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Oxidative stability of corn oil with elevated tocotrienols

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Oxidative stability of corn oil with elevated tocotrienols

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ABSTRACT

Oxidation of tocotrienol enriched corn oil was measured for primary oxidative products, lipid hydroperoxides and quantified by peroxide value (PV). Oxidative Stability Index (OSI) was used to determine the induction period (hrs) by indirect measurement of volatile secondary oxidation products, mainly formic acid. Vitamin E stripped corn oil samples were spiked with individual tocotrienols or tocopherols (collectively, tocols) at concentrations between 100 and 5,000 ppm. A positive relationship was observed between the concentration of all tocols and inhibition of the formation of secondary products. Gamma tocotrienol (γ-T3) provided the most protection, with delta tocopherol (δ-T) and delta tocotrienol (δ-T3) providing similar protection. Alpha tocotrienol (α-T3) and alpha tocopherol (α-T) followed a similar trend but with diminishing capacity at concentrations higher than 700 ppm. The change in mean daily peroxide value increased as α- tocopherol and α- tocotrienol concentrations increased. When compared against the non-spiked, stripped control oil, both α- tocopherol and α- tocotrienol demonstrated better antioxidant effects at lower concentrations and actually promoted oxidation at concentrations at 700 ppm and above. These effects were not observed with the γ- and δ- tocols.

Crude oils from corn kernels, both control and that expressing a homogentisate geranylgeranyl transferase (HGGT) gene, were tested for oxidative stability. No pro-oxidant effects were observed in the modified crude corn oil containing up to 5,000 ppm tocotrienols (6,200 ppm total tocotrienols and tocopherols) when compared to the control crude corn oil containing 300 ppm tocotrienols (1,500 ppm total tocols).

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Project Significance

As a product of biotechnology, oil extracted from kernels of homogentisic acid geranylgeranyl transferase modified corn can now contain elevated concentrations of tocotrienols, exceeding twenty fold the concentration in unmodified corn [1]. The potential oxidative impact of these active lipophilic, phenolic compounds must be better understood to determine the food value of corn oil with significantly increased tocotrienols. As both the composition and concentrations of tocotrienols can be manipulated through biotechnology and processing, the effects of specific tocotrienols and their concentration should also be examined. It is the goal of this research to elucidate the role tocotrienols have in corn oil stability and oxidation. Recent publications have identified the potential health benefit of tocotrienols *in vivo* [2, 3]. The possible nutritional or therapeutic benefits are beyond the scope of this project, but the research offers insight into the oxidative properties of using this domestically supplied oil as a feedstock for other processes, products or prepared foods.

General Background

Tocotrienols and tocopherols comprise a group of eight natural, plant produced, homologs that are exploited for their ability to quench free radicals and retard oxidation in lipids and lipid systems [4-6]. Their structures consist of a chromanol head and non polar hydrocarbon tail. Tocotrienols (T3) have an isoprenoid tail with three double bonds. The tocopherol phytyl tail is fully saturated. The degree and position of methylation on the phenolic ring is denoted by α- (5, 7, 8 trimethyl), β (5, 8-dimethyl), γ- (7, 8-dimethyl) and δ- (8-methyl) (Figure 1). Although these eight, fat soluble compounds are commonly referred to as vitamin E or vitamin E complex, only α- tocopherol is recognized currently as vitamin

E due to the discovery of the biologically active α- tocopherol transport protein [7]. The other tocols show varying degrees of vitamin E activity as measured in the rat resorption-gestation test relative to α- tocopherol, but none as high [8]. Beta tocopherol has a relative biological activity of 50%, α- tocotrienol of 30% and the others are lower still with δ-tocopherol of 3% of that of α- tocopherol. The values for γ- and δ- tocotrienols are unknown or have not been reported.

Tocopherols and tocotrienols, collectively known as tocols or chromanols [8] are commonly found in many plants. Due to their lipid solubility, tocols are concentrated in the extracted oils of vegetative tissue, grains and seeds, including canola, sunflower, corn, cottonseed, palm, palm kernel, soybean and peanut [5, 9, 10]. These vegetable oils are the primary sources of vitamin E (α- tocopherol), an essential nutrient in the human diet. Tocopherols are expressed preferentially in most plants including corn, while higher concentrations of tocotrienols are found in palm, rice, barley and some other monocots [11, 12].

The lack of tocotrienols, relative to tocopherols, in corn represents a limitation to, as well as an opportunity for, enhanced antioxidative and nutritional properties of the oil. Genetic modification is a viable tool to introduce significant amounts of tocotrienols in corn [13, 14]. Flux of carbon into tocotrienols is regulated by the substrate specificity of homogentisic acid geranylgeranyl transferase (HGGT). Thus, introducing the gene encoding HGGT from barley, a crop that produces tocotrienols, should result in significant accumulation of these tocols in corn. This was indeed the case following the work of Cahoon and coworkers [1]. A six-fold increase in total tocols, mostly tocotrienols, was reported in HGGT-expressing corn kernels. This increase did not come at the expense of tocopherol content that remained relatively stable in the modified material.

Due to the many nutritional and health claims surrounding tocols, tocopherols and tocotrienols are found widely in dietary supplements. Tocopherols have been studied for

their health benefits for many years [3]. There is also growing interest in the benefits of tocotrienols due to their recently reported efficacies over tocopherol to reduce cholesterol, suppress tumors and inhibit platelet aggregation [2, 15]. Because tocotrienols are functionally unique *in vivo* from tocopherols [16], it has also been advocated that tocotrienols be classified, in the broadest definition, as nutrients [17].

The main function of the endogenous tocols is antioxidation within the plant system. Vegetable oils also benefit from the tocols as the tocols at low concentration disrupt autoxidation of the double bonds contained in unsaturated fatty acids. The mechanisms of oxidation of unsaturated lipids have been well studied and characterized and most often occur as a free radical chain reactions [5, 8, 18]. This reaction proceeds in three steps: initiation, propagation and termination. Tocols can be classified as propagation inhibitors that stabilize the lipid peroxyl radicals [5].

Thesis Organization

This thesis is organized into four chapters. Chapter 1 is a general introduction and literature review. Chapters 2 and 3 are two individual manuscripts to be submitted to the Journal of the American Oil Chemists' Society. Chapter 4 contains general conclusions.

Literature Review

History

Herbert Evans and Katherine Scott Bishop first reported in 1922 on the essential nutritive and fertility properties of a dietary factor in rats and later designated it as vitamin E [19]. The compounds were isolated by Evans and others from wheat germ oil and lettuce leaf [20]. The compounds were characterized as alcohols and the trivial name of tocopherols, as suggested by George M. Calhoun, was used by Evans and his research group. The structure of α-tocopherol was elucidated by E. Fernholz and published in 1938 [21]. The distinction between the tocotrienols and tocopherols would come 26 years later, as reported by Bunyan and others [22]. They suggested the nomenclature for tocotrienols and continued to study the biological potency of these compounds and tocol activity in rats. The structure and chromatographic separation of the tocotrienols from tocopherols were reported by Pennock in 1964 [23]. In 1981, the International Union of Pure and Applied Chemistry (IUPAC) set the standard for nomenclature (http://www.chem.qmul.ac.uk/iupac/misc/toc.html, 10/25/2009) but trivial names are still commonly used [24].

The research on antioxidant properties of tocotrienols is intertwined with that of tocopherols due to the structural similarities of the chromanol head group and the testing of natural products that often contained mixtures of differing tocols in dissimilar proportions or synthetic products that contained numerous isomerisms. Tocotrienols have been at times, relegated to afterthoughts. Often reported as vitamin E or an impurity of tocopherols, the literature and study of tocotrienols in non-biological systems are extremely sparse when compared to membrane systems, *in vivo* research or to the volume of work with tocopherols [2, 4, 25]. As research found that the bio-kinetics and bio-potency of tocotrienols offered unique function properties not found in tocopherols as a result of the unsaturated tail of the

former, considerably more resources were being devoted to the research of tocotrienols, mostly from a biological or health perspective.

Similar to the different iso-forms or homologues of tocopherols, tocotrienol homologues exhibit different antioxidative properties based on degree of methylation. The antioxidative properties of tocols and mechanisms have been well studied in model lipid systems, but models are limited in their usefulness to real world applications [7]. The kinetics of oil oxidation with added antioxidants become very complicated and dynamic as subtle differences in environmental parameters and substrates can shift the kinetics, making extrapolation to complex food systems or biological systems less than straightforward [5].

Effects of tocotrienols on oil oxidation *in vitro*

The relative rates of lipid oxidation and tocol breakdown in model lipid systems and stripped oils have been studied, but full consensus has yet to be reached on absolute rates in complex systems. None of the previous literature has explored the effects of the high concentrations of tocotrienols in oils now available in products of genetic modification on lipid oxidation. Most of the experiments that have been conducted used concentrations typically found in natural products although a recent dissertation by Kim [26] explored oxidative properties of tocotrienol concentrations up to 1,000 ppm spiked in lard. Coconut fat with 1,000 ppm of tocotrienols has also been studied [27].

Chow and Draper [28] measured lipid hydroperoxide formation and tocol degradation of native tocols in corn oil and found no significant differences in oil oxidation rates between the tocopherol and tocotrienol homologues in both the α- and γ- form. As percentage of initial concentration, α- homologues were degraded more rapidly than γ-, but in terms of absolute amount, more γ- was destroyed than the α- form.

Similar results were reported by Lehmann and Slover using model systems spiked with individual tocols at 500 ppm and tocol mixtures with 500 ppm of each individual

compound [29]. At elevated temperatures, the model lipids, methyl myristate and methyl linoleate, were both photo-oxidized and autoxidized with increased oxygen exposure. Lipid oxidation was measured as peroxide value and degradation/loss of the added tocols. They observed that the α-homologues were the most active antioxidant followed by the β-, γ- and δ-forms. In methyl myristate, there were no differences in the stabilities of α-tocopherol and α-tocotrienol. In methyl linoleate, α-tocopherol was slightly less stable than α-tocotrienol [29].

