# 3-Chloropropane-1,2-Diol Fatty Acid Esters in Potato Products

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**Abstract**

Ilko V., Zelinková Z., Doležal M., Velíšek J. (2011): **3-Chloropropane-1,2-diol fatty acid esters in potato products.** Czech J. Food Sci., **29**: 411–419.

The levels of 3-chloropropane-1,2-diol (3-CPD) and fatty acid esters in raw potatoes, potato flakes, instant mashed potatoes, 2 potato dumplings, 32 French fries, and 61 potato crisps are reported. In a majority of the samples, free 3-CPD amount was under the limit of detection (< 3 μg/kg) or under the limit of quantification (< 9 μg/kg). Higher concentrations of free 3-CPD were found in potato flakes (37 μg/kg) and seven samples of potato crisps (10.4–46.2 μg/kg). Low concentrations of bound 3-CPD were present in raw potatoes (2 μg/kg), potato flakes (18 μg/kg), mashed potatoes (38 μg/kg) and potato dumplings (10–13 μg/kg). Higher levels of bound 3-CPD occurred in fried potato products. The amount of bound 3-CPD in pre-fried French fries was 27–64 μg/kg, in fried French fries its content was 100–258 μg/kg. In potato crisps, the bound 3-CPD amount was 98–2201 μg/kg. A striking difference was observed between the levels of bound 3-CPD in crisps fried in rapeseed oil and in fractionated palm oil (palmolein). For that reason, the changes were studied of the bound 3-CPD levels in palm oil after heating and in blends of rapeseed and palm oil during frying. It was proven that the frying oils absorbed into the fried potato products represent the main source of bound 3-CPD.

**Keywords**: 3-chloropropane-1,2-diol; 3-CPD; 3-chloropropane-1,2-diol esters; bound 3-CPD; potato products; potato chips; potato crisps; French fries

Chloropropanols and their fatty acid esters are contaminants formed during the processing and manufacture of certain foods and food ingredients. The presence of these compounds in foods is of concern because the results of toxicological studies showed that they could endanger human health (EC 2001, BfR 2007, EFSA 2008, Joint FAO/WHO 2008, 2009). Velíšek *et al*. (1979, 2002) and Davídek *et al*. (1982) were the first to report the discovery of CPD esters together with other chloropropanols in acid hydrolysed proteins (Velíšek *et al.* 1980; Velíšek 2009) and in a cultivar of processed foods (Svejkovská *et al*. 2004). The most important precursors of chloropropanol esters identified in foods are fats derived from glycerol (glycerolipids) such as triacylglycerols, partial acylglycerols, glycerophospholipids, and chlorides either naturally present or added as sodium chloride. The main physical factors influencing the formation of chloropropanol esters are the water activity, temperature, and time.

The most prevalent chloropropanol esters in thermally processed foods are 3-chloropropane-1,2-diol (3-CPD) esters, however, 2-CPD, 1,3-dichloropropan-2-ol, and 2,3-dichloropropan-1-ol

Partly supported by the Ministry of Education and Youth of the Czech Republic, Projects No. MSM 6046137305 and No. 2B06168.

esters might also occur at lower or insignificant concentrations. Subsequently, chloropropanol esters can be hydrolysed to free chloropropanols (Hamlet *et al.* 2002; Hamlet & Sadd 2009). Generally, the amount of 3-CPD in any food or food ingredient released from 3-CPD esters by hydrolysis may exceed that of free 3-CPD by a factor of 15–20. High concentrations of 3-CPD esters have been found in some edible oils, notably in refined palm and olive oils (Zelinková *et al.* 2006; Karšulínová *et al.* 2007). 3-CPD fatty acid esters were also found in infant and baby foods containing refined vegetable oils (Zelinková *et al.* 2009a) and in breast milk (Zelinková *et al.* 2008a), which prompted further interest in these compounds.

The published data on 3-CPD ester concentrations in potato products are fragmentary and need to be verified and extended. A very high concentration of bound 3-CPD in French fries (6100 µg/kg) was reported by Svejkovská *et al.* (2004), while Zelinková *et al.* (2009b) found bound 3-CPD concentrations at the level of 100–258 µg/kg and Hamlet and Asuncion (2009) found concentrations of 35–397 µg/kg. The content of bound 3-CPD in crisps was 48–1186 µg/kg (Hamlet & Asuncion 2009) and 229–1008 µg/kg (Zelinková *et al.* 2009b), respectively.

