1998

Determination of antioxidant activity of polyphenol extract from artichoke leaves

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DETERMINATION OF ANTIOXIDANT ACTIVITY OF POLYPHENOL EXTRACT

FROM ARTICHOKE LEAVES

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

Manjiri Ghate

December 1998

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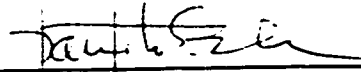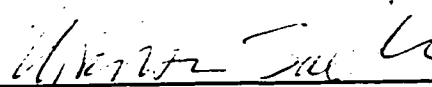
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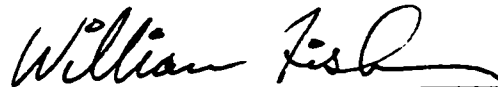Dr. Panfilo Belo, Ph.D., Graduate Advisor

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ABSTRACT

DETERMINATION OF ANTIOXIDANT ACTIVITY OF POLYPHENOL EXTRACT FROM ARTICHOKE LEAVES

by Manjiri Ghate

This study was initiated to determine the antioxidant activity of crude polyphenol extract from artichoke leaves (AE) as compared to that of commercial antioxidants propyl gallate (PG) and ascorbyl palmitate (AP). Concentrations of 200 ppm and 500 ppm polyphenols from AE, 200/100 ppm of PG/AP, 200/100 ppm of AE/AP, and 100 ppm of AP were added to 200 ml of pure canola oil. Canola oil with no added antioxidant served as the control. The treated and control canola oils were subjected to accelerated autoxidation (at 71^0C with continuous aeration) for eight days. Samples were withdrawn every 24 hours for peroxide value (PV), thiobarbituric acid value (TBA), and anisidine value (AV).

Analysis of variance indicated that AE, at the tested concentrations, did not exhibit any significant antioxidant properties as compared to PG and AP. Increasing the AE concentration from 200 ppm to 500 ppm polyphenols did not increase its antioxidant activity.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank my advisor, Dr. Belo for his time and patience during my entire thesis. I am also grateful to my committee members, Dr. Saltmarch and Dr. McProud, for their valuable time and for helping me to organize my manuscript. Last, but not the least, my special thanks to Judith Schallberger for her timely help in the laboratory.

DEDICATION

This thesis is dedicated to my husband, Sanjiv, whose love and support encouraged me throughout my graduate program at San Jose State University. This endeavour would not have been possible without him.

PREFACE

This thesis is written in publication style. The second chapter is written in journal format and will be submitted to <u>Journal of Agricultural and Food Chemistry</u>. The first and third chapters are written according to the guidelines outlined in the <u>Publication Manual of the American Psychological Association</u>, fourth edition, 1994.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

The deleterious effects of oxidation in food products are numerous. Oxidative changes are responsible for changes in flavor, color, texture and nutritional value, due to the destruction of fat-soluble vitamins and essential fatty acids such as linoleic acid. The oxidative deterioration of food lipids involves, primarily, autoxidative reactions which are accompanied by various secondary reactions having oxidative and nonoxidative character (Gray, 1978). These reactions are generally enhanced by a number of factors such as presence of oxygen, light, heat, heavy metals, pigments and degree of unsaturation of lipid fatty acids. The unsaturated fatty acids of food lipids are the primary target of oxidation accompanied by various secondary reactions leading to off-flavor and rancidity (Arumughan, Bhat, & Sen, 1984). The state of oxidation increases with the number of double bonds (Gray, 1978).

The acceptability of a food product depends on the extent to which deterioration has occurred. Researchers are also interested in determining the effects of certain processes or antioxidants on the stability of the product. Thus some criterion for assessing the extent of oxidation is required. Although sensory analysis is one of the most sensitive methods available, it is subjective and not practical for routine analyses. Consequently, many chemical and physical methods have been developed to quantify oxidative deterioration with the object of correlating the data with off-flavor development (Gray, 1978).

Antioxidants are added to lipid containing foods to prevent the formation of various off flavors and development of oxidative rancidity. They are substances that can delay onset, or slow the rate, of oxidation of autoxidizable materials. Literally hundreds of compounds, both natural and synthetic, have been reported to possess antioxidant properties (Nawar, 1985). Commonly used synthetic antioxidants, like BHA and BHT, have proven effective in retarding lipid oxidation in foods. They have unsurpassed efficacy in various food systems besides their high stability, low cost, and other practical advantages. However, their use in food has been falling off due to their suspected action as promoters of carcinogenesis as well as being due to a general rejection of synthetic food additives (Namiki, 1990). Moreover, the present trend in food processing is to use natural ingredients.

The artichoke is popular for its pleasant bitter taste which is attributed to phytochemicals found in the green parts of the plant called cynaropicrin and cynarin (http://www.rain-tree.com/artichoke.htm). The phytochemicals in the artichoke have been well documented and the leaves rather than the flower heads have been found to be in higher medicinal value (Dranik & Chernobai, 1966). The medicinal activity of leaves are attributed to the presence of caffeoylquinic acid derivatives including neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, cynarin, and luteolin (Monograph Artichoke, 1992). The leaf extracts have demonstrated a beneficial effect on the gallbladder, to stimulate secretion of bile in the liver, detoxify the liver, and lower the level of cholesterol in the blood (Stary, 1992). In addition, other compounds in the leaves have been shown to possess a hypoglycemic effect (Bianchini, 1975). However, there is

not any systematic study regarding the antioxidant nature of polyphenols in artichoke leaves and their effects in food.

Objective

The objective of the present study was to determine the antioxidant properties of the crude polyphenol extract from artichoke leaves. The activity of the crude extract was compared to that of commercially used antioxidants, propyl gallate and ascorbyl palmitate, in canola oil.

Significance of the Study

The general public concern with the safety of chemical additives has stimulated a continuing search for naturally occurring antioxidants. The most important natural antioxidants which are commercially exploited are tocopherols. Tocopherols have a potent ability to inhibit lipid peroxidation *in vivo* by trapping peroxy radicals (Hughes, Burton, Ingold, Slabey, & Foster, 1992). Unfortunately, tocopherols are much less effective as food antioxidants *in vitro*. The search and development of other antioxidants of natural origins is highly desirable.

Most of the antioxidants are phenolic in nature. Phenolic compounds are ubiquitous in plant foods, and therefore, a significant quantity is consumed in our daily diet. They are closely associated with the sensory and nutritional quality of fresh and processed plant foods such as odor and flavor and may offer an alternative method of food preservation. Extending the shelf-life of the products would have a definite positive effect in the operation of institutional food services, retail stores, and fast food chains. Work exploring natural antioxidants and their efficiency is deemed necessary. The

antioxidant activities of phenolic compounds have been recognized for decades, and research and development on the use of natural substances or food ingredients containing phenolic antioxidants will continue to be of great interest to the food industry (Ho, 1992).

Artichoke leaves have been widely used for medicinal and therapeutic purposes. The leaves of the artichoke, which are a major waste product in the artichoke industry, contain polyphenols like chlorogenic acid, caffeoylquinic acid, cryptochlorogenic, neochlorogenic, caffeic acid and three flavonoids (Bombardelli, Gabetta, & Martinelli, 1977). If these polyphenols are shown to be effective antioxidants like the currently used synthetic antioxidants, the result would be of great economic significance to the food and artichoke industry. The results of the present study would contribute to the knowledge of artichoke leaves and open a whole new world of a natural source of antioxidants.

Review of Literature

Autoxidation

The autoxidation of unsaturated fatty acids occurs via a free radical mechanism that consists of three basic steps: initiation, propagation, and termination.

Initiation

$$RH \longrightarrow R^\cdot + H^\cdot$$

Propagation

$$R^\cdot + O_2 \longrightarrow ROO^\cdot$$
$$ROO^\cdot + RH \longrightarrow ROOH + R^\cdot$$

Termination

$$R^{\cdot} + R^{\cdot} \longrightarrow RR$$

$$R^{\cdot} + ROO^{\cdot} \longrightarrow ROOR$$

$$ROO^{\cdot} + ROO^{\cdot} \longrightarrow ROOR + O2$$
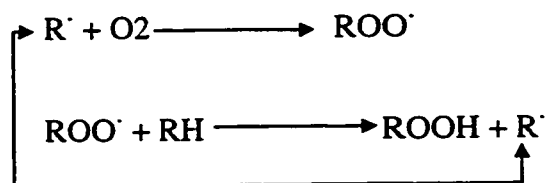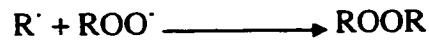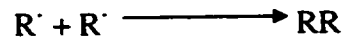
During initiation, the hydrogen atom (RH) adjacent to the double bond in the fatty acid is abstracted by exposure to light, heat, or metal catalysts to form a free radical. The resultant free radical ($R^{\cdot}$) reacts with atmospheric oxygen to form an unstable peroxy free radical ($ROO^{\cdot}$), 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.

The propagation step of the autoxidative process includes an induction period when hydroperoxide formation is minimal (Perkins, 1967; Labuza 1971; Sherwin 1978; Warner & Frankel, 1985). The rate of oxidation of fatty acids increases in relation to their degrees of unsaturation. Therefore, the relatively high linolenic acid content of canola oil poses stability problems.

Thermal Oxidation

The chemistry of lipid oxidation at elevated temperatures is complicated by the fact that in the presence of air, both thermolytic and oxidative events are superimposed. Studies (Gordon, 1986) have shown that the rate of lipid oxidation is greatly accelerated

by an increase in temperature. During heating, an oil is subjected to elevated temperatures

in the presence of air. Under such conditions, oil oxidation, hydrolysis, and thermal

degradation are relatively rapid and no induction period is detected. As the oxidation,

hydrolysis, and thermal reactions proceed, the functional, sensory, and nutritional quality

of fats are changed (Stevenson et al., 1984).

Phenolic Antioxidants

Phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated

hydroxytoluene (BHT), and propyl gallate (PG) are commonly used to prevent those

changes in flavor quality and nutritive value that result from oxidation of unsaturated fats

and fat-containing products (Raccach, 1983). These phenolic compounds react with free

radicals to form relatively stable antioxidant free radicals that, in turn, can be degraded to

form quinones, thereby terminating the chain reaction (deMan, 1980). BHA, BHT, PG are

low molecular weight (166-220) compounds. Phenolic antioxidants are practically

insoluble in water, but soluble in organic solvents such as ethanol and propylene glycol

(Raccach, 1983).

Phenolic antioxidants delay the formation of rancidity by donating a proton or an

electron. They act as free radical traps leading to resonance-stabilized intermediates

which increase the likelihood of chain termination (Raccach, 1983). The action of most

antioxidants can be described as $R^{\cdot} + AH \rightarrow RH + A$, where $R^{\cdot}$ depicts the fatty acid

free radical and AH is the antioxidant. The resultant antioxidant free radical (A) is a

"stable resonance hybrid" that is unable to initiate or propagate further oxidation (Sherwin, 1985).

Preservatives, such as ascorbyl palmitate (AP) and tocopherols, have been found to be effective in increasing the autoxidative and thermal stability of oils (Sedlacek, 1975). AP functions both as an inhibitor of free radical formation and as an oxygen scavenger to hamper the autoxidation process (Cort 1974).

