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Antioxidative effect of green tea extract on lipid oxidation of canola oil

Harveen Sethi
San Jose State University

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ANTIOXIDATIVE EFFECT OF GREEN TEA EXTRACT
ON LIPID OXIDATION OF CANOLA OIL

A Thesis

Presented to

The Faculty of the Department of
Nutrition and Food Science
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

By

Harveen Sethi

December 1997

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APPROVED FOR THE DEPARTMENT OF
NUTRITION AND FOOD SCIENCE

Dr. Miriam Saltmarch, Ph.D., Graduate Advisor
Department of Nutrition and Food Science.

Dr. Panfilo S. Belo Ph.D., Committee Member,
Department of Nutrition and Food Science.

Dr. Lucy McProud, Ph.D., R.D. Department Chairperson.
Department of Nutrition and Food Science.

APPROVED FOR THE UNIVERSITY

ABSTRACT

ANTIOXIDATIVE EFFECT OF GREEN TEA EXTRACT
ON LIPID OXIDATION OF CANOLA OIL

by Harveen Sethi

The purpose of the research was to investigate the antioxidative properties of an extract of green tea (GTE), and compare it with those of butylated hydroxyanisole (BHA) and α -tocopherol. Pure canola oil (1.75 L) and canola oils (1.75 L) treated with 200ppm BHA, 500ppm α -tocopherol and 200ppm GTE respectively were heated at 190° C for 16 hours. The pure canola oil, without any added antioxidants served as the control. Evaluation of antioxidative properties was conducted by means of sensory evaluation of odor, color, and overall acceptability of oil samples by using a semi-trained panel of 16 members, by thiobarbituric acid (TBA) testing, and by viscosity measurements.

Viscosity measurements did not correlate well with the average overall acceptance scores and the TBA results. Results of sensory evaluation and TBA testing correlated well and indicated that GTE (200ppm) and α -tocopherol (500ppm) showed no particular antioxidative effect compared to BHA (200ppm).

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DEDICATION

This thesis is dedicated to my husband, Manmeet Singh, whose support and encouragement helped me throughout the course of my studies at San Jose State University.

PREFACE

This thesis is written in publication style. The second chapter is written in journal format according to the online style guide for research papers and will be submitted to the Journal of Food Science. The first and third chapters are written according to the guidelines outlined in the Publication Manual of the American Psychological Association, fourth edition, 1994.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Antioxidants are food additives, which help in extending the shelf life of oils and fatty foods during storage and processing, by delaying the oxidation of autoxidizable materials. Natural antioxidants are present in virtually all food commodities providing them with a valuable degree of protection against oxidation. When food commodities are subject to processing, such natural antioxidants are often depleted. Therefore, food processors routinely add synthetic antioxidants to processed food products in order to delay the onset of oxidation.

Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroxyquinone (TBHQ), and α -tocopherol have been used to improve the stability of fats & oils and a wide variety of edible products containing them. However, synthetic antioxidants such as BHA and BHT have decreased in popularity due to their suspected action as promoters of carcinogenesis as well as a general rejection of synthetic food additives by the public. Natural antioxidants such as tocopherols, found in almost every vegetable oil have the ability to inhibit lipid peroxidation *in vivo* by scavenging peroxy radicals. Unfortunately they are much less effective as food

antioxidants (Xie, Huang, Chen, & Ho, 1993). As a result, the search for better antioxidants of natural origin continues unabated.

Tea is one of the most popular beverages of the world. It is believed to have a wide range of physiological properties including being a stimulant, anti-depressant, anti-inflammatory, anti-hypertensive, anti-atherosclerotic and hypocholesteremic agent. Tea also contains large quantities of phenolic compounds, which are believed to have excellent antioxidant properties (Zhu, Qiao, Zhang & Lu, 1992). Green tea contains about 30% total phenols of which the most important are the flavanols or tea catechins (Lunder, 1992, p. 116).

Several studies have been done to investigate the antioxidant properties of various fractions of green tea and their major phenolic components. Results of most of these studies indicate that the phenolic components of green tea have strong antioxidant activities (Chen, Chen, Ma, Fung, & Wang, 1996; Ho, Chen, Huang, Zhang, & Rosen, 1992; Tanizama, Toda, Sazuka, Taniyama, Hayashi, Arichi, & Takino, 1984; Wu, 1993; Xie et al., 1993; Zhao, Li, He, Cheng, & Xin, 1989). However, not much information is currently available about their antioxidative effects on cooking/frying oils and fatty foods.

Objectives

The objective of this study was to investigate the

antioxidative effect of an extract of green tea on oxidation of heated canola oil as compared to BHA and α -tocopherol.

Significance of the Study

The ability of fats and oils to carry odors & flavors and to serve as a convenient means of rapid heat transfer has lead to their increasing use in commercial frying operations. However prolonged use at elevated temperatures can lead to oxidative and thermal degradative changes in the cooking/ frying oils. Synthetic antioxidants are presently being used in the stabilization of fats and oils and a wide variety of edible products containing them. However, because of consumer concerns, synthetic additives are becoming less popular these days.

Green tea is an excellent dietary resource for natural antioxidants, but not much information is currently available about its antioxidative effects on cooking/ frying oils and fatty foods. If the antioxidative effect of green tea extract on lipid oxidation of oils is shown to be similar to that of synthetic antioxidants currently in use or greater than tocopherols, this could be of great economic benefit to the food industry. The results of this study will contribute to the available information on the antioxidative effect of green tea on cooking/frying oils.

Review of Literature

Fats have at least three important functions in foods:

culinary, physiological and nutritional. The quality of oil as a heating/frying medium and the quality of the food produced are closely linked. Vegetable oils are frequently used in bulk quantities as a heat transfer medium, therefore their cooking performance and quality at elevated temperatures are of great interest to oil processors, commercial frying operators as well as individual consumers.

Chemistry of Heating of Oils

Prolonged heating of oils and fats at high temperatures ($\sim 180^{\circ}$ - 190° C) in the presence of oxygen can lead to development of lipid oxidation which can further result in the formation of several volatile and non-volatile decomposition products (Nawar, 1985). According to Sherwin (1978), lipid oxidation can result in rancidity in finished products, making them unacceptable to consumers, and can also cause other degrading effects such as discoloration, vitamin destruction, nutritional losses, polymerization, and possible toxic effects.

The unsaturated sites of the fatty acids in fats and oils are the most susceptible sites for heat-induced and radical-based fat oxidation. It is widely agreed that the autoxidation of unsaturated fatty acids occurs via a free radical mechanism that consists of three basic steps: initiation, chain propagation, and termination.

Initiation: -

Propagation: -

Termination: -

During initiation, a hydrogen atom (RH) is removed from the methylene carbon atom adjacent to the double bond in the fatty acid. This reaction results in the formation of a free radical ($\text{R}^\bullet$) and can be catalyzed by light, heat, trace metals, pigments such as chlorophyll, or enzymes such as lipoxigenases. The free radical ($\text{R}^\bullet$) reacts with atmospheric oxygen to form an unstable peroxy free radical ($\text{ROO}^\bullet$), which may in turn abstract hydrogen from another unsaturated fatty acid to form a hydroperoxide (ROOH) and a new free radical. The new free radical initiates further oxidation and contributes to the chain reaction. The chain reaction (or propagation) may be terminated by formation of nonradical products from two free radicals.

According to Nawar (1985), the initiation step of autoxidation is thermodynamically difficult with activation energy of about 35 Kcal/mole. Hence, the production of first few radicals necessary to start the chain reaction must occur by some catalytic means. It has been postulated that singlet oxygen is the active species involved in the initiation of autoxidation and this can be generated in a variety of ways, probably the most important is via photosensitization by the natural pigments in foods. Singlet oxygen being highly electrophilic, can react with moieties of high electron density such as C = C bonds. The resulting hydroperoxides can then cleave to initiate a conventional free radical chain reaction.

Also, the propagation step of the autoxidative process includes an induction period when hydroperoxide formation is minimum. These induction periods can be dramatically altered by the use of pro- and antioxidants or synergists (Labuza, 1971; Perkins, 1967; Sherwin, 1978; Warner & Frankel, 1985).

