

**CHOLESTEROL OXIDATION IN ROASTED SALMON FISH WITH  
DIFFERENT COOKING OILS**

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Ting Zhang  
B.S. Beijing University of Chemical Technology, 2000  
M.S., Louisiana State University, 2003  
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## **Abstract**

The effects of plant source cooking oils on cholesterol oxidation during heating in cholesterol standard and salmon meat model were investigated. A GC-MS system was used to identify and quantify of cholesterol and cholesterol oxidation products. The capabilities of different plant source cooking oils in preventing cholesterol oxidation were compared. Commercial plant oils used in this experiment included corn oil, canola oil, olive oil, soybean oil, and rice bran oil. Two lab prepared crude soybean and rice bran oils were also used in this study to evaluate their capabilities of preventing cholesterol oxidation. The tocopherols and tocotrienols antioxidants in those oils were measured by a HPLC system as well. In both cholesterol and salmon meat models, it was found that the cholesterol level decreased with increasing heating temperature and time. The cholesterol decreasing in the salmon meat model was not as fast as in the cholesterol model. Ketocholesterol was the major cholesterol oxidation product in the two models. Soybean oil had the highest capability in preventing cholesterol oxidation, while rice bran oil had the lowest capability among these oils. The soybean oil had the highest alpha and gamma-tocopherol levels, which were considered to be the major antioxidant components of preventing cholesterol oxidation. The commercial oils had lower level of tocopherol and tocotrienol than the lab prepared oil due to the loss of those antioxidants during oil refining procedures. The different capacities between lab prepared soybean oil and rice bran oil in salmon samples were not as significant as in cholesterol model since the salmon meat may contain antioxidants in itself and reduce the total amount of cholesterol oxidation.

## Chapter 1 Introduction

Cholesterol is an essential molecule for humans as a component of cell membrane and as a precursor of steroid hormones and bile acids. Many foods containing high level of cholesterol, such as eggs, seafood, milk, and meat, are important sources of cholesterol. However, cholesterol in foods is readily oxidized to form cholesterol oxidation products (COPs) when exposed to light, oxygen, active chemicals and high temperature. High intake of COPs from foods could result in cardiovascular diseases and formation of certain types of cancers. Therefore, lowering the COPs level in daily foods is very important. Cooking oils from plants are considered a rich source of tocopherols and tocotrienols, which are important antioxidants for preventing lipid oxidation. The antioxidants may also have capabilities of preventing cholesterol oxidation and reducing COPs production during heating. However, information on prevention of cholesterol oxidation by various cooking oils during heating is limited. As many studies indicated the health beneficial effects of consuming fish meat, fish and fish oil are becoming more and more important food in our diet. The advantages of fish and fish oil have been related with the fat composition that is rich in long-chain  $\omega$ -3 fatty acids, particularly eicosapentaenoic fatty acid (EPA) and docosahexaenoic fatty acids (DHA). High-fat fish species, such as salmon, which are the main supply of  $\omega$ -3 PUFAs in the diet, also show significant cholesterol content. The cholesterol in salmon may readily to be oxidized during cooking and forms COPs. In this study, oils with higher capability of preventing cholesterol oxidation were mixed with salmon

meat. The protective capability of those oils against cholesterol oxidation in salmon meat during heating were also investigated

In this study, the capabilities of cooking oils were investigated by using cholesterol and salmon meat model. The results could help us to understand the relationships between cholesterol oxidation and COPs production and the role of different cooking oils in prevention of cholesterol oxidation and COPs production in food systems during heating. This study may provide guidance for preparing cholesterol-rich foods that would minimize COPs and reduce the possible risk of heart diseases and cancer.

The hypothesis of this study is that cholesterol will be oxidized significantly with increasing either the heating temperature or time. Plant source cooking oils have the capacity of protecting cholesterol oxidation and the formation of COPs during heating. The protective capability of plant source cooking oils in the cholesterol model will protect the cholesterol in roasted salmon meat as well.

The research objectives include, developing a model for evaluating cholesterol oxidation and COPs, investigating capabilities of different cooking oils in preventing cholesterol oxidation during heating, and investigating cholesterol oxidation in control and roasted salmon samples with selected cooking oils. A GC-MS system was used to evaluate the cholesterol oxidation and monitor the formation of cholesterol oxidation products.

## Chapter 2. Review of Literature

### 2.1. Cholesterol Oxidation

#### 2.1.1. Chemical Structure of Cholesterol and Cholesterol Oxidation Products

Cholesterol consists of four fused rings, an aliphatic side chain branched to the D ring at C-17, a hydroxyl group attached to the A-ring at C-3, and a double bond between C-5 and C-6 of B ring. This double bond makes both C-4 of A-ring and C-7 of B ring on the same plane. Figure 2.1 shows the chemical structure of cholesterol.

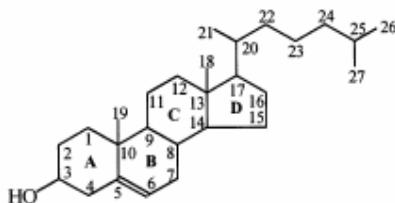
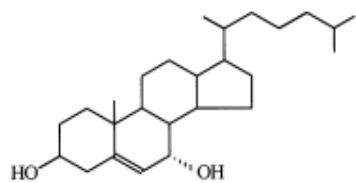
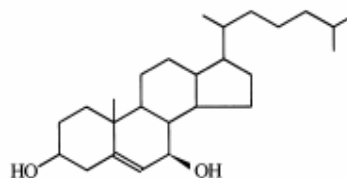


Figure 2.1. The Chemical Structure of Cholesterol.

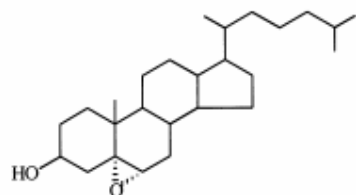
One may expect that both the C-4 and C-7 position should have an equal opportunity for an oxidative attack to occur. However, C-7 is indeed a common position for oxidants to react. In contrast, the attack rarely occurs at C-4 because of the possible shielding effect provided by the neighboring hydroxyl group at C-3 and the trialkyl substituted C-5 (Smith, 1981). Both the 20- and 25-C of the aliphatic side chain are at a tertiary position, and are, therefore, more susceptible to oxidative attack than the other carbons (Maerker, 1987). Figure 2.2 and 2.3 shows the chemical structures of some COPs.



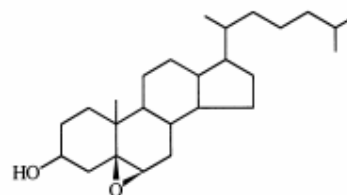
7 $\alpha$ -Hydroxycholesterol (7 $\alpha$ -OH)



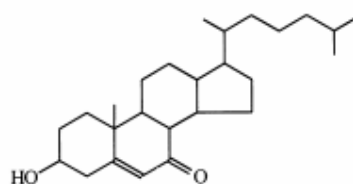
7 $\beta$ -Hydroxycholesterol (7 $\beta$ -OH)



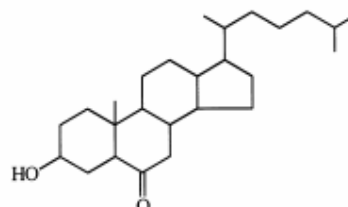
5,6 $\alpha$ -Epoxycholesterol (5,6 $\alpha$ -EP)



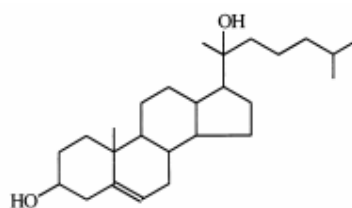
5,6 $\beta$ -Epoxycholesterol (5,6 $\beta$ -EP)



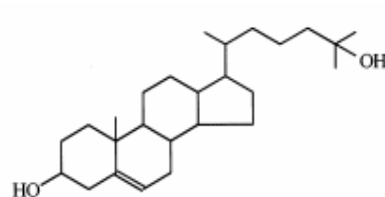
7-Ketocholesterol (7-keto)



6-Ketocholesterol (6-keto)



20-Hydroxycholesterol (20-OH)



25-Hydroxycholesterol (25-OH)

Figure 2.2 The Chemical Structures of Some Cops—Hydroxycholesterol, 5,6-Epoxycholesterol and Ketocholesterol.

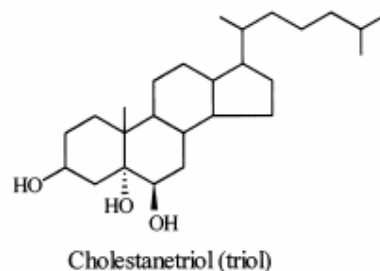


Figure 2.3 The Chemical Structures of Cholestanetriol.

## 2.1.2. Autoxidation

### 2.1.2.1. Kinetic Model of Cholesterol Autoxidation During Heating

The susceptibility of cholesterol to oxidation has been recognized and investigated for more than several decades (Maerker, 1987). However, the formation pathways of certain COPs still have not been fully clarified. Cholesterol oxidation was reported to be similar to that of lipid oxidation (Smith, 1981), i.e., it can be initiated in the presence of oxygen (air) at elevated temperatures or under UV light resulting in autoxidation. The autoxidation of unsaturated fatty acid such as oleic acid can be initiated at the carbon with double bond by oxygen attracting. Because of the ring structure of cholesterol, the oxidation products of lipids can be more complex than cholesterol. The cholesterol autoxidation can be probably initiated at C-7. Since cholesterol-containing phospholipids, fatty acids and cholesterol are associated closely as the integral part of the lipid bilayer of the cell membrane, the hydroperoxides derived from oxidation of unsaturated fatty acids are believed to play an important role in facilitating cholesterol oxidation (Smith, 1981).

The oxidation pathways of cholesterol have been well proposed by Smith (1981) and Maerker (1987). The mechanism of cholesterol oxidation is reported to be similar

to that of lipid oxidation because both can undergo a series of free radical chain reactions to form peroxides and other degradation products (Smith, 1981). It has been established that the initiation period of lipid oxidation belongs to the first-order reaction, and the second-order reaction follows afterward (Bateman et al., 1953).

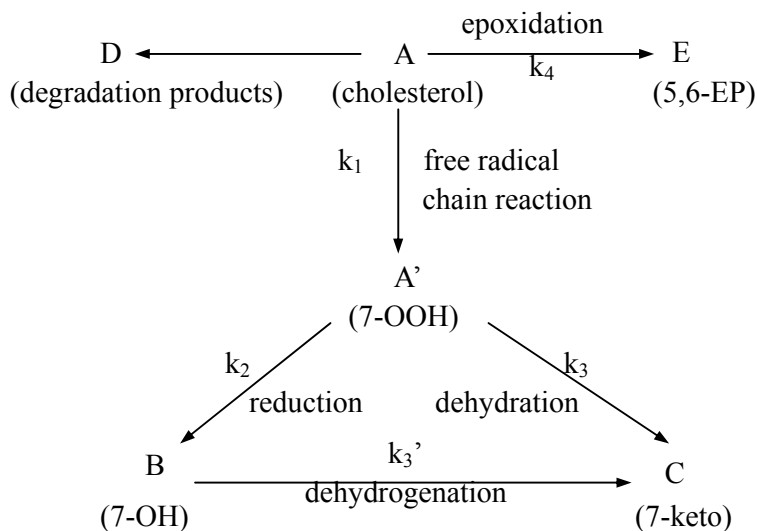


Figure 2.4. Major Pathways of Cholesterol Oxidation: A, cholesterol; A', 7-hydroperoxycholesterol (7-OOH); B, 7-hydroxycholesterol (7-OH); C, 7-ketocholesterol (7-keto); E, 5,6-epoxycholesterol (5,6-EP); D, degradation products.  $k_1$ - $k_4$ ,  $k_3'$  rate constants of the reactions of cholesterol oxidation (Smith, 1981).

Figure 2.4. shows the major pathways of cholesterol oxidation. The reaction can be divided into three major routes of C-7 oxidation degradation and epoxidation. The group changing of cholesterol at C-7 implies that cholesterol oxidation is initiated by hydrogen abstraction, predominantly at C-7. The C-7 oxidation pathway includes (1) formation of 7-OOH (A') from cholesterol (A) through free radical chain reaction (A-A'), (2) formation of 7-OH (B) from 7-OOH (A') through reduction (A'-B), (3)

formation of 7-keto (C) from 7-OOH (A') through dehydration (A'-C), and (4) formation of 7-keto (C) from 7-OH (B) through dehydrogenation (B-C). The epoxidation pathway includes the formation of 5,6-EP (E) from cholesterol through 7-OOH (A') (A+A'-E). In cholesterol degradation pathway, cholesterol may also be decomposed to form other products (D) such as short-chain hydrocarbons, aldehydes, ketones, or alcohols.

However, the information of cholesterol degradation reaction was complicated and very limited because these degraded compounds were not identified. By comparing the reaction rate of the major reaction pathways of cholesterol oxidation, it can be found that  $k_1 > k_4 > k_3 > k_2 > k_3'$ , that is free radical chain reaction > epoxidation > dehydration > reduction > dehydrogenation (Chien et al., 1998).

#### **2.1.2.2. Formation of Cholesterol Oxidation Products during Heating**

Cholesterol is reported to be more stable in solid form than in liquid form, and the latter is more susceptible to oxidation than the former (Nawar et al., 1991; Kim and Nawar, 1993). Cholesterol was oxidized in fast rate when it was heated over 200 °C (Park and Addis, 1986). In Chien and Wang's study (1998), it was found that the percentages of 7-OOH (7 $\alpha$ -OOH and 7 $\beta$ -OOH) increased sharply during the initial heating period. Then they reached a plateau after heating for 6 minutes or more. Similarly, the percentage of 7-OH (7 $\alpha$ -OH and 7 $\beta$ -OH) also increased sharply during the first 10 minutes of heating and reached a plateau thereafter. The percentages of both 7-keto and 5,6-EP (5,6 $\alpha$ -EP and 5,6 $\beta$ -EP) increased with increasing heating time over a 30-minute period. The formation of 7-keto can be attributed to the dehydration

of 7-OOH or dehydrogenation of 7-OH under dry and oxygen-rich conditions (Teng et al., 1973; Kim and Nawar, 1993; Nielsen et al., 1996).

For 5,6-EP formation, it has been reported that when there is enough reaction of epoxidation, 7-OOH formed during heating (Smith, 1981, 1987; Maerker and Bunick, 1986). In Chien and Wang's study (1998), the epoxide formation was observed at the beginning period of heating. It suggested that the 7-OOH was formed in adequate amounts for the reaction of epoxide formation to proceed at the beginning of heating. Maerker and Bunick's study (1986) also demonstrated that the 5,6 EP concentration increased along with increasing of 7-OOH concentration during heating of cholesterol at 80 °C in an aqueous dispersion at pH 8 for 24 hours. These results implied that the differences in heating treatment of cholesterol may greatly affect the formation and degradation rates of 7-OOH. The 5,6-EP was formed in the greatest amount, followed by 7-keto, 7-OH, and 7-OOH.

The side chain oxidation occurs when cholesterol in the solid phase or in the crystalline form during heating. The oxygen of free radical attack at tertiary C-20 and C-25 position generates 20-OOH and 25-OOH, respectively (Smith, 1987). These hydroperoxides can be further degraded to 20-OH and 25-OH, which are quite stable and can sustain consecutive heating at 100°C for 6 months. However, this type of oxidation is not observed in solution or in aqueous dispersion (Maerker, 1987).

### **2.1.3. Photooxidation**

Photosensitizers, such as chlorophyll and hematoporphyrin, can absorb energy in the form of radiation and transfer it to from the singlet oxygen so that the more active

singlet oxygen is formed. The singlet oxygen reacts with the double bond of the B ring of cholesterol, and cause in migration of one double bond and formation of 5-OOH (Kulig, 1973). The 5-OOH can be further converted to the more stable 7-OOH or 6-OOH, which present in minor amount. Irradiation of 7-keto in aqueous dispersion results in formation of 7-ketocholestanol, indicating that hydrogenation may occur through the interaction between 7-keto and the radiolysis products of water (Smith, 1987). However, with increasing light intensity, both isomeric 5,6-EP and 7-OH can be further converted to 6-ketocholesterol (6- keto) and 7-keto, respectively (Maerker, 1993; Hwang, 1993). The formation of a minor amount of the isomeric 7-OH was also observed. This result implied that both 7-OH and 7-keto may be convertible depending on the illumination conditions (Nielson, 1996). The formation pathways of some COPs during irradiation are summarized in Figure 2.5.

