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#### -RESEARCH ARTICLE-

# Determination of Fatty Acid Profile and 3-Monochloropropane-1,2-diol (3-MCPD) Levels in Bakery Products

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#### **Abstract**

Thermally processed foods and refined oils are the most significant sources of 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters. The International Agency for Research on Cancer (IARC) classified 3-MCPD as a "possible human carcinogen (group 2B)" and the UK Food Advisory Committee has recommended reducing its level to minimum in foods. In this study, firstly the fatty acid contents of many foods such as cakes, biscuits, waffles, chocolates, cookies products consumed frequently in daily life were determined by GC-MS. The main fatty acids were determined as palmitic acid and stearic acid. The amounts of 3-MCPD esters were found to be between 0.06 and 0.60 mg kg<sup>-1</sup>, and the amounts of glycidyl esters were found to be between 0.07 and 8.80 mg kg<sup>-1</sup>.

## **Keywords:**

Fatty acid, 3-monochloropropane-1,2-diol (3-MCPD), bakery products, GC-MS.

## **Article history:**

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## Introduction

3-monochloropropane-1,2-diol (3-MCPD) is a food processing contaminant formed by heat as a reaction product of triacylglycerols, phospholipids or glycerol and hydrochloric acid in fat-based or fat-containing foods. It occurs in foods in its free (diol) form as well as in the bound esterified (with fatty acids) forms. Depending on the type of food it may occur as a free substance, in the form of an ester with fatty acids or in both forms (Svejkovska et al., 2004).

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Toxicological animal studies have shown that the main target organ for 3-MCPD toxicity is the kidney, with chronic oral exposure resulting in nephropathy and tubular hyperplasia and adenomas (JECFA, 2002). The International Agency for Research on Cancer has classified 3-MCPD as a "possible human carcinogen (group 2B)" (IARC, 2012). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established for the free compound a provisional maximum tolerable daily intake of 2  $\mu$ g/kg b.w.

Recent studies showed that fatty acid esters of 3-MCPD, i.e., bound 3-MCPD, were found in various foodstuffs such as pickled olives, roasted and green coffee, crisp bread, soda crackers, potato crisps and French fries, salami, certain types of ham, smoked and pickled fish, as well as some cheeses. 3-MCPD has been shown to release under certain processing conditions, such as enzymatic hydrolysis by bakery grade lipase during the baking process (FAO/WHO, 2007).

A number of studies reporting levels of 3-MCPD esters in different food commodities have been published in the literature. High levels of 3-MCPD esters have been reported in edible refined plant oils and fats; especially palm oil, for which levels up to 10 mg kg<sup>-1</sup> (Weißhaar, 2011). Coffee creamers, cream aerosols and bouillon cubes presented concentrations in ranges of 130–730, 50–730 and 380–670  $\mu$ g kg<sup>-1</sup>, respectively (Karsulinová et al., 2007). In fried potato products, the amount of 3-MCPD esters was 27–64  $\mu$ g kg<sup>-1</sup> in pre-fried French fries, 100–258  $\mu$ g kg<sup>-1</sup> in fried French fries and 98–2201  $\mu$ g kg<sup>-1</sup> in potato crisps (Ilko et al., 2011). Moreover, levels between 62 and 588  $\mu$ g kg<sup>-1</sup> were reported in infant formula (Zelinková et al. 2009). Samaras et al. (2016) were detected 250  $\mu$ g kg<sup>-1</sup> 3-MCPD in waffle samples while 3-MCPD levels were found in the range of 29 – 470 ng g<sup>-1</sup> in cookies by Becalski et al. (2015).

The aim of the study is to determine fatty acid profile and 3-MCPD levels in bakery products sold in Turkish markets.

#### **Materials and Methods**

## Reagents and standards

3-monochloropropane-1,2-diol (98%, 3-MCPD) and d5-3-MCPD (98%) were purchased from Sigma Aldrich (Gillingham, UK). All chemicals were obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical grade and were at least 99.5 % pure.

