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Determination of chloropropanols in protein hydrolysates

C. A. van Bergen, P. D. Collier*, D. D. O. Cromie, R. A. Lucas, H. D. Preston and D. J. Sissons

Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford MK44 ILQ (UK)

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ABSTRACT

Protein hydrolysates, widely used as seasonings and savoury flavours, have been reported to contain traces of chloropropanols. Methods of analysis have now been developed for identification and determination of a full range of chloropropanols in protein hydrolysates and composite savoury food products. Based on capillary gas chromatography of heptafluorobutyrate derivatives with electron-capture and mass spectrometric detection, the methods show that the main chloropropanols found in traditionally produced hydrolysates are (in order of abundance) 3-chloro-1,2-propanediol, 2-chloro-1,3-propanediol, 1,3-dichloro-2-propanol and 2,3-dichloro-1-propanol. The limits of detection are 50–100 $\mu g\ kg^{-1}$ for the diols and 10 $\mu g\ kg^{-1}$ for the dichloropropanols.

INTRODUCTION

Protein hydrolysates are widely used as seasonings and ingredients in processed savoury food products. They are commonly produced by hydrochloric acid hydrolysis of proteinaceous by-products from edible oil or starch extraction, such as soy bean and rape seed meals and maize gluten. Studies by Velisek and co-workers [1–6] have demonstrated the presence in protein hydrolysates of several chloropropanols and their fatty acid esters, and their formation in model hydrolysis systems from lipids (both synthetic and residual lipids extracted from raw materials used in hydrolysate manufacture). The main chloropropanol detected by Velisek and co-workers in protein hydrolysates was 1,3-dichloro-2-propanol (1,3DCP), together with smaller amounts of 2,3-dichloro-1-propanol (2,3DCP) and 3-chloro-1-propanol [4,6]. Model system studies with lipids [3] and glycerol [2] strongly suggest that 3-chloro-1,2-propanediol (3MCPD) and 2-chloro-1,3-propanediol (2MCPD) may also be expected in commercial protein hydrolysates, but to date no methods for their determination or data on their levels in protein hydrolysates have been published.

We now report methods of analysis suitable for the confirmed determination of a range of chloropropanols in protein hydrolysates and derived food products. Based on capillary gas chromatography of heptafluorobutyrate derivatives with electron-capture (GC–ECD) and mass spectrometric detection (GC–MS), the methods permit the determination of 3MCPD, 2MCPD, 1,3DCP, 2,3DCP and three isomeric monochloropropanols.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade unless otherwise stated. Sodium chloride, sodium hydroxide and anhydrous sodium sulphate were obtained from BDH. Diethyl ether and ethyl acetate (HPLC grade) were supplied by Fisons and hexane (glass-distilled) by Rathburns. Extrelut 20 and Extrelut 3 columns and refill material were obtained from Merck, glycidol (2,3-epoxy-1-propanol) from Sigma and heptafluorobutyrylimidazole (HFBI) from Pierce.

Standards

1-Chloro-2-propanol, 2-chloro-1-propanol and *p*-dichlorobenzene (PDCB) internal standard were

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obtained from Aldrich. 3-Chloro-1-propanol, 1,3-dichloro-2-propanol (1,3DCP) and 2,3-dichloro-1-propanol (2,3DCP) were supplied by Pfalz and Bauer. 3-Chloro-1,2-propanediol (3MCPD) was obtained from Fluka. A small sample of NMR-pure 2-chloro-1,3-propanediol was provided by Dr. F. Ruf (Maizena, Heilbronn, Germany).

Calibration solutions

A stock mixed standard solution containing 1 g l^{-1} of each chloropropanol in diethyl ether or ethyl acetate as appropriate (see *Sample preparation*) was prepared fresh for each batch of samples to be analysed. Calibration solutions covering the range 0.02–100 mg l^{-1} were prepared by serial dilution of the stock solution with diethyl ether or ethyl acetate as appropriate. A working strength (50 mg l^{-1}) solution of PDCB was obtained by dilution of a stock solution with hexane. Stock PDCB solutions were prepared monthly (0.1 g of PDCB in 100 ml of hexane). All stock and working strength solutions were kept refrigerated (0–5°C).

Sample preparation

Liquid protein hydrolysate (20 g) or solid hydrolysate (8 g dissolved to 20 g with 5 *M* sodium chloride solution) was applied to an Extrelut 20 column and allowed to equilibrate for 15 min (note: viscous samples may be mixed with Extrelut refill material before it is packed into a column). Chloropropanols were recovered from Extrelut columns by one of the following methods.

