Formation and Decomposition of 3-Chloropropane-1,2-diol Esters in Models Simulating Processed Foods

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Abstract

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The formation of 3-chloropropane-1,2-diol (3-MCPD) released from its esters with higher fatty acids was studied using the recognised precursors of 3-MCPD (tripalmitin, 1,3-dipalmitin, 1-monopalmitin and soybean oil) in the presence of sodium chloride. The precursors were reacted with sodium chloride in an emulsion stabilised with an emulsifier under conditions which modelled the thermal treatment of foods during processing. The highest amount of bound 3-MCPD (released from its esters) was formed from 1-monopalmitin followed by 1,3-dipalmitin, whereas tripalmitin and soybean oil yielded the lowest levels of bound 3-MCPD. Four sets of experiments were then carried out aimed at monitoring the influence of various factors (soybean oil amount, NaCl content, water content, and temperature) on the yield of bound 3-MCPD. The formation of bound 3-MCPD was directly proportional to the concentration of either oil or NaCl. The highest amount of bound 3-MCPD was formed in media containing approximately 20% water. The amount of bound 3-MCPD decreased with increasing temperature over the range 100–230°C and reached its highest value at 100°C. Models with 1,2-dipalmitoyl-3-chloropropane-1,2-diol showed that the decomposition rate of this ester rapidly increased with increasing temperature over the range 100–230°C being the lowest at 100°C and the highest at 230°C.

Keywords: chloropropanols; 3-chloropropane-1,2-diol; 3-MCPD; bound 3-MCPD, 3-MCPD esters; lipids; contaminants

During frying as well as during other thermal processes fats thermally and oxidatively decompose to a great number of various products. Water and steam hydrolyse triacylglycerols and phospholipids, producing diacylglycerols, monoacylglycerols, free fatty acids, glycerol and other products. Other non-volatile polar products include partial acylglycerols containing chain scission products, triacylglycerol oligomers, and oxidised triacylglycerols. Water partly evaporates, especially in the surface layer of food. Glycerol partially decomposes, partially evaporates and the reaction equilibrium is thus shifted in favour of the hydrolysis products. The extent of hydrolysis is a function of various factors, such as the oil temperature, the interface area between oil and the aqueous phase, the amount of water and steam (since water hydrolyses lipids more quickly than steam) (Warner 1998). Moreover, various lipids (acylglycerols), glycerol, and naturally present and/or intentionally added

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sodium chloride may become the precursors to chlorinated propanols, particularly to 3-chloropropane-1,2-diol (3-MCPD), in thermally processed foods such as meat, diary and cereal products (Hamlet *et al.* 2002).

Fatty acid esters of 3-MCPD are known precursors to the formation of 3-MCPD in model mixtures consisting of hydrochloric acid and triacylglycerols, phospholipids, soybean oil, soybean meal, wheat lipids, and maize gluten lipids (Davídek *et al.* 1980; Velíšek *et al.* 1982). They were also found as the constituents of acid-HVP (Velíšek *et al.* 1980) and reported in the neutral fraction of goat's milk lipids (Cerbulis *et al.* 1984), where their occurrence was tentatively ascribed to the use of chlorine-based sanitisers.

