Formation of Acylglycerol Chloro Derivatives in Vegetable Oils and Mitigation Strategy

Jan ŠMIDRKAL[1], Vojtěch ILKO[2], Vladimír FILIP[1], Marek DOLEŽAL[2], Zuzana ZELINKOVÁ[2], Jan KYSELKA[1], Iveta HRÁDKOVÁ[1] and Jan VELÍŠEK[2]

[1]Department of Dairy and Fat Technology and [2]Department of Food Chemistry and Analysis, Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Prague, Prague, Czech Republic

Abstract

Šmidrkal J., Ilko V., Filip V., Doležal M., Zelinková Z., Kyselka J., Hrádková I., Velíšek J. (2011): **Formation of acylglycerol chloro derivatives in vegetable oils and mitigation strategy**. Czech J. Food Sci., **29**: 448–456.

The most important acylglycerol chloroderivatives identified in foods are 3-chlorpropane-1,2-diol fatty acid esters (3-CPD esters) that are accompanied by epoxypropanol fatty acid esters formed in processed foods and, particularly, during the deodorisation of vegetable oils. Their content in refined vegetable oils is influenced by the oil composition, refining process conditions and process conditions of hydrogenation. Described and discussed here are the main pathways that lead to the formation of acylglycerols chloroderivatives and epoxypropanol fatty acid esters. The article offers detailed explanation of the reaction mechanisms using the well-known chemical principals based on experimental data. The conditions suitable for removing the unwanted products from the refined vegetable oils were studied in models containing variable proportions of agents (bicarbonates or carbonates) causing the decomposition of 3-CPD fatty acid esters.

Keywords: 3-chloropropane-1,2-diol; 3-CPD, 3-MCPD; 3-chloropropane-1,2-diol esters; bound 3-CPD; processing contaminants; mitigation strategy

3-Chloropropane-1,2-diol (3-CPD) is one of a series of chemically related contaminants collectively known as chloropropanols. Free 3-CPD and 3-CPD esters with fatty acids (bound 3-CPD) are present in a number of foods, especially in foods with high contents of salt and fat, in sour foods, and, especially, in baked, fried, and grilled foods that are processed at high temperatures (Svejkovská *et al*. 2004). It should be noted that the formation of 3-CPD and 3-CPD esters in foods cannot be fully avoided, nevertheless various measures, such as low temperature processing and low salt additions, can be taken to reduce their concentrations. The most prevalent chloropropanol esters widespread in thermally processed foods are 3-chloropropane-1,2-diol (3-CPD) esters followed by 2-CPD esters, 1,3-dichloropropan-2-ol esters, 2,3- dichloropropan-1-ol esters, free chloropropanediols and free chloropropanols that occur in lower or insignificant concentrations. High concentrations of 3-CPD esters have been found in certain processed foods (Svejkovská *et al*. 2004) and some edible oils, notably in refined palm and olive oils (Zelinková *et al*. 2006; Karšulínová *et al*. 2007; Franke *et al*. 2009; Weisshaar 2011). Consequently, high concentrations of 3-CPD esters were present in products containing refined vegetable oils, such as infant and baby foods (Zelinková *et al*. 2009; Weisshaar 2011), deep-fried potato

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products (Ilko *et al*. 2011), coffee creamers, cream aerosols, bouillon cubes (Karšulínová *et al*. 2007), breakfast cereals, biscuits (including infant biscuits), and crackers (Svejkovská *et al*. 2004; Hamlet *et al*. 2011), which prompted further interest in these compounds.

The respective studies have linked free 3-CPD with infertility in rats, suppression of the immune function and possible carcinogenicity. In 2001, the EC Scientific Committee on Food established a Tolerable Daily Intake (TDI) of 2 µg/kg body weight for 3-CPD (European Commission 2001a) while maximum concentration level of 20 µg/kg has been specified in EU legislation for 3-CPD in hydrolysed vegetable protein and soy sauce (European Commission 2001b). Based on the opinion on CPD esters (BfR 2007) by the German Institute for Risk Assessment (BfR), the EFSA COMTAM panel has assumed a 100% release of 3-CPD from its esters in the gut (EFSA 2008). 3-CPD esters as such have previously been evaluated on a limited scale for their anti-tumor properties (Langen *et al*. 1979) and mutagenicity (Šilhánková *et al*. 1982) and are widely used as substrates for the chemical synthesis of e.g. biologically active chemicals (Stamatov & Stawinski 2006); otherwise, they appear to find no direct industrial application. The toxicological significance of 3-CPD esters and their potential contribution to the dietary intake of non-esterified 3-CPD is not yet known.