For analysis of all the chloropropanols (mono- and diols) a two-stage extraction separating the less abundant monools from the more abundant diols and other interfering compounds proved necessary. The Extrelut 20 column was first eluted with hexane–diethyl ether (90:10, v/v) to collect 50 ml (extract 1) containing the monools, followed by diethyl ether to collect 250 ml (extract 2) containing the chloropropanediols.

For analysis only of the chloropropanediols, the Extrelut column was eluted with diethyl ether only to collect 250 ml (extract 3). An alternative, faster procedure for analysis of chloropropanediols consisted of adding 3 g of hydrolysate liquor to a small (2 g, 1 cm diameter) Extrelut 3 column, followed by equilibration, then elution with ethyl acetate to collect 25 ml (extract 4).

Derivatization

Extract 1 (5 ml), or extract 2, 3 or 4 (1 ml), or the calibration solution (1 ml), was pipetted into a 25-ml volumetric flask. PDCB working solution (1 ml) was added and the solution made up to 25 ml with hexane. HFBI (200 μl) was added and the solution was mixed and allowed to stand at ambient temperature (20°C) for 15 min with intermittent shaking.

The mixture was then transferred to a screw-capped vial containing distilled water (2 ml), shaken (1 min), the separated organic layer washed twice more and a 10-ml aliquot filtered through a 4-cm column of anhydrous sodium sulphate (4 g) before analysis by GLC–ECD. Dried derivatized solutions were found to be stable for up to 3 days under refrigeration. When necessary, limits of determination were improved by taking a larger aliquot of the appropriate Extrelut extract for derivatization. Ether extracts 2 and 3 must (when aliquots greater than 2 ml are taken) be blown dry under a gentle stream of nitrogen before derivatization, to overcome sporadic ether-based interference problems.

Analysis by GC–ECD

Heptafluorobutyrate esters of the chloropropanols were separated and determined by capillary GC–ECD using a Perkin-Elmer Model 8320, Varian Model 3400 or Philips PU 4550 gas chromatograph fitted with a splitless injection port and a 25 m × 0.2 mm I.D. fused-silica column of immobilized OV-1 (0.33 μm) (Hewlett-Packard, part no. 19091Z-102). Optimum operating conditions were as follows: injector temperature, 280°C (vent opened after 0.6 min); injection volume, 3 μl maximum; carrier gas, helium, 12 p.s.i.g.; temperature programme, 50°C held for 2 min, then increased to 85°C at 1°C min^{-1} and to 250°C at 20°C min^{-1}, held at 250°C for 20 min; detector temperature, 350°C (Perkin-Elmer Model 8320). Detector responses to derivatized chloropropanols and the PDCB internal standard vary with temperature in an instrument-dependent fashion. The detector should be operated within a temperature range over which the response is least affected by temperature. Retention time data are presented in Table I. Chloropropanol levels were calculated from the height of the appropriate peak relative to that of the PDCB internal standard.

Confirmation by GC–MS

Identities of both derivatized and underivatized chloropropanols were confirmed by GC–MS using a VG70 HSE double-focusing mass spectrometer linked to a Hewlett-Packard Model 5890A gas chromatograph and operating in the electron impact (EI) mode at a beam energy of 70 eV, an emission of 100 μA and a source temperature of 200°C.

Underivatized Extrelut extracts were separated on a 15-m DB Wax fused-silica capillary column (J & W Scientific) under the following conditions: injector temperature, 220°C (splitless mode); carrier gas, helium, flow-rate, 1 ml min^{-1}; temperature programme, 65°C, held for 1 min, then increased at 15°C min^{-1} to 200°C. Retention times were as follows [min (′) s (″)]: 1-chloro-2-propanol, 1′49″; 2-chloro-1-propanol, 2′06″; 3-chloro-1-propanol, 3′10″; 1,3DCP, 4′19″; 2,3DCP, 4′43″; 3MCPD, 5′53″; 2MCPD, 6′14″. Quantitative analysis was carried out by selected-ion recording (SIR) at a resolution of 1 in 5000, monitoring characteristic ions at *m/z* 79 and 81 (1-chloro-2-propanol and 1,3DCP), 62 and 64 (2-chloro-1-propanol, 2MCPD and 2,3DCP), 58 and 76 (3-chloro-1-propanol) and 61, 79 and 81 (3MCPD).

HFBI-derivatized extracts were separated on a 25-m BP1 fused-silica capillary column (SGE) programmed from 50 to 85°C at 1°C min^{-1} and to 260°C at 30°C min^{-1} with a helium carrier gas flow-rate of 1.5 ml min^{-1}. Retention times were as follows: 2-chloro-1-propanol, 4′50″; 1-chloro-2-propanol, 5′00″; 3-chloro-1-propanol, 7′45″; 1,3DCP, 13′25″; 2,3DCP, 14′24″; 3MCPD, 20′30″; 2MCPD, 21′10″.

