

TOCOPHEROL AND TOCOTRIENOL CONTENT OF FAST FOODS AND PIZZAS

by

DEBOLINA CHATTERJEE

(Under the direction of Ronald. R. Eitenmiller)

ABSTRACT

The objective of this study is to measure the amount of vitamin E in various fast foods and determine the effects of baking on the vitamin E content of pizzas. Tocopherol and tocotrienol contents of various fast foods were determined by saponification and normal phase liquid chromatography. Samples were collected through the USDA National Food and Nutrient Analysis Program and from a local pizzeria. Ten baked and ten uncooked pizzas were locally collected to study the retention of vitamin E after baking. The values of vitamin E for fast foods and pizzas ranged from 8mg/100g to 0.3mg/100g. γ -Tocotrienols were detectable in all the samples but at levels less than 0.1mg/100g. α -Tocopherol in supreme pizza was statistically higher at the 95% confidence interval level in baked pizza compared to raw pizza. The study provides data on vitamin E content of fast foods in the U.S. diet.

INDEX WORDS: Vitamin E content, Retention time, Tocopherols, Tocotrienols, Fast foods,
Pizzas

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DEDICATION

Dedicated to

My beloved husband, Santanu Chatterjee, and my Parents

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CHAPTER 1

INTRODUCTION

Food consumption patterns have changed throughout the 20th century. Fast foods are a growing component of the American diet. The frequency of fast food consumption has increased dramatically since the 1970's. Recent estimates show that in 2001 there were about 222,000 fast food locations in the United States and sales totaled more than \$ 125 billion. The percentage of individuals who reported eating fast foods was higher among those 10 to 39 years of age and declined among older individuals. The reported use of fast food was lowest among people 60 years of age and older and among those with a household income below poverty threshold. The children and adolescents who reported eating fast foods had a significantly lower intake of breads and cereals and significantly higher intake of fried potatoes. The children who reported eating higher amount of fast foods also had a significant higher intake of energy and fat and very low levels of healthy nutrients. This widespread fast food consumption which adds up to energy and fat intake in individuals can lead to child and adult obesity. There is a large increase in the intake of pizza, lasagna, crackers, popcorns, pretzels and corn chips. Total fat intake has increased, but the percentage of calories from fat has declined as energy intake has increased. The total amount of fat from dairy products has remained constant, as cheese consumption has increased as compared to the 1970's (Johnson 2000, Paeratakul 2003). Given the fast-paced American lifestyle, lack of time is a big factor and, therefore, there is also an increase in the percentage of food eaten away from home. About half of family income used for food is spent on food and beverages outside home with 34 percent on fast foods (Johnson 2000). There is also an

increase in ready-to-eat prepared foods aimed mainly for the younger generation. Families also choose fast food restaurants depending on playgrounds and toys offered by the meal and franchise for children (Johnson 2000). All these factors have added to the increase in consumption of fast foods and have also changed the pattern of per capita food consumption in the United States. There is an increase in per capita consumption of cheese, poultry and soft drinks. Moreover, the food consumed in the American diet ranges from fast food burgers and fries to tacos, fajitas, Chinese rice and noodles, pasta and pizza and many other types of foods (Regmi 2001).

Neilson et al. carried out a study in 2002 to see the change in trend in energy intake in the U.S. between 1977 and 1996. This study included data on subjects aged 2 years and above from four nationally representative surveys of the U.S. population. A total of 63,380 individuals participated in different surveys like the Nationwide Food Consumption Survey (NFCS77), and Continuing Survey of Food Intake by Individuals (CSFII 89 and 96). The study showed that energy intake from home cooked meals decreased by 11% and 20.8% for all age groups from 1977 to 1996. Moreover, there was an increase in energy intake from restaurants/fast foods by 91.2% and 208% for all age groups. There was also a relative increase in energy consumed as snacks. The shift was from 11.3% (1979) to 17.7% (1996). The average American increased restaurant/fast food consumption for meals from 9.6% to 23.5% between 1977 and 1996. The study also showed that there was an increase in consumption of salty snacks and pizzas between 132 to 143% from 1977 to 1996. This increase was mainly seen between the age groups of 2-18 years and 19 to 39 years. Overall, Americans increased their energy intake from French fries, hamburgers, cheeseburgers, pizzas and Mexican food as part of meals from 3.9% to 9%.

There has also been an increase in cheese consumption of 146 % from 1970 to 1997. Most of these cheeses come from commercially manufactured and prepared foods such as pizza, tacos, nachos, fast food sandwiches, and packaged snack foods. Per capita consumption of mozzarella cheese, which is the main cheese pizza, has increased by more than 7 times since the 1970s to 8.4 pounds in 1997 (Putnam and Allshouse 1999).

Pizza is one of America's most important and popular foods. According to a survey conducted by the top pizza chain restaurants in 2002, about 94 percent of the American population eats pizza. According to a Gallup poll, children between ages 3 and 11 prefer pizzas to all other food groups for both lunch and dinner (Top Market Share Pizza Chain Restaurants Survey, 2002). Although pizza and fast foods form one of the most important parts of the American diet, there is no available data on their nutrient composition. Therefore, it is important to make this information available to consumers on energy and macro and micronutrient content of fast foods by the fast food restaurants. Consumers on the other hand should also utilize the available information on foods and beverages they are purchasing (Bowman et al., 2004). An important aspect of the food composition of pizzas and fast foods is the content of vitamin E, which is a natural antioxidant with several known health benefits. Since pizzas and fast foods are becoming an increasingly dominant part of the American diet, it is important to determine the vitamin E intake in that diet. The USDA National Nutrient Database for Standard Reference (SR 17, 2004) has little or no information in food composition tables on pizza and other fast foods. Therefore, it is important to produce enough information and food composition data on pizza and other fast foods to better define the significance of foods to the U.S. consumer.

The objective of this study is two-fold: (i) to provide reliable data on the content of vitamin E in pizzas and fast foods in their cooked and processed forms present in the American diet, and (ii) to find out the amount of vitamin E retention after the process of baking pizza.

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CHAPTER 2

TOCOPHEROL AND TOCOTRIENOL CONTENT OF FAST FOODS AND PIZZAS

2.1. LITERATURE REVIEW

The USDA National Nutrient Database for Standard Reference is the major source of food composition data in United States. The data compiled in the standard reference are from published and unpublished sources. Published sources include the scientific and technical literature. Unpublished data include those obtained from the food industry, other government agencies, and research conducted under the National Food and Nutrient Analysis Program (NFNAP) (USDA National Nutrient Database for SR (17), 2004). The NFNAP is an integrated system for identifying foods and nutrients, food sampling, food preparation and compositing, sample preparation, chemical analysis, and data dissemination. The program has structured five objectives to achieve this goal:

- Evaluation of existing data.
- Identification of key foods and nutrients for analysis.
- Development of a nationally based sampling plan.
- Analysis of samples.
- Compilation and calculation of representative food composition data (Pehrsson et al., 2003).

Values that are published are from results of laboratory analysis or are calculated by using appropriate algorithms, factors, or recipes from the source code in the Nutrient Data File (Hayowitz et al., 2002).

The NFNAP has identified approximately 1000 foods, which includes 660 key foods, ethnic foods, mixed dishes, foods used in metabolic studies, and food ingredients that are important contributors of critical nutrients in the U.S. food supply or to the diet of specific populations (Pehrsson et al., 2000, Hayowitz et al., 2002).

The Nutrient Data Laboratory (NDL) has focused on emerging nutrients or nutrients like individual carotenoids, vitamin K, procyanidins and anthocyanidins, flavonoids, fluorine, choline and sphingolipids, trans fatty acids, and n-3 fatty acids since scientific interest in these areas has increased dramatically in recent years. In addition to the nutrient analysis, new dietary reference intakes have been developed by the National Academy of Science's Food and Nutrition Board (Pehrsson et al., 2003).

Among the other foods that have been recently analyzed under the NFNAP are eggs and fast foods. Fast foods that were sampled and analyzed included hamburgers, cheeseburgers, French fries, chicken sandwiches, fish sandwiches, shakes, carbonated beverages, and coffee. These results are undergoing quality control review prior to their incorporation into the database (Pehrsson et al., 2003).

The NDL has used the key foods approach for over 20 years for its nutrient analysis (Hepburn, 1987; Hayowitz et al., 1996). This approach has allowed the NDL to concentrate analytical resources on those foods that contribute significant amounts of nutrients of public

health interest to the diet. In addition to the 660 key foods, the NDL has analyzed mixed dishes, ethnic foods, and ingredients, as well as foods used in clinical studies (Hayowitz et al., 2002).

Food is made of chemicals. According to McCance & Widdowson (1940), “knowledge of the chemical composition of foods is the first essential in the dietary treatment of disease or in any quantitative study of human nutrition.” The data on food composition is essential for a diversity of purposes in many fields of activity. The primary use of food composition data is assessment and planning of the nutrient intakes in an individual or populations (physiological, socioeconomic or clinical groups). Food composition tables are used to calculate the average personal intake of different nutrients from food. Food composition databanks can also provide information for product labels. Data obtained from analytical methods are most reliable but is more costly and time consuming (Leclercq et al., 2001). Determination of composition using analytical methods is known as the direct method. Another method is the indirect method which is more cost effective but less reliable. It uses data from published literature and unpublished lab reports. In some cases, values are imputed from incomplete or partial analysis of food (www.ifis.co.uk). The limitations of food composition tables or databanks are that they are not sufficiently understood by people. Moreover, information about the samples is limited, i.e., not all types of food are included. The NFNAP has analyzed about 500 foods till spring 2002 which have been included in the databank. They have a target of completing 1000 foods by 2003–2004. Another limiting factor is that for a number of foods groups namely fish, meat, poultry, and snacks, relatively few foods have been analyzed (Pehrsson et al., 2003).

According to Rand et al. (1991), there are 5 major ways to obtain data on nutrient content of foods:

1. Direct analysis based on analytical measurement.
2. Calculated as representative value (e.g. weighted means of several samples).
3. Gathered from other sources (e.g. taken from other tables or the literature).
4. Estimated from similar foods (e.g. substitution of data)
5. Estimated from ingredients (e.g. recipe calculation).

Nutrient databases provide food composition data that are used in variety of ways. Health researchers and epidemiologists use databases to correlate food components with causes or prevention of diseases. Dietitians recommend dietary changes based on analysis of their usual dietary habits. Food manufacturers determine the nutrient content of their products for food labels. All of these uses of nutrient databases require the database for each food to be complete so that nutrient content is not misunderstood. A study was conducted by Schakel et al. (1996) on the procedures for estimating nutrient values for the food composition database. There are different ways of obtaining values for nutrient databases. Chemical analysis of nutrients in foods is the most efficient but also most costly and is often unavailable for foods not commonly consumed. Another way is to calculate nutrient values from a different form of the same food. Other methods, which can be used, are conversion of nutrient values from nutrient label information of a commercial food product or calculation of nutrient values from a product standard. According to Schakel et al. (1997), the update of nutrient values in a food composition database requires implementation of quality control procedures to verify that appropriate data entries were used. It is also important to validate the estimated values as their derivation often depends on assumptions made by the database compiler as well as series of calculation procedures.

To estimate the nutrient content of the cooked food, consideration must be given to the cooked yield nutrient retention (McCarthy, 1992). Nutrient retention factors for vitamins and minerals have been determined for groups of foods and various cooking methods by the USDA, using data from paired samples of raw and cooked food and the following formula (Murphy et al., 1975):

$$\% \text{ True Nutrient Retention} = \frac{(\text{Nutrient content per g of cooked food})(\text{g cooked food})}{(\text{Nutrient content per g of raw food})(\text{g raw food})}(100)$$

The retention factors include nutrient losses due to heating and losses due to draining. Another way is to calculate nutrient values from other components in the same food. Vitamin E expressed as alpha-tocopherol equivalents can be calculated from the activity of tocopherols and tocotrienols using various conversion factors derived from different assay methods. Two common methods, which were used to calculate vitamin E are as follows:

1. For individual foods (McLaughlin and Weihrauch, 1979):

$$\text{mg alpha tocopherol equivalents} = \text{mg alpha tocopherol} + 0.04(\text{mg } \beta\text{-T}) + 0.1(\text{mg } \gamma\text{-T}) + 0.01(\text{mg } \sigma\text{-T}) + 0.3(\text{mg } \alpha\text{-T}_3) + 0.05(\text{mg } \beta\text{-T}_3) + 0.01(\text{mg } \gamma\text{-T}_3).$$

2. For mixed diets (National Research Council, 1989):

$$\text{mg alpha tocopherol equivalents} = \text{mg alpha tocopherol} + 0.5(\text{mg } \beta\text{-T}) + 0.1(\text{mg } \gamma\text{-T}) + 0.3(\text{mg } \alpha\text{-T}_3).$$

The mg α -tocopherol equivalents (mg α TEs) have been defined for recommending dietary intakes of vitamin E on the basis of biological activity of tocopherols and tocotrienols

determined by the rat fetal absorption test (Eitenmiller and Lee, 2004). One mg of α -TE is the activity of 1 mg of RRR- α -T. Total α -TEs (mg) of food containing only the RRR-isomers are calculated by multiplying the amount (mg) of α -T by 1.0, of β -T by 0.5, of γ -T by 0.1, of α -T3 by 0.3, and of γ -T3 by 0.05. In fortified foods, the conversion factors for all-rac- α -T and all-rac- α -tocopheryl acetate are 0.74 and 0.67, respectively.

Prior to 1980, 1 IU of vitamin E activity was defined as 1 mg of all rac- α -tocopheryl acetate by the United States Pharmacopeia (USP) (DRI, 2000). However, after 1980, the IU was changed to the USP unit where where 1 USP of vitamin E was still defined as equivalent to 1 mg of all rac- α -tocopheryl acetate, 0.67 mg of RRR- α -tocopherol, or 0.74 mg of RRR- α -tocopheryl acetate. In the more recently published DRI (Food and Nutrition Board, 2000); the definition of vitamin E is limited to the 2R-stereoisomeric forms of α -T in order to establish recommended intakes. Given this definition, all-rac- α -T has 50 % of the activity of RRR- α -T found in foods or present in the 2R-stereoisomeric forms of α -T in fortified foods and supplements. Therefore, to achieve an RDA of 15 mg/day of α -tocopherol, a person must consume 15 mg/day of RRR- α -tocopherol or 15 mg/day of the 2R-stereoisomeric forms of α -T, or some combination of the two.

