Review Article

Cholesterol oxides: their occurrence and methods to prevent their generation in foods

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Eight cholesterol oxides are commonly found in foods with high cholesterol content, such as meat, egg yolk and full fat dairy products. Factors known to increase the production of cholesterol oxides in foods are heat, light, radiation, oxygen, moisture, low pH, certain pro-oxidising agents and the storage of food at room temperature. Processes, such as pre-cooking, freeze-drying, dehydration and irradiation, have all been reported to result in increased production of cholesterol oxides in meats. As prepared consumer foods are becoming increasingly popular, the consumption of higher levels of cholesterol oxides in foods is inevitable. An understanding of the mechanisms involved in the generation of cholesterol oxides may assist in their reduction in foods and possibly reduce the impact of these compounds on human health.

Key words: cholesterol oxides, dairy foods, eggs, egg products, food processing, food storage, meat, meat products, sea food.

Introduction

Cholesterol oxides are a group of sterols similar to cholesterol, which contain an additional functional group, such as a hydroxyl, ketone or an epoxide group in the sterol nucleus and/or on the side chain of the molecule. While Fig. 1 shows the structure of eight common cholesterol oxides, more than 60 have been identified.[1] Some cholesterol oxides are produced endogenously in human tissues during conversion of cholesterol into bile acids and steroid hormones;[2] autooxidation of cholesterol also occurs *in vivo.*[3]

Cholesterol oxides are also present in our diet and have been identified in foods high in cholesterol content. The presence of cholesterol oxides in a range of foods has been extensively reviewed.[2,4–7] As a rule, fresh foods contain very low levels of cholesterol oxides. Storage, cooking and processing all tend to increase the cholesterol oxide content of cholesterol-containing foods. Cholesterol oxides occur in relatively high concentrations (range 10–150 μg/g dry weight) in egg yolk,[7,8,9] stored frozen meat,[10] butter, cheese,[11] cream[5] and heated tallow.[12] Egg-based products (cakes, sweet biscuits and mayonnaise) will also contain cholesterol oxides if fresh materials are not used in their manufacture.

It is important to note that high cholesterol-containing foods show an increase in cholesterol oxide content after heating, spray-drying and deep-frying.[5,13] For instance, in heat-treated clarified butter (ghee), 12% of the total sterol content is cholesterol oxides.[14] The high levels of cholesterol oxides in ghee is not surprising, since butter is commonly heated at 150°C in an open vessel for 20–25 min without any antioxidant, to make this Indian product.

Literature reports on the levels of cholesterol oxides in the same type of foods are extremely difficult to compare. The quantification of cholesterol oxides in foods is difficult, because their isolation is frequently hindered by the large amounts of interfering cholesterol, triglycerides, phospholipids and other lipids present in the food.[15] The inconsistencies observed in the cholesterol oxide contents of foods are mainly due to differences in the analytical methods and failure to validate the methods correctly.[15] The procedures currently used to extract, purify and quantify cholesterol oxides have been well summarized.[3,16]

An attempt has been made to harmonize the methods used for the analysis of cholesterol oxides, and two interlaboratory round robin analyses have been performed on standard egg powder and milk powder.[16,17] A summary of two studies, one carried out in 1995 (study 1) and the second in 1997 (study 2), is shown in Tables 1 and 2. The reduction in data variability in study 2 may be due to the use of better packaging and improved methods. In the first study, some of the samples were held up in US Customs and possibly stored

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Accepted 7 September 2001

in uncontrolled conditions. However, even in the second study, the range of results reported for each cholesterol oxide for the same food sample was much wider than is desirable.

Dairy foods

Cholesterol oxides have not been reported in fresh, traditional and ultra-high temperature pasteurized, condensed and skimmed milk. Full cream milk may contain 20–30 μg/g[18] while commercial milk powders and infant formulas contain very low levels of cholesterol oxides.[19] Freshly opened full cream powders that had been packed under inert gas only contained traces of cholesterol oxides (25 ng/g total fat). Full fat cream powders that had been stored unopened for 1 year also contained very low levels of cholesterol oxides. The values obtained by Rose-Sallin *et al.*[19] were comparable to the results obtained by Nourooz-Zadeh and Appelqvist[20] who demonstrated that fresh milk powders produced by low or medium heat spray-drying contained very low amounts of cholesterol oxides.