Using individual tocotrienols from palm oil distillate, Yamaoka and others [30] used weight gain of methyl linoleate spiked with 200 and 500 ppm α-tocols at and 500 ppm γ-tocols at to determine rates of oxidation. The induction period, as rate of O_2 uptake and subsequent weight gain of the tocotrienol spiked material, was equal to or greater than the same tocopherol homologue at the same concentration. γ-Tocols at 500 ppm were far more effective than α-tocols at 200 or 500 ppm. α-Tocol at 200 ppm were the least effective but superior to the untreated control.

The OSI induction period of tocotrienol-spiked coconut fat at 160^{o}C increased as tocotrienol concentrations increased up to 1,000 ppm, implying a positive contribution to frying oil stability [27]. At 60^{o}C, the addition of α- and β-tocotrienols increased fat oxidation as measured by PV. This prooxidant effect increased with increasing tocotrienol concentrations. Both γ- and δ-tocotrienols had the opposite effect and reduced lipid hydroperoxide formation, although the effect was not correlated with γ-tocotrienol concentration [27].

In the dissertation of Hyun Jung Kim [26], the role of tocotrienols as antioxidants in lard was investigated. Lard, spiked with individual tocotrienols at 0, 100, 200, 300, 500 and 1,000 ppm concentrations, was sealed with headspace and oxidized in the dark at 55^{o}C for seven days. PV and percent oxygen in the headspace were measured daily. With the exception of the PV for 1,000 ppm α-tocotrienol, all the spiked fat samples were more

resistant to oxidation than the control. The α- and the β-tocotrienols were most effective at 100 ppm concentration with insignificant differences at higher concentrations. The concentrations of γ- and δ-tocotrienols did not exhibit significant differences on antioxidant properties under the conditions reported. α-Tocotrienol was less effective than the others. Thus, 100 ppm of tocotrienols could be the optimum and most economical level in lard [26].

Effects of tocotrienols *in vivo*

Tocols are an essential nutrient to preserve fertility in mammals and are an effective antioxidant in membranes. Vitamin E must be obtained from the diet. In general nutrition, the term vitamin E is often used to encompass these eight specific tocol compounds [9]. The ability to act as chain-breaking antioxidants *in vivo* suggests that the damage by free radical oxidation and their products is reduced by dietary tocols [31]. Vitamin E may also help ameliorate the effects of some chronic diseases, especially cardiovascular disease.

Differences in bioavailability and biopotency between the homologues of the individual compounds suggest highly specific biological transport. Beyond antioxidative properties, individual tocols and specifically α-tocopherol exhibit cell signaling functions and affect protein expression. T3 has garnered recent interest as a number of studies reported health benefits for tocotrienols that are not found with tocopherols [2, 3, 25].

The reported benefits of tocotrienols include the inhibition of cholesterogenesis and associated reduction in coronary heart disease [25, 32]. By lowering plasma triacylglycerides, tocotrienols may reduce the effects of metabolic syndrome. Tocotrienols have shown promise to control tumor growth and suppress growth of specific melanoma cells *in vitro* and in animal studies. There may also be synergistic advantages to using tocotrienols with other chemotherapies [33].

Sourcing

As health claims for tocopherols and tocotrienols become more widespread, these compounds or nutraceuticals have become readily available in many health and retail stores as well as on the worldwide internet. These supplements vary in formulation and sourcing feedstock, such as palm oil and rice bran oil. Although generally considered not to be a safety concern for the general population at current usage, extensive data for tocotrienols are lacking [34]. Beyond supplements, tocopherols, and increasingly tocotrienols, are being used as functional ingredients in processed food and cosmetics [35, 36].

There are many dietary sources of tocols but all tocols are synthesized originally in plants. Although found in all plant tissues, tocols are most abundant in plant seeds and green tissue [37]. Main sources of tocols are tree nuts, such as walnuts and pecans, and peanuts. High concentrations of tocotrienols can be found in palm fruit, rice bran, barley, oat and wheat germ and their oils. Due to their lipophilic properties, tocols become concentrated in vegetable oils during extraction, but losses may occur during processing, including deodorization of the refined oil. Tocopherols represent most of the tocols in the US and European diets [34, 37].

Using molecular biology to manipulate metabolic pathways in plants for increased tocol production and varying their compositions has been reviewed and accomplished by Cahoon and others in 2003 [1, 14]. The assumption is that genetic modification of the tocopherol and tocotrienol biosynthetic pathways can enhance the plant, the seed and products derived from the grain. These benefits include reducing the oxidative stress of the plant, increasing crop productivity, increasing storage duration of seed and plant material, improving shelf life of vegetable oils, and meet the increasing demands for nutraceutical or therapeutic markets [1]. As research continues on the genetics and biochemistry of tocol biosynthesis, further enhancements to tocol fortified-seeds are expected.

References

1. Cahoon EB, Hall SE, Ripp KG, Ganzke TS, Hitz WD, Coughlan SJ (2003) Metabolic Redesign of Vitamin E Biosynthesis in Plants for Tocotrienol Production and Increased Antioxidant Content. Nature Biotechnology 21:1082-1087
2. Theriault A, Chao JT, Wang Q, Gapor A, Adeli K (1999) Tocotrienol: A Review of Its Therapeutic Potential. Clinical Biochemistry 32:309-319
3. Watson RR, Preedy VR (2009) Tocotrienols: Vitamin E Beyond Tocopherols. CRC Press, Boca Raton, FL
4. Gunstone FD (1994) Chemical Properties. In: Gunstone FD, Harwood JL, Padley FB (eds) The Lipid Handbook, 2nd edn. Chapman & Hall, London, pp 566-571
5. Frankel EN (1998) Lipid Oxidation The Oily Press, Dundee
6. Choe E, Min DB (2009) Mechanisms of Antioxidants in the Oxidation of Foods. Comprehensive Reviews in Food Science and Food Safety 8:345-358
7. Frankel EN (2007) Antioxidants in Food and Biology: Facts and Fiction. The Oily Press, PJ Barnes and Associates, Bridgewater
8. Kamal-Eldin A, Appelqvist LA (1996) The Chemistry and Antioxidant Properties of Tocopherols and Tocotrienols. Lipids 31:671-701
9. Akoh CC, Min DB (2002) Food Lipids: Chemistry Nutrition and Biotechnology, 2nd edn. Marcel Dekker, Inc., New York
10. Stone WL, Papas A (2003) Tocopherols, tocotrienols and vitamin E. In: Gunstone FD (ed) Lipids For Functional Foods And Nutraceuticals. The Oily Press, PJ Barnes and Associates, Bridgewater
11. Collakova E, Dellapenna D (2003) The Role of Homogentisate Phytyltransferase and Other Tocopherol Pathway Enzymes in the Regulation of Tocopherol Synthesis during Abiotic Stress. Plant Physiology 133:930-940
12. Li Y, Wang ZN, Sun XF, Tang KX (2008) Current Opinions on the Functions of Tocopherol Based On the Genetic Manipulation of Tocopherol Biosynthesis in Plants. Journal of Integrative Plant Biology 50:1057-1069

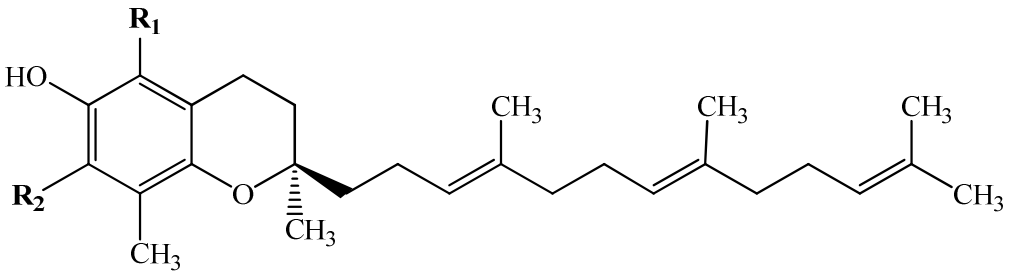
13. Dörmann P (2003) Corn with Enhanced Antioxidant Potential. Nature Biotechnology 21:1015-1016
14. Hunter SC, Cahoon EB (2007) Enhancing Vitamin E in Oilseeds: Unraveling Tocopherol and Tocotrienol Biosynthesis. Lipids 42:97-108
15. Nesaretnam K, Yew WW, Wahid MB (2007) Tocotrienols and Cancer: Beyond Antioxidant Activity. Eur J Lipid Sci Technol 109:445-452
16. Serbinova E, Kagan V, Han D, Packer L (1991) Free-Radical Recycling and Intramembrane Mobility in the Antioxidant Properties of Alpha-Tocopherol and Alpha-Tocotrienol. Free Radical Biology and Medicine 10:263-275
17. Hendrich S, Lee KW, Xu X, Wang HJ, Murphy PA (1994) Defining Food Components as New Nutrients. Journal of Nutrition 124:S1789-S1792
18. Peers KE, Coxon DT, Chan HW S (1981) Autoxidation of Methyl Linolenate and Methyl Linoleate - The Effect of Alpha-Tocopherol. J Sci Food Agric 32:898-904
19. Evans HM, Bishop KS (1922) On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction. Science 51:650-651
20. Evans HM, Emerson OH, Emerson GA (1936) The Isolation From Wheat Germ Oil of an Alcohol α-Tocopherol Having the Properties of Vitamin E. J Bio Chem 113:319-332
21. Fernholz E (1938) On the Constitution of α-Tocopherol J Am Chem Soc 60:700-705
22. Bunyan J, Marcinkiewicz S, McHale D, Green J (1961) Biological Potencies of Epsilon- And Zeta1-Tocopherol and 5-Methyltocol. Brit J of Nutr 15:253-257
23. Pennock JF, Hemming FW, Kerr JD (1964) A Reassessment of Tocopherol Chemistry. Biochemical and Biophysical Research Communications 17:542-548
24. International Union of Pure and Applied Chemistry (1981) International Union of Biochemistry Joint Commission on Biochemical Nomenclature: Nomenclature of Tocopherols and Related Compounds Recommendations. Molecular and Cellular Biochemistry 49:183-185
25. Sen CK, Khanna S, Roy S (2006) Tocotrienols: Vitamin E Beyond Tocopherols. Life Sciences 78:2088-2098

