Application of Modern Technology in Food Safety : Chloropropanols in Sauces and Condiments

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Abstract

This paper reports the development of a selective and sensitive method for the determination of the possible carcinogen, 1,3-dichloropropanol and the related compound 3-monochloropropan-1,3-diol in commercial food ingredients and retail food products. Samples of soya sauce, oyster sauce and condiments were collected from the retail market in Hong Kong. Samples were spiked with deuterium labeled standards and homogenized with 5M sodium chloride solution. Extraction was effected with organic solvents on Extrelut 20 SPE columns. The concentrated extract was derivatized with heptafluorobutyrylimidazole and analyzed by capillary gas chromatography with mass spectrometric detection. Results obtained on about 80 commercial samples were reported and discussed.

1. Introduction

Acid Hydrolyzed Vegetable Protein (acid-HVP) is produced by acid hydrolysis of plant-protein-containing raw materials, such as wheat and rice gluten and roughly ground soybean, palm kernels or peanuts. Hydrolysis of HVP normally proceeds at temperatures above 100°C and at appropriate pressure in presence of hydrochloric acid. Fatty acid esters (glycerol) residue present in the raw material will also undergo hydrolysis forming chloropropanols [1,2]. Amongst others, 1,3-dichloropropan-2-ol (1,3-DCP) and 3-chloropropane-1,2-ol (3-MCPD) are two of the most toxic chloropropanols found as contaminants in acid-hydrolysed vegetable protein (acid-HVP) [3-5], and a range of other foods and ingredients, most notably in soy sauce [6,7]. The United Kingdom's Committee on Mutagenicity found 3-MCPD to be a non-genotoxic carcinogen [8]. The Joint FAO/WHO Expert Committee on Food Additives found 1,3-DCP to be a genotoxic carcinogen [9].

Based on technological feasibility and a preliminary quantitative cancer risk assessment by the Food and Drug Administration of United States, specifications of 1 mg/kg 3-MCPD and 0.05 mg/kg 1,3-DCP in acid-hydrolysed vegetable proteins (on dry basis) were established by the Food chemicals codex (FCC) in December of 1997 [10]. Since 1997, the UK's Food Advisory Committee has recommended that the industry take steps to ensure that 3-MCPD is undetectable in foods or food ingredients at a level of 0.01 ppm [6]. In 2001, the

Commission of the European Communities established a maximum level of 0.02 ppm 3-MCPD in HVP and soy sauce [11]. In 2001, the Joint Food and Agricultural Organization (FAO) and World Health Organization (WHO) Expert Committee on Food Additives (JECFA) had evaluated the safety of 3-MCPD and 1,3-DCP, JECFA concluded, as 3-MCPD is found infrequently in foods, a regulatory limit would be unlikely to have much effect on the overall intake of non-consumers of soya sauce. However, because the distribution of residual 3-MCPD in soya sauce is highly skewed and because it is likely that brand loyalty could result in regular consumption of highly contaminated brands, a regulatory limit on the concentration of 3-MCPD in soya sauce could markedly reduce the intake of 3-MCPD by consumers of this condiment. JECFA had allocated a Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 μg/kg body weight to 3-MCPD. JECFA also considered it inappropriate to set a tolerable intake for 1,3-DCP; however, noted that the margin between the estimated dietary exposure the level that results in tumour formation in animal studies is very large. Codex is considering a maximum level of 0.4 mg/kg for 3-MCPD in liquid condiments containing acid-HVP (currently at step 3). In 2005, the 37th Session of the Codex Committee on Food Additives and Contaminants (CCFAC) agreed to request JECFA to conduct an exposure assessment for chloropropanols from all sources.

