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3-MCPD and glycidol levels in edible oils and fats obtained from local markets in Türkiye

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SUMMARY: In this study, it was aimed to determine the 3-MCPD and glycidol levels in 9 types (46 brands) of edible fat and oil offered for sale in markets located in Türkiye. 3-MCPD and glycidol levels were determined by making some modifications to the DGF C VI 18 (10) method. The highest levels of 3-MCPD and glycidol levels were detected in hazelnut oils, riviera olive oils, margarines, and shortenings. As expected, these contaminants were not observed in extra-virgin olive oils, while they were detected at low levels in fish oils. The highest 3-MCPD levels were found in the range of 0.06-2.12 mg·kg^{-1} in hazelnut oil, 0.16-1.69 mg·kg^{-1} in riviera olive oils, and 0.17-1.17 mg·kg^{-1} in margarines. The highest glycidol levels were found in the shortenings in the range of 1.98-6.46 mg·kg^{-1}, followed by hazelnut oil (0.54-2.63 mg·kg^{-1}) and riviera olive oil (0.19-3.53 mg·kg^{-1}).

KEYWORDS: *3-MCPD; Edible oils; Glycidol; Margarine; Olive oil.*

RESUMEN: ***Niveles de 3-MCPD y glicidol en aceites y grasas comestibles obtenidos de mercados locales en Turquía.*** En este estudio, el objetivo fue determinar los niveles de 3-MCPD y glicidol en 9 tipos (46 marcas) de grasas y aceites comestibles ofrecidos a la venta en mercados ubicados en Turquía. Los niveles de 3-MCPD y glicidol se determinaron haciendo algunas modificaciones al método DGF C VI 18 (10). Los niveles más altos de 3-MCPD y glicidol se detectaron en aceites de avellana, aceites de oliva riviera, margarinas y mantecas. Como era de esperar, estos contaminantes no se observaron en los aceites de oliva virgen extra, mientras que se detectaron en niveles bajos en los aceites de pescado. Los niveles más altos de 3-MCPD se encontraron en el rango de 0,06-2,12 mg·kg^{-1} en aceite de avellana, 0,16-1,69 mg·kg^{-1} en aceites de oliva riviera y 0,17-1,17 mg·kg^{-1} en margarinas. Los niveles más altos de glicidol se encontraron en las mantecas en el rango de 1,98-6,46 mg·kg^{-1}. Le siguieron el aceite de avellana (0,54-2,63 mg·kg^{-1}) y el aceite de oliva riviera (0,19-3,53 mg·kg^{-1}).

PALABRAS CLAVE: *3-MCPD; Aceites comestibles; Aceite de oliva; Glicidol; Margarina.*

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1. INTRODUCTION

Most of the edible oils on the market shelves are made ready for consumption by refining. With the help of the refining process, sensory characteristics such as taste, smell, appearance, and shelf stability are provided (Pudel *et al.,* 2011). Due to the application of high temperatures, process contaminants may be formed as a result of some reactions (hydrolysis, oxidation) (Shahidi, 2005). 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl esters (GE) are formed during several processes including frying, cooking, distillation, and refining (Zhou *et al.,* 2014). 3-MCPD and GE could be formed during the refining of crude oils, especially in the deodorization stage where high temperatures are applied (230-250 °C) (Özdikicierler *et al.,* 2016). These researchers stated that the formation of contaminants accelerated with the increase in monoglyceride, diglyceride, and chloride ion concentrations in the environment. Cyclic acyloxonium ions and glycidyl esters are formed when mono and diglycerides are formed as a result of triglyceride hydrolysis when edible fats and oils are exposed to high temperatures. These compounds are converted into 3-MCPD with the presence of chloride ions (Hamlet *et al.,* 2011). In the presence of water and at high temperatures, triglyceride is hydrolyzed to diglyceride and monoglyceride. In glycerol and glycerides, 3-MCPD ester formation increases with increasing salt concentration and reaches its maximum level with approximately 15% water content (EFSA, 2016). 3-MCPD are formed as a result of the replacement of the acyl or hydroxyl groups in the acylglycerol molecule with the chloride ion (Svejkovska *et al.,* 2006). In the absence of water in the environment, pre-hydrolysis of the acyl groups cannot occur, but the hydroxyl group of the glycerol molecule and the chlorine are directly replaced (EFSA, 2016). It has been stated that precursors such as monoglycerides, diglycerides, and chloride ions which cause 3-MCPD formation, as well as parameters such as processing temperature and duration, play a significant role in 3-MCPD formation (Sevindirici *et al.,* 2018). In addition, it was observed by Shimizu *et al.* (2012) that glycidol was formed from di- and monoacyl glycerols in the absence of chlorine ions at temperatures of 240 °C and above. However, Destaillats *et al.* (2012) determined that the majority of MCPD diesters are formed above 200 °C by the reaction of organochlorines with triacylglycerols. Additional experiments by these researchers confirmed that this reaction could be initiated during palm oil deodorization with hydrogen chloride (HCl) gas released through the thermal degradation of the organochlorines present in the oil. Since toxicological studies have shown that the free form of 3-MCPD is carcinogenic (Cho *et al.,* 2008), 3-MCPD has possible health risks. Similar to 3-MCPD, glycidol is also highly risky for health because of its epoxy ring structure (IARC, 2013). The International Agency for Research on Cancer has classified 3-MCPD as a "potential carcinogen to humans" in group 2B. Glycidyl, on the other hand, was stated to have mutagenic and carcinogenic properties and classified in group 2A, which means that it was "probably carcinogenic to humans" (IARC 2013). According to the report published in 2018, the tolerable daily intake of 3-MCPD was recommended as 2 $\mu g \cdot kg^{-1}$ body weight (EFSA 2018).