Quantitative analysis was carried out by monitoring characteristic ions at *m/z* 241 (1-chloro-2-propanol), 228 (2-chloro-1-propanol), 254 (3-chloro-1-propanol), 275 and 277 (1,3DCP), 253 (2,3DCP), 453, 289 and 291 (3MCPD) and 289 and 253 (2MCPD).

Hydrolysis of glycidol

Glycidol (1 g) was heated with 5 *M* hydrochloric acid (10 ml) in a sealed vial at 105°C for 16 h. The cooled mixture was neutralized with sodium hydroxide solution and cleaned up on an Extrelut 20 column using diethyl ether. The ether extract (containing a mixture of the isomeric chloropropanediols) was examined by the GC–ECD and GC–MS procedures described above.

Recovery and cross-over tests

Duplicate (20 g) solutions of 5 *M* sodium chloride containing 0.05, 0.10 and 100 mg kg^{-1} each of 1,3DCP, 2,3DCP and 3MCDP were analysed by the two-stage Extrelut clean-up procedure followed by HFBI derivatization and GC–ECD to establish the recoveries in each extract and through the method as a whole.

Aliquots (20 g) of a maize gluten liquid hydrolysate were spiked with 0.5–10 mg kg^{-1} of 3MCPD and 2MCPD and analysed by the two-stage Extrelut clean-up procedure followed by HFBI derivatization and GC–ECD. The original liquid hydrolysate was analysed (ten times) in two independent laboratories.

RESULTS AND DISCUSSION

Chromatography of HFBI derivatives

The separation of HFBI derivatives of commercially available chloropropanol standards under optimum conditions is shown in Fig. 1A. 2MCPD is not commercially available; accordingly, a mixture of 3MCPD and 2MCPD was obtained by hydrochloric acid treatment of glycidol; the separation of the HFBI-derivatized mixture is shown in Fig. 1B. Confirmation of the retention time of 2MCPD was finally obtained using a sample of pure 2MCPD kindly provided by Dr. F. Ruf (Maizena) (see Fig. 1C). The products of hydrolysis of glycidol were unambiguously identified as 3MCPD and 2MCPD by GC–MS comparison with authentic standards before and after HFBI derivatization. Retention time data demonstrating column stability are presented in Table I.

Calibration data confirmed the excellent linearity of the detector response to all the chloropropanols examined over the concentration range 0.01–1.0 mg l^{-1}. 1,3DCP and 2,3DCP gave almost identical responses, as did 3MCPD and 2MCPD. HFBI derivatization proved to be complete under the conditions used over the dichloropropanol concentration range 0.01–100 mg l^{-1}, and with 3MCPD up to at least 500 mg l^{-1}.

Sample preparation

Two-stage Extrelut clean-up. Preliminary examination of a number of commercially available protein hydrolysates using the three Extrelut clean-up methods described indicated that the chloropropa-

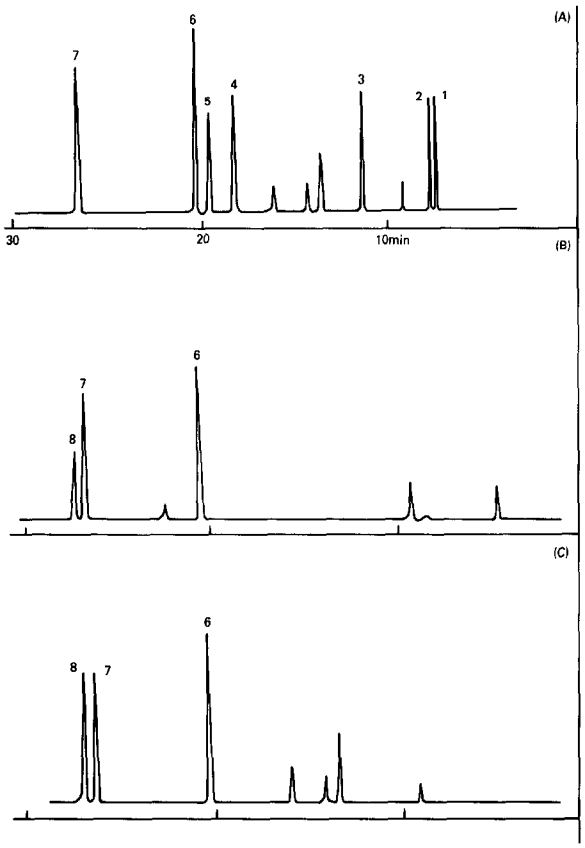

Fig. 1. Separation of (A) chloropropanol standards as HFB derivatives, (B) glycidol reaction products as HFB derivatives and (C) chloropropane diol standards as HFB derivatives. Column: 25 m × 0.2 mm I.D. OV-1- Peaks: 1 = 2-chloro-1-propanol; 2 = 1-chloro-2-propanol; 3 = 3-chloro-1-propanol; 4 = 1,3 DCP; 5 = 2,3DCP; 6 = PDCB (internal standard); 7 = 3MCPD; 8 = 2MCPD.

