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Analysis, Formation and Inhibition of Cholesterol Oxidation Products in Foods: An Overview (Part II)

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This article is the second in a two-part series. Part I appeared previously in the issue of December 1999.

FACTORS AFFECTING FORMATION OF COPS

I. *Processing Method*

(I) *Heating*

1. *Cholesterol Standards*

Cholesterol can undergo oxidation to form a large amount of COPs after heating at an elevated temperature for a certain period of time. Fioriti and Sim[66] heated the cholesterol solution at 82°C for 9 weeks and a total of 13 COPs was formed. The long heating period probably plays an important role for COPs formation because it has been reported that cholesterol is very stable at 100°C[68]. Meanwhile, the application of different heating facilities may influence the production of COPs in various forms. The amount of COPs in the egg mix with direct heat treatment utilizing a gas fired oven to generate hot air, was found to be three times higher than the indirect heat treatment using a steam convection process[43]. Of the various COPs, 7-keto, 5,6α-EP and 5,6β-EP were present in the highest amount. The ratio of 5,6α-EP/5,6β-EP was reported to be between 1 and 4[67]. The reason that the direct heating treatment produced a higher amount of COPs can be attributed to the formation of nitrogen oxide, which is a by-product of organic combustion. Nitrogen oxide is known to be able to initiate the autoxidation of unsaturated fatty acids[43]. Osada *et al*.[68] studied the stability of cholesterol during heating at 100, 120, 150 and 200°C for 24 hrs in an oven. Results showed that approximately 60% of cholesterol was destroyed after 150°C heating for 24 hrs, while the complete destruction of cholesterol was observed after 200°C heating for 6 hrs. Likewise, under this condition, all of the COPs formed were also degraded completely. Interestingly, no oxidized cholesterol was formed at 100°C for 24 hrs. In view of this result, certain cooking methods such as boiling and steaming are likely to limit the formation of COPs. A total of six COPs was generated, including 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto and triol in the heated cholesterol[68]. In a recent study, Chien *et al*.[69] studied the kinetics of cholesterol oxidation during heating of cholesterol at 150°C for up to 30 min. The COPs concentration was found to increase with increasing heating time. During the early stage of oxidation, the highest rate constant (hr^{-1}) was observed for 7-OOH formation, followed by epoxidation (cholesterol ← 5,6α-EP and 5,6β-EP), dehydration (7-OOH ← 7-keto), reduction (7-OOH ← 7-OH) and dehydrogenation (7-OH ← 7-keto). These results agree with most studies, showing that 5,6α-EP and 7-keto are the most common COPs formed in

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either food or model systems. The production of 7-OOH in a large amount is expected because it is a precursor of several COPs such as 7-keto and 7-OH.

2. *Egg and Egg Products*

Eggs have been widely used in cooking or as ingredients for processed food products, and the presence of COPs in egg products has become a major concern for consumers. Tsai and Hudson[41] reported that both 5,6α-EP and 5,6β-EP were present in freshly dehydrated whole egg powder and yolk powder, however, no COPs were identified in fresh shell eggs or lyophilized eggs. Apparently, both 5,6α-EP and 5,6β-EP are generated during the commercial drying process. In a more recent study, several other COPs were also identified in spray-dried eggs. Likewise, Nourooz-Zadeh and Appelqvist[70] did not detect COPs in fresh or freeze dried egg yolks. After undergoing the spray drying process, a trace amount of COPs was found and the quantity increased with longer storage time. The total COPs concentration reached 36 ppm after one year of storage. The authors identified 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 20α-OH, 7-keto, 25-OH and triol in dehydrated egg yolk and egg mixes. Similarly, Pie and coworkers[37] also observed the same COPs in some commercial egg powders and egg mixes, and the total COP content in egg powders was much higher than that in dehydrated egg yolk[70]. It is evident that spray drying is a key step to the production of COPs in egg powders[37,41,70]. Since egg powders are commonly used as ingredients of many food products, some additional heat treatments such as baking may further accelerate the oxidation rate. Zunin *et al*.[71] used 7-keto as an indicator to quantify COPs in baked foods containing fresh eggs or egg powders. The authors reported that commercial biscuits generally contained a higher amount of 7-keto than sweet snacks. The difference may be due to the variety of ingredients used. In the same study, Zunin and co-researchers[71] further demonstrated that with the use of fresh eggs or egg powder as the only cholesterol-containing component in biscuit making, the cholesterol oxidation proceeded much faster than when egg powder was used as an ingredient. Kou and Holmes[42] found that the highly toxic 25-OH was not present in egg yolk and spray-dried yolk powder. However, a significant amount of 25-OH was found in yolk powder after heating at 110˚C for 4 days. A relatively high concentration of 25-OH (10.4 ppm) was also found in an 8-year-old dehydrated egg yolk sample[70]. Both cases indicate that 25-OH is likely to be formed under drastic conditions. However, from a practical point of view, these conditions are not expected to occur in a pilot plant.