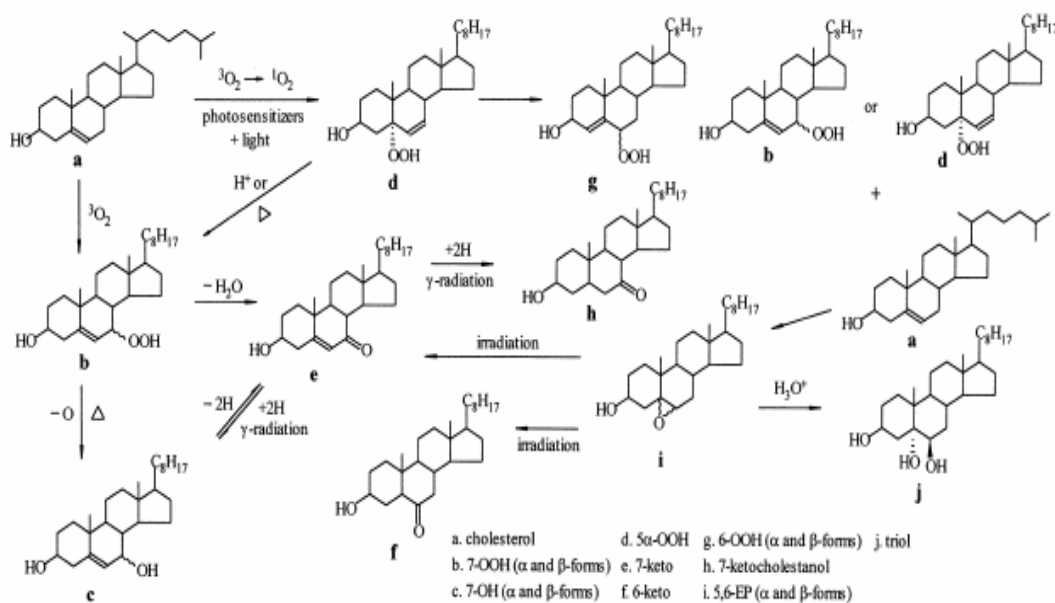


Figure 2.5. The Formation Pathways of Some COPs During Irradiation (Nielson, 1996)

## **2.2 Toxicity of Cholesterol Oxidation Products**

In recent years, COPs have drawn much attention mainly because of their potential health implications. Numerous studies have shown that COPs may possess biological effects such as mutagenicity (Sevanian, 1986), angiotoxicity (Dorset, 1992), carcinogenicity (Morin, 1991), cytotoxicity, atherogenicity and cell membrane damage (Guardiola, 1996), and inhibition of cholesterol biosynthesis (Addis, 1990, 1991). In addition, COPs may induce atherosclerosis (Imai, 1976).

Foods rich in cholesterol content such as dairy products, eggs and meat products, are prone to undergo autoxidation or enzymatic oxidation and form COPs. COPs can also be generated during food preparation when exposed to heat, air, light and radiation (Smith, 1992). Moreover, inappropriate storage conditions will also facilitate COPs formation (Paniangvait, 1995).

Due to the complexity of food itself, it is difficult to study cholesterol oxidation in real food systems. The results observed in the pure cholesterol samples may not be identical to those in real food systems because of numerous factors such as the presence of water, protein, fat, carbohydrate, and other components in foods. For instance, Kim and Nawar (1991) demonstrated that the stability of cholesterol in complex mixtures is influenced by interaction among lipid components and their decomposition products. Yan and White (1990) also concluded that the accumulation of COPs in a high temperature, cholesterol-rich food system is a dynamic one, depending upon the amount of cholesterol present, the treatment of oil (intermittent or continuous heating), and the severity of the heat treatment.

## 2.3. Effect of Food Components on Cholesterol Oxidation

### 2.3.1. Lipids in Foods

Park and Addis (1992) studied the formation of COPs in tallow heated at 135, 150, 165 or 180°C for 70, 144 and 216 hrs. Only 7-keto and 5,6 $\alpha$ -EP were found, and the amount of 7-keto formed during frying increased linearly with heating time. In a similar study, Park and Addis (1986) isolated five COPs from tallow heated at 155 °C for 400 hrs. These COPs were identified as 7 $\alpha$ -OH,  $\beta$ -OH, 7-keto, 5,6 $\alpha$ -EP and 5,6 $\beta$ -EP. Approximately 25% cholesterol was lost, and five COPs, triol, 7-keto, 7-oxo-cholesta-3, 5 -diene, 7 $\alpha$ -OH and 5,6 $\alpha$ -EP (5,6 $\beta$ -EP) were detected in tallow heated at 142-184 °C for 56-70 hrs (Csiky, 1982). Likewise, Park and Addis (1986) also observed a 40-50% cholesterol loss in tallow heated at 155 and 190 °C for 300 hrs and identified 7 $\alpha$ -OH, 7 $\beta$ -OH, 7-keto and 5,6 $\alpha$ -EP. Zhang et al. (1991) investigated the COPs contents in fried oil from fast food restaurants. It was found that after continuous frying for 15 days, a high amount of COPs (50 ppm) was present, however, it began to decline thereafter. This is probably because COPs can undergo degradation after prolonged heating.

Yan and White (1990) studied cholesterol oxidation in heated lard enriched with 2 and 10 times the amount of the cholesterol contained originally in lard. Both 7-keto and 5,6 $\alpha$ -EP were the predominant COPs found, while 7 $\alpha$ -OH, 7 $\beta$ -OH and triol were formed in minor amounts. Results also indicated that after heating at 180 °C (10 hrs per day) for 24 days, lard enriched with cholesterol at 10 times was more susceptible to the formation of COPs than that enriched with cholesterol at 2 times. However, the

degradation rate of cholesterol of the former lower level was slower than that of the high level. Chen et al. (1996) analyzed the COPs in heated lard, and five COPs, 7-keto, cholesta-4,6-diene-3-one, 5,6 $\alpha$ -EP (5,6 $\beta$ -EP), 7 $\beta$ -OH, and triol were detected. In most cases, the COPs contents increase with increasing heating time, however, several COPs, 5,6 $\alpha$ -EP, 5,6 $\beta$ -EP and 7 $\beta$ -OH, increased in the first 100 hrs and then declined thereafter. The highly toxic cholesteroltriol could not be detected until heating time reached 20 hrs. The long heating period or high temperature may cause the degradation of COPs to a great extent in foods. Some other harmful compounds may be produced at high level as well.

Polyunsaturated triglycerides components, such as salt, triglyceride,  $\beta$ -carotene and chlorophyll have been reported to have a great impact on the rate of cholesterol oxidation. Kim and Nawar (1991) found that in the presence of triglyceride, cholesterol oxidation was facilitated, and the rate of cholesterol oxidation should be dependent upon the degree of unsaturation of triglyceride. This is because the unsaturated fatty acid can be oxidized to form free radicals and peroxides during heating, both of which can promote oxidation of cholesterol (Korytowski et al., 1992). This phenomenon is often referred to as “cooxidation”. Likewise, the stability of lipid in foods during heating may also be affected by cholesterol. Osada et al. (1993) reported that the levels of COPs in processed seafood were much higher than other food products because that fish contains a relatively higher amount of polyunsaturated fatty acids

Zhang et al. (1991) detected six COPs, 7 $\beta$ -OH, 5,6 $\beta$ -EP, 5,6 $\alpha$ -EP, triol, 7-keto and 25-OH, in French-fried potatoes, which were fried in animal-vegetable shortenings

at local restaurants. Surprisingly, triol, derived from isomeric 5,6-EP, was formed in the largest amount, followed by 7-keto and 25-OH. Since triol was rarely found in foods, this result further demonstrated that some drastic heat treatments could produce triol. This is probably because that during frying, both steam and free fatty acids are formed through water evaporation and triglyceride hydrolysis, which in turn results in the formation of triol from 5,6 $\alpha$ -EP or 5,6 $\beta$ -EP.

### **2.3.2. Antioxidants in Foods**

Generally, heat, pH, light, oxygen, water activity, and the presence of unsaturated fatty acids are the major factors that influence COPs formation during food processing or storage (Dionisi, 1998). Due to the potential health risk of consumption of COPs-containing foods, it is important to learn more about the formation and inhibition of COPs in foods during heating or illumination.

Several researchers reported that some antioxidants sufficiently inhibited cholesterol oxidation, which was induced by oxidants such as hydrogen peroxide and nitrogen oxide, or prooxidants such as metal ions (Guardiola, 1997). Rankin and Pike (1993) examined the inhibition of COPs formation by some antioxidants in an aqueous meat model system at pH 5.5 and 80 °C. Results showed that with the exception of tocopherols, rosemary oleoresin, quercetin, myricetin and BHA were not effective against 7-keto formation. Similarly, Maerker and Unruh (1986) also showed that BHT was not effective against cholesterol oxidation. However, an opposite result was reported by Madhavi (1995), who revealed that BHA, BHT, propyl gallate, tertiary butylhydroquinone and  $\alpha$ -tocopherol exhibited an inhibitory effect for cholesterol

autoxidation in an aqueous model system. This difference may be due to the variety of buffer systems and surfactants, the concentrations of antioxidants, and the presence of prooxidants.

#### **2.3.2.1. Ascorbyl Palmitate and $\alpha$ -Tocopherol**

As the mechanism of the cholesterol oxidation has been demonstrated to be similar to that of lipid oxidation (Smith, 1981), the incorporation of antioxidants to a cholesterol-containing food should retard cholesterol oxidation effectively. Park and Addis (1986) studied the effects of ascorbyl palmitate and  $\alpha$ -tocopherol on the inhibition of cholesterol oxidation in tallow during heating. Results showed that both possessed the ability of inhibiting cholesterol oxidation at 135 °C. However, at 165 and 180 °C, only a minor inhibition effect occurred. This is probably because both ascorbyl palmitate and  $\alpha$ -tocopherol may undergo degradation during heating at high temperatures. Yan and White (1990) also found that cholesterol oxidation could be inhibited by incorporation of methyl silicone into lard during heating.

The concentration of antioxidants affects the ability to decrease cholesterol oxidation as well. Tocopherols are known to delay the rate of cholesterol oxidation. Of the various isomers,  $\gamma$ - and  $\delta$ -tocopherols were more effective in inhibiting cholesterol oxidation than  $\alpha$ -tocopherol in an aqueous model system (Labuza, 1991). Some studies have also suggested that  $\alpha$ -tocopherol possesses an inhibitory effect against cholesterol autoxidation at a level of 0.02% or 0.2%, but exhibit prooxidant activity at a level of 2% (Rankin and Pike, 1993). Park and Addis (1986) reported that a combination of ascorbyl palmitate and  $\alpha$ -tocopherol could prevent the formation of COPs in tallow

heated at 135 °C for 70 hrs. In addition, this type of combination exerted a synergistic effect in protecting deep-frying fats and oils from cholesterol oxidation (91-93).

In contrast, Guardiola et al. (1997) found that the mixture of ascorbyl palmitate and  $\alpha$ -tocopherol showed a slight cooxidant effect of cholesterol oxidation during storage of spray-dried egg yolk powder. Li et al. (1996) also reported that tocopherols might significantly suppress the formation of COPs in fish oil during storage or heating. However, the tocopherol concentration may be too low to inhibit COPs formation in flax, sunflower and palm oils. It has been well established that tocopherols are effective antioxidants when used at a relatively low concentrations (100-300 ppm), however, the antioxidant activity may undergo loss at high levels (> 500 ppm) (Dougherty, 1988).

### 2.3.2.2. Antioxidant in Plant Oils

Table. 2.1 Fatty Acids Content (g/100g Fat) of Cooking Oils (Gunstone and Padley, 1997).

Plant Oil	Unsat. /Sat. ratio	Saturated			Mono unsaturated	Poly unsaturated
		Palmitic Acid C16:0	Stearic Acid C18:0	Oleic Acid C18:1	Linoleic Acid ( $\omega$ 6) C18:2	Linolenic Acid ( $\omega$ 3) C18:3
Canola Oil	15.7	4	2	62	22	10
Corn (Maize) Oil	6.7	11	2	28	58	1
Olive Oil	4.6	13	3	71	10	1
Soybean Oil	5.7	11	4	24	54	7
Rice bran Oil	13.2	16	2	41	37	1.3

As discussed above, unsaturated fatty acids can affect cholesterol oxidation. Plant oils contain high level of unsaturated fatty acids. Most vegetable oils contain high levels of oleic and linoleic acid, while palmitic acid is the major saturated acid. Table

2.1 shows the fatty acid profile of some cooking oils. Olive and canola oil has over 50% oleic acid, soybean and corn oil have more linoleic acid than oleic acid. Both fatty acids levels are high in rice bran oil (Gunstone and Padley, 1997).

The tocopherols and tocotrienols in plant oils, though in low levels, attract attention because the antioxidant activities. The tocopherols are a series of benzopyranols with one, two, or three methyl groups attached to the phenolic ring. The molecules also have a C16 side chain on the pyran ring. The saturated side chain structures are tocophreols, and three double bonds in the tocotrienols. The chemical structures of  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  tocopherols and tocotrienols show in Figure 2.6. The antioxidant activity of tocopherols are  $\delta > \gamma > \beta > \alpha$  (Sanhueza and Nieto, 2002).

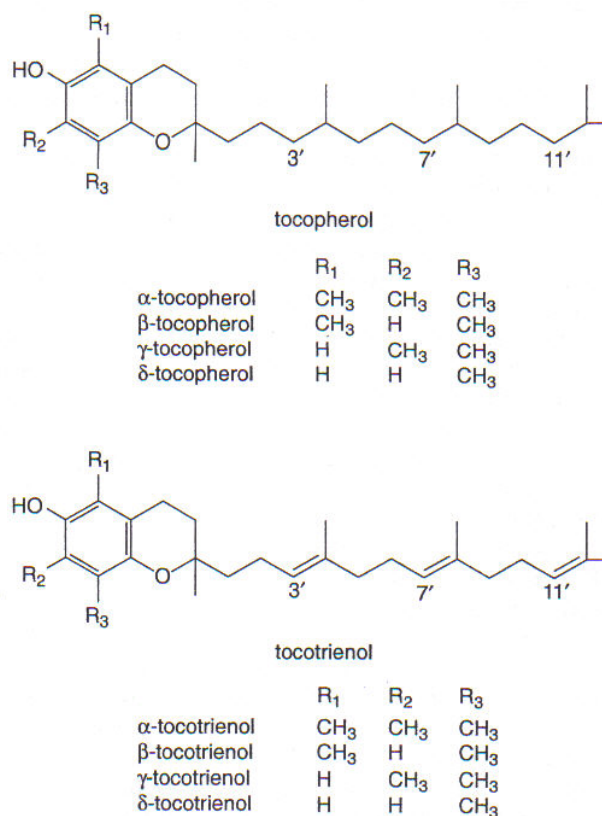


Figure 2.6. Chemical Structure of Tocopherols and Tocotrienols

Walnut oil and wheatgerm are good sources of tocopherols. Others with high vitamin E activity include sunflower, cottonseed, safflower, and palm oils. Antioxidant activity is high in soybean oil, corn oil, and walnut oil. The tocopherols are partially removed during oil refining. This reduced oxidative stability but can furnish a concentrate that can be added back. Much natural antioxidant comes from a by-product of soybean refining. Soy is now the prime source of oil. The bean contains high quality protein (38-42%) and oil (18-22%), which is usually recovered by hexane extraction (Gunstone and Padley, 1997).

