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Review

Oxidised cholesterol in milk and dairy products

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Abstract

Cholesterol is found in animal foods. It can be oxidised in various ways and cholesterol oxidation products (COPs) are formed. Such products are often found in animal foods including dairy products. Recently published results suggest that the contents of COPs in milk and dairy products is very small and does not confirm the results of earlier studies. A higher concentration of COPs can be found only in processed dairy products exposed to harsh storage conditions where the impact of oxygen and light or oxygen and low water activity are concomitant.

Keywords: Cholesterol oxidation products; Oxidised cholesterol; Milk; Dairy products; Heat treatment; Storage; Light exposure

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1. Introduction

When Imai, Werthessen, Taylor, and Lee (1976) published their results, cholesterol oxidation products (COPs) prompted great interest within the medical science and food science communities. They had fed rabbits with 5-year-old United States Pharmacopeia (USP)-grade cholesterol, thereby inducing severe arterial damage, whereas purified cholesterol did not. By concentrating the impurities of this sample, they found several COPs Cholestanetriol (cholestane-3β, 5α, 6β-triol) and 25-hydroxy-cholesterol (cholest-5-en-3β, 25-diol) were the most atherogenic compounds (Taylor, Peng, Werthessen, Tham, & Lee, 1979). Individual cases of such substances had already been reported in milk products prior to this date (Flanagan, Ferretti, Schwartz, & Ruth, 1975; Kuzdzal–Savoie, Langlois, & Krobicka, 1978; Parks, Schwartz, Keeney, & Damico, 1996). Further COPs, which are important from a toxicological point of view, have been identified. They are cholesterol-5α,6α-epoxide (5,6α-epoxy-5α-cholestan-3β-ol), cholesterol-5β,6β-epoxide (5,6β-epoxy-5β-cholestan-3β-ol), 7α- and 7β-hydroxycholesterol (cholest-5-en-3β,7α-diol, cholest-5-en-3β,7β-diol), 7-ketocholesterol (3β-hydroxycholest-5-en-7-one) and cholesta-3,5-dien-7-one. At present, a total of more than 80 different COPs have been reported (Bösinger, Luf, & Brandl, 1993; Smith, 1981). COPs have many biological effects (Bösinger et al., 1993; Guardiola, Codony, Addis, Rafecas, & Boatella, 1996; Linseisen & Wolfram, 1998a; Morel & Lin, 1996; O'Brien, O'Callaghan, Lyons, & Woods, 2000). They are not only atherogenic (Imai et al., 1980; Peng, Hu, & Morin, 1991a), but also cytotoxic (Peng, Sevanian, & Morin, 1991b; Sevanian & Peterson, 1986), mutagenic (Sevanian & Peterson, 1986), carcinogenic (Morin, Hu, Peng, & Sevanian, 1991b) and they are potent inhibitors of cholesterol biosynthesis (Morin, Hu, & Peng, 1991a). From a toxicological viewpoint, the intake of COPs by humans should be avoided. However, there are also indications that COPs may represent normal constituents of physiological processes and they are physiological mediators in many cholesterol-induced metabolic effects and also potential chemotherapeutic agents (Björkhem & Diczfalusy, 2002; Morel & Lin, 1996; Schroepfer, 2000).

Several reviews have already been published on the presence of COPs in foods of animal origin (Bösinger et al., 1993; Finocchiaro & Richardson, 1983; Paniangvait, King, Jones, & German, 1995; Sieber, 1986; Tai, Chen, & Chen, 1999, 2000; Yan, 1999). COPs may be formed during heating, dehydration, storage and irradiation. Their formation needs the presence of several reactive oxygen species, unsaturated fatty acids, cholesterol, transition metals and, in rare cases, enzymes (Rose–-Sallin, Sieber, Bosset, & Tabacchi, 1996b). The possibility of removing cholesterol from milk or degrading it using enzymes has also been evaluated (Smith, Sullivan, & Goodman, 1991; Xiansheng, Hung, Drew, & Versteeg, 1990). The present review describes the presence of COPs in milk and dairy products and discusses the potential and limitations of the current analytical detection methods.

2. Methods for determination of COPs in milk and dairy products

Although there are many methods for determining COPs in foods, no generally accepted standardised method is available. Variability between methods is observed with respect to analytical indicators such as linearity range, detection limit, repeatability and recovery rate (Mc Cluskey & Devery, 1993). Because COPs are present at very low concentrations in foods and COP artifacts are easily formed and information on their chromatographic behavior is lacking, their determination requires a sensitive method, which covers several steps: extraction of lipids from foods, saponification of extracted lipids, subsequent enrichment of COPs and quantification (Shan et al., 2003; Schroepfer, 2000; Guardiola, Bou, Boatella, & Codony, 2004). During these processes it is possible that the COPs are degraded or artifacts formed during oxidation of cholesterol. The identity of the separated compounds need to be confirmed by mass spectrometry and, if possible, verified by labeled cholesterol. Several solid-phase extraction methods can be used for the separation of COPs. Ulberth and Rössler (1998) found the combination of a silica cartridge followed by an NH_2 cartridge optimal for removing matrix components.

Various analytical methods have been developed for quantifying COPs in food: thin-layer chromatography (TLC) (Bican, 1984; Cleveland, 1987; Cleveland & Harris, 1987; Stenzel & Wunderlich, 1995), high-pressure liquid chromatography (HPLC) (Bican, 1984; Bösinger, 1991; Caboni, Capella, Lercker, & Bortolomeazzi, 1989; Csiky, 1982; Lakritz & Jones, 1997; Osada, Ravandi, & Kuksis, 1999; Penazzi et al., 1995), HPLC with atmospheric pressure chemical ionisation (Razzazi–Fazeli, Kleineisen, & Luf, 2000), gas chromatographic (GC) separation (van de Bovenkamp, Kosmeijer-Schuil, & Katan, 1988; Calvo, Ramos, & Fontecha, 2003; Cleveland, 1987; Cleveland & Harris, 1987; Nielsen, Olsen, Duedahl, & Skibsted, 1995; Rodriguez-Estrada, Caboni, Costa, & Lercker, 1998; Rose-Sallin, 1996; Rose-Sallin et al., 1995; Rose-Sallin, Sieber, & Bosset, 1994; Rose-Sallin, Sieber, Bosset, & Tabacchi, 1996a; Ubhayasekera, Verleyen, & Dutta, 2004) with mass spectrometric identification (Calvo et al., 2003; Nielsen et al., 1995; Rose-Sallin et al., 1995; Ubhayasekera et al., 2004), ^{1}H-NMR (Fontana, Antoniazzi, Ciavatta, Trivellone, & Cimino, 1993) and

atmospheric pressure chemical ionisation liquid chromatography/mass spectrometry (Manini, Andreoli, Careri, Elviri, & Musci, 1998). Some of these methods are also applicable to milk and dairy products. An enzymatic method for determination of COPs (Lebovics, Antal, & Gaal, 1996) was also described. 7-ketocholesterol can also serve as an indicator of cholesterol oxidation in routine analysis (Nielsen, Olsen, Jensen, & Skibsted, 1996a).

Some problems can occur during the analysis of COPs. 7-ketocholesterol is extensively degraded by hot saponification in comparison to cold saponification. For example, saponification at 75 °C for 30 min caused a loss of nearly 70% of the 7-ketocholesterol and formed the dehydration product cholesta-3,5-dien-7-one, whereas cold saponification without use of heating resulted in a 96% recovery (Park, Guardiola, Park, & Addis, 1996). Four frequently used methods for COP analysis in milk powder (one with direct saponification and three with preliminary fat extraction) have been compared. The method offering the best result was found to be direct saponification of the milk powder, without preliminary fat extraction, followed by aminopropyl-SPE (solid phase extraction) and GC-MS quantification (Dionisi, Golay, Aeschlimann, & Fay, 1998). For the enrichment of COPs, cold saponification with 1 M KOH/95% EtOH is more suitable for the analysis of very low levels in foods than cold saponification with KOH/MeOH or hot saponification with 1 M KOH/95% EtOH or transesterification (Ubhayasekera et al., 2004).