Cholesterol oxides have been reported in blancmange powder, pancake powder and cheese stored at room temperature.[6] Fresh butter manufactured by batch or continuous methods contains only traces of 7-ketocholesterol at the detection limit,[20] as the cholesterol in butter appears to be quite stable to oxidation because most fatty acids are saturated. Storage of the butter at 4°C for up to 4 months caused an increase in the levels of isomeric 5,6-epoxycholesterols, while heating the butter for 10 min between 150 and 200°C, under conditions similar to shallow pan frying, caused a gradual increase in total cholesterol oxides from 0 to 2.5 μg/g in the butter.[20]

Dairy spreads tend to contain higher amounts of unsaturated fatty acids, which favour cholesterol oxide formation.[21] This is a real problem, since there has been an increase in the consumption of dairy spreads as nutritionists have advised people to decrease their intake of saturated fats. Nielson *et al.*[21] also noted that there was a lag phase of 7 weeks in cholesterol oxidation in dairy spread stored at 4°C, after which there was a rapid rise in the cholesterol oxide content. This could well be a problem as dairy spread is normally considered to have a shelf life of up to 10 weeks at 4°C. Very low levels of cholesterol oxides could be observed when the dairy spread was stored at –18°C, even after 13 weeks.

Soft and hard cheeses contain only traces of some cholesterol oxides, while grated cheeses (which have a high surface area) contain 0.5–2.2 μg/g of cholesterol oxides in

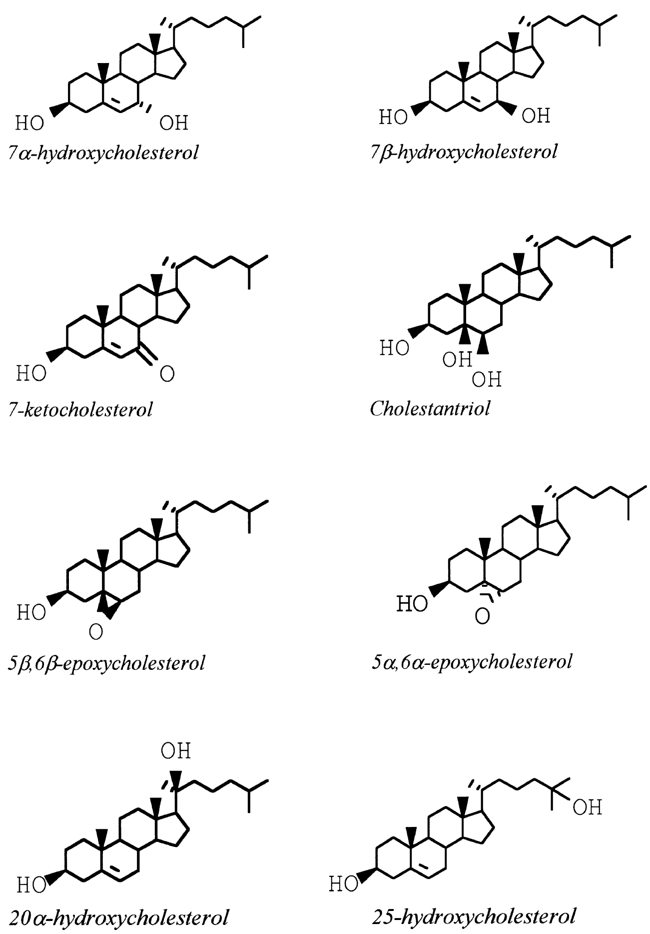

Figure 1. Structure of the eight most common cholesterol oxides.

Table 1. Interlaboratory variation in content of cholesterol oxides in egg powder determined in two ring-test studies[17]

	Study	No. laboratories (μg/g)	Total range	Mean ± SD
7α-Hydroxycholesterol	1	10	0.3–12.6	5.8 ± 4.8
	2	7	2.2–5.5	3.6 ± 1.3
7β-Hydroxycholesterol	1	13	0.3–157.1	18.6 ± 43.1
	2	8	1.2–4.9	2.7 ± 1.4
7-Ketocholesterol	1	15	0.4–24.6	6.0 ± 8.1
	2	8	1.5–5.9	2.6 ± 1.32
5α,6-α-Epoxycholesterol	1	11	0.3–54.4	13.7 ± 19.5
	2	6	0.4–1.6	1.0 ± 0.4
5β,6β-Epoxycholesterol	1	10	0.4–30.6	10.5 ± 12.0
	2	6	1.5–3.5	2.7 ± 0.8
Cholestantriol	1	9	0.1–14.5	2.7 ± 4.6
	2	4	0.09–0.25	0.17 ± 0.06

the lipid fraction.[20] Generally speaking, fresh cheese contains very low levels of cholesterol oxides but grated cheeses (with a high surface area), such as Parmesan and Romano, contain between 6 and 32 μg/g.[22]

