연사 6

Formation of Cholesterol Oxidation Products(COPs) in Animal Products

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I. Introduction

Cholesterol is a monounsaturated lipid with a double bond on carbon-5, and is susceptible to oxidation in the presence of oxygen, light, heat, radiation, free radicals, metal ions, and other factors. Cholesterol oxidation products (COPs) are group of sterols that are similar in structure to cholesterol but contain an additional hydroxy, ketone or epoxide group on the sterol nucleus and /or a hydroxyl group on the side chain of their molecules. Animal products are a complex food with a highly structured nutritional composition and major source of cholesterol in the diet. It becomes edible and more digestible when it is subjected to cooking. However, heat treatment can lead to undesirable modifications, such as the loss of the nutritional value of meats mainly due to lipid oxidation and changes in some components of the cholesterol. The degree of oxide formation is related to processing temperature, heating time, storage conditions, level of activator present, packaging and most of the oxides found in foods were subjected to processing conditions or exposure to heat (Paniangvait et al., 1995). COPs have been known to be more injurious to arterial cells than pure cholesterol and are more directly connected to the development of atherosclerosis and coronary heart disease (Addis, 1986). COPs deteriorated the bioavailability of cholesterol by inhibiting cholesterol biosynthesis (Lund and Bjorkhem, 1994) and dietary uptake of cholesterol (Peng et al., 1985). COPs also impaired a membrane function, which results in altered membrane permeability. The most predominant oxidized cholesterol detected was 7-ketocholesterol, as well as β-epoxycholesterol and α-epoxycholesterol. Many studies demonstrated that the amount of COPs in foods could frequently reach 1% of total cholesterol and occasionally 10% or more (Addis, 1986). The lipid oxidation has been associated with

quality deterioration caused by the development of off-flavors and off-odors during storage (Kumar and Singhal, 1992). More recently, interest in the possible toxicological effects of lipid oxidation products, particularly cholesterol oxidation products (COPs), has increased. Thus, these researches are important for human health and meat quality.

II. Oxidation of Cholesterol

Cholesterol is a eukaryotic sterol that in higher animals is the precursor of bile acids, vitamin D_3 and steroid hormones, and a key constituent of cell membranes, mediating their fluidity and permeability. Most is synthesized by the liver and other tissues, but some is absorbed from dietary sources. Dietary cholesterol enters the body by way of the chylomicron pathway and is removed from the plasma by the liver as a component of chylomicron remnants. Cholesterol is a molecule with an unsaturated or double bond; therefore, it is prone to oxidation. It is sensitive to free radical oxidation by diatomic molecular oxygen (O_2) in the air. Cholesterol molecules are arranged in double layers with the 3-hydroxyl groups in juxtaposition and side chains exposed (Smith, 1987). COPs are a group of sterols that are similar in structure to cholesterol but contain an additional hydroxy, ketone or epoxide group on the sterol nucleus and /or a hydroxyl group on the side chain of their molecules. As cholesterol contains one Δ5-double bond, formation of any oxygen radical or free radical is expected to initiate cholesterol oxidation (Smith, 1987). The hydroperoxides of polyunsaturated fatty acid formed during lipid oxidation may be necessary to initiate cholesterol oxidation. The high polyunsaturated fatty acid content of phospholipids in food and their considerable vulnerability to attack by oxidizing species generated within cells and close to the cell membrane have led to the probability that lipid oxidation is initiated at the subcellular membrane level (Igene et al., 1979). It is conceivable, therefore, that cholesterol oxidation should proceed in a way analogous to fatty acid oxidation. Smith et al (1987) also suggested that the hydroperoxides of polyunsaturated fatty acids formed during lipid oxidation may be necessary to initiate cholesterol oxidation. In intermolecular systems, hydrogen is extracted from cholesterol by peroxy or oxy radicals of oxidized neighboring polyunsaturated fatty acid in the membrane. In intramolecular systems, the oxidized fatty acyl portion attacks the cholesteryl portion of the same cholesteryl ester molecule. The oxidation of cholesterol in aqueous alkali is much faster than that of cholesterol fatty acyl esters (Smith, 1987). Cholesterol molecules function as an integral part of the lipid bilayer of cell membranes and are closely associated with membranal phospholipids. The intermolecular free-radical processes, involving hydrogen extraction from cholesterol by

peroxy or oxy radicals of polyunsaturated fatty acids, may promote cholesterol oxidation. Alternatively, intramolecular oxidation between the oxidized fatty acyl portion and the cholesterol portion of cholesterol ester may occur. Both mechanisms could operate within muscle cell membranes. COPs may be generated within tissues through non-enzymatic oxidation of cholesterol, in general mediated by reactive pro-oxidant species (Osada et al., 1994) or via enzymatic catalysis (Bjorkhem et al., 1994). In addition to the reactive oxygen species involved in the autoxidation of exogenous cholesterol, in animal tissues autoxidation may also be supported by peroxyl ($ROO^{\cdot}$) or alkoxyl (RO) radicals derived from lipid peroxidation (Smith, 1987). Other reactive species may derive from activation within macrophages and neutrophils (Smith, 1996). It appears credible that non-enzymatic routes play some role in the production of oxysterol, since plasma or tissue levels decrease in animal fed antioxidants such as vitamin E, butylated hydroxytoluene or probucol (Mol et al., 1997). However, further investigation in needed to properly evaluate the full impact of autoxidantion in the endogenous production of cholesterol oxides. Some of the most abundant COPs found in vivo are enzymatic products of cholesterol catabolism. In mammals, the main enzymatic pathway involving cholesterol is its mono-hydroxylation in the synthesis of bile acid biosynthesis are 27-hydroxylase and 7α-hydroxylase (Russell and Setchell, 1992). The enzyme sterol 27-hydroxylase plays an important role in the hepatic degradation of the side-chain of cholesterol, during the formation of bile acids (Bjorkhem et al., 1994). This enzyme is present not only in hepatocytes but also in several other cell types, including leukocytes and endothelial cell (Reiss et al., 1994) where it appears involved in the elimination of cholesterol (Bjorkhem et al., 1994). The most predominant oxidized cholesterol detected was 7-ketocholesterol, 20-hydroxycholesterol, 25-hydroxycholesterol, 7α -hydroxycholesterol, 7β-hydroxycholesterol, 5, 6α-epoxide, 5, 6β-epoxide and triol. Many studies demonstrated that the amount of COPs in foods can frequently reach 1% of total cholesterol and occasionally 10% or more (Kumar and Singhal, 1992). Cholesterol oxidation is initiated by the abstraction of the allylic C-7 hydrogen, with the subsequent formation of C-7 COPs such as 7α- and 7β-hydroxycholesterols and 7-ketocholesterol (Smith, 1987). Cholesterol α- and β-epoxides are the products of attack by cholesterol 7-hydroperoxide on the 5,6-double bond of cholesterol and therefore are the secondary oxidation product (Maerker, 1987). Thus, these oxygenated compounds apparently originated from hydroperoxidation of cholesterol.

