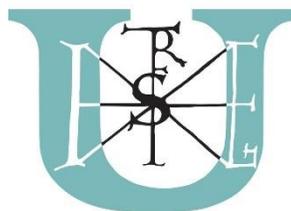


THESIS OF THE DOCTORAL DISSERTATION

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SZENT ISTVÁN UNIVERSITY

DOCTORAL SCHOOL OF FOOD SCIENCE

THESIS OF THE DOCTORAL DISSERTATION

**Investigation of MCPD- and glycidyl ester formation in seed oils
during refining and domestic cooking**

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BUDAPEST

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1 BACKGROUND AND OBJECTIVES

Last decades the plant oil industry focused on the topic of 3-monochloro-1,2-propanediol fatty acid esters (3-MCPD) and glycidyl fatty acid esters (GE). Since the first detection in oils, both components were proved to form during the deodorization step of plant oil refining technology due to the high temperature conditions. The researches so far aimed attention at three fields: formation mechanism, mitigation and analytical developments. The results of these investigations revealed the precursors and the possibility of their mitigation, which oils and fats are critical in terms of formation, which deodorization parameters affect the formation of 3-MCPD-esters and GE, and how to remove them after refining. The developments of analytical methods continuously support the research and practical quality assurance, as well.

The presence of 3-MCPD-esters and GEs is an issue not only in cooking oils reaching the final consumer, but also in other food industrial sectors that use cooking oils and fats as raw materials. As a result of a long evaluation process, maximum levels of 3-MCPD-esters and GEs were determined by European Union depending on the application of oils with special attention on baby food and foods for young children and infants. There may be difficulties in complying with the limit values: on the one hand due to the added contaminated oil and on the other hand due to the possibility of endogenous formation during some technological step – for example industrial frying technology.

Cooking oils intended for the final consumer may contact the temperature that ensures the formation of 3-MCPD esters and GEs during kitchen practice, as well. An example is frying in oil at a temperature of 150-190 ° C. The frying system is very complex in terms of composition, and may contain components that catalyse certain reactions. Therefore, the critical temperature of formation – for example 200 °C for GE formation – could be reduced by these catalytic compounds. In addition to the complexity of the fried product, atmospheric oxygen is also involved in the high-temperature process, so the system of reactions during frying process will be complex as well, ensuring the formation of numerous intermediates and reaction products.

My PhD researches aimed to investigate these technologic contaminants especially in seed oils. That means on the one hand, laboratory study of refining operations (bleaching, deodorization), and on the other hand, the observation of the effect of different chlorine compounds in the framework of thermal treatment experiments at frying temperature.

The objectives of the research

Deodorization process

- Deodorization of different bleached seed oils and comparison of them depending on the raw material (sunflower, rapeseed and soybean)
- Investigation of effect of contact time and deodorization temperature on the formation of MCPD-esters and GE

Thermal treatments

- Comparison of traditional sunflower oil and high oleic sunflower oil in terms of MCPD-ester and GE formation
- Study of different chlorine salts in terms of MCPD-ester formation
- Determination of critical concentration of FeCl_3 at 180°C in terms of MCPD-esters and GE limit values
- Study of temperature effect in presence of 0.1% FeCl_3 in terms MCPD-ester and GE formation

Bleaching process

- Study of MCPD- and GE formation in high oleic sunflower oil under bleaching conditions in presence of 0.1% FeCl_3

Frying process

- Quantitative determination of MCPD-esters and GE formed during experiments conducted under real frying conditions

2 MATERIALS AND METHODS

Deodorization

During the deodorization experiments the three most widely produced seed oils in the world (sunflower, rapeseed and soybean) were investigated. The industrially bleached oils were deodorized in lab-scale deodorization system in two parallel experiments with the following parameters:

- Quantity of oil: 180 g
- Temperature: 220/230/240/250/260°C
- Sampling: at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min
- Absolute pressure: 3-4 mbar
- Stripping gas: N₂

The results of GE were evaluated in 3² full factorial design, as well.

