Monitoring of heat-induced carcinogenic compounds (3-monochloropropane-1,2-diol esters and glycidyl esters) in fries

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3-Monochloropropane-1,2-diol (3-MCPD) esters and glycidyl esters (GE) are heat-induced contaminants which form during oil refining process, particularly at the high temperature deodorization stage. It is worth to investigate the content of 3-MCPD and GE in fries which also involved high temperature. The content of 3-MCPD esters and GE were monitored in fries. The factors that been chosen were temperature and duration of frying, and different concentration of salt (NaCl). The results in our study showed that the effect was in the order of concentration of sodium chloride < frying duration < frying temperature. The content of 3-MCPD esters was significantly increased whereas GE was significantly decreased, when prolong the frying duration. A high temperature results in a high 3-MCPD ester level but a low GE level in fries. The present of salt had contributed significant influence to the generation of 3-MCPD. The soaking of potato chips in salt showed no significant effect on the level of GE during the frying. The oil oxidation tests showed that all the fries were below the safety limit. Hence, the frying cycle, temperature and the added salt to carbohydrate-based food during frying should be monitored.

Deep-fat frying is commonly being used to process food. During the process, heat transfer between the fried food and oil is occurs. The frying oil replaced the moisture in food during the dehydration process. Thus, the frying oil quality is highly influencing the fried food. During deep-fat frying, free fatty acids, aldehydes, ketones, hydrocarbons, diacylglycerol and monoacylglycerol are formed from numerous complex interrelation reactions induced by hydrolysis, oxidation, isomerization and polymerization. Palm olein is widely being used for deep-fat frying because of the stability of the oil and high smoke point. This is due to the unique properties of palm olein, which contain the relative balance of unsaturated and saturated fatty acids. It is suitable for repeated frying within a certain number of frying cycles.

3-Monochloropropane-1,2-diol (3-MCPD) esters and glycidyl esters (GE) are trace contaminants formed during the high-temperature refining of oils, especially during the deodorization process. The temperature for deep-fat frying usually is within 150–190 °C, which fall within the temperature range of refining process. Researchers believe that the 3-MCPD esters are from the conversion of triacylglycerol to cyclic acyloxonium, together with chloride ions. 3-Monochloropropane-1,2-diol is in vivo carcinogenic and in vitro genotoxic. Glycidyl esters are suggested to be formed through an intermediate stage during 3-MCPD ester formation via monoacylglycerol-derived acyloxonium ion pathway. The free form of glycidyl is a carcinogenic directly acting multisite mutagen in animal studies that reacts readily with cellular nucleophiles.

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Frying oils that are transferred into the fried potatoes can be the main sources of 3-MCPD esters in fried potatoes. A potato, which contains carbohydrates, protein, fat, vitamins and other minor constituents, can undergo a very complex chemical reaction with salt under prolonged high-temperature deep frying. Therefore, the influence of the frying temperature, frying time and salt was monitored. The concentration of salt used for soaking were from 1 to 5%. The chosen temperature were 160 °C and 180 °C, and continue the frying for 5 days. The parameters chosen were to simulate the most common frying condition.

**Results**

**Influence of the temperature during frying.** The 3-monochloropropane-1,2-diol (3-MCPD) ester levels of fries prepared by frying at 160 and 180 °C with controls (distilled water) and three different salt solutions (1%, 3% and 5%) are shown in Fig. 1. The 3-MCPD esters contents ranged from 0.60 to 2.14 mg/kg oil for 160 °C and 0.89 to 2.51 mg/kg for 180 °C after each day's frying. Generally, the 3-MCPD esters in fries under 180 °C are higher than 160 °C. The GE contents ranged from 5.23 to 6.94 mg/kg oil for 160 °C and 4.56 to 6.30 mg/kg for 180 °C after each day's frying. For glycidyl esters (GE), the fries under 160 °C was higher as compared with fries under 180 °C. Besides that, all the oxidation parameters were increasing with the increasing of frying temperature.

**Influence of the frying duration.** The influence of frying time on the level of 3-MCPD esters in fries was examined from Day 0 to Day 5. The increasing of the heating duration to Day 5 leads to the reduction of 3-MCPD esters and increase of GE. The 3-MCPD esters of fresh oil in this study was 3.5 mg/kg. The 3-MCPD esters was 0.60 mg/kg oil after 25 frying cycles at 160 °C and 0.89 mg/kg at 180 °C. In other words, the percent decreases were 82.86% for 160 °C and 74.57% for 180 °C. The GE content in the fresh oil before frying was approximately 4.0 mg/kg. The GE contents for all tested fries was 9.31 mg/kg oil after 25 frying cycles at 160 °C and 6.71 mg/kg at 180 °C. The percent increases were 73.50% for 160 °C and 57.5% for 180 °C.

