Free and Bound 3-Chloropropane-1,2-diol in Coffee Surrogates and Malts

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Abstract

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The levels are reported of the free 3-chloropropane-1,2-diol (3-MCPD), its bound forms, the recognised precursors of 3-MCPD, and the factors influencing its formation in 5 selected coffee surrogates and 18 malts in the Czech Republic. The coffee surrogates had the free 3-MCPD level in the range of < 9.0 to 32 μg/kg while the highest amount was found in roasted barley. In malts, the free 3-MCPD levels were similarly low (< 9.0 to 45 μg/kg) being the highest in roasted malts (16–45 μg/kg). Nevertheless, the values found in either surrogates or malts, calculated after normalisation to 40% dry matter content, did not exceed the European Union limit of 20 μg/kg adopted for soy sauces and acid-HVP. The risk for consumers could arise from the bound 3-MCPD, its elevated levels having been found in both coffee surrogates and malts. In coffee surrogates, the bound 3-MCPD levels varied between 145–1184 μg/kg product; the highest level was found in roasted barley. The bound 3-MCPD levels exceeded the free 3-MCPD levels 32 to 81 times. In malts, the bound 3-MCPD levels ranged from 4.0 to 650 μg/kg, the highest amount having been found in roasted malts (463–650 μg/kg). The bound 3-MCPD levels exceeded the free 3-MCPD levels 0.4 to 36 times.

Keywords: chloropropanols; 3-chloropropane-1,2-diol; 3-MCPD; bound 3-MCPD, 3-MCPD esters; contaminants; coffee surrogate; malt

3-Chloropropane-1,2-diol (better known as 3-MCPD) was identified in acid-hydrolysed vegetable proteins (acid-HVP) in 1981 (Davídek *et al*. 1982) where it is formed as a reaction product of phospholipids, acylglycerols, and glycerol with hydrochloric acid. Later, it was shown that 3-MCPD occurs as a racemic mixture of its enantiomers, (*R*)-3-MCPD and (*S*)-3-MCPD (Velíšek *et al*. 2002). 3-MCPD is a representative of the so called endogenous, food borne or food processing contaminants.

In view of its toxicity, the European Commission's Scientific Committee on Food (SCF) has proposed a provisional Total Daily Intake (TDI) level of 2 μg/kg body weight/day for the amount of 3-MCPD that can be consumed daily over a lifetime without any appreciable harm to health (SCF 2001). The TDI was adopted on 8 March 2001 and applies from 5 April 2002. Similarly, the Joint FAO/WHO Expert Group on Food Additives (JECFA) set a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight per

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day in 2001 (JECFA 2001). A regulatory limit of 20 µg/kg, based on the 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 (EC 2001).

Several studies have shown that elevated levels of 3-MCPD can occur not only in acid-HVP, soy sauces, and related products but also in a wide range of home-made and retail outlet foods as well as in various food ingredients (Crews *et al*. 2001, 2002; Hamlet & Sadd 2004; Hamlet *et al*. 2002, 2004; Divinová *et al*. 2004b; Svejkovská *et al*. 2004; Zelinková *et al*. 2006). Coffee surrogates and malts may be potential foodstuffs with high 3-MCPD levels caused by their high content of roasted cereals, low water content, and the high temperatures employed during the roasting process.

Recently, 10 samples of different coffee surrogates were analysed for 3-MCPD content by Kurzrock and Speer (2005). The contents in samples from the German market varied between 43 µg/kg and 759 µg/kg. The lowest amounts of 3-MCPD, between 43 and 120 µg/kg, were determined in the insoluble samples. In the soluble coffee surrogates, the amounts from 274 to 759 µg/kg were found; no significant differences between normal soluble samples and soluble bio-products (containing 3-MCPD at the level of 80–483 µg/kg) could be observed. Further, it was shown that 3-MCPD content in coffee surrogates can be influenced by the roasting conditions and by the amount of individual non-cereal ingredients.

On the opposite, the level of 3-MCPD in coffee was relatively low, possibly due to its relatively high content of sulphur-containing substances. Green coffees contained only traces of 3-MCPD. In roasted coffees, 3-MCPD was found at the level of 10.1–18.5 µg/kg. The highest 3-MCPD level of 18.5 µg/kg was found in one instant coffee sample and in coffees roasted for a very long time. The final colour of the roasted coffee beans was directly linked to the 3-MCPD formed, the darker beans having the highest concentration, and arabica coffees containing lower 3-MCPD levels than robusta coffees (Doležal *et al*. 2005).

