

The effect of pan frying on 3-monochloropropane-1,2-diol and glycidyl ester contents of vegetable oils

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Abstract: The aim of this work was to determine the effect of the pan-frying process on 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl ester (GE) amounts of vegetable oils. For this purpose, potatoes were pan-fried with refined sunflower, corn, hazelnut oils, virgin olive oil and margarine for 5, 10 and 15 minutes at 160, 180 and 200°C. The fried oil samples were analysed for their 3-MCPD and glycidyl ester levels. Results have shown that 3-MCPD ester concentrations were higher than the GEs' for all types of oils. Virgin olive oil was found to be devoid of the contaminants and no endogenous formation was observed throughout frying cycles for any of the matrices tested. Margarine was determined to have the highest content of both esters. The GE amounts of margarine were found to increase by the intensity of the frying process. The 3-MCPD-E levels of hazelnut oil did not vary significantly, whereas slight differences were observed for sunflower and corn oils by varying the process parameters.

Keywords: 3-monochloropropane-1,2-diol ester; Glycidyl ester; Margarine; Pan-frying

1. INTRODUCTION

Frying is used as a traditional technique for food preparation worldwide. Both deep-fried and pan-fried foods are preferred by the consumers for the palatable taste, unique flavor, and texture of the product. During frying, food is in contact with hot oil (150-190°C) and both the heat and mass transfer occur simultaneously [1]. Besides, a series of chemical, physical and thermal changes including hydrolysis, oxidation and polymerisation take place during frying [2]. As the reactions progress, the nutritional and sensorial quality of the frying medium, the oil, degenerates continuously, accompanying with a formation of free fatty acids, the darkening in colour and the off flavoring [3]. The quality and the stability of the frying oil is notably important since it is absorbed by the fried product.

The 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl esters are heat-induced chemical contaminants that occur in refined vegetable oils and oil containing foods. These contaminants have gained increased interest in recent years for their potential toxicity due to the release of 3-MCPD and glycidol, the hydrolysates of their parent esters (3-MCPD esters and glycidyl esters) during digestion [4]. 3-MCPD has been classified as "possible human carcinogens" (group 2B) and glycidol has been grouped as "probably carcinogenic to humans" (group 2A) by the International Agency for Research on Cancer [5]. A critical tolerable daily intake for the protection of human health of 2 µg·kg⁻¹ body weight for 3-MCPD and its fatty acid esters was suggested by CONTAM Panel [6]. Moreover, the European Commission defined a maximum limit of 1 ppm for glycidyl fatty acid esters in vegetable oils and fats, fish oils and oils from other marine organisms placed on the market for the final consumer or for use as an ingredient in food. Additionally, the maximum level of 1.25 ppm was established for the sum of 3-monochloropropanediol (3-

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MCPD) and 3-MCPD fatty acid esters for vegetable oils and fats from coconut, maize, rapeseed, sunflower, soybean, palm kernel and olive oils (composed of refined olive oil and virgin olive oil) and mixtures of oils and fats. In addition, the maximum tolerable limit of 3-MCPD and fatty acid esters was determined as 2.5 ppm for other vegetable oils (including olive pomace olive oil), fish oils and oils obtained from other marine organisms [7].

The formation of 3-MCPD esters (3-MCPD-Es) and glycidyl esters (GEs) occur mainly during vegetable oil refining process. The high temperatures achieved at the deodorisation step encourage the formation of the esters in the presence of precursors such as organic and inorganic chlorinated compounds and partial acylglycerols [8]. Certain food preparation techniques such as frying, grilling, and baking with high temperatures may also cause the formation of these undesired process contaminants. The temperatures used for deep-fat frying vary in 160-190°C and fall within the temperature range of refining. In addition, fried foods are sometimes salted or coated before frying which causes the inclusion of chloride that may promote the formation of 3-MCPD in the frying medium. Hence, the effect of frying on 3-MCPD and glycidyl ester levels has received attraction from researchers and some reports have been published recently on the behaviors of 3-MCPD-E and GEs when exposed to different frying parameters. Although factors such as frying oil, temperature, time, chlorine source, moisture, the composition of the food were reported to influence the 3-MCPD and glycidyl ester content of fried product [9], no consistent conclusion has been drawn yet [10]. The majority of the earlier works on the topic mainly focused on the changes during deep-fat frying; however, there is a lack of research on the variation of these process contaminants during pan-frying. To the authors' knowledge, the unique study was published by Raczyk et al. [11], who reported remarkable formation of 3-MCPD esters and moderate increases of glycidyl esters in margarine samples pan-fried over 15 minutes. The objective of this work was to study the formation of 3-MCPD and glycidyl esters during the pan-frying process conducted under varying conditions of temperature and time.