Egg and egg products

Eggs contain a relatively high content of cholesterol (approximately 200 mg/egg) and fresh egg yolks are virtually free of cholesterol oxides.[6] Unfortunately, cooking and dehydration considerably increase the cholesterol oxide content. Spray-dried egg yolk powder contains the highest levels of total cholesterol oxides (55–113 mg/100 g); epoxides were the most abundant cholesterol oxide in this study.[18] Sarantinos *et al*.[18] went on to show that domestic frying of eggs also resulted in cholesterol oxide production. Commercially prepared foods that contain egg products, such as egg pasta, cakes, sweet biscuits and mayonnaise, may have significant cholesterol oxide contents.[6,23,24]

Meat and meat products

Only trace amounts of cholesterol oxides have been found in fresh meat,[25] but meat products can be one of the most important sources of cholesterol oxides in the diet.[2,4,6] An increase in cholesterol oxidation does occur in beef, veal and pork after being stored frozen for three months.[10] The generation of cholesterol oxides in prepared food is influenced by many factors, for instance, contact with air/oxygen, temperature treatment during processing, exposure to light, presence of metal ions, level of antioxidants, packaging methods and storage conditions.[6,7,17] Beef hamburgers contained higher levels of total cholesterol oxides when compared to meatballs containing 50% pork/50% beef (Table 3).[26] Larkeson *et al*.[26] also found that the cholesterol oxide content of the raw and prefried products increased when they were fried. The levels of cholesterol oxide content of all products increased if they were stored in the dark for either 1 or 2 weeks at 4°C; the greatest increase was seen in the prefried hamburgers (Table 3).

Rodriguez-Estrada *et al*.[7] also evaluated the effect of different cooking methods on cholesterol oxidation of beef hamburgers, detecting a higher content of 7-ketocholesterol in the combination of roasting and microwave heating, as compared with other cooking treatments (Table 4). However, the storage conditions in the supermarket, where refrigerated meat is subjected to continuous lighting, could be a major factor in the development of lipid oxidation in raw ground meat.[7]

Processes such as freeze-drying, dehydration and irradiation have also been reported to lead to the increased production of cholesterol oxides in meats.[6] The effects of processing technology and cholesterol oxidation of salami were studied.[27] It was found that the amount of 7-ketocholesterol ranged from 1.2 to 2.7 μg/g in lipids, reaching its highest value one week after the oven treatment for the activation of the fermentation process. The effect of UV irradiation (1800 μwatt/cm^2 for 1 min) on cholesterol oxidation of

Table 2. Interlaboratory variation in content of cholesterol oxides in milk powder determined in two ring-test studies[17]

	Study	No. laboratories	Total range (μg/g)	Mean ± SD
7α-Hydroxycholesterol	1	11	0.04–9.7	1.2 ± 2.9
	2	6	0.03–0.8	0.2 ± 0.3
7β-Hydroxycholesterol	1	14	0.03–2.6	0.5 ± 0.8
	2	8	0.02–0.7	0.2 ± 0.3
7-Ketocholesterol	1	14	0.05–3.1	0.6 ± 0.8
	2	8	0.05–1.9	0.4 ± 0.6
5α,6-α-Epoxycholesterol	1	12	0.02–7.3	1.1 ± 2.2
	2	4	0.1–0.7	0.4 ± 0.3
5β,6β-Epoxycholesterol	1	10	0.003–5.8	1.3 ± 2.0
	2	5	0.09–0.6	0.3 ± 0.2
Cholestantriol	1	6	0.005–2.2	0.6 ± 0.9
	2	5	0.008–1.6	0.4 ± 0.7

Table 3. Total cholesterol oxide content (μg/g lipids) in raw and cooked meat balls and hamburgers

	Raw		Prefried	
	Meat balls 50% beef/50% pork	Hamburgers 100% beef	Meat balls 50% beef/50% pork	Hamburgers 100% beef
Fresh	3.3	5.5	5.5	8.4
Fried	10.4	6.7	8.2	29.4
Storage 1 week	11.0	6.9	14.3	41.9
Storage 2 weeks	10.8	7.2	16.1	49.5