High-temperature dehydration is an indispensable procedure for the production of some low-moisture food products. Under such a process, it is difficult to prevent COPs formation in certain products with high cholesterol levels. Moreover, these food items tend to be treated for a longer storage time and thus the generation of COPs can be enhanced. Freeze drying is another alternative for the replacement of high-temperature drying to prevent COPs formation, however, because of high cost, this method is not practical for commercial production of dehydrated foods.

3. *Dairy Products*

The presence of COPs in milk powder and infant formula is another major concern since dehydration is a key step for the production of these products. As weak or sick people are likely to be the consumers of milk powder and infant formula, the issue of COPs formation in these products may need extra caution. Nourooz-Zadeh and Appelqvist[48] examined COPs in some commercial milk powders made from various pre-concentrated milks and retained over extended storage periods. Milk powders were classified as "low-heat", "medium-heat" or "high-heat" depending on the temperature used during the concentration stage of pre-concentrated milk. The authors reported that no COPs were detected in the fresh low-heat-, medium-heat-skim or whole milk powders, but several COPs, 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP and 7-keto, were identified in the fresh high-heat milk powders. Infant formula

Table 2. Formation of COPs in food products as affected by various treatments

Food	Treatment	COPs formed	Amount (ppm)	Ref.
Dairy products:				
Skim milk powder	Fresh	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	Trace-2.6	48
Skim milk powder	Storage (13-37 mo)	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20α-OH, 25-OH, triol	Trace-23.3	48
Whole milk powder	Fresh	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	Trace-1.4	48
Whole milk powder	Storage (12 mo)	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	Trace-9.2	48
Milk powder	Commercial	7α-OH, 7β-OH, 7-keto, 25-OH, triol	0.005-22.415	4
Infant formula powder	Commercial	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 25-OH, triol	2-46	35
Butter	Fresh	7-keto	Trace	37
Butter	Storage (-20°C, 3 or 6 mo)	7α-OH, 5,6β-EP, 7-keto	0.22-1.52	37
Butter	Heating (170 or 180°C, 10 or 20 min)	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20α-OH, 25-OH, triol	Trace-18.45	37
Butter	Illumination	7α-OH, 7β-OH	-	78
Butteroil	Bleaching, storage	7α-OH, 7β-OH, 5,6-EP	20-90	52
Butter powder	Commercial	7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	3-26	35
Sour cream powder	Commercial	7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	3-13	35
Cheese	Bleaching	7α-OH, 7β-OH, 5,6-EP, triol	4-110	52
Cheddar cheese powder	Commercial	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 25-OH, triol	3-17	35
Blue cheese powder	Commercial	5,6α-EP, 7-keto	3 and 4	35
Parmesan cheese powder	Commercial	7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 25-OH, triol	2-16	35
Romano cheese powder	Commercial	7β-OH, 5,6α-EP	2 and 5	35
Parmesan cheese	Commercial	7α-OH, 7β-OH, 19-OH, 20α-OH, 25-OH, 5,6α-EP, 5,6β-EP, 7-keto	0.31-2.81	49
Cheese spread	Commercial	7α-OH, 7β-OH, 19-OH, 25-OH, 5,6α-EP, 5,6β-EP, 7-keto, 3β,5-dihydroxy-5α-cholestan-6-one	0.44-0.75	49
Cream cheese	Commercial	5,6α-EP, 7-keto	9 and 3	35
Powdered cheese	Storage	7α-OH, 7β-OH, 25-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	1-42	36
Powdered cheese	Illumination	5,6α-EP, 5,6β-EP, 7β-OH, 7-keto	1-20	36
Egg products:				
Egg yolk powder	Commercial	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto	2-79	35
Egg yolk	Spray-dried	5,6α-EP, 5,6β-EP	-	41
Whole egg powder	Commercial	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	2-111	35
Egg nog mix	Illumination	7α-OH, 7β-OH	0-85	59

