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## Fatty acids esters of 3-chloropropane-1,2-diol in edible oils

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### Abstract

A series of 25 virgin and refined edible oils, obtained from the retail market was analyzed for their content of free 3-MCPD and 3-MCPD released from its esters with higher fatty acids (bound 3-MCPD). The oils contained free 3-MCPD ranging from  $< 3 \mu\text{g}/\text{kg}$  (LOD) to  $24 \mu\text{g}/\text{kg}$ . Surprisingly, the bound 3-MCPD level was much higher and varied between  $< 100 \mu\text{g}/\text{kg}$  (LOD) and  $2462 \mu\text{g}/\text{kg}$ . On average, virgin oils had relatively low levels of bound 3-MCPD ranging from  $< 100 \mu\text{g}/\text{kg}$  (LOD) to  $< 300 \mu\text{g}/\text{kg}$  (LOQ). Higher levels of bound 3-MCPD were found in oils obtained from roasted oilseeds ( $337 \mu\text{g}/\text{kg}$ ) and in the majority of refined oils ( $< 300 \mu\text{g}/\text{kg}$  to  $2462 \mu\text{g}/\text{kg}$ ), including refined olive oils. In general, it appears that the formation of bound 3-MCPD in oils is linked with the preliminary heat treatment of oilseeds and with the process of oil refining. The analysis of unrefined, de-gummed, bleached, and deodorized rapeseed oil showed that the level of bound MCPD decreased during the refining process. However, additional heating of seed oils for 30 min at temperatures ranging from  $100^\circ\text{C}$  to  $280^\circ\text{C}$ , and heating at  $230^\circ\text{C}$  ( $260^\circ\text{C}$ ) for up to 8 hours led to an increase of the bound 3-MCPD level. On the contrary, heating of olive oil resulted in a decrease of the bound 3-MCPD level. For comparison, the fat isolated from salami was analyzed for the intact fatty acid esters of 3-MCPD. This fat contained the bound 3-MCPD at the level of  $1670 \mu\text{g}/\text{kg}$  and the fatty acid esters of 3-MCPD mainly consisted of 3-MCPD diesters, monoesters of 3-MCPD were present in smaller amounts. The major types of 3-MCPD diesters (about 85%) were mixed diesters of palmitic acid with C18 fatty acids (stearic, oleic, linoleic acids). These diesters were followed by 3-MCPD distearate (11%) and 3-MCPD dipalmitate (4%). Generally, very little 3-MCPD existed as the free compound ( $31 \mu\text{g}/\text{kg}$ ).

**Key words:** *3-Chloropropane-1,2-diol, 3-MCPD, bound 3-MCPD, 3-MCPD fatty acid esters, edible oils, contaminants*

## Introduction

3-Chloropropane-1,2-diol (also known as 3-MCPD) is a representative of the so called food borne or food processing contaminants. It was first identified in acid-hydrolyzed vegetable protein (acid-HVP) in 1981 (Davídek et al. 1982) as a reaction product of either acylglycerols or glycerol with hydrochloric acid and quite recently shown to be a racemic mixture of the corresponding enantiomers, (*R*)-3-MCPD and (*S*)-3-MCPD (Velíšek et al. 2002). 3-MCPD has been extensively monitored in acid-HVP because of its suspected carcinogenicity. In view of its toxicity, a provisional maximum tolerable daily intake of 2 µg per kg of body mass has been set (European Commission 2001) and a regulatory limit of 20 µg/kg, based on a 40% dry matter content, has been adopted for 3-MCPD in acid-HVP and soy sauce and came into force in the European Union in 2002 (European Commission 2001).

Recent studies (Crews 2002; Hamlet et al. 2002a; Hamlet et al. 2002b; Divinová 2004) have shown that elevated levels of 3-MCPD (< 5 to 134 µg/kg) can occur not even in acid-HVP, soy sauces and related products but also in a wide range of retail processed foods such as meat, dairy and cereal products. Cereal products, particularly biscuits, crackers, doughnuts and bread, accounted for the highest levels of 3-MCPD reported (Hamlet et al. 2002b). Furthermore, 3-MCPD was found in malt products comprising brewing malts and malt extracts. The typical levels of 3-MCPD for specialist malts (having typical EBC colour units > 20) ranged from < 30 to < 500 µg/kg (Hamlet et al. 2002a), which is well over the maximum level for acid-HVP.

While levels in retail foods have not been found to exceed 134 µg/kg (Crews 2002; Hamlet et al. 2002a; Hamlet et al. 2002b; Divinová et al. 2004a), the potential for additional generation of 3-MCPD from < 5 to 275 µg/kg, resulting from domestic cooking procedures, has been also reported (Crews 2001; Breitling-Utzmann 2003; Breitling-Utzmann 2004). Significant levels of 3-MCPD were found notably in the high temperature crust region of grilled cheeses (Crews 2001) and toasted breads (Crews 2001; Breitling-Utzmann 2003; Breitling-Utzmann 2004). More than 500 µg/kg 3-MCPD can be found in well-toasted breads (Breitling-Utzmann 2003; Breitling-Utzmann 2004). However, at the moment there are no limits for any food product or food ingredient other than acid-HVP and soy sauce.

