Esters of 3-Chloropropane-1,2-Diol in Foodstuffs

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Abstract

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We provide here for the first time the evidence that 3-chloropropane-1,2-diol (3-MCPD) occurs in foodstuffs in its free form and also in the form of esters with higher fatty acids. These esters represent a new class of food contaminants as 3-MCPD may be released *in vivo* by a lipase-catalysed hydrolysis reaction. We analysed 20 samples of selected retail food products for their free and bound 3-MCPD content. All samples contained free 3-MCPD at approximately 9.6–82.7 μg/kg food (3 replications, RSD = 0.4–7.0%). The levels of bound 3-MCPD (monoesters and diesters of 3-MCPD with higher fatty acids) found in the foodstuffs analysed varied between the LOD (1.1 mg per kg of fat) and 36.8 mg/kg fat with RSD = 0.3–3.3%. Five foodstuffs of plant origin processed at high temperatures contained elevated levels of bound 3-MCPD (0.14–6.10 mg/kg). A high level of bound 3-MCPD (0.28 mg/kg) was also found in a sample of pickled fish. Some variables potentially influencing the levels of either free or bound 3-MCPD in foodstuffs were determined (pH, water, chlorides, glycerol, fat and its components) and their significance was discussed.

Keywords: 3-chloropropane-1,2-diol (3-MCPD); chloropropanediols; chlorolipids; 3-MCPD esters; 3-MCPD monoesters; 3-MCPD diesters

3-Chloropropane-1,2-diol (3-MCPD) is a food borne contaminant that was identified in acid-hydrolysed vegetable protein (acid-HVP) in 1981 (Davídek *et al*. 1982) and recently showed to be a racemic mixture of (*R*)- and (*S*)-enantiomers (Velíšek *et al*. 2002). 3-MCPD was extensively monitored in acid-HVP and soy sauces because of its suspected carcinogenicity (MacArthur *et al*. 2000; Jin *et al*. 2001; Crews *et al*. 2003; Nyman *et al*. 2003; Divinová *et al*. 2004a, b). In view of 3-MCPD toxicity, a regulatory limit of 0.02 mg/kg, based on a 40% dry matter content, was adopted for 3-MCPD in soy sauce and acid-HVP and came into force in the European Union in 2002 (European Commission 2001). During the last few years, several other countries established maximum levels for 3-MCPD.

Recent studies revealed that elevated levels of 3-MCPD can occur not even in soy sauces and related products but also in a wide range of foodstuffs (e.g. in bakery products, cakes, malt, meat and dairy products) formulated without acid-HVP (Crews *et al*. 2001, 2002; Hamlet *et al*. 2002a,b; Breitling-Utzmann *et al*. 2003). Domestic cooking procedures including toasting, microwaving, and grilling were shown to produce elevated 3-MCPD levels in some foods, especially in toasts (Crews *et al*. 2001). At the moment there are no limits for any other food product but acid-HVP and soy sauce.

The question about the occurrence of the bound forms of 3-MCPD in acid-HVP was first raised by workers at the Institute of Chemical Technology in Prague. Fatty acid esters of 3-MCPD were

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identified in model mixtures of hydrochloric acid with triacylglycerols, phospholipids, soybean oil, and lipids isolated from soybean meal and wheat gluten (Davídek *et al*. 1980). Esters of 3-MCPD with palmitic, stearic, oleic, linoleic, and several minor fatty acids were later found also in unripe acid-HVP and waste humins (Velíšek 1989; Velíšek & Ledahudcová 1993). The amount of esters of 3-MCPD (diesters and monoesters of higher fatty acids) in unripe acid-HVP was 39 mg/kg, i.e. 0.004% (w/w). These esters were removed during filtration of the ripe hydrolysate to such extent that only traces were found in the final commercial product. However, the waste humins contained 7 to 9 times higher levels of these esters in comparison with the unripe hydrolysate (diesters of 3-MCPD: 35–1890 mg/kg, monoesters of 3-MCPD: 4–205 mg/kg).