Table 2. Continued

Food	Treatment	COPs formed	Amount (ppm)	Ref.
Dried powdered egg mix	Dried by a direct heating source or indirect heating source	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	1.4-50.0	43
Fresh egg	Commercial	7-keto	Trace	70
Omelet mix	Commercial	7β-OH, 5,6β-EP, 7-keto,	2-10	35
Dehydrated egg mix	Storage (2-18 mo)	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	0.2-3.2	70
Dehydrated egg yolk	Storage (2 mo-8 yr)	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 20α-OH, 7-keto, triol	Trace-46.8	70
Egg yolk	Spray-dried	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	4.93-9.13	53
Fish products:				
Anchovy	Salted-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	0.2-48.8	63
Northern cod	Salted-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	0.2-9.7	63
Pacific cod	Salted-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	0.2-5.9	63
Japanese whiting	Salted-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	3.4-24.9	63
Pacific saury	Salted-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	Trace-9.9	63
Pacific herring	Salted-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	3.5-8.4	63
Anchovy	Boiled-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	1.8-60.6	63
Shrimp	Boiled-dried	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	Trace-4.0	63
Keta salmon	Smoked	7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	2.4-7.3	63
Squid	Canned boiled	7β-OH, 5,6α-EP, 5,6β-EP	0.02-0.28	72
Squid	Dried	7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	0.14-0.55	72
Dried squid	Baking	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	0.8-15.5	73
Alaskan pollack roe	Pickled, spiced	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	0.03-0.58	72
Salame	Commercial	7α-OH, 7β-OH, 20α-OH, 5,6α-EP, 5,6β-EP, 7-keto	0.35-2.21	49
Meat products:				
Minced beef	Raw	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20-OH, 25-OH	0.14-1.06	62
Minced beef	Cooked	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20-OH, 25-OH	0.23-2.11	62
Beef	unirradiated	5,6α-EP, 5,6β-EP, 7-keto, 4-cholesten-3-one, 4,6-cholestadien-3-one,	1.9-60.1	30

Table 2. Continued

Food	Treatment	COPs formed	Amount (ppm)	Ref.
		4-cholestene-3,6-dione		
Beef	Irradiated	5,6α-EP, 5,6β-EP, 7-keto, 4-cholesten-3-one, 4,6-cholestadien-3-one, 4-cholestene-3,6-dione	2.3-443	30
Beef	Raw	5,6α-EP, 5,6β-EP, 7-keto	13.4-82.9	47
Freeze-dried beef	Commercial	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	1-27	35
Veal	Raw	5,6α-EP, 5,6β-EP, 7-keto	3.3-22	47
Veal	Unirradiated	5,6α-EP, 5,6β-EP, 7-keto, 4-cholesten-3-one, 4,6-cholestadien-3-one, 4-cholestene-3,6-dione	Trace-31.6	30
Veal	Irradiated	5,6α-EP, 5,6β-EP, 7-keto, 4-cholesten-3-one, 4,6-cholestadien-3-one, 4-cholestene-3,6-dione	2.7-183	30
Minced veal	Raw	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20-OH, 25-OH	0.04-0.71	62
Minced veal	Cooked	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20-OH, 25-OH, triol	0.07-0.84	62
Pork	Raw	5,6α-EP, 5,6β-EP, 7-keto	2.3-6.3	47
Freeze-dried pork	Stored at 22°C with air (3 yr)	7α-OH, 7β-OH, 5,6α-EP, 7-keto, triol	12.5-259.8	34
Pork	Unirradiated	5,6α-EP, 5,6β-EP, 7-keto, 4-cholesten-3-one, 4,6-cholestadien-3-one, 4-cholestene-3,6-dione	Trace-45.5	30
Pork	Irradiated	5,6α-EP, 5,6β-EP, 7-keto, 4-cholesten-3-one, 4,6-cholestadien-3-one, 4-cholestene-3,6-dione	Trace-108	30
Minced pork	Raw	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 25-OH, triol	0.04-0.92	62
Minced pork	Cooked	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20-OH, 25-OH, triol	0.06-2.25	62
Pork links	Commercial	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	0.55-3.84	49
Chicken	Raw	5,6α-EP, 5,6β-EP, 7-keto	5.8-12.9	47
Chicken	Storage (50°C, 8-18 days)	7α-OH, 7β-OH, 7-keto, 5,6α-EP, 5,6β-EP	Trace-6.6	74
Freeze-dried chicken	Commercial	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	2-43	35
Freeze-dried turkey	Commercial	7β-OH, 5,6α-EP, 5,6β-EP, 7-keto	6-21	35
Bovine raw brain concentrate	Commercial	7-keto, 7α-OH, 7β-OH	31.6-71.1	44
Bovine raw liver concentrate	Commercial	7-keto	13.8	44
Liverwurst	Commercial	7α-OH, 7β-OH, 20α-OH, 25-OH,	0.86-10.88	49