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3 Model experiments simulating processed foods (Velíšek et al. 2003; Calta et al. 2004) showed  
4 that 3-MCPD evidently arised from either acylglycerols or glycerol in a reaction with  
5 naturally present or intentionally added sodium chloride. The 3-MCPD level strongly depends  
6 on temperature and the content of lipids, glycerol, salt and water. It is believed that the  
7 precursors (acylglycerols and glycerol) react with negatively charged nucleophiles, hydroxyl  
8 and chloride anions, which leads first to 3-MCPD esters and finally to free 3-MCPD.  
9 Generation of 3-MCPD in model dough systems showed that free glycerol is the key  
10 precursor of 3-MCPD in leavened doughs (Hamlet et al. 2004a). Furthermore,  
11 monoacylglycerols, lysophospholipids, and phosphatidylglycerols present in flour improvers  
12 were important precursors of 3-MCPD in leavened doughs (Hamlet et al. 2004b).

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23 Recent findings (Rétho and Blanchard 2005) indicate that also bromine anions react with  
24 acylglycerols and glycerol yielding 3-bromopropane-1,2-diol and 2-bromo-1,3-propanediol  
25 that were found in vegetable oils at the level ranging from 7 to 45  $\mu\text{g.kg}^{-1}$ .

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30 The mechanisms of 3-MCPD formation in processed foods are still very little understood.  
31 However, there have been recent studies of mechanism of its formation in cereal products e.g.  
32 from glycerol (Hamlet et al. 2004a), mono- and diacylglycerols and glycerol phosphate  
33 (Hamlet et al. 2004b).

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

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51 Our recent findings indicate that the formation of 3-MCPD esters (monoesters and diesters  
52 with higher fatty acids) is characteristic of a variety of processed foods (Divinová et al.  
53 2004b; Svejková et al. 2004; Doležal et al. 2005). These compounds represent a new class  
54 of food contaminants, the bound form of 3-MCPD, from which free 3-MCPD could be  
55 released by a lipase-catalyzed hydrolysis reaction. In many cases the amount of bound 3-  
56 MCPD exceeded that of the free 3-MCPD.

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3 We have analyzed 20 samples of selected retail food products for their bound 3-MCPD  
4 content. The levels of bound 3-MCPD found in the analyzed foods varied between traces (140  
5  $\mu\text{g}/\text{kg}$ ) and 6100  $\mu\text{g}/\text{kg}$  and were 10 to 396 times higher than the free 3-MCPD levels. Five  
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7 foods of plant origin that were processed at high temperatures (particularly crisp bread, salty  
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9 crackers, doughnuts, French fries and dark malt) contained elevated levels of bound 3-MCPD.  
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11 The highest level of bound 3-MCPD (6100  $\mu\text{g}/\text{kg}$ ) was found in a sample of French fries  
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13 (Svejkovská et al. 2004). The level of bound 3-MCPD in roast coffee was relatively low and  
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15 varied between 6  $\mu\text{g}/\text{kg}$  (soluble coffees) and 390  $\mu\text{g}/\text{kg}$  (decaffeinated coffee) and exceeded  
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17 the free 3-MCPD level 8 to 33 times (Doležal et al. 2005). The presence of bound 3-MCPD in  
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19 pickled olives and herrings suggests that these compounds can also form at relatively low  
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21 temperatures and even in acid media (Svejkovská et al. 2004).  
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25 Analysis of white bread showed (Hamlet and Sadd 2004) that the highest levels of bound 3-  
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27 MCPD were found in the crust (547  $\mu\text{g}/\text{kg}$ ) and toast (160  $\mu\text{g}/\text{kg}$ ) while in crumb they were  
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29 found in much lower amount (26  $\mu\text{g}/\text{kg}$ ). The corresponding levels of the free 3-MCPD were  
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31 477  $\mu\text{g}/\text{kg}$ , 93  $\mu\text{g}/\text{kg}$  and  $< 0.5 \mu\text{g}/\text{kg}$ , respectively, i.e. 6 to 52 times lower. It was also  
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33 showed that free 3-MCPD can be released from its esters by a commercial lipase (from  
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35 *Aspergillus oryzae*) hydrolysis.  
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39 The vegetable oils recently analyzed by Rétho and Blanchard (2005) contained very low  
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41 levels of 3-MCPD, between 3 and 7  $\mu\text{g}/\text{kg}$ . The possible formation of 3-MCPD in oils by a  
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43 lipase-catalyzed transesterification of triacylglycerols has recently been reported (Robert et al.  
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45 2004). It is not yet known whether this phenomenon is due to hydrolysis of 3-MCPD esters or  
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47 lipase catalyzed formation from triacylglycerols and chlorides.  
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51 Due to the occurrence of 3-MCPD esters in a variety of retail and homemade foods, we  
52  
53 assumed that these lipophilic compounds could also arise in edible oils during the refining  
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55 process. Described herein are our investigations into the determination of 3-MCPD esters in a  
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57 variety of oils and fats. Our attention was mainly focused on the role of various factors that  
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59 can influence the level of 3-MCPD esters during industrial processing of oils and their  
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61 culinary use.

