yields, but the increase in the yields may lower the stability and concentration of nutrients in the grains. Recent studies have indicated that the genetic method (selected and used in agriculture) mostly results in strong appearance of trade-offs between the grain yields and mineral concentration (iron, zinc, copper, selenium, phosphorus, sulfur) in the grains (Davis, 2005).

Wheat Origin

As one of the first cereal grains to be cultivated, wheat had been cultivated in the Eastern Mediterranean and Mesopotamia for at least 5,000-6,000 years and became the primary food for the ancient civilizations of Babylon, Egypt, Crete, Greece, and Rome. Around 3,000 BC, the primitive wheat reached Europe and southern Russia. The primitive wheat is fragile, breaks easily into segments when it is threshed, and does not separate readily from its enveloping structures when it becomes mature. Later, this primitive wheat was replaced by another cultivated grain species called *T. aestivum*. This species resists breakage, and has characteristics that allow the plant to have efficient harvesting and threshing. In 1520, Spaniards brought the wheat to North American continent for the first time (Matz, 1991).

Hybrid Wheat

Successful crossbreeding techniques applied on corn, sorghum, and sunflower cultivation have encouraged scientists to start the development of hybrid wheat (Matz,

1991). In addition, the interest of scientists in improving wheat quality (such as yield of wheat, ease of processing, adaptation of wheat to soil and climate condition, and stability of nutritional composition) has resulted in developing a wide variety of hybridization techniques (Kent & Evers, 1994). The phenomenal success of creating hybrid wheat was reported in 1962. Today, almost all wheat seed planted results in a variety of wheat products that have better quality (Matz, 1991).

When compared to other grains, wheat is more complicated to hybridize. The breeding techniques used on wheat are labor-intensive and time consuming. The difficulty of applying the techniques on wheat is due to the nature of the wheat plants: male and female parts are located in the same flower. This kind of characteristic has caused difficulties in isolation of the parts from one another, and in trying to selectively fertilize the flower (Matz, 1991).

Chemical Composition of Wheat Kernel

Wheat endosperm contains carbohydrates and proteins. During the growth of the plant, concentration of sucrose, reducing sugars, and pentosans in the endosperm rapidly decrease, while starch content rapidly increases. In addition, the protein concentration is nearly stable during the wheat kernel growth (Matz, 1991).

In wheat, the germ is laterally placed outside the endosperm (see Figure 4) and has a slight protuberance (Godon & Willm, 1994). The germ (see Figure 4) contains lipids, vitamins, and minerals. The lipids mostly consist of linoleic (55 Wt.%), oleic (18 Wt.%), and linolenic acid (2.3 Wt.%) (Kulp & Ponte, 2000). Vitamin E is the major fat-soluble vitamin present in the germ. The germ contains tocopherols 2.6 mg/g of wheat

grain (Kent & Evers, 1994). In addition, zinc, iron, copper, and manganese are minerals commonly found in the germ (Matz, 1991).

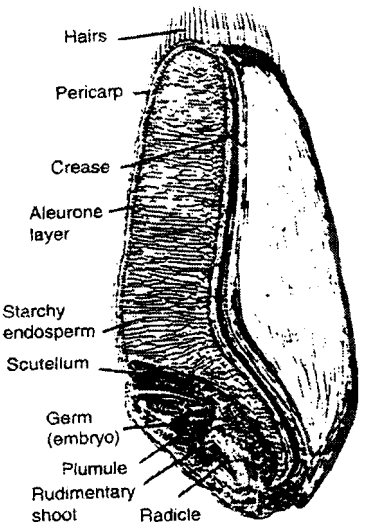

Figure 4. Longitudinal section and parts of whole wheat kernel

Source: Godon & Willm, 1994

Corn Origin

Corn (*Zea mays*) originated in central America (including southern Mexico and highlands of Peru). Corn was the first cereal plant that was systematically cultivated by American Indians. Modern corn that we now know, is the result of repeated seed selection from corn plants with larger kernels, more kernels on each cob, and other desirable characteristics. The ancestor of modern corn can no longer be found because the original corn was completely displaced by domesticated corn. For several thousand years, corn has evolved under cultivation. The evolution has resulted in change of the size of kernels and productivity (Matz, 1991). In many countries around the world, corn became an important commodity for development of various food products because of its grain size, high yield, ease of cultivation, versatile food uses, and stability during storage (Inglett, 1970).

Hybrid Corn

The genetic properties of corn allow great potential for easy improvement of the stability of its chemical composition: carbohydrates, protein, and oil. Therefore, it is easy for scientists to develop a crossbreeding technique that focuses on minimizing nutritional loss during food processing. Many crossbreeding techniques have been developed to achieve more desirable corn quality, including higher yield, stronger kernels for preventing breakage during the harvesting process, and early maturing of the corn (producing dry grain) that consequently requires less drying processes and reduces production costs (Inglett, 1970).

Modern technology has led to the development of gene-altered corn in the late 1980s. Genetically engineered corn is more resistant to insect attack, has a higher yield, and has a higher nutritional content. For example, inserting a gene which would encode a high concentration of the amino acid lysine to the hybrid corn would increase the production level of the amino acid in the grain (Matz, 1991).

Chemical Composition of Corn Kernel

In the corn kernel, carbohydrates and proteins can be found in the endosperm and germ (see Figure 5). Sucrose is the major sugar component present in the kernel; approximately three-fourths of total sucrose is in the germ and the remaining one-fourth of it is in the endosperm (Inglett, 1970). In the kernel, over 50% of the protein is in the form of amino acids. Concentrations of the amino acids in the endosperm and the germ are almost equal (Matz, 1991).