The recent Dietary Reference Intake (DRI) approach to calculate vitamin E are as follows (Eitenmiller and Lee, 2004):

- Milligrams (mg) of RRR- α -T in a meal = mg of α -TE \times 0.8
- Milligrams (mg) of RRR- α -T in a food, fortified food, or multivitamin = IU (USP unit) of all-rac- α -T \times 0.45.

The next section will discuss the importance of vitamin E to human health and its relationship to the prevention or cure of several ailments like cardiovascular disease, cancer, neurodegenerative diseases, and ageing.

Vitamin E as an Antioxidant

Vitamin E is a natural antioxidant and is a fat-soluble vitamin that exists in eight different forms. The chemical definition of oxidation is that part of an oxidation-reduction reaction characterized by electron loss or by an increase in valance state. The oxidation that occurs in the body is seen in the aging process; however, it can also lead to the development of degenerative diseases. The oxidation of food occurs when oxygen is added to unsaturated sites of organic molecules. Oxygen, light, heat, heavy metals, pigments, and alkaline conditions are catalysts in this process. Oxidation can be slowed down by the addition of antioxidants. Antioxidants are defined as chemicals that specifically retard deterioration, rancidity, or discoloration due to oxidation. The use of antioxidants prevents or minimizes the phenomenon of oxidation in foods. There are two classes of antioxidants: metal sequestrants and free-radical scavengers. Metal sequestrants precipitate a metal or suppress its reactivity by occupying all coordination sites. Free-radical scavengers include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), the tocopherols and tocotrienols (vitamin E), and ascorbic acid (vitamin C). Oxidation in the body usually occurs via naturally-produced free radicals. Radicals are compounds that contain one or more unpaired electrons and consequently are unstable. To achieve stability, free radicals "borrow" or "steal" electrons from stable compounds. Consequently, the formerly stable compounds become reactive and oxidation occurs. The result is the formation of a chain reaction.

One of the ways the body controls oxidation is through the antioxidant action of vitamin E (Higdon, 2004).

Vitamin E is often used to denote any mixture of biologically active tocopherols and tocotrienols. Each form of vitamin E has its own biological activity, measure of potency, and functional use in the body. Vitamin E is fat-soluble and, therefore, acts as an antioxidant in the lipid part of the cells. It acts as a chain-breaking antioxidant that prevents the propagation of radical reaction (Burton and Ingold, 1986). Vitamin E is a peroxy radical scavenger and especially protects polyunsaturated fatty acids (PUFA) within membrane phospholipids and in plasma lipoprotein (Burton et. al, 1983). When lipids undergo oxidation, they form peroxide radicals, which are highly reactive. Peroxy radicals react with vitamin E 1000 times more rapidly than they do with PUFA. Vitamin E slows this process by donating one of its hydrogen atoms to the peroxide radical, which then becomes stable and non-reactive. As a result, vitamin E becomes a non-reactive free radical (Packer, 1994).

Vitamin E deficiency in the human body can occur through conditions that affect the absorption of fat and/or vitamin E. Malabsorption can be caused due to pancreatic, liver, intestinal, and as well as genetic abnormalities. At the cellular level, vitamin E deficiency produces increased oxidation of cellular membranes. Related symptoms might include decreased energy production by mitochondria, DNA mutation, and changes in plasma membrane transport mechanisms. As Olson and Munson (1994) point out, these phenomena can cause reproductive disorders, abnormalities of muscle, liver, bone marrow, and brain functioning.

α -Tocopherol is the most active form of vitamin E in humans as emphasized in the DRI report (Food and Nutrition, 2000). Antioxidants act to protect the cells against the effects of free

radicals, which are potentially damaging by-products of the body's metabolism. Oxidative stress damage to the human body includes the onset of diseases including cancer (through initiation of carcinogenesis, mutagenesis), cardiovascular diseases (through oxidation of blood lipoproteins and the development of atherosclerosis), and cataracts (through oxidative damage to the lens of the eye). Evidence indicates that antioxidant components of the diet are beneficial for the prevention of various diseases like cancers, cardiovascular disease, and cataracts, as well as the other disorders mentioned above. The hypothesis that antioxidant protection could modulate the outcome of such disorders is the basis for many clinical intervention studies with vitamin E supplements. Block and Langseth (1994) stressed that new knowledge on disease preventing effects of micronutrients will lead to new views on supplementation to close "the gap between the amounts of antioxidant nutrients found in typical diets and higher levels needed for optimal protection against chronic diseases."

Dietary Reference Intake (DRI)

The Institute of Medicine, Panel on Dietary Antioxidants and Related Compounds considered hydrogen peroxide induced hemolysis the best biomarker used in conjunction with plasma α -T concentrations to estimate adult human requirements for α -T (Food and Nutrition Board, 2000, Eitenmiller and Lee, 2004). The latest recommended vitamin E requirements and the dietary reference intakes were published by the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, 2000. These new requirements were based largely on the studies by Horwitt in 1950s and 1960s (Traber, 2001). Horwitt suggested that the daily requirement of vitamin E is less than 15 mg. The Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for adults are 12 and 15 mg, respectively (Food and

Nutrition Board, 2000, Eitenmiller and Lee, 2004).). On the basis of biological activity of tocopherols and tocotrienols determined by the rat fetal absorption test, Milligrams α -Tocopherol Equivalents (mg α -TEs) were defined to recommend dietary intake of vitamin E (Bieri et al, 1974, McLaughlin et al 1979., Murphy et al, 2002.). One mg of α -TE is the activity of 1 mg of RRR- α -T. Total α -TEs (mg) of food containing only RRR-isomers are determined by multiplying the amount (mg) of α -T by 1.0, β -T by 0.5, γ -T by 0.1, α -T3 by 0.3, γ -T3 by 0.05. The Panel on Antioxidants and Related Compounds determined from USDA food intake survey data shows that 80% of the mg α -TE from food arises from RRR- α -T. Therefore the conversion factor is 0.8 (Eitenmiller and Lee, 2004). Results of two national surveys, the National Health and Nutrition Examination Survey (NHANES III 1988-91) and the Continuing Survey of Food Intakes of Individuals (1994 CSFII) indicated that the dietary intake of most Americans does not provide the recommended intake for vitamin E. It was emphasized that the estimates of vitamin E intake from food intake surveys are usually underreported. Further, there are problems with the assessment of fats and oils added during food preparation, uncertainty about the type of fat added, and the variability of food composition tables.

Vitamin E and Cardiovascular Diseases

Atherosclerosis and its complications such as coronary heart disease, myocardial infarction, and stroke are the leading causes of death in the developed world. Several epidemiological studies and intervention trials have been performed with vitamin E and some of them have shown that it prevents atherosclerosis. There are two hypotheses that explain the initiation of the atherogenic process: the oxidative theory (Mitchinson, 1983 and Steinberg et al.,

1989) and the theory of “injury response” (Ross, 1993). Both theories consider the oxidative modification of LDL as the key event in atherosclerosis induction and or progression.

A study was conducted by Stampfer et al. (1993) to determine the influence of vitamin E supplements in reducing heart diseases in women. It showed that among middle-aged women, the use of vitamin E is associated with a reduced risk of coronary heart disease. Cigny et al. (1994) studied vitamin E metabolism and its role in arterioscleroses. Based on the influence of vitamin E on cell response and the modification of lipoproteins (especially LDL), they demonstrated that vitamin E might act to prevent the initiation or progression of spontaneous arterioscleroses. Torun et al. (1995) conducted a study on serum levels of vitamin E in relation to cardiovascular diseases. The results showed that the serum vitamin E levels were lower in the group with cardiovascular disease than in the group without such diseases (control group).

As seen in the previous paragraph, in recent years vitamin E has been thought of as a cardio-protective agent. Experimental studies have identified potential mechanisms by which vitamin E may inhibit the development of cardiovascular disease, and observational studies of individuals without cardiovascular disease suggest that vitamin E may prevent future cardiovascular events. However, examination and interpretation of large-scale epidemiological studies and clinical trials by the DRI committee have shown that there is little benefit from vitamin E supplementation. Based on these findings, the DRI did not recommend supplementation of vitamin E for the general public (Eitenmiller and Lee, 2004).

Researchers at the Cleveland Clinic Foundation found the following when they reviewed seven vitamin E studies involving a total of more than 80,000 people. Subjects took in the range of 50-800 International Units (IU) of vitamin E per day and were followed for as many as six

years. The researchers concluded that no dose of vitamin E proved to reduce the occurrence of cardiovascular disease. The outcome was so strong that researchers “discouraged the inclusion of vitamin E in future... trials in patients at high risk of coronary artery disease.”

One of these studies was the Heart Outcome Prevention Evaluation (HOPE), which followed 2,545 women and 6,996 men at high risk for cardiovascular disease for 4.5 years. In this intervention study, the subjects received 265mg (400 IU) of vitamin E daily and did not experience significantly fewer cardiovascular events or hospitalizations for heart failure or of angina when compared to those subjects who received sugar pills (Yusuf, 2000). This supports the DRI panel conclusion: It is unlikely that vitamin E supplementation provides any cardio-protective agents.

The Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study investigated the effect of a combination of RRR- α -tocopheryl-acetate and vitamin C on the progression in intima-media thickness (IMT) of the common carotid artery. Only male smokers treated with both vitamins showed a relevant decrease in the rate of IMT progression (Salonen et al., 2000).

A large source of controversy involving vitamin E and cardiovascular disease is in the area of health claims on nutritional labels of foods and supplements. The Food and Drug Administration (FDA) must determine whether a dietary supplement has a “qualified” health claim, or an “unqualified” health claim. The qualified health claim must meet the Significant Scientific Agreement (SSA) standard set forth by Congress in the Nutrition Labeling and Education Act (NLEA) of 1990. Manufacturers have the right to make claims about their product’s health benefits, but if science does not support the SSA standard then to be a

“qualified” product, there must also be a disclaimer. In 2003, the FDA formed a Task Force to focus on the food and supplement labels of health claims and dietary guidance for consumers. This gives guidelines and a reviewing process for filing applications for qualified health claims (Eitenmiller and Lee, 2004).

In the case of vitamin E, FDA was petitioned to authorize a claim that showed a positive relationship between vitamin E and reduced risk of heart disease. The claim stated that individuals with a diet low in saturated fat and cholesterol might see a decrease in the risk of heart disease with intakes of 400 IU of vitamin E from natural sources and 200-800 IU from synthetic sources. Based the 2003 procedures for evaluation, FDA concluded that “..there is no significant agreement for a relationship between vitamin E supplements and cardiovascular risk, and that the scientific evidence for a relationship is outweighed by the scientific evidence against the relationship.” (Eitenmiller and Lee, 2004).

Vitamin E and Cancer

The role of vitamin E along with other antioxidants in preventing various types of cancers has also received attention among researchers. The relationship between vitamin E intake and the risk of developing several types of cancers was thought for years to be understood, but a number of recent clinical studies have called that relationship into question. The α -T and β -Carotene Cancer Prevention Study looked at the effects of dietary supplements taken over an eight-year period (1985-1993) on lung cancer in male smokers. The results of the study showed no significant relationship between the use of tocopherol dietary supplements and cancer prevention for several types of cancer (urinary tract, colon, and prostate). The United States Health Professional Study followed over 45,000 men for 11 years and found no correlation

between Vitamin E supplementation and a decrease in prostate cancer (Willis and Wians, 2003). The Cancer Prevention Study II had mixed results depending on the type of cancer being examined. Vitamin E supplementation had no effect on colon and stomach cancers. However, long term supplementation did decrease the mortality due to bladder cancer (Rodriguez et al., 2004). In 2001, the Selenium and Vitamin E Cancer Prevention Trial began. The study is set to follow over 30,000 men for 12 years in order to determine, among other things, if a correlation between Vitamin E supplementation and cancer exists (Willis and Wians, 2003, Lippman et al., 2005). This study started in the year 2001 and the final results are expected by the year 2013 (Klein et al., 2003). Bjelakovic et al. (2004) studied the effect of antioxidant supplementation on gastrointestinal cancers and mortality. They identified 14 randomized trials. There were no significant effects of supplementation of vitamin E, A, C and selenium on either of the fixed or randomized trials. In seven high quality trials the fixed effect model showed significant increase of mortality due to antioxidant supplementation while low quality trials showed no significant effect. Beta-carotene and vitamin E together significantly increased mortality, whereas selenium had significant beneficial effects on gastrointestinal cancer. They concluded that there was no evidence of preventive action of antioxidants on cancer and on the contrary, they tended to increase the mortality rate. They also recommended studying the potential preventive effect of selenium.

Better understanding of biochemical mechanisms has recently drawn attention to γ -tocopherol, which is the primary form of dietary vitamin E. γ -T is in fact a better antioxidant than α -T, which is the primary form of supplemental vitamin E, and the form that has traditionally been the most studied (Hensley et al., 2003; Willis and Wians, 2003). Some research (as well as common sense) has shown that diets high in fruits, vegetables and other

plant materials may help reduce the risk of developing certain types of cancer. It has been suggested by some researchers that γ -tocopherol be studied more closely, but the superficial conclusion one could draw is that a healthy diet that includes sources of natural vitamin E (i.e. lots of fruit and vegetables) and plant oils helps reduce the risk of developing cancer more than supplementation of the vitamin (Hensley et al., 2003).

γ -Tocopherol, the predominant form of vitamin E in diets, inhibits proliferation of prostate cancer cells (LNCaP and PC-3) and lung cancer cells (A549). In this study, γ -T or its combination with δ -T induced apoptosis in androgen-sensitive prostate LNCaP, by the induction of cytochrome c release. This study also showed that γ -T and mixed vitamin E forms induce cell death by interrupting the *de novo* sphingolipid pathway in a prostate cancer cell line. Thus, certain vitamin E forms may be valuable as anticancer agents (Jiang et al , 2004).

A study was conducted by Link et al. (2004) to find out whether men affected with prostate cancer can adhere to a low fat diet. Men with elevated prostate-specific antigen levels, most of whom were recently treated for prostate cancer, were randomized to one of four dietary regimens for which they received nutritional counseling: a low-fat diet (15% fat or less) with supplements (vitamin E and selenium), a low-fat diet (15% fat or less) without the supplements, the supplements alone, and a control group. Adherence was evaluated by the change in weight, fat intake, free fatty acids, cholesterol, high-density and low-density lipoproteins, and triglycerides during a 12-month period. Three months after starting the intervention, those randomized to low-fat dietary counseling had lost 2 kg, on average, compared with 0.8 kg lost by those who did not receive this counseling. At 12 months, those receiving low-fat counseling had lost 2.8 kg on average, compared with 0.5 kg gained among the other group. With appropriate

counseling, men with prostate cancer can adhere to a low-fat dietary intervention for a 12-month period. (Link et al., 2004).

