ANTIOXIDANT ACTIVITY OF *AMPELOPSIS GROSSEDENTATA* CRUDE EXTRACT AND ITS MAJOR COMPONENT DIHYDROMYRICETIN

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science in Life Science
In
Food Science and Technology

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July 12th, 2011
Blacksburg, VA

Keywords: antioxidant, *Ampelopsis grossedentata*, Teng Cha, dihydromyricetin, soybean oil oxidation, DPPH, peroxide value, anisidine value, Totox value, headspace volatiles, headspace oxygen content, TBARS

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ABSTRACT

Oxidation limits the shelf life of many food products. Adding antioxidants to foods is the most common way to solve this problem. Reports on safety issues of synthetic food additives have raised consumer interest in "all natural" foods, without added antioxidants or with synthetic replaced with natural antioxidants. The natural antioxidants now in use are much more expensive and less potent than the synthetic antioxidants. Thus, effective and economical natural antioxidants are of great interest to researchers. Teng Cha is a type of herbal tea found in China that has reported high levels of antioxidants. Antioxidant activity of Teng Cha extract and its major component dihydromyricetin has been reported, but no studies have provided clear evidence for the antioxidant effectiveness of Tech Cha extracts. The goal of this study was to measure the antioxidant activity of Teng Cha extract and dihydromyricetin (DHM), a major component of Tech Cha extract. The DPPH assay was conducted and antioxidant activities of the crude extract and dihydromyricetin were evaluated in soybean oil based on the peroxide value, anisidine value, Totox value, headspace volatiles and headspace oxygen. Antioxidant effectiveness was also evaluated in a cooked beef model system. DHM was more potent than BHA in preventing soybean oil oxidation. The crude extract was not as effective as BHA and DHM, possibly because it contained transition metals. In cooked beef, DHM and the crude extract showed lower activity than BHA, possibly due to their low solubility. Overall, Teng Cha extract and DHM are potential natural food antioxidants for future applications.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my great advisor Sean O'Keefe for his patience, magnanimity and inspiration throughout my study at Virginia Tech. It has been an honor to work in his research group. I would also like to thank Dr. Duncan, Dr. Eigel and Dr. Wang for serving on my advisory committee.

I would like to thank the staff members, Harriet Williams, Joe Bowling, Terry Rakestraw and Walter Hartman, for always giving freely of their time to help. Special thanks to Harriet Williams for her kind help and sweet smile over the past two years.

A warm thanks to my brother Yumin Dai who is always there for my questions; to my officemates for all of the discussions and laughter, especially my original office buddies Kevin Holland, Paul Sarnoski and Silvia Peralta, whose amazing personalities affected me a lot; to my fellow graduate students in Food Science for their support; to Lingxiao Xue for his assistance with formatting the documents; to my dear friends Luman Chen, Qin Li, Hanqing Zhang, Lei Pan for their companies in my life.

I would like to thank my family for supporting me throughout my study. I was a naughty and troublesome teenager in many teachers' eyes before I went to college. My parents got lots of complaints from them. But they never stopped believing me and gave me enough freedom to adjust myself.

I would like to thank the Food Science department for financially supporting me during the past two years and made this all possible.

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LIST OF ABBREVIATIONS AND TERMS

AAPH	2,2’-azobis(2-amidino-propane) dihydrochloride
AnV	anisidine value
AUC	area under a curve
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
DHM	dihydromyricetin
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	ethylenediaminetetraacetic acid
ESI-MS	electrospray ionization
GC	gas chromatography
HPLC	high performance liquid chromatography
IC_{50}	concentration of sample to cause 50% inhibition
IR	ionizing radiation
IUPAC	International Union of Pure and Applied Chemists
MDA	malondialdehyde
NMR	nuclear magnetic resonance
PG	propyl gallate
PV	peroxide value
SDS	sodium dodecyl sulfate
SPME-GC-MS	solid phase microextraction-gas chromatograph-mass spectrometer
TBA	thiobarbituric acid
TBHQ	tertiary butylhydroxyquinone
TEAC	trolox equivalent antioxidant activity assay
TMP	tetramethoxypropane
TPC	total phenolic content
TRAP	total radical-trapping parameter assay
UV	ultraviolet
AOCS	American Oil Chemists’ Society

CHAPTER 1: INTRODUCTION

Lipid Oxidation and Antioxidants

Lipid oxidation is one of the most important factors limiting the shelf life of many food products. The traditional way to extend the shelf life in the modern food industry is to add antioxidants into the food matrices. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroxyquinone (TBHQ), and propyl gallate (PG) are very commonly employed. However, in recent decades, the reported safety issues of synthetic additives raised consumers' concern on these food ingredients. Products with less and natural additives are preferred. Currently, natural antioxidants in use include rosemary and sage extracts. Their antioxidant effect has been demonstrated in many food substrates (Madhavi, Singhal, & Kulkarni, 1996), but they are expensive and still not as potent as some synthetic compounds. Thus, researchers continue to look for new and effective natural antioxidants.

Ampelopsis grossedentata and Dihydromyricetin

Ampelopsis grossedentata, which belongs to the family *Vitaceae* and the genus *Ampelopsis Michx*., is a wild plant growing in mountain areas of southern China. Its dried leaves and stems, called Mao Yan Mei or Teng Cha, have been consumed as health tea and herbal medicine for hundreds of years. Research has been conducted on the nutritional profile (Chen, Dong, Chen, Xiao, Li, & Luo, 2007; Zhang, Yang, & Xiong, 2001), health benefits and toxicity (Zhong, Zhou, & Chen, 2003; Zhou, Hu, & Jiang, 2001) of Teng Cha. Dihydromyricetin is the most abundant bioactive component in Teng Cha extract. Its structure is very close to that of quercitin, and

dihydromyricetin was reported to be an effective antioxidant (Reische, Lillard, & Eitenmiller, 2008).

Gao and others (2009) measured the DPPH radical scavenging activity of Teng Cha extract and dihydromyricetin, and found dihydromyricetin had significant higher antioxidant capacity than TBHQ. Radical scavenging tests became very popular in the past decade. But the biological and practical significance of the results has been questioned. Radical scavenging tests are suitable for screening and predicting antioxidant activity in preliminary studies. Antioxidant assays in food models are necessary to verify the effectiveness in real applications (Frankel & Meyer, 2000).

Wang tested the antioxidant activity of Teng Cha extract in lard, and found the lard samples treated with the crude extract had significantly lower peroxide value than those with TBHQ and the controls (Wang, Tian, Xiong, & Zhang, 2004). However, peroxide value alone cannot indicate the effectiveness of the antioxidants. In 2005, Decker and others published a paper on measuring antioxidant effectiveness in foods (Decker, Warner, Richards, & Shahidi, 2005). It was recommended that standard oxidation conditions and protocols should be used, so that the data obtained are comparable with that from other works. It was also mentioned that both primary and secondary oxidation products should be measured.

Research Objectives

As an effort to verify the antioxidant activity of Teng Cha extract and dihydromyricetin, this study was conducted with several specific objectives.

Objective 1: Analyze the DHM content in Teng Cha. 70% v/v aqueous ethanol was used as the extracting solution. DHM content in the crude extract was measure by using an Agilent HPLC system and running a series of DHM standards.

Objective 2: Verify the DPPH radical scavenging activity of Teng Cha extract and DHM. DPPH radical scavenging activity of the crude extract, DHM and BHA was measured. IC_{50} was calculated.

Objective 3: Examine antioxidant effect of Teng Cha extract and DHM in soybean oil. Soybean oil samples with BHA, DHM, extract and BHA+DHM and soybean oil controls were oxidized at 60 ℃ for 15 days. On Day 0, Day 3, Day 6, Day 9, Day 12 and Day 15, peroxide value, anisidine value, headspace volatiles and headspace oxygen content were measured for each treatment in triplicate. 200 ppm BHA and 200 ppm DHM was used together in soybean oils in order to see if there is any synergistic effect of these two compounds.

Objective 4: Examine antioxidant activity of Teng Cha extract and DHM in cooked beef. Cooked beef with 200 ppm BHA, 200 ppm DHM, 200 ppm extract and a control were stored in a 4 ℃ refrigerator for two weeks. On Day 1, Day 7 and Day 14, TBARS test was conducted on each treatment in triplicates.

References

Chen, X., Dong, B., Chen, J., Xiao, C., Li, B., & Luo, D. (2007). Study on the medicinal value of Teng Cha. *Hunan Agricultural Sciences* (6), 180-181.

Decker, E. A., Warner, K., Richards, M. P., & Shahidi, F. (2005). Measuring antioxidant effectiveness in food. *Journal of Agricultural and Food Chemistry, 53*(10), 4303-4310.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*(13), 1925-1941.

Gao, J., Liu, B., Ning, Z., Zhao, R., Zhang, A., & Wu, Q. (2009). Characterization and antioxidant activity of flavonoid-rich extracts from lleaves of *Amelopsis grossedentata*. *Journal of Food Biochemistry, 33*(6), 808-820.

Madhavi, D. L., Singhal, R. S., & Kulkarni, P. R. (1996). Technological aspects of food antioxidants. In D. L. Madhavi, S. S. Deshpande & D. K. Salunkhe (Eds.), *Food Antioxidants,* (pp. 159-266). New York: Marcel Dekker.

Reische, D. W., Lillard, D. A., & Eitenmiller, R. R. (2008). Antioxidants. In C. C. Akoh & D. B. Min (Eds.), *Food Lipids* 3rd ed., (pp. 409-434). Boca Raton, FL: CRC Press.

Wang, W., Tian, Z., Xiong, D., & Zhang, F. (2004). The antioxidation effects of the extracts of *Ampelopsis grossedentata*. *Chinese Wild Plant Resources, 23*(4), 46-47, 53.

Zhang, Y., Yang, W., & Xiong, H. (2001). Basic constituent of *Ampelopsis grossedentata*. *Natural Product Research and Development, 13*(5), 46-48.

Zhong, Z., Zhou, G., & Chen, X. (2003). The rat chronic toxicity test of total flavone of *Ampelopsis grossedentata* from Guangxi. *Lishzhen Medicine and Materia Medica Research, 14*(4), 193-195.

Zhou, Y., Hu, Y., & Jian, B. (2001). Toxicological assessment of *Ampelopsis grossedentata* and its immune regulation study. *Practical Preventive Medicine, 8*(6), 412-414.

CHAPTER 2: LITERATURE REVIEW

LIPID OXIDATION

Carbohydrate, lipid and protein are the three major caloric components of most foods. Theoretically, all three of these classes of compounds could be oxidized under certain conditions. Since lipid is more susceptible to oxidation during storage and processing and this oxidation can cause significant quality deterioration in foods, research on lipid oxidation is of particular interest.

Mechanisms

Based on the state of the oxygen involved, there are two types of lipid oxidation. Triplet oxygen oxidation is any oxidation with triplet oxygen, which is the most abundant and stable oxygen form in the air. Singlet oxygen oxidation occurs when singlet oxygen exists. Singlet oxygen can be formed in the presence of a photosensitizer, light and triplet oxygen. Mechanisms of these two types of oxidation are different.

In some reviews, mechanisms of lipid oxidation were discussed under different categories, such as autoxidation, photosensitized oxidation and thermal oxidation. Autoxidation is any oxidation that occurs in open air or in the presence of oxygen or UV radiation and forms peroxides and hydroperoxides. It is featured by the free-radical chain reaction. Photosensitized oxidation happens when there is a suitable sensitizer and sufficient light exposure. Oxygen is added to the double bond directly with a subsequent shift in the position of the double bond (Wong, 1989). Photosensitization is one of the most important mechanisms for the formation of

singlet oxygen. Thus, photosensitized oxidation takes a significant part in the singlet oxygen oxidation. Thermal oxidation is the lipid oxidation at elevated temperatures. The evidence accumulated to date indicates that the principal reaction pathways of thermal oxidation and autoxidation are essentially the same within the temperature range from 25 ℃ to 80 ℃ (Nawar, 1985). At higher temperatures, the formation and destruction of hydroperoxides is extremely rapid. The decomposition products are unstable and rapidly undergo further oxidative decomposition, which is different from the oxidation pathway at low temperatures.

Triplet Oxygen Oxidation

In the absence of photosensitizer or light, both free fatty acids and fatty acids esterified to triacylglycerols and phospholipids could be oxidized in the following pathway (MeClements & Decker, 2008).

1) Initiation: abstraction of a hydrogen from a fatty acid (LH) and formation of a fatty acid radical known as the alkyl radical (L ·).

$$LH \rightarrow L\cdot + H\cdot$$

2) Propagation: a. addition of oxygen to the alkyl radical and formation of peroxyl radical (LOO ·); b. addition of a hydrogen from another fatty acid to the peroxyl radical and formation of a fatty acid hydroperoxide (LOOH) and a new alkyl radical; c. repeat a.

a. $L_1 + O_2 \rightarrow L_1OO\cdot$

b. $L_1OO\cdot + L_2H \rightarrow L_1OOH + L_2\cdot$

c. $L_2 + O_2 \rightarrow L_2OO \cdot$

3) Termination: combination of two radicals (R ·) to form nonradical species.

$R_1 \cdot + R_2 \cdot = R_1R_2$

For example,

$L_1OO \cdot + L_2OO \cdot \rightarrow L_1OOOOL_2$

$L_1OO \cdot + L_2 \cdot \rightarrow L_1OOL_2$

$L_1 \cdot + L_2 \cdot \rightarrow L_1L_2$

Linoleic acid could be used as an example to demonstrate the three steps above (MeClements & Decker, 2008).

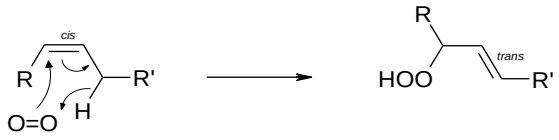
1) Initiation: After the abstraction of a hydrogen, alkyl radical forms (Figure 2.1). This polyunsaturated alkyl radical is then stabilized by the double bond shifting and formation of conjugated double bonds.

2) Propagation: Free radicals on oxygen react with the alkyl radical and form a peroxyl radical at a diffusion-limited rate (Figure 2.2). This high energy peroxyl radical could easily abstract a hydrogen from another unsaturated fatty acid and initiate a new oxidation process. Thus the reaction is propagated.

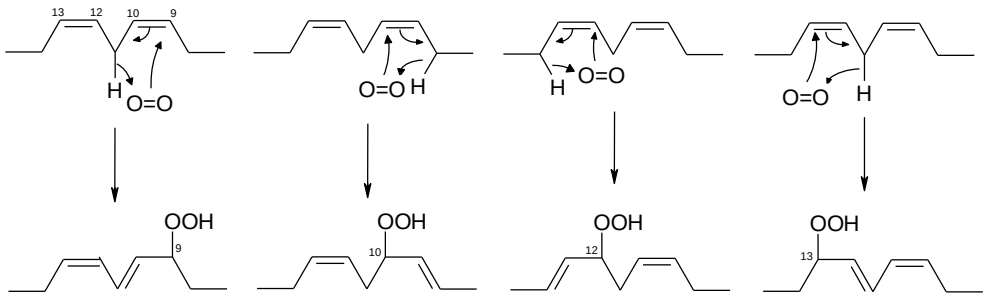
3) Termination: Under low oxygen conditions, termination reactions prone to occur between alkyl radicals as the following (Figure 2.3). If there is sufficient oxygen, termination reactions may occur between peroxyl and alkoxyl radicals.

Singlet Oxygen Oxidation

Photosensitizers such as chlorophyll, pheophytins, porphyrins, riboflavin, myoglobin, and some synthetic colorants in food can absorb energy from light and transfer it to triplet oxygen to form singlet oxygen (Kim & Min, 2008). Because of its high electrophilicity, singlet oxygen reacts with unsaturated fatty acids at a much faster rate than triplet oxygen. Singlet oxygen oxidizes fatty acids by direct reaction with double bonds as shown below. This process (1) involves no free radicals, (2) results in changes from *cis* to *trans* configuration, (3) is independent of oxygen pressure, (4) shows no measurable inductive period, and (5) is inhibited by singlet oxygen quenchers such as b-carotene and tocopherols (Wong, 1989).



Again, linoleate can be used as an example to demonstrate the mechanism (Wong, 1989).

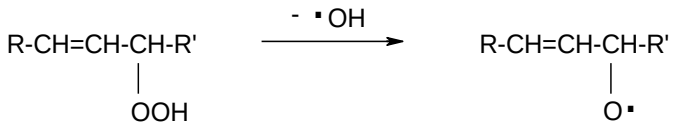


Hydroperoxides of fatty acids formed by triplet and singlet oxygen oxidation is different. Neff and Frankel compared the hydroperoxides composition of these two types of oxidation (Table 2.1) (Neff, 1980).

Formation of Secondary Oxidation Products

Lipid hydroperoxides are the primary oxidation products, which are relatively stable at room temperature in the absence of metals. However, under certain conditions, they are readily decomposed and form various volatile compounds such as aldehydes, ketones, acids, esters, alcohols, and short-chain hydrocarbons.

1) Decomposition of hydroperoxides produces alkoxyl radicals.



2) Alkoxyl radicals are not stable and may undergo β-scission to form short chain radicals and non-radical compounds (Figure 2.4).

3) The short chain radicals may abstract hydrogen from fatty acids and form new alkyl radicals and non-radical short chain compounds (Figure 2.4). They may also react with other radicals to form non radical substances.

Because of the large number of hydroperoxide types, radical types, scission and combination pathways, numerous secondary oxidation products could be formed in real food systems.

Negative Effect of Lipid Oxidation

Lipid oxidation is one of the most important factors limiting the shelf life of many food products. Firstly, it leads to significant changes in the sensory properties including odor, flavor, color and texture, which are easily detected by the consumers. Secondly, lipid oxidation reduces the nutritional value of lipids and gives rise to the formation of unhealthy compounds such as free radicals and reactive aldehydes.

Negative Effect on Sensory Properites

Secondary oxidation products formed during lipid oxidation are directly responsible for most of the undesirable changes in the sensory properties of foods. Composition of secondary oxidation products such as aldehydes, ketones, alcohols and hydrocarbons varies in different food products and gives rise to different sensory impressions (Jacobsen, 2010). For example, off-flavors of oxidized neat oils range from nutty through green, grass, cucumber to rancid and synthetic. Oxidized fish oils are described with the term “train oil”. In extremely advanced lipid oxidation, a painty odor may be developed. More research is needed to fully understand the

quantitative relationship between off-flavor formation and the concentrations of the specific volatile oxidation compounds. Besides causing off-odors and off-flavors, lipid oxidation may go hand in hand with oxidation of proteins and pigments and lead to texture and color changes (Jacobsen, 2010).

Negative Effect on Health

Lipid oxidation in food may cause negative effects on health through the nutritional quality loss of food components and formation of toxic compounds. Nutritional quality changes include loss of polyunsaturated fatty acids, loss of amino acids and loss of non-nutritive components such as vitamins, carotenoids, polyphenols, phytosterols and squalene (Eder, 2010). As some amino acids, such as sulphur, are nutritionally limiting in many proteins, a reduction of these amino acids induced by lipid oxidation will lead to a significant loss in the nutritional quality of the food. Most of the non-nutritive components lost during lipid oxidation are antioxidants which might be beneficial to human health.

