Food Chemistry 171 (2015) 233–240

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Cholesterol transformations during heat treatment

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ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form 18 August 2014
Accepted 27 August 2014
Available online 7 September 2014

Keywords:
Cholesterol degradation
Oxysterols
Polymers
Volatile compounds
Cholestadienes
Fragmented cholesterol molecules

ABSTRACT

The aim of the study was to characterise products of cholesterol standard changes during thermal processing. Cholesterol was heated at 120 °C, 150 °C, 180 °C and 220 °C from 30 to 180 min. The highest losses of cholesterol content were found during thermal processing at 220 °C, whereas the highest content of cholesterol oxidation products was observed at temperature of 150 °C. The production of volatile compounds was stimulated by the increase of temperature. Treatment of cholesterol at higher temperatures i.e. 180 °C and 220 °C led to the formation of polymers and other products e.g. cholestadienes and fragmented cholesterol molecules. Further studies are required to identify the structure of cholesterol oligomers and to establish volatile compounds, which are markers of cholesterol transformations, mainly oxidation.

1. Introduction

Cholesterol is the most known sterol present in food. This steroid alcohol due to unsaturation in the ring B is susceptible to oxidation. Cholesterol in foods is present mainly in free or esterified form. During thermal processing and storage cholesterol and other sterols undergo changes like: degradation, oxidation and polymerisation. Thermo-oxidation degradation of phytosterol was investigated by Rudzińska, Przybylski, and Wąsowicz (2009). They showed that not only oxidation products but also volatile compounds and polymers of phytosterol are formed during thermal oxidation. There is a lack of publications about cholesterol alteration during thermal processing, that present such holistic approach as Rudzińska et al. (2009) proposed.

Heat induced cholesterol degradation was widely investigated. Park and Addis (1986) reported, that cholesterol losses during lard processing at 155 °C lasting 250 h was 50%, which is consistent with the notion of a linear loss of cholesterol during heating. During lard heating at the same temperature lasting 2 h cholesterol degraded about 4.3–7.6%, depending on the thickness of the heated fat layer (Derewiaka & Obiedziński, 2010a).

Oxidation of cholesterol during heating was investigated by many researchers. The pioneer in this field was Smith (1981, chap. 4). This process is similar to hydrocarbons oxidation. Autoxidation of sterols is free radical chain reaction, which leads to the formation of a variety of oxides (Lethonen, Lampi, Ollilainen, Struijs, & Piironen, 2011). The oxidation of sterol is strongly related to the temperature of processing and other factors like moisture, acidity index etc. Cholesterol oxidation products have been proven to have an adverse effects on human body e.g. cytotoxic, apoptotic and pro-inflammatory and also some investigations have shown that take part in a atherosclerotic and neurodegenerative process (Otaegui-Arrazola, Menéndez-Carreño, Ansorena, & Astiasarán, 2010). They have been found at low concentrations in foodstuffs e.g. meat, fish, eggs and milk and their products (Calderón-Santiago, Peralbo-Molina, Priego-Capote, & Luque de Castro, 2012; Derewiaka & Obiedziński, 2010b; Orczewska-Dudek, Bederska-Łojewska, Pieszka, & Pietras, 2012; Ubhayasekera, Jayasinghe, Ekanayake, & Dutta, 2012).

Polymerisation of sterols was mainly described in studies involving phytosterols. Struijs, Lampi, Ollilainen, and Piironen (2010) presented formation of stigmasterol polymers after heating at 180 °C. Similar investigation was published in the same year by Rudzińska, Przybylski, Zhao, & Curtis (2010) describing thermal oxidation of sitosterol and production of dimers, trimers and tetramers. Lethonen, Lampi, Agalga, Struijs, and Piironen (2011) indicated that formation of sterol oligomers (e.g. pentamers and hexamers) was induced by the presence of acyl moiety and its

Abbreviations: NMR, nuclear magnetic resonance; IR, infrared spectroscopy; SEC, size exclusion chromatography; APCI, atmospheric pressure chemical ionisation; MS, mass spectrometry.

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http://dx.doi.org/10.1016/j.foodchem.2014.08.117
0308-8146/

unsaturation. The characteristics of sitosterol oligomers produced during thermal processing were presented by Sosińska, Przybylski, Hazendonk, Zhao, and Curtis (2013). They applied spectroscopic methods such NMR, IR, Raman and SEC/APCI/MS to establish sitosterol dimers structure.

There are only a few studies describing formation of volatile compounds due to thermal-oxidation of sterols. In Van Lier, de Costa, & Smith, 1975 determined at least fourteen volatile compounds that were produced during decomposition of cholesterol. The amount of volatiles, formed during heating of phytosterol in different temperatures, was discussed by Rudzińska et al. (2009). They found mainly hydrocarbons, ketones, aledehydes and acids in the profile of volatile fraction formed during heating.

The aim of the study was to evaluate the intensity of cholesterol degradation, oxidation, polymerisation and formation of volatile compounds during thermal treatment at temperatures typical for food processing.

2. Materials and methods

2.1. Materials

Cholesterol and 5α-cholestane standards were purchased from Sigma–Aldrich (St. Louis, MO, USA) and 19-hydroxycholesterol from Steraloids (Newport, RI, USA). Solvents (acetone, hexane, methanol, diethyl ether, potassium hydroxide, tetrahydrofuran) were purchased from POCH (Gliwice, Poland), while 1,2-dichlorometane from Aldrich (Dorset, England). A sylilation agents BSTFA (N,O-Bis(trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane) and anhydrous pyridine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cholesterol oxidation standards: 7β-hydroxycholesterol, 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, 5α-cholestane-3β, 5β,6β-triol (triol), 7-ketocholesterol, 25-hydroxycholesterol came from Sigma-Aldrich Co. (Poznań, Poland). SPE DSC-Si Silica tubes (1 g/6 mL) and SPME Fibre divinylbenzene/carboxene/.

polydimtheylsiloxane (DVB/CAR/PDMS) (2 cm) were purchased from Supelco (Bellefonte, PA, USA).

