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ARTICLE

Influence of Storage Conditions on Cholesterol Oxidation in Dried Egg Pasta

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The oxidative characteristics of three different egg coproducts, namely, pasteurized eggs obtained from hens bred with organic methods (POE), pasteurized eggs from conventional breeding (PCE) and pasteurized spray-dried eggs (SPCE) obtained from conventional breeding, were analyzed. SPCE samples showed the highest content of peroxide (PV) and cholesterol oxides (COPs). In contrast, pasteurized eggs from organic breeding had the lowest index of oxidation. The three egg coproducts were used to produce dried egg pasta (spaghetti). The spaghetti was stored for 12 months at room temperature using typical pasta packaging (polypropylene foil) both under light, typical of retail conditions, and in the dark. Peroxide values and cholesterol oxidation were monitored at time 0 and then quarterly for 12 months. The oxidative parameters were significantly different in various egg coproducts, but the peroxide values of pasta were in the range of 3.0−3.5 mequiv of O_2/kg of fat, with no differences in the types of pasta prepared with the various egg coproducts. Samples stored in the dark did not show any significant variations in peroxide values. However, PCE, SPCE and POE spaghetti stored in typical packaging increased the PV by 742.8, 846.7 and 625.7%, respectively. The pasta at time 0 showed COP values of about 50 μg of COPs/g of fat. During storage, COP values increased significantly. PCE, SPCE and POE spaghetti stored in the dark showed a content of total cholesterol oxides that was 2.0, 2.0, and 1.5 times lower than that of samples stored with typical pasta packaging.

KEYWORDS: Photosensitized oxidation; spaghetti; egg pasta; cholesterol oxides (COPs); peroxide value (PV); shelf life

INTRODUCTION

Many foods, such as eggs, seafood, milk and meat, contain higher levels of dietary cholesterol than other foods. However, cholesterol in foods can be readily oxidized to form cholesterol oxidation products (COPs) when exposed to light, oxygen, active chemicals and high temperature. COP intake from foods can lead to cardiovascular disease and certain types of cancers. Therefore, lowering the level of COPs in foods is of interest. The susceptibility of cholesterol to oxidation has been recognized and investigated for several decades (*1*). Cholesterol is reported to be more stable in a solid than a liquid form, and the latter is more susceptible to oxidation than the former (*2*).

In recent years, COPs have drawn much attention because of their potential health implications (*3*−*5*). Numerous studies have shown that COPs may possess biological effects such as cytotoxicity and mutagenicity (*6*), carcinogenicity (*7*), atherogenicity and cell membrane damage (*8*). COPs can also be generated during food preparation when exposed to heat, air, light and radiation (*9*). Moreover, inappropriate storage conditions also facilitate formation of COPs (*10*).

Eggs have a cholesterol content of about 400 mg/100 g edible portion, which is among the highest found in foods (about 2 times that of butter and 5 times that of meat), and heat, oxygen, light, UV light, γ-radiation, water activity, technologically related events influence the formation of COPs (*11*).

The presence of COPs, the major cholesterol oxidation products in pasteurized eggs and egg powders, has been deeply investigated (*10*, *12*, *13*). The most abundant COPs in egg are 7α-hydroxycholesterol, 7β-hydroxycholesterol, and the products of their dehydrogenation, namely, 7-ketocholesterol, and 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol and the product of its hydratation, cholestane-3β,5α,6β-triol, as well as 20-hydroxycholesterol and 25-hydroxycholesterol.

In egg pasta, cholesterol can undergo oxidation that depends on the quality of the raw materials, the drying cycle and storage conditions of the finished product (*14*).

To date, no research studying the effects of light on cholesterol oxidation in egg pasta during storage has been carried out. Considering the interest in photo-oxidation of pasta lipids, the

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aim of the present study was to assess the effect of two packaging conditions on the accumulation of oxysterols in egg pasta prepared with different egg products during 12 months of storage at room temperature that mimics commercial conditions.

MATERIALS AND METHODS

Chemicals. The standards used for identification of COPs were supplied by Steraloids (Wilton, NH): 5-cholesten-3β-ol-7-one (7-KC), 5-cholestene-3β,7α-diol (7-α-HC), 5-cholestene-3β,7β-diol (7-β-HC), 5-cholestan-5α,6α-epoxy-3β-ol (α-CE), 5-cholestan- 5β,6β-epoxy-3β-ol (β-CE), 5-cholestene-3β,19-diol (19-HC). All solvents were analytical grade, and unless specified were purchased from Merck (Darmstadt, Germany).