Furthermore, 3-MCPD was found in 9 of 24 malt products (38%) comprising brewing malts, malt extracts, and malt flour, at the level of > 10 µg/kg (Hamlet *et al*. 2002). The highest level found in 4 malt products was in the range of 250–500 µg/kg. All the samples were, without exception, products used for colouring and flavouring applications. The additional heat treatment to which these malts were subjected to produce the desired colour and flavour appeared to be a significant factor in the formation of 3-MCPD from endogenous precursors. The typical levels of 3-MCPD in white malted barley (with typical colour range in EBC units < 9) were < 10 µg/kg (MAGB 2003). For specialist malts (EBC colour units > 20), the 3-MCPD levels ranged from < 30 to < 500 µg/kg. The higher the desired final specialist malt colour, the higher the 3-MCPD level in the finished product. With roasted barley (EBC colour units 900–1400), the 3-MCPD level was < 500 µg/kg, which is the maximum limit of 3-MCPD for the typical dilution in products 1:100 but still well over the maximum level for acid-HVP.

Fatty acid esters of 3-MCPD were the known precursors in the formation of 3-MCPD in model mixtures consisting of hydrochloric acid and triacylglycerols, phospholipids, soybean oil, soybean meal, wheat and maize lipids (Velíšek *et al*. 1980; Davídek *et al*. 1982). They were also found as the constituents of raw 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 of a variety of processed foods and food ingredients (Divinová *et al*. 2004b; Svejkovská *et al*. 2004; Doležal *et al*. 2005; Zelinková *et al*. 2006). These compounds represent the bound form of 3-MCPD, from which free 3-MCPD could be released by a lipase-catalysed hydrolysis reaction. In many cases the amount of the bound 3-MCPD exceeded those of the free 3-MCPD.

The level of the bound 3-MCPD in roasted coffee was relatively low and varied between 6 µg/kg (soluble coffee) and 390 µg/kg (decaffeinated coffee) and exceeded the free 3-MCPD level 8 to 33 times (Doležal *et al*. 2005). As concerns coffee surrogates and malts, no data on their bound 3-MCPD contents are available, hence our analysis of coffee surrogates and Czech malts. Our attention was also focused on the role of various factors (water, fat, glycerol and chloride amount) that can influence the levels of the bound 3-MCPD during the processing of these commodities.

MATERIAL AND METHODS

Chemicals. 3-Chloropropane-1,2-diol (98%), hexane for organic trace analysis and tetrahydrofurane p.a. (99.5%) were obtained from Merck (Darmstadt, Germany), phenylboronic acid and sulphuric acid for organic trace analysis (> 95%) were from Fluka Chemie (Buchs, Switzerland), 3-chloropropane-1,2-diol-d_5 (99.4%) was from Dr. Ehrenstorfer (Augsburg, Germany). Acetone p.a. (99.5%), methanol p.a. and butane-1,3-diol p.a. were products of Lach-Ner (Neratovice, Czech Republic), diethyl ether p.a. (99.7%), petroleum ether p.a. (b.p. 40–60°C), sodium bicarbonate (99.5%), and sodium chloride p.a. (99.9%) were products of Penta (Chrudim, Czech Republic), silver nitrate p.a. was produced by Safina (Vestec, Czech Republic). All other reagents and solvents were of analytical purity.

Materials. Coffee surrogates were produced by Kávoviny a.s. (Pardubice, Czech Republic). Four samples of coffee surrogates were analysed and designated as sample A (roasted rye), sample B (roasted chicory root), sample C (roasted barley), sample D (a mixture of roasted barley, rye, chicory root and sugar beet), and sample E (a mixture of roasted barley, rye, and chicory root).

The samples of malts were supplied by Sladovny Soufflet ČR (Prostějov, Czech Republic). These samples included Pilsner malt, Munich malt, crystal malt, roasted malt, wheat malt, and Karapils malt. Furthermore, the supplier roasted a series of malts at the maximum temperature of about 150°C for 100, 115, 130, and 145 min to obtain roasted malts of different colour intensities and of different 3-MCPD contents.