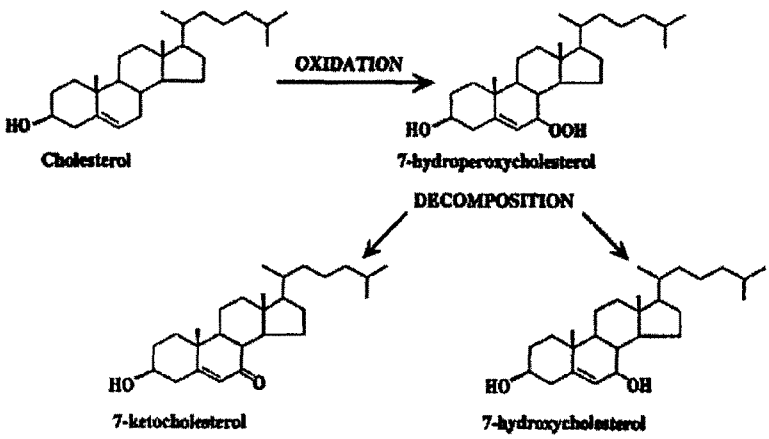
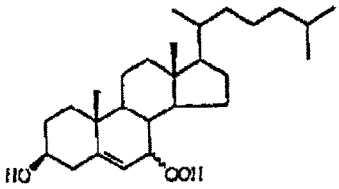
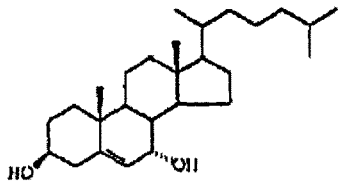


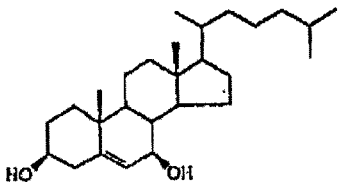
Figure 1. Cholesterol oxidation pathways



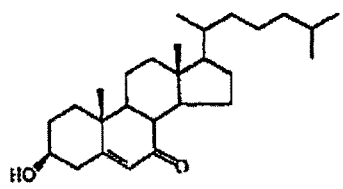
7-Hydroperoxycholesterol



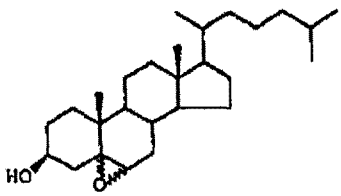
7α-Hydroxycholesterol



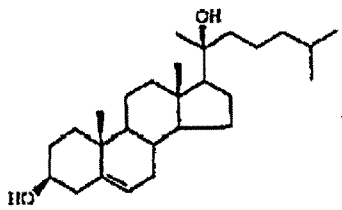
7β-Hydroxycholesterol



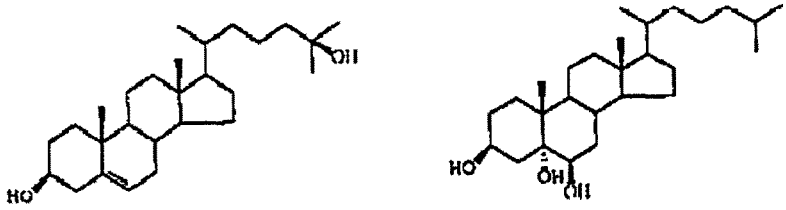
7-Ketocholesterol



5,6-Epoxycholesterol



20-Hydroxycholesterol



25-Hydroxycholesterol　　Cholestanetriol

Figure 2. The structure of cholesterol oxidation products

III. Factors of Cholesterol Oxidation

Many foods have been analyzed because of their cholesterol content and exposure to conditions known to promote oxidation of cholesterol. Those conditions include application of heat, exposure to light and prolonged storage. The degree of oxide formation is related to processing temperature, length of heating time, storage conditions, level of activator present, and packaging. The effect of ionizing radiation on cholesterol has also been examined in various model systems. The factors studied have included processing or heating, storage and packaging, and irradiation. Cholesterol oxidation occurred via singlet oxygen attack as well as free radical mechanisms. It was a light-induced surface phenomenon. In muscle food, lipid oxidation has been associated with quality deterioration caused by the development of off-flavors and off-odors during storage. A variety of aldehydes, ketones, and organic acids arising from the breakdown of lipid hydroperoxides contribute to the sensory properties of meat particularly after cooking (Mottram, 1987).