Described herein are our investigations into the determination of 3-CPD esters in a large set of deep-fried potato products, French fries, and crisps, produced in four successive years 2006–2009. Both products might contain elevated concentrations of 3-CPD esters as they have a low water activity and are usually deep-fried in refined vegetable oils at high temperatures. Our attention was mainly focused on the role of various factors that might influence the amounts of 3-CPD esters during industrial processing of potatoes. The aim of this work was to determine free 3-CPD and 3-CPD fatty acid ester amounts and evaluate the role of the oil type employed.

## MATERIAL AND METHODS

***Chemicals***. 3-Chloropropane-1,2-diol (3-CPD, > 98%), diethyl ether p.a. (99.7%), hexane for organic trace analysis, and tetrahydrofurane p.a. (99.5%) were purchased from Merck (Darmstadt, Germany). 3-CPD-$d_5$ (97%) was obtained from Sigma Aldrich Izotech (St. Louis, USA), phenylboronic acid (PBA, ≥ 97%) and sulphuric acid (> 95%) from Fluka Chemie (Buchs, Switzerland), acetone p.a. (99.5%), sodium bicarbonate p.a. (99.5%), methanol p.a. (99.8%) and sodium sulphate p.a. (99%) from Penta (Nové Mesto n. Metují, Czech Republic). Diester of 3-CPD with palmitic acid and deuterium labelled diester of 3-CPD-$d_5$ with palmitic acid were synthesized 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 used were of analytical grade.

***Materials***. Raw potato tubers, instant mashed potatoes, two samples of potato dumplings, and five samples of potato crisps were purchased from a local retail market in Prague (Czech Republic). The sample of potato flakes was supplied by Vitana a.s. (Bíšice, Czech Republic). Seven different cultivars of potatoes (Karin, Granola, Pirol, Crispy, Saturna, Lady Clare, and Opal) for the preparation of deep fried potato products were grown in two localities, Valečov and Velhartice (Czech Republic), in 2006–2009. After the harvest, a set of 32 samples of French fries (16 pre-fried and 16 fried products, in 2007) and 56 samples of potato crisps (No. 1–30 in 2006, No. 31–46 in 2007, No. 47–49 in 2008, No. 50–56 in 2009) were produced under industrial conditions in Fritagro Nížkov s.r.o. (Nížkov, Czech Republic).

Refined rapeseed oil, RBD palm oil, palmolein, and palmstearin were supplied by STZ a.s. (Ústí n. L., Czech Republic), frying oil Forte (80% rapeseed oil, 20% palm oil) was obtained from Pribina a.s. (Přibyslav, Czech Republic), palm oil based product Master Frit, (fractionated palm oil for frying, palmolein) was a product of Master Martini CE s.r.o. (Ústí n. L., Czech Republic).The pre-fried French fries were fried at a temperature of 158°C for 2 min in Master Frit oil and then deep-frozen to –18°C. The final frying was carried out at 180°C for 3 min in Master Frit oil. Potato crisps were fried at 170°C for 2 min in either rapeseed (samples No. 1–30) or Master Frit (samples No. 31–56) oils.

***Methods***. The determination of the fat content was carried out by the Soxhlet method (IUPAC 1964) using 150 ml of diethyl ether (8 h). Free and bound 3-CPD were analysed by GC/MS according to the method of Divinová (2004) modified by Zelinková (2006). Deuterated diester of 3-CPD with palmitic acid was used as the internal standard for the determination of bound 3-CPD. Three parallel examinations were made of each sample.

***GC/MS analysis***. Capillary GC/MS analysis of free and 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) and data processing system (MSD ChemStation, G1701CA, Agilent Technolgies, Palo Alto, USA). 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 splittless injection), 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 finally 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 (derivative of 3-CPD) and at *m/z* 150 (derivative of 3-CPD-$d_5$). To confirm the identity, the ions at *m/z* 196 (derivative of 3-CPD) and at *m/z* 201 (derivative of 3-CPD-$d_5$) were used.

***Statistical methods***. Descriptive statistics and ANOVA analysis were performed using the software SPSS for Windows (Release 11.0.0, SPSS Inc., Chicago, USA).

## RESULTS AND DISCUSSION

3-CPD and its fatty acid esters are typical technological (processing) contaminants that form from food lipids during processing at higher temperatures. Furthermore, it is known that refined vegetable oils, especially palm oil, contain high amounts of 3-CPD esters formed during deodorisation and may thus contaminate deep-fried foods (Hamlet *et al.* 2011).