Our recent findings indicate that the formation of 3-MCPD esters (monoesters and diesters with higher fatty acids) is characteristic for a variety of processed foods (Divinová *et al.* 2004; Svejkovská *et al.* 2004; Doležal *et al.* 2005). The esters of 3-MCPD with higher fatty acids represent a new class of food contaminants as they are the bound form of 3-MCPD. Subsequently, 3-MCPD can be released from these esters by a lipase-catalysed hydrolysis reaction *in vivo*. In many cases, the amount of 3-MCPD thus released (called bound 3-MCPD) exceeded the level of free 3-MCPD. The levels of bound 3-MCPD found in the retail foods varied between traces (140 μg/kg) and 6100 μg/kg and were 10 to 396 times higher than the free 3-MCPD levels. Five foods of plant origin (particularly crisp bread, salty crackers, doughnuts, french fries and dark malt) that were processed at high temperatures contained elevated levels of bound 3-MCPD. The highest level of bound 3-MCPD (6100 μg/kg) was found in a sample of french fries (Svejkovská *et al.* 2004). The level of bound 3-MCPD in roast coffee was relatively low and varied between 6 μg/kg (soluble coffees) and 390 μg/kg (decaffeinated coffee) and exceeded the free 3-MCPD level 8 to 33 times (Doležal *et al.* 2005). The presence of bound 3-MCPD in pickled olives and herrings suggests that these compounds can also form at relatively low temperatures and even in acid media (Svejkovská *et al.* 2004). A series of virgin and refined edible oils obtained from the retail market and a local producer were analysed for the contents of free and bound 3-MCPD. These oils contained free 3-MCPD ranging from < 3 μg/kg (LOD) to 24 μg per kg. The level of bound 3-MCPD varied between < 100 μg/kg (LOD) and 2460 μg/kg. On average, virgin oils had lower levels of bound 3-MCPD than refined oils. Higher levels of bound 3-MCPD were found in oils obtained from roasted oilseeds, germ oils, and the majority of refined oils including refined olive oils. It appears that the formation of 3-MCPD esters in oils is linked with the process of oil refining. Heating of oils also led to an increase of the bound 3-MCPD level (Zelinková *et al.* 2006).

The analysis of white bread showed (Hamlet *et al.* 2004) that the highest levels of 3-MCPD esters were found in the crust (2916 μg/kg) and toast (853 μg/kg) while in crumb they were found in much lower amount (26 μg/kg). The corresponding levels of free 3-MCPD were 477 μg/kg, 93 μg/kg and < 0.5 μg/kg, respectively, i.e. 6 to 52 times lower. It was also shown that free 3-MCPD can be released from its esters by hydrolysis with a commercial lipase (from *Aspergillus oryzae*). The possible formation of 3-MCPD by a lipase-catalysed transesterification of triacylglycerols has recently been reported (Robert *et al.* 2004). It is not yet known whether this phenomenon is due to hydrolysis of 3-MCPD esters or lipase catalysed formation from triacylglycerols and chlorides.

It is evident that further studies are needed to investigate the formation of 3-MCPD from lipids and sodium chloride under conditions more typical of baking, roasting, and toasting processes. Described herein are models using systems with low water activity simulating surface layers of foods, systems with various classes of lipids and sodium chloride (the recognised precursors of 3-MCPD) and heated to high temperatures corresponding to those encountered during the thermal processing of foods.

MATERIAL AND METHODS

Chemicals and materials. 3-Chloropropane-1,2-diol (98%) was obtained from Merck (Darmstadt, Germany), phenylboronic acid, tripalmitin (1,2,3-tripalmitoyl-*sn*-glycerol, 99%) were from Fluka Chemie (Buchs, Switzerland), 3-chloropropane-1,2-diol-d_5 (99.4%) was from Dr. Ehrenstorfer (Augsburg, Germany), Tween 80, sodium chloride and paraffin were from Lachema (Brno, Czech Republic). DL-α-Monopalmitin (1-monopalmitoyl-*sn*-glycerol, 98%) and 1,3-dipalmitoylglycerol (1,3-dipalmitoyl-*sn*-glycerol, 99%) were a gift from the Department of Dairy and Fat Technology, Institute of Chemical Technology, Prague, Czech Republic. 1,2-Dipalmitoyl-3-chloro-1,2-propanediol (97%) was synthesised (Kraft *et al.* 1979) and purified on a silica gel column using light petroleum ether/diethyl ether mixtures. Soybean oil was a retail market product (Brölio, Hamm, Germany). All other reagents and solvents were of analytical purity.

Model systems with soybean oil. The basic reaction mixture consisted of soybean oil (100 mg), NaCl (7.5 mg), the inert emulsifier Tween 80 (15 mg), and paraffin (12.5 mg) that were placed in a 5 ml glass tube together with water (15 mg). The tube was sealed, sonicated and heated in an oven at 100°C, 140°C, 170°C, 200°C, and 230°C for 30 min and then cooled to room temperature. The influence of oil, salt and water amounts was studied in reaction mixtures with modified compositions at 100°C for 30 min. Using the basic reaction mixture, the amount of soybean oil was changed at the expense of paraffin ranging from 15 to 100 mg. The amount of sodium chloride ranged from 0.06 to 15 mg and that of water from 0 to 27 mg. A modified system was used to study the influence of higher amounts of water. In this case the reaction mixture consisted of soybean oil (100 mg), NaCl (5 mg), Tween 80 (20 mg), water (0 up to 125 mg), and paraffin (125 mg down to 0).

