Chloropropanols and their Esters in Cereal Products

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Abstract: Chloropropanols, in particular monochloropropanediols (MCPDs) are food contaminants that can form in the high temperature crust region of cereal products. Previous studies have indicated that MCPD-esters may be formed from a reaction between chloride ions and some lipids, although, to date, these compounds have not been reported in cereal products. MCPD-esters were extracted into an organic solvent and cleaned up by a preparative thin-layer chromatography procedure. Sample extracts were analysed using gas chromatography/mass spectrometry (GC/MS) and the data compared with that obtained from a reference MCPD-ester, prepared by a custom synthesis. A faster method using enzyme hydrolysis and GC/MS showed that MCPD-esters could also be determined as the amount of 3-MCPD released by a commercial lipase from *Aspergillus oryzae*. Under the conditions of enzyme hydrolysis, model system studies indicated that low levels of MCPDs might also be generated by a lipase catalysed reaction between short chain triacylglycerols and chloride ions.

Keywords: cereal products; chloropropanol-esters; chloropropanols; lipase; 3-MCPD-esters

INTRODUCTION

Chloropropanols are food borne contaminants that can occur in a wide range of foodstuffs [1]. Among this group, 3-chloropropane-1,2-diol (3-MCPD) has been extensively monitored because of its suspected carcinogenicity [2]. It has been shown that specific glycero-lipids can react with chloride ions from added cooking salt to generate MCPDs in baked dough [3]. This study showed that 3-MCPD-esters might be generated as stable intermediates or by-products of the formation reaction from mono- and diacylglycerol precursors (Figure 1). It is known that mono- and diesters of MCPDs can be generated by the treatment of triacylglycerols with concentrated hydrochloric acid [4, 5]. However, the origin of these compounds when found in goats milk and milk fat [6–8] is not known. MCPD-esters represent a bound form of 3-MCPD that could be released *in vivo* by a lipase-catalysed hydrolysis reaction.

EXPERIMENTAL

Preparation of 3-MCPD-dipalmitate reference compound. Hexadecanoic acid 2-chloro-1-hexadecanoyloxymethyl-ethyl ester, prepared according to procedure of Hartman [9], was recrystallised twice from petroleum ether 40–60 (purity 98% by GC/MS and LC/MS).

Preparation of toast and bread samples for analysis. Sliced white bread was toasted (single side) in duplicate using a radiant electric grill. Temperature was recorded using fine type k thermocouples

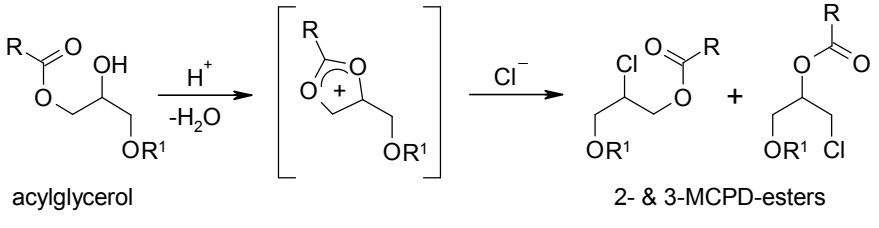

Figure 1. Possible formation mechanism for MCPD-esters from mono- and diacylglycerol precursors in cereal products: R^1 = H or COR

(Labfacility, UK; 7.6×10^{-3} mm diameter) placed in the outer (1 mm) surface layer. Samples of bread top crust were obtained from commercial white bread using a domestic cheese grater. All samples were dried to approximately 10% moisture and homogenised to a fine powder.

Isolation and quantification of MCPD-esters. Air-dried samples were extracted by shaking overnight with ethyl acetate. MCPD-esters were isolated by preparative TLC according to the procedure of Davídek *et al*. [4]. Sample extracts were analysed by GC/MS using a 10 m × 0.25 mm i.d. × 0.1 μm DB1HT capillary column. Mono-, and diesters of MCPDs were identified using reference mass spectral data [*ibid*]: all MCPD-esters were quantified as 3-MCPD-dipalmitate using 5-α-cholestane as an internal standard.

MCPD-esters by enzyme hydrolysis. Samples (4 g) were dispersed in 30 ml of 0.1 M phosphate buffer (pH 7.0). LIPOPAN 50 BG, 50 mg (Novozymes; activity, 50 kilo lipase units per gram), was added and the sample incubated at 23°C ± 1°C for 24 h. MCPD-esters were determined from MCPD released by lipase: MCPDs were analysed as the heptafluorobutyryl esters by GC/MS according the procedure of Hamlet [10].

Generation of MCPDs from reference triacylglycerols. High-purity reference triacylglycerols (Sigma, UK; 50 mg) were pre-adsorbed onto 710 μm glass beads in a 50 ml centrifuge tube. Sodium chloride (50 mg), LIPOPAN 50 BG (50 mg) and phosphate buffer (20 ml of 0.1M, pH 7.0) were added, and the tubes were incubated (gentle agitation) at 23 ± 1°C.