During lipid oxidation, several classes of compounds may be formed including lipid peroxides, hydroxyl fatty acids, carbonyl compounds, cyclic monomers, dimers and polymers, polycyclic aromatic hydrocarbons and oxidized sterols. These products could cause both acute and chronic effects on health (Sanders, 1989). Examples of chronic effects are poor growth rate, myopathy, hepatomegaly, steatitis, haemolytic anaemia and secondary deficiencies of vitamins A and E. Diarrhea is a common acute symptom.

ANTIOXIDANTS IN USE

Antioxidants could be defined as any compounds which inhibit the oxidative process that deteriorate lipids and food quality (Decker, Chen, & Panya, 2010). These substances can occur as natural constituents of foods, but they also may be added to the products intentionally (Reische, Lillard, & Eitenmiller, 2008). Antioxidants added to foods must be inexpensive, nontoxic, effective at low concentrations, stable, and with minimal color, flavor and odor. Besides these basic rules, the choice of antioxidants also depends on product compatibility and regulatory guidelines.

Synthetic Antioxidants

The most important synthetic antioxidants used in food industry include butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroxyquinone, ethoxyquin, gallates, tocopherols, erythorbic acid and ascorbyl palmitate. Because of the suspicion of the carcinogenic effect of synthetic antioxidants, their use has decreased in recent years (Berdahl, Nahas, & Barren, 2010). The chemical structures (Figure 2.5), characteristics and applications of the four most commonly used synthetic antioxidants in the food industry are briefly summarized below.

Butylated Hydroxyanisole (BHA)

BHA is a light tan to light gray crystal with a slight phenolic odor (Madhavi, Singhal, & Kulkarni, 1996). It is highly soluble in fats and oils and insoluble in water. Commercial BHA is a mixture of 2-BHA and 3-BHA (Reische, Lillard, & Eitenmiller, 2008). BHA exhibits considerable carry-through properties and thus it is used extensively in baked and fried foods. It

also functions synergistically with other antioxidants, such as gallates, tocopherols, BHT, TBHQ, citric acid, and phosphoric acid (Madhavi, Singhal, & Kulkarni, 1996). According to Code of Federal Regulations, maximum amount of BHA allowed in foods is 0.02% based on the total weight of lipids.

Butylated Hydroxytoluene (BHT)

BHT is a white granular crystalline solid with a slight phenol odor (Madhavi, Singhal, & Kulkarni, 1996). It is similar to BHA in many of its properties. However, BHT is not as effective as BHA, mainly because of the presence of two t-butyl groups, which offer greater steric hindrance than BHA. Also, it is not as stable as BHA and has less carry-through (Reische, Lillard, & Eitenmiller, 2008). BHT is typically used together with BHA to provide synergy. BHT effectively acts as a hydrogen replenisher of BHA, allowing BHA to regenerate its effectiveness. Foods could contain BHA and BHT mixtures at levels up to 0.02%.

Tertiary Butylhydroxyquinone (TBHQ)

TBHQ is a white to light tan crystalline powder with little odor. It is very effective in stabilizing fats and oils, especially polyunsaturated crude vegetable oils (Madhavi, Singhal, & Kulkarni, 1996). TBHQ is commonly used in frying applications with highly unsaturated vegetable oils, where it is generally considered to be more effective than BHA and BHT (Reische, Lillard, & Eitenmiller, 2008). It shows excellent synergism with citric acid in vegetable oils. TBHQ is not allowed for use in foods in Canada and European Union and not permitted to be combined with propyl gallate in United States (Mikova, 2001).

Propyl Gallate (PG)

PG is a slightly water-soluble, white crystalline powder (Madhavi, Singhal, & Kulkarni, 1996). Compared with BHA and BHT, PG is less stable at high temperatures. Thus, it is not suitable for frying applications (Reische, Lillard, & Eitenmiller, 2008). Gallates are sold as mixtures with metal chelators because they will form undesirable, dark-colored complex with iron and copper (Berger, 1989). Gallates have synergistic effects with many of other antioxidants and are often included in mixed antioxidant preparations.

The synthesis of novel antioxidants for food use is limited by rising costs of research and development, costs associated with safety assessment, and the time required to obtain regulatory approval of additives (Reische, Lillard, & Eitenmiller, 2008). Although there is no concrete evidence for any negative effect of the synthetic antioxidants on human health and no guarantee for the safety of all the natural ones, consumers are trying to avoid the artificial antioxidants. Because of consumer preference, safety consideration from food producers and food regulations, there seems to be a small drop in the total sales percentage of synthetic antioxidants in the market (Berdahl, Nahas, & Barren, 2010). For food processors, the application of natural antioxidants allows them to mark the products as all-natural and may shorten the waiting time for approval from the regulatory agencies. All these facts lead to the growing research work on the development of natural antioxidants.

Natural Antioxidants

Due to the potential hazardous effects of synthetic antioxidants, special attention has been given to the identification, isolation and application of natural antioxidants. Numerous natural

antioxidants are under investigation and some of them are already in application, including natural phenolics, ascorbic acid, carotenoids, proteins, peptides, phospholipids, sterols, gums and Maillard reaction products. These compounds are derived from plants, animal products and microbial sources. According to Boskou, plant phenols are the most intriguing source for natural antioxidants (Boskou, 2006). Plant phenolics occur in various chemical forms, such as phenolic acids, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, anthocyanins, tannins, lignans, stilbenes, coumarins and essential oils (Pokorny, 2007). Some natural antioxidants in use contain only one specific phenolics or one class of phenolics; some are mixtures of these compounds.

Rosemary Extract

Rosemary extract is the dominant spice extract antioxidant in the market (Berdahl, Nahas, & Barren, 2010). It has been used in culinary applications since ancient times and the reports on its antioxidant activity date back to the 1950s (Chipault, 1956). The major active phenolics in rosemary are shown below (Figure 2.6). Like most phenolic compounds, these active ingredients function as radical scavengers by donating hydrogen to reactive radicals and forming more stable radicals. Carnosic acid and carnosol are good peroxyl radical scavengers; rosmarinic acid is a good superoxide scavenger (Nakamura, 1998). Due to its high potency, it has found its way into numerous food applications including oils, fats, baked goods, fried foods, meat and beverages. Synergistic effects have been demonstrated between rosemay extracts and other antioxidants such as tocopherols and citric acid (Irwandi, 2004).

Sage

Sage extract has many similarities with rosemary but it is less attractive because of its lower antioxidant activity. The most effective antioxidants in sage are also carnosic acid, carnosol and rosmarinic acid but it also contains other antioxidants including 9-ethylrosmanol ether and luteolin-7-o-b-glucopyanoside (Yanishlieva, Marinova, & Pokorn, 2006). It is used in various foods such as oils, fats, meat and fried foods.

Oregano

Oregano also has similar chemical composition with rosemary and sage but the carnosic acid and carnosol levels are much lower (Hernandez-Hernandez, 2009). It contains essential oils such as carvacrol and thymol, which are also very active but the distinct flavor and aroma of these components greatly limits applications. Oregano is also rich in less polar antioxidants such as flavones, flavanon, dihydroflavonol and dihydroflavonol, which showed high efficacy against lard and oil oxidation (Vekiari, 1993).

Tea

Tea antioxidants have been patented for use in several food products (Reische, Lillard, & Eitenmiller, 2008). There are three major polyphenol groups in teas: catechins, theaflavins, and thearubigins. The chemical structures of catechins and theaflavins have been identified (Yanishlieva & Heinonen, 2001). (Figure 2.7 & Figure 2.8) The major active components in green tea are the catechins including epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate and gallocatechins. Green tea catechin extract was much more effective

than rosemary extract against lipid oxidation in canola oil, lard, and chicken fat at 100C (Chen, Wang, Chan, Zhang, Chung, & Ling, 1998).

With the rising consumer demand for natural food additives, the use of natural phenolics is increasing. Extracts of herbs, spices and teas are good source for these phenolic antioxidants. Researchers have been trying to identify and isolate new active components from them. So far, only rosemary and sage are commercially available as flavorless, odorless, and colorless antioxidant extracts at present. The substantially higher cost and lower efficiency prevents them from being used as widely as synthetic antioxidants. Thus, research and development of new natural phenolic antioxidants is still of great interest. The other reason for the continuous interest is the growing understanding of the relationship between dietary natural antioxidants and biological oxidative stress in human body, which is believed to be relevant to some degenerative diseases.

EVALUATING ANTIOXIDANT EFFECTIVENESS

A large amount of effort has been invested in identifying new natural antioxidants and developing methods to evaluate their effectiveness. However, different tests and different protocols of the same tests led to the accumulation of a tremendous amount of data which are difficult to compare or sometimes conflicting (Nenadis & Tsimidou, 2010). As the knowledge in the mechanisms of lipid oxidation and antioxidant activities expanded, researchers gradually realized the importance of standardizing protocols and using multiple methods to evaluate the antioxidant effectiveness. Methods currently in use could be divided into two classes; those accessing free radical scavenging activity and those evaluating changes in a lipid substrate.

Assessing Radical Scavenging Activity

Antioxidants may function through numerous possible pathways, such as scavenging radicals, chelating prooxidant metals, deactivating singlet oxygen, replenishing hydrogen, decomposing hydroperoxides, deactivating singlet oxygen, absorbing ultraviolet radiation and scavenging oxygen. Phenolics, which are the most widely distributed natural antioxidants and the most important class of antioxidants under investigation, mainly act through their radical scavenging activity. Thus, focus on evaluating radical scavenging activity was given (Nenadis & Tsimidou, 2010).

DPPH Radical Scavenging Capacity Assay

This widely used decoloration assay was first reported by Brand-Williams (Brand-Williams, 1995). The DPPH radical is synthetic nitrogen radical with a deep purple color. It is commercially available and does not have to be generated before assay. During this assay, the purple radical is reduced by antioxidants to the corresponding pale yellow hydrazine. The loss of DPPH is measured by electron spin resonance or by monitoring the absorbance decrease at 515-528 nm (Karadag, 2009). Several parameters have been used to express the antioxidant capacity of test compounds in DPPH assay, such as EC_{50}, T_{EC50} and AE_{50} (Brand-Williams, 1995). The concentration that causes a decrease in the initial DPPH concentration by 50% is defined as EC_{50}. The time needed to reach the steady state with EC_{50} is defined as T_{EC50}. AE_{50} means antiradical efficiency. It was defined as $AE = (1/EC_{50})T_{EC50}$ (Huang, 2005). The test is simple, rapid, and only needs a UV-vis spectrophotometer to perform. However, DPPH is a stable synthetic nitrogen radical that bears no similarity to the peroxyl radicals involved in lipid

peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly to DPPH (Prior, 2005). Other concerned disadvantages of DPPH assay include that 1) spectra overlap of DPPH and test compounds at 515 nm; 2) DPPH color could also be lost via reaction other than radical reaction or reduction; 3) steric accessibility is a major determinant of the reaction; 4) DPPH could only be dissolved in organic media, not in aqueous media; 5) reaction mechanism is not well known.

Oxygen Radical Absorbance Capacity (ORAC) Assay

This assay is based on the early work of Ghiselli (Ghiselli, 1995), Glazer (Glazer, 1990) and Cao (Cao, 1993). It measures antioxidant inhibition of peroxyl radical induced oxidations and reflects classical radical chain breaking antioxidant activity by hydrogen atom transfer (Ou, 2001). In ORAC assay, peroxyl radical generated by 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) decomposition reacts with a fluorescent probe to form a nonfluorescent product, which can be quantified easily by fluorescence. In the presence of an antioxidant, the fluorescence decay is inhibited as the antioxidant competes for peroxyl radicals. Evaluation of the antioxidant capacity is based on the difference of integrated areas under the decay curves. The merits of this method include applicability to both hydrophilic and lipophilic compounds, automation potential, good linearity of area under a curve (AUC) vs antioxidant concentration, and good precision (Nenadis & Tsimidou, 2010). Although ORAC assay was criticized in that it assumed the mechanism mimics critical biological substrates (Frankel, & Meyer, 2000), it has so many advantages that it has been proposed for standardization during the first international congress on antioxidant methods (Prior, 2005).

Several other free radical scavenging tests have also been developed to measure the antioxidant capacity of natural phenolics from various plant extracts. The most commonly used ones are the total radical-trapping parameter assay (TRAP), the superoxide anion scavenging assay and the trolox equivalent antioxidant activity assay (TEAC). Their principles, advantages and disadvantages are well summarized in some reviews (Antolovich, 2001; Becker, 2004; Frankel, 2000; Huang, 2005; Karadag, 2009; Prior, 2005). Compared to the tests conducted on a lipid substrate or a real food system, these tests are economical, fast and simple. However, most of them were criticized for neglecting the complex multistep mechanism of antioxidants and their multiple actions in complex food systems (Frankel & Meyer, 2000), which lead to some confusion in understanding the significance of the results.

Measuring Antioxidant Effectiveness in a Lipid Substrate

To accurately evaluate the potential of antioxidants in foods, models must be developed that have the chemical, physical, and environmental conditions expected in food products (Decker, 2005). The models commonly employed in lipid oxidation studies are bulk oils, dispersed systems and complex food matrices. Standard oxidation conditions and measurement of the oxidation levels in these models were recommended for easy comparison between papers.

Measuring Antioxidant Effectiveness in Edible Oils

For autoxidation, oven storage at 60 ℃ in the dark was recommended. Extreme conditions, such as high temperatures, using initiators or exposure to intense light, were criticized in some review papers (Decker, 2005; Frankel, 2000), because they could not reflect the real food systems and may change the behavior of the test antioxidants. Storage temperatures higher than

60 ℃ may introduce thermal oxidation, which has a different mechanism with oxidation at lower temperatures. High temperatures also can cause decomposition or deactivation of antioxidants. Sampling frequency and endpoint should be set ahead depending on the stability of the oil (Decker, 2005).

It was recommended that each antioxidant evaluation should be carried out using several methods to measure different products of oxidation (Frankel & Meyer, 2000). To effectively analyze the antioxidant activity, both primary oxidation products (e.g. hydroperoxides, conjugated dienes) and secondary oxidation products (e.g. carbonyls, volatiles) should be measured. Some compounds, such as tocopherol, can increase the primary oxidation products and decrease the secondary oxidation products (Decker, 2005). Some other compounds, such as transition metals, may catalyze the decomposition of hydroperoxides and the formation of secondary products. Thus, primary or secondary products alone could not indicate the degree of oxidation.

Peroxide Value (PV). Hydroperoxides, which are generally referred to as peroxides, are the primary products of lipid oxidation (Gray, 1977). Numerous procedures with different experimental conditions and reducing agents for the measurement of lipid hydroperoxides were described in the literature. Most methods rely on the ability of the hydroperoxides to oxidize an indicator compound (MeClements & Decker, 2008). The AOCS (American Oil Chemists' Society) method for peroxide value measures the hydroperoxide-promoted conversion of iodide to iodine by titration with sodium thiosulfite in the presence of a starch indicator. This method has several disadvantages that should be known before doing this test. First, the concentrations of hydroperoxides in lipid substrates decrease during the latter stages of oxidation because the

formation rates become slower than the decomposition rates. Thus, peroxide value alone cannot indicate the degree of oxidation. Second, the primary oxidation products are not volatile and do not contribute to off-flavors and off-aromas, which are directly related to food qualities. Third, in the presence of transition metal or at high temperatures, the decomposition rate of hydroperoxides is very high. In this case, peroxide value may produce misleading results if no other tests are conducted.

Anisidine Value (AnV). During the deodorization stages of refining, lipid hydroperoxides are broken down and volatile compounds are removed. Thus, badly oxidized oil may have a low peroxide value and an acceptable flavor after this process. However, the damaged oil has a lower level of natural antioxidants present. In addition, the nonvolatile secondary products left in the oil may catalyze further oxidation. Anisidine value test measures nonvolatile, high molecular weight carbonyls, principally 2-alkenals and 2,4-dienals (Tompkins, 1999). Thus, it is useful in detecting damaged but refined oils. The Anisidine value (AnV) is defined as 100 times the absorbance of a solution resulting from the reaction of 1 g of fat or oil in 100 ml of a mixture of solvent and p-anisidine, measured at 350 nm in a 10 mm cell under the conditions of the test (Rossell, 1989). Holm introduced the term total oxidation value, or Totox Value: Totox Value = 2PV + AnV. Using this combination of tests, slightly higher correlations with flavor were obtained (Jackson, 1981).

Headspace Volatile Compounds. Volatile compounds, such as alcohols, aldehydes, ketones, alkanes, esters, and short chain acids, are formed as secondary metabolites from the hydroperoxides of unsaturated fatty acids (Pawliszyn, 2001). They are typically measured by gas chromatography using direct injection, static or dynamic headspace, or solid-phase

microextraction (MeClements & Decker, 2008). Depending on the substrate compositions and oxidation conditions, different products are selected as indicators. For example, 2,4-decadienal and hexanal are derived from the oxidation of omega-6 fatty acids and, thus, are commonly used as makers of the oxidation of linoleic acid. Propanal and nonanal are usually measured to predict the oxidation of linolenic acids and oleic acid, respectively. Solid phase microextraction (SPME) is a solvent-free sample preconcentration technique developed by Arthur and Pawliszyn. The most common SPME utilizes a small fused silica fiber coated with a polymeric phase (Pawliszyn, 2001). The analytes are absorbed by the fiber at equilibrium, followed by gas chromatography (GC) analysis. The assay has extended its application to numerous fields including lipid oxidation analysis because it has strong correlation with sensory analysis (Vichi, 2002). The disadvantage lies in the expense of instrumentation and the difficulty in analyzing large amounts of samples at a same time.

Headspace Oxygen Content. Oxygen uptake or residual oxygen has long been used to estimate lipid oxidation degree in closed systems that do not allow any gas transfer. Like all the other oxidative stability evaluation tests, oxygen content assay is rarely used alone. It has been used together with peroxide value assay, fatty acids analysis, and sensory tests (Chen, Ratnayake, & Cunnane, 1994; Fakourelis, 1987; Frankel, 1993). The most commonly used method in the past is the one developed by Chen and Nawar (Chen & Nawar, 1991). In brief, the headspace oxygen was sampled only once per package with a gas-tight syringe and analyzed in a gas chromatography. There are some new methods that do not require punching the packaging. For example, with the OxySense 4000B system, oxygen concentration can be measured through reading the fluorescence of the oxygen-sensitive dot placed inside the packaging. It is a fast, accurate, reproducible and non-destructive measuring technique.

Measuring Antioxidant Effectiveness in Muscle Foods

Complex food matrices are also often used as models to study antioxidant activity with the goal of creating systems more close to actual foods. Muscle foods, which are composed of various natural antioxidants and prooxidant factors, are a good example of complex food systems. Protein in the muscle foods can significantly affect the antioxidant behavior through hydrogen bonding and ionic interactions with phenols. Studies carried out in the presence and absence of protein could have huge differences. However, muscle food substrate has its own disadvantages. An assessment with a muscle food model is often time-consuming because the endogenous antioxidants provide a substantial lag phase. Microbial growth may occur prior to oxidative rancidity. Besides, determining the antioxidant activity is difficult because of the complexity of the muscle food system (Decker, 2005). Thus, it is tempting to assess "antioxidant capacity" using non-lipid free radical scavenging assays first and then extrapolate the findings to activity in muscle foods.