The cold tube was opened, its content dissolved in tetrahydrofurane (1 ml) and transferred to a volumetric flask (10 ml) using the same solvent. An aliquot (1 ml) of this solution with internal standard solution added (1 ml) was evaporated to dryness in a vacuum rotary evaporator at 45°C, the residue was derivatised with phenylboronic acid and used for the determination of free 3-MCPD according to the method of Divinová *et al.* (2004). All determinations were repeated three times. Another aliquot (1 ml) of the solution was allowed to react with an interesterification reagent (1.8 ml of 1.8% sulphuric acid in methanol) at 40°C for 16 h, neutralised, evaporated to dryness, derivatised with phenylboronic acid and used for the determination of bound 3-MCPD, i.e. 3-MCPD release by the interesterification reaction from its esters with higher fatty acids (Divinová *et al.* 2004).

Model systems with 1,2-dipalmitoyl-3-chloropropane-1,2-diol. The basic reaction mixture in a glass tube (5 ml) contained 1,2-dipalmitoyl-3-chloropropane-1,2-diol (30 µl, 0.312 mg/ml) in tetrahydrofurane. The solvent was evaporated in a stream of nitrogen and NaCl (7.5 mg), paraffin (112.5 mg), emulsifier (15 mg) and water (15 ml) were added. The sealed tube was then heated at 100°C, 140°C, 170°C, 200°C and 230°C and further processed as given above. Three replicates of each determination were made.

Methods. GC/MS analyses were carried out on an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a quadrupole mass selective detector Agilent 5973 MSD (70 eV) and data processing system (MSD ChemStation, G1701CA version C.00.00). Gas chromatography was performed on a capillary column SPB-1 (30 m × 0.25 mm i.d., thickness of 1 µm, Supelco, PA, USA). The injector was held at 250°C (splitless), the column temperature was programmed from 80°C (1 min) to 300°C (37 min) at a rate of 10°C/min. Helium at a flow rate of 0.8 ml/min was used as the carrier gas, 1 µl sample was injected. For the quantification purposes, single ion monitoring was used to monitor ions at *m/z* 147 (3-MCPD) and at *m/z* 150 (3-MCPD-d_5).

RESULTS AND DISCUSSION

Preliminary experiments were carried out using mixtures of soybean oil, tripalmitine, 1,3-dipalmitine and 1-monopalmitine with NaCl (5%) and water (9.4%) to simulate the formation of 3-MCPD in the surface layers of thermally processed foods containing naturally present or intentionally added salt. An inert emulsifier was added to make a dispersed system of these immiscible substances. The individual mixtures were heated at 230°C for 30 min and then analysed for the bound 3-MCPD levels. The original level of bound 3-MCPD in the soybean oil used throughout these experiments was under the limit of detection (LOD = < 100 µg/kg).

It has been shown that the highest amount of 3-MCPD was released from 1-monopalmitin (238 µmole/mole), followed by 1,3-dipalmitin (99 µmole/mole), whereas tripalmitin (28 µmole per mole) and soybean oil (approximately 45 µmole per mole) reaction products yielded the lowest levels of 3-MCPD. The amount of bound 3-MCPD produced from 1-monopalmitin was almost 9 times higher than that produced from tripalmitin.

The extent to which the various pathways lead from acylglycerols to 3-MCPD esters is still unknown. The overall reactions that can proceed