The nature, proportion, and degree of unsaturation of fatty acids present in animal product systems will indicate the approximate susceptibility of those products to oxidative deterioration. Generally, the higher the proportion and degree of unsaturation in fatty acids. the more labile the lipid system in to oxidation. Free ionic iron plays a major role in the initiation of lipid oxidation. Ionic iron plays an important role in oxidation by catalyzing the degradation by hydroperoxides, the primary by-products of lipid oxidation. Heavy transition metals such as cobalt, copper, iron, manganese, and nickel possessing two or greater valance states generally increased rate of oxidation of food lipids (Ingold, 1962). Their basic function is also to increase the formation of peroxy radicals. Obara et al. (2005) reported that water in foods may function as a substrate or medium of many reactions. Water significantly influences lipid oxidation

processes in food products. It can either stimulate or inhibit lipid oxidation reaction (Duckworth, 1975). The inhibition of lipid oxidation in food may be caused by lowering oxygen diffusion to the sites of oxidation, by lowering catalytic properties of metal ions as a result of their chelation and also by the effect of binding of hydroperoxides (Obara et al., 2005). They suggested that water can promote lipid oxidation through lowering viscosity and facilitating the movement of the molecules. In food with very low water activity its increase causes the inhibition of the rate of lipid oxidation. Thus, water content of animal products can be influence the cholesterol oxidation.

1. Heating

Overall results from several sources showed that fresh food contained none or undetectable levels of COPs. Most of the oxides found were in foods subjected to processing conditions or exposure to heat. Cooking of food under standard domestic conditions increased production of COPs. (Morgan and Armstrong, 1992). COPs formation needs the presence of several reactive oxygen species, unsaturated fatty acid, cholesterol, transition metals and in rare cases, enzyme (Rose-Sallin et al., 1996). The protein denaturation by cooking, which can lead to the loss of antioxidant enzyme activeity or the release of catalytically-active iron from metallo-proteins (mainly myoglobin); disruption of cell membranes, which bring polyunsaturated fatty acids into contact with prooxidants: transformation of myoglobin from an antioxidant to a prooxidant species; and thermal decomposition of hydroperoxides to prooxidant species, such as alkoxyl hydroxyl radicals. Thus, cooking lead to significantly increased oxidation, as reflected by COPs and TBARS values (Grau et al., 2001). These radicals will accelerate the chain reaction of lipid oxidation, including COPs. Monahan et al. (1992) demonstrated that the rate of cholesterol oxidation in pork was greatly accelerated during storage following cooking and appeared to follow the same trend as lipid oxidation in general. Sarantinos et al. (1993) reported that prolonging cooking time increased the COPs content of fried and boiled eggs. They suggested that total COPs were usually under 5% of total cholesterol in processed or heated egg or milk products. Park and Addis (1986) reported that cholesterol was oxidized to isomeric 7-hydroxycholesterols, 7-ketocholesterol and epimeric epoxides in tallow heated at 155℃ for 376hr. 7-ketocholesterol was produced without formation of epimeric 7-hydroxycholesterol as a decomposition product of 7-hydroperoxides because 7-hydroxycholesterol was readily dehydrated in the absence of water at elevated temperatures, such as 155℃. Park and Addis (1987) studied rare and well done beef steaks, 7β-hydroxycholesterol and 7-ketocholesterol were suspected to be present, but

concentrations were low. Thermal decomposition of C-7 hydroperoxides has been reported to give rise to stable autoxidation products, like 7α-hydroxy and 7β -hydroxycholesterol and the dehydration product, 7-ketocholesterol. Park and Addis (1986) showed that the level of 7-ketocholesterol increased linearly with heating time but not with temperature. Studies on the effects of heating were mostly conducted in frying oil such as tallow and lard. The formation of 7-ketocholesterol was nearly linear with heating time, reaching about 10% of the initial cholesterol content at 376hr heating 155℃. Park and Addis (1986) noted that cholesterol loss ceased in 2 heated tallow samples when 40-45% of the initial cholesterol was gone. Heating tallow at 155℃ or 190℃ resulted in loss of half the initial content of cholesterol at 250hr, and samples heated at 190℃ were affected slightly more than those at 155℃. After 250hr at 155℃, 7% of the cholesterol had been converted to 7-ketocholesterol with lesser qualities of 7α - and 7β-hydroxy and α-epoxide derivatives of cholesterol, with only 50% of the original cholesterol remaining. 1.2 and 1.1% of the cholesterol into 7-keto and α-epoxide derivatives, respectively, after 70hr at 135℃. The rates of cholesterol loss for lard with 10 times the normal cholesterol level heated at 180℃ for 10hr/day for 24days were compared with lard with 2 times the cholesterol level heated at 180℃ for 10hr/day for 16 days. Accumulation of COPs occurred during both heating tests. Consistent with other research, the amount of COPs formed did not equal the cholesterol loss. The reason for this may be thermal degradation. During heating, thermal degradation of cholesterol likely occurred, but degradation products were not detected or identified.

2. Illumination and irradiation

The various COPs formed through oxidation of cholesterol during illumination. The COPs were increased with increasing illumination time. Especially, COPs were more concentrated at the surface than throughout the entire meats during illumination. The COPs involves free radicals, formed by triplet sensitizer-reducing substrate interaction. Storage of unirradiated beef at 0-4℃ for 2 weeks increased the COPs content considerably (Hwang and Maerker, 1993). In meat, free ionic iron released from iron-binding macromolecules may react with hydrogen peroxide and forms hydroxy radicals. The presence of oxygen facilitates the propagation step of lipid oxidation as well as generation of susperoxide anion and hydrogen peroxide (Diehl, 1995). Therefore, oxygen greatly accelerates irradiation-induced cholesterol oxidation. UV-light also generates free radicals by photolysis of water (McCord and Fridovich, 1973), and the reaction is similar to that of the irradiation. When molecules absorb ionizing energy they become very reactive and form ions or free radicals. These ions and free radicals react

and form stable radiolytic products (Woods and Pikaev, 1994). Irradiation could produce a large amount of hydroxy radicals in meat because over 75% of muscle cells are composed of water (Thakur and Singh, 1994). The primary autoxidation is followed by a series of secondary reactions that lead to oxidation of cholesterol. Unirradiated pork and veal during storage also showed an increase in most COPs, although increases were smaller than with beef. Irradiation of the meats followed by 2 weeks storage at 1-4℃ raised the COPs level considerably over that of unirradiated, stored samples, with exception of 4, 6-cholestadien-3-one (Hwang and Maerker, 1993). Hwang and Maerker (1993) indicated that the amount of COPs in meat increased substantially with irradiation at 10kGy dose and with storage in the presence of air. Lebovics et al (1992) reported that the chemical changes in cholesterol induced by ionizing radiation were similar in nature to those occurring during autoxidation. Maerker and Jones (1991) reported that irradiation produced a large amount of 7-hydroxycholesterol by oxidizing cholesterol in liposomes. They showed that the ratio of 7-ketocholesterol/choelsterol 5,6-epoxides generated by irradiation was less than 1, much lower than that by autoxidation, and suggested using this product ratio as an indicator of irradiation. Thus, the use of aluminium foil as a light and gas barrier is important for preventing light-induced cholesterol oxidation in foods. Paper-based packaging materials cannot prevent this sufficiently (Luby et al., 1986).

