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Monitoring changes in 3-monochloropropanediol, 2-monochloropropanediol, and glycidyl esters of palm olein after repeated deep frying of commercial chicken nuggets

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Abstract

The present work examined the changes in 3-monochloropropanediol ester (3-MCPDE), 2-monochloropropanediol ester (2-MCPDE), and glycidyl ester (GE) in palm olein after repeated deep frying of commercial chicken nugget samples at 160 and 180°C. Free fatty acid (FFA) composition, *p*-anisidine value (*p*-AV), and specific extinction at 232 and 268 nm (K_{232} and K_{268}) were determined. Results indicated that the concentrations of MCPDEs and GE in the frying oil after the fourth frying cycle were higher than the initial concentrations, except for the GE concentration when fried at 180°C. The content of 3-MCPDE for fried chicken nugget samples increased during deep frying at 160 and 180°C, but its 2-MCPDE and GE levels decreased. FFA contents in frying oil were found to increase over time; however, the increases were determined to be non-significant. *p*-AV increased linearly with higher values in the 180°C frying system. No significant relationship was found between the concentration of MCPDEs and GE formed in the frying oil with oil oxidation in the repeated deep frying of up to four cycles. All of the oil qualities were within safe consumption levels.

List of abbreviations

3 MCPDE: 3-monochloropropanediol ester; 2 MCPDE: 2-monochloropropanediol ester; GE: glycidyl ester; FFA: free fatty acid; *p*-AV: *p*-anisidine value; MAG: monoacylglycerol; DAG: diacylglycerol; PP-3-MCPD: rac 1,2-bis-palmitoyl-3-chloropropanediol; PP-2-MCPD: 1,3-dipimitoyl-2-chloropropanediol; Gly-P: glycidyl palmitate; PP-3-MCPD-d5: rac 1,2-bis-palmitoyls-3-chloropropanediol-d5; Gly-P-d5: glycidyl palmitate-d5; RBD: refined, bleached, and deodorised; LOD: limit of detection; LOQ: limit of quantification; 3-MBPD: 3-monobromopropanediol; NaOH: sodium hydroxide; GC-MS/MS: gas chromatography-tandem mass spectrometry; MRM: multiple reaction monitoring; *R*: correlation coefficients; R^2: coefficient of determination; NaCl: sodium chloride; and TAG: triacylglycerol.

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Introduction

Monochloropropanediol esters (MCPDEs) and glycidyl ester (GE) are known heat-induced contaminants that are present in various frying foods and food ingredients. MCPDEs are the esterified form of MCPD, whereas GE is the esterified form of glycidol. The presence of 3-MCPDE, 2-MCPDE, and GE in the diet is a health concern. These esters are effectively hydrolysed in the gastrointestinal tract, producing 3-MCPD, 2-MCPD, and glycidol. 3-MCPD was first discovered in acid-hydrolysed vegetable protein and soy sauce in 1978 (Velisek *et al*., 1978). It was subsequently found in edible oils and oil-based food products, especially in palm oil (Svejkovska *et al*., 2004; Ermacora and Hrnčiřík, 2014; Aniołowska and Kita, 2016a; 2016b; Abd Razak *et al*., 2019; Custodio-Mendoza *et al*., 2019).

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The free forms of 3-MCPD and glycidol have been categorised as group 2B (possible human carcinogens) and group 2A (probably carcinogenic to humans) carcinogens, respectively, by the International Agency for Research on Cancer (IARC) (IARC, 2000; 2013). In 2016 and 2018, the European Food Safety Authority (EFSA) investigated the potential health effects of 3-MCPDE and GE. Animal studies have shown renal and reproductive toxicity of 3-MCPDE (EFSA, 2016; 2018), and glycidol is also known to be genotoxic, promoting gene mutations leading to spontaneous DNA synthesis (BfR, 2009). As a result of insufficient toxicological data available on 2-MCPD, its potential hazard is considered equal to that of 3-MCPD (EFSA, 2016).

The EFSA has set maximum allowed amounts of 3-MCPD in food products, especially refined vegetable oils and infant formulae. On the basis of its toxicological hazards, EFSA determined a tolerated daily intake of 2 µg/kg body weight for 3-MCPD (EFSA, 2016). Research is still underway to determine the safe exposure limits, and many regulations that restrict 3-MCPD levels also attempt to include 2-MCPD. For GE, the European Union announced tough restrictions for infant formulae and other products for vulnerable populations. In March 2018, a maximum level of 1,000 µg/kg for GEs in vegetable oils and fats intended for use in food has been set.

The formation of 3-MCPDE, 2-MCPDE, and GE occurs predominantly during the refining process of vegetable oil. The use of refined vegetable oils as part of daily food processing or for cooking introduces these contaminants into different food varieties. 3-MCPD has been detected in many processed foods in their free form and esters with increased fatty acid levels. They are formed mainly when fat-containing foods with salt are heated to high temperatures, that is, when lipid components (glycerol or glycerol lipids) react with salt (chloride ions) (Haile and Satheesh, 2017). In other words, chloride is a necessary precursor to form MCPDEs. By contrast, the chloride content does not affect the concentration of GE in a heating system because chlorine is not found in the chemical structure of GE. In processed edible oils, GE has been discovered during the deodorisation portion of the refinery process when the oils are subjected to high temperatures (up to 200°C). GE has also been found to be related to the synthesis of 3-MCPDE, suggesting that GE might act as the primary precursor during food processing (Habermeyer *et al*., 2011).