#### **2.3.2.3. Phytosterol**

Phytosterol contained in vegetable oils is known to exert a hypocholesterolemic function. The antioxidant effects of phytosterol and its components, beta-sitosterol, stigmasterol, and campesterol, against lipid peroxidation were examined by making a comparison with 2,2,5,7,8-pentamethyl-6-chromanol (PMC) (Yoshida and Niki, 2003). Phytosterol also suppressed the oxidation and consumption of alpha-tocopherol. Stigmasterol accelerated the oxidation of both methyl linoleate in solution and PLPC liposomal membranes in aqueous dispersions, which was ascribed to the oxidation of allylic hydrogens at the 21- and 24-positions. The study shows that phytosterol chemically acts as an antioxidant, a modest radical scavenger, and physically as a stabilizer in the membranes (Yoshida and Niki, 2003).

#### **2.4. Fish and Fish Products**

Osada et al. (1993) reported that raw fish contains essentially no COPs, however, following air-drying, the content of COPs largely increased in sardine and squid (287

and 146 ppm). Ohshima et al. (1993) also found a high amount of COPs (9.6-138 ppm) in commercial salted and dried anchovies, Northern cod, Pacific cod, Japanese whiting and Pacific herring. These levels are probably higher than those generated in most food items. Kao and Hwang (1997) analyzed COPs in dried squid and found that  $7\alpha$ -OH,  $7\beta$ -OH, 5,6 $\beta$ -EP, 5,6 $\alpha$ -EP, 7-keto, 20 $\alpha$ -OH, 25-OH and triol were present. When dried squid was baked at 200 °C for 10 min, the cholesterol level dropped from 7300 to 6020 ppm while the total COPs level increased from 12.07 to 43.46 ppm, significantly lower than that reported by Osada et al. (1993). The loss of cholesterol was at a much larger magnitude than the formation of COPs, revealing that most of the cholesterol was degraded to other compounds. The total cholesterol level in fresh fish does not exceed that of milk or eggs but the COPs formed in processed fish are more than those formed in milk and egg products. The high content of unsaturated fatty acids in fish is the main cause of such a phenomenon.

The study of Li et al. (1996) revealed that a mixture of cholesterol and fish oil generated much more COPs than that of cholesterol and flax oil, sunflower oil or palm oil. Compared to a mixture of cholesterol and palm oil, cholesterol and fish oil produced COPs more than 30 folds over a storage period of 35 days. This result is expected, since fish oil contained fatty acid with a higher degree of unsaturation than that of palm oil. Theoretically speaking, both flax and sunflower oils also contain high levels of polyunsaturated fatty acid (> 66%) and should be prone to COPs formation. However, the amount of COPs formed in both oils were less than that in fish oil. This is probably because that fish oil contains a high amount of long-chain unsaturated fatty

acids such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), which should facilitate the oxidation rate of cholesterol. In addition, the presence of antioxidants in flax and sunflower oils, such as tocopherols, may also delay cholesterol oxidation. It has been well established that tocopherols are able to delay cholesterol oxidation and protect lipids against autoxidation at an appropriate concentration (Nawar, 1996).

## **2.5. Cholesterol Extraction and Analysis**

### **2.5.1. Saponification and Extraction**

Saponification is a vital step, which is conducted routinely to remove triglyceride, free fatty acid and water soluble impurities during extraction of COPs from foods (Finocchiaro, 1983; Park and Addis, 1986). Two saponification procedures, cold and hot saponification, are often employed. Many reports have shown that high recovery can be achieved by cold saponification at 25°C (Yan and White, 1990; Pie, 1990), and the formation of COPs artifacts can be retarded. However, the saponification time is too long (18-20 h). Thus, some authors used hot saponification (56°C) to facilitate the extraction of COPs from foods (Maerker and Uniu, 1990). Nevertheless, it has been reported that hot saponification may degrade 7-keto and isomeric epoxides to form artifacts (Peng et al. 1979).

To remedy these problems several authors used silica gel- or C18 cartridges to extract COPs from foods (Missler, 1985). Nourooz-Zadeh and Appelqvist (1988) employed a Sep-Pak silica cartridge to enrich COPs. The cartridge was washed with hexane-1,2-dichloroethane (9:1, v/v) to remove triacylglycerols, followed by 1,2-dichloroethane-methanol (1:1, v/v) to elute COPs and polar lipids. This method was

rapid in removing excessive amount of lipids and the recoveries of both radio labeled 3H-cholesterol and 3H-triol reached 90% and 97%, respectively. Therefore, the method is suitable for lipid-rich products such as milk powders. Hwang and Maerker (1993) applied a solid phase extraction (SPE) column to extract COPs from meats. The lipid extract was loaded in the column and eluted serially with hexane-ethyl acetate of 100:2 (v/v), 85:15 (v/v) and 4:6 (v/v), respectively. The first fraction contained triacylglycerol and lipid-soluble impurities, while the second fraction contained 4-cholesten-3-one, 4, 6-cholestadien-3-one and 4-cholestene-3,6-dione. The other COPs, including  $\alpha$ -EP,  $\beta$ -EP and 7-keto, were eluted in the third fraction.

Penazzi et al. (1995) used an SPE florisil cartridge to purify 7-keto in several foods. The sample-loaded cartridge was washed with 2-propanol-heptane (2:98, v/v) to remove triacylglycerols and cholesterol, and 7-keto was eventually recovered by acetone. Also, the authors used an SPE silica cartridge to isolate 7-keto from the same food items. The cartridge was first washed by hexane-diethyl ether (8:2, v/v) to remove impurities, followed by elution of 7-keto by two solvents, hexane-diethyl ether (1:1, v/v) and methanol (100%). The last two fractions containing enriched 7-keto were pooled for further analysis. The application of an SPE cartridge has been considered an easier and faster method than the cold saponification method (Penazzi et al, 1995).

### **2.5.2. HPLC Analysis**

HPLC is one of the most widely used techniques for separation of COPs and has been shown to elute COPs effectively (Rose-Sallin, 1995; Fontana, 1992). Both reversed- and normal-phase columns have been applied to the separation of COPs.

The reversed-phase column is currently used more often than the normalphase column. Ansari and Smith (1979) developed two HPLC methods using a silica gel or a C18 column, respectively, to resolve 10 COPs by using an isocratic solvent system of hexane-isopropanol (24:1, v/v) or acetonitrile-water (9:1, v/v), with detection at 212 nm. A C18 column provides a better resolution than a silica gel column.

Tsai and Hudson (1981) also developed a HPLC method to resolve 10 COPs by using a binary solvent system of hexane-2-propanol (100:3, v/v), with a flow rate at 3.0 ml/min and by refractive index detection (RI). Separation is complete within 28 min., however, several COPs are not adequately resolved. Tsai and Hudson (1984) used HPLC to purify COPs in egg yolk by employing a solvent system of 2-propanol-hexane (2:98, v/v), with RI detection and flow rate 1 ml/min. Two COPs, 5,6 $\alpha$ -EP and 5,6 $\beta$ -EP, are adequately resolved, however, the separation time (50 min.) is too long. Kou and Holmes (1985) further used a reversed-phase HPLC column to purify 25-OH, followed by quantitation on a silicic acid column, with detection at 205 nm and flow rate at 1.7 ml/min. The authors reported that the application of two columns is necessary to obtain the consistent baseline resolution of 25-OH from the other contaminating peaks. Chen and Chen (1994) evaluated both CN- and C18-columns for the separation of COPs. Hexane-2-propanol (95:5, v/v) was used as the mobile phase for the former, which resolved 8 COPs standards within 18 min.

### **2.5.3. GC Analysis**

Numerous GC methods have been developed to separate the various COPs in foods (Zubillaga, 1991; Nourooz-Zadeh, 1990). The combination of GC and the flame

ionization detector (FID) provides a powerful tool to precisely quantify COPs. For cholesterol and COPs analyses, the conversion of these compounds to trimethylsilyl (TMS) ether derivatives is a frequently applied step to stabilize some of the diols (Maerker, 1987). Li et al. (1996) separated cholesterol and 8 COPs with good resolution by using a fused silica capillary (dimethyl polysiloxane) column. With the help of the purified standards, they identified and quantified several COPs in baked dried squid. The application of GC-MS with a selected ion monitoring mode (SIM) and a capillary column is generally regarded as the most rapid and sensitive mean to identify COPs (Parks and Addis 1992).

By comparing the mass spectrum with the established chemical library, COPs can be easily identified with high accuracy. Park and Addis (1986) used a GC-MS with a fused silica capillary column to identify and quantify  $7\beta$ -OH, 25-OH,  $5,6\alpha$ -EP,  $5,6\beta$ -EP, 7-keto and triol in heated tallow. Ohshima et al. (1993) analyzed COPs in fish products by conversion of them to TMS-derivatives and subsequent separation and detection by GC with a flame ionization detector (GC-FID). The identification of these COPs was carried out in a quadruple MS fitted with an electron ionization source, and six COPs,  $7\beta$ -OH, 7-keto,  $5,6\alpha$ -EP,  $5,6\beta$ -EP, 25-OH and triol were quantified.

Schmarr et al. (1996) determined COPs in several food products using GC-FID and GC-MS, and a high recovery was found for the moderate polar and polar oxysterols, however, the less polar COPs may undergo partial loss by this method. The identified COPs include  $7\alpha$ -OH,  $7\beta$ -OH, 19-OH,  $20\beta$ -OH, 25-OH,  $5,6\alpha$ -EP,  $5,6\beta$ -EP, 7-keto, 3,5-dihydroxy- $5\alpha$ -cholestan-6-one and triol. Among these COPs, some (19-OH and

20 $\beta$ -OH) were rarely identified by HPLC. Due to the chemical properties of COPs, most of the GC columns used are with low polarity.

#### **2.5.4. Comparison of HPLC and GC-MS**

HPLC and GC-MS are the most frequently used methods for COPs analysis. HPLC is often carried out under ambient temperatures while GC is under high temperatures. Although the resolution power of HPLC is theoretically inferior to that of GC, the former can provide an ideal means for sample recovery and purification. Meanwhile, HPLC can simplify the quantification procedure, shorten the analysis time and introduce fewer artifacts (Tsai and Hudson, 1981). HPLC is commonly equipped with an UV detector to monitor COPs, however, some double bond-free COPs such as the isomeric 5,6-EP and triol can not be detected.

The narrow absorbance range of most COPs may result in interference with detection as well as limitation of the choice of solvents. In addition, the production of solvent waste by HPLC analysis poses a disposal problem. On the other hand, GC can be used to resolve geometric isomers, which can not be separated by HPLC. As mentioned, the combination of GC and MS is an effective tool for COPs analysis, which includes not only quantification but also identification. The mass spectrums derived from GC-MS assist elucidation of the chemical structures of COPs and further contribute to their identification. However, the major drawback of GC is that it may also thermally destroy cholesterol and B-ring hydroperoxide to form artifacts (Finocchiaro, 1984). In addition, COPs need a derivatization step for GC separation, which extend the analysis time and affect the quantization accuracy.

## **Chapter 3 Methodology**

### **3.1 Cholesterol and Cholesterol Oxidation Products Analysis Using GC-MS**

#### **3.1.1. GC-MS Operating Condition**

The cholesterol and cholesterol oxidation products in cholesterol and salmon models were determined using a GC-MS system. The GC-MS system consisted of Varian CP 3800 GC and Saturn 2000 MS (Varian, Inc., Walnut Creek, CA) with a SAC-5 fused silica capillary column (30m x 0.25 mm x 0.25 mm film thickness) (Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas at flow rate of 2ml/min. The injection port temperature was set at 300°C. The GC oven temperature was increased by 15°C/min to temperature 250°C from initial temperature of 200°C. The MS detector was turned on after 4 min of running time. The split ratio was set at 1:20. Before injecting sample, water/air level in the GC-MS system was checked to below 20%.

#### **3.1.3. Determination of Cholesterol and Cholesterol Oxidation Products**

##### **3.1.3.1. Identification**

Cholesterol and cholesterol oxidation products in samples were identified by matching the retention times and typical ion spectrums with their standards. The retention time and ion spectrum of each compound was obtained by injecting standard mixture into the GC-MS system. The standard mixture solution consisted of 5 $\alpha$ -cholestane (internal standards), cholesterol, and 7-ketocholesterol. These compounds in unknown samples were identified by matching their retention times with the standard compounds. Because the components in nature food systems were much

more complex than the compounds in cholesterol model, other compounds may have similar retention time interfere the interested compounds. These interferences were eliminated by using selective ion method. The MS fingerprint of 5 $\alpha$ -cholestane, cholesterol, and 7-ketocholesterol that was shown in Figure 3.1. A typical ion of 5 $\alpha$ -cholestane was 218 that of cholesterol and ketocholesterol were 386 and 400, respectively. These ions are unique for each compound, and were used for quantifying cholesterol and cholesterol oxidation products to get reliable results.

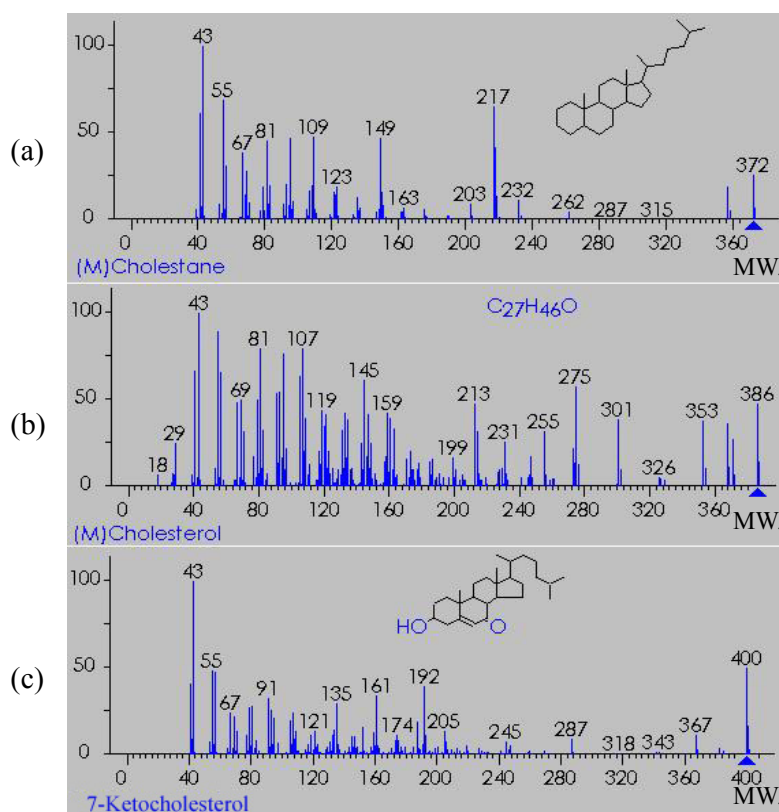


Figure 3.1 Mass-Spectrum Fingerprint of (a) 5 $\alpha$ -Cholestane, (b) Cholesterol, and (c) 7-Ketocholesterol

### 3.1.3.2. Standard Curves and Quantification

5 $\alpha$ -Cholestane was used as an internal standard. Standard curves of 5 $\alpha$ -cholestane, cholesterol, and 7-ketocholesterol were made to determine the concentration of cholesterol and 7-ketocholesterol in control and treated samples.

Table 3.1 The Amount of Injection Used to Make Standard Curves of 5A-Cholestane, Cholesterol and 7-Ketocholesterol

	Amount of Injection (10 <sup>-3</sup> $\mu$ g)	Solution Concentration (ppm)
5 $\alpha$ -cholestane	1	1
	2	2
	5	5
	10	10
	20	20
cholesterol	20	20
	100	100
	200	200
	500	500
7-ketocholesterol	20	20
	100	100
	500	500

Because the injection volume for GC analysis was very small, a little inconsistency in injection volume from sample would result a great variety. Also, the evaporating rate in injection port of each injection may not be exactly the same. By comparing the resulting concentration of internal standard (5 $\alpha$ -cholestane) to that of the added known quantity concentration, an injection corrective coefficient could be obtained. This coefficient was used to adjust the injection inconsistent error and obtain more reliable results for other interested compounds. Table 3.1 showed the amount of

injection to make each standard curve at the injection volume of 1 $\mu$ g. Concentration and –peak height standard curves of the three compounds were made.