The Regulation of Phenolic Antioxidants

In the U.S. the use of phenolic antioxidants in foods is regulated by the Food and Drug Administration for all food products except meat and poultry products which are regulated by the Department of Agriculture (Code of Federal Regulations Titles 9 and 21). In general, phenolic antioxidants are allowed in food products up to 200 ppm based on their fat and oil content (Code of Federal Regulations, Title 21). In meat and poultry products, the phenolic antioxidants are allowed up to 100 ppm based on the fat content of the product, but two or more phenolic antioxidants can be added if the total does not exceed 200 ppm (Code of Federal Regulation, Title 9). In the United States their use is subject to regulation under the Federal Food Drug and Cosmetic Act. Antioxidants for food products are also regulated under the Meat Inspection Act, the Poultry Inspection Act, and various state laws. The regulatory status of phenolic antioxidants is being modified continually. It is advisable to consult a recent government publication or the manufacturer of the compound under consideration.

Natural Antioxidants

Antioxidants are added to fats and oils or foods containing fats to prevent the formation of various off-flavors and other objectionable compounds that result from the oxidation of lipids. Natural antioxidants perform the same function, but these antioxidative compounds are obtained from various plant or animal tissues, and the amount of active ingredient may vary with the source. The most common natural antioxidants are flavonoids, cinnamic acid derivatives, tocopherols, and polyfunctional organic acids (Pratt, 1992). Many endogenous plant compounds retard oxidative processes in their environment and in products to which they have been added (Pratt, 1992). There is an implied assumption of safety for such compounds. One might avoid prolonged and expensive safety studies that are associated with synthetic materials (Pratt, 1992). But their potential as mutagens, carcinogens, teratogens, or as other pathogens must be investigated.

Certain problems other than toxicity, may be associated with the use of natural antioxidants: (a) the amount of active ingredient may vary with the source and method of extraction; (b) some may impart off-colors and off-odors; (c) the cost of extraction may be nearly inhibitive; and (d) undesirable reactions may occur with nutrients in the product (Pratt, 1992).

One reason for the interest in natural antioxidants is that there is some question as to the safety of presently used synthetic antioxidants. An important study by Branen (1975) examines the pathological effects of BHA and BHT. Some sources of natural

antioxidants are shown in Table 1. This is a general and abbreviated list. These materials

contain several types of compounds that possess antioxidant activity (Table 2).

Plant Sources

Natural antioxidants occur in all parts of the plant - wood, bark, stems, pods,

leaves, fruits, roots, flowers, pollen, and seeds (Pratt, 1992). These are usually, phenolic

and polyphenolic compounds. Typical compounds that possess antioxidant activity

include tocopherols, flavonoids, cinnamic acid derivatives, phosphatides, and

polyfunctional organic acids. The flavonoids include flavones, flavonols, isoflavones,

catechins, flavonones, and chalcones. The cinnamic acid derivatives include caffeic acid,

ferulic acid, chlorogenic acid and several others (Pratt, 1992).

Flavonoids

Flavonoids are naturally occurring compounds that normally contain a number of

aromatic substituted hydroxy or alkoxy groups. These compounds are found in a variety

of fruits, vegetables, leaves, and flowers. Of all the naturally occurring flavones, quercetin

is the most widespread. The abundance of quercetin was brought to light in two studies

(Pratt & Watts, 1964; Pratt, 1965). In both investigations hot water extracts of several

vegetable plants were tested for antioxidative effects. In the first study (Pratt & Watts,

1964), almost all extracts had some activity, but those from green onion tops and green

pepper seeds exhibited particularly high antioxidant behavior.

Tea Leaves

From tea leaves, Hara and Matsuzaki (1985) isolated epicatechin (EC),

epigallocatechin (ECG), epicatechin gallate (ECg), and epigallacatechin gallate (EGCg)

homologues in pure form by HPLC and examined their antioxidant activities. They showed activities stronger than BHA or dl-tocopherol in a lard autoxidation system; the order was EC < ECg < EGC < EGCg on molar bases. A strong synergistic effect of EGCg was seen with ascorbic acid and α-tocopherol, and also with citric acid and tartaric acid, but not with amino acids. Recently, the scavenging effects of green tea extracts and other natural antioxidants on active oxygen radicals have been studied by Wenjuan, Zhao, Li, He, and Cheng (1989) using a spin trapping method. In stimulated polymorphonuclear leukocytes system, water extract fraction of green tea and green tea polyphenols showed the most strong scavenging activity, followed by ascorbic acid, rosemary antioxidant, and others.

Extracts from the following seeds, oilseeds, and herbs have been shown to have antioxidant properties.

Sesame. The antioxidant properties of sesame have been related to a phenolic compound present in sesame oil called sesamol (Lyon, 1972). It has been reported that sesamol is more effective in lard than BHA and BHT (Lyon, 1972). The high resistance of sesame oil to oxidation compared with other vegetable oils has long been known, but has not been fully explained on a scientific basis. It has been attributed to the presence of a phenolic compound, sesamol, which is produced from sesamolin, a lignan characteristic to sesame (Bodowski, 1964).

Canary Seed. A study by Tagaki and Iida (1980) showed that an ether extract of canary seeds was a potent antioxidant in lard and sardine oil. The antioxidant compounds were the esters of caffeic acid with sitosterol, gramisterol, campesterol, and cycloartenol.

Rapeseed. Phenolic compounds isolated from rapeseed processing in the preparation of protein concentrates contained a large percentage of phenolic acids. The extracted phenolics in ethanol, after fractionation, possessed strong antioxidant effects in linoleate/β—carotene systems which were, in some cases, comparable to the effectiveness of approved food phenolics (Zadernowski, Nowak, & Kozlowska, 1991).

Spices and Herbs. Chipault, Mizuno, Hawkins, and Lundberg (1952) showed that 32 spices (at 0.02%) possessed some antioxidant activity when added to prime steam lard at 98^0 C. Of all the spices tested in this substrate, rosemary and sage were particularly impressive antioxidants. Allspice, cloves, sage, oregano, rosemary, and thyme increased the stability of all fat substrates in which they were tested (Chipault, Mizuno, & Lundberg, 1956). When the spices were added to ground pork, it was discovered that cloves exhibited the greatest antioxygenic effects. The greatest antioxidant activity in mayonnaise and French dressing was produced by oregano.

Thus, naturally-occurring antioxidants, found in different plant materials have been exploited in different processing applications (Shahidi, Rubin, & Wood, 1987). Their possible use in product formulations, as such or in the form of protein extenders and binders as well as spices and condiments may present an effective and attractive means of retarding lipid oxidation in foods. Some of the polyphenols found in artichoke leaves, such as flavonoids and tannins, have been found to have antioxidant properties. Hence, artichoke leaves could also be a possible source of natural antioxidants.

Artichoke

Scientific name: *Cynara scolymus* L. Family: Compositae or Asteraceae.

Common name: Globe artichoke

Botany

The artichoke was developed by lengthy selective breeding from the Cardoon (*Cynara cardunculus*), which still grows wild in the Mediterranean region and has also been cultivated as a vegetable since ancient times (Stary, 1992). It is a large, perennial herb with upright, unbranched, felted stem, growing to a height of approximately five feet. The large leaves are without spines and are grey-felted on the underside; the lower stem leaves are stalked, the upper ones stalkless. The flower heads are large and violet, blooming from July to August.

History

Artichokes were known as a vegetable to the ancient Egyptians, Greeks and Romans, and in Rome they were an important item of the menu at feasts. They are readily destroyed by frosts. The botanical name for artichoke, *Cynara scolymus*, is derived partly from the custom of fertilizing the plant with ashes, and partly from the Greek *skolymus*, meaning 'thistle', from the spines which are found in the involucral bracts (they are not leaves) which enclose the flowering heads and form the edible part (Bianchini, 1975).

The artichoke is widely cultivated for food use, the edible parts being the fleshy bases of immature flower heads, as well as fleshy bases of the large bracts. The flowering heads contain inulin which is very valuable in the diet of diabetics (Chopra, Nagar, & Chopra, 1956). The flower head is cooked and eaten as a delicacy. It contains a sweetener that enhances flavor perception, while the leaves contain bitter principles that are used in the preparation of aperitif liqueurs. Artichoke extracts have ancient traditional uses in

Folk medicine, especially in European countries due to their choleretic (increase in the

flow of bile from the gall bladder), ureolytic, and hypocholesterolemic (decrease in the

rate of cholesterol synthesis) activities and these extracts are still used for therapeutic

purposes (Lietti, 1977).

<u>Chemical Composition</u>

The leaves, which are odorless and have a bitter taste, are gathered during the

flowering period. The constituents include the pleasant tasting glycosidic bitter principles

cynarin & cynaropicrine, flavonoids with an anti-inflammatory action, and tannins. The

active principles are polyphenols, the chief being cynarine, flavonoids, and enzymes

(Schauenberg & Paris, 1977).

The artichoke possesses cholagogic, liver-protective, nutritive, tonic, stomachic,

astringent, diuretic and hypoglycaemicizing properties. Much of the pharmacologic

activity of the leaves has been attributed to the presence of caffeoylquinic acid derivatives

including neochlorogenic acid, cryptochlorogenic acid, chlorogenic acid, cynarin, and

luteolin (Monograph Artichoke, 1992). The relative proportions of these compounds

varies with the strain, age, and generation of the plant (Hammouda, 1991; Puigmacia,

1986).

The organic acids, glyceric, malic, citric, glycolic, lactic and succinic were

detected in artichokes (Bogaert, Mortier, Jouany, Plet, & Delaveau, 1972). Luteolin,

cynaroside (luteolin-7-glucoside) and scolymoside (luteolin-7-rutionoside) were

chromatographically detected in the leaves of artichoke (Constantinescu, Platon, & Pavel,

1967). A flavonoidal compound, cynarotrioside, was also isolated from the leaves of artichokes (Dranik & Chernobai, 1966).

Pharmacology

Various preparations such as wines, tinctures and pills are made from artichokes. In combination they have a beneficial effect on a diseased and weakened gall bladder and stimulate the secretion of bile by the liver as well as provide detoxifying action. They lower the level of cholesterol in blood and prevent excessive fatty deposits in the liver tissue and the blood, thereby warding off arteriosclerosis. The drug prevents the formation of gall stones and lowers the level of sugar in the blood in incipient diabetes and diabetes in the elderly (Bianchini, 1975).

The choleraic, cholagogue and also recently hepatoprotector action of artichoke leaves has been attributed to the caffeoylquinic acid derivatives. The above structures are highly unstable and during extraction may undergo isomerization phenomena through hydrolysis and posterior intramolecular transesterification which modify the composition of the extract; this effect has been revealed by means of HPLC (Adzet & Puigmacia 1985).

Assessment of Oil Quality

Autoxidation is a major cause of deterioration of fats and oils and fatty foods. The rate of oxidation increases geometrically with the number of double bonds (Arumughan et al., 1984). The rate of oxidation also depends on storage conditions of fats and oils. A single limit value based on a particular method is inadequate to detect rancidity in all edible oils (Arumughan et al., 1984). In order to determine the oxidative stability or

quality of an oil/fat, a number of stability test methods have been developed. Chemical, instrumental, and sensory techniques are employed to determine the extent of autooxidation and thermal degradation in canola oils subjected to stability tests.