## Total fat and fatty acid analysis

Total fat (TF) was determined gravimetrically by extraction with petroleum ether at 65–80 °C (Official Methods 960.39) (AOAC, 1999). The extracted lipid residue was dried at 40 °C under a stream of nitrogen. For the preparation of fatty acid methyl esters (FAME), a cold method with hexane and 2N KOH in methanol (Bannon et al., 1982) was used. FAME were quantified using a gas chromatograph (Shimatzu QP-2010, Shimadzu Corporation, Kyoto, Japan) fitted with a capillary column Rt-2560 (fused silica), (100 m x 0.25mm id) and flame ionization detector. The oven was held at an initial temperature of 60 °C for 2 min, then increased to 220 °C at the rate of 3 °C per minute, the end temperature of 220 °C held for 12 minute. The entire time of analysis was 67 minute (TSE 4664 EN ISO 5508).

## Determination of 3-MCPD and glycidyl ester contents

3-MCPD and glycidyl ester were determined according to the DGF method C-VI 18 (10) which is based on alkaline trans esterification. One analysis was comprised of two assays (A and B). For each assay, about 100 mg of sample was weighed in a tube. After adding 250  $\mu$ L of methyl tertbutyl ether (MTBE) and 100  $\mu$ L internal standard solutions, the sample was shaken vigorously. For the saponification of MCPD and glycidyl esters, 350  $\mu$ L of a methanol/NaOH solution was added. The sample was shaken slowly for 10 minutes. The assay A reaction was quenched with 600  $\mu$ L of acidic NaCl solution while a chlorine free NaBr solution was used for assay B to avoid the formation of additional MCPD from glycidol.

The following preparation steps were similar for both assays. After the addition of 600  $\mu$ L hexane, the sample was vigorously shaken and incubated for 10 minutes. The sample was again shaken vigorously and the organic hexane layer was dispensed to waste. This step was repeated twice to remove matrix. Free 3-MCPD was extracted by 600  $\mu$ L MTBE/EtAc (3/2 v/v). The extract was collected in a new 2 mL vial pre-filled with sodium sulfate as drying agent. After adding 30  $\mu$ L of phenylboronic acid the sample was evaporated to dryness. The phenylboronic acid derivates were redissolved in isooctane and transferred to a new vial with  $\mu$ -vial insert ready for injection. The fact that phenylboronic acid was not very soluble in isooctane helps reduce the amount of derivatization agent injected. The evaporation step was therefore used both to increase the sensitivity of the analysis and also to remove excess phenylboronic acid in order to protect the MSD. Quantification was performed in Agilent 6890 system equipped with Rxi-17 sil ms (Restek) column (30 m x 0.25 mm x 0.25 mm) (Agilent Technologies, USA).

## Analysis conditions:

MPS: 3 µL injection volume

PTV: baffled liner, deactivated solvent vent

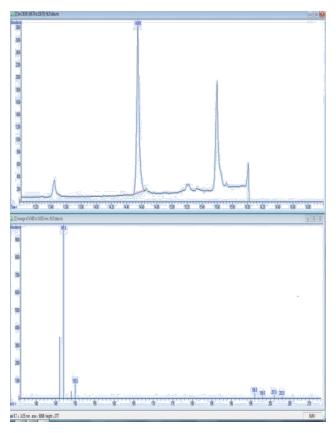
40°C (0 min); 12°C/s; 300°C (5 min)

Pneumatics: He, constant flow = 1 mL/min

Oven: 50°C (2 min); 10°C/min; 200°C (0 min) 20°C/min; 300°C (5 min)

MSD: Selected ion monitoring SIM

3-MCPD: 196/198/147 amu 3-MCPD-d5: 201/203/150 amu



The GC chromatogram and MS spectrum of 3-MCPD are given in Figure 1.

Figure 1. The GC chromatogram and MS spectrum of 3-MCPD.