Thermal treatments of oils in presence of chlorine sources

The thermal treatments were conducted under laboratory conditions in two parallel experiments, in a beaker on a hot plate with magnetic stirring (250 rpm). The temperature was monitored and registered through the whole reaction time. The investigated chlorine salts:

- 3% NaCl – Sodium-chloride, table salt
- 3% KCl – Potassium-chloride, analytical grade
- 3% CaCl₂ – Calcium-chloride, analytical grade
- 0,1% NH₄Cl – Ammonium-chloride, analytical grade
- 0,1% FeCl₃ – Ferric(III)-chloride, analytical grade

In the first block of experimental design high oleic (HOSO) and traditional sunflower oil were investigated. The aim of these experiments was to compare the two kinds of oils and the effect of the five different chlorine salts at 180 °C.

The second block focused on the effect of FeCl₃ concentration (0.001-0.5%) in HOSO oil at 180 °C.

In the third block, the effect of temperature (130-180 °C) was studied in HOSO oil in presence of 0.1% FeCl₃.

Bleaching

The predewaxed, washed high oleic sunflower oil was bleached in a laboratory bleaching system in two parallel experiments. Parameters:

- Quantity of oil: 100 g
- Temperature: 100/110 °C
- Bleaching earth, dosage: Tonsil 112 FF (activated), 0.5%
- Bleaching time: 30 min
- FeCl₃ dosage: 0.1%

Frying

The frying experiments were conducted under laboratory conditions with high oleic sunflower oil, in a beaker on a hot plate with magnetic stirring. Parameters:

- Quantity of oil: 200 g
- Temperature: 160 °C
- Fried object: pre-fried, breaded deep frozen pork chop and chicken liver
- Frying circles, time: 3 circles, 5 min
- Ratio of fried object/oil: 1/15

Determination of MCPD- and glycidyl-esters – AOCS 29b-13 method

The quantity of MCPD- and glycidyl-esters was determined by an indirect officially AOCS method. The hydrolysis is conducted under mild alkaline conditions at -22 °C. The reaction is stopped by addition of acidified sodium bromide solution, and the released glycidol is transformed to 3-monobromo-1,2-propanediol in this step. After cleaning the samples with iso-hexane, the components are transferred to organic phase (ethyl-acetate) with two-step liquid-liquid extraction. Volatile derivatives are formed with phenylboronic acid. Finally, the analytes are dissolved in heptane.

Deuterated inner standards:

- Assay A (GE determination): glycidyl-d₅-palmitate, 3-MCPD-d₅
- Assay B (determination of 2- and 3-MCPD esters): 2-MCPD-d₅-palmitate, 3-MCPD-d₅-palmitate

GC-MS parameters:

- Coupled system: Agilent 6890 – GC, 5973 – MS
- Column type: Ultra Inert DB-35MS (35% phenyl-methyl siloxane 30 m×0.25 mm×0.25 µm)
- Carrier gas: Helium 5.0 (1.2 ml/min)
- MS mode: SIM
- Injection: 2 µl with autosampler, splitless mode
- Gradient temperature program

3 RESULTS AND DISCUSSION

Deodorization

The deodorization of soybean and sunflower oil showed similar results for 3-MCPD-esters, the maximum concentration of contaminants was 0.4 mg/kg in both oils. In contrast, the concentration of 3-MCPD-esters in rapeseed oil reached the limit of quantification (LOQ=0.1 mg/kg) only at 260 °C. Most of the 3-MCPD esters were formed at the beginning of the process, during the heating-up period, and during the first 15-45 minutes of the deodorization time. The quantity of 2-MCPD-esters in soybean and sunflower oil reached the LOQ only at 250 and 260 °C, while in rapeseed oil no formation was observed. Based on these results, the chlorine precursors were present only at a low level in the bleached rapeseed oil. The calculated ratio of 3- and 2-MCPD-esters decreased during the deodorization time in case of the sunflower oil and stagnated in case of the soybean oil.

Studying the concentration of GEs, the results of rapeseed and sunflower oil were similar, the maximum levels were 1.4 and 1.6 mg/kg, respectively. In contrast, higher amount (5.5 mg/kg) of GEs formed in soybean oil at 260 °C after 180 minutes, which can be traced back to the level of di- and monoacylglycerols as precursors in the raw material. The level of GEs was under 0.5 mg/kg in all three oils at 230 °C for 90 minutes, so the critical temperature was about 240 °C for the seed oils. The effects of two independent factors – temperature and residence time – and their interaction as a full factorial experimental design were evaluated by analysis of variance. For all three oils, the effect of temperature was dominant, the effect of time was notable only at higher temperatures.