The determinations were performed on the fresh products, after frying and after storage for 1 or 2 weeks at 4°C.[26]

three sliced meat products (Milan-type salami, mortadella and cooked ham), after storage at 4°C under modified atmosphere, has also been evaluated.[28] Sampling was performed at three different stages: (i) just after the treatment (0 weeks); (ii) at the average commercial shelf life time of the corresponding untreated products (12, 6 and 4 weeks for salami, mortadella and cooked ham, respectively); (iii) when modifications on the sensory profile of the products were detected by an expert panel (20, 8 and 8 weeks for salami, mortadella and cooked ham, respectively). Treated samples were compared with untreated ones (controls). As can be observed in Table 5, UV irradiation enhanced cholesterol oxidation resulting, in general, in higher levels of 7-ketocholesterol in the UV-irradiated products, as compared to those of the untreated ones. However, the untreated cooked products (mortadella and cooked ham) already presented a certain degree of oxidation at the beginning of the study, which is probably due to the processing technology. In addition, the cooking procedure partially masked the oxidizing effects of UV irradiation in these cooked products.

Table 4. Effect of cooking treatments on the total 7-ketocholesterol content and the ratio of total 7-ketocholesterol to total cholesterol[7]

Treatment	7-Ketocholesterol (μg/g in lipids)*	7-Ketocholesterol/ total cholesterol (%)*
Raw	25.2[d]	0.5[b]
Roasted	18.0[b]	0.5[b]
Microwave	19.6[c]	0.4[a]
Microwave and roasted	22.0[e]	0.5[b]
Barbecue	19.5[c]	0.4[a]
Boiling	16.4[a]	0.4[a]
Frying pan	18.6[b]	0.4[a]

* Mean values of three replicates. [a–e] Means with different superscripts within the same category are significantly different ($P = 0.05$).

Table 5. Mean contents of total 7-ketocholesterol found in control and UV-irradiated sliced salami, mortadella and cooked ham[28]

Product	Time (weeks)	Sample treatment	7-Ketocholesterol (μg/g in lipids)*
Salami	0	Control	1.2
		UV	4.5
	12	Control	4.2
		UV	4.3
	20	Control	2.9
		UV	7.8
Mortadella	0	Control	6.6
		UV	1.8
	6	Control	5.9
		UV	10.9
	8	Control	11.2
		UV	5.5
Cooked ham	0	Control	16.8
		UV	7.7
	4	Control	11.2
		UV	11.3
	8	Control	18.8
		UV	12.3

* Data are the mean values of three replicates.

In contrast, Novelli *et al.*[29] found that the cholesterol oxide content of fresh and frozen pork was very low and despite the fact that mincing, storage and cooking are all considered processes that might induce oxidative changes in meat, the levels found in salame Milano and mortadella were again very low. It is possible that additives such as nitrites and ascorbic acid may have a positive role in reducing oxidative processes in these processed foods. One of the main problems in comparing the cholesterol oxide content of meat and meat products is the wide range of methods used to measure them, as mentioned above.[6]

The potential to consume more cholesterol oxides increases as prepared precooked products are becoming more popular in modern Western diets, where prepared foods are in great demand because they take little time to cook at home.

Sea food

Low levels of cholesterol oxides have been observed in fresh, frozen and smoked herring, ranging from 5.5 to 9.2 μg total cholesterol oxides/g lipids and rising to 10.4 μg/g in fried herring.[30] In contrast, the cholesterol oxide content of salt-dried fish varied according to the fish species. In fact, Pacific cod, Northern cod and anchovy displayed a mean of 15.2 μg/g, 20.8 μg/g and 127.3 μg/g, respectively.[31]

Many locally caught Japanese fish contain relatively low levels of cholesterol oxides.[32] It was observed that Japanese whiting and Pacific round herring exhibited small increases in cholesterol oxides after grilling, whereas other fish, such as squid, displayed no change after grilling. When both boiled and dried anchovies were grilled for 6 min at 220°C, the cholesterol oxide content increased markedly when compared to the levels found in the boiled and dried anchovies; however, it appeared to decrease slightly on further grilling.[33] Dietary supplementation of α-tocopherol or the treatment of the Rainbow trout fillets with an oleoresin rosemary dip significantly reduced the formation of cholesterol oxides during subsequent cooking.[34] The levels of cholesterol oxides found in various processed marine products raise some questions about the potential safety of these products, which are normally considered beneficial for health.