Due to the lack of “significant scientific agreement,” the FDA currently allows products to carry a health claim that vitamin E “may reduce the risk of certain types of cancer” only if it is followed by a disclaimer indicating that FDA considers the available evidence supporting this claim to be “limited and not conclusive” (FDA, 2004). However, recent scientific evidence seems to strengthen the link between vitamin E and cancer. A study was conducted by Weinstein et al. (2005) to find out the relation of serum α -T and γ -T to prostate cancer risk. The Alpha-Tocopherol, Beta-carotene Cancer Prevention Study demonstrated a 32 % reduction in prostate cancer incidence in response to daily α -T supplementation. The study examined baseline serum concentrations of α -T and γ -T and compared their baseline association with prostate cancer risk. Two hundred and forty six case patients were selected randomly through the Finnish Cancer Registry. Serum α -T and γ -T concentrations were determined by reverse phase high performance liquid chromatography. Case patients had lower intake of vitamin E than control subjects otherwise comparable to control subjects. Men with higher circulating levels of α - and γ -tocopherols had lower prostate cancer risk. Therefore, in the study they concluded that major vitamin E fraction i.e. α - and γ -T are associated with substantially lower risk of prostate cancer. They also concluded that serum α - and γ -T were not associated with the risk.

Neurodegenerative Diseases

There are several neurodegenerative diseases that increase in incidence as individuals age. It is believed that these diseases are at least partially a result of a lifetime of cell damage

due to free radical damage. Therefore, several studies have examined the link between antioxidants, such as vitamin E, and risk of developing a neurodegenerative disease. Evidence that free radicals may contribute to the pathological processes in Alzheimer's disease (AD) has led to interest in the use of vitamin E in the treatment of this disorder.

One recent study on Alzheimer patients administered a micronutrient supplement, which included vitamin E, but failed to see any slowing in the progression of the disease. The authors of the study state that more research into the vitamin E-Alzheimer link is necessary as it is currently “ambiguous” (Planas et al., 2004). Parkinson’s disease, another degenerative neurological disease usually with a late-life onset, has also been studied to determine if a relationship with antioxidants exists. The Rotterdam Study administered a semi-quantitative food frequency questionnaire to over 5,300 subjects in the Netherlands. The results of the study indicate that high dietary vitamin E levels correlated with a lower incidence of Parkinson’s disease. The same conclusion could also be drawn from the results of this study for AD. Similar to cancer, this evidence suggests γ -T may be the more protective tocopherol, and further emphasizes the importance of a well-rounded healthy diet rich in vegetables and other plant foods in maintaining long-term good health (deRijik et al., 1997). A study was done to compare CSF and serum levels, and the CSF/serum ratio of α -T (vitamin E), measured by HPLC, in 44 apparently well-nourished patients with AD and 37 matched controls. CSF and serum vitamin E levels were correlated, both in AD patients and in controls. The mean CSF and serum vitamin E levels were significantly lower in AD patients, and the CSF/serum ratio of AD patients did not differ significantly between the 2 study groups. CSF vitamin E levels did not correlate with age, age at onset, duration of the disease and score of the Minimental State Examination in the AD group. Weight and body mass index were significantly lower in AD patients than in controls.

These results suggest that low CSF and serum vitamin E concentrations in AD patients could be related with a deficiency of dietary intake of vitamin E. (Jimenez-Jimenez et al., 1997).

Laurin et al. (2004) examined the association of midlife dietary intake of antioxidants to late-life dementia and its subtypes. Data were obtained from the Honolulu-Asia Aging Study, a prospective community-based study of Japanese-American men who were aged 45-68 years in 1965-1968. The analysis included 2,459 men with complete dietary data who were dementia-free at the first assessment in 1991-1993 and were examined up to two times for dementia between 1991 and 1999. The sample included 235 incident cases of dementia (102 cases of AD, 38 cases of AD with contributing cerebrovascular disease, and 44 cases of vascular dementia). Intakes of beta-carotene, flavonoids, and vitamins E and C were not associated with the risk of dementia or its subtypes. This analysis suggests that midlife dietary intake of antioxidants does not modify the risk of late-life dementia or its most prevalent subtypes.

Aging

Cardiovascular disease, some cancers, and neurodegenerative disease are often found in older individuals. Further scientific research in a variety of areas has led to many theories of aging. Current understanding of the evolution of and the processes involved in aging lead to the primary conclusion that species are designed to survive long enough to ensure successful procreation. Once individuals have reproduced, nature has no reason to select genes, which will extend their lifespan further. It has been shown in theory that accumulation of free radical damage and its catalysis by various oxidants including quinones and other age pigments, metal ions, lipid peroxides, prostaglandins and components released from cells, increase with age.

Animal and human health studies have demonstrated protective effects of vitamin E and other antioxidants on free radical reactions and peroxidative changes in the aging process (Eitenmiller and Lee 2004). However, not enough evidence has been found to fully support the idea that vitamin E is beneficial to health and aging. The results of some studies conducted on the relationship between antioxidants vitamins and degenerative diseases have been inconclusive. Currently, the DRI committee has not recommended supplementation for vitamin E. The guidelines as of right now include a diet rich in various antioxidants, not just vitamin E, which will provide increased longevity with increased maintenance of good functionality (Eitenmiller and Lee, 2004).

A category of aging that may also be affected by vitamin E intake is eye health. More than 22 million Americans suffer from cataracts and age-related macular degeneration (AMD), which are the two leading causes of visual loss and blindness. Since cataracts require costly surgery and treatment options for AMD are currently limited, preventive measures play a particularly important role. Studies have suggested that six nutrients- the antioxidants lutein, zexanthen, beta-carotene, vitamin C, vitamin E, and zinc- are associated with maintaining eye health. However, according to the DRI panel, evaluation of data specifically relating to vitamin E and cataract development provided “mixed” results (<http://www.transceiver.com/eye/>). For example, a recent study of middle-aged male smokers did not demonstrate any effect from vitamin E supplements on the incidence of cataract formation (Teikari et al., 1997). The effects of smoking, a major risk factor for developing cataracts, may have overridden any potential benefit from the vitamin E, but the conflicting results also indicate a need for further studies before researchers can confidently recommend extra vitamin E for the prevention of cataracts

Food composition

Research on vitamin E has focused on food composition due to the following reasons: (i) increased understanding of human and animal requirements for vitamin E, (ii) understanding of the relationship of requirements to the level of intake of poly-unsaturated fatty acids, and (iii) clearer understanding of oxidative aspects of the stability and vitamin E content of oils.

Tocopherols are natural antioxidants of physiological importance. They occur as four derivatives, with α -T being the most efficient. A recent study (Matthaus et al., 2003) was conducted to find out the fatty acid and tocopherol composition of Vietnamese seeds and to determine the correlation between PUFA and tocopherol /tocotrienol compositions. The HPLC analysis method was used to determine the amount of vitamin E in 40 plant seeds and a t-test was done at the 0.01 and 0.05 levels to test for statistical significance. The results indicated a negative correlation ($r = -0.31$) between oil content and δ -tocotrienols and a strong positive correlation between linolenic acid and γ -T ($r = 0.51$). γ -Tocopherol was the predominant tocopherol found in the seeds. It's part of total vitamin E varied from 53.4 to 95.9 percent. β -Tocopherol ranged from 65 to 107 mg/kg. In eight seed oils, the tocotrienol content ranged from 57.2 to 82 percent of total vitamin E content. This shows that oilseeds containing high amounts of PUFA produces more active antioxidants (γ -T). The contents of oleic acid were negatively correlated with both α and γ -T and other forms of vitamin E. The study also predicted that tocopherols are unnecessary for protecting oils with high oleic acid content against oxidative deterioration. This study, however, could not confirm any strong correlation between oil content and γ -T. This variation may be attributed to the 23 different varieties of seeds that were studied.

Velasco et al. (2002) investigated the genetic and environmental variation of tocopherol contents and composition in commercial hybrids of sunflower cultivated in southern Spain. High performance liquid chromatography was used to determine tocopherol content and composition. Thirty-three commercial hybrids of sunflower from 16 seeds, along with 3 experimental hybrids were used in this study. Variation of tocopherol content ranges from 314.5-1024.5 mg/kg of seed and from 562.8 to 1872.8 mg/kg of oil. The results showed that α -T was the major fraction of the total tocopherol content; the rest were β and γ -T, while there was no trace of δ -T. For α and β -T, the genotypic variance was greater than the environmental variance, while for γ -T, the relationship was opposite. The study also concluded that the environment significantly influenced the total tocopherol content. The greater magnitude of the genotypic effect in comparison with the genotype \times environment suggests the feasibility of selecting for increased tocopherol content in sunflower.

Another study, conducted by Goffman and Becker (2002), analyzed the genetic variation of tocopherol content and its composition in rapeseed. Different breeding lines (87) were used. HPLC was used to determine the tocopherol level. The tocopherol composition was expressed as a ratio of α to γ -T contents. The results showed that genotypic effect was highly significant for both tocopherol content and composition. Environmental effects were highly significant for the tocopherol contents and for the α/γ T ratio. The study also showed that the negative correlation between oil and the α/γ T ratio, together with the fact that α and γ -T are differently localized, suggest the possibility that the α -T content is related to plastid development and γ -T to oil accumulation. However, further investigations are required to confirm this hypothesis.

All of the vitamin E found in eggs is in the yolk. Dial and Eitenmiller (1995) assayed 6 whole egg samples collected from various geographical areas and reported α -T levels of 0.6 mg/100g. Muscle foods almost only contain α -T at a level less than 1 mg/100g. Supplementation of the diets of many species can increase the vitamin E levels in muscles and organs, which in turn increase the oxidative stability and nutritive value of raw and processed foods (Eitenmiller and Lee, 2004).

Zanini et al. (2003) evaluated the dietary inclusion of oil sources and vitamin E supplements on the concentration of total lipids, cholesterol, and vitamin E in cockerel-thigh and breast meat. The experiment was performed on 240 cockerels (30 weeks old). The antioxidant used was Lutavit E50 and the diet was supplemented in soybean oil. Using gas chromatography, the authors showed that the use of fish and canola oil in the diet reduced the content of total lipids in thigh meat, while the total lipid content of breast meat increased when the cockerels were fed with canola oil based diet (supplemented with 400 mg of vitamin E). The authors suggested that the vitamin E supplementation of this diet caused an increase in the deposition of MUFA present in this oil. They also concluded that the amount of saturated fat in the diet had a direct connection with the cholesterol deposited on the carcass, except when there is a supply of n3-fatty acids. Finally, the study also showed that the vitamin E deposition in the thigh meat was different from that in breast meat due to the difference in the fatty acids deposited in each type of meat.

In another study, Ng et al. (2003) evaluated the nutritive value of various dietary lipids and palm oil sources as the only lipid source of African catfish, using a casein-gelatin based semi-purified diet. Also, the influence of dietary lipids on growth, food utilization efficiency,

body proximate composition, muscle fatty acid composition, and α -tocopherols were examined. African catfish fingerlings were fed with a lipid-free conditioning diet for 2 weeks. Each diet was fed to an apparent saturation of 4 percent of their body weight per day in two equal feedings. The fish were skinned and muscle tissues were removed, pooled, and stored frozen for subsequent analysis. The procedure outlined by Bligh and Dyer (1959) was used for the analysis and extraction of lipids and α -T from the muscle samples. The African catfish fed with sunflower oil (SFO) or various palm oils showed significantly higher weight gain. An earlier study by Hoffman and Prinsloo (1995) reported that diets with cod liver oil (CLO) led to much lower weight gain in African catfish. Therefore, this study records a distinct improvement of growth performance, at least in tropical fish, when fish oil (CLO) was replaced by plant-based oils. The results also indicate that African catfish required n-6 fatty acids for maximum growth. Also, palm oil should be a better replacement compared to other vegetable oils due to its low polyunsaturated fatty acid (PUFA) content, especially that of 18:2n-6. Palm oil products such as crude palm oil (CPO), crude palm kernel oil (CPKO), and refined, bleached, deodorized palm olein (RBDPO) are also rich sources of vitamin E as a natural antioxidant and its bio-accumulation in fish fillets can impart antioxidative properties which, in turn, would translate into longer shelf-life for seafood products (Lim et al., 2001). The authors further concluded that since western diets already contain too much 18:2n-6 compared to n-3 PUFA, a good fish oil substitute should maintain levels of healthy eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), while at the same time limiting 18:2n-6 deposition. This makes palm oil a good candidate as an alternative to fish oil, compared to other vegetable oils.

Recently, Kriese et al. (2004) conducted a study to find oil content, tocopherol composition, and fatty acid patterns in the seeds of 51 *Cannabis sativa* L. genotypes. Hemp,

which is an annual herbaceous crop, is traditionally used for fiber and oil production. It is also an important source of Δ^9 -tetrahydrocannabinol (THC). The oil extracted from hemp seeds can be used for human nutrition and several components of hemp oil have been known to have beneficial effects on human health. Hemp oil contains PUFA, with linoleic and α -linolenic acid being important components. According to this study, the variability of oil content, tocopherol composition, and fatty acid patterns in hemp is limited. Hemp seed oil was extracted by the supercritical fluid extraction (SFE), following the procedures described by Bruhl and Matthaus (1999). HPLC analysis was performed for tocopherols. Fatty acid composition was determined according to the method of ISO/DIS 5509 (ISO 1998) by gas liquid chromatography.

The oil content of hemp seeds ranged from 26.25-32.5 percent. The oil content and composition of hemp seed is largely affected by environmental factors. The authors also suggested that the oil content of hemp seeds might be increased by classical breeding methods. Tocopherols and plastochromanol (P-8) are among the most important anti-oxidants that have a positive effect on the oxidative stability of oils. Analyzing hemp seeds just after two months of harvest, the authors found that the ratio of γ -, α -, σ - and β -T was 87:7:1:5. Thereby, indicating that γ -T was the dominant form in hemp seeds. Further, P-8 was more closely correlated to unsaturated fatty acids than γ -T. Therefore, the authors concluded that P-8 may be more effective in protecting unsaturated fatty acids than tocopherols in hemp seeds.