Thermal treatments of oils in presence of chlorine sources

The first block of thermal treatments focused on comparing the two kinds of oils (traditional and high oleic sunflower oil /HOSO/) and the five kinds of chlorine sources (NaCl, KCl, CaCl₂, NH₄Cl, FeCl₃). There was no significant difference between the traditional and HOSO oils (p=0.13), so the further thermal experiments were performed with HOSO.

In presence of different chlorine salts the contaminants formed in different amounts depending on the salt type. The formation of MCPD-esters was catalysed by each salt. Based on the conversion ratio of chlorine to MCPD esters, the order of the salts catalytic effect is as follows: FeCl₃ >> NH₄Cl > CaCl₂ > KCl > NaCl. FeCl₃ had the strongest catalytic effect: 242 mg/kg 3-MCPD ester was formed in the 8 hour long thermal treatment of HOSO at 180 °C in presence of 0.1 % FeCl₃. The dynamic of 2- and 3-MCPD ester formation was different. The formation of 3-MCPD esters was faster in the first half of the experiments in the presence of most chlorine sources, as described in deodorization experiments above: 3-MCPD

ester concentration peaked after 2–4 hours and then decreased or stagnated. In contrast, the concentration of 2-MCPD esters increased steadily until the end of the experiments. The ratio of 3- and 2-MCPD esters typically decreased as the reaction time progressed, while the amount of total MCPD esters stagnated from half of the reaction time. It may be explained with the different availability of the carbon atoms on the glycerol backbone. The sn-2 position is more reactive than the sn-1 and sn-3, but the bond of chlorine on sn-2 is more stable. In the first part of the thermal treatment the formation of 3-MCPD esters is faster, because carbon atoms at external positions are more available for the chlorine.

Despite the fact that chlorine is not a precursor of GEs, the formation of them was catalysed by each salt except for NaCl, the order is as follows: $\text{FeCl}_3 > \text{CaCl}_2 > \text{NH}_4\text{Cl} > \text{KCl}$. The FeCl_3 had the strongest catalytic effect in case of GE formation, as well.

In the second block of the thermal experiments, the effect of FeCl_3 dosage (0.001-0.5%) on the formation of contaminants was investigated. Based on the experimental results, the conversion ratio of chlorine to 3-MCPD esters was lower at lower doses of FeCl_3 , typically 5-7% of chlorine was converted to 3-MCPD esters. In case of higher doses (0.2-0.5% FeCl_3), the conversion ratio reached almost 30%. The ratio of 3- and 2-MCPD esters decreased as the reaction time progressed (as presented in the experiments above), but the initial value (at T_0 : reaching the target temperature) and the rate of decreasing showed relationship with the FeCl_3 dosage. In the experiments with higher doses of FeCl_3 , the ratio of MCPD esters was higher at time T_0 and decreased to a lower value at the end of 2-hour long treatments than in the experiment with lower doses of FeCl_3 .

The determination of threshold concentration was based on the limit values for 3-MCPD esters coming into force from January 2021, namely 1.25 mg/kg. Since this value was exceeded in all cases of my experiments, it was not possible to determine the threshold concentration empirically. The threshold was estimated in two different ways. First, I used the extreme values of the chlorine conversion ratios calculated for the lower FeCl_3 doses (0.005-0.05%), with which I was able to determine a concentration range. In the same dosing range, there is a strong linear relationship between the concentration of 3-MCPD esters and the added FeCl_3 at 1 and 2 hour of reaction time. Using the regression line equations, I calculated the threshold concentration of FeCl_3 , the results are shown in Table 1.

The threshold of FeCl_3 for GE formation taking into account the valid limit value (1 mg/kg) of GEs was determined experimentally. The linear relationship between GE concentration and added FeCl_3 was strong at lower doses of FeCl_3 , so using the regression line equations, I calculated the threshold concentration of FeCl_3 , the results are shown in Table 1.