In corn, the germ is located inside the endosperm (see Figure 5) and its end is covered by a cap that can be seen externally (Godon & Willm, 1994). The germ contains

lipids, vitamins, and minerals. The lipids mostly consist of linoleic (58.7 Wt.%), oleic (26.6 Wt.%), and linolenic acid (0.8 Wt.%) (Kulp & Ponte, 2000). Vitamin E is the major fat-soluble vitamin present in the germ. The germ contains tocopherols 0.8-0.9 mg/g of corn grain (Kent & Evers, 1994). In addition, zinc, iron, copper, and manganese are minerals commonly found in the germ (Matz, 1991). Concentration levels of the minerals in the corn are lower than the levels in wheat (Kulp & Ponte, 2000).

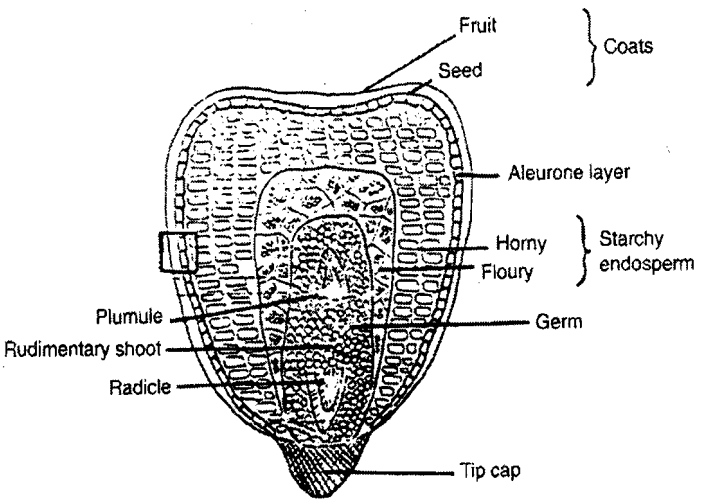

Figure 5. Longitudinal section and parts of whole corn kernel

Source: Godon & Willm, 1994

High Performance Liquid Chromatography (HPLC)

HPLC is a more advanced version of liquid chromatography (LC). It is pretty simple to operate and takes a relatively short time to complete the analysis. In addition, HPLC is the best and most popular method of choice for routine quantification of vitamin E content in foods (Eitenmiller & Lee, 2004).

An HPLC system has seven basic components (see Figure 6):

- Container for the mobile phase
- Pump to get the eluent and sample into the system

- Automatic injector for sample introduction
- Column to separate the chemical components of the sample
- Detector for visualizing and identifying targeted components
- Waste container for the used solvent
- Computer and software to save and provide interpretation of the collected data

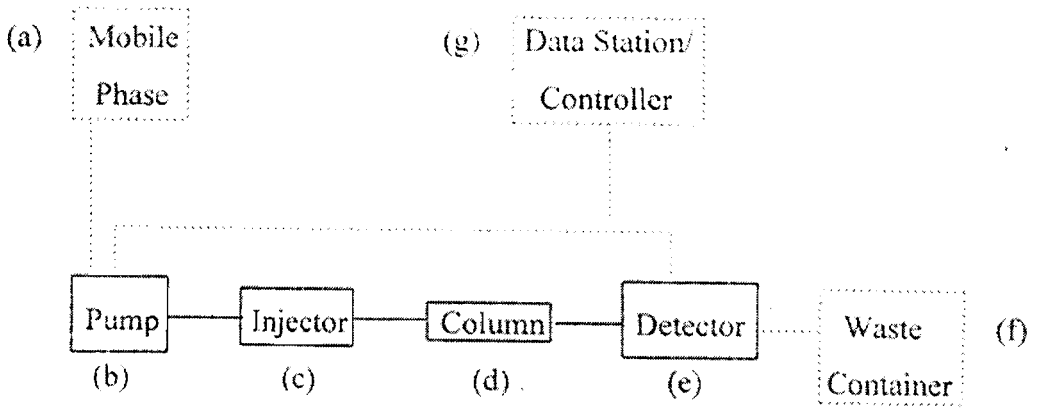

Figure 6. Schematic diagram of seven basic components of HPLC

Source: Weston & Brown, 1997

The flow rate of the solvent inside the pump can be adjusted. The range of the flow rate that can be set depends on the type of the pump being used. There are three types of pumps: microbore (1-250 μL/min), standard bore (100 μL/min – 10 mL/min), and preparative (>10 mL/min). In microbore HPLC, a syringe pump is used to deliver the eluent through the chromatograph with a smooth flow (Weston & Brown, 1997).

Automatic sample injection is used to obtain a precise and consistent injection volume. The autosampler allows the operator to select a specific number of samples and run time for HPLC analysis. It automatically introduces a sample from the vial (held in a carousel) to the column before examination by a detector (Weston & Brown, 1997).

The column is the most important part in chromatography; it separates the solvent containing the sample, which goes through it, into components. Silica is the most commonly used material in the main stationary phase for adsorption, because it can withstand the high pressures applied to deliver the mixture through the column. The efficiency of the column depends on the polarity of the components and the type of phase used in HPLC. Normal-phase chromatography is very well suited to the analysis of samples (such as edible oils, fat-soluble vitamins in corn, cereal, and feeds) that are soluble in non-polar solvents (Eitenmiller & Landen, 1999). The chromatography has a polar stationary phase (column packing material) and a nonpolar mobile phase (solvent). Fat-soluble vitamins and hydrocarbons are samples that can be easily separated into components if silica (stationary phase) and hexane (mobile phase) are used in the chromatography (Weston & Brown, 1997).