RESULTS AND DISCUSSION

Bread was toasted to generate MCPDs and hence MCPD-esters. Mono- and diesters of 3-MCPD, corresponding to combinations of 16:0, 18:0, 18:1 and 18:2 fatty-acyl groups, were identified and quantified in prepared sample extracts using diagnostic ions from their reference mass spectra [4], i.e. $[M\text{-}RCO_2H]^+$ and $[M\text{-}RCO_2]^+$, respectively. The values given in Table 1 represent best estimates as not all components of the mass chromatograms could be identified.

Using enzyme hydrolysis and GC/MS, bound MCPDs could be determined as MCPD released from MCPD-esters by a commercial lipase from *Aspergillus oryzae* (Figure 2). The recovery of 3-MCPD from 3-MCPD-dipalmitate reference standard, adsorbed onto fine glass beads at the equivalent of 250 and 750 μg/kg (sample basis), was 106% and 91% in less than 24 h. The repeatability of the method, expressed as a coefficient of variation, was 3.7%.

To compare methods, a sample of the toasted bread of known MCPD-ester levels (Table 1) was prepared by the method of enzyme hydrolysis. The level of bound MCPDs released (Table 2) in the toasted bread, and hence by calculation MCPD-esters, showed reasonable agreement with that determined by direct analysis (Table 1).

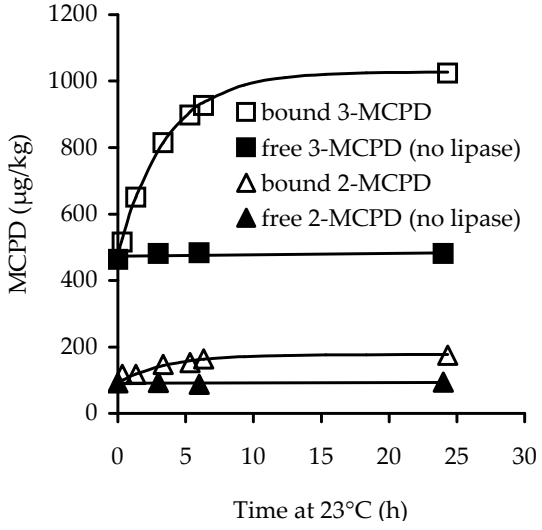

Figure 2. Release of bound MCPDs in bread crust by enzyme hydrolysis

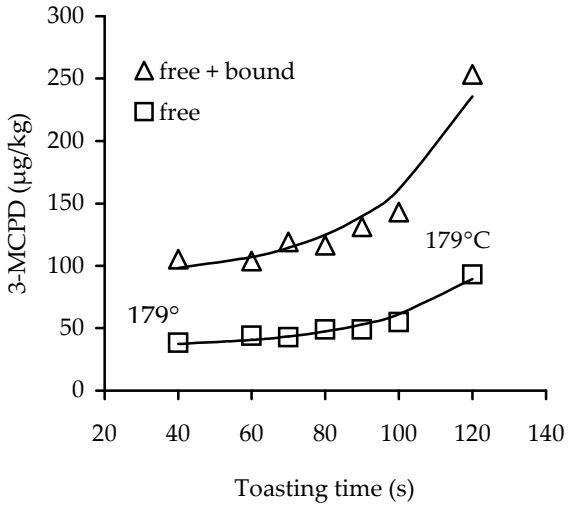

Figure 3. Effect of toasting time and temperature on free and bound MCPDs

Table 1. Analysis of MCPD-esters in toasted bread by solvent extraction and GC/MS

Class of 3-MCPD-ester	3-MCPD-esters[a] (μg/kg)	Bound 3-MCPD[b] (μg/kg)
Monoesters	289	91
Diesters	312	59
Total	**601**	**150**

[a]measured, [b]calculated: mole equivalents expressed as 3-MCPD

Analysis of white bread showed that the highest levels of both free and bound MCPDs were found in the hottest region of the loaf, i.e. the crust (Table 2). The presence of bound MCPDs in breadcrumb as well suggests that MCPD-esters can form at relatively low temperatures, i.e. < 96°C, possibly *via* the mechanism given in Figure 1.

To determine the effect of temperature on the production of free and bound MCPDs, samples of sliced white bread were toasted over 40–120 s. Figure 3 shows that the levels of free & bound MCPDs increased together with increasing toasting time/temperature. Although the correlation between the free and the bound levels of 3-MCPD generated is not yet understood (Figure 4), it may indicate a common formation mechanism.

The possible formation of MCPDs by a lipase-catalysed transesterification of triacylglycerols has recently been reported [11]. To investigate this, high purity reference triacylglycerols of increasing fatty acid chain lengths (4:0, 13:0, 16:0 and 18:0) were incubated with lipase.