Benitez-Sanchez et al. (2003) compared the oil composition of hazelnuts with other vegetable oils, mainly olive oil. Hazelnuts are thick-shelled tree nuts whose oils are either consumed crude, preferably from roasted seeds, or refined. The authors examined hazelnut oil from different geographical origins. The HPLC method was used for analyzing tocopherols and

tocotrienols, while fatty acids were detected by gas chromatography, and triacylglycerides were quantified by the International Union of Pure and Applied Chemistry (IUPAC) method. Four tocopherols (α , β , γ , and δ) were found, while α , β , and γ tocotrienols were found in only some of the samples. These results were invariant to the geographical origin of the nuts and whether they were roasted or not. The authors also reported that the highest concentration of α -T belonged to sunflower oil, while the lowest concentration of β -T was in hazelnut oil. Further, neither hazelnuts nor olive oil have high amounts of α , β , and γ tocotrienols. Finally, roasted nuts were found to have a higher content total sterols, aliphatic and triterpenic alcohols.

Vitamin E Analysis

Tocopherols and tocotrienols are a group of dietary plant constituents that are believed to have beneficial effects on human health. There are four tocopherol and tocotrienol vitamers. These vitamers are distinguished by the number and location of their methyl group on their chromanol ring. It is possible to accurately analyze all forms of tocopherols and tocotrienols with modern chromatographic methods (Eitenmiller and Landen, 1999). There are three major preparation approaches for food samples: (i) simple dilution of oil samples in organic solvent, (ii) direct extraction of the vitamers with the solvent, and (iii) extraction of the vitamers after saponification. Tocopherols and tocotrienols are relatively unstable in alkaline conditions. They are protected by using antioxidants, flushing the saponification vessel with nitrogen and working under subdued light (Eitenmiller and Landen, 1999). Saponification is the most widely used and generally accepted method for extraction of vitamin E. It is an alkaline hydrolysis performed by potassium hydroxide. In this process the tocopherols and tocotrienols are freed from the sample matrix while carbohydrates, protein, lipids are destroyed and other interfering substances are

removed. There are certain parameters like time, temperature, volume, concentrations and sample size can be varied to optimize the digestion (Eitenmiller and Lee, 2004). The detection of tocopherols and tocotrienols can be achieved by UV/visible fluorescence detectors, fluorescence, electrochemical and evaporative light scattering detector (Eitenmiller and Lee, 2004). Fluorescence detectors are considerably more sensitive than UV detectors (Hoehler et al., 1998). Vitamin E analysis is generally conducted with normal phase high performance liquid chromatography with fluorescence detection. Other means of separation include reversed phase HPLC, gas chromatography and most recently capillary electro chromatography (Abidi et al., 2002). Gas chromatography can be used to identify analytes from complex matrices that include tocopherols and tocotrienols (Eitenmiller and Lee, 2004). The use of solid phase extraction has proven to be an efficient technique for simplifying sample clean up prior to HPLC analysis (Huo et al., 1996, Eitenmiller et al., 1999, Bonvehi et al., 2000). Adsorption chromatography using silica is commonly used in separating isomers, as silica is capable of discriminating different isomeric forms of the same molecule. Silica is commonly used as the stationary phase in many of the chromatographic methods as reversed-phased columns are not capable of separating β and γ isomers. The main advantages of reversed-phase chromatography compared to normal phase chromatography are fast equilibration time and better reproducibility of retention times. Reversed-phase columns are preferred when the separation of β - and γ -T is not important. Reversed-phase HPLC usually resolves most of the analytical problems related to tocopherol analysis in more simple and rapid ways than other methods (Strohschein et al, 1998).

Gliszczynska-swiglo and Sikorska (2004) studied the simple reversed-phase liquid chromatography method for determination of tocopherols in edible plant oils. The reverse phase columns are usually octadecylsilane modified silica which do not completely separate β - and γ -

T. Recently, however, some satisfactory separation was achieved with polymeric octadecyl polyvinyl alcohol, ODS-2 [20] and a C30 column. In this study a simple, rapid and precise reversed-phase HPLC method performed at room temperature and using fluorescence detection was proposed for the determination of α -, (β + γ)-, and δ -T in edible plant oils. Samples of olive, corn, grape seed, rapeseed, sunflower and soybean oils were weighed, ranging from 0.04 to 0.12 g. All HPLC analyses of tocopherols were performed at room temperature. For determination of tocopherols in oils, a mobile phase consisting of 50 % of acetonitrile and 50 % of methanol was used with a flow rate of 1ml/min. Tocopherols were identified by comparing their retention times with those of corresponding standards. A linearity of fluorescence signals for α -, (β + γ)-, and δ -T with increasing amount of oil was obtained at least to 150mg/ml for sunflower oil and 100mg/ml for soybean oil. The reversed phase elution system was designed to achieve optimal separation of α -, (β + γ)-, and δ -T within a reasonable time period. The reliability of these methods was tested for linearity, precision, and sensitivity. All curves were linear to at least 25 μ g/ml for γ - and δ -tocopherols and 100 μ g/ml for α -T. Plant oil contains relatively small quantities of beta-T as compared to the other homologues and isomers. Therefore, the lack of separation of β - and γ -T did not introduce any significant error in quantification. The authors thus concluded that this method can be used for analyzing α -, (β + γ)-, and δ -T in plant oils.

Research conducted by Hewavitharana (2003) studied simple solutions to problems encountered in quantitative analysis of tocopherols and tocotrienols using silica columns. In this study, chromatography was performed at ambient temperature and the flow rate was 1ml/min. The hypothesis of this study was that tocopherols and tocotrienols were irreversibly adsorbed on some of the sites on silica and when these sites were blocked out by tocopherols and tocotrienols, normal chromatographic peak sizes are restored. The author found that addition of 0.02mg/L α -T

to mobile phase and injection and elution of two 200 μ L aliquots of 2000mg/L α -T were adequate to block the extra active sites in the column. The standard mixtures and sample extracts produced consistent peak areas confirming that there is no loss due to irreversible adsorption. Tocopherols and tocotrienols eluted from the columns in the order of increasing polarity suggesting that they were retained on the silanol sites rather than siloxanes.

A study conducted by Ryynanen et al. (2004) reported that saponification is an efficient means to liberate tocopherols and tocotrienols from cereal matrix prior to lipid extraction, but the saponification conditions should be carefully examined and controlled to avoid decomposition of the analytes. The materials used for the development of this method were 10 varieties of rye flour. HPLC analysis was performed and three critical experimental factors were optimized: saponification time, temperature, and amount of KOH. The saponification time and amount of KOH showed a significant effect on the output factor. The effect of saponification time on the output factor was less than that of the amount of KOH. Saponification temperatures showed insignificant effects on tocopherol and tocotrienol values. Therefore, they chose the highest settings of 100 $^{\circ}$ C to enhance the decomposition of cereal matrix. The total content of vitamin E was 28.7 μ g/g and amount of α - and β -T and tocotrienols were 8.2, 8.1, 2.9 and 9.3 μ g/g, respectively. Hot saponification for 25 min can reduce the time needed for sample preparation. Moreover, scaling down the sample size to 0.5 g can also reduce the amounts of solvent needed. They concluded that rye grains possess a beneficial ratio of tocotrienols and tocopherols as well as high amount of tocotrienols and tocopherols, although there was variation among the varieties.

2.2. MATERIALS AND METHODS

Samples

All the samples (253) were collected through the USDA National Food and Nutrient Analysis Program (NFNAP) and from local pizza houses (20). Frozen cheese pizzas (10) were bought from a retail store, Athens, GA. The USDA samples consisted of 63 breakfast sandwiches, 4 cheese nachos, 14 fast food sandwiches, 4 French fries, 16 breakfast pastries, 32 tacos & burritos. Pizza samples consisted of 37 fast food, 25 mixed dishes, 25 cheese, 8 pepperoni, 6 meat and 7 meat and vegetable pizzas. Fast food pizzas were collected from different pizza companies and mixed dish pizzas were collected from a local retailer. Ten baked and ten uncooked pizzas were locally collected to study retention of vitamin E after baking. Five of the frozen pizzas were baked at a temperature of 450⁰C for 8 min. Samples obtained from the local pizza houses and retail store (cooked and uncooked) were ground to make the sample homogenous.

Analysis of Vitamin E

High Performance Liquid Chromatography:

The normal phase HPLC system consisted of a Shimadzu LC-6A pump equipped with a Shimadzu RF-10A spectrofluometric detector (Shimadzu Corp.), a SpectraSeries AS100 autosampler (Thermo Separation Products Inc., CA), and a 25 cm × 4mm 5 µm LiChrosorb Si60 column (Hibar Fertigsäule RT. Darmstadt, F.R.Germany) equipped with a precolumn packed with Perisorb A 30-40 µm (Darmstadt, F.R.Germany). The isocratic mobile phase contained 0.9% isopropanol in n-hexane (J.T. Baker Chemical Co., Philipsburg, NJ) and the flow rate was

1 mL/min. The mobile phase was filtered using a 0.22 μ m nylon membrane filter (MSI Inc., Westboro, MA) and degassed by stirring under vacuum. The wavelengths were set at 290 nm for excitation and 330 nm for emission.

Sonicator:

FS30 (Fisher Scientific Pittsburgh, PA, USA).

Oven:

Mechanical convection oven (Precision Scientific Group, G.C.A. Corporation., Chicago, IL)

Saponification and Extraction

All extraction procedures were performed under gold fluorescent light (Sylvania, F40/GO, Gold, 40W) to protect from sunlight and fluorescence lights throughout the experiment. 1.5g – 2g of samples were weighed into an extracting tube (ground glass tube connected to the air condenser measuring 20.32 cm x 3.81 cm x 2.54 cm (I.D)). Each sample was assayed in duplicate. Ten milliliters of pyrogallol (6% w/v) solution were added to each sample and agitated to avoid agglomeration. For recovery samples, 0.3 mL of spike solution was added. Each of the tubes was then flushed with nitrogen gas for about 1 min and then sonicated for 10 min. 3.2 mL – 3.5 mL of 60 % potassium hydroxide was then added to each tube prior to connection to an air condenser. Again, each tube was flushed with nitrogen gas for 1 min. The contents were then digested at 70⁰C in a shaker water bath and were sonicated for 5 min. Following cooling in an ice bath, 20mL of 2% sodium chloride in deionized water was added and extracted 2 times with 30 mL of extracting solvent (hexane:ethyl acetate, 90:10, v/v) containing 0.01% BHT. The

extracting solvents were filtered through a fritted glass funnel containing 3 g of magnesium sulfate into a 50 mL volumetric flask and the volume was adjusted to 50 mL with the extracting solvent. The hexane layer was collected into a 50 mL volumetric flask. The volume was adjusted to 50 mL with n-hexane.

Solid Phase Extraction

The samples were cleaned through silica gel disposable (solid phase extraction, SPE) columns (J.T. Baker Chemical Co., Phillipsburg, NJ). The silica cartridge was first washed with 5 mL acetone followed by 2 mL of hexane/ethyl acetate (90/10). The cartridge was then drained with a vacuum manifold system. Two milliliters of sample extract was added to the column, and eluate was collected. The eluate consisted of the tocopherol-tocotrienol fraction with 1.0 mL volume consisting of two 0.5ml additions of hexane:ethyl acetate (80:20). Vacuum SPE was used to completely elute added solvent. Combined eluates were then evaporated to dryness under nitrogen gas and then re-dissolved in hexane. For recovery, 1.0 mL aliquot of the sample was used. The hexane containing the unsaponifiable matter was filtered through a 0.45µm nylon filter (MSI Inc., Westboro, MA) and 20 µL was injected onto the HPLC system. All solvents used were HPLC grade.

Reagents

All reagents are of analytical purity. The following chemicals were needed:

n-hexane. HPLC grade (J.T. Baker, Phillipsburg, NJ, USA)

Isopropanol. HPLC grade (Fisher Scientific, Pittsburg, PA, USA)

2,6-di-tert-butyl-4-methyl phenol (BHT) (Sigma, St. Louis, MO, USA)

Magnesium sulfate (anhydrous, powder) (J.T. Baker)

Ethanol, HPLC grade (Sigma)

all-rac- α -tocopherol (USP)

all-rac- γ -tocopherol (Sigma)

all-rac- δ -tocopherol (Sigma)

Hexane-BHT solution (0.1%BHT). Dissolve 1g BHT in 1 liter n-hexane

Tocopherol Standard Solution

(1) Tocopherol pre-solution (10 mg/5 mL): Accurately weigh 20 mg α -T and dissolve in n-hexane. Transfer to a 10 mL volumetric flask and dilute to volume with hexane. Similarly prepare tocopherol pre-solutions for γ - and δ -T.

(2) Standard intermediate solution (1.2 mg/100mL): Prepare mixed standard solution containing α -, γ -, and δ -T. Pipette 2.0 mL of each stock solution into a 100 mL volumetric flask and dilute to volume with hexane-BHT solution. Prepare solution monthly and store at -20°C.

(3) Standard working solution (containing 14, 48 and 1200 ng tocopherol/mL): Pipette 1.0 mL the intermediate standard solution into a 10 mL volumetric flask and dilute to volume with hexane-BHT solution to obtain standard 1 (1200ng tocopherol /mL). Pipette 1.0 mL standard1 into 25 mL volumetric flasks and dilute to volume with hexane-BHT solution to obtain

standard 2 (48 ng tocopherol/mL). Pipette 3.0 mL standard 2 into a 5 mL volumetric flask and dilute to volume with hexane-BHT to obtain standard 3 (14 ng tocopherol/mL). Prepare fresh on day of use.

(4) The purity of standard solutions (2 mg/25mL): Pipette 1.0mL tocopherol pre-solution into a 25 mL volumetric flask and dilute to volume with ethanol. Similarly, prepare working solutions for the other standards. Determine absorbance difference ($A - A^0$) for each standard working solution with a spectrophotometer at suitable wavelengths, using settings give in Table 1. A is absorbance of the standard solution and A^0 is absorbance of the blank (ethanol). Calculate concentration of each standard working solution from $E_{1\text{cm}}^{1\%}$ data.

LC mobile phase: n-Hexane-isopropyl alcohol (99.1+0.9). Dilute 991 mL n-hexane with 9 mL isopropyl alcohol and degas.