In HPLC, the detector converts the detected physical or chemical property of eluent (solute) into a signal. The signal from the detector is sent to a computer. Data processing system of the computer records the information, and develops a chromatogram with peak areas and peak heights. For a fluorescence detector, the compounds having multiple double bonds and electron-donating groups, such as $-NH_2$, -OH, -F, $-OCH_3$, and $-N(CH_3)_2$ can be well detected because those compounds are able to absorb light at specific wavelength and reemit it in all directions at a longer wavelength (lower energy) (Weston & Brown, 1997).

Chapter III: Methodology

The purpose of this research was to investigate significant differences in the loss rate of alpha-tocopherol concentration in whole-wheat flour and corn meal after exposing those flour samples to heat within certain time periods. This chapter includes a description of the materials, sample preparation, instrumentation, and statistical analysis used in this vitamin E stability study.

Materials

Whole-wheat (bread) flour was obtained from Archer Daniels Midland (ADM) Milling Company (Minneapolis, MN), and whole-corn flour was obtained from ADM Milling Company (Jackson, TN). DL-alpha-tocopherol (100 mg) with minimum purity 99.6% (MW=430) was purchased by the Chemistry Department at the University of Wisconsin-Stout from Supelco Chemical Company (Bellefonte, PA). Absolute 200 proof ethanol was purchased from AAPER Alcohol and Chemical Company. HPLC grade hexane and certified ACS potassium hydroxide were purchased from Fisher Scientific Company. Certified ACS petroleum ether was purchased from EM Science Company. Diisopropyl ether (98+%) was purchased from Alfa Aesar Company. Certified ACS pyrogallol was purchased from Acros Company. Those reagents were provided by the Chemistry Department at the University of Wisconsin-Stout.

Standard Solutions and Calibration Curves

Alpha-tocopherol stock solution was prepared by dissolving 70 mg of alpha-tocopherol in mixed petroleum ether and diisopropyl ether solution (3:1) in a 100-mL volumetric flask to produce a concentrated solution (700 μg/mL). A series of standard solutions was prepared by diluting the stock solution (with mixed petroleum ether and

diisopropyl ether solution (3:1) in 25-mL volumetric flask) to concentrations of 56, 112, 168, 224, and 280 μg/mL (see Table 4). Calibration curves produced from these standards indicated a high degree of linearity (r=0.969) upon plotting standard concentration (μg/mL) versus peak area (mV-sec) obtained from HPLC analyses with 100 μL injections. Each concentration of the standards was analyzed by triplicate injections.

Table 4

Concentration of Alpha-Tocopherol in Standard Solutions

Standard	Stock Solution Added (mL)	Concentration of α-T (μg/mL)
1	2	56
2	4	112
3	6	168
4	8	224
5	10	280

Note: α-T = alpha-tocopherol. The amount of stock solution added to each standard (in 25 mL flask) was diluted to the mark with petroleum ether and diisopropyl ether solution (3:1).

The linear graph of standard solution was made by preparing a range of the tocopherol standards with specified concentrations and peak areas (see Table 5).

Table 5

Concentration of Alpha-Tocopherol in Standards & Peak Area of the Standards

Standard	Concentration of α-T (μg/mL)	Peak Area (mV-sec)
1	56	182684
2	112	305399
3	168	417903
4	224	517595
5	280	665461

Note: α-T = alpha-tocopherol.

The standard curve of the alpha-tocopherol (see Figure 7) was obtained by using Microsoft® Excel 2000 Edition Software.