Methods

Free 3-MCPD. To the sample (about 5 g) placed in a 100 ml beaker, 30 ml of hexane/acetone mixture (1:1, v/v) and 50 µl of the internal standard solution in water (0.1 mg/ml) were added. The mixture was homogenised for 3 min using the homogenisator Ultra-Thurrax T25 (Janke and Kunkel, IKA-Labortechnik, Switzerland), and then filtered through a Büchner funnel. The solid residue in the beaker was washed with two 10 ml portions of the hexane/acetone mixture and the filtrate was transferred into a separatory funnel containing 10 ml water. The lower aqueous layer was separated, the organic layer re-extracted with another 10 ml portion of water, and the combined extracts collected in a 100 ml distillation flask were evaporated under vacuum at 55°C to dryness. The residue was dissolved in 2 ml of 20% NaCl solution, 0.4 ml of the phenylboronic acid solution (0.25 mg/ml) was added and the flask was heated in a water bath at 90°C for 20 min. After cooling to room temperature, hexane (2 ml) was added and the 4-chloromethyl-2-phenyl 1,3,2-dioxaborolane formed was extracted by vigorous shaking. One µl of the hexane layer was analysed by GC/MS (Divinová *et al*. 2004a).

Bound 3-MCPD. The homogenised sample (about 20 g) was extracted with diethyl ether (1:4, w/v) using the Soxhlet apparatus and the extract was filtered through a Büchner funnel. The residue was washed with 2 portions of diethyl ether (1:1, w/v). The filtrate was extracted with water (5:1, v/v) in a separatory funnel. The solvent was dried over anhydrous sodium sulphate and evaporated to dryness using a vacuum rotary evaporator. About 100 mg of the residue obtained was dissolved in tetrahydrofurane (1 ml) and transmethylated, using 1.8 ml of 1.8% sulphuric acid solution, at 40°C for 16 hours. The mixture was then cooled to room temperature, neutralised with 0.5 ml of saturated $NaHCO_3$ and evaporated under vacuum at 55°C to dryness. The residue was dissolved in 2 ml of 20% NaCl solution, and before the derivatisation described above, it was extracted with two 2 ml portion of hexane (Divinová *et al*. 2004a).

Determination of pH, water, chlorides, glycerol and fat. *A*pproximately 2.5 g of the homogenised sample was mixed with 25 ml of distilled water and pH value of the mixture was measured by the pH meter Radiometer (Copenhagen NV, Denmark) with the electrode THETA 90, type RE 413. The water content was determined by drying the homogenised sample (5–10 g) in an oven at 103–105°C. Chlorides were determined in the extract obtained as follows: Hot distilled water (50 ml) was added to 5 g of sample placed in a beaker; the beaker was covered with a watch glass, its content was boiled for 2 min and cooled to room temperature; the resulting suspension was filtered through a Büchner funnel and the filtrate was titrated with 0.1M $AgNO_3$ using the above pH meter equipped with a chloride-selective electrode ISE. For the determination of glycerol, 10 g of the homogenised sample was mixed with 50 ml of butane-1,3-diol (internal standard) in methanol (0.1 mg/l). The suspension was filtered and the

solvent evaporated. The residue was dissolved in 10 ml of methanol and 1 µl of the solution was analysed by GC. The fat content was determined by the Soxhlet method using 150 ml of light petroleum ether (8 h). The solvent was evaporated on a rotary vacuum evaporator and the residue was dried in an oven at 103–105°C.

GC/MS analyses. The GC/MS analysis of the free and the bound 3-MCPD 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 × 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 200°C (37 min) at a rate of 5°C/min, and then at a rate 10°C/min to 300°C with hold for 17 min. Helium at a flow rate of 0.8 ml/min was used as the carrier gas, 1 µl sample was injected. Quantitative analysis was carried out by monitoring characteristic ions at *m/z* 147 (3-MCPD) and at *m/z* 150 (3-MCPD-d_5). Ions at *m/z* 91 and 196 (3-MCPD) and at *m/z* 201 (3-MCPD-d_5) were used as qualifiers (Divinová *et al*. 2004a).

The GC analysis of glycerol was carried out using an HP 4890A apparatus (Hewlett Packard, USA) equipped with a flame ionisation detector, data processing system (CSW 1.7), and an HP-20M capillary column (15 m, 530 µm i.d., 1.3 µm film thickness). The oven was initially set to 120°C, kept for 2 min, then programmed for the rate of 10°C/min to 180°C and kept at this temperature for 14 min. The injection port (split 1:1) and the detector were held at 220°C and 280°C, respectively. Helium at a flow rate of 15 ml/min was used as a carrier gas.