## Quality assurance

- The linearity of the method was verified by analyzing palm oil spiked at five different levels. This was performed for both assays. The excellent linearity ( $R^2 > 0.9998$ ) achieved for both assays.
- Reagent blanks of less than 0.005 mg L<sup>-1</sup> for 3-MCPD.
- Recovery of spiked sample fell within 90–110 %.
- Relative response for repeat injections of calibration standards to be within  $\pm$  10 % the relative response of calibration standards.
- All the qualifier ion abundance ratios to be within  $\pm$  20 % the mean of the ion abundance ratios of the calibration standards.

## Results

The fatty acid compositions of the studied bakery products (cake, biscuit, chocolate, waffle, and cookie) are shown in Table 1-5, respectively. The major fatty acids in cake samples were palmitic acid (35.88 – 41.58 %) and stearic acid (28.85 – 40.34 %). Total saturated fatty acid ratios of the cake samples were determined between 77.49 - 88.38 %. Unsaturated fatty acid ratios were much lower (9.98 - 22.41 %). Unsaturated fatty acid was not detected in any of the examined cake samples. The total trans-fatty acid content of the samples ranged from 0.15 to 1.64 %. Turkish food codex indicated that 100 grams of total fat in foodstuffs could contain less than 1 gram of trans-

fatty acid. Only one of the detected trans-fat levels in the samples examined was above the limit value.

Table 1. The fatty acid composition of the studied cakes (N=3).

E-44- A-23-	Color	Color	C-l	Color	C-l
Fatty Acids	Cake <sub>1</sub>	Cake <sub>2</sub>	Cake₃	Cake₄	Cake₅
(%)					
C 8:0	nd	nd	$0.21 \pm 0.01$	nd	$0.34 \pm 0.01$
C 10:0	$1.97 \pm 0.01$	$1.04 \pm 0.01$	$0.96 \pm 0.01$	$1.08 \pm 0.01$	$1.33 \pm 0.01$
C 12:0	$0.87 \pm 0.01$	$6.93 \pm 0.03$	$0.55 \pm 0.01$	$3.38 \pm 0.02$	$4.25 \pm 0.04$
C 14:0	$1.16 \pm 0.01$	$3.29 \pm 0.02$	$1.08 \pm 0.01$	$2.27 \pm 0.01$	$1.36 \pm 0.01$
C 16:0	$41.58 \pm 0.08$	$36.78 \pm 0.07$	$38.56 \pm 0.07$	$40.12 \pm 0.08$	$35.88 \pm 0.07$
C 17:0	$1.00 \pm 0.01$	nd	$0.88 \pm 0.01$	nd	nd
C 18:0	$31.69 \pm 0.06$	$40.34 \pm 0.08$	$35.35 \pm 0.06$	$30.64 \pm 0.05$	$33.46 \pm 0.06$
C 20:0	$1.39 \pm 0.01$	nd	nd	nd	$1.05 \pm 0.01$
Σ SFA*	$79.66 \pm 0.10$	$88.38 \pm 0.11$	$77.59 \pm 0.09$	$77.49 \pm 0.09$	$77.67 \pm 0.10$
C 16:1	nd	nd	$0.25 \pm 0.01$	$0.32 \pm 0.01$	nd
C 18:1	$20.34 \pm 0.05$	$9.98 \pm 0.04$	$22.08 \pm 0.05$	$22.04 \pm 0.05$	$21.75 \pm 0.05$
C 20:1	nd	nd	$0.08 \pm 0.001$	nd	$0.10 \pm 0.001$
Σ USFA*	$20.34 \pm 0.05$	$9.98 \pm 0.04$	$22.41 \pm 0.05$	$22.36 \pm 0.05$	$21.85 \pm 0.05$
C 18:2	nd	nd	nd	nd	nd
C 18:3	nd	nd	nd	nd	nd
$\Sigma OSFA*$					
C 18:1 t9	nd	$1.64 \pm 0.01$	nd	nd	$0.48 \pm 0.01$
C 18:2 t9t12	nd	nd	nd	$0.15 \pm 0.01$	nd
ΣTFA*		$1.64 \pm 0.01$		$0.15 \pm 0.01$	$0.48 \pm 0.01$

\*SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; OSFA: Over saturated fatty acid;