Decker and his coworkers listed some factors that should be considered before measuring antioxidant effectiveness in muscle foods. These include animal-to animal variations, fat content and composition of muscle samples, partitioning of antioxidants in the samples and representative sampling (Decker, 2005). A variety of methods could be employed for monitoring lipid oxidation in muscle foods. Methods measuring primary changes such as oxygen uptake, loss of polyunsaturated fatty acids and formation of hydroperoxides are more suited to measuring low levels of oxidation in uncooked products stored at low temperatures (Coxon, 1987). Methods involving measurement of secondary products, such as carbonyls, hydrocarbons and fluorescent products have also been used (Melton, 1983). Among all these methods, the thiobarbituric acid

assay (TBA) appears to be the most popular one besides the headspace volatiles analysis discussed above (Melton, 1983).

Thiobarbituric Acid Assay. TBA assay is based on the reaction between TBA and carbonyls to form red, fluorescent adducts under certain conditions (Yu, 1967). Malondialdehyde (MDA), which is a dialdehyde produced by a two-step oxidative degradation of fatty acids with three or more double bonds, is the primary lipid oxidation product detected by TBA. However, TBA can also react with nonlipid carbonyls such as ascorbic acid, sugars, and nonenzymatic browning products and form compounds that also absorb at the testing wavelength. Besides, MDA yield varies with fatty acid composition. Thus, it is inappropriate to compare oxidative changes in products with different fatty acid compositions or with high concentration of interfering compounds. TBA assay has very good correlation with sensory analysis to detect rancidity in animal foods (Fernandez, 1995), such as pork meat (Turner, 1954) and chicken meat (Salih, 1987).

AMPELOPSIS GROSSEDENTATA AND ITS MAJOR COMPONENT DIHYDROMYRICETIN (DHM)

Ampelopsis grossedentata.

Ampelopsis grossedentata., which belongs to the family *Vitaceae* and the genus *Ampelopsis Michx*., is a wild plant growing in lots of mountain areas of southern China. Its dried leaves and stems, called Mao Yan Mei or Teng Cha, have been consumed as health tea and herbal medicine for hundreds of years. The earliest record of Teng Cha dates back to the year of 937, which is more than one thousand years ago. In 1279, Hou Qin waged a war against the

tyranny of the Min government in a remote area where the Tujia minority group resides. The emperor worried about losing his control over the country and called for 250,000 soldiers to fight against the insurgent. The battle continued for 7 years. During that period, the insurgent suffered injuries and some diseases but many were cured by the *Ampelopsis* grossedentata provided by an old Tujia man. From then on, Tujia people call it magic tea and consume it on a daily basis to prevent diseases. It is said that this minority has the highest rate of old people over one hundred years old, who have never developed hypertension, hyperlipemia, obesity or cancer.

Zhang and others studied the composition of Ampelopsis grossedentata and found it is rich in crude protein, soluble protein, amino acids and phenolics (Zhang, Yang, & Xiong, 2001). The Institute of Nutrition and Food Safety of the Chinese Center for Disease Control and Prevention also worked on the nutritional profile of Teng Cha. The profile showed that in 100 gram (g) Tengcha there was 9.4 g moisture, 20.4 g protein, 0.8 g lipid, 5.23 mg carotene, 6.32 mg vitamin E, 10.1 mg iron, 1.58 mg zinc, 411.3 mg calcium, 0.67 copper, 121.6 mg magnesium, 68.9 mg manganese, 468.9 mg potassium, 344.1 mg phosphorous, 14.6 g dietary fiber, and 10.62 ug selenium (Chen, Dong, Chen, Xiao, Li, & Luo, 2007). Toxicological assessment has been done on Teng Cha by animal experiments (Zhong, Zhou, & Chen, 2003; Zhou, Hu, & Jian, 2001; Zhou, Hu, & Zang, 2001).The result showed that Teng Cha was safe for drinking, and that it could enhance immunologic function in mice. Other health beneficial effects, such as lowering blood pressure, lowing blood sugar, and protecting liver, were also well studied. Findings were well summarized in some reviews (Chen, Dong, Chen, Xiao, Li, & Luo, 2007; Yi, 2004; Zhang & Chen, 2006; Zheng & Liu, 2006).

Teng Cha, like other common teas, is commercially available in China now. Different processing methods were studied by Yi and the best process with key parameters is published (Yi, 2003). The most common Teng Cha products are those packed in single serving tea bags. There are also some mixed herbal tea products containing Teng Cha (Yang, Zhang, & Zhang, 2005). Some innovative products, such as Teng Cha beverages (Yao, Cao, & Li, 2004), dietary supplements and fruit flavored jelly (Zhang, 2002; Zhang, Zhang, & Zhang, 2004), have also been developed.

Dihydromyricetin

Dihydromyricetin (DHM) is the major component of *Ampelopsis grossedentata* leaf extract. HPLC analysis showed that the content of DHM in leaves was higher than 75% (Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009). Some other works also found a high content of DHM on the basis of dry leave weight (Tian, Zhang, Yang, Yang, & Gong, 2002). As an effort to identify and isolate specific compounds that are more potent than the crude extract, many tests done on Teng Cha were also tried on DHM.

Lin and others reported that DHM was still stable after heating for 30 min at 100 C (Lin, Gao, Guo, & Ning, 2004). He and coworkers reported that DHM was stable in weak acid solution and was unstable under the conditions of basic solution (He, Pei, & Li, 2007). Based on this information, a great variety of methods and conditions have been used for the extraction of DHM or total flavonoids from Teng Cha. Chen and others built a model to study the effects of temperature, time, ratio of Teng Cha to water on the extraction of DHM and total flavonoids

(Chen, Li, & Meng, 2009). The optimum condition was found to be: solvent-74% ethanol, temperature-65 C, heating time-94min and ration of Teng Cha to water-1:35 (w/v).

The structure of DHM (Figure 2.9) was identified using UV, IR (ionizing radiation), 13C and 1H NMR (nuclear magnetic resonance), and ESI-MS (electrospray ionization) (Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009). It is very close to that of quercitin, which has gained attention as a very potent antioxidant (Reische, Lillard, & Eitenmiller, 2008). DHM was also investigated in the area of polymer science (Zheng, Xu, Zhu, Chen, Liu, Chen, Xu & Chen, 2010). Wu and others tested the radical scavenging activity of DHM-Zn complex in depth with DPPH radical, hydroxyl radical, superoxide radical and alkane radical (Wu, Zheng, & Chen, 2011). The results suggested that the complex would be an effective free radical scavenger. Gao and coworkers studied the antioxidant capacity of DHM and AG crude extract by DPPH assay and reducing power test (Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009). DHM showed higher antioxidant activity than TBHQ in both tests. Linoleic acid system was employed as a model to evaluate the antioxidant activity of DHM and the crude extract. However, only the primary oxidation products were measured and only one testing method was used, both of which made the conclusion of this test not reliable. Another effort to measure the antioxidant activity of DHM in linoleic acid system was made by Zhang (Zhang, Ning, Yang, & Wu, 2003). In this study, $FeSO_4$-edetic acid was used as the catalyst, which is not recommended by researchers today. Lard was used as a lipid substrate to analyze the antioxidant activity of DHM and DHM-lecithin complex. However, in Wang's work, only peroxide value was measured for the evaluation of the antioxidant activity (Wang, Tian, Xiong, & Zhang, 2004). In Liu's work, only rancimat was employed (Liu, 2009).

TABLES

Table 2.1. Hydroperoxides of fatty acids formed by triplet and singlet oxygen oxidation.

		Oleate	Linoleate	Linolenate
$^3O_2$		8-OOH 9-OOH 10-OOH 11-OOH		
	Conjugated hydroperoxides		9-OOH 13-OOH	9-OOH 12-OOH 13-OOH 16-OOH
$^1O_2$		9-OOH 10-OOH		
	Conjugated hydroperoxides		9-OOH 13-OOH	9-OOH 12-OOH 13-OOH 16-OOH
	Nonconjugated		10-OOH 12-OOH	10-OOH 15-OOH

FIGURES

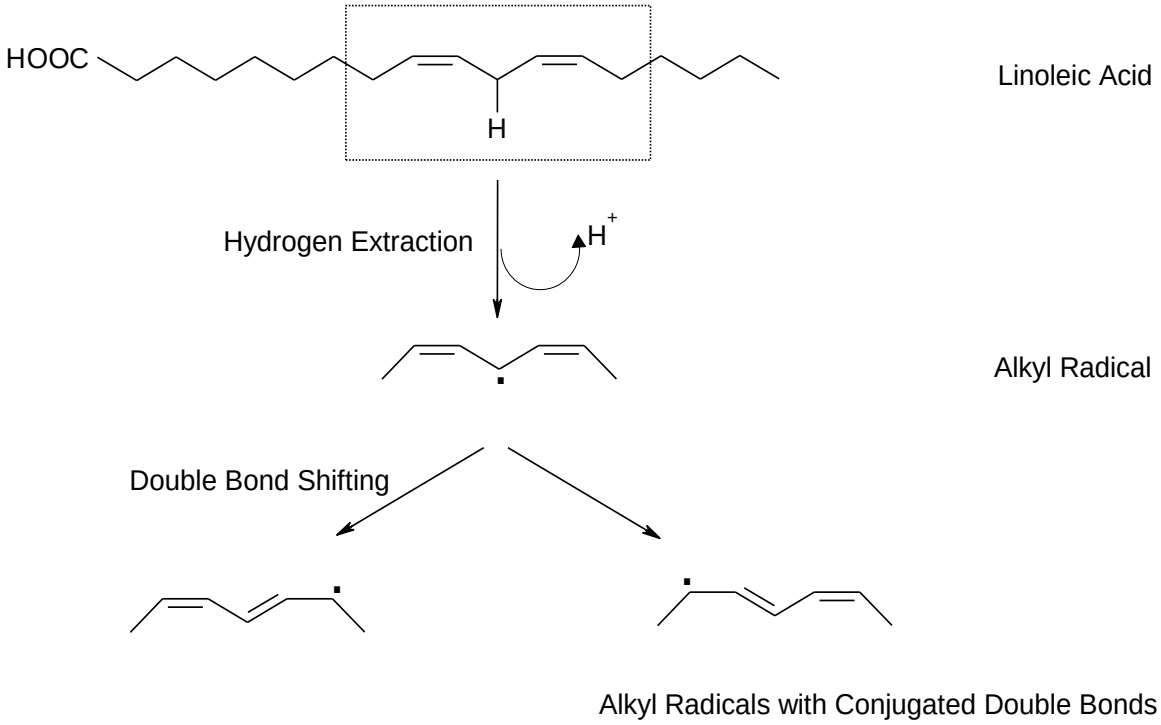


Figure 2.1. The initiation step of lipid oxidation for linoleic acid. (As modified from McClements and Decker, 2008)

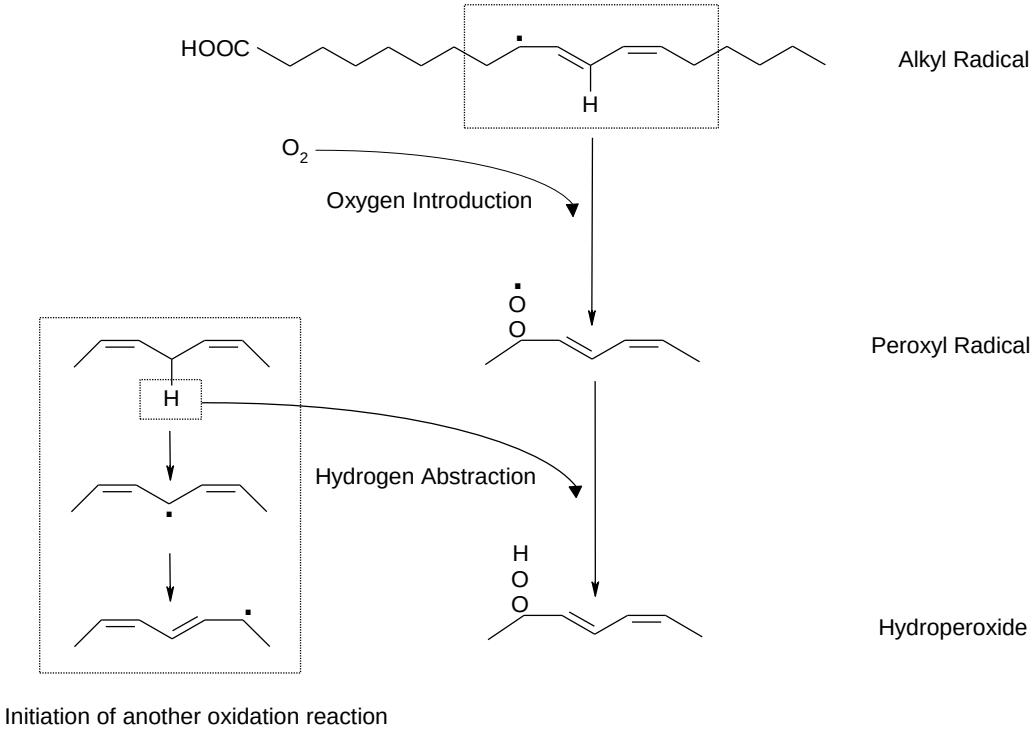


Figure 2.2. The propagation step of lipid oxidation for linoleic acid. (As modified from McClements and Decker, 2008)

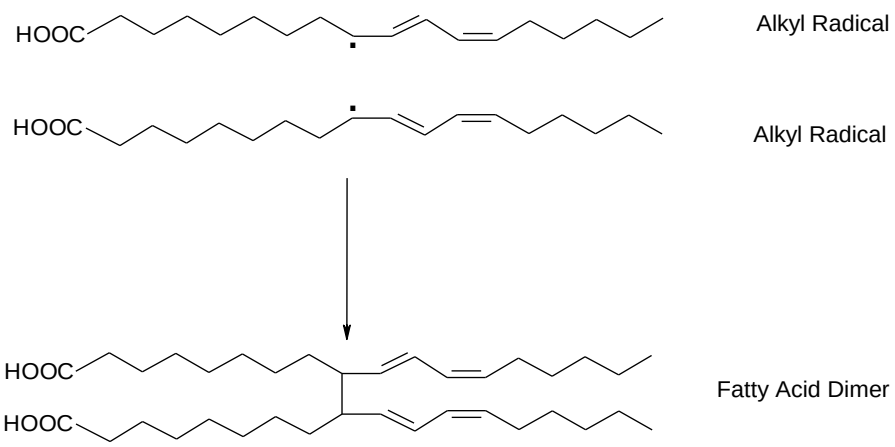


Figure 2.3. The termination step of lipid oxidation for linoleic acid. (As modified from McClements and Decker, 2008)

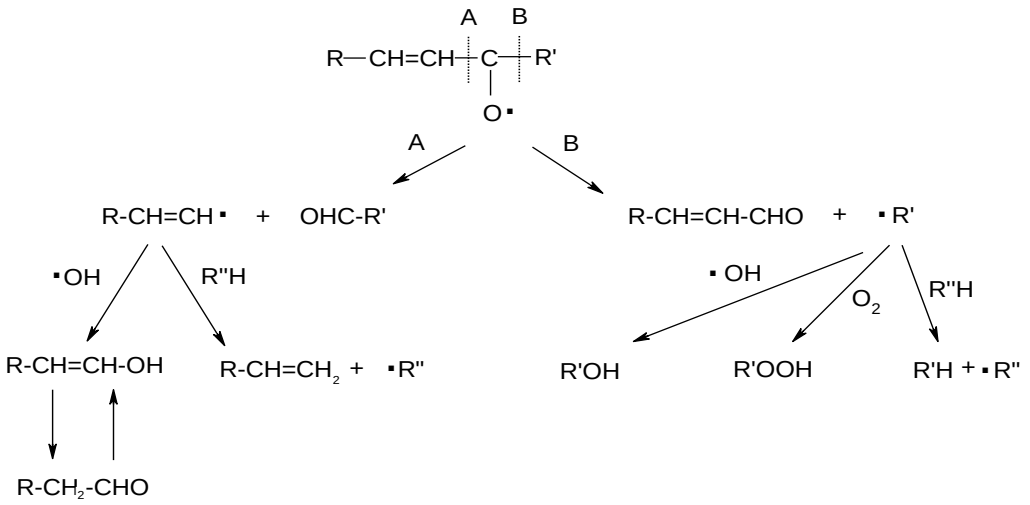
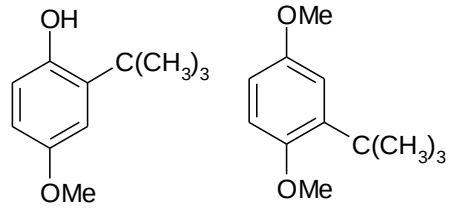
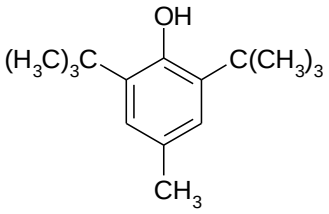


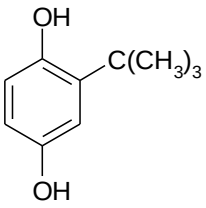
Figure 2.4. β-Scission of alkoxyl radicals and formation of the secondary oxidation products. (As modified from Frankel, 1998)



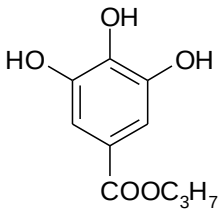
Butylated hydroxyanisole (BHA)



Butylated hydroxytoluene (BHT)

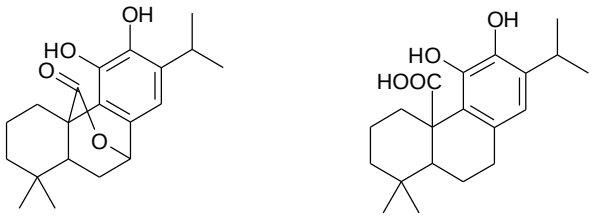


tert-butyl hydroquinone (TBHQ)



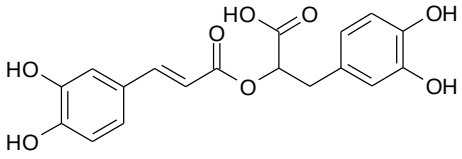
Propyl gallate (PG)

Figure 2.5. Synthetic antioxidants in use.



Carnosol

Carnosic acid



Rosmarinic acid

Figure 2.6. Active phenolics in rosemary.

