

ORIGINAL ARTICLE

Fatty acid compositions, trans-fatty acids and cholesterol contents of cheese-flavored crackers

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Keywords

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Abstract

Introduction Today, cholesterol and trans-fatty acids are main concerns in the diet of consumers. It is thought that it is important to determine the cholesterol and trans-fatty acids contents of snack foods which are widely consumed. *Objective* Fatty acid compositions, trans-fatty acids and cholesterol contents of cheese-flavored crackers sold in Turkish markets were determined. *Method* A gas chromatographic method was used to determine the amounts of these compounds in cheese-flavored cracker samples. *Results* It was determined that C18:1cis had the highest (40.2%) amount of unsaturated fatty acid followed by C18:2cis (14.6%), whereas the amounts of trans-fatty acids in C18:1tr and C18:2tr were 0.2% and 0.4% of total fatty acids, respectively. The average cholesterol content of samples was 0.59 mg/100 g. *Conclusion* The results from this study indicate that cheese-flavored crackers sold in Turkish markets do not contain considerable amounts of trans-fatty acids. The PUFA/SFA ratios of the samples were under the limit of 0.45% and nearly half of the fat fraction of the crackers was composed of saturated fatty acids (44.0%).

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Introduction

Trans-fatty acid is defined as 'unsaturated fatty acids containing one or more isolated double bonds in a trans configuration'. Trans-fatty acids in the diet come from meat and milk and in small amounts through biological hydrogenation process of unsaturated fatty acids (Norhayati *et al.*, 2011).

Dietary sources of trans-fatty acids in a diet are vegetable oil-based margarines, shortenings and cooking oils that have been subjected to hydrogenation. Dietary fats having different structures also have different effects on metabolism of human beings. For example, saturated fatty acids have negative effects on the cardiovascular system by increasing the amount of low-density lipoprotein cholesterol in blood. On the other hand, monounsaturated fatty acids are known as having a neutral health effect, whereas polyunsaturated fatty acids are believed to have beneficial health effects on human beings (Hu *et al.*, 2002). It is well-known by several authors that trans-fatty acid intake increases the risk of cardiovascular diseases (Hu *et al.*, 1997; Vijver *et al.*, 2000; Oomen *et al.*,

2001) and trans-fatty acids also increase the plasma triglyceride amounts (Nestel *et al.*, 1992).

Saulo (2006) reported that dietary supplements with at least 0.5 g of trans fat must also declare trans fat on the products label. And it was stated that this declaration was designed to help consumers while making food choices and determining their healthfulness.

From that point of view, it was planned to determine the fatty acid profiles, trans-fatty acid and cholesterol contents of cheese-flavored crackers sold in Turkish markets. To our knowledge, there is no published paper regarding the determination of these characteristics of cheese-flavored crackers consumed in Turkey.

Materials and methods**Sampling**

Crackers sold under seven different trademarks were purchased from local markets in İzmir for two times at 1 week intervals in their own packages. Cracker samples were kept

under refrigerator conditions at 4 ± 1 °C until they were analysed.

Reagents

Diethyl ether and methanol were obtained from Riedel (Riedelde Haen, Seeize, Hanover, Germany), and KOH and hexane from Merck (Darmstadt, Germany). The reference standards for cholesterol and standard fatty acid methyl ester mixtures were purchased from Sigma Chemical (St. Louis, MO, USA).

Determination of fat contents of cheesy cracker samples

Cracker samples were ground well in a mortar and 10 g of sample was taken and the total fat amount was determined by using a Soxhlet extraction apparatus (AOAC, 1984).

Lipid extraction and preparation of fatty acid methyl esters

Lipids were extracted with diethyl ether as described by Renner (1993). Crackers were crushed into small pieces and then diethyl ether (Merck) was added to the cracker samples and mixed well. After waiting for 5 min, the mixture was filtered through filter paper (Whatman no. 2) from a funnel. The filtrate was centrifuged for 2 min at 6000 rpm to remove the undesired particles originating from the cracker. Liquid phase of diethyl ether and oil was taken into centrifuge test tube and diethyl ether was removed using a rotary evaporator (Rv 05-St, IKA Labortechnik, Staufen, Germany) at 40 ± 1 °C. Then, the sample was flushed with nitrogen to remove the remaining ether from the oil from the cracker. Fatty acid methyl esters were prepared according to AOCS (1997). The sample (200 mg) was weighed into a stoppered-glass centrifuge vial. About 0.5 mL of 2 N methanolic KOH and 2.5 mL of pure hexane were added into the tube. The tube was shaken well for about 30 s and centrifuged for 2 min at 6000 rpm. The upper phase was taken into a vial to be analysed by gas chromatography (GC).