Before 1997, gas chromatography with different detection was shown to be the method of choice for the quantitation of 3-MCPD. Most of the methods relied on the detection of either the underivatised 3-MCPD [13] or the derivatives of 3-MCPD with phyenylboronic acid [14,15], butaneboronic acid [16], N,O-bis-(trimethylsilyl)trifluoroacetamide [17] and toluene-4-sulfonic acid [14]. However, none of these methods are of sufficient sensitivity or selectivity for the determination of μg/kg levels of 3-MCPD in foodstuffs. In 1997, Hamlet et al. [18,19] reported an analytical method for determination of 3-MCPD and 2-MCPD at μg/kg levels in HVP, seasonings and food products using gas chromatography/ion trap tandem mass spectrometry. Since then, AOAC started to adopt a modified Hamlet's method as the official method for the quantitation of 3-MCPD in HVP. Meierhans et al. [20], Dayrit et al. [21] and Retho et al. [22] developed an alternative gas chromatographic/mass spectrometric (GC-MS) method with other derviatization agents for the determination of 3-MCPD.

However, analytical method for the quantitation of 1,3-DCP of μg/kg levels is quite limited. Chung et al. reported the first GC-MS method for the simultaneous separation and quantitation of 1,3-DCP and 3-MCPD in 2002 [23]. Nyman et al. [24] and Bogusz [25] reported similar method with modification mainly in sample extraction or cleanup and addition of internal standard for better quantitation of 1,3-DCP in the following years. Besides, Crews et al. developed an alternative headspace gas chromatography-mass

spectrometry for the determination of 1,3-DCP in soy sauces [26].

In view of lacking local data on levels of chloropropanols, namely 3-MCPD and 1,3-DCP, in sauces and condiments, a study on 3-MCPD and 1,3-DCP is conducted. Our laboratory has modified Nyman's method to determine 1,3-DCP and 3-MCPD in soya sauce and related products with gas chromatography – negative chemical ionization – mass spectrometric detection to obtain the best detection limits.

2. Experimental

Sample

Eighty-four samples were obtained from supermarkets and retailers in Hong Kong. The samples were divided into four classes.

a) Seasoning: 38 samples comprising soya sauces and other seasoning in liquid form.
b) Oyster sauces: 14 samples comprising oyster sauces and oyster flavouring sauces.
c) Soup base powders: 13 samples comprising of chicken powder and noodle soup bases.
d) Miscellaneous: 19 samples comprising chicken broths, gravy mix and other sauces.

Sample preparation

Samples of liquids and pastes form without solid were analyzed directly. Powders and other sauces with meats and/or vegetables were processed in a blender, gain was milled and, in each case, a sub-sample was taken for analysis.

A test portion of liquid sauce (8 g) or viscous liquid sauce (such as oyster sauce) (6 g) or solid sample (5 g) was diluted to 20 g with 5M sodium chloride solution, spiked with internal standards solution, sonicated for 10 minutes and mixed thoroughly with a 20 mL Extrelut^{TM} refill pack. The Extrelut^{TM} mixture was added to an Extrelut 20 column, topped with 1 cm anhydrous sodium sulphate and left to stand for 20 minutes. The column was eluted with diethyl ether/hexane (1:9) with the flow rate about 8 mL/min. Discarded the first 90 mL eluant and collected 1,3-DCP in the subsequent 50 mL of eluant. 3-MCPD was then eluted with 250 mL diethyl ether at a flow rate of approximately 8mL/min. The 1,3-DCP eluate was partitioned with two 25 mL portions of acetonitrile and the acetonitrile extract was concentrated carefully to 1 mL by rotary-evaporation system with the condenser at 5°C and the water bath at 70°C. The 3-MCPD eluate was concentrated at temperature not above 35℃ to about 5mL and makeup to 10 mL with diethyl ether.

Derivatisation

Transferred quantitatively the 1,3-DCP extract or 1 mL of the 3-MCPD extract into a 13mL culture tube, then added 1 mL 2,2,4-trimethylpentane and 0.05 mL HFBI into the sample extract. Capped the tube and mixed well with a vortex mixer. Placed the tube in 70°C water bath for 20 min. and shook occasionally. Cooled the derivatized extract to room temperature and add 1 mL water to deactivate the residual HFBI. Added 1 mL of iso-octane into the mixture and shook on a vortex mixer for about 1 minute. Let the organic and aqueous phases to separate. Transferred the organic phase to another sample vial for GC/NCI/MS analysis.