Deep frying is the most commonly used method for preparing food at high temperatures (> 150°C) worldwide (Li *et al*., 2017). Edible oils are often used repeatedly as a cost-cutting measure, and to enhance the flavour of fried foods. Palm oil is the primary source of fat in most Malaysian dishes and meals. Its stability at high temperatures, affordability, and widespread availability in the market make it a popular choice as a food product and ingredient domestically. Chicken nuggets made from chicken meat, wheat flour, water, and food additives are amongst the most popular deep-fried food. Commercially prepared chicken nuggets always contain some amount of salt or sodium chloride (NaCl; 0.5 - 2%, w/w). The effect of deep frying and the development of 3-MCPDE and GE have garnered considerable attention from researchers and consumers due to its common use in most street-fried foods and restaurant chains (Xu *et al*., 2022). Repeated deep frying subjects oil to thermal oxidation, polymerisation, and hydrolysis in the presence of oxygen and water. This results in the development of polar components monoacylglycerol (MAG) and diacylglycerol (DAG), as well as certain degradation products (Li *et al*., 2016). Theoretically, in such a high temperature setting with chloride ions present, MCPDEs can potentially form. DAG, a product of oil degradation, may react at high temperatures typical of the frying process to create GE and degrade into glycidol (BfR, 2009). In general, high DAG content is associated with high MCPDE and GE. The percentage of DAG in palm oil ranges from 6 to 10%, and is significantly higher than other DAG contents in vegetable oils, which are typically 1 - 3% (Goh *et al*., 2021). In the last ten years, several conflicting findings relating to the development of 3-MCPD and GE in deep-frying oils have been reported (Arisseto *et al*., 2015; Aniołowska and Kita, 2016a; Yıldırım and Yorulmaz, 2018; Abd Razak *et al*., 2019; Turan *et al*., 2019; Goh *et al*., 2021).

Therefore, the present work aimed to determine the effect of repeated frying of commercial chicken nuggets in palm olein on the formation of 3-MCPDE, 2-MCPDE, and GE, as well as analyse the changes in the oil quality after repeated deep frying. The present work also investigated the correlation between the formation of these contaminants, before and after the deep-frying processes.

Materials and methods

Chemicals and reagents

The individual external standards of rac 1,2-bis-palmitoyl-3-chloropropanediol (PP-3-MCPD), 1,3-dipimitoyl-2-chloropropanediol (PP-2-MCPD), and glycidyl palmitate (Gly-P), and internal standards of rac 1,2-bis-palmitoyls-3-chloropropanediol-d5 (PP-3-MCPD-d5) and glycidyl palmitate-d5 (Gly-P-d5) were procured from Toronto Research Chemical (Toronto, Ontario, Canada). Other reagents and chemicals used were of reagent grade. Tetrahydrofuran (anhydrous), methanol, *n*-heptane, acetone, toluene, sulphuric acid (purity ≥ 95%), sodium hydrogen carbonate (purity ≥ 99%), and sodium sulphate (purity ≥ 99%) were acquired from Friendemann Schmidt Chemical (Washington, USA). Phenylboronic acid (purity ≥ 97%) and sodium bromide (purity ≥ 99.5%) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Petroleum ether (40 - 60°C) from Fisher Scientific (Malaysia) was used as the solvent for fat extraction.

Sample collection and preparation

Frozen chicken nugget samples were purchased from a local supermarket in Penang. The samples were selected based on common brands found in the market. Preliminary chloride content tests were carried out before the experiment. The acquired nugget samples contained 1.72% (w/w) chloride content as analysed by the standard method (ISO, 1996). Each batch of the nugget sample was weighed to ~200 g. Refined, bleached, and deodorised (RBD) palm olein of known 3-MCPD concentration from the same production batch was used as the frying oil (courtesy of Yee Lee Oil Sdn. Bhd., Perak, Malaysia).

Method validation

Six standard solution concentrations of 3-MCPD and 2-MCPD (0.03 - 0.72 µg/g) and GE (0.06 - 1.66 µg/g) were prepared. The calibration curves were constructed using spiked standards prepared in matrix-matched derivatisation and extra virgin olive oil as the blank. The quantification limits for 3-MCPDE, 2-MCPDE, and GE were determined using the signal-to-noise ratio (S/N) of at least 10:1. The recovery of the analytes was between 90 and 124%, and precision expressed as percent coefficient variation (% CV) was less than 10% from the assigned values.

Frying experiment of commercial chicken nugget

The overview of the experiment is shown in Figure 1. The experiment was conducted in two frying systems at different temperatures (160 and