Table 1: Threshold concentration of FeCl₃ for formation of 3-MCPD and glycidyl esters

Time (hour)	Threshold concentration of FeCl ₃ for 3-MCPD esters (mg/kg)		Threshold concentration of FeCl ₃ for GEs (mg/kg)
	Based on chlorine conversion ratio	Based on regression line equation	Based on regression line equation
1	10.9-12.4	10.2	123.7
2	8.7-11.1	9.5	101.2

In the third block of thermal treatments the effect of temperature between 130 and 180 °C was investigated in presence of 0.1% FeCl₃, the oil was sampled every 15 minutes for 1 hour. This temperature range covers, on the one hand, the usual frying temperatures and, on the other hand, the threshold temperature (140 °C) of the 3-MCPD ester formation under deodorization conditions. The amount of 3-MCPD esters reached 22 mg/kg at 130 °C during the heating-up period, therefore I found that the concentration of 3-MCPD esters is definitely above the limit value with the addition of 0.1% FeCl₃ in the tested temperature range. Based on the results, the ratio of 3 and 2-MCPD esters and its change over time depends on the temperature. The ratio of the two groups of components stagnated up to 165 °C during the 1-hour heating time, while it decreased between 170 and 180 °C over time.

The concentration of GEs exceeded the limit of 1 mg/kg already at 130 °C during the heating-up period, therefore I concluded that with the addition of 0.1% FeCl₃, the concentration of GEs in this temperature range is definitely above the limit.

Bleaching

According to the results presented above, a significant amount of contaminants was formed even at 130 °C in the presence of 0.1% FeCl₃, therefore it is worthwhile to study the process of bleaching, which is in second place in terms of operating temperature during the vegetable oil refining process. The concentrations of 3-MCPD esters exceeded the limit at both 100 and 110 °C in both heating (0.1% FeCl₃) and laboratory bleaching experiments (0.1% FeCl₃ and 0.5% activated bleaching earth). Under atmospheric conditions, the concentration of 3-MCPD esters was about twice as high as the amount formed under bleaching conditions with reduced pressure. Consequently, the presence of atmospheric oxygen may also play a role in the formation of 3-MCPD esters, and intermediates formed in several FeCl₃-catalyzed reactions, such as oxidation intermediates, may react with the chlorine of FeCl₃.

At bleaching temperature, the formation GEs was suppressed, the concentrations were below the limit in each experiment.

Frying

Frying experiments were performed at a medium frying temperature of 160 °C. The concentration of 3-MCPD esters in the oil reached the highest value during the frying of pork chop after the 3rd frying cycle (0.52 mg / kg), the values were slightly lower with the frying of chicken liver. During the heating experiment with 1% NaCl, 0.14 mg/kg of 3-MCPD ester was formed after the third frying cycle. Statistical tests (paired t-tests) showed that the results of 1% salt and pork chop ($p=0.016$) as well as 1% salt and chicken liver ($p=0.004$) differed significantly, but there was no significant difference between frying pork chop or chicken liver ($p=0.29$). These results demonstrate that table salt alone is not sufficient for formation of 3-MCPD esters in high concentrations.

The 3-MCPD ester concentrations obtained during the frying pre-fried, breaded deep frozen products were still well below the limit. Based on the average oil intake of fried foods, these 3-MCPD ester concentrations pose little risk to consumers.

The concentrations GEs ranged from 0.26 to 0.33 mg/kg regardless of treatments without trend.

4 NEW SCIENTIFIC RESULTS

1. It was proved that the formation of MCPD-esters is catalysed by each salt, the order of the catalytic effect is as follows: $\text{FeCl}_3 > \text{NH}_4\text{Cl} > \text{CaCl}_2 > \text{KCl} > \text{NaCl}$.
2. It was stated that the formation of GEs is catalysed by each salt except for NaCl, the order of the catalytic effect is as follows: $\text{FeCl}_3 > \text{CaCl}_2 > \text{NH}_4\text{Cl} > \text{KCl}$.
3. It was verified that the dynamics of 2- and 3-MCPD ester formation are different: the concentration of 3-MCPD esters increases rapidly in the initial phase of the thermal treatments, then stagnates or decreases, while the amount of 2-MCPD ester increases continuously.
4. Threshold concentration was determined for FeCl_3 dosage at 180 °C in terms of 3-MCPD and GE formation.
5. It was stated that the concentration of 3-MCPD esters and GEs in oil exceed their maximum limits at frying temperatures between 130-180 °C in the presence of 1000 mg/kg FeCl_3 .
6. It was verified that in presence of 1000 mg/kg FeCl_3 the bleaching temperature applied during industrial refining is high enough for 3-MCPD ester formation at a concentration above the maximum limit, while GE formation under these conditions is suppressed.
7. The ambient oxygen was proved to play a role in the FeCl_3 catalysed 3-MCPD ester formation, as under atmospheric conditions the concentration of 3-MCPD esters reached higher level than under reduced pressure during bleaching.