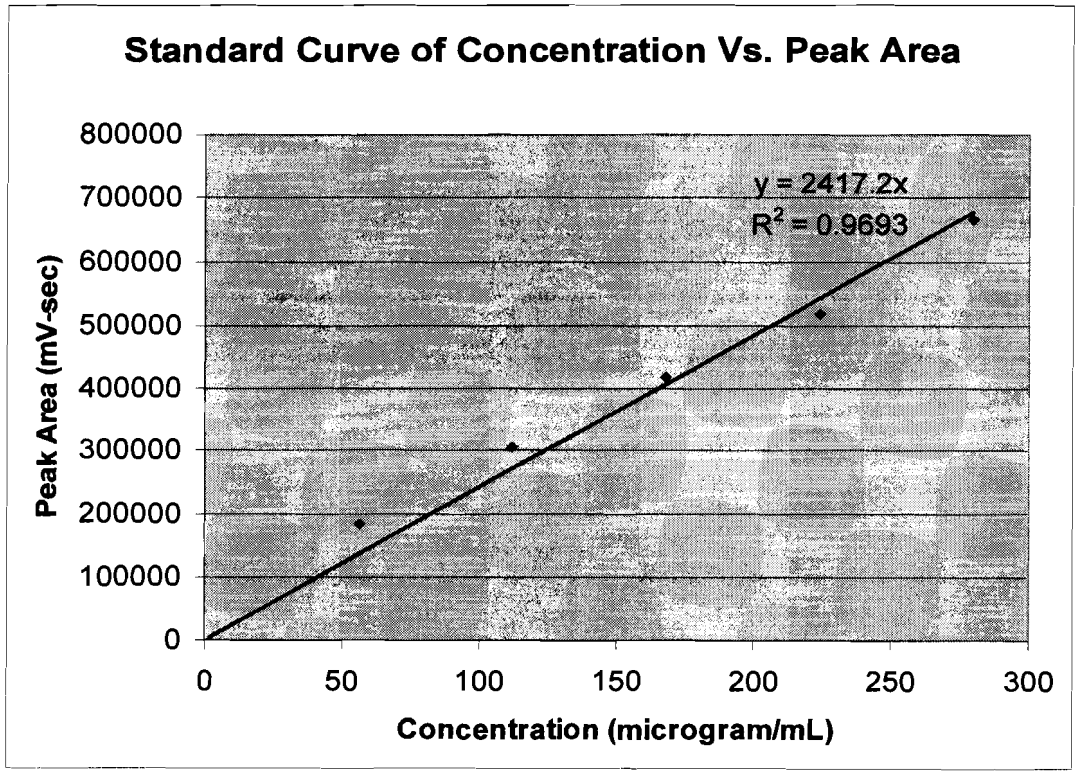

Figure 7. Standard curve of alpha-tocopherol concentration vs. peak area

Procedure

The whole wheat and whole corn flour were divided into four different samples (Control, A, B, and C). Both types of flour were prepared in triplicate. Flour samples A, B, and C (about 10 g) were weighed by using an analytical balance (Mettler Toledo), put in a glass bowl, and heated in an isotemp oven (Fisher Scientific) at 95° C for various amounts of time. For example, samples A, B, and C were heated for 3, 6, and 9 hours respectively while the control was not heated; the control was in a sealed plastic bag, and stored in a drawer (at room temperature).

After the heated whole wheat and whole corn flour samples were cooled to room temperature (25° C), the vitamin E contents of the samples were extracted by the following procedure. Each sample (about 2 g) of both kinds of flour was weighed by using an analytical balance (Mettler Toledo) and put in a 250 mL centrifuge plastic bottle (Nalgene). About 20 mL of ethanol containing 2% pyrogallol was poured into the plastic bottle. Alpha-tocopherol stock solution (0.4 mL) with concentration 700 μg/mL and 50% potassium hydroxide solution (4 mL) were added. Then, the solution in the bottle was heated in an isotemp water bath (Fisher Scientific) at 70° C for 12 minutes. During the heating, the solution was agitated every 3 minutes. After the heating was done, the solution was allowed to cool to room temperature. Then, about 10 mL of the mix of petroleum and diisopropyl ether solution (3:1) was added. After that, the sample solution in the plastic bottle was shaken mechanically for 8 minutes by putting the plastic bottle on the top of an orbit shaker (Lab-Line). Before the shaker was turned on, the speed was set to 200 rpm. After the shaking was done, Milli-Q water (30 mL) was added. The

plastic bottle containing the sample solution was inverted ten times and centrifuged for 10 minutes at 1800 rpm by using a centrifuge (Sorvall) equipped with superspeed fixed-angle rotor: GSA (six-place rotor). The clear upper layer part of the sample solution was transferred into a small HPLC vial and analyzed in triplicate (see Figure 8).