Earlier results need to be critically evaluated. In the opinion of Nourooz-Zadeh and Appleqvist (1988b), the analytical data of Fischer, Laskawy, and Grosch (1985) who saponified total lipids, include esterified sterol oxides as some artefacts were generated. The method (TLC analysis) used by Finocchiaro, Lee, and Richardson (1984) also appears to yield values which are higher than results using other methods.

3. COPs in fresh milk and dairy products

Milk contains approximately 12 mg cholesterol per 100 g or 3 mg per g milk fat and the content depends on several factors (Walte, 1994). 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. Furthermore, milk has a low level of polyunsaturated fatty acids and of prooxidant trace elements such as iron and copper.

The determination of oxidised cholesterol in fresh milk and dairy products has been the subject of several investigations (Table 1). Bican (1984) did not detect COPs in raw milk using TLC and HPLC. Later, Cleveland and Harris (1987) confirmed these results in raw milk using TLC. However, Sander, Smith, and Addis (1988) detected 9 mg α-epoxycholesterol and 1 mg β-epoxycholesterol kg^{-1} in milk used for cheese manufacture. In human milk, the 7-ketocholesterol content in the lipid extract was at the detection limit of 0.5 mg kg^{-1} (Scopesi et al., 2002). Liquid milk and other liquid dairy products therefore contain little or no COPs, with the exception of vanilla yoghurt (Sander, Addis, Park, & Smith, 1989a).

In cheese (Parmesan) and cheese spreads, COPs were detected only in a range of a few mg kg^{-1} fat (Schmarr, Gross, & Shibamoto, 1996). In another study, only 7-ketocholesterol and traces of 7α- and 7β-hydroxycholesterol were found in one of five samples of processed cheese and in Raclette cheese (Rose-Sallin, Sieber, Bosset, & Tabacchi, 1997). Similar results were obtained for sliced yellow and grated yellow cheese (Nielsen et al., 1995) as well as for unbleached feta cheese (Nielsen, Olsen, Lyndon, Sorensen, & Skibsted, 1996b).

Fresh cream (Kou & Holmes, 1995) as well as fresh butter did not contain detectable COPs (Bican, 1984; Jacobson, 1987; Luby, Gray, & Harte, 1986a; Luby, Gray, Harte, & Ryan, 1986b; Pie, Spahis, & Seillan, 1990). However, in other studies, traces (at the detection limit of 0.1 mg kg^{-1} in the lipids) or low levels of 7 β-hydroxy- and 5,6α-epoxycholesterol (Kumar & Singhal, 1992; Sander et al., 1988; Sander et al., 1989a) and 7-ketocholesterol (Nourooz-Zadeh & Appleqvist, 1988b; Pie et al., 1990) were detected. According to Nielsen et al. (1996a), the total COPs content of butter and dairy spread were 1.4 (without 7-ketocholesterol) and 2.7 mg kg^{-1} lipid, respectively.

4. COPs in heat-treated dairy products

Milk heated under different time–temperature conditions, varying from pasteurisation to UHT-treatment, showed no (Bican, 1984; Cleveland & Harris, 1987) or only a small formation of COPs (Table 2). Sander et al. (1989a) found 7 mg kg^{-1} α-epoxycholesterol in one of two commercial (assumed heat-treated) samples of whole milk, while in the second sample no COPs were detectable.

Raclette cheese heated for 5 min to a temperature of 134 °C 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 amounts of COPs (Rose-Sallin et al., 1997).

COPs were detected in butter after different heat treatments (Table 2). Heating of fresh butter for 10 min at 170 °C and for 10 and 20 min at 180 °C resulted in total COPs amounting to 13.9, 14.6 and 27.3 mg kg^{-1}, respectively (Pie et al., 1990). These results were contrary to the values reported by Nourooz-Zadeh

Table 1
COP levels in fresh milk and dairy products (mg kg^{-1} fresh product)

Product	*N*	7α	7β	α-ep	β-ep	7-k	20-OH	25-OH	Triol	Ref.
Milk raw	1	nd	nd	nd		nd		nd	nd	1
Milk evaporated	1	nd	nd	nd	nd	nd		nd	nd	2
Vanilla yogurt	1	2	1	2	3	4		nd	1	2
Vanilla ice cream	1	nd	nd	6	1	nd		nd	nd	2
Sour cream	1	nd	nd	3	nd	nd		nd	nd	2
Milkfat	1	nd	7	nd	nd	nd		nd	nd	2
Cream	3	nd	nd		nd	nd	nd	nd	nd	3
Cream buffalo	3	nd	nd		nd	nd	nd	nd	nd	3
Butter	1	nd	nd			nd		2.6		4
Butter	1	nd	nd	nd		<0.2		<0.2	nd	5
Butter	3	nd	nd	nd	nd	tr		nd	nd	6
Butter	1		2	7						2
Butter	3	nd	<1.0		<1.0	nd	nd	nd	nd	3
Butter[1]	2	0.40	0.26	0.34	0.19	nq			0.23	7
Butter buffalo	3	nd	<1.0		<1.0	nd	nd	nd	nd	3
Ghee	14	73±37	67±28		tr		61±40	58±23		8
Ghee	6	71						295[a]		9
Ghee	3		0.92±0.21		7.2±0.27	2.4±0.22	6.6±0.08	3.2±0.03	nd	10
Ghee cow	3	2.1±1.2	4.2±1.8		5.6±1.8	3.1±0.2	nd	nd	nd	11
Ghee buffalo	3	3.6±0.8	5.9±1.0		4.6±0.9	4.1±0.9	2.7±1.0	nd	nd	11
Dairy spread	2	0.33	0.20	0.22	0.16	1.68		nd	0	7
Cheese spread	3	nd	12/3/nd	3/nd	nd	nd		nd	nd	2
Cheese spread[1]	3	0.59±0.02	0.75±0.01	0.68±0.04	0.81±0.03	0.48±0.03		0.51±0.02	nd	12
Cottage cheese	1	nd	nd	nd	nd	nd		nd	nd	2
Cream cheese	1	nd	nd	9	nd	3		nd	nd	2
Parmesan[1]	3	1.16±0.05	1.31±0.04	0.93±0.08	0.71±0.21	1.22±0.01	2.8±0.27	0.57±0.05	nd	12
Blue cheese	1	<0.1	0.2		0.1			nd	nd	13
Parmesan grated	3	0.53	0.6		0.6			nd	nd	13
Grana type grated[1]	5					0.8–4.3				14
Raclette	1	<0.1	<0.1	nd		0.1±0.01		nd	nd	5
Processed cheese	1	<0.1	<0.1	nd		0.1±0.0		nd	nd	5
Processed cheese	4	nd	nd	nd		nd		nd	nd	5
Condensed milk	1	0.3	0.3		0.2			nd	nd	13
Butter oil	1	nd	nd	nd		<0.20		<0.20	nd	5
Butter oil	3	0.27	0.67		0.15			tr	tr	13
Butter oil	1	10.8	nd	nd		7.3		nd	0.5	15