End Products of Autoxidation

Numerous degradation products are formed during the autoxidation process. These products include a series of aldehydes, ketones, acid esters, alcohols, hydrocarbons, lactones, cyclic compounds, dimers and polymers. Several other compounds may also be formed from the many possible interactions among the lipid decomposition products or

between these products and other non-lipid food components. The relative amounts of these compounds may vary widely depending on the nature of the substrate and the parameters of the heat treatment.

Methods to measure oxidation

A measure of the heat abuse taking place in an oil and an understanding of the products formed during deep-fat frying are of great interest and importance to the consumer and to the food industry. Therefore, many methods ranging from simple to complex have been developed for analyzing heat abuse of a fat or oil. The method(s) chosen may depend on a number of factors such as accuracy of measurement desired, resources available for establishing and running the tests, and the time allowed.

According to Perkins (1992), the degree of oxidation of oils can be measured/monitored by:

(a) Physical methods - such as molecular weight, refractive index, viscosity, specific gravity and dielectric constant, all of which increase with heating time. This type of data indicates that polymerization is taking place and in the case of dielectric constant means that there is oxygen incorporation into the fat.

(b) Chemical methods - such as the peroxide value, iodine value (which decreases as a result of destruction of unsaturation), TBA test and measurement of polar materials.

(c) Sensory analyses.

According to Warner and Frankel (1985), although chemical and physical measurements are useful in determining the oxidative stability of oils, sensory evaluation of the odor and flavor quality of vegetable oils is considered to be the ultimate method of assessing oil quality. Objective tests determine oil autoxidation, but sensory analysis measures oxidized flavors as well as those from other sources (Warner, 1985). A number of researchers (Dobbs, Vaisey-Genser & Diamant, 1978; Hawrysh, Erin & Lin, 1989a; Hawrysh, Shand, Tokarska & Lin, 1988; Vaisey-Genser & Ylimaki, 1985) have used trained panelists to assess the oxidative stability of canola oils via measurements of odor and flavor intensity. According to Jackson (1981), combined with chemical and/or instrumental data, sensory analysis is the final judgment of oil quality.

Food Antioxidants

Antioxidants are of great interest to food scientists and food manufacturers. Control of nonenzymatic lipid oxidation, which often limits the shelf life of manufactured foods, is usually achieved by adding antioxidants to food. The antioxidants to be used are determined by various factors including legislation, effectiveness and cost. The effectiveness of antioxidants may vary depending on the food and conditions of processing and storage (Gordon &

Kourimská, 1995).

Antioxidants are added to fats and oils to retard the oxidation of fatty acids and thus to decrease the development of rancidity. However, it is important to remember that an antioxidant cannot improve the flavor of already damaged fat or oil. According to Dugan (1976), Sherwin (1976), Sherwin (1978), and Frankel (1985), antioxidants can inhibit or terminate the autoxidation "chain" reaction by acting as hydrogen donors and forming inactive non-radical products. The action of most antioxidants can be described as $R^{\bullet} + AH \longrightarrow RH + A^{\bullet}$, where $R^{\bullet}$ depicts the fatty acid free radical and AH is the antioxidant. The resultant antioxidant free radical ($A^{\bullet}$) is a "stable resonance hybrid" that is unable to initiate or propagate further oxidation (Sherwin, 1985).

The antioxidants most commonly added to fats and oils and a wide variety of edible products containing them are synthetic phenolic compounds such as butylated hydroxyanisole(BHA), butylated hydroxytoluene(BHT), propyl gallate(PG), and tertiary butylhydroxyquinone(TBHQ). These phenolic compounds react with lipid free radicals to form antioxidant free radicals that can in turn be degraded to form quinones, thereby terminating the chain reaction (DeMan, 1980).

According to Gordon and Kourimská (1995), BHA has good

stability and is an effective antioxidant in fried foods, but it does not contribute much towards increasing the stability of oils containing tocopherols. BHT is very effective in animal fats, but is less effective in vegetable oils and may be lost during frying because of its steam volatility. TBHQ is known to be a very effective antioxidant for vegetable oils and is stable at high temperatures, but its antioxidant activity in fried foods is little better than that of BHA. Recently BHA and BHT, the most widely used synthetic antioxidants in the food industry have decreased in popularity due to their suspected action as promoters of carcinogenesis as well as a general rejection of synthetic food additives by the public (Xie et al., 1993).

Tocopherols are the most important natural antioxidants commercially available. They are naturally present in almost all vegetable oils and are believed to inhibit lipid peroxidation *in vivo* by scavenging peroxy radicals (Burton, 1994). Unfortunately, tocopherols are much less effective than BHA or BHT as food antioxidants (Xie et al., 1993). Therefore, the search for other antioxidants of natural origin is highly desirable. A variety of compounds with oxidation inhibition activity have been studied and proposed for use in foods. These compounds include the natural flavorings such as extracts of rosemary and sage, and compounds found in wood smoke. More recent research in this

area has investigated items such as rice bran oil and the water-soluble and fat-insoluble catechins present in green tea for use as food antioxidants. Since tea is believed to have other health benefits such as being a stimulant, anti-depressant, anti-inflammatory etc., besides antioxidant capabilities, the use of tea extracts may possibly have some advantages over traditional antioxidants.

Federal Regulations on the Application of Antioxidants to Fats and Oils

According to the Office of the Federal Register National Archives and Records Administration (1996), in the United States the current approved levels for use of BHA, BHT, and TBHQ in foods is 200ppm, mixed or alone. Since α -tocopherol is a natural antioxidant, initially there was no limitation for its addition to food (Nawar, 1985, p.200) but in 1987, the Codex Committee on fats and oils recommended the following maximum for food additives: 75mg/kg for BHT, 175mg/kg for BHA, and 500mg/kg for tocopherols (Patterson, 1989).

Green Tea

Tea beverages are prepared from the leaves of *Camellia sinensis*. Second only to water, tea is the most widely consumed beverage in the world today with annual per capita consumption exceeding 40 L (International Tea Committee Ltd. Annual Bulletin of Statistics, 1990, London). There are

three major types of tea produced - green (unfermented), oolong (partially fermented), and black (fully fermented). Green teas are mostly consumed in Asian countries, whereas black teas are more popular in western countries.

Manufacture of Tea

The manufacturing process determines the type of tea to be produced. The manufacturing process of green tea is designed to prevent the oxidation of phenolic components by catalytic enzymes, whereas the manufacturing of oolong and black teas involves the partial or complete enzymatic oxidation of green tea flavan-3-ols contained in the leaf cytoplasm, to a number of condensed polyphenols such as theaflavins, thearubigens, bisflavonols, etc. (Balentine, 1992).

Composition of Tea

Tea composition varies with climate, growing conditions, genetic strain and the age of the leaf. The phenolic compounds, especially the catechin group of flavanols, constitute the most interesting group of tea leaf components (Graham, 1992). The fresh tea leaf is very rich in catechins, which may constitute up to 30% of the dry leaf weight. Green tea composition is very similar to that of the fresh leaf except for a very few enzymatically catalyzed changes, which occur extremely rapidly following plucking; hence catechins are the predominant group of substances in green tea also.

Antioxidative Components of Green Tea

The catechins are colorless, astringent, water-soluble compounds. They are readily oxidizable with varying oxidation potentials and this property has been exploited in their use as food antioxidants. Graham (1992) suggested that catechins may retard rancidity in fats and oils by quenching free radical peroxide activity brought about by aerobic oxidation.

According to Lunder (1992), the principal phenolic components in green tea are the catechins, which include (+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin (EC), (-)-Epigallocatechin (EGC), (-)-Epicatechingallate (ECG) and (-)-Epigallocatechingallate (EGCG). He also suggested that the antioxidant activity of green tea may be positively related to the content of epigallocatechin.