TFA: Trans fattyacid; nd: not detected

While the ratio of palmitic acid in the biscuit samples ranged from 27.82 to 43.29 %, the stearic acid ratio was in the range of 10.48 - 19.09 %. Total saturated fatty acid ratios of biscuit samples were determined between 53.83 - 73.60 %. Unsaturated fatty acid ratios are much lower (26.40 - 35.15 %). Some 9,97 - 12,89 % of unsaturated fatty acid (linoleic acid) was detected in some of the biscuit samples examined. In one of the samples, linolelaidic acid (0.38 %) was determined from trans-fatty acids, whereas in no sample elaidic acid was found. The amount of trans-fat detected in the samples examined was below the limit value.

Table 2. The	fatty acid	composition	of the s	tudied	biscuits	(N=3).

Fatty Acids	Biscuit <sub>1</sub>	Biscuit <sub>2</sub>	Biscuit <sub>3</sub>	Biscuit₄	Biscuit <sub>5</sub>
(%)					
C 8:0	nd	$0.46 \pm 0.01$	$0.88 \pm 0.01$	nd	nd
C 10:0	$1.17 \pm 0.01$	$0.50 \pm 0.01$	$2.19 \pm 0.01$	$3.12 \pm 0.01$	$5.53 \pm 0.03$
C 11:0	nd	nd	$0.43 \pm 0.01$	nd	nd
C 12:0	$0.53 \pm 0.01$	$5.26 \pm 0.03$	$7.80 \pm 0.06$	$4.06 \pm 0.02$	$4.51 \pm 0.02$
C 14:0	$1.23 \pm 0.01$	$3.41 \pm 0.01$	$4.58 \pm 0.02$	$3.24 \pm 0.01$	$3.92 \pm 0.01$
C 15:0	nd	$0.07 \pm 0.001$	nd	nd	$2.99 \pm 0.01$
C 16:0	$40.63 \pm 0.08$	$32.49 \pm 0.07$	$27.82 \pm 0.06$	$43.29 \pm 0.08$	$39.57 \pm 0.07$
C 17:0	nd	$0.17 \pm 0.001$	nd	nd	$8.78 \pm 0.04$
C 18:0	$14.09 \pm 0.05$	$10.60 \pm 0.04$	$10.48 \pm 0.04$	$19.09 \pm 0.05$	nd
C 20:0	$0.56 \pm 0.01$	$0.74 \pm 0.01$	nd	$0.80 \pm 0.01$	$4.98 \pm 0.02$
C 22:0	nd	$0.13 \pm 0.001$	$0.72 \pm 0.01$	nd	nd
ΣSFA*	$58.21 \pm 0.09$	$53.83 \pm 0.09$	$54.90 \pm 0.09$	$73.60 \pm 0.09$	$70.28 \pm 0.10$
C 16:1	nd	$0.03 \pm 0.001$	nd	nd	nd
C 18:1	$31.44 \pm 0.07$	$35.12 \pm 0.07$	$32.17 \pm 0.07$	$26.40 \pm 0.06$	$29.72 \pm 0.06$
Σ USFA*	$31.44 \pm 0.07$	$35.15 \pm 0.07$	$32.17 \pm 0.07$	$26.40 \pm 0.06$	$29.72 \pm 0.06$
C 18:2	$9.97 \pm 0.05$	$11.02 \pm 0.04$	$12.89 \pm 0.04$	nd	nd
ΣOSFA*	$9.97 \pm 0.05$	$11.02 \pm 0.04$	$12.89 \pm 0.04$		
C 18:2 t9t12	$0.38 \pm 0.01$	nd	nd	nd	nd
ΣTFA*	$0.38 \pm 0.01$				

\*SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; OSFA: Over saturated fatty acid;

TFA: Trans fattyacid; nd: not detected

The percentage of palmitic acid in chocolate samples ranged from 21.89% to 29.04%, while the ratio of stearic acid was between 0.69% and 32.05%. Total saturated fatty acid ratios of chocolate samples were determined between 37.42 - 68.81%. Unsaturated fatty acid ratios were lower (22.18% - 46.51%). In all of the samples examined was found linoleic acid (8.53-16.07%) as unsaturated fatty acid. Trans -fatty acids were not detected in any of the samples.