2. MATERIALS AND METHODS

2.1. MATERIALS AND CHEMICALS

Frying oils (refined sunflower oil, refined corn oil, refined hazelnut oil, virgin olive oil, margarine) and potatoes were purchased from local markets in Aydın. Chemicals used during analyses, namely, 3-monochloropropane-1,2-diol-d₅, diethyl ether, ethyl acetate, glycidol, *n*-hexane, isooctane, methanol, phenyl boronic acid, sodium hydroxide, sodium methoxide, *tert*-butyl methyl ether and toluene were purchased from Sigma-Aldrich (St-Louis, ABD). Sodium bromide, sodium chloride and sulfuric acid were pur-

chased from Merck (Darmstadt, Germany).

2.2. METHODS

Preparation and pan-frying of potatoes

In the first step, the potatoes were washed and peeled. Each potato was sliced to equal dimensions (0.5 cm × 2 cm × 5 cm). The frying process was carried out in the Teflon pan (Tefal-24 cm, France) and 100 g of potatoes were fried in 100 ml oil. Samples were fried in five different edible oils (sunflower oil, corn oil, hazelnut oil, virgin olive oil and margarine) for 5, 10 and 15 minutes at 160, 180 and 200°C. The frying was performed using a conventional electric kitchen cooker. Each frying process was performed in duplicate. There was a total of 18 (3 times × 3 temperatures × 2 replicates) different frying processes for each oil. Some of the frying oil was absorbed by the potatoes and the remaining oil was transferred to 100 ml dark colored glass bottles and kept at +4°C until analysis. Margarine samples were melted at 50°C before analysis.

Determination of 3-MCPD and glycidyl esters contents

3-MCPD and glycidyl ester content analysis was carried out according to the DGF C-VI 18 (10) standard method [12] which is an indirect analysis method and based on alkaline transesterification. 3-MCPD-d₅ was used as the reference standard. Quantification was performed according to the method of Cheng et al. [13] and quantitative analysis of 3-MCPD and glycidyl esters were performed in gas chromatography (Shimadzu QP2020 system-Shimadzu, Kyoto, Japan) with mass spectrometry (GC-MS). Separation of the target components was performed with HP-5MS capillary column (30 m length, 0.32 mm inner diameter and 0.25 μm film thickness, Agilent Technologies, USA). The oven temperature was programmed from 80°C to 155°C at a rate of 5°C/min then temperature was increased to 300°C at a rate of 60°C/min and held for 5 min. Helium was used as carrier gas at a flow rate of 1.18 ml/min. 1 μl of oil sample was injected in splitless mode for each analysis. The mass spectrometer detector was operated in selected ion monitoring mode (SIM mode) with positive electron ionisation (EI+) at a 70 eV ionisation voltage. The temperature of the ion source and interface in the mass spectrometer was 200 and 280°C, respectively. The quantitative analysis of the derivatised 3-MCPD compound was examined by monitoring characteristic ions. The ion traces m/z 147 was selected for 3-MCPD and m/z 150 for 3-MCPD-d₅. The limit of detection of the method was 0.07 mg/kg.