Regulation EU 2020/1322, amending Regulation EC No. 1881/2006, applies as of January 1st 2021 with regard to foods in the EU. This specifies maximum quantities for free 3-MCPD in hydrolyzed plant protein and soya sauce as well as maximum quantities for glycidyl fatty acid esters, expressed as glycidol, in vegetable oils and fats, fish oils and other marine oils which are placed on the market for consumers or for use as an ingredient in foods (maximum quantity 1 $mg \cdot kg^{-1}$) (EU, 2020). In addition, EU regulation set the maximum level for GE at 1 $mg \cdot kg^{-1}$ in vegetable oils and fats which are aimed for the final consumer or as an ingredient in food. In addition, the level of GE for vegetable oils and fats destined for baby food and processed cereal-based food is set at 0.5 $mg \cdot kg^{-1}$ (EU, 2020). With the same regulation, maximum quantities for the sum of free and fatty acid bound 3-MCPD (analyte group) are set for the same foods for which maximum levels for GE are established. Due to the low 3-MCPD formation potentials, a stricter maximum level (1.25 $mg \cdot kg^{-1}$) for the sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD is set for oils and fats from coconut, maize, rapeseed, sunflower, soybean, palm kernel and olive oils and mixtures of oils and fats with oils and fats only from this category. A higher maximum level of 2.5 $mg \cdot kg^{-1}$ applies to other vegetable oils (including olive pomace oils), fish oils and oils from other marine organisms and mixtures of oils and fats with oils and fats

only from this category. For oil mixtures from both categories with quantitatively known ingredients, the category-related maximum levels apply to single ingredients. In the case of oil and fat mixtures of unknown composition, the higher maximum level of 2.5 $mg \cdot kg^{-1}$ applies. For vegetable oils and fats, fish oils and other marine oils which are destined for the production of baby food and processed cereal-based food for infants and young children the maximum level for the sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD is set to 0.75 $mg \cdot kg^{-1}$. Finally, for infant formula, follow-on formula and foods for special medicinal purposes for infants and young children as powders the maximum level is addressed as 0.125 $mg \cdot kg^{-1}$ for powders and as 15 $\mu g \cdot kg^{-1}$ for liquids. These limits set by the EU are valid for European countries.

Although there are many studies in the literature on the detection of 3-MCPD and glycidol in different food products, few studies have been conducted in Türkiye on the determination of 3-MCPD and glycidol in potato chips (Önal *et al.,* 2016), in steam distillation of olive oils (Özdikicierler *et al.,* 2016), in baking in biscuit making (Mogol 2014), or in frying stages (Deniz Şirinyıldız *et al.,* 2019). These processes applied to the oils mentioned above may differ from region to region. In this respect, we think that it is important to examine the edible fats and oils offered for sale in the country's markets in terms of these process contaminants. Considering the results of this study, the daily intake limits determined by the relevant authorities for consumers may not be exceeded. In addition, manufacturers can make efforts to limit these compounds. Within the scope of this study, sunflower oil (7 brands), hazelnut oil (4 brands), corn oil (6 brands), natural extra virgin olive oil (7 brands), riviera olive oil (7 brands), margarine and shortening (9 brands), peanut oil (2 brands), and fish capsules (4 brands) which are sold and consumed widely in the markets of Türkiye were investigated. In total, 3-MCPD and glycidol levels were detected in 46 samples.

2. MATERIALS AND METHODS

2.1. Materials

The different cooking oils, fish oils, margarines and shortenings used in the study were obtained from local markets in Türkiye in 2019. Their names and number of brands are shown in Table 1. All of these oils, except for virgin olive oil and fish oils, are refined oils. Virgin olive oil is obtained by cold pressing., that is, it is not refined. Riviera oil consists of 20% virgin olive oil and 80% refined olive oil. 3-MCPD and 3-chloro-1,2-propane-1,1,2,3,3-d5-diol (3-MCPD-d5), glycidyl stearate, diethyl ether, methanol, sodium hydroxide, sodium bromide, ethyl acetate, phenylboronic acid (PBA), acetone and toluene were obtained from Sigma-Aldrich (Steinheim, Germany). The purity of the chemicals was ensured.

Table 1. The sample names and number of brands.

Sample names	Number of brands
Sunflower oil (Refined)	7
Hazelnut oil (Refined)	4
Peanut oil (Refined)	2
Corn oil (Refined)	6
Virgin Olive Oil (Cold-pressed)	7
Riviera olive oil (Blend from refined and cold pressed)	7
Pastry oil (Shortening) (Refined)	3
Margarine (Refined)	6
Fish oil (capsule) (Native-Refined)	4
Total number of samples	46

2.2. Methods

2.2.1. Preparation of samples

The lipid fractions of margarine and fats were obtained by centrifugation of the molten product, then filtered through anhydrous sodium sulfate by removing the upper oil phase. In margarines and shortenings, analyses were performed only for the lipid phase. Encapsulated fish oils were removed from the capsules with a sterile needle. Bottled edible oils were taken with a straw directly from the bottle in which they were kept in the market. All oil samples were stored at 4 °C in a dark environment until the experiments.