3. Storage and packaging

The content of COPs in raw ground beef and turkey was essentially zero before storage. Pack and Addis (1987) reported that the content of COPs in raw ground beef and turkey was essentially zero before storage. However, the length of storage time should be affect the formation of COPs in foods. Conchillo et al. (2005) reported that total COPs levels were 1.6, 5.9 and 1.94 fold higher in aerobically stored than in vacuum stored raw, grilled and roasted samples respectively. The efficiency of vacuum conditions in reducing cholesterol oxidation during frozen storage was higher for cooked than for raw samples. Cooked samples stored aerobically showed the highest COPs amounts, especially roasted samples. This result indicate that cooking enhances cholesterol oxidation during storage (Conchillo et al., 2005). Dry eggnog mix developed increasing levels of 7α- and 7β-hydroxycholesterol up to 80 days storage, after exposure to fluorescent light (Herian and Lee, 1985). Li et al. (1996) reported that the concentration of COPs in egg powders increases with time and temperature of storage - the longer the time and the higher the temperature of storage - the bigger the amounts of oxysterols produced. The presence of oxygen and light also promote oxysterol formation

(Guardiola et al., 1997). Oxidized cholesterol in ppm have also been detected in longterm stored and cooked egg and dehydrated egg products. Because the major unsaturated fatty acids in egg are oleic acid and linoleic acid, it is likely that peroxy radicals of these unsaturated fatty acids attack cholesterol. The extent of cholesterol oxidation in meat samples during storage was represented by total COPs level and related to percent of residual cholesterol content in each samples as well as corresponding TBARS.

Ⅳ. COPs in Animal Products

1. Meat products

The mean lipid content of lean meat is 10%, wet weight basis, of which triglycerides and phospholipids are major components and cholesterol is a lesser component, ranging from 50 to 89mg% (Rhee et al., 1982; Pikul et al., 1984). Lercker and Rodriguez-Estrada (2000) suggested that no significant differences in the 7-ketocholesterol content were found among the different cooking treatments, but the raw meat already presented a considerably high initial 7-ketocholesterol level (3.5 ppm). This result might be a direct consequence of the holding period to which meat is usually subjected; during this process, meat is often kept at 4-6℃ for 10-15 days in order to increase the meat tenderness and to promote the flavor formation (Lercker and Rodriguez-Estrada, 2000). Park and Addis (1986) reported that cholesterol was oxidized to isomeric 7-hydroxycholesterols, 7-ketocholesterol and epimeric epoxides in tallow heated at 155℃ for 376 hr. In raw pork chops initial cholesterol oxidation products were nondetectable (limit of detection was 5-10ng of COPs), and even after 8 days of refrigerated storage detectable COPs were only present in some sample (Monahan et al., 1992). Pie et al. (1991) detected 0.99mg of cholesterol/g of cooked ground pork. Conchillo et al. (2005) reported that β-epoxycholesterol was the most abundant COPs in raw samples and in vacuum-stored grilled beef, while 7-ketocholesterol was the most abundant COPs in the rest of the samples. Baggio et al. (2002) found 7-ketocholesterol levels of about 330㎍ /kg sample in raw turkey breast meats after 16 month of frozen storage. Amounts of 7α -hydroxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholesterol and β-epoxycholesterol were lower in vacuum-stored than in aerobically stored samples, probably owing to the lack of oxygen under vacuum, which would protect cholesterol from oxidation (Conchillo et al., 2005). Monahan et al. (1992) demonstrated that the rate of cholesterol oxidation in pork is greatly accelerated during storage following cooking and appears to follow the same trend as lipid oxidation in general. Zubillaga and Maerker (1991) reported that the predominant species was reported to be 7-ketocholesterol, accounting for almost half of

the total COPs, followed by 7α- and 7β-hydroxycholesterol and both epoxides in order of decreasing concentration. Oxidation reactions are initiated in the highly susceptible membrane bound phospholipids, which contain relative large amounts of polyunsaturated fatty acids. This process particularly affects unsaturated lipids, with poultry meat being one of the most susceptible, because, according to food composition tables, the ratio between unsaturated and saturated fatty acids in chicken is higher than in other meats such as pork, beef and mutton (Moreiras et al., 2001). Verleyen et al. (2003) reported that the amount of 7β-hydroxycholesterol and β-epoxide in crude tallow were 0.2 and 0.6 μg/g, respectively. It is noteworthy that other COPs were not present in quantifiable concentrations in crude tallow samples, which may be due very low oxidative stress applied to the crude tallow during production (Verleyen et al., 2003).

On the other hand, oxidation in meat products depends the quality of the raw materials, the amount of antioxidants added, the processing conditions and the length of ripening and storage. In general, great discrepanices have been observed in the COPs data obtained from different laboratories for the various matrices analyzed, which may be due to differences in the extraction procedures or the chromatographic methods. In any case, these large discrepancies underline, once more, the necessity of comparing the most promising methods for determination of COPs in food (Dionisi et al., 1998).

