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Cholesterol oxidation in tallow during processing

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Abstract

The oxidation of cholesterol during the bleaching and deodorization process of tallow was studied. Eight polar cholesterol oxidation products were targeted, but only cholest-5-en-3β,7β-diol (7β-HC) and 5β, 6β-epoxy-5β-cholestan-3β-ol (β-CE) could be quantified in the samples analysed. The content of 7β-HC was slightly higher in the processed tallow (0.6–0.7 μg/g) compared with natural tallow (0.2 μg/g). However, the content of β-CE was considerably increased during processing, ranging from 0.8 to 3.4 μg/g, compared with 0.6 μg/g in the natural tallow. The general observation was that the content of β-CE was influenced by temperature, duration and type of bleaching earth.

Keywords: Bleaching; Cholesterol oxidation products; COPs; Deodorization; Processing; Tallow

1. Introduction

In order to become suitable for human consumption, animal fats require processing. After rendering of animal fats, bleaching and deodorisation are the two most important processes. During these processes, components that give undesirable colour and flavour are removed from fats and oils during treatment with bleaching earth and heat, and dry steam (Love, 1996). During bleaching and deodorisation, the total sterol content decreases considerably, depending on type and amount of bleaching earth, temperature and duration and level of steam and this can generate a number of undesirable compounds (Dutta, Przybylski, Appelqvist, & Eskin, 1996).

Various types of sterol degradation products have been analysed, mainly from vegetable oils, generated during processing. Among the known degradation products are sterenes or steradienes, the dehydration products of sterols produced mainly during bleaching and deodorisation. In addition to the sterenes, which are relatively non-polar compounds, a number of polar oxidation products of sterols are known to be present in processed edible vegetable fats and oils; however, data on animal fats are scarce (Dutta et al., 1996).

Cholesterol is the main sterol in animal fats and oils and can generate many oxidation products (COPs) under prevailing conditions (Savage, Dutta, & Rodriguez-Estrada, 2002). Much attention has been paid to the levels of the polar COPs due to their possible adverse health effects and their levels in foods (Paniangvait, King, Jones, & German, 1995; Schroepfer, 2000).

It has been reported that small amounts of some COPs are present in natural and processed lard samples, collected from two producers (Nourooz-Zadeh & Appelqvist, 1989). No literature reports on the effects of processing on levels of COP in tallow could, however, be found by the authors.

In order to learn which parameters have an effect on the oxidation of cholesterol, some preliminary experiments have been carried out on bleaching and deodorization of beef tallow. Emphasis was placed on the influence of the bleaching process parameters, including temperature, time, degree of bleaching earth, and bleaching earth acid activation, on the formation of COPs in tallow.

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2. Materials and methods

2.1. Materials

Crude beef tallow fat was kindly donated by Baeten (Overmere, Belgium) and bleaching earths were obtained from Südchemie (Germany). All samples were stored in the refrigerator at −20 °C until analysis. Industrially used bleaching earths were obtained from Sudchemie (Munchen, Germany) and their main characteristics are presented in Table 1.

2.2. Methods

Refining procedure. All refining steps were performed on the laboratory scale. For bleaching, the crude tallow fat was preheated to 95 °C under reduced pressure (50 mbar) in a rotavapor. After addition of bleaching earth, the oil was mixed at 85 rpm for 30 min followed by filtration over a Buchner filter. The deodorization process was carried out according to the procedure described previously (Petrauskaite, De Greyt, & Kellens, 2000). The bleaching and deodorization procedures are reported elsewhere (Verleyen et al., 2002). Industrially used bleaching earths were obtained from Sudchemie (Munchen, Germany) and their characteristics are given in Table 1.

In order to isolate the COP, tallow samples were subjected to cold saponification according to methods published previously, after minor modification (Larkeson, Dutta, & Hansson, 2000). Briefly, a fat sample (0.20–0.25 g), weighed into a glass tube with a stopper, was dissolved in dichloromethane (3 ml) and 5 ml of potassium hydroxide solution, dissolved in 95% ethanol, (2 M) were added. The mixture was left overnight in the dark at room temperature for 18–20 h. Water (10 ml) and dichloromethane (7 ml) were added and the tube was shaken vigorously. The dichloromethane layer was pipetted into a clean tube and washed once with 0.5 N KOH (5 ml). After centrifugation (2 min at 3000 rpm), the water layer was removed and the organic fraction was washed repeatedly until a clear solution was obtained. The dichloromethane was evaporated under nitrogen and the unsaponfiables obtained were dissolved in 1 ml *n*-hexane/diethyl ether (75/25 v/v).