Determination of fatty acid composition by gas chromatography

The instrumentation used for the analyses was as follows: Agilent GC (Model 6890N, China) equipped with Supelco SP-2380 fused silica capillary column (60 m \times 0.25 mm i.d., 0.2 mm film thickness; Supelco, Bellefonte, PA, USA) and a flame ionisation detector. The injection volume was 2 mL.

The temperature of a GC oven was programmed from 180 °C to 220 °C at the rate of 4 °C min⁻¹. The injector and detector temperatures were 250 °C. Nitrogen was used as the carrier gas and the flow rate was 1 mL min⁻¹. The split ratio was set at 1:100 (Dönmez *et al.*, 2005). The identification of the peaks was achieved by retention times and by comparing them with authentic standards analysed under the same conditions. Peak areas of duplicate injections were measured with a HP computing integrator.

Determination of cholesterol

Cholesterol was determined by the modification of the procedure described by Fletouris *et al.* (1998). One gram of cracker was taken into a test tube and 5 mL of 2 N KOH was added into it. The tube was shaken well for 15 s then kept in a water bath at 80 °C for 30 min and shaken at 5 min intervals. The tube was cooled down under tap water and 1 mL of distilled water and 5 mL of hexane were added, then shaken for 1 min and centrifuged for 1 min at 2000 rpm. The upper phase was taken into a vial and analysed with an Agilent GC (Model 6890N, China). For preparation of cholesterol standards, the stock solution (2 mg mL⁻¹) was prepared by dissolving 20 mg of reference standard (Sigma Chemical Company) with hexane in a 10-mL volumetric flask. Working solutions were prepared by appropriately diluting aliquots from the stock solution with hexane to obtain solutions in the range of 10–80 mg mL⁻¹. GC conditions used for analyses were as follows: ZB-1 silica capillary column (30 m \times 0.25 mm i.d., 0.1 mm film thickness; Phenomenex, Inc., USA). Oven temperature was set at 285 °C, injection port temperature at 300 °C and flame ionisation detector temperature at 300 °C. The flow rates were 2 mL min⁻¹ for nitrogen, 30 mL min⁻¹ for hydrogen and 300 mL min⁻¹ for air. The injection volume was 2 mL with a split ratio of 20:1. The concentration of cholesterol (C) in the analysed samples was calculated according to the equation $C = M \times V \times 2.5$, where M is the computed mass (ng) of the analytic in the injected extract (1 mL), V the dilution factor, if any, that was applied.

Results and discussion

The average fat contents of cheese-flavored cracker samples are given at Table 1. As seen from the table, fat contents of

Table 1 Average fat and cholesterol contents of cheesy cracker samples

Sample (n = 14)	Fat (%)	Cholesterol (mg/100 g)
Mean	10.8 (6.9–15.0)	0.60 (0.42–1.22)
SD	2.50	0.30

Table 2 Unsaturated fatty acids profile of cheesy cracker samples (% of total fatty acids)

Sample (n = 14)	C14:1	C16:1	C17:1	C18:1tr	C18:1cis	C18:2tr	C18:2cis	C18:3	TFA ¹ (%)
Mean	0.1 (ND-0.1)	0.1 (0.1–0.3)	0.1 (ND-0.1)	0.2 (ND-0.6)	40.2 (37.4–48.7)	0.4 (0.1–0.7)	14.6 (12.3–19.2)	0.7 (0.5–1.0)	0.59 (0.1–1.2)
SD	0.01	0.07	0.07	0.22	3.87	0.26	2.46	0.16	0.43

¹Trans-fatty acids.**Table 3** Saturated fatty acids profile of cheesy cracker samples ((% of total fatty acids)