### Raw potatoes, potato flakes, mashed potatoes, and potato dumplings

The content of total lipids (fat) in raw potato tubers is very low, approximately 0.1% (Doležal *et al.* 2008a; USDA 2010) (Table 1). Therefore, it was not surprising that the concentration of free 3-CPD in raw potatoes was not detected (< 3 μg/kg) and the amount of bound 3-CPD reached only 2 μg/kg (Doležal *et al.* 2008a). Somewhat higher concentrations of 3-CPD were found in the potato products processed at relatively low temperatures. For example, potato flakes contained free 3-CPD at the level of 37 μg/kg and bound 3-CPD at the level of 18 μg/kg. Free 3-CPD concentrations in instant mashed potato and potato dumpling were under the limit of detection (< 3 μg/kg), but the content of bound 3-CPD in these products was 38 μg/kg and 10–13 μg/kg, respectively (Doležal *et al*. 2008b). It seems that the bound 3-CPD in potato flakes can form during drying and its increased level in mashed potato could be ascribed to the addition of vegetable fat.

Potato crisps and French fries (potato chips) are products manufactured by deep-frying using various oil blends based mainly on refined, bleached, and deodorised palm oil (RBD palm oil). As long as the oil is hot enough and the food is not immersed in the oil for too long, oil penetration will be confined to the outer surface. However, if the food is cooked in the oil for too long, much of the water will be lost and the oil will begin to penetrate the food. As a result, the total lipid content in French fries was 13.37–17.40%, in crisps it was 27.20–36.40%, while in reduced fat products the fat content was approximately 20.80% (USDA 2010). Furthermore, it is evident that the fat found in these products was almost exclusively that used for deep-frying.

### French fries

In 2007, analysed 32 samples of French fries (pre-fried and fried) manufactured on an industrial scale using two potato cultivars grown in

Table 1. Content of fat, free and bound 3-CPD in raw potatoes, potato flakes, mashed potatoes and potato dumplings

| Sample | Fat (%) | RSD (%) | Free 3-CPD (μg/kg) | RSD (%) | Bound 3-CPD* (μg/kg) | RSD (%) |
|---|---|---|---|---|---|---|
| Raw potato tubers | 0.1 | 16.1 | < 3 | – | 2 | 1.8 |
| Potato flakes | 0.4 | 1.3 | 37.0 | 1,6 | 18 | 1.2 |
| Mashed potatoes | 0.4 | 5.6 | < 3 | – | 38 | 0.4 |
| Potato dumplings | 0.7 | 6.2 | < 3 | – | 10 | 1.6 |
| Potato dumplings | 0.6 | 1.6 | < 3 | – | 13 | 1.9 |

*The concentration of bound 3-CPD in these low fat products were not given

two localities. Analogously to potato crisps, all samples were analysed for their contents of fat, and free and bound 3-CPD (Table 2).

The fat content of pre-fried and fried French fries, regardless of the cultivar and locality, was 2.7–4.3% (average value of 3.3%) and 8.7–10.9% (average value 9.8%), respectively. The average amounts of fat in pre-fried and fried products manufactured from the Karin cultivar were 3.5% and 9.1% (locality Valečov), while the same cultivar from the locality Velhartice contained 3.3% and 10.2% of fat, respectively. The average amounts of fat in pre-fried and fried products manufactured from the Granola cultivar were 3.2% and 10.1% (locality Valečov) and the same cultivar from the locality Velhartice contained 3.4% and 9.9% of fat, respectively. The fat content of fried French fries was significantly lower than that declared by USDA (2010).

All samples of pre-fried and fried French fries contained free 3-CPD below the limit of detection (< 3 μg/kg) or the limit of quantification (< 9 μg/kg).

The amount of bound 3-CPD in pre-fried samples ranged from 27 μg/kg (sample No. 16) to 64 μg/kg