Other processed foods

If a food product is cooked in butter or tallow, the possibility of cholesterol oxide absorption exists. Total cholesterol oxides ranged from 20 to 24 μg/g in French-fried potatoes, and other deep-fried foods cooked in animal/vegetable fat were a major source of cholesterol in the US diet. Fortunately, tallow is no longer widely used in many countries.[35] The amount of cholesterol oxides in fried foods depend on a number of factors, such as the composition of the frying fat, the length of frying time and the cooking temperature. If the food is battered, then the absorption of fat into this layer can be considerable. Recently, a study on cholesterol oxidation of chicken cutlets was performed, in which the raw meat, the breaded raw pieces and the fast-fried cutlets obtained from four different producers were monitored.[36] The results showed that 7-ketocholesterol was already present in the raw meat that was used for the cutlets, but it increased markedly after being subjected to the thermal treatment (Table 6). No 7-ketocholesterol was detected in the frying oil, even though a small amount of cholesterol did solubilize in the oil during frying.

Storage of foods

Cholesterol-containing foods that are heated during processing, as well as those that are dried and then stored, have a high cholesterol oxide content.[6] Storage in non-ideal conditions (O_2 permeability, exposure to heat and light) can lead to a further increase in their cholesterol oxides levels.[6,37] For instance, storage of whole milk powders in oxygen impermeable materials, such as glass, reduced the rate of cholesterol oxide production, when compared to polyethylene pouches.[38]

On the other hand, data on the effect of storage on the level of cholesterol oxides in presently available dairy spreads, has not been reported in the literature. It would be expected to be relatively low as these mixed spreads (containing butter and margarine) are packaged in cream or white containers, which reduces the amount of light reaching the product. Many of these products have a metal foil cover over the top, which would also protect the product from light exposure. In any case, it is also clear that the larger companies do not allow their product to remain in supermarket shelves long enough for the development of cholesterol oxides to become a major problem.

Table 6. 7-Ketocholesterol content of raw chicken meat, breaded raw pieces and fast-fried chicken cutlets[36]

Sample*	7-Ketocholesterol (μg/100 g)†
V 1a	1.6
V 1b	1.9
V 2a	6.4
X 1a	2.7
X 1b	2.2
X 2a	7.9
Y 1a	3.8
Y 1b	2.0
Y 2a	6.4
Z 1a	0.8
Z 1b	0.6
Z 2a	7.5

* The letters V, X, Y and Z refer to 4 different chicken cutlets producers. The samples are identified as follows: 1a, raw meat; 1b, breaded raw pieces; 2a, fast fried cutlets (sampled at the beginning of the daily frying cycle).
† 7-ketocholesterol expressed in μg/100 g of non-lipid food matrix.

Why are cholesterol oxides so important in human nutrition?

Cholesterol oxides in foods are efficiently absorbed into the blood stream.[39,40] Endogenous and food-sourced cholesterol oxides are transported in the low density lipoprotein (LDL) to the liver.[41–43] More recent studies have shown that unesterified cholesterol oxides associate readily with serum albumin.[44]

Most sterols, including cholesterol and cholesterol oxides, are eliminated from the body through the bile secretions, even though it is not clear that cholesterol oxides share the same pathways for bile synthesis as cholesterol. Javitt has shown that a separate group of p-450 7α-hydrolysases catalyze cholesterol oxides separately.

Many cholesterol oxides exhibit atherogenic properties and an ability to modulate cholesterol metabolism. In animal feeding studies, where cholesterol oxides were fed at relatively high levels, cholesterol oxides were more potent than pure cholesterol in causing aortic endothelial damage and inducing arteriosclerosis.[46] Parasassi *et al.*[47] in fact, have proven that LDL hydroperoxides induce proteolysis of the aorta fibers.

The 7β-hydroxycholesterol concentration of the serum was the strongest predictor of rapid progression of carotid arteriosclerosis in humans.[48] In studies where healthy young men were fed salami and Parmesan cheese, it is interesting to note that 90% of the cholesterol oxides in the plasma were acyl esters and less than 10% were non-esterified cholesterol oxides.[40] This suggests that great care has to be taken in the analysis of cholesterol oxides in food materials to make sure that the acyl esters are completely hydrolysed before separation and analysis. Linseisen and Wolfram[40] did highlight the reported wide range of the cholesterol oxide contents of salami and Parmesan cheese, which relates to the manufacturing and analysis methods utilized.