Abbreviations: 7α = 7α-hydroxycholesterol; 7β = 7β-hydroxycholesterol; α-ep = α-epoxycholesterol; β-ep = β-epoxycholesterol; 7-k = 7-ketocholesterol; 20-OH = 20-hydroxycholesterol; 25-OH = 25-hydroxycholesterol; Triol = cholestanetriol
N = number of samples; nd = not detected; nq = not quantified or not quantifiable; tr = trace
References: (1) Cleveland and Harris (1987); (2) Sander et al. (1989a); (3) Kumar and Singhal (1992); (4) Csiky (1982); (5) Rose-Sallin et al. (1997); (6) Pie et al. (1990); (7) Nielsen et al. (1996a); (8) Jacobson (1987); (9) Sen et al. (1994); (10) Kumar et al. (1999); (11) Kumar and Singhal (1992); (12) Schmarr et al. (1996); (13) Fischer et al. (1985); (14) Lercker and Rodriguez-Estrada (2000); (15) Angulo et al. (1997)
[1] mg kg^{-1} lipid
[a] Cholesta-3,5-dien-7-one

and Appleqvist (1988b) and Rose-Sallin et al. (1997). A 2-month old butter heated at 150 °C for 10 min (Nourooz-Zadeh & Appleqvist, 1988b) or at 170–175 °C for 5 and 15 min (Rose-Sallin et al., 1997) contained no detectable levels of COPs (Nourooz-Zadeh & Appleqvist, 1988b) or only traces of some COPs (Rose-Sallin et al., 1997). However, after 10 min at 160, 170, 180, 190 and 200 °C, measurable levels of some COPs were detected, but the total amount of COPs was below 2.3 mg kg^{-1} in lipids and heating at 200 °C for 10 min produced a lower amount of total COPs than at 190 °C for the same duration (Nourooz-Zadeh & Appleqvist, 1988b). After long-term heating of butter-fat in the presence of air at 185 °C for 8 and 16 h, the following COPs were found: 3,5-cholestadien-7-one, 4-cholesten-3-one, cholesterol-5α,6α- and -5β,6β-epoxide, 7α- and 7β-hydroxycholesterol and 7-ketocholesterol (Amer, Gharavy, & Kupranycz, 1986).

Butter oil is manufactured by heating or centrifuging butter to remove the water. Only traces of 7-keto- and 25-hydroxycholesterol (Rose-Sallin et al., 1997) or low levels of 7α-, 7β-hydroxy- and α-epoxycholesterol (Fischer et al., 1985) were found in fresh butter oil. After 30 min of heating of butter oil at 170–175 °C, a

Table 2
COP levels in heat-treated dairy products (mg kg^{-1} fresh product)

Product	Treatment	N	7α	7β	α-ep	β-ep	7-k	20-OH	25-OH	Triol	Ref.
Milk	pasteurised/UHT		nd	nd	nd		nd		nd	nd	1
Milk whole	commercial	2	nd	nd	7/nd	nd	nd		nd	nd	2
Milk	85 °C, 12 h		nd	nd	nd		nd		nd	nd	1
Milk, canned	evaporated		nd	nd	nd		nd	nd		nd	1
Butter	180 °C, 5′	1	19.6	8.4			5.2		34.0		3
Butter	180 °C, 10′	1	52.2	19.7			11.2		nd		3
Butter	170 °C, 10′	3	1.2±0.02	1.7±0.05	1.0±0.14	4.8±0.15	5.2±0.14	tr	tr	tr	4
	180 °C, 10′	3	1.6±0.01	2.3±0.07	1.3±0.07	4.3±0.18	5.1±0.25	tr	tr	tr	4
	180 °C, 20′	3	3.9±0.04	4.6±0.31	2.9±0.31	7.3±0.25	8.6±0.01	tr	tr	tr	4
Butter[1]	150 °C, 10′	1	nd	nd	nd	nd	nd	nd	nd		5
	180 °C, 10′	1	0.2	0.1	0.2	0.4	0.5	nd	0.2		5
	190 °C, 10′	1	0.3	0.4	0.1	0.6	0.9	nd	nd		5
	200 °C, 10′	1	0.1	0.2	0.3	0.4	0.2	0.4	nd		5
Butter cake	not specified	4	0.32	0.29	0.34	1.18	1.62	0.10	0.08	0.03	4
Butter cookie	not specified	2	0.17	0.13	0.14	0.64	0.72	tr	tr	tr	4
Butter oil	170–175 °C, 5′	1	nd	nd	nd		<0.20		<0.20	nd	6
	170–175 °C, 30′	1	0.26±0.02	0.33±0.02	0.20±0.01		0.82±0.02		nd	nd	6
	205–210 °C, 5′	1	nd	nd	nd		<0.20		<0.20	nd	6
	205–210 °C, 10′	1	<0.20	<0.20	nd		<0.26±0.0		<0.20	nd	6
	205–210 °C, 30′	1	3.87±0.03	6.49±0.03	2.81±0.05		5.68±0.05		0.36±0.0	nd	6
Ghee	heat-damaged	6	164						466[a]		7
Ghee cow	control		3.7±1.4	6.4±1.8	7.6±1.4		4.1±1.6	nd	nd	nd	8
	225 °C, 30′, 3d[b]		22.3±3.7	42.4±5.2	60.1±4.7		45.3±3.6	7.7±1.5	10.2±0.4	7.6±0.9	8
Ghee buffalo	control		3.1±1.1	5.5±1.0	7.5±0.8		3.3±0.7	1.6±0.7	nd	nd	8
	225 °C, 30′, 3d[b]		22.9±1.6	45.9±3.8	68.8±1.6		47.4±2.1	7.3±1.6	10.8±1.1	6.1±0.2	8
Ghee	control	3		0.92±0.2	7.2±0.3		2.4±0.2	6.6±0.08	3.2±0.03	nd	9
	120 °C, ≈50 h	3		18±2.3	30.5±0.5		80.5±3.4	84.3±1.3	6.9±0.05	4±0.8	9
Raclette	134 °C, 5′	2	<0.1	<0.1	nd		0.17±0.01		nd	nd	6
Cheese fondue	98 °C, 4.5′	2	0.12±0.00	0.14±0.01	nd		0.15±0.01		nd	nd	6

Abbreviations: see table 1
References: (1) Cleveland and Harris (1987); (2) Sander et al. (1989a); (3) Csiky (1982); (4) Pie et al. (1990); (5) Nourooz-Zadeh and Appleqvist (1988b); (6) Rose-Sallin et al. (1997); (7) Sen et al. (1994); (8) Kumar and Singhal (1992); (9) Kumar et al. (1999)
[1]mg kg^{-1} lipid
[a]cholesta-3,5-dien-7-one;
[b]intermittent heating

total amount of COPs of 1.7 mg kg^{-1} fresh product was produced (Table 2). A more intense treatment at 205–210 °C for 30 min yielded a COP total of nearly 20 mg kg^{-1} (Rose-Sallin et al., 1997). Butter oils prepared from salted and unsalted butter were heated at 110 °C for 24 days. After this treatment, unsalted butter oil contained more than 300 mg kg^{-1} ketocholesterol and 200 mg kg^{-1} 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, Smith, Addis, & Park, 1989b).

Ghee (clarified butter) is produced from butter by removing water at a clarification temperature between 105 and 118 °C (Sserunjogi, Abrahamsen, & Narvhus, 1998). At this temperature, the formation of various COPs can be observed (Jacobson, 1987; Kumar, Sambaiah, & Lokesh, 1999; Kumar & Singhal, 1992; Prasad & Subramanian, 1992; Sen, Banerjee, Murherjee, & Sen, 1994). Very high amounts of COPs were detected in Indian ghee: 12.3% of the initial cholesterol (2100 mg kg^{-1}) was oxidised to COPs (Jacobson, 1987; Sen et al., 1994). However, Kumar and Singhal (1992) reported a total COP level of only 15.0 mg kg^{-1} in cow's ghee and of 20.9 mg kg^{-1} in buffalo's ghee and Kumar et al. (1999) 21.4 mg kg^{-1} in cow's ghee, but after heat treatment the amount of COPs was increased (Table 2). A total COP content of 285 mg kg^{-1}–equivalent to 17.6% of total sterols-was found in ghee heated at 120 °C for 45–50 h (Kumar et al., 1999). Nath, Usha, and Murthy (1996) found no COPs in ghee, but after 15 min of frying. The data of Jacobson (1987) and Sen et al. (1994) were inconclusive, as the COPs were separated only by TLC and quantified by densitometry and the samples were heated for long periods. Jacobson (1987) raised the hypothesis that high levels of COPs in ghee

account for the high prevalence of atherosclerotic heart disease in migrant Indian populations. According to Peterson (1987) and Raheja (1987) there are several other potential explanations and consumption of ghee containing COPs may be only one factor.