Table 2. Continued

Food	Treatment	COPs formed	Amount (ppm)	Ref.
		5,6α-EP, 5,6β-EP, 7-keto, 3β,5-dihydroxy-5α-cholestan-6-one, triol		
Bacon	Fried	7α-OH, 7β-OH, 7-keto, 5,6α-EP, 25-OH	0.2-0.5	74
Lipids:				
Fish oil, flax oil, sunflower oil, palm oil	Heating, storage, antioxidant addition	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	0.62-56.5	65
Tallow	Heating (135-180°C)	5,6α-EP, 7-keto	9.1-43.7	33
Tallow	Heating	7α-OH, 7β-OH, 5,6α-EP, 7-keto	-	32
Lard	Refined, deodorized	7α-OH, 7-keto, 5,6α-EP, 20α-OH, 25-OH	Trace-0.4	74
Others:				
Protein powder	Commercial	7α-OH, 7β-OH, 5,6β-EP, 7-keto, triol, 25-OH	2-24	35
Vanilla ice cream	Commercial	5,6α-EP, 5,6β-EP	6 and 1	35
Vanilla yogurt	Commercial	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, triol	1-4	35
Pancake mix	Commercial	7-keto	1.1	44
French fries	Commercial	7-keto, 7β-OH	4.1-58.8	44
French fries	Commercial	7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 25-OH, 7-keto, triol	1-17	61

powders have also been found to contain several COPs, including 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 25-OH and triol[35] (Table 2).

Butter is commonly used as an ingredient in western-style cooking. However, Sander *et al.*[36] reported that heating butter might cause formation of COPs. These researchers detected five COPs, 7-keto, 7α-OH, 7β-OH, 5,6α-EP and 5,6β-EP, in butter heated at 110°C for 24 days. Nevertheless, the heating time is too long to represent actual cooking conditions. In another study, Pie *et al.*[37] found that nearly no COPs were present in fresh butter, but the quantity increased greatly after heating at 170 or 180°C for 10 or 20 min. The identified COPs included 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20α-OH, 25-OH and triol. Both 25-OH and triol, the highly toxic COPs, were produced in significant levels when the stored butter was used for cooking, while only a trace amount was generated when fresh butter was used. The same authors[37] also demonstrated that COPs were readily formed when cooking with butter as an ingredient. In addition, dehydrated butter powder was found to contain COPs. Sander *et al.*[35] identified 7β-OH, 5,6α-EP, 5,6β-EP, and 7-keto in commercial butter powder.

4. *Fish and Fish Products*

Osada *et al.*[72] 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.*[63] 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[73] analyzed COPs in dried squid and found that 7α-OH, 7β-OH, 5,6β-EP, 5,6α-EP, 7-keto, 20α-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.*[72]. 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, which will be discussed in a later section.

5. *Meat and Meat Products*

Several researchers[34] found that no or only a trace amount of COPs was present in fresh meat, but some other workers[30,62] identified a high amount of COPs with a more sensitive technique. The presence of cholesterol in meat and its processed products are responsible for formation of COPs during cooking. Nourooz-Zadeh and Appelqvist[74] determined the COPs in rind and lean portions of bacon. No COPs were found in raw rind, however, both 7α-OH and 7β-OH were detected when the bacon was subjected to frying at 170°C for 10 min. Other COPs, 5,6α-EP, 7-keto and 25-OH, were formed with the frying temperature at 200°C. Interestingly, COPs were detected in lean portions of either raw or fried samples. Park and Addis[44] did not detect 7-keto in raw beef and beef products, however, it was identified in broiled beef steak[34]. Pie *et al.*[62] compared the contents of COPs in raw and cooked minced meats (beef, veal, and pork), which were heated in an electric skillet (135°C) for 10 min each side. The COPs levels of cooked beef, veal and pork increased by 1.7, 2.3 and 2.5 fold, respectively. When beef, pork and veal were oven-cooked at 220°C for 60, 80 and 90 min, respectively, the COPs levels increased by 3.5, 5.4 and 4.2 fold. The identified COPs included 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 7-keto, 20α-OH, 25-OH and triol. Also, the primary oxidation products (5,6α-EP, 5,6β-EP, 7-keto, 20α-OH or 25-OH) were formed at a greater amount than the secondary products (7α-OH, 7β-OH or triol) during cooking of meat products[62]. Sander *et al.*[35] observed 7-keto, 7β-OH, 5,6α-EP, 5,6β-EP, 25-OH and triol in dehydrated beef, chicken and turkey in ppm levels.

6. *Lipid*

Park and Addis[32] 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 5,6α-EP were found, and the amount of 7-keto formed during frying increased linearly with heating time. In a similar study, Park and Addis[33] used the same technique to isolate five COPs from tallow heated at 155°C for 400 hrs. These COPs were identified as 7α-OH, 7β-OH, 7-keto, 5,6α-EP and 5,6β-EP. Approximately 25% cholesterol was lost, and five COPs, triol, 7-keto, 7-oxo-cholesta-3,5-diene, 7α-OH and 5,6α-EP (5,6β-EP) were detected in tallow heated at 142-184˚C for 56-70 hrs[75]. Likewise, Park and Addis[33] also observed a 40-50% cholesterol loss in tallow heated at 155 and 190°C for 300 hrs and identified 7α-OH, 7β-OH, 7-keto and 5,6α-EP. Zhang *et al.*[61] 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[38] 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 5,6α-EP were the predominant COPs found, while 7α-OH, 7β-OH and triol were formed in minor amounts. Results also showed that after heating at 180°C (10 hrs per day) for 24 days, lard enriched with cholesterol at 10 times was more susceptible to COPs formation than that enriched with cholesterol at 2 times. However, the degradation rate of cholesterol of the former was slower than the latter. Chen *et al.*[56] analyzed the COPs in heated lard, and five COPs, 7-keto, cholesta-4,6-diene-3-one, 5,6α-EP (5,6β-EP), 7β-OH, and triol were detected. In most cases, the COPs contents rose with increasing heating time,