In Table 1, the higher temperature frying system showed significantly higher (p < 0.05) FFA contents, which causes the negative effect to the taste of fries. The conjugated dienes and trienes shows the trends of increasing for both of the 160 °C and 180 °C frying systems when prolong frying duration. The lower temperature frying system showed significant (p < 0.05) lower p-anisidine value, representing a clear reduction in the rate of secondary oxidation due to the high frying temperature.

**Influence of the salt (NaCl).** Figure 1 shows the content of 3-MCPD with the influence of salt. When the concentration of salt was increasing, the content of 3-MCPD esters were significantly (p < 0.05) increased. This finding is in agreement with another study that reported that the generation of 3-MCPD esters increased with the increasing sodium chloride concentration. The influences of salt on the GE in different frying systems are shown in Fig. 2. There was some increase in the GE with the increased concentration of salt. However, there was no significant difference (p > 0.05) between different concentrations of salt. The increases of p-anisidine, CD and CT were observed when the amount of salt increased.

**Discussion**

The formation of 3-monochloropropane-1,2-diol (3-MCPD) esters and glycidyl esters (GE) are a multivariate problem, which is affected by fat and salt content, processing temperature and time. This study is focused on the content of 3-MCPD esters and GE in fries which undergone different frying parameters. The frying oil was absorbed in the fried potatoes during frying. The content of lipids in the fried potatoes was approximately 40%. Fresh potatoes are mainly made up of water, with an average content of 75%. During the frying, the high temperatures result in a partial evaporation of the moisture in the fries, and travels through the surrounding oil. The oil replaces the water lost in fries. A higher frying temperature under atmospheric conditions causes more oil to accumulate in the fries. This is because the higher frying temperature leads to the rapid formation of a
Table 1. Oxidative analyses of fries from different frying systems. Each value in the table represents the mean ± standard deviation of six analyses from two replications. Means within each row with different superscripts are significantly (p < 0.05) different. Means within each column with different subscripts are significantly (p < 0.05) different.

![Glycidyl esters in fries](image_url)

Figure 2. Glycidyl esters in fries in different frying systems.

solid crust, which is favorable for oil absorption. Therefore, it is necessary to monitor the amounts of 3-MCPD esters and GE in fries during repeated frying.

In this study, the temperature of 160 °C and 180 °C were selected because they are commonly used deep frying temperatures. A higher temperature results in a higher 3-MCPD esters level in the fries, in agreement with other studies, but the degree of increase was not the same for the different frying parameters, which was attributed to the different contents of salt and frying temperatures. For 3-MCPD esters, the rate of degradation is lower than the formation rate under high temperatures. It is believed that the level of GE is affected by the competitive reactions between generation and decomposition. In contrast with 3-MCPD, for GE, the rate of degradation is higher than the formation rate under high temperatures.

Five days frying durations did not contribute to significant differences in the acylglycerol composition. The longer the heating time, the more 3-MCPD esters were degraded. The heat of frying reduces, oxidizes, and degrades the generated 3-MCPD esters during the process. The GE was stable under a long frying duration at a constant heating temperature, and therefore the content of GE gradually increased.

The mechanisms of 3-MCPD esters and GE are little understood, but it is commonly agreed that acylglycerols are one of the precursors. A few studies have suggested that the formation pathway of 3-MCPD esters is
Table 2. Acylglycerol composition of fries in different frying systems. Each value in the table represents the mean ± standard deviation of six analyses from two replications. Means within each row with different superscripts are significantly (p < 0.05) different. Means within each column with different subscripts are significantly (p < 0.05) different.