Moisture Content

The moisture content was calculated for purchased pizza samples (10 cooked and 10 uncooked). AOAC Official Method - 984.25 for moisture in frozen french fried potatoes was used (Official Methods of Analysis, 17th Edition). Samples were dried at $103^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 16 hrs. Samples (5-6 g) (in duplicate) were weighed in an aluminum-weighing dish and placed in a mechanical convection oven at $100^{\circ}\text{C} \pm 4^{\circ}\text{C}$ for 16 hrs. The dried samples were weighed again and moisture content was calculated by the following formula:

$$\left[\frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying} - \text{Weight of aluminium dish}} \right] \times 100$$

2.3 RESULTS AND DISCUSSION

Evaluation of the Vitamin E Assay

In-House Control Sample (Crisco Soybean Oil)

An in-house control sample (soybean oil) was assayed over the course of this study to determine the effect that minor changes in the analytical parameters (e.g. eluent composition, temperature, and column quality) might have on separation and quantitation. The quality control oil was assayed over the same period of time (duration of this study) and the results obtained were consistent. Figures 2.1, 2.2 and 2.3 provide quality control charts for the chromatography of the vitamin E forms based on the analysis of soybean oil. Data for α - and γ -, and δ -T are presented in these figures, respectively. Each data point in Fig.1 indicates the content of γ -T in the QC sample (soybean oil). The average content (mean) of α -, γ - and δ -T in soybean oil was 12.04, 73.16, and 27.45 mg/100g, respectively. All data in the control charts were close to their respective means, lying between the upper control line (UCL) and lower control line (LCL), which are set by ± 10 % of the mean. Normally, all the data should fall within this range, which shows that the chromatography system is stable. No changes are apparent in the concentration of the tocopherols over approximately a one-year time frame. All values remained within the laboratory quality control parameters of ± 10 %.

Separation conditions

Figures 2.4, 2.5 and 2.6 provide charts for the chromatographic parameters: column capacity factor (k), column selectivity (α), and resolution (R) for γ -T. Each point in Fig.2.4 indicates the calculated k values for γ -T in the standard. α and R values were calculated between

the adjacent peaks, β - and γ -T, in the standard solution (refer to Fig. 2.5 and 2.6). The mean values of k , α , and R of HPLC system used in this study are 2.18, 1.12 and 1.69, respectively. The capacity factor (k) is a measure of a compound's (γ -T) retention in terms of the column volumes. The column capacity factor (k) is measured by the following formula:

$$(t_R - t_0) / t_0.$$

where,

t_R = retention time of the solute.

t_0 = retention time of the unretained components.

A low k value is desired for a rapid analysis but a high k value is required for a good separation of components to be assayed. According to Pomeranz and Meloan (1994), a k value of 2 to 6 is preferable. The k value in this study is 2.18, which is within the preferable range. Column selectivity (α) is also referred to as the separation factor. It is the ratio of the net time for any two components in a column. It is measured by the following formula:

$$((t_{R2} - t_0) / (t_{R1} - t_0))$$

where,

t_0 = retention time of the unretained components.

t_{R1} and t_{R2} = retention time of component 1 and 2 respectively.

A column selectivity value above 1.0 indicates peak resolution while an α value of less than 1 indicates incomplete separation of the peak. The value of α (1.12) is greater than 1 and thereby shows that there is a good separation between the peaks. A larger R -value means better separation; baseline separation requires R -values equal to or greater than 1.5. It is calculated by using the following formula:

$$2\Delta t / (w_1 + w_2)$$

where,

Δt = differences between retention times of peak 1 and 2.

w_1 and w_2 = width of the peak 1 and 2 at baseline, respectively.

The value of R obtained (1.69) shows good separation between the β - and γ -T peaks, which is an advantage of normal phase compared to reversed-phase HPLC. Therefore, from the above data it can be concluded that the HPLC system used in this study is stable and useful for the quantitation of the different forms of vitamin E in pizza and other fast foods.

Recovery

Recoveries for tocopherols vary depending on the sample matrix. The recovery values for most of the pizzas and fast foods were around 90 % (Table 2.2). The percentage mean recoveries \pm S.D. in 6 types of pizzas ($n = 19$) were 90.5 ± 4.80 , 92.6 ± 3.95 and 88.6 ± 2.99 for α -, γ -, and δ -T, respectively. The percentage mean recoveries \pm S.D in 5 types of fast foods ($n = 16$) were 92.2 ± 9.43 for α -, 100.7 ± 8.20 for γ -, and 92.4 ± 8.61 for δ -T. The percentage mean recoveries \pm S.D in 2 types of uncooked pizzas ($n = 4$) were 83.6 ± 6.65 for α -, 87.6 ± 11.95 for γ -, and 84.5

± 8.06 for δ -T and in 2 types of cooked pizzas ($n = 4$) were 88.5 ± 10.32 for α -, 95.5 ± 1.91 for γ -, and 87.4 ± 7.00 for δ -T. These recoveries were used to correct the contents of tocopherols and tocotrienols in the samples assayed in this study. The percentage recovery values determined in our laboratory for various USDA food samples (248) assayed range from $109.1 - 80.4$ for α -, $111.54 - 80.8$ for γ -, and $119.9 - 81.47$ for δ -T. Samples consisted of a large variety ranging from baby foods, cheese to tomato, yogurt etc. The percentage mean recovery values for the samples assayed in this study are similar to those obtained from the USDA samples. Also, since all percentage recovery values were quite high (greater than 80 %) indicates that vitamin E isomers appeared stable to the saponification conditions and recoverable from the specific matrices.

Moisture Content

Moisture content varies depending on the sample matrix. Table 2.4 shows moisture content in cooked and uncooked pizzas. Moisture loss occurred in each pizza type after baking. The percentage mean moisture content for supreme uncooked pizza was 54.89 ± 2.01 while for the cooked supreme pizza it was 49.69 ± 2.51 . The means moisture content for uncooked vegetable pizza was 46.87 ± 6.71 percent and for the cooked pizza it was 46.40 ± 6.19 percent. The mean moisture content for uncooked cheese pizza was 49.7 ± 1.20 percent and cooked pizza it was 45.5 ± 0.25 percent. The moisture loss from raw to baked form was significantly higher in supreme and cheese pizzas while there was no significant loss in vegetable pizza ($p \geq 0.05$). These moisture contents were used to correct the tocopherol and tocotrienol contents of the samples to a dry weight basis, allowing calculation of vitamin E retention.

Tocopherols and Tocotrienols in Pizza

The tocopherols and tocotrienol compositions of the pizzas are shown in Tables 2.5. β - and δ -Tocotrienols were not detectable in any of the samples. α -Tocopherol and γ -T were detectable in all of the products. In pizza, γ -T values ranged from 0.9 to 2.1 mg/100g. Pepperoni showed the highest γ -T content (2.1 mg/100g), followed by mixed dish (1.9 mg/100g), and fast food pizzas (1.8 mg/100g). The γ -T content of the fast food, cheese, mixed dish, pepperoni and meat pizzas were not statistically different at the 95% confidence interval level. The value of γ -T in meat and vegetable pizza were significantly lower than cheese, fat food and meat pizzas. It is difficult to make assertive conclusions about such differences in the results, since the ingredient information of the samples are lacking. However, the larger γ -T levels would likely indicate a somewhat higher fat content originating from the crust consisting of vegetable oil.

In pizzas, α -T ranged from 0.7 mg/100g to 1.1 mg/100g. The α -T value for fast food pizza (1.1 mg/100g) was higher compared to the other pizza categories ($p \geq 0.05$). Due to the lack of specific ingredient usage in the samples classified as fast food pizzas, no definitive conclusion can be reached regarding the noted statistical difference in the α -T content of fast food. α - and γ -Tocotrienols were detectable in all the samples, but at levels less than 0.1 mg/100g. The overall mean and standard deviation ($n = 120$) for pizza was 0.9 ± 0.33 mg/100g (α -T) and 1.8 ± 1.11 mg/100g (γ -T). Figure 2.7 shows a bar graph with different mean values of various kinds of pizzas. With reference to the total vitamin E, the values ranged from 2.0 to 3.7 mg/100g. Similar levels were present in cheese, fast food, mixed dish, pepperoni and meat pizzas. The meat and vegetable pizza were lower ($p \leq 0.05$) in total tocopherol content. This can be explained by the fact that vegetables such as onions and peppers have lower vitamin E

levels than other ingredients and fat sources. Relatively large weights of vegetables used as ingredients in such pizzas would decrease the contribution of the crust to the overall weight of the pizza and lessen the contribution of the fat source in the crust to the total vitamin E content. The amount of total vitamin E in the meat and vegetable category was lower (2.0 mg/100g) compared to fast food, mixed dish and pepperoni pizzas ($p \leq 0.05$).

Tocopherols and Tocotrienols in Fast foods

The tocopherols and tocotrienol compositions of various fast foods are shown in Table 2.6. β - and δ -Tocotrienols were not detectable in any of the samples. α -Tocopherol and γ -Tl were detectable in all of the products. α -Tocopherol ranged from 1.3 ± 0.10 mg/100g in French fries to 0.3 ± 0.18 mg/100g in fast food sandwiches. α -Tocopherol values of French fries followed by cheese nachos are much higher in comparison to other categories of fast foods at the 95% confidence interval. γ -T levels were relatively high in cheese nachos (8 ± 0.72 mg/100g), followed by breakfast pastries (6.5 ± 1.61 mg/100g), french fries (3.9 ± 0.96 mg/100g), and fast food sandwiches (0.8 ± 0.83 mg/100g) at $p < 0.05$. α - and γ -Tocotrienols were detectable in all the samples. γ -Tocotrienol was highest in cheese nachos (0.5 ± 0.03 mg). Figure 2.8 shows a bar graph with different mean values of various kinds of fast foods. Total content of vitamin E was significantly higher in cheese nachos followed by breakfast pastries and french fries ($p < 0.05$). Since nachos, and french fries are deep fried in soybean oil they have a high content γ -T along with a higher level of total vitamin E. In full fat cheese, the α -T value ranges from 0.3 to 0.5 mg/100g (Hogarty et al., 1989). According to Carlson and Tabacch (1986), vitamin E loss depends on the type of food being fried, the duration of the use of oil and also replacement of the oil during operation. They also showed that the retention of γ -T was higher than α -T in french

fries fried in soybean oil. Studies on canola oil and its varieties showed that intermittent frying of french fries produces different rates of degradation of vitamin E among the oils (Normand et al., 2001).

Tables 2.8 and 2.9 provide data on the Nutrient Density of pizzas and fast foods, respectively. Nutrient density (ND) is the amount of a particular nutrient (carbohydrate, protein, fat, etc.) per unit of energy in a given food. The ND of total tocopherol content is higher in mixed dish (13.83 mg/kcal) followed by vegetable (13.77 mg/kcal) and supreme pizza (13.72 mg/kcal). The most popular pizza choice is considered to be supreme pizza. According to the Food Industry News, each American eats an average of 23 pounds (46 slices) of pizza per year. They also enlisted that 94% of all American population eats pizza. According to the Gallop poll, children from ages 3 to 11 prefer pizza to all other food groups. Therefore, it can be seen that pizza plays an important role as a good source of vitamin E. The ND of both γ - and α -T are high in pizzas compared to other food groups like white bread (1.11 mg/kcal), peppers (3.73 mg/kcal), raw onions (0.48 mg/kcal) and mozzarella cheese (0.63 mg/kcal). Therefore, pizzas provide considerable amounts of γ - and α -T, which are not only the two most biologically active forms of vitamin E, but also act as antioxidants and play a major role in the prevention or treatment of certain diseases.

Compared to pizza, cheese nachos (43.46 mg/kcal) have much higher ND of total vitamin E followed by breakfast pastries (24.64 mg/kcal) and french fries (18.86 mg/kcal). In comparison with other food groups such as white bread (1.11 mg/kcal), raw beef ground meat (1.54 mg/kcal), mozzarella cheese (0.63 mg/kcal), fast foods have much higher ND. Fast food consumption in US has increased considerably in recent years. According to Bowman et al.

(2004), from the adult U.S. population more African Americans (31%) than other race ethnic group reported eating fast food. Adults living in mid west (29%) or in south (29%) were more likely to be fast food eaters than adults living in the northeast (20%) or the west (22%). Fast Food Consumption Status CSFII 1994-1996 showed that more than one third of the day's energy intakes were provided by fast foods. It is also seen from table 2.9 that fast food like French fries, nachos, etc. contributes a high amount of γ - and α -T per kcal. These antioxidants help to promote good health and prevent certain stress-related disease.

Retention of Tocopherols and Tocotrienols After Baking

The tocopherol and tocotrienol content of cooked and uncooked pizza are shown in Table 2.7. The α -T content of uncooked vegetable pizza is 1.5 ± 0.43 mg/100g; whereas, for the cooked pizza it is 0.8 ± 0.07 mg/100g. The γ -T content of uncooked vegetable pizza is 1.7 ± 0.71 mg/100g; whereas for the cooked pizza it is 1.1 ± 0.08 mg/100g. For vegetable pizza, the retention percentage upon baking was 53.1 for α -T and 68 for γ -T. The decrease in amount of moisture for vegetable pizza after baking was not statistically significant. The α -T content of uncooked supreme pizza was 1.3 ± 0.27 mg/100g. For the cooked pizza, it was 0.8 ± 0.07 mg/100g. The γ -T content of uncooked supreme pizza was 1.5 ± 0.29 mg/100g compared to 1.1 ± 0.08 mg/100g in cooked pizza. For supreme pizza the retention percentage upon baking was 59.7 for α -T and 73 for γ -T. The moisture loss after baking in the supreme and cheese pizzas was statistically significant ($p < 0.05$). There was also a considerable decline in the amount of α - and γ -T after baking. The α -T values for uncooked cheese pizza was 1.0 ± 0.03 mg/100g and 0.9 ± 0.08 mg/100g after baking. The γ -T values for uncooked cheese pizza was 2.0 ± 0.32 mg/100g and 1.8 ± 0.33 mg/100g after baking. The difference in retention value of α -T and γ -T between

the baked and raw variety of supreme, vegetable and cheese pizzas were statistically significant at the 95% confidence interval level. Vitamin E is susceptible to oxidation and heat. The varying stability of the various forms of tocopherols is a complex function of the matrix and processing conditions (Suknark et al., 2001). Therefore, during the baking process, the application of heat reduces the total amount of vitamin E in pizzas. An insignificant loss of all other tocopherol forms (β -T, δ -T and T3s) was found in other pizza varieties. Although vitamin E destruction occurred, the lowest retention value noted in the study was 66% in vegetable pizza for total vitamin E. Therefore, quite a good percentage of the original vitamin E is present at nutritionally significant levels.