Statistical analysis

Each frying process was performed in duplicate and the measurements were replicated twice. The results were expressed as mean ± standard deviation of four measurements for the analytical determination. Statistical analysis was carried out using SPSS 15.0 statistical software (SPSS Inc., Chicago, USA). Data

were evaluated by the single factor analysis of variance (one-way ANOVA) procedure using the Duncan multiple range test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. 3-MCPD ESTER CONTENT OF FRYING OILS

The effects of frying time and temperature on 3-MCPD-E content of various vegetable oils is given in Table I. 3-MCPD esters of fried and non-fried virgin olive oil samples were below the detectable limit, proposing that the virgin olive oil has none or negligible amount of these contaminants. 3-MCPD and glycidyl esters are known to be formed during refining, especially at the deodorisation step of the process. Virgin olive oils are obtained only by mechanical techniques and can be consumed without further refining. Like current findings, virgin olive oil has been mainly reported to be devoid of these contaminants in previous literature [14]. Thus, it can be hypothesised that pan-fried potatoes in virgin olive oil are more likely to include lower 3-MCPD-E amounts.

The 3-MCPD ester content of fresh sunflower oil was $0.18 \text{ mg}\cdot\text{kg}^{-1}$. European Food Safety Authority (EFSA) determined the mean concentration of 3-MCPD esters in fresh sunflower oils (refined-non fried) as $0.521 \text{ mg}\cdot\text{kg}^{-1}$ in 2016 [15]. The frying time was found to be ineffective at three frying temperatures. Merkle et al. [16] emphasised that pre-frying temperature and heating time were the most important parameters regarding the MCPD-E contents during deep-fat frying of frozen fish products with sunflower oil. Dingel and Matissek [17] reported non-formation of these undesirable contaminants in high oleic sunflower oil during deep-fat frying of potato crisps at large scale production.

Corn oil was the other linoleic rich seed oil in the current work and had $0.43 \text{ mg}\cdot\text{kg}^{-1}$ 3-MCPD-E in fresh form, higher than both sunflower and hazelnut oils. The pan-frying process at 200°C caused slight de-

creases in 3-MCPD-E content of corn oil by the increase in process time. The frying processes at 180°C caused significant increases in 3-MCPD-E contents in comparison with fresh oil. Ariseto et al. [18] reported no endogenous formation of bound 3-MCPD during frying, when corn oil containing non-significant levels of the contaminant at the beginning of the process was used.

The 3-MCPD-E content of non-fried hazelnut oil was found to be $0.19 \text{ mg}\cdot\text{kg}^{-1}$. The frying time and temperature were found to be ineffective on bound 3-MCPD content of the hazelnut oils during frying. Arslan et al. [19] detected $0.44 \text{ mg}\cdot\text{kg}^{-1}$ of 3-MCPD-E in fresh hazelnut oil and reported a reduction in the concentration of the contaminant with frying temperature and time. Similarly, Wong et al. [20] described a decrease in bound 3-MCPD with an increase in frying time.

The highest contents of 3-MCPD-Es were detected in margarine samples that ranged in 1.50 and $1.65 \text{ mg}\cdot\text{kg}^{-1}$, which may be attributed to palm oil and chlorinated compounds present in the formulation. Palm oil has been reported to be a critical source of 3-MCPD and glycidyl esters [21, 22], whereas chlorinated components have been defined as principal precursors of the contaminants [17]. Several works, investigating the 3-MCPD-E content of margarines purchased from various markets, have been published. Goh et al. [23] described up to $3.83 \text{ mg}\cdot\text{kg}^{-1}$ 3-MCPD-E in five different margarine samples, Jedrkiewicz et al. [22] found a range of 1.3 - $7.3 \text{ mg}\cdot\text{kg}^{-1}$ of 3-MCPD-E in the lipid fraction of five margarines for different brands, Şirinıldız et al. [24] reported a range of 0.57 - $4.54 \text{ mg}\cdot\text{kg}^{-1}$ for 26 different margarines sold in market and Custodio-Mendoza et al. [14] determined up to $8.09 \text{ mg}\cdot\text{kg}^{-1}$ of the contaminant in lipid part of margarines. The results indicate that margarines are important contributors to dietary exposure of 3-MCPD-Es. In the current work, the pan-frying process caused a slight increase in 3-MCPD-E content of the samples fried at 160°C for 10 minutes.