26. Kim HJ (2007) Oxidation Mechanism of Riboflavin Destruction and Antioxidant Mechanism of Tocotrienols. Food Science and Nutrition, The Ohio State University, Columbus
27. Wagner KH, Wotruba F, Elmadfa I (2001) Antioxidative Potential of Tocotrienols and Tocopherols in Coconut Fat at Different Oxidation Temperatures. Eur J Lipid Sci Technol 103:746-751
28. Chow CK, Draper HH (1974) Oxidative Stability and Antioxidant Activity of Tocopherols in Corn and Soybean Oils. International Journal for Vitamin and Nutrition Research 44:396-403
29. Lehmann J, Slover HT (1976) Relative Autoxidative and Photolytic Stabilities of Tocols and Tocotrienols. Lipids 11:853-857
30. Yamaoka M, Tanaka A, Kato A (1985) Antioxidative Activity of Tocotrienols. Yukagaku 34:120-122
31. Jiang Q, Christen S, Shigenaga MK, Ames BN (2001) γ-Tocopherol The Major Form of Vitamin E in the US Diet Deserves More Attention. American Journal of Clinical Nutrition 74:714-722
32. Cao J, Qi W, Song, BL (2009) Tocotrienols and the Regulation of Cholesterol Biosynthesis Tocotrienols. In: Watson RR, Preedy VR (eds) Vitamin E Beyond Tocopherols CRC Press, Boca Raton pp 237-256
33. Mo H, Elson C (2009) Role of the Mevalonate Pathway in Tocotrienol-Mediated Tumor. In: Watson RR, Preedy VR (eds) Vitamin E Beyond Tocopherols CRC Press, Boca Raton pp 185-208
34. The Commission on Mixed Tocopherols (2008) Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. The EFSA Journal 640:1-34
35. Jun L (2006) (accessed October 2009) Tocotrienols: Vitamin E beyond Tocopherols in Skin Protection. Davos Life Science Pte Ltd, Singapore http://www.davoslife.com/pdf/skinprotection_Web.pdf

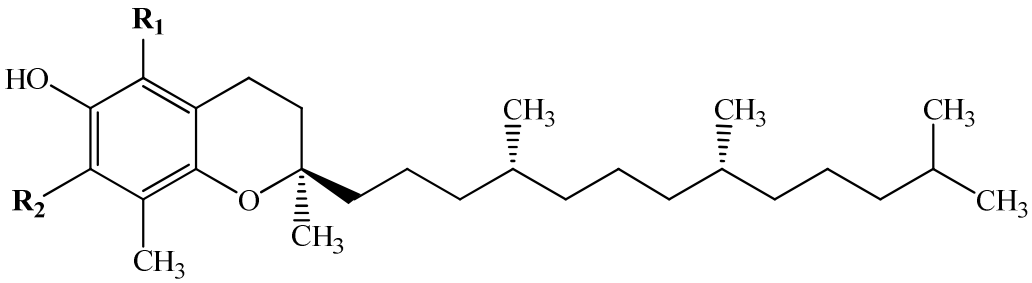
36. Jun L (2006) (accessed October 2009) Tocotrienols - Healthy Ingredient for Healthier Food. Davos Life Science Pte Ltd, Singapore http://www.davoslife.com/pdf/Healthy%20Ingredient%20Rev.pdf

37. Sheppard AJ, Weihrauch JL, Pennington JAT (1993) Analysis and Distribution of Vitamin E in Vegetable Oils and Food. In: Packer L, Fuchs J (edn) Vitamin E in Health and Disease. Marcel Dekker, Inc., New York pp 9-31

Tocotrienol



Tocopherol



	R_1	R_2
Alpha (α)	CH_2	CH_2
Beta (β)	CH_2	H
Gamma (γ)	H	CH_2
Delta (δ)	H	H

Figure 1. Structures of tocotrienols and tocopherols.

CHAPTER 2. OXIDATION OF CORN OILS WITH SPIKED TOCOLS

A paper to be submitted to the Journal of the American Oil Chemists' Society

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Abstract

Stripped corn oil with added tocopherols and tocotrienols, at various concentrations between 100 and 5,000 ppm, was used to test antioxidant activity of these compounds. The formation of lipid hydroperoxides was accelerated at 60°C in the dark for five days and measured as peroxide values (PV). Resistance to oxidation as induction period (IP) was measured using an Oxidative Stability Index (OSI). The PVs of the oils containing α- tocols exhibited decreasing effectiveness with α-tocol concentration. At day five, samples with 100 ppm α-tocols had the lowest PVs of 40 and samples containing 5,000 ppm had the highest from 150 to 200 meg/kg. At concentrations above 700 ppm of α-tocols, there was an inversion of antioxidative properties as α-tocols promoted oxidation. The opposite concentration effect was observed with δ-tocols and γ-tocotrienol (T3) for which antioxidant effectiveness increased with concentration. OSI-IP hour at 100°C increased with increasing tocol concentrations of all tocol homologs, although with diminishing effectiveness at greater than 700 ppm. The α-homologues were less effective in extending the induction period (~9 hrs at 5,000 ppm) than the δ-tocols and γ-T3, (13-14 hrs at 5,000 ppm). The induction period became less well defined with increasing concentration across all tocols indicating a slight shift in the oxidations and breakdown reaction kinetics.

Keywords: Antioxidants, corn oil, oxidative stability index, peroxide value, tocopherol, tocotrienol.

Introduction

Tocopherols and tocotrienols have long been recognized for their antioxidative properties in both food systems and *in vivo*. Although structurally similar to tocopherols, tocotrienols have not been as well characterized in either biological systems or food products and oils. A number of preliminary findings of unique biological functions of tocotrienol homologues have spurred research on the healthful or nutritive effects of these compounds [1, 2]. Still, very little research has been performed on the oxidative effects of individual tocotrienol homologues and their corresponding tocopherols in foods and edible oils [2].

Because most natural sources of tocols contain various proportions or concentrations of the eight compounds that comprise the tocol group, a model system of corn oil stripped of endogenous tocols and then spiked with different concentrations of individual tocotrienols or tocopherols was used in the present experiments.

It was expected that the oxidative or antioxidative contribution of individual tocol homologues at various concentrations will be more clearly delineated in a model system than in a natural product. A model system helps eliminate and reduce the complexity of food systems, such as emulsions and extensive processing that may have other contributing factors that affect lipid oxidation.

Material and Methods

Materials: Stripped corn oil was purchased from Dyets, Inc (Bethlehem, PA) and had a residual tocol content of less than 10 ppm. α-, γ- and δ-Tocotrienol were obtained from Davos Life Sciences, PTE Ltd (Singapore). α- and δ-Tocopherol were purchased from MP Biomedicals, LLC (Solon, OH). Acetic acid, chloroform, potassium iodide, sodium thiosulfate, potato starch, salicylic acid were all purchased through VWR International, LLC (West Chester, PA).

Sample preparation: Stripped corn oil was spiked directly with individual tocotrienols (α-, γ- and δ-) and tocopherols (α- and δ-) to achieve the desired concentrations of 100, 250, 700, 2,000 and 5,000 ppm of each tocol. Non-spiked, stripped corn oil was used as the control for these 25 samples.

For primary oxidation as measured by PV, three 1-mL aliquots of the bulk samples and controls were each placed in 1.8-mL amber vials for each of the five days of accelerated oxidation. Seventy-eight vials were removed from a 60°C oven each day at the same time over five subsequent days. The samples were blanketed with argon, capped and stored at -23°C until analyzed for PV. Day zero samples were also placed in amber vials and stored under argon at -23°C.

Resistance to oxidation as IP or conductance of secondary products of oxidation was measured in triplicate subsamples for each of the 25 samples and control using the Oxidative Stability Index Instrument.

Fatty acid composition: The fatty acid composition for the major fatty acids of the stripped corn oil was determined by gas chromatography using a ZB-wax column (Phenomenex, Torrance, CA) at 220°C. About 50 uL of oil was diluted in 1 mL hexane and transmethylated with trimethylsulphonium hydroxide [3, 4].

Primary lipid oxidation products determined by peroxide value: Samples were oxidized at 60°C in the dark over five days and analyzed for lipid hydroperoxides by using the AOCS Cd 8-53 method adapted by Crowe and White [5]. Three replicate samples from each day, and tocol type and concentrations were analyzed.

Resistance to oxidation as IP determined oil stability index (OSI): Five g of each sample were analyzed in triplicate by using an ADM OSI unit (the Oxidative Stability

Instruments, Omnion, Rockland, MA). The samples and controls were analyzed at 100°C. Both the induction period in hrs and the actual plot of conductivity by time were recorded.

Statistical Analysis: All experiments were conducted with triplicate treatments unless otherwise noted. Data analyses were done by using Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA). One-way Analysis of Variance (ANOVA) was used and least significant difference was calculated at $P < 0.05$ ($LSD_{0.05}$).