however, several COPs, 5,6α-EP, 5,6β-EP and 7β-OH, increased in the first 100 hrs and then declined thereafter. The highly toxic triol could not be detected until heating time reached 20 hrs. The long heating period or high temperature may degrade COPs to a great extent, but some other harmful compounds may be produced as well.

(II) *Bleaching and Sterilization*

Bleaching is commonly applied in the dairy industry to improve the color quality of milk products. Benzoyl peroxide, a prooxidant and a free radical generating agent, has been widely used to process blue, Swiss or Italian cheese. As a consequence, bleaching may induce COPs formation. Fortunately, no COPs were detected in ungrated cheeses made from bleached milk and freshly bleached butter oil. However, COPs were identified in stored bleached butter oils and commercial grated cheeses. These COPs include 7α-OH, 7β-OH, 5,6α-EP and 5,6β-EP[52]. Similarly, hydrogen peroxide, a strong oxidizing agent, has been used to improve the color quality of many foods. Furthermore, hydrogen peroxide has been employed in the dairy industry as a "cold sterilant" for milk to be used in certain kinds of cheese making[98]. The interaction between oxidizing agents and cholesterol-containing foods may potentially facilitate the formation of COPs.

II. *Storage Conditions*

Sander *et al*.[36] showed that the amounts of COPs increased both with increasing storage temperature and time in cheese powder. Similar results were observed for milk powder stored for 37 months[48,70]. However, the amount formed during storage of cheese powder at 4°C for 18 months was found to be 3 times greater than that stored at 21 or 38°C for 6 months[36]. This result suggests that storage time might have a greater impact than storage temperature on the formation of COPs under mild conditions. Pie *et al*.[62] further reported that only a minor increase of COPs in various minced meats occurred after storage at -20°C for 3 months. In another study, Nourooz-Zadeh and Appelqvist[70] found that only traces of COPs were formed in spray-dried egg yolk powder during storage at 4°C for 2 months. However, several COPs, 7-keto, 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP, 20-OH and 25-OH were detected after prolonged storage for one year. Controlling storage temperature along with a proper storage period can effectively prohibit COPs formation. Apparently, both considerations are the key points in preventing COPs formation in food system. In an extreme case, when both the storage temperature and time are abused, the content of COPs mounts to several hundreds of ppm. With freeze-dried pork storage at 22°C for 3 years, 7-keto was formed in highest amount (260 ppm), followed by 7α-OH, 7β-OH, triol and 5,6α-EP[34]. Nourooz-Zadeh and Appelqvist[70] found that the quantity of COPs showed an increased trend in spray-dried egg yolk powder during storage. Among these COPs, 5,6β-EP, 7α-OH and 7β-OH were the most abundant compounds over a 12-mo storage period. Likewise, 5,6β-EP, 7-keto and 7α-OH were the major COPs in dehydrated egg mixes over the same storage period.

The occurrence of oxidation reaction is the major blame for the formation of COPs. Therefore, to minimize the contact between foods and air is always an effort for food manufacturers. Finocchiaro *et al*.[52] investigated the influence of nitrogen or air on COP formation in stored butter oils. Both 7α- and 7β-OH were identified in butter oils in the air and the content of COPs was about 1.5-3 times higher than that under nitrogen. These oils were then transferred to -20°C and the storage time was extended for an additional year. After storage, four COPs, 5,6-EP, triol, 7α-OH and 7β-OH were present in the oils. For 5,6-EP, 7α-OH and 7β-OH, the levels increased moderately for the oil stored under nitrogen and greatly in air. For cheeses, the same COPs were identified but 5,6-EP became the most abundant compound. The concentrations of these COPs were also found to vary with different types of cheeses. Sander *et al*.[36] investigated the COPs formation in two commercial powdered cheeses under prolonged and adverse storage conditions, and five COPs, 5,6β-EP, 5,6α-EP, 7β-OH, 7-keto and 25-OH were

detected. In general, little or no change in COPs levels was noted for both cheeses stored at 4, 21, or 38°C for up to 6 months[36]. This result is somewhat unexpected since the storage temperatures (21 and 38°C) are relatively higher than most of the other studies and the change of COPs content is minor. When irradiated meats or produces have been introduced into daily life, the safety of those foods is always a controversial issue for consumers and food manufacturers. In the aspect of COPs formation, the irradiation of cholesterol-rich foods should be considered. Hwang and Maerker[30] found that irradiated meats were more susceptible to oxidation than unirradiated ones, and COPs formation was observed during storage at 0-4°C for 2 weeks. After storage, the COPs content increased more than 3 and 4 times in irradiated veal and pork, respectively, while in unirradiated ones, the increased amount was less than 1.6 times. However, this phenomenon was not so obvious in beef since both irradiated and unirradiated ones increased about 5.5 times after storage.