## Materials and methods

### *Chemicals and samples*

3-Chloropropane-1,2-diol (> 98%, 3-MCPD) was purchased from E. Merck (Darmstadt, Germany), 3-MCPD-*d*<sub>5</sub> (99.4%) from Dr. Ehrenstorfer (Augsburg, Germany), phenylboronic acid (PBA, ≥ 97%) from Fluka Chemie (Buchs, Switzerland). 1,2-Diacyl-3-chloro-1,2-propanediols were synthesised according to KRAFT et al. 1979 and purified on a silica gel column using light petroleum ether/diethyl ether mixtures. All other reagents and solvents were of analytical purity.

Vegetable oils as well as roasted almonds and peanuts were obtained from retail outlets in Prague. The sample of salami was provided by Central Science Laboratory, York, UK. Unrefined (mixture of pressed and extracted oil), degummed, bleached, deodorized rapeseed oils were supplied by Setuza a.s. (Ústí n. L., Czechia).

### *Instrumentation*

GC/MS analysis of 4-chloromethyl-2-phenyl 1,3,2-dioxaborolane, obtained by derivatization of 3-MCPD using phenylboronic acid, was carried out on an Agilent Technologies 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Series 5973 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 Equity™-1 (30 m x 0.25 mm i.d., thickness of 1 µm, Supelco, PA, USA). The injector was held at 250°C (splittles), 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 quantification purposes, single ion monitoring was used to monitor ions at *m/z* 147 (3-MCPD) and at *m/z* 150 (3-MCPD-*d*<sub>5</sub>). Ions at *m/z* 91 and 196 (3-MCPD) and at *m/z* 93 and 201 (3-MCPD-*d*<sub>5</sub>) were used as qualifiers.

The fat isolated from salami was separated by preparative TLC (Davídek et al. 1980) into the individual fraction and the fractions containing diesters and monoesters of 3-MCPD were analyzed by GC using the same instrument as above and a DB-1HT fused silica capillary column (15 m x 0.25 mm i.d., thickness of 1 µm, J&W Scientific, CA, USA). One 1 µl sample was injected into the split injection port (1:4) held at 300°C with a solvent delay of 2

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3 min. The oven was initially set to 100°C, then programmed to 340°C at a rate of 10°C/min and  
4 kept at 340°C for 26 min. The helium carrier gas was supplied at a constant rate of 0.7  
5 ml/min. The analytes were ionized by EI at 70 eV and the obtained mass spectra of 3-MCPD  
6 esters with fatty acids were consistent with the spectra of the synthesized standards (KRAFT et  
7 al. 1979, Velíšek et al. 1980). The individual esters were quantified using 1,2-dipalmitoyl-3-  
8 chloro-1,2-propanediol (purity of 96.9) as the external standard.  
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#### 15 16 *Isolation of fat from nuts and salami*

17 To obtain fat from the roasted almonds, roasted peanuts, and salami the material (20 g) was  
18 extracted with diethyl ether (80 ml) using Ultra Turrax T25 (Janke and Kunkel, IKA-  
19 Labortechnik, Switzerland). The obtained extract was filtered off and the residue again  
20 extracted with two portions of diethyl ether (20 ml each). The combined extracts were washed  
21 with water (20 ml), dried with anhydrous sodium sulfate and the solvent evaporated to  
22 dryness. The oil content was determined using the Soxhlet method (extraction with either  
23 diethyl ether - nuts or hexane - salami).  
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#### 30 *Heating of oils*

31 The oil (20 g) was placed to a beaker and heated in an oven at the desiderative temperature  
32 (100°C, 150°C, 200°C, 230°C, 260, and 280°C) for 30 minutes. Other portions of the oil were  
33 heated at either 260°C or 280°C for 0.5, 1, 2, 4, and 8 hours. The oils were let to cool to room  
34 temperature and analyzed for the content of free and bound 3-MCPD.  
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#### 39 *Free 3-MCPD*

40 The oil (5 g) placed in a 100 ml Erlenmeyer flask was dissolved in a hexane : acetone mixture  
41 (1:1, v/v, 30 ml) and 50 µl of an aqueous solution of 3-MCPD-*d*<sub>5</sub> (internal standard, 0.5 µg)  
42 were added. The solution was transferred to a 100 ml separatory funnel and the Erlenmeyer  
43 flask was washed again with two 10 ml portions of the hexane/acetone mixture. The  
44 combined extracts was transferred to a separatory funnel and extracted with water (10 ml).  
45 The aqueous phase was evaporated to dryness using a vacuum rotary evaporator at 55°C. The  
46 residue was dissolved in 20% sodium chloride (2 ml), derivatised with phenylboronic acid  
47 (0.4 ml) and used for the determination of free 3-MCPD according to the procedure of  
48 Divinová et al. (2004b). Three parallel determinations of each sample were made.  
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#### 56 *Bound 3-MCPD*