Samples. Three egg coproducts, namely, pasteurized eggs obtained from hens bred with organic methods (POE), pasteurized eggs from conventional breeding (PCE) and spray-dried pasteurized eggs (SPCE) obtained from conventional breeding, were obtained from a local factory (Eurovo, Occhiobello, RO, Italy). Samples of egg coproducts were analyzed and used to prepare spaghetti pasta.

Pasta was produced at the pilot plant at Scientific and Technological Park Moliseinnovazione (CB, Italy). Both the pasta formulated with pasteurized egg and that made with spray dried egg contain the same percentage of egg product (4 eggs/kg of semolina). Spaghetti was manufactured by means of an experimental pasta making apparatus (NAMAD, Roma, Italy). Spaghetti was dried using low temperature LT, according to Marconi et al. (*15*). LT was a 20 h total drying cycle. Relative humidity was held at 90% RH for 60 min and then decreased to 80% RH in 1 h and followed by a 18 h linear gradient from 80 to 70%. The drying temperature was a 1 h linear gradient from 50 to 60 °C and from 60 to 50 °C and was held at 50 °C for another 17 h; finally it was cooled to 40 °C in 60 min. All the spaghetti was equilibrated to room temperature overnight.

The diameter of the spaghetti was about 1.4 mm. Extruded spaghetti was packed with commercial transparent polypropylene film with high oxygen permeability and stored in different modalities described as follows:

(1) At room temperature for 12 months, under conditions of light exposure with a daylight lamp from Osram (Milan, Italy), at a temperature and power of 6000 K and 32 W, respectively. The packages stored under light conditions were placed on a table with a light tube installed 55 cm over the table.
(2) At room temperature for 12 months under dark conditions.

Analyses were carried out at time 0 and after 3, 6, 9, and 12 months of storage. Each sample was analyzed in duplicate, and the average value of the determinations is reported.

Lipid Extraction. Pasta samples were ground with an Ika-Werke food processor (Staufen, Germany). The lipid fraction of the egg products (3 g) and pasta samples (15 g) was extracted using the procedure described by Folch et al. (*16*), which has been slightly modified as reported elsewhere (*17*).

Peroxide Evaluation. The International Dairy Federation method of Shantha and Decker (*18*) was used to determinate peroxide values (PV). Specifically, 0.05 g of fat was added to an Fe(II) and ammonium thiocyanate solution, and the intensity of a red-violet complex at 500 nm was evaluated.

COPs Determination. Cholesterol oxides (COPs) were collected by cold saponification (*19*) at room temperature, after addition of an exact volume of the internal standard solutions, 25 μL of 19-hydroxy-cholesterol (0.5 mg/mL) to 250 mg of lipids. COPs were purified from the unsaponifiable matter, obtained as described above, by NH_2 solid-phase extraction (SPE) according to Rose-Sallin and co-workers (*20*). Successively, the dried eluate was silylated (*21*) and dried again under gentle nitrogen flow. After redissolution in 100 μL of hexane, 1 μL was analyzed by capillary gas chromatography (GC-FID), using a Clarus 500 Perkin-Elmer instrument (Waltham, MA) equipped with a split−splitless injector and a flame ionization detector (FID). The capillary column was a ZB-5 (30 m × 0.25 mm i.d., low bleeding for MS) from Phenomenex (Torrance, CA) coated with a 0.25 μm film of 5% phenylpolysiloxane and of 95% dimethylpolysiloxane. The samples were injected in split mode (1 μL) with a split ratio of 1:10; the carrier gas was helium at a flow of 1 mL/min; the oven temperature was programmed from 260 to 315 °C at a rate of 2 °C/min and from 315 to 325 °C at rate of 5 °C/min; the injector and detector temperature were set at 325 °C. COPs identification was confirmed by gas chromatography−mass spectrometry (GC−MS) analysis. A GCMS-QP2010 Plus (Shimadzu Corp., Tokyo, Japan) was used. The column, the split and the temperature programmed were the same used in GC-FID; the helium flow was 1.4 mL/min and the injector and detector were set to 250 and 300 °C, respectively. The quadrupole was used in the electronic impact mode (70 eV), and a mass range of 40−650 m/z was monitored.