Table 2. Content of fat, free and bound 3-CPD in French fries

| Sample No. | Cultivar | Locality | | Fat (%) | RSD (%) | Free 3-CPD (μg/kg) | RSD (%) | Bound 3-CPD (μg/kg fat) | RSD (%) | Bound 3-CPD (μg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Karin | Velhartice | pre-fried | 2.7 | 0.6 | < 3 | - | 1145 | 1.6 | 30 |
| | | | fried | 10.5 | 10.7 | < 9 | - | 2384 | 6.3 | 249 |
| 2 | | Velhartice | pre-fried | 2.9 | 1.4 | < 3 | - | 1165 | 1.9 | 33 |
| | | | fried | 10.2 | 0.8 | < 9 | - | 2370 | 8.4 | 242 |
| 3 | | Velhartice | pre-fried | 3.3 | 2.9 | < 3 | - | 1450 | 1.7 | 48 |
| | | | fried | 9.6 | 4.4 | < 9 | - | 2431 | 9.8 | 234 |
| 4 | | Velhartice | pre-fried | 4.3 | 3.2 | < 3 | – | 1495 | 1.6 | 64 |
| | | | fried | 10.7 | 1.3 | < 9 | – | 2420 | 4.5 | 258 |
| 5 | | Valečov | pre-fried | 4.1 | 2.2 | < 3 | – | 1228 | 6.6 | 51 |
| | | | fried | 8.8 | 1.5 | < 9 | – | 2074 | 3.7 | 183 |
| 6 | | Valečov | pre-fried | 4.0 | 3.6 | < 3 | – | 1277 | 0.8 | 51 |
| | | | fried | 9.4 | 4.8 | < 9 | – | 2681 | 2.0 | 253 |
| 7 | | Valečov | pre-fried | 3.0 | 0.8 | < 3 | – | 1260 | 2.5 | 37 |
| | | | fried | 8.7 | 4.4 | < 9 | – | 1140 | 7.5 | 100 |
| 8 | | Valečov | pre-fried | 3.0 | 4.2 | < 3 | – | 1338 | 1.4 | 41 |
| | | | fried | 9.3 | 0.2 | < 9 | – | 1233 | 7.7 | 114 |
| 9 | Granola | Velhartice | pre-fried | 3.6 | 4.0 | < 3 | – | 1328 | 3.9 | 48 |
| | | | fried | 10.3 | 2.6 | < 9 | – | 1401 | 3.2 | 145 |
| 10 | | Velhartice | pre-fried | 3.5 | 3.7 | < 3 | – | 1291 | 2.7 | 45 |
| | | | fried | 9.6 | 1.5 | < 3 | – | 1891 | 4.9 | 182 |
| 11 | | Velhartice | pre-fried | 3.1 | 6.1 | < 3 | – | 1585 | 15.4 | 49 |
| | | | fried | 10.1 | 0.3 | < 3 | – | 1753 | 4.1 | 177 |
| 12 | | Velhartice | pre-fried | 3.2 | 0.9 | < 3 | – | 1235 | 2.9 | 39 |
| | | | fried | 9.6 | 1.1 | < 3 | – | 1715 | 2.5 | 165 |
| 13 | | Valečov | pre-fried | 3.5 | 1.2 | < 3 | – | 1203 | 8.1 | 43 |
| | | | fried | 10.3 | 2.3 | < 9 | – | 1349 | 3.2 | 139 |
| 14 | | Valečov | pre-fried | 3.3 | 1.1 | < 3 | – | 1143 | 1.8 | 38 |
| | | | fried | 10.9 | 3.6 | < 3 | – | 1551 | 0.8 | 168 |
| 15 | | Valečov | pre-fried | 2.7 | 2.2 | < 3 | – | 1073 | 5.6 | 29 |
| | | | fried | 9.3 | 1.1 | < 9 | – | 1265 | 3.5 | 118 |
| 16 | | Valečov | pre-fried | 3.2 | 0.4 | < 3 | – | 838 | 1.3 | 27 |
| | | | fried | 10.0 | 2.0 | < 9 | – | 1493 | 9.5 | 149 |

(No. 4) regardless of the cultivar and locality. The products made from the cultivar Karin grown in Valečov contained 45 μg/kg (1276 μg/kg fat); the same cultivar grown in Velhartice contained 44 μg/kg (1314 μg/kg fat). French fries manufactured from the cultivar Granola grown in Valečov contained 34 μg/kg (1064 μg/kg fat); the same cultivar grown in Velhartice contained 45 μg/kg (1360 μg/kg fat). The content of bound 3-CPD in fried French fries was 100 μg/kg (No. 7) – 258 μg/kg (sample No. 4). The products made from the cultivar Karin grown in Valečov contained 163 μg/kg (1782 μg/kg fat); the same cultivar grown in Velhartice contained 246 μg/kg (2401 μg/kg fat). French fries manufactured from the cultivar Granola grown in Valečov contained 144 μg/kg (1415 μg/kg fat); the same cultivar grown in Velhartice contained 167 μg/kg (1690 μg/kg fat).

The results obtained were evaluated using the analysis of variance (ANOVA) to assess whether the measured values of bound 3-CPD differed from each other within the pre-defined groups of two different potato cultivars and localities. It was found that bound 3-CPD amounts expressed in either μg/kg (*P* value, significance 0.364) or in μg/kg fat (significance 0.364) obtained from the Karin and Granola cultivars did not differ at the significance level of 5% (0.05). The same results were achieved by analysing the products made from the potatoes grown in Valečov and Velhartice localities (significance 0.338 and 0.054, respectively).