2.2.2. Analysis of 3-MCPD and glycidol

3-MCPD and glycidol levels were determined based on the standard method of DGF C VI 18 (10) (DGF, 2011) with some modifications. In part A of

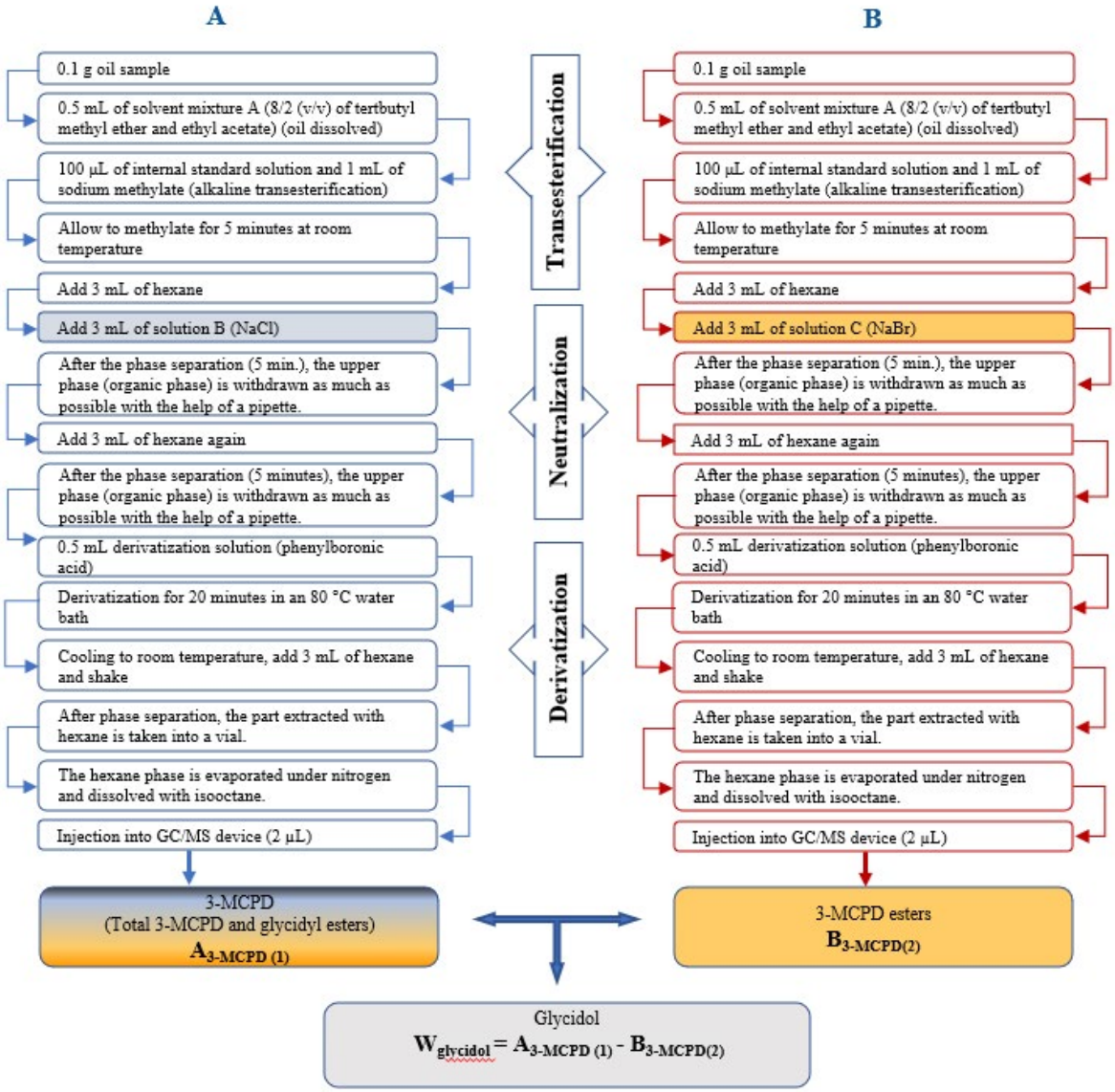

Figure 1. 3-MCPD and Glycidol analysis flow chart.

this method, 3-MCPD, corresponding to the sum of bound 3-MCPD and bound glycidol was determined, as in the DGF C VI 17 (10) method. We modified the DGF C VI 18 (10) method by applying the DGF C VI 17 (10) method in part A. We modified the DGF C VI 18 (10) method part B so that only the amount of ester-bound 3-MCPD was determined. We did not intend to determine 2-MCPD in this part B. Therefore, the relevant standard was not used. Both methods which form the basis of our method, namely C-VI 17 (10) and C-VI 18 (10), have been developed and approved only for the analysis of edible oils and fats. The flow chart of processing steps is shown in Figure 1. According to this method, isotope-labeled 3-MCPD-d5 was used as an internal standard. All oil samples were derivatized after esterification and neutralization processes and injected into the GC/MS (Shimadzu GC-2010 Gas Chromatography-QP-2010 Ultra Mass Spectrometry System, Shimadzu Corporation, Kyoto, Japan) device. The operating conditions of the GC/MS device are provided in Table 2.

Solutions used in esterification, neutralization, and derivatization in analysis. A sodium methylate solution with methanol ($NaOCH_3$): 0.5 molar methanol solution of sodium methylate (27 grams of sodium methylate tart) was dissolved in 1 liter of methanol.

Table 2. Operating conditions of the GC/MS device.

Device:	Shimadzu GC-2010
Detector:	EI+, SIM Mode
Internal standard Mass:	m/z = 201 or 150 (3-MCPD- d5) m/z = 196 or 147 (3-MCPD)
Column:	TRB - 5MS, 30m × 0.25mm × 0.25µm
Gases:	Helium 2.1 mL/min.
Temperature:	Oven Program: 60 °C (1 min), 6 °C·min^{-1} to 190 °C 190 °C - 280 °C to 20 °C /min (10–30 min)
Split: **Splitless time:**	20 mL·min^{-1}. 1.5 min.