nediols (3MCPD and 2MCPD) are invariably much more abundant than the chloropropane monools and that extract 3 (diethyl ether) and extract 4 (ethyl acetate) contain species whose HFBI derivatives interfere with those of the chloropropane monools, and confirmed that hexane–diethyl ether extraction gives very poor recoveries of 3MCPD and 2MCPD. In view of these findings, attention was focused on two-stage extraction with hexane–diethyl ether followed by diethyl ether as a flexible and selective procedure for the analysis of both chloropropane monools and diols.

Recoveries of chloropropanols in extract 1 [(hexane–diethyl ether (90:10)] and extract 2 (diethyl ether) obtained from standard chloropropanol solutions in 5 *M* sodium chloride ranged from 90 to 106% monools in extract 1 and from 98 to 115% diols in extract 2, thus confirming that two-stage extraction is an efficient method for separating chloropropane monools from diols prior to their separate derivatization and analysis by GLC. The variable and sometimes high recoveries (100–218%) observed for 1-chloro-2-propanol in extract 1 are thought to arise from sporadic reagent-based interferences, which make this method unsuitable for analysis of low levels of 1-chloro-2-propanol unless mass spectrometric detection is employed.

Analysis of HFBI-derivatized extracts 1 and 2 obtained from commercial protein hydrolysates produced by traditional processes prior to 1986 indicate that the four main chloropropanols found in traditional hydrolysates are (in order of abundance) 3MCPD, 2MCPD, 1,3-DCP and 2,3-DCP (Table

TABLE I

RETENTION TIMES OF CHLOROPROPANOL HFBI DERIVATIVES

Chloropropanol	Retention time (min)			Retention time relative to PDCB		
	8.11.86	21.10.88	12.5.87	8.11.86	21.10.88	12.5.87
2-Chloro-1-propanol	8.20	8.16		0.39	0.40	
1-Chloro-2-propanol	8.45	8.41		0.41	0.41	
3-Chloro-1-propanol	11.80	11.77		0.57	0.57	
1,3-DCP	18.55	18.45		0.89	0.90	
2,3-DCP	19.65	19.60		0.95	0.96	
PDCB	20.77	20.50	21.40			
3MCPD	26.55	26.59	27.80	1.28	1.30	1.30
2MCPD			28.40			1.33

TABLE II

REPLICATION OF TWO-STAGE EXTRELUT EXTRACTION AND HFBI DERIVATIZATION PROCEDURE FOR PROTEIN HYDROLYSATES

Sample	Chloropropanol level found (mg kg^{-1})			
	1,3-DCP	2,3-DCP	3MCPD	2MCPD
1	3.9	0.44	239	43
	4.05	0.45	241	48
	4.07	0.45	273	42
	4.1	0.46	276	49
Mean:	4.03	0.45	257	45.5
2	0.19	0.02	20.8	3.1
	0.18	0.02	20.6	3.3
	0.22	0.02	16.8	3.0
Mean:	0.20	0.02	19.4	3.1
3	0.07	<0.01	238	31.9
	0.07	<0.01	244	32.3
	nd[a]	nd	215	27.7
	nd	nd	221	29.6
	nd	nd	226	30.3
	nd	nd	241	34.0
Mean:	0.07	<0.01	231	31.0
4	<0.01	<0.01	574	88.0
	<0.01	<0.01	564	86.4
	<0.01	<0.01	520	87.6
	<0.01	<0.01	534	86.4
	<0.01	<0.01	565	87.8
	<0.01	<0.01	575	86.2
	<0.01	<0.01	535	87.5
	<0.01	<0.01	525	86.1
Mean:	<0.01	<0.01	549	87.0

[a] nd = Not determined.

II). Indications of slight cross-over of chloropropanediols into eluate 1, and recoveries of the main chloropropanols from the two-stage extraction procedure were checked by analysis of a series of spiked hydrolysates. The typical results presented in Table III confirm that cross-over of the diols into extract 1 is less than 2% of the total diols recovered: excellent recoveries (93–120%) over a wide range of levels (0.05–100 mg/kg for the chloropropane monools and 0.5–350 mg kg^{-1} for the chloropropanediols) were obtained.