The substantial amount of cholesterol oxides found in Indian ghee (12.3% of the total cholesterol content) would explain the high risk of arteriosclerosis observed in Indian immigrant populations in the USA and in the UK, since this is a food material consumed in significant amounts by this subgroup of the population.[14]

Conclusions

Until recently, the understanding of the physiological importance of cholesterol oxides has been limited by the lack of analytical procedures to analyse foods with sufficient sensitivity and accuracy. The important issue now is to establish which of the several methods available gives the most reliable results. Until this has been established with confidence, it is not easy to compare the results that are being published by a number of well-established research groups. In any case, there is no doubt that cholesterol oxides have a considerable negative effect on human metabolism far above the levels found in the tissues. Van de Bovenkamp *et al.*,[9] using a duplicate diet technique, were able to calculate that a person eating an average diet in the Netherlands would consume 1 mg of 7-β-hydroxycholesterol and 0.5 mg of

Table 7. Methods to reduce cholesterol oxidation in foods

Reduce the total cholesterol content of the food
Do not cook food in cholesterol-containing fats
Feed animals antioxidants such as α-tocopherol prior to slaughter and processing
Where permitted, add antioxidants during processing of the food
Process food at as low a temperature as possible
Package the food more effectively to exclude O_2
Store the food in the dark

cholesterol-α-epoxide per day. It is not possible to say whether this level of intake is acceptable or not but most authors suggest overall that the levels of intake of cholesterol oxides should be reduced.

The prevention of cholesterol oxidation in foods could be reduced in a number of ways (Table 7). It is clear that a reduction in total cholesterol content would have a significant effect on the amount of cholesterol oxides in a food. Another approach would be to incorporate antioxidants, such as α-tocopherol, into the diet prior to slaughter and processing.[49] Addition of antioxidants into food during processing, processing food at as low a temperature as possible, packaging food to exclude O_2[38] and storage in low light conditions[50] would all have the effect of reducing the rate of cholesterol oxidation.

It is clear that cholesterol oxides are formed in high cholesterol processed foods during storage. Manufacturers of these products need to consider all the ways that can be used to prevent the development of these unnecessary atherogenic compounds in food. In many cases, greater attention to packaging and storage conditions would make a significant contribution.