**Potato crisps**

In four consecutive years 2006–2009, we analysed 56 samples of potato crisps manufactured on an industrial scale using seven potato cultivars grown in two localities. All samples were analysed for their contents of total lipids (fat), and free and bound 3-CPD (Table 3).

The fat content of potato crisps, regardless of the cultivar and locality, was 34.5–52.5%, with the average value of 41.1%, which was significantly higher than the contents declared by USDA (2010). The average fat content was 40.0% in 2006, 41.7% in 2007, 40.8% in 2008, and 44.9% in 2009. In 2006, the average amounts of fat in the crisps produced from cv. Karin (samples No. 1–12) and cv. Granola (No. 19–30) grown in Valečov were 37.4% and 42.7%, respectively. In the crisps obtained from the cv. Karin grown in Velhartice (samples No. 13–18), the average amount of fat was 39.5%. The crisps manufactured on an industrial scale from four potato cultivars in 2007 were fried in two consecutive days using fresh oil each day. The first day products (samples No. 31–39) had the fat content 41.6% and almost the same fat content (41.7%) was found in the second day products (No. 40–46). Furthermore, potato chips obtained in 2006 (samples No. 1–30) were fried using rapeseed oil and contained 40.0% fat, on average, while the rest of the samples (No. 31–56) were fried in palmolein and contained 42.4% fat. The fat content of potato crisps obtained from the local market (samples No. 57–61) was 26.6–39.1% (average 32.7%). The results concerning the fat content in potato crisps were not evaluated statistically.

In most samples, the amount of free 3-CPD in potato crisps was either below the limit of detection (< 3 μg/kg) or the limit of quantification (< 9 μg/kg). Somewhat elevated concentrations of 10.4–46.2 μg per kg were found only in seven samples analysed in 2006 (samples No. 7, 8, 9, 21, 24, 29, and 30).

Bound 3-CPD concentrations found in potato crisps were expressed per kg of sample and also per kg of fat. The lowest amount in the samples manufactured on an industrial scale was 98 (249 μg/kg fat) in sample No. 11 while the highest amount was 2201 μg/kg (5028 μg/kg fat) in sample No. 48. The average concentration was 510 μg/kg (1213 μg/kg fat). The average amount of bound 3-CPD was 228 μg/kg (563 μg/kg fat) in 2006 (fried in rapeseed oil), 616 μg/kg (1485 μg/kg fat) in 2007, 1769 μg/kg (4244 μg/kg fat) in 2008, and 935 μg/kg (2079 μg/kg fat) in 2009 (fried in Master Frit oil). In 2006, the amounts of bound 3-CPD in the crisps produced from cv. Karin (samples No. 1–12) and cv. Granola (samples No. 19–30) grown in Valečov were 166 μg/kg (435 μg/kg fat) and 266 μg/kg (623 μg/kg fat), respectively. In the crisps obtained from the Karin cultivar grown in Velhartice (samples No. 13–18), the amount of bound 3-CPD was 277 μg/kg (699 μg/kg fat). The crisps manufactured on an industrial scale from four potato cultivars in 2007 were fried in two consecutive days using fresh oil each day. The first day products (samples No. 31–39) had their bound 3-CPD content 779 μg/kg (1883 μg/kg fat) and in the second day products (samples No. 40–46) the bound 3-CPD amount was 406 μg/kg (1973 μg/kg fat). The potato chips fried in rapeseed oil (samples No. 1–30) contained bound 3-CPD at the level of 228 μg/kg (563 μg/kg fat), while the samples fried in palmolein (samples No. 31–56) contained bound 3-CPD at the level of 835 μg/kg