Both 3-MCPD and 2-MCPD were formed from tributyrin only (isomer ratios ≈ 3:1) and the rate of generation was the same with and without added chloride over the first 48 h (Figure 5). It is not yet known whether these results are due to hydrolysis

Table 2. Analysis of MCPD-esters (in μg/kg) in cereal products by the method of enzyme hydrolyis

Sample	Bound MCPDs[a]		MCPD-esters[b]		Free MCPDs[a]	
	3-MCPD	2-MCPD	3-MCPD	2-MCPD	3-MCPD	2-MCPD
White flour	< 0.5	< 0.5	< 2.7	< 2.7	< 0.5	< 0.5
White bread	6.7	0.9	35	4.6	6.1	1.0
crumb	4.9	0.9	26	4.5	<0.5	<0.5
crust	547	83	2916	442	477	92
toast	**160**	59	**853**	315	93	17
DATEM	66	18	352	96	54	6.2

[a]measured, [b]calculated: mole equivalents expressed as MCPD-dipalmitate

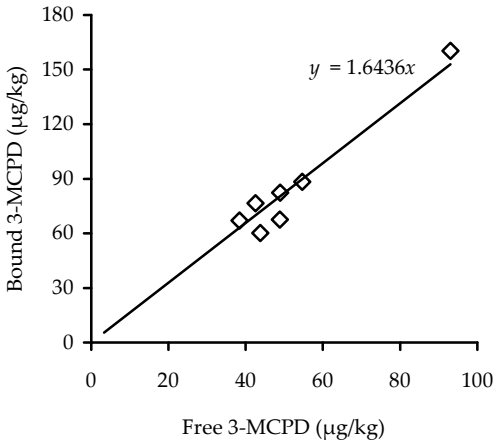

Figure 4. Correlation betwee free and bound 3-MCPD

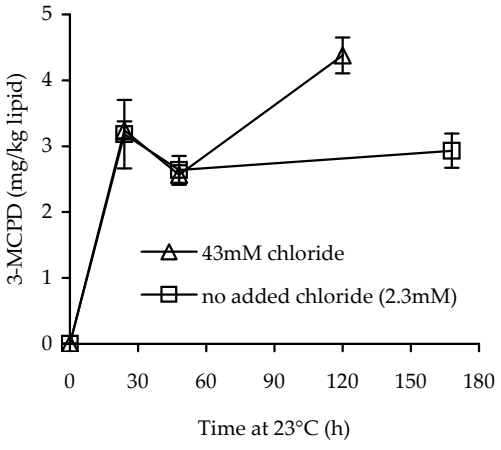

Figure 5. Generation of 3-MCPD (mean ± 2 SD) from tributyrin

of residual MCPD-esters in tributyrin or lipase catalysed formation from the triacylglycerol. In the case of the latter, the data suggests that the lipase may be specific for triacylglycerols with short-chain fatty acids, i.e. less than 13:0. This mechanism could account for the levels of MCPDs found in some dairy products [12] that are known to contain significant levels of short-chain triacylglycerols. Although the lipase used in this study showed hydrolytic activity on medium and long-chain triacylglycerols e.g. 16:0 and 18:0, consistent with that reported elsewhere [13], it is known that *Aspergillus oryzae* produces at least two other lipolytic enzymes [*ibid*.]: the first shows high activity on short-chain triacylglycerols while the second preferentially hydrolyses mono- and diacylglycerols. The relative proportions of each of these enzymes could be significant in this instance.

CONCLUSIONS

MCPD-esters are present in baked cereal products and MCPDs can be released from them by a lipase-catalysed hydrolysis using e.g. a lipase from *Aspergillus oryzae*. MCPDs can also be generated by the action of a lipase on a short-chain reference triacylglycerol and this may account for prior results on dairy products [12].

References

[1] Hamlet C.G., Jayaratne S.M., Matthews W. (2002): Food Addit. Contam., **19**: 619.

[2] Lynch B.S., Bryant D.W., Hook G.J., Nestmann E.R., Munro I.C. (1998): Int. J. Toxicol., **17**: 47.

[3] Hamlet C.G., Sadd P.A., Gray D.A. (2004): J. Agric. Food Chem., **52**: 2067.

[4] Davídek J., Velíšek J., Kubelka V., Janíček G., Šimicová Z. (1980): Z. Lebensm.-Unters. Forsch., **171**: 14.

[5] Gardner A., Yurawecz M., Cunningham W., Diachenko G.W., Mazzola E., Brumley W. (1983): Bull. Environ. Contam. Toxicol., **31**: 625.

[6] Cerbulis J., Parks O., Liu R., Piotrowski G., Farrell H. (1984): J. Agric. Food Chem., **32**: 474.

[7] Kuksis A., Marai L., Myher J.J., Cerbulis J., Farrell H.M., Jr. (1986): Lipids, **21**: 183.

[8] Myher J.J., Kuksis A., Marai L., Cerbulis J. (1986): Lipids, **21**: 309.

[9] Hartman L. (1957): J. Chem. Soc.: 3572

[10] Hamlet C.G. (1998): Food Addit. Contam., **15**: 451.

[11] Robert M.-C., Stadler R.H. (2003): In: Eklund T. *et al*. (eds): Proc. Euro Food Chem XII, KVCV, Brugge, Belgium, Vol. 2: 527.

[12]. Crews C., Hough P., Brereton P., Harvey D., Matthews W. (2002): Food Addit. Contam., **19**: 22.

[13] Toida J., Fukuzawa M., Kobayashi G., Ito K., Sekiguchi J. (2000): FEMS Microbiol. Lett., **189**: 159.