Several review articles covering the literature on chloropropanols and their esters since 1991 to the end of 2010 have been already published (Collier *et al*. 1991; Hamlet *et al*. 2002, 2011; Hamlet & Sadd 2009; Velíšek 2009). The most important precursors of chloropropanol esters are the fats derived from glycerol, i.e. glycerolipids (such as TAG, partial acylglycerols e.g. DAG, MAG and glycerophospholipids) and chloride ions, that can be naturally present, added to foods as sodium chloride or contained in the bleaching earth used for oil refining (Hamlet & Sadd 2009; Velíšek 2009; Yaylayan 2009; Hamlet *et al*. 2011). The main physical factors influencing the formation of chloropropanol esters are the water activity, reaction temperature, and time. Due to the fragmentary data available, the major pathways of chloropropanol esters formation in foods are still unclear as all details are not known and the mechanisms suggested need to be verified.

Described herein are our investigations into the mitigation of 3-CPD esters effects in models consisting of oils containing a high quantity of 3-CPD esters. Our attention was mainly focused on the role of various factors that might influence the amount of 3-CPD esters found in commercial refined oils. Besides, the aim of this work was also aimed at the reaction mechanisms using the well-known chemical principals and the accessible experimental data.

MATERIAL AND METHODS

Chemicals. 3-Chloropropane-1,2-diol (3-CPD, > 98%), hexane for organic trace analysis and tetrahydrofurane p.a. were purchased from Merck (Darmstadt, Germany), 3-CPD-d_5 (97%) from Sigma Aldrich Izotech (St. Louis, USA), phenylboronic acid (PBA, ≥ 97%) and sulphuric acid (> 95%) from Fluka Chemie (Buchs, Switzerland), sodium bicarbonate p.a. (99.5%) methanol p.a. (99.8%) and sodium suplhate p.a. (99%) from Penta (Strakonice, Czech Republic). The ester of 3-CPD with palmitic acid and deuterium labelled ester of 3-CPD-d_5 with palmitic acid were synthesised according to Kraft *et al*. (1979). All other reagents and solvents were of analytical grade.

Oil sample. The Sunflower oil Fritol (acid value 0.14 mg KOH/g, saponification value 211.2 mg/g, < 0.1% MAG, 0.4% DAG) was obtained from the Slovak Republic. MAG and DAG contents were determined by TLC-FID using the apparatus Iatroscan (Iatron Laboratories, Inc., Tokyo, Japan) and Chromarod S II using the development in a mixture of hexane:diethyl ether:formic acid (92:8:1, v/v/v).

Oil deodorisation. The oil (150 g) was deodorised in a reactor (glass tube, height 160 mm, inner diameter 45 mm) provided with a sintered glass (S4) in the lower part and gas exhaust in the upper part, placed in a thermostat and held at 100°C. Following the addition of sodium chloride (0.12 g NaCl in 0.36 ml of water), the temperature was raised to 160°C and water was removed with a gentle stream of argon. After 5 min, the basic compound (0 or 37.5 mg $KHCO_3$ or 31.4 mg $NaHCO_3$ or 19.8 mg Na_2CO_3) was added (time = 0) and the temperature was raised to 240°C during 10 minutes. At the given intervals, aliquots (5 g) were taken for analysis. The amount of the basic substance corresponded to the KOH equivalent calculated using the oil acid value.