The results of replication studies are summarized in Tables II and IV and the results of an inter-laboratory multi-level spiking study on 3MCPD and 2MCPD are presented in Table V.

On the basis of all these data, the two-stage Extrelut extraction procedure coupled with HFBI derivatization and capillary GLC–ECD is clearly an accurate and sensitive method for the determination of dichloropropanols and monochloropropanediols in protein hydrolysates. Its limits of detection are 10 $\mu g\ kg^{-1}$ for 1,3-DCP and 2,3-DCP and 50–100 $\mu g\ kg^{-1}$ for 3MCPD and 2MCPD.

The method has been used to elucidate the mechanisms of formation of the chloropropanols [7]. It has been routinely and successfully used since 1986 to guide the development and monitor the performance of new processes designed to minimize the levels of chloropropanols in hydrolysates. In this period, chloropropanol levels in hydrolysates produced in Europe have substantially diminished.

The dichloropropanols have been virtually eliminated: 1,3-DCP levels are now below 50 $\mu g\ kg^{-1}$, in most hydrolysates below 20 $\mu g\ kg^{-1}$, and in many instances 1,3-DCP is no longer detectable (less than 10 $\mu g\ kg^{-1}$). 3MCPD levels have been reduced from up to 700 mg kg^{-1} to 10–20 mg kg^{-1}, and hydrolysates containing less than 2 mg kg^{-1} are now beginning to appear. 2MCPD levels have been reduced from up to 150 mg kg^{-1} to less than 20 mg kg^{-1} and hydrolysates containing less than 10 mg kg^{-1} are beginning to appear. The method has proved satisfactory for the analysis of these hydrolysates provided that precautions are taken to check for and eliminate sporadic reagent and solvent-based interferences which can occasionally interfere with the determination of low levels (less than 2 mg

TABLE III

LEVELS OF 3MCPD AND 2MCPD IN EXTRELUT EXTRACTS 1 AND 2 (E_1 AND E_2) FROM PROTEIN HYDROLYSATES

Results in mg kg^{-1} on original sample.

Sample	3MCPD		2MCPD	
	E_1	E_2	E_1	E_2
1	0.67	516	0.11	69.8
2	0.61	577	0.09	86.0
3	1.52	215	0.19	27.7
4	1.66	240	0.20	34.0
5	<0.05	1.7	<0.05	1.93

TABLE IV

INTER-LABORATORY REPLICATION STUDY: ANALYSIS OF HYDROLYSATE FOR 3MCPD AND 2MCPD BY TWO-STAGE EXTRELUT EXTRACTION AND HFBI DERIVATIZATION

	3MCPD found (mg kg^{-1})		2MCPD found (mg kg^{-1})	
	Lab. 1	Lab. 2	Lab. 1	Lab. 2
	1.36	1.22	1.11	1.14
	1.28	1.22	1.03	1.20
	1.15	0.95	0.92	1.06
	1.36	1.04	1.10	1.14
	1.38	0.98	1.13	1.12
	1.06	1.20	0.84	1.14
	1.16	1.13	0.93	1.06
	1.22	1.16	1.01	1.18
	0.96	1.09	0.74	1.20
	0.95	1.09	0.65	1.13
Mean:	1.19	1.11	0.95	1.14
95% confidence limits:	±0.109	±0.065	±0.110	±0.034
Standard deviation:	0.1526	0.0913	0.1542	0.047
Repeatability:	0.427	0.256	0.432	0.131

kg^{-1}) of 2MCPD. In these circumstances, drying of the washed derivatized extract 2 is essential.

Attempts to apply the method to composite food products (*e.g.*, sauces and soups) and flavours containing protein hydrolysates have been less successful. Chromatograms are frequently complex; matrix-derived peaks sometimes interfere with the comparatively small chloropropanol peaks derived from the low levels of hydrolysates normally present in such products, and lead to falsely high results or false positives. The GC–MS methods described under Experimental are essential for the reliable determination of chloropropanols in composite food products and flavours.

Single-stage Extrelut clean-up. The two-stage Extrelut extraction procedure is time consuming. In view of the diminishing need to monitor hydrolysates for 1,3DCP, the suitability of simpler and faster diethyl ether and ethyl acetate single-stage extraction procedures for the analysis of chloropropanediols as their HFBI derivatives was investigated.