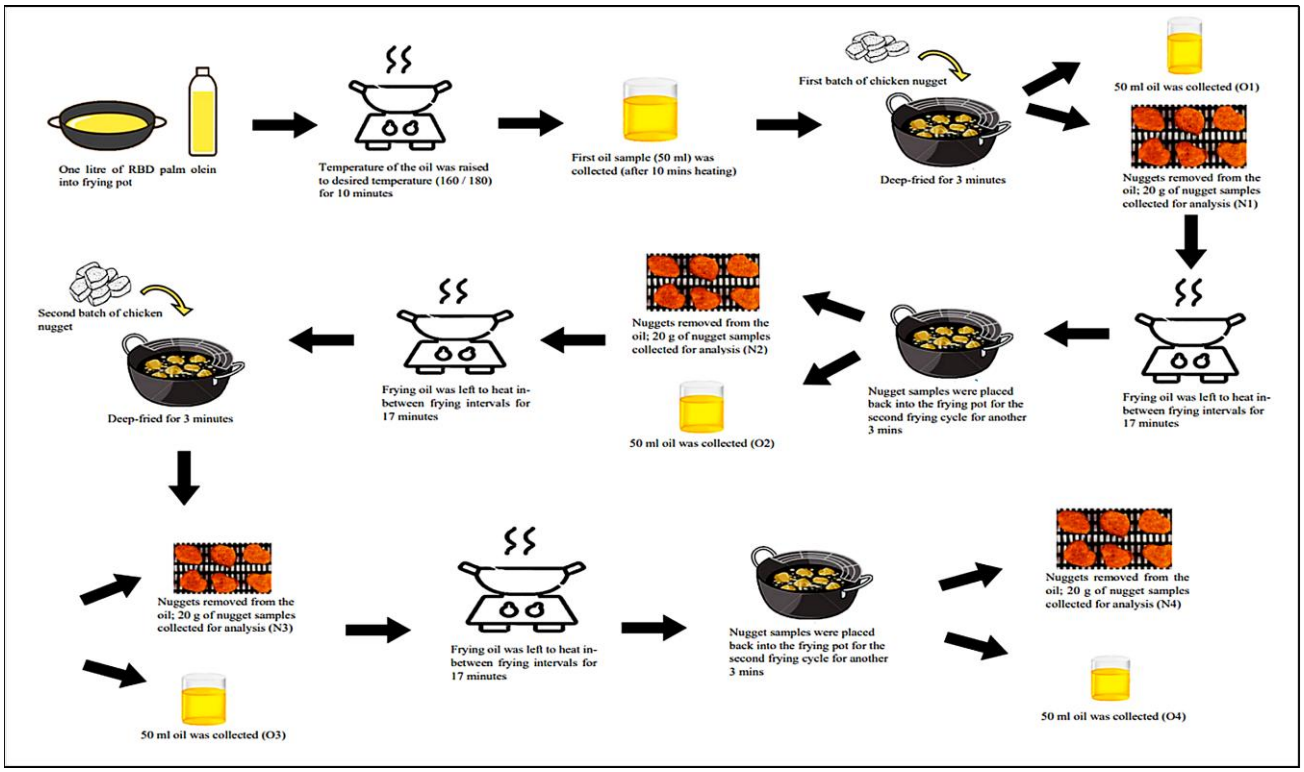

Figure 1. Overall flowchart of experimental design for repeated frying of commercial chicken nuggets.

180°C). The temperatures were selected to achieve the desired and distinct quality of fried foods from the simultaneous heat and mass transfer of oil, food, and air during frying (Choe and Min, 2007). First, RBD palm olein (1 L) was measured into the frying utensil. Then, the oil was heated approximately for 10 min until it reached the intended temperature. The first oil sample (50 mL) was collected prior to frying. For the frying process, 200 g nugget samples were placed in the heated oil, and cooked for 3 min as instructed on the packaging. After 17 min interval, the oil was allowed to remain at the intended temperature, and another frying cycle was carried out. A sample of the second oil was collected before the fried nuggets were returned into the cooking utensil for a second frying cycle. After the third oil sample collection, a new batch of nugget samples was used. The frying process continued for another two cycles. In total, there were four frying cycles: five oils and four nugget samples were collected for each frying system. Two frying cycles using the same batch of nuggets were intended to simulate common practices of reheating. In Malaysia, households and commercial settings commonly reuse frying oil to save costs. Research indicates that food handlers typically discard the reused cooking oil after using it twice, and they rarely exceed four cycles with the same batch of oil (Azman *et al*., 2012; Sivananthan *et al*., 2013; Zainah *et al*., 2023). The same frying process was repeated at 180°C. The experiments were conducted in duplicates, and all samples (oil from every cycle and fried nugget samples) were stored in the freezer before analysis.

Determination of oil quality parameters for repeated frying oils

Free fatty acid content

The free fatty acid (FFA) content was determined following the AOCS Method Ca 5a-40 (AOCS, 2017). Test samples (10 g) were weighed into an Erlenmeyer flask before adding 50 mL of neutralised ethanol, and heated until boiled. The mixture was then left to cool prior to adding 2 mL of phenolphthalein indicator. The sample was titrated with standardised 0.1 M sodium hydroxide (NaOH) solution, and shaken vigorously until a permanent pink colour appeared and persisted for 30 s. The FFAs in the sample were expressed as palmitic acid and calculated using Eq. 1:

$$\text{FFAs as palmitic, \%} = (V \times M \times 25.6) / W \quad \text{(Eq. 1)}$$

where, V = volume of NaOH solution (mL); M = molarity of the standardised NaOH solution; 25.6 = equivalence factor of palmitic acid (the most predominant fatty acid found in palm oil); and W = weight of the test portion (g).

p-Anisidine value

The *p*-Anisidine value (*p*-AV) was determined following the AOCS Official Method Cd 18-90 (AOCS, 1996). Briefly, approximately 1 g of oil sample was weighed into a volumetric flask of 25 mL. The oil sample was dissolved with isooctane to volume. The oil solution was then measured for absorbance at 350 nm against solvent as the reference. Aliquots of the oil solution (5 mL) and solvent (5 mL) were placed into two different test tubes, which were labelled as test tubes A and B. Then, *p*-Anisidine reagents (1 mL) were added into both test tubes, and allowed to stand for 10 min. The absorbance of the solutions in test tube A was measured against the solvent from test tube B at 350 nm. The *p*-AVs were then calculated using Eq. 2:

$$p\text{-AV} = (25 \times [(1.2 \times A) - A_b]) / W \quad \text{(Eq. 2)}$$

where, A = absorbance of oil solution after addition of *p*-anisidine reagent; A_b = absorbance value of oil solution before the addition of *p*-anisidine reagent; and W = weight of the oil sample (g).