Results and Discussion

Fatty acid composition: The major fatty acid components as molar percentage of total of the control corn oil were palmitic (10.4%), stearic (1.9%), oleic (28.3%), linoleic (57.3%) and linolenic (0.9%) acids. This was a typical profile for corn oil and well within the expected range [6]. Because of the higher degree of unsaturation, corn oil is more prone to oxidation than other oils such as coconut, lard or hydrogenated fats [7-9], but less prone to oxidation than other oils with higher concentration of polyunsaturated fatty acids, such as fish oils [10]. Although the fatty acid composition of oil is usually the main determinate of oxidative stability, among different oil types, stability can be enhanced by elevated amounts of endogenous antioxidants or with the addition of natural or synthetic antioxidants [11]. The fatty acid composition was constant throughout this experiment, so this variable did not influence the differential oxidative stability observed.

Oxidative stability as measured by PV over time: The rate of lipid oxidation was calculated as the change of the PV over six days. These changes were unusually linear during the five days of sampling. A representative graph showing the PVs of the control and oils with five concentrations of added α-tocotrienol is shown in Figure 1. The R^2 of PV over time (day) linear line was 0.965 for 100 ppm α-tocotrienol-spiked oil, 0.953 for 250 ppm,

0.934 for 700 ppm, 0.934 for 2,000 ppm, and 0.939 for the 5,000 ppm spiked oil. The R^2 for the control linear line was 0.941. Unlike typical RBD oils in which lipid hydroperoxides slowly build up and then rapidly increase after the induction period in a nearly exponential manner, the rate of PV increase was linear across the five days.

The rate of hydroperoxide development per day for the stripped corn oil with added tocols as measured by PV (meq/kg) is given in Table 1. The change in PV demonstrates the effects of both tocol concentration and the degree of methylation. All samples at the lower two concentrations (100 and 250 ppm) were significantly better at inhibiting hydroperoxide formation than the stripped control oil with no added tocols. The α-forms of both the tocopherol and tocotrienol exhibited better antioxidant properties at 100 ppm than at any of the higher concentrations. The antioxidative effects diminished at concentrations above 100 ppm. At concentrations of 700 ppm and above, the α-tocols promoted hydroperoxide formation compared with the control and thus acted as prooxidants under the test conditions. The daily rate of PV development was highest (38.35 meq/kg day) for 5,000 ppm α-tocotrienol followed by 5,000 ppm α-tocopherol (30.28 meq/kg day). Oil with 2,000 ppm δ-tocopherol had the most stability with a low PV accumulation rate of 4.02 meq/kg day. The prooxidant effects of α-tocopherol at higher concentrations have been noted before by Frankel and Warner [11, 12]. Recently, the prooxidant properties of α- tocotrienol have also been reported in lard and coconut fat [7, 8]. The PV of lard with 1,000 ppm α-tocotrienol exceeded the PV of the control at day six. The optimum concentration to prevent formation of hydroperoxides was reported to be 100 ppm α-tocotrienol [7]. In coconut fat, the α-tocotrienol reduced the stability of the fat as measured by PV at three spiked concentrations, 100, 500 and 1,000 ppm [8]. The decreased protection of oils from the formation of primary oxidation products was well correlated with tocol concentration over the range 100 to 5,000 ppm for both α- tocols, with R^2 of 0.95 and 0.96 for α-tocopherol and α-tocotrienol, respectively. The opposite effect was observed for δ-tocotrienol. Although less well

correlated ($R^2 = 0.73$), the higher concentrations improved oil stability. There was little correlation between the concentrations of δ-tocopherol or γ-tocotrienol and oil stability, indicating higher concentrations do not give better protection.

The results of this experiment show that higher concentrations of the α-tocols act as prooxidants in this oil model. Since the α-tocols have lower bond energies for the cleavage of phenolic OH, it would be predicted that they have the highest antioxidant activity [13]. Thus, α-tocols would form free radicals preferentially over the other tocol homologues found in a mixture. Since the model system lacks any additional antioxidants that could act synergistically, there are no other mechanisms for proton transfer other than back to the fatty acids. Although tocols are believed to act by reducing free radical propagation, at higher concentrations the α-tocols or the excess tocol free radicals appear to initiate or catalyze lipid oxidation or continue to propagate oxidation [11]. Similar effects for α-tocopherol were reported in a purified rapeseed triacylglycerol system at 40^{o}C under low oxygen environment [14]. There is also increased chance of direct interaction at the oil/air interface at higher α-tocols concentrations [11].

Oxidative stability as measured by OSI: Resistance to oxidation was evaluated by using the oil stability index (OSI) with an oxidative stability instrument at 100^{o}C. The induction period (IP) is the time, in hrs, at the tangent of the conductivity slope as determined by the instrument. The OSI hrs are listed in Table 2. Unlike measuring of primary oxidation products, there were no prooxidant effects of the tocols compared to the stripped control in this experiment. Across all tocol additions, IP duration increased with increasing tocol concentration. There was, however, diminished effectiveness with higher concentrations as shown in Table 2. As with PV, the α-tocols were less effective at increasing the resistance to generating secondary oxidation products than the other tocols assayed. Because this analysis is secondary to hydroperoxide formation and is a degradation measurement, the higher PV

formation observed in the first experiment explains the lower IP with the α-tocols. This result, however, would not explain the relatively lower production of volatile secondary compounds at higher concentrations of tocols. It appears increased tocol concentration may inhibit formation of secondary oxidation products from hydroperoxides. The oil with 5,000 ppm δ-T3 added exhibited the longest induction period at 14.05 hrs, extending the IP by over 9 hrs compared to the control oil (IP 4.82 hrs.). The optimum or most effective antioxidant concentration was < 700 ppm for the α-tocols and < 2000 ppm for the other tocols.

Synergy of tocotrienols: Although individual kinetics vary with different tocols, the matrix in which they are placed, other compounds in the matrix, and the environmental conditions [11], the mechanisms of oxidation do not suggest synergistic effects within different tocol compositions [15, 16]. This does not preclude the sparing effect of individual tocols, specifically α-tocopherol, which is more rapidly degraded than γ- and δ-tocopherol in a mixed tocol matrix [17]. A preliminary evaluation of possible synergy for two different tocotrienol mixtures in stripped corn oil was performed in duplicate. The IPs of the oils was determined using OSI. The ratio of the first mixture was 1:1:1 α-, γ- and δ- tocotrienols at total concentrations of 100, 700 and 5,000 ppm, and the second mixture was 31% α-, 1.9 % β-, 45.8% γ- and 21.3% δ- tocotrienol at 250 and 2,000 ppm total concentrations. Mean IPs were 6.6, 9.5 and 12.0 hrs for the first experiment and 8.1 and 11.0 hrs for the second experiment. There was little indication of synergy when compared against the IPs of the individual tocotrienols. If calculated from the data for individual tocols to a similar mix, IPs would be 6.7, 9.2, and 12.1 hrs for the first experiment and 7.7 and 11.6 hrs for the second. No further investigation on synergy was performed.

Conclusion

The addition of lower concentrations of 100 and 250 ppm of individual tocotrienols and tocopherols to corn oil stripped of natural tocols provide stability to the oil with both reduced hydroperoxide formation and extended IP. As concentration increased, the effectiveness of the tocols as antioxidants diminished. The α-homologues provided less protection against lipid oxidation than the δ- homologues or γ- tocopherol and actually promote hydroperoxide formation at concentrations at and above 700 ppm. This prooxidant effect increased with concentrations up to the experiment limit of 5,000 ppm. Increasing the concentration of the δ- homologues or γ- tocopherol above 700 ppm in the oil had little effect.

References

1. Theriault A, Chao JT, Wang Q, Gapor A, Adeli K (1999) Tocotrienol: A Review of Its Therapeutic Potential. Clinical Biochemistry 32:309-319
2. Watson RR, Preedy VR (2009) Tocotrienols: Vitamin E Beyond Tocopherols. CRC Press, Boca Raton
3. Butte W, Eilers J, Kirsch M (1982) Trialkylsulfonium-Hydroxides and Trialkylselenoniumhydroxides for the Pyrolytic Alkylation of Acidic Compounds. Analytical Letters Part a-Chemical Analysis 15:841-850
4. Dolde D, Vlahakis C, Hazebroek J (1999) Tocopherols in Breeding Lines and Effects of Planting Location Fatty Acid Composition and Temperature during Development. J Am Oil Chem Soc 76:349-355
5. Crowe TD, White PJ (2001) Adaptation of the AOCS Official Method for Measuring Hydroperoxides from Small-Scale Oil Samples. J Am Oil Chem Soc 78:1267-1269
6. Padley FB, Gunstone FD, Harwood JL, (1994) Occurrence and Characteristics of Oils and Fats. In: (eds) The Lipid Handbook, 2nd edn. Chapman & Hall, London, pp 47-224