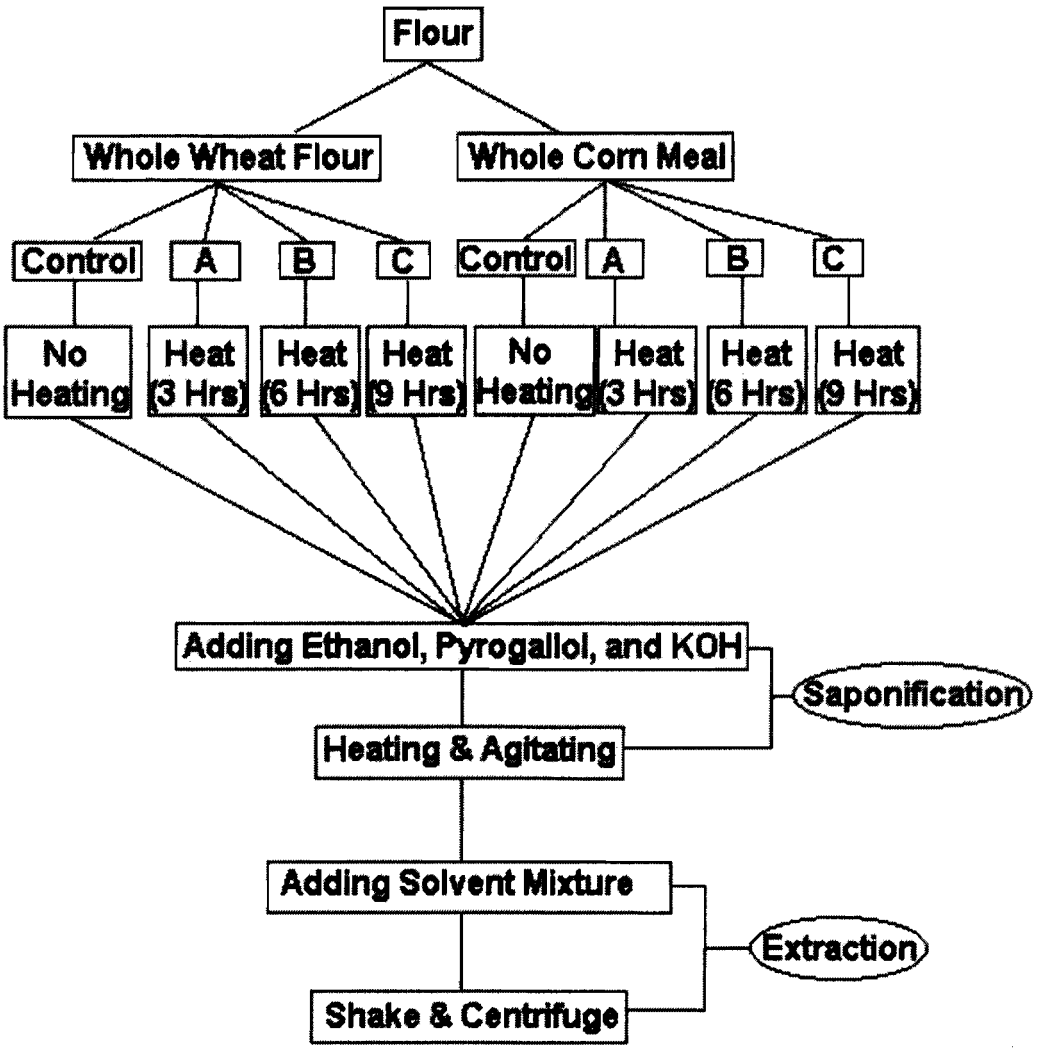

Figure 8. The flow diagram of vitamin E (alpha-tocopherol) extraction

Instrumentation

The HPLC used in this research was equipped with a 710B WISP autosampler (Millipore/Waters Associates) and a Waters M6000A chromatography pump

(Millipore/Waters Associates) with an operating range from 0 to 6000 psi. A Shimadzu model RF-530 fluorescence detector (equipped with Xe lamp) was interfaced with a Macintosh PowerBook computer equipped with Dynamax MacIntegrator Version 1.4.3 software. A 250 mm x 4.6 mm column of 10 micron Li Chro Sorb Si 60 (Supelco) was used in this study. The instrument conditions and parameters for the detection of the alpha-tocopherol at ambient temperature were:

- Mobile phase: hexane solution that contains 475 mL water-saturated hexane, 475 mL dry hexane and 50 mL diethyl ether (Thompson & Hatina, 1979). The saturated hexane was prepared by adding 5 mL Milli-Q water to 1 L hexane in a bottle capped little bit loosely, and stirring the moist hexane on stirrer plate for 2 days.
- Flow rate: 2.5 mL/minute.
- Injection volume: 150 microliters for sample analysis and 100 microliters for standard solution analysis.
- Detection at 290 nm (excitation) and 330 nm (emission) (Thompson & Hatina, 1979).
- Run time: 10 minutes.

Data Analysis

Data were analyzed using SPSS V.14 software. A T-test was performed in order to determine whether tocopherol loss rate (mg/hr) mean value of whole-wheat flour and corn meal were significantly different at a specified confidence level ($p \leq .05$). The 2-factor analysis of variance (ANOVA) was used to determine if the effect of type of flour used in this research and heating time periods, as well as the effect of interactions

between type of flour and heating time periods on tocopherol loss (mg) mean value of wheat and corn flour (in three different heating time periods) were significant ($p \leq .05$).

Chapter IV: Results and Discussion

The study was conducted in order to investigate if there was a significant difference in the loss rate of alpha-tocopherol concentration in whole-wheat flour and corn meal after heating within certain periods. Therefore, peak area and retention time of alpha-tocopherol on chromatogram, and data of the tocopherol concentration in each flour sample in three different heating time periods (including the control) were needed. In addition, the statistical analysis (T-test and ANOVA) on the loss rate of alpha-tocopherol in both flour samples after each heating period was very necessary to perform in order to test the hypothesis of this research.

Item Analysis

HPLC analysis of alpha-tocopherol in standard solutions was performed in order to estimate the retention time of the tocopherol compound. The Chromatograms (see Figure 9 and 10) showed that the tocopherol compound eluted at about 7 minutes.