The GC retention time and fragmentation pattern of the identified peaks was compared with those of pure standard substances.

STATISTICAL ANALYSIS

The Statistica 6.0 software package from StatSoft (Tulsa, OK) was employed. Tukey's honest significant difference (HSD) test was considered significant, unless otherwise specified, when $p < 0.05$; factorial ANOVA univariate analysis was employed to evaluate the effects of storage packaging and egg ingredients on PV and COPs of pasta samples.

RESULTS AND DISCUSSION

Lipid Oxidation of Raw Ingredients. Three different raw egg materials were used to produce spaghetti pasta, namely, pasteurized eggs from hens bred with organic methods (POE), pasteurized eggs (PCE) and spray-dried pasteurized eggs (SPCE) from conventional breeding. Prior to pasta production, the stability of lipids in egg ingredients was evaluated by peroxide values (PV) and cholesterol oxide content (COPs).

The different COPs were identified by mass spectrometry and compared with the retention time of the standard solution. The LOD for the COPs concentration was 0.11 mg/mL silanized extracts and the LOQ was 0.35 mg/mL silanized extracts. The hydroxyl oxidized compounds, such as 7-α-HC and 7-β-HC, showed a target ion at 456 m/z and a molecular ion at m/z 546. The epoxy derivative oxides (α-CE and β-CE) reported the molecular ion at 474 m/z and a parent ion at m/z 384. A base peak at m/z 472 was identified as 7-ketocholesterol (7-KC) (**Figure 1**). The same mass data were reported by Menendez-Carreño et al. (*22*).

The different egg samples did not show any significant differences in fat content, with fat values on dry weight of 43.2, 43.8, and 43.1 g/100 g for PCE, SPCE and POE, respectively, according to Caboni et al. (*10*).

PV of all egg samples did not exceed the upper limit of 5 mequiv of O_2/kg of lipid. However, PV increased significantly ($p < 0.05$) in the spray-dried sample (**Table 1**).

Table 1 shows the values for COPs in raw ingredients. In agreement with Caboni et al. (*10*) and Guardiola et al. (*23*), the drying treatment caused substantial chemical modifications in processed egg. In fact the spray-dried samples reported the highest content of oxycholesterols (COPs) (76.4 ± 3.2 μg/g of fat). In contrast, the lowest content of COPs was in POE samples (18.2 ± 2.1 μg/g of fat) due to the high content of dietary natural antioxidants (data not shown). As reported by other authors, 7-keto is one of the first COPs to form and one of the oxides found in the highest amounts (*24*, *25*).

Lipid Oxidation of Spaghetti during Storage. The progression of lipid oxidation in spaghetti was monitored by PV and COP determinations. The initial peroxide values of PCE, SPCE and POE pasta were 3.5 ± 0.0, 3.0 ± 0.1, 3.5 ± 0.1 mequiv of O_2/kg of fat, respectively. The initial peroxide values in pasta at time 0 were not different despite the differences observed in raw materials; the production process of pasta and the interactions among the ingredients would be expected to cause degradation or reaction of both peroxides and COPs with other compounds such as proteins and phospholipids.