## **3.2. Cholesterol Oxidation Model**

### **3.2.1. Stock Solution**

One g of 5 $\alpha$ -cholestane was weighed and diluted by hexane in a 1000ml volumetric flask. Cholesterol stock solution was made by dissolving 5.00 g standard cholesterol sample in 1000 ml hexane in a volumetric flask. The concentrations of stock solutions were 1000 ppm for 5 $\alpha$ -cholestane and 5000 ppm for cholesterol. The stock solutions were stored in dark at 4 $^{\circ}$ C before use. The sample solutions used for cholesterol oxidation model were diluted from the stock solution.

### **3.2.2. Cholesterol Oxidation Test Tube Preparation**

Cholesterol solution was made from the cholesterol stock solution by diluting 1 ml of the stock solution in hexane to a 100 ml. One ml of cholesterol solution was added into a 25 ml test tube. The hexane in the test tube was evaporated under vacuum by a CentriVap Mobil System (Labconco, Kansas City, MO). Thus, 50  $\mu$ g of cholesterol was coating on the bottom of the test tube uniformly. The test tube coating with cholesterol could be heated directly or mixed with other substance to monitor the degree of the cholesterol oxidation during heating.

Vacuum evaporating under was fast and safe way to dry and coat cholesterol in the test tube. CentriVip system consists of a centrifugation and a vacuume. Centrifugal force with heat and vacuum are used to rapidly dry samples. A vacuum was applied in the chamber to lower solvent boiling point to a low temperature, the lower temperature was avoid to change the sample properties. Use centrifugation eliminates bumping

caused by vacuum and concentrates the solute in the bottom of the tube. This allows total solutes to be recovered during the solvent evaporation. The evaporated solvent was trapped into a container placed in a low temperature chamber. The trapped solvent could be reused if necessary.

### 3.2.3. Heating Conditions

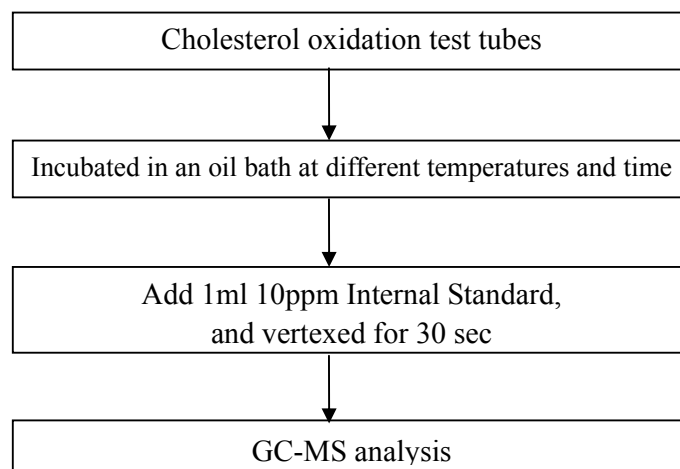


Figure 3.2 Procedures of Preparing Cholesterol Oxidation Test Sample During Heating

Figure 3.2 shows method to prepare sample of cholesterol oxidation model. The cholesterol oxidation test tubes were incubated into an oil bath at different temperatures and times. The incubated temperature and time are shown in the Table 3.2.

Table 3.2 Cholesterol sample heating times and temperatures

Heating Temperature (°C)	Heating Time (min)
125, 150, 175, 200	10
	20
	30

After the test tubes were cooled down, 1 ml of internal standard (10 ppm of 5 $\alpha$ -cholestane) was added into the test tube. The tube was vortexed for 30 sec to

dissolve the cholesterol and cholesterol oxidation products produced during heating, and mixed them with the internal standard solution well. One  $\mu\text{l}$  of this solution was injected into the GC-MS system for determining the cholesterol and cholesterol oxidation products.

### 3.3 Lab Prepared Soybean or Rice Bran Oil

Soybean and rice bran oil were made in our lab in order to test their capability of preventing the cholesterol oxidation. These oils could contain compounds having antioxidation removed during refining commercial oils, such as purification, clarification, winterization, and deodorization, and so on. Figure 3.3 is the flow chart of preparing lab made soybean or rice bran oil.

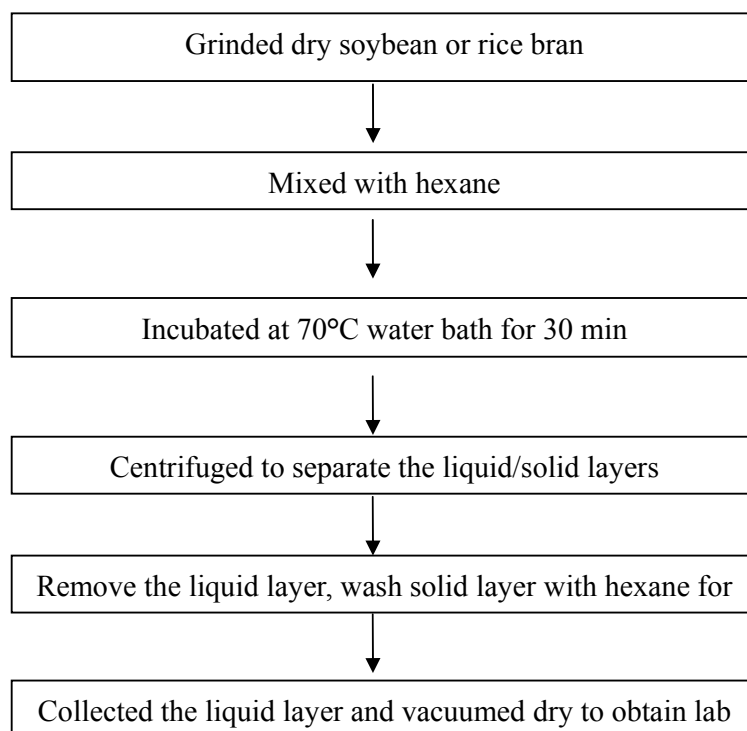


Figure 3.3 Flowchart of Preparing Lab Made Soybean and Rice Bran Oil

Soybean and rice bran was dried prior to solvent extraction, because many organic solvents could not easily penetrate into foods containing high level of water and result in inefficient. Dried samples were finely grinded to produce a more homogeneous sample and to increase the surface area of lipid exposed to extraction as well. One hundred g of grinded soybean or rice bran was mixed with 500 ml hexane. The mixture was incubated into a water bath at 70 °C and shock for 30 min. The flask that contained grinded soybean/rice bran and hexane was shaken vigorously during extraction so the organic solvent and aqueous phase were allowed to separate. After the mixture was cooled down, it was centrifuged by a Hermle Labortechnik Centrifuge (Wehingen, German) at 4000 rpm for 10 min to separate the hexane layer from solid phases. The hexane layer was then transferred out to a clean 500 ml flask. This procedure was repeated another two times with 100 ml of fresh hexane to improve the efficiency of the extraction. All the solvent fractions were collected together. The lab prepared soybean and rice bran oil were obtained after all hexane was evaporated by vacuum drying. The CentriVap Mobile System was used to evaporate hexane undervacuum.

#### **3.4. Treatments Groups with Various Cooking Oils**

Figure 3.4 shows the procedures of preparing cholesterol oxidation samples mixing with different cooking oils during heating. One ml of cook oil was dissolved in 100 ml of hexane to oil solution. Then 1 ml of the oil solution was added into the cholesterol oxidation test tubes. The solution of cooking oil and cholesterol were vortexed for 30 second to mix cholesterol and oil well. The homogenous mixture in the

test tube was dried by evaporating under vacuum at 30°C. The test tubes were then incubated in the oil bath at 150°C and 175°C for 10min, 20min, and 30min, respectively. One ml of 10 ppm cholestane solution was added into the tubes as internal standard after the test tubes were cooled down. The solution of cholesterol, cooking oil, and internal standard was vortexed for 30 second, 1 µl of this solution was injected into the GC-MS system.

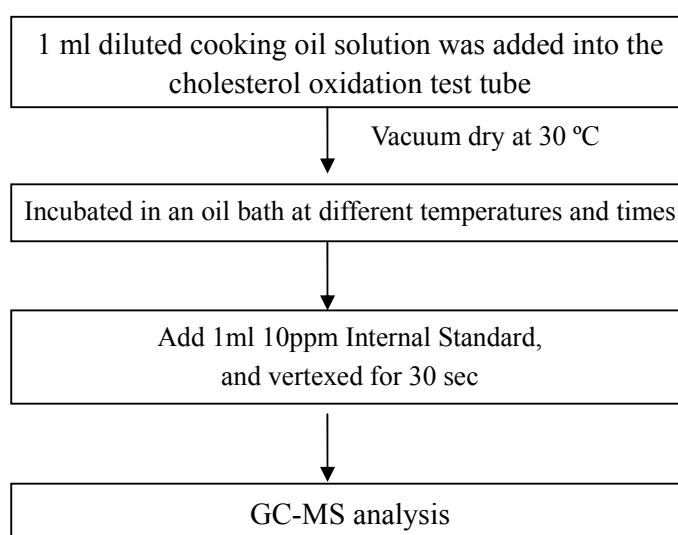


Figure 3.4 Procedures of Preparing Cholesterol Oxidation Samples with Different Cooking Oils During Heating

Temperatures of 125°C and 200°C were not selected in cholesterol oxidation with cooking oil study. According to the results was obtained from the cholesterol oxidation model, at 125°C, the cholesterol degradation was not as significant. At 200°C, the cholesterol was oxidized too fast. The differences after 10 min of heating times at 200°C were not significant.

### **3.5. Cholesterol Oxidation in Salmon Fish Meat with and Without Cooking Oils**

#### **3.5.1. Salmon Sample Preparation**

Salmon fish fillet were bought from local market. The fillet skin was removed. The salmon fish meat was homogenized to avoid cholesterol level variable due to the different lipid contents in different part of the fish fillet. For the salmon fish meat without adding cooking oil (control group), each sample contained 3.0 g of salmon sample. For the salmon fish meat with cooking oil (the treatment group), 0.3 g of rice bran oil or lab made soybean oil was mixed with 3 g of salmon samples evenly to prevent the formation of oil coat. The salmon meat of control and treatment were pressed to form approximate 5 mm thickness and 25 mm diameter when heating.

#### **3.5.2. Moisture Content**

Ten grams of fresh salmon fish was weighted and put into the 55°C oven for 24 hours. The sample was spreaded as uniformly thin as possible. The salmon sample was weighted again after heating (ASTM).

The moisture content =  $100\% \times (\text{Original weight} - \text{Dried weight}) / \text{Original weight}$

#### **3.5.3. Roasting Control and Treatment of Salmon Fish Meat**

The control and treatment salmon samples were put into kitchen oven (Hotpoint automatic oven) the temperature and time indicated in the Table 3.3. Normal roasting temperatures for cooking salmon were selected for heating control and treatment samples. Generally, the roasting time very from 325°F to 425°F, and the cooking times are from 12 min to 25 min. Although the temperature of 200°C was not selected in cholesterol oxidation with cooking oils model due to the cholesterol degraded too fast. The differences after 10 min heating at 200°C were not significant. However, the

Table 3.3 Control and Treated Salmon Sample Heating Times and Temperatures

Heating Temperature (°C)	Heating Temperature (°F)	Heating Time (min)
150	305	10
		20
		30
175	347	10
		20
		30
200	394	10
		20
		30

temperature was selected in salmon study because not only it was widely used in cooking salmon, but also, with the protection of sample salmon meat, the cholesterol oxidation in salmon sample would not as fast as that of the cholesterol oxidation model. So the differences among the heating time would be enlarged.

#### 3.5.4. Oil Extraction from Salmon Samples

Procedures of extraction cholesterol from salmon samples is shown in Figure 3.5. The control, treatment, and fresh salmon samples were homogenized using Ultra-Turrax T8 (IKA-Werke, Wilmington, NC). Then each salmon sample was put into a 25 ml test tube and mixed with 9 ml of chloroform/methanol 2:1 (v/v) solution. Four ml of internal standard (10 ppm of 5 $\alpha$ -cholestane) was added to the mixture for obtaining extraction and injection efficiency coefficient. The test tube was vortexed for 30 seconds. After centrifugation, there were three layers in the test tube. The upper liquid layer was methanol and remained water in the salmon sample. The middle layer was the solid layer, which was salmon meat residual. The lower layer was the chloroform solution with lipids, such as, triacylglycerols, diacylglycerols,

monoacylglycerols, free fatty acids, phospholipids, carotenoids, vitamins, cholesterol and cholesterol oxidation products. The salmon meat fractions were almost the same density with chloroform solution; it was not easy to separate them completely with centrifugation. So the lower layer was further filtered by a funnel under vacuum. Pure salmon oil was obtained by evaporating the solvent under vacuum. The extracted oil was weighted to calculate the extraction yield.

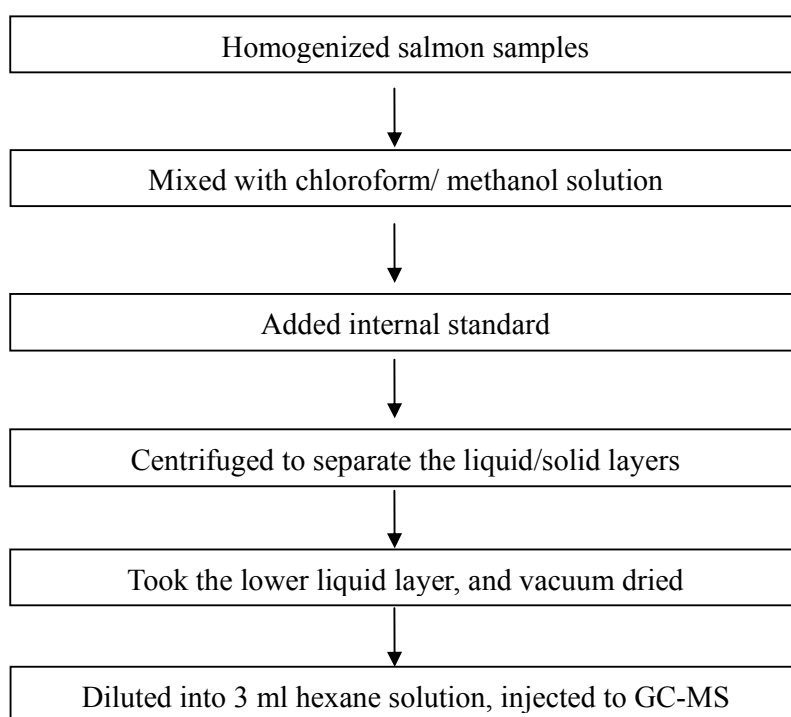


Figure 3.5 Procedures of Extraction Cholesterol from Salmon Samples

### 3.6. Vitamin E Analysis Using HPLC

One hundred mg cooking and salmon oil was weighed, and diluted into 1 ml with hexane. The oil solutions were transfer into 2 ml HPLC vials for HPLC analysis. The concentrations of Vitamin E in different cooking oils and salmon samples were determined by HPLC system. The system consisted a Waters 510 HPLC pump,

Waters 717 Plus Autosampler, Discovery C18 HPLC column (25 cm x 3 mm, 5  $\mu$ m) (Supelco Inc., Bellefonte, PA), and Waters 474 Scanning Fluorescence Detector. The mobile phase was a mixture solution of 0.8% acetate acid, 0.8% ethyl acetate, and 98.4% of hexane, and the flow rate was 2 ml/min. The mobile phase solution was degassed with very low pressure of helium before pumped into the HPLC system. The sample injection volume was 25  $\mu$ l. The HPLC system without the column was first flashed using methanol for 20 min to eliminate any contamination in the system. After flashing, the mobile phase was pumped into the system without column to get rid of the methanol in the system. The column was connected after 10 min of flushing with mobile phase. Disconnection the column was to prevent washing solution entering the column, thus would largely increase the balance time. Equinity the column with mobile phase for 30 min to fill the whole column with mobile phase. This could be monitored on the output from the computer that connected with the detector. A straight base line indicated the finishing of balance.