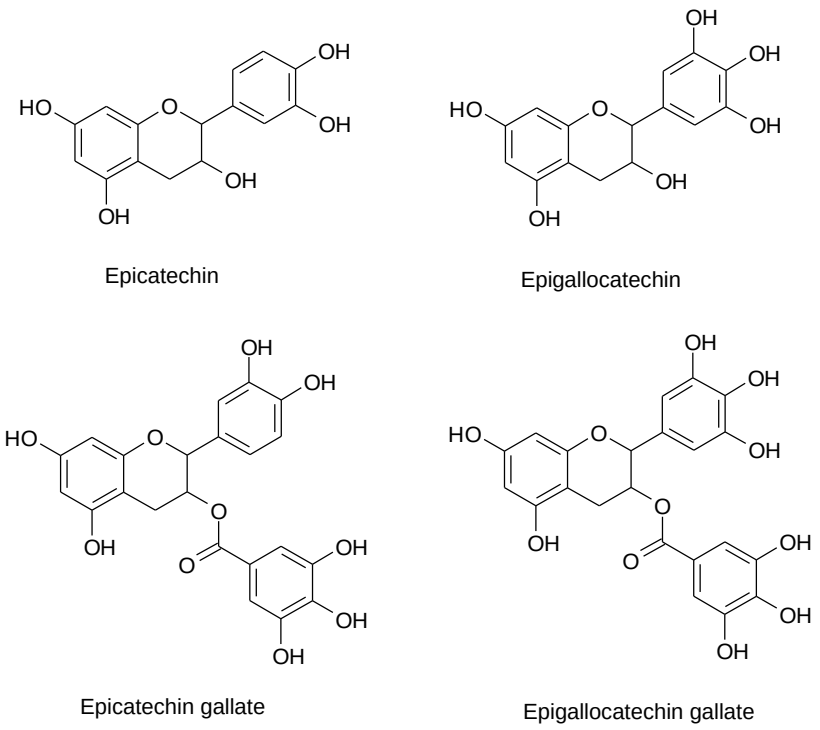
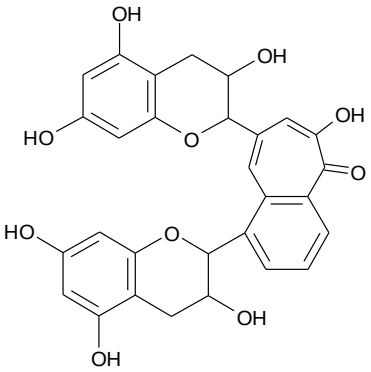
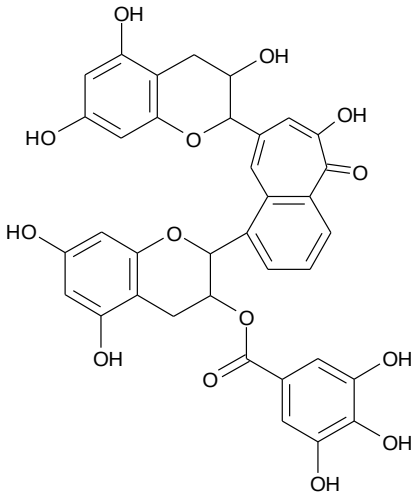


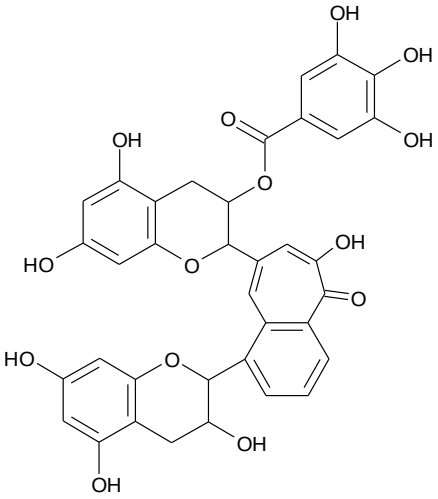
Figure 2.7. Catechins in teas.



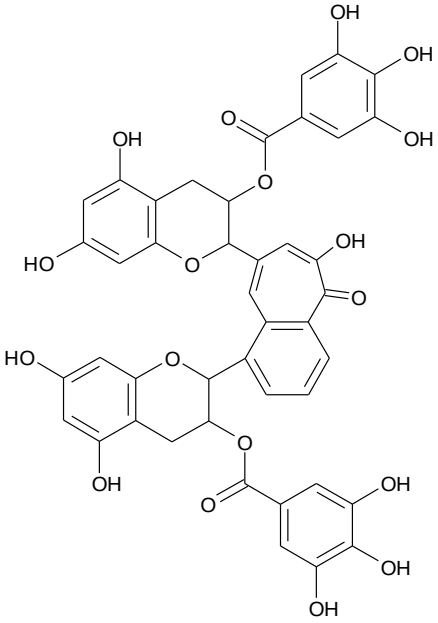
Theaflavin



Theaflavin monogallate

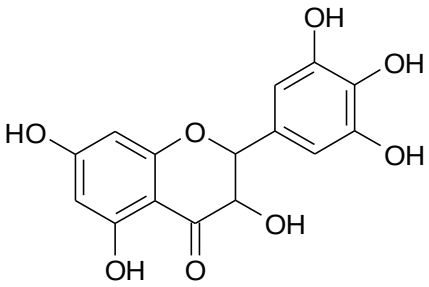


Theaflavin monogallate

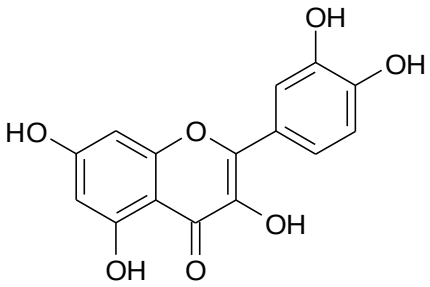


Theaflavin digallate

Figure 2.8. Theaflavins in teas.



Dihydromyricetin



Quercetin

Figure 2.9. Structure of dihydromyricetin and quercetin.

REFERENCES

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst, 127*(1), 183-198.

Becker, E. M., Nissen, L. R., & Skibsted, L. H. (2004). Antioxidant evaluation protocols: Food quality or health effects. *European Food Research and Technology, 219*(6), 561-571.

Berdahl, D. R., Nahas, R. I., & Barren, J. P. (2010). Synthetic and natural antioxidant additives in food stabilization: Current applications and future research. In E. A. Decker, B. Chen & A. Panya (Eds.), *Oxidation in Foods and Beverages and Antioxidant Applications*, vol. 1 (pp. 272-320). Cambridge, UK: Woodhead Publishing.

Berger, K. G. (1989). The use of antioxidants. In J. C. Allen & R. J. Hamilton (Eds.), *Rancidity in Foods* 2nd ed., (pp. 83-104). Essex, England: Elsevier Science Publishing.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology, 28*(1), 25-30.

Cao, G., Alessio, H. M., & Cutler, R. G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine, 14*(3), 303-311.

Chen, X., Dong, B., Chen, J., Xiao, C., Li, B., & Luo, D. (2007). Study on the medicinal value of Teng Cha. *Hunan Agricultural Sciences*(6), 180-181.

Chen, Z.-Y., Wang, L.-Y., Chan, P., Zhang, Z., Chung, H., & Liang, C. (1998). Antioxidative activity of green tea catechin extract compared with that of rosemary extract. *Journal of the American Oil Chemists' Society, 75*(9), 1141-1145.

Chen, Z., Ratnayake, W., & Cunnane, S. (1994). Oxidative stability of flaxseed lipids during baking. *Journal of the American Oil Chemists' Society, 71*(6), 629-632.

Dimitrios, B. (2006). Sources of natural phenolic antioxidants. *Trends in Food Science and Technology, 17*(9), 505-512.

Decker, E. A., Chen, B., & Panya, A. (2010). Understanding antioxidant mechanisms in preventing oxidation in foods. In E. A. Decker, R. J. Elias & D. J. McClements (Eds.), *Oxidation in Foods and Beverages and Antioxidant Applications*, vol. 1 (pp. 225-248). Cambridge, UK: Woodhead Publishing.

Decker, E. A., Warner, K., Richards, M. P., & Shahidi, F. (2005). Measuring antioxidant effectiveness in food. *Journal of Agricultural and Food Chemistry, 53*(10), 4303-4310.

Eder, K. (2010). Health aspects of oxidized dietary fats. In E. A. Decker, R. J. Elias & D. J. McClements (Eds.), *Oxidation in Foods and Beverages and Antioxidant Applications*, vol. 1 (pp. 143-180). Cambridge, UK: Woodhead Publishing.

Fakourelis, N., Lee, E. C., & Min, D. B. (1987). Effects of chlorophyll and β-carotene on the oxidation stability of olive oil. *Journal of Food Science, 52*(1), 234-235.

Fernández, J., Pérez-Álvarez, J. A., & Fernández-López, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry, 59*(3), 345-353.

Frankel, E. N. (1993). In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends in Food Science and Technology, 4*(7), 220-225.

Frankel, E. N., & Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80*(13), 1925-1941.

Gao, J., Liu, B., Ning, Z., Zhao, R., Zhang, A., & Wu, Q. (2009). Characterization and antioxidant activity of flavonoid-rich extracts from leaves of Amelopsis grossedentata. *Journal of Food Biochemistry, 33*(6), 808-820.

Ghiselli, A., Serafini, M., Maiani, G., Azzini, E., & Ferro-Luzzi, A. (1995). A fluorescence-based method for measuring total plasma antioxidant capability. *Free Radical Biology and Medicine, 18*(1), 29-36.

Glazer, A. (1988). Fluorescence-based assay for reactive oxygen species: a protective role for creatinine. *The FASEB Journal, 2*(9), 2487-2491.

Gray, J. (1978). Measurement of lipid oxidation: A review. *Journal of the American Oil Chemists' Society, 55*(6), 539-546.

He, G., Pei, G., & Li, B. (2007). Studies on stability of dihydromyricetin. *Chinese New Drugs Journal, 16*(22), 1888-1890.

Hernández-Hernández, E., Ponce-Alquicira, E., Jaramillo-Flores, M. E., & Guerrero Legarreta, I. (2009). Antioxidant effect rosemary (*Rosmarinus officinalis L.*) and oregano (*Origanum vulgare L.*) extracts on TBARS and colour of model raw pork batters. *Meat Science, 81*(2), 410-417.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841-1856.

Jackson, H. (1981). Techniques for flavor and odor evaluation of soy oil. *Journal of the American Oil Chemists' Society, 58*(3), 227-231.

Jacobsen, C. (2010). Understanding and reducing oxidative flavour deterioration in foods. In E. A. Decker, R. J. Elias & D. J. McClements (Eds.), *Oxidation in Foods and Beverages and Antioxidant Applications*, vol. 1 (pp. 122-142). Cambridge, UK: Woodhead Publishing.

Jaswir, I., Kitts, D. D., Man, Y. B. C. H. E., & Hassan, T. H. (2004). Synergistic effect of rosemary, sage and citric acid on fatty acid retention of heated flaxseed oil. *Journal of Oleo Science, 53*(12), 581-591.

Karadag, A., Ozcelik, B., & Saner, S. (2009). Review of methods to determine antioxidant capacities. *Food Analytical Methods, 2*(1), 41-60.

Lin, S., Gao, J., Guo, Q., & Ning, Z. (2004). Stability of dihydromyricetin and factors affecting the stability. *Journal of Wuxi University of Light Industry, 23*(2), 17-20.

Liu, B., Du, J., Zeng, J., Chen, C., & Niu, S. (2009). Characterization and antioxidant activity of dihydromyricetin–lecithin complex. *European Food Research and Technology*, *230*(2), 325-331.

Madhavi, D. L., Singhal, R. S., & Kulkarni, P. R. (1996). Technological aspects of food antioxidants. In D. L. Madhavi, S. S. Deshpande & D. K. Salunkhe (Eds.), *Food Antioxidants*, (pp. 159-266). New York: Marcel Dekker.

McClements, D. J., & Decker, E. A. (2008). Lipids. In S. Damodaran, K. L. Parkin & O. R. Fennema (Eds.), *Fennema's Food Chemistry* 4th ed., (pp. 155-216). Boca Raton, FL: CRC Press.

Mikova, K. (2001). The regulation of antioxidants in food. In J. Pokorny, N. Yanishlieva & M. Gordon (Eds.), *Antioxidants in Food*, (pp. 267-284). Cambridge, England: CRC Press.

Nakamura, Y., Ohto, Y., Murakami, A., & Ohigashi, H. (1998). Superoxide scavenging activity of rosmarinic acid from *Perilla frutescens Britton Var. acuta f. viridis*. *Journal of Agricultural and Food Chemistry, 46*(11), 4545-4550.

Nawar, W. W. (1985). Chemistry of thermal oxidation of lipids. In D. B. Min & T. H. Smouse (Eds.), *Flavor Chemistry of Fats and Oils*. Urbana, IL: AOCS Press.

Neff, W., & Frankel, E. (1980). Quantitative analyses of hydroxystearate isomers from hydroperoxides by high pressure liquid chromatography of autoxidized and photosensitized-oxidized fatty esters. *Lipids, 15*(8), 587-590.

Nenadis, N., & Tsimidou, M. Z. (2010). Assessing the activity of natural food antioxidants. In E. A. Decker, R. J. Elias & D. J. McClements (Eds.), *Oxidation in Foods and Beverages and Antioxidant Applications*, vol. 1 (pp. 332-367). Cambridge, UK: Woodhead Publishing.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*(10), 4619-4626.

Pawliszyn. (2001). Solid phase microextraction. In R. L. Rouseff & K. R. Cadwallader (Eds.), *Headspace Analysis of Foods and Flavors*, (pp. 73-88). New York: Plenum Publishers.

Pokorný, J. (2007). Are natural antioxidants better – and safer – than synthetic antioxidants? *European Journal of Lipid Science and Technology, 109*(6), 629-642.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290-4302.

Reische, D. W., Lillard, D. A., & Eitenmiller, R. R. (2008). Antioxidants. In C. C. Akoh & D. B. Min (Eds.), *Food Lipids* 3rd ed., (pp. 409-434). Boca Raton, FL: CRC Press.

Rossell, J. B. (1989). Measurement of rancidity. In J. C. Allen & R. J. Hamilton (Eds.), *Rancidity in Foods* 2nd ed., (pp. 23-52). Essex, England: Elsevier Science Publishing.

Salih, A. M., Smith, D. M., Price, J. F., & Dawson, L. E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Science, 66*(9), 1483-1488.

Sanders, T. A. B. (1989). Nutritional aspects of rancidity. In J. C. Allen & R. J. Hamilton (Eds.), *Rancidity in Foods* 2nd ed., (pp. 125-140). New York: Elsevier Science Publishing.

Tian, S., Zhang, Y., Yang, Y., Yang, W., & Gong, Y. (2002). Dihydromyricetin in Ampelopsis grossedentataby reversed-phase high performance liquid chromatograpohy. *Journal of Hunan Agricultural University, 28*(1), 32-34.

Tompkins, C., & Perkins, E. (1999). The evaluation of frying oils with the Anisidine value. *Journal of the American Oil Chemists' Society, 76*(8), 945-947.

Turner, E. W. (1954). Use of the 2-thiobarbituric acid reagent to measure rancidity in frozen pork. *Food Technology (Chicago), 8*, 326.

Vekiari, S., Oreopoulou, V., Tzia, C., & Thomopoulos, C. (1993). Oregano flavonoids as lipid antioxidants. *Journal of the American Oil Chemists' Society, 70*(5), 483-487.

Vichi, S., Castellote, A. I., Pizzale, L., Conte, L. S., Buxaderas, S., & López-Tamames, E. (2003). Analysis of virgin olive oil volatile compounds by headspace solid-phase microextraction coupled to gas chromatography with mass spectrometric and flame ionization detection. *Journal of Chromatography A, 983*(1-2), 19-33.

Wang, W., Tian, Z., Xiong, D., & Zhang, F. (2004). The antioxidation effects of the extracts of *Ampelopsis grossedentata*. *Chinese Wild Plant Resources, 23*(4), 46-47, 53.

Wong, D. W. S. (1989). *Mechanism and Theory in Food Chemistry*. New York: Van Nostrand Reinhold.

Wu, C., Zheng, X., & Chen, L. (2011). Study on antioxidant activity of Dihydromyricetin-Zinc(II) complex. *Advanced Materials Research, 183-185*, 863-867.

Yang, Y., Zhang, R., & Zhang, Y. (2005). Teng Cha products development and their manufacturing process. *Guangdong Agricultural Science*(2), 70-80.

Yanishlieva, N. V., & Heinonen, I. M. (2001). Sources of natural antioxidants: vegetables, fruits, herbs, spices and teas. In J. Pokorny, N. Yanishlieva & M. Gordon (Eds.), *Antioxidants in Food,* (pp. 210-263). Cambridge, England: CRC Press.

Yao, M., Cao, Y., & Li, G. (2004). Manufacturing process of sugar free Teng Cha products. *Food and Fermentation Industries, 30*(11), 110-113.

Yi, C. (2003). Preliminary study on Teng Cha manufacturing process. *Forest By-Product and Speciality in China*(2), 53-54.

Yi, C. (2004). Research development of *Ampelopsis grossedentata*. *Nonwood Forest Research, 22*(3), 51-56.

Yu, T., & Sinnhuber, R. (1967). An improved 2-thiobarbituric acid (TBA) procedure for the measurement of autoxidation in fish oils. *Journal of the American Oil Chemists' Society, 44*(4), 256-258.

Zhang, Q., & Chen, L. (2006). Research on bioactivity of flavons in *Ampelopsis grossedentata*: a review. *Central South Pharmacy, 4*(4), 302-304.

Zhang, Y. (2002). Preparation of healthy *Ampelopsis grossedentata* jelly. *Food Science and Technology* (7), 22-23.

Zhang, Y., H., Z., & X., Z. (2004). Development of healthy tablet of *Ampelopsis grossedentata*. *Chinese Wild Plant Resources, 23*(5), 33-34.

Zhang, Y. S., Ning, Z. X., Yang, S. Z., & Wu, H. (2003). Antioxidation properties and mechanism of action of dihydromyricetin from *Ampelopsis grossedentata*. *Yao Xue Xue Bao, 38*(4), 241-244.

Zhang, Y., Yang, W., & Xiong, H. (2001). Basic constituent of *Ampelopsis grossedentata*. *Natural Product Research and Development, 13*(5), 46-48.

Zheng, D., & Liu, G. (2006). Research and development of Chinese Teng Cha resources. *Agriculture Network Information*(6), 136-142.

Zheng, Q., Xu, L., Zhu, L., Chen, J., Liu, F., Chen, D., Xu, K., & Chen, M. (2010). Preliminary investigations of antioxidation of dihydromyricetin in polymers. *Bulletin of Materials Science, 33*(3), 273-275.

Zhong, Z., Zhou, G., & Chen, X. (2003). The rat chronic toxicity test of total flavone of *Ampelopsis grossedentata* from Guangxi. *Lishzhen Medicine and Materia Medica Research, 14*(4), 193-195.