Peroxide Value

The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides. Therefore, it is reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. However, this theory is limited to the transitory nature of peroxides (Gray, 1978). Since hydroperoxides are the primary products of lipid oxidation, measurement of peroxide value (PV) is often used as an indicator of initial oxidation. Peroxides decompose readily during storage and heating; thus PV may not indicate the actual extent of oil degradation (Sherwin, 1968). Numerous analytical procedures for the measurement of PV are described in the literature. The results and suitability of the test depend on the experimental conditions and the reducing agent employed. Since peroxides are vulnerable to further reaction, the complete oxidative history of the oil may not be revealed (Gray, 1978).

Thiobarbituric Acid Test

The thiobarbituric acid (TBA) test is commonly used to measure oil stability (Gray, 1978, 1985). Peroxidation of polyunsaturated fatty acids produces malonaldehyde which reacts with the TBA reagent to form a pink-colored pigment. However, no evidence was presented that malonaldehyde could be found in all oxidizing systems. Dahle, Hill, and Holman (1962) postulated a mechanism for the formation of malonaldehyde, a secondary product in the oxidation of polyunsaturatred fatty acids. This

mechanism was based on the investigations which showed that no color developed for linoleate even at peroxide value of 2000 or greater, but that for fatty acids with three or more double bonds the molar yield of the TBA value increased with the degree of unsaturation. It therefore becomes imperative to know the fatty acid profile of the sample to be tested (Gray, 1978). TBA values should be considered in conjunction with other chemical methods and organoleptic analysis.

Anisidine Value

Anisidine values (AV) have been measured to determine the total amounts of carbonyl compounds in oxidized canola oils. The analysis is based on the reaction of anisidine and unsaturated aldehydes and the formation of a yellowish pigment (International Union of Pure and Applied Chemistry, 1987). McMullen (1988) reported good correlations between AV and sensory data for canola oil subjected to shallow-pan heating and deep-fat frying. List et al., (1974) reported a highly significant correlation between the anisidine values of salad oils processed from undamaged soybeans and their flavor scores.

Canola Oil as a Medium

Canola oil enjoys a nutritional advantage in health-conscious North American markets because of its low levels of saturated fatty acids and its high level of unsaturated fatty acid, oleic acid (Malcolmson, Vaisey-Genser, Przybylski, & Eskin, 1994). Canola oil is an almost perfectly balanced mixture of fatty acids for human nutrition and health. In 1985, the U.S. Food and Drug Administration granted 'generally recognized as safe' (GRAS) status to canola oil and it is widely used in salad oils, tablespreads, and

shortenings. In Canada, refined canola oil has been widely used in both salad oil and cooking oil products, and is also very acceptable (Vaisey-Genser & Eskin, 1987) in hydrogenated products such as margarine and shortenings. Acceptance of canola oil for GRAS status in the United States should extend this use into new foods as well as for purely oil uses. Safety has been demonstrated and not merely assumed for canola oil.

The quality of canola oils has been criticized frequently because of undesirable odors and flavors developed during storage and heating (Tokarska, Hawrysh, & Clandinin, 1986). Canola oil has a higher oleic acid content (55%), lower linoleic acid content (26%), and a higher linolenic acid content (8-12%) than most other vegetable oils. The high unsaturated fatty acid content, especially C18:3, in canola oil influences its stability and quality. The unpleasant flavor is derived from primary and secondary oxidation products of these fatty acids (Jackson & Giacherio, 1977).

Antioxidant Efficacy in Canola Oil. Propyl gallate (PG) is considered to be a potent phenolic antioxidant and has consistently shown high performance in vegetable oils when compared to BHA and BHT (Sherwin & Thompson 1967; Sherwin 1972; Cort 1974). A storage stability study (Pinkowski, Witherly, Harvey, & Tadjalli, 1986) of oils, including canola, noted that PG was one of the most effective antioxidants.

CHAPTER 2

JOURNAL ARTICLE

Authors' Title Page

DETERMINATION OF ANTIOXIDANT ACTIVITY OF POLYPHENOL EXTRACT FROM ARTICHOKE LEAVES

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ABSTRACT

This study was initiated to determine the antioxidant activity of crude polyphenol extract from artichoke leaves (AE) as compared to that of commercial antioxidants propyl gallate (PG) and ascorbyl palmitate (AP). Concentrations of 200 ppm and 500 ppm polyphenols from AE, 200/100 ppm of PG/AP, 200/100 ppm of AE/AP, and 100 ppm of AP were added to 200 ml of pure canola oil. Canola oil with no added antioxidant served as the control. The treated and control canola oils were subjected to accelerated autoxidation (at 71^0C with continuous aeration) for eight days. Samples were withdrawn every 24 hours for peroxide value (PV), thiobarbituric acid value (TBA), and anisidine value (AV).

Analysis of variance indicated that AE, at the tested concentrations, did not exhibit any significant antioxidant properties as compared to PG and AP. Increasing the AE concentration from 200 ppm to 500 ppm polyphenols did not increase its antioxidant activity.

Keywords: *antioxidant, polyphenols, artichoke extract, accelerated autoxidation, canola oil.*

INTRODUCTION

The deleterious effects of oxidation in food products are numerous. Oxidative changes are responsible for changes in flavor, color, texture and nutritional value, due to the destruction of fat-soluble vitamins and essential fatty acids such as linoleic acid. The oxidative deterioration of food lipids involves, primarily, autoxidative reactions which are accompanied by various secondary reactions having oxidative and nonoxidative character (Gray, 1978). These reactions are generally enhanced by a number of factors such as presence of oxygen, light, heat, heavy metals, pigments and degree of unsaturation of lipid fatty acids. The unsaturated fatty acids of food lipids are the primary target of oxidation accompanied by various secondary reactions leading to off-flavor and rancidity (Arumughan et al., 1984). The state of oxidation increases with the number of double bonds (Gray, 1978).

The acceptability of a food product depends on the extent to which deterioration has occurred. Researchers are also interested in determining the effects of certain processes or antioxidants on the stability of the product. Thus some criterion for assessing the extent of oxidation is required. Although sensory analysis is one of the most sensitive methods available, it is subjective and not practical for routine analyses. Consequently, many chemical and physical methods have been developed to quantify oxidative deterioration with the object of correlating the data with off-flavor development (Gray, 1978).

Antioxidants are added to lipid containing foods to prevent the formation of various off flavors and development of rancidity. They are the substances that can delay

the onset, or slow the rate, of oxidation of autoxidizable materials. Literally hundreds of compounds, both natural and synthetic, have been reported to possess antioxidant properties (Nawar, 1985). Commonly used synthetic antioxidants, like BHA and BHT, have proven effective in retarding lipid oxidation in foods. They have unsurpassed efficacy in various food systems besides their high stability, low cost, and other practical advantages. However, their use in food has been falling off due to their suspected action as promoters of carcinogenesis as well as being due to a general rejection of synthetic food additives (Namiki, 1990). Moreover, the present trend in food processing is to use natural ingredients.

Phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are commonly used to prevent those changes in flavor quality and nutritive value that result from oxidation of unsaturated fats and fat-containing products (Raccach, 1983). These phenolic compounds react with free radicals to form relatively stable antioxidant free radicals that, in turn, can be degraded to form quinones, thereby terminating the chain reaction (deMan, 1980). Preservatives, such as ascorbyl palmitate (AP) and tocopherols, have been found to be effective in increasing the autoxidative and thermal stability of oils (Sedlacek, 1975). AP functions both as an inhibitor of free radical formation and as an oxygen scavenger to hamper the autoxidation process (Cort, 1974).

Natural antioxidants perform the same function as synthetic antioxidants, but these antioxidative compounds are obtained from various plant or animal tissues, and the amount of active ingredient may vary with the source. Many endogenous plant

compounds retard oxidative processes in their environment and in products to which they have been added. There is an implied assumption of safety for such compounds. One might avoid prolonged and expensive safety studies that are associated with synthetic materials (Pratt, 1992).

One reason for renewed interest in natural antioxidants is that there is some question as to the safety of presently used synthetic antioxidants. An important study by Branen (1975) examines the pathological effects of BHA and BHT.

The artichoke is popular for its pleasant bitter taste which is attributed to phytochemicals found in the green parts of the plant called cynaropicrin and cynarin. The phytochemicals in the artichoke have been well documented (Dranik and Chernobai, 1966) and the leaves rather than the flower heads have been found to be of higher medicinal value. The medicinal activity of leaves are attributed to the presence of caffeoylquinic acids and acid derivatives, cynarin, and luteolin (Monograph Artichoke, 1992). In combination, leaf extracts have demonstrated a beneficial effect on the gallbladder by stimulating secretion of bile in the liver, detoxifying the liver, and lowering the level of cholesterol in the blood (Stary, 1992). In addition, other compounds in the leaves have been shown to possess a hypoglycemic effect (Bianchini, 1975). However, there is not any systematic study regarding the antioxidant nature of polyphenols in artichoke leaves and their effects in food.

In order to determine the oxidative stability or quality of an oil/fat, a number of stability test methods have been developed. Chemical, instrumental, and sensory techniques are employed to determine the extent of autoxidation and thermal degradation

in canola oils subjected to stability tests. The primary products of lipid oxidation are hydroperoxides which are generally referred to as peroxides (Gray, 1978). Therefore, it is reasonable to determine the concentration of peroxides as a measure of the extent of oxidation.

The thiobarbituric acid (TBA) test is commonly used to measure oil stability (Gray, 1978, 1985). Peroxidation of polyunsaturated fatty acids produces malonaldehyde which reacts with the TBA reagent to form a pink-colored pigment. TBA values should be considered in conjunction with other chemical methods and organoleptic analysis (Gray, 1978).

Anisidine values (AV) have been measured to determine the total amounts of carbonyl compounds in oxidized canola oils. The analysis is based on the reaction of anisidine and unsaturated aldehydes and the formation of a yellowish pigment (IUPAC, 1987). McMullen (1988) reported good correlations between AV and sensory data for canola oil subjected to shallow-pan heating and deep-fat frying.

The artichoke leaves, which are odorless and have a bitter taste, are gathered during the flowering period. The constituents include the pleasant tasting glycosidic bitter principles cynarin and cynaropicrine, flavonoids with an anti-inflammatory action, and tannins. The active principles are polyphenols, the chief being cynarine, flavonoids and enzymes (Schauenberg and Paris, 1977).

The artichoke possesses cholagogic, liver-protective, nutritive, tonic, stomachic, astringent, diuretic and hypoglycaemicizing properties. Much of the pharmacologic activity of the leaves has been attributed to the presence of caffeoylquinic acid derivatives

including neochlorogenic acid, cryptochlorogenic acid, chlorogenic acid, cynarin, and luteolin (Monograph Artichoke, 1992). The caffeoylquinic acid derivatives are highly unstable and during extraction may undergo isomerization phenomena through hydrolysis and posterior intramolecular transesterification which modify the composition of the extract; this effect has been revealed by means of HPLC (Adzet and Puigmacia, 1985). The relative proportions of these compounds varies with the strain, age, and generation of the plant (Hammouda, 1991; Puigmacia, 1986).

Canola oil enjoys a nutritional advantage in health-conscious North American markets because of its low levels of saturated fatty acids and its high level of the unsaturated fatty acid, oleic acid (Malcolmson et al., 1994). Canola oil is an almost perfectly balanced mixture of fatty acids for human nutrition and health. In 1985, the U.S. Food and Drug Administration granted GRAS status to canola oil and it is widely used in salad oils, tablespreads, and shortenings. Propyl gallate (PG) is considered to be a potent phenolic antioxidant and has consistently shown high performance in vegetable oils when compared to BHA and BHT (Sherwin, 1972; Cort, 1974).

