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Thermo-oxidation of cholesterol: Effect of the unsaturation degree of the lipid matrix

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ABSTRACT

The influence of the unsaturation degree of different triacylglycerols (tristearin, triolein, trilinolein and trilinolenin) on cholesterol oxidation at 180 °C, was evaluated. Cholesterol degraded faster when heated alone than in the presence of triacylglycerols; moreover, the more unsaturated the matrix, the slower the degradation of cholesterol. Both cholesterol and triacylglycerols degradation fit a first order kinetic model (R^2 > 0.9), except for the tristearin sample. Cholesterol oxidation products (COPs) and peroxides were formed during the heating treatment. The presence of any type of lipid matrix postponed and decreased the maximum concentration of both oxidation parameters. Maximum total COPs concentrations were achieved at 20 min in neat cholesterol, 120 min in tristearin and triolein and 180 min in polyunsaturated matrix samples. 7-Ketocholesterol was the main COP in most cases during the whole heating treatment. Both the presence of triacylglycerols and their unsaturation degree inhibited cholesterol thermooxidation at 180 °C.

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1. Introduction

Cholesterol present in animal food products has been reported to degrade under certain conditions, such as high temperature, long-term storage and/or light exposure (Boselli, Cardenia, & Rodriguez-Estrada, 2012; Cardenia, Rodriguez-Estrada, Baldacci, Savioli, & Lercker, 2012; Mazalli & Bragagnolo, 2007; Saldanha & Bragagnolo, 2008). In this process, cholesterol oxidation products (known as COPs) are formed among other compounds. COPs have been related to atherosclerosis, mutagenesis, neurodegenerative diseases and some other harmful effects for human health (Otaegui-Arrazola, Menéndez-Carreño, Ansorena, & Astiasarán, 2010).

Food matrix and its chemical composition are key factors in cholesterol oxidation. In particular, the unsaturation degree of the lipid fraction may play an important role towards cholesterol degradation and COPs formation. Recent studies carried out in real food systems (horse meat and eggs) concluded that higher unsaturation degree of the lipid matrix promotes cholesterol oxidation (Boselli, Rodríguez-Estrada, Ferioli, Caboni, & Lercker, 2010; Pignoli et al., 2009), due to the generation of a pro-oxidant environment with the presence of free radicals and hydroperoxides (Ohshima, 2002). Previous work (Kim & Nawar, 1991) stated that, during heating, the presence of unsaturated fatty acids should promote cholesterol oxidation more readily than the presence of saturated fatty acids. However, in photoxidised model systems containing fatty acid methyl esters and cholesterol, Hu and Chen (2002) observed that stearate resulted in a higher loss of cholesterol than linoleate and docosahexaenoate.

As it has been shown, there is no general agreement on the role of the unsaturation degree of fatty acids in cholesterol oxidation, as some authors have concluded that other factors could be strongly decisive on the oxidative behaviour, such as temperature and heating time (Soupas, Juntunen, Lampi, & Piironen, 2004), being the effect of fatty acids on cholesterol oxidation unlikely related to their unsaturation level (Xu, Sun, Liang, Yang, & Chen, 2011).

Most of the studies dealing with cholesterol oxidation have been directly performed on food products (Boselli et al., 2012; Cardenia, Rodríguez-Estrada, Boselli, & Lercker, 2013; Orczewska-Dudek, Bederska-Lojewska, Pieszka, & Pietras, 2012; Vicente & Sampaio, 2012), so interferences with other components of the sample are assumed. On the other hand, model systems have been recognised as a good means to obtain precise information about kinetic behaviour of sterols subjected to heating (Barriuso, Otaegui-Arrazola, Menéndez-Carreño, Astiasarán, & Ansorena,

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2012; Chien, Hsu, Inbaraj, & Chen, 2010). Until now, few of them have directly tackled the effect of lipid unsaturation. Various strategies, such as purifying vegetable oils (Soupas et al., 2004) or using synthetic free fatty acids (Kim & Nawar, 1991; Xu et al., 2011), have been applied as an attempt to minimise the influence of other interfering factors different from the lipid unsaturation degree. However, to our knowledge, no information is available on the effect of synthetic triacylglycerols (TAG), which could be different from that of free fatty acids, since the later are more prone to oxidation and the former are more abundant in food. For a better understanding of the oxidative process, more studies with model systems would be required, so that a clear trend can be elucidated, with no interferences.

In this context, the aim of the present study was to evaluate the influence of the unsaturation degree of different TAG (tristearin (TS), triolein (TO), trilinolein (TL) and trilinolenin (TLn)) on cholesterol oxidation susceptibility and COPs formation, under heating conditions.

2. Materials and methods

2.1. Materials and reagents

All solvents used were analytical grade. Reagents were supplied by Carlo Erba Reagenti (Rodano, Italy) and Merck (Darmstadt, Germany). Commercial standards of triacylglycerols (TS, TO, TL and TLn) were purchased from Nu-Check (Elysian, MN, USA). 19-Hydroxycholesterol, 5α-cholestane and 7α-hydroxycholesterol (7α-HC) were supplied by Steraloids (Newport, RI, USA). Cholesterol, 7β-hydroxycholesterol (7β-HC), 5,6α-cholesterol epoxide (α-CE), 5,6β-cholesterol epoxide (β-CE), 3,5,6-cholestanetriol (CT) and 7-ketocholesterol (7-KC) were purchased from Sigma (St. Louis, MO, USA). The purity of sterols and COPs standards was controlled by GC-FID. NH_2 solid-phase extraction (SPE) cartridges (500 mg/3 ml Strata cartridges) from Phenomenex (Torrence, CA, USA), were utilised for COPs purification. The silylation mixture was prepared with dried pyridine, hexamethyldisilazane and trimethylchlorosilane at a ratio of 5:2:1 by volume.