### **3.7. Statistical Analysis**

Each of control and treatment groups was replicated three times at the same heating temperature and time. T test procedure (Excel Data Analysis, Microsoft Inc., Seattle, WA) was used to compare the loss of cholesterol and production of 7-ketocholesterol between control and each treatment group. Significant difference among means was considered at  $P < 0.05$ .

## Chapter 4 Results and Discussions

### 4.1. Cholesterol and Cholesterol Oxidation Products Analysis Method

#### 4.1.1. GC Column Selection for the Cholesterol Analysis

Column selection is based on five primary factors: sample, stationary phase type, column ID, stationary phase film thickness, and column length. The stationary phase is a polymeric film coated on the inner wall of the capillary column. Differences of the chemical and physical properties of injected organic compounds and their interactions with the stationary phase are the very critical principles for the successful separation. Retention time is that how long the time of analyzed compound is retained in the column. The most important characteristic of stationary phase is polarity, because it dictates selectivity, or the ability of the column to separate sample components. A nonpolar column is best for analyses of nonpolar compounds. Interactions between nonpolar compounds and a nonpolar phase are dispersive, thus separation is based exclusively on the boiling points of the molecules (Supelco, 1999; Restek, 2004). Cholesterol and most of its COPs are low polarity. A fused silica column with coating film of poly (5% diphenyl/95% dimethylsiloxane) was selected in this study. The tolerance temperature limits of this column is -60°C to 320°C, which is high enough be used in the reproducible analyses of plant sterols, cholesterol, and COPs. A Rtx-5 (phenyl/ methylsiloxane) column was used by Echarte (2001) and Al-Saghir et al. (2004) to test cholesterol and COPs. However, chemical derivatization was needed to apply to the cholesterol samples before injection.

When selecting the capillary column inside diameter (ID), two factors need to be considered: efficiency (number of theoretical plates) and sample capacity (the amount of any one sample component that can be applied to the column without causing an overload). Narrower columns (0.10mm-0.32mm ID) provide the best resolution, while wider columns (0.53mm and 0.75mm ID) provide the greatest sample capacity (VWR International). In our study, the efficiency of the column is much more important, so a narrow column with 0.25 mm ID was selected. As the concentration of the cholesterol sample in this study was low and the injection volume was small, the overloading problem for the narrow column could be avoided.

#### **4.1.2. GC-MS Operating Conditions**

Factors considered for a GC operation include sample concentration, injection volume, column temperature, and flow rate of carrier gas. The optimal parameters were developed after numeric trials of standard and actual samples. Figure 4.1 is the chromatogram of 5-cholestane, cholesterol, and 7-ketoncholesterol using the optimized operation condition. The retention time of 5 $\alpha$ -cholestane, cholesterol, and 7-ketoncholesterol was 7.024min, 9.998min, and 14.986 min, respectively. Narrow peaks of each component without overlap were obtained, the differences of retention time (resolution) among the components were big enough. The peak of the highest component of the sample (cholesterol) was not much different from that of the internal standard.

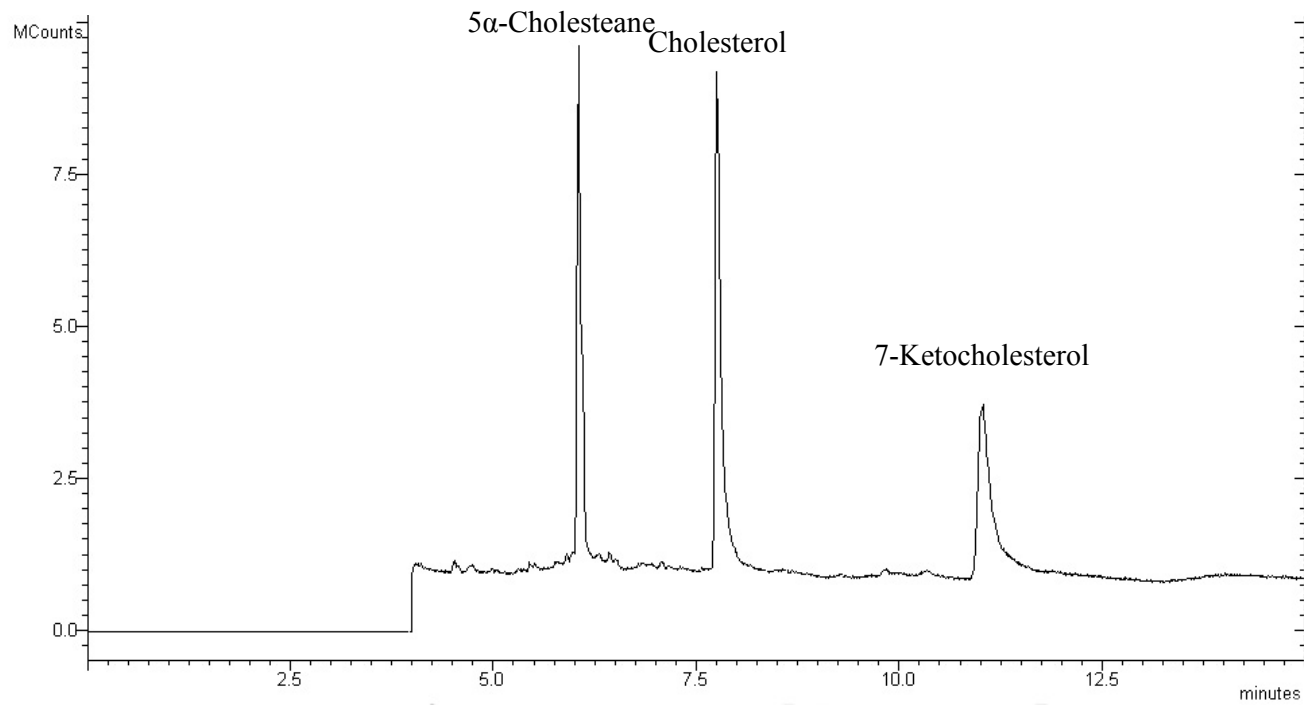


Figure 4.1 GC-MS Chromatogram of the Standard Mixture of 5 $\alpha$ -Cholesteane (Internal Standard), Cholesterol, and 7-Ketocholesterol

The concentration of the sample was selected. The sample could not be vaporized completely at the injection port, if the concentration was too high. The unvaporized sample was not sent into the column and resulted low injection efficiency. The left over sample was carbonized by the high temperature; the carbonized compounds could stick on the injection port, and cause contaminations. In case, the peak heights of sample solutions at different concentrations were not linear. Injection volume has to be increased to make the peaks detectable and analyzable if the sample concentration was too low. Increasing injection volume might reduce the vaporization efficiency and cause over load of the injection port and. In this study, when the injection volume is 1  $\mu\text{l}$ , the suitable concentration of internal standard (5 $\alpha$ -cholestane), cholesterol, and 7-ketocholesterol was 10 ppm, 50 ppm, and 100 ppm, respectively.

The column temperature was optimized when sensitivity and resolution of a complex mixture was achieved in the shortest possible run time. Several temperature programs were tested in this study. The condition for the highest sensitivity was: the GC oven temperature was increased by 15 $^{\circ}\text{C}/\text{min}$  to a final temperature of 250 $^{\circ}\text{C}$  from the initial temperature of 200 $^{\circ}\text{C}$ . The detector was closed at the first 2.5 min to avoid the solvent peak. Too much amount of ions went into the detector, such as the solvent, would reduce the life time of the detector. Because the flow rate of carrier gas could be changed by increasing column temperature, the pressure control is set up to ensure the constant flow rate.

#### **4.1.3. Other GC Method**

Chemical derivatization was done in sample preparation in Fletours et al. (2000)'s study. The reason for chemical derivatization is to impart nonvolatility to volatile compounds. The low volatility may result from the size of the molecule and the resultant large dispersion forces holding the molecule together. Higher molecules may

have a low volatility due to the strong intermolecular attractions between polar groups. In the latter case, masking the polar groups or modifying polar by chemical derivatization can dramatically increase the volatility. However, yield of chemical derivatization could be lower for some compounds. It could lower the sensitivity of GC analysis. With the operating conditions used in this study, the cholesterol and cholesterol oxidation product could be separated very well and determined without using chemical derivatization by using the GC column as shown in Figure 4.1.

A fused silica capillary column (15 m x 0.32 mm id), coated with SPB-1 with higher 1.0-mm film thickness, was used in Fletours et al. (2000)'s study. The drawbacks of increasing the film thickness include, reducing column efficiency, increasing retention times and the temperature at which a sample component will elute from the column, and reducing the upper temperature limit (Supelco, 1999). A shorter column also has less resolution than a longer column. Furthermore, as a chemical derivatization is needed in their study, the sample preparation for GC analysis was more complicated.

#### **4.1.3.2. TLC Method**

Thin-layer chromatography (TLC) has been used previously for separation of COPs (Herian and Lee, 1985; Lube et al., 1986). Although TLC can readily separate some side-chain and B-ring hydroxycholesterols, the separation of cholesterol hydroperoxides has been difficult (Teng et al. 1973). TLC is used mainly to separate and determine the concentration of different types of lipid groups in foods, e.g. triacylglycerols, diacylglycerols, monoacylglycerols, cholesterol, cholesterol oxides and phospholipids. This procedure is inexpensive and allows rapid analysis of and is popular in testing cholesterol and COPs (Maerker and Bunick, 1986). TLC can be used to confirm the identity of COPs based on their distinctive color development after

spraying with sulfuric acid and observation under UV light (Maerker and Bunick, 1986). The disadvantage of the TLC technique is that the resolution is low and precise orientation of the separated components on the plate may vary from one run to another. No standard sample can be used to appreciate the variations.

#### **4.1.3.3. HPLC Method**

HPLC is one of the most powerful analytical procedures for separating and analyzing the lipids. It has been used to determine cholesterol and its oxidation products (Teng, 1991; Chen, 1994). Compared to TLC, it allows detection and determination of a larger range of compounds at higher sensitivity. The HPLC method shows very good separation power and selectivity for cholesterol and COPs. Kou and Holmes (1985) used a reversed-phase HPLC column to purify 25-OH, followed by quantization on a silicic acid column, with detection at 205 nm and flow rate at 1.7 ml/min. The authors reported that the application of two columns is necessary to obtain the consistent baseline resolution of 25-OH from the other contaminating peaks. The major disadvantages of HPLC methods are the low sensitivity for cholesterol and COPs, and running time is relatively longer. The concentration needed for HPLC is at least 1000 ppm (Chen, 1994). That study also evaluated C18-columns for the separation of COPs. A gradient system of acetonitrile-methanol in various proportions was used within 60 min running time.

#### **4.1.3.4. Advantages of Our GC-MS Method**

GC can be used to resolve geometric isomers, which can not be separated by HPLC. The combination of GC and MS is an effective tool for COPs analysis, which includes not only quantification but also identification. The mass spectrums obtained from GC-MS assist elucidation of the chemical structures of COPs and further contribute to their identification. The MS fingerprint is shown in Figure 3.1. The advantages of our

method including simple sample preparation (a chemical derivatization is not necessary), higher sensitivity and resolution, and could select ion for quantification in case of overlap problem to interested peaks.

#### 4.1.4. Standard Curves

The standard curves of 5 $\alpha$ -cholestane, cholesterol, and 7-ketocholesterol was  $y=215.89x$  ( $R^2 = 0.9424$ ),  $y=12.839x$  ( $R^2 = 0.9945$ ), and  $y=76.03x$  ( $R^2 = 0.9228$ ), respectively. The typical ion of cholesterol (386) was also picked to make standard curve,  $y=1.0642x$  ( $R^2 = 0.9712$ ). For each injection, the real concentration of the internal standard in that specific injection was calculated by using the peak height of the 5 $\alpha$ -cholestane and its standard curve. This calculated concentration was then divided by the solution concentration (10 ppm), so the ratio of the amount that really injected into the GC column to the amount of injection was obtained. The injection ratio was later multiplied to the concentrations of cholesterol and other COPs obtained from their standard curves, to get the true concentration of these compounds.

For example, a concentration- peak height standard curves of internal standard and cholesterol are:

$$\text{Height}_{\text{IS}} = 215.89 \times \text{Conc}_{\text{IS}}$$

$$\text{Height}_{\text{chol}} = 12.839 \times \text{Conc}_{\text{chol}}$$

An unknown cholesterol oxidation sample with the internal standard concentration of 10 ppm is injected into the GC system. The peak height of internal standard and cholesterol is 1960 KCount and 540 KCount. According the standard curve of internal standard, the true internal standard concentration should be

$$1960/215.89 = 9.08 \text{ ppm.}$$

So the injection corrective coefficient is

$$100 \times 9.08/10 = 90.8\%.$$

The peak height of cholesterol is corrected to

$$540 \times 90.8\% = 485.2 \text{ KCount.}$$

So the cholesterol concentration of the unknown sample is

$$485.2/12.839 = 37.7 \text{ ppm.}$$

## **4.2. Oil Extraction**

### **4.2.1. Extraction Yield**

An ideal solvent for extraction would completely extract all interested components from a food, while leaving all other components behind. In practice, the efficiency of solvent extraction depends on the polarity of the interested compound compared to the polarity of the solvent. Polar lipids, such as glycolipids or phospholipids, are more soluble in polar solvents, such as alcohols, than in non-polar solvents, such as hexane. Different compounds have different polarities means that it is impossible to select a single organic solvent to extract them all. Thus extraction yield of different lipids, such as triglyceride and cholesterol, depends on the polarity of the organic solvent used to in extraction: the total lipid/cholesterol content determined using one solvent may be different from that determined using another solvent. In addition, a solvent should also have a relatively low boiling point, so that it can easily be removed by evaporation (Gunstone and Padley, 1997). Ethyl ether and petroleum ether are the most commonly used solvents, but pentane and hexane are also used for some foods. Chloroform is a popular solvent, particularly for compounds of intermediate polarity such as cholesterol and most COPs. Hexane is a good solvent only for lipids of low polarity. We used hexane to extract soybean and rice bran oil, so some antioxidant, such as Vitamin E. Phytochemicals could be extracted along with lipid. The salmon oil was extracted by chloroform/methanol solution, which was more able to extract on cholesterol and cholesterol oxidation products.

The extraction rates of soybean and rice bran oil were 19.2% and 18.34%, respectively. Soybean contains appropriately 18%-21% lipid that varies from species (Erickson, 1980). Rice bran which is obtained as a by-product in the milling of brown rice kernel to yield the familiar white rice, has an oil content that varies from 12 to 23 %, depending on variety of rice and degree of milling (Gupta, 1989; Saunders, 1986). Amarasinghe and Gangodavilage (2002) extracted rice bran oil with the extraction ratio of 16.7%.

#### **4.2.2. Comparison of Lab Prepared and Commercial Oils**

##### **4.2.2.1. Color, Physical and Chemical Differences**

The color and viscosity differences between the commercial and lab prepared oils were compared by visual observation. The commercial oils had bright and light yellow color. The oils were very clear and semi-translucent. The lab prepared oils had darker yellow color. They were not as clear and translucent as commercial oils, and not as fluid as commercial oil at room temperature. Because lab prepared oil also contains triglycerides, phospholipids, phenolic compounds, and phytosterols. The other compounds increased the viscosity of lab oils and made the oils darker color.

##### **4.2.2.2. Industry Process of Refining Oil**

Oil is first degummed by a more aggressive medium, such as citric or phosphoric acid to hydrate the phosphatides, and permit their separation from the oil. The next step is neutralization with sodium hydroxide. The primary objective of the neutralization is to remove the free fatty acids in the crude oil. These two procedures may destroy some phenolic compounds by reacting with acid or alkyl. Neutral refined oil is then washed with hot soft water and dried under a moderate vacuum at an oil temperature of 90-100°C. The oil is then bleached by bleaching clays, which are naturally occurring bentonite and montmorillonite clays. Bleaching clays can also remove trace metals and

phosphatides in the oil. The final step of refining is deodorization, which is a steam distillation process that is carried out at high temperature (200-260°C). This step removes partial of natural tocopherol and tocotrienol antioxidants (Norris, 1985). These compounds may also be reduced by thermal degradation during the refining procedure (Gunstone and Padley, 1997).