Antioxidant Mechanism for Phenolic Compounds

The antioxidant mechanism recognized for phenolic compounds involves quenching of peroxy (ROO°) radicals in the propagation step leading to the formation of hydroperoxides in the free radical sequence (Satue, Huang, & Frankel, 1995). According to the same researchers, phenolic compounds can also inhibit further decomposition of hydroperoxides by reacting with alkoxyl radicals (RO°), which are responsible for the generation of volatile compounds such as hexanal contributing to rancidity.

Antioxidant Effects of Green Tea Extracts

Several studies have been conducted on the antioxidative effects of tea extracts in various model systems. Ho et al. (1992) studied the antioxidant activities of twelve types of Chinese teas using Rancimat method and pure lard as substrate. They found that green tea extract (GTE) showed stronger antioxidant activity, compared to oolong and black tea extracts, possibly due to its higher EGCG content. In 1996, Chen et al. examined antioxidant activities of ethanol extracts of six types of teas against oxidation of heated canola oil. The researchers observed that GTE strongly inhibited oxidation of canola oil compared to BHT. In contrast, the examined oolong teas exhibited only moderate antioxidative activity possibly due to the partial destruction of natural phenolic components by semifermentation. The ethanol extracts of black, dark-green, and ginseng teas studied showed little or no protection to canola oil from lipid oxidation, possibly due to the complete destruction of natural phenolic compounds by fermentation during their manufacturing processes.

In 1993, Wu examined (a) scavenging action of GTE on singlet oxygen and (b) preventive effect of GTE on lipid peroxidation. He found that singlet oxygen (1O_2) produced by illuminated photosensitizer Rose Bengal could be scavenged by GTE, with the scavenging action being obvious at the

early stage of photosensitized oxidation. He also observed that the higher the concentration of GTE in the photo-oxidation reaction system, the stronger was the singlet oxygen scavenging action. The scavenging effect of the fresh GTE solution on singlet oxygen was better than that of old solution.

Studies have also been conducted to compare the antioxidative effects of the major phenolic components of tea. Tanizama et al. (1984) isolated and identified the antioxidative components in the leaves of green tea and found that 1-epicatechin (EC) was the main antioxidative component of tea leaf, based on the air oxidation of linoleic acid. The inhibitory concentration of EC was nearly equal to that of BHA and lower than that of dl- α -tocopherol. Xie et al. (1993) examined the antioxidant properties of fractions and phenolic constituents from green, oolong, and black teas using the Rancimat method and pure lard as substrate. These researchers observed that the antioxidant activities of tea fractions depended mainly on their flavanol contents. In another study, Zhao et al. (1989) reported that in stimulated polymorphonuclear leukocytes system, the water extract fraction from green tea and phenolic compounds in green tea had a much stronger scavenging effect on the active oxygen radicals compared to the effects of vitamin C and vitamin E.

From the above mentioned studies it is evident that several researchers have demonstrated that GTE can impart stability to pure oils in model systems, however much more work needs to be done to clarify the antioxidative function of GTE in complex foods during commercial food processing operations such as cooking in oils at elevated temperatures and deep-fat frying.

Canola Oil

Definition of Canola Oil

Canola is referred to as a rapeseed cultivar that contains less than 2% erucic acid in its oil and less than 30 $\mu\text{mol/g}$ of one or more combinations of the four aliphatic glucosinolates (gluconapin, progoitrin, glucobrassicinapin, and napoleiferin) in its defatted meal. In 1985, the United States Food and Drug Administration (FDA) recognized that rapeseed and canola were different species and thus granted GRAS (Generally Recognized As Safe) status to canola (Shahidi, 1990).

Polyunsaturated Fatty Acid Composition of Canola Oil

Canola oil has a unique polyunsaturated fatty acid composition that differentiates it from other oils. In general, canola oil has a higher oleic acid (C18:1) content (60%) and lower linoleic acid (C18:2) content (21%) than most other vegetable oils. Canola oil also has a higher linolenic acid (C18:3) content (8-12%) as compared to

vegetable oils such as soybean, sunflower, olive, and corn, which have 8.0%, 0.2%, 0.8%, and 0.7%, respectively (Anonymous, 1979; Sheppard, Iverson & Weihrauch, 1978).

It is well known that the rate of oxidation of lipid fatty acids increases in relation to their degree of unsaturation. In 1971, Labuza stated that the rate of oxidation of linolenic acid (C18:3) is twice that of linoleic acid (C18:2) and 25 times that of oleic acid (C18:1). Forss (1972) reported that C18:2, C18:3, and arachidonic acid (C20:4) are important precursors for off-odor and off-flavor development in foods since they readily form hydroperoxides. Hence, the high unsaturated fatty acid content of canola oil, especially linolenic acid can influence its stability and quality.

Effect of Antioxidants on Stability of Canola Oil

Canola oil undergoes oxidation and thermal degradation when it is heated at high temperatures in the presence of air. In addition, heated canola oil develops an unpleasant odor that has been described as painty with buttery, sweet, sulfurlike, and fishy notes (Dobbs, Vaisey-Genser, & Diamant, 1978; Eskin, Vaisey-Genser, Durance-Todd, & Przbylski, 1989). Initially, Niewiadomski (1970) observed this phenomenon in heated rapeseed oil and attributed this unpleasant odor to the oxidation of unsaturated fatty acids. Later, McKeag (1977) suggested that the heated room odor of canola oil, i.e. the odor of a room in which canola oil is

being heated, may be due to oxidation of linolenic acid.

Phenolic antioxidants such as BHA and BHT, either in combination or individually, are commonly added to canola oil during processing to retard oxidative changes due to storage and heat. Recently, questions regarding the efficacy of these phenolic antioxidants have arisen. Several researchers (Hawrysh, et al., 1988; Tokarska, Hawrysh, & Clandinin, 1986; Vaisey-Genser & Ylimaki, 1985) have concluded that BHA/BHT is not effective in promoting canola oil stability. Thus, the addition of BHA/BHT to canola oils at allowed maximum levels does not appear to be beneficial for either canola oil processors or consumers.

Antioxidants such as ascorbyl palmitate (AP) and α -tocopherol, either in combination or individually, may offer advantages over other antioxidants in terms of efficacy, safety, and positive labeling connotations. Hawrysh, et al. (1989a), found that combinations of dl α -tocopherol and AP (100ppm each) extended canola oil stability for up to eight days of Schaal storage, whereas dl α -tocopherol at 200ppm was generally ineffective in retarding oxidation in stored canola oils. Schaal storage test involves putting a small amount of the fat in a beaker and placing it into an oven under standardized conditions at 60°C to oxidize the samples. Samples are then taken periodically and peroxide values are determined on them (Perkins, 1992). Evaluations

of the efficacy of dl α -tocopherol alone or together with AP in light-exposed canola oils also suggested that these antioxidants did not delay off-odor and off-flavor development (Hawrysh, Erin & Lin, 1989b).

According to Hawrysh et al. (1988, 1989a), changes in the flavor and oxidative stability of antioxidant (TBHQ or ascorbyl palmitate) treated canola oil that occur during accelerated storage tests are not indicative of those that take place during actual storage or those that result from heating (McMullen, 1988; Vaisey-Genser & Ylimaki, 1985). Therefore, further studies on the quality and stability of antioxidant treated canola oils exposed to a variety of processing conditions are needed to determine the cause(s) for differences in antioxidant efficacy.

Antioxidants such as BHA, BHT, TBHQ and α -tocopherol are compounds used in foods to prevent deterioration, rancidity or discoloration caused by oxidation. However, synthetic antioxidants such as BHA and BHT have decreased in popularity because of consumer concerns about their suspected actions as promoters of carcinogenesis. Health practitioners and dietitians have praised the fatty acid profile of canola, labeling it the oil with an almost perfectly balanced mixture of fatty acids for human nutrition and health. Research also indicates that the fatty acid composition of canola oil is most favorable in

terms of health benefits and as a part of a nutritionally balanced diet. As a result, canola oil is becoming a popular medium for cooking and frying operations in the food industry these days. However, the relatively high unsaturated fatty acid content of canola oil makes it more susceptible to oxidation and thermal degradation when it is heated at high temperatures in the presence of air. Phenolic antioxidants such as BHA and BHT have been added to canola oil to retard these oxidative changes; however, recently several researchers have concluded that these antioxidants are not very effective in promoting canola oil stability. As a result, a variety of compounds with oxidation inhibition activity such as rosemary extracts and polyphenolic catechins present in green tea have been studied and proposed for use in foods. Studies conducted by several researchers have indicated that phenolic components of green tea have strong antioxidant activities. However detailed information about the antioxidative effects of these compounds on the lipid oxidation of cooking/frying oils and fatty foods is lacking.