7. Wagner KH, Wotruba F, Elmadfa I (2001) Antioxidative Potential of Tocotrienols and Tocopherols in Coconut Fat at Different Oxidation Temperatures. Eur J Lipid Sci Technol 103:746-751
8. Kim HJ (2007) Oxidation Mechanism of Riboflavin Destruction and Antioxidant Mechanism of Tocotrienols. Food Science and Nutrition, The Ohio State University, Columbus
9. Nogala-Kalucka M, Korczak J, Elmadfa I, Wagner KH (2005) Effect of Alpha- and Delta-Tocopherol on the Oxidative Stability of a Mixed Hydrogenated Fat Under Frying Conditions. European Food Research and Technology 221:291-297
10. Zuta PC, Simpson BK, Zhao X, LeClerc L (2007) The Effect of Alpha-Tocopherol on the Oxidation of Mackerel Oil. Food Chemistry 100:800-807
11. Frankel EN (1998) Lipid Oxidation The Oily Press, Dundee
12. Warner K (2005) Effects on the Flavor and Oxidative Stability of Stripped Soybean and Sunflower Oils with Added Pure Tocopherols. J Agric Food Chem 53:9906-9910
13. Wright J S, Carpenter D J, Mckay D J, Ingold K U (1997) Theoretical Calculation of Substituent Effects on the O-H Bond Strength of Phenolic Antioxidants Related to Vitamin E. J Am Chem Soc 119:4245-4252
14. Isnardy B, Wagner KH, Elmadfa I (2003) Effects of α- γ- and δ-Tocopherols on the Autoxidation of Purified Rapeseed Oil Triacylglycerols in a System Containing Low Oxygen. J Agric Food Chem 51:7775-7780
15. Decker EA, Warner K, Richards MP, Shahidi F (2005) Measuring Antioxidant Effectiveness in Food. J of Agric Food Chem 53:4303-4310
16. Choe E, Min D B (2009) Mechanisms of Antioxidants in the Oxidation of Foods Comprehensive. Reviews in Food Science and Food Safety 8:345-358
17. Lehmann J, Slover HT (1976) Relative Autoxidative and Photolytic Stabilities of Tocols and Tocotrienols. Lipids 11:853-857

Table 1. Change in average daily peroxide value (Δ-PV/day) ± one standard deviation over five days in stripped corn oil with added tocols at 60°C in the dark

Concentration	α-Toco	α-T3	δ-Toco	δ-T3	γ-T3
100 ppm	6.89 ± 0.17^{e}	6.73 ± 0.02^{e}	5.34 ± 0.08^{a}	7.26 ± 0.52^{a}	5.43 ± 0.58^{b}
250 ppm	8.87 ± 0.09^{d}	8.37 ± 0.70^{d}	4.98 ± 0.14^{c}	6.88 ± 0.24^{b}	5.01 ± 0.33^{cd}
700 ppm	13.51 ± 0.10^{c}	12.85 ± 0.64^{c}	5.21 ± 0.10^{b}	6.42 ± 0.46^{c}	4.73 ± 0.56^{d}
2,000 ppm	20.68 ± 0.59^{b}	25.43 ± 1.02^{b}	4.02 ± 0.08^{e}	5.48 ± 0.46^{d}	7.26 ± 0.43^{a}
5,000 ppm	30.28 ± 0.81^{a}	38.35 ± 2.04^{a}	4.63 ± 0.05^{d}	5.36 ± 0.38^{d}	5.25 ± 0.08^{bc}
$LSD_{0.05}$	0.37	0.90	0.08	0.34	0.36

Stripped control 10.09 ± 0.35 Δ-PV/day
[1]Values not sharing a common superscript in each column are significantly different ($P < 0.05$)

Table 2. OSI induction period ± one standard deviation (hrs) at 100°C for stripped corn oil spiked with individual tocols at various concentrations

Treatment	α-Toco	α-T3	δ-Toco	δ-T3	γ-T3
Control	4.82 ± 0.41[d]	4.82 ± 0.41[e]	4.82 ± 0.41[f]	4.82 ± 0.41[f]	4.82 ± 0.41[e]
100 ppm	5.93 ± 0.08[cd]	6.58 ± 0.24[d]	6.45 ± 0.09[e]	6.17 ± 0.10[e]	7.32 ± 0.19[d]
250 ppm	6.93 ± 0.08[bc]	7.34 ± 0.57[cd]	8.18 ± 0.58[d]	7.28 ± 0.18[d]	8.72 ± 0.08[c]
700 ppm	8.15 ± 0.33[ab]	8.05 ± 0.71[bc]	10.23 ± 0.46[c]	8.60 ± 0.30[c]	10.82 ± 0.43[b]
2,000 ppm	8.60 ± 1.35[a]	8.91 ± 0.85[ab]	12.85 ± 0.70[b]	11.42 ± 0.65[b]	13.28 ± 1.02[a]
5,000 ppm	9.30 ± 1.34[a]	9.40 ± 1.12[a]	14.05 ± 0.98[a]	12.95 ± 0.30[a]	13.95 ± 0.36[a]
$LSD_{0.05}$	1.29	1.03	0.91	0.61	0.98

[1]Values not sharing a common superscript in each column are significantly different, ($P < 0.05$)

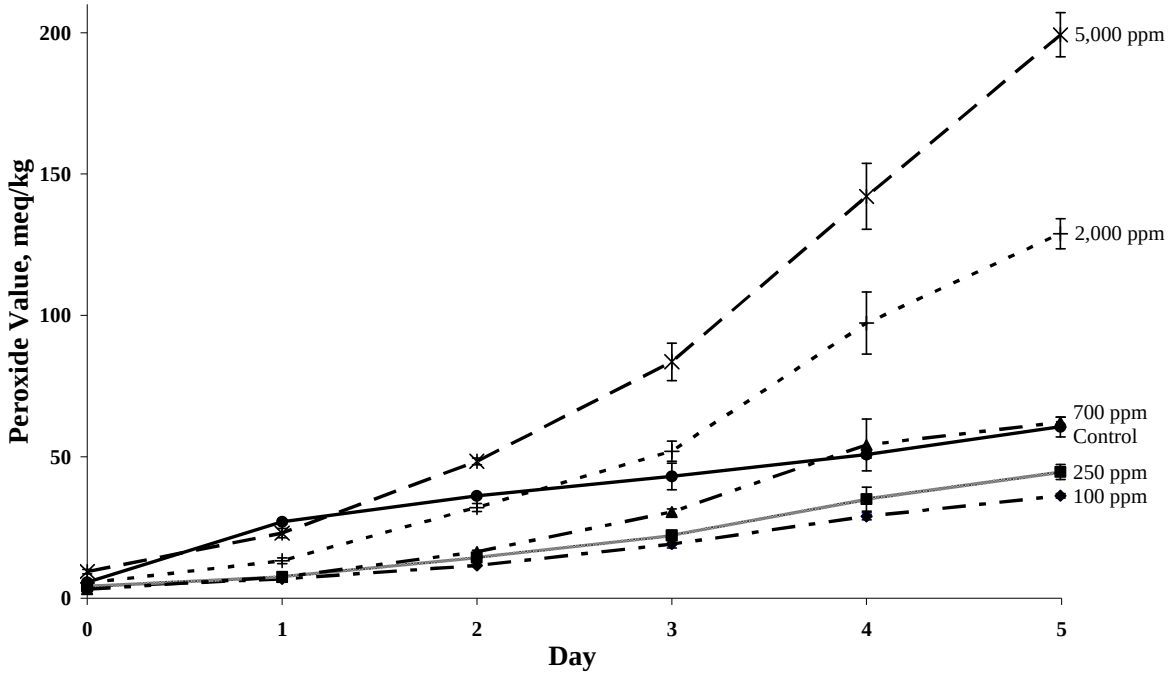

Figure 1. Peroxide value of corn oil at 60°C with added α-tocotrienol compared with stripped corn oil control.

CHAPTER 3. OXIDATION OF CRUDE CORN OIL WITH AND WITHOUT ELEVATED TOCOTRIENOLS

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Abstract

Kernels and oil from corn with increased concentrations of tocotrienols (T3) due to the transgenic expression of a homogentisic acid geranylgeranyl transferase (HGGT) gene from two growing years were characterized for tocol and fatty acid compositions. The crude extracted oil was analyzed for oxidative properties and compared to non-transformed material derived from the plants grown at the same location and during the same year. No significant difference was observed in composition of major fatty acids. Both the seed (kernel) and extracted crude oil from the HGGT expressing corn contained more than a 17-fold increase in tocotrienol content from 300 to 5,400 ppm in oil. There was a concurrent 20% decrease in tocopherol content, 1,150 ppm total tocopherols in control oil declining to 940 ppm in HGGT oil. Although tocopherols and tocotrienols are generally considered antioxidants, they may exhibit prooxidant effects at higher concentrations. Crude oil was extracted from control and transformed corn produced during 2005 and 2006 yielding four oil samples that were evaluated for their oxidative properties. The formation of lipid hydroperoxides, a primary oxidation product, was evaluated at 60^{o}C over nine days by measuring PV. Resistance to oxidation or induction period (IP) was measured using an Oxidative Stability Instrument. There was a slight decrease in hydroperoxide formation in the HGGT oil compared to the corresponding control but was less than the year to year differences. The induction period was the same for the 2005 oils, with or without the increased tocotrienol content, but the crude oil with enhanced tocotrienol had a longer IP than the control crude oil in the 2006 samples.

Keywords: Antioxidants, corn oil, oxidative stability index, homogentisic acid geranylgeranyl transferase, peroxide value, tocopherol, tocotrienol.

Introduction

Tocotrienols and tocopherols are lipophilic, plant-produced antioxidants exhibiting vitamin E activity. The composition of the eight tocol homologues differs considerably in different plant species. Common sources of tocopherols, such as nuts and oils of soy, corn, canola and sunflower, contain little or no tocotrienols. Because of their similar phenolic structure, all tocols exhibit antioxidative properties at parts per million (ppm) concentrations in oil or lipid systems. Recent research suggests individual tocotrienols provide benefits beyond these antioxidation properties [1, 2].

The reported benefits of tocotrienols include inhibiting cholesterogenesis and the coronary heart disease [1-3]. By lowering plasma triacylglycerides, tocotrienols may reduce the effects of metabolic syndrome. Tocotrienols have shown promise to control tumor growth and suppress growth of specific melanoma cells *in vitro* and in animal studies. There may also be synergistic advantages to using tocotrienols with other chemotherapies [4].