Determination of bound 3-CPD. The bound 3-CPD was determined according to the method of Divinová *et al*. (2004), modified by Zelinková *et al*. (2006), which is based on the derivatisation of

Table 1. Formation of 3-CPD esters in sunflower oil at 240°C

Composition	Time (h)					
	0.0	0.5	1.0	1.5	2.5	4.5
Oil	0.30	0.30	0.31	0.33	0.33	0.30
Oil + NaCl	0.30	1.42	2.10	1.72	1.60	2.12
Oil + NaCl + $NaHCO_3$	0.30	0.45	0.52	0.42	0.48	0.47
Oil + NaCl + Na_2CO_3	0.30	0.35	0.35	0.44	0.55	0.51
Oil + NaCl + $KHCO_3$	0.30	0.41	0.30	0.30	0.38	0.54

Relative standard deviations are < 5%

3-CPD with phenylboronic acid and determination of the volatile 4-chloromethyl-2-phenyl-1,3,2-dioxaborolane using GC/MS analysis. Deuterated 3-CPD-d_5 diester with palmitic acid was used as the internal standard. Three parallel examinations of each sample were made.

GC/MS analysis. Capillary GC/MS analysis of the bound 3-CPD was carried out on an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Palo Alto, USA) equipped with a quadrupole mass selective detector Agilent 5975 MSD (70 eV). Gas chromatography was performed on a capillary column Equity-1 (30 m × 0.25 mm × 1 µm; Supelco, Bellefonte, USA). The injector was held at 250°C (pulsed splittles), the column temperature was programmed from 80°C (1 min) to 170°C at a rate of 10°C/min, then to 200°C at a rate of 3°C/min, and then to 300°C (15 min) at a rate of 10°C/min. Helium was used as the carrier gas (flow rate 0.8 ml/min), 1 µl sample was injected. For quantification purposes, single-ion monitoring was used to monitor ions at *m/z* 147 (3-CPD) and at *m/z* 150 (3-CPD-d_5). Ions at *m/z* 196 and 198 (3-CPD) and at *m/z* 201 and 203 (3-CPD-d_5) were used as qualifiers.

RESULTS AND DISCUSSION

During thermal processes, fats (neutral lipids and phospholipids) thermally and oxidatively decompose to a great number of various products and react with chlorides yielding esters of chloropropanols, among which 3-CPD esters predominate. It is not surprising that 3-CPD esters form in relatively high amounts during the refining of edible oils, notably in refined palm (Karšulínová *et al*. 2007) and olive pomace oils (Zelinková *et al*. 2006). These oils contain relatively high concentrations of partial acylglycerols and are processed at high temperatures in the deodorisation step and during physical refining(Franke 2009). During the deodorisation step, the oil is heated to 180-260°C under reduced pressure (3–20 mbar) for the removal of free fatty acids and flavour-active volatiles.

Reaction mechanisms

The most important precursors of chloropropanol esters identified in foods and vegetable oils are the fats derived from glycerol (glycerolipids), such as triacylglycerols (TAG) and partial acylglycerols, glycerophospholipids, and chloride ions (Hamlet & Sadd 2009; Velíšek 2009; Yaylayan 2009; Hamlet *et al*. 2011). Raw vegetable oils always contain partial acylglycerols (diacylglycerols and monoacylglycerols), phospholipids, free fatty acids, and chlorides that are present either as anions derived from the plant pulp or as hydrochloric acid derived from the bleaching earth (Franke *et al.* 2009; Yaylayan 2009; Collison 2010). The mechanism of the formation of 3-CPD esters from triacylglycerols was first proposed by Collier *et al*. (1991) and was later revised by Yaylayan (2009) and Hamlet *et al*. (2011). According to Yaylayan (2009), TAG may undergo acid hydrolysis resulting in the formation of an acylated cyclic acyloxonium ion intermediate (2-alkyl-1,3-dioxolane 4-hydroxymethylester) under acidic conditions. The ring structure may be then opened by chloride ion yielding 3-CPD diester, which may be hydrolysed to 3-CPD. Analogously, cyclic acyloxonium ion intermediate with unesterified hydroxymethyl group (4-hydroxymethyl-2-alkyl-1,3-dioxolane) may be formed by hydrolysis of 3-CPD diester, which is subsequently opened by either water to yield 3-CPD monoester or by chloride anion, which leads to dichloropropanol esters and to dichloropropanols.