Table 3. The fatty acid composition of the studied chocolates (N=3).

Fatty Acids	Choc olate <sub>1</sub>	Chocolate <sub>2</sub>	Chocolate <sub>3</sub>	Chocolate <sub>4</sub>	Chocolate <sub>5</sub>
(%)					
C 8:0	$0.61 \pm 0.01$	$2.52 \pm 0.01$	$0.19 \pm 0.001$	$0.45 \pm 0.01$	$0.52 \pm 0.01$
C 10:0	$0.69 \pm 0.01$	$2.93 \pm 0.01$	$0.45 \pm 0.01$	$0.58 \pm 0.01$	$0.74 \pm 0.01$
C 11:0	nd	$0.16 \pm 0.001$	nd	nd	$0.05 \pm 0.001$
C 12:0	$6.09 \pm 0.03$	$13.95 \pm 0.04$	$1.61 \pm 0.01$	$1.59 \pm 0.01$	$4.08 \pm 0.02$
C 13:0	nd	$0.24 \pm 0.001$	nd	nd	nd
C 14:0	$3.83 \pm 0.01$	$7.87 \pm 0.03$	$3.47 \pm 0.01$	$3.02 \pm 0.01$	$2.99 \pm 0.01$
C 15:0	$0.08 \pm 0.001$	nd	nd	nd	nd
C 16:0	$27.28 \pm 0.06$	$21.89 \pm 0.06$	$29.04 \pm 0.07$	$26.33 \pm 0.06$	$28.14 \pm 0.07$
C 17:0	nd	nd	$0.38 \pm 0.01$	nd	nd
C 18:0	$15.30 \pm 0.04$	$17.26 \pm 0.05$	$0.69 \pm 0.01$	$24.58 \pm 0.06$	$32.05 \pm 0.07$
C 20:0	$1.01 \pm 0.01$	$1.99 \pm 0.01$	$1.57 \pm 0.01$	nd	nd
C 22:0	$0.15 \pm 0.001$	nd	$0.02 \pm 0.001$	nd	nd
Σ SFA*	$55.04 \pm 0.08$	$68.81 \pm 0.09$	$37.42 \pm 0.07$	$56.55 \pm 0.08$	$68.57 \pm 0.10$
C 16:1	$0.05 \pm 0.001$	nd	nd	$0.16 \pm 0.001$	nd
C 18:1	$31.72 \pm 0.07$	$20.43 \pm 0.06$	$46.51 \pm 0.08$	$30.48 \pm 0.07$	$22.18 \pm 0.06$
C 20:1	nd	$2.23 \pm 0.01$	nd	nd	nd
Σ USFA*	$31.77 \pm 0.07$	$22.66 \pm 0.06$	$46.51 \pm 0.08$	$30.64 \pm 0.07$	$22.18 \pm 0.06$
C 18:2	$12.89 \pm 0.04$	$8.53 \pm 0.03$	$16.07 \pm 0.04$	$12.81 \pm 0.04$	$9.25 \pm 0.03$
C 18:3	$0.30 \pm 0.01$	nd	nd	nd	nd
ΣOSFA	$13.19 \pm 0.04$	$8.53 \pm 0.03$	$16.07 \pm 0.04$	$12.81 \pm 0.04$	$9.25 \pm 0.03$
*CEA : Catamata d fatta ani d. LICEA : Lincotropted fatta ani d. OCEA : Ocean automata d fatta ani d.					

\*SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; OSFA: Over saturated fatty acid;

nd: not detected

While the ratio of palmitic acid in the wafer samples ranged from 23.58 to 30.01 %, the ratio of stearic acid was 9.36 - 37.29 %. Total saturated fatty acid ratios of the wafer samples were determined between 49.79 - 78.95 %. Unsaturated fatty acid ratios are observed to be lower (21.05 - 45.70 %). Linoleic acid was found to be between 3.34 - 12.76 % of the unsaturated fatty acids in the wafer samples. In one of the samples, linolelaidic acid (0.86%) was determined from trans-fatty and its amount was below the limit value.