Table 3. Content of fat, free and bound 3-CPD in potato crisps

| Sample No. | Year of harvest | Cultivar | Locality | Fat (%) | RSD (%) | Free 3-CPD (μg/kg) | RSD (%) | Bound 3-CPD (μg/kg fat) | RSD (%) | Bound 3-CPD (μg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | 37.8 | 1.5 | < 3 | – | 534 | 49.1 | 202 |
| 2 | | | | 38.9 | 3.1 | < 3 | – | 401 | 31.6 | 156 |
| 3 | | | | 34.5 | 2.9 | < 9 | – | 325 | 24.2 | 112 |
| 4 | | | | 34.7 | 2.2 | < 3 | – | 483 | 39.9 | 167 |
| 5 | | | | 37.8 | 5.7 | < 3 | – | 461 | 26.1 | 174 |
| 6 | | | Valečov | 35.1 | 1.0 | < 3 | – | 339 | 19.3 | 119 |
| 7 | | | | 38.7 | 1.8 | 31.7 | 12.4 | 462 | 8.4 | 178 |
| 8 | | | | 38.7 | 6.9 | 39.9 | 5.2 | 304 | 13.5 | 118 |
| 9 | | Karin | | 34.5 | 0.8 | 10.4 | 10.6 | 397 | 45.7 | 137 |
| 10 | | | | 35.8 | 1.0 | < 9 | – | 303 | 25.9 | 108 |
| 11 | | | | 39.2 | 2.1 | < 9 | – | 249 | 7.5 | 98 |
| 12 | | | | 43.7 | 1.5 | < 9 | – | 965 | 1.6 | 421 |
| 13 | | | | 41.8 | 1.2 | < 3 | – | 532 | 12.9 | 222 |
| 14 | | | | 40.9 | 1.4 | < 3 | – | 763 | 18.6 | 312 |
| 15 | | | | 36.7 | 1.7 | < 3 | – | 589 | 7.5 | 216 |
| 16 | 2006 | | Velhartice | 37.9 | 2.5 | < 3 | – | 649 | 7.0 | 246 |
| 17 | | | | 40.7 | 3.1 | < 3 | – | 880 | 4.9 | 358 |
| 18 | | | | 39.3 | 3.1 | < 3 | – | 781 | 3.2 | 307 |
| 19 | | | | 40.8 | 2.2 | < 3 | – | 296 | 15.7 | 121 |
| 20 | | | | 39.5 | 0.4 | < 9 | – | 384 | 2.2 | 152 |
| 21 | | | | 40.6 | 1.5 | 21.7 | 10.5 | 442 | 0.2 | 179 |
| 22 | | | | 37.8 | 2.0 | < 3 | – | 1271 | 11.3 | 481 |
| 23 | | | | 39.2 | 2.5 | < 3 | – | 385 | 16.0 | 151 |
| 24 | | | | 39.6 | 2.2 | 46.2 | 19.9 | 383 | 23.9 | 152 |
| 25 | | Granola | Valečov | 46.7 | 1.0 | < 3 | – | 1298 | 5.6 | 606 |
| 26 | | | | 40.5 | 1.4 | < 9 | – | 877 | 3.4 | 355 |
| 27 | | | | 44.4 | 2.6 | < 3 | – | 838 | 11.3 | 372 |
| 28 | | | | 45.2 | 3.5 | < 3 | – | 369 | 24.9 | 167 |
| 29 | | | | 46.4 | 2.5 | 15.1 | 15.5 | 447 | 19.0 | 208 |
| 30 | | | | 51.9 | 1.3 | 11.3 | 22.5 | 485 | 14.9 | 252 |
| 31 | | Pirol | | 37.1 | 0.3 | – | – | 2620 | 2.3 | 971 |
| 32 | | | | 39.9 | 0.1 | – | – | 2525 | 5.1 | 1008 |
| 33 | | | | 44.2 | 0.1 | – | – | 1919 | 0.6 | 848 |
| 34 | | | | 41.9 | 0.4 | – | – | 1870 | 2.7 | 784 |
| 35 | | Crispy | | 44.0 | 0.5 | – | – | 1597 | 0.7 | 703 |
| 36 | | | | 42.8 | 0.4 | – | – | 1996 | 6.4 | 854 |
| 37 | | | | 43.3 | 0.5 | – | – | 1952 | 7.6 | 846 |
| 38 | 2007 | | | 40.0 | 0.1 | – | – | 1294 | 2.0 | 518 |
| 39 | | Saturna | | 41.1 | 0.2 | – | – | 1171 | 0.1 | 481 |
| 40 | | | Valečov | 41.8 | 0.1 | – | – | 599 | 1.7 | 250 |
| 41 | | | | 41.4 | 0.2 | – | – | 1577 | 3.4 | 652 |
| 42 | | | | 40.7 | 2.8 | – | – | 1367 | 1.4 | 557 |
| 43 | | Lady Clare | | 42.3 | 1.6 | – | – | 754 | 3.2 | 319 |
| 44 | | | | 42.3 | 0.1 | – | – | 624 | 1.2 | 264 |
| 45 | | | | 42.9 | 0.2 | – | – | 1324 | 4.4 | 568 |
| 46 | | | | 40.2 | 0.5 | – | – | 569 | 2.1 | 229 |
| 47 | | Pirol | | 42.8 | 0.7 | – | – | 4903 | 7.6 | 2100 |
| 48 | 2008 | Crispy | | 43.8 | 1.8 | – | – | 5028 | 2.5 | 2201 |
| 49 | | Lady Clare | | 35.9 | 6.3 | – | – | 2803 | 2.7 | 1007 |
| 50 | | | | 44.7 | 0.7 | – | – | 2553 | 4.3 | 1141 |
| 51 | | Pirol | | 42.9 | 1.1 | – | – | 2832 | 3.8 | 1215 |
| 52 | | | | 42.5 | 3.6 | – | – | 2647 | 2.2 | 1126 |
| 53 | 2009 | | Valečov | 44.0 | 2.6 | – | – | 2174 | 4.3 | 956 |
| 54 | | Lady Clare | | 44.7 | 0.6 | – | – | 975 | 4.4 | 436 |
| 55 | | Opal | | 43.2 | 2.1 | – | – | 1076 | 2.8 | 465 |
| 56 | | Opal | | 52.5 | 1.6 | – | – | 2296 | 4.5 | 1205 |
| 57 | | | | 29.0 | 0.6 | < 3 | – | 1761 | 5.4 | 511 |
| 58 | | | | 33.3 | 0.5 | < 3 | – | 1344 | 1.8 | 447 |
| 59 | – | – | – | 39.1 | 1.2 | < 3 | – | 1846 | 4.4 | 722 |
| 60 | | | | 35.3 | 0.6 | < 3 | – | 1605 | 1.8 | 567 |
| 61 | | | | 26.6 | 0.2 | < 3 | – | 1337 | 2.7 | 355 |