Fatty acid esters of 3-MCPD were also found in Spanish cooking rape seed oils adulterated with aniline, then refined with hydrochloric acid and mixed with edible oil (Gardner *et al*. 1983). Diesters and monoesters of C16 and C18 fatty acid were identified in 3 samples of this oil having the highest levels of chlorine. The sample containing chlorine at the level at 440 mg/kg (0.044% w/w) contained diesters of 3-MCPD at the level of 3800 mg/kg (0.38% w/w).

Low levels of 3-MCPD diacyl esters (< 1% of total neutral lipids, total fat 4.5%) were found in the neutral lipid fraction of fresh goat milk by Cerbulis *et al*. (1984). These diesters were found to be composed of molecular species containing C10–C18 fatty acids and to correspond closely in the carbon number to *sn*-1,2-diacylglycerol moieties of milk triacylglycerols (Kuksis *et al*. 1986). Their results first demonstrated the occurrence of this class of halolipids in a natural unprocessed food. However, it was not clear whether these compounds were dietary substances merely passed through the organism, or were synthesised from anthropogenic chlorine-containing compounds, or *de novo* synthesised from 3-MCPD in the mammary gland.

Esters of chloropropanols have not been identified in processed foods up to now. Nevertheless, they undoubtedly arise in processed foods as primary reaction products of lipids with sodium chloride (chloride ions). Their occurrence could be expected at least in the surface layers of foods processed at high temperatures, in foods having low water contents and high levels of sodium chloride, and in foods stored for long periods. Therefore, the current study aimed to report the results of analyses of a range of selected foods purchased from retail outlets in Prague in 2004 for their contents of 3-MCPD esters. The aim was also to develop an understanding of the major factors affecting the formation of 3-MCPD esters and free 3-MCPD in foodstuffs.

MATERIAL AND METHODS

Analysed foods. Malt samples were produced by Obchodní sladovny, a. s. (Prostějov, CZ), all other food samples were purchased from retail outlets in Prague. The samples were stored according to the recommendations of the producers or analysed immediately.

Chemicals. The chemicals used were specified by Divinová *et al*. (2004a).

Determination of pH, water, chlorides, glycerol, fat and fat composition. The analytical methods previously described were employed in this work (Divinová *et al*. 2004a). The fat composition was determined by HPLC (Réblová 1998).

Determination of free and bound 3-MCPD. Free 3-MCPD was determined using capillary gas chromatography with mass spectrometric detection and deuterated 3-chloropropane-1,2-diol as the internal standard (Divinová *et al*. 2004b). 3-MCPD esters were isolated as fat, subjected to methanolysis, and the generated 3-MCPD was quantified using the same method.

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

RESULTS AND DISCUSSION

We analysed 20 samples of selected retail food products for their free and bound 3-MCPD contents (Table 1). The foodstuffs of plant and animal origin, processed at high temperatures or stored for a long period, were selected according to their contents of water, salt and fat, and according to their pH values. All samples contained free 3-MCPD at approximately 9.6–82.7 μg/kg food (3 replications, RSD = 0.4–7.0%). High levels exceeding 20 μg/kg food were found in some cheeses, dark malt, roasted peanuts, fermented salami, ham, grilled chicken, and pickled herring. After normalisation to 40% dry matter content, 3-MCPD levels in 6 samples (30%) were still above the European Union limit of 20 μg/kg adopted for soy sauces and acid-HVP.