2. Egg products

An average egg contains 213mg cholesterol (Anon, 1990). This is about twice the cholesterol content of butter and freeze-dried meat products and about 5-10 times more cholesterol than is found in most dairy products (Nourooz-Zadeh and Appelqvist, 1988). Concentrations of total COPs in egg products have ranged from trace amount to 200ppm, but COPs have not been reported in fresh eggs. α and β-epoxide in egg were most predominant oxidized cholesterol. Sander et al. (1989) showed that the α -epoxysterols were the main accumulating products of cholesterol oxidation in during egg powders storage. They found the average levels of COPs in eggs in decreasing order as follows: α-epoxide, 7β-hydroxycholesterol, β-epoxide, and 7-ketocholesterol. Nourooz-Zaheh and Appelqvist (1988) found higher β-epoxides than α-epoxides with the β to α ratio equal to 5-10 to 1 depending on storage time. Fontana et al. (1993) reported that 25-hydroxycholesterol and cholestanetriol were found only in the egg powder that had been heated at 90℃ for 6 to 24hr. They found that 7-ketocholesterol (from 2.2 ppm to 317ppm) also increased by heating. Generally, cholesterol content in moisturized powders had lower values than in not moisturized ones. Obara et al. (2005) reported that the total level of COPs was higher in spray-dried powders than in freeze-dried one.

Water content in powders had a highly significant influence on oxysterol accumulation during storage (Obara et al., 2005). They estimated five COPs of spray-dried powder and 5α, 6α-epoxide content accumulated in the highest amounts in egg powders was dependent on the level of water. Before storage, 5α, 6α-epoxide constituted 37% of total oxysterols in egg powders. After 3 months of storage, the 5α, 6α-epoxide content increased to 47% of total COPs (Obara et al., 2005). Caboni et al. (2005) reported that cholesterol in egg samples was 2.6g/100g fat: after 12 months of storage at 20℃ the oxidation affected about 0.6% of cholesterol since COPs concentration reached 167μg/g fat. They found that the main COPs were 7α, 7β-hydroxycholesterol, α,β-epoxide, 7-ketocholesterol and triol in spray-dried egg during storage. 7-ketocholesterol represented about 17% of total COPs, both in pasteurised egg and immediately after spray-drying. However, although 7-ketocholesterol increased during 12 month of storage at 20℃, at the end of the storage it represented only 10% of total COPs (Caboni et al., 2005). In fresh and preserved meat, the 7-ketocholesterol amount was 30% of total COPs, whereas, in food containing eggs, the percentage decreased, such as in egg noodle, where 7-ketocholesterol was not the main COPs (Boseli et al., 2004). The fact that 7-ketocholesterol was not the most represented COPs, although the sample preparation causes the formation of additional amount of 7-ketocholesterol from hydroperoxide degradation (Rodriguez-Estrada et al., 2004), suggests that the ketonic function has a selective reactivity, leading to formation of still unidentified compounds. The sum of 7α, 7β-hydroxycholesterol represented 52% of total COPs at the end of the storage at room temperature. A the same stage, the sum of α, β-epoxide and triol is about 35% of total COPs in spray-drying eggs (Caboni et al., 2005). However, Guardiola et al. (1995) determined only α-epoxide and 7β-hydroxycholesterol, and 20-hydroxycholesterol was also identified, but its amount reached 3.5μg/g fat and no significant variation was observed during storage. Sarantinos et al. (1993) reported that the COPs were not detected in fresh egg yolk. They suggested that prolonging cooking time increased the COPs content of fried and boiled eggs. An increase in production of COPs was related to cooking time in lyophilized (phospholipid-free) egg yolk. van de Bovenkamp et al. (1988). reported that the very high level of COPs in dry egg yolk powder after 1 year storage followed by irradiation with UV light for 3 weeks. The trend of COPs was peculiar and different with respect to results observed in other experiments. These differences may result from differences of experimental design and difference of analysis methods. Most COPs are readily absorbed and carried to tissues where they can exert their negative biological effect (Caboni et al., 2005). Thus, more research is needed to how to reduce the COPs in foods. and their relation to the

biological effect and disease in animal models or human.

3. Dairy products

Milk contains approximately 12mg cholesterol/100g or 3mg/g milk fat and the content depends on several factors (Walte, 1994), and several dairy products are reported to contain COPs (Pie et al., 1990). Nourooz-Zadeh and Appelqvist (1988) reported that the quantitative pattern for concentration of the 8 COPs in milk powder products was fairly similar to that observed in analysis of dehydrated egg yolk and its mix products. In Parmesan cheese and cheese spreads, COPs were detected only in a range of a few mg/kg fat (Schmarr et al., 1996). In other study, only 7-ketocholesterol and traces of 7α and 7β-hydroxycholesterol were found in one of five samples of processed cheese and Raclette cheese (Rose-Sallin et al., 1997). Similar results were obtained for sliced yellow and grated yellow cheese (Nielsen et al., 1995). Raclette cheese heated for 5 min to a temperature of 134℃ contained a detectable amount of 7-ketocholesterol and 7-ketocholesterol as well as 7α and 7β-hydroxycholesterol were found in a cheese fondue treated in an infrared oven for 4.5 min. However, these heat-intense treatments produced only small amount of COPs (Rose-Sallin et al., 1997). Butter oils prepared from salted and unsalted butter were heated at 110℃ for 24 days. After this treatment, unsalted butter oil contained more than 300mg/kg ketocholesterol and 200mg/kg α-epoxycholesterol, levels of COPs, which were two to three times higher than in salted butter oil. Salt seems to have an antioxidant effect on cholesterol oxidation (Sander et al., 1989). Gallina et al. (1995) reported that milk products usually show predominant concentration of 7-ketocholesterol over all the other COPs. 7-ketocholesterol was in relatively higher concentration in milk powder products than in egg yolk powder. It is highly likely that differences in mineral composition of the products govern the proportion of 7-ketocholesterol to 7-hydroxycholesterol (Smith, 1987). Fresh cream as well as fresh butter did not contain detectable COPs (Pie et al., 1990). Saneder et al. (1989) also showed that fresh milk and milk products contained none or only trace amount of COPs. They also reported that cheese spread, cottage cheese, evaporated milk, and whole milk did not contain any of the COPs. The probability of COPs forming in fresh milk or fresh dairy products is very low since the medium is liquid and the oxygen content is low (Sieber, 2005). Furthermore, milk has a low level of polyunsaturated fatty acids and of prooxidant trace elements such as iron and copper. In other studies, traces or low levels of 7β-hydroxycholesterol and 5, 6α-epoxycholesterol (Kumer and Singhal, 1992) and 7-ketocholesterol (Pie et al., 1990) were detected. Nielsen et al. (1996) reported the total COPs content of butter and dairy spread were 1.4 and

2.7mg/kg lipid, respectively. However, these studies were questionable because not all samples were confirmed by MS.