5. COPs in dried milk and dairy products

Different powders (skim milk, whole milk, casein, lactalbumin, infant formula, cheese, sour cream, butter) were analysed for COP content (Table 3). No COPs were detected either in fresh whole milk powder (Chan et al., 1993) nor in roller-dried whole milk (one sample), spray-dried whole (one sample) and skim milk powders (two samples) (Nourooz-Zadeh & Appelqvist, 1988a). These four Swedish samples were classified as "low"- or "medium"-heat powders. On the other hand, "high"-heat powders prepared from whole milk or skim milk contained measurable amounts of COPs (2.8 and 7.6 mg kg^{-1} total COPs in lipids). Spray-dried whole and skim milk powder samples obtained from New Zealand had similar amounts of COPs (Nourooz-Zadeh & Appelqvist, 1988a). Mc Cluskey et al. (1997) and Mc

Table 3
COP levels in powdered dairy products (mg kg^{-1} fresh product)

Powder	Process	*N*	7α	7β	α-ep	β-ep	7-k	25-OH	Triol	Total	Ref.
Skim milk			nd	tr	nd		nd	nd	nd		1
Whole milk		1	0.2	0.2		0.2		nd	nd		2
Whole milk			nd	nd	nd	nd	nd				3
Whole milk[1]		7					1.1-3.2				4
Whole milk, low heat[1]	spray-dried	1	nd	nd	nd	nd	nd	nd			5
Whole milk, low heat[1]	roller-dried	1	nd	nd	nd	nd	nd	nd			5
Whole milk, high heat[1]	spray-dried	1	1.4	0.6	0.6	0.2	tr	nd			5
Whole milk (NZ)[1]	spray-dried	1	nd	nd	0.6	0.3	tr	nd			5
Whole milk	spray-dried	1	nd	nd	0.6		0.4	nd	0.1		6
Whole milk	spray-dried A	3	14	2		nd	nd	nd	1		7
Whole milk	spray-dried B	3	7	3		2	nd	nd	nd		7
Whole milk	spray-dried C	3	2	nd		8	5	nd	nd		7
Whole milk, low heat[1]		14								0.66/0.43	8
Whole milk, high heat[1]		14								1.6/1.07	8
Whole milk[1]		1					1.36	~1			9
Skim milk, low heat[1]	spray-dried	1	nd	nd	nd	nd	nd	nd			5
Skim milk, low or medium heat[1]	spray-dried	1	nd	nd	nd	nd	nd	nd			5
Skim milk, high heat[1]	spray-dried	1	2.0	2.1	2.6	0.9	tr	nd			5
Skim milk[1]	spray-dried	1	1.0	2.3	0.8	0.9	2.5	nd			5
Skim milk	spray-dried	6	nd	nd		nd	nd	nd	nd		7
Skim milk	spray-dried	1	0.1	nd	nd		nd	nd	nd		6
Milk[1]		3	0.8	0.5	2.0		1.9	0.6	nd		10
Casein		1	nd	6.2	0.4		2.6	nd	nd		6
Lactalbumin		1	0.1	3.8	0.3		2.0	nd	nd		6
Infant formula[2]		3	8/nd	6/8/7	3/2/nd	46/5/4	13/13/18	18/19/5	4/4/35		11
Infant formula + Fe[2]		2	6/8	8/9	3/3	7/nd	11/17	8.5	3/nd		11
Infant formula[1]		5	1.0	0.9	2.5		2.0	0.7	nd		10
Adapted formula	reconstituted	10					3.6±3.7				12
Adapted milk formula[1]		6					nd-11.2				13
Adapted milk formula[1]		2					0.9/0.28	2.53/0			9
Follow-up milk formula[1]		6					nd-4.1				13
Follow-up milk formula[1]		1					0.38	0			9
Cheddar cheese[2]	dehydrated	11	6	7/9	7/3/5/3/9/6	4/3/3	14/3	3/3	17/4		11
Blue cheese[2]	dehydrated	4	nd	nd	3	nd	4	nd	nd		11
Parmesan[2]	dehydrated	6	nd	6/9	4/3/4/5/4	2/6	16	4	9		11
Romano[2]	dehydrated	6	nd	2/nd	5	nd	nd	nd	nd		11
Cream[1]	spray-dried	1	nd	nd	nd	nd	nd	nd			5
Sour cream[2]	dehydrated	3	nd	7/4	4/13	3/3	6/3	nd	nd		11
Butter[2]		2	nd	8/nd	26/nd	3/nd	3/nd	nd	nd		11

Abbreviations: see table 1

References: (1) Cleveland and Harris (1987); (2) Fischer et al. (1985); (3) Chan et al. (1993); (4) Lercker and Rodriguez-Estrada (2000); (5) Nourooz-Zadeh and Appleqvist (1988a); (6) Angulo et al. (1997); (7) Sarantinos et al. (1993); (8) McCluskey (1997); (9) Calvo et al. (2003); (10) Przygonski et al. (2000); (11) Sander et al. (1989a); (12) Scopesi et al. (2002); (13) Zunin et al. (1998).

[1] mg kg^{-1} lipid

[2] single value

Cluskey (1997) confirmed these results. They found significantly higher COP levels in high-heat than in low-heat milk powder. Fresh milk powders from cows of "restricted" or "supplemented" herds did not differ in their COP levels (Mc Cluskey, 1997; Mc Cluskey et al., 1997). COPs were not detected in Australian freshly spray-dried skim milk powders, but total amounts of COPs (data of 22-keto- and 20α-hydroxycholesterol are not shown in Table 3) of 34 ± 8 (brand A, $n = 3$), 21 ± 4 (brand B, $n = 3$) and 31 ± 6 (brand C, $n = 3$) mg kg^{-1} edible portion were observed in spray-dried full cream milk powders, which correspond to between 3.4% and 4.9% of total cholesterol (Sarantinos, Odea, & Sinclair, 1993). Levels of 5.85 and 7.48 mg kg^{-1} lipid extract of total COPs were found in Polish milk powders and fresh infant formulas (Przygonski, Jelen, & Wasowicz, 2000). Five samples of milk powder analysed by HPLC contained 0.6 mg 7-ketocholesterol kg^{-1} (Gallina Toschi et al., 1994). The air-heating system of the spray-driers (direct-fired heating with high or low levels of nitrogen NO_x (these compounds are known initiators of lipid oxidation) or indirect heating) initially had no influence on the presence of COPs (Chan et al., 1993).

The surface composition of a powder is of major importance for the oxidation of cholesterol. A milk-resembling powder with high-melting pure tristearin as an oil-phase showed low COP values for a period of 6 months. A powder with dry technical tristearin emulsion had the largest amount of COPs, whereas the powder, which had liquid triolein as the oil phase, lay in between the COPs amount for the other two oil phases. This phenomenon can be explained by the liquid oil phase acting as a barrier to oxygen diffusion and to some degree protecting the cholesterol from oxidation (Granelli, Fäldt, Appelqvist, & Bergenstahl, 1996).

6. COPs in stored dairy products

The effects of storage conditions on COP formation are shown in Table 4. Commercial milk powders stored in the original packaging oxidised only to a small extent (Rose-Sallin et al., 1995). Fourteen samples of spray-dried skim milk powders were analysed after storage of 13–37 months at different warehouses. They contained substantial amounts of total COPs (between 20 and 78 mg kg^{-1} in total lipids; while the lipid content was only 10 g kg^{-1} using the IDF-method) (Nourooz-Zadeh & Appelqvist, 1988a). In contrast to fresh milk powders, high-heat powders showed a smaller amount of COPs than low-heat powders after being stored for 12 months at 15 or 30 °C (Mc Cluskey, 1997; Mc Cluskey et al., 1997). After storage for between 2 and 23 months after repacking, seven small consumer packages of the same type of powder contained variable amounts of some COPs. Three samples of spray-dried whole milk powders stored for 12 months at room temperature (about 20 °C) contained between 8.3 and 16.7 mg kg^{-1} total COPs in lipids (Nourooz-Zadeh & Appelqvist, 1988a). Whole milk powder, stored for 2 and 7 years, showed total COPs below 10 mg kg^{-1} in contrast to higher concentrations found in egg yolk and whole egg powder (van de Bovenkamp et al., 1988; Cleveland, 1987). Adapted milk formulas contained more 7-ketocholesterol than human milk (Scopesi et al., 2002) and infant milk formulas (Zunin, Calcagno, & Evangelisti, 1998).