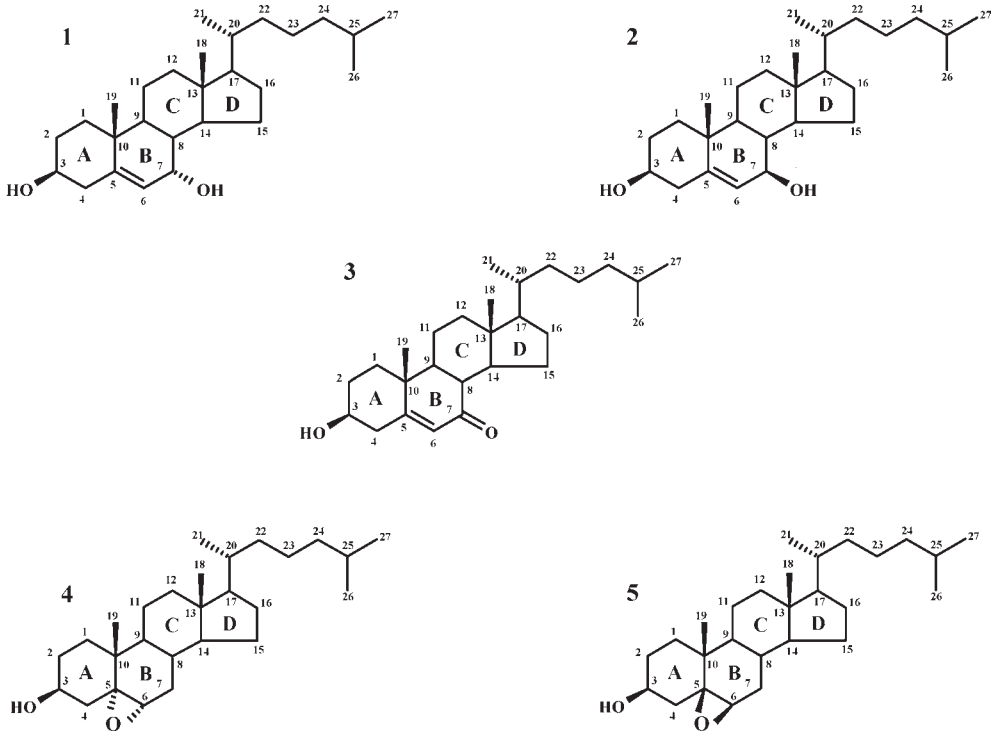

Figure 1. Structures of five forms of COPs found in egg coproducts and spaghetti: (**1**) 7-α-HC (5-cholestene-3β,7α-diol), (**2**) 7-β-HC (5-cholestene-3β, 7β-diol), (**3**) 7-KC (5-cholesten-3β-ol-7-one), (**4**) α-CE (5-cholestan-5α,6α-epoxy-3β-ol), (**5**) β-CE (5-cholestan-5β,6β-epoxy-3β-ol).

Table 1. Evaluation of Lipid Oxidation in Egg Ingredients[a]

raw egg products	PV[b]	7-α-HC[c]	7-β-HC[c]	β-CE[c]	α-CE[c]	7-KC[c]	sum[c]
PCE[d]	2.4 b	6.3 b	4.5 b	6.8 b	3.5 b	8.0 b	29.2 b
SPCE[e]	4.4 a	17.2 a	15.2 a	13.0 a	7.2 a	23.8 a	76.4 a
POE[f]	2.8 b	4.2 c	3.1 b,c	3.6 c	1.7 c	5.6 c	18.2 c

[a] Means in the same column with different letters are significantly different ($p \leq 0.05$). [b] Units: mequiv of O_2/kg of fat. [c] Units: μg/g of fat. [d] PCE = pasteurized egg from conventional breeding hens. [e] SPCE = spray-dried pasteurized egg from conventional breeding hens. [f] POE = pasteurized egg from organic breeding hens.

Table 2 shows the peroxide values for 12 months for different pasta under retail conditions in the dark and during storage under light. Pasta stored in the dark did not show a different content of primary oxidation products, independently of the raw egg ingredient.

It is clear that light-exposed samples had a significantly ($p < 0.05$) higher oxidation than samples stored in the dark. Differences in PV values can be attributed to differences in both the egg coproduct in samples and storage conditions ($p < 0.01$). Peroxide values increased in all pasta samples exposed to light during 12 months of storage.

When oxidation was induced by exposure to light, the primary oxidation products were significantly higher ($p < 0.05$) after 6−9 months depending on the egg type, although between 9 and 12 months a significant increase was detected in peroxide values from 8.4 to 26.0 mequiv of O_2/kg of fat. Generally, samples containing POE had lower peroxide values than samples with PCE and SPCE. Similarly, secondary lipid oxidation products of formulated pasta were assessed by determination of cholesterol oxidation products (COPs).