(2219 µg/kg fat). The bound 3-CPD content of the potato crisps obtained from the local market (samples No. 57–61) was 355 µg/kg (1337 µg/kg fat) in sample No. 61 and 722 µg/kg (1846 µg/kg fat) (average 520 µg/kg and 1579 µg/kg fat).

The results obtained were again evaluated using the analysis of variance (ANOVA). The results revealed that the bound 3-CPD amounts expressed in either µg/kg (*P*-value, significance 0.062) or in µg/kg fat (significance 0.122) gained from the Karin and Granola cultivars grown in Valečov in 2006 did not differ at the significance level of 5% (0.05). The crisps obtained from the cv. Karin grown in Valečov and Velhartice (significance 0.012 and 0.007, respectively) and those fried on consecutive days (significance 0.001 and 0.002, respectively) differed at the significance level of 5%. These differences may have been caused by several factors such as water content, differences in temperature and time of frying. The greatest difference at the significance level of 5% was found between the crisps fried in rapeseed oil and those fried in Master Frit oil (significance 0.000 and 0.000, respectively).

Sixteen samples of Master Frit oil were analysed for the bound 3-CPD content (Table 4). The oil samples were taken in two consecutive days (samples No. 31–39 and No. 40–46, resp.) during frying of potato crisps in 2007. The levels of bound 3-CPD in frying oils from the first day varied between 1043 µg/kg and 1920 µg/kg, the average content was 1488 µg/kg. The bound 3-CPD concentrations of the second day oils were 654–1291 µg/kg, the average amount being 1061 µg/kg. No difference in the bound 3-CPD levels was observed between the two days of frying.

Table 4. Content of bound 3-CPD in oils from potato crisps frying

| Sample No. | Frying day | Bound 3-CPD* (µg/kg) | RSD (%) |
|---|---|---|---|
| 1 | 1 | 1787 | 2.9 |
| 2 | 1 | 1870 | 5.0 |
| 3 | 1 | 1516 | 1.7 |
| 4 | 1 | 1920 | 2.1 |
| 5 | 1 | 1697 | 3.2 |
| 6 | 1 | 1206 | 3.3 |
| 7 | 1 | 1277 | 2.5 |
| 8 | 1 | 1080 | 5.9 |
| 9 | 1 | 1043 | 4.4 |
| 10 | 2 | 1065 | 4.7 |
| 11 | 2 | 1176 | 3.9 |
| 12 | 2 | 1291 | 1.8 |
| 13 | 2 | 801 | 4.1 |
| 14 | 2 | 1247 | 1.5 |
| 15 | 2 | 1196 | 1.6 |
| 16 | 2 | 654 | 3.2 |