57 The oil (100 mg) placed in a 10 ml distillation flask was dissolved in tetrahydrofuran (1 ml).  
58 To this solution 1.8 ml sulphuric acid solution (98%, 1.8 ml in 100 ml methanol) was added  
59 and the mixture was heated at 40°C for 16 hours. The cooled mixture was then neutralised  
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3 with saturated NaHCO<sub>3</sub> solution (0.5 ml), 1 ml of 3-MCPD-*d*<sub>5</sub> (internal standard, 1 µg) was  
4 added and the solution was evaporated to dryness using a vacuum rotary evaporator at 55°C.  
5 The residue dissolved in 20% sodium chloride (2 ml), extracted with two portions of hexane  
6 (2 ml), derivatised with phenylboronic acid (0.2 ml) and used for the determination of bound  
7 3-MCPD according to the above procedure (Divinová et al. 2004b). Three parallel  
8 determinations of each sample were made.  
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## 14 15 16 **Results and discussion**

17 Our recent experiments simulating processed foods have revealed that the models consisting  
18 of the recognized 3-MCPD precursors (i.e. glycerol, triolein, lecithin) and heated (230°C, 30  
19 min) with sodium chloride at a level typically present in foods yielded both enantiomers of 3-  
20 MCPD, i.e. (*R*)-3-MCPD and (*S*)-3-MCPD, in equimolar amounts (Velíšek et al. 2002).  
21 Stereospecific analysis of diesters of 3-MCPD found in goat's milk also showed that they  
22 were a racemic mixture (Myher et al. 1986). In total, six different compounds, four  
23 monoesters and two diesters, can be derived from racemic 3-MCPD and a fatty acid  
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32 **(Figure 1).**