Table 1. Levels of free and bound 3-MCPD in foodstuffs

Foodstuff	Free 3-MCPD		Free 3-MCPDn	Bound 3-MCPD		
	μg/kg	RSD	μg/kg	mg/kg fat	RSD	mg/kg
Pickled olives	13.93	1.0	23.70	< 3.30	–	< 0.28
Roasted coffee	16.15	4.3	6.56	< 3.30	–	< 0.33
Roasted peanuts	22.10	2.0	9.05	nd	–	nd
Light malt (Pilsener type)	9.55	5.2	4.00	< 3.30	–	< 0.05
Dark malt	27.90	2.6	11.34	36.51	1.7	0.58
Crisp bread	11.05	2.0	4.60	6.31	1.3	0.42
Salty crackers	10.72	2.5	4.44	12.52	0.3	0.14
Doughnuts	16.58	2.3	8.53	3.95	0.9	1.21
Potato crisps	14.48	2.4	5.91	< 3.30	–	< 1.21
French fries	15.41	1.3	9.10	36.77	2.42	6.10
Soft salami	14.03	3.8	12.73	< 3.30	–	< 0.93
Fermented salami	47.63	1.0	24.75	< 3.30	–	< 1.49
Smoked ham	23.18	1.8	15.65	< 3.30	–	< 1.62
Bologna ham	26.24	0.7	16.63	nd	–	nd
Grilled chicken	26.04	2.2	24.07	< 3.30	–	< 0.43
Smoked mackerel	18.62	1.4	19.27	< 3.30	–	< 0.53
Pickled herring	28.19	7.0	38.86	3.57	3.3	0.28
Parmesan cheese	82.67	0.4	43.06	nd	–	nd
Processed cheese	29.83	2.8	26.53	< 3.30	–	< 0.77
Feta cheese	12.98	1.2	12.39	< 3.30	–	< 0.58

RSD = relative standard deviation (%); 3-MCPDn = normalised to 40% dry matter content; nd = below LOD (not detected); < 3.30 mg/kg fat = below LOQ

Up to now, the esters of chloropropanols (a group of the recognised precursors of 3-MCPD in acid-HVP) have not been identified in processed foods. Undoubtedly, they arise in processed foods as primary reaction products of lipids (e.g. triacylglycerols or partial acylglycerols) with salt (chloride ions). The reaction proceeds either *via* direct nucleophilic substitution of acyl and/or hydroxyl groups by chloride ions (March 1992), or by opening the cyclic acyloxonium ion intermediates of diacylglycerols with chloride ions (Collier *et al*. 1991). The reaction is promoted by high contents of fat and chlorides, low water activity, and low pH. Diesters and monoesters of 3-MCPD occurring in acid-HVP contained (*R*)- and (*S*)-enantiomers of 3-MCPD in the ratio of 1:1 (Velíšek *et al*. 2002). Stereospecific analysis of 3-MCPD diesters found in goat milk (Myher *et al*. 1986) also showed that both 3-MCPD isomers were present as a racemate. Based on these results and also on our preliminary experiments, it can be concluded that esters of 3-MCPD are present in processed foodstuffs as racemic mixtures of the corresponding enantiomers.

The levels of the bound 3-MCPD (monoesters and diesters of 3-MCPD with higher fatty acids) found in the foodstuffs analysed varied between the LOD (1.1 mg/kg fat) and 36.8 mg/kg fat with RSD = 0.3–3.3%. Five foodstuffs of plant origin processed at high temperatures (i.e. dark malt, crisp bread, salty crackers, doughnuts and French fries) contained elevated levels of 3-MCPD esters (0.14–6.10 mg/kg). A high level of 3-MCPD esters (0.28 mg/kg) was also found in pickled herring. Most striking is that the bound 3-MCPD contents exceeded the free 3-MCPD levels at least 5 to 396 times. The Joint WHO/FAO Expert Committee on Food

Additives (JECFA 2001) has recommended a provisional maximum tolerable daily intake (TDI) of 2 μg/kg body weight for 3-MCPD. For example, if our doughnut with a bound 3-MCPD content of 1.21 mg/kg is eaten by an adult weighing 70 kg and the esters are totally hydrolysed in the body by enzymes, his or her TDI of 140 μg is reached after the consumption of 116 g doughnut, disregarding the free 3-MCPD level and the 3-MCPD level in other foodstuffs. But will it really harm us? Is every one at risk?

Identification of the individual esters of 3-MCPD has also been done. For example, in salty crackers, palmitate and oleate were the dominant monoesters of 3-MCPD. The level of diesters of 3-MCPD was a little higher than that of monoesters and the major components were diesters containing palmitate and one of C18 fatty acids (oleic, stearic, linoleic).