Contribution of Pizza to the Dietary Reference Intake (DRI)

Recent statistics indicate that the average U.S. consumer eats 23 lbs of pizza per year. Using the following procedure, the contribution of pizza to the intake of α -T can be calculated:

$$\begin{aligned}
 23 \text{ lbs} \times 454 \text{ g / lbs} &= 10432.57 \text{ g pizza / year} \\
 10432.57 \text{ g pizza / year} \times \text{year} / 365 \text{ day} &= 28.58238 \text{ g pizza / day} \\
 [28.58 \text{ g pizza / day} \times 0.81 \text{ mg } \alpha\text{-T} / 100 \text{ g}] / 100 &= 0.23 \text{ mg } \alpha\text{-T} / \text{day} \\
 [0.23 \text{ mg } \alpha\text{-T} / \text{day} \div 15 \text{ mg / day}] \times 100 &= 1.54 \% \text{ DRI}
 \end{aligned}$$

The value of 1.54 % DRI shows that pizza consumption is an important source of α -T in the U.S. diet. The leading sources of vitamin E are margarine, salad dressing, oil, cereals, oil (vegetable oil), shortening, salad dressing, peanut butter, snacks and eggs ranging from 5.53 % to 1.55 % (Haytowitz, 2003, personal communication). These values are % contribution to the total vitamin E available in the US diet, which are based on total tocopherol content. It is evident that the

contribution of α -T (from pizza) to the DRI is significant. Therefore, pizza ranks as a leading source of vitamin E to the US consumer.

2.4 CONCLUSIONS

Fast foods are a growing component of the American diet. Pizza is one of the most important and popular American foods. Although pizza and other fast foods form an integral part of the American diet, there is no available data on their nutrient composition. Therefore, it is important to make this information available to consumers, especially with reference to energy and macro and micronutrient content of fast foods provided by fast food restaurant franchises. This study has made an attempt to provide information about vitamin E content in pizzas and fast foods. Vitamin E is not only a major antioxidant, but research has shown that it helps to prevent and to some extent even cure many stress-related diseases. The analytical results thus obtained from this study will help to generate a reliable databank on the tocopherol and tocotrienol content of pizzas and fast food.

The objective of this study is two-fold. The first is to provide reliable data on the content of vitamin E in pizzas and fast foods, in their cooked and processed forms, present in the American diet. Secondly, it attempts to find out the amount of vitamin E retention after the process of baking pizza.

Different types of fast foods and pizzas were assayed in order to find out their tocopherol and tocotrienol contents.

1. α -, γ -, δ -Tocopherols was detectable in all the samples. α -, γ -Tocotrienols was also detectable in all samples but at a level less than 0.1mg/100g.

2. Mean % recoveries (n=19) for 6 types of pizzas were 90.5, 92.6 and 88.6 for α -, γ -, and δ -T, respectively. In 5 types of fast foods (n = 16) they were 92.2 for α -, 100.7 for γ -, and 92.4 for δ -T.
3. The γ -T was present at higher levels in pepperoni pizza (2.1 mg/100g) and cheese nachos (8 mg/100g) compared to other types of pizzas and fast foods ($p \leq 0.05$).
4. The value of α -T was higher in fast food pizza (1.1mg/100g) and French fries (1.3 mg/100g) than any other category of pizzas or fast foods ($p \leq 0.05$).
5. In pizzas, α -T ranged from 0.7 mg/100g to 1.1 mg/100g and γ -T values ranged from 0.9 to 2.1 mg/100g.
6. In fast foods, α -Tocopherol ranged from 1.3 ± 0.10 mg/100g to 0.3 ± 0.18 mg/100g and γ -T values ranged from 8mg/100g to 0.8mg/100g.
7. The total content of vitamin E was significantly higher in cheese nachos followed by breakfast pastries ($p \leq 0.05$).
8. γ -Tocotrienols were higher in cheese nachos (0.5 mg/100g) compared to other fats foods.
9. The Nutrient Density of total tocopherol content is higher in mixed dish (13.83 mg/kcal) followed by vegetable (13.77 mg/kcal) and supreme pizza (13.72 mg/kcal). In case of fast foods, cheese nachos (43.46 mg/kcal) have much higher ND of total vitamin E followed by breakfast pastries (24.64 mg/kcal) and french fries (18.86 mg/kcal).
10. There was also a significant moisture loss in supreme and cheese pizzas ($p \leq 0.05$). There was also a significant decline in the amount of α - and γ -T after baking ($p \leq 0.05$).

11. With regard to the retention of vitamin E, the lowest retention value noted in the study was 66% in vegetable pizza for total vitamin E. Although there is some loss in the content of vitamin E due to baking, the cooked pizza still retains a significant amount of vitamin E.

Analysis of fast foods and pizzas shows that these types of food are valuable source of α -T, γ -T and other forms of tocopherols and tocotrienols (vitamin E) to the American consumer's diet.

Table 2.1

Specific Absorption Coefficients ($E_{1\text{cm}}^{1\%}$) and Maximum Wavelengths (λ_{max}) for
Tocopherols in 96% (v/v) Ethanol Solutions.

Tocopherol	λ_{max} nm	$E_{1\text{cm}}^{1\%}$
α -T	292	70.8
γ -T	298	92.8
δ - T	298	91.2

Source: Ball (1998)

Table 2.2

Analytical Recovery Values for Pizza and Various Fast Food Products.

Sample	n	Recovery (%)			
		α -Tocopherol	β -Tocopherol	γ -Tocopherol	δ -Tocopherol
Cheese pizza	3	94.1	-	95.9	92.3
Fast Food Pizza	6	87.7	-	92.9	89.3
Meat and Vegetable Pizza	2	85.9	-	87.8	85.9
Meat Pizza	2	85.9	-	87.8	85.9
Mixed Dish Pizza	4	97.6	-	97.0	91.8
Pepperoni Pizza	2	91.8	-	93.9	86.2
Mean \pm S.D	19	90.50 \pm 4.80	---	92.55 \pm 3.95	88.57 \pm 2.99
Breakfast Sandwiches	7	87.3 \pm 6.96	-	107.2 \pm 98.5	82.6 \pm 8.58
Breakfast Pastries	2	91.0	-	106.8	89.6
Tacos	3	84.7 \pm 4.75	-	105.1 \pm 5.24	87.7 \pm 5.17
Toaster Pastries	2	108.5	-	88.5	104.3
French Fries	2	89.3	--	96.1	97.8
Mean \pm S.D	16	92.16 \pm 9.43	--	100.74 \pm 8.20	92.4 \pm 8.61

Table 2.3

Analytical Recovery Values for Uncooked and Cooked Pizzas.

Sample	n	Recovery (%)			
		α -Tocopherol	β -Tocopherol	γ -Tocopherol	δ -Tocopherol
Supreme Pizza Raw	2	88.3	--	96.0	90.2
Vegetable Pizza Raw	2	78.9	--	79.1	78.8
Cheese Pizza Raw	2	97.2	--	96.4	99.95
Mean \pm S.D (Uncooked)		88.13 \pm 9.15	---	90.50 \pm 9.87	89.65 \pm 10.50
Vegetable Pizza Baked.	2	81.2	--	96.8	92.3
Supreme Pizza Baked	2	95.8	---	94.1	82.4
Cheese Pizza Baked	2	104.10	----	103.56	99.97
Mean \pm S.D (Cooked)		93.70 \pm 11.59		98.45 \pm 4.87	91.56 \pm 8.81

Table 2.4

Moisture Content of Cooked and Uncooked Pizzas.

Sample	n	Mean (%)
Supreme raw pizza	5	54.9 ± 2.01
Supreme baked pizza	5	49.7 ± 2.51
Vegetable raw pizza	5	46.9 ± 6.71
Vegetable baked pizza	5	46.4 ± 6.19
Cheese raw pizza	5	49.7 ± 1.20
Cheese baked pizza	5	45.5 ± 0.25

Table 2.5Tocopherol and Tocotrienol Contents of Pizzas (mg/100g) ^a

Pizzas	No	α -T ^b	β -T	γ -T	δ -T	α -T3 ^c	γ -T3	Total	α -TE
Cheese	25	0.8 ^B	0.1 ^A	1.7 ^A	0.5 ^{AB}	0.1 ^A	0.02 ^A	3.0 ^{AB}	1.0 ^A
Fast Food ¹	37	1.1 ^A	0.1 ^A	1.8 ^A	0.6 ^{AB}	0.1 ^A	0.01 ^A	3.7 ^A	1.3 ^A
Mixed Dish ²	25	0.8 ^B	0.04 ^A	1.9 ^{AB}	0.6 ^A	0.03 ^A	-----	3.5 ^A	1.0 ^A
Meat and Vegetable	7	0.7 ^B	0.04 ^A	0.9 ^B	0.3 ^B	0.04 ^A	0.01 ^A	2.0 ^B	0.9 ^A
Pepperoni	8	0.7 ^B	0.1 ^A	2.1 ^{AB}	0.7 ^A	0.03 ^A	0.02 ^A	3.6 ^A	1.0 ^A
Meat	6	0.7 ^B	0.1 ^A	1.2 ^A	0.4 ^{AB}	0.1 ^A	0.01 ^A	2.4 ^{AB}	1.0 ^A

^a Mean \pm S.D; ^b T= tocopherol; ^c T3= tocotrienol;¹ Sample collected from fast food restaurants like Papa Johns, Pizza Hut, Dominos etc.² Sample collected from local retail stores.^{A, B} Means with same letters within columns are not significantly different (statistically) in a F-test ($p \leq 0.05$).

Table 2.6Tocopherol and Tocotrienol Contents of Fast Food Products (mg/100g) ^a

Fast Foods	No	α -T ^b	β -T	γ -T	δ -T	α -T3 ^c	γ -T3	Total	α -TE
Breakfast Sandwiches	63	0.8 ^C	0.04 ^A	2.6 ^{CD}	1.2 ^B	0.05 ^A	-----	4.6 ^{DC}	1.1 ^A
Cheese Nachos	4	1.1 ^B	0.04 ^A	8.0 ^A	3.2 ^A	0.2 ^A	0.5 ^A	13.3 ^A	2.0 ^A
Fast Food Sandwiches	14	0.3 ^D	0.02 ^A	0.8 ^E	0.4 ^C	0.1 ^A	-----	1.5 ^E	0.4 ^A
French Fries	4	1.3 ^A	0.04 ^A	3.9 ^C	1.2 ^B	-----	0.1 ^A	6.5 ^C	1.8 ^A
	16	1.0 ^{BC}	0.1 ^A	6.4 ^B	2.7 ^A	0.1 ^A	-----	10.2 ^B	1.7 ^A
Breakfast Pastries	32	0.4 ^D	0.03 ^A	1.6 ^{DE}	0.7 ^{BC}	0.1 ^A	0.1 ^A	2.8 ^{DE}	0.6 ^A
Tacos									

^a Mean \pm S.D; ^b T= tocopherol; ^c T3= tocotrienol;^{A,B,C,D,E} Means with same letters within columns are not significantly different (statistically) in a F-test ($p \leq 0.05$).

Table 2.7Tocopherols and Tocotrienols in Uncooked and Cooked Pizza (mg/100g) ^a

Pizzas	No	α -T ^b	β -T	γ -T	δ -T	α -T3 ^c	γ -T3	Total	α -TE
Vegetable, Uncooked	5	1.8 ^A	0.1 ^A	1.9 ^A	0.3 ^A	0.1 ^A	0.1 ^A	4.4 ^A	2.1 ^A
Vegetable, Cooked	5	1.0 ^B	0.1 ^A	1.4 ^B	0.2 ^B	0.1 ^A	0.1 ^A	2.9 ^B	1.2 ^A
Supreme, Uncooked	5	1.3 ^A	0.1 ^A	1.5 ^A	0.3 ^A	0.1 ^A	0.1 ^A	3.4 ^A	1.6 ^A
Supreme, Cooked	5	0.8 ^B	0.1 ^A	1.1 ^A	0.2 ^A	0.1 ^A	0.1 ^A	2.3 ^B	0.9 ^A
Cheese, Uncooked	5	1.0 ^A	0.1 ^A	2.0 ^A	0.6 ^A	0.1 ^A	-----	4.0 ^A	1.3 ^A
Cheese, Cooked	5	0.9 ^A	0.01 ^A	1.8 ^A	0.5 ^A	0.1 ^A	-----	3.6 ^A	1.2 ^A

^a Mean \pm S.D; ^b T= tocopherol; ^c T3= tocotrienol;^{A, B} Means with same letters within columns are not significantly different (statistically) in a F-test ($p \leq 0.05$).

Table 2.8Nutrient Density of Different Types of Pizzas (mg vitamin E/kcal)¹

Pizzas	Kcal/100g ^a	α -T ^b	β -T	γ -T	δ -T	α -T	γ -T3	Total	α -TE
Cheese	286	2.76	0.17	5.77	1.82	0.17	0.07	10.63	3.53
Fast Food ²	286	3.88	0.21	6.43	1.96	0.28	0.03	12.76	4.76
Mixed Dish ³	253	3.20	0.16	7.71	2.61	0.12	0.00	13.83	4.19
Meat and Vegetable	276	2.64	0.14	3.33	1.01	0.14	0.04	7.28	3.12
Pepperoni	287	2.37	0.17	7.39	2.40	0.10	0.07	12.54	3.31
Meat	276	2.68	0.18	4.38	1.30	0.25	0.04	8.80	3.33
Supreme	253	5.26	0.32	6.09	1.15	0.43	0.43	13.72	6.21
Vegetable	276	5.33	0.29	6.30	1.09	0.43	0.33	13.77	6.27

^akcal/100g derived from USDA Nutrient Databank Values (USDA National Nutrient Database for SR (17), 2004); ^bT = Tocopherol; ^cT3 = Tocotrienol;

¹ Nutrient density of Tocopherol = mg Tocopherol/kcal * 1000

² Sample collected from fast food restaurants like Papa Johns, Pizza Hut, Dominos etc.

³ Sample collected from local retail stores.

Table 2.9Nutrient Density of Different Types of Fast Foods (mg vitamin E/kcal)¹

Fast Foods	Kcal/100g	α -Tb	β -T	γ -T	δ -T	α -T	γ -T3	Total	α -TE
Breakfast Sandwiches	401	2.02	0.09	6.55	2.86	0.12	0.00	11.50	2.50
Cheese Nachos	306	3.43	0.13	26.18	10.62	0.65	1.63	43.46	6.70
Fast Food Sandwiches	243	1.11	0.08	3.29	1.69	0.21	0.21	6.17	1.52
French Fries	342	3.83	0.12	11.26	3.51	0.00	0.00	18.86	5.12
Breakfast Pastries	412	2.15	0.22	15.56	6.56	0.14	0.00	24.64	4.07
Tacos	216	2.04	0.14	7.22	3.19	0.37	0.37	13.01	2.96

^akcal/100g derived from USDA Nutrient Databank Values(USDA National Nutrient Database for SR (17), 2004);; ^bT = Tocopherol; ^cT3 = Tocotrienol;

¹ Nutrient density of Tocopherol = mg Tocopherol/kcal * 1000.