Statistical methods. Statistical evaluation of the results achieved was done employing the computer program SPSS for Windows, Release 11.0.0, Standard Version.

RESULTS AND DISCUSSION

Coffee surrogates

The samples of insoluble coffee surrogates were analysed for their free and bound 3-MCPD contents (Table 1). As can be seen, the naturally present chlorides and lipids (the recognised precursors of 3-MCPD in processed foodstuffs) were sufficient for the 3-MCPD formation during the roasting process. Surprisingly, only the sample of roasted barley (sample C) contained an elevated level of free 3-MCPD (31.6 µg/kg) whereas the lowest level of free 3-MCPD was found in roasted rye (sample A). Nevertheless, the values calculated after normalisation to 40% dry matter content did not exceed the European Union limit of 20 µg/kg adopted for soy sauces and acid-HVP (EC 2001). Higher levels of the free 3-MCPD (43 µg/kg in roasted barley malt and 120 µg/kg in a mixture of roasted malt, barley, rye, and chicory root) were determined in the insoluble coffee surrogates (Kurzrock & Speer 2005).

As expected, the levels of the bound 3-MCPD found in fats isolated from the coffee surrogates were very high and varied between 19 300–50 600 µg/kg fat, i.e. 145–1184 µg/kg product. Again, the highest level of the bound 3-MCPD was found in the fat extracted from roasted barley (sample C). On the

Table 1. Contents of free and bound 3-MCPD in coffee surrogates

Sample	Free 3-MCPD		Free 3-MCPDn (µg/kg)	Bound 3-MCPD		
	µg/kg	RSD		µg/kg fat	µg/kg	RSD
A	< 9.0	3.2	< 9	19 300	145	0.3
B	11.8	5.1	< 9	50 300	957	0.3
C	31.6	1.5	13	50 600	1184	1.7
D	10.9	2.1	< 9	34 300	460	0.1
E	13.9	1.0	< 9	38 700	859	0.1

3-MCPDn = normalisation to 40% dry matter content; Free 3-MCPD = 9 µg/kg; Bound 3-MCPD = 300 µg/kg fat; RSD = relative standard deviation (%)

Table 2. pH values and contents of fat, glycerol, chlorides, and water in coffee surrogates

Sample	pH	Fat		Glycerol		Chlorides		Water	
		%	RSD	%	RSD	%	RSD	%	RSD
A	5.9	0.8	8.0	0.009	0.1	0.03	3.18	2.6	1.3
B	4.6	1.9	0.5	0.042	0.2	0.11	0.51	3.2	0.7
C	5.0	2.4	0.5	0.005	1.0	0.13	0.33	2.8	0.5
D	4.9	1.3	2.7	0.032	0.0	0.07	2.73	1.5	0.4
E	5.0	2.2	2.0	0.015	0.4	0.11	1.75	3.3	0.2

RSD = relative standard deviation (%)

contrary, the lowest level of the bound 3-MCPD (19 300 µg/kg fat, 145 µg/kg product) was found in roasted rye (sample A). However, the bound 3-MCPD levels exceeded the free 3-MCPD levels 32 to 81 times (one half of the value of LOQ for free 3-MCPD was used for the calculation), being the highest in roasted chicory root (sample B) and the lowest in roasted rye (sample A).

Table 2 summarises pH values and the levels of fat, glycerol, chlorides, and water of the coffee surrogates analysed. As expected, the lowest pH value was in the sample of roasted chicory root (sample B) due to the formation of saccharinic and other acids during the Maillard reaction (Barlianto & Maier 1995). The fat content in our coffee surrogate samples varied between 0.8 and 2.4%, the highest fat content having been found in roasted barley (sample C). The levels of chlorides were in the range of 0.03 to 0.13% and, again, the highest amount of chlorides was found in roasted barley (sample C). These phenomena could explain the relatively high content of either the free 3-MCPD or the bound 3-MCPD in roasted barley. The glycerol content of our samples varied between 0.005 and 0.042%, being the highest in roasted chicory root (sample B), which could correlate with its relatively high content of the bound 3-MCPD. Furthermore, the roots of the chicory plant are often roasted with the addition of sugar beet, low amounts of edible fats or oils, and salt (Belitz *et al*. 2004), which could result in an increased level of 3-MCPD, and with alkali carbonates that decompose 3-MCPD (Velíšek *et al*. 2003).