| Acylglycerol composition (%) | Day | 160 °C | 160 °C with 1% NaCl | 160 °C with 3% NaCl | 160 °C with 5% NaCl | 180 °C | 180 °C with 1% NaCl | 180 °C with 3% NaCl | 180 °C with 5% NaCl |
|-----------------------------|-----|--------|---------------------|---------------------|---------------------|-------|---------------------|---------------------|---------------------|
| Free fatty acid/ monoacylglycerol (FFA/MAG) | 1   | 0.11 ± 0.07*a | 0.13 ± 0.04*a     | 0.13 ± 0.03*a     | 0.14 ± 0.03*a     | 0.21 ± 0.04*a | 0.22 ± 0.06*a     | 0.23 ± 0.02*a     | 0.25 ± 0.06*a     |
|                            | 2   | 0.13 ± 0.07*a | 0.15 ± 0.06*a     | 0.15 ± 0.03*a     | 0.19 ± 0.03*a     | 0.25 ± 0.05*a | 0.28 ± 0.05*a     | 0.28 ± 0.07*a     | 0.32 ± 0.05*a     |
|                            | 3   | 0.14 ± 0.08*a | 0.15 ± 0.05*a     | 0.17 ± 0.06*a     | 0.20 ± 0.05*a     | 0.29 ± 0.05*a | 0.31 ± 0.05*a     | 0.32 ± 0.05*a     | 0.33 ± 0.08*a     |
|                            | 4   | 0.16 ± 0.05*a | 0.17 ± 0.05*a     | 0.17 ± 0.05*a     | 0.26 ± 0.07*a     | 0.31 ± 0.04*a | 0.34 ± 0.07*a     | 0.33 ± 0.07*a     | 0.34 ± 0.08*a     |
|                            | 5   | 0.16 ± 0.01* | 0.18 ± 0.01*      | 0.22 ± 0.12*      | 0.35 ± 0.07*      | 0.37 ± 0.01*  | 0.37 ± 0.06*      | 0.39 ± 0.03*      | 0.39 ± 0.04*      |
| Diacylglycerol (DAG) | 1   | 2.32 ± 0.34*a | 2.54 ± 0.76*a     | 2.65 ± 0.51*a     | 2.98 ± 0.36*a     | 3.10 ± 0.46*a | 3.32 ± 0.27*a     | 3.58 ± 0.29*a     | 3.64 ± 0.40*a     |
|                            | 2   | 2.63 ± 0.46*a | 2.70 ± 0.44*a     | 2.68 ± 0.36*a     | 3.27 ± 0.49*a     | 3.83 ± 0.57*a | 4.02 ± 0.75*a     | 3.99 ± 0.31*a     | 4.06 ± 0.35*a     |
|                            | 3   | 3.04 ± 0.61*a | 2.91 ± 0.65*a     | 2.92 ± 0.31*a     | 3.45 ± 0.61*a     | 3.85 ± 0.39*a | 4.01 ± 0.52*a     | 4.36 ± 0.94*a     | 4.55 ± 0.63*a     |
|                            | 4   | 3.33 ± 0.37*a | 3.41 ± 0.83*a     | 3.64 ± 0.21*a     | 3.87 ± 0.53*a     | 3.91 ± 0.49*a | 4.15 ± 0.12*a     | 4.39 ± 0.43*a     | 4.68 ± 0.37*a     |
|                            | 5   | 3.58 ± 0.54*a | 3.77 ± 0.54*a     | 3.85 ± 0.54*a     | 4.33 ± 0.24*a     | 4.01 ± 0.43*a | 4.37 ± 0.60*a     | 5.05 ± 0.77*a     | 5.47 ± 0.33*a     |
| Tricylglycerol (TAG) | 1   | 97.56 ± 0.26*a | 97.33 ± 0.71*a    | 97.21 ± 0.62*a    | 96.87 ± 0.34*a    | 96.69 ± 0.38*a | 96.46 ± 0.60*a    | 96.19 ± 0.47*a    | 96.29 ± 0.34*a    |
|                            | 2   | 97.24 ± 0.39* | 97.15 ± 0.38*     | 97.16 ± 0.61*     | 96.54 ± 0.46*     | 95.91 ± 0.48* | 95.69 ± 0.77*     | 95.73 ± 0.33*     | 95.62 ± 0.48*     |
|                            | 3   | 96.83 ± 0.52* | 96.94 ± 0.61*     | 96.90 ± 0.22*     | 96.35 ± 0.90*     | 95.86 ± 0.34* | 95.68 ± 0.47*     | 95.31 ± 0.89*     | 95.12 ± 0.59*     |
|                            | 4   | 96.50 ± 0.42* | 96.43 ± 0.36*     | 96.18 ± 0.60*     | 95.87 ± 0.83*     | 95.78 ± 0.45* | 95.51 ± 0.43*     | 95.27 ± 0.31*     | 94.98 ± 0.53*     |
|                            | 5   | 96.26 ± 0.67* | 96.05 ± 0.57*     | 95.93 ± 0.90*     | 95.32 ± 0.67*     | 95.62 ± 0.43* | 95.26 ± 0.54*     | 94.56 ± 0.75*     | 94.14 ± 0.57*     |

Methods

Materials and chemicals. The frying oil, Refined Bleached Deodorized palm olein was purchased from a Malaysia oil production company (Selangor, Malaysia). Potatoes (Russet var.) that used to prepare potato chips were obtained from a local market. The 3-MPCD and GE standards and internal standards were bought from Toronto Research Chemicals, Inc. (Toronto, Canada). The solvents were from Merck (Darmstadt, Germany). Potatoes of 2 mm thickness were prepared from fresh potatoes using a hand slicer. The potato chips were blotted on tissue paper and weighed into each batches of 100 g.

Experiment methods. Preparation of fries. There was a total of eight frying parameters, with different combination of temperature (160 °C and 180 °C) and salt concentration (0%, 1%, 3% and 5%). The frying with each set of parameters was duplicated using an electric deep fryer (Cornell, Malaysia).