2.2. Heating treatment

For each type of TAG, a stock solution of TAG:cholesterol (100:1) was prepared in chloroform. Samples (200 mg) were put into open glass tubes (18 mm diameter, 100 mm height), dried under N_2 stream (40 °C) until constant weight. The unsealed tubes were then placed open in an oven (Memmert GmbH+ Co.KG, Nurnberg, Germany) previously heated at 180 °C, in duplicate.). They were taken out from the oven after different heating times (0, 5, 10, 20, 30, 60, 90, 120, 180 and 240 min) and cooled down for 2 min. One millilitre of chloroform was added to each tube, and samples were shaken vigorously for 40 s and kept under −20 °C until analysis. The heating experiment was run twice. TL and TLn mixtures sampled at 240 min were not analysed due to the incomplete solubility in chloroform. A similar experimental set up was applied to cholesterol alone (2 mg/tube).

2.3. Analysis of remaining cholesterol and TAG

An aliquot (50 μl), equivalent to approximately 10 mg of the heated sample, were transferred to a test tube. The solvent was evaporated and the exact lipid weight was registered. Chloroform (1 ml) and 5α-cholestane as internal standard (IS, 50 μl of a 1 mg/ml solution) were added. The mixture was vigorously shaken and 1 μl was injected into a TRACE gas chromatograph coupled to a flame ionisation detector (GC-FID) (Thermo Finnigan, Milan, Italy), which was interfaced with a computerised system for data acquisition (Chromcard Data System, ver. 2.3.1, Fisons Instruments). A SE52 MEGA fused-silica column (10 m × 0.32 mm × 0.1 μm) (Mega s.n.c., Legnano (Milan), Italy) coated with 5% phenyl-95% dimethyl-polysiloxane, was used. The oven temperature was programmed from 100 to 350 °C at 6 °C/min, and kept at 350 °C for 20 min; the injector and detector temperatures were both set at 325 °C. Helium was used as carrier gas at a flow of 1.0 ml/min; the split ratio was 1:20. Areas for cholesterol, TAG and IS were integrated. Remaining cholesterol and TAG for each time of analysis were calculated considering the initial amount ($t = 0$) as 100%.

2.4. COPs purification and analysis

To determine COPs, it was first necessary to remove TAG and cholesterol from heated samples by NH_2-SPE, as suggested by Rose-Sallin, Hugget, Bosset, Tabacchi, and Fay (1995). COPs were eluted with acetone, and then silylated by adding 0.1 ml of the derivatizing mixture (pyridine:hexamethyldisilazane:trimethylchlorosilane, 5:2:1, v/v/v) at 40 °C for 15 min (Sweeley, Bentley, Makita, & Wells, 1963); thereafter, silylated COPs were dried under nitrogen stream and dissolved in 100 μl of *n*-hexane. One microlitre of the silylated COPs was analysed by Fast GC–MS, as suggested by Cardenia et al. (2012). Fast GC/MS analysis was performed using a GC Shimadzu QP 2010 Plus (Kyoto, Japan) equipped with a split–splitless injector and coupled to a EI mass spectrometric detector. A fused silica capillary column Restek RTX-5 (10 m × 0.1 mm i.d. × 0.1 μm film thickness; Bellafonte, PA, USA) coated with 95% dimethyl- and 5% diphenyl-polysiloxane, was used. The temperature was programmed from 250 to 325 °C at 20 °C/min. The injector temperature was set at 325 °C and the ion source temperature was set at 230 °C. Helium was used as the carrier gas and linear velocity was 43 cm/s. Helium inlet pressure was 426.7 kPa. The injection was performed in the split system at 1:50 splitting ratio. The electron energy was 70 eV. A mass range from m/z 40 to 650 was scanned at a rate of 3333 amu/s, which corresponded to 0.2 event time.

The acquisition and integration modes were Full Scan (TIC) and Single Ion Monitoring (SIM), respectively. COPs were recognised and quantified by their corresponding characteristic ions with a high abundance: m/z 353 (19-HC); m/z 456 (7α-HC and 7β-HC); m/z 384 (α-CE and β-CE); m/z 403 (CT); m/z 131 (25-HC) and m/z 472 (7-KC). COPs quantification was performed by using calibration curves in the SIM mode (Cardenia et al., 2012), plotting the ratio of a COP area to the internal standard area as function of the ratio of COP standard concentration to internal standard concentration. The COPs concentration range of the calibration curves was 0.01–45 μg/ml ($r^2 = 0.996$–1.000). GC–MS LOD and LOQ of COPs were 2.18–5.07 ng/ml and 6.93–16.90 ng/ml, respectively (Cardenia et al., 2012).

2.5. Peroxides analysis

Peroxide value (PV) was determined in 10 mg of sample, as suggested by Shantha and Decker (1994). Briefly, this method is based on the ability of peroxides to oxidise ferrous ions to ferric ions, which react with ammonium thiocyanate and give rise to the formation of a coloured complex. PV was evaluated at 500 nm with a double-beam UV–visible spectrophotometer (Jasco model V-550, Jasco International, Tokyo, Japan), and it was calculated from the absorbance. For the quantitative determination of PV, a Fe(III) standard calibration curve was used with a concentration range of 0.1–5 μg/ml ($y = 0.0311x - 0.0003$; $r^2 = 0.999$). PV was expressed as meq O_2/kg of fat. Three replicates were run per sample.

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