If, for example, a brew is prepared from a mixture of roasted rye (sample A, 24 g/l) and roasted chicory root (sample B, 8 g/l), according to the suggestions of the producer, then the brew contains 1.67 µg 3-MCPD/cup (150 ml). If this brew, with the bound 3-MCPD content of 11.1 µg/l, is consumed by an adult weighing 70 kg, all 3-MCPD esters having been totally extracted into the brew and totally hydrolysed in the body by enzymes, then his/her TDI level of 140 µg is reached after the consumption of 12.6 l of the brew, disregarding the free 3-MCPD level in the brew and the 3-MCPD level in other foodstuffs. Analogous values can be calculated for the other coffee surrogates. For example, brews prepared from our sample D (a mixture of roasted barley rye, chicory root and sugar beet) and E (a mixture of roasted barley, rye and chicory root) contained 2.2 and 0.8 µg 3-MCPD/cup (150 ml), respectively.

The levels of the bound 3-MCPD found in coffee samples by Doležal *et al*. (2005) varied between 6 µg/kg (soluble coffees) and 390 µg/kg (decaffeinated coffee) and exceeded the free 3-MCPD level 8 to 33 times. It is obvious that coffee is less prone to the formation of 3-MCPD than barley, rye, and cereal products (Hamlet *et al*. 2002), probably due to its higher content of sulphur-containing compounds that can react with 3-MCPD under elimination of hydrochloric acid (Velíšek *et al*. 2003). It is known that green coffee beans contain higher levels of cysteine (about 3 times more then barley or rye grains). Furthermore, several volatile thiols have been identified as constituents of roasted coffee aroma. A considerable part of these thiols is present in roasted coffee as disulfide bound to cysteine, SH-peptides and proteins (Belitz *et al*. 2004).

Malts

Fourteen different malt samples were analysed for their contents of both the free and the bound 3-MCPD (Table 3). Two Pilsner malts (raw materials for most beers), 2 Munich malts (for Munich

Table 3. Content of free and bound 3-MCPD in malts

Sample		Free 3-MCPD		Free 3-MCPDn (µg/kg)	Bound 3-MCPD		
		µg/kg	RSD		µg/kg fat	µg/kg	RSD
Pilsner malt	1	9.6	5.2	3.8	700	11.1	1.4
	2	9.4	11.3	3.7	300	5.2	3.7
Munich malt	1	6.8	7.3	2.7	300	4.8	2.0
	2	9.2	8.5	3.7	300	4.0	1.7
Crystal malt	1	9.9	3.3	< 9	700	9.9	3.3
	2	14.9	12.4	< 9	500	7.3	5.1
	3	< 9	–	< 9	1 900	29.6	7.1
	4	11.2	1.9	< 9	500	8.5	4.9
Roasted malt	1	28.0	2.2	11.2	29 100	463	1.0
	2	44.7	9.5	17.9	30 300	493	5.9
	3	15.6	6.0	< 9	38 900	544	2.5
	4	18.2	2.2	< 9	43 000	650	2.0
Wheat malt		< 9	–	< 9	500	6.7	12.4
Karapils malt		< 9	–	< 9	1 500	21.7	10.1

3-MCPDn = normalisation to 40% dry matter content; Free 3-MCPD = 9 µg/kg; Bound 3-MCPD = 300 µg/kg fat; RSD = relative standard deviation (%)

beer production), 4 crystal malts (for the production of dark Munich beer, used in baking and confectionery industry and for lemonade production), 4 roasted malts (for the production of dark Munich beer and used in baking and confectionery industry), 1 wheat malt (for the production of special wheat beers), and 1 sample of Karapils malt (for the production of light alcohol-free beer) were