The influence of three methods of spray drying (indirect electrical; direct, low NO_x; direct, high NO_x) and of three packaging conditions (PE pouches, glass vials with and without oxygen absorbers) on the formation of COPs in stored whole milk powder was studied by Chan et al. (1993). The methods of drying did not affect the concentrations of COPs in whole milk powder stored at 20 °C for 6 months, but significantly affected them at 40 °C. A COP concentration up to 540 μg kg^{-1} lipid was found in a sample manufactured by the direct high NO_x gas-fired heating process and packaged in polyethylene pouches. A smaller concentration was found in samples manufactured by the direct low NO_x gas-fired heating process and only a minimal concentration in powders produced by indirect electrical heating. Milk powders processed by direct high NO_x gas-fired heating and packaged in PE pouches had a total COPs content of 15.6% of the original cholesterol content, whereas samples packaged in glass vials with and without oxygen absorbers had COPs contents of 0.7% and 5.9% of the original cholesterol, respectively. According to this study, the oxygen content in the headspace plays an important role in the formation of COPs. A study by Nielsen, Olsen, and Skibsted (1996c) in a model system showed that, besides the presence of oxygen, water-soluble radicals are as important for the oxidation of cholesterol because in the reaction sequence from 7β-hydroxycholesterol to 7-ketocholesterol, dissolved oxygen is not incorporated in the formed 7-ketocholesterol.

Rose-Sallin et al. (1997) studied the effect of oxygen, air or nitrogen at different water activities on the COPs content of whole milk powder stored at 30 °C for one year. Nitrogen and air protected the milk powder from oxidation or only small amounts were produced at a_w-values of 0.11 in the presence of air. However, harsh conditions such as an oxygen content of 660 mL L^{-1} and a low water activity of 0.11 lead to a considerable formation of total COPs (120 mg kg^{-1}), but only small amounts were found at a_w-values of 0.22 and 0.33. The storage of vacuum-packed whole milk powders for 12 months at 15 or 30 °C led to lower COPs levels than the storage of sachet-packed whole milk powders (Mc Cluskey, 1997; Mc Cluskey et al., 1997).

Table 4
COP levels in stored dairy products (mg kg^{-1} fresh product)

Product	Storage	N	7α	7β	α-ep	β-ep	7-k	25-OH	Triol	Ref.
Full cream powder	3 m	1	0.08	0.03			<0.1		0.01	1
Full cream powder	1 yr	2	0.41	0.63			<0.1		0.04	1
Full cream powder	2 yr (1 yr opened)	1	0.59	0.88			<0.1		0.02	1
Whole milk powder	2 yr	1	3.9		4.1		1.5	0.1	0.3	2
Whole milk powder	7 yr	1	2.9		1.2		0.5	0.8	<0.1	2
Whole milk powder	32 °C, air, 12 mo	1	6.9	12.1	0.1		11.9		1.8	3
	32 °C, N_2, 12 mo	1	0.2	1.6	0.1		3.2		nd	3
	55 °C, air, 12 mo	1	13.1	35.4	6.5		53.4		10.8	3
	55 °C, N_2, 12 mo	1	0.5	1.3	0.4		2.1		0.3	3
Whole milk powder[1]	open can, 6/24 mo	1					1.53/2.7	~1.1/3.3		4
Skim milk powder[1]	13 mo in a warehouse	1	4.5	6.6	1.8	0.7	5.0	0.3		5
Skim milk powder[1]	13 mo	1	4.5	6.6	1.8	0.7	5.0	0.3		5
Skim milk powder[1]	37 mo	3	12.3	13.4	2.5	8.3	17.0	0.4		5
Skim milk powder	32 °C, air, 12 mo	1	1.0	2.1	0.7		2.8		0.7	3
	32 °C, N_2, 12 mo	1	0.4	1.1	0.1		1.6		0.4	3
	55 °C, air, 12 mo	1	0.1	3.3	nd		4.1		1.1	3
	55 °C, N_2, 12 mo	1	6.0	8.2	0.2		7.0		8.4	3
Butter[1,2]	−18 °C, 32 wk	2	0.6	0.5	0.1	0.23	2.1		0	6
	4 °C, 11 wk	2	0.3	0.32	0.27	0.29	1.3		0.14	6
	20 °C, 13 wk	2	1.2	0.75	0.38	0.24	2.9		0.24	6
Butter	cold store, 2 wk	3	<0.1	0.1	0.9			nd	nd	7
	cold store, 2 mo		<0.1	<0.1	0.4					7
	cold store, 18 mo		<0.1	<0.1	0.5					7
Butter[1]	8,10 d	2	nd	nd	nd	nd	<0.1	nd		8
	1 mo	4	nd	nd	0.3	nd	<0.1	nd		8
	4 °C, 2 mo	1	0.2	0.3	0.3	0.5	0.2	nd		8
	4 mo	2	0.3	0.25	0.8	1.0	0.55	nd		8
Butter	−26 °C, 2/24 wk	1/1	nd/nd	7/7	5/2	5/nd	2/nd	nd/nd	8/nd	9
	4 °C, 2/24 wk	1/1	nd/2	7/2	3/10	3/4	4/3	nd/nd	nd/nd	9
	16 °C, 2/24 wk	1/1	nd/nd	6/4	5/13	nd/6	4/3	nd/nd	nd/nd	9
Butter	−20 °C, 3 mo	3	nd	nd	nd	nd	1.0	nd	nd	10
	−20 °C, 6 mo	3	0.2	nd	nd	1.5	0.4	nd	nd	10
Butter oil	N_2, 15 °C, 90 d	4	10±3	20±3		<3			<3	11
	N_2, −20 °C, +1 yr	4	20±6	30±6		20±3			nq	11
Butter oil	air, 15 °C, 90 d	4	30±2	30±2		nd			<3	11
	air, −20 °C, +1 yr	4	60±4	90±5		30±5			nq	11
Butter oil	−24 °C, 30 mo	1	<0.20	<0.20	<0.20		0.28	<0.20	<0.20	12
Dairy spread[1,2]	−18 °C, 13 wk	2	1.3	0.56	0.48	0.35	2.0		0.31	6
	4 °C/20 °C, 13 wk	2/2	1.1/1.3	0.49/1.6	1.6/1.3	0.49/0.67	5.7/5.3		0.97/0.96	6
Parmesan brand A	clear glass bottle	4	6±0.6	6±0.6	32±1.9				2±0.6	11
brand A	cardboard shaker box	4	3±1.2	3±1.2	9±1.2				nq	11
brand D	cardboard shaker box	4	nq	nq	6±1.2				nq	11
Parmesan	initial	1	nd	nd	2	2	3	2	nd	13
	21/38 °C, 6 mo	1/1	nd/nd	nd/nd	7/10	nd/nd	5/3	5/4	nd/nd	13
Cheddar	7 mo	1	nd	3	2	1	nd	nd	nd	8
Cheddar	initial	2	nd	8.5	2	1.5	1.5	nd	nd	13
	21/38 °C, 6 mo	2/2	nd/nd	3/3	5/9	1/2.5	2/2.5	nd/nd	nd/nd	13
Romano	clear glass bottle	4	3±0.6	3±0.6	16±1.6				nq	11
Cheese	6 °C, 63 d	1	0.11±0.0	0.17±0.01	nd		0.29±0.02	nd	nd	12
Cheese hard melted[1]	4 mo	1	<0.1	nd	<0.1	0.1	nd			8
Cheese soft melted[1]	18 mo	1	nd	0.2	<0.1	nd	nd			8
Cheese grated[1]	18 mo	1	0.3	0.6	0.5	nq	0.4			8
Parmesan grated	−24 °C, 25 mo	1	nd	nd	nd		<0.10	nd	nd	12
Parmesan grated	4 °C, 3 mo	1	nd	nd	nd		<0.10	nd	nd	12
Cheddar powder	4 °C, 0/18 mo	7	0.9/nd	2.3/3.4	3/6.3	0.4/14.1	2.4/10.4	0.4/0.1	3/4.3	13
Blue powder	4 °C, 0/18 mo	4	nd/nd	nd/4.3	0.8/6.8	nd/4.3	1/1.8	nd/nd	nd/nd	13
Parmesan powder	4 °C, 0/18 mo	4	nd/nd	2.3/3.3	3/10	nd/4	4/4.3	1/1	2.3/2.3	13
Romano powder	4 °C, 0/18 mo	2	nd/nd	1 2/4 2	2.5/7	nd/4.5	nd/3.5	nd/nd	nd/nd	13
Processed cheese	37 °C, light, 1 yr	2					3.3			14
Sour cream powder	4 °C, 0/18 mo	2	nd/nd	3.5/5 6	2/11	1.5/4	3/2.5	nd/nd	nd/nd	13
Butter powder	4 °C, 0/18 mo	2	nd/6.5	4 8/2.5	13/28	1.5/9	1.5/6	nd/3.5	nd/7	13
Infant formula	1 yr	1	<0.1	0.09			<0.1	<0.1	<0.01	1