Table 4. The fatty acid composition of the studied waffles (N=3).

Fatty Acids	Waffle <sub>1</sub>	Waffle <sub>2</sub>	Waffle₃	$Waffle_4$	Waffle₅
(%)					
C 8:0	$1.86 \pm 0.01$	$0.42 \pm 0.01$	$0.75 \pm 0.01$	$1.15 \pm 0.01$	$1.91 \pm 0.01$
C 10:0	$1.93 \pm 0.01$	$1.57 \pm 0.01$	$2.18 \pm 0.01$	$1.33 \pm 0.01$	$2.75 \pm 0.01$
C 12:0	$15.06 \pm 0.04$	$3.19 \pm 0.01$	$6.10 \pm 0.02$	$4.97 \pm 0.02$	$8.80 \pm 0.03$
C 13:0	nd	nd	nd	$0.31 \pm 0.01$	$0.08 \pm 0.001$
C 14:0	$8.43 \pm 0.03$	$2.33 \pm 0.01$	$2.55 \pm 0.01$	$3.71 \pm 0.01$	$9.93 \pm 0.03$
C 15:0	$0.05 \pm 0.001$	$0.57 \pm 0.01$	nd	$0.39 \pm 0.01$	$0.31 \pm 0.01$
C 16:0	$25.50 \pm 0.06$	$29.00 \pm 0.06$	$30.01 \pm 0.06$	$25.59 \pm 0.06$	$23.58 \pm 0.06$
C 17:0	$0.15 \pm 0.001$	nd	nd	nd	$1.57 \pm 0.01$
C 18:0	$9.36 \pm 0.03$	$13.50 \pm 0.04$	$11.83 \pm 0.04$	$37.29 \pm 0.07$	$4.51 \pm 0.01$
C 20:0	$0.59 \pm 0.01$	$2.94 \pm 0.01$	nd	$2.59 \pm 0.01$	-
C 22:0	$0.19 \pm 0.001$	$0.54 \pm 0.01$	nd	$1.62 \pm 0.01$	$0.86 \pm 0.01$
Σ SFA*	$63.12 \pm 0.09$	$54.13 \pm 0.08$	$53.42 \pm 0.08$	$78.95 \pm 0.09$	$49.79 \pm 0.08$
C 16:1	$0.15 \pm 0.001$	$2.06 \pm 0.01$	nd	nd	$1.96 \pm 0.01$
C 18:1	$23.17 \pm 0.06$	$32.23 \pm 0.07$	$40.95 \pm 0.07$	$21.05 \pm 0.06$	$43.74 \pm 0.08$
C 20:1	$0.28 \pm 0.01$	nd	$2.29 \pm 0.01$	nd	nd
Σ USFA*	$23.60 \pm 0.06$	$34.29 \pm 0.07$	$43.24 \pm 0.07$	$21.05 \pm 0.06$	$45.70 \pm 0.08$
C 18:2	$12.76 \pm 0.04$	$10.72 \pm 0.04$	$3.34 \pm 0.01$	nd	nd
C 18:3	$0.52 \pm 0.01$	nd	nd	nd	nd
ΣOSFA*	$13.28 \pm 0.04$	$10.72 \pm 0.04$	$3.34 \pm 0.01$		
C 18:2 t9t12	nd	$0.86 \pm 0.01$	nd	nd	nd
ΣTFA*		$0.86 \pm 0.01$			

\*SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; OSFA: Over saturated fatty acid;

TFA: Trans fattyacid; nd: not detected

The ratio of palmitic acid in cracker samples ranged from 28.90 - 40.08 %, while the ratio of stearic acid was in the range of 14.98 - 25.15 %. Total saturated fatty acid ratios of cracker samples were determined between 50.90 - 65.97 %. Unsaturated fatty acid ratios were lower (28.74 - 38.80 %). Unsaturated fatty acid ratios were lower (28.74 - 38.80 %). Linoleic acid was found to be between 2.95 - 12.80 % of the unsaturated fatty acids in the wafer samples. Trans -fatty acids were not detected in any of the samples.