The pH values and the amounts of water, chlorides and fat found in our foodstuffs are summarised in Table 2 and their basic fat composition is given in Table 3. As it can be seen, the pH value of the respective foodstuff certainly plays an important role as the only foodstuff of animal origin with a relatively high content of 3-MCPD esters, pickled herring, has the lowest pH value of all the foodstuffs analysed. The foodstuffs with high contents of bound 3-MCPD contained 0.09–0.29% salt. The only exception was salty crackers with 1.8% salt. On the contrary, foodstuffs with comparable or higher salt contents (e.g. light malt and grilled chicken) had lower levels of 3-MCPD esters. The foodstuffs with the highest content of 3-MCPD esters had the fat content in the range of 1.1–30.7% while smoked ham with 49.0% fat contained a significantly lower amount of 3-MCPD esters. The

Table 2. pH and levels of water, chlorides, glycerol and fat in foodstuffs

Foodstuff	pH	Water		Chlorides		Glycerol		Fat	
		% (w/w)	RSD	% (w/w)	RSD	% (w/w)	RSD	% (w/w)	RSD
Pickled olives	3.70	76.49	0.0	3.68	3.1	0.011	8.5	8.60	0.8
Roasted coffee	5.85	1.56	0.5	0.06	12.9	0.026	0.6	10.00	0.2
Roasted peanuts	6.55	2.32	3.7	0.39	1.1	0.001	12.6	48.80	0.0
Light malt (Pilsener type)	5.90	4.45	7.4	0.14	6.4	0.002	5.5	1.60	1.0
Dark malt	4.55	1.60	0.4	0.09	8.3	0.019	0.4	1.60	0.0
Crisp bread	6.15	3.93	1.1	0.29	14.6	0.004	7.6	6.60	1.1
Salty crackers	8.28	3.36	0.2	1.80	7.5	0.009	2.4	1.10	5.9
Doughnuts	6.60	22.23	0.7	0.20	10.9	0.314	3.6	30.70	4.9
Potato crisps	5.98	1.93	2.6	0.80	3.5	tr	–	36.80	0.5
French fries	5.78	32.24	0.4	0.13	0.0	0.005	14.1	16.60	0.9
Soft salami	6.68	55.92	0.2	1.76	2.4	0.039	5.4	28.10	0.2
Fermented salami	6.13	23.03	0.2	4.62	0.8	0.576	3.7	45.20	0.9
Smoked ham	6.03	40.77	0.2	3.01	0.5	0.014	7.3	49.00	1.2
Bologna ham	5.98	36.89	0.3	4.66	1.1	0.577	4.9	23.40	1.5
Grilled chicken	6.68	56.72	0.2	0.65	6.5	0.018	5.8	13.10	4.3
Smoked mackerel	6.60	61.34	0.5	1.73	3.3	0.056	6.7	16.10	2.2
Pickled herring	3.90	70.98	0.1	0.12	1.4	0.004	16.4	7.70	0.3
Parmesan cheese	5.50	23.21	0.6	1.89	0.8	1.835	1.1	29.70	0.5
Processed cheese	6.45	55.03	0.0	0.18	0.0	0.028	10.2	23.30	0.9
Feta cheese	4.88	58.09	0.0	3.84	0.2	0.035	2.1	17.60	0.8