The focus of the present study was the determination of antioxidative properties of the crude polyphenol extract from artichoke leaves in canola oil.

MATERIALS AND METHODS

Artichoke Leaves and Canola Oil. The artichoke leaves were provided by Artichoke Industries, Inc (Castroville, CA). The leaves, which were kept frozen during storage, were cut, trimmed, and freeze-dried for three days to remove all traces of water. The freeze-dried leaves were reduced to a fine powder in a blender and later used for

extraction. Canola oil was purchased from a local grocery market (Wesson brand with no added antioxidants).

Chemicals. Chloroform, folin-ciocalteau, p-anisidine, gallic acid, propyl gallate, and ascorbyl palmitate were purchased from Sigma Chemicals Co. (MO, U.S.A). Sodium carbonate, methanol, and 1-butanol were purchased from Fisher Scientific. All of the reagents were of analytical grade.

Preparation of Artichoke Extract. The extract was prepared by the method of Adzet and Puigmacia (1985). A freeze-dried artichoke sample (10 g) was extracted with boiling water for 2 hours and filtered while warm. Boiling water extraction was repeated three times. The combined water extracts were concentrated to a minimum volume and the polyphenols in the extract were precipitated using 10 volumes of methanol. The precipitation process was repeated twice. The methanolic fractions were dried in a vacuum oven at 43^0C for 5 days until most of the methanol had evaporated.

Total Phenol Analysis. A total of three extracts were prepared, at different times, for determination of polyphenols. Analysis of total phenols was performed according to the method of Singleton and Rossi (1965) using gallic acid as the standard solution. The method consisted of heating 2 ml of the sample plus 10 ml of 1/10 diluted folin-ciocalteau reagent, and 8 ml of sodium carbonate (75 g/l) at 50^0C for 5 minutes and reading the absorbance at 765 nm.

Accelerated Oxidation Test. To detect the antioxidant properties of the extract of artichoke leaves, a total of four experiments were carried out, at different times, involving accelerated lipid oxidation of canola oil. The different treatments used during the

experiments are shown in Table 3. A schematic diagram of the accelerated test used is shown in Figure 1. The test was carried out in a 250 ml conical flask containing 200 ml of canola oil. The conical flasks were kept in a drying oven maintained at 71^0C with constant aeration. This continuous heating and aeration was maintained for a period of eight days and oil samples from each flask were withdrawn every 24 hours for PV, AV, and TBA analysis. No fresh oil was added back to the flasks to replace the oil lost during sampling.

The activity of artichoke extract was compared with the synthetic antioxidant, propyl gallate, and the synergist, ascorbyl palmitate. Propyl gallate is considered to be a potent phenolic antioxidant and has shown consistently high performance in vegetable oils when compared to BHA and BHT (Sherwin, 1972; Cort, 1974). Ascorbyl palmitate is also an effective antioxidant in lipid systems. It prevents the formation of free radicals and thereby delays the initiation of the chain reaction that leads to fat deterioration (Liao and Seib, 1987). Ascorbyl palmitate is used in vegetable oils because it acts synergistically with naturally occurring tocopherols.

Peroxide values are expressed as milliequivalent of oxygen per kg of fat (Official and Tentative Methods of the American Oil Chemists' Society, 1980). PV was performed according to American Oil Chemists' Society method (1990). TBA test was performed according to an interlaboratory study and their standardized method (Collaborative study, 1989). This standard describes a method for the direct determination of TBA value in oils and fats without preliminary isolation of secondary oxidation products. According to this method TBA values are expressed as the increase in the absorbance measured at 530 nm

due to the reaction of the equivalent of 1 mg of sample per 1 ml. volume with 2-thiobarbituric acid (Collaborative study, 1989).

The AV was determined according to the IUPAC method (Hendrikse and Harwood, 1986). The AV is equivalent to 100 times the absorbance, (measured in a 1 cm cell), of a solution resulting from the reaction of 1 g of oil or fat in 100 ml of solvent with 'anisidine reagent' (Hendrikse and Harwood, 1986). In the presence of acetic acid, p-anisidine reacts with aldehydic components in oils or fats. The intensity of the color of the yellowish reaction product formed depends not only on the amount of aldehydic components present, but also on their structure. It has been found that a double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four to five times. This means that 2-alkenals, in particular, will substantially contribute to the value found (Hendrikse and Harwood, 1986).

Statistical Analysis. Analysis of variance (ANOVA) was carried out, using Microsoft Excel 7.0, to determine whether significant differences existed in PV, AV, and TBA values between different treatments and time of oxidation at a 95% confidence level. Least significant differences (LSD) were calculated to compare means when the F-ratios were significant. In addition , correlation coefficients, indicating a relationship between the chemical methods and period of accelerated oxidation, were also calculated. According to Burgard and Kuznicki (1990), correlation coefficients in the range of 0.4 to 0.9 were considered as moderate to good correlations between the two groups of data evaluated.

RESULTS

Total Phenol Analysis of Crude Artichoke Extract. It was observed that each

of the three extracts, prepared at different times, had a different yield of total phenols. The

yields of the three extracts were 35%, 24%, and 40% respectively. Based on these yields,

appropriate weights of the crude AE were added to the canola oil to make the final

concentration to 200 ppm and 500 ppm polyphenols.

Experiment 1. The PV, TBA, and AV of the canola oil treated with C, AE (200

ppm), and PG/AP (200/100 ppm) are shown in Figures 2-4. In this experiment it was

noted that AE was not completely miscible in canola oil. Therefore 5 ml of methanol was

mixed with AE and added in the oil to incorporate the extract. Analysis of variance

indicated significant differences between treatments and oxidation time. A typical

ANOVA table representing the PV results is shown in Table 4. It was noted that AE does

not exhibit any antioxidative property compared to PG/AP. In fact, LSD calculations

showed that the activities of AE and C were not statistically significant, but they were

significantly different from PG/AP. The only exception was for AV where AE exhibited

very high values as compared to C and PG/AP.

The PV, TBA, and AV of PG/AP treated oil were the lowest as compared to C

and AE during the entire oxidation process. There was a strong positive correlation

between the oxidation values and oxidation time (Table 8).

Experiment 2. The PV, TBA, and AV of canola oil treated with C, AE (200

ppm), and AE/AP (200/100 ppm) are shown in Figures 5-7. ANOVA results showed

statistical significant differences between treatments and oxidation time. A typical ANOVA table representing the TBA results is shown in Table 5.

The PV, TBA, and AV of AE/AP treated oil were the lowest compared to C and AE during the entire oxidation process. A statistically significant increase in the values of PV and AV were observed on the sixth day but the TBA values were steady until the eighth day. A strong positive correlation was observed between the oxidation values and oxidation time indicating that the degree of oil deterioration increased as period of oxidation increased (Table 8).

Experiment 3. The PV, TBA, and AV of canola oil treated with AE (200 ppm), AP (100 ppm), and AE/AP (200/100 ppm) are shown in Figures 8-10. It was observed from the ANOVA results that there were significant differences in the activities exhibited by the three treatments, but none in the oxidation time. A typical ANOVA table representing the AV results is shown in Table 6.

The oil treated with AE had higher PV, TBA, and AV values as compared to AP and AE/AP. The PV and TBA values of AP and AE/AP treated canola oil were similar until the fifth day, while the AV were similar until the fourth day. But later the oxidation values of AE/AP increased compared to those of AP treated canola oil, though this increase was not statistically significant. A strong positive correlation was observed between the oxidation values and oxidation time when the oil was treated with AE and AE/AP. However, with regards to AP, a weak correlation with PV and a negative correlation with TBA and AV was observed (Table 8).

Experiment 4. A fourth and last experiment was carried out to determine whether a higher concentration (500 ppm) would behave as an antioxidant. For this experiment only three samples were withdrawn in a period of 8 days for chemical analyses. PV, TBA, and AV of canola oil treated with AE (200 ppm) and AE (500 ppm) are shown in Figures 11-13. ANOVA results showed that there were no significant differences in the activities of the two treatments. A typical ANOVA table representing the PV results is shown in Table 7.

DISCUSSION

Total Phenol Analysis of Crude Artichoke Extract. The artichoke extract was prepared in methanol to precipitate the polyphenols. Artichoke leaves also contain a variety of fatty acids, other than polyphenols, like lauric acid, linoleic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, and stearic acid. (http://www.rain-tree.com/artichoke.htm). These acids are soluble in methanol, especially at temperatures nearing the boiling point of methanol (Singleton, 1960). Some of the other phytochemicals present in artichokes include beta-carotene, decanal, eugenol, phenylacetaldehyde, folacin etc. (http://www.rain-tree.com/artichoke.htm). Thus, the remaining precipitate might be due to the presence of phytochemicals that are soluble in methanol. This may have reduced the yield of polyphenols from artichoke leaves.

Experiment 1. It was observed from the figures that the oxidation values of canola oil treated with AE peaked on the fifth day. The LSD calculations indicated that, at day five, the PV, TBA, and AV of AE treated oil were significantly different from those of C and PG/AP. But there was no statistical significant difference between the oxidation

values of C and PG/AP. The reason for the above trend could be the poor solubility of AE in canola oil and the presence of some proxidants, like chlorophyll, in the extract. A second experiment was carried out to determine whether AE could behave as an antioxidant with the synergist, AP.

Experiment 2. Since AE did not fare well as compared to the synthetic antioxidant, more methanol (25 ml) was added to the oil for better incorporation. Increasing the volume of methanol did not affect the chemical analyses. The activity exhibited by AE was not significantly different from that of C, but they were significantly different from those of AE/AP. Thus, addition of more methanol in the oil containing AE resulted in similar activity exhibited by C and AE. This led to a third experiment to observe whether the improvement in the oil containing AE/AP was due to the synergist alone.

Experiment 3. Of the three treatments used in this experiment, AP and AE/AP had significantly lower oxidation values compared to AE. Hence the average oxidation values of the three treatments, on any given day, remained low. Since the change in the average was lower than the LSD, there were no significant differences between the oxidation times.

Experiment 4. Increasing the AE concentration from 200 ppm to 500 ppm polyphenols did not lower the PV, TBA, and AV of the oil samples.

CONCLUSION

Polyphenol extract from artichoke leaves did not exhibit any significant antioxidant activity in the accelerated lipid oxidation of canola oil as compared to propyl

gallate and ascorbyl palmitate. In fact, there were no statistically significant differences between the activities of control and artichoke extract. Increasing the concentration of AE from 200 ppm to 500 ppm did not result in an increase in its ability to behave as an antioxidant. The poor solubility of AE in canola oil might have affected its activity as compared to other treatments.

It is recommended that various concentrations of AE (500 ppm to 1000 ppm) be used during practical storage conditions, to test its efficacy as an antioxidant. A modification in the extraction procedure, wherein a high yield of polyphenols is obtained, may be desired. It is also suggested that the individual polyphenols of the extract be tested as antioxidants. The extract should also be analyzed for the presence of pro-oxidants, if any.