The frying was started with 3 kg of RBD palm olein. The temperature was raised and hold at the target temperature for 10 min. Fifty milliliters of oil sample was kept before heating. 100 g of fresh potato chips were fried for 2.5 min and followed with 17.5 min intervals for 5 cycles every day. It was conducted for 5 days to comprise a total of 25 frying cycles. There was no oil replenishment for the 5 days frying. The fryer was uncovered when the frying was carried out. After the end of 5th cycle of the day, 50 mL of oil was transferred to an amber glass...
bottle and the fryer was turned off. Then, the oil was kept in the electric fryer, and continue at the next day. Lastly, all the collected oil samples were kept in chiller at – 20 °C until analysed.

**Oil extraction.** The oil from fries was extracted by an automated fat extractor, Soxtec 8000 (Foss Analytical AB, Sweden, Denmark). Prior to extraction, 10 g of fries was cut and dry in an oven for 2 h at 80 °C. After that, dried fries was put in the thimble and cover with cotton wool. Each extraction cup was filled with 80 mL of n-hexane. The extraction program was started with 20 min boiling, subsequently followed by 40 min of rinsing, and lastly 10 min of recovery to remove the solvent.

**Conjugated dienes (K232) and trienes (K268).** AOCS Method Ch 5-91 was used to determine the conjugated dienes and trienes. The oil samples were prepared with isooctane and the absorbance at 232 nm for dienes and 268 nm for trienes were measured.

**P-anisidine value.** AOCS Official Method Cd 18-90 was used to determine the p-Anisidine value.

**Free fatty acid content.** AOCS Method Ca 5a-40 was used to determine the free fatty acids (FFA). The data were presented in palmitic acid percentage.

**Acylglycerol composition.** The acyglycerol compositions in fries were analyzed using an Alliance model Waters 22695 Separation Module RP-HPLC system equipped with an evaporative light scattering detector (model 242) as described by Wong et al.13. A Merck silica capillary column (Darmstadt, Germany; Purospher STAR RP-18, 22695 Separation Module RP-HPLC system equipped with an evaporative light scattering detector (model 242)) was used for separation. Before the analysis, oil samples in 10 mg/mL were prepared with acetone. After that, the sample solutions were pass through a PTFE 0.45 µm filter. To determine acyglycerol compositions, a dual-solvent gradient was used, where solvent A was acetone and solvent B was acetonitrile. The column was developed at a 1 mL/min gradient flow with 15 min of 1:1 A/B to 5.5:4.5, 15 min to 6:4 A/B, and 15 min to 6.5:3.5 A/B. The column temperature was 35 °C. The nebulizer temperature was programmed at 36 °C with nitrogen gas at 35 psi. The drift tube temperature was 45 °C. FFA/MAG were monitored at 3 to 5 min, DAG at 6–12 min and lastly TAG at 20–38 min.

**Determination of 3-MCPD esters and glycidyl esters.** The modified AOCS method Cd 29a-13 was used. The 3-MCPD and glycidol contents of the oil samples were analyzed using a gas chromatography—mass spectrometry (5975C Agilent Technologies, USA). The separation capillary column used was HP-5MS capillary column (Agilent Technologies, USA) with 30 m length, 0.25 mm internal diameter and the film thickness of 0.25 µm. The injector was set at splitless mode at 250 °C. Besides, the column temperature was started from 80 °C for 1 min and increased to 170 °C at 10 °C/min, to 200 °C at 3 °C/min and lastly to 300 °C at 15 °C/min and holding for 15 min. Helium (0.8 mL/min) was used as the carrier gas. A 1 µl sample was injected. In the quantitative analysis of the derivatized 3-MCPD, the quantifier ions were monitored at m/z 147 for 3-MCPD and m/z 150 for 3-MCPD-d5. The qualifiers for 3-MCPD were ions at m/z 196 and 198, and for 3-MCPD-d5 were m/z 201. In the quantitative analysis of the 3-MBPD compound, the qualifier ions were m/z 147 for 3-MBPD and m/z 150 for 3-MBPD-d5. For the qualifiers, 3-MBPD was monitored at m/z 240 and 3-MBPD-d5 at m/z 245.

**Statistical analysis.** Two parallel examinations were made of each sample, and the measurements were duplicated twice. All the results were presented as the mean ± SD. One way analysis of variance (ANOVA) was used to compared between the means. A probability value of 95% (p < 0.05) was used to demonstrate the significance.

**Conclusions**

Our study found that the effects of deep-fat frying on potato chips were in order of sodium chloride < frying duration < frying temperature. A high frying temperature resulted in high 3-MCPD esters but lower GE. A long frying duration caused a reduction of 3-MCPD esters and an increase in GE. The soaking of potato chips in salt solution significantly increases 3-MCPD esters, but no significant difference was observed in the GE content. The qualities of fries were fall within the safety range.

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Competing interests
The authors declare no competing interests.

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