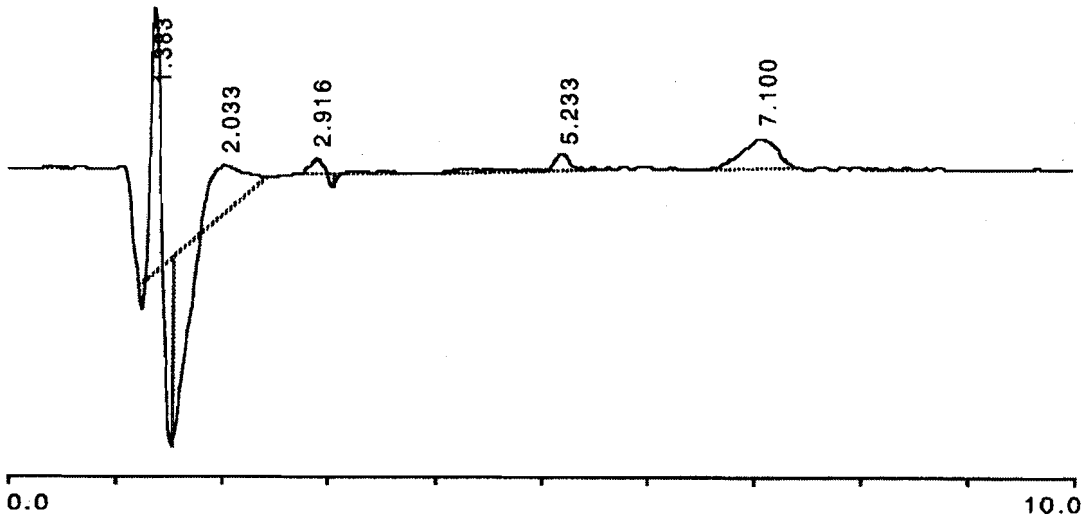

Figure 9. The chromatogram of alpha-tocopherol standard 1

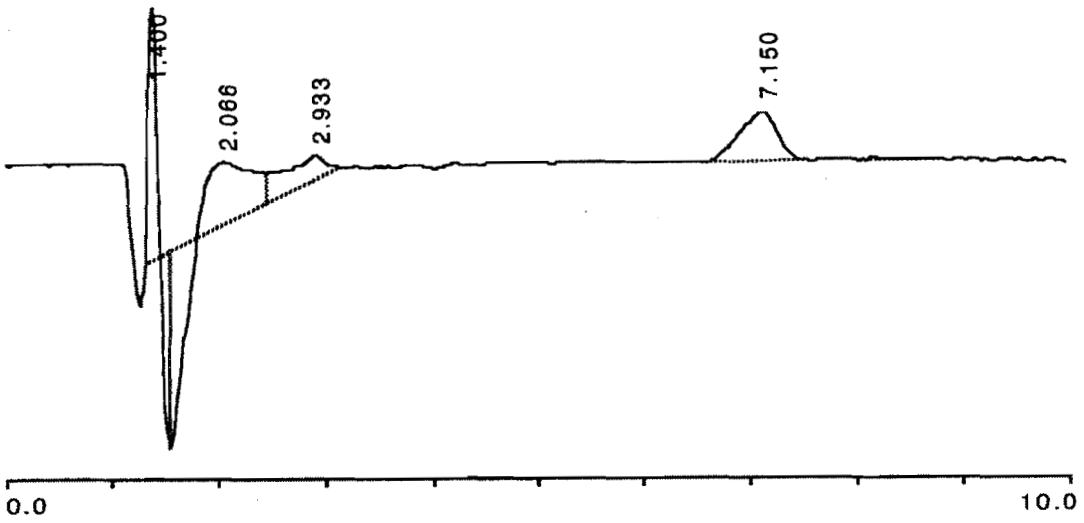

Figure 10. The chromatogram of alpha-tocopherol standard 2

Identification of the tocopherol peaks (peak areas) of flour samples was done by finding the spikes that occurred at around 7 minutes on the chromatograms (see Figure 11 and 12) produced by HPLC analysis of the samples.

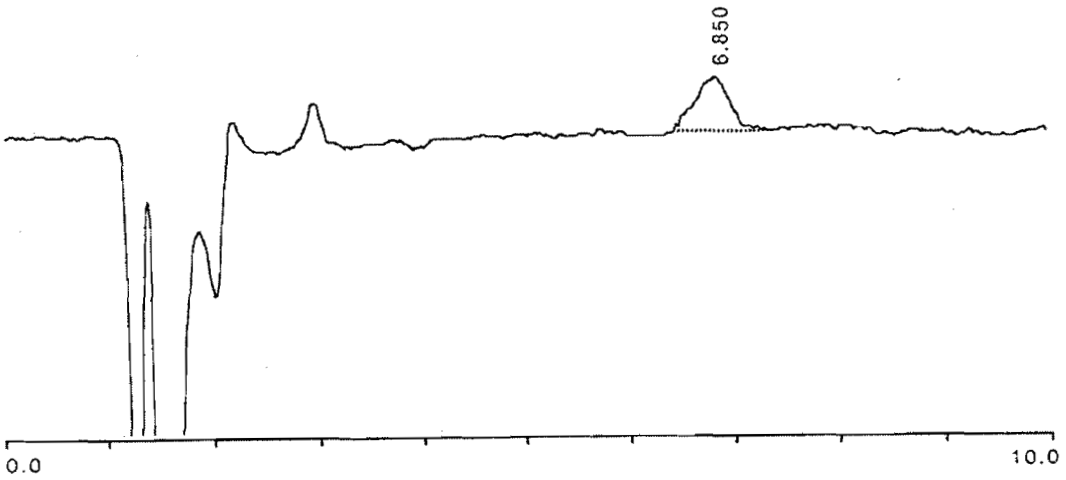

Figure 11. The chromatogram of alpha-tocopherol in wheat flour (control)

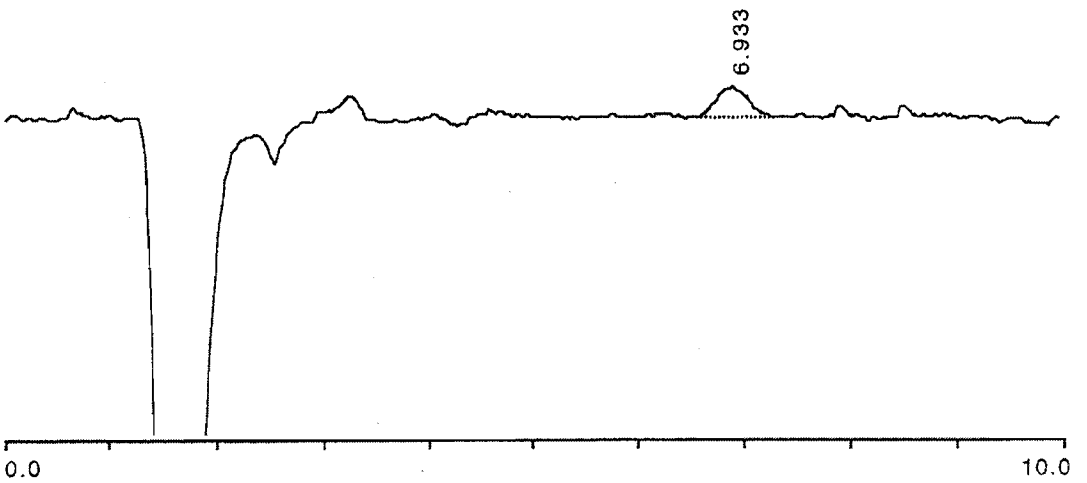

Figure 12. The chromatogram of alpha-tocopherol in corn meal (control)

The alpha-tocopherol concentrations (see Table 6, 7, and Figure 13) in whole-wheat and corn flour sample in each heating time were calculated by using the linear equation (y = 2417.2x) derived from the standard curve of alpha-tocopherol.