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35 The method used throughout our research and focused on the occurrence of 3-MCPD esters in  
36 foods (Divinová et al. 2004b) was based on the determination of 3-MCPD released from the  
37 esters by methanolysis and thus did not have the potential to differentiate between the  
38 monoesters and diesters of 3-MCPD or the esters derived from its individual enantiomers. As  
39 a part of the present work, the above method has been optimized to increase its sensitivity so  
40 that the limit of detection (LOD) and limit of quantification (LOQ) of 3-MCPD released from  
41 the esters, when expressed as 3-MCPD (so called bound 3-MCPD), was 100 µg/kg oil and  
42 300 µg/kg oil, respectively. The LOD and LOQ for the method used to determine the free 3-  
43 MCPD was 3 µg/kg oil and 9 µg/kg oil, respectively. Using these two methods, a series of  
44 retail market oils, oils isolated from nuts, and oils obtained from our industrial partner was  
45 analyses for the levels of free and bound 3-MCPD. Groups of retail market oils were selected  
46 to focus on virgin oils produced by different producers and from different raw materials, as  
47 well as on various grades of oils that might contain 3-MCPD esters resulting from heat  
48 processing during the refining process (Table I).  
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3 It can be seen that the analyzed oils, with only three exceptions, contained free 3-MCPD at  
4 the levels of either LOD or LOQ (3 replications). The levels of 3-MCPD higher than the LOQ  
5 were only found in virgin hazel nut oil (10 µg/kg), virgin soybean oil (12 µg/kg), and virgin  
6 wheat germ oil (24 µg/kg) and they did not exceed the level of 50 µg/kg dry matter adopted  
7 for 3-MCPD in acid-HVP and soy sauce (European Commission 2001).  
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14 The bound 3-MCPD was found in oils at levels ranging from < 100 µg/kg (LOD) to  
15 2462 µg/kg. As it can be seen (Figure 2), the average bound 3-MCPD level of oils (half of the  
16 value of either LOD or LOQ was used to calculate the average values) is low in virgin seed  
17 oils obtained from unroasted seeds (63 µg/kg), and increases in the following sequence: virgin  
18 olive oils (75 µg/kg), virgin germ oils (100 µg/kg), virgin seed oils from gained roasted seeds  
19 (337 µg/kg), refined seed oils (524 µg/kg), and refined olive oils (1464 µg/kg).  
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27 Within the sets of virgin oils, levels exceeding 300 µg/kg (LOQ) were only found in sesame  
28 oil obtained from roasted seeds (337 µg/kg). Refined seed oils (< 300-1234 µg/kg) and  
29 refined olive oils (< 300-2462 µg/kg) had higher levels of bound 3-MCPD. The amount of  
30 2462 µg of bound 3-MCPD/kg found in refined olive oil then corresponds e.g. to 7780 µg/kg  
31 of 3-MCPD monooleate or to 13110 µg/kg of 3-MCPD dioleate and exceeds by 500% the  
32 level adopted for 3-MCPD in acid-HVP and soy sauce (European Commission 2001).  
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40 The average ratio of free 3-MCPD to bound 3-MCPD was 19 within the set of virgin seed oils  
41 and even 12 if the roasted sesame seed oil was not included. Virgin olive oils (extra virgin  
42 olive oils have a free acidity, expressed as oleic acid, of not more than 1 gram per 100 grams,  
43 virgin olive oils have a free acidity of less than 2 grams per 100 grams) had their average free  
44 3-MCPD/bound 3-MCPD ratio 22, while this ratio increased to 150 within the set of refined  
45 seed oils and even to 325 within the set of refined olive oils.  
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52 It appears that the formation of 3-MCPD esters in oils is linked either with the preliminary  
53 heat treatment of seeds (e.g. roasting as in the case of roasted sesame seed oil) or with the  
54 process of oil refining (refined seed and olive oils). Moreover, there is certain dissimilarity  
55 between seed oils and olive oils as the latter have been obtained from the pulp. The pulp oils  
56 have high water content and active lipases yielding by hydrolysis partial acylglycerols during  
57 handling of fruits and their subsequent storage prior processing. Partial acylglycerols may  
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3 then become the precursors of 3-MCPD esters (Calta et al. 2004). Virgin olive oils are  
4 produced by thrifty procedures where temperature does not exceed 40°C while refined olive  
5 oils are commonly obtained by physical refination (degumming and bleaching). Moreover, the  
6 diatomaceous earth used for bleaching is sometimes purified by hydrochloric acid, which  
7 results in the formation of metal chlorides that may become the precursors of MCPD esters.  
8 Olive oil is a blend of virgin oil with refined oil and olive pomace oil is a blend of refined  
9 pomace olive oil and possibly some virgin oil (Firestone 2005).  
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17 To check the influence of heat treatment on oilseeds, we have analyzed the level of free and  
18 bound 3-MCPD in retail roasted almonds and roasted peanuts, both unsalted and salted. The  
19 obtained results are summarized in Table II. For example, the amount of bound 3-MCPD in  
20 the oil extracted from roasted salted almonds was 1035 µg/kg while the level of bound 3-  
21 MCPD in virgin almond oil was < 100 µg/kg. Similarly, roasted salted peanuts contained  
22 bound 3-MCPD at the level of 883 µg/kg, which is much higher than the level (< 100 µg/kg)  
23 found in virgin peanut oil (Table I). The presence of salt did not markedly influence the level  
24 of 3-MCPD esters as it is usually added to the cooled roasted product.  
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33 We have also analyzed unrefined, degummed, bleached and deodorized rapeseed oil obtained  
34 from our industrial partner and, surprisingly, the level of bound MCPD esters decreased  
35 during the refining process (Table III). Bleaching was done at about 90-100°C for 30 min and  
36 deodorization at 230–250°C for approximately 30 min. Therefore, it seems that the chemical  
37 composition of the oil given by its origin (pulp *versus* seed) plays a crucial role in the  
38 formation of MCPD esters.  
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46 We were further interested how different batches of oil from the same producer differed in  
47 their bound 3-MCPD level. Therefore, 6 batches of rapeseed oil produced in 2005 and 2006  
48 were analyzed and it was found that the bound 3-MCPD levels ranged from 381 to 484 µg/kg  
49 (Table I).  
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55 We were also interested whether the thermal treatment of commercial oils exerts influence  
56 upon the level of bound 3-MCPD. Therefore, samples of refined rapeseed oil (with the  
57 original amount of 3-MCPD esters of 381 µg/kg), virgin wheat germ oil (<100 µg/kg), and  
58 refined olive oil (2462 µg/kg) were heated for 2 hrs at temperatures ranging from 100°C to  
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3 280°C, then at 230°C and 260°C for 0.5, 1, 2, 4 and 8 hrs and analyzed for the content of  
4 bound 3-MCPD. The extreme temperatures of 260°C and 280°C and very long times of  
5 heating were done for comparison. The achieved results can be seen in Figure 3 and Figure 4,  
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7 respectively.  
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11 Our findings indicate that the level of bound 3-MCPD in rapeseed and wheat germ oils  
12 increased with temperature and time of heating while the level of bound 3-MCPD in olive oil  
13 decreased (Figure 3 and Figure 4). The increase in bound 3-MCPD level is probably due to the  
14 formation of 3-MCPD esters *de novo* while its decrease in olive oil is mainly caused by rapid  
15 decomposition, oxidation, and polymerization of 3-MCPD esters (Svejkovská et al. 2006).  
16 Heating of oils at 260°C (Figure 5 and Figure 6) first leads to an increase of the bound 3-  
17 MCPD level in olive and rapeseed oils (achieved in about one hour), which is followed by a  
18 decrease with prolonged heating. Again, the bound forms of 3-MCPD in olive oil are more  
19 susceptible to decomposition, oxidation, and polymerization. The amount of free 3-MCPD did  
20 not change under the experimental conditions and remained at the level of < 9 µg/kg.  
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31 Since the 3-MCPD fatty acid esters derived from fats and oils seem to be universally  
32 distributed in processed foods (Divinová et al. 2004, Hamlet and Sadd 2004, Doležal et al.  
33 2005), the individual naturally occurring esters of 3-MCPD were investigated in the last stage  
34 of this study. We have analyzed the fat isolated from salami (fermented for 3 days at 62°C,  
35 water 28.0%, protein 19.3%, fat 45.2%, salt 4.31%, carbohydrates 1.9%) that contained free  
36 and bound 3-MCPD at the level of 31 µg/kg and 1670 µg/kg, respectively. The bound 3-  
37 MCPD mainly consisted of 3-MCPD diesters, monoesters of 3-MCPD were present in smaller  
38 amounts. The major types of 3-MCPD diesters (about 85%) were mixed diesters of palmitic  
39 acid with C18 fatty acids (stearic, oleic, linoleic acids). These diesters were followed by 3-  
40 MCPD distearate (11%) and 3-MCPD dipalmitate (4%). Analogous results were obtained  
41 analyzing bakery products.  
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53 Further research aimed at the reaction pathways leading to the formation of 3-MCPD esters  
54 (that seem to be universally distributed in processed foods) and conditions under which these  
55 compounds form is ongoing.  
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Table I. The content of bound 3-MCPD in retail edible oils.