Table 2.10Retention (%) of Tocopherols and Tocotrienols in Pizzas¹

Pizza	α -T ^a	γ -T	δ -T	Total
Vegetable	56	74	67	66
Supreme	62	73	67	68
Cheese	90	90	83	90

^aT = Tocopherol

¹ Percentage value of retention

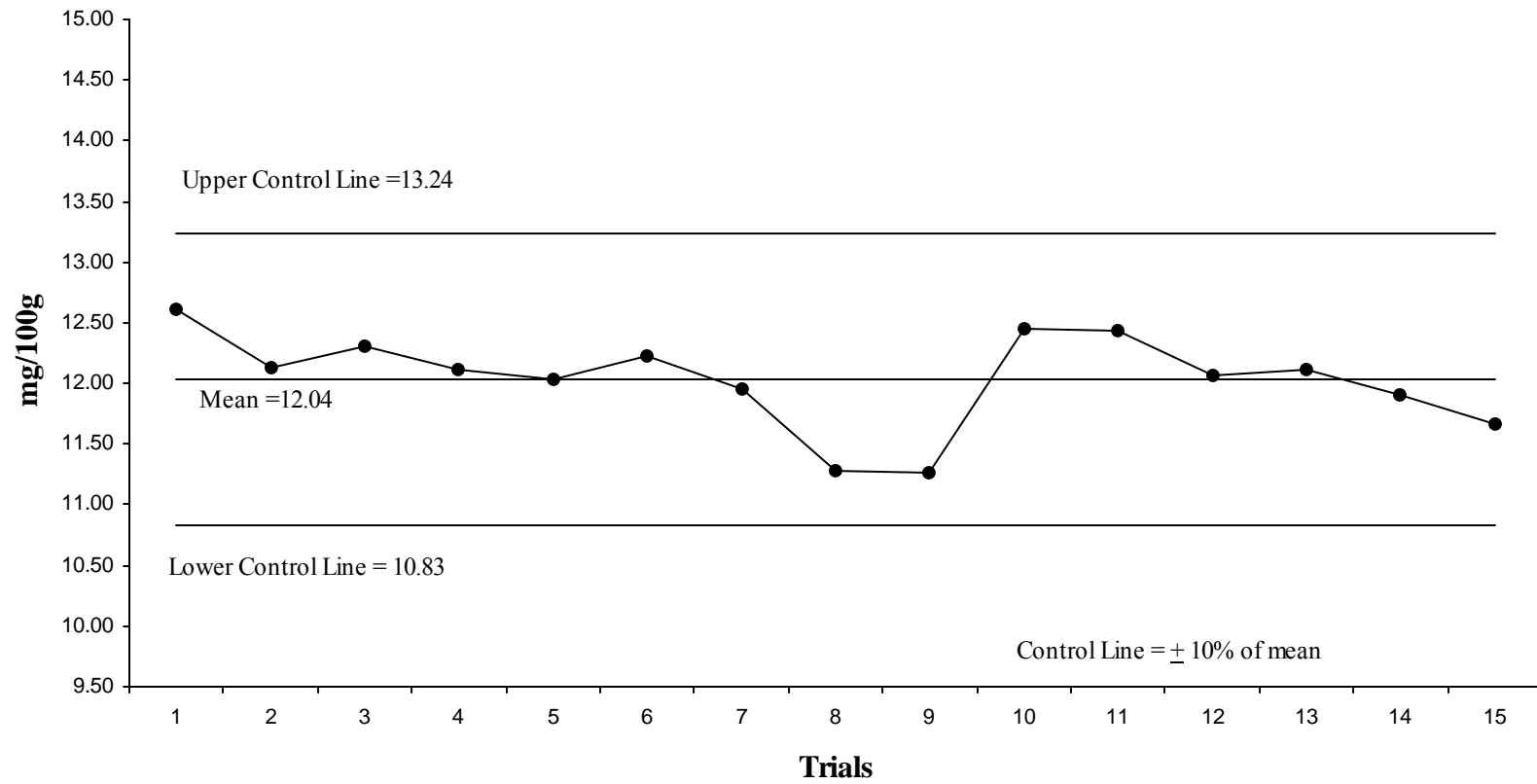


Figure 2.1
Control Chart for Alpha-Tocopherol in Soybean Oil

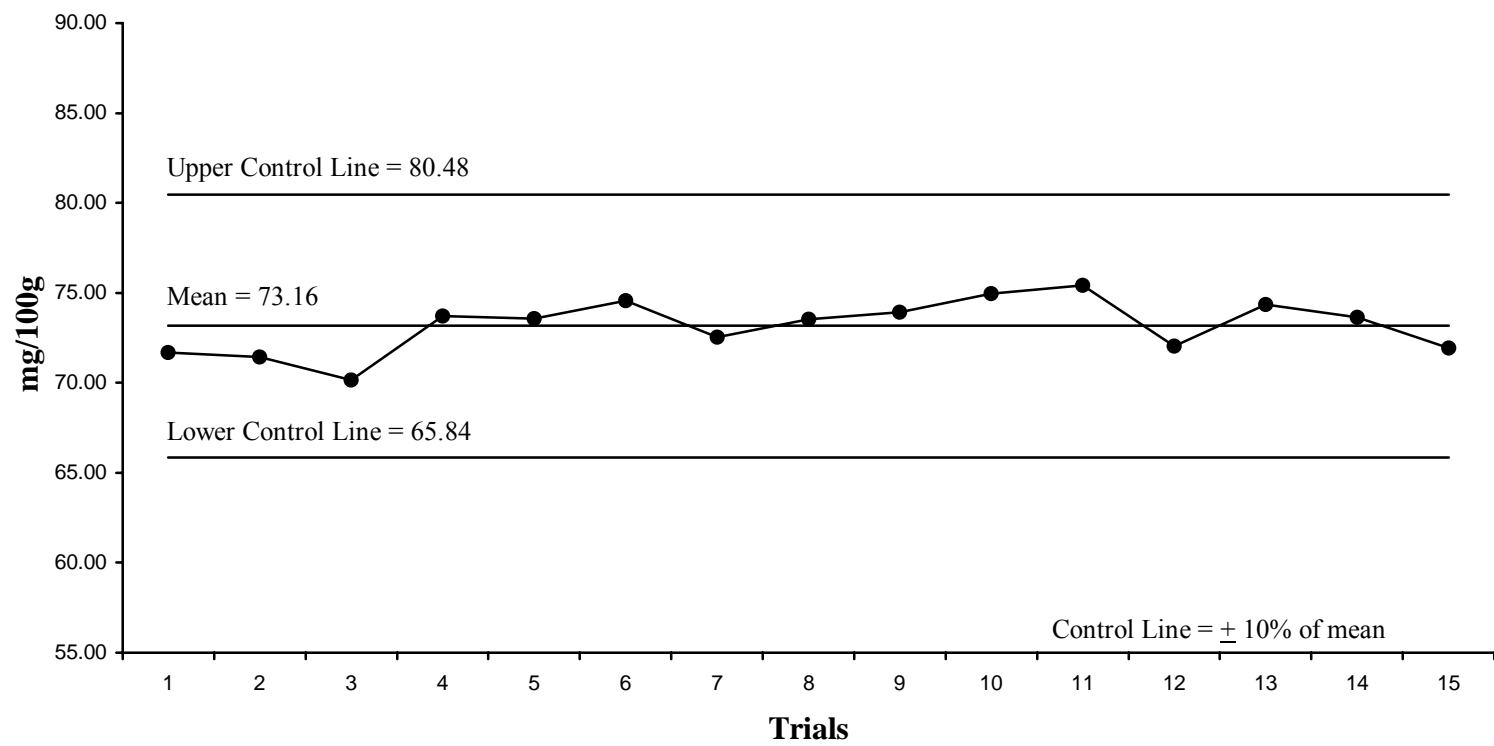


Figure 2.2
Control Chart for Gamma-Tocopherol in Soybean Oil

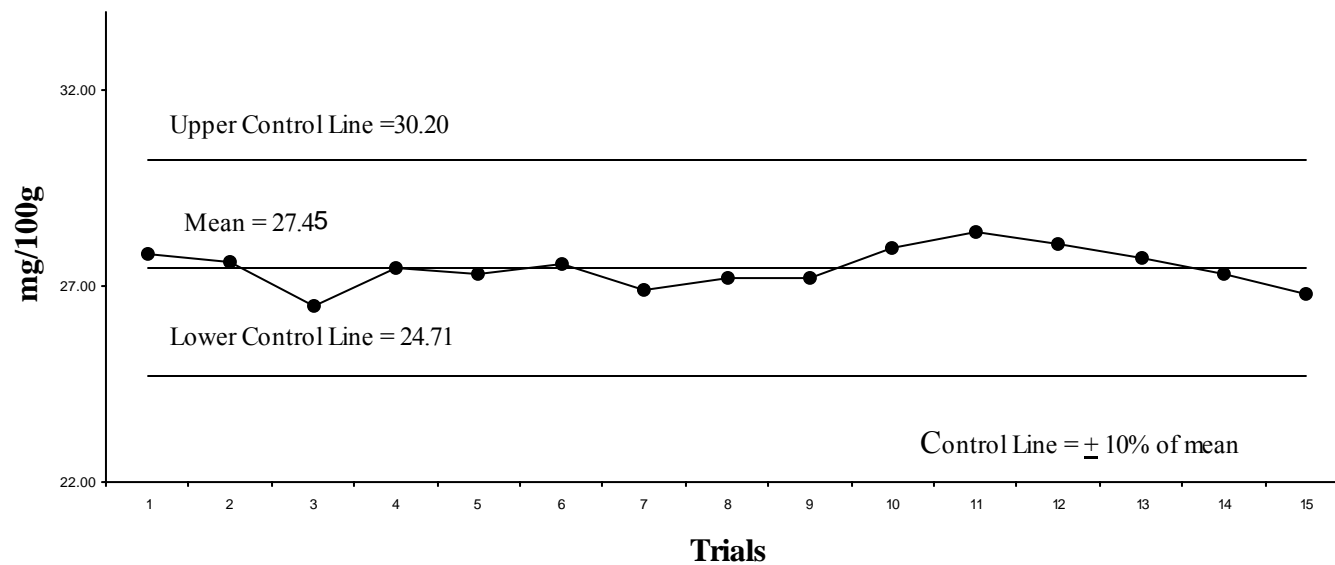


Figure 2.3
Control Chart for Delta-Tocopherol in Soybean Oil