Table 4. pH values and contents of fat, glycerol, chlorides and water in malts

Sample		EBC unit	pH	Fat		Glycerol		Chlorides		Water	
				%	RSD	%	RSD	%	RSD	%	RSD
Pilsner malt	1	21	5.9	1.6	1.0	0.002	5.5	0.06	3.70	4.5	0.3
	2	5	5.9	1.7	0.8	0.004	0.5	0.05	3.28	4.7	0.2
Munich malt	1	18	5.8	1.6	0.6	0.006	0.7	0.07	0.20	3.5	0.6
	2	23	5.9	1.4	1.4	0.010	0.3	0.06	1.02	2.9	0.4
Crystal malt	1	200	5.0	1.5	0.8	0.011	0.2	0.06	3.25	5.1	0.0
	2	124	5.0	1.4	0.3	0.007	1.0	0.07	1.68	5.2	0.4
	3	105	5.1	1.6	1.4	0.004	0.6	0.08	1.52	6.5	1.0
	4	212	4.9	1.6	0.9	0.004	0.2	0.08	0.57	4.5	1.0
Roasted malt	1	1600	4.6	1.6	0.3	0.019	0.2	0.07	3.90	1.6	0.3
	2	798	4.7	1.6	0.5	0.023	0.8	0.08	0.42	3.3	0.0
	3	1350	4.9	1.4	0.5	0.001	4.0	0.08	1.79	1.8	8.7
	4	1550	4.8	1.5	0.5	0.001	5.9	0.06	0.60	2.4	1.9
Wheat malt		5	6.3	1.4	1.0	0.001	1.1	0.07	0.32	5.5	1.3
Karapils Malt		29	5.4	1.5	1.0	0.003	0.3	0.09	0.73	7.2	0.3

RSD = relative standard deviation (%)

analysed. Their EBC colour units, pH values, and their contents of fat, glycerol, chlorides and water, characterised the malts (Table 4).

The free 3-MCPD levels were relatively low (< 9.0 to 45 μg/kg), lower than the findings given by MAGB (2003), being probably caused by different technological conditions used for the malts production. As expected, the highest free 3-MCPD levels were found in roasted malts (16–45 μg/kg). In no malt sample did even the values calculated after normalisation to 40% dry matter content exceed the European Union limit of 20 μg/kg adopted for soy sauces and acid-HVP (EC 2001). The bound 3-MCPD levels in the analysed malts ranged from 4.0 μg/kg to 650 μg/kg, being the lowest in the Munich malts (4.0–4.8 μg/kg), wheat malt (6.7 μg/kg), Pilsner malts (5.2–11.1 μg/kg) and some crystal malts (7.3–29.6 μg/kg). The highest levels of the bound 3-MCPD were found in the roasted malts (463–650 μg/kg). The bound 3-MCPD levels exceeded the free 3-MCPD levels 0.4 to 36 times (one half of the value of LOQ for free 3-MCPD was used for the calculations), being the lowest in one of the Munich malts (sample 2) and the highest in one of the roasted malts (sample 4).

The differences between the pH values of the individual malt samples as well as between their contents of fat, glycerol, chlorides and water samples were not very distinctive. To visualise the relationships between the malt samples and the individual analytes, partial correlation analysis was done using the data presented in Tables 2 and 4. A positive correlation significant at the 0.05 level (2-tailed) was found e.g. between the free 3-MCPD level and the EBC colour unit, and a negative correlation, significant at the same level of significance, was found between the free 3-MCPD level and the pH value, and between the free 3-MCPD level and the water content (Table 5). A positive correlation significant at the 0.01 levels was found between the free 3-MCPD level and the content of glycerol that is formed from lipids by the action of lipolytic enzymes. The amount of the bound 3-MCPD negatively correlated with the pH value at the 0.05 level of significance, and with the water content at the 0.01 level of significance. A positive correlation significant at the 0.01 level of significance was found between the bound 3-MCPD level and the EBC colour unit, and between the bound 3-MCPD amount and the free 3-MCPD concentration.

Table 5. Pearson correlations and significance (2-tailed) for all variables

Variables		EBC	MCPD_F	MCPD_B	pH	Fat	Glycerol	Chlorides	Water
EBC	corr.	1	0.623*	0.954**	–0.693**	–0.058	0.281	0.081	–0.755**
	sign.	0	0.017	0.000	0.006	0.843	0.331	0.783	0.002
MCPD_F	corr.	0.623*	1	0.708**	–0.624*	0.175	0.784**	0.122	–0.557*
	sign.	0.017	0	0.005	0.017	0.550	0.001	0.678	0.039
MCPD_B	corr.	0.954**	0.708**	1	–0.641*	–0.058	0.288	0.120	–0.720**
	sign.	0.000	0.005	0	0.013	0.844	0.319	0.683	0.004
pH	corr.	–0.693**	–0.624*	–0.641*	1	–0.070	–0.433	–0.376	0.331
	sign.	0.006	0.017	0.013	0	0.812	0.122	0.185	0.247
Fat	corr.	–0.058	0.175	–0.058	–0.070	1	0.208	–0.139	0.052
	sign.	0.843	0.550	0.844	0.812	0	0.475	0.634	0.860
Glycerol	corr.	0.281	0.784**	0.288	–0.433	0.208	1	0.071	–0.347
	sign.	0.331	0.001	0.319	0.122	0.475	0	0.809	0.224
Chlorides	corr.	0.081	0.122	0.120	–0.376	–0.139	0.071	1	0.255
	sign.	0.783	0.678	0.683	0.185	0.634	0.809	0	0.379
Water	corr.	–0.755**	–0.557*	–0.720**	0.331	0.052	–0.347	0.255	1
	sign.	0.002	0.039	0.004	0.247	0.860	0.224	0.379	0