CHAPTER 2
JOURNAL ARTICLE

Authors' Title Page

ANTIOXIDATIVE EFFECT OF GREEN TEA EXTRACT
ON LIPID OXIDATION OF CANOLA OIL

Harveen Sethi, Miriam Saltmarch, Lucy McProud

and Panfilo Belo

Department of Nutrition and Food Science

San Jose State University

1 Washington Square

San Jose, CA 95192

Address questions and comments to: Dr. Miriam Saltmarch,
Department of Nutrition and Food Science, San Jose State
University, 1 Washington Square, San Jose, CA 95192,
(408) 924-3118.

ABSTRACT

The effectiveness of an extract of green tea (GTE) as an antioxidant was compared with that of butylated hydroxyanisole (BHA) and α -tocopherol during heating of canola oil at deep-fat frying temperatures. Pure canola oil (1.75 L) and canola oils (1.75 L) treated with 200ppm BHA, 500ppm α -tocopherol, and 200ppm GTE respectively were subjected to 16 hours of heating at 190°C. Evaluation of antioxidative properties was conducted by means of sensory evaluation of odor, color, and overall acceptability of oil samples by using a semi-trained panel of 16 members; by thiobarbituric acid (TBA) testing; and by viscosity measurements. Results of sensory evaluation and TBA testing showed that GTE (200ppm) and α -tocopherol (500ppm) exhibited no particular antioxidative effect against thermal oxidation of canola oil, compared to BHA (200ppm).

Key Words: green tea extract, BHA, antioxidant, thermal oxidation, canola oil

INTRODUCTION

Antioxidants are food additives, which help in extending the shelf life of oils and fatty foods during storage and processing, by delaying the oxidation of autoxidizable materials. Natural antioxidants are present in virtually all food commodities providing them with a valuable degree of protection against oxidation. When food commodities are subject to processing, such natural antioxidants are often depleted. Therefore, food processors routinely add synthetic antioxidants to processed food products in order to delay the onset of oxidation.

Antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroxyquinone (TBHQ), and α -tocopherol have been used to improve the stability of fats & oils and a wide variety of edible products containing them. However, synthetic antioxidants such as BHA and BHT have decreased in popularity due to their suspected action as promoters of carcinogenesis as well as a general rejection of synthetic food additives by the public. Natural antioxidants such as tocopherols, found in almost every vegetable oil have the ability to inhibit lipid peroxidation *in vivo* by scavenging peroxy radicals. Unfortunately they are much less effective as food antioxidants (Xie, Huang, Chen, & Ho, 1993). As a result, the search for better antioxidants of natural origin

continues unabated.

Tea is one of the most popular beverages of the world. It is believed to have a wide range of physiological properties including being a stimulant, anti-depressant, anti-inflammatory, anti-hypertensive, anti-atherosclerotic and hypocholesteremic agent. Tea also contains large quantities of phenolic compounds, which are believed to have excellent antioxidant properties (Zhu, Qiao, Zhang & Lu, 1992). Green tea contains about 30% total phenols, most important of which are the flavanols or tea catechins (Lunder, 1992).

Several studies have been done to investigate the antioxidant properties of various fractions of green tea and their major phenolic components. The results of most of these studies indicate that the phenolic components of green tea have strong antioxidant activities (Chen, Chen, Ma, Fung, & Wang, 1996; Ho, Chen, Huang, Zhang, & Rosen, 1992; Tanizama, Toda, Sazuka, Taniyama, Hayashi, Arichi, & Takino, 1984; Wu, 1993; Xie et al., 1993; Zhao, Li, He, Cheng, & Xin, 1989). However, not much information is currently available about their antioxidative effects in cooking/frying oils and fried foods. If the antioxidative effect of green tea extract on lipid oxidation of oils is proven to be similar to that of synthetic antioxidants, currently in use or greater than tocopherols, this could benefit the food industry.

The objectives of this study were to investigate the antioxidative effect of an extract of green tea on oxidation of heated canola oil and compare it with that of BHA and α -tocopherol.

The results of this study are intended to contribute to the available information on the antioxidative effect of green tea in cooking/frying oils.

LITERATURE REVIEW

Fats and oils have at least three important functions in foods: culinary, physiological and nutritional. The quality of oil as a heating/frying medium and the quality of the food produced are closely bound. Vegetable oils are frequently used in bulk quantities as heat transfer medium, hence their cooking performance and quality at elevated temperatures are of interest to oil processors and commercial frying operators as well as individual consumers.

The processes, which take place when an oil or fat is heated at high temperature in the presence of oxygen, may be termed as thermal oxidation. Such changes are analogous to those which probably take place in a deep fat fryer under normal operating conditions; i.e., a surface layer of hot fat is in contact with oxygen in the atmosphere above the fryer (Perkins, 1967). These changes may involve a complex pattern of thermolytic and oxidative reactions and may result in several physical and chemical changes. The

visible changes taking place in a fat or oil during heating and frying include darkened color, increased viscosity, decreased smoke points, and increased foaming (Rock & Roth, 1967; Tangel, Leeder, & Chang, 1977). Morton (1997) suggested that these visible changes and other chemical changes could be attributed to three different types of reactions that can occur in heated fats:

1. Fats can hydrolyze to form free fatty acids and mono- and diglycerides.
2. Fats can oxidize to form hydroperoxide, epoxides, hydroxides, conjugated dienoic acids, aldehydes, and ketones. These compounds may undergo fission into smaller fragments or may remain in the triglyceride molecule and cross-link with each other, leading to formation of dimeric and higher polymeric triglycerides. Also thiobarbituric acid (TBA)- reactive material, such as malondialdehyde (MDA), may be formed from the decomposition of hydroperoxides produced by the oxidation of unsaturated fatty acids.
3. Fats can form new carbon-carbon linkages in the absence of oxygen resulting in the formation of cyclic fatty acids, dimeric acids, or polymers with higher molecular weights.

The relative amounts of these products can vary widely depending on the nature of the substrate and the parameters of the heat treatment. These products have been studied by

researchers for many years to determine the relationship of these compounds to (1) nutritive value and toxic effects of the heated oil and (2) sensory quality of the oil and food fried in it (White, 1991).

Researchers have used many methods to assess the heat abuse and/or lipid oxidation of a fat or oil. Examples include physical methods such as measurement of refractive index, viscosity and dielectric constant; chemical methods such as peroxide value, iodine value, TBA test and measurement of polar materials; and sensory evaluation of oils and fats. The method(s) chosen depend on a number of factors such as accuracy of the measurement desired, resources available for establishing and running the tests, and the time allowed.

According to Nawar (1985), the formation of malondialdehyde (MDA) is the basis of the well-known TBA method used for measuring oxidation of oils and fats. MDA is one of the oxidation products of unsaturated systems and it can produce a color reaction with TBA. The TBA test is one of the most widely used tests for evaluating the extent of lipid oxidation. It is believed that the chromagen results from condensation of two molecules of TBA with one molecule of MDA. Various compounds, other than those found in oxidized systems, have been found to interfere with the TBA test by producing the characteristic red pigment upon reaction with the reagents. However, in many cases the TBA

test is applicable for comparison of samples of a single material at different states of oxidation.

Antioxidants are of great interest to food scientists and food manufacturers. Control of nonenzymatic lipid oxidation, which often limits the shelf life of manufactured foods, is usually achieved by adding antioxidants to food. The antioxidants to be used are determined by various factors including legislation, effectiveness and cost. The effectiveness of antioxidants varies depending on the food and conditions of processing and storage (Gordon & Kourimská, 1995).