| Oil type                    | Country | Free $\mu\text{g}/\text{kg}$ | Bound $\mu\text{g}/\text{kg}$ | Ratio bound/free * | Oil type                     | Country | Free $\mu\text{g}/\text{kg}$ | Bound $\mu\text{g}/\text{kg}$ | Ratio bound/free * |
|-----------------------------|---------|------------------------------|-------------------------------|--------------------|------------------------------|---------|------------------------------|-------------------------------|--------------------|
| <i>Virgin seed oils</i>     |         |                              |                               |                    | <i>Virgin germ oils</i>      |         |                              |                               |                    |
| Almond                      | France  | < 9                          | < 100                         | 11.1               | Wheat                        | France  | 24                           | < 100                         | 2.1                |
| Soybean                     | France  | 12                           | < 100                         | 4.2                | Maize                        | France  | < 3                          | < 300                         | 100.0              |
| Rapeseed                    | Germany | < 9                          | < 100                         | 11.1               | <i>Refined seed oils</i>     |         |                              |                               |                    |
| Sunflower                   | France  | < 9                          | < 100                         | 11.1               | Soybean                      | Czechia | < 9                          | 1234                          | 274.2              |
| Sesame (unroasted seed)     | France  | < 9                          | < 300                         | 33.3               | Rapeseed **                  | Czechia | < 9                          | 484                           | 107.6              |
| Sesame (roasted seed)       | Germany | < 9                          | 337                           | 74.9               | Rapeseed **                  | Czechia | < 9                          | 381                           | 84.7               |
| Hazelnut                    | France  | 10                           | < 100                         | 5.0                | Sunflower                    | Hungary | < 9                          | < 300                         | 33.3               |
| Peanut                      | Germany | < 9                          | < 100                         | 11.1               | Maize                        | Hungary | < 3                          | 372                           | 248.0              |
| Pumpkin                     | France  | < 9                          | < 100                         | 11.1               | <i>Refined olive oils***</i> |         |                              |                               |                    |
| <i>Virgin olive oils***</i> |         |                              |                               |                    | Olive oil                    | Spain   | < 9                          | < 300                         | 33.3               |
| Extra virgin olive oil      | Spain   | < 9                          | < 100                         | 11.1               | Olive oil                    | Italy   | < 9                          | 1333                          | 296.2              |
| Extra virgin olive oil      | Italy   | < 9                          | < 300                         | 33.3               | Olive oil                    | Italy   | < 9                          | 2462                          | 546.7              |
| Virgin olive oil            | France  | < 3                          | < 100                         | 33.3               | Olive pomace oil             | Italy   | < 9                          | 1047                          | 232.7              |
| Virgin olive oil            | Greece  | < 9                          | < 100                         | 11.1               | Olive pomace oil             | Spain   | < 9                          | 2332                          | 517.2              |

\* Half of the value of LOQ (or LOD) was used. \*\* Different batches from the same producer. \*\*\* Retail grades according to The International Olive Oil Council (IOOC) standards.

Table II. The content of oil, free and bound 3-MCPD in roasted almonds and peanuts.

| <b>Roasted nuts</b> | <b>Oil<br/>%</b> | <b>Free<br/>µg/kg</b> | <b>Bound<br/>µg/kg</b> | <b>Bound<br/>µg/kg oil</b> | <b>Ratio<br/>bound/free</b> |
|---------------------|------------------|-----------------------|------------------------|----------------------------|-----------------------------|
| Almonds (salted)    | 48.35            | 31                    | 500                    | 1035                       | 16                          |
| Peanuts (unsalted)  | 49.55            | 21                    | 437                    | 882                        | 21                          |
| Peanuts (salted)    | 49.02            | 20                    | 433                    | 883                        | 22                          |

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Table III. Changes in the content of free and bound 3-MCPD in rapeseed oil during physical refining

| <b>Rapeseed oil</b> | <b>Free<br/>µg/kg</b> | <b>Bound<br/>µg/kg</b> | <b>Ratio<br/>bound/free*</b> |
|---------------------|-----------------------|------------------------|------------------------------|
| Unrefined           | 20                    | 990                    | 50                           |
| Degummed            | 11                    | < 300                  | 14                           |
| Bleached            | < 9                   | < 300                  | 33                           |
| Deodorized          | < 9                   | < 300                  | 33                           |

\* Half of the value of LOQ was used.