V. Prevention of Cholesterol Oxidation

Lipid oxidations are dependent on several factors, the most important being the level of polyunsaturated fatty acids present (Allen and Foegeding, 1981). Triacylglycerol and phospholipids were important in the development of lipid oxidation in chicken (Igene et al., 1980). The influence of triacylglycerols on development of rancidity was shown to depend on the degree of unsaturation. The polyunsaturated fatty acid content of muscle varies between species and decreases in the order fish>poultry>pork>beef>lamb. Susceptibility to lipid oxidation and cholesterol oxidation were shown to be in the same order. Lipid oxidation is catalyzed by myoglobin, hemoglobin, cytochromes, non-heme iron and other heavy transition metals. Apt and Morrissey (1987) clearly showed that the ferritin fraction contributed significantly to lipid oxidation in heated meat systems. The susceptibility of muscle to lipid oxidation is influenced by the presence of antioxidant. Dietary vitamin E supplementation improved the oxidative stability of muscle from meat. Galvin et al. (2002) analysing the effect of dietary vitamin E supplementation on cholesterol oxidation in vacuum-packaged cooked beef steaks, observed that 7-ketocholesterol decreased in both some refrigerated and frozen samples. Lai et al. (1995) reported that oleoresin rosemary reduced the formation of COPs by 36% in the egg powders. Morgan and Armstrong (1987) showed that elevated temperature and pro-oxidizing agent (H_2O_2) markedly increased cholesterol-5, 6-epoxides production. However, the antioxidants butylated hydroxyanisole and butylated hydroxytoluene displayed and inhibitory effect. Several researchers have suggested that prevention of cholesterol oxidation in processed foods should be similar to procedures to prevent lipid oxidation. Overall, the formation of COPs in animal products can be minimized by the application of low processing temperatures, that is through minimal processing, by the use of oxygen-proof packaging and a protective atmosphere as well as by low-temperature and light-free storage, by the dietary antioxidants to animals or antioxidants addition to foods.

VI. Effect of COPs on Atherosclerosis

Animal products are the major source of cholesterol in the diet. The effects of cholesterol-containing foods in the human diet has been the subject of many

investigations in recent years, largely due to the hypothesized link between cholesterol and coronary heart disease. There no longer is any doubt that hypercholesterolemia is one of the major causative factors in atherosclerosis because cholesterol is a major ingredient of atherosclerotic plaque and cholesterol feeding has been shown to induce atherosclerosis in animals. Dietary COPs are incorporated as acyl esters (Bonded COPs) in chylomicrons and transported via lymph into the blood stream (Vine et al., 1997). Results of several investigations demonstrated that 25-hydroxycholesterol and cholestane-3β, 5α, 6β-triol (cholestanetriol) were the most toxic agents. Peng et al. (1991) demonstrated that 25-hydroxycholesterol and cholestanetriol are toxic agents linked to atherosclerosis. Cholesterol in the American diet has been indicated as contributing to the high death rate from cancer and coronary heart disease. Several researchers have focused on the association between cholesterol and atherosclerosis (Grundy, 1986; Ross, 1986; Allred et al., 1990; Kumar and Singhan, 1991). Animal studies showed a positive correlation between hypercholesterolemia and atherosclerosis (Chaikoff et al., 1948; Faggiotto and Ross, 1984; Higley et al., 1986). They noted that free radical attack on exogenous cholesterol resulted in the accumulation of oxides in tissue. One toxic COPs, 25-hydroxycholesterol, is absorbed in mammals when included in the diet and causes defects in the aortic surface as revealed by scanning electron microscopy (Peng et al., 1982). Various hydroxylated cholesterol derivatives are potent inhibitors of HMG-CoA reductase in the aortic cells to some extent (Peng et al., 1979; Kumar and Singhal, 1991; Lund and Bjorkhem, 1994). Inhibition of cholesterol biosynthesis by these compounds may cause cell death because of membrane dysfunction. These dead cells could be the primary entry for lipid infiltration which ultimately led to atherosclerosis (Morin and Peng, 1989; Peng et al., 1991).

Table 1. Cholesterol oxidation products in a variety animal products.