corr. – significant *at 0.05 level (2-tailed) and **at 0.01 level (2-tailed)

sign. = significance; EBC = EBC colour units; MCPD_F = free 3-MCPD; MCPD_B = bound 3-MCPD

Table 6. Content of free and bound 3-MCPD in roasted malts

Sample/time of roasting	Free 3-MCPD		Free 3-MCPDn (μg/kg)	Bound 3-MCPD		
	μg/kg	RSD		μg/kg fat	μg/kg	RSD
A/100 min	15.0	0.6	< 9	14 000	236	0.8
B/115 min	16.5	0.9	< 9	26 500	429	0.3
C/130 min	16.8	2.6	< 9	34 100	545	0.4
D/145 min	19.6	1.0	< 9	39 700	631	0.2

3-MCPDn = normalisation to 40% dry matter content; Free 3-MCPD) = 9 μg/kg; Bound 3-MCPD) = 300 μg/kg fat; RSD = relative standard deviation (%)

Table 7. pH values and contents of fat, glycerol, chlorides and water in roasted malts

Sample/time of roasting	EBC unit	pH	Fat		Glycerol		Chlorides		Water	
			%	RSD	%	RSD	%	RSD	%	RSD
A/100 min	202	5.0	1.7	0.9	0.028	6.5	0.06	0.58	4.6	0.5
B/115 min	439	4.8	1.6	0.9	0.033	2.1	0.07	1.48	4.5	0.3
C/130 min	775	4.9	1.6	1.4	0.036	1.7	0.07	1.13	4.3	0.1
D/145 min	1280	4.8	1.6	0.7	0.048	0.1	0.07	3.06	4.7	0.6

In another set of experiments, we analysed a series of 4 malts obtained by roasting the raw material for different time intervals ranging from 100 to 145 min (Tables 6 and 7). As can be seen, the level of free 3-MCPD increased from 15 μg/kg to almost 20 μg/kg with the time of roasting, nevertheless, the corresponding values calculated after normalisation to 40% dry matter content were lover than the LOQ. Similarly, the bound 3-MCPD level increased from 236 μg/kg to 631 μg/kg and exceeded the free 3-MCPD levels 16 to 32 times. As could be expected, the EBC units increased from 202 to 1280, similarly as the content of glycerol which increased from 0.028% to 0.048% with prolonged roasting. The amounts of the free 3-MCPD positively correlated with the EBC units at the 0.05 level (Pearson correlation = 0.972). The Pearson correlation between the bound 3-MCPD levels and EBC units was less significant being 0.941. On the other hand, the amounts of fat, chlorides, and water remained almost constant.

CONCLUSIONS

Malting is considered a low risk process, however, 3-MCPD and especially its bound form represent a potential hazard. Luckily, the free and the bound 3-MCPD occur in higher amounts only in high colour malts and roasted barley, i.e. in products exposed to very high temperatures and a long time application during the roasting operations. This contamination can be minimised by choosing proper conditions for the roasting processes. Furthermore, the typical dilution of malts in foodstuffs ranges from 1:10 (i.e. 1 kg malt per 10 kg product) to 1:100.

References

Barlianto H., Maier H.G. (1995): Acids in chicory roots and malt. II. Determination of acids derived from saccharides. Zeitschrift für Lebensmittel-Untersuchung- und Forschung, **200**: 273–278.

Belitz H.D., Grosch W., Schieberle P. (2004): Food Chemistry. 3rd Rev. Ed. Springer, Berlin.

Cerbulis J., Parks O.W., Liu R.H., Piotrowski E.G., Farrell H.M., Jr. (1984): Occurrence of diesters of 3-chloro-1,2-propanediol in the neutral lipid fraction of goat's milk. Journal of Agricultural and Food Chemistry, **32**: 474–476.

Crews C., Brereton P., Davies A. (2001): Effect of domestic cooking on the levels of 3-monochloropropane-1,2-diol in food. Food Additives & Contaminants, **18**: 271–280.