Thus, the crude polyphenol extract from artichoke leaves was unable to behave as an antioxidant under the tested concentrations and conditions of oxidation. Canola oil treated with commercial antioxidants had a significantly lower oxidation value as compared to AE throughout the accelerated oxidation test.

Table 1. Some sources of natural antioxidants

Algae	Oil seed
Amla	Olives
Citrus poly and peel	Osage orange
Cocoa powder or shell	Plant (extracts)
Heated products	Protein hydrolysate
Herbs and Spices	Resin
Microbial products	Soy products
Oat flour	Tempeh

Note. From "Phenolic Compounds in Foods and Their Effects on Health II" by M. Huang,

C. Ho, and C. Y. Lee (eds), p. 56, American Chemical Society, Washington, DC, 1992.

Table 2. Some components of natural antioxidants

Amino acid	Other organic acids
Ascorbic acid	Reductions
Carotenoids	Peptides
Flavonoids	Tannins
Melanoidin	Tocopherols

Note. From "Phenolic Compounds in Foods and Their Effects on Health II" by M. Huang, C. Ho, and C. Y. Lee (eds), p. 56, American Chemical Society, Washington, DC, 1992.

Table 3. Different experimental treatments

	Experiment 1			Experiment 2			Experiment 3			Experiment 4	
Treatment*	C	AE	PG/AP	C	AE	AE/AP	AE	AP	AE/AP	AE	AE
Conc (ppm)	-	200	200/100	-	200	200/100	200	100	200/100	200	500

* C - control; AE - artichoke extract; PG - propyl gallate; AP - ascorbyl palmitate.

Table 4. ANOVA table for peroxide value of canola oil (Experiment 1)

Source of Variation	SS	df	MS	F	P-value	F - crit
Antioxidant treatment*	36321.34	2	18160.67	10.077	0.001471	3.6337
Accelerated oxidation time	43033.97	8	5379.246	2.984839	0.029854	2.5911
Error	28835.03	16	1802.1869			
Total	108190.3	26				

* C, AE-200 ppm, PG/AP-200/100 ppm

Table 5. ANOVA table for TBA value of canola oil (Experiment 2)

Source of Variation	SS	df	MS	F	P-value	F - crit
Antioxidant treatment*	0.032033	2	0.016016	3.742176	0.046423	3.6337
Accelerated oxidation time	0.292514	8	0.036564	8.543179	0.000159	2.5911
Error	0.068479	16	0.004280			
Total	0.393026	26				

* C. AE-200 ppm, AE/AP-200/100 ppm

Table 6. ANOVA table for anisidine value of canola oil (Experiment 3)

Source of Variation	SS	df	MS	F	P-value	F - crit
Antioxidant treatment*	1974.397	2	987.1985	6.400281	0.009073	3.6337
Accelerated oxidation time	1496.416	8	187.052	1.212713	0.351953	2.5911
Error	2467.888	16	154.243			
Total	5938.701	26				

* AE-200 ppm, AP-100 ppm, AE/AP-200/100 ppm

Table 7. ANOVA table for peroxide value of canola oil (Experiment 4)

Source of Variation	SS	df	MS	F	P-value	F - crit
Antioxidant treatment*	84.05281	1	84.05281	1.426304	0.354802	18.51276
Accelerated oxidation time	34123.55	2	17061.78	289.5237	0.003442	19.00003
Error	117.861	2	58.9305			
Total	34325.47	5				

* AE-200 ppm, AE-500 ppm

Table 8. Correlation coefficients of different treatments between oxidation values and oxidation time

Chemical analysis	Expt 1			Expt 2		
	C	AE (200 ppm)	PG/AP (200/100 ppm)	C	AE (200 ppm)	AE/AP (200/100 ppm
PV	0.9063	0.9194	0.9337	0.9375	0.9582	0.8077
TBA	0.9387	0.7932	0.8745	0.7331	0.7134	0.6707
AV	0.8747	0.9603	0.3905	0.8874	0.8924	0.7394

Chemical analysis	Expt 3			Expt 4	
	AE (100 ppm)	AP (100 ppm)	AE/AP (200/100 ppm)	AE(200 ppm)	AE(500 ppm)
PV	0.9393	0.0007	0.7776	0.9708	0.9922
TBA	0.8545	-0.6672	0.2695	0.9999	0.7029
AV	0.9105	-0.4063	0.6115	0.9626	0.9886