Food Products	Cholesterol oxidation products						Reference
	7α-hy[a] 7β-hy	7-keto	α-ep β-ep	25-OH	triol	Total COPs	
Raw beef	nt[b]	3.5 (mg/kg)	nt	nt	nt	0.5-3.4 (mg/kg)	Hwang and Maercker (1993)
Beef tallow	β-0.17 (μg/g)	nd	β 0.60 (μg/g)	nd	nd	nt	Verleyen et al. (2003)
Raw ground beef	nd	tr-0.6 (mg/g)	nd	nd	nd	nt	Paniangvait et al. (1995)
Fresh pork	nd	nd	nt	nd	nt	nt	
Freeze dried pork (3years stored)	α-28.98 (mg/g) β-21.05 (mg/g)	126.56 (mg/g)	nt	nd	nt	nt	
Cooked beef	nt	nt	nt	nt	nt	3.1-5.9 (mg/kg)	Engeseth and Gray (1994)
Chicken meat (beef tallow fed)	nt	nt	nt	nt	nt	3.70 (μg/g)	Grau et al. (2001)
Chicken meat (after irradiation)	α,β-43.2 (μg/g fat)	6.0 (μg/g fat)	α-3.9 (μg/g fat)	nt	nd	53.4 (μg/g fat)	Nam et al. (2001)
Raw chicken meat (3months stored)	α-1.31 (μg/g fat) β-1.49 (μg/g fat)	0.55 (μg/g fat)	α-0.20 (μg/g fat) β-2.69 (μg/g fat)	0.23 (μg/g fat)	0.92 (μg/g fat)	7.40 (μg/g fat)	Conchillo et al (2005)
Raw turkey meat	α-3.6 (mg/kg) β-4.8 (mg/kg)	5.1 (mg/kg)	α-1.6 (mg/kg) β-7.0 (mg/kg)	nt	1.5 (mg/kg)	23.7 (mg/kg fat)	Boselı et al. (2005)
Buffalo meat (6days stored)	α-5.41(%)	10.07(%)	α-17.81(%) β-1067(%)	nt	4.86 (%)	nt	Rao et al. (1996)
Salami	nt	0.2-1.1 (mg/kg)	nt	nt	nt	tr-16.6 (mg/kg)	Novelli et al. (1998)
Cured ham(fat) (12months stored)	β-0.4 (mg/g)	1.1 (mg/g)	α,β-0.4 (mg/g)	0.2 (mg/g)	0.7 (mg/g)	nt	Vestergaard et al (1999)
Raw milk	nd	nd	nd	nd	nd	nt	Sıeber (2005)
Vanılla yogurt	α-2 (mg/kg) β-1 (mg/kg)	4 (mg/kg)	α-2 (mg/kg) β-3 (mg/kg)	nd	1 (mg/kg)	nt	
Grated cheese	nt	0.2-0.8 (mg/kg)	nt	nt	nt	4-46 (mg/kg)	Fınocchıaro et al (1984)
Whole milk powder	nt	0.3-0.7 (mg/kg)	nt	nt	nt	tr-6.8 (mg/kg)	Zunin et al (1998)
Whole egg powder	nt	1.3-4.6 (mg/kg)	nt	nt	nt	8-311 (mg/kg)	Laı et al. (1995)
Spray-dried egg (1month stored)	nt	nt	nt	nt	nt	47.8 (μg/g fat)	Caboni et al (2005)
Egg yolk powder	α-3.95 (mg/100g fat) β-5.92 (mg/100g fat)	7.94 (mg/100g fat)	α-12.75 (mg/100g fat) β-13.55 (mg/100g fat)	nt	nt	44.11 (mg/100g fat)	Obara et al. (2005)

[a] 7α-hy = 7α-hydroxycholesterol, 7β-hy=7β-hydroxycholesterol, 7-keto=7-ketocholesterol, α-ep = α-epoxycholesterol, β-ep = β-epoxycholesterol, 25-OH = 25-Hydroxycholestrol, triol = Cholestanetriol.

[b] nt= not tested, nd=not detected

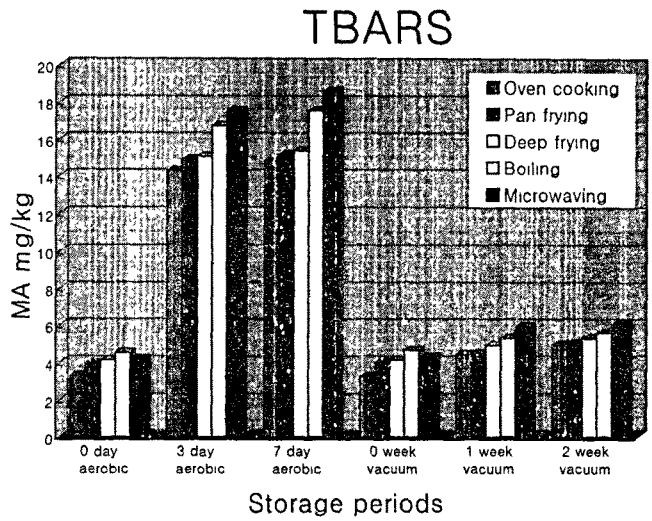


Figure 3. Effect of cooking methods and packaging conditions on the TBARS of turkey thigh meat patties during storage.

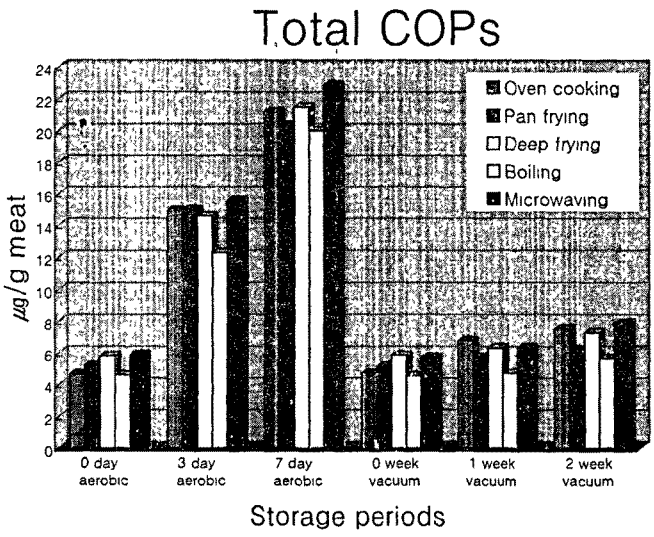


Figure 4. Effect of cooking methods and packaging conditions on the total COPs of turkey thigh meat patties during storage.