According to Dugan (1976), Sherwin (1976), Sherwin (1978) and Frankel (1985), antioxidants can inhibit or terminate the autooxidation "chain" reaction by acting as hydrogen donors and forming inactive non radical products. The action of most antioxidants can be described as $R^\bullet + AH \longrightarrow RH + A^\bullet$, where $R^\bullet$ depicts the fatty acid free radical and AH is the antioxidant. The resultant antioxidant free radical ($A^\bullet$) is a "stable resonance hybrid" that is unable to initiate or propagate further oxidation (Sherwin, 1985). According to DeMan (1980), phenolic antioxidants can react with lipid free radicals to form antioxidant free radicals that can, in turn be degraded to form quinones, thereby terminating the chain reaction.

The antioxidants most commonly added to fats and oils

and a wide variety of edible products containing them are synthetic phenolic compounds such as BHA, BHT, propyl gallate(PG), and TBHQ. In 1987, the Codex Committee on fats and oils recommended the following maximum for food additives: 75mg/kg for BHT, 175mg/kg for BHA, and 500mg/kg for tocopherols (Patterson, 1989).

In recent years significant attention has been focused on the potencies of natural antioxidant compounds as popularity of BHA and BHT, the most widely used synthetic antioxidants in food systems, has decreased due to their suspected action as promoters of carcinogenesis, as well as a general rejection of synthetic food additives by the public (Xie et al., 1993).

Tocopherols are the most important natural antioxidants commercially available. They are naturally present in almost all vegetable oils and are believed to inhibit lipid peroxidation *in vivo* by scavenging peroxy radicals (Burton, 1994). Unfortunately, tocopherols are much less effective than BHA or BHT as food antioxidants (Xie et al., 1993). Therefore, the search for other antioxidants of natural origin continued unabated.

According to Zhu, Qiau, Zhang and Lu (1992), teas are believed to have a wide range of physiological properties including being a stimulant, anti-depressant, anti-inflammatory, anti-hypertensive, anti-atherosclerotic and hypocholesteremic agent. Teas are also excellent dietary

resources for natural antioxidants such as phenolic compounds. Green tea contains about 30% total phenols, most important of which are the flavanols or tea catechins. According to Lunder (1992), the principal phenolic compounds in green tea are the catechins, which include (+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin (EC), (-)-Epigallocatechin (EGC), (-)-Epicatechingallate (ECG), and (-)-Epigallocatechingallate (EGCG). Lunder (1992) also suggested that the antioxidant activity of green tea may be positively related to the content of epigallocatechin.

Several studies have been done to investigate the antioxidant properties of various fractions of green tea and their major phenolic components. Results of most of these studies indicate that the phenolic components of green tea have strong antioxidant activities (Chen et al., 1996; Ho et al., 1992; Tanizama et al., 1984; Wu, 1993; Xie et al., 1993; Zhao et al., 1989).

The antioxidant mechanism recognized for phenolic compounds involves quenching of peroxy ($\text{ROO}^\bullet$) radicals in the propagation step leading to the formation of hydroperoxides in the free radical sequence (Satue, Huang, & Frankel, 1995). According to the same researchers, phenolic compounds can also inhibit further decomposition of hydroperoxides by reacting with alkoxy radicals ($\text{RO}^\bullet$), which are responsible for the generation of volatile

compounds such as hexanal contributing to rancidity.

Canola is referred to as a rapeseed cultivar that contains less than 2% erucic acid in its oil and less than 30 $\mu\text{mol/g}$ of one or more combinations of the four aliphatic glucosinolates (gluconapin, progoitrin, glucobrassicinapin, and napoleiferin) in its defatted meal. In 1985, the United States Food and Drug Administration (FDA) recognized that rapeseed and canola were different species and thus granted GRAS (generally recognized as safe) status to canola (Shahidi, 1990).

Canola oil has a unique polyunsaturated fatty acid composition, which differentiates it from other oils. In general, canola oil has a higher oleic acid (C18:1) content (60%) and lower linoleic acid (C18:2) content (21%) than most other vegetable oils. Canola oil also has a high content (8-12%) of linolenic acid (C18:3) compared to vegetable oils such as soybean, sunflower, olive, and corn, which have 8.0%, 0.2%, 0.8%, and 0.7%, respectively (Anonymous, 1979; Sheppard, Iverson & Weihrauch, 1978).

Canola oil undergoes oxidation and thermal degradation when it is heated at high temperatures in the presence of air. In addition, heated canola oil develops an unpleasant odor that has been described as painty with buttery, sweet, sulfurlike, and fishy notes (Dobbs, Vaisey-Genser, & Diamant, 1978; Eskin, Vaisey-Genser, Durance-Todd, &

Przbylski, 1989). Initially, Niewiadomski (1970) observed this phenomenon in heated rapeseed oil and attributed this unpleasant odor to the oxidation of unsaturated fatty acids. Later, McKeag (1977) suggested that the heated room odor of canola oil may be due to oxidation of linolenic acid.

Phenolic antioxidants such as BHA and BHT, either in combination or individually, are commonly added to canola oil during processing to retard oxidative changes due to storage and heat. Recently, questions regarding the efficacy of these phenolic antioxidants have arisen. Researchers (Hawrysh, Shand, Tokarska, & Lin, 1988; Tokarska, Hawrysh, & Clandinin, 1986; Vaisey-Genser & Ylimaki, 1985) have concluded that BHA/BHT is not effective in promoting canola oil stability. Thus, the addition of BHA/BHT to canola oils at allowed maximum levels does not appear to be beneficial for either canola oil processors or consumers.

The use of canola oil by consumers and in commercial food preparation is becoming increasingly popular these days as nutrition experts have suggested that canola oil is an almost perfectly balanced mixture of fatty acids for human nutrition and health. However, the high unsaturated fatty acid content, especially C18:3, in canola oil can influence its stability and quality. The need therefore arises, to examine the efficacy of other alternate antioxidants such as extracts of green tea, on the storage and heat stability,

and quality of canola oil.

MATERIALS AND METHODS

Materials. The heating medium used in this study was 100% pure canola oil with no added antioxidants (Wesson brand), purchased from a local market. BHA and α -tocopherol were purchased from Sigma Chemicals, and the green tea (Long Jing brand) was purchased from a local Chinese tea store.

Preparation of Green Tea Extract (GTE). GTE was prepared according to the method described by Ho et al. (1992) with some modifications. Specifically, 100g of dried green tea was ground and soaked in 200ml Ethanol (Spectrum, 200 proof) overnight and then homogenized for five minutes. The homogenized green tea was mixed with 200ml of ethanol and was shaken in an automatic shaker-bath set at 50°C, for two hours, followed by filtration. The residue was mixed with 400ml of ethanol and subjected to further shaking for two hours, followed by filtration. The combined filtrate was dried in a rotary evaporator at 60°C. The residue was dried overnight in a vacuum oven at 63°C and 17"Hg to obtain the dried green tea extract, which was then dissolved in distilled water (0.4g/100ml) and each 100ml of the solution was extracted three times with 200ml Hexane to remove chlorophyll. The aqueous phase was freeze-dried for four days to obtain the final GTE. The total phenolic content of the GTE was determined spectrophotometrically according to the

method described by Lau, Lu, and Huang (1989).

The final green tea extract was pale yellow in color and had a slight detectable odor. Its total phenolic content was determined to be 92.05% and it was incorporated into canola oil via 200 proof Ethanol, which served as a carrier to make the otherwise insoluble GTE soluble in oil.

Experimental Design. The oxidation of canola oil was studied using three antioxidant treatments. These were: 100% pure canola oil treated with 200ppm BHA; 100% pure canola oil treated with 500ppm α -tocopherol and 100% pure canola oil treated with 200ppm GTE. Canola oil, 100% pure i.e. without any added antioxidant served as a control.

A pilot study conducted with 100% pure canola oil indicated that heating of the oil for 12 hours at 190°C was sufficient to develop distinct off odors in canola oil samples. However, for the actual experiment, heating time period of 16 hours was selected in order to collect more number of samples for the measurement of lipid oxidation by subjective and objective means.