Table I. The effect of time and temperature on 3-MCPD ester content of frying oils ($\text{mg}\cdot\text{kg}^{-1}$)

Frying temperature ($^\circ\text{C}$)	Frying time (min)	Sunflower oil	Corn oil	Hazelnut oil	Virgin olive oil	Margarine
Fresh oil		0.18 ± 0.00^a	0.43 ± 0.00^{ab}	0.19 ± 0.00	ND	1.50 ± 0.03^a
160	5	0.18 ± 0.00^{ab}	0.44 ± 0.01^{abc}	0.19 ± 0.01	ND	1.56 ± 0.02^{ab}
	10	0.20 ± 0.02^b	0.43 ± 0.02^{ab}	0.19 ± 0.01	ND	1.65 ± 0.12^b
	15	0.20 ± 0.01^{ab}	0.46 ± 0.02^{bcd}	0.19 ± 0.01	ND	1.54 ± 0.08^a
180	5	0.18 ± 0.01^a	0.49 ± 0.03^e	0.19 ± 0.00	ND	1.59 ± 0.04^{ab}
	10	0.19 ± 0.00^{ab}	0.47 ± 0.04^{cde}	0.19 ± 0.00	ND	1.59 ± 0.06^{ab}
	15	0.18 ± 0.01^a	0.48 ± 0.01^{de}	0.19 ± 0.01	ND	1.52 ± 0.05^a
200	5	0.18 ± 0.01^a	0.48 ± 0.02^{de}	0.19 ± 0.00	ND	1.54 ± 0.01^a
	10	0.18 ± 0.02^{ab}	0.42 ± 0.01^a	0.19 ± 0.01	ND	1.56 ± 0.06^{ab}
	15	0.19 ± 0.01^{ab}	0.43 ± 0.01^{abc}	0.20 ± 0.01	ND	1.50 ± 0.07^a

Mean values followed by the same letter in each column are not significant different at $p < 0.05$ by ANOVA and Duncan's test. ND: Not detected.

Raczyk et al. [11] reported significant increases by 15 minutes of pan-frying in 3-MCPD-E content of margarine samples by pan-frying.

3.2. GLYCIDYL ESTER CONTENT OF FRYING OILS

The effects of frying time and temperature on glycidyl ester contents of different vegetable oils is given in Table II. The GE content of the oils were found to be lower than 3-MCPD esters' and no significant correlation was detected between the two contaminants due to different formation mechanisms [25]. Virgin olive oil samples had no glycidyl ester contents in fresh forms and no formation of GE was determined throughout pan-frying cycles. Unrefined virgin vegetable oils have been reported to contain undetectable contents of 3-MCPD and glycidyl esters [26].

The glycidyl ester content of non-fried sunflower oil was $0.09 \text{ mg}\cdot\text{kg}^{-1}$ and the level of glycidyl esters increased with frying at 180° for 10 minutes and 200°C for 10 and 15 minutes of processes. Yıldırım and Yorulmaz [27] reported GE contents that vary between 0.19 and $0.48 \text{ mg}\cdot\text{kg}^{-1}$ for sunflower oils used in deep-fat frying of potatoes. Xu et al. [28] determined $0.58 \text{ mg}\cdot\text{kg}^{-1}$ of GE in fresh high oleic sunflower oil and reported a degradation rate for GEs by deep-fat frying process, decreasing to $0.05 \text{ mg}\cdot\text{kg}^{-1}$ at the end of the frying. Kalkan et al. [29] investigated the formation of GEs in sunflower oil by frying using central composite design. The process parameters were temperature, duration, salinity, and the results have shown that the formation of GEs was observed in hardest process conditions (180°C , 40 minutes, $300 \text{ mg NaCl}/100 \text{ ml}$).

The glycidyl ester content of fresh corn oil was $0.10 \text{ mg}\cdot\text{kg}^{-1}$. Xu et al. [30] reported $0.25 \text{ mg}\cdot\text{kg}^{-1}$ and MacMahon et al. [26] reported $0.68 \text{ mg}\cdot\text{kg}^{-1}$ of GE for corn oils. The corn oil samples of the current work that were pan-fried at 160 for 15 minutes, at 180°C for 10 and 15 minutes and at 200°C for 5 and 15 minutes were found to be devoid of the glycidyl esters.