Table 4 (*continued*)

Product	Storage	*N*	7α	7β	α-ep	β-ep	7-k	25-OH	Triol	Ref.
Infant formula, milk-based	initial/32, 55 °C, 1 yr						7.1/10.0/19.2			15
Infant formula, soy-based	initial/32, 55 °C, 1 yr						12.7/19.9/43.7			15
Infant formula, hypoallergenic	initial/32, 55 °C, 1 yr						10/11.9/21.8			15

Abbreviations: see table 1; mo = month; wk = week; yr = year;
References: (1) Rose-Sallin et al. (1995); (2) van de Bovenkamp et al. (1988); (3) Angulo et al. (1997); (4) Calvo et al. (2003); (5) Nourooz-Zadeh and Appleqvist (1988a); (6) Nielsen et al. (1996a); (7) Fischer et al. (1985); (8) Nourooz-Zadeh and Appleqvist (1988b); (9) Sander et al. (1988); (10) Pie et al. (1990); (11) Finocchiaro et al. (1984); (12) Rose-Sallin et al. (1997); (13) Sander et al. (1989b); (14) Kristensen et al. (2001); (15) Angulo et al. (1998)
[1]mg kg^{-1} lipid;
[2]values for week 0 are in Table 1

Storage of grated Parmesan and Cheddar cheese in tightly capped glass tubes in the dark at 21 and 38 °C for 6 months showed little change in the COP levels. The initial total COP levels were 9 mg kg^{-1} for Parmesan and 13 and 14 mg kg^{-1} for the two Cheddar cheese samples. After 6 months at 21 and 38 °C, grated Parmesan cheese contained 17 and 17 mg kg^{-1} total COPs and the two Cheddar samples stored at 21 °C contained 13 and 9 mg kg^{-1} COPs, whereas the two samples stored at 38 °C had levels of 20 and 17 mg kg^{-1}. α-epoxide occurred more frequently and at higher levels than other COPs (Sander et al., 1989b). Milk- and soy-based as well as hypoallergic infant formulas were stored for one year at 32 and 55 °C. The 7-ketocholesterol level of the infant formulas at these two storage temperatures doubled and more than doubled (Angulo, Romera, Ramirez, & Gil, 1998). This COP was found in five grated cheese samples (no indication of storage time) at a level of 0.4 mg kg^{-1} (Gallina Toschi et al., 1994) and in processed cheese only after exposure to light for one year at 37 °C, but not in the dark and not at 5 °C (Kristensen, Hansen, Arndal, Appelgren Trinderup, & Skibsted, 2001).

Butter stored at 4 °C for 1 month contained 5α,6α-epoxycholesterol in quantities between 0.1 and 0.4 mg kg^{-1} lipids, and traces of 7-keto-, 7α- and 7β-hydroxycholesterol. A further storage period of 3 month increased the amount of several COPs, but no higher than 1 mg kg^{-1} lipids (Nourooz-Zadeh & Appleqvist, 1988b). The COP concentration was in the range of 0.14–1.3 mg kg^{-1} lipid in butter stored at 4 °C for 11 weeks, whereas butter stored at 20 °C for 13 weeks had COPs levels of 0.24–2.9 mg kg^{-1} lipid (Nielsen et al., 1996a). During storage of butter oil for 30 months at −24 °C only small amounts of 7-ketocholesterol and traces (<0.2 mg kg^{-1}) of 7α- and 7β-, 25-hydroxycholesterol and cholestanetriol were found (Rose-Sallin et al., 1997). Fischer et al. (1985) as well as Fischer and Grosch (1985) detected only traces of some of the predominant COPs in three butter samples after cold storage for 14 days, 2 and 18 months. Storage of butter at −20 °C can also result in the formation of COPs; 0.97 mg ketocholesterol kg^{-1} after 3 months and 0.22 mg kg^{-1} α-hydroxycholesterol, 1.52 mg kg^{-1} β-epoxide and 0.42 mg kg^{-1} 7-ketocholesterol after 6 months (Pie et al., 1990). The accumulation of COPs as a function of storage time was studied by Nielsen et al. (1996a) for butter and dairy spread at different storage temperatures. After storage of butter at −18 and 4 °C for 13 weeks, there was no accumulation of COPs. However, at 20 °C cholesterol oxidation occurred at a constant rate. Total COPs did not accumulate for dairy spread based on milk fat and rapeseed oil (3:1) stored at −18 °C. For storage at 4 °C, the accumulation of COPs was clearly delayed in the first weeks but after 7 weeks increased formation was found. At 20 °C, COP oxidation was high during the first weeks of storage and was constant after 7 weeks. In dairy spread the most abundant COP was 7-ketocholesterol, which has the highest concentration (5.7 mg kg^{-1} lipid) after storage at 4 °C for 13 weeks.

Several powders of dehydrated cheese, sour cream and butter were stored at 4 °C for 18 months. This procedure caused increased levels of COPs in 19 out of 21 products. Seven of the 17 cheese powders, one of two sour cream powders and one of two butter powders contained no COPs prior to storage. The average total content of COPs prior to storage for the 17 cheese powders was 9.0 mg kg^{-1} and after storage 23.9 mg kg^{-1} (range 3–70 mg kg^{-1}). This study shows that the cholesterol in stored powdered dairy products is relatively stable (Sander et al., 1989b).