At time 0, all pasta samples formulated with different egg products showed no significant differences in COP content, independently of the initial oxidation state of raw materials. It is apparent that total COPs are reduced in pasta produced with dried eggs and the amount of 7-KC increases, while both

Table 2. Peroxide Values in Formulated Spaghetti (mequiv of O_2/kg of Fat)[a]

	PCE spaghetti		SPCE spaghetti		POE spaghetti	
month	light storage	dark storage	light storage	dark storage	light storage	dark storage
0	3.5 c	3.5 a	3.0 e	3.0 a	3.5 d	3.5 a
3	4.0 c	3.1 a,b	4.2 d	3.0 a	4.6 c	3.5 a
6	4.6 c	2.5 b	6.2 c	2.4 a	5.5 c	3.0 a
9	15.3 b	3.0 a,b	19.2 b	3.0 a	8.4 b	3.1 a
12	26.0 a	3.2 a,b	25.4 a	5.2 a	21.9 a	4.6 a

source of variation	probability
storage conditions	*
egg coproducts utilized	*
storage packaging × egg coproducts utilized	*

[a] Means in the same column with different letters are significantly different ($p \leq 0.05$). *$p < 0.01$.

hydroxyl and epoxy decrease. This suggests that the drying process accelerates the decomposition of these compounds, probably through dehydradation with formation of less polar compounds, such as ketosteradiens or other volatile demolition products (*1*), from the opening of oxiranic rings (*26*) and cannot be determined using the applied analytical method. 7-KC is the only COP that increased after drying, as previously observed in cooked meat (*27*). Alterations in COPs during storage of pasta are shown in **Table 3**. COPs values showed important differences depending on the storage conditions. Immediately after production, the three types of spaghetti samples did not show any significant differences in the level of total COPs. After 3 months of storage, COPs remained practically constant due to the higher evolution of these compounds' products with respect to their formation (*28*). The increase of these compounds was observed from 6 to 12 months of storage. These high values can be explained taking into account that lipid oxidation in spaghetti can occur due to factors such as the storage conditions and egg

Table 3. Cholesterol Oxidized Evaluation in Formulated Spaghetti (μg/g of Fat)[a]

month	7-α-HC	7-β-HC	β-CE	α-CE	7-KC	sum
			PCE Spaghetti			
			Light Storage			
0	4.1 d	2.5 e	4.8 b,c	2.1 c,d	38.4 b	52.0 d
3	6.0 d	4.0 e	2.5 c	1.4 c,d	34.0 b,c	47.8 d
6	5.3 d	4.5 e	2.7 c	1.5 c,d	31.6 b,c	45.6 d
9	52.0 a	58.1 b,c	13.1 a	7.1 a	70.9 a	201.2 a
12	53.5 a	47.6 c	11.4 a	7.0 a	45.2 a,b	164.7 b
			Dark Storage			
0	4.1 d	2.5 e	4.8 b,c	2.1 c,d	38.4 b	52.0 d
3	4.2 d	2.5 e	4.7 b,c	2.2 c,d	37.6 b	51.2 d
6	4.5 d	3.4 e	5.0 b,c	1.8 c,d	43.5 b	58.1 d
9	15.3 c	15.3 d	12.9 a	7.2 a	70.7 a	121.4 b
12	5.0 d	4.3 e	4.4 b,c	1.2 d	54.8 a,b	69.8 c,d
			SPCE Spaghetti			
			Light Storage			
0	4.8 d	4.5 e	3.3 c	1.2 d	30.0 b	43.8 d
3	9.6 c,d	7.2 d,e	4.8 b,c	3.1 c,d	36.3 b	61.0 c,d
6	12.8 c	12.0 d	4.5 b,c	2.3 c,d	37.8 b	69.4 c
9	64.5 a	70.7 b	13.4 a	7.9 a	53.0 a,b	209.6 a
12	46.2 b	44.5 c	10.2 a	6.1 a	45.3 a,b	152.3 b
			Dark Storage			
0	4.8 d	4.5 e	3.3 c	1.2 d	30.0 b	43.8 d
3	4.8 d	4.5 e	3.2 c	1.2 d	29.9 b	43.7 d
6	10.9 c	10.3 d	7.8 a,b	5.0 a,b	63.2 a	97.3 b
9	16.5 c	17.7 d	9.6 a	6.4 a	67.1 a	117.3 b
12	8.1 c,d	7.5 d,e	5.8 b	1.5 d	37.3 a,b	60.2 c
			POE Spaghetti			
			Light Storage			
0	3.3 d	1.9 e	3.8 c	1.2 d	40.3 b	50.6 d
3	6.7 d	5.1 e	5.5 b,c	3.1 c	40.0 b	60.3 c
6	6.8 d	5.2 e	3.1 c	2.2 c,d	43.4 b	60.7 c
9	34.2 b	34.9 c,d	13.0 a	6.6 a	70.7 a	159.3 b
12	32.3 b	29.9 c,d	9.8 a	4.1 b	86.9 a	163.1 b
			Dark Storage			
0	3.3 d	1.9 e	3.8 c	1.2 d	40.3 b	50.6 d
3	3.2 d	1.9 e	3.7 c	1.2 d	39.6 b	49.6 d
6	6.5 d	4.8 e	7.3 a,b	2.7 c	62.8 a	84.0 b,c
9	13.3 c	14.2 d	3.0 c	2.2 c,d	73.4 a	106.0 b
12	3.9 d	3.1 e	3.6 c	1.1 d	30.5 b	42.3 d