References

1. Smith LL. Cholesterol autoxidation 1981–1986. Chem Phys Lipids 1987; 44: 87–125.
2. Bösinger S, Luf W, Brandl E. Oxysterols: Their occurrence and biological effects. Int Dairy J 1993; 3: 1–33.
3. Addis PB, Park PW, Guardiola F, Codony R. Analysis and health effects of cholesterol oxides. In: McDonald RE, Min DB, eds. Food Lipids and Health. New York: Marcel Dekker, 1996.
4. Finocchiaro ET, Richardson T. Sterol oxides in foodstuffs: A review. J Food Prod 1983; 46: 917–925.
5. Kumar N, Singhal OP. Cholesterol oxides and arteriosclerosis: a review. J Sci Food Agric 1991; 55: 497–510.
6. Paniangvait P, King AJ, Jones AD, German BG. Cholesterol oxides in foods of animal origin. J Food Sci 1995; 60: 1159–1174.
7. Rodriguez-Estrada MT, Penazzi G, Caboni MF, Bertacco G, Lercker G. Effect of different cooking methods on some lipid and protein components of hamburgers. Meat Sci 1997; 45: 365–375.
8. Nabber EC, Biggert MD. Cholesterol oxidation products in fresh and heated egg yolk lipids. Fed Proc 1982; 41: 531.
9. van de Bovenkamp P, Kosmejer-Schuil TG, Katan MB. Quantitation of oxysterols in Dutch foods: egg products and mixed diets. Lipids 1988; 23: 1079–1085.
10. Pie JE, Spahis K, Seillan C. Cholesterol oxidation in meat products during cooking and frozen storage. J Agric Food Chem 1991; 39: 250–254.
11. Sander BD, Smith DE, Addis PB. Effects of processing and storage conditions on cholesterol oxidation products in butter and cheddar cheese. J Dairy Sci 1988; 71: 3173–3178.
12. Ryan TC, Gray JI, Morton LD. Oxidation of cholesterol in heated tallow. J Sci Food Agric 1981; 32: 305–308.
13. Sander BD, Addis PB, Park SW, Smith DE. Quantification of cholesterol oxidation products in a variety of foods. J Food Prot 1988; 52: 109–114.
14. Jacobson MS. Cholesterol oxides in Indian ghee: possible cause of unexpected high risk of atherosclerosis in Indian immigrant populations. Lancet 1987; I: 656–658.
15. McCuskey S, Devery R. Validation of chromatographic analysis of cholesterol oxides in dried foods. Trends Food Sci Technol 1993; 4: 175–178.
16. Dutta PC, Przybylski R, Appelqvist LÄ, Eskin NAM. Formation and analysis of oxidized sterols in frying fat. In: Perkins EG, Erickson MD, eds. Deep frying Chemistry, Nutrition, and Practical Applications. Champaign, IL: AOCS Press, 1996.
17. Dutta PC, Caboni MF, Diczfalusy U, Dionisi F, Dzeletovic S, Grandgirard A, Guardiola F, Kumpulainen J, Lebovics VK, Pihlava JM, Rodriguez-Estrada MT, Ulberth, F. Measurements of cholesterol oxides in foods: results of an interlaboratory comparison study. In: Kumpulainen JT, Salonen JT, eds. Natural antioxidants and anticarcinogens in nutrition, health and disease. London: Royal Society of Chemistry, 1999.
18. Sarantinos J, O'Dea K, Sinclair AJ. Cholesterol oxides in Australian foods: identification and quantification. Food Aust 1993; 45: 485–490.
19. Rose-Sallin C, Huggett AC, Bosset JO, Tabacchi R, Fay LB. Quantification of cholesterol oxidation products in milk powders using [$^2H_7$] cholesterol to monitor cholesterol autooxidation artefacts. J Agric Food Chem 1995; 43: 935–941.
20. Nourooz-Zadeh J, Appelqvist LA. Cholesterol oxides in Swedish foods and food ingredients: Butter and cheese. J Am Oil Chem Soc 1998; 65: 1635–1641.
21. Nielson JH, Olsen CE, Jensen C, Skibsted LH. Cholesterol oxidation of butter and dairy spread during storage. J Dairy Res 1996; 63:159–167.
22. Finocchiaro ET, Lee K, Richardson T. Identification and quantification of cholesterol oxides in grated cheese and bleached butteroil. J Am Oil Chem Soc 1984; 61: 977–883.
23. Penazzi G, Caboni MF, Zunin P, Evangelisti F, Tiscornia E, Gallina Toschi T, Lercker G. Routine HPLC determination of 7-ketocholesterol in some foods, by two different analytical methods. J Am Oil Chem Soc 1995; 71: 1523–1527.
24. Zunin P, Evangelisti F, Calcagno C, Tiscornia E. Cholesterol oxidation products in dried egg pasta: detecting 7-ketocholesterol constituent. Cereal Chem 1996; 73: 691–694.
25. Hwang KT, Maerker G. Quantitation of cholesterol oxidation products in unirradiated and irradiated meats. J Am Oil Chem Soc 1993; 70: 371–376.
26. Larkeson B, Dutta PC, Hansson I. Effects of frying and storage on cholesterol oxidation in minced meat products. J Am Oil Chem Soc 2000; 77: 675–680.
27. Rodríguez-Estrada MT. Effect of Processing Technology and Ripening on Lipid Hydrolysis and Oxidation of Salami. Proceedings of the 1st Workshop on the Developments in the Italian PhD Research in Food Biotechnology, Università della Tuscia, Viterbo, Italy, September 23–24, 1996. Viterbo: Università della Tuscia, 1996; 54–57.
28. Rodríguez-Estrada MT, Capucci F, Giovanardi C, Lercker G. Gas chromatographic study on the extent of lipid hydrolysis and oxidation on UV-irradiated meat products. In: Sandra P, ed. Proceedings of the 23rd International Symposium on Capillary Chromatography, Riva del Garda, Italy, June 5–10, 2000; M06 (CD-ROM). Riva del Garda: IOPMS, 2000.
29. Novelli E, Zanardi E, Ghiretti GP, Campanini G, Dazzi G, Madarena G, Chizzolini R. Lipid and cholesterol oxidation in frozen stored pork, salame Milano and mortadella. Meat Sci 1998; 48: 29–40.