Figure 1. Fatty acid monoesters and diesters of 3-MCPD.

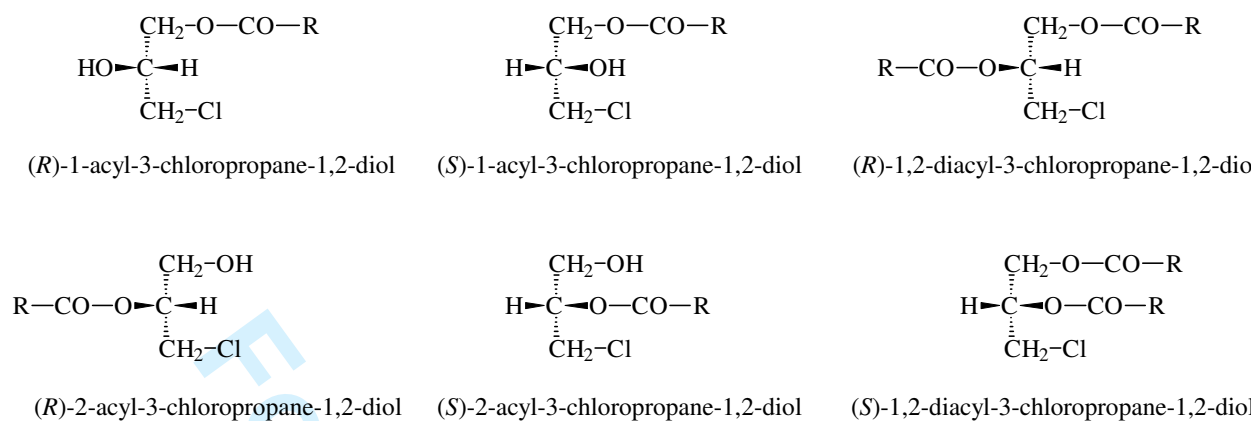
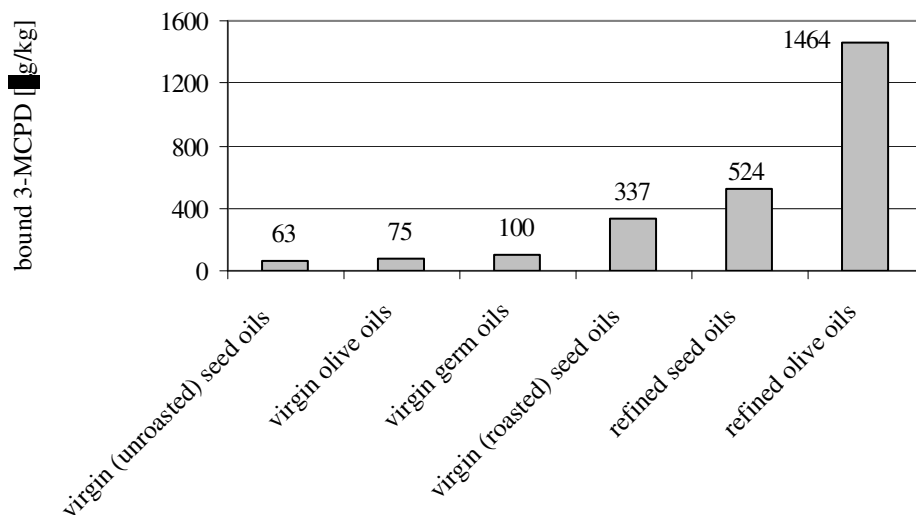


Figure 2. Average bound 3-MCPD level in oils.



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Figure 3. Changes in bound 3-MCPD level in rapeseed, wheat germ, and olive oils heated to various temperatures for 30 min.