Solvent mixture A: 8/2 (v/v) mixture of tertbutyl methyl ether and ethyl acetate

Sodium chloride (NaCl) solution: 20% (m/v) aqueous solution of sodium chloride

Sodium bromide (NaBr) solution: 60% (m/v) aqueous solution of sodium bromide

Solvent mixture B: A mixture of 30 mL of sodium chloride solution and 1 mL of acetic acid (v/v) (prepared daily)

Solvent mixture C: A mixture of 30 mL of sodium bromide solution and 1 mL of acetic acid (v/v) (prepared daily)

Derivatization solution: 2.5 grams of phenylboronic acid dissolved in a mixture of 19 mL of acetone and 1 mL (m/v) distilled water

Internal standard stock solution: 2000 mg·L^{-1} (m/v) of 3-MCPD-d5 dissolved in ethanol

Internal standard solution: Prepared from the internal standard stock solution by dissolving in tert-butyl methyl ether (20 mg·L^{-1}).

The first step in the determination of 3-MCPD and glycidol based on the indirect DGF C-VI 18 (10) method was to evaluate the efficiency of the conversion from glycidol to 3-MCPD following the method used for Assay A. Figure 2 shows the amount of 3-MCPD formed as a function of the amount of glycidol (in the form of glycidyl stearate) in a spiked blank oil (olive oil) at seven different levels (0.05; 0.1; 0.25; 0.5; 1; 2.5 and 5 mg·kg^{-1}). A linear regression of the type y = mx + b was performed, the reciprocal slope (1/m) provided the conversion factor (t) (Lucas *et al.,* 2017).

Two-stage analysis was performed. In the first step (A), the total amount of 3-MCPD and glycidol in the sample, expressed as 3-MCPD, was calculated according to Eq.1. Here, 3-MCPD and glycidol were not separated. Therefore, the solvent was prepared with sodium bromide instead of sodium chloride in the second step (B) of the analysis in order to prevent the conversion of glycidol to 3-MCPD (Karl *et al.,* 2016). In the second step, pure or bound 3-MCPD was determined according to the Eq.2. The glycidol levels were calculated by subtracting the result from the second stage from the result from the first stage and multiplying the conversion rate of glycidol to 3-MCPD (Eq.3).

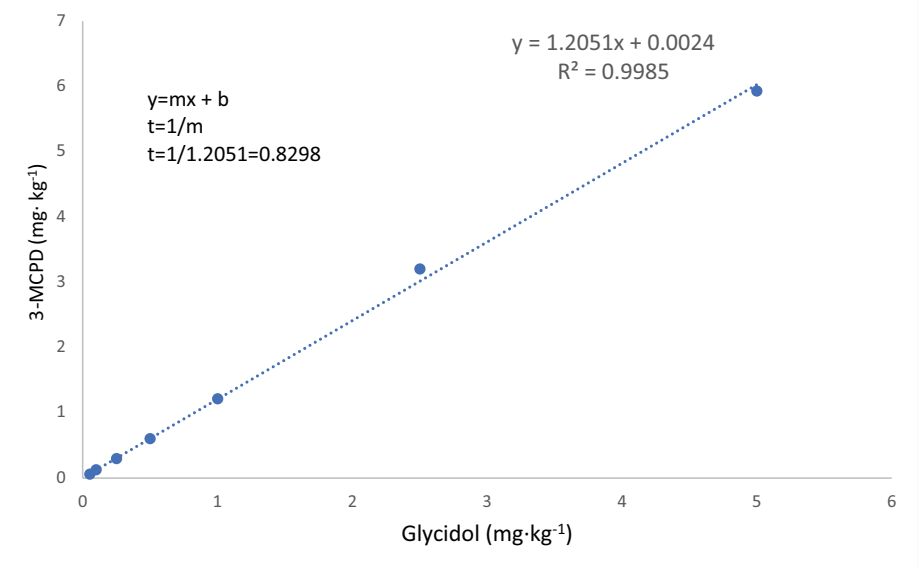

Figure 2. The amount of 3-MCPD formed as a function of the amount of Glycidol at seven different levels. A linear regression of the type y = mx + b was performed, the reciprocal value of the slope (1/m) provides the conversion factor (t).

$$A_{3\text{-MCPD (1)}} = [Q\,(147) \times C_{d5-3-MCPD\,(1)}] / Q\,(150) \quad \text{(Eq. 1)}$$

$A_{3\text{-MCPD (1)}}$ = Mass fraction of 3-MCPD (mg·kg^{-1}) detected in the first step
Q (147) = Peak area of 3-MCPD determined in the first step
Q (150) = Peak area of 3-MCPD – d5 determined in the first step
$C_{d5-3-MCPD\,(1)}$ = Concentration of internal standard (mg·kg^{-1}) used in the first step

$$B_{3\text{-MCPD (2)}} = [Q\,(147) \times C_{d5-3-MCPD\,(2)}] / Q\,(150) \quad \text{(Eq. 2)}$$

$B_{3\text{-MCPD (2)}}$ = Mass fraction of 3-MCPD (mg·kg^{-1}) detected in the second step
Q (147) = Peak area of 3-MCPD determined in the second step
Q (150) = Peak area of 3-MCPD-d5 determined in the second step
$C_{d5-3-\mathbf{MCPD\,(2)}}$ = Concentration of internal standard (mg·kg^{-1}) used in the second step

$$W_{Glycidol} = t \times (A_{3\text{-MCPD (1)}} - B_{3\text{-MCPD (2)}}) \quad \text{(Eq. 3)}$$

$W_{Glycidol}$ = Mass fraction of glycidol in samples (mg·kg^{-1})
t = In the equation in the created calibration graph (Figure 2), the ratio of 1/m (y=1.2051x+0.0024, R^2=0.9984).
$A_{3\text{-MCPD (1)}}$ = Mass fraction calculated in the first step
$B_{3\text{-MCPD (2)}}$ = Mass fraction calculated in the second step

2.3. Statistical analysis

All data were statistically analyzed using SPSS (version 20.0 for Windows, SPSS Inc., Chicago, Illinois) package program by conducting one-way analysis of variance (ANOVA), and defining a significant difference at $P < 0.05$ by Duncan's test. All measurements were performed with triplicate fresh samples, and values were expressed as means ± SD of triplicates from each independent experiment.