Zhou, Y., Hu, Y., & Jian, B. (2001). Toxicological assessment of *Ampelopsis grossedentata* and its immune regulation study. *Practical Preventive Medicine, 8*(6), 412-414

CHAPTER 3: ANTIOXIDANT ACTIVITY OF *AMPELOPSIS GROSSEDENTATA* CRUDE EXTRACT AND ITS MAJOR COMPONENT DIHYDROMYRICETIN

ABSTRACT

Antioxidant activity of Teng Cha extract and its major component dihydromyricetin has been reported in a few works, but none of these works provided clear evidence for their antioxidant effects. The goal of this study is to verify the antioxidant activity of Teng Cha extract and its major component dihydromyricetin. Antioxidant activity of the crude extract and dihydromyricetin in soybean oil were evaluated based on the peroxide value, anisidine value, Totox value, headspace volatiles and headspace oxygen. Cooked beef was used as another substrate to test their antioxidant effectiveness. It was found that dihydromyricetin (DHM) was more potent than BHA in preventing soybean oil oxidation. The crude extract was not as effective as BHA and DHM, mainly because it contained transition metals, which would lead to the decomposition of hydroperoxides. Synergistic effects of BHA and DHM were not observed. In cooked beef, DHM and the crude extract showed lower antioxidant activity than BHA, possibly due to their low solubility. Future study is needed to demonstrate the overall antioxidant activity of Teng Cha extract and DHM as potential natural food antioxidants.

KEYWORDS

Antioxidant, *Ampelopsis grossedentata*, Teng Cha, dihydromyricetin, soybean oil oxidation, DPPH, peroxide value, anisidine value, Totox value, headspace volatiles, headspace oxygen content, TBARS

INTRODUCTION

Lipid oxidation is one of the most important factors limiting the shelf life of many food products. The traditional way to extend the shelf life in the modern food industry is to add antioxidants into food matrices. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroxyquinone (TBHQ), and propyl gallate (PG) are very commonly employed. However, in recent decades, the reported safety issues of synthetic additives have raised consumer concerns about food ingredients. Products with fewer total additives and utilizing natural additives are preferred. Currently, natural phenolic antioxidants in use include rosemary and sage extract. Their antioxidant effect has been demonstrated in many food substrates (Madhavi, Singhal, & Kulkarni, 1996), but they are expensive and still not as potent as some synthetic compounds. Thus researchers are still looking for new and effective natural antioxidants.

Ampelopsis grossedentata, which belongs to the family *Vitaceae* and the genus *Ampelopsis Michx*., is a wild plant growing in many mountain areas of southern China. The dried leaves and stems, called Mao Yan Mei or Teng Cha, have been consumed as health tea and herbal medicine for hundreds of years. Significant research work has been conducted on the nutritional profile (Chen, Dong, Chen, Xiao, Li, & Luo, 2007; Zhang, Yang, & Xiong, 2001), health benefits and toxicity (Zhong, Zhou, & Chen, 2003; Zhou, 2001) of Teng Cha. Dihydromyricetin (DHM) is the most abundant bioactive component in Teng Cha extract. Its structure is very close to that of quercitin, and DHM is reportedly an effective antioxidant (Reische, Lillard, & Eitenmiller, 2008).

Gao and others compared the DPPH radical scavenging activity of Teng Cha extract and DHM to tertiary butylhydroquinone (TBHQ) and reported significantly higher antioxidant capacity for DHM than for TBHQ (Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009). The antioxidant activity of Teng Cha extract in lard was reported to have significantly lower peroxide value than lard treated with TBHQ and control lard samples (Wang, Tian, Xiong, & Zhang, 2004).

As an effort to verify the antioxidant activity of Teng Cha extract and its active antioxidant component, DHM, this study was conducted to evaluate the antioxidant capacity of Teng Cha extract and DHM, in comparison to BHT, in solvent systems and to determine the effectiveness in two different products (soybean oil and ground beef). Specific objectives include: 1) analyze the DHM content in Teng Cha; 2) verify the DPPH radical scavenging activity of Teng Cha extract and DHM in solvent systems, 3) examine antioxidant effects of Teng Cha extract and DHM in soybean oil over time as compared to BHA; and 4) examine antioxidant effectiveness of Teng Cha extract and DHM in cooked beef over time compared to BHA.

MATERIALS AND METHODS

Materials

Teng Cha was purchased from a tea store in Zhangjiajie, China. DHM was supplied by ChromaDex (Irvine, CA). BHA (96% purity) was purchased from Acros Organics (Pittsburgh, PA), as well as Trolox standard and potassium iodide of 99% purity. All Natural Crisco Pure Vegetable Oil (soybean oil) was purchased from a local grocery store. Raw ground beef was donated by Virginia Tech Meat Lab (Blacksburg, VA). Starch indicator (1%) and ethanol of USP grade and 95.5% purity were purchased from Ricca Chemical Company (Arlington, TX). Acetic

acid, chloroform, acetonitrile, methanol and iso-octane of HPLC grade; sodium thiosulfate, *p*-anisidine, propyl gallate (PG), ethylenediaminetetraacetic acid (EDTA), tetramethoxypropane (TMP), thiobarbituric acid (TBA) and sodium dodecyl sulfate (SDS) were purchased from Fisher Scientific. ASTM D2887 Quantitative Calibration Solutions were obtained from Supelco (Bellefonte, PA). Gallic acid, 2 N Folin-Ciocalteau's phenol reagent and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical were supplied by Sigma (St. Louis, MO).

Part I. Extraction and Analysis

The experimental design of this part is demonstrated in Figure 3.1. Teng Cha was extracted in triplicate. DHM content of the three freeze-dried extracts was analyzed by using an HPLC and compared with DHM standards. Total phenolic content of the three extracts were measured and compared with the commercial DHM powder. DPPH radical scavenging activities of the crude extracts and DHM powders were measured to evaluate the antioxidant capacity.

Extraction and Lyopilization

Teng Cha (10 g) was extracted with 200 mL 74% (v/v) aqueous ethanol in a 250 mL screw cap Pyrex Erlenmeyer flask by shaking at 65 ℃ for 30 minutes in a Precision Scientific 360 Orbital Shaker Bath (Precision Scientific Inc., Chicago, IL). The extract was filtered through a stomacher bag into a 1000 mL round bottom flask. Ethanol in the extract was evaporated by a Buchi Rotavapor R-3000 (Buchi Laboratory Equipment, Flawil, Switzerland) at 60 ℃ until most ethanol was removed. The extract was transferred from the round bottom flask into a 70 mm × 50mm crystallizing dish. The round bottom flask was rinsed with 50 mL distilled water, which was then combined with the extract in the crystallizing dish. The extract in crystallizing dishes

was frozen and then freeze dried at -50 ℃ for 3 days in a Labconco Freezone18 Freeze Dryer (Labconco Co., Kansas City, MO). The freeze-dried extract was weighed on an analytical balance and stored at -20 ℃ for later use. Extracts were prepared in triplicate.

HPLC Analysis

An Agilent 1260 Infinity Series HPLC (Agilent Technologies, Richmond, VA) consisting of solvent pump, autosampler with refrigeration, thermostat column compartment, diode array and multiple wavelength detector, and an Agilent Porshell 120 EC-C18 column (5 cm x 0.46 cm, 2.7 um particle size) was employed to analyze the DHM content in the crude extract. The mobile phase with a flow rate of 0.5 mL/min consisted of 90% (v/v) distilled water (containing 0.1% acetic acid) and 10% acetonitrile (containing 0.1% acetic acid). The sample injection volume was 5.0 uL. UV absorbance was recorded at 290 nanometers and UV absorption spectra were obtained from 190 to 400 nanometers.

Two sets of standards that consisted of 0 mg/mL, 0.25 mg/mL, 0.50 mg/mL, 0.75 mg/mL, 1.00 mg/mL DHM in methanol were prepared for making standard curves. Teng Cha extract samples in methanol (1.00 mg/mL) were made in triplicates from the three free dried extracts. One milliliter from each standard and extract sample was pipetted into a 2 mL HPLC autosampler vial for DHM analysis as described above.

Total Phenolic Content

Total phenolics were determined by a Folin-Phenol method, as reported by O'Keefe and Wang (2005). Standard solutions with six concentrations (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 g/L) of gallic

acid were prepared immediately before use. Three extract samples and three DHM solutions with concentrations close to 0.5 mg/mL were prepared in 100 mL volumetric flasks. The weight of the extract powder and DHM was measured on an analytical balance and recorded to 0.0001g.

Aliquots (100 μL) of samples or standards were mixed with 900 uL distilled water in 10 mL test tubes followed by addition of 2.5 mL 0.2 N Folin-Ciocalteau reagent and mixing on a Fisher Vortex Genie2 mixer (Fisher Scientific, Pittsburgh, PA). Saturated sodium carbonate (2.0 mL) was added into each tube, which was vortexed again. After 2 hours reaction, absorbance was read at 765 nm in a Shimadzu UV-2550 UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of different concentrations of Teng Cha extract (Ext.), DHM and BHA were measured with a UV-VIS spectrophotometer following a previously reported method (Hogan, Zhang, Li, Zoecklein, & Zhou, 2009). Trolox standards (0, 10, 20, 40, 60, 80, 100 uM in 50% ethanol) were prepared immediately before use. Six concentrations (2, 4, 6, 8, 10, 12 ppm) of Ext., DHM and BHA were also made freshly in 50% ethanol. DPPH (2.5 mL of 0.2 mM in ethanol) was mixed with 2.5 mL of sample or Trolox standard in a 10 mL test tube. The absorbance was read at 515 nm after 30 min reaction at room temperature. DPPH scavenging activity was calculated as $[Abs_{control}-(Abs_{sample}-Abs_{blank})]/Abs_{control}*100\%$. It was plotted against the sample concentration to obtain IC_{50}, which is the concentration of sample to cause 50% inhibition.

Part II. Evaluating Antioxidant Activity of *Ampelopsis Grossedentata* Crude Extract and Dihydromyricetin in Soybean Oil

Preparation of Soybean Oil Samples

Soybean oil samples with different antioxidant treatments (BHA, crude extract, DHM, and DHM+BHA, control) were prepared (Table 3.1). Since there was no significant difference in DHM content and total phenolic content between the three extracts obtained in Part I, only one extract was picked randomly and used in the following experiments. The volume of each treatment was brought up to 200 mL in a volumetric flask with fresh soybean oil (Figure 3.2). Each flask was then put in a Fisher FS20 Ultrasonic Bath (Fisher Scientific, Pittsburgh, PA) for 5 minutes followed by stirring on a Corning Stirrer Plate (Corning INC., NY) for 10 minutes to assist the dissolving of the antioxidant compounds. For each treatment, thirty-six 10 mL glass centrifuge vials with 5 mL oil sample inside were prepared. A total of 180 vials (36 vials * 5 treatments = 180) were randomly distributed in a Precision Gravity Convection Incubator (Precision Scientific Inc., Chicago, IL) with the temperature set at 60 ℃. On Day 0, 3, 6, 9, 12, and 15, six vials from each treatment were picked randomly for headspace volatile analysis and peroxide value assay in triplicate.

Due to the limited volume of soybean oil in one bottle, another bottle of soybean oil with the same lot number was used for the other two oxidation tests. Oil samples with different antioxidant treatments were prepared in volumetric flasks as described above (Figure 3.3). For each treatment, eighteen 10 mL glass centrifuge vials with 5 mL oil sample inside were prepared. On day 0, 3, 6, 9, 12 and 15, three vials were picked randomly for anisidine value assay. For the

headspace oxygen content assay, another set of samples were prepared in headspace vials. Oil samples (5 mL each) were dispensed into 10 ml headspace vials and hermetically sealed with a Teflon-lined silicone crimp cap. For each of the 5 treatments, triplicates were made. These samples were also stored in the 60 ℃ incubator. On Day 0, 3, 6, 9, 12, 15, they were taken out for the oxygen content test and then put back without disruption.

Peroxide Value

Peroxide values of the oil samples with different antioxidant treatments were determined using the AOCS method. Around 5 g of oil was weighed into a 125 mL glass stopper Erlenmeyer flask. The weight was recorded to the nearest 0.01 g. Acetic acid – chloroform (3:2) (v/v) solution (30 mL) was added to dissolve the oil. Saturated potassium iodide solution (0.5 mL) was added using a 0.1ml Mohr pipette. The mixture was swirled gently for exactly one minute. After 30 ml of distilled water added, the flask was stoppered and shaken vigorously to liberate the iodine from the organic layer. Starch solution (1 mL) was added into the solution as an indicator. The mixture was titrated with 0.1 N sodium thiosulfate until the blue gray color disappeared. The volume of the titrant used was recorded to the nearest 0.01 mL.

Headspace Volatile Compounds

Headspace volatiles were analyzed with a solid phase microextraction-gas chromatograph-mass spectrometer (SPME-GC-MS) system. The system consists of a Hewlett Packard 5890A gas chromatograph (Agilent Technologies, Richmond, VA), a Hewlett Packard 5972 Mass Selective mass spectrometer (Agilent Technologies, Richmond, VA) and a CTC Analytics CombiPal autosampler (Leap Technologies, Inc., Carrboro, NC). A Supelco

divinylbenzene/carboxen/polydimethylsiloxane fiber (Bellefonte, PA) was employed to absorb volatiles in the headspace. After heating in the sample vial for 30 minutes at 40 ℃, the fiber was desorbed in the inlet of the gas chromatograph for 12 minutes. A HP-5MS column (30 m ×0.25 mm i.d. × 0.25 μm film thickness) was used. The inlet temperature was 250 °C and the detector temperature was 220 ℃. The oven was held at 45 ℃ for 1 minute, heated from 45 ℃ to 210 ℃ at 10 ℃ per minute and held at 210 C for 5 minutes. The total run was 22.5 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min (~25 cm/sec linear flow velocity). The mass spectrometer was operated in scan mode from 40 m/z to 550 m/z. ASTM D2887 calibration solution was also analyzed by this system to obtain the Kovats index of compounds, which then were be used to verify the compounds identified by the mass spectrometer.

Anisidine Value

p-Anisidine value of oil samples was determined with a method similar to the IUPAC standard method. An oil sample (2 mL) was weighed into a 50 mL plastic centrifuge tube on an analytical balance. The weight was recorded to the nearest 0.001 g. Iso-octane (23 mL) was pipetted into the centrifuge tube to dissolve and dilute the oil sample. The absorbance of the solution was measured at 350 nm with a spectrophotometer, using iso-octane as the blank. Each oil solution (5 mL) and 5 mL iso-octane were mixed with 1 mL of *p*-anisidine reagent (2.5 g/L *p*-anisidine in glacial acetic acid) in 15 ml plastic centrifuge tubes. After exactly 10 minutes, the absorbance of each reaction mixture was measured at 350 nm, with the mixture consisting of 5 mL iso-octane and 1 ml *p*-anisidine reagent as the blank in the reference cell. The anisidine value was calculated by the formula: $AnP = 25 * (1.2\ A_s - A_b)/m$, where A_s is the absorbance of the reaction mixture, A_b is the absorbance of the oil solution, m is the oil mass.

Headspace Oxygen Content

Headspace oxygen content was measure by an OxySense 4000B (OxySence, Inc., Dallas, TX). The instrument was calibrated using 0% and 21% oxygen standards following the instructions provided by the manufacture. A fluorescent O_2xyDot was glued to the interior surface of a headspace vial with a translucent adhesive. After the glue between the dot and the vial surface was cured, 5 mL oil sample was dispensed into the vial and hermetically sealed with a crimp cap. On day 0, 3, 6, 9, 12, 15, the vials of oil samples were taken out of the 60 C incubator for oxygen content measurement. After the vials were cooled to room temperature, each vial was placed in front of the reader pen with the aperture directly over the O_2xyDot. With the timer and the logging on, the fluorescence was measured 5 times for each vial and the data was recorded in an Excel worksheet automatically. The measurement was performed in a dark room to achieve the best signal-to-noise ratio.

Part III. Evaluating Antioxidant Activity of *Ampelopsis Grossedentata* Crude Extract and Dihydromyricetin in Cooked Ground Beef

Preparation and Oxidation of Cooked Beef

Four ground beef samples (3 antioxidant treated samples and one control) were prepared as listed below (Table 3.2). The ground beef used in this study was frozen during storage and thawed before use. For each treatment, 44 mg of extract, BHA or DHM was mixed with 20 ml 20% (v/v) aqueous ethanol on a stir plate before added into the 200 g ground beef to obtain samples with 200 ppm antioxidants. The ground beef samples with 20% ethanol solutions were then homogenized by using a KitchenAid Classic Mixer (KitchenAid Inc., Saint Louis, MO) for

3 min. Beef samples were weighed into 50 mL centrifuge tubes (45 g per tube, triplicate for each sample). Tubes were sealed and centrifuged for 1 minute at 1000 RPM in an IEC International Centrifuge (International Equipment Co., Needham, MA) to make beef samples into a sausage shape. Samples in the tubes were cooked in hot water bath (95 ℃) till the inner temperature reached 160 ℉ (71 ℃) then cooled to room temperature. The liquid was drained. The surface of the cooked beef was dried on clean paper towels. The sausage-like samples were then placed in trays, covered with PVC film and stored in a 4 ℃ refrigerator. On Day 1, Day 7 and Day 14, TBARS value was measured for each treatment in triplicate.

TBARS Test

TBARS test was conducted using the method developed by Spanier and Traylor (Spanier & Traylor, 1991) as modified by O'Keefe and Wang (O'Keefe & Wang, 2006). Around 5.0 g beef sample was weighed into a 150 mL beaker on a balance and recorded to the nearest 0.01 g. Beef samples were homogenized with 65 ml distilled water, 0.1 mL 10% SDS and 10.0 mL solution III (made with 0.05 g PG, 0.10g EDTA, 500 mL distilled water) using a Virtis Homogenizer (The Virtis Company, Inc., NY) until there was no visible chunks. Distilled water (20 mL) was used to rinse the homogenizer head and combined with the homogenate. To reduce the oxidation during this process, all the beakers were kept in a box filled with ice. 6-tetramethoxypropane (TMP) standard solutions were prepared at the concentrations of 0.0, 2.5, 5.0, 7.5 and 10.0 uM. Sample homogenates (1.0 mL) and 1.0 mL of the standard solutions were transferred into 15 mL centrifuge tubes. Solution I (0.375% TBA, 0.506% SDS, 9.370% acetic acid, pH 3.4; 4.0 mL) was added into each tube. The tube was capped, vortexed and incubated in a 95 ℃ Fisher Water Bath (Fisher Scientific, Pittsburgh, PA) for 60 min. After the tubes were

cooled down to room temperature, 5.0 mL of solution II (n-butanol and pyridine at the ratio of 15:1 v/v) were added into each tube and mixed on a vortex mixer for 10 seconds. The solutions were centrifuged at 3500 RPM (1,000 × g) for 25 minutes at room temperature. 2.5 mL top-layer organic solution was transferred to a cuvette. The absorbance was read at 532 nm under a hood within 1 hour.

Statistical Analysis

Statistical analysis of antioxidant activities in soybean oil on each testing day, antioxidant activities in ground beef on each testing day, and antioxidant capacities of each concentration were performed by a one-way analysis of variance with the general linear model in SAS (Version 9.1.3, 2003, SAS, Cary, NC). If the model was significant ($p < 0.05$), the main effects of antioxidants were separated by using Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Dihydromyricetin (DHM) Content in Teng Cha

After extraction and lyopilization, an average of 3.49 g powder with a light yellow color was obtained from 10 g of Teng Cha. HPLC analysis of this powder showed that it contained 64.7% (w/w) DHM on average. Assuming that all the DHM dissolved into the extracting solution, this Teng Cha product contained around 22.6% (w/w) DHM on dry weight basis. This means 10 g Teng Cha could yield 2.26 g DHM with this extraction method. Instead of a complicated extraction process with several organic solvents involved, a fast and simple method with 74% ethanol as the extracting solution was used in our work, because it's more practical to

employ nontoxic solvents in the food industry. Although it's more economical to extract DHM from fresh plants, commercially available Teng Cha was used in this study because of its easy availability.