Using molecular biology to manipulate metabolic pathways in plants for increased tocol production and varying their compositions have been reviewed and accomplished by Cahoon and others in 2003 [5-7]. The assumption is that genetic modification of the tocopherol and tocotrienol biosynthetic pathways can reduce oxidative stress in plants, increase crop productivity, increase storage duration of seed and plant material, improve shelf life of vegetable oils, and meet increasing demands for nutraceutical or therapeutic markets [7]. As research continues on the genetics and biochemistry of tocol biosynthesis, further enhancements to tocol-fortified seeds are expected.

Tocols at higher concentrations, however, may act as prooxidants [8]. As little as 250 ppm of α-tocopherol has been reported as having prooxidative effects in the formation of hydroperoxides [9]. In tocotrienol-spiked coconut fat, at 60^{o}C, the addition of α- and β-tocotrienols at 100, 500 and 1,000 ppm increased fat oxidation as measured by PV compared to the control. This prooxidant effect increased with increased tocotrienol concentration [10].

Both γ- and δ-tocotrienols had the opposite effect and reduced lipid hydroperoxide formation, although the effect was not correlated with γ-tocotrienol concentration [10]. The primary goal of this research was to determine the oxidative effects of very high levels of tocotrienols (> 5,000 ppm) in crude oil from corn genetically transformed with a homogentisate geranylgeranyl transferase gene expressing increased tocotrienols.

Materials and Methods

Material: Four bulk samples of approximately 8 kilograms of corn each were provided by Pioneer Hi-Bred International, Inc (Johnston, IA). Two samples from each of 2005 and 2006 were provided. One sample was genetically transformed with the barley homogentisate geranylgeranyl transferase (HGGT) gene to express increased amounts of tocotrienols, and the second was a sample control with a similar pedigree but lacking the transgene. No field or yield data was provided.

α-, γ- and δ-Tocotrienol standards were obtained from Davos Life Sciences, PTE Ltd (Singapore). α- and δ-Tocopherol were purchased from MP Biomedicals, LLC (Solon, OH). Acetic acid, chloroform, hexanes, potassium iodide, sodium thiosulfate, potato starch, salicylic acid were all purchased through VWR International, LLC (West Chester, PA).

Oil extraction sample preparation: The corn was ground with a Thomas Wiley lab mill with a 1-mm screen. Sixty g batches were extracted with hexanes by using a Buchi B811 (New Castle, DE), an automated Soxhlet extraction system. The extraction efficiency was 97% by using a minimum of 10 cycles and 1-hour extraction. The mean percentages of oil recovered was 2.63, 2.66, 2.99 and 2.91 for the 2005 control, 2005 HGGT, 2006 control and 2006 HGGT material, respectively. The crude extracted oils were stored under argon in amber glass at -23^{o}C until tested.

Three 1-mL aliquots of the crude oils were each placed in 1.8-mL uncapped amber vials for each of the nine days of accelerated oxidation. Twelve vials were removed from a 60^{o}C oven each day at the same time over nine subsequent days. The samples were blanketed with argon, capped and stored at -23^{o}C until analyzed for peroxide value. Day zero samples were also placed in amber vials and stored under argon at -23^{o}C.

Tocopherol and tocotrienol content: The tocotrienols and tocopherols were determined by using a modification of AOCS Official Method Ce 8-89 [11]. Tocols were separated by using a Waters HPLC Alliance 2695 (Milford, MA) with a 3μ NH_2 100A, 150 mm x 3.0 mm column and detected by fluorescence (Waters 2475) with EXλ = 292 nm and EMλ = 335 nm. An external calibration curve of 0.05, 0.1, 0.2, 0.5, 1.0, 2.5 and 5 ppm of each tocol was used for quantification.

Fatty acid composition: The fatty acid compositions for the major fatty acids of the crude corn oils were determined by gas chromatography by using a ZB- wax column (Phenomenex, Torrance, CA) at 220^{o}C. About 50 uL of oil was diluted in 1 mL of hexane and methylated with trimethylsulphonium hydroxide [12, 13].

Free fatty acid content: The free fatty acid percentages in the crude oils were determined by using AOCS method Ca 5a-40 [11]. Percentage free fatty acid (FFA) was expressed as percentage oleic acid.

Primary lipid oxidation products determined by peroxide value: Three subsamples of each extracted crude oil from the four bulk corn samples were oxidized at 60^{o}C for each of nine days and analyzed by using the AOCS Cd 8-53 method adapted by Crowe and White [14].

Resistance to oxidation as by IP determined oil stability index (OSI): Five subsamples of each extracted crude oil from the four bulk corn samples were analyzed by using an ADM OSI unit (Oxidative Stability Instruments, Omnion, Rockland, MA). The samples were oxidized at 100^{o}C with airflow of 110 mL/min. Both the induction period and the actual plot of conductivity by time were recorded.

Statistical Analysis: All experiments were conducted with replicate treatments as noted. Data analyses were done by using Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA). One-way Analysis of Variance (ANOVA) was used and least significant difference was calculated at $P < 0.05$ ($LSD_{0.05}$).

Results and Discussion

Fatty acid composition: The major fatty acids in the crude extracted corn oil as molar percentages are given in Table 1. There were minimal differences in the profile of the four crude oils, and modification of the tocol pathway did not appreciably affect fatty acid composition beyond the minor differences normally attributed to environmental conditions. Thus, fatty acid composition, one of the main determinants of oxidative stability, was not expected to influence the PV and OSI values obtained.

Free fatty acids: The free fatty acid percentages in the crude oils expressed as oleic acid, were 10.69 for the 2005 control oil, 10.97 for the 2005 HGGT oil, 10.42 for the 2006 control oil and 12.30 for the 2006 HGGT oil. All values were much higher than expected for oils extracted from high quality, undamaged grain. A commodity corn grain sample extracted using the same equipment and process had a more reasonable FFA of 2.44%, indicating that the high FFA percentages observed did not result from the extraction process.

No yield data was provided with the corn, therefore, the cause is unknown. The initial hydroperoxide values for the crude oils were 1.45, 1.32, 1.10 and 1.04 meq/kg for the 2005 control, 2005 HGGT, 2006 control and 2006 HGGT, respectively, indicating no major damage or degradation of the seed during storage. The high FFA is a concern as free fatty acids can be prooxidants [15-17]. Thus, the oxidative stability of the crude oils may not reflect the stability of the refined, bleached and deodorized final product after the free fatty acids have been removed. However, as both the control oils and the HGGT oils had similar FFA content, their influence on oxidative stability should also be similar. Ideally, the crude oil should have been fully refined and then evaluated for oxidative stability. However, due to the limited amount of oil obtained, refining was not possible.

Tocol content: Both samples from each year were grown at the same location. No significant differences were observed in the single kernel analysis of the control corn compared with HGGT corn for moisture, oil content, kernel weight and fatty acid composition. As expected, there were slight year-to-year differences. There were, however, significant differences in tocol amounts and tocol distribution between the control and the HGGT corn. The 2005 HGGT kernels segregated into two distinct populations, one with high T3 concentration (~75% total analyzed population) and one with low T3 concentration, similar to that of the control sample. This finding suggests that the dominant HGGT transgene was segregating in the 2005 HGGT sample. This phenomenon was not seen in the 2006 HGGT sample (Fig 1).

The concentrations of tocopherols and tocotrienols in the oils studied are listed in Table 2. Modification of the tocol biosynthetic pathway in HGGT corn resulted in dramatic increases of all tocotrienols, especially γ-tocotrienol. There was a slight decrease in α- and γ-tocopherols and a slight increase in β- and δ-tocopherols in the modified HGGT corn oil compared with the unmodified control oil. Major tocotrienols were less abundant in the 2005

HGGT oil than the 2006 HGGT oil. The reduction may have been a result of the segregating population in 2005.

Accumulation of primary oxidation products: The rates of hydroperoxide development in the crude corn oils are given in Table 3. Due to the linearity of PV development during the sampling period, expressing the rate of PV change as Δ-PV/day was reasonable (Fig 2). The R^2 for the linear relationship ranged from a low value of 0.9826 for the 2006 control crude to a high value of 0.9976 for the 2005 control crude (Fig 2). The rate of PV development of the HGGT crude corn oil was statistically lower ($P<0.05$) than that in the control crude oils of the same year. Thus, even at unusually high tocol concentrations there appears to be an antioxidant effect in the crude oil. Results from our model system (unpublished data) and research by others suggest, however, that the optimum levels of tocotrienols for antioxidation in bulk oils should be lower [10, 18, 19]. The higher concentrations used in these other experiments promoted primary lipid oxidation products in the purified fats and oils but this was not observed with the crude extracted corn oils. Other native antioxidants in the crude oils, such as carotenoids, may provide synergies not found in highly purified oils [17, 20]. This would allow the tocotrienols and tocopherols to retain their effectiveness at very high levels. These endogenous compounds provide an alternate path for sequestering free radicals resulting from tocol oxidation beyond the lipids [20, 21].

Oxidative stability of crude oils as measured by OSI: The IP and conductivity at the IP time are given in Table 4. Although IP time is typically used to differentiate oil stabilities, a better understanding of the stability index and development of secondary products is deduced from the graph of the actual conductance of the five subsamples as seen in Figure 3. Both the control and HGGT crude oils from 2005 had the same IP but exhibited different curve shapes and different conductivities at the IP time. The control oil had a

lower, much sharper and well-defined inflection point than the HGGT crude oil containing increased tocotrienol levels. The 2006 samples also exhibited the same pattern but this similarity is less obvious as the IPs were significantly different from each other (Fig 3). Similar findings of a smoothing of the curve and less defined inflection point of high tocol oils has also been reported when hexanal formation at lower temperatures was used to track secondary oxidation [21]. Because of the high temperature and artificial conditions used to generate the IP time, the value of this type of testing has caused considerable controversy, but it continues to be widely used.