30. Dutta PC, Pickova J. Cholesterol oxides in herring lipids and in a sample of refined menhaden oil. In: O'Connor CJ, ed. Pacific Oils 2000. Proceedings of the International Conference on Plant Oils and Marine Lipids, Auckland, NZ, November 25–28, 1997. Auckland: The Oils and Fats Specialist Group of the New Zealand Institute of Chemistry, 2000; 151–152.
31. Ohshima T, Li N, Koizumi C. Oxidative decomposition of cholesterol in fish products. J Am Oil Chem Soc 1993; 70: 595–599.
32. Shozen KI, Ohshima T, Ushio H, Koizumi C. Formation of cholesterol oxides in marine fish products induced by grilling. Fish Sci 1995; 61: 817–821.
33. Ohshima T, Shozen KI, Ushio H, Koizumi C. Effects of grilling on the formation of cholesterol oxides in seafood products rich in polyunsaturated fatty acids. Lebens-Wissen Technol 1996; 29: 94–99.
34. Akhtar P, Gray JI, Booren AM, Gomaa A. The effects of dietary α-tocopherol and surface application of oleoresin rosemary on lipid oxidation and cholesterol oxide formation in cooked rainbow trout (*Oncorhynchus mykiss*) muscle. J Food Lipids 1998; 5: 59–71.
35. Zhang WB, Addis PB, Krick TP. Quantification of 5α-cholestane-3β,5,6β-triol and other cholesterol oxidation products in fast food French fried potatoes. J Food Sci 1991; 56: 716–718.
36 Rodríguez-Estrada MT, Penazzi G, Caboni MF, Vandi L, Lercker G. Quality of Fried Chicken Cutlets. In: Cavalchini LG, Baroli D, eds. Proceedings of the XIV European Symposium on the Quality of Poultry Meat. VII European Symposium on the Quality of Eggs and Egg Products, Bologna, Italy, September 19–23, 1999. Milan: Associazione Italiana di Avicoltura Scientifica, 1999; 467–471.
37. Yan SP. Cholesterol oxidation products. Their occurrence and detection in our foodstuffs. In: Jackson LS, Knize MG, Morgan JN, eds. Impact of processing on food safety. New York: Kluwer Academic, Plenum Publishers, 1999.
38. Chan SH, Gray JI, Gomaa EA. Cholesterol oxidation in whole milk powders as influenced by processing and packaging. Food Chem 1993; 47: 321–328.
39. Emmanuel HA, Hassel CA, Addis PB, Bergmann SD, Zavoral JH. Plasma cholesterol oxidation products (oxysterols) in human subjects fed a meal rich in oxysterols. J Food Sci 1991; 56: 843–847.
40. Linseisen J, Wolfram G. Absorption of cholesterol oxidation products from ordinary foodstuff in humans. Ann Nutr Metab 1998; 42: 221–230.
41. Javitt NB, Kok E, Burstein S, Cohen B, Kutscher J. 26-Hydroxycholesterol: Identification and quantification in human serum. J Biol Chem 1981; 256: 12644–12646.
42. Peng SK, Taylor CB, Hill JC, Morin RJ. Cholesterol oxidation derivatives and arterial endothelial damage. Atherosclerosis 1985; 41: 395–402.
43. Sevanian A, Bittolo-Bon G, Cazzolato G, Hodis H, Hwang J, Zamburlini A, Maiorino M, Ursini F. LDL is a lipid hydroperoxide-enriching circulating lipoprotein. J Lipid Res 1997; 38: 419–428.
44. Lin CY, Morel DW. Distribution of oxysterols in human serum characterization of 25-hydroxycholesterol association with serum albumin. J Nutr Biochem 1995; 6: 618–625.
45. Javitt NB. 26-Hydroxycholesterol synthesis, metabolism and biologic activities. J Lipid Res 1990; 31: 1527–1533.
46. Schroepfer GJ. Oxysterols: Modulators of cholesterol metabolism and other processes. Physiol Rev 2000; 80: 361–554.
47. Parasassi T, Yu W, Durbin D, Kuriashkina L, Gratton E, Maeda N, Usini F. Two-photon microscopy of aorta fibers shows proteolysis induced by LDL hydroperoxides. Free Rad Biol Medical 2000; 28: 1589–1597.
48. Salonen JT, Nyyssonen K, Salonen R, Porkkala-Sarataho E, Tuomainen TP, Diczfalusy U, Bjorkhem I. Lipoprotein oxidation and progression of carotenid atherosclerosis. Circulation 1997; 95: 840–845.
49. Monahan FJ, Buckley DJ, Grey JI, Morrissey PA, Asghar A, Hanrahan TJ, Lynch PB. Effect of dietary vitamin E on the stability of raw and cooked pork. Meat Sci 1990; 27: 99–108.
50. Luby JM, Gray JI, Harte BR. Effects of packaging and light source on the oxidation stability of cholesterol in butter. J Food Sci 1986; 51: 908–911.