Table 6

Concentration of Alpha-Tocopherol in the Whole-Wheat Flour Samples in Each Heating Time (at 95° C)

Heating Time	Amount of Alpha-Tocopherol in 100 g WWF (mg)
Control (0 hour)	16.0 ± 0.297
3 hours	8.39 ± 0.102
6 hours	4.78 ± 0.278
9 hours	1.90 ± 0.267

Note: WWF = Whole Wheat Flour. The values are the mean of tocopherol contents of triplicate samples ± standard deviation.

Table 7

Concentration of Alpha-Tocopherol in the Whole-Corn Meal Samples in Each Heating Time (at 95° C)

Heating Time	Amount of Alpha-Tocopherol in 100 g WCM (mg)
Control (0 hour)	3.41 ± 0.229
3 hours	2.17 ± 0.192
6 hours	1.87 ± 0.380
9 hours	0.625 ± 0.301

Note: WCM = Whole Corn Meal. The values are the mean of tocopherol contents of triplicate samples ± standard deviation.

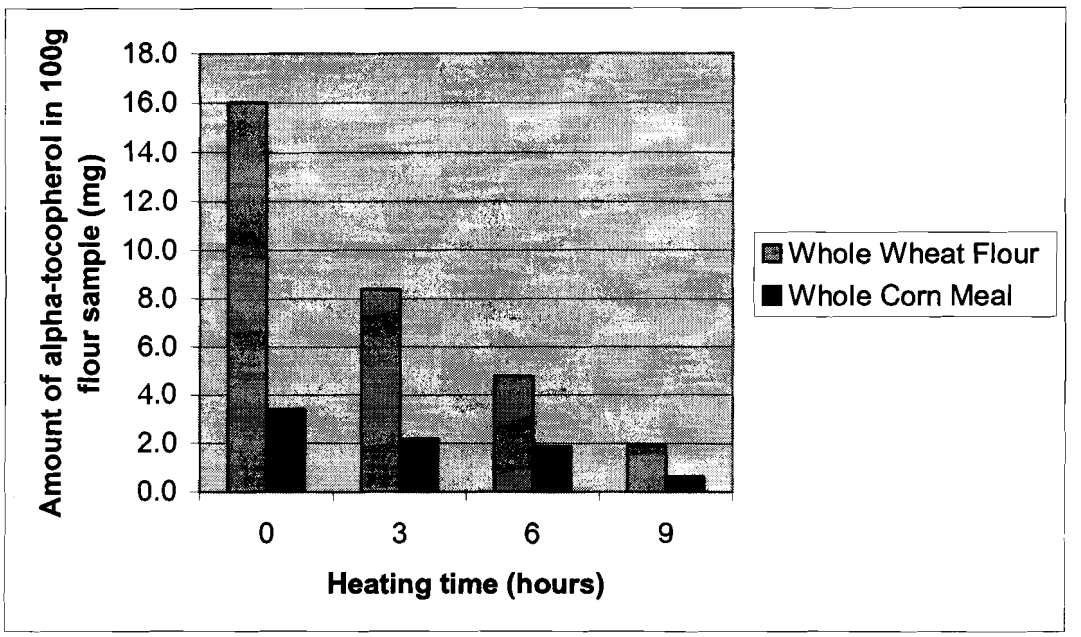

Figure 13. Concentration of alpha-tocopherol in flour in each heating time

The decrease in alpha-tocopherol content of whole-wheat and corn flour samples during heating were analyzed statistically by using a T-test method. It was found that the

loss rate (in mg/hr) of the tocopherol of both flour samples during the heating were significantly different ($p \leq .05$) (see Table 8). The loss rate of the tocopherol in 100 g of whole-wheat flour was 1.53 ± 0.0252 mg/hr, while the one in 100 g of whole-corn meal was 0.288 ± 0.0436 mg/hr.

Table 8

Results of Statistical Analysis (t-Test)

Tocopherol Loss Rate	MD	SED	df	t	t-Critical Value
Wheat & Corn	1.24	0.0290	4	42.8	2.13

Note: MD = Mean Difference, SED = Standard Error Difference, df = degree of freedom.

Based on the result from the statistical analysis (the 2-factor ANOVA), it was found that the type of flour used in the research and the heating time periods had significant effects ($p \leq .05$) on the amount of alpha-tocopherol lost. The interaction (type of flour and heating time periods) also significantly affected the amount of the tocopherol lost (see Table 9 and 10).

Table 9

The Amount of Tocopherol Lost in Whole-Wheat Flour and Corn Meal in Each Heating Period (at 95° C)

Heating Period	Tocopherol Lost in WWF (mg)	Tocopherol Lost in WCM (mg)
0-3 hours	7.61 ± 0.216	1.24 ± 0.211
3-6 hours	3.61 ± 0.290	0.30 ± 0.305
6-9 hours	2.88 ± 0.350	1.25 ± 0.655

Note: WWF = Whole Wheat Flour, WCM = Whole Corn Meal. The values are the mean of the amount of tocopherol lost in triplicate samples ± standard deviation.