The phenolic compounds in the lab prepared oil may have antioxidant function and reinforce other antioxidants in the oil to inhibit the cholesterol oxidation during heating (Karakaya et al., 2001; Fukumoto and Mazza, 2000). Processing removes over 30% of the vitamin E. In the lab prepared oils, phenolic compounds, which are mostly removed during the bleaching step of commercial oil refining, were most likely retained. Thus, the loss of antioxidants during oil refining may also be a critical factor for those oils having lower capability in preventing cholesterol oxidation. In this study, the level of 7-ketocholesterol in the lab prepared rice bran oil and soybean oil treatment is much lower than that of commercial rice bran oil and soybean oil treatment, respectively (Figure 4.6 and 4.7). The tocopherols and tocotrienols in the commercial and lab prepared soybean and rice bran oils were tested by the HPLC and will be discussed below.

### **4.3. Cholesterol Oxidation Model**

#### **4.3.1. Cholesterol Oxidation**

Percentages of retained cholesterol during heating at different temperatures and times were depicted in Figure 4.2. Less than 30% of cholesterol was lost after 30 min of heating at 125°C. At 150°C heating temperature, the loss of cholesterol increased to 70% after 30 min. This was in agreement with the result reported by Chien et al. (1998), where 64.8% of cholesterol was lost at the same heating temperature (150°C) for 30 min. At 175°C heating temperature, the losses of cholesterol were 61, 87, and 88% at 10, 20, and 30 min, respectively.

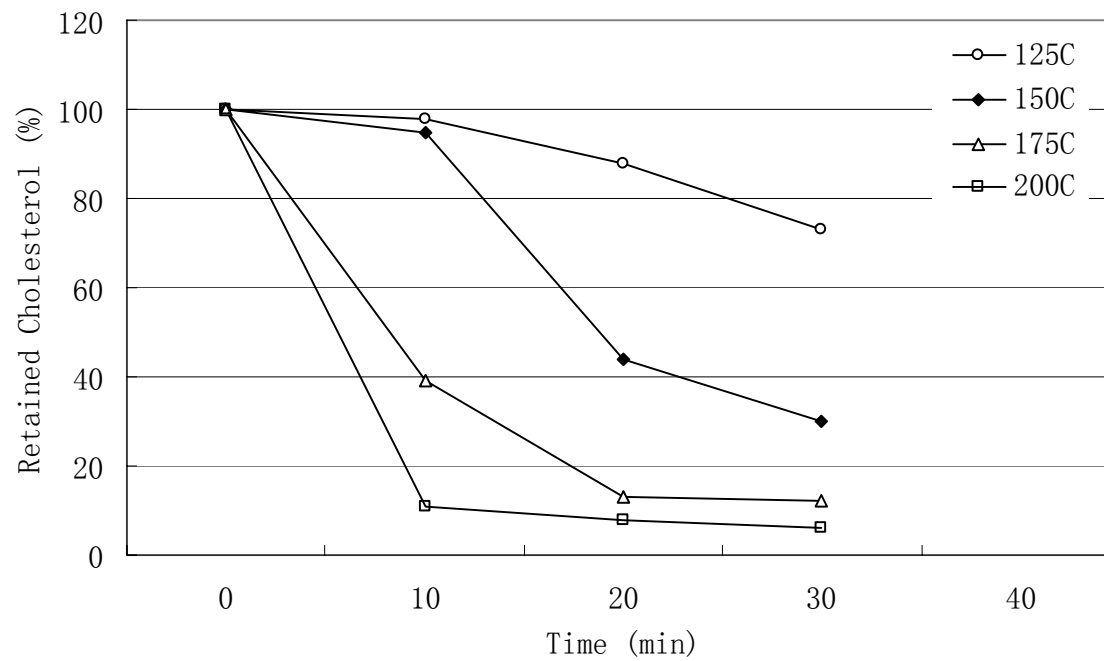


Figure 4.2. Changes of Retained Cholesterol during Heating at 125, 150, 175, and 200°C

Only 11% of cholesterol was retained after heating at 200°C for 10 min. These results indicated that the loss of cholesterol by heating was extremely rapid when the heating temperature was over 175 °C.

#### **4.3.2. COP Formation**

7-Ketocholesterol has been found as a primary COP in many foods (Echarte, 2001; Petron et al., 2003). It was also the principal COP in this study even in the low concentration cholesterol model that was used. Figure 4.3 indicated the changes of 7-ketocholesterol when the cholesterol was heated at different temperatures and times. 7-ketocholesterol was not detected even after cholesterol had been heated at 125°C for 30 min. However, it was found when the heating temperature was raised to 150°C. At 150°C, the concentration of 7-ketocholesterol was drastically increased to 2.11 µg/ml during 30 min of heating. At 175°C heating, it rapidly increased to 2.06 µg/ml in 10 min and then leveled off. After 10 min heating at 200°C, the 7-ketocholesterol concentration continuously decreased after it reached the highest concentration (2.46 µg/ml). The production of 7-ketocholesterol did not correspond to the loss of cholesterol at 200 °C. And the rate of degradation of cholesterol at 200°C was less than the rate of degradation of 7-ketocholesterol after 10 min heating.

This result suggested that the production of 7-ketocholesterol took place at 150°C and was greater than its degradation at the temperature. When the heating temperature was raised over 175 °C, this production rate could be suppressed by its degradation. The degradation rate of 7-ketocholesterol could be less or higher than its production under different heating temperatures, time, and concentrations of cholesterol. It may be the reason why the different production rates of 7-ketocholesterol were reported in different model studies (Osada, 1994).

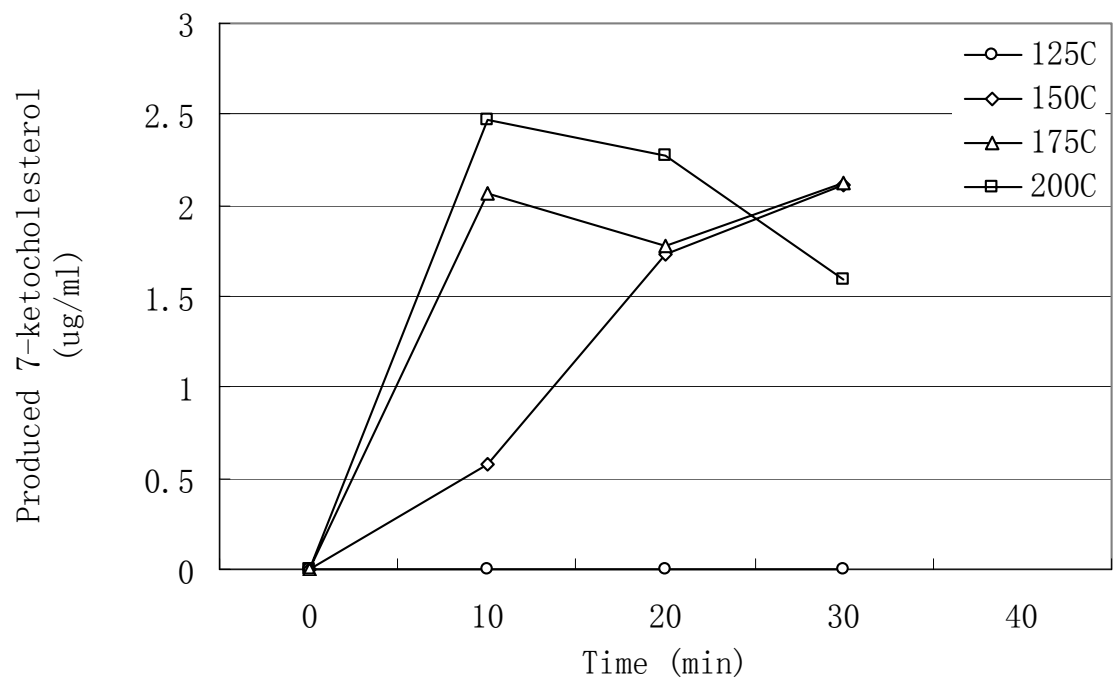


Figure 4.3. Production of 7-Ketocholesterol in Pure Cholesterol Samples During Heating at 125, 150, 175, and 200°C

#### 4.4. Vitamin E Contents in Cooking Oils

The HPLC chromatograph in Figure 4.4 shows rice bran oil sample chromatogram in tocopheral-tocotrienol analysis using the HPLC system. In the normal-phase HPLC, the order of elution of tocopherols and tocotrienols was  $\alpha$ -tocopherol,  $\alpha$ -tocotrienol,  $\gamma$ -tocopherol,  $\gamma$ -tocotrienol, and  $\delta$ -tocopherol. Since the tocopheral-tocotrienol standard curve was not made, the content of vitamin E isomers in each cooking oil were compared by peak area.

The peak areas of the vitamin E isomers in different cooking oils is shown in Figure 4.5. Lab soybean oil showed the highest  $\gamma$ -tocopherol, followed by Corn oil and commercial soybean oil, these oils all had relatively low level of  $\alpha$ -tocopherol. However, Rice bran oil (lab and commercial), canola, and olive oil all had high level of  $\alpha$ -tocopherol, but very low  $\gamma$ -tocopherol. Soybean oils also contain high level of  $\delta$ -tocopherol, this compound was in very low level in corn oil and not found in the other oils. The  $\gamma$ -tocotrienol levels of all oils were very low to a trace only level except rice bran oil. Rice bran oil had very high level of  $\gamma$ -tocotrienol. Lab prepared soybean oil contained 21.7% more  $\gamma$ -tocopherol, and 18.7 % more  $\alpha$ -tocopherol than commercial soybean oil. And lab prepared rice bran oil had 19.04% more  $\gamma$ -tocotrienol, 22.5 % more  $\alpha$ -tocopherol, 21.6%  $\gamma$ -tocopherol, and 20% more  $\delta$ -tocopherol than commercial rice bran oil.

According to our results, soybean and corn oil had better cholesterol protection capacity than the other oils, rice bran oil had the lowest capacity. It suggested that  $\alpha$ -tocopherol and  $\gamma$ -tocopherol maybe the major compounds that contribute to the protection.

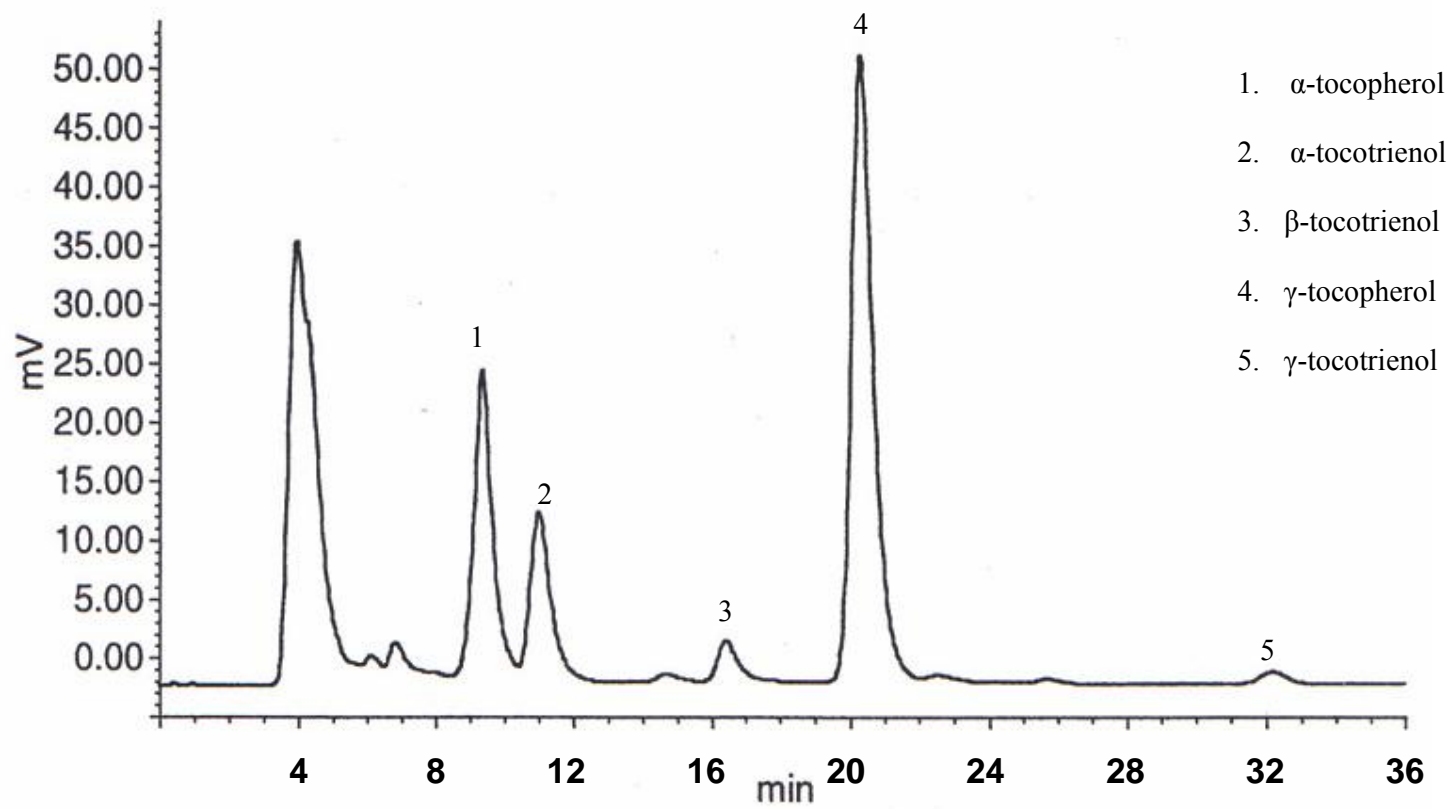


Figure 4.4. HPLC Chromatogram of Tocopherols and Tocotrienols of High Concentrated Rice Bran Oil