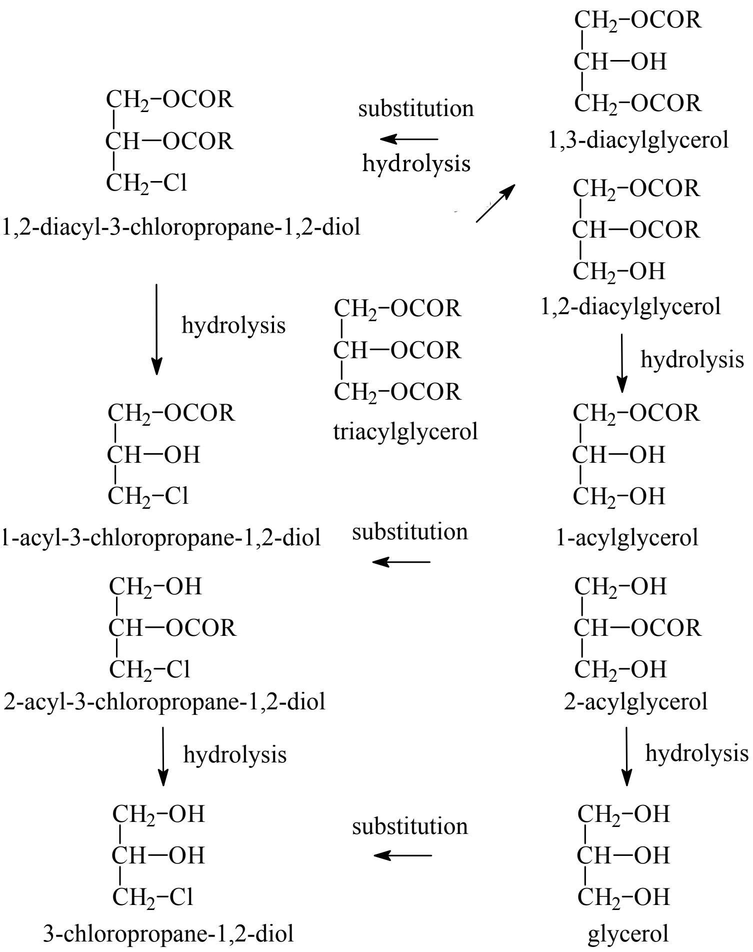

Figure 1. Formation of 3-MCPD and its esters from acylglycerols

during the heating of triacylglycerols with NaCl are schematically outlined in Figure 1. To simplify the reaction scheme, only the hydrolytic reactions leading from triacylglycerols to partial acylglycerols (diacylglycerols and monoacylglycerols), glycerol, 3-MCPD monoesters, and 3-MCPD are given as well as the major substitution reactions leading to diesters and monoesters of 3-MCPD. The formation of 2-chloropropane-1,3-diol (2-MCPD) and dichloropropanols (1,3-dichloropropan-2-ol and 2,3-dichloropropan-1-ol) was not taken into consideration. Possible mechanisms of 3-MCPD formation from monoacylglycerols and diacylglycerols have been proposed (Hamlet *et al.* 2004).

Starting from triacylglycerols, their hydrolysis leads first to diacylglycerols that are further hydrolysed to monoacylglycerols and finally to glycerol. It is supposed that 3-MCPD esters form by nucleophilic substitution of the acyl group by chloride anion. The formation of these esters was explained, in accord with the theory, as proceeding via the partial acylglycerols with the ester group providing anchimeric assistance through the formation of a cyclic acyloxonium ion intermediates (Collier *et al.* 1991; Hamlet & Sadd 2002). This cyclic acyloxonium ion intermediates readily form by the elimination of hydroxyl groups from either diacylglycerols or monoacylglycerols (Figure 2).

The reaction of acyloxonium ion intermediate derived from diacylglycerols with chloride anion leads to 3-MCPD diesters whereas the intermediate derived from monoacylglycerols leads mostly to 1-acyl-3-chloropropane-1,2-diol and the corresponding 2-acyl-3-chloro-propane-1,2-diol is a minor product. The steric and electronic effects arising from the terminal ester group, which pref-

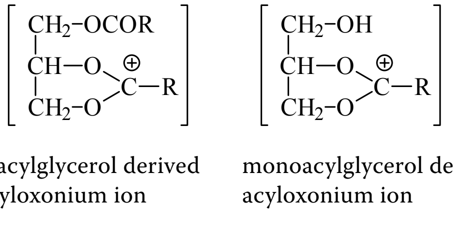

Figure 2. Structure of cyclic acyloxonium ion intermediates derived from diacylglycerols and monoacylglycerols