2.2. Sample heat treatment

Cholesterol standard (10–20 mg) was placed in a glass ampoule of 20 mL capacity and the glass neck of the ampoule was closed over the burner flame. Closed ampoules were heated at 120, 150, 180 and 220 °C for 30, 60, 120 and 180 min. Heating procedures were done in triplicate. A control sample was a sample of non-heated cholesterol standard.

2.3. Determination of cholesterol, oxysterols and cholestadienes

Cholesterol, oxysterols and cholestadienes were separated on SPE cartridges. 2 mg of heated cholesterol sample, diluted in hexane, was applied on SPE column (conditioned with 2 mL of hexane). Column was washed with 4 mL of hexane:diethyl ether (75:25; v/v), cholesterol was eluted with 15 mL of hexane: diethyl ether (60:40; v/v) (I fraction) whereas oxysterols and cholestadienes with 10 mL of acetone (II fraction). To the first fraction 0.2 mL of 5α-cholestane solution (10 mg/25 mL of chloroform) was added and to the second fraction 0.2 mL of 19-hydroxycholesterol solution (8.5 mg/25 mL of chloroform) and 0.2 mL of α-cholestane solution (10 mg/25 mL of chloroform) was added. Fractions were evaporated under a stream of nitrogen. Samples were dissolved in 2 mL of hexane and saponified with addition of 0.5 mL of 2 N potassium methoxide for 1 h at room temperature. Afterwards, solvents were removed under a stream of nitrogen and samples were derivatized for 24 h at room temperature due to addition of 100 μL of anhydrous pyridine and 100 μL of sylilation agents. Cholesterol, oxysterols and cholestadienes were analysed on gas chromatograph equipped with a mass spectrometer (GCMS-QP2010S) Shimadzu Corporation (Shim-Pol A. M. Borzymowski, Poland) using. DB5ms (30 m × 0.25 mm × 0.25 μm) capillary column Phenomenex (Torrance, CA, USA). Helium was used as a carrier gas at a flow rate of 0.61 mL/min. The injector temperature was set at 250 °C, and the column temperature was programmed as follows: 200 °C for 1 min, subsequent increase to 250 °C at the rate of 15 °C/min, then to 310 °C at the rate of 3 °C/min for 6 min. The interface temperature for GC–MS was 260 °C. Temperature of ion source was 250 °C and ionisation energy was 70 V. The split ratio was 50:1. The total ion monitoring (TIC) was used to detect sterols, oxysterols and cholestadienes (m/z ranged 100–600). The internal standard 5α-cholestane was used to quantify cholesterol and cholestadienes, 19-hydroxycholesterol to quantify oxysterols. Regression coefficient of oxysterols curves were between $R^2 = 0.94$ for 5β,6β-epoxycholesterol to $R^2 = 0.997$ for triol. Cholesterol and cholestadienes content was expressed as equivalents of 5α-cholestane in mg per g of heated cholesterol standard, where as oxysterols content was expressed as mg of 19-hydroxycholesterol per g of heated cholesterol standard. Identification of compounds was made on the basis of mass spectral libraries (NIST 47, NIST 147 and Wiley 175) as well as data from literature and by comparison of their retention times with authentic standards, including cholesterol, 7β-hydroxycholesterol, 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, triol, 7-ketocholesterol, 25-hydroxycholesterol. Three replicates per each sample were analysed.

2.4. Determination of fragmented cholesterol molecules

Fragmented cholesterol molecules were separated on SPE cartridges. 2 mg of heated cholesterol sample, diluted in hexane, was applied on SPE column (conditioned with 2 mL of hexane). Column was washed firstly with 4 mL of hexane: diethyl ether (75:25; v/v) and 15 mL of hexane: diethyl ether (60:40; v/v) was used to collect sterol (I fraction) and than fragmented cholesterol molecules were eluted with 10 mL of acetone (II fraction). To the second fraction 0.2 mL of 5α-cholestane solution (10 mg/25 mL of chloroform) was added Fraction was evaporated under a stream of nitrogen. Samples were dissolved in 2 mL of hexane and saponified with addition of 0.5 mL of 2 N potassium methoxide for 1 h at room temperature. Afterwards, solvents were removed under a stream of nitrogen and samples were derivatized for 24 h at room temperature due to addition of 100 μL of anhydrous pyridine and 100 μL of sylilation agents. Fragmented cholesterol molecules were analysed on gas chromatograph equipped with a mass spectrometer (GCMS-QP2010S) Shimadzu Corporation (Shim-Pol A. M. Borzymowski, Poland) using. DB5ms (30 m × 0.25 mm × 0.25 μm) capillary column Phenomenex (Torrance, CA, USA). Helium was used as a carrier gas at a flow rate of 0.61 mL/min. The injector temperature was set at 250 °C, and the column temperature was programmed as follows: 200 °C for 1 min, subsequent increase to 250 °C at the rate of 15 °C/min, then to 310 °C at the rate of 3 °C/min for 6 min. The interface temperature for GC–MS was 260 °C. Temperature of ion source was 250 °C and ionisation energy was 70 V. The split ratio was 50:1. The total ion monitoring (TIC) was used to detect fragmented cholesterol molecules (m/z ranged 100–600). The internal standard 5α-cholestane was used to semi-quantify fragmented cholesterol molecules. Fragmented sterol molecules content was expressed as equivalents of α-cholestane in mg per g of heated cholesterol standard. Identification of compounds was made on the basis of mass spectral libraries (NIST