A volume of 1.75 liters of control i.e. 100% pure canola oil and each of the above mentioned treated canola oils was heated at 190°C for 16 hours (8 hr./day for 2 days) in an electric fryer of 3.5 l capacity. Starting with 0 hour time, 50 ml oil samples were withdrawn at one hour intervals for the 16 hour heating period. The samples were

poured into glass bottles and stored at refrigeration temperatures until being evaluated by sensory, TBA and viscosity analysis. No fresh oil was added to the fryers during the entire heating process.

Evaluation of Lipid Oxidation of Heated Canola Oil.

Lipid oxidation was measured/ monitored by conducting sensory evaluation, TBA testing, and viscosity measurements on the oil samples. Previous studies on antioxidative properties of green tea did not evaluate the sensory qualities of the test samples. However, in this study, sensory evaluation of the oil samples was also conducted along with objective testing, as lipid oxidation can adversely affect the consumer acceptability of oils/ fats and fatty foods by causing a marked flavor deterioration in these products.

A sensory panel consisting of 16 members conducted evaluation of odor, color and overall acceptance of the oil samples. The panel members were graduate & undergraduate students from the Department of Nutrition and Food Science at San Jose State University. Written approval for the use of human subjects in this study was obtained from the Human Subjects - Institutional Review Board, San Jose State University and then the panelists were approached personally for voluntary participation in this study. All of the panelists had prior experience in the use of sensory evaluation techniques. The panel was also trained to detect

oxidized odors from other odors. Panelists were served coded samples (10 ml oil samples at room temperature) in capped, clear plastic cups on white trays and were asked to evaluate odor, color and overall acceptance of the oil samples according to the scales depicted on the score cards in appendices - B, C and D. The results were the mean of 16 evaluations.

TBA testing of oil samples followed the sensory evaluation. The TBA values of the oil samples expressed as micrograms of MDA per gram of oil were determined according to the method outlined by Botsoglou, Fletouris, Papageorgiou, Vassilopoulos, Mantis, and Trakatellis (1994). The results were the mean of duplicated measurements.

Viscosity measurement is a traditional, non-specific method used by several researchers to assess heat damage in oils. A Brookfield Synchro-Lectric LVT Viscometer (Brookfield Engineering Laboratories, Stoughton, Massachusetts) was used to measure viscosities of the oil samples. The viscosity measurements in the current study were conducted at 60 RPM, using a #2 LV cylindrical spindle.

Statistical Analysis. Trend line analyses were performed for samples from each antioxidant treatment. The slopes of the trend lines were calculated to compare antioxidative effects of the different antioxidant treatments. Two-factor analysis of variance (ANOVA) was applied to analyze the

sensory data, TBA values and viscosity values. F-ratios were used to determine significant difference at $p \leq .05$. Least Square Means Procedure was applied to compare means when analysis of variance indicated a significant ($p \leq .05$) effect.

RESULTS AND DISCUSSION

Sensory Evaluation. The average sensory evaluation scores for odor, color and overall acceptability are shown in Figures 1 to 3. The numeric ratings for all three sensory attributes measured, showed a difference with progress in heating time, and these ratings are presented in Tables 1 to 3.

The average odor scores for the BHA treated oil samples were highest during the entire heating process, followed by α -tocopherol, control and GTE treated oil samples until four hours. After four hours, GTE treated oil samples had higher odor scores than α -tocopherol and control oil samples respectively (Figure 1). Plots of the average odor scores vs. time indicated that the control samples had the steepest decrease in acceptability, possibly indicating that the rate of formation of volatile oxidation products was fastest in the control.

In the scale used for odor evaluation for oil samples, an average odor score of "3" was considered "disagreeable off odor." This score was reached at around 15 hours of

heating for the control oil samples whereas for the GTE, α -tocopherol and BHA treated oil samples the scores were greater than "3" till the end of heating period of 16 hours. This indicated that even though panel members perceived off odors in the antioxidant treated oil samples, they didn't consider the odors to be disagreeable until the end of the entire heating period.

The average color scores for the entire heating process were highest for the GTE treated oil samples, followed by BHA-treated oil samples, α -tocopherol treated oil samples and control oil samples respectively (Figure 2). The plot of average color scores vs. time also showed the steepest increase in color for the GTE treated oil samples, indicating the fastest rate of change in color for these oil samples. The comparatively higher color scores for the GTE treated oil samples throughout the heating period can possibly be attributed to the initial pale yellow color of green tea extract.

The average overall acceptance scores for the BHA-treated oil samples were better (highest) during the entire heating process, followed by the control, α -tocopherol and GTE treated oil samples (Figure 3). The pale yellow color and faint detectable odor of GTE may have influenced the sensory panel members' judgment about its overall acceptance, thereby resulting in lowest average overall

acceptance scores for the GTE treated oil samples. The panel members considered BHA treated oil samples acceptable, based on odor and color, until 12 hours of heating; control samples and α -tocopherol treated samples until 10 hours, and GTE treated oil samples until ~ 11 hours of heating time.

The measured sensory attributes showed significant changes as the heating time increased for all three treatments and the control. Table 6 illustrates the magnitudes of change with heating time for the odor, color, and overall acceptance scores for the oil samples.

TBA Values. The results for the TBA testing of the oil samples showed that for all three treatments and the control, the TBA values (TBAV) expressed as micrograms of malondialdehyde (MDA)/gram of oil increased as heating progressed from 0 to 16 hours. The MDA concentration was highest in the GTE treated oil samples and lowest in the BHA treated canola oil samples during the entire heating process of 16 hours (Table 4). The MDA concentration ($\mu\text{g/g}$ of oil) at the time of heating when the samples became unacceptable to the sensory panel, was determined to be approximately 0.47 for control oil samples, and 0.36, 0.5, and 0.55 for BHA, α -tocopherol and GTE treated oil samples respectively.

MDA is a secondary oxidation product of autoxidation and its rate of formation can be used to assess the extent

of lipid oxidation in a fat/oil. Thus from figure 4 and table 7, it can be seen that the rate of thermal oxidation was lowest in the BHA treated canola oil, followed by control (100% pure canola oil), GTE treated canola oil, and α -tocopherol treated canola oil respectively.

An induction period of five hours was observed for the BHA treated canola oil whereas, no significant induction periods were observed for the control, GTE treated, and α -tocopherol treated canola oils. Other researchers have also observed that phenolic antioxidants are effective in extending the induction period when added to oil which has not deteriorated to a great extent (Mabrouk & Dugan, 1961; Scott, 1965).

Viscosity Changes. The viscosities for all of the oil samples increased as heating progressed from 0 to 16 hours (Table 5). During the entire heating period of 16 hours, the viscosities of BHA treated oil samples were higher than those of the α -tocopherol treated oil samples, which in turn had higher viscosities than the control samples. The GTE treated oil samples, on the other hand, showed the lowest viscosities until 12 hours of heating. Beyond 12 hours, their viscosities were higher than the control samples, but less than the BHA and α -tocopherol treated oil samples (Figure 5). As mentioned earlier in the materials and methods section, the green tea extract was insoluble in

oil and was therefore dissolved in ethanol prior to its addition to canola oil. Thus, unlike the control, BHA or α -tocopherol treated oils, the GTE treated oil had been diluted with ~ 215 ml of ethanol. This could possibly account for the comparatively lower viscosity values of GTE treated oil samples for the first 12 hours of heating.

Table 7 illustrates that TBA and viscosity values changed significantly with increases in heating times for all three treatments and the control. It was also observed that the TBA results correlated with the overall acceptance scores whereas the viscosity values did not correlate well with the overall acceptability of the oil samples.

Analysis of Variance. Tables 8-12 illustrate the two-factor ANOVA results for sensory, TBA, and viscosity data of the oil samples. The F -ratio values in these ANOVA tables show significant differences in the sensory scores, TBA values, and viscosity values respectively of the different treatments at different hours of heating ($p \leq .05$). LSD procedure on ANOVA results of TBA data showed that, at the time of heating when oil samples became unacceptable to the sensory panel, the MDA concentration ($\mu\text{g/g}$ of oil) of control oil samples and α -tocopherol treated oil samples were not significantly different from each other but were significantly different from the MDA concentration of BHA

and GTE treated oil samples ($p \leq .05$).