These simpler procedures were found to give results in good agreement with those of two-stage extraction over the diol range 2–500 mg kg^{-1}. Replication studies of two-stage extraction and one-stage ethyl acetate extraction (Table VI) confirm that over the diol range 1–50 mg kg^{-1} these two methods give closely similar results with similar precision.

The results of an inter-laboratory replication test of the ethyl acetate extraction procedure (Table VII) indicate that it is suitable for the determination of 3MCPD in hydrolysates at levels below 1 mg kg^{-1}. It appears, however, that interference problems may limit its accurate application to 2MCPD determination to levels above 1 mg kg^{-1}, and that its use at lower 2MCPD levels should be approached with caution unless GC–MS facilities are available. Nevertheless, the ethyl acetate–HFBI–GC–ECD method is a rapid, precise and sensitive method for chloropropanediol analysis. Used in conjunction with screening methods based on other derivatives (*e.g.*, phenylboronates [8]), it could provide confirmed diol results without resort to GC–MS procedures.

GC–MS confirmation procedures

The identities and levels of chloropropanols found by GC–ECD of HFBI-derivatized Extrelut extracts were confirmed by GC–MS of both unde-

TABLE V

INTER-LABORATORY SPIKING STUDY: ANALYSIS OF HYDROLYSATE FOR 3MCPD AND 2MCPD BY TWO-STAGE EXTRELUT EXTRACTION AND HFBI DERIVATIZATION

Laboratory	3MCPD level (mg kg^{-1})			Recovery (%)
	Spiked	Found	Difference	
1	0	1.23		
	1.97	3.48	1.51	109
	4.96	5.97	1.01	96
	9.69	10.99	1.30	101
		Mean:	1.27	102
2	0	1.1		
	0.5	1.54	1.04	96
	0.5	1.59	1.09	99
	1.0	2.15	1.15	102
	1.0	2.23	1.23	106
	2.0	3.06	1.06	98
	2.0	3.28	1.28	106
		Mean:	1.14	101
	2MCPD level (mg kg^{-1})			
	Spiked	Found	Difference	
2	0	1.14		
	0.5	1.48	0.98	90
	0.5	1.53	1.03	93
	1.0	2.12	1.12	99
	1.0	2.18	1.18	102
	2.0	2.96	0.96	94
	2.0	3.15	1.15	100
		Mean:	1.07	96

Least mean squares correlation of MCPD found *vs.* MCPD spiked gave the following results:

Laboratory	Base level MCPD (mg kg^{-1})				Recovery (slope)	
	3MCPD		2MCPD		3 MCPD	2MCPD
	I[a]	II[a]	I[a]	II[a]		
1	1.23	1.29			0.992	
2	1.11	1.14	1.14	1.09	1.02	0.99

[a] I = Directly measured; II = calculated intercept of least mean squares plot.

rivatized and HFBI-derivatized standards and extracts 1–4.

Underivatized extracts and standards. The EI mass spectra of the underivatized chloropropanols can be readily characterized in terms of molecular structure and, in conjunction with GC retention times, provide unambiguous identification down to the low ng μl^{-1} level. Table VIII lists the major diagnostic and base ions present in these spectra: as expected, no molecular ions were observed. The

TABLE VI

REPLICATION OF 3MCPD AND 2MCPD ANALYSIS BY (A) TWO-STAGE AND (B) ONE-STAGE (ETHYL ACETATE) EXTRELUT ELUTION

Sample	3MCPD level (mg kg^{-1})		2MCPD level (mg kg^{-1})	
	A	B	A	B
1	49.9	53.1	1.3	1.8
	51.1	53.2	1.3	1.7
	51.0	53.9	1.3	1.6
	51.0	54.2	1.5	1.6
	50.0	53.9	1.3	1.7
Mean:	50.6	53.7	1.3	1.7
95% confidence limits:	±0.66	±0.54	±0.10	±0.09
Standard deviation:	0.533	0.432	0.08	0.07
2	10.9	11.3	1.0	1.0
	11.2	11.2	1.0	1.0
	11.2	11.8	0.9	1.1
	11.5	11.1	1.0	0.9
	11.3	12.7	1.1	1.2
Mean:	11.2	11.6	1.0	1.0
95% confidence limits:	±0.24	±0.73	±0.07	±0.12
Standard deviation:	0.194	0.591	0.06	0.10

TABLE VII

INTER-LABORATORY REPLICATION STUDY: ANALYSIS OF HYDROLYSATES FOR 3MCPD AND 2MCPD BY ETHYL ACETATE EXTRELUT EXTRACTION AND HFBI DERIVATIZATION