The reaction mechanism proposed by Yaylayan (2009) suggests the formation of chloropropanol

esters from TAG but does not take into consideration the recent experimental results available (Matthäus 2009; Matthäus & Vosmann 2009) that proved the dependence of 3-CPD esters in the deodorised oil on the content of diacylglycerols (monoacylglycerols) in the raw oil. When the partial acylglycerols were not present in the oil, 3-CPD esters were not detected (Matthäus 2009). The concentration of 3-CPD esters also depends on the oil origin, which reflects its composition. For example, in the rapeseed oil containing 2% diacylglycerols and 0.1% free fatty acids, the bound 3-CPD amount increased from 400 μg/kg to 1000 μg/kg on deodorisation, while in the palm oil (diacylglycerol content of 5%, free fatty acid content of 0.24%), the bound 3-CPD level increased from 1000 μg/kg to 4400 μg/kg (Franke *et al.* 2009), which supports the mechanism based on their formation from partial acylglycerols. Furthermore, the main products are always 3-CPD diesters followed by 2-CPD diesters (Franke *et al.* 2009). In fat mixes, the amount of 3-CPD bound in diesters ranged from 897 μg/kg to 2435 μg/kg, and in monoesters it reached 61–299 μg/kg (Seefelder *et al.* 2008). However, in deodorised oils, 3-CPD monoesters were not found (Collison 2010; Weisshaar & Perz 2010). Their amount will be under the limit of detection as the content of their precursors (monoacylglycerols) is usually below 0.1% (Franke *et al.* 2009). The concentrations of free 3-CPD and 2-CPD in refined oils are always negligible (Zelinková *et al.* 2006).

In commercial refined palm oils, esters of glycidol have been recently identified (Weisshaar & Perz 20110; Collison 2010). For example, oils deodorised at 210°C, 230°C, and 250°C contained glycidol esters at the level of 300, 800, and 2900 μg/kg, respectively. The amounts of 3-CPD esters in these oils were 2800, 2700, and 3300 μg/kg, respectively.

Generally, it is known that the esterification of carboxylic acids by alcohols is catalysed by strong acids. Fatty acids are week acids that do not dissociate completely and cannot be esterified by alcohols. In aprotic solvents, oligomers may be formed by hydrogen bonding. This process has the effect of enhancing their acidity. Furthermore, their dissociation (ionisation) constants rise steeply with increasing temperature, and at temperatures of 150–250°C these acids behave as strong acids and may be esterified by alcohols without the presence of a catalyst (Ernest *et al.* 1959). Fatty acids then react with sodium chloride in a reversible reaction yielding hydrogen chloride and fatty acid salts (Figure 1).

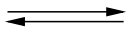

Figure 1. Hydrochloric acid formation from sodium chloride and fatty acid (according to Ernest *et al.* 1959)

Based on the well-known chemical principals and the experimental results published, triacylglycerol (TAG) does not act as direct precursors of 3-CPD esters in oils as they do not hydrolyse during deodorisation (Franke *et al.* 2009) The low electron density found around the acyl oxygen at the *sn*-2 position of TAG (x labeled) disables cyclisation, i.e. the formation of the five-member intramolecular ring intermediate (cyclic ketal, substituted 1,3-dioxolane). Instead, the first reaction leading to CPD esters starts with enzymatic (and chemical) hydrolysis of TAG to diacylglycerol (1,3-DAG and 1,2-DAG) prior to the refining process (Hamlet *et al.* 2011) (Figure 2). The acyl carbonyl group at *sn*-1 or *sn*-3 in 1,3-DAG or at *sn*-2 in 1,2-DAG is then activated by protonation by hydrochloric acid, which leads to a decrease of electron density on the carbonyl group carbon atom. The nucleophilic attack of the vicinal hydroxyl group results in the formation of a protonised acylated cyclic acyloxonium ion intermediate with 1,3-dioxolane skeleton, which is in equilibrium with its tautomeric form. The elimination of water yields 2-alkyl-1,3-dioxolane 4-hydroxymethylester that exists in two resonance stabilised structures. The ring opening of these structures by chloride in two positions yields either 1,2-diacyl-3-CPD (less sterically hindered carbon atom) or 1,3-diacyl-2-CPD, which is a minor product.