7. COPs in heat-treated and stored dairy products

Only two publications have reported on the influence of storage conditions on heat-treated dairy products (Table 5). After heat treatment at different temperatures, butter samples were stored at −20 °C for 3 and 6 months. The higher the temperature used and the longer the storage time, the more total COPs were produced

Table 5
COP levels in heat-treated and stored butter (mg kg^{-1} fresh product) and cheese (mg kg^{-1} lipid)

Product	Heat treatment	Storage	N	7α	7β	α-ep	β-ep	7-k	20-OH	25-OH	Triol	Ref.
Butter	Unheated		3	nd	nd	nd	nd	0.97±0.01	nd	tr	tr	1
	170 °C, 10 min	−20 °C, 3 mo	3	1.95±0.04	2.49±0.02	1.65±0.12	4.52±0.02	8.48±0.04	tr	tr	nd	1
	180 °C, 10 min	−20 °C, 3 mo	3	2.88±0.07	3.84±0.11	2.13±0.02	6.55±0.02	9.50±0.07	0.57±0.01	0.49±0.01	0.33±0.01	1
	180 °C, 20 min	−20 °C, 3 mo	3	4.13±0.07	5.73±0.15	2.83±0.04	9.21±0.03	10.78±0.23	0.62±0.01	0.61±0.01	0.44±0.02	1
Butter	Unheated		3	0.22±0.02	nd	nd	1.52±0.01	0.42±0.18	nd	nd	nd	1
	170 °C, 10 min	−20 °C, 6 mo	3	1.64±0.10	1.93±0.04	1.00±0.10	4.38±0.55	5.01±0.16	tr	tr	tr	1
	180°C, 10 min	−20 °C, 6 mo	3	4.49±0.11	5.60±0.26	2.94±0.18	8.18±0.35	9.70±0.14	tr	tr	tr	1
	180°C, 20 min	−20 °C, 6 mo	3	8.88±0.34	14.88±0.40	7.40±0.65	18.45±2.07	14.3± 0.42	tr	tr	tr	1
Feta	Control			0.6	0.3	0.3	0.1	1.2	0.1		0.3	2
Feta	265 °C, 2.4 min	4 °C, 5 mo		1.6	1.0	0.7	0.1	3.7	0.1		0.5	2
	265 °C, 3.1 min	4 °C, 5 mo		1.0	1.2	0.7	0.1	4.6	0.1		1.1	2
	265 °C, 4.3 min	4 °C, 5 mo		0.9	1.3	0.6	0.1	4.9	0		1.2	2
Feta	280 °C, 2.4 min	4 °C, 5 mo		2.7	4.0	1.2	0.2	13.1	-		2.2	2
	280 °C, 3.1 min	4 °C, 5 mo		2.5	3.3	1.5	0.3	11.4	0.2		2.3	2
	280 °C, 4.3 min	4 °C, 5 mo		2.1	2.9	1.6	0.2	10.4	0		1.5	2

Abbreviations: see Table 1.
References: (1) Pie et al. (1990); (2) Nielsen et al. (1996b).

Table 6
COP levels in dairy products after exposure to light (mg kg^{-1} fresh product)

Product	Conditions	N	7α	7β	α-ep	β-ep	7-k	25-OH	Triol	Ref.
Butter	1500 lx, 8 d		present	present						1
Cheese	control, 6 °C, 63 d	2	0.11±0.0	0.17±0.01	nd		0.29±0.02	nd	nd	2
Cheese	5600 lx, 6 °C, 63 d	2	0.36±0.03	0.47±0.05	<0.10		1.12±0.05	<0.10	nd	2
Sliced yellow ch.[a]	0 d		1.2	0.9	nd	nd	5.5	nd	nd	2
	fl.l, 4 °C, 55 d		1.0	1.2	0.1	0.2	5.8	nd	nd	2
Grated yellow ch.[a]	0 d		0.4	0.4	0.3	0.4	3.5	nd	nd	2
	fl.l., 4 °C, 72 d		11	5.1	0.8	1.4	17	nd	nd	2
Feta[a]	0 d		0.8	1.2	nd	nd	5.5	nd	nd	2
	fl.l., 4 °C, 30 d		4.0	3.8	8.2	9.7	220	nd	nd	2
Cheddar	fl.l., 12 w	1	nd	4	9	2	4	nd	nd	3
Cheese fondue[b]	control, 6 °C, 63 d	2	<0.10	<0.10	nd		0.11±0.01	nd	nd	4
Cheese fondue[b]	5600 lx, 6 °C, 63 d	2	23.0±0.3	23.1±0.1	3.2±0.1		27.3±0.4	1.5±0.2	0.24±0.04	4
Cheese fondue	5600 lx, 6 °C, 113 d	2	nd	nd	nd		<0.10	nd	nd	4
Nabulsi	light protected, r.t. 0/3/6/9 mo	1					1.2/1.1/1.8/2.3			5
Nabulsi	transparent, r.t. 0/3/6/9 mo	1					1.2/1.2/2.9/5.2			5
Nabulsi	fluorescent + day, r.t. 0/1/2/3 wk						1.4/3.1/5.6/8.8			5
Nabulsi commercial	light exposed	8					8.3±0.3			5

Abbreviations: see Table 1, lx = lux (light intensity), r.t. = room temperature; fl.l. = fluorescent light.
References: (1) Luby et al. (1986b); (2) Nielsen et al. (1995); (3) Sander et al. (1989b); (4) Rose-Sallin et al. (1997); (5) Al-Ismail and Humied (2003).
[a] mg kg^{-1} lipid.
[b] In smooth slices.

with one exception (10 min, 170 °C, 6 month):

10 min. at 170 °C after 0, 3 and 6 months of storage: 13.7, 19.3 and 14.0 mg kg^{-1},

10 min. at 180 °C after 0, 3 and 6 months of storage: 14.6, 26.3 and 30.9 mg kg^{-1},

20 min. at 180 °C after 0, 3 and 6 months of storage: 27.3, 34.6 and 64.0 mg kg^{-1} (Pie et al., 1990).

Feta cheeses were produced from bovine milk and butter oil bleached at different temperatures were investigated by Nielsen et al. (1996b). The bleaching of butter oil can be performed at temperatures up to 280 °C. After 11 weeks of storage at 4 °C, feta cheese with butter oil bleached at 265 or 280 °C for 2.4, 3.1 and 4.3 min showed increasing amounts of COPs depending on the bleaching conditions. At the end of a 20-week storage period, feta cheese bleached at 280 °C contained the highest concentration of each of the different COPs, whereas the non-bleached had the lowest COPs levels. The dominant COP was 7-ketocholesterol, which formed a constant fraction of ~50% of the COPs (Table 5).

8. COPs in dairy products after light exposure

Foods containing oil or fat exposed to light undergo accelerated oxidative reactions. In the presence of riboflavin and fatty acid methyl esters, cholesterol can be photo-oxidised to various COPs (Chien, Lu, Hu, & Chen, 2003; Hu & Chen, 2002). Cholesterol in butter was oxidised during exposure to fluorescent light (light intensity approximately 1500 lux) (Luby et al., 1986a) and more intensely by daylight (Luby et al., 1986b). After 20-day of treatment with fluorescent light, at least five cholesterol-like components were visible on a TLC plate. 7α- and 7β-hydroxycholesterol were tentatively identified as COPs (Table 6). The COPs were more concentrated at the surface than throughout the entire butter block, which underlines the light-induced effect (Luby et al., 1986b. The use of aluminium foil as a light and gas barrier is important for preventing light-induced cholesterol oxidation in butter. Paper-based packaging materials cannot prevent this sufficiently (Luby et al., 1986a). Three types of butter (sour cream butter that had been frozen for 6 months, fresh sweet cream butter and fresh sour cream butter) were stored for 6 weeks under different light and temperature conditions (Table 7). After 23 days of continuous exposure to daylight or light for food, COP levels remained fairly low in all samples. After 6 weeks at room temperature, similarly high levels of COPs were found for both types of light exposure. However, at refrigerator temperature, the special light for food significantly accelerated the formation of COPs in comparison to the daylight lamp. Four-day exposure to UV light at room temperature leads to detectable amounts of COPs (Hiesberger & Luf, 2000).

A very marked accumulation of 7-ketocholesterol (Table 6) was seen in Feta cheese exposed to fluorescent light at 4 °C for 30 days compared to day 0 (Nielsen et al., 1995). Nabulsi cheese stored at room temperature in transparent jars contained more 7-ketocholesterol than when stored in light-protected jars (Al-Ismail & Humied, 2003). Cheese illuminated for 63 days at 5600 lux produced more 7-ketocholesterol, 7α- and 7β-hydroxycholesterol than the samples stored in the dark (Table 6). Cheese fondue packaged in a glass pot and illuminated for 113 days showed no COPs in the outer zone and only small amounts of total COPs in the de-emulsified fat-layer at the surface. High amounts of total COPs were also detected in a cheese sample with a defect in the packaging or in a transparent dish containing individually packaged slices of cheese (nearly 70 or 80 mg kg^{-1} against 0.57 or 0.11 mg kg^{-1} in the control cheeses) (Rose-Sallin et al., 1997).