Specific extinction at 232 (K_{232}) and 268 nm (K_{268})

Specific extinction helps in measuring the conjugated diene and triene content in the oils. The conjugated diene and triene contents were determined following the Official AOCS Method Ch 5-91 (AOCS, 1998). Briefly, 0.25 g of oil samples was placed into a 25 mL volumetric flask. The oil samples were then dissolved with isooctane until the required volume was achieved, and the samples were homogenised. The baseline of the UV-Vis spectrophotometer was corrected (220 - 280 nm) with solvent in both cells. The sample cell was then filled with the test solution, and the extinctions were measured at 232 and 268 nm against isooctane as the reference. The specific extinctions at both wavelengths were calculated using Eq. 3:

$$K_\lambda = E_\lambda / (c \times s) \quad \text{(Eq. 3)}$$

where, K_λ = specific extinction at wavelength λ; E_λ = extinction measured at wavelength λ; c =

concentration of the solution (in g/100 mL); and s = path length of the quartz cell (in cm).

Extraction of MCPDEs and GE from food samples

The extraction method was adapted from the AOCS Official Method Cd 29a-13 (AOCS, 2013), which is an indirect method where GE is converted to 3-monobromopropanediol (3-MBPD) monoesters in an acid solution containing a bromide salt. In acid methanolic solution, 3-MBPD esters, as well as 2- and 3-MCPDE, are converted to the free (non-esterified) form. The fatty acid methyl esters produced during the reaction were extracted from the sample, followed by the derivatisation of 2- and 3-MCPD, as well as 3-MBPD, using phenylboronic acid before gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis. Results of 3-MCPDE, 2-MCPDE, and GE are reported in micrograms per gram (μg/g) of oil.

Approximately 100 - 110 mg of extracted fat was weighed in a screw-capped glass tube, and 50 μL of internal standard solutions 3-MCPD-d5 and Gly-P-d5 was added to it. For calibration samples, external standard working solutions (PP-3-MCPD, PP-2-MCPD, and Gly-P) were added based on the level of concentrations needed. Tetrahydrofuran (2 mL) was pipetted into the mixture before being mixed vigorously using a vortex for 15 s. The acid aqueous solution of sodium bromide (30 μL) was inserted into the mixture, vortexed, and incubated in the oven at 50°C for 15 min. The reaction was stopped by the addition of 3 mL of 0.6% aqueous solution of sodium hydrogen carbonate. The addition of 2 mL of *n*-heptane to the mixture caused two distinct layers to form after being vigorously shaken. The upper layer was transferred to another glass tube, and the solvents were evaporated to dryness using a nitrogen stream (40°C for 15 - 20 min). The residue was dissolved in 1 mL of tetrahydrofuran.

The esterification step was introduced to the mixture by adding 1.8 mL of sulphuric acid/methanol solution, and shaken vigorously for 10 s. The mixture was then incubated for 16 h at 40°C. After the incubation period, 0.5 mL of saturated sodium hydrogen carbonate solution was added to end the reaction. The mixture was vortexed for 10 s, and the organic solvents were evaporated using nitrogen gas until an oil layer formed at the surface, and the volume of the mixture was approximately 1 mL. Sodium sulphate solution (2 mL) and *n*-heptane (2 mL) were added, and the mixture was shaken vigorously. Two separate layers formed, and the upper layer containing fatty acid methyl esters in *n*-heptane was removed. The extraction process using *n*-heptane was repeated.

The mixture was derivatised by adding 250 μL of the phenylboronic acid solution prior to vortexing, and incubated in an ultrasonic bath at room temperature for 5 min. The derivatives of 3-MCPD, 2-MCPD, and 3-MBPD were extracted with 2 mL of *n*-heptane. The mixture was vortexed for 10 s for the formation of two layers. The upper layer was transferred to another glass tube before the remaining extract was evaporated to dryness with nitrogen gas. The white residue was dissolved in 400 μL of *n*-heptane, and vortexed. The supernatant was then transferred to a GC vial prior to injection.

GC-MS/MS analysis

The AOCS Official Method Cd 29a-13 (AOCS, 2013) was adapted for the quantitative analysis of MCPDEs and GE. For each derivatised sample, 1 μL of solution was injected into the GC-MS/MS instrument (GCMS-TQ8040, Shimadzu Corporation) in splitless mode, with the MS operating in the multiple reaction monitoring (MRM) mode. SH-Rxi-1MS capillary column (30 m × 0.25 mm I.D. × 1 μm d_f; Shimadzu Corporation) was used to chromatographically separate the targeted compounds. The injection temperature was set at 250°C, and helium was used as the carrier gas with a flow rate of 0.8 mL/min. The column oven temperature was programmed at 80°C for 1 min, and increased to 170°C at a rate of 10°C/min, followed by 3°C/min to 200°C. The temperature further increased to 300°C at 15°C/min, and held for 15 min. The transfer line, ion source, and quadruple temperatures for the MS detector were set at 300, 230, and 150°C, respectively. Analysis was performed by observing each compound's targeted ion pairs and reference ion pairs. The results were obtained and processed in GCMS Solutions software (version 4.50, Shimadzu Corporation).

Statistical analysis

Statistical analysis was performed using SPSS software (version 28, IBM SPSS, New York, USA). Prior to analysis, data were tested for normality using Shapiro-Wilk statistics. Data were analysed using ANOVA test and Tukey's *post-hoc* test. The results

were presented as mean ± standard deviation, and the probability value of 0.05 was used to determine statistical significance.

Results and discussion

Method validation

The constructed calibration curves for all the analytes were found to be linear with the coefficient of determination (R^2) of at least 0.996 (Figure 2). The LOQ on the instrument was 0.10 µg/g for 3-MCPDE, 2-MCPDE, and GE, with a percent accuracy of 90.1, 111.1, and 123.2%, respectively.

Formation of MCPDEs and GE in frying oil and commercial chicken nuggets

The RBD palm olein oil investigated in the present work is commonly used for frying in households and industries, and has a low melting point that prevents a waxy or greasy taste in fried food. Adding sodium chloride (NaCl) to food before frying is a common practice that provides a saltish flavour, and controls microbial growth and changes in food texture. During the frying process, complex chemical reactions occur within the food, NaCl, and frying oil. Although the quality of frying oil has been studied extensively, research on the formation of MCPDEs and GE in palm oil during repeated deep frying remains limited.