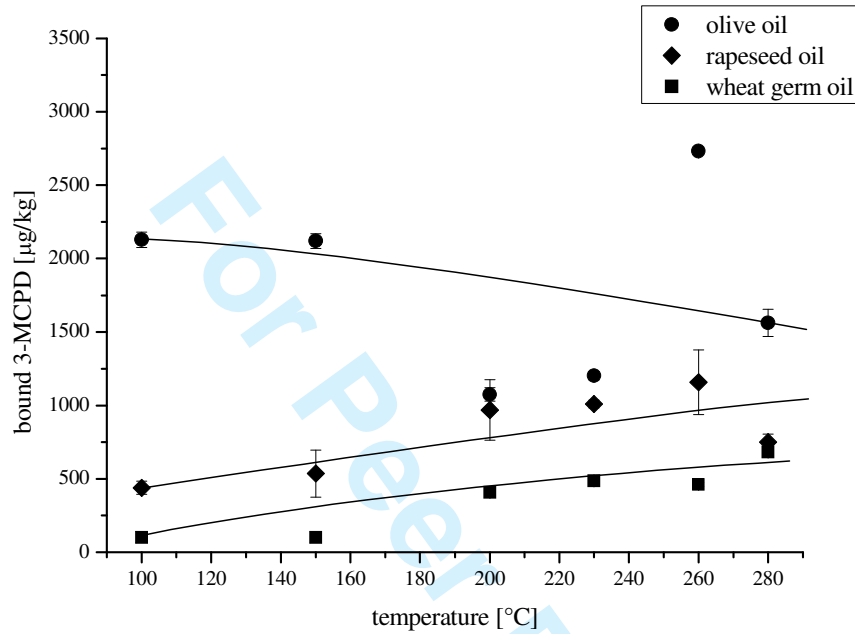


Figure 4. Changes in bound 3-MCPD level in rapeseed, wheat germ, and olive oils with time of heating at 230°C.

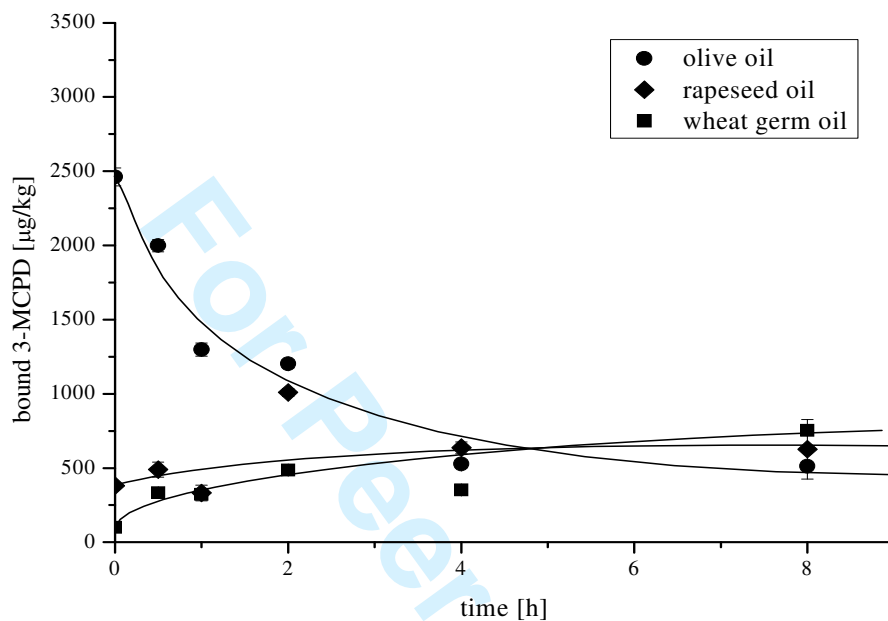




Figure 5. Changes in bound 3-MCPD level in olive oils with time of heating at 230°C and 260°C.

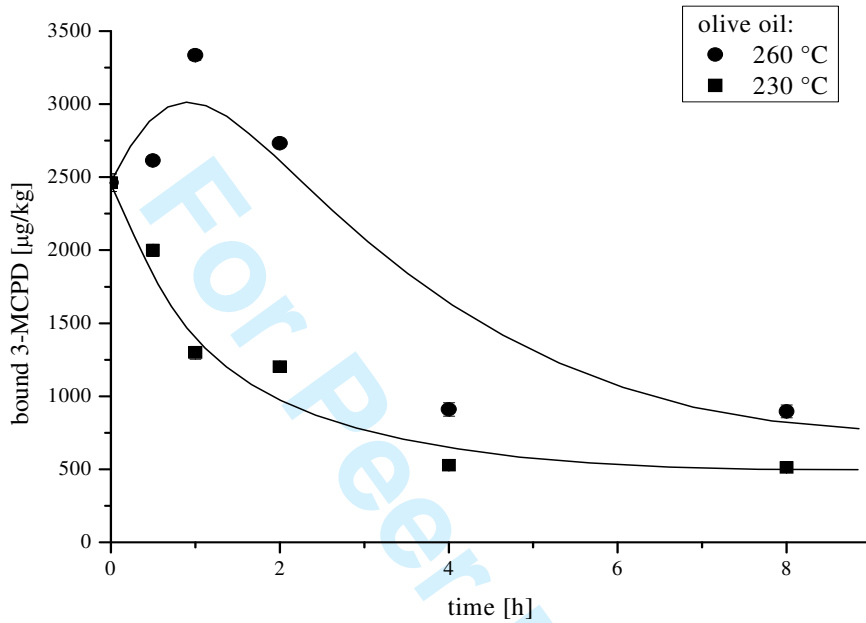


Figure 6. Changes in bound 3-MCPD level in rapeseed oils with time of heating at 230°C and 260°C.