Comparison of Present Study with Previous Research.

Results of this study indicate a weak antioxidative effect for the phenolic components of green tea, in contrast to previous studies, which have reported strong antioxidative properties for green tea phenolic compounds. This difference between previous studies and the present one may be due to differences in the methods used for extraction of green tea phenolic compounds, methods used to induce lipid oxidation in test samples, and/or oxidation measurement methods. Previous research demonstrating strong antioxidant activity of green tea involved the use of purified phenolic extracts of green tea or purified fractions of phenolic components of green tea.

In 1996, Chen et al. evaluated antioxidative properties of ethanol extract of green tea, and in 1992, Ho et al. studied polyphenol green tea extract and purified components of green tea such as gallic acid, EC, ECG, EGC, and EGCG for their antioxidant activities. Tanizawa et al. (1984) used commercial d-catechin and l-epicatechin in tests of antioxidative effects of green tea and in 1993, Xie et al. separated pure EC, ECG, EGC, and EGCG from green tea extract by column chromatography and then studied the antioxidant activities of these pure components.

The methods used by these researchers for inducing

lipid oxidation and for measuring its progress were also different than those employed in this study. Chen et al. (1996) conducted oxidation of canola oil in a sealed test tube at 100°C, with subsequent headspace oxygen analysis. Ho et al. (1992) and Xie et al. (1993) heated pure lard substrate in a sealed reaction vessel to 100°C, under 20 L/hr air supply, and measured lipid oxidation by Rancimat method. Tanizawa et al. (1984) conducted air-oxidation of linoleic acid, instead of pure oil or fat, at 60°C for two hours, with an air supply of 500 ml/min, followed by measurement of the peroxide value (POV) and thiobarbituric acid value (TBAV) of the test samples.

These researchers conducted only objective testing and did not consider the sensory qualities of the tea extract in heated oils. In the present study, consumer acceptability of GTE treated oil samples (based on odor and color) was also evaluated along with objective testing. This was done, as sensory acceptability is still considered to be the ultimate test for assessing oils/fats in any lipid oxidation study.

In addition, the present study constituted an experimental field study, which investigated the antioxidative properties of phenolic components of green tea under realistic conditions (i.e. prolonged heating of canola oil for 16 hr., at 190°C, in an open electric fryer) similar to those employed in commercial deep fat frying operations.

Under such conditions, many chemical reactions occurring in the oil such as oxidative degradation, thermal decomposition, polymerization etc. may affect/ alter the manifestation of antioxidative properties by green tea phenolic components.

CONCLUSIONS

Sensory analysis on the oil samples showed a decrease in average odor and average overall acceptance scores and an increase in average color scores under tested conditions. The comparatively lower average overall acceptance scores of GTE treated oil samples could possibly be attributed to the pale yellow color and faint detectable odor of green tea extract. TBA testing showed that concentration of malondialdehyde was highest in GTE treated oil samples and lowest in BHA treated oil samples during the entire heating period. Results of sensory evaluation and TBA testing indicated that green tea extract at 200ppm concentration exhibited no particular antioxidative effect on the thermal oxidation of canola oil, as compared to BHA (200ppm).

Based on these conclusions it is recommended that antioxidative effects of higher concentrations of GTE (i.e. higher than 200ppm) should be investigated in future trials of this study, as many researchers have observed the antioxidative effects of green tea in model systems.

Table 1. Sensory Evaluation Scores* For Odor (Scores are mean of 16 evaluations)

Treatment	Heating Time(hours)								
	0	2	4	6	8	10	12	14	16
Control	8.6	6.5	6.0	5.6	5.4	5.0	4.1	3.4	2.5
BHA	8.6	6.8	6.2	5.8	5.7	5.4	4.9	4.3	4.1
α -tocopherol	8.6	6.6	6.3	5.8	5.4	5.3	4.6	3.8	3.2
GTE	8.6	6.1	5.9	5.7	5.6	5.2	4.8	4.2	3.5

* The sensory evaluation score sheet used for odor evaluation of oil samples is shown in Appendix B.

Table 2. Sensory Evaluation Scores* For Color (Scores are mean of 16 evaluations)

Treatment	Heating Time(hours)								
	0	2	4	6	8	10	12	14	16
Control	10.4	15.2	18.5	22.3	28.4	34.8	38.2	43.1	49.7
BHA	11.8	16.4	22.7	27.8	33.1	38.0	43.9	48.1	53.5
α -Tocopherol	11.4	15.5	19.4	25.6	27.6	33.0	37.8	44.7	49.4
GTE	19.9	27.3	32.8	39.2	42.3	48.3	54.9	58.4	64.3

* The sensory evaluation score sheet used for color evaluation of oil samples is shown in Appendix C.

Table 3. Sensory Evaluation Scores* For Overall Acceptance (Scores are mean of 16 evaluations)

Treatment	Heating Time(hours)								
	0	2	4	6	8	10	12	14	16
Control	8.7	6.7	6.3	5.5	4.7	4.1	3.8	2.9	2.3
BHA	8.8	7.2	6.6	5.6	4.9	4.5	4.0	3.6	3.1
α -Tocopherol	8.5	6.9	6.1	5.3	4.4	4.1	3.7	3.3	2.5
GTE	5.2	5.0	5.0	4.9	4.9	4.4	3.7	3.1	2.6

* The sensory evaluation score sheet used for evaluation of overall acceptance of oil samples is shown in Appendix D.

Table 4. Thiobarbituric Acid Values of Canola Oil samples expressed as μg of MDA/g of oil. (Values are mean of duplicate measurements)

Heating Time (hours)	MDA Concentration (μg /g of oil)			
	Control	BHA	α - Tocopherol	GTE
0	0.05	0.04	0.03	0.05
1	0.31	0.27	0.25	0.37
2	0.38	0.26	0.33	0.41
3	0.36	0.27	0.36	0.43
4	0.37	0.26	0.39	0.46
5	0.44	0.27	0.41	0.51
6	0.43	0.29	0.38	0.47
7	0.42	0.3	0.4	0.5
8	0.47	0.31	0.44	0.46
9	0.47	0.3	0.51	0.5
10	0.47	0.34	0.5	0.51
11	0.53	0.36	0.56	0.55
12	0.54	0.36	0.6	0.67
13	0.55	0.39	0.62	0.65
14	0.57	0.4	0.61	0.7
15	0.58	0.44	0.67	0.7
16	0.58	0.46	0.67	0.73

Table 5. Viscosity Values of Canola Oil Samples
(Values are mean of duplicate measurements)

Heating Time (hours)	Viscosity (centipoise)			
	Control	BHA	α - Tocopherol	GTE
0	75	77.5	77.5	55
2	77.5	80	82.5	65
4	82.5	90	85	77.5
6	85	100	87.5	80
8	90	110	87.5	82.5
10	95	115	90	92.5
12	100	120	110	100
14	107.5	150	120	110
16	113	155	125	117.5

Table 6. Slopes of Change With Time for Sensory Attributes*

Attribute	Treatment			
	Control	BHA	α -Tocopherol	GTE
Degree of Odor	-0.632	-0.477	-0.561	-0.484
Degree of Color	4.882	5.244	4.724	5.405
Degree of Overall Acceptability	-0.723	-0.666	-0.680	-0.326

* A negative slope denotes a decrease in the attribute with time.

Table 7. Slopes of Change With Time for TBA and Viscosity Values

Assay Method	Treatment			
	Control	BHA	α -Tocopherol	GTE
TBA	0.023	0.017	0.031	0.029
Viscosity	2.392	4.958	2.958	3.688

Table 8. Analysis of Variance for Odor Evaluation.

Source	SS	df	F
Treatment	1.36	3	7.55*
Hr. of heating	76.36	8	158.52**
Error	1.44	24	(0.06)

Note. Value enclosed in parentheses represents mean square error.