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Fatty Acids (%)	Cookie <sub>1</sub>	Cookie <sub>2</sub>	Cookie <sub>3</sub>	Cookie <sub>4</sub>	Cookie <sub>5</sub>
C 8:0	$0.12 \pm 0.001$	nd	$0.23 \pm 0.01$	nd	$0.31 \pm 0.01$
C 10:0	$0.26 \pm 0.01$	$0.08 \pm 0.001$	$0.12 \pm 0.001$	$0.27 \pm 0.01$	0.16 ± 0.001
C 12:0	$1.17 \pm 0.01$	$2.08 \pm 0.01$	$1.98 \pm 0.01$	$1.48 \pm 0.01$	$2.19 \pm 0.01$
C 14:0	$1.58 \pm 0.01$	$1.25 \pm 0.01$	$1.33 \pm 0.01$	$1.42 \pm 0.01$	$1.54 \pm 0.01$
C 15:0	$0.10 \pm 0.001$	$0.05 \pm 0.001$	nd	$0.02 \pm 0.001$	nd
C 16:0	$28.90 \pm 0.06$	$32.16 \pm 0.07$	$40.08 \pm 0.07$	$34.50 \pm 0.07$	$30.88 \pm 0.07$
C 17:0	$0.29 \pm 0.01$	$0.08 \pm 0.001$	nd	nd	nd
C 18:0	$25.15 \pm 0.06$	$14.98 \pm 0.04$	$22.08 \pm 0.06$	$20.56 \pm 0.05$	$18.44 \pm 0.05$
C 20:0	$0.16 \pm 0.001$	$0.22 \pm 0.01$	nd	nd	nd
C 22:0	$0.22 \pm 0.01$	nd	$0.15 \pm 0.001$	nd	$0.12 \pm 0.001$
Σ SFA*	$57.95 \pm 0.09$	$50.90 \pm 0.08$	$65.97 \pm 0.09$	$58.25 \pm 0.09$	$53.64 \pm 0.09$
C 16:1	$0.41 \pm 0.01$	$0.16 \pm 0.001$	$0.32 \pm 0.01$	$0.28 \pm 0.01$	$0.14 \pm 0.001$
C 18:1	$33.98 \pm 0.07$	$36.14 \pm 0.07$	$28.42 \pm 0.06$	$38.52 \pm 0.07$	$34.56 \pm 0.07$
ΣUSFA*	$34.39 \pm 0.07$	$36.30 \pm 0.07$	$28.74 \pm 0.06$	$38.80 \pm 0.07$	$34.70 \pm 0.07$
C 18:2	$7.56 \pm 0.02$	$12.80 \pm 0.04$	$5.29 \pm 0.02$	$2.95 \pm 0.01$	$11.66 \pm 0.04$
C 18:3	$0.10 \pm 0.001$	nd	nd	nd	nd

Table 5. The fatty acid composition of the studied cookies (N=3).

\*SFA: Saturated fatty acid; USFA: Unsaturated fatty acid; OSFA: Over saturated fatty acid; nd: not detected

 $5.29 \pm 0.02$ 

 $7.66 \pm 0.02$   $12.80 \pm 0.04$ 

After the all bakery products examined in the study were blended in itself, 3-MCPD esters and glycidyl esters were determined and the obtained values are given in Table 6. As can be seen from the table, the highest 3-MCPD amounts were found in biscuit and cake samples while the lowest 3-MCPD values were determined in cracker samples. Glycidyl ester amounts were generally close to each other, they were found to be high in biscuit samples but not in chocolate samples.

Table 6. Levels of 3-MCPD Esters and Glycidyl Esters (N=3).