Table 10

Results of Statistical Analysis (2-Factor ANOVA)

Source of Variation	SS	df	MS	F	F-critical
Type of Flour	64.3	1	64.3	470.9	4.49
Heat Period	23.8	2	11.9	87.13	3.68
Type of Flour x Heat Period	17.6	2	8.79	64.41	3.34

Note: SS = Sum of Squares, MS = Mean Square, df = degree of freedom.

According to Wennermark and Jagerstad (1992), the heating process of flour could cause a problem in the extraction of alpha-tocopherol in the flour samples. When exposed to heat, the tocopherol compound could form complexes with starch and protein, and it would be broken up more easily during saponification process of the vitamin E extraction method. The change in the extractibility of vitamin E could affect the accuracy in determination of the amount of tocopherol lost during certain heating periods.

Piironen, Varo, and Koivistoinen (1988) stated that the loss rate of alpha-tocopherol in flour was influenced by storage temperature (heating), lipid content, and the presence of minerals, such as iron and copper. In flour, linolenic acid is the most unstable unsaturated fatty acid during heating (deMan, 1999). When compared to wheat flour, corn meal has lower linolenic acid content. Wheat flour has 2.3 Wt.% linolenic acid, while corn meal has 0.8 Wt.% linolenic acid (Kulp & Ponte, 2000). Because the degradation of alpha-tocopherol is due to its role as an antioxidant in lipid (linolenic acid) oxidation in a high temperature environment, the loss of the tocopherol in wheat flour is faster than the one in corn meal. The regenerating mechanisms of alpha-tocopherol will not occur if oxidative stress overloads the antioxidant defense. In this condition, the loss

of the tocopherol cannot be prevented (Fuchs & Packer, 1993). When compared to wheat flour, corn meal has lower iron and copper content (see Table 11) (Kulp & Ponte, 2000).

Table 11

The Amount of Iron and Copper Present in Wheat and Corn

Grains	Fe (mg/100g)	Cu (mg/100g)
Wheat	6	0.8
Corn	2	0.2

Source: Kulp & Ponte, 2000

Iron and copper can catalyze the lipid oxidation process by breaking up the lipid hydroperoxides to produce alkoxy radicals. As an antioxidant, alpha-tocopherol scavenges the radicals that will result in the decrease in the amount of the tocopherol (Fuchs & Packer, 1993). Because the amount of iron and copper in the wheat is higher (compared to the corn), it is possible that the loss of the tocopherol in the wheat flour is faster than the one in the corn meal.

According to Wijewickreme and Kitts (1998), Maillard reaction products (MRPs) produced by heating reducing sugars and amino acids could slow down the lipid oxidation in the presence of copper. During the growth of wheat kernel, reducing sugars and free amino acids decrease rapidly (Matz 1991). Breeding techniques applied on corn in the past have led to the rapid increase in the amount of amino acids and high level of stability of the sugars during the maturation of the corn (Inglett, 1970). The higher reducing sugar and amino acid contents in corn (compared to wheat) could contribute to the low loss rate of alpha-tocopherol in the corn flour.

Chapter V: Conclusion

Summary

This study was conducted to examine the stability of alpha-tocopherol, the most effective antioxidant in vitamin E, in whole-wheat flour and corn meal in a high temperature environment (95° C). The study mainly focused on:

1. Testing the stability of alpha-tocopherol in both types of flour by heating them at 95° C in an oven for 3, 6, and 9 hours.
2. Evaluating and comparing the amount of the tocopherol in both kinds of flour after the heating.

The HPLC and statistical analysis methods (T-test and 2-factor ANOVA) were used in order to compare data on the tocopherol concentration in whole-wheat and corn flour for each heating period. It was found that the loss rate (in mg/hr) of the tocopherol in both flour samples during the heating was significantly different ($p \leq .05$). Compared to whole-wheat flour, the tocopherol in whole-corn meal was more stable. In addition, the two different types of flour used and the heating time periods had significant effects ($p \leq .05$) on the amount of alpha-tocopherol lost. The decrease in the tocopherol content in both kinds of flour showed different patterns. This might be related to the change in the extractibility of the tocopherol in the flour after each heating period.

The loss of alpha-tocopherol in whole-wheat flour and corn meal is primarily related to the lipid oxidation that occurs during heating. The decrease in the tocopherol content of both types of flour is caused by the lipid oxidation that induces the tocopherol, as an antioxidant, to give a hydrogen atom to free radicals produced during the oxidation process. The difference in the loss rate of the tocopherol content of both kinds of flour

may be caused by several factors, such as fatty acid composition, mineral content, and genetic property of those types of flour.

This study indicates that the difference in chemical composition of whole-wheat and corn meal can affect the loss rate of alpha-tocopherol in both types of flour during the heating. DNA of the cereal plants, controlling the production of certain chemical components inside the grains, plays a very important role in minimizing the loss of the tocopherol during the heating. Therefore, breeding techniques (gene modifications) on the plants in order to slow down the loss of the nutrient in the flour dough during baking may be an option.

Recommendations for further study

The study suggest that further analysis on alpha-tocopherol could be conducted. Here are some recommendations:

1. Increase the total of heating periods of both types of flour in order to obtain a clear pattern of the decrease in the tocopherol in the flour samples.
2. Examine the stability of alpha-tocopherol in other types of flour (oat, rye, barley and rice) to find out which type of flour has the highest stability of the alpha-tocopherol.
3. Compare the loss rate of the tocopherol in several kinds of flour from different varieties of a type of cereal plant in order to discover the best breeding technique that should be applied on the plants.
4. Use different extraction methods to identify which method is able to give better results.

5. Fortify the flour by adding a specific chemical compound or nutrient in order to learn more about the interactions between alpha-tocopherol and the compound.

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