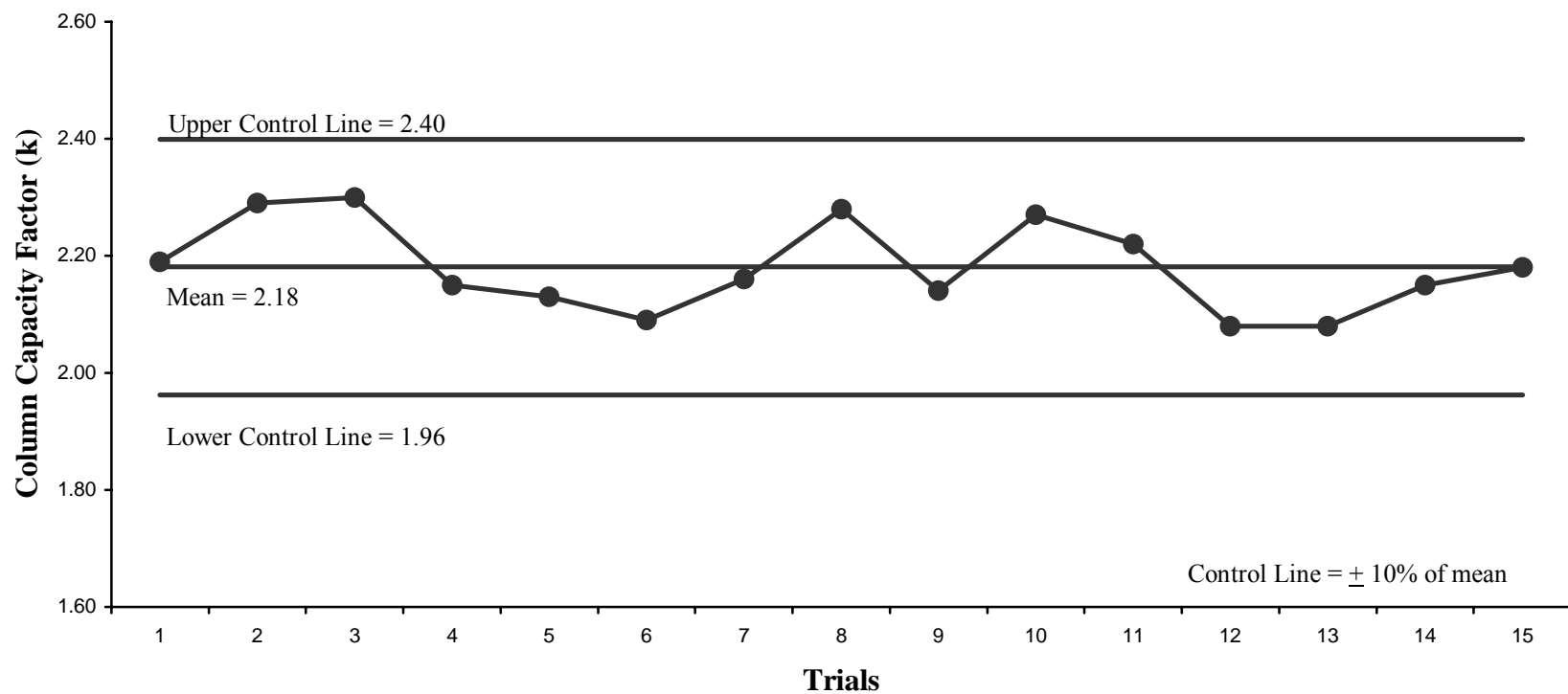


Figure 2.4
Control Chart for Column Capacity Factor (k) of Gamma-Tocopherol

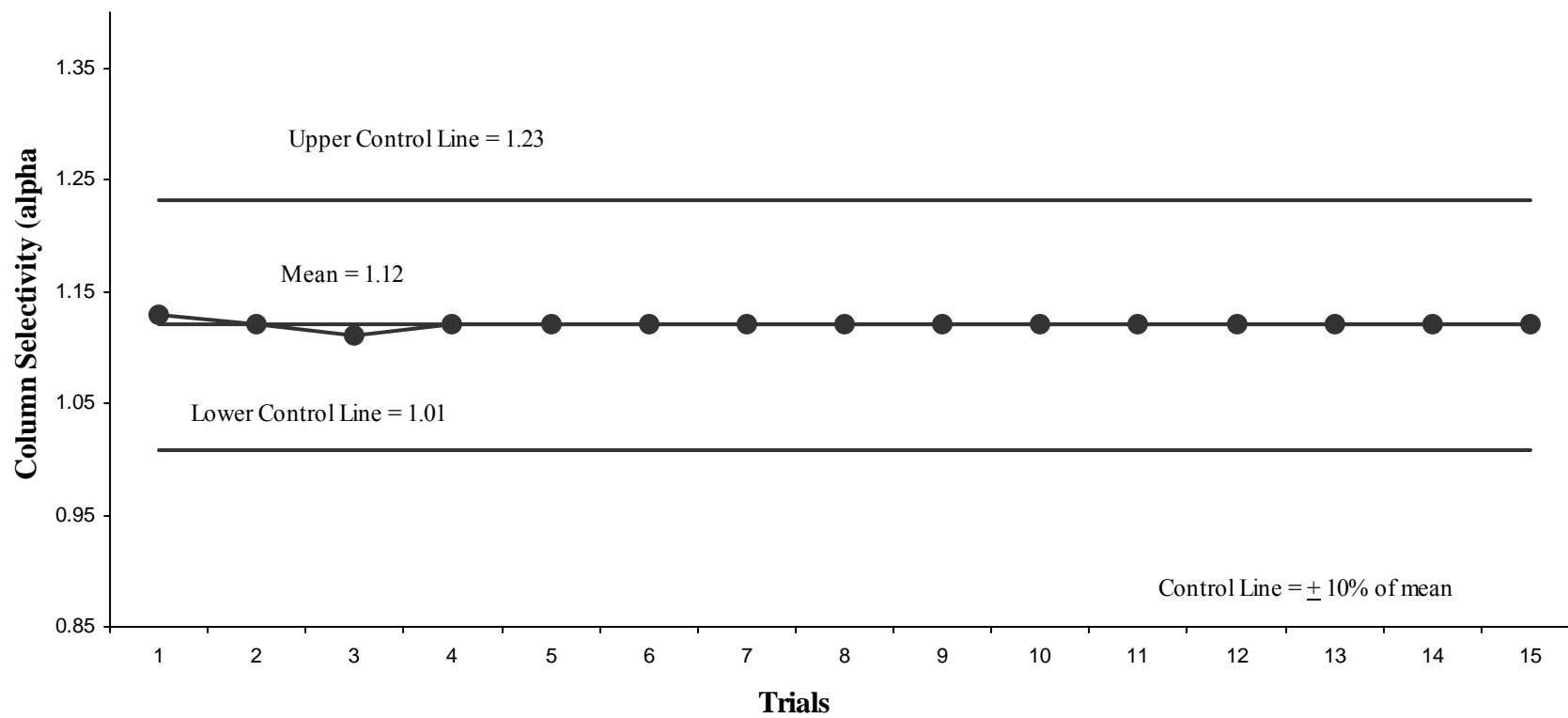


Figure 2.5
Control Chart for Column Selectivity (alpha) of Gamma-Tocopherol

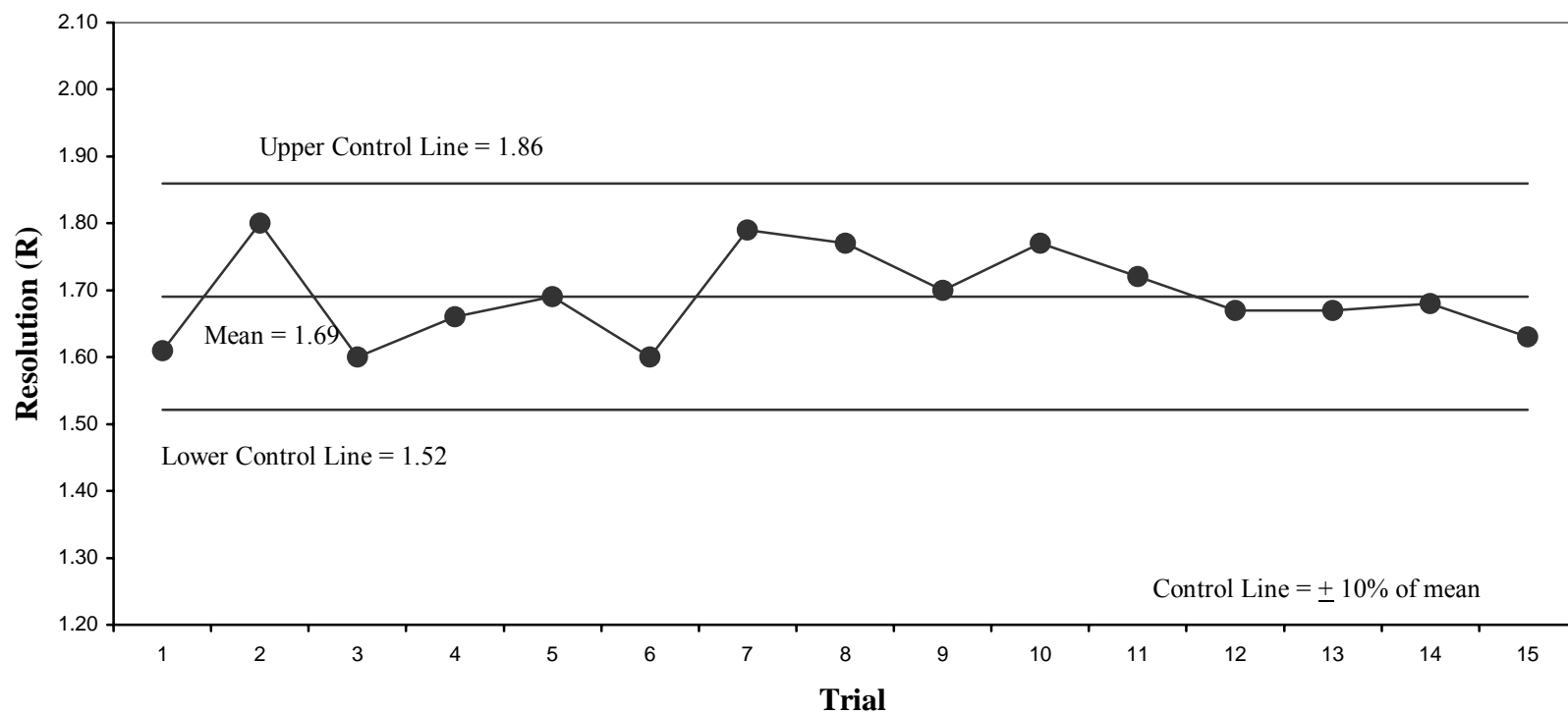


Figure 2.6
Control Chart for Resolution (R) of Gamma-Tocopherol

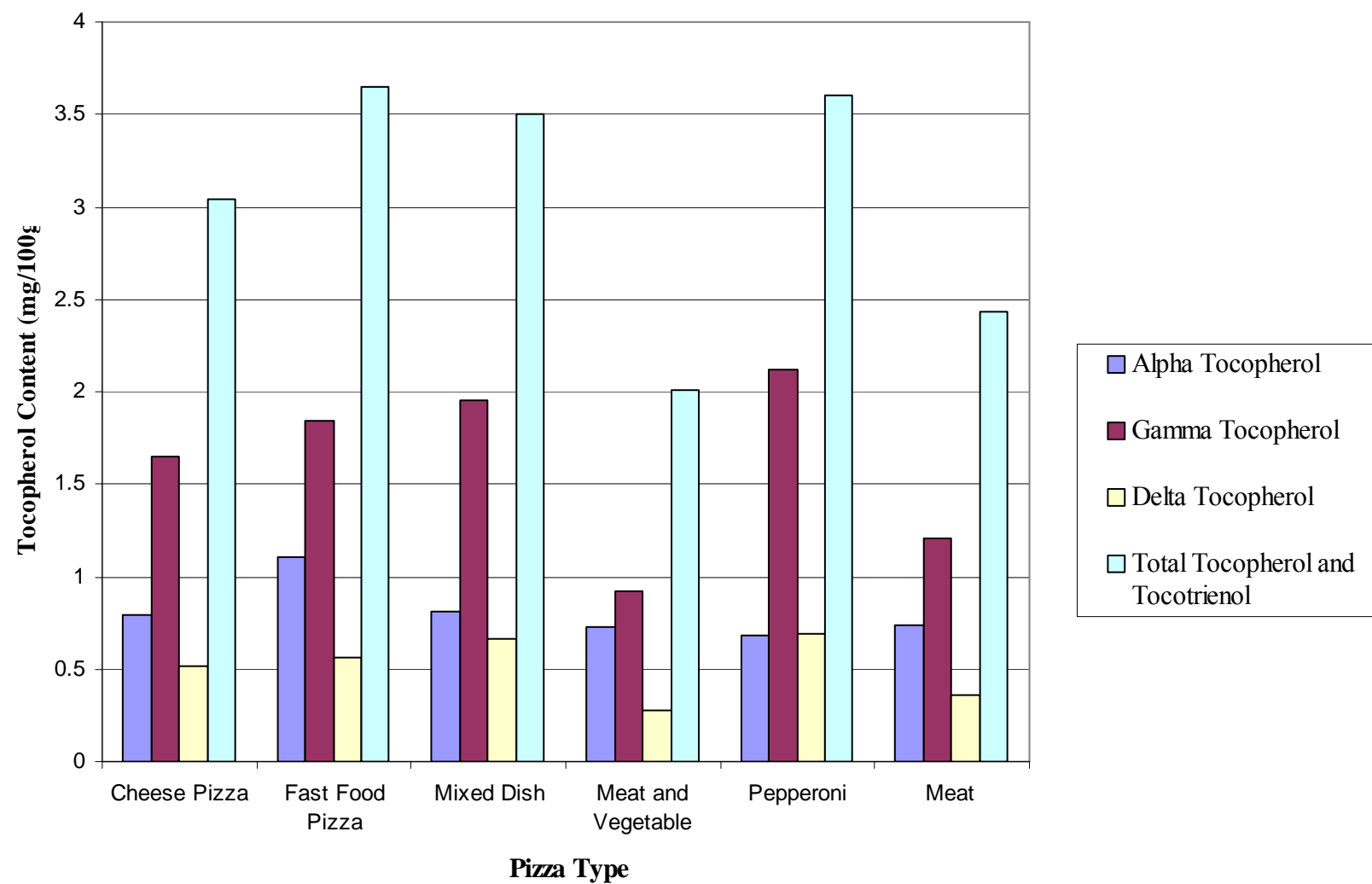


Figure 2.7
Tocopherols in Pizza

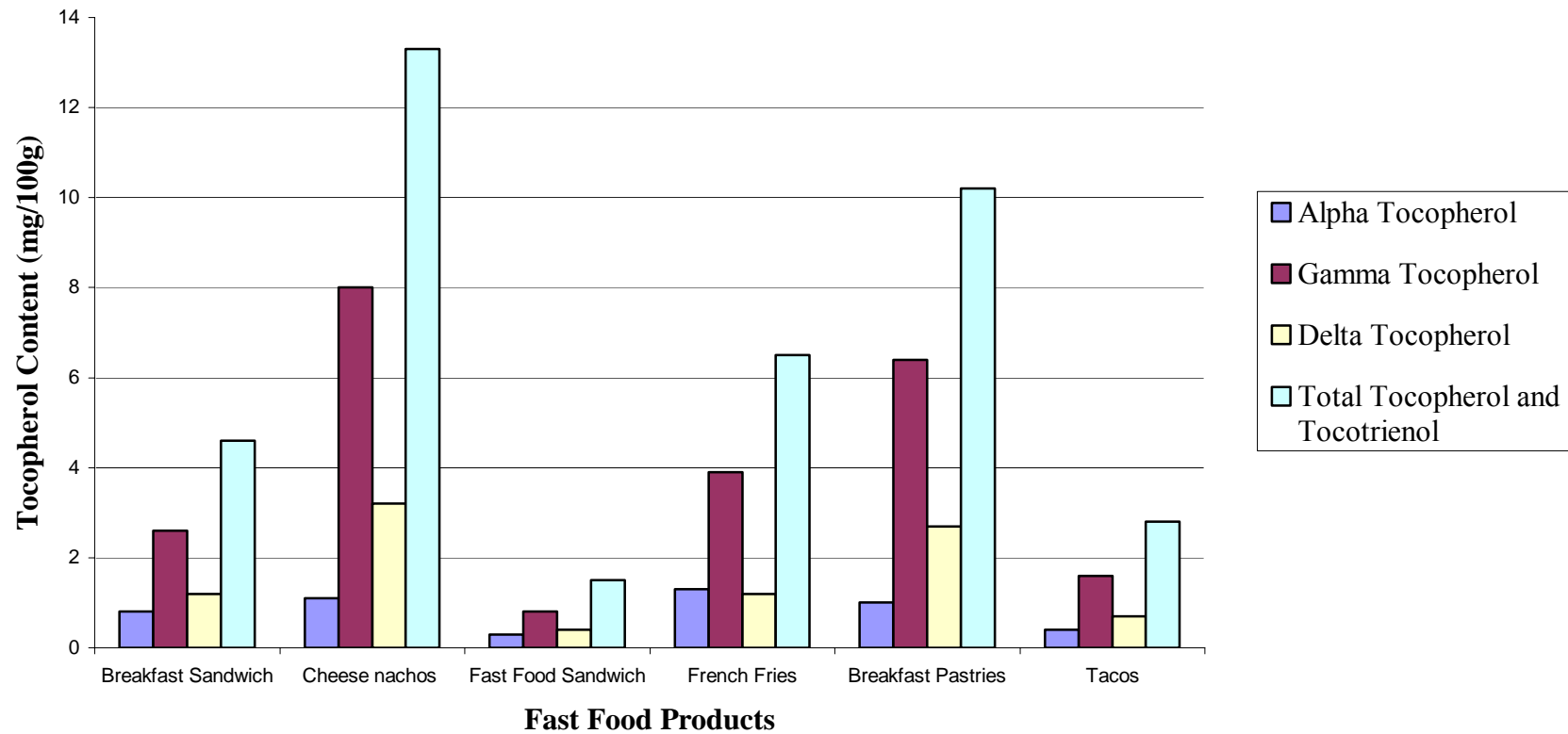


Figure 2.8
Tocopherols in Fast Foods.

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