Two temperatures of 160 and 180°C were selected in the present work. These temperatures (160 - 180°C) are commonly used for deep frying, and resemble the deodorisation process occurring at similar temperatures (Oke *et al*., 2018). The utilisation of high temperatures in thermal food processing can lead to the development of 3-MCPDE, 2-MCPDE, and GE. Figure 3A compares the levels of 3-MCPDE, 2-MCPDE, and GE in the oils of both frying systems (160 and 180°C). The initial concentrations of 3-MCPDE, 2-MCPDE, and GE in the oils before heating (room temperature) were 2.40 ± 0.09, 1.08 ± 0.05, and 5.26 ± 0.11 µg/g oil, respectively. We observed that after the oils were heated for 10 min, all the assessed contaminants increased in both frying systems. The concentrations of all contaminants in both frying systems were unstable after the first frying cycle, but 3-MCPDE in the 160°C system showed a decreasing trend, and 2-MCPDE in the 180°C system showed an increasing trend. However, the changes were not significantly different ($p > 0.05$) from the first frying cycle onwards.

The 3-MCPDE content was found to increase by 18% when we compared the 160 and 180°C frying systems. This result was consistent with the findings by Wong *et al*. (2017a) of a 15% increase. A high temperature led to an increase in 3-MCPDE levels, consistent with other studies (Rahn and Yaylayan, 2011). The degradation rate of 3-MCPDE was lower than its formation rate at high temperatures. The formation of 3-MCPDE is associated with triacylglycerol (TAG), which can hydrolyse into monoacylglycerol (MAG) and diacylglycerol (DAG) that further transform into GE during the frying process (Craft *et al*., 2013). The overall GE content was found to be higher than 3-MCPDE and 2-MCPDE in each frying system. Compared with the 160°C frying system, the GE level was lower at higher temperatures. These findings were comparable to Wong *et al*. (2017a), who further hypothesised that the increased amount of GE in the 160°C frying system could be due to the decomposition of MAG and DAG, which were used to generate GE in the frying system. According to Cheng *et al*. (2017), a reactive epoxide group in GE makes it less thermally stable during deep frying. In contrast to 3-MCPD, at high temperatures, the rate of GE degradation is substantially more rapid than that of its formation, and its level is the result of competitive reactions between generation and decomposition (Shimizu *et al*., 2013). The degradation of GEs is considerably accelerated by increasing heat energy, which increases reaction rates. The reaction kinetics follow the Arrhenius equation, which states that even a minor increase in temperature can result in an exponential increase in decomposition rate (Cheng *et al.*, 2020). GEs degrade rapidly at 180°C into glycidol and other secondary products, giving these molecules a short half-life in oil. At 160°C, breakdown proceeds slowly, allowing the GE to stay in the oil for a long period. As a result, there is less development of toxic substances, and the frying oil has an extended shelf life. The 2-MCPDE concentrations from all the frying cycles in both systems were significantly different ($p < 0.05$) from that of fresh oil. Given the lack of reports on 2-MCPDE, a detailed comparison was not possible.

Carbohydrates, proteins, and micronutrients in the food matrix increase the production of 3-MCPDE during the frying process through a series of chemical

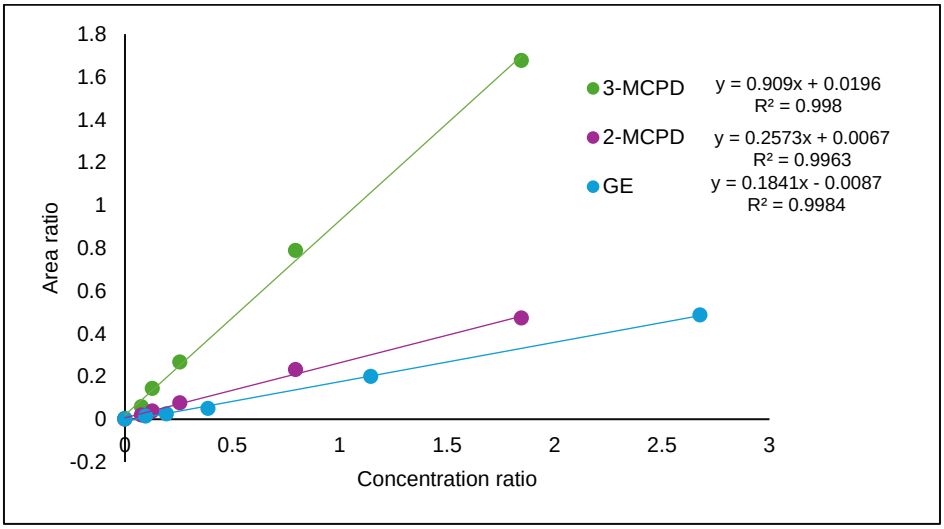

Figure 2. Calibration curve of 3-MCPDE (green line) (0.03 to 0.72 µg/g), 2-MCPDE (blue line) (0.03 to 0.72 µg/g), and GE (yellow line) (0.06 to 1.66 µg/g).