Analogously to the formation of 3(2)-CPD diesters, cyclic acyloxonium ion intermediates with free (unesterified) hydroxymethyl group (4-hydroxymethyl-2-alkyl-1,3-dioxolanes) may be formed by cyclisation of either 1-MAG or 2-MAG (Figure 3), being subsequently opened by chloride anion to yield 3-CPD-2-monoester and 2-CPD-1-ester in a lower yield. The isomerisation of 3-CPD-2-monoester may yield 3-CPD-1-monoester as a minor product. The amount of CPD esters is supposed to be under the limit of detection as the MAG concentration in oils is one order lower than that of DAG. CPD monoesters may be otherwise formed by hydrolysis of CPD diesters. The ring opening of the acyloxonium ion resonance structures derived from 3-CPD-2-monoester by chloride may yield negligible amounts of 1,3-DCP and 2,3-DCP esters.

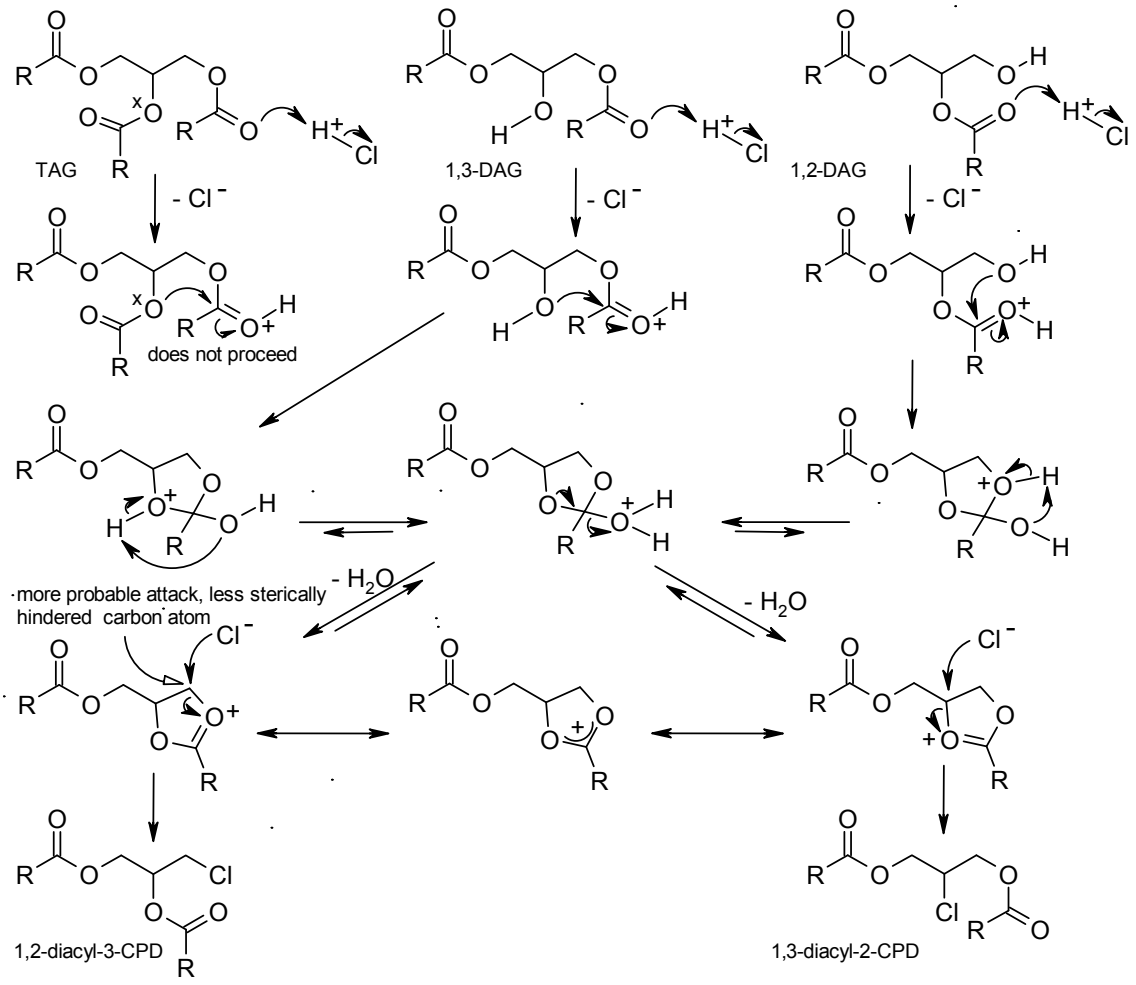

Figure 2. Mechanism of CPD diesters formation

In oils deodorised at lower temperatures (210°C and 240°C), glycidol esters are probably formed by the mechanism shown in Figure 3, but at higher temperatures (e.g. at 270°C) they may arise by dehydration of MAG.

Mitigation