3. RESULTS AND DISCUSSION

3.1. The levels of 3-MCPD and glycidol in sunflower oils

The levels of 3-MCPD and glycidol determined in the sunflower oil samples are presented in Figure 3A. The amounts of 3-MCPD in the sunflower oil samples belonging to 7 different brands were determined in the range of 0.02-0.44 mg·kg^{-1}. The highest 3-MCPD were determined in SO6, the lowest 3-MCPD were obtained from the SO2 sample. On the other hand, while the lowest glycidol was found in the SO5 sample, the highest glycidol was detected in the SO4 sample. It is noteworthy that the amount of glycidol was higher compared to 3-MCPD (Figure 3A). The levels of

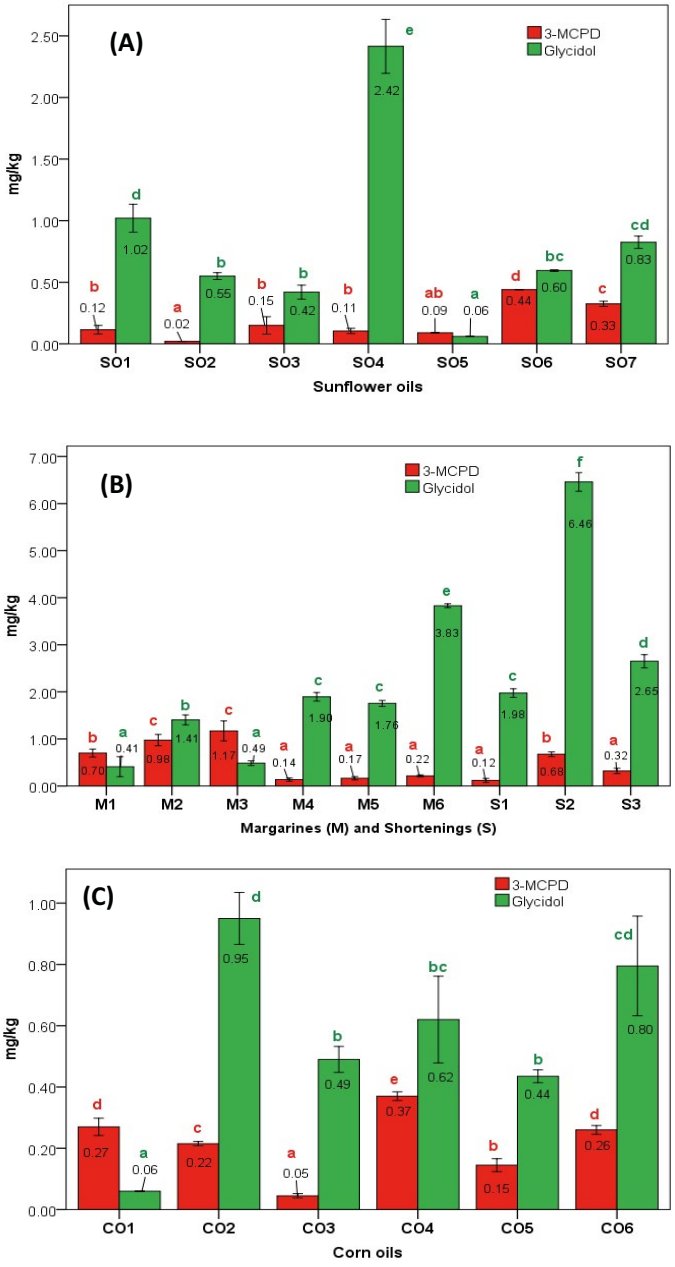

Figure 3. 3-MCPD and glycidol levels in sunflower oils (A), margarines and shortenings (B), corn oils (C). Means followed by similar letters of the same color on the bars are not significantly different at 5% probability level by the Duncan test. All treatments were performed in three replicates.

3-MCPD in the sunflower oil samples were found to be SO6 > SO7 > SO3 > SO1 > SO4 > SO5 >SO2. On the other hand, the levels of glycidol were found to be SO4 > SO1 > SO7 > SO6 > SO2 > SO3 < SO5. Kuhlmann (2011) found 3-MCPD values in sunflower oils in the range of 0.1-2.1 mg·kg^{-1} and glycidol values in the range of 0.1-0.4 mg·kg^{-1}. The same author found 3-MCPD levels in

the range of 0.08-0.96 mg·kg^{-1} and glycidol levels in the range of 0.02-0.90 mg·kg^{-1} in sunflower oil in another study (Kuhlmann 2016). Zelinkova *et al.* (2006) determined 3-MCPD values of less than 0.1 and 0.3 mg·kg^{-1} in two types of sunflower oil. Weißhaar and Perz (2010) found 3-MCPD values for sunflower oils of less than 1.0 mg·kg^{-1} and glycidol values of less than 0.4 mg·kg^{-1}. The results of the current study partially overlap with these literature findings. The majority of our glycidol values were higher than those. The possible reason for this variation in glycidol levels may be the difference in parameters such as temperature and time applied in refining. The glycidol in SO1 and SO4 samples were determined as 1.02 and 2.42 mg·kg^{-1}, respectively. They were determined in the range of 0.06-0.83 mg·kg^{-1} in other samples. Similarly, Kalkan *et al.* (2021) determined glycidol in the range of 0.06-0.72 mg·kg^{-1} in sunflower oil during the frying of french fries in their research.