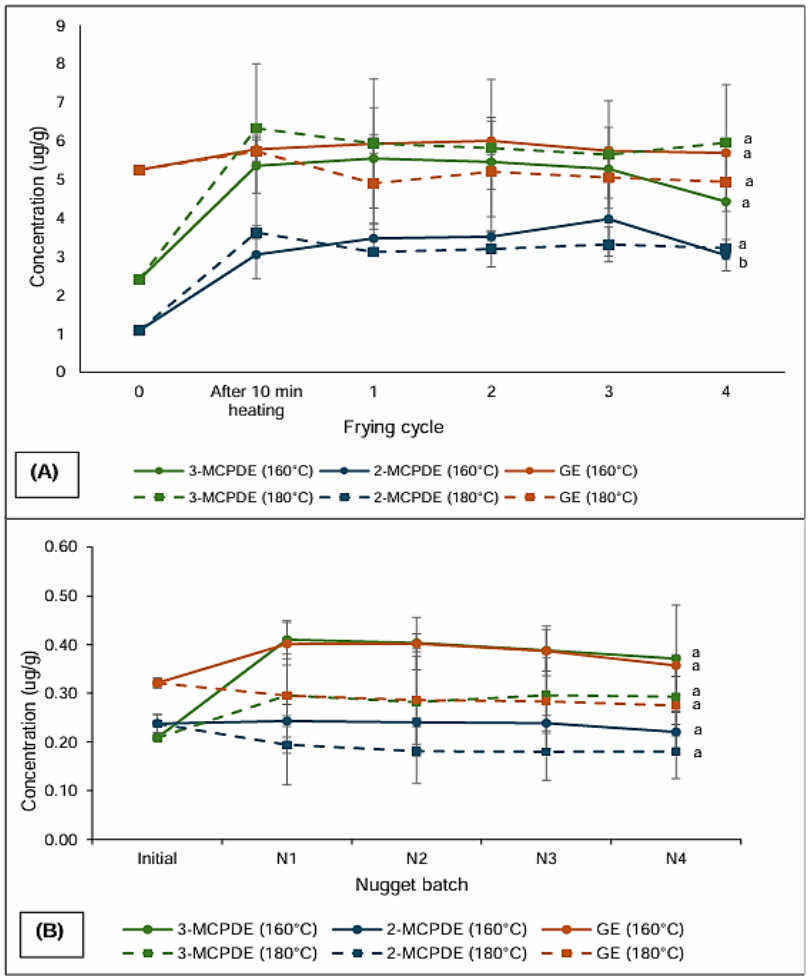

Figure 3. (A) Levels of 3-MCPDE, 2-MCPDE, and GE in frying oil of different frying systems (n = 22). Analysis was done in duplicates. Different lowercase letters indicate significant difference ($p < 0.05$) between each frying cycle. **(B)** Levels of 3-MCPDE, 2-MCPDE, and GE in fried chicken nuggets in different frying systems (n = 18). Analysis was done in duplicates. Different lowercase letters indicate significant difference ($p < 0.05$) between each nugget batch.

reactions that generate free radicals in the frying oil (Kalogianni *et al*., 2009; Goh *et al*., 2021). In 2020, Xu *et al*. (2020) found that the presence of the food matrix is correlated with the indicators of oxidation state. They also found that the increase in free radicals in the frying system enhanced the formation of 3-MCPDE during repeated deep frying of French fries. In the presence of chloride ions, some of the polar compounds, such as glycerol, may convert into 3-MCPDE through pathways, including direct nucleophilic attack or formation of acyloxonium ion (Rahn and Yaylayan, 2011). The concentrations of MCPDEs and GE in the fried chicken nuggets were also measured (Figure 3B). The contamination of fried foods with 3-MCPDE was mainly due to the carry-over effect attributed to the use of contaminated oil during frying (Dingel and Matissek, 2015; Arisseto *et al*., 2017). Given that the nugget samples were processed commercially, the initial concentrations of 3-MCPDE, 2-MCPDE, and GE in the frozen nuggets must be determined. The presence of these compounds in frozen chicken nugget samples indicated that they were introduced during the manufacturing process (Figure 3B). The concentration of 3-MCPDE in chicken nuggets significantly increased ($p < 0.05$) after the first frying cycle at 160°C, when the concentration increased by 85% from 0.21 μg/g. Subsequent frying processes did not significantly change the amount of 3-MCPDEs in nugget samples for both frying systems. A similar observation was reported in the repeated frying of French fries (Xu *et al*., 2022). The present work also noted an increase in GE in fried nugget samples in the 160°C frying system. At 180°C, the 3-MCPDE concentration was generally found to increase compared with initial (frozen) nugget samples. The decrease in 2-MCPDE and GE was also observed in the fried nugget samples at 160 and 180°C, respectively, but the differences were not statistically significant ($p > 0.05$) compared with those before frying.

The levels of MCPDEs and GE in food products in various frying processes and food matrices have been reported. For instance, Wong *et al*. (2020) documented that the contents of 3-MCPD and GE in fries were influenced by NaCl, frying duration, and frying temperature, in ascending order. Merkle *et al*. (2018) found that after frying with sunflower oil for 7 h at 185°C, salted fish fingers with 0.16 and 1.5% NaCl had increased levels of 2- and 3-MCPDE, respectively, but decreased levels of GE. Xu *et al*. (2022) reported the highest concentration of 3-MCPDE (1.14 μg/g oil) in the 16th batch (1 d) of French fries, which was approximately 15.5 μg/100 g fries. Similarly, the content of GE in French fries was 0.13 μg/g oil in the same batch. In the present work, the highest 3-MCPDE, 2-MCPDE, and GE concentrations were determined at 0.43, 0.25, and 0.44 μg/g extracted oil from fried nuggets, respectively.