Sample (n = 14)	C8:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C22:0	C24:0
Mean	0.1 (0.1–0.1)	1.4 (0.5–2.5)	1.9 (1.4–2.3)	0.1 (ND-0.2)	35.1 (30.2–39.4)	0.1 (0.1–0.2)	5.1 (4.6–5.3)	0.1 (ND-0.2)	0.1 (0.07–0.11)	0.1 (ND-0.1)
SD	0.05	0.7	0.33	0.05	3.49	0.04	0.29	0.1	0.01	0.03

Table 4 SFA, MUFA and PUFA profiles of cheesy cracker samples ((% of total fatty acids)

Sample (n = 14)	SFA	MUFA	PUFA	PUFA/SFA ratio
Mean	44.0 (39.3–47.8)	40.7 (38.1–49.3)	15.7 (13.4–20.2)	0.36 (0.28–0.51)
SD	3.4	3.9	2.6	0.1

the samples ranged from 6.9% to 15.0%, and the average fat content was 10.8%. Cholesterol contents of the samples can be seen in the same table. It was determined that cholesterol content of the samples varied between 0.42 mg/100 g and 1.42 mg/100 g with an average cholesterol content of 0.60 mg/100 g sample. According to Han *et al.* (2010), the average cholesterol content of gluten-free cracker samples was 1.53 mg/100 g.

Fatty acids profiles of cracker samples are given in Table 2–4. As seen from Tables 1 and 2, fatty acids C8:0, C12:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1tr, C18:1cis, C18:2tr, C18:2cis, C18:3, C20:0, C22:0 and C24:0 were detected in the fat proportion of the samples. Fatty acids levels, which were less than 0.1% of total fatty acids, are not given in the tables.

The average unsaturated fatty acid profile of the samples is given in Table 2. C18:1cis was the highest (40.2% of total fatty acids) unsaturated fatty acid followed by C18:2cis (14.6% of total fatty acids), whereas the amount of C18:1tr and C18:2 tr were 0.2% and 0.4% of total fatty acids, respectively. Total trans-fatty acid contents of the samples were between 0.1% and 1.2% of total fatty acids, with an average value of 0.59%. Dağlıoğlu *et al.* (2002) revealed that the total trans-fatty acid contents of cracker samples was 2.1% of total fatty acids in their study. According to Martin *et al.* (2005), average trans-fatty acid contents of Brazilian biscuits was 20.1%, whereas this value was found as 2.0% of total fatty acids for biscuits purchased from New Zealand markets

(Lake *et al.*, 1996). Tavella *et al.* (2000) reported that trans-fatty acid amount of fat obtained from cheese-flavored cracker sticks was 3.74% of total fatty acids, whereas average fat content of the samples were given as 38%, which was considerably higher than our findings. On the other hand, Richter *et al.* (2009) declared that trans-fatty acid contents of the fat fractions of snacks, cakes and biscuits range from 0.64% to 12.26% of total fatty acids. These results show that the amount of trans-fatty acids may considerably vary from one food to another because of the hydrogenation conditions of the oil used in the formulation (Karabulut *et al.*, 2003).

Among the saturated fatty acids (SFA) of the samples, palmitic acid (C16:0) presented the highest value ranging from 30.2% to 39.4%, followed by stearic acid (C18:0) that varied from 4.6% to 5.3% of total fatty acids (Table 3).

In Table 4, the average saturated, monounsaturated (MUFA) and polyunsaturated (PUFA) fat level of cracker samples are given. Because essential fatty acids are present in this group, the PUFA content is very important in terms of biological and nutritional value. As seen from the table, the PUFA contents of the samples were between 13.4% and 20.2% of total fatty acids, whereas SFA and MUFA contents were on average 44.0% and 40.7% of total fatty acids, respectively. According to the UK Department of Health (Anon, 1994), the minimum value of the ratio of PUFA/SFA should be 0.45% of the total fatty acids. As seen from Table 4, the average SFA/PUFA ratio of our samples was 0.36 of the total

fatty acids, which is lower than this value. PUFA/SFA ratios of the samples ranged between 0.28% and 0.51% of total fatty acids, and at this ratio, only one sample (0.51%) was over the limit of 0.45% of total fatty acids.

Conclusion

The results from this study indicate that cheese-flavored crackers sold in Turkish markets do not contain considerable amounts of trans-fatty acids. The PUFA/SFA ratios of the samples were under the limit of 0.45% and nearly half of the fat fraction of the crackers was composed of saturated fatty acids (44.0%).

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