III. *pH Value*

Maerker and Bunick[25] studied the stability of cholesterol by heating at 80°C in an aqueous dispersion under various pH values for 24 hrs. It was found that with pH 8.0, six COPs, 7-keto, 7α-OH, 7β-OH, 5,6α-EP, 5,6β-EP and triol, were formed. Triol was readily formed through hydrolysis of 5,6α-EP or 5,6β-EP under acidic condition. With pH 12, both 5,6α-EP and 5,6β-EP were not formed until heating time reached 24 hrs. However, with pH between 5.5 and 8.0, the amount of isomeric 5,6-EP increased with increasing heating time. No 5,6α-EP or 5,6β-EP could be detected because of acid hydrolysis when pH was less than 3.0. The ratio of 5,6α-EP/5,6β-EP remained constant with pH was at 8.0 or 12.0; however, the ratio increased with extended heating time at pH 5.5, because the β form is more susceptible to hydrolysis than the α form. At pH 3, the ratio of 5,6α-EP/5,6β-EP showed inconsistent change mainly because the hydrolysis rate is too fast. Kim and Nawar[76] studied the effect of pH on cholesterol oxidation in film fragments suspended in buffer at 75°C. The percentage of residual cholesterol was 90, 55, and 43% at pH 6.3, 6.9 and 7.4, respectively, after heating for one day. However, after prolonged heating for 2-6 days, the percentage of residual cholesterol was between 40 and 50% under any pH value. The ratio of 7-keto/7-OH at pH 6 and 7.4 was found to be 7 and 3, respectively. This result implied that the formation of 7-keto from 7-OH through oxidation was more likely to proceed under low pH values. In addition, it was observed that the ratio of 5,6β-EP/5,6α-EP declined with decreasing pH value. Again, this study shows the α-form of 5,6-EP is more acid-resistant that the β-form.

IV. *Irradiation*

The rate of cholesterol oxidation can be facilitated in the presence of light, and the oxidation pathway is reported to be different from that during heating[18]. The extent of cholesterol oxidation can be dependent upon many factors, such as light source, light intensity, illumination time and illumination mode. In an early study, Chicoye *et al*.[77] observed that only 5,6β-EP but no 5,6α-EP was present in spray-dried egg yolk when exposed to sun or fluorescent light. However, in a later study, Tsai and Hudson[54] suggested that the absence of 5,6α-EP is probably due to an improper use of the separation technique. Five COPs, 7-keto, 7α-OH and 7β-OH, 5,6β-EP and triol were detected in spray-dried yolk powder after exposure to fluorescent light for 280 hrs or sunlight for 5 hrs[77]. Similar results were reported by Herian and Lee[59]. Luby *et al*.[78] placed butter blocks under illumination at 22°C (1500 lux) for 20 days. COPs became detectable after 8 days of storage and were more prevalent on the surface. The identified COPs included 7α-OH, 7β-OH and a compound suspected to be 6-cholesten-3β,5α-diol. To remedy this problem, a proper packaging method should be employed to minimize cholesterol oxidation in butter under light storage[79]. However, in cheese powder, only two COPs, 7-keto and 5,6α-EP, were detected after storage under fluorescent light (1611 lux) for 3 weeks[36]. Moriarity

and Maerker[28] studied the effect of γ-irradiation on COPs formation in liposome, and several COPs, including isomeric 5,6-EP, 7-OH, cholest-4-en-3-one, cholest-4-ene-3,6-dione and cholesta-4,6-dien-3-one, were detected.

Irradiation has been incorporated into the preparation procedure of meat products to extend their shelf lives. Hwang and Maerker[30] investigated the contents of COPs formed in irradiated meats including beef, pork and veal. The A-ring oxidation products, which had been found to form in a significant amount in irradiated liposomes, were suggested to become an indicator of irradiated meats[29]. A total of six COPs was identified in irradiated (10 kGy) meats, including 5,6α-EP, 5,6β-EP, 7-keto, 4-cholesten-3-one, 4,6-cholestadien-3-one and 4-cholestene-3,6-dione. Irradiation did significantly promote formation of COPs in all samples. However, among A-ring compounds, only 4-cholesten-3-one was formed at a higher amount, while minor traces of 4,6-cholestadien-3-one and 4-cholestene-3,6-dione were also formed.