Tian and coworkers evaluated the DHM content in different parts of the plant *Ampelopsis grossedentata* (Tian, Zhang, Yang, Yang, & Gong, 2002). On a dry weight basis, the leaves contained 27.35%±3.61% DHM, while the mixture of leaves and stems contained 23.40%±1.62% DHM. The Teng Cha sample used in our experiment contained both leaves and stems. Although a different extraction method was used in our work, the DHM content result is within the range provided by Tian and others (2002). Gao and others also worked on the extraction of DHM from *Ampelopsis grossedentata*, using only the leaves (Gao, Liu, Ning, Zhao, Zhang, & Wu, 2009). The crude extract yield (3.89g dry extract ÷ 10.08g A. g. leaves = 38.6%) is a little higher than that we obtained (3.49g ÷ 10.00g = 34.9%).

DHM could also be extracted from other plants. The fruit and seed of *Hovenia dulcis* has been well studied by researchers in Japan and South Korea. DHM was found in this plant (Yoo, Mun, & Kim, 2005) and was referred to as the most important component responsible for the hepatoprotective effect of the *Hovenia dulcis* extract (Hase & Xiong, 1997). *Ampelopsis cantoniensis*, which is from the same genus as *Ampelopsis grossedentata*, also contains a high level of DHM (25.2% on dry weight basis) as reported (Chen, Cai, & Chen, 1997).

Total Phenolic Content (TPC)

Folin-Phenol analysis results showed that the DHM powder contains 78.3% (w/w) gallic acid equivalents. Considering the HPLC analysis results, total phenolic content from DHM in the

crude extract should be 78.3% * 64.7% = 50.7% (w/w). Folin-Phenol analysis on the dry extract reveals that it contains 64.9% (w/w) gallic acid equivalent. This suggests that there are other phenolics present in the dry extract. For easy comparison with other herbs, the result can be converted to the total phenolics content in Teng Cha, which is 227 mg GAE /g Teng Cha (64.9% * 34.9% = 22.7%).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of different concentrations of BHA, DHM and the crude extract are shown below (Figure 3.4). At the same concentration, the crude extract has a little lower activity than BHA and DHM. DHM with levels higher than 3 ppm has a relatively higher activity than BHA. IC_{50} of BHA, DHM and crude extract are 2.83 ppm, 2.76 ppm and 3.86 ppm respectively.

Antioxidant Activity in Soybean Oil

Soybean oil is widely used by the food industry in a variety of products such as margarine, shortening, salad dressing, imitation dairy products, baked goods and snack foods. It also has been used as the oxidation substrate to test the effects of processing (Jung, Yoon, & Min, 1989) and efficacy of antioxidants (Nenadis & Tsimidou, 2010). Compared with other bulk oil substrates, soybean oil has several advantages as an oxidation substrate. First, it contains a relatively higher level of polyunsaturated fatty acids, which are more susceptible to oxidation (McClements & Decker, 2008). Canola oil and olive oil are also common edible oils in the market, but they have much lower levels of polyunsaturated fatty acids than soybean oil (Table 3.3). Thus, under the same condition, the oxidation period of canola oil and olive oil will be

much longer than that of soybean oil to reach the same level of oxidation. Second, soybean oil does not contain the photosensitizers such as chlorophyll, which can be found in olive oils. With light exposure, these compounds may accelerate the oxidation speed to a level that oxidation measurement needs to be done every several hours. Since antioxidant assessments usually involve several treatments and replicates, the time budget is tight. The interval should be long enough to finish all the oxidation tests. Preliminary tests with different oils and oxidation conditions were conducted before setting up the final test. At the recommended oxidation temperature of 60 ℃ (Decker, Warner, Richards, & Shahidi, 2005), peroxide value of soybean oil reached 20 after 9 days, which is a perfect length for oxidation tests. Besides, soybean oil is commonly replenished with antioxidants such as Vitamin E and BHA after refining. Searching potent antioxidants for soybean oil are also of great economic significance for the soybean oil industry. Thus, soybean oil was chosen as the substrate for antioxidant test in this work. Peroxide value, headspace volatiles, anisidine value and headspace oxygen content were measured for evaluating the antioxidants.

Peroxide Value

Peroxide value was measured for each treatment on Day 0, Day 3, Day 6, Day 9, Day 12 and Day 15 (Figure 3.5). Soybean oil without antioxidants had the highest peroxide value before Day 12. After Day 12, the hydroperoxides content started to decrease, because the decomposition rate exceeded the formation rate. Soybean oil with 200 ppm BHA had a little bit lower peroxide value than the control within the first 12 days. However, on Day 15, its peroxide value exceeded that of control. It is possible that BHA has an inhibition effect on hydroperoxides decomposition so the rate of hydroperoxides formation was still higher than that of decomposition. This may

lead to the continued increase in peroxide value. Soybean oil samples with the treatment of DHM, extract and BHA+DHM had much lower peroxide value. Samples with 200 ppm DHM had the lowest peroxide value. However, peroxide value alone could not demonstrate the overall antioxidant activity of the compounds. Other tests on the secondary oxidation products are needed.

Headspace Volatiles

Headspace volatiles in the soybean oil samples that had been oxidized for 0, 3, 6, 9, 12 and 15 days were analyzed by an SPME-GC-MS system.

Level of total volatiles for each sample was calculated based on the volatile GC peak area (Figure 3.6). Total volatiles of soybean oils with BHA, DHM and BHA+DHM were significantly lower than that of the control. Although BHA and DHM had similar effect against volatiles formation, BHA had much less effect against the formation of hydroperoxides. The oil containing crude extract had the highest level of volatiles. This may be explained by the transition metals content in the crude extract. It was reported that Teng Cha contains several important trace elements including the transition metals iron and copper (Xue, 2004; Yi & Bin, 2002; Zheng & Liu, 2006). Transition metals are one of the major food prooxidants that promote decomposition of hydroperoxides (McClements & Decker, 2008).

Seven volatile compounds were chosen as indicators. An overlaid chromatograph containing the peaks of these compounds is given as an example (Figure 3.7 and Figure 3.8). Volatile levels in the soybean oil samples with 15 days oxidation were summarized (Figure 3.9 and Figure 3.10). Levels of ethene were much higher than the other six compounds. Thus a

separate graph was created for ethene (Figure 3.9). All the other detected volatiles were omitted from this report for specific reasons (pentane has been used as an indicator in earlier work (Selke & Frankel, 1987; Snyder, Frankel, Selke, & Warner, 1988), however, pentane levels in this experiment did not change much over the 15 days. Some other compounds had significant change over the oxidation period, but their peaks were too close together and overlapped with each other and made the identification difficult.

Anisidine Value

p-Anisidine value (AnV) assay measures the nonvolatile secondary oxidation products in oils. Oil samples with DHM, crude extract and BHA+DHM had significantly lower anisidine values than the control and BHA treated oils (Figure 3.11). AnV is often used in conjunction with the peroxide value (PV) to calculate the total oxidation value (Totox Value) (Rossell, 1989; Wai, Saad, & Lim, 2009) by using the formula: Totox Value = 2PV + AnV. Totox values of the soybean oil samples with different treatments were also compared (Figure 3.12). Totox values agreed well with the peroxide values.

Headspace Oxygen Content

There was a significant drop in the headspace oxygen content of the control and BHA treated soybean oils (Figure 3.13). Oil samples with DHM, crude extract and BHA+DHM had much higher oxygen content in the headspace. This result agreed well with that of the peroxide value assay and AnV assay. Oils with DHM had the highest headspace oxygen level, which is in accordance with the lowest peroxide value of DHM treated oil samples.

Antioxidant Activity in Cooked Beef

In this test, beef samples with BHA, DHM and crude extract had much lower TBARS value than the control (Figure 3.14). However, unlike the bulk oil ssample, BHA in cooked beef showed higher antioxidant activity than DHM and the crude extract. Also, the extract was more effective than the DHM against the oxidation of beef.

During this experiment, it was observed that the solubility of DHM in the 20% ethanol solution was very low. When mixed with the ground beef, there were still visible DHM granules in the solution. Thus, the relatively lower antioxidant activity of DHM and the extract in cooked beef might be explained by poor solubility.

CONCLUSION

Antioxidant activity of *Ampelopsis grossedentata* extract and its major component dihydromyricetin were studied. Dihyromyricetin showed significantly better inhibition effect on soybean oil oxidation than BHA in all four oxidation tests used in this work. The crude extract showed higher antioxidant activity than BHA in only some of the oxidation assays. The extract treated soybean oil had much higher levels of headspace volatiles than all the other treatments, possibly because of the transition metal content. In the oxidation test with cooked beef substrates, BHA showed higher antioxidant activity than DHM and the extract. However in this experiment, physicochemical properties such as decomposition temperature and solubility of DHM and the extract were not considered. Future work is needed to explore the sensory characteristics, solubility and carry-through properties of the extract and dihydromyricetin. It is

also of great interest to search for more economic natural sources containing dihydromyricetin in the US.

ABBREVIATIONS USED

DHM, dihydromyricetin; DPPH, 2,2-diphenyl-1-picrylhydrazyl; PV, peroxide value; AnV, anisidine value; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; TBHQ, tertiary butylhydroxyquinone; PG, propyl gallate; EDTA, ethylenediaminetetraacetic acid; TMP, tetramethoxypropane; TBA, thiobarbituric acid; SDS, sodium dodecyl sulfate; HPLC, high performance liquid chromatography; UV, ultraviolet; AOCS, American Oil Chemists' Society; SPME-GC-MS, solid phase microextraction-gas chromatograph-mass spectrometer; IUPAC, International Union of Pure and Applied Chemistry; TPC, total phenolic content

TABLES

Table 3.1. Preparation of soybean oil samples with different treatments to evaluate the antioxidant effectiveness of Teng Cha crude extract (Ext.), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and synergistic effect of DHM and BHA.

Treatment	Content	
C	200 mL soybean oil only	
B	200 mL soybean oil	40.0 mg BHA
D	200 mL soybean oil	40.0 mg DHM
BD	200 mL soybean oil	40.0 mg BHA and 40.0 mg DHM
E	200 mL soybean oil	61.54mg Ext.

*Soybean oil source: Crisco Pure Vegetable Oil

Table 3.2. Preparation of raw ground beef samples with different treatments to evaluate the antioxidant effectiveness of Teng Cha crude extract (Ext.), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and synergistic effect of DHM and BHA.

Treatment	Description	Content		
C	Control	200 g ground beef	20 ml 20% ethanol	--
E	200 PPM Ext.			44 mg Ext.
B	200 PPM BHA			44 mg BHA
D	200 PPM DHM			44 mg DHM

Table 3.3. Fatty acids profile of olive oil, soybean oil and canola oil. (As modified from McClements and Decker, 2008)

Fatty acids		Olive oil (%)	Soybean oil (%)	Canola oil (%)
Myristic	14:0	--	0.1	--
Palmitic	16:0	13.7	11.0	3.9
Palmitoleic	16:1w7	1.2	0.1	0.2
Stearic	18:0	2.5	4.0	1.9
Oleic	18:1w9	71.1	23.4	64.1
Linoleic	18:2w6	10.0	53.2	18.7
Linolenic	18:3w3	0.6	7.8	9.2

FIGURES

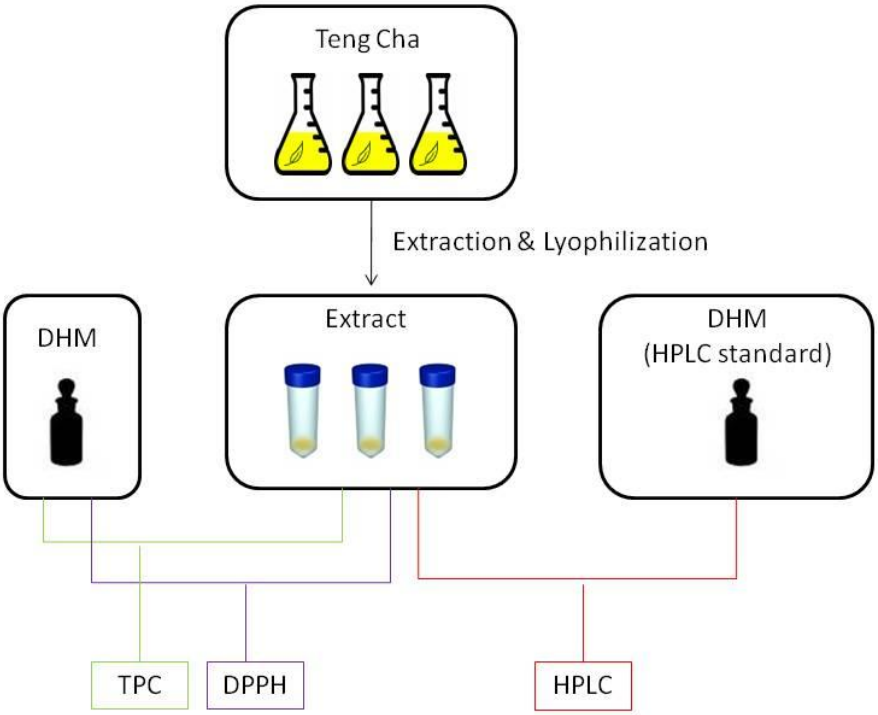


Figure 3. 1. Experimental design of Part I: Extraction and Analysis.

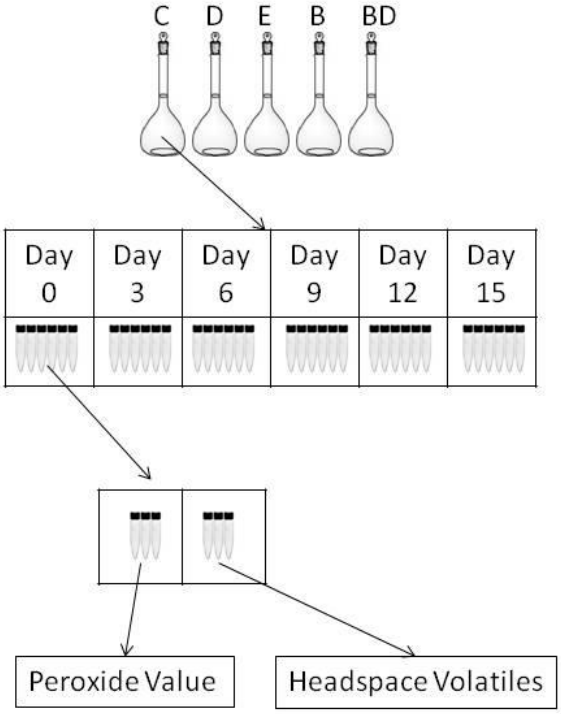


Figure 3. 2. Preparation of soybean oil samples for peroxide value assay and headspace volatile analysis.

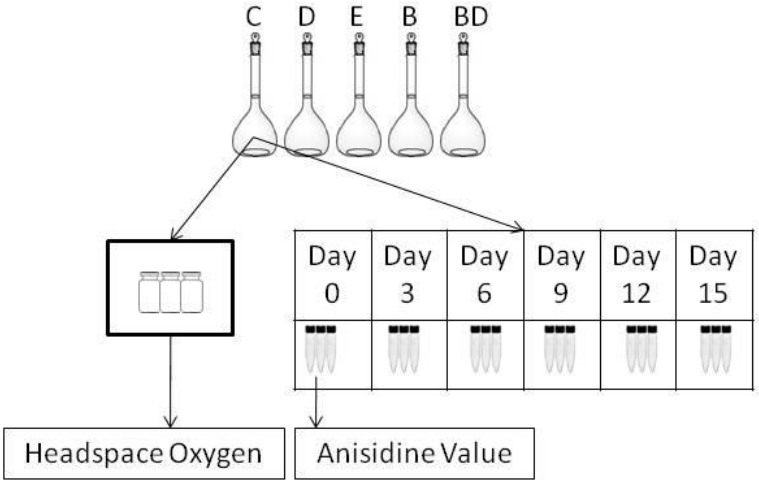


Figure 3. 3. Preparation of samples for anisidine value assay and headspace oxygen test.

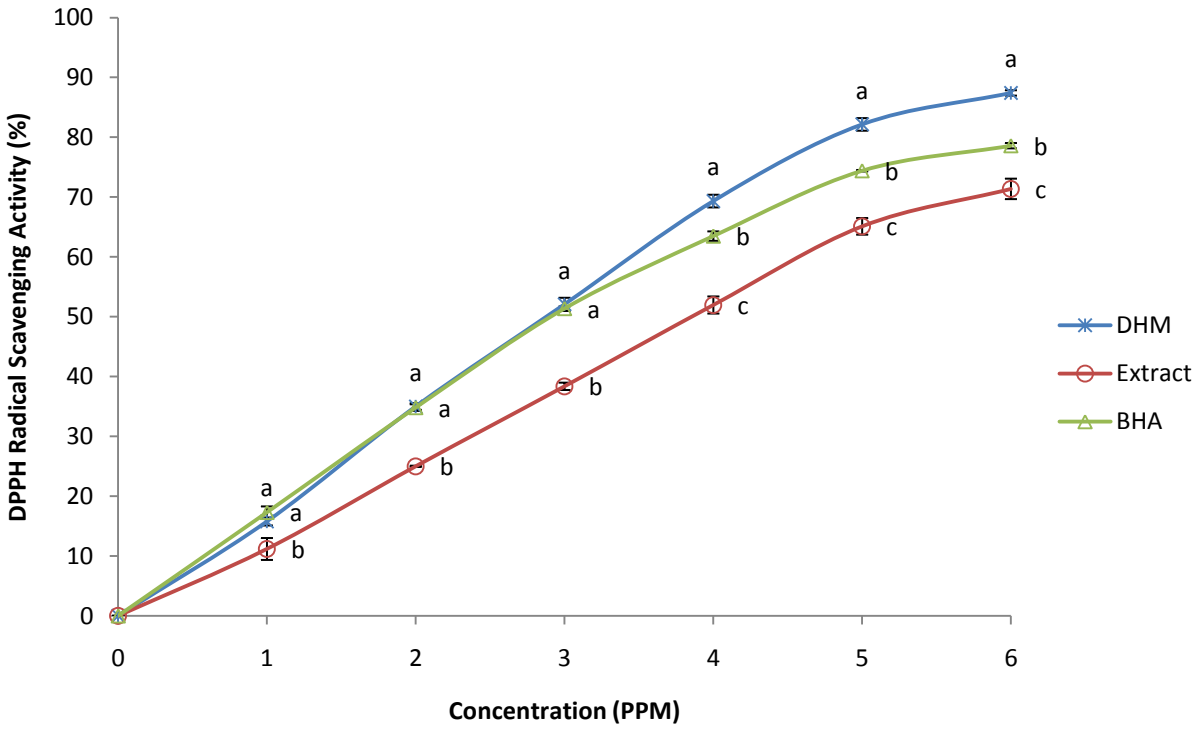


Figure 3.4. DPPH radical scavenging activity of Teng Cha crude extract (Extract), dihydromyricetin (DHM) and butylated hydroxyanisole (BHA) in 50% ethanol at room temperature (Test was conducted in triplicate; error bars indicate standard deviations).