5 CONCLUSIONS AND SUGGESTIONS

In the field of 3-MCPD esters and GE, previous research has focused primarily on optimizing the refining of palm oil at both the laboratory and industrial scales. The results of my doctoral research confirmed that the formation of contaminants should be controlled in seed oils as well.

The deodorization experiments revealed that a simultaneous increase in temperature and time could result in extremely high levels of GEs in oils, however the formation can be controlled (<1 mg/kg) under industrial deodorization conditions in case of the tested oil types. The formation of 3-MCPD esters cannot be prevented by optimizing the deodorization parameters. For both contaminants, the quality of raw material plays a key role because of the presence of precursors: the cultivation and harvesting of oilseed, the oil extraction operations and the oil refining steps prior to deodorization are also important.

Investigation of different chlorine sources also contributes to the understanding of the processes taking place in the production and application of cooking oils. Primarily, inorganic chlorine sources have been selected that the oil plant can naturally absorb from the soil, fertilizer and irrigation water, or the fried food may contain them. The role of table salt in kitchen practice is essential, and KCl and CaCl₂ are additives approved to use in food in the European Union. Previous studies investigating the effect of chlorine sources on the formation of 3-MCPD esters under other experimental conditions were also taken into account in the selection of chlorine sources.

Based on the results a clear order of chlorine salts was established in terms of catalytic effect. Although in the experiments I worked with extremely high doses (0.1 or 3%), which cannot occur either in the crude oil, during the refining steps or during the application of the oils, but from a practical point of view it should be emphasized that these substances are able to catalyse the formation reactions. In addition, the threshold concentration of FeCl₃ is about 10 mg/kg for the formation of 3-MCPD esters under the examined reaction conditions, which is more likely to occur naturally. The threshold concentration of FeCl₃ for 3-MCPD esters could not be determined experimentally on a laboratory scale, so a scaling up could be a solution. On a pilot scale with 10-40 kg batches of oil, the lower dosage of FeCl₃ would involve less weighing uncertainty. In terms of temperature, at a concentration of 0.1% FeCl₃, even at the temperature of the oil bleaching, the formation of contaminants cannot be suppressed: 3-MCPD esters are formed at 100 °C.

My experiments, which represent real frying conditions, have shown that frying meat results in a significantly higher concentration of 3-MCPD ester than the addition of table salt alone. Based on the results, the amount of 3-MCPD esters formed at the chosen frying temperature does not pose a health risk in the case of a balanced and varied food consumption.

Overall, the results of my empirical thermal treatment experiments in oil refining and domestic cooking contribute to the understanding of the formation of MCPD and glycidyl esters. The effects of the investigated parameters (origin of chlorine, concentration of chlorine source, temperature, presence of oxygen, real frying conditions) can be well evaluated based on my results and help further research of these contaminants.

6 PUBLICATIONS RELATED TO THE THESIS

Articles with impact factor

Erzsébet Bognár, Gabriella Hellner, Andrea Radnóti, László Somogyi, Zsolt Kemény (2020): Effect of Different Chlorine Sources on the Formation of 3-Monochloro-1,2-Propanediol and 2-Monochloro-1,3-Propanediol Fatty Acid Esters during Frying. *Periodica Polytechnica Chemical Engineering*. 64(4): 523–529.

DOI: <https://doi.org/10.3311/PPch.14137> **IF: 1,257**

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DOI: <https://doi.org/10.1556/066.2020.49.2.7> **IF: 0,384**

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