C - control; AE - artichoke extract; PG - propyl gallate; AP - ascorbyl palmitate.
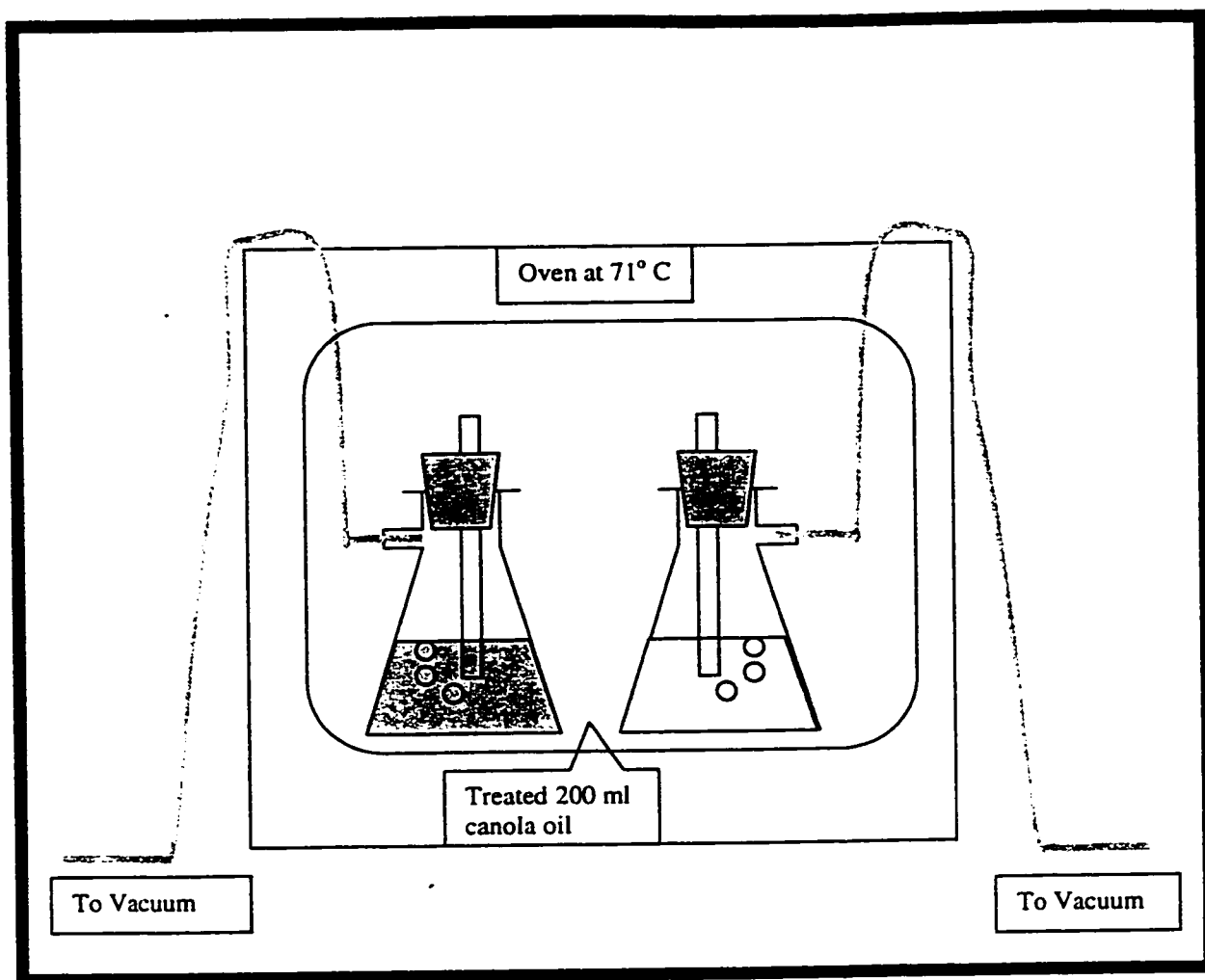
Figure 1. Schematic for accelerated oxidation test.
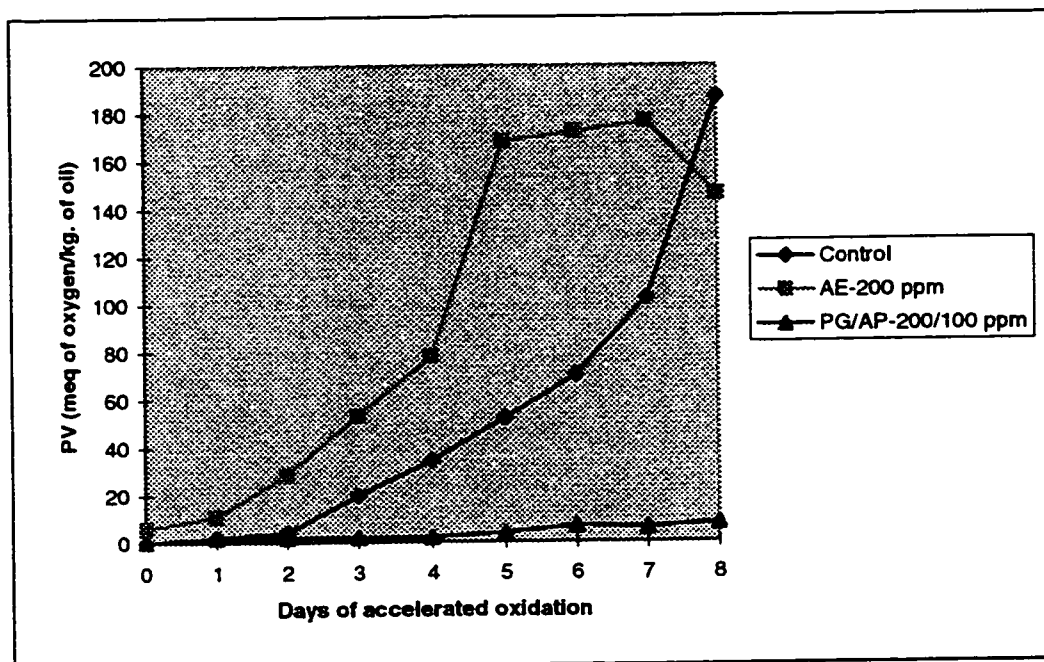
Figure 2. Peroxide values (milliequivalent of oxygen per kg of oil) of canola oil
during experiment 1.
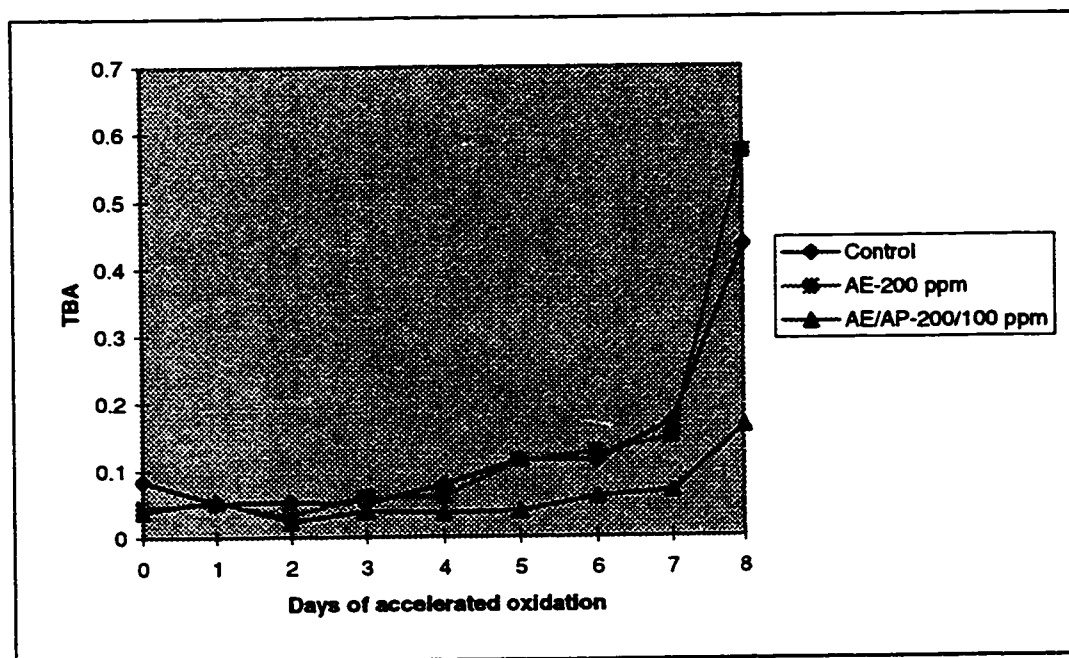Values are the means of duplicated samples.
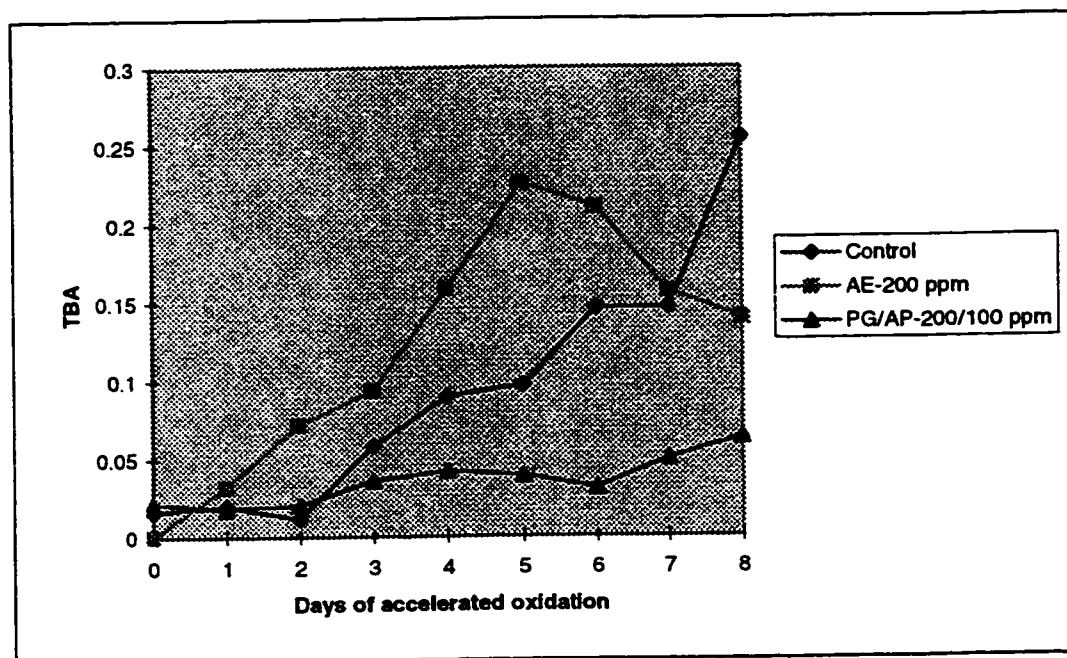
Figure 3. TBA values(increase of absorbance due to the reaction of 1 mg of oil per 1 ml volume with 2-TBA) of canola oil during experiment 1.
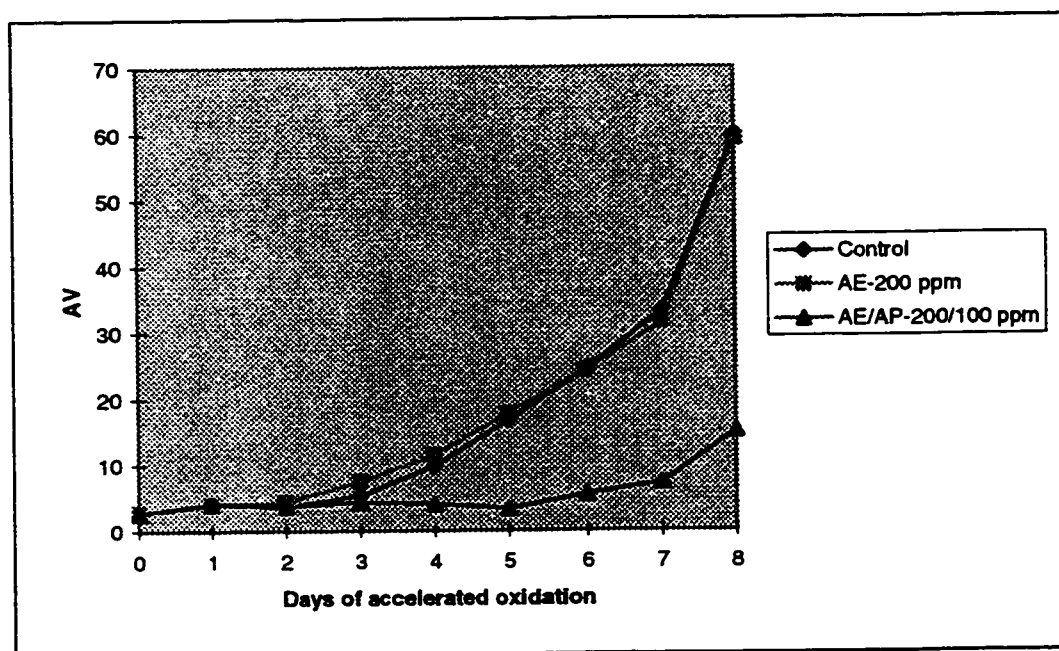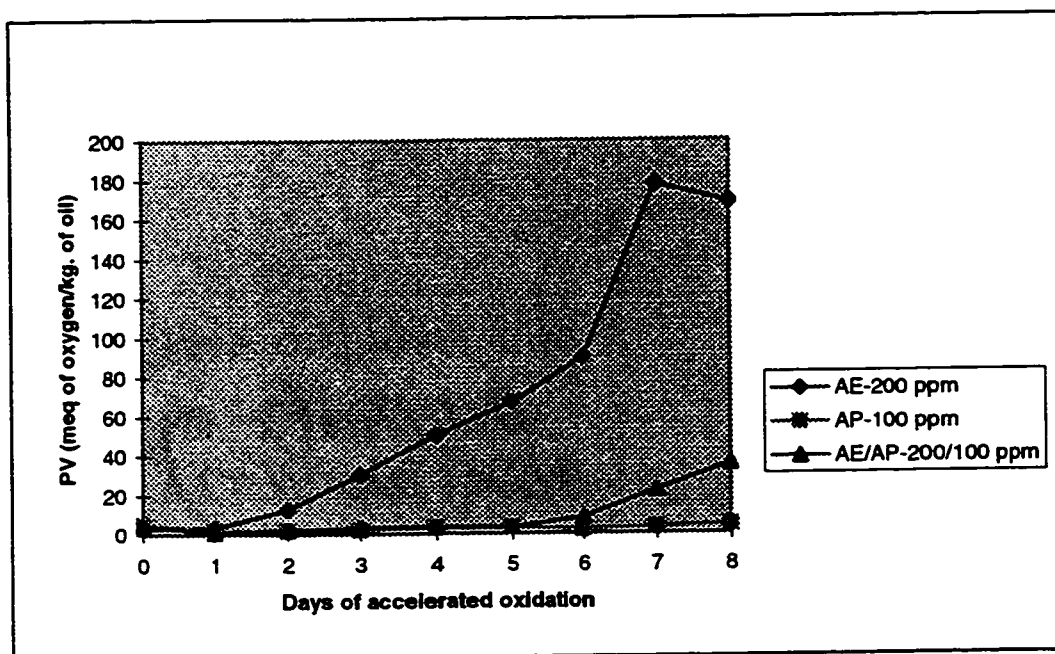Values are the means of duplicated samples.
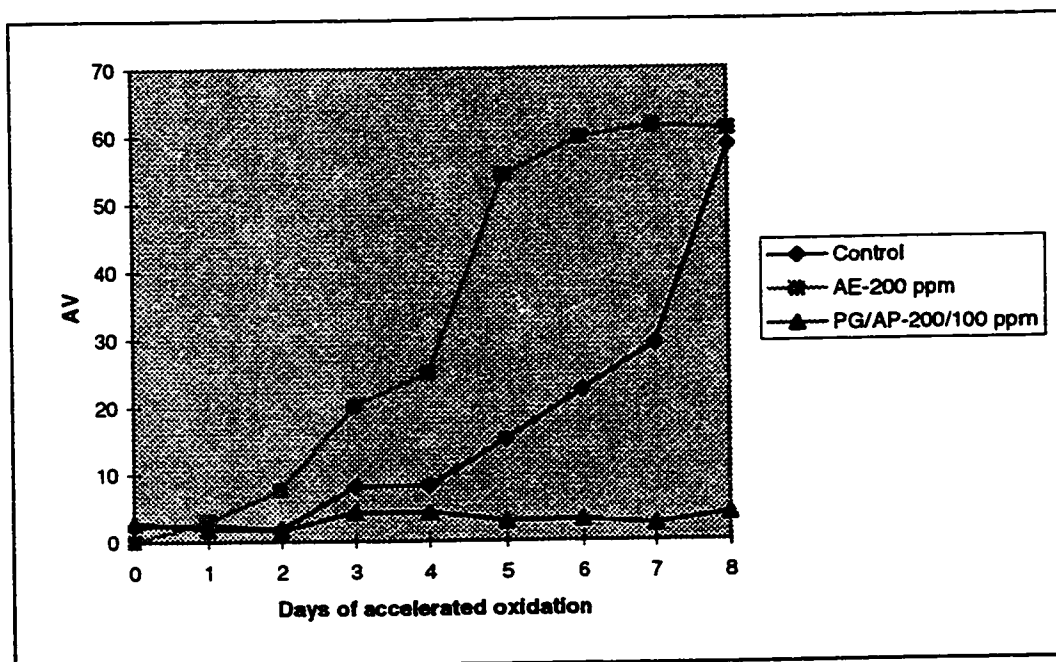
Figure 4. Anisidine values (100 times the absorbance of a solution resulting from the reaction of 1 g of oil in 100 ml of solvent with anisidine reagent) of canola oil during experiment 1.
Values are the means of duplicated samples.
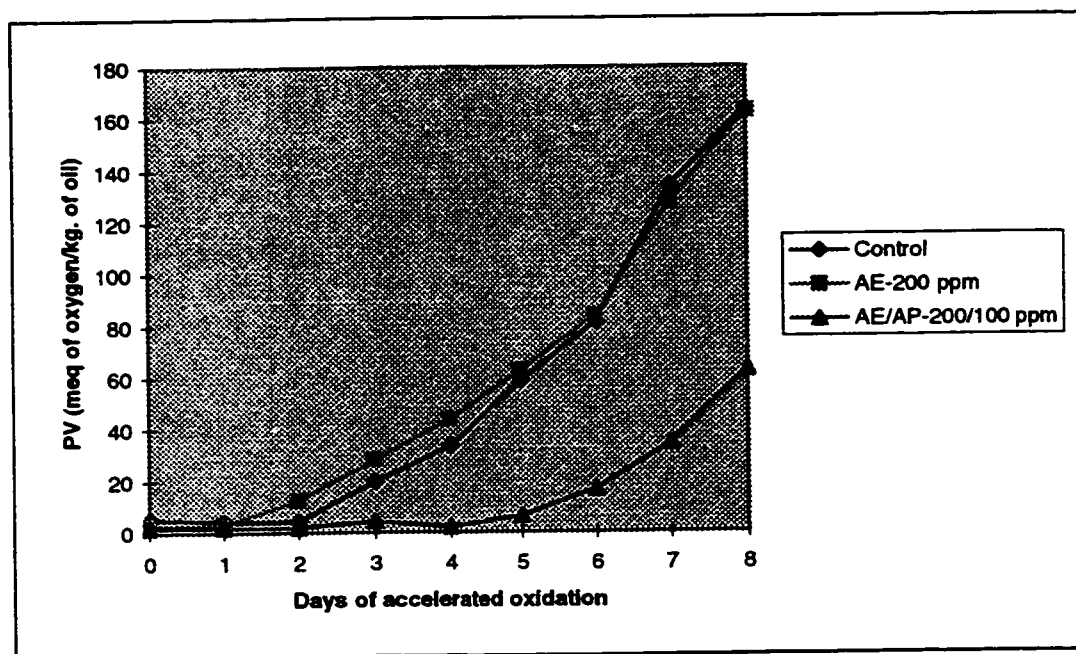
Figure 5. Peroxide values (milliequivalent of oxygen per kg of oil) of canola oil
during experiment 2.
Values are the means of duplicated samples.