Since several reports were recently published on the contamination of foods and edible oils with 3-CPD, its fatty acid esters, and related compounds, there was been a great demand to remove these contaminants. Different strategies have been considered for e.g. edible oils: removing the critical reactants from the raw material, changing the refining process conditions, and the removal of the formed 3-CPD esters and related compounds from the refined oil. However, not all of these procedures are applicable on the industrial scale.

An enzymic approach to remove 3-CPD and its esters from aqueous and biphasic systems is based on its conversion to glycerol by an enzyme cascade consisting of halohydrin dehalogenase from *Arthrobacter* sp. AD2 and epoxide hydrolase from *Agrobacterium radiobacter* AD1 (Bornscheuer & Hesseler 2010). The oleic acid monoester of 3-CPD was converted in a biphasic system in the presence of edible oil by *Candida antarctica* lipase A to yield free 3-CPD and the corresponding fatty acid. A process for making deodorised edible oil or fat containing a low level of bound CPD was recently described (Bertoli & Cauville 2011). The process involves a step of stripping the vegetable oil or fat with nitrogen. Another mitigation process comprises a step of contacting the oil or fat with carboxymethylcellulose, a cation exchange resin (Bertoli *et al.* 2011), or a silicate adsorbent selected from the group consisting of magnesium silicate, calcium silicate, aluminum silicate, and