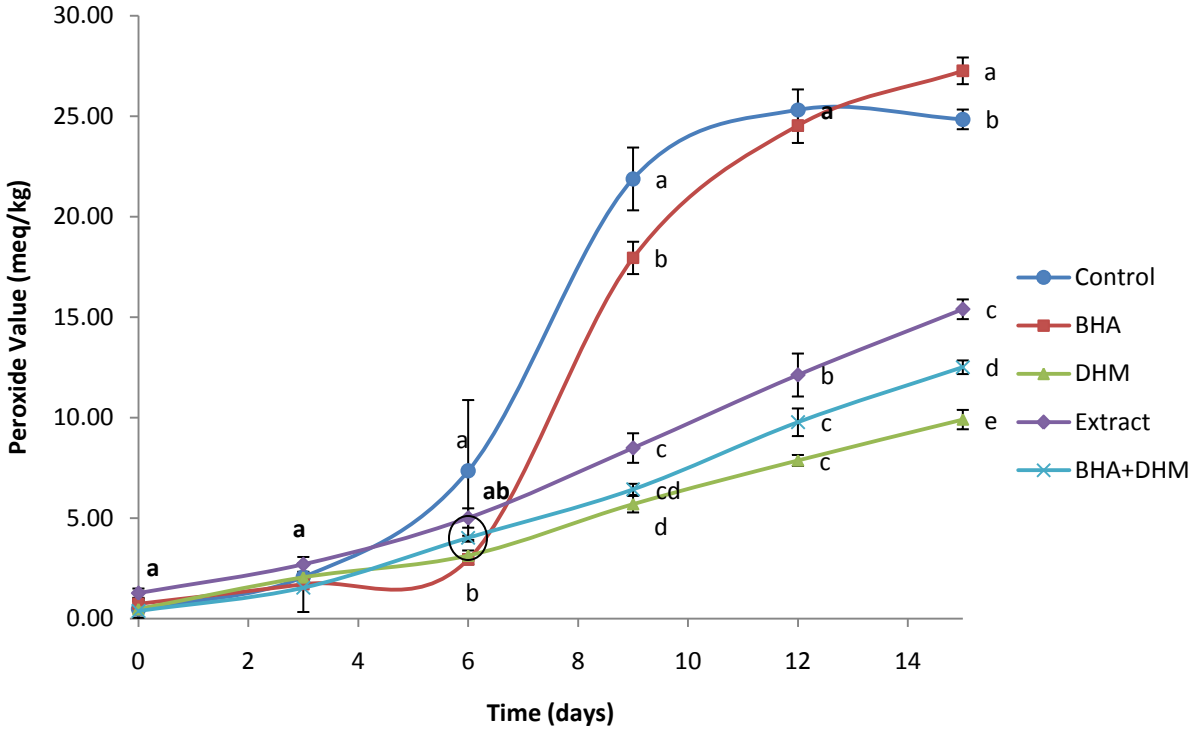


Figure 3.5. Peroxide value (meq/kg) of soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after stored at 60 ℃ for 0, 3, 6, 9, 12 and 15 days (Test was conducted in triplicate; error bars indicate standard deviations).

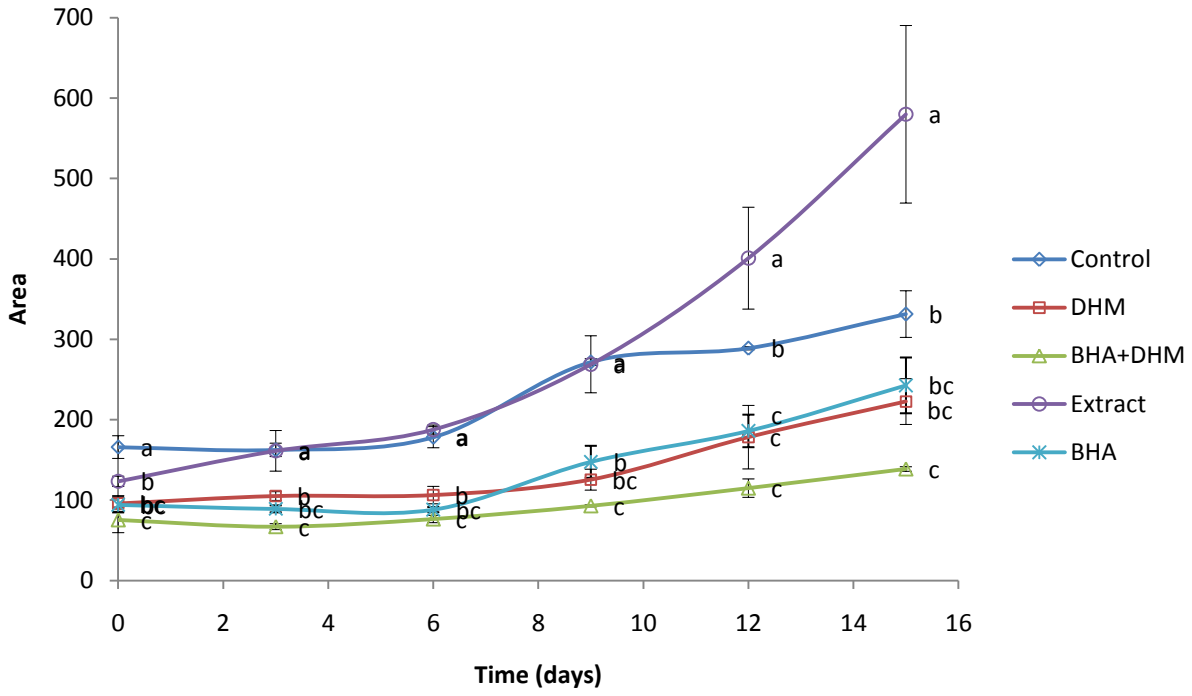


Figure 3.6. Total volatiles in soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after stored at 60 ℃ for 0, 3, 6, 9, 12 and 15 days (Test was conducted in triplicate; error bars indicate standard deviations).

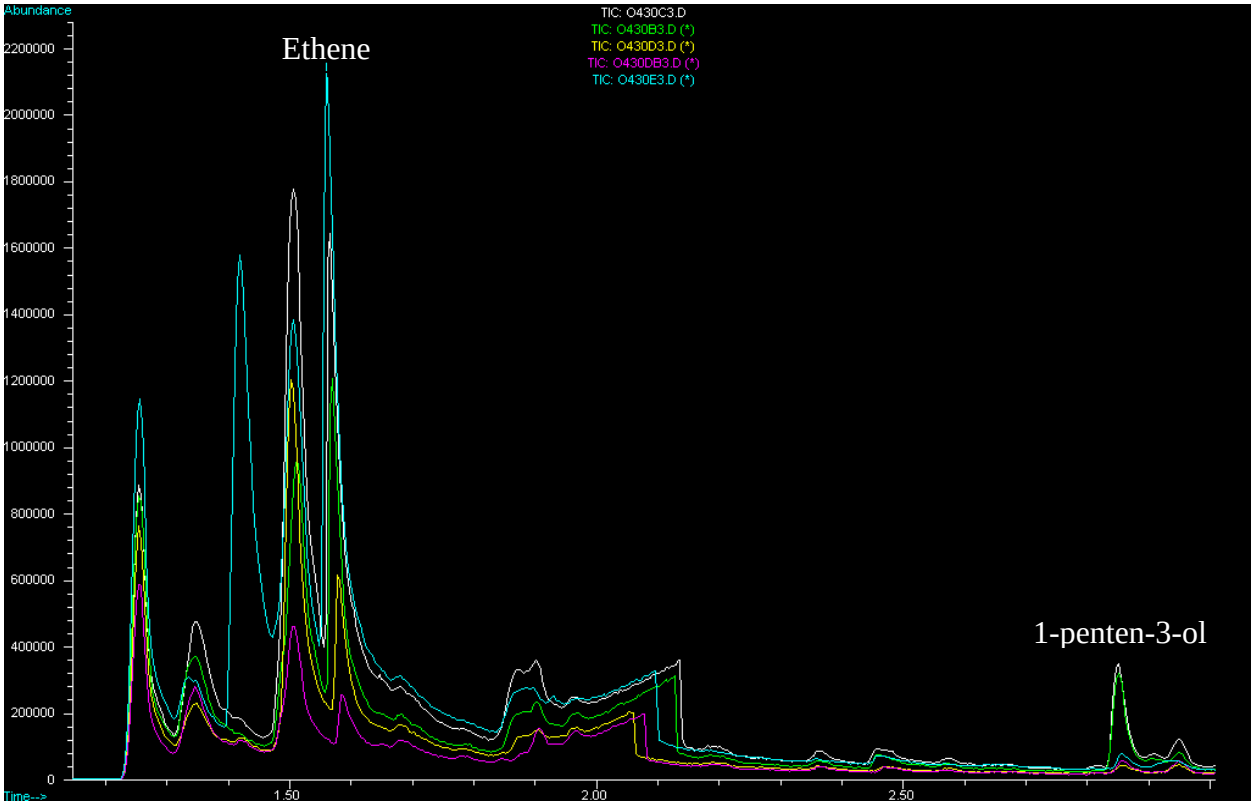


Figure 3.7. Peaks of ethene and 1-penten-3-ol in overlaid SPME-GC/MS chromatograph of soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after 15 day storage at 60 ℃ (Control-TIC 0430C3, BHA-TIC 0430B3, DHM-TIC 0430D3, Extract-TIC 0430E3, DHM+BHA-TIC 0430DB3; test was conducted in triplicate for each treatment but only one chromatograph was randomly picked to show the difference between treatments in this figure).

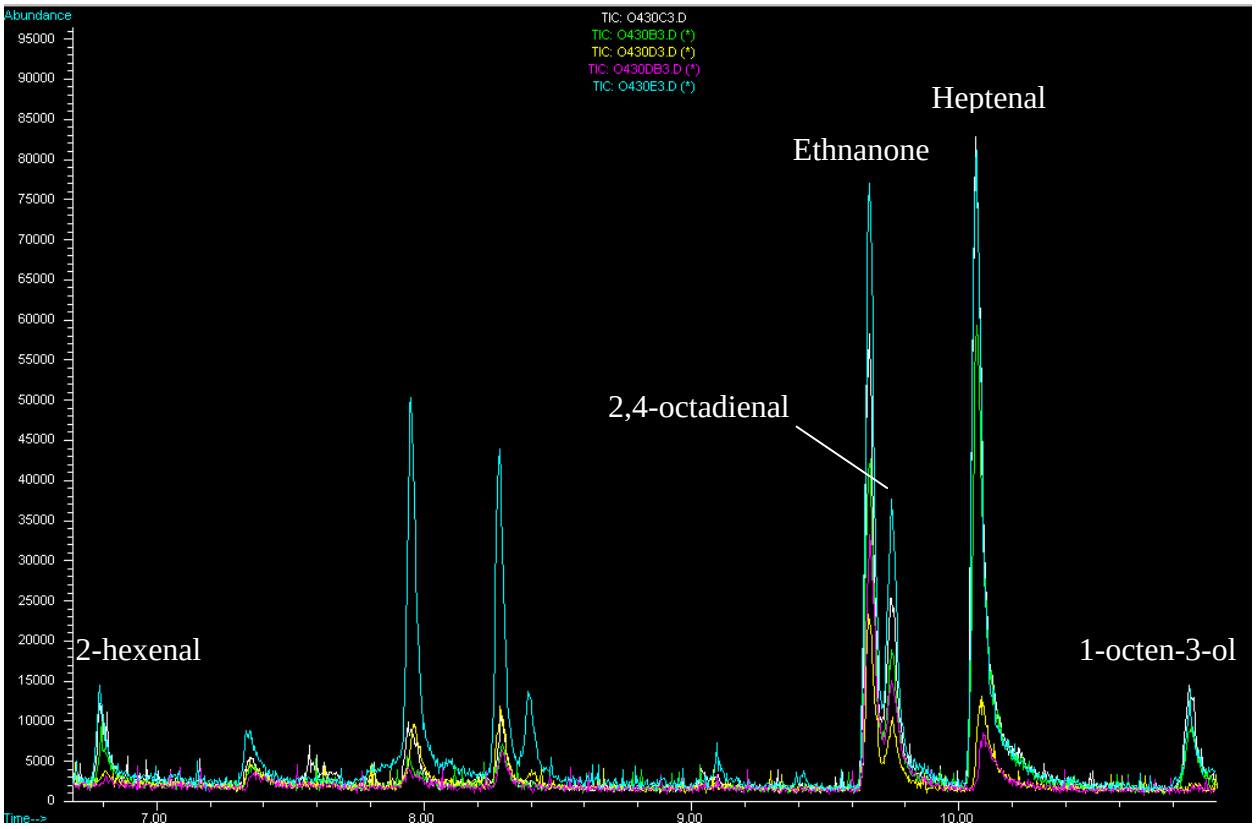


Figure 3.8. Peaks of 2-hexenal, ethnanone, 2,4-octadienal, heptenal and 1-octen-3-ol in overlaid SPME-GC/MS chromatograph of soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after 15 day storage at 60 ℃ (Control-TIC 0430C3, BHA-TIC 0430B3, DHM-TIC 0430D3, Extract-TIC 0430E3, DHM+BHA-TIC 0430DB3; test was conducted in triplicate for each treatment but only one chromatograph was randomly picked to show the difference between treatments in this figure).

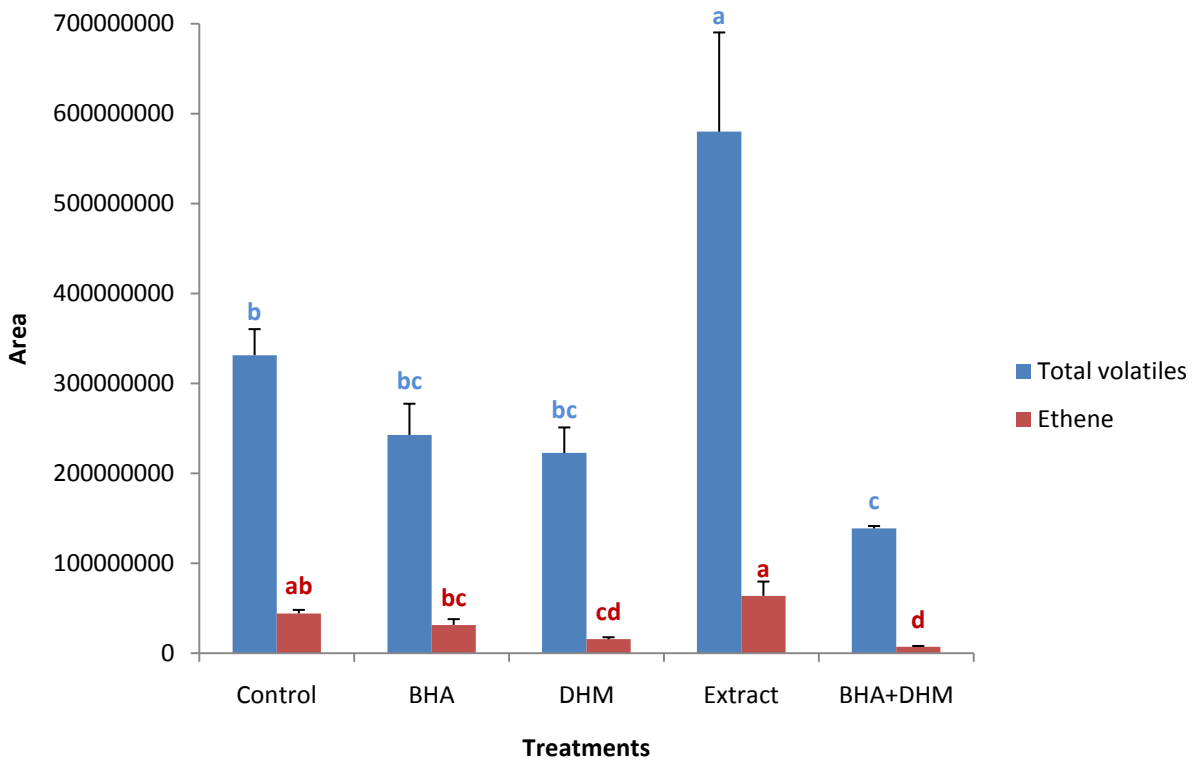


Figure 3.9. Levels of total volatiles and ethene in soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after 15 day storage at 60 ℃ (Test was conducted in triplicate; error bars indicate standard deviations; Tukey test was used to separate the effects of antioxidants).

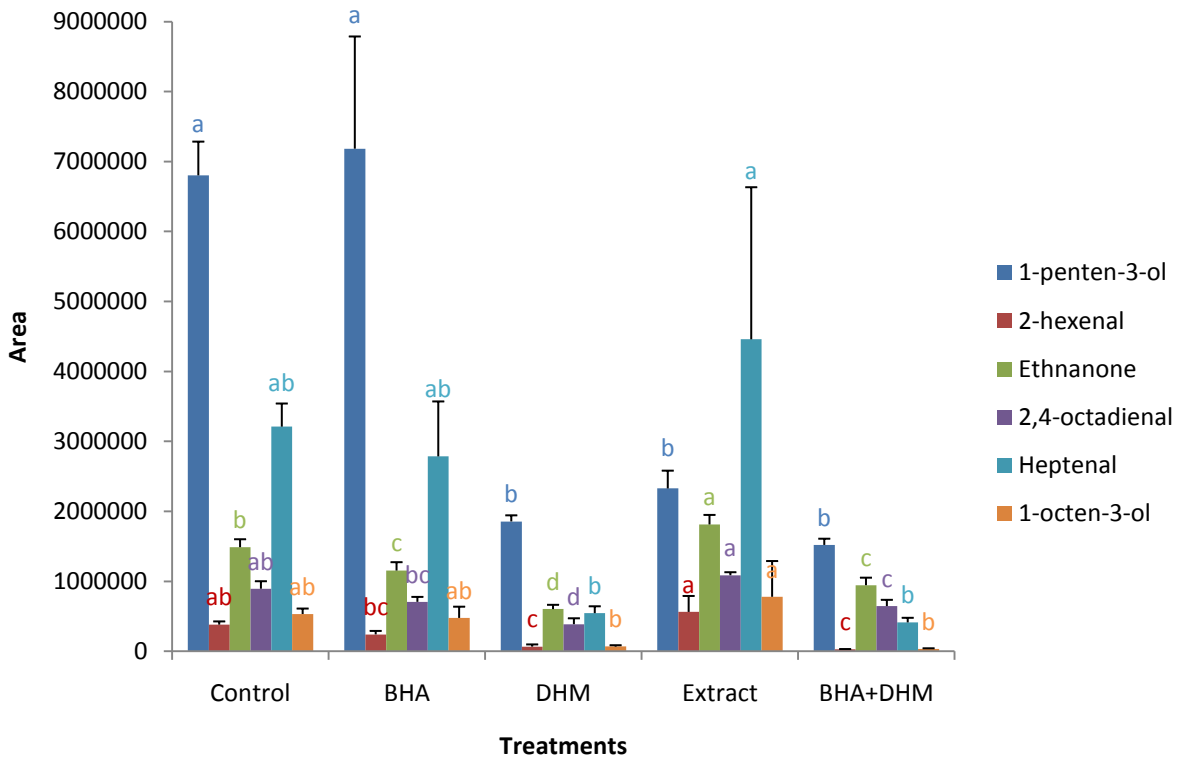


Figure 3.10. Levels of 1-penten-3-ol, 2-hexenal, ethnanone, 2,4-octadienal, heptenal and 1-octen-3-ol in soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after 15 day storage at 60 ℃ (Test was conducted in triplicate; error bars indicate standard deviations; Tukey test was used to separate the effects of antioxidants on the formation of each volatile).