In contrast to this, the exposure of sliced yellow cheese to fluorescent light for 55 days did not significantly change the concentration of COPs. The situation changed when grated cheese was exposed to light for a longer time. In the first 20 days, only a small amount of COPs was found in grated cheese under fluorescent light, the formation of 7-ketocholesterol was faster than 7α- and 7β-hydroxycholesterol whereas α- and β-epoxide remained nearly constant for 72 days (Nielsen et al., 1995). According to Finocchiaro et al. (1984), the levels of 5,6-epoxycholesterol, 7α- and 7β-hydroxycholesterol were higher in grated Parmesan cheese packaged in glass than the same grated cheese packaged in a cardboard box. Exposure of cheese powders to fluorescent light (1611 lux) for 12 weeks increased the levels of α-epoxide and 7-ketocholesterol,

Table 7
COP levels (mg kg^{-1} fresh product) in stored butter after exposure to light at cooling and room temperature (Hiesberger & Luf, 2000)

Butter type	COPs		Day light lamp[a]				Light for food[a]				UV-light[a]	
			Refrigerator[b]		Room[b]		Refrigerator[b]		Room[b]		Room[b]	
Days		0	23	42	23	42	23	42	23	42	2	4
Storage butter[c]	7-k	0.12	0.12	0.87	0.24	17.7	0.12	7.3	0.22	16.1	0.36	2.0
	7α	nd	nd	nd	nd	7.5	nd	5.8	nd	9.1	nd	2.4
	7β	nd	nd	nd	nd	10.5	nd	10.5	nd	14.4	nd	3.9
Sweet cream butter[d]	7-k	0.10	0.10	0.44	0.10	24.7	0.12	5.1	0.10	16.6	0.44	2.4
	7α	nd	nd	nd	nd	18.0	nd	3.1	nd	13.3	nd	2.4
	7β	nd	nd	nd	nd	26.2	nd	4.9	nd	20.0	nd	3.5
Sour cream butter[d]	7-k	0.10	0.10	0.41	1.35	36.2	1.66	8.7	1.6	27.9	0.41	2.2
	7α	nd	nd	nd	nd	14.6	nd	5.0	nd	14.1	nd	2.3
	7β	nd	nd	nd	nd	28.7	nd	12.5	nd	23.4	nd	3.9

Abbreviations: see Table 1.

[a]Day light lamp = fluorescent tube Osram L 18W/11 Lumilux Daylight; Light for food = fluorescent tube Osram L 18W/76 Natura de luxe; UV lamp = fluorescent lamp Osram Eversun L 40W/79K.

[b]Refrigerator temperature = 4 ± 2 °C, room temperature = 20 ± 2 °C.

[c]Sour cream butter that had been frozen for 6 months.

[d]Fresh butter.

with the levels being highest after 3 weeks and then decreasing throughout the remaining weeks to about half (Sander et al., 1989b).

9. Significance for human nutrition

The impact of COP consumption on human health has been investigated in a number of studies. According to van de Bovenkamp et al. (1988), in the Netherlands, mixed diets (raw or baked/fried/grilled or the latter plus extra fruit and vegetables) contained 4.2, 3.6 and 6.2 mg kg^{-1} total COPs. With an average daily food intake of 500 g, the average consumer can consume up to 1 mg 7β-hydroxy- and 0.5 mg of both α-epoxy- and 7-ketocholesterol. Emanuel, Hassel, Addis, Bergmann, and Zavoral (1991) have shown that humans absorb oxycholesterols from their food. After a meal with powdered egg containing COPs (0.7 g kg^{-1} body weight; α-epoxy- 90, β-epoxy- 50, 7 β-hydroxy- 60, 7-ketocholesterol 30 mg kg^{-1}), they observed a postprandial rise in COPs from 200 to 1500 $\mu g\,dL^{-1}$ in the plasma, but only a slight rise after eating fresh egg. Linseisen and Wolfram (1998b) were also able to confirm this finding in 5 young men after a meal rich in COPs. In a recently published study, Staprans, Pan, Rapp, and Feingold (2003) gave 400 mg α-epoxy-cholesterol to six controls and three subjects with type III hyperlipoproteinemia. After 4 h, this substance reached a concentration in the serum of more than 250 $\mu g\,dL^{-1}$ and could still be detected after 72 h. After 10 h, varying amounts of this COP could be found in chylomicrons/chylomicron remnants, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). Two hours after consumption this COP was already transferred to lipoproteins from chylomicrons/chylomicron remnants. However, there are still no data on the absorption rate in humans. The absorption of COPs in rats and monkeys has also been studied (Bascoul, Domergue, Mourot, Debry, & Crastes de Paulet, 1986; Fornas, Martinz-Sales, Camanas, & Baguena, 1984; Peng et al., 1982).

In in vitro tests, the presence of copper or lipoxygenase promotes the formation of oxycholesterols in LDL (Dzeletovic, Babiker, Lund, & Diczfalusy, 1995). This could also be explained by the fact that apart from their dietary origin, COPs can also be formed endogenously in the human organism. Thus, feeding a diet which is rich in iron and polyunsaturated fatty acids enhanced the formation of COPs in the liver of adult rats (Brandsch, Ringseis, & Eder, 2002). A distinction must be made here between enzymatic and non-enzymatic pathways. In the first case, the enzymes cholesterol 7α-hydroxylase, 27-hydroxylase, 7-ketone dehydrogenase and 5α,6α-epoxidase are involved in the formation of COPs and in the second case, the cholesterol may be converted into COPs by autoxidation (attack by reactive oxygen species, attack by peroxyl and alkoxyl radicals from lipid peroxidation and leukocyte/H_2O_2/HOCl system) (Brown & Jessup, 1999; Leonarduzzi, Sottero, & Poli, 2002; Linseisen & Wolfram, 1998a). According to Meaney et al. (2001) the three major oxycholesterols in the human circulation are 7α-, 27- and (24S)-hydroxycholesterol and are formed enzymatically from cholesterol. COPs also have a different role in humans. They are obligatory intermediates in bile acid synthesis and they may be regarded as transport forms of cholesterol (Björkhem, Meaney, & Diczfalusy, 2002). The occurrence of COPs in humans raises the question if a link between COPs and atherosclerosis can be established, but their role is still controversial (Brown & Jessup, 1999; Björkhem, & Diczfalusy, 2002; Carpenter, 2002; Schroepfer, 2000). In ApoE-deficient mice Staprans, Pan, Rapp, Grunfeld, and Feingold (2000) showed an increased aortic lesion area after feeding COPs, whereas Ando, Tomoyori, and Imaizumi (2002) provided evidence in the same animal model and Sandner, Dahme, Liebich, & Giesecke (1990) in swine that dietary COPS are non-atherogenic. Also according to Lyons, Samman, Gatto, and Brown (1999) diet may not be a major source of COPs in atherosclerotic plaques. Although according to Brown and Jessup (1999) further studies are necessary in order to definitely determine the role of COPs in the pathogenesis of atherosclerotic lesions, it seems sensible to keep the level of COPs in foodstuffs to a minimum.

10. Conclusions

The findings obtained in recent years lead to the conclusion that fresh milk and milk products contain little or no COPs. Formation of COPs in milk and dairy products can only occur under harsh conditions, such as the application of high heating temperatures for a long period or long storage at high temperatures, and in the case of foods in the dehydrated state or at low water activities. However, bleaching milk fat in the manufacture of feta cheese (Nielsen et al., 1996b), shows that use of an unfavourable processing technique can have unfavourable consequences for human health. Therefore, Nielsen et al. (1996b) postulated that if thermal bleaching of butter oil were considered necessary, bleaching in feta cheese manufacture should be carried out at the lowest possible temperature. Overall, the formation of COPs in food can be minimised 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. From an analytical point of view there is still a need for better

standardised methods with improved sensitivity and reproducibility for measurement of COPs due to the low concentrations in lipid extracts.

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