* $p = .001$. ** $p < .001$.

Table 9. Analysis of Variance for Color Evaluation.

Source	SS	df	<u>F</u>
Treatment	1163.92	3	219.80*
Hr. of heating	6157.25	8	436.04*
Error	42.36	24	(1.77)

Note. Value enclosed in parentheses represents mean square error.

* $p < .001$.

Table 10. Analysis of Variance for Overall Acceptability Evaluation.

Source	SS	df	<u>F</u>
Treatment	5.23	3	4.56*
Hr. of heating	87.49	8	28.56**
Error	9.19	24	(0.38)

Note. Value enclosed in parentheses represents mean square error.

* $p = .011$. ** $p < .001$.

Table 11. Analysis of Variance for TBA Testing.

Source	SS	df	F
Treatment	0.35	3	70.67*
Hr. of heating	1.21	16	45.62*
Error	0.08	48	(0.002)

Note. Value enclosed in parentheses represents mean square error.

* $p < .001$.

Table 12. Analysis of Variance for Viscosity.

Source	SS	df	F
Treatment	2925.06	3	19.02*
Hr. of heating	12129.5	8	29.57*
Error	1230.44	24	(51.27)

Note. Value enclosed in parentheses represents mean square error.

* $p < .001$.

Figure 1. Sensory evaluation odor scores over time. Average scores of 16 evaluations are plotted.

Figure 2. Sensory evaluation color scores over time. Average scores of 16 evaluations are plotted.

Figure 3. Sensory evaluation scores for overall acceptability over time. Average scores of 16 evaluations are plotted.

Figure 4. Thiobarbituric acid values of canola oil samples. Average values of duplicate measurements are plotted.

Figure 5. Viscosity of canola oil samples. Average values of duplicate measurements are plotted.

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CHAPTER 3

SUMMARY and RECOMMENDATIONS

Summary

This study was initiated to investigate the antioxidative properties of an extract of green tea and compare it with those of butylated hydroxyanisole (BHA) and α -tocopherol. Pure canola oil without any added antioxidant (1.75 L) and canola oil treated with 200ppm BHA, 500ppm α -tocopherol, and 200ppm GTE respectively was subjected to 16 hours of heating at 190°C. Evaluation of antioxidant activity was conducted by means of sensory evaluation of odor, color, and overall acceptability of oil samples by a semi-trained panel of 16 members; by TBA testing; and by viscosity measurement.

Sensory evaluation data indicated that GTE treated oil samples had lower average overall acceptance scores based on odor and color, compared to other treatments and the control. The pale yellow color and faint, detectable odor of the green tea extract may have influenced the panel's judgment regarding its overall acceptance, thereby resulting in comparatively lower average overall acceptance scores for the GTE treated oil samples. Results of TBA testing indicated that the concentration of malondialdehyde, which is a secondary product of lipid oxidation, was highest in GTE treated oil samples and lowest in BHA treated oil

samples during the entire heating period of 16 hours. Results of sensory evaluation and TBA testing indicated that green tea extract exhibited no particular antioxidative effect against thermal oxidation of canola oil, compared to BHA in the tested concentrations.

Recommendations

In this experimental study canola oil samples were subjected to heating at 190°C rather than deep fat frying because it was considered important to investigate and compare the antioxidative properties of GTE, BHA, and α -tocopherol in a pure state prior to actual testing in a commercial situation. However, deep-fat frying is a more common procedure in commercial food operations as compared to plain heating of oils hence, it is therefore recommended that a similar study involving deep-fat frying should be conducted with the same and/or higher concentrations of GTE.

The green tea extract used in this study had a pale yellow color and a slight detectable odor. These factors may possibly have affected the overall acceptability scores of GTE treated canola oil samples during sensory evaluation. It is thus recommended that in future trials of this study, the GTE should be purified further in order to improve the sensory quality of GTE treated oil samples.

The phenolic components of green tea, which are believed to be responsible for its antioxidative properties

are colorless, astringent, water-soluble compounds, with poor solubility in oils. Ethanol was therefore used in this study as a carrier, to make the green tea extract soluble in oil. The boiling point of ethanol however, is much lower than the heating temperatures used in this study ($\sim 190^{\circ}\text{C}$). Hence it may be possible that GTE was not uniformly dispersed in the oil and therefore it could not exhibit its antioxidative effects. Hence it is recommended that better methods for the incorporation of GTE into the oil should be researched, and the rate of thermal oxidation of such a system be investigated in future trials of this study.

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APPENCICES

APPENDIX A

Human Consent Form

AGREEMENT TO PARTICIPATE IN RESEARCH

AT SAN JOSE STATE UNIVERSITY

RESPONSIBLE INVESTIGATOR: HARVEEN SETHI
GRADUATE STUDENT,
FOOD SCIENCE

TITLE OF PROTOCOL: THE EFFECT OF POLYPHENOL EXTRACT FROM
GREEN TEA ON LIPID OXIDATION OF HEATED
CANOLA OIL

1. I have been asked to participate in a research study investigating the effect of polyphenol extract from green tea (GTE) on lipid oxidation of heated canola oil.
2. I will be asked to evaluate the odor, color and overall acceptance of butylated hydroxyanisole (BHA), α -tocopherol and GTE treated canola oils, heated for 16 hr each.
3. I understand that the BHA, α -tocopherol, and GTE added to the canola oil are at or below safe levels set by the FDA. I also understand that the probability and magnitude of harm or discomfort are no greater than those encountered in daily life, as wholesome samples shall be prepared by the researcher under clean and hygienic conditions.
4. No discernible benefits are expected, from my participation in this study.
5. The results of the study may be published but no information that could identify me will be included.
6. Questions about the research may be addressed to the principal investigator, Harveen Sethi at (408) 296-2248. Complaints about the research may be presented to the Nutrition & Food Science Department Chair, Dr. Lucy McProud at (408) 924-3100. Questions or complaints about research, subjects' rights, or research related injury may be presented to Serena Stanford, Ph.D., Associate Academic Vice President for Graduate Studies and Research, at (408) 924-2480.

APPENDIX A (continued)

7. No service of any kind, to which I am otherwise entitled, will be lost or jeopardized if I choose to "not participate" in the study.
 8. I am participating in this study voluntarily. I may refuse to participate in the study or in any part of the study. If I decide to participate in the study, I will be free to withdraw at anytime without prejudice to my relations with San Jose State University or any other participating institutions.
 9. I have received a signed and dated copy of the consent form.
- **The signature of a subject on this document indicates agreement to participate in the study.**
 - **The Signature of a researcher on this document indicates agreement to include the above named subject in the research and attestation that the subject has been fully informed of his or her rights.**

Subject's Signature

Date

Investigator's Signature

Date

APPENDIX B

Sensory Evaluation Scoresheet

Odor Evaluation Of Canola Oil
(Category Scale)

Judge Name: _____

Date: _____

Please smell the reference first. The odor of the reference has been assigned a value of "9". Then, assign values to the samples according to the following descriptions: -

Value assigned to odor	Description of the odor
9 (desirable)	Completely bland.
8	Suspicion of OFF -ODOR
7	Slight OFF-ODOR
6	Noticeable OFF-ODOR
5	More noticeable OFF-ODOR
4	Distinct OFF-ODOR
3	Disagreeable OFF-ODOR
2	More Disagreeable OFF-ODOR
1 (undesirable)	Extremely Disagreeable OFF-ODOR (V. Rancid)

Sample #:									
Value assigned:									

APPENDIX D

Sensory Evaluation Scoresheet

OVERALL ACCEPTANCE OF CANOLA OIL
(Category Scale)

Judge Name: _____
Date: _____

Please evaluate the reference first. The overall acceptance of the reference has been assigned a value of "9". Then, rate the overall acceptance of the samples from 1 to 9 on the scale provided below, by putting a cross mark at any position on the scale. If you perceive the overall acceptance of a sample to be greater than that of the reference, please assign an approximate value and note it down next to the right end of the line scale.

Sample code: **Scale: 9-6 Acceptable; 5-marginal; 4-1 unacceptable**

[illegible]