V. *Food Components*

Many food components, such as salt, triglyceride, β-carotene and chlorophyll have been reported to have a great impact on the rate of cholesterol oxidation. Kim and Nawar[80] 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[51,81]. This phenomenon is often referred to as "cooxidation". Likewise, the stability of lipid in foods during heating may also be affected by cholesterol. In the presence of cholesterol, the amount of unsaturated fatty acid was found to decrease sharply with increasing heating time[80]. In addition, the triglyceride may be decomposed to form free fatty acid during heating, which in turn may lower the pH value of foods. This outcome often leads to formation of triol through hydration of 5,6α-EP or 5,6β-EP[18,38,39]. Fish contains a relatively higher amount of long-chain polyunsaturated fatty acids than most other food products, therefore, it is more susceptible to the formation of COPs. Osada *et al*.[72] reported that the levels of COPs in processed seafood were much higher than other food products.

Sander *et al*.[36] observed that the amount of COPs formed in salted butter was less than in unsalted butter. This result suggests that salt may possess the ability to retard cholesterol oxidation. It has been reported that salt may reduce the free water content in food systems by bonding with water[82], thus reducing the mobility of metal ions (prooxidants) and retarding the oxidation of cholesterol.

Zhang *et al*.[61] detected six COPs, 7β-OH, 5,6β-EP, 5,6α-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 such as frying is necessary for triol formation. 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 5,6α-EP or 5,6β-EP. In another study, Bascoul *et al*.[75] detected triol in restaurant-prepared French-fried potatoes as well, however, this result was not confirmed by mass spectrometry. Park and Addis[34] also identified triol from the adversely treated freeze-dried pork muscle (stored in contact with air at 22°C for ca. 3 years) at the level of 28 ppm.

In general, the degree of fatty acid unsaturation has a profound influence on COPs formation, and the coexistence of cholesterol and unsaturated fatty acids is quite common in food systems. Osada *et al*.[68] observed that no COPs were found when cholesterol was heated alone. However, the cholesterol level was readily reduced and COPs were generated as soon as heating started when fats and cholesterol were heated together. The formation rate of COPs was further facilitated in the

presence of unsaturated fats. The study of Li *et al*.[65] 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[83-85]. Similar results were observed when cholesterol and those oils shown above were heated at 110°C for 22 hrs[65]. Six COPs, including 7α-OH, 7β-OH, 5,6β-EP, 5,6α-EP, triol and 7-keto, were identified. The mixture of fish oil and cholesterol was found to contain the highest level of COPs (152 ppm), followed by sunflower oil and cholesterol (56 ppm), palm oil and cholesterol (31 ppm), and flax oil and cholesterol (28 ppm).

INHIBITION OF COPS

I. *Food Additives*

(I) *Antioxidants*

As the mechanism of the cholesterol oxidation has been demonstrated to be similar to that of lipid oxidation[19], the incorporation of antioxidants to a cholesterol-containing food should retard cholesterol oxidation effectively. Park and Addis[32] studied the effects of ascorbyl palmitate and α-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 α-tocopherol may undergo degradation during heating at high temperatures. Yan and White[38] also found that cholesterol oxidation could be inhibited by incorporation of methyl silicone into lard during heating. 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[86-89]. Rankin and Pike[83] 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[40] also showed that BHT was not effective against cholesterol oxidation. However, an opposite result was reported by Sagers[90], who revealed that BHA, BHT, propyl gallate, tertiary butylhydroquinone and α-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.

The concentration of antioxidants affects the ability to decrease cholesterol oxidation as well. Morgan and Armstrong[86] reported that 67 ppm propyl gallate was slightly more effective than at 200 ppm for reduction of COPs formation in spray-dried egg yolk. Some authors also found that the COPs formation could be retarded in the presence of 100 ppm propyl gallate[89]. This result agrees with a report by Madhavi *et al*.[91], who pointed out that propyl gallate possessed antioxidant activity at optimum concentrations and might act as a prooxidant at high levels. Tocopherols are known to delay the role of cholesterol oxidation.

Of the various isomers, γ- and δ-tocopherols were more effective in inhibiting cholesterol oxidation than α-tocopherol in an aqueous model system [83-85]. Some studies have also suggested that α-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%[83,90].

Park and Addis[32] reported that a combination of ascorbyl palmitate and α-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.*[89] found that the mixture of ascorbyl palmitate and α-tocopherol showed a slight prooxidant effect of cholesterol oxidation during storage of spray-dried egg yolk powder. Li *et al.*[65] 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)[94].