3.2. The levels of 3-MCPD and glycidol in margarines and shortenings

The levels of 3-MCPD and glycidol determined in different kinds of margarine and shortenings are presented in Figure 3B. The 3-MCPD concentration was found in the range of 0.14-1.17 mg·kg^{-1}, and the glycidol concentration in the range of 0.41-3.83 mg·kg^{-1} for 6 different kinds of margarine. The results obtained for margarine and shortening are valid only for the oil phase. The concentrations of 3-MCPD and glycidol in three shortenings were in the range of 0.12-0.68 mg·kg^{-1} and 1.98-6.46 mg·kg^{-1}, respectively. It was observed that the glycidol level was higher than 3-MCPD in all kinds of margarine except for M1 and M3 samples (Figure 3B). The glycidol level was found significantly higher in M6 samples compared to the other samples ($p < 0.05$). No significant changes were observed among the M4, M5, and M6 samples in terms of 3-MCPD values. Similarly, the 3-MCPD values in M2 and M3 samples were not statistically different. In terms of glycidol, there was no significant difference between M1 and M3, or M4 and M5. By looking at the shortenings, the amounts of glycidol were found significantly higher than the 3-MCPD ($p < 0.05$). The amount of glycidol in S2 shortening samples was approximately 2.5-3 times higher than the other samples. Custodio-Mendoza *et al.* (2019) determined that the total bound 3-MCPD concentrations in the lipid fractions of margarines were in the range of 0.11-2.61 mg·kg^{-1}. The 3-MCPD values were found in the range of 0.79-1.60 mg·kg^{-1} in five samples (Li *et al.,* 2015), 0.4-4.5 mg·kg^{-1} in 37 samples (Weißhaar 2011), and 0.09-0.43 mg·kg^{-1} in four kinds of margarine (fat portion) and 0.50 mg·kg^{-1} in a vegetable shortening (Becalski *et al.,* 2015). In a report published in the Netherlands (Boon and te Biesebeek 2016), 3-MCPD concentrations were reported as 0.16-1.8 mg·kg^{-1} in seven margarines and shortenings. These findings are in agreement with the current results. The glycidol concentrations (0.15-5.5 mg·kg^{-1}) determined by Weißhaar (2011) in 22 margarines (fat portion) were similar to our findings. Deniz Şirinyıldız *et al.* (2019) found 3-MCPD concentrations in the range of 0.57-4.54 mg·kg^{-1} in 14 margarines obtained from the market in Türkiye. The possible reason why these values were higher in margarines compared to many other oils may be salt and water contents. As known, palm oil is generally used to produce margarine. Palm oil is a fruit oil and contains more water than seed oils. Therefore, it is more sensitive to hydrolysis reactions. The high levels of monoglyceride and diglyceride formed as a result of these reactions cause the formation of 3-MCPD and glycidol (Shahidi and Zhong, 2005). On the other hand, the presence of organochlorine compounds in palm oil has been implicated as a potential source of chlorine for the formation of 3-MCPD (Nagy *et al.,* 2011). While the palm tree is growing, it absorbs chloride ions from the soil and water, which paves the way for the formation of 3-MCPD (Anonymous, 2018). In addition, the formation of 3-MCPD in palm oil is directly related to the oil's exposure to high temperatures during refining (Franke *et al.,* 2009; Weißhaar, 2008).

3.3. The levels of 3-MCPD and glycidol in corn oils

The levels of 3-MCPD and glycidol determined in corn oils are presented in Figure 3C. The 3-MCPD and glycidol values were determined to be in the range of 0.05-0.37 mg·kg^{-1}, and 0.06-0.95 mg·kg^{-1}, respectively in six corn oils. The glycidol levels were found higher than 3-MCPD in corn oil samples except for CO1 samples.

While the mean value of 3-MCPD in corn oils was 0.22 mg·kg^{-1}, the mean glycidol value was 0.56 mg·kg^{-1}. The difference between the values of the samples was found statistically significant ($p < 0.05$). Among the samples, the CO1 sample appears to be more stable in terms of these process contaminants. Kuhlmann (2011) found that the 3-MCPD value in corn oil was 0.2 mg·kg^{-1} and the glycidol value was 0.7 mg·kg^{-1}. The result of the current study was similar to these findings. In another study, the amounts of 3-MCPD and glycidol levels in corn oils were found to be < 1.7 and < 0.6 mg·kg^{-1}, respectively (Weißhaar 2011). Zelinkova *et al.* (2006) reported 3-MCPD values of less than 0.3 and 0.372 mg·kg^{-1} in crude and refined corn oils, respectively. These results are also in good agreement with our findings. Deniz Şirinyıldız *et al.* (2019) found that the 3-MCPD concentration was 0.51-2.49 mg·kg^{-1} in five corn oil samples. The values determined in the current study were lower than those findings.