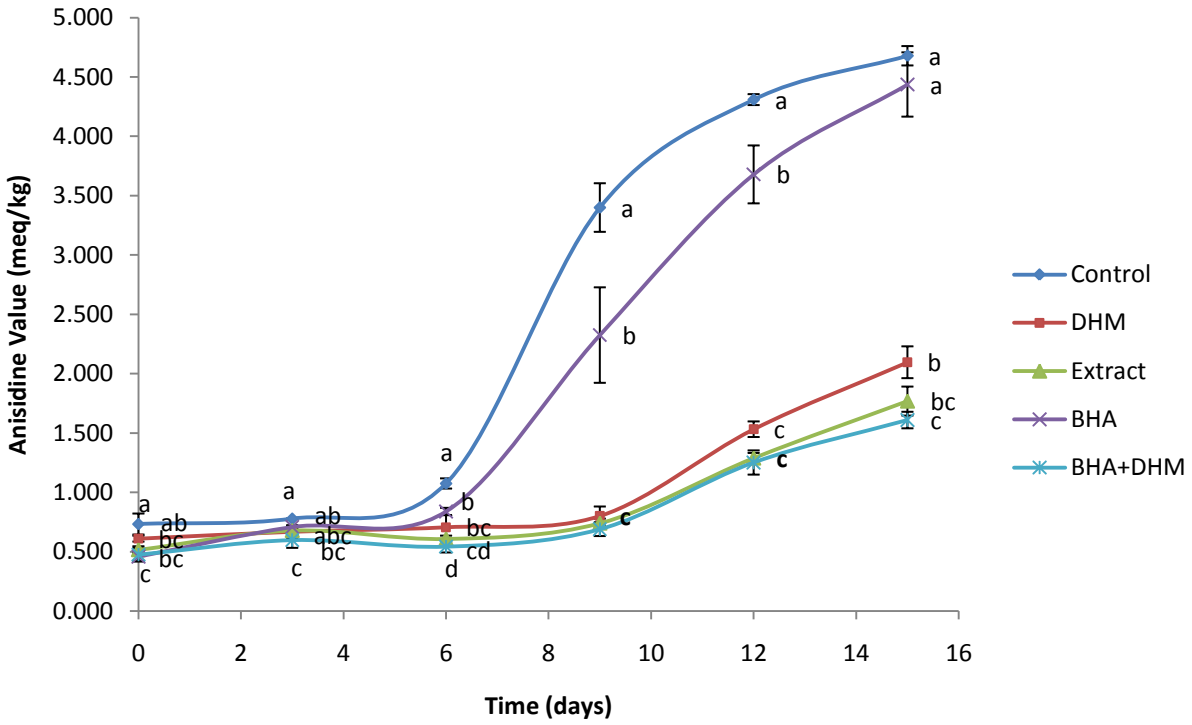


Figure 3.11. Anisidine value (meq/kg) of oxidized soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after stored at 60 ℃ for 0, 3, 6, 9, 12 and 15 days (Test was conducted in triplicate; error bars indicate standard deviations).

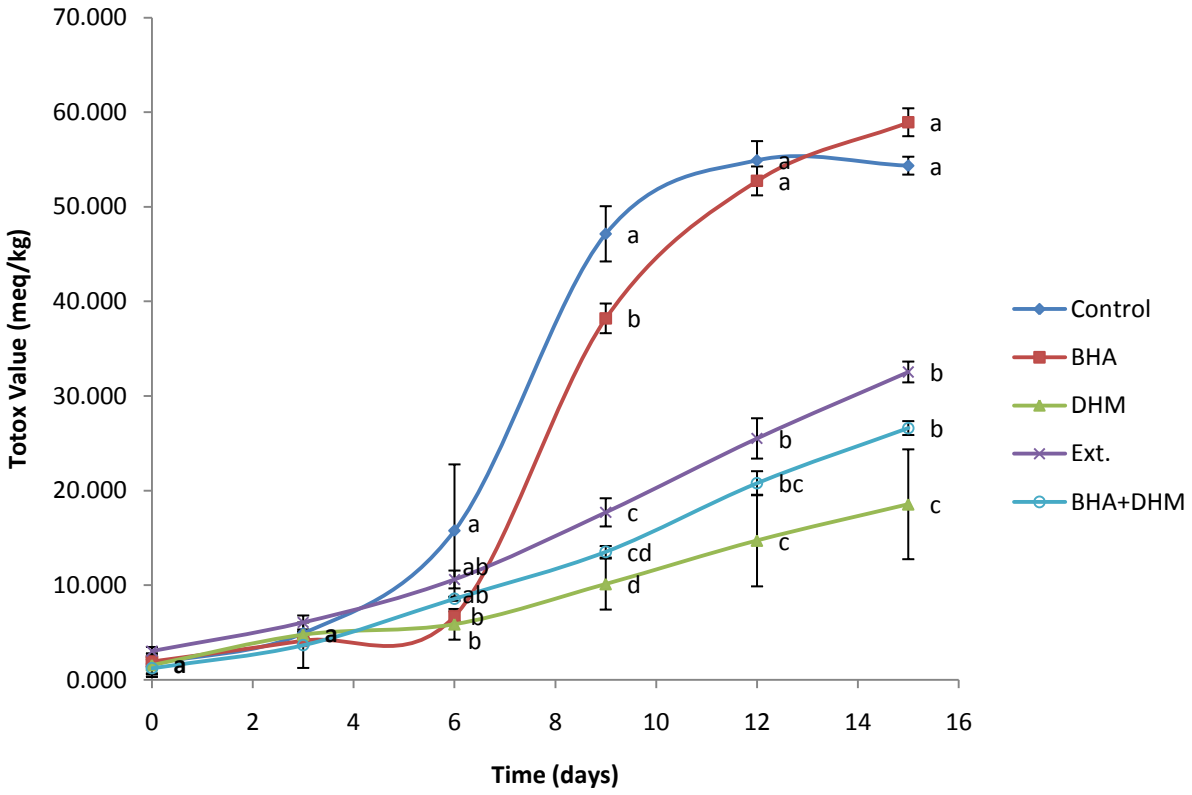


Figure 3.12. Totox value (meq/kg) of oxidized soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after stored at 60 ℃ for 0, 3, 6, 9, 12 and 15 days (Test was conducted in triplicate; error bars indicate standard deviations).

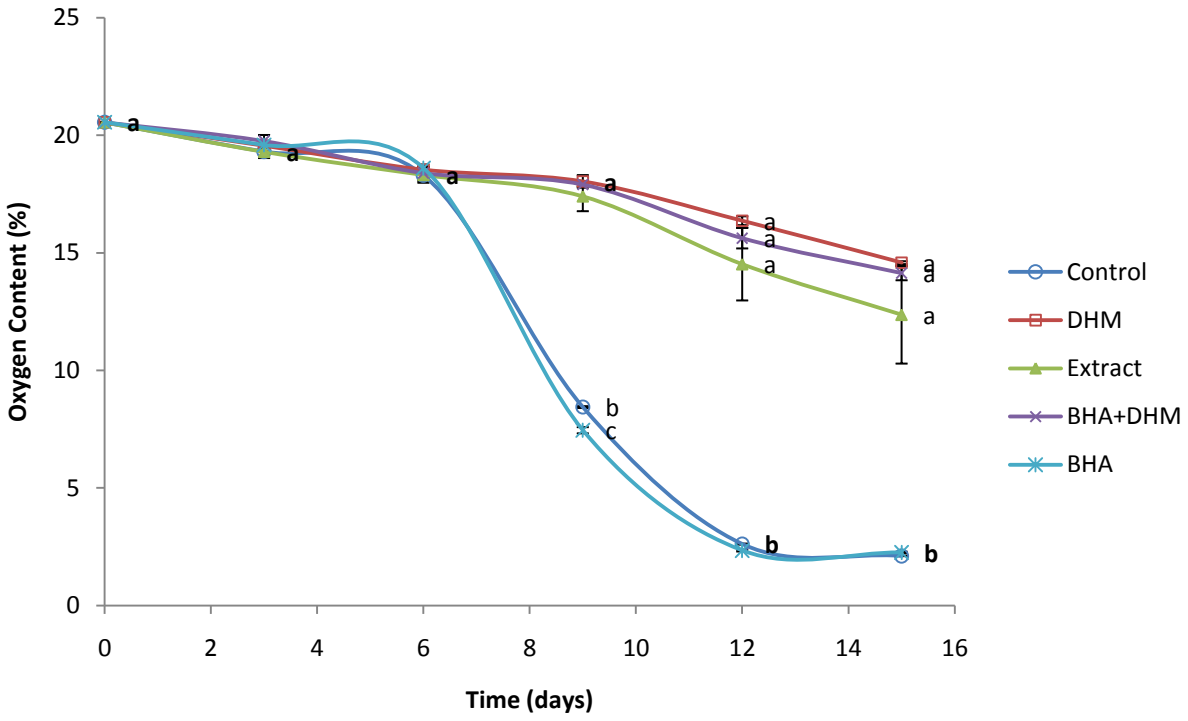


Figure 3.13. Headspace oxygen content (%) of oxidized soybean oil controls (Control) and samples with Teng Cha crude extract (Extract), dihydromyricetin (DHM), butylated hydroxyanisole (BHA) and mixture of DHM and BHA (DHM + BHA) after stored at 60 ℃ for 0, 3, 6, 9, 12 and 15 days (Test was conducted in triplicate; error bars indicate standard deviations).

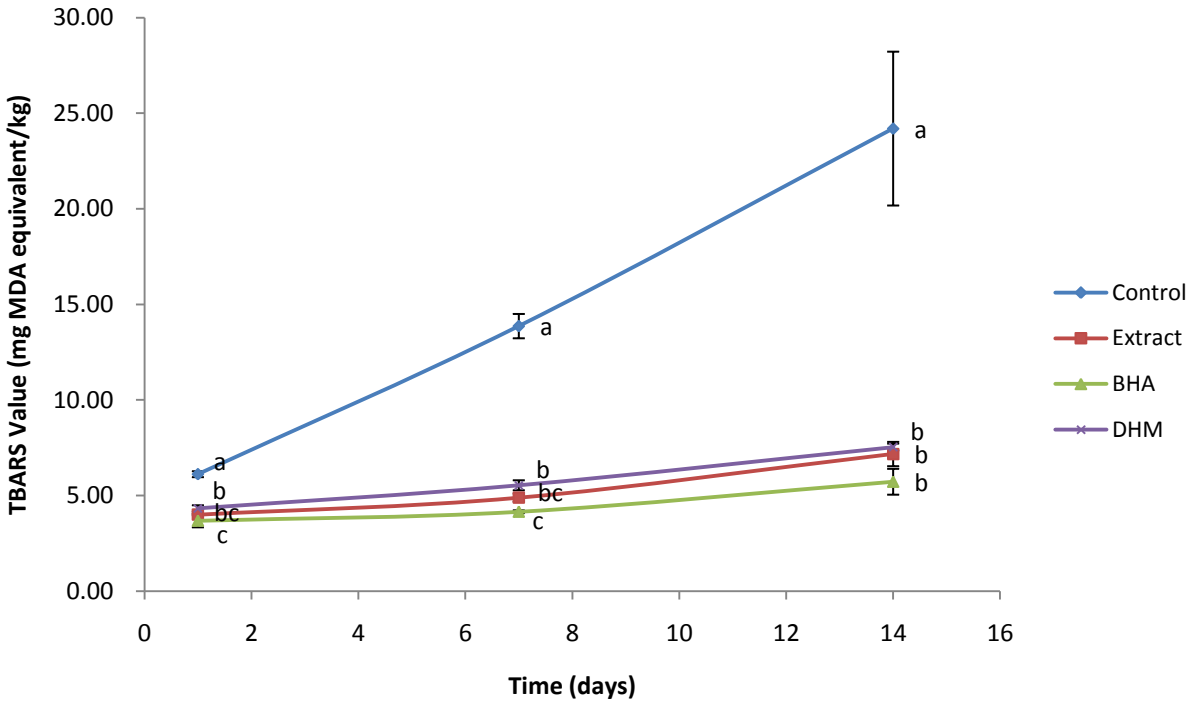


Figure 3.14. TBARS value (mg MDA equivalent/kg) of oxidized cooked beef controls (Control) and samples treated with Teng Cha crude extract (Extract), dihydromyricetin (DHM) and butylated hydroxyanisole (BHA) after stored in 4 ℃ refrigerator for 1, 7 and 14 days (Test was conducted in triplicate; error bars indicate standard deviations).

REFERENCES

Chen, X., Dong, B., Chen, J., Xiao, C., Li, B., & Luo, D. (2007). Study on the medicinal value of Teng Cha *Hunan Agricultural Sciences*(6), 180-181.

Chen Z, W. M., Cai P, Chen X. (1997). Determination of ampelopsin and myricetin in *Ampelopsis cantoniensis*. *Zhong Yao Cai*, *20*(1), 23-25.

Decker, E. A., Warner, K., Richards, M. P., & Shahidi, F. (2005). Measuring antioxidant effectiveness in food. *Journal of Agricultural and Food Chemistry, 53*, 4303-4310.

Gao, J., Liu, B., Ning, Z., Zhao, R., Zhang, A., & Wu, Q. (2009). Characterization and antioxidant activity of flavonoid-rich extracts from leaves of *Ampelopsis grossedentata*. *Journal of Food Biochemistry*, *33*(6), 808-820.

Hase, K., Ohsugi, M., Xiong, Q., Basnet, P., Kadota, S., & Namba, T. (1997). Hepatoprotective effect of Hovenia dulcis thunb. on experimental liver injuries induced by carbon tetrachioride or D-galactosamine/lipopolysaccharide. *Biological and Pharmaceutical Bulletin*, *20*(4), 381-385.

Hogan, S., Zhang, L., Li, J., Zoecklein, B., & Zhou, K. (2009). Antioxidant properties and bioactive components of Norton (*Vitis aestivalis*) and Cabernet Franc (*Vitis vinifera*) wine grapes. *LWT - Food Science and Technology, 42*(7), 1269-1274.z

Jung, M., Yoon, S., & Min, D. (1989). Effects of processing steps on the contents of minor compounds and oxidation of soybean oil. *Journal of the American Oil Chemists' Society, 66*(1), 118-120.

Madhavi, D. L., Singhal, R. S., & Kulkarni, P. R. (1996). Technological aspects of food antioxidants. In D. L. Madhavi, S. S. Deshpande & D. K. Salunkhe (Eds.), *Food Antioxidants,* (pp. 159-266). New York: Marcel Dekker.

MeClements, D. J., & Decker, E. A. (2008). Lipids. In S. Damodaran, K. L. Parkin & O. R. Fennema (Eds.), *Fennema's Food Chemistry* 4th ed., (pp. 155-216). Boca Raton, FL: CRC Press.

Nenadis, N., & Tsimidou, M. Z. (2010). Assessing the activity of natural food antioxidants. In E. A. Decker, R. J. Elias & D. J. McClements (Eds.), *Oxidation in Foods and Beverages and Antioxidant Applications*, vol. 1 (pp. 332-367). Cambridge, UK: Woodhead Publishing.

O'Keefe, S. F., & Wang, H. (2006). Effects of peanut skin extract on quality and storage stability of beef products. *Meat Science, 73*(2), 278-286.

Reische, D. W., Lillard, D. A., & Eitenmiller, R. R. (2008). Antioxidants. In C. C. Akoh & D. B. Min (Eds.), *Food Lipids* 3rd ed., (pp. 409-434). Boca Raton, FL: CRC Press.

Rossell, J. B. (1989). Measurement of rancidity. In J. C. Allen & R. J. Hamilton (Eds.), *Rancidity in Foods* 2nd ed., (pp. 23-52). Essex, England: Elsevier Science Publishing.

Selke, E., & Frankel, E. (1987). Dynamic headspace capillary gas chromatographic analysis of soybean oil volatiles. *Journal of the American Oil Chemists' Society*, *64*(5), 749-753.

Snyder, J. M., Franke, E. N., Selke, E., & Warner, K. (1988). Comparison of gas chromatographic methods for volatile lipid oxidation compounds in soybean oil. *Journal of the American Oil Chemists' Society, 65*(10), 1617-1620.

Spanier, A. M., & Traylor, R. D. (1991). A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. *Journal of Muscle Foods*, *2*(3), 165-176.

Tian, S., Zhang, Y., Yang, Y., Yang, W., & Gong, Y. (2002). Dihydromyricetin in *Ampelopsis grossedentata* by reversed-phase high performance liquid chromatograpohy. *Journal of Hunan Agricultural University*, *28*(1), 32-34.

Wai, W. T., Saad, B., & Lim, B. P. (2009). Determination of TOTOX value in palm oleins using a FI-potentiometric analyzer. *Food Chemistry*, *113*(1), 285-290.

Wang, W., Tian, Z., Xiong, D., & Zhang, F. (2004). The antioxidation effects of the extracts of *Ampelopsis grossedentata*. *Chinese Wild Plant Resources*, *23*(4), 46-47, 53.

Xue, H. (2004). Determination of trace elements and study on health protection of *Ampelopsis grossedentata*. *Guangdong Trace Elements Science*, *11*(8), 56-58.

Yi, C., & Bin, D. (2002). The nutritional and health keeping roles of Tengcha tea. *Quarterly of Forest By-Product and Specialty in China*(1), 53-54.

Yoo, S. M., Mun, S., & Kim, J. H. (2005). Recovery and pre-purification of (+)-dihydromyricetin from *Hovenia dulcis*. *Process Biochemistry, 41*, 56-570.

Zhang, Y., Yang, W., & Xiong, H. (2001). Basic constituent of *Ampelopsis grossedentata*. *Natural Product Research and Development, 13*(5), 46-48.

Zheng, D., & Liu, G. (2006). Research and development of Chinese Teng Cha resources. *Agriculture Network Information*(6), 136-142.

Zhong, Z., Zhou, G., & Chen, X. (2003). The rat chronic toxicity test of total flavone of *Ampelopsis grossedentata* from Guangxi. *Lishzhen Medicine and Materia Medica Research, 14*(4), 193-195.

Zhou, Y., Hu, Y., Zang, B., Hu, Y., Qiu, F., Liu, X., Nie, Y., & Wu, L. (2001). Toxicological assessment on *Ampelopsis grossedentata* and its immune regulation study. *Practical Preventive Medicine, 8*(6), 412-414.