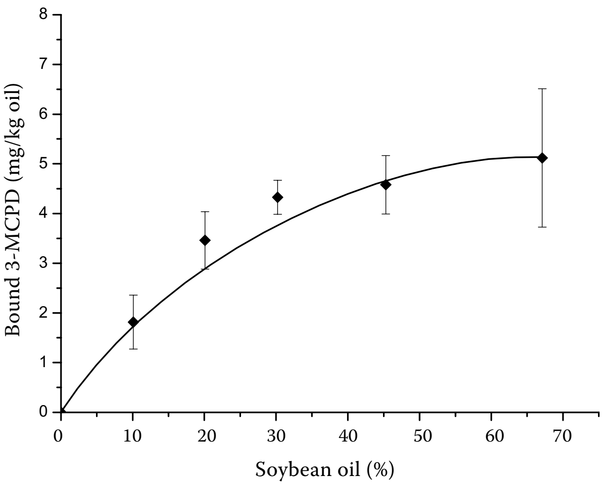

Figure 3. Formation of bound 3-MCPD influenced by soybean oil amount at 100°C

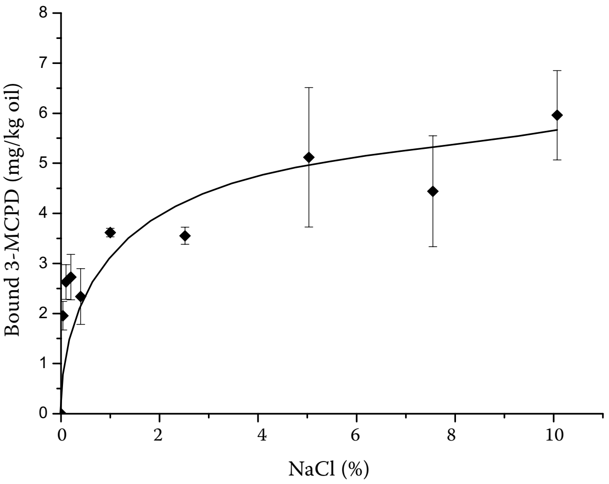

Figure 4. Effect of sodium chloride on bound 3-MCPD content at 100°C

erentially directs substitution to the CH_2 carbon atom, control the ratio of 3-MCPD and 2-MCPD esters. 3-MCPD diesters are finally hydrolysed to the corresponding monoesters, and monoesters to free 3-MCPD. Another way in which chloropropanediol esters can arise is a direct substitution of acyl or hydroxyl groups in acylglycerols by chloride anions.

Subsequently, models consisting of soybean oil, NaCl and water were used throughout all further experiments. Four sets of experiments were carried out with the aim to monitor the influence of soybean oil amount, NaCl level, water content, and temperature on the yield of bound 3-MCPD (however, the employed method did not differentiate between 3-MCPD diesters and 3-MCPD monoesters). As it could be expected (Figure 3), the amount (expressed in mg/kg soybean oil) of bound 3-MCPD increased with the amount of soybean oil present in the model.

The results of the experiments aimed at studying the formation of bound 3-MCPD from soybean oil influenced by the amount of NaCl are given in Figure 4. As can be seen, the quantity of bound 3-MCPD formed depended on the concentration of NaCl and increased with its concentration in the reaction mixture. It seems probable that in the presence of higher amounts of NaCl, even higher amounts of bound 3-MCPD would form.

The amount of water present in the reaction mixture also affected the formation of bound 3-MCPD from soybean oil. It can be seen (Fig-