(II) *Salt*

Salt is also an effective inhibitor of COPs in butter oil. Sander *et al.*[36] demonstrated that salt rendered an inhibitory effect against COP formation in butter oil. The COPs generated in unsalted samples were found to be 2 to 3 folds more than those in salted samples. This phenomenon can be attributed to the reduction of water activity of food samples in the presence of salt, and hence the reaction rate of cholesterol oxidation is minimized.

II. *Processing Method*

(I) *Processing Condition*

The formation of COPs in dried food products can be inhibited by employing a more approprite processing method. Tsai and Hudson[41] and Missler *et al.*[43] proposed that the direct heating of drying air by gas combustion enhanced cholesterol oxidation in egg powders. Chan *et al.*[95] also demonstrated that the indirect electrical heating method was more efficient than the direct method for reduction of COPs formation in whole milk powder during spray drying. Therefore, the COPs formation can also be controlled under mild processing conditions.

(II) *Packaging*

Physical barriers such as packaging materials are able to inhibit or reduce the permeation of air and light into foods, and consequently minimize cholesterol oxidation as well. Luby *et al.*[78] examined the effect of light transmission of packaging material on the oxidation stability of cholesterol in butter exposed to fluorescent light, and found that after 15 days of exposure to light, only aluminum foil was more effective than argarine wrap, opaque parchment, wet strength dry wax paper or polyethylene film for prevention of cholesterol oxidation.

Reducing oxygen availability is also useful for inhibiting cholesterol oxidation. Chan *et al.*[95] showed that a crimped-sealed glass vial is a better packaging material than a polyethylene pouch. Vacuum packaging or oxygen absorbers such as ferric carbonate and calcium hydroxide are also highly effective to reduce oxygen availability in foods.

(III) *Storage Condition*

Low temperature and darkness are able to minimize lipid oxidation in foods[78,79] and thus reduce cholesterol oxidation. Luby *et al.*[79] found that the cholesterol oxidation in butter proceeded faster at 22°C than at 4°C. In addition, both daylight and roomlight may prohibit the photooxidation of cholesterol, and the former appeared to be more detrimental to butter than the latter.

CONCLUSION

From the preceding discussion it may be con-

cluded that COPs are formed in most processed products containing cholesterol. Therefore, it is difficult to prevent COPs formation in foods unless they are cholesterol-free. Numerous methods have been developed to analyze COPs over the past several decades. TLC, HPLC and GC are the most commonly employed techniques for COPs analysis. However, there are problems to overcome in order to establish a method with the merits of precision, accuracy, low cost, short analysis time and high sensitivity. Heating is the most common causing factor, and other factors such as dehydration, irradiation, pH, food components, storage conditions and the bleaching process may more or less impart an influence on COPs formation. Nevertheless, the inhibition of COPs is possible with antioxidants such as tocopherols and propyl gallate at an appropriate level. This overview summarizes the accomplishments concerning COPs research in the past, and indicates areas where future progress is needed. Further efforts are still necessary in order to minimize or prevent COPs formation in foods.

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食品中膽固醇氧化物的分析，形成與抑制：綜論(第二部分)

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摘　要

膽固醇受熱或光照時會進行一系列自氧化或光氧化作用形成膽固醇氧化產物(cholesterol oxidation products, COPs)。許多研究指出，COPs可能與許多生理不良反應有關，如細胞突變、致癌、血管腫瘤、細胞毒害、動脈硬化、細胞損傷及抑制膽固醇合成等。因此，COPs的安全性已經是大眾所關切的問題之一。本文主要針對食品中COPs的分析方法及其生成與抑制作一綜合性的回顧及討論。食品中的COPs一般使用有機溶劑萃取，經皂化及固相萃取法純化濃縮後，再利用薄層層析(TLC)、高效率液相層析(HPLC)或氣相層析質譜儀(GC-MS)加以定性及定量。GC-MS測定COPs含量具有快速及靈敏度高的優點，但因高溫操作而可能產生其他異構物是其主要缺點。對HPLC而言，則無法分離某些幾何異構物及無法以紫外光偵測不含雙鍵的COPs，如5,6-epoxide異構物及triol。許多因素會影響食品中膽固醇的氧化作用，如加熱、光照、貯存環境及食品組成等。食品中主要的COPs包含7α-OH、7β-OH、5,6α-EP、5,6β-EP、7-keto、20α-OH、25-OH及triol等。其中5,6α-EP、5,6β-EP、7-keto、20α-OH、25-OH爲一級產物，而7α-OH、7β-OH及triol則爲次級產物。部分抗氧化劑已被証實在適當濃度下具有抑制膽固醇氧化的效果。此外，適當的包裝亦可降低光和空氣對食品中膽固醇的氧化傷害，減少COPs的產生。更多的研究必須進行以瞭解如何有效抑制食品中COPs的生成。

關鍵詞：膽固醇氧化物，高效液相層析，氣相層析-質譜儀，加工方法。