The actual slopes or curve shapes generated by OSI suggest that the mechanism and antioxidative properties shift as the concentrations of tocols increase in bulk oils. The initial rate of the evolution of secondary oxidation products increases slightly more rapidly in high tocol oils than in those oils containing less tocols. Yet the high tocol oils continue to inhibit secondary product formation for a longer time. The classical model of a long induction period of little secondary product formation followed by a very rapid increase in these compounds is less useful for oils having very high tocol concentrations. Using IP time alone to compare crude oils with high tocol levels is insufficient to determine the actual oxidative effects.

Conclusion

Crude oil from corn expressing tocotrienols at 4,000 to 5,400 ppm exhibited no prooxidant effects. High tocotrienol concentrations displayed slight antioxidative properties in the reduction of the formation of hydroperoxides in crude oils. The induction period was the same or extended for these oils but the curve of the oxidative stability index shifted away from a sharp inflection point indicating a small shift in the oxidation kinetics. Crude corn oil with increased tocol levels remains as oxidatively viable as crude oil from non-enhanced corn.

References

1. Theriault A, Chao JT, Wang Q, Gapor A, Adeli K (1999) Tocotrienol: A Review of Its Therapeutic Potential. Clinical Biochemistry 32:309-319
2. Watson RR, Preedy VR (2009) Tocotrienols: Vitamin E Beyond Tocopherols. CRC Press, Boca Raton
3. Sen CK, Khanna S, Roy S (2006) Tocotrienols: Vitamin E beyond Tocopherols. Life Sciences 78:2088-2098
4. Mo H, Elson C (2009) Role of the Mevalonate Pathway in Tocotrienol-Mediated Tumor. In: Watson RR, Preedy VR (eds) Vitamin E Beyond Tocopherols CRC Press, Boca Raton pp 185-208
5. Chen S, Li H, Liu G (2006) Progress of Vitamin E Metabolic Engineering in Plants. Transgenic Research 15:655-665
6. Hunter SC, Cahoon EB (2007) Enhancing Vitamin E in Oilseeds: Unraveling Tocopherol and Tocotrienol Biosynthesis. Lipids 42:97-108
7. Cahoon EB, Hall SE, Ripp KG, Ganzke TS, Hitz WD, Coughlan SJ (2003) Metabolic Redesign of Vitamin E Biosynthesis in Plants for Tocotrienol Production and Increased Antioxidant Content. Nature Biotechnology 21:1082-1087
8. Cillard J, Cillard P, Cormier M (1980) Effect of Experimental Factors on the Prooxidant Behavior of Alpha-Tocopherol. J Am Oil Chem Soc 57:255-261
9. Huang SW, Frankel EN, German JB (1995) Effects of Individual Tocopherols and Tocopherol Mixtures on the Oxidative Stability of Corn Oil Triglycerides. J Agric Food Chem 43:2345-2350
10. Wagner KH, Wotruba F, Elmadfa I (2001) Antioxidative Potential of Tocotrienols and Tocopherols in Coconut Fat at Different Oxidation Temperatures. Eur J Lipid Sci Technol 103:746-751
11. American Oil Chemists' Society (1990) Official methods and recommended practices of the American Oil Chemists' Society, 4th edn. American Oil Chemists' Society, Champaign 1990

12. Butte W, Eilers J, Kirsch M (1982) Trialkylsulfonium-Hydroxides and Trialkylselenoniumhydroxides for the Pyrolytic Alkylation of Acidic Compounds. Analytical Letters Part a-Chemical Analysis 15:841-850
13. Dolde D, Vlahakis C, Hazebroek J (1999) Tocopherols in Breeding Lines and Effects of Planting Location Fatty Acid Composition and Temperature during Development. J Am Oil Chem Soc 76:349-355
14. Crowe TD, White PJ (2001) Adaptation of the AOCS Official Method for Measuring Hydroperoxides from Small-Scale Oil Samples. J Am Oil Chem Soc 78:1267-1269
15. Frega N, Mozzon M, Lercker G (1999) Effects of Free Fatty Acids on Oxidative Stability of Vegetable Oil. J Am Oil Chem Soc 76:325-329
16. Miyashita K, Takagi T (1986) Study on the Oxidative Rate and Prooxidant Activity of Free Fatty Acids. J Am Oil Chem Soc 63:1380-1384
17. Choe E, Min DB (2009) Mechanisms of Antioxidants in the Oxidation of Foods. Comprehensive Reviews in Food Science and Food Safety 8:345-358
18. Lehmann J, Slover HT (1976) Relative Autoxidative and Photolytic Stabilities of Tocols and Tocotrienols. Lipids 11:853-857
19. Kim HJ (2007) Oxidation Mechanism of Riboflavin Destruction and Antioxidant Mechanism of Tocotrienols. Food Science and Nutrition, The Ohio State University, Columbus
20. Schroeder MT, Becker EM, Skibsted LH (2006) Molecular Mechanism of Antioxidant Synergism of Tocotrienols and Carotenoids in Palm Oil. J Am Oil Chem Soc 54:3445-3453
21. Frankel EN (1998) Lipid Oxidation The Oily Press, Dundee

Table 1. Fatty acid composition (molar %) of crude corn oils

	16:0	18:0	18:1	18:2	18:3
Control Corn Oil 2005	12.40	1.93	23.15	59.53	1.35
HGGT Corn Oil 2005	12.07	1.96	23.65	59.37	1.28
Control Corn Oil 2006	11.47	1.76	24.29	59.77	1.27
HGGT Corn Oil 2006	11.69	1.91	24.28	59.53	1.23

Table 2. Tocol composition of extracted crude corn oil in ppm and relative %

	Tocotrienols (ppm)					Tocopherols (ppm)				
	α-	β-	γ-	δ-	Total	α-	β-	γ-	δ-	Total
Control 2005	192	ND	55	1	248	501	20	730	28	1,279
HGGT 2005	643	18	3,081	442	4,184	301	26	641	56	1,024
Control 2006	187	1	108	7	303	373	29	647	23	1,072
HGGT 2006	690	20	4,051	618	5,379	185	31	565	60	841

	Tocotrienols (%)					Tocopherols (%)				
	α-	β-	γ-	δ-	Total	α-	β-	γ-	δ-	Total
Control 2005	12.6	ND	3.6	0.1	16.2	32.8	1.3	47.8	1.8	83.8
HGGT 2005	12.3	0.3	59.2	8.5	80.3	5.8	.5	12.3	1.1	19.7
Control 2006	13.6	0.1	7.9	0.5	22.0	27.1	2.1	47.1	1.7	78.0
HGGT 2006	11.1	0.3	65.1	9.9	86.5	3.0	0.5	9.1	1.0	13.5

	Tocol homologues (%)				Tocol homologues (ppm)				
	α-	β-	γ-	δ-	α-	β-	γ-	δ-	Total
Control 2005	45.4	0.0	51.4	1.9	693	ND	785	29	1,527
HGGT 2005	18.1	0.8	71.5	9.6	944	44	3,722	498	5,208
Control 2006	40.7	2.2	54.9	2.2	560	30	755	30	1,375
HGGT 2006	14.1	0.8	74.2	10.9	875	51	4,616	678	6,220

Table 3. Change in average daily peroxide value ± one standard deviation over nine days in crude corn oil at 60°C in the dark

Δ-PV/day	2005	2006
Control	11.96 ± 1.06	9.19 ± 0.34
HGGT	9.83 ± 0.32	8.48 ± 0.27

Table 4. OSI induction period (IP) (hrs) and conductivity (OSI value) ± one standard deviation at IP of extracted crude corn oil[1, 2]

	OSI Induction period, hrs	OSI conductivity at induction time
Control Corn Oil 2005	11.57 ± 0.22[b]	6,650 ± 403[c]
HGGT Corn Oil 2005	11.61 ± 0.51[b]	10,548 ± 756[b]
Control Corn Oil 2006	9.86 ± 0.57[c]	6,869 ± 1,550[c]
HGGT Corn Oil 2006	13.82 ± 1.04[a]	13,015 ± 1,804[a]
$LSD_{0.05}$	0.87	1,673

[1]Values not sharing a common superscript are significantly different
[2]OSI Temperature, 100°C

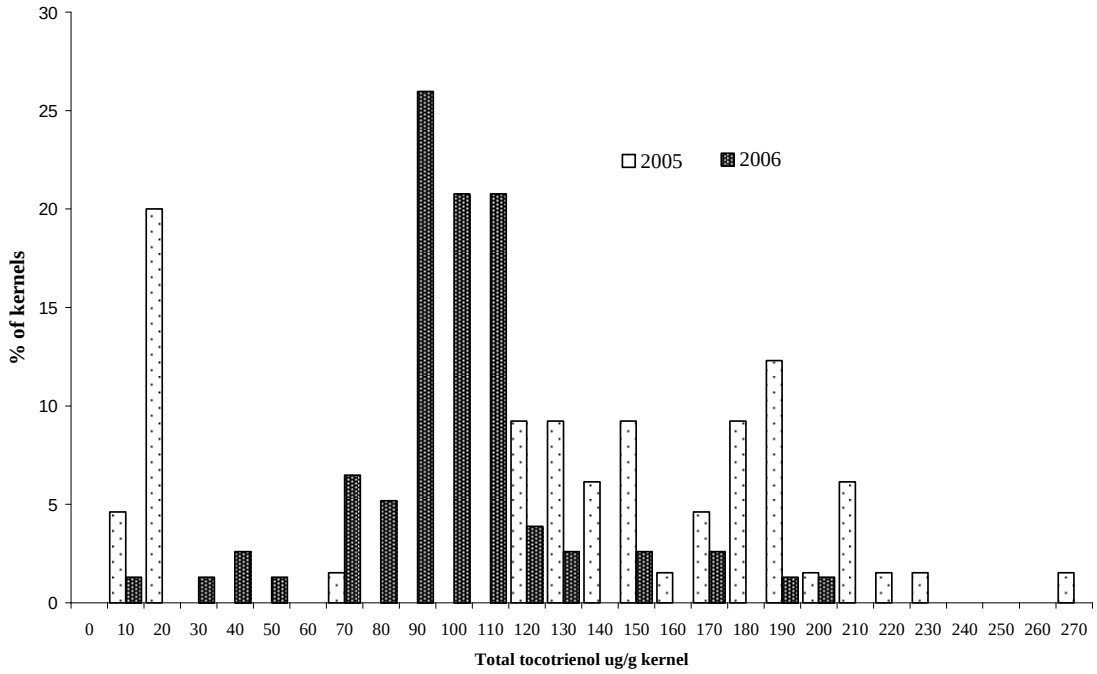

Figure 1. Histogram of total tocotrienols in corn kernels modified with a homogentisate geranylgeranyl transferase (HGGT) gene.