The COP fraction was further enriched by solid phase extraction (SPE), derivatised to trimethylsilyl-ether derivatives, and quantified by GC-FID, as essentially described previously (Larkeson et al., 2000). Content of cholesterol was determined following a method described previously (Larkeson et al., 2000). Duplicate analyses of COPs and cholesterol were carried through all the steps and mean values are reported in the Table 1.

2.3. Gas chromatography–mass spectrometry (GC–MS)

For identification purpose, a GC 8000 Top Series gas chromatograph (ThermoQuest Italia S. p. A., Rodano, Italy) coupled to a Voyager mass spectrometer with a MassLab data system version 1.4V (Finnigan, Manchester, England) was used. The COPs were separated on the same column as used in the GC analysis. Helium was used as carrier gas at an inlet pressure of 80 kpa. The injector temperature was 250 °C and the samples were injected in a splitless mode of injection. Oven temperature was at 60 °C for 0.5 min and then raised to 290 °C at a rate of 50 °C/min, and finally the temperature was raised to 300 °C at a rate of 0.5 °C/min. The

Table 1
Content of two cholesterol oxidation products (μg/g, ±SEM) in crude-, bleached-, and deodorised tallow

Sample No.	Bleaching earth			Time (min)	Temperature (°C)	Steam (%)	Pressure (mbar)	Cholesterol	7β-OH[a]	β-CE[b]
	Type	pH suspension	Surface area (m^2/g)					μg/g tallow±SEM		
Natural tallow										
1								502±3	0.17±0.03	0.60±0.15
Bleached tallow										
2	1% Optimum 215[c]	2.8	230	30	100	–	–	–	0.69±0.02	2.65±0.60
3	1% Optimum 215	–	–	60	80	–	–	–	0.69±0.07	3.37±0.23
4	2% Optimim 215	–	–	30	80	–	–	–	0.49±0.01	1.48±0.29
5	1% Standard 310[d]	3.6	330	30	100	–	–	–	0.60±0.09	1.98±0.45
6	1% Ex 640[e]	6.0	140	30	100	–	–	–	0.69±0.08	0.81±0.12
Deodorised tallow										
7[f]				45	230	1	2	–	0.60±0.07	2.09±0.36

[a] Cholest-5-en-3β,7β-diol.
[b] 5β, 6β-Epoxy-5β-cholestan-3β-ol.
[c] Very acid-activated bleaching earth.
[d] Standard acid-activated bleaching earth.
[e] Natural bleaching earth.
[f] Bleached according to condition of sample No. 2.

mass spectra were recorded at an electron energy of 70 eV and the ion source temperature was at 200 °C.

3. Results and discussion

Eight common COPs that are generally reported in foods were targeted in this study. These COPs are; cholest-5-en-3β,7α-diol (7α-HC), cholest-5-en-3β, 7β-diol (7β-HC), 5α,6α-epoxy- 5α-cholestan-3β-ol (α-CE), 5 β,6β-epoxy- 5β-cholestan-3β-ol (β-CE), 5α-cholestan-3β,5,6β-triol (CT), cholest-5-en-3β,20α-diol (20α-HC), cholest-5-en-3β,25-diol (25-HC) and 3β-hydroxycholest-5-en-7-one (7-KC) (Larkeson et al., 2000). The level of quantification of the COP was 0.1 μg/g tallow under the analytical conditions used in this study. Results of duplicate analyses are reported only for those COP quantified at, or more than, 0.1 μg/g tallow.

The crude tallow was also analysed for the content of cholesterol (Larkeson et al., 2000) and the amount was found to be 500 μg/g (Table 1). Literature reports on the level of cholesterol in crude tallow range from 700 to 1400 μg/g, depending on the source, and the result obtained in this study was even lower than those published reports (Dutta et al.,1996; Park & Addis,1986).