Figure 6. TBA values (increase of absorbance due to the reaction of 1 mg of oil per 1 ml volume with 2-TBA) of canola oil during experiment 2. Values are the means of duplicated samples.
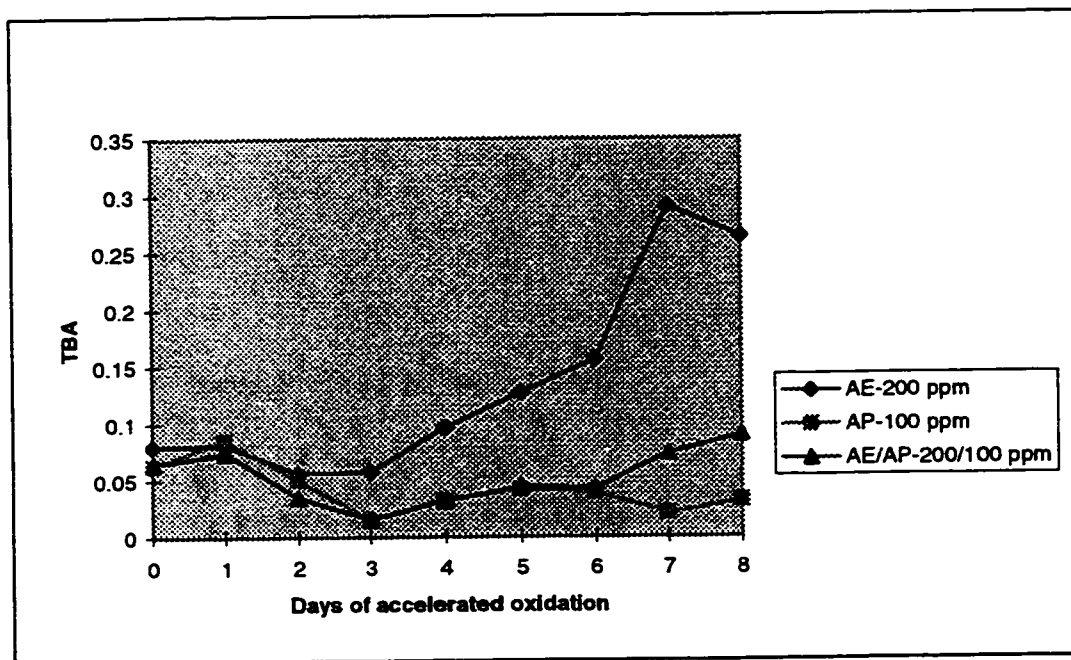
Figure 7. Anisidine value (100 times the absorbance of a solution resulting from the reaction of 1 g of oil in 100 ml of solvent with anisidine reagent) of canola oil during experiment 2.
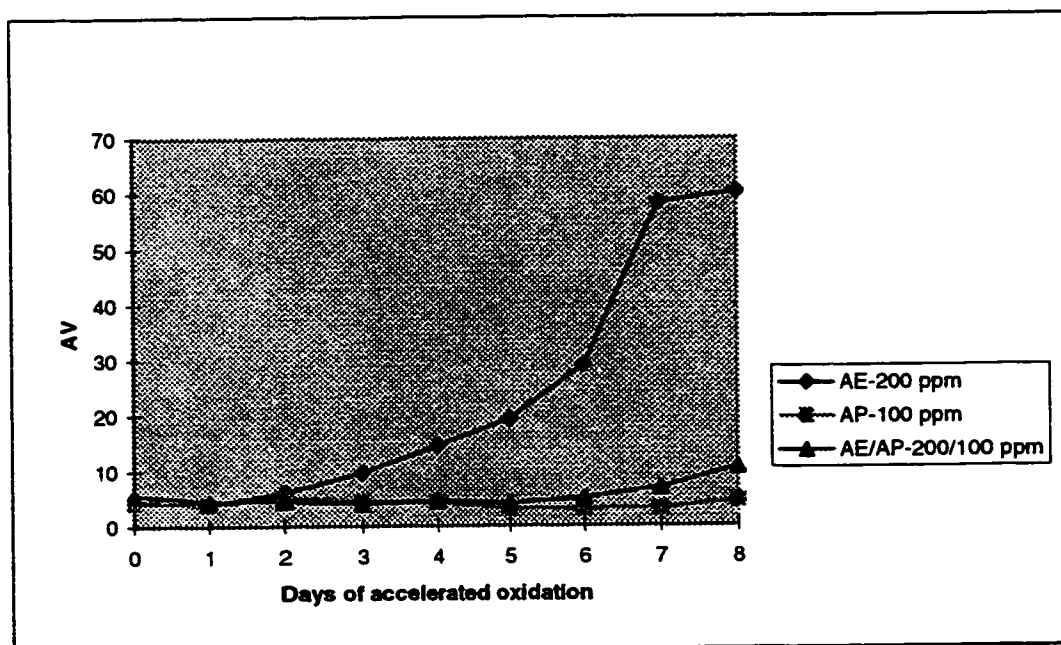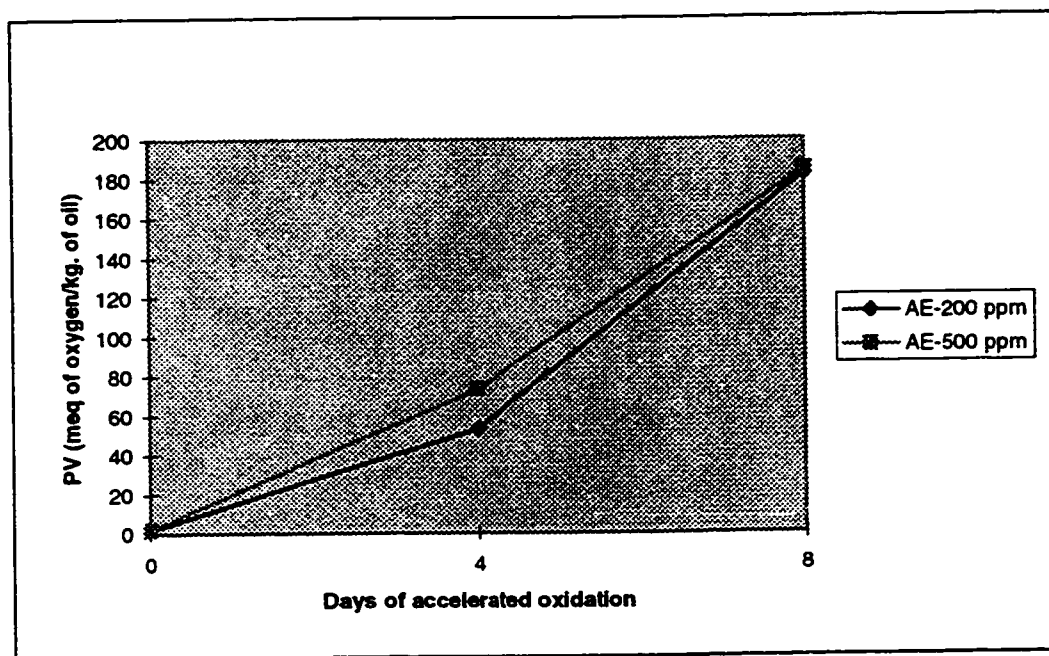Values are the means of duplicated samples.

Figure 8. Peroxide values (milliequivalent of oxygen per kg of oil) of canola oil during experiment 3.
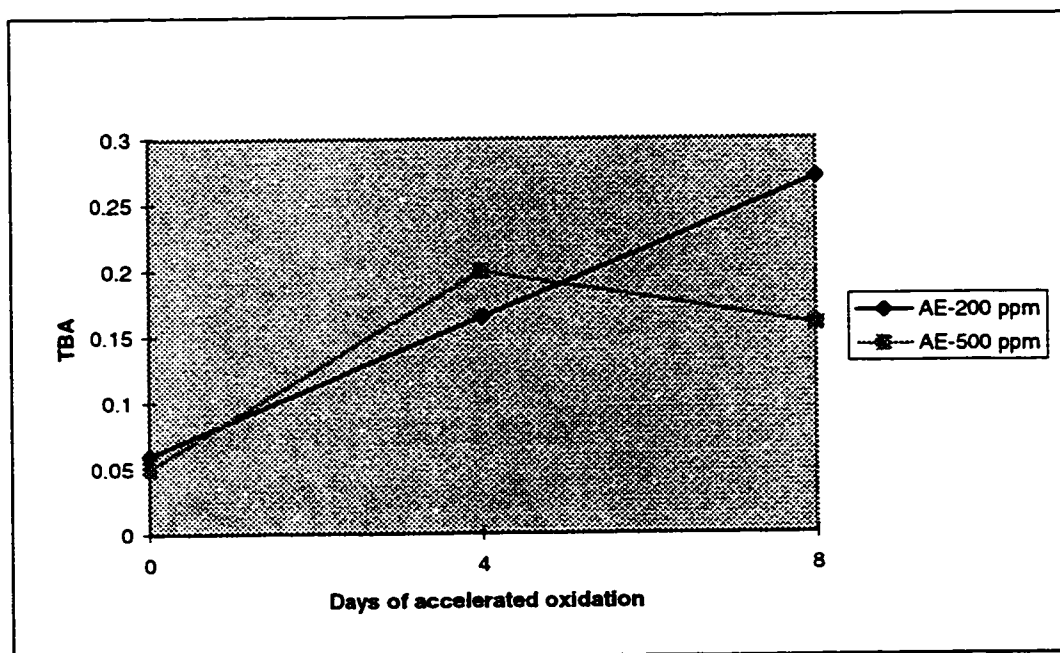Values are the means of duplicated measurements.