Crews C., Hough P., Brereton P., Harvey D., Macarthur R., Matthews W. (2002): Survey of

3-monochloropropane-1,2-diol (3-MCPD) in selected food groups, 1999–2000. Food Additives & Contaminants, **19**: 22–27.

Davídek J., Velíšek J., Kubelka V., Janíček G. (1982): New chlorine-containing compounds in protein hydrolysates. In: Baltes W., Czedik-Eysenberg P.B., Pfannhauser W. (eds): Recent Developments in Food Analysis. Proceedings Euro Food Chem I. Vienna, Austria, 17–20 Feb, 1981, Weinheim, Deerfield Beach, Florida: 322–325.

Divinová V., Svejkovská B., Doležal M., Velíšek J. (2004a): Determination of free and bound 3-chloropropane-1,2-diol by gas chromatography with mass spectrometric detection using deuterated 3-chloropropane-1,2-diol as internal standard. Czech Journal of Food Sciences, **22**: 182–189.

Divinová V., Svejkovská B., Novotný O., Velíšek J. (2004b): Survey of 3-chloropropane-1,2-diol and its precursors in foods in the Czech Republic. Czech Journal of Food Sciences, Special Issue, **22**: 230–234.

Doležal M., Chaloupská M., Divinová V., Svejkovská B., Velíšek J. (2005): Occurrence of 3-chloropropane-1,2-diol and its esters in coffee. European Food Research & Technology, **221**: 221–225.

EC 2001: European Commission Regulation No. 466/2001. Setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities L77/1, 16 March, Luxembourg: Office for Official Publications of the European Communities.

Hamlet C.G., Sadd P.A. (2004): Chloropropanols and their esters in cereal products. Czech Journal of Food Sciences, Special Issue, **22**: 229–262.

Hamlet C.G., Jayaratne S.M., Matthews W. (2002): 3-Monochloropropane-1,2-diol (3-MCPD) in food ingredients from UK food producers and ingredient suppliers. Food Additives & Contaminants, **19**: 15–21.

Hamlet C.G., Sadd P.A., Crews C., Velíšek J., Baxter D.E. (2002): Occurrence of 3-chloro-propane-1,2-diol (3-MCPD) and related compounds in foods: a review. Food Additives & Contaminants, **19**: 619–631.

JECFA (2001): Joint FAO/WHO Expert Committee on Food Additives. In: 57th Meeting, Rome, 5–14 June 2001.

Kurzrock T., Speer K. (2005): Determination of 3-monochloropropane-1,2-diol in coffee surrogates. In: Eklund T., Schwarz M., Steinhart H., Thier H.-P., Winterhalter P. (eds): Proceedings of the EURO FOOD CHEM XIII Conference: Macromolecules and their degradation products in food – physiological, analytical and technological aspects. Volume 2. Geselschaft Deutscher Chemiker e.V., Hamburg: 479–482.

MAGB (2003): The MAGB HACCP Guide for Malting. Version 2.0, July 4th 2003, accessed as www.ukmalt.com.

SCF (2001): Opinion of the Scientific Committee on food on 3-monochloro-propane-1,2-diol (3-MCPD) updating the SCF opinion of 1994. Adopted on 30 May 2001.

Svejkovská B., Novotný O., Divinová V., Réblová Z., Doležal M., Velíšek J. (2004). Esters of 3-chloropropane-1,2-diol in foodstuffs. Czech Journal of Food Sciences, **22**: 190–196.

Velíšek J., Davídek J., Kubelka V., Janíček G., Svobodová Z., Šimicová Z. (1980): New chlorine-containing organic compounds in protein hydrolysates. Journal of Agricultural and Food Chemistry, **28**: 1142–1144.

Velíšek J., Doležal M., Crews C., Dvořák T. (2002): Optical isomers of chloropropanediols: mechanisms of their formation and decomposition in protein hydrolysates. Czech Journal of Food Sciences, **20**: 161–170.

Velíšek J., Calta P., Crews C., Hasnip S., Doležal M. (2003): 3-Chloropropane-1,2-diol in models simulating processed foods: precursors and agents causing its decomposition. Czech Journal of Food Sciences, **21**: 153–161.

Zelinková Z., Svejkovská B., Velíšek J., Doležal M. (2006): Fatty acids esters of 3-chloropropane-1,2-diol in edible oils. Food Additives & Contaminants, 21: submited.

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