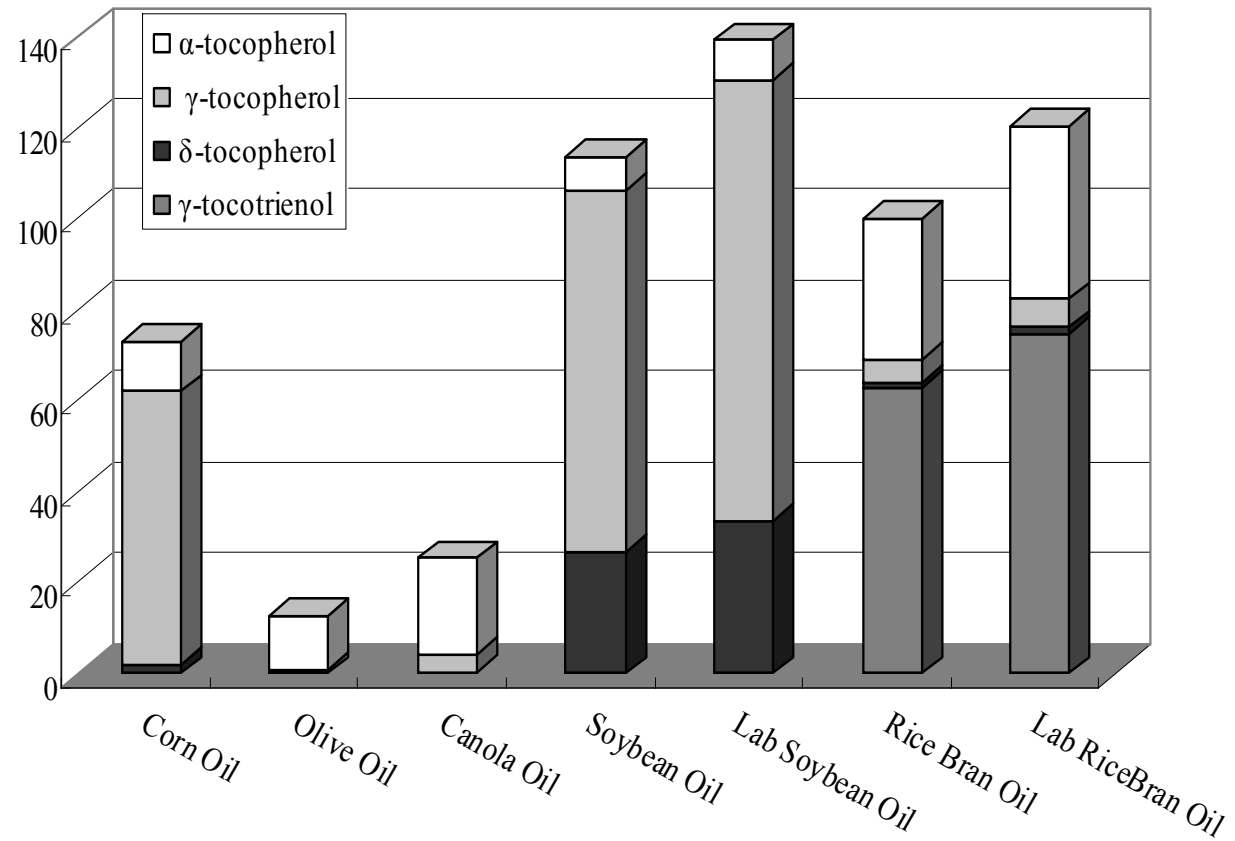


Figure 4.5. Content of Vitamin E Isomers in Cooking Oils

#### **4.5. Cholesterol Oxidation with Different Cooking Oils Treatment**

150°C and 175°C were chosen to evaluate the role of cooking oil in preventing cholesterol oxidation and 7-ketocholesterol production because the changes of cholesterol concentration were drastic in each 10 min interval at the heating temperatures, and these temperatures are normally applied for roasting, baking, and frying muscle foods and seafoods. Seven different cooking oils from plant sources (soybean oil, lab prepared soybean oil, corn oil, canola oil, rice bran oil, lab prepared rice bran oil, and olive oil) were used in this study. The percentages of retained cholesterol during heating at 150°C and 175 °C in different cooking oil treatments were shown in Figure 4.6 and 4.7. After 20 min of heating at 150°C, the percentage of retained cholesterol in each group of cooking oil treatment was significantly higher than that in the control group (Figure 4.6). At 175°C, the cholesterol loss was significantly inhibited in each treatment group after 10 min, except rice bran oil (Figure 4.7). The capability of rice bran oil to stabilize cholesterol disappeared after 20 min of 175°C heating. Cholesterol stability with corn, soybean, and lab prepared soybean oil treatments was significantly higher than canola, olive, rice bran, and lab prepared rice bran oil treatment during 30 min of heating at 150°C and 175°C. At different heating time, the oils showed different protective capability order. For example, at 150 °C, after 10 min heating, olive oil had the highest protection than all the other oils, but after 20 min, the protective capability of olive oil was less than soybean and rice bran oil, then it was showed better capability than rice bran oil again after 30 min heating. This may be because that different oils had different lipid profile which can effect the cholesterol degradation, and the lipid profile of oils kept changing during heating. For instant, rice bran and canola oil had higher unsaturated/saturated lipid ratio than soybean and olive oil. The high unsaturated oil oxidized faster than the saturated oil

during heating. Because the changes of oils themselves were different during heating, their protective capability to the cholesterol changed at different ratio as well. The capabilities of cooking oils to inhibit cholesterol loss were also reported by Echarte et al. (2001). In that study, higher retained cholesterol was found in salmon samples fried with olive or soybean oils than in the roasted sample without oil. In addition, the retained cholesterol in the salmon sample fried with soybean oil was higher than that fried with olive oil. The result that soybean oil had greater capability to inhibit cholesterol loss is in agreement with our study.

Unlike the control group, 7-ketocholesterol was not detected in the corn, lab prepared soybean, and lab prepared rice bran oil treatment group during 30 min of heating at 150°C (Figure 4.8). At 150 °C, the level of 7-ketocholesterol in the canola, soybean, and olive oil treatments was significantly lower than that of the control group. At 175 °C, the concentration of 7-ketocholesterol in each treatment group generally increased consistently during 30 min heating (Figure 4.9). Among the treatment groups, the level of 7-ketocholesterol in corn, lab soybean, and lab rice bran oil treatment was significantly lower than that in canola, soybean, and olive oil treatments. These results were similar to those of Echarte et al. (2001), who reported that the level of 7-ketocholesterol in the salmon sample fried with soybean oil was two and four times lower than that fried using olive oil and roasted, respectively.

In our study, there was in cholesterol degradation in the cholesterol oxidation model than oil mixed model, but oil mixed cholesterol sample produced more 7-ketocholesterol than cholesterol oxidation model. The levels 7-ketocholesterol in oil mixed samples did not decrease after 10 min heating at 175 °C as in cholesterol oxidation model. This should suggested that the oils protected not only the cholesterol but the cholesterol oxidation products as well.

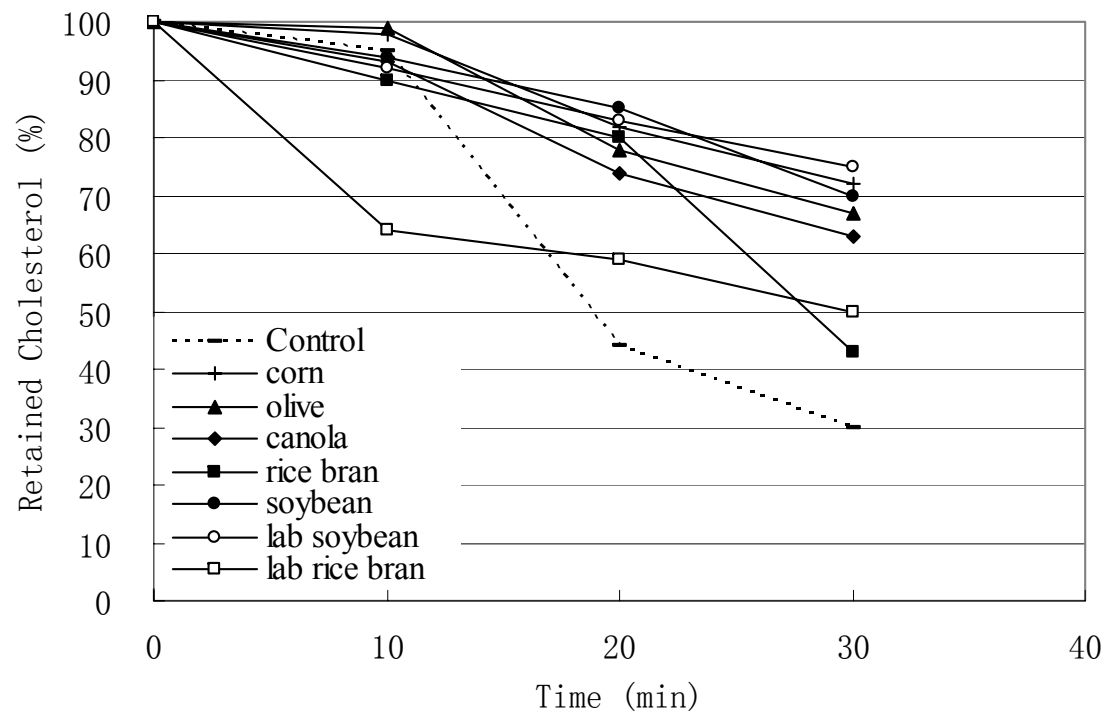


Figure 4.6. Changes of Retained Cholesterol in Pure Cholesterol and Oil Mixed Samples at 150°C

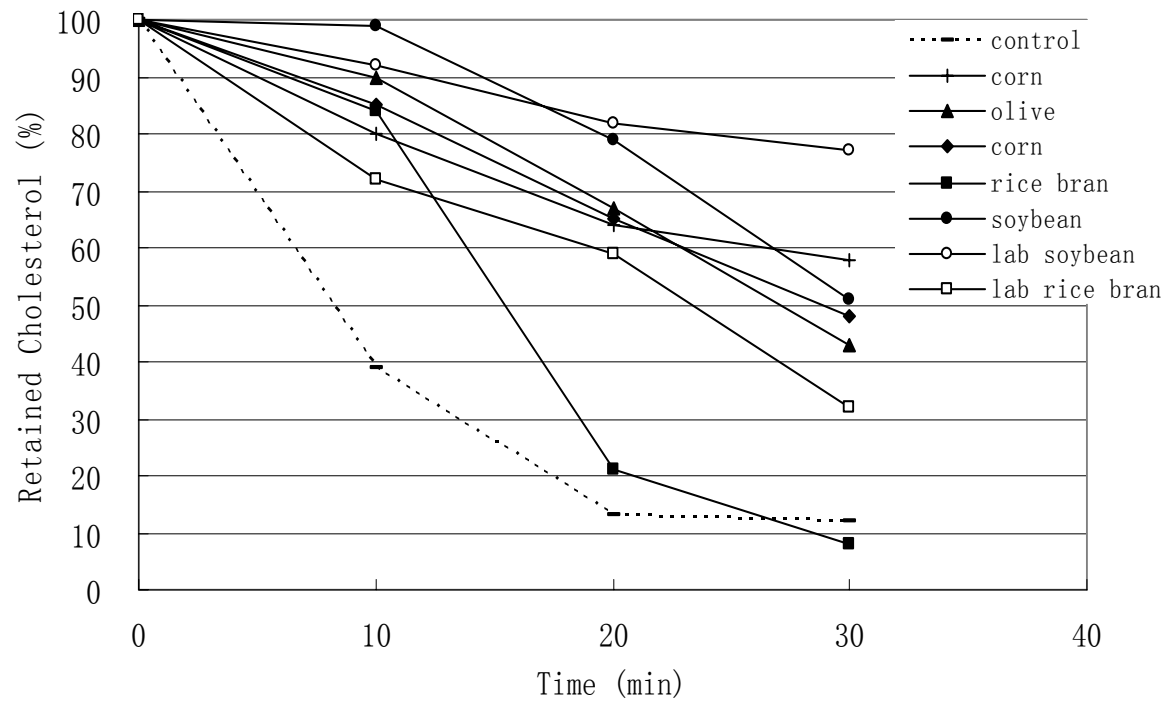


Figure 4.7. Changes of Retained Cholesterol in Pure Cholesterol and Oil Mixed Samples at 175°C

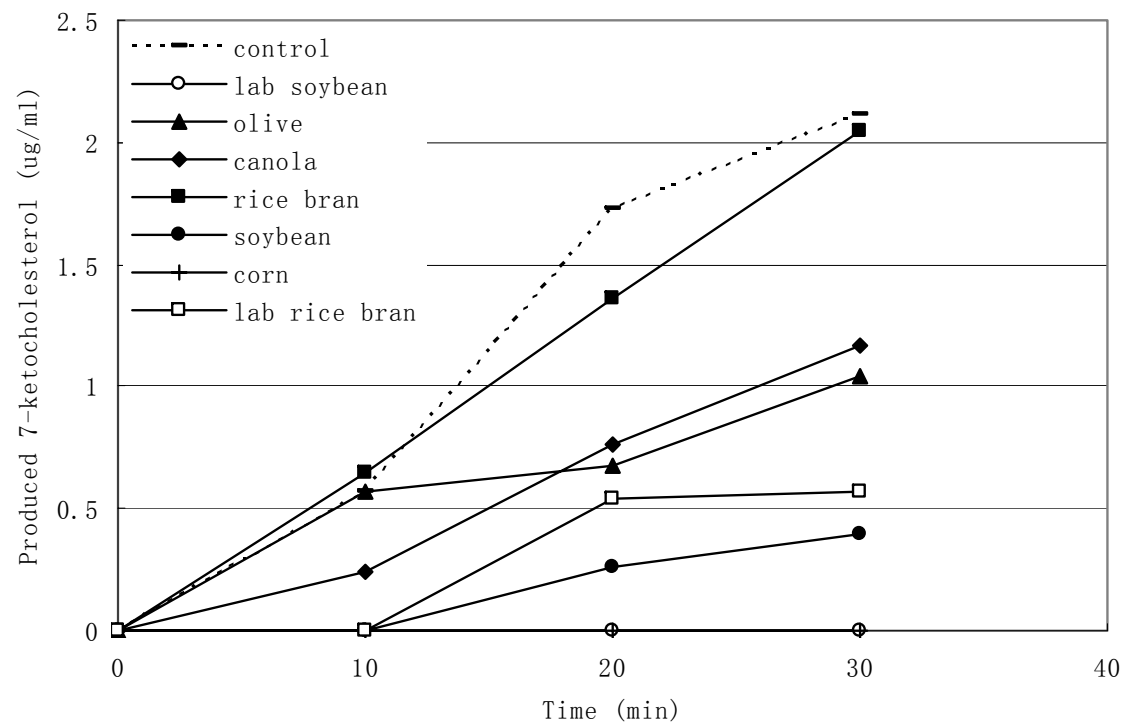


Figure 4.8. Production of 7-Ketocholesterol in Pure Cholesterol and Oil Mixed Sample During Heating at 150°C

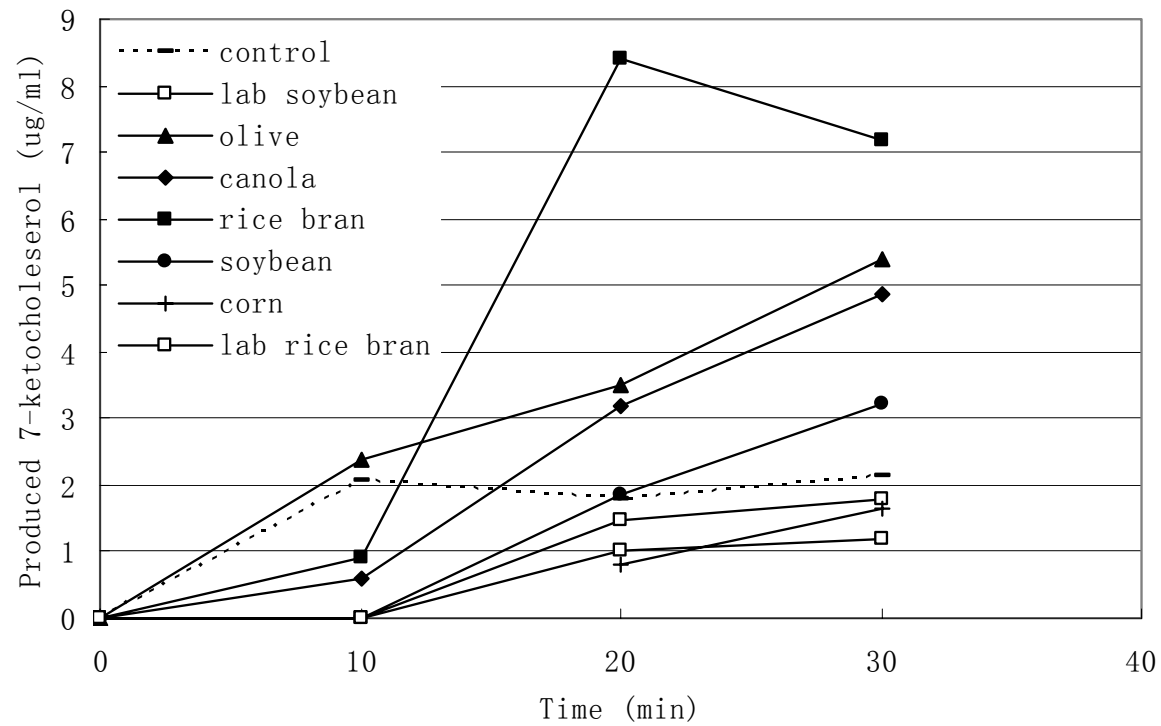


Figure 4.9. Production of 7-Ketocholesterol in Pure Cholesterol and Oil Mixed Sample During Heating at 175°C

## 4.6. Cholesterol and Cholesterol Oxidation Products in Salmon Fish Meat with and without Cooking Oils

### 4.6.1 Salmon Samples

Table 4.1 shows the moisture, fat, and cholesterol content of salmon sample. It was showed in the cholesterol oxidation model that lab prepared soybean oil have the highest capability of inhibiting cholesterol oxidation, while that capability of rice bran oil was the lowest. These two oils were selected to be used in adding in salmon fish.