Figure 9. TBA values (increase of absorbance due to the reaction of 1 mg of oil per 1 ml volume with 2-TBA) of canola oil during experiment 3.
Values are the means of duplicated measurements
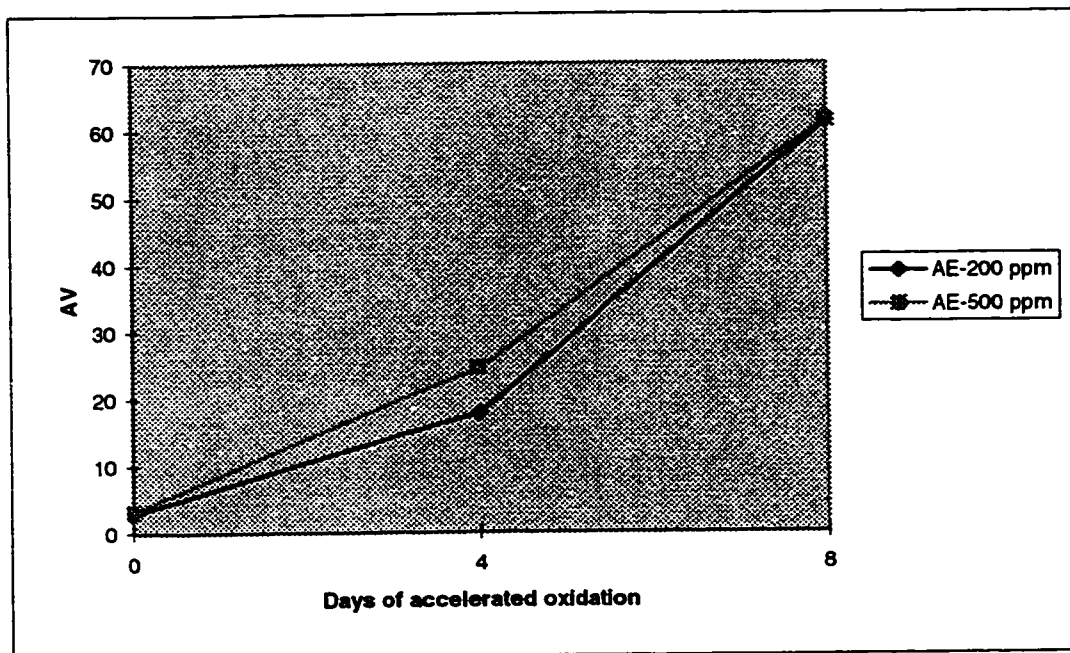
Figure 10. Anisidine values (100 times the absorbance of a solution resulting from the reaction of 1 g of oil in 100 ml of solvent with anisidine reagent) of canola oil during experiment 3.
Values are the means of duplicated samples.

Figure 11. Peroxide values (milliequivalent of oxygen per kg of oil) of canola oil
during experiment 4.
Values are the means of duplicated measurements

Figure 12. TBA values (increase of absorbance due to the reaction of 1 mg of oil per 1 ml volume with 2-TBA) of canola oil during experiment 4. Values are the means of duplicated samples.

Figure 13. Anisidine values (100 times the absorbance of a solution resulting from the reaction of 1 g of oil in 100 ml of solvent with anisidine reagent) of canola oil during experiment 4. Values are the means of duplicated measurements.

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CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

The present study was initiated to determine whether the polyphenols in artichoke leaves had any antioxidant properties as compared to PG and AP. A volume of 200 ml of pure canola oil without any added antioxidant and canola oil treated with AE (200 ppm and 500 ppm), PG/AP (200/100 ppm), AE/AP (200/100 ppm), and AP (100 ppm) were subjected to accelerated oxidation for eight days by continuous aeration and heating at 71^0C. The oil quality was assessed by PV, TBA, and AV every 24 hours. Results of the study indicated that AE did not possess significant antioxidant activity as compared to PG and AP. A high concentration of AE did not improve its efficacy as an antioxidant.

Recommendations

Selection of the correct type and amount of antioxidant(s) for use in a particular oil and under specific processing conditions necessitates careful consideration as each antioxidant functions in a unique way to delay autoxidation, photoxidation, and thermal degradation. Obviously, for (an) antioxidant(s) to terminate autoxidation and function with maximum efficiency in a vegetable oil, the antioxidant(s) must be completely incorporated into the oil. Further studies of the quality and stability of AE treated canola oils exposed to a variety of storage conditions are needed to determine the cause(s) for difference in antioxidant efficacy. A modification in the extraction procedure of the artichoke leaves would be desirable to increase not only its polyphenol yield, but also its solubility in canola oil. Specific polyphenols from the extract should be isolated to test

their efficacy as antioxidants. Furthermore, the extract should also be analyzed for the presence of prooxidants, if any.

The efficacy of specific antioxidants in retarding oxidative changes in heated canola oils may vary depending on the particular stability test selected and the analytical procedures employed. The most common storage conditions for canola oil include room temperature storage, storage in dark or fluorescent lights, and storage in plastic or glass containers. The test conditions employed here were extreme and are rarely encountered in daily routines. Changes in oxidative stability of antioxidant treated canola oil that occur during accelerated storage tests are not indicative of those that take place during practical storage (Hawrysh, Shand, Tokarska, & Lin, 1988). Both autoxidation and antiozidant behavior proceed by different mechanisms at higher temperatures and oxygen concentrations than at lower temperature and oxygen concentration. Therefore, it is difficult to predict with accuracy, the shelf life storage stability.

The analytical methods used here are based on certain chemical changes brought about during primary and secondary stages of the oxidation. These analytical methods are designed to analyze for one product only or for one type of product. For example, in the TBA test the rancid oil is tested for a single oxidation product, malonaldehyde. These tests are not always precise, and sometimes fail to correlate with organoleptic measurements of rancidity (Scholz & Ptak, 1966).

Standardization of the methods used for accelerated lipid oxidation would be desirable. In particular, the container size in relation to sample size requires standardization because this affects oxygen accessibility through the ratio of surface area

exposed to oxygen to oil volume (Malcolmson et al., 1994). However, the rancidity of

any oil determined by chemical methods must be corroborated by organoleptic evaluation

before it is adopted for practical purposes (Arumughan et al., 1984).

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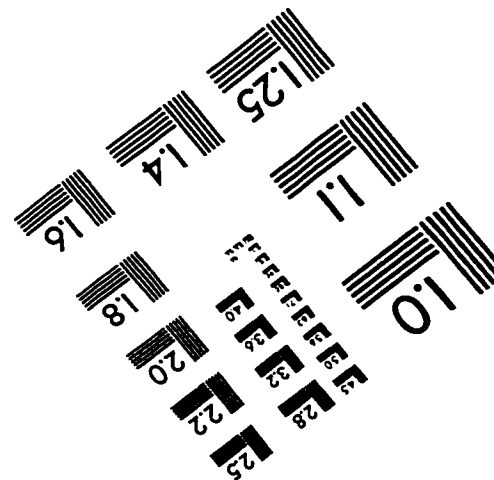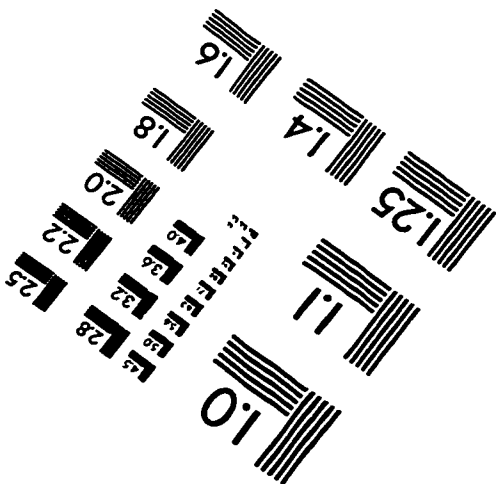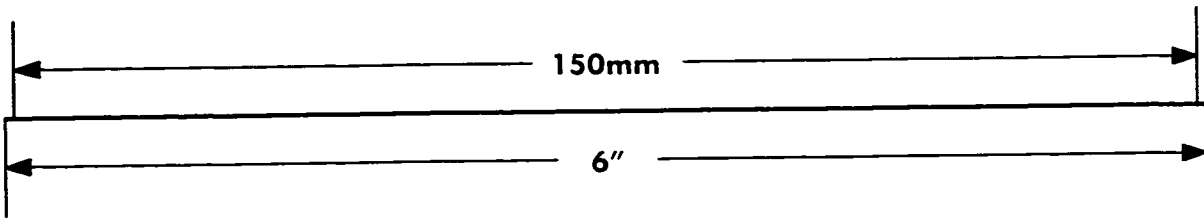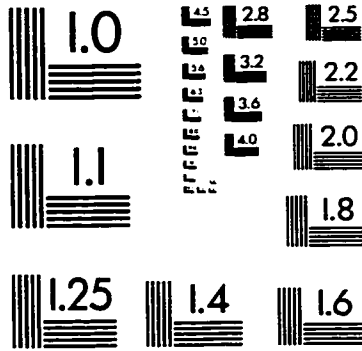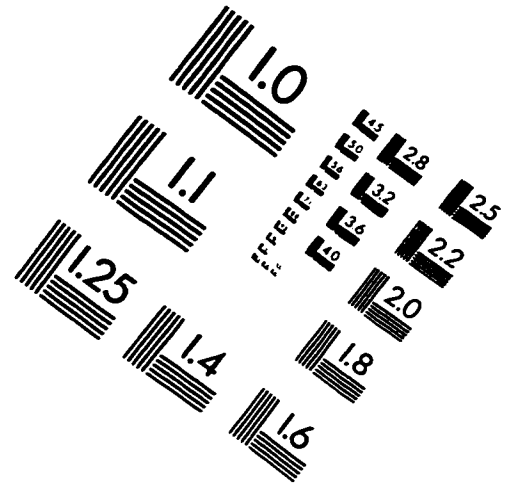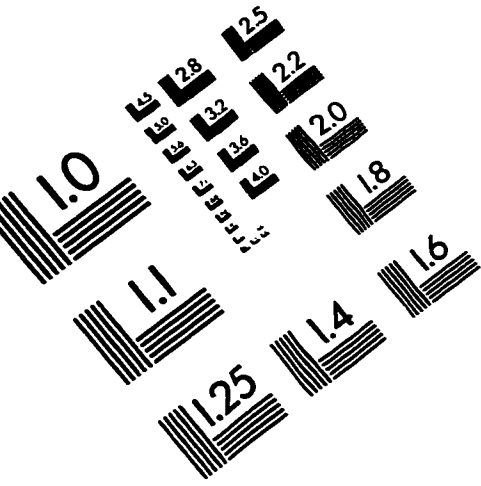
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IMAGE EVALUATION
TEST TARGET (QA-3)

150mm

6"