Sample	3-MCPD Esters (mg kg <sup>-1</sup> )	Glycidyl Esters (mg kg <sup>-1</sup> )
Cake	$0.50\pm0.01$	$0.10 \pm 0.001$
Biscuit	$0.60 \pm 0.01$	$8.80 \pm 0.05$
Chocolate	$0.20 \pm 0.001$	nd
Waffle	$0.20 \pm 0.001$	$0.10 \pm 0.001$
Cookie	$0.06 \pm 0.001$	$0.07 \pm 0.001$

nd: not detected

#### **Discussion**

In the study of Othman et al. (2018), the major fatty acids in the cakes were determined as palmitic (31.10 - 37.85 %), stearic (5.08 - 12.57 %), myristic (2.90 - 13.86 %) and lauric acid (1.46 - 6.34 %). They did not find trans- fatty acid in the samples they examined. In comparison with our results, palmitic acid and lauric acid values were similar, stearic acid values were much lower and myristic acid values were higher.

In the study of Dias et al. (2015), the major fatty acids in the salty and sweet biscuits were determined as palmitic (35.31 - 50.64 %; 22.73 - 44.43 %), oleic (27.50 - 48.94 %; 17.10 - 59.49

%), linoleic (10.87 - 31.18 %; 8.51 - 22.60 %), respectively. In our study, the percentages of fatty acids in saline samples were higher than those of sweet samples.

Suzuki et al. (2011) studied fatty acid composition of chocolates consumed in Brazil and determined that the essential fatty acids were palmitic (4.86 - 6.93 %), stearic (5.63 - 8.05 %) and oleic acid (5.81 - 8.42 %). They found only elaidic acid (0.06 - 0.1 %) from trans - fatty acids in the samples. The results are much lower than the values we determined in our study.

In the study of Stroher et al. (2012), the main fatty acids were palmitic acid (11.52 - 38.72 %), stearic acid (8.73 - 17.37 %), oleic acid (2080 - 33.81 %) and linoleic acid (2.18 - 15.57 %) in the wafer samples. Total trans- fat content was found between 0.055 - 0.154 %. The values obtained are close to our results.

In the study of Santos and colleagues (2015) in Portugal, total saturated fatty acid content in cracker samples was between 43.1 - 59.6 %, total monounsaturated fatty acid content was between 28.7 - 41.0 % and total polyunsaturated fatty acid content was between 9.8 - 15.5 %. Total transfatty acid content was between 9.44 and 9.4

The composition of fatty acids of fatty plants not fixed. The synthesis of fatty acids is changed depending on the genetic, ecological, morphological, physiological and cultural applications. Thus, the type of fat used in food products has a great effect on the fatty acid compositions. The difference between the results and the literature can be weldable from this effect.

Mogol et al. (2014) investigated 3-MCPD formation in biscuits baked at different times (5, 10, 15 and 20 minutes) and temperatures (180, 200 and 220 °C) and the effects of salt and oil type on 3-MCPD formation. It was found that 3-MCPD levels in biscuits ranged from 0.018 to 0.074 mg kg-1, 3-MCPD formation increased as temperature increased, after 11 minutes at 220 °C, these values increased 4-fold, and 3-MCPD levels were higher than salt-added biscuits. The values they find are lower than our results. Becalski et al. (2015) found 3-MCPD esters levels in crackers between 29 - 470 ng g<sup>-1</sup> and glycidyl esters levels between 17 and 339 ng g<sup>-1</sup>. In our study, the mean 3-MCPD level in the cracker samples was 0.06 mg kg<sup>-1</sup>, and the average glycidyl ester level was 0.07 mg kg<sup>-1</sup> and it was close to the minimum values determined by Becalski et al. Samaras et al. (2016) examined 3-MCPD esters levels in samples such as waffles, potato chips, corn flakes, margarine. They determined the average 3-MCPD levels of the waffle samples as 250 μg kg<sup>-1</sup>. This value is very close to the value we found.

Although the values obtained for a package of samples are below the limit values, the limit value can easily be exceeded when consumed a little too much. This type of food is especially risky because it is frequently consumed by children. In order to be able to demonstrate the effects of these species on health more clearly and to take necessary measures, more samples should be examined.

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