In the present work, the concentration of 2-MCPDE was consistently lower than that of 3-MCPDE for the oil and nugget samples. The 2-MCPDE levels in the oil and nugget samples fell in the range of 45 - 75% and 58 - 67%, respectively. Typically, the concentration of 2-MCPDE is between 40 and 80% of the concentration of 3-MCPDE (Kuhlmann, 2011). Overall, the percentage of increase in 3-MCPDE, 2-MCPDE, and GE from the initial stage to heating for 10 min in oil was higher than those in nugget samples. 3-MCPDE levels in oil increased by more than 120% from 2.4 μg/g to the highest points of 5.55 (at frying cycle 1) and 6.33 μg/g (after 10 min of heating) for the frying system at 160 and 180°C, respectively. As for 2-MCPDE, it increased to around 200% from the fresh oil (1.8 to 3.98 μg/g at frying cycle 3 in the 160°C frying system), whereas GE only showed around 10% increment (from 5.26 to 5.79 μg/g for both frying systems). By contrast, nugget samples demonstrated only up to 37.5% increase in GE concentration from the initial (0.32 μg/g), static, or decreasing trend in 2-MCPDE (initial: 0.24 μg/g), and maximum of 85% increment in 3-MCPDE levels (initial: 0.21 μg/g). These results agreed with findings that frying did not elevate 3- and 2-MCPDE contamination at relatively low initial concentrations (< 1 mg/kg) (Xu *et al*., 2020).

Oil quality evaluation after repeated frying of commercial chicken nuggets

Heat is transferred from oil to food between 140 and 180°C. Simultaneously, water is evaporated from the food while oil is absorbed into it, causing the product to contain a significant portion of the oil. As a result, oil quality significantly affects the quality of the fried product. According to Márquez-Ruiz *et al*. (2014), complex chemical reactions including oxidation, hydrolysis, polymerisation, cyclisation, and isomerisation occur during frying. High amounts

of FFA, mono- and diacylglycerols, and glycerol are present in oil due to hydrolysis. In frying oils, the oxidation rate is even faster than the rate of hydrolysis (Choe and Min, 2007; Aniołowska and Kita, 2015). The thermal oxidation and polymerisation of oils can be accelerated by repeated high-temperature frying (Aladedunye, 2014). The changes in oil due to repeated frying are often deteriorative, and fatty acids undergo chemical changes, causing the fried food to be an unsuitable product in terms of nutritional value (Sebedio *et al*., 1996).

The results of the evaluation of oil quality after repeated frying are shown in Table 1. The content of FFA illustrates the extent of oil deterioration due to the hydrolysis reactions of lipids, cleavage, and oxidation of the double bonds of unsaturated fatty acids. The present work found that the FFA (expressed in percentage of palmitic acid) in oil samples increased over time, although the changes were not significant ($p > 0.05$). These findings were similar to the results of Sharoba and Ramadan (2017), in which the increment of FFA content was observed after frying French fries for 16 h, indicating that polyunsaturated fatty acid levels decreased, but saturated fatty acid increased during frying. FFAs are also a good indicator of hydrolytic rancidity for frying fats and oils. Generally, as the cycles increase, the content of FFA also increases. FFA in frying oil contributes to the development of off-flavours and odours. However, the FFA values in the present work ranged between 0.07 and 0.15%, which were much lower than the acceptable limit for food application of 5% (Bahadi *et al*., 2016). The unsaturated aldehydes formed during secondary oxidation were measured using *p*-AV. The trend in the two systems' *p*-AV levels showed an increasing trend, with higher *p*-AV in the 180°C frying system than in the 160°C frying system. These findings confirmed that palm olein frying oil was prone to oxidation at high temperatures and after long periods of frying. The *p*-AV levels increased due to the less stable hydroperoxides from primary oxidation decomposing further to form secondary oxidation products, namely, aldehydic compounds (Abdulkarim *et al*., 2007).

Table 1. Free fatty acid contents, *p*-anisidine values, and specific extinctions at 232 and 268 nm (K_{232} and K_{268}) of frying oil ($n = 22$).

Temperature	Frying cycle	FFA (%)	*p*-AV	K_{232}	K_{268}
Initial	0	0.07 ± 0.007^{a}	5.76 ± 0.04^{f}	2.51 ± 0.03^{d}	0.42 ± 0.009^{f}
160°C	10 min heated	0.11 ± 0.02^{a}	6.20 ± 0.57^{def}	2.51 ± 0.21^{cd}	0.53 ± 0.07^{de}
	1	0.10 ± 0.04^{a}	9.05 ± 0.77^{de}	2.96 ± 0.28^{bcd}	0.71 ± 0.09^{d}
	2	0.11 ± 0.0001^{a}	13.84 ± 1.1^{c}	3.07 ± 0.07^{bc}	1.01 ± 0.03^{c}
	3	0.12 ± 0.03^{a}	20.46 ± 1.11^{b}	3.34 ± 0.06^{ab}	1.40 ± 0.06^{b}
	4	0.14 ± 0.02^{a}	26.36 ± 1.19^{a}	3.87 ± 0.03^{a}	1.64 ± 0.08^{a}
180°C	10 min heated	0.10 ± 0.009^{ab}	9.37 ± 0.11^{e}	2.39 ± 0.14^{d}	0.69 ± 0.004^{e}
	1	0.09 ± 0.0003^{a}	14.64 ± 0.87^{d}	3.04 ± 0.10^{cd}	1.02 ± 0.01^{d}
	2	0.11 ± 0.02^{ab}	19.12 ± 0.87^{c}	3.42 ± 0.07^{c}	1.34 ± 0.06^{c}
	3	0.12 ± 0.009^{ab}	24.32 ± 0.62^{b}	4.01 ± 0.08^{b}	1.63 ± 0.04^{ab}
	4	0.15 ± 0.03^{b}	27.88 ± 0.20^{a}	4.69 ± 0.20^{a}	1.78 ± 0.08^{a}

Analysis was done in duplicates, and results are expressed as mean ± standard deviation. Means within each column followed by different lowercase superscripts are significantly different ($p < 0.05$).