Sample	3MCPD level (mg kg^{-1})		2MCPD level (mg kg^{-1})	
	Lab. 1	Lab. 2	Lab. 1	Lab. 2
1	0.58	0.72	<0.5[a]	nd[b]
	0.57	0.68	<0.5[a]	nd
	0.68	0.72	<0.5[a]	nd
	0.58	0.72	<0.5[a]	nd
	0.51	0.72	<0.5[a]	nd
Mean:	0.58	0.71	<0.5[a]	nd
95% confidence limits:	±0.068	±0.02		
Standard deviation:	0.055	0.016		
2	10.9	10.4	<0.5[a]	nd
	9.7	10.6	<0.5[a]	nd
	9.0	10.6	<0.5[a]	nd
	9.7	10.6	<0.5[a]	nd
	9.5	10.3	<0.5[a]	nd
Mean:	9.76	10.5	<0.05[a]	nd
95% confidence limits:	±0.776	±0.156		
Standard deviation:	0.625	0.126		

[a] Interference with 2MCPD peak prevented accurate measurement.
[b] nd = Not determined.

TABLE VIII

CHARACTERISTIC IONS IN EI MASS SPECTRA OF CHLOROPROPANOLS

Chloropropanol	Molar mass	Ion (*m/z*) resulting from loss of					Base ion (*m/z*)
		H_2O	CH_2OH	HCl	CH_2Cl	CH_3	
3-Chloro-1-propanol	94	76/78		58			58 ($M^+ - HCl$)
2-Chloro-1-propanol	94		62/64	58			31 (CH_2OH^+)
1-Chloro-2-propanol	94				45	79/81	45 ($M^+ - CH_2Cl$)
1,3-DCP	128				79/81		79/81 ($M^+ - CH_2Cl$)
2,3-DCP	128			92/94			62/64 ($M^+ - CHOH - HCl$)
3MCPD	110		79/81		61		44 ($M^+ - CH_2Cl - OH$)
2MCPD	110	92/94					62/64 ($M^+ - CHOH - H_2O$)

spectra of the 3- and 2-chloropropane diols (Fig. 2) illustrate the value of mass spectrometry in distinguishing between isomeric structures and of Cl^{35}/Cl^{37} isotope ratios in determining fragmentation pathways.

HFBI-derivatized extracts and standards. EI mass spectra of the HFBI derivatives of the chloropropanols are dominated by ions derived from charge retention on the heptafluorobutyryl moiety, *e.g.*, *m/z* 69 (CF_3) 100 (C_2F_4), 119 (C_2F_5), 169 (C_3F_7) and 197 (C_3F_7 CO). Diagnostically significant ions are in general much less intense but, as shown in Table IX, permit differentiation between the various isomers. Although superficially more alike than those derived from underivatized materials, the HFBI derivative spectra contain characteristic fragments that permit unequivocal assignment of structure (Fig. 3).

Quantification. Quantitative analysis was performed by selected ion recording in which the characteristic ions were monitored at a resolution of 1 in 5000. Peaks detected at the appropriate retention time were quantified with reference to external standards interspersed with the experimental extracts: peak area responses of relevant ions were compared with those obtained from the appropriate standard chloropropanol, with particular emphasis being placed on the relative intensities of chlorine isotope ions. The responses of standards were found to be linear over the range 0.04–3 ng injected onto the

TABLE IX

CHARACTERISTIC IONS IN THE EI MASS SPECTRA OF THE HFBI DERIVATIVES OF CHLOROPROPANOLS

Chloropropanol HFBI derivative	Molar mass	Ion (*m/z*) resulting from loss of					Other structurally significant ions (*m/z*)
		$C_3F_7CO_2$	HCl	$C_3F_7CO_2$ + HCl	CH_2Cl	$C_3F_7CO_2$ + CH_2Cl	
3-Chloro-1-propanol	290	77/79[a]	254				
2-Chloro-1-propanol	290	77/79[a]					228 ($M^+ - CH_2 = CHCl$)
1-Chloro-2-propanol	290	77/89			241[a]		275/77 ($M^+ - CH_3$)
1,3-DCP	324	111		75/77[a]	275/77		
2,3-DCP	324	111		75/77[a]		62/66	253 ($M^+ - Cl - HCl$)
3MCPD	502	289		253	453	240	75/77[a] ($M^+ - C_3F_7COOH - C_3F_7COO$)
2MCPD	502	289		253			

[a] Base ion (excluding HFB fragments).