RSD = relative standard deviation (%); tr = traces

Table 3. Fat composition of foodstuffs

Foodstuff	Relative composition (%)									
	Polymers	RSD	TAG	RSD	DAG	RSD	MAG	RSD	FFA	RSD
Pickled olives	tr	–	95.41	0.5	tr	–	2.11	11.9	2.48	7.9
Roasted coffee	tr	–	80.10	0.6	7.34	3.5	10.01	0.7	2.55	5.9
Roasted peanuts	0.15	15.7	96.49	0.2	2.51	7.5	0.09	89.6	0.77	7.2
Light malt (Pilsener type)	0.57	20.4	78.89	0.3	12.13	2.0	tr	–	8.41	2.9
Dark malt	4.14	18.9	68.60	1.5	19.85	1.1	1.90	7.3	5.51	6.8
Crisp bread	0.13	57.1	89.67	1.0	4.74	2.3	3.01	11.9	2.45	15.3
Salty crackers	0.26	8.1	84.90	0.4	12.67	3.4	tr	–	2.17	13.2
Doughnuts	3.40	4.4	87.10	0.3	8.78	0.5	0.30	35.7	0.42	4.1
Potato crisps	1.42	13.9	89.08	0.3	7.73	1.1	tr	–	1.77	10.2
French fries	0.95	8.2	82.50	1.2	4.35	11.9	tr	–	12.20	4.0
Soft salami	0.19	15.8	98.70	0.4	tr	–	tr	–	1.11	36.3
Fermented salami	tr	–	86.70	1.2	3.63	7.0	tr	–	9.67	8.2
Smoked ham	tr	–	99.11	0.1	tr	–	tr	–	0.89	15.2
Bologna ham	0.61	11.7	81.94	0.4	6.00	3.3	tr	–	11.46	3.7
Grilled chicken	tr	–	96.83	0.7	tr	–	tr	–	3.17	21.6
Smoked mackerel	tr	–	95.65	0.2	tr	–	tr	–	4.35	3.5
Pickled herring	0.21	20.1	78.66	0.3	5.15	5.4	3.34	8.2	12.65	2.0
Parmesan cheese	tr	–	51.84	1.0	44.05	0.7	tr	–	4.10	11.7
Processed cheese	0.13	173.2	46.40	1.0	52.29	1.0	tr	–	1.18	12.7
Feta cheese	0.02	173.2	51.38	1.2	47.25	1.2	tr	–	1.35	7.7

RSD = relative standard deviation (%); TAG = triacylglycerols, DAG = diacylglycerols; MAG = monoacylglycerols; FFA = free fatty acids; tr = traces

correlation between the level of 3-MCPD esters and any of these variables estimated by correlation analysis and principal components analysis was not significant. Changes of 3-MCPD esters are complex as their formation is influenced by several factors and, furthermore, they undergo hydrolytic reactions during processing and storage. Some other variables such as the temperature and the duration of the thermal processes and the time of storage evidently play a crucial role, too. For example, the significance of some of these variables can be seen if the level of bound 3-MCPD is compared for light and dark malt.

CONCLUSION

Our findings indicate that 3-MCPD occurs in foodstuffs either as a free compound or bound to fatty acids. We provide here for the first time the evidence that 3-MCPD occurs in foodstuffs also in the form of esters with higher fatty acids. Esters of 3-MCPD thus represent a new class of food contaminants, a bound form of 3-MCPD, which may be released *in vivo* by a lipase-catalysed hydrolysis reaction. In many cases their amount exceeds that of the free 3-MCPD.

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Souhrn

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Výzkum poprvé prokázal, že 3-chloropropan-1,2-diol (3-MCPD) se vyskytuje v potravinách nejen jako volná látka, ale i ve formě esterů s vyššími mastnými kyselinami. Tyto estery představují novou skupinu potravinářských kontaminantů, neboť 3-MCPD by mohl být *in vivo* uvolněn reakcemi katalyzovanými lipasami. Analyzovali jsme 20 vzorků vybraných potravinářských produktů na obsah volného a vázaného 3-MCPD. Všechny tyto vzorky obsahovaly volný 3-MCPD zhruba v mezích 9,6–82,7 μg/kg potraviny (3 opakování, relativní směrodatná odchylka – RSD = 0,4–7,0 %). Hladiny vázaného 3-MCPD (monoestery a diestery 3-MCPD) nalezené v analyzovaných potravinách se pohybovaly mezi limitem detekce (1,1 mg/kg tuku) a 36,8 mg/kg tuku (RDS = 0,3–3,3 %). Pět vyorků analyzovaných potravin rostlinného původu zpracovaných při vyšších teplotách mělo zvýšené hladiny esterů 3-MCPD (0,14–6,10 mg/kg). Vyšší hladiny esterů 3-MCPD (0,28 mg/kg) byly též nalezeny ve vzorku nakládaných ryb. Byly stanoveny některé proměnné potenciálně ovlivňující množství 3-MCPD nebo jeho esterů (pH, obsah vody, chloridů, glycerolu, tuku, složení tuku) a byl diskutován jejich význam.

Klíčová slova: 3-chloropropan-1,2-diol (3-MCPD); chloropropandioly; chlorované lipidy; estery 3-MCPD; monoestery 3-MCPD; diestery 3-MCPD

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