The GE levels of hazelnut oils used in pan-frying trials of the current work ranged in 0.09 - $0.14 \text{ mg}\cdot\text{kg}^{-1}$. Arslan et al. [18] previously described 0.04 - $0.14 \text{ mg}\cdot\text{kg}^{-1}$ levels of GE for hazelnut oils. The oil sample used for pan frying at 160°C for 10 minutes had slightly lower GE level than other samples.

Margarine was detected to be the richest fat in terms of GEs in the present study. The fresh margarine sample contained a mean of $0.54 \text{ mg}\cdot\text{kg}^{-1}$ of GE lower than the findings of Goh et al. [26] and higher than the results of Hidalgo-Ruiz et al. [31]. The GE levels of margarine samples were found to increase as the intensity of the process was increased. Raczyk et al. [11] reported a moderate increase for margarines pan-fried over 15 minutes.

4. CONCLUSION

The study reports the changes in 3-MCPD and GE contents of different vegetable oils by frying time and temperature during pan-frying process. 3-MCPD ester contents of the margarine samples were detected to be over the regulatory limit of 1.25 ppm in both fresh and fried samples, whereas corn, sunflower and hazelnut oils' concentrations were within the established value. The GE levels of all frying oil samples were within the maximum regulatory limit of 1 ppm . Margarines are possible important contributors to dietary exposure of 3-MCPD-Es and GEs. Fresh virgin olive oil was found to be free of the contaminants and no endogenous formation was observed during pan-frying. Hence, potatoes pan-fried in virgin olive oil sound to be safer in terms of bound 3-MCPD and glycidol amounts. The 3-MCPD and GE content of the initial fresh oil seem to be more effective on the content of final oil and consequently the fried product than the formation or reduction of the contaminants throughout the frying cycles. A number of works have been published on the effect of frying on the formation of these undesired heat-induced contaminants.

Table II. The effect of time and temperature on glycidyl ester content of frying oils ($\text{mg}\cdot\text{kg}^{-1}$)

Frying temperature ($^\circ\text{C}$)	Frying time (min)	Sunflower oil	Corn oil	Hazelnut oil	Virgin olive oil	Margarine
Fresh oil		0.09 ± 0.01^a	0.10 ± 0.03^a	0.14 ± 0.01^a	ND	0.54 ± 0.02^a
160	5	0.11 ± 0.02^{ab}	0.09 ± 0.02^a	0.12 ± 0.03^{ab}	ND	0.66 ± 0.09^c
	10	0.10 ± 0.02^{ab}	0.10 ± 0.01^a	0.09 ± 0.02^b	ND	0.65 ± 0.04^c
	15	0.11 ± 0.01^{ab}	ND	0.14 ± 0.01^a	ND	0.67 ± 0.01^c
180	5	0.12 ± 0.01^{ab}	0.08 ± 0.03^a	0.14 ± 0.02^a	ND	0.56 ± 0.01^{ab}
	10	0.11 ± 0.01^b	ND	0.14 ± 0.01^a	ND	0.67 ± 0.05^c
	15	0.10 ± 0.00^{ab}	ND	0.13 ± 0.02^a	ND	0.76 ± 0.06^d
200	5	0.11 ± 0.01^{ab}	ND	0.14 ± 0.03^a	ND	0.63 ± 0.03^{bc}
	10	0.12 ± 0.01^b	0.08 ± 0.02^a	0.14 ± 0.02^a	ND	0.65 ± 0.02^c
	15	0.12 ± 0.01^b	ND	0.13 ± 0.02^a	ND	0.66 ± 0.05^c

Mean values followed by the same letter in each column are not significant different at $p < 0.05$ by ANOVA and Duncan's test.

ND: Not detected.

Yet, the present work is the first one investigating the effect of frying temperature and time on 3-MCPD and glycidyl ester levels of different vegetable oils during pan-frying. More studies should be conducted to better understand the contamination routes of the fried foods to reduce dietary human exposure.

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Conflict of interest

The authors declare they have no conflict of interest.

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