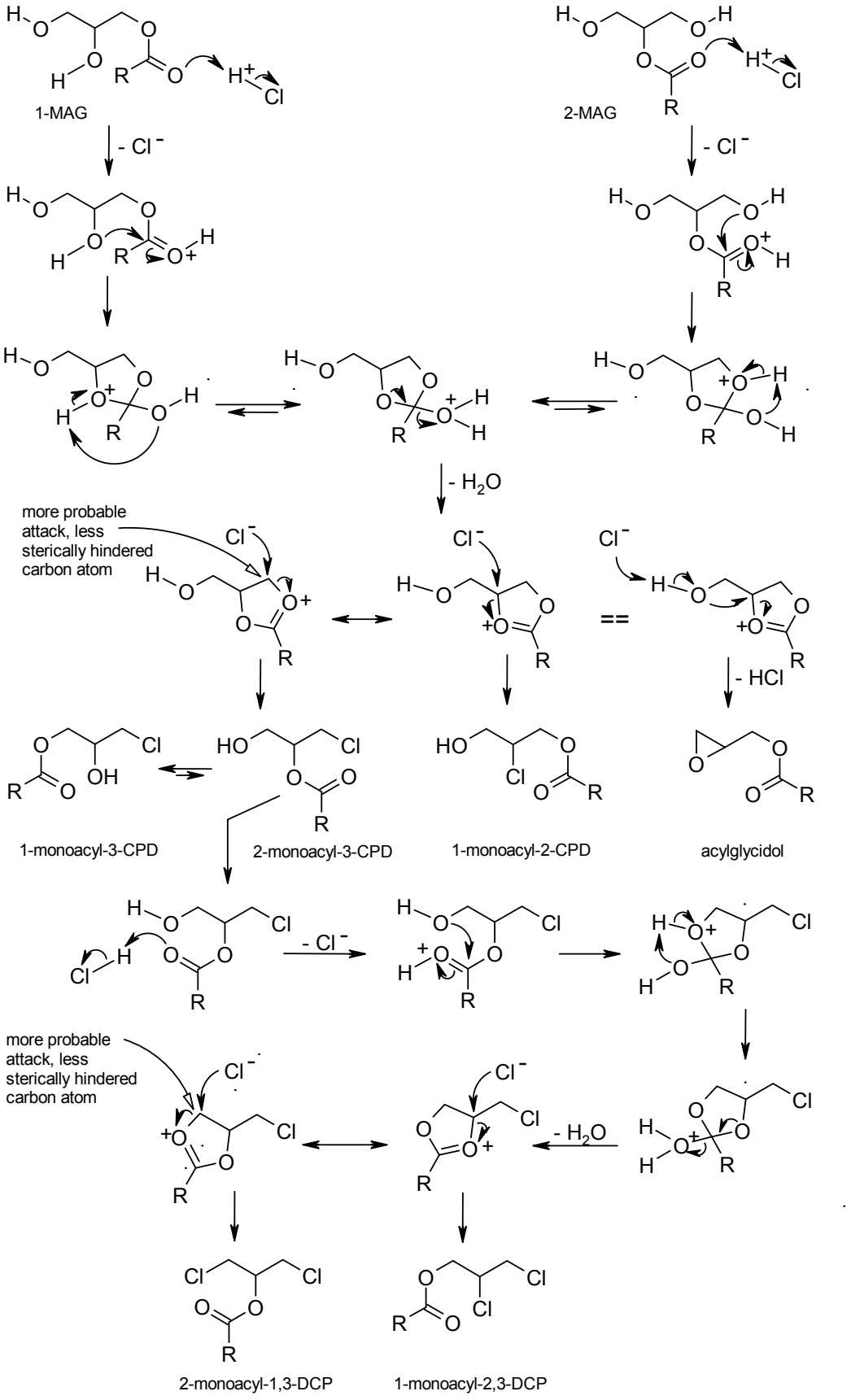

Figure 3. Mechanism of CPD monoesters, DCP and glycidol esters formation

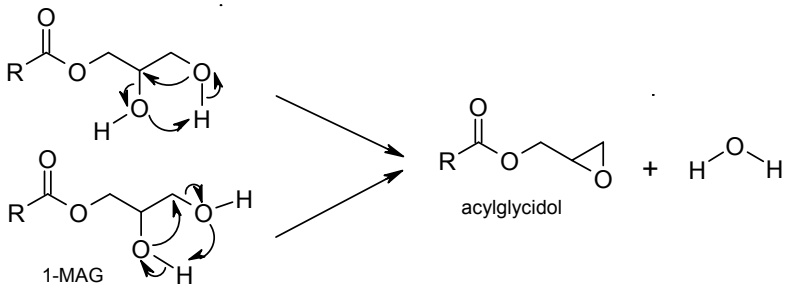

Figure 4. Proposed mechanism of glycidol esters formation at high temperature

combinations of these silicates. The invention further provides deodorised oil selected from palm oil, a palm oil fraction or a combination thereof. The deodorised oil is characterised by the bound 3-CPD content below 300 µg/kg (Strijowski *et al.* 2010; Zieverink *et al.* 2011a). A similar process comprises fractionation of crude palm oil having free fatty acid content < 1.5% and DAG content < 5.5% to produce a deodorised palm oil fraction with the content of 3-CPD esters < 1 µg/kg (Zieverink *et al.* 2011b). Another invention employs crude oil which is first degummed (without the addition of acid at a temperature < 70°C and separated from the aqueous phase), the degummed oil is subsequently heated to 80–100°C, mixed with bleaching earth (1.5%) and bleached, the bleaching earth is then separated from the bleached oil by filtration, and the filtered oil is deodorised (Schurz 2010a,b).