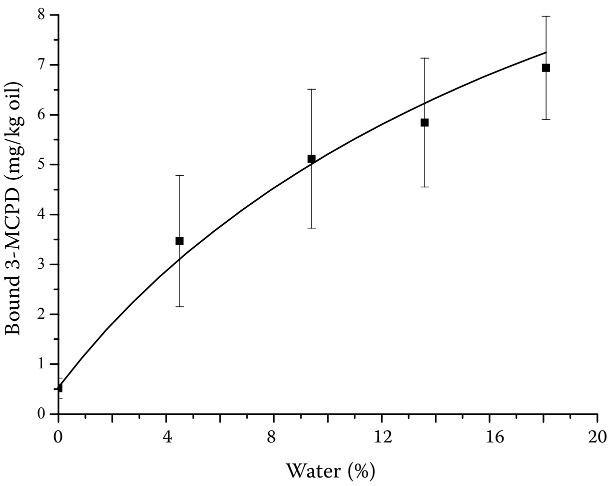

Figure 5. Effect of water (0–20%) on bound 3-MCPD content at 100°C

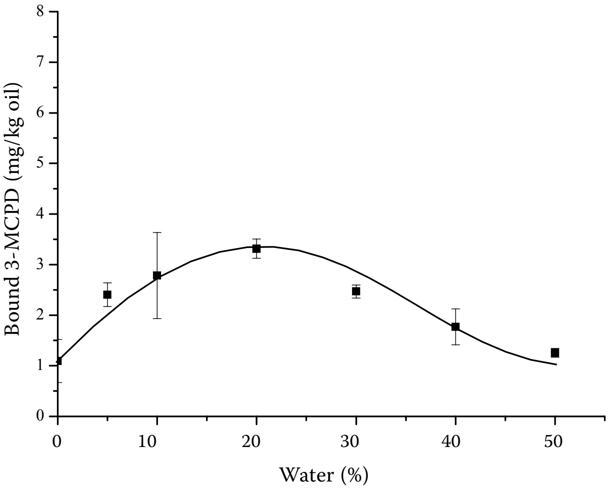

Figure 6. Effect of water (0–50%) on bound 3-MCPD content at 100°C

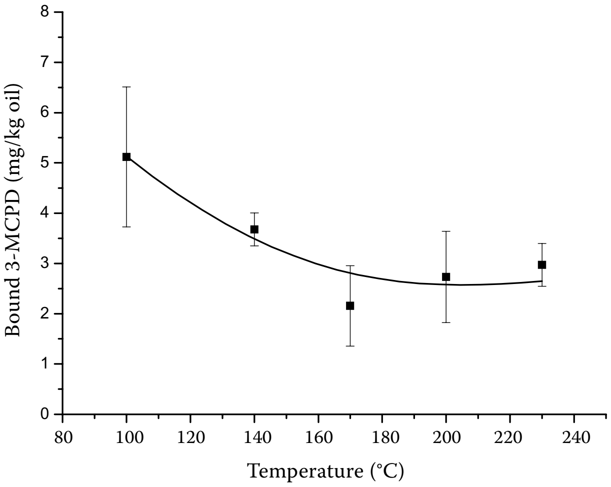

Figure 7. Influence of temperature on bound 3-MCPD

ure 5) that in the systems with low water levels (where the hydrolysis of the acyl group can hardly proceed) the direct nucleophilic substitution of the acyl group in soybean oil triacylglycerols by chloride ion to form 3-MCPD esters is very slow. In systems containing more water, triacylglycerols can be firstly hydrolysed to partial acylglycerols that then more easily react with chloride ions yielding 3-MCPD esters.

To study this phenomenon, model systems with soybean oil containing different amounts of water were evaluated and it was found that the highest amount of bound 3-MCPD formed in models having the highest water content. However, the actual model used throughout this study did not allow to use higher amounts of water. Therefore, a different model having the water content up to 50% was employed and it was found that the largest amount of bound 3-MCPD formed in media containing approximately 20% water. At higher water levels the amount of bound 3-MCPD decreased (Figure 6).

The influence of temperature on the formation of bound 3-MCPD was studied in the range of 100–230°C. The results illustrating the formation of bound 3-MCPD are given in Figure 7. It can be seen that the amount of bound 3-MCPD decreased with increasing temperature mainly due to polymerisation of 3-MCPD esters (Zelinková *et al.* 2006). The highest level of 3-MCPD was released from the corresponding esters at 100°C.

As it was shown, the formation of bound 3-MCPD is a multivariate problem as it depends (at a given temperature) on water, fat, and salt contents as well as on the fat composition. The aim of the following part of our research was to study the simultaneous formation and decomposition of bound 3-MCPD from soybean oil under different temperatures (100–230°C). The results can be seen in Figure 8.