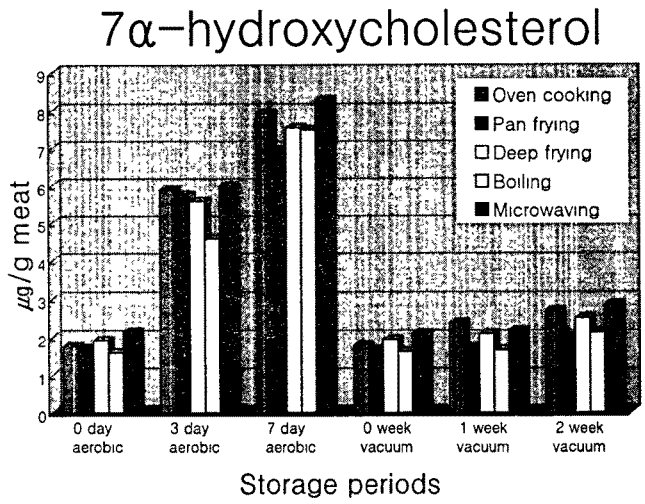


Figure 5. Effect of cooking methods and packaging conditions on the 7α-hydroxycholesterol of turkey thigh meat patties during storage.

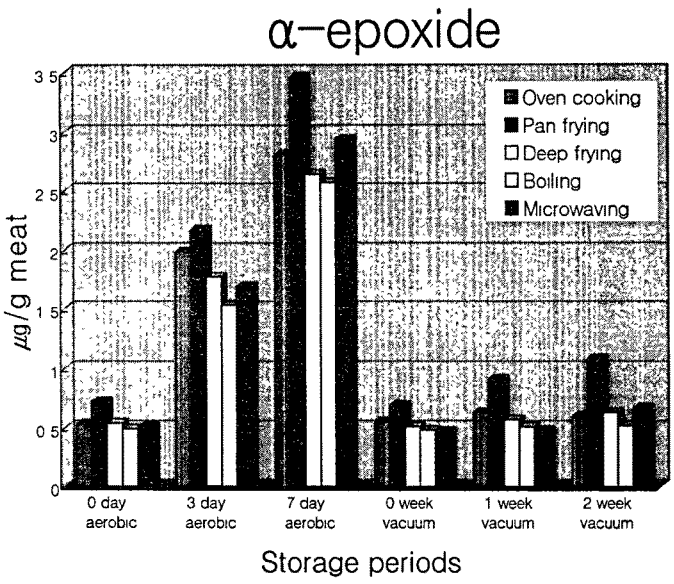


Figure 6. Effect of cooking methods and packaging conditions on the β-epoxide of turkey thigh meat patties during storage.

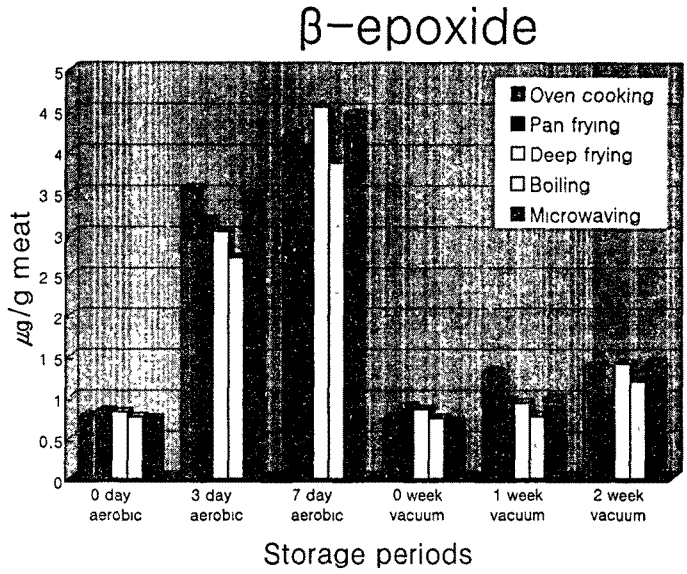


Figure 7. Effect of cooking methods and packaging conditions on the α-epoxide of turkey thigh meat patties during storage.

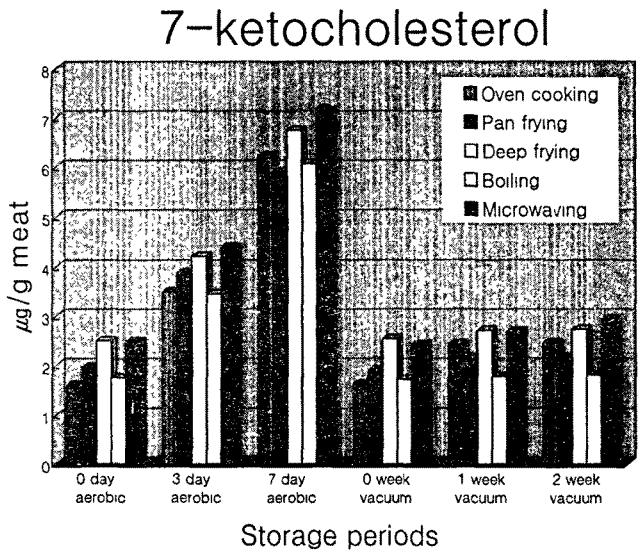


Figure 8. Effect of cooking methods and packaging conditions on the 7-ketocholesterol of turkey thigh meat patties during storage.

Table 2. Effect of dietary cholesterol and cholesterol oxidation products on total cholesterol oxidation products in rabbits liver.

Dietary chol[1] + COPs g/kg diet	COPs in liver		
	6 week	12 week	SEM
	μg/g		
Experiment 1			
0 g	trace^{Eb}	0.048^{Ea}	0.003
1g chol	0.030^{Db}	0.068^{Da}	0.005
0.9chol + 0.1COPs	0.042^{Cb}	0.074^{Da}	0.004
0.8chol + 0.2COPs	0.055^{Bb}	0.094^{Ca}	0.005
0.5chol + 0.5COPs	0.055^{Bb}	0.096^{BCa}	0.004
Experiment 2			
2g chol	0.053^{BCb}	0.109^{BCa}	0.006
1.6chol + 0.4COPs	0.054^{BCb}	0.112^{Ba}	0.005
1.2chol + 0.8COPs	0.082^{Ab}	0.146^{Aa}	0.006
SEM	0.004	0.006	

^{A,B,C,D} Different letters within a column are significantly different ($P<0.05$).

^{a,b,c,d,e} Different letters within a row are significantly different ($P<0.05$).

[1] chol: natural cholesterol

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