To remove mono- and diacylglycerols from deodorised oils and thus prevent the formation of 3-CPD esters is impossible on the industrial scale. However, the mitigation of the effects of CPD esters may be achieved by neutralisation of the free hydrogen chloride during the neutralisation of free fatty acids to prevent the formation of hydrogen chloride from chlorides. Such procedures, based on our previous model experiments (Velíšek *et al.* 2003), employed a suitable base, e.g. potassium or sodium bicarbonates or sodium carbonate, and can completely prevent the formation of CPD esters in oils during refinement as illustrated in Figure 5. The model mixture consisted of sunflower oil to which a solution of sodium chloride was added to insure its good dispersal in the system. The added water was evaporated at 160°C, bicarbonate/carbonate was added, the mixture was heated to 240°C and analysed at the given intervals. The potency of the individual bases to mitigate 3-CPD formation was evaluated as well. It was shown that the most effective bases were bicarbonates or carbonates of alkali metals ($NaHCO_3$, $KHCO_3$ or Na_2CO_3 or K_2CO_3), that acted even in the amounts corresponding to the equivalents of KOH used for neutralisation of free fatty acids present in the oil. The soap formed was removed by extraction with water and the oil was dried at 80°C under a pressure of 3 kPa. As potassium soaps are more soluble than sodium soaps, potassium bicarbonate or potassium carbonate are the preferred agents.

CONCLUSION

Edible refined oils (especially palm oils) represent the main source of 3-CPD esters in foods. Therefore, further studies should be focused on the strategies to reduce the amounts of these compounds in edible oils, selection of oils with low levels of these contaminants and to mitigation

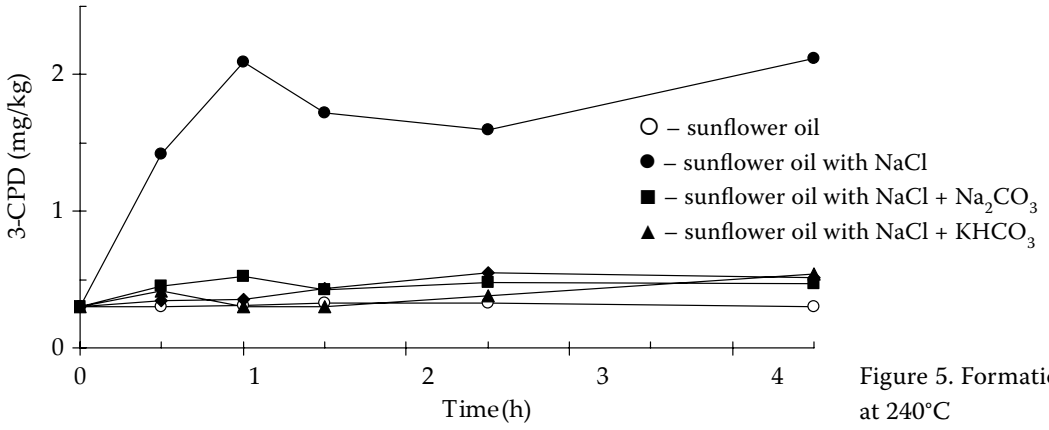

Figure 5. Formation of 3-CPD esters at 240°C

strategies employed. Neutralisation of free fatty acids by either alkali metal bicarbonate or carbonate (one mole of $NaHCO_3$ or $KHCO_3$ or 0.5 mole of Na_2CO_3 or K_2CO_3 per one mole of free fatty acids) prevents the formation of CPD esters. This procedure might be used on an industrial scale for the deodorisation of edible plant oils including those designated for infant and baby foods.

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Corresponding author:

Prof. Ing. Jan Šmidrkal, Vysoká škola chemicko-technologická v Praze. Fakulta potravinářské a biochemické technologie, Ústav technologie mléka a tuků, Technická 5, 160 28 Praha 6, Česká republika
tel.: + 420 220 443 822, e-mail: jan.smidrkal@vscht.cz