As expected, at all temperatures bound 3-MCPD formed and decomposed simultaneously so that the maximum levels at the lowest temperatures (100°C and 140°C) were achieved in about 2 h of heating whereas at higher temperatures (e.g. 200°C) the maximum levels were reached at about 13 h and later. Absolutely, the highest amount of bound 3-MCPD was produced at 230°C. It was already shown (Figure 1) that the reaction pathways lead-

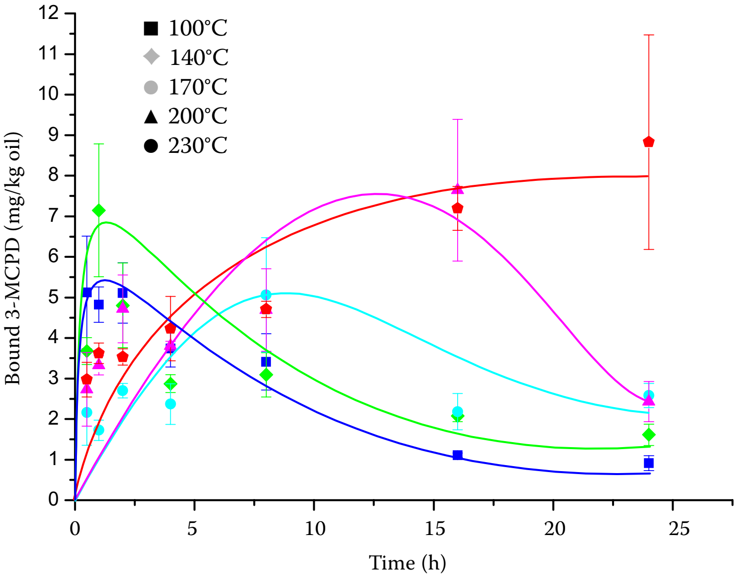

Figure 8. Bound 3-MCPD formed from soybean oil at different temperatures

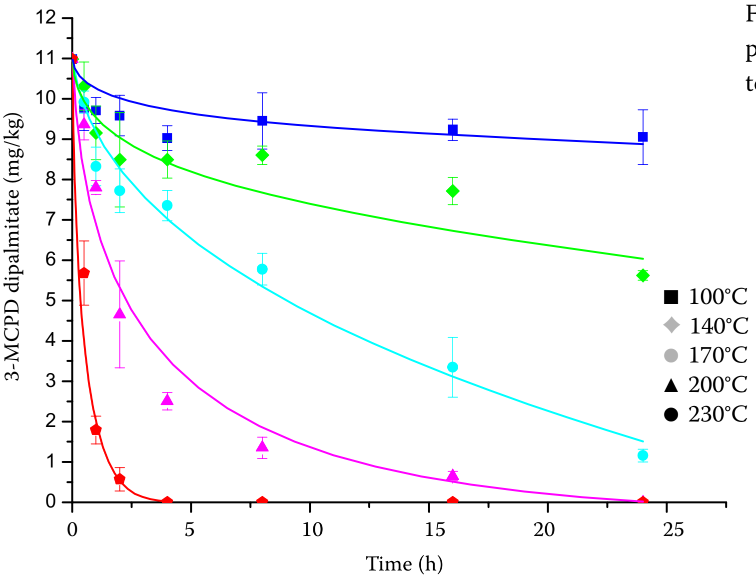

Figure 9. Bound 3-MCPD formed from 1,2-dipalmitoyl-3-chloropropane-1,2-diol at different temperatures

ing to 3-MCPD esters are very complex and the results obtained do not enable to calculate the corresponding rate constants. Therefore, another set of experiments was prepared to follow the stability of 3-MCPD esters at higher temperatures. The models were again mixtures of water and soybean oil with only an emulsifier and an inert material (paraffin). Each of the mixtures was heated at temperatures of 100°C to 230°C for up to 24 h. 1,2-Dipalmitoyl-3-chloropropane-1,2-diol was used throughout this study as a typical representative of 3-MCPD esters arising in processed foods (Zelinková *et al.* 2006). It is evident (Figure 9) that the decomposition rate of 1,2-dipalmitoyl-3-chloropropane-1,2-diol rapidly increased with increasing temperature, being the lowest at 100°C and the highest at 230°C.

CONCLUSION

The experiments conducted with model mixtures that contained soybean oil, sodium chloride, and water has shown that bound 3-MCPD (occurring as mono- and diesters with higher fatty acids) levels depend on the amount of the respective component. The bound 3-MCPD levels were directly proportional to the amounts of soybean oil and sodium chloride, the maximum levels having been achieved in models containing 20% water. The bound MCPD levels were also proportional to the temperature employed. It was shown that the MCPD esters are not stable compounds as they relatively readily decompose on heating.

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