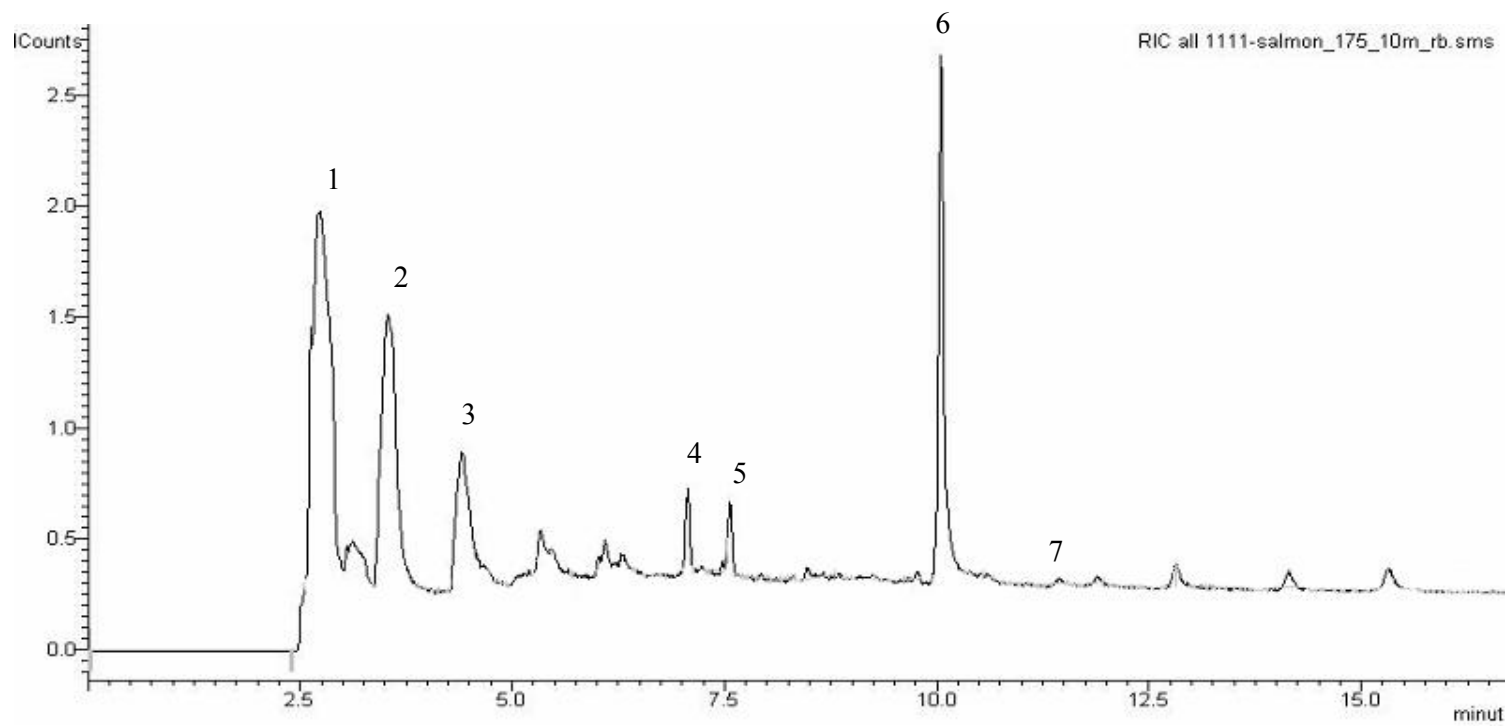
Table 4.1 Moisture, Fat, and Cholesterol Content of Salmon Samples

Moisture %	Fat %	Cholesterol	
		mg/100g Sample	mg/g Fat
65.94	7.76	35	4.51

We used 3.0 g of homogenized salmon, and 0.3 g of oil. The amount of cholesterol in the 3.0 g of salmon was about 105 mg, so the oil added to cholesterol ratio was about 3:1. In the cholesterol oxidation model, the oil to cholesterol ratio was 80:1. The amount of oil higher than 0.3 g would cause the salmon meat soak into the oil.

Too much oil would just flow around the meat. In this case, the degradation of cholesterol would definitely decrease significantly, because the excessive oil could form a physical barrier around the salmon to block the contact of oxygen and cholesterol. This could not be counted to the chemical protection of cooking oils.

Figure 4.10 showed the GC-MS gas chromatography of salmon roasted with rice bran oil at 175°C for 10 min. The 5 $\alpha$ -cholestane showed relatively lower peak than cholesterol, but cholestane had similar extraction rate as cholesterol by chloroform/methanol solution. When the cholesterol peak was enlarged, different MS fingerprint other than cholesterol was showed on the right shoulder of the big peak. This suggested that other component in salmon fish had the close retention time as



- |   |                |                                    |                                       |
|---|----------------|------------------------------------|---------------------------------------|
| 1. n-Hexadecanoic Acid                      | 2. Oleic Acid  | 3. 5,8,11,14-Eicosatetraenoic Acid | 4. 2,6,10,14,18-Eicosapentaenoic Acid |
| 5. $\alpha$ -Cholestane (internal standard) | 6. Cholesterol | 7. 22-Ketocholesterol              |                                       |

Figure 4.10. Gas Chromatography of Roasted Salmon Oil with Rice Bran Oil

cholesterol had under current GC-MS condition. To solve this problem, only the typical cholesterol ion (386) was used in quantifying the amount of cholesterol.

#### **4.6.2. Cholesterol Oxidation in Salmon Fish Meat**

Percentages of retained cholesterol in control and treatment salmon sample during heating at different temperatures, times, and oils were depicted in Figure 4.11. According to the standard curves, the cholesterol level in unheated salmon was about 350 µg/g. No COPs were detected in raw samples. In the control group, about 25% of cholesterol was lost after 30 min of heating at 150°C. The loss of cholesterol at 175°C, and 200°C were 38% and 55%, respectively, which were much less than the loss in pure cholesterol oxidation model. After roasted with the oils, the losses of cholesterol at these three temperatures were reduced to 16%, 35%, and 48%, respectively. The loss of cholesterol was more than those reported by Echarte et al. (2001). In their study, cholesterol level decreased about 24% after roasted at 200°C for 30 min. One important reason to cause the difference was that they used whole salmon fillet in the study, while we used the homogenized salmon sample. Our samples had more surface to contact and react with oxygen, thus caused more oxidation than their study.

Less cholesterol was decreased when compare with the data that pure cholesterol oxidation heated with cooking oils. The loss of cholesterol heated with lab prepared soybean oil for 30 min at 150°C and 175°C were 25% and 40%, respectively, the loss of cholesterol heated with rice bran oil were 55% and 90% at the two temperatures. In roasted salmon samples, cholesterol decreased by 16% (lab soybean oil, 150°C), 31% (lab soybean oil, 175°C), 19% (rice bran oil, 150°C), and 35% (rice bran oil, 175°C). And the difference of protect capabilities between lab soybean oil and rice bran oil were not as much as in the pure cholesterol oxidation

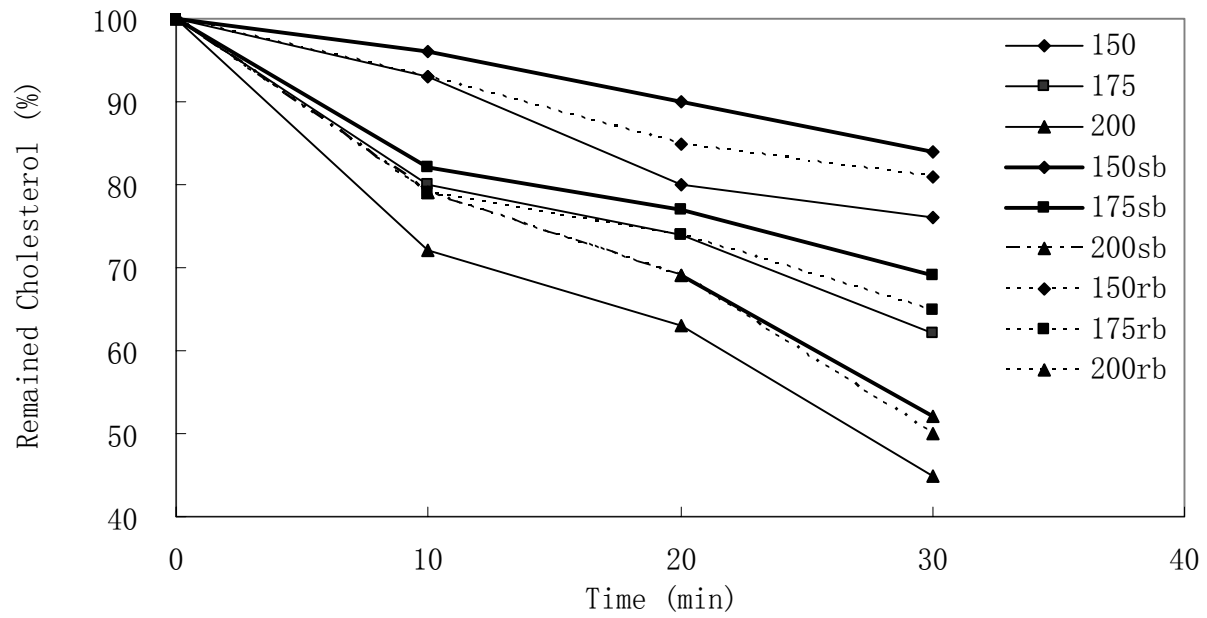


Figure 4.11. Change of Retained Cholesterol in The Control and Treatments during Heating at 150°C, 175°C, and 200°C.

model. This might be because that salmon fish contains its own antioxidants. One hundred grams of salmon fish contains about 1.4 mg of alpha-tocopherol, which was almost the same as olive oil has. Another reason was that the tissues in salmon meat formed a physical barrier during roasting to protect cholesterol. Because the total amount of cholesterol that participated in oxidation was reduced, the protection capability of different oils could not be fully displayed in the roasted salmon model.

#### **4.6.3. Cholesterol Oxidation Product Formation**

Al-Saghir et al. (2004) reported to have found 7-hydroxycholesterol, 5,6-epoxicholesterol, and 7-ketocholesterol in fried and steamed salmon samples. No COPs were found in our control samples, 22-ketocholesterol and 5 $\beta$ -cholest-3-ene were found in oil-blended samples above 175°C. The amount of 5 $\beta$ -cholest-3-ene was not high enough to compare among the samples. The production of 22-ketocholesterol at 150, 175, and 200°C was shown in Figure 4.12. The highest level of 22-ketocholesterol was found in the rice bran oil-blended sample that roasted at 200°C for 10 min, the amount was 0.98  $\mu\text{g/g}$ . Actually, the highest content of 22-ketocholesterol in each roasted temperature were all found in 10 min of roasting in spite of the type of cooking oil. At 30 min of roasting with different oils at 175°C and 200°C, the levels of 22-ketocholesterol were almost the same. Though COP was not found in the control group, it did not suggest that the control group had less cholesterol oxidation. As discussed above, the ketocholesterol was not stable in high temperature as well. At 150°C of roasting, the produced COPs might not be high enough to be detected in the salmon sample. At a temperature higher than 175°C, COPs formed and soon degraded at the high temperature in the control group. In oil-blended groups, the oil could prevent both cholesterol against oxidation and produced COP. That is the reason why COP was not found in treatment groups.

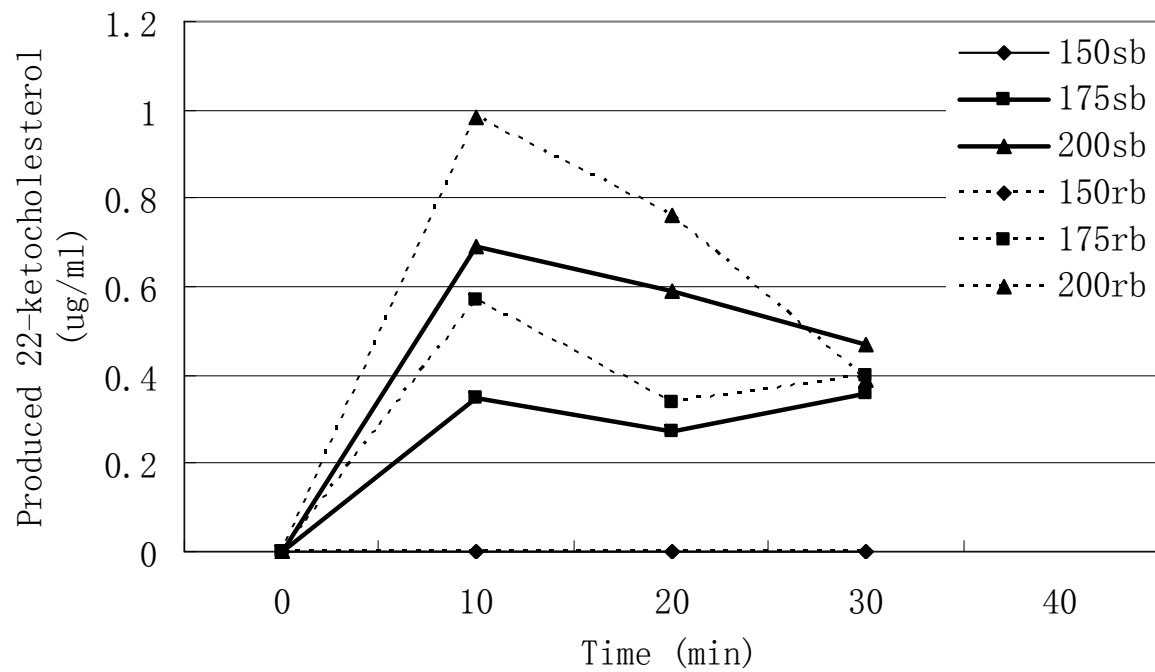


Figure 4.12 Production of 22-Ketocholesterol in The Treated Salmon Samples During Heating at 175°C and 200°C (No COP was found in control group)

## Chapter 5 Conclusions

Some plant source cooking oils could reduce cholesterol loss and prevent ketocholesterol production during heating in both pure cholesterol oxidation and salmon fish meat model. In the cholesterol oxidation model, the concentration of cholesterol decreased with increasing heating temperature and heating time. The level of 7-ketocholesterol was increased during heating, but decreased after the highest level was reached at heating temperature above 175°C. It suggested that 7-ketocholesterol was not stable at high temperature either.

The soybean oil had the highest capacity of preventing cholesterol oxidation, and rice bran oil had the lowest capability. Soybean oil contained the highest level of alpha and gamma-tocopherol, which was considered as the major compounds against cholesterol oxidation among those oils. The lab prepared oils have higher capability than commercial oil in preventing cholesterol oxidation. About 19-25% more tocopherols and tocotrienols in the lab prepared oils than those amount in commercial oils.

Cholesterol oxidation in roasted salmon meat model were not as much as, but exhibited the same trend as that in cholesterol oxidation model. It suggested that the protection of meat tissues and the antioxidants in salmon itself could play an important role in preventing cholesterol oxidation. Ketocholesterol was found only in oil mixed salmon samples at the roasting temperature higher than 175°C since the oils stabilized not only the cholesterol but also the COPs. The difference between the protective capacity of lab soybean and rice bran oil were not significant as in pure cholesterol oxidation model, because that the cholesterol oxidation in salmon meat were low which reduced the difference.

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## **Vita**

Ting Zhang was born in Dalian, China, on September, 1977. In the year 1996, she attended the School of Material Science and Technology, Beijing University of Chemical Technology, and pursued a number of interests both within and without the university curriculum. These interests eventually led her to make a decision to study abroad in the United States. In the year of 2003, she got her master's degree in textile science in the School of Human Ecology at Louisiana State University. She began her master's degree in the Department of Food Science at Louisiana State University in the same year.