Results on the levels of COPs found in non-processed and processed tallow samples are listed in Table 1. In all the samples analysed, only 7β-HC and β-CE were found in quantifiable amounts and their chemical structures were confirmed by GC–MS. In the crude tallow, the amounts of 7β-HC and β-CE were 0.2 and 0.6 μg/g, respectively. It is noteworthy that other COPs were not present in quantifiable concentrations in crude tallow samples, which may be due very low oxidative stress applied to the crude tallow during production. Nevertheless, small amounts of COP might naturally be present in tissues or they might be formed by autoxidation during the rendering process (Smith, 1987).

Similar to crude tallow, the bleached tallow samples contained quantifiable amounts only of 7β-OH and β-CE. All samples bleached under various conditions had rather similar amounts of 7β-OH, ca. 0.6 μg/g, slightly higher than natural tallow, which contained 0.2 μg/g of this compound (Table 1). This level was not influenced by bleaching conditions, e.g. time, temperature and type of bleaching earth used.

The bleached samples contained β-CE ranging from 0.8 to 3.4 μg/g, which was considerably more than in the crude tallow sample (0.6 μg/g). It is known that β-CE predominates over α-CE as an autoxidation product of cholesterol and, under acidic systems, at pH below pH 5.5, β-CE is more sensitive than α-CE (Smith, 1987). However, the influence of the acid-activated bleaching earth on the pH of the tallow during bleaching could not be ascertained in this study. From the results (Table 1), it is seen that 1% highly acid-activated bleaching earth contributed to generation of larger amounts of β-CE, both at higher temperature and during longer periods of bleaching. However, higher amounts of the same bleaching earth did not generate a greater effect of β-CE in sample No. 4 (Table 1).

In general, bleaching with an acid-activated bleaching earth promotes the formation of β-CE. Little or no increase in the level of β-CE was observed in the sample bleached with a natural bleaching earth (No. 6) whereas a gradual increase in the level of β-CE was observed upon bleaching with an acid-activated bleaching earth (Nos. 2–5). Natural bleaching earths are only slightly acidic and have a small surface area and porosity, which restricts their adsorptive capacities. In order to increase the adsorptive capacities of a bleaching earth it is acid-activated (Zschau, 2000). Due to the acid activation, the surface of the bleaching earth becomes very reactive, which may facilitate the oxidation of cholesterol.

A sample of tallow bleached under similar conditions as sample No. 2 (Table 1) was further deodorised (No. 7). Compared with sample No. 2, the amount of 7β-OH was virtually unchanged, but the amount of β-CE was slightly decreased. It is recognised that CT is a potent angiotoxic agent which can be formed upon hydrolysis of epimers of CE (Smith, 1987, 1996), but it was was not present in quantifiable amounts in this or other samples analysed.

As mentioned earlier, to our knowledge, similar work on tallow processing has not been reported; however, a few publications are known where formation of COPs in tallow was studied after heat treatment (Park & Addis, 1986; Ryan, Gray, & Morton, 1981; Zhang & Addis, 1990). In a study on refined edible beef tallow, it was reported that, before heat treatment, it did not contain any traceable amount of COPs (Ryan et al., 1981). In another study, a similar result was reported on refined edible beef tallow, where no quantifiable amounts of COPs were detected in the unheated sample, although several COPs were detected after prolonged heating at 155 and 190 °C (Park & Addis, 1986). It was, however, reported later that 4 μg/g of 7α-OH was present in the 0 time sample of 90% tallow and 10% cotton seed oil mixture (Zhang & Addis, 1990). From these reports, it can be concluded that processed edible beef tallow does not contain, or contains only minor amounts, of COPs, and our results concur with those reports.

4. Conclusions

A few COPs were formed in the processed tallow sample used in this study, albeit in negligible amounts compared with other processed foods. During bleaching, an increase in the content of β-CE was observed, which seems to be influenced to some extent by the bleaching process conditions. It would, however, be of interest to study, in the future, the formation of COPs in

tallow having various levels of cholesterol, as well as the fatty acid profile during processing.

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