3.4. The levels of 3-MCPD and glycidol in hazelnut and peanut oils

The amount of 3-MCPD and glycidol levels in hazelnut and peanut oils are exhibited in Figure 4A. While the 3-MCPD concentration was found as 0.06 mg·kg^{-1} in HO4 samples, it was found as 1.13-2.12 mg·kg^{-1} in the other three hazelnut oils (HO1, HO2, and HO3). Deniz Şirinyıldız *et al.* (2019) found that the 3-MCPD concentration in three hazelnut oils was in the range of 0.24-0.45 mg·kg^{-1}. Our results (except HO4) were higher than these values. The possible reason for this variation may be the difference in parameters such as temperature and time applied in refining. The amounts of glycidol in hazelnut oil samples were determined to be in the range of 0.54-2.63 mg·kg^{-1}. While there was no significant difference between the 3-MCPD values of HO2 and HO3 samples, the difference between 3-MCPD amounts in these samples and others was found statistically significant ($p < 0.05$). The same trend was followed for the glycidol amounts in the samples. The order of 3-MCPD and glycidol levels determined in hazelnut oil samples were found to be HO2 > HO3 > HO1 > HO4. Kuhlmann (2011) found the 3-MCPD value at 19 mg·kg^{-1} and the glycidol value at 0.5 mg·kg^{-1} for hazelnut oil. Zelinkova *et al.* (2006) reported that the amount of 3-MCPD in unrefined crude hazelnut oil was less than 0.1 mg·kg^{-1}. According to the results obtained in the current study, it was important to indicate that these compounds were formed in higher concentrations in hazelnut oils compared to the other oil samples analyzed in the study. It is well known

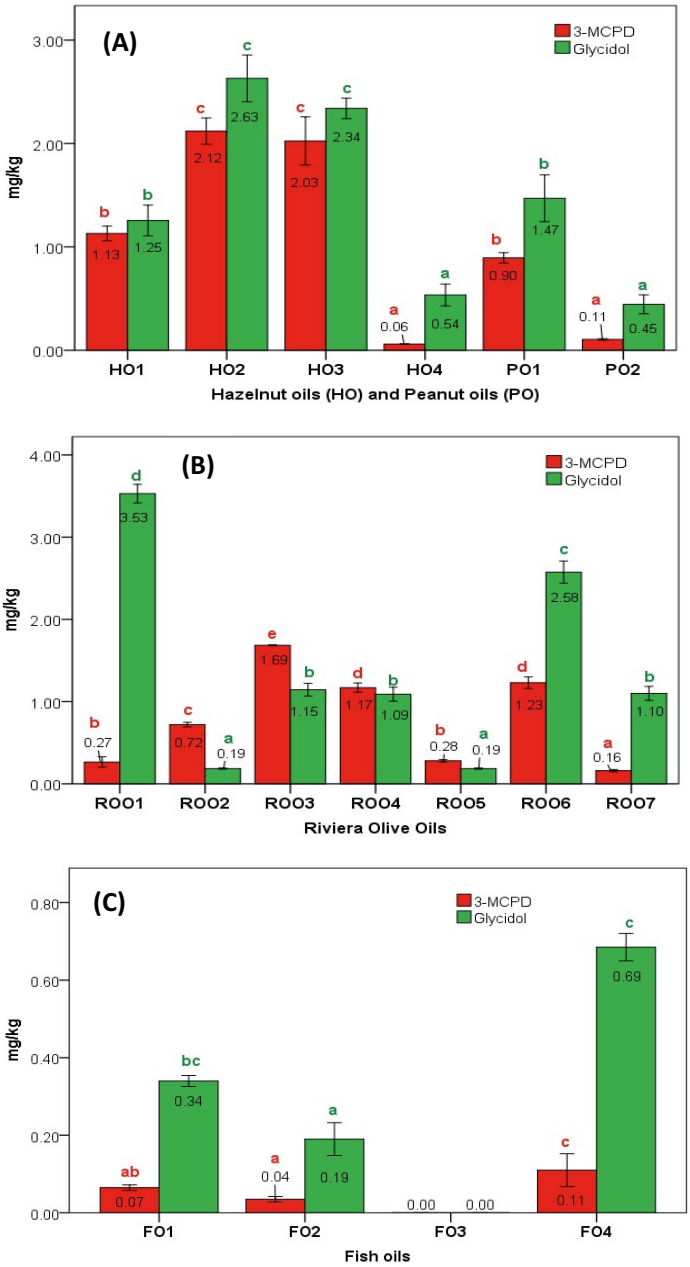

Figure 4. 3-MCPD and glycidol levels in hazelnut and peanut oils (A), riviera olive oils (B), fish oils (C). Means followed by similar letters of the same color on the bars are not significantly different at 5% probability level by the Duncan test. All treatments were performed in three replicates.

that fruit oils such as hazelnut oil are more prone to hydrolysis because of their high water content compared to seeds. It has been determined that the risk of 3-MCPD formation during the refining of these oils is quite high because of the formation of mono and diglycerides as a result of the hydrolysis reaction and the chlorine ion in the structure of the fruits which pass into the oil (Xu *et al.,* 2016).

In two peanut oils, 3-MCPD concentrations were found to be 0.90 and 0.11 mg·kg^{-1}, and glycidol concentrations were found at 1.47 and 0.45 mg·kg^{-1} (Figure 4A). Li *et al.* (2016) found that the 3-MCPD contents in 3 different peanut oils after deodorization to be 0.43-0.62 mg·kg^{-1}. Kuhlmann (2011) determined that the 3-MCPD content was 0.1-0.9 mg·kg^{-1} and glycidol content was 0.4-1.1 mg·kg^{-1} in peanut oils. Similar results were found for the 3-MCPD level by Zelinkova *et al.* (2006) at < 0.1 mg·kg^{-1} and Li *et al.* (2015) at 0.45-1.18 mg·kg^{-1}. The results of the studies given above are similar to our observations. The amounts of 3-MCPD and glycidol levels determined in these peanut oils we used in our study showed a significant difference ($p < 0.05$). The amount of 3-MCPD in PO1 oil was 8 times higher than that of PO2, and the amount of glycidol was approximately 3 times higher.