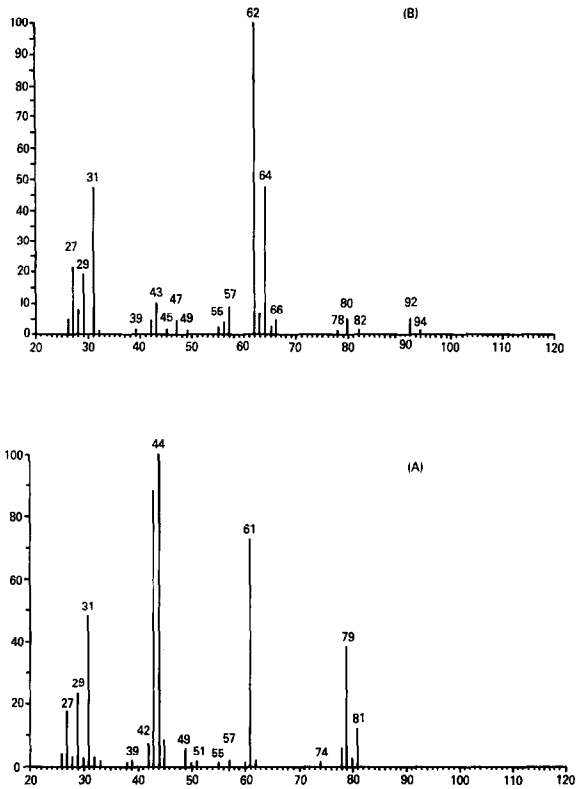

Fig. 2. Mass spectra of chloropropane diols: (A) 3MCPD; (B) 2MCPD.

GC column, and more concentrated extracts were diluted to within this range for quantitative analysis. Underivatized extracts gave the better sensitivity, owing to both shorter retention times and significantly greater intensities of the ions monitored. For straightforward protein hydrolysates, results obtained by the GC–MS and HFBI–GC–ECD methods were in reasonable agreement, with GC–MS detection limits of 10–20 μg kg^{-1} for chloropropane monools and 20–80 μg kg^{-1} for the diols. For more complex products (*e.g.* sauces, soup mixes, composite flavours, stock cubes), the GC–MS method often failed to confirm the presence of chloropropanols detected (often at substantial levels) by the HFBI–GC–ECD method, thus confirming the need to use the GC–MS method for the analysis of composite food products and flavours. Detection limits for the analysis of such complex products

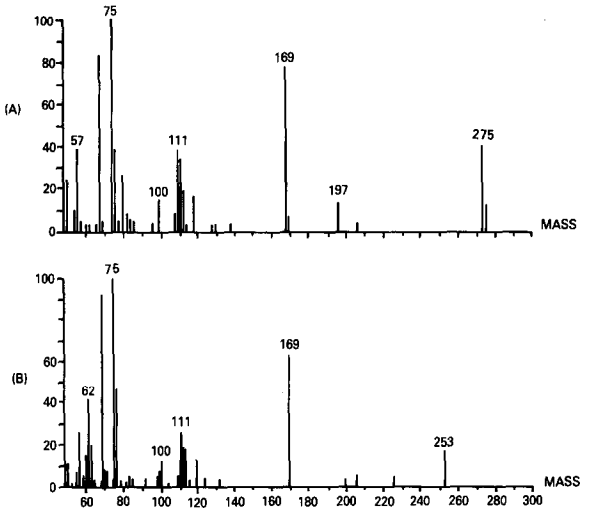

Fig. 3. Mass spectra of dichloropropanol HFB derivatives: (A) 1,3DCP; (B) 2,3DCP.

ranged from 30 to 40 μg kg^{-1} for the chloropropane monools and from 100 to 300 μg kg^{-1} for the diols.

CONCLUSIONS

Analysis of protein hydrolysates for a complete range of chloropropanols can be carried out with good recoveries, precision and sensitivity (limits of detection 10–100 μg kg^{-1}) by two-stage solid-phase extraction on diatomaceous earth (Extrelut columns) followed by HFBI derivatization and capillary GC ECD. Analysis of protein hydrolysates for chloropropanediols (the principal chloropropanols found) can be more conveniently carried out by small-scale single-stage solid-phase extraction using ethyl acetate. Whilst giving results of comparable precision and sensitivity for 3MCPD (limit of detection 50–100 μg kg^{-1}), the method is less sensitive for 2 MCPD and may not be suitable for hydrolysates containing less than 1 mg kg^{-1} 2MCPD unless used with MS detection.

Unambigous confirmation of results obtained by these methods can be obtained with similar sensitivities by capillary GC–MS of underivatized solid-phase extracts.

Analysis of composite food products and flavours for chloropropanols by the HFBI–GC–ECD

procedure gives false positives and high results: the GC–MS procedure is essential for the reliable analysis of such products.

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