*LOD = 100 µg/kg oil, LOQ = 300 µg/kg oil

Table 5. Content of free and bound 3-CPD in heated palm oil

| | Free 3-CPD (µg/kg) | Bound 3-CPD* (µg/kg) | Ratio bound/free** |
|---|---|---|---|
| Hours of heating at 230°C | | | |
| 0 | < 9 | 4170 | 927 |
| 0.5 | < 9 | 3860 | 858 |
| 1 | < 9 | 3340 | 742 |
| 2 | < 9 | 1750 | 389 |
| 4 | < 9 | 960 | 213 |
| Temperature (°C) | | | |
| 100 | < 9 | 4600 | 1022 |
| 150 | < 9 | 4490 | 998 |
| 200 | < 9 | 2070 | 460 |
| 230 | < 9 | 1750 | 389 |
| 260 | < 9 | 1800 | 400 |

*LOD = 100 µg/kg oil, LOQ = 300 µg/kg oil; **half of the value of LOQ was used

These findings closely correlate with the amount of bound 3-CPD occurring in refined vegetable oils. The analysis of virgin rapeseed oil revealed only traces of 3-CPD esters (< 100 µg/kg), while levels of 381–484 µg/kg (Zelinková *et al*. 2006) and of 500–1500 µg/kg (Weisshaar 2009) were found in refined rapeseed oils. Generally, native, unrefined vegetable oils concentrations of bound 3-CPD were < 100–140 µg/kg, while in refined vegetable oils 3-CPD esters were nearly always present at the levels of 200–2150 µg/kg Weisshaar (2009). Zelinková *et al*. (2006) analysed heated rapeseed oils and found that the level of bound 3-CPD increased with temperature and time of heating being, for example, 381 µg/kg in fresh oil and 675 µg/kg after heating at 170°C for 30 minutes.

In RBD palm oils, the levels of bound 3-CPD were 1390-2730 µg/kg (Karšulínová *et al*. 2007) and often exceeded 4000 µg/kg (Weisshaar 2009). The levels of bound 3-CPD in palm oil fractions, palmolein and palmstearin, were 2550 µg/kg and 1760 µg/kg, respectively (Zelinková *et al*. 2008b). The RBD oil obtained from the producer and deodorised for the second time before use had the level of bound 3-CPD 4170 µg/kg (Karšulínová *et al*.

Table 6. Content of free and bound 3-CPD in frying fats

| Frying | Free 3-CPD (μg/kg) | Bound 3-CPD (μg/kg) | Ratio bound/free* |
|---|---|---|---|
| Fresh fat | < 9 | 750 | 167 |
| Used for 1 day | < 9 | 1420 | 315 |
| 2 days | 11 | 2030 | 185 |
| 3 days | 9 | 2050 | 228 |
| 4 days | 11 | 1550 | 141 |
| 5 days | 10 | 1790 | 179 |

*half of the value of limit of quantification (LOQ) was used

2007). Thus, we were interested in the changes of the bound 3-CPD in this palm oil after heating at different temperatures for different times. The results obtained are summarised in Table 5 and show that the amount of bound 3-CPD increases upon heating at lower temperatures (100°C and 120°C) while at higher temperatures, a decrease of the bound 3-CPD levels can be observed. Nevertheless, these levels are high enough to be able to contaminate the deep-fried products.

As a result, the bound 3-CPD levels in fresh or heated rapeseed oil are relatively low to influence the levels of these esters in potato crisps, but palm oil, palm oil fractions and blends have a potential to do so. It is known that unused frying fats contained 600–2650 μg/kg of bound 3-CPD while in used frying fats, the levels of 3-CPD esters were lower than in the corresponding fresh frying fats (Weisshaar 2009). Thus, our attention was drawn to blends consisting of rapeseed oil and palm oil (frying oil Forte, 80% rapeseed oil, 20% palm oil) that were used for deep frying of potato crisps samples No. 1–30. Fresh fat contained free 3-CPD at the level of < 9 μg/kg and bound 3-CPD, due to the presence of palm oil, at the level of 750 μg/kg. The fat was used for frying for five days and each day the used fat was mixed with a fresh one to keep the final fat volume constant. Apparently, the maximum level of bound 3-CPD was reached after 3 days of frying and then the level of bound 3-MCPD decreased (Table 6). The results thus proved that high levels of 3-CPD esters can be found not only in palm oils but also in blends of palm and rapeseed oils.

## CONCLUSION

Edible refined oils (especially palm oils) represent the main source of 3-CPD esters in fried 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 on the mitigation strategies in fried products production.

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Received for publication December 1, 2010
Accepted after corrections January 27, 2011

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