3.5. The levels of 3-MCPD and glycidol in extra virgin olive oils

The 3-MCPD and glycidol levels were not detected in 7 different extra virgin olive oils used in the study. It was expected that extra virgin olive oil does not contain any traces of 3-MCPD or glycidol since it is mechanically extracted without any heat treatments. The 3-MCPD in edible oils were studied by Jedrkiewicz *et al.* (2016) and according to the obtained results from that study, MCPD were not detected in cold-pressed or unrefined edible oils. Similarly, the 3-MCPD and glycidol in extra virgin olive oils were not determined in the study by Custodio-Mendoza *et al.* (2019). On the other hand, Zelinkova *et al.* (2006) determined the amount of 3-MCPD to be less than 0.1 mg·kg^{-1} in virgin olive oils and in the range of 0.3-2.4 mg·kg^{-1} in refined olive oils.

3.6. The levels of 3-MCPD and glycidol in riviera olive oils

According to the International Olive Council classification, riviera olive oil is an oil consisting of a mixture of refined olive oil and extra virgin olive oil, and is suitable for consumption. It has a free acidity, expressed as oleic acid, of not more than 1.00 gram per 100 grams and its other physico-chemical and organoleptic characteristics correspond to those fixed for this category in this standard (IOOC, 2021). Riviera olive oil is the oil whose properties are improved by mixing natural olive oil in different proportions ranging from 5 to 20% with refined olive oil (Türkoğlu *et al.,* 2012). The amounts of 3-MCPD and glycidol levels determined in riviera olive oil samples are shown in Figure 4B. The 3-MCPD values varied between 0.16-1.69 mg·kg^{-1} ($p < 0.05$). The highest level was determined in ROO3 oil, the lowest level was determined for ROO7 oil. The amounts of glycidol were found in the range of 0.19-3.53 mg·kg^{-1} ($p < 0.05$). It is noteworthy that the variation in glycidol concentration was higher than the variation in 3-MCPD concentration. Kuhlmann (2011) found 3-MCPD amounts in the range of 0.3-1.2 mg·kg^{-1} and glycidol amounts in the range of 0.1-0.4 mg·kg^{-1} in riviera olive oils. While the amounts of 3-MCPD in this study were similar to our findings, the amounts of glycidol only partially matched our results. Weißhaar and Perz (2010) determined that the average 3-MCPD value was 1.2 mg·kg^{-1} and glycidol was 0.3 mg·kg^{-1} in six riviera olive oil samples.

3.7. The levels of 3-MCPD and glycidol in fish oils

The 3-MCPD and glycidol contents detected in four different brands of fish oil are shown in Figure 4C. The 3-MCPD and glycidol values were not determined in the FO3 sample. The highest amount of 3-MCPD was obtained for the FO4 sample at 0.11 mg·kg^{-1}. While the glycidol could not be detected in the FO3 sample, the highest amount was found in the FO4 sample at 0.69 mg·kg^{-1}. Kuhlmann (2011) reported that the amounts of 3-MCPD and glycidol levels in different fish oils were less than 0.05 and 0.025 mg·kg^{-1}, respectively. Jedrkiewicz *et al.* (2016) determined that the 3-MCPD content in refined fish oils was in the range of 1.5-5.5 mg·kg^{-1}. The results of the present study were lower than these findings. The reason for this might be that the refining processes changed according to the fish type.

4. CONCLUSIONS

This study surveyed the contamination levels of 3-MCPD and glycidol in edible oils collected in 2019 in Türkiye. The results for different oils and

fats showed that 3-MCPD and glycidol levels did not occur in crude or natural oils and fats. 3-MCPD and glycidol were commonly detected in different amounts in refined oils and fats. The highest 3-MCPD amounts were found in some hazelnut and riviera olive oils. Glycidol was found above the limit values in most margarine, shortening, riviera and hazelnut oils. It is known that fruit oils such as hazelnut oil, olive oil and palm oil are more prone to hydrolysis because of their highwater content compared to seeds. It is thought that 3-MCPD and glycidol formation may be higher during the refining of these oils because of the hydrolysis reaction of triglycerides to form mono and diglycerides and the chlorine ion in the structure of the fruits, which pass into the oil. It was observed that the amounts of 3-MCPD did not exceed a certain level while the glycidol presented significantly higher maximum amounts. As expected, 3-MCPD and glycidol levels were not found in extra virgin olive oils as they were not subjected to refining processes. While these compounds were not detected in one brand in fish oils, 3-MCPD and glycidol were determined at 0.12 $mg \cdot kg^{-1}$ and less than 0.69 $mg \cdot kg^{-1}$, respectively in other fish oil samples. 3-MCPD and glycidol limit values in Türkiye are generally compatible with European regulations. When evaluated in this respect, the majority of margarine, shortening, hazelnut and riviera oils are above the glycidol limit value. 3-MCPD was above the limit in most of the hazelnut oils. The formation of 3-MCPD and glycidol can be limited by reducing their precursors such as monoglycerides and diglycerides in hazelnut, olive and palm oils before high temperature processing, as well as by reducing the palm oil content in margarine and shortening. A dietary risk assessment for 3-MCPD and glycidol was not performed in this study. In future studies, in the light of these data, the risk assessments of edible oils and fat-containing foods should be made.

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