source of variation	probability
storage packaging	*
egg coproducts utilized	*
storage packaging × egg coproducts utilized	*

[a] Means in the same column with different letters are significantly different ($p \leq 0.05$). *$p < 0.01$.

coproduct utilized. As reported by Boselli et al. (*14*), when a low drying cycle was used the most abundant COPs were 7-KC and 7-α-HC.

Cholesterol oxidation is initiated by the abstraction of the allylic C-7 hydrogen, with the subsequent formation of C-7 COPs such as 7α- and 7β-hydroxycholesterols and 7-ketocholesterol (*29*). Cholesterol α- and β-epoxides are the products of attack by cholesterol 7-hydroperoxide on the 5,6-double bond of cholesterol, and are therefore the secondary oxidation products (*1*). Accordingly, their concentrations were not lower than other COPs.

Moreover, according to Caboni et al. (*10*), the formation of hydroxy derivatives of cholesterol (7-α-HC) was higher in egg pasta obtained with spray-dried eggs.

The total COP content in light-exposed samples increased significantly ($p < 0.05$) after 9 months by 483, 478 and 314% in PCE, SPCE and POE pasta, respectively. The level of COPs in the samples stored in the dark increased significantly ($p < 0.05$) after 9 months to 233, 267 and 209% in PCE, SPCE and POE pasta, respectively.

After 12 months of storage the total COP decrease was probably due to decomposition or secondary reactions (*17*, *30*) of COPs during storage, since oxidation in cholesterol-rich foods is a dynamic reaction.

Pasta produced with different egg products showed significant differences, and in particular spaghetti produced with PCE and SPCE showed an oxidation ratio of cholesterol that was twice as high in conventional packaging than in dark conditions. However, spaghetti with POE had an oxidation ratio (COP conventional packaging/COP dark conditions) of 1.5.

Unexpectedly, the COP content in PCE and SPCE spaghetti was not different. Thus, the double technological treatment such as spray drying of eggs and drying to obtain the SPCE sample does not apparently lead to higher formation of COPs.

The three different spaghetti samples studied showed the same oxidation state at time 0. This demonstrates that the oxidation state at time 0 was influenced only by the technological processing of pasta production, while it was not influenced by the oxidation state of egg coproducts.

Hydroperoxides (PV) were measured to determine the initial rate of oxidation. During storage of pasta products, primary oxidation products were formed as a function of time, raw egg ingredient and storage conditions. The PCE and SPCE egg coproduct showed a different content of total COPs, but the samples formulated with them did not show significant variation of COP content during their storage. Nonetheless, POE spaghetti showed the lowest content of cholesterol oxides. Thus, the use of a light barrier is important for preventing light-induced cholesterol oxidation in pasta. Notwithstanding, typical packaging materials do not prevent this in a sufficient manner, and particular attention should be given to different packaging materials and food processing in order to generate a minimum amount of COP oxidation, which would help to increase the overall quality of the product.

Future studies will be focused on the identification of nonpolar oxidation lipid products to identify a possible marker of oxidation. As reported by Boselli et al. (*31*), COPs may be intermediate products of degradation of cholesterol and the nature of the final products still needs investigation. Additional experiments with this model system will be required to establish the sensitizer that reacts with cholesterol to form a radical.

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