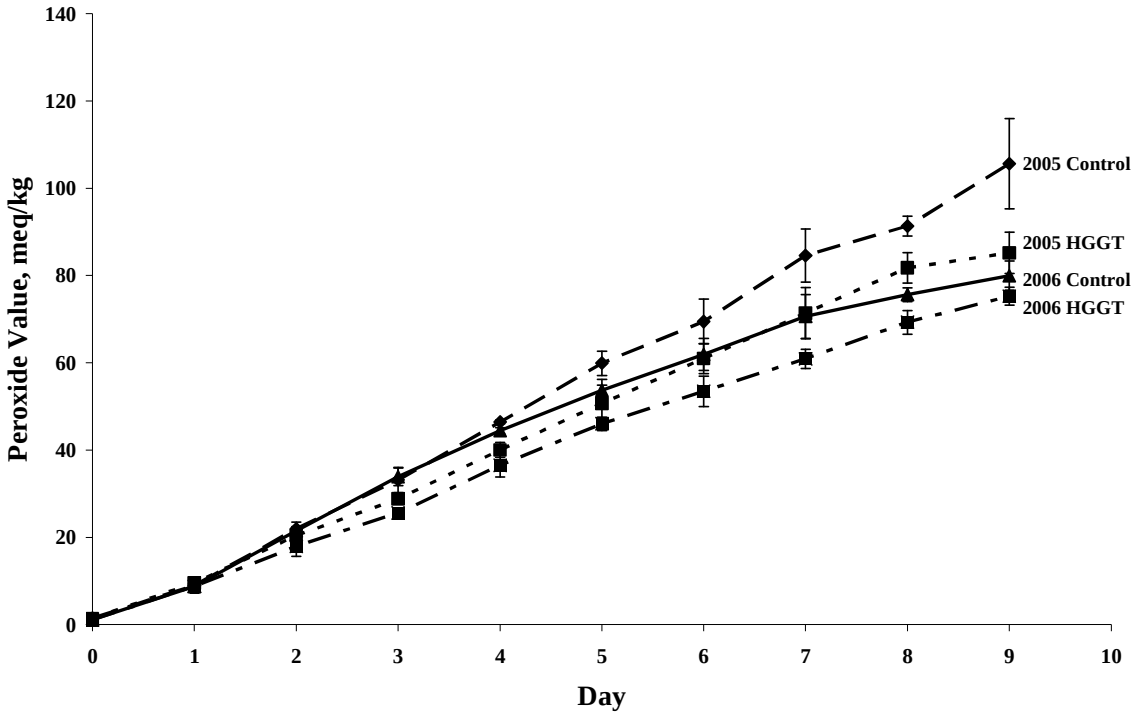

Figure 2. Peroxide values for extracted crude corn oil from control kernels and kernels modified with homogentisate geranylgeranyl transferase (HGGT) from 2005 and 2006 at 60^{o}C and in the dark.

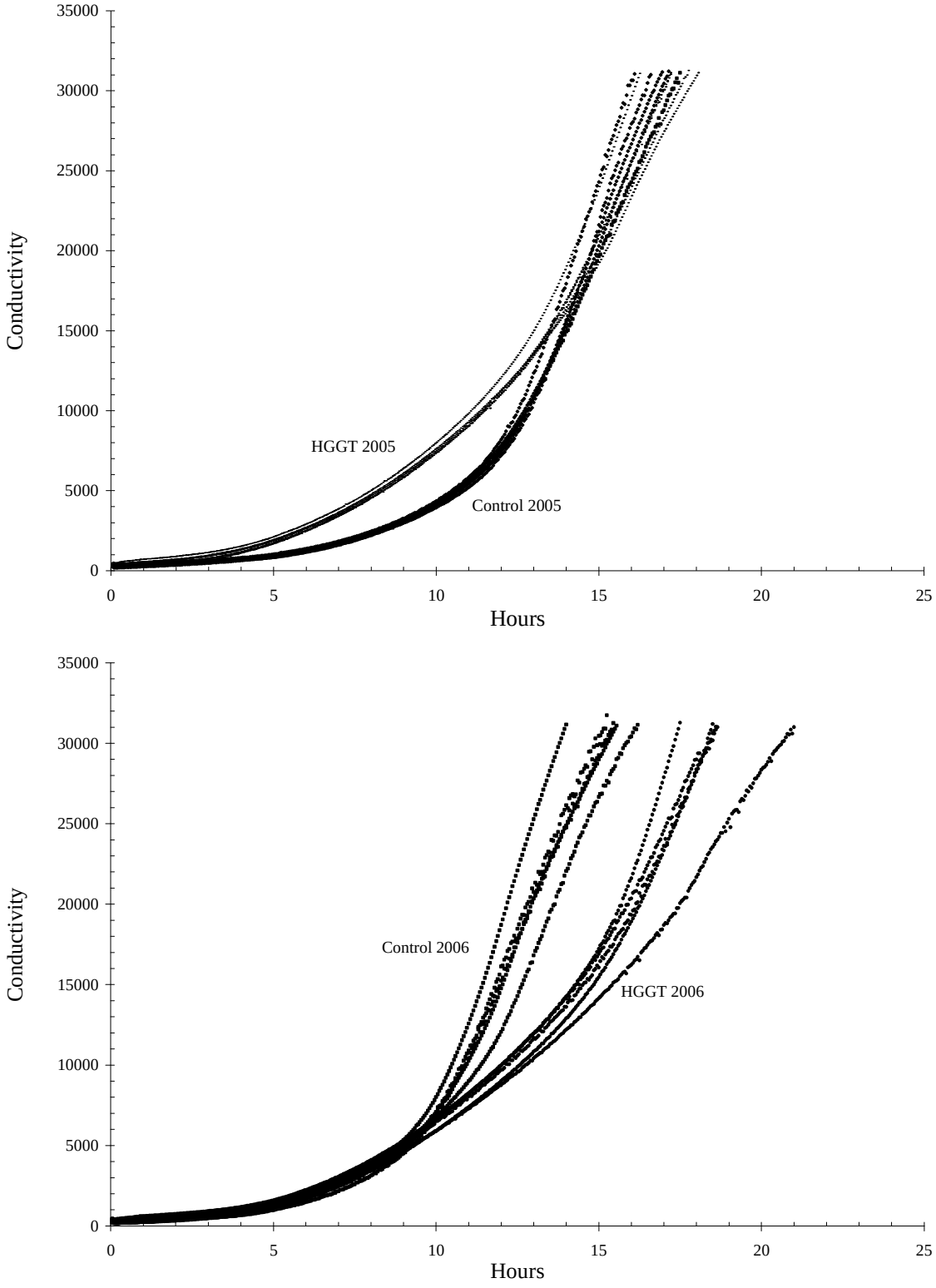

Figure 3. Oxidative stability index at 100°C of five subsamples each of extracted crude corn oil from control kernels and kernels modified with homogentisate geranylgeranyl transferase.

CHAPTER 4. GENERAL CONCLUSIONS

Tocotrienols affect the oxidative stability of corn oil in both a model system as well as in crude corn oils. The stability, as measured by peroxide value and oxidative stability index, of the oils was increased or reduced, depending on the concentration of the individual tocols and the initial condition of the oils. The prooxidant effect of very high tocols in crude oils may have been mitigated by endogenous compounds and thus, was less apparent than in the model RBD and stripped oils. Under the experimental conditions used, the degree of tocol methylation of the phenolic ring had a greater influence on oxidation than saturation or unsaturation of the hydrocarbon tail.

As the α-homologues increased in concentration from the 100 to 5,000 ppm, they lost their ability to retard oxidation. At concentrations of ≥ 700 ppm, the α-tocols began to increase primary oxidation and the rate of oxidation increased with higher levels. There was little or no effect with concentration for γ-tocopherol or the δ-tocols. However, the rate of change of the peroxide values was lower in γ- and δ-tocol amended oils than in the stripped control oil with no added tocols.

All tocols at all concentrations investigated extended the induction period (IP) as measured by OSI against the control oil. Diminishing returns were observed in inhibiting secondary products of oxidations. Although the IP increased with increasing tocol concentrations, a plateau was reached with individual tocols at concentrations of 700 ppm and above. Again, γ-tocopherol and δ-tocols were superior in extending oxidative stability of the corn oil.

Despite the very high levels of tocotrienols, 4,200 to 5,300 ppm, in the HGGT crude corn oil versus 250 to 300 ppm in the non HGGT crude corn oil, there was no prooxidive effect of these high levels of tocotrienol. Enhancement to the stability of the high T3 oil was minimal or nonexistent considering the dramatic increase in tocotrienols. Results of the

model oil experiments suggest that the optimal concentration for oil stability is considerably less than the endogenous levels found in the modified corn oils. Crude corn oils with these high concentrations of tocotrienols must be further manipulated or processed to maximize the impact of this modified material or the value capture elsewhere.

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