The magnitude of changes in specific extinction coefficients at 232 and 268 nm (K_{232} and K_{268}) using ultraviolet spectrophotometric analysis could be used as a relative measure of oxidation degree of frying oil (Gray, 1978). Changes in ultraviolet absorption at 232 and 268 nm were correlated with changes in the conjugated dienes and trienes formed by polyunsaturated fatty acid oxidation. During the first 10 min of heating prior to frying, we observed a negligible increase in the content of conjugated dienes. After one frying cycle, the formation of conjugated dienes was proven by the change in the K_{232} value in the oil of both frying systems. This could be due to the formation of peroxides and decomposition during oil heating at high temperatures. The K_{232} values reflect the formation of conjugated dienes, which are early-stage oxidation products of polyunsaturated fatty acids. These compounds indicate the initiation of oil oxidation. The K_{268} values, representing conjugated trienes, indicate advanced stages of oil degradation. Increased ultraviolet absorption is associated with

polyunsaturated fatty acid oxidation. Both frying systems exhibited an increasing trend as the frying cycle increased in K_{232} and K_{268}. As the number of frying cycles increased, the changes in K_{232} and K_{268} revealed that conjugated dienes and trienes steadily formed. Higher K_{232} and K_{268} values were determined in the 180°C frying system compared with the 160°C frying system.

Relationship between formation of 3-MCPDE, 2-MCPDE, and GE in frying oil and oil oxidation

Pearson correlation test was used to investigate the relationship between MCPDEs and GE concentrations in frying oil and oil quality parameters after repeated frying. Table 2 demonstrates a strong positive correlation (R = 0.812) between 3-MCPDE and 2-MCPDE, which indicated that the formation of these two contaminants occurred simultaneously because 2-MCPD is a constitutional isomer of 3-MCPD (Fry *et al*., 2013). The present work noted that GE had a weak correlation with 2-MCPDE (R = 0.132) and a negative correlation with 3-MCPDE (R = 0.313). The 3- and 2-MCPDE concentrations showed a weak negative correlation with FFA content (R = 0.150 and 0.004, respectively). 3-MCPDE also had a weak correlation with *p*-AV (R = 0.346) and specific extinction at 232 nm (R = 0.251). A similar correlation (R = 0.281) was found between 2-MCPDE and K_{232}. The increase in FFA content could be due to oil hydrolysis, including TAG, and the formation of partial acylglycerols (DAG and MAG) could further lead to the formation of MCPDEs (Wong *et al*., 2017b). The initial mechanism of free radical intermediates mediating the production of 3-MCPD diesters was reported by Zhang *et al*. (2013). Based on their studies, the free radicals in this mechanism may have originated from the oxidation of lipids. Free radicals were reduced, and their oxidation increased in the oil during the frying process, which increased *p*-AV but decreased the MCPDE concentration.

Table 2. Pearson correlation analysis between MCPDEs of frying oil and oil quality parameters after repeated frying of chicken nuggets.

	3-MCPDE	2-MCPDE	GE	FFA	p-AV	K_{232}	K_{268}
3-MCPDE	1						
2-MCPDE	0.812[a]	1					
GE	-0.313	0.132	1				
FFA	-0.150	-0.004	0.017	1			
p-AV	0.346	0.473	-0.258	0.499	1		
K_{232}	0.251	0.281	-0.373	0.532[a]	0.925[a]	1	
K_{268}	0.400	0.517[a]	-0.268	0.466	0.992[a]	0.919[a]	1

[a]Correlation is significant at $p < 0.01$.

Implications of 3-MCPD, 2-MCPD, and GE for human health

Deep-fat frying alters the flavour, colour, texture, and nutritional value of foods. Hydrolysis, oxidation, and polymerisation are frequent chemical processes in frying oil that create volatile or non-volatile chemicals (Choe and Min, 2007). As frying oils oxidise, they generate hazardous substances such as free radicals, aldehydes, hydroperoxides, and *trans*-fatty acids. These substances increase the oil's overall toxicity, which has several effects, including an increase in oxidative stress. High amounts of free radicals can overwhelm the body's antioxidant defences, causing cellular damage and inflammation, which can lead to chronic diseases such as cancers, cardiovascular diseases, and diabetes. Lipid peroxidation, which is caused by the oxidation of fats, yields harmful by-products such as malondialdehyde, which has been connected to carcinogenic and mutagenic properties (Almoselhy, 2021). The synergistic impact of 3-MCPD, 2-MCPD, and GEs with the oxidation products of frying oils can increase the health hazards associated with them. These substances can intensify the toxicity effects of 3-MCPD, 2-MCPD, and GE by inducing oxidative stress and inflammation.

Conclusion

The obtained results indicated that the levels of contaminants in the nuggets and frying oil changed as the frying cycle was extended. The final concentration of MCPDEs and GE in the frying oil after the fourth frying cycle was higher than the initial concentration, except for the GE concentration when fried at 180°C. The oil quality changed with each

frying process, due to hydrolysis and oxidation. Although the change was not significant, oil degradation could create a dynamic change in its chemical composition when used repeatedly for frying, resulting in the formation and degradation of MCPDEs and GE. Weak to moderate correlations were found between the oil quality parameters and the concentration of MCPDEs and GE in the frying oil. The changes and effects of frying oils may be more significant if the frying duration and cycle were extended and involved the use of various food samples with different ingredients and compositions. Food manufacturers and consumers are encouraged to adopt several key practices to reduce the formation of these contaminants. These practices include using stable and fresh oils, avoiding overheating (below 180°C), limiting the use of recycled cooking oil, and regularly monitoring oil quality parameters. Additionally, the type of food must be considered when adjusting frying techniques. Kitchen staff should receive training and follow standard operating procedures to ensure proper oil handling, appropriate frying temperatures, and maintenance of frying equipment. Consumers are advised to explore alternative cooking methods instead of deep frying. By implementing these recommendations, the formation of harmful substances can be significantly reduced, thereby minimising potential health risks.

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