Calibration

1 mL of mixed calibration standards, containing 0, 0.1, 0.2, 0.5 and 1.6 mL of each stock 1,3-DCP and 3-MCPD solution and 1 mL of each stock d_5-1,3-DCP and d_5-3-MCPD solution were concentrated to about 0.2 mL at room temperature under a gentle stream of nitrogen. The standards were derivatised according to the same procedures as described for the sample. 1.0 μL of each of the standard solutions were injected into the GC-NCI-MS system and the retention times and peak areas of 1,3-DCP, 3-MCPD, d_5-1,3-DCP and d_5-3-MCPD were recorded respectively. The peak area ratios of the analytes against corresponding internal standard (m/z 304 to m/z 308 for 1,3-DCP and m/z 482 to m/z 486 for 3-MCPD) were determined. Two 5-points calibration graphs were obtained by plotting the respective peak-are ratios against the concentrations of 1,3-DCP and 3-MCPD using unweighted least-squares linear fitting.

Gas chromatography – negative chemical ionization – mass spectrometry (GC-NCI-MS)

Capillary GC-NCI-MS analysis was carried out on an Agilent 6890 Network gas chromatograph equipped with a Series 5973 Network mass selective detector, a Series 7683 automatic sampler and a data processing system (Agilent). Gas chromatography was performed on a DB-5MS fused-silica capillary column (30m, 0.25mm I.D., 0.25μm film thickness). Ultra-high-purity Helium was used as the carrier gas at constant flow of 1.0 mL/min.

A split-splitless injection system operated in the splitless mode with quartz 2 mm id, 250 μL, deactivated injector liner was employed. 1 minute after the injection, the septum purge was activated to a flow-rate of 23 mL/min. for 1 min. Afterwards, the total flow was set to 20 mL/min. The initial column temperature was set at 50°C. After the

sample injected for 1 min., it was increased at 2°C/min to 90°C, maintained for 1 min., then increased at 40°C/min to 270°C and hold for 10 min. The temperature of the injector was at 270°C. The mass spectrometer was operated in the negative chemical ionization mode with methane as reagent gas and the ion source temperature was set at 150°C.

Qualitative and quantitative analysis was carried out by selectively monitored the detector response of characteristic ions for the heptafluorobutyryl derivative of 1,3-DCP at m/z 304, 268 and 306; 3-MCPD at m/z of 482, 502 and 446 ; d_5-1,3-DCP at m/z of 308; and d_5-3-MCPD at m/z at 486.

3. Results and Discussion

Method

HFBI was reported to react selectivity with chloropropanols to give stable heptafluorobutyrate derivatives that undergo fragmentation into characteristic ions for characterization and quantitation under electronic impact mode. Prest reported that GC-NCI-MS could be used to determine 3-MCPD in HVP [27]. However, hitherto, similar analytical method for the quantitation of 1,3-DCP is not available in the literature. The full scan NCI mass spectrum of 1,3-DCP made over the mass range of m/z 50 – 350 at 1 scan/sec was shown in Figure 1. Apart from some major ion derivatised from the heptafluorobutyryl moiety such as (m/z 194 and 213), the NCI mass spectrum of the heptafluorobutyryl derivative of 1,3-DCP exhibit a number of characteristic ions including m/z 268, 304, 306 and 308. Though these characteristic ions are less intense than the ion arising from the heptafluorobutyryl moiety, they provide evidence to support the equivocal assignment and confirmation of presence of 1,3-DCP.

To enhance the sensitivity of measurement, selective ion monitoring was used to monitor characteristic ions (**bold** denotes the ions used for quantitation) at m/z 268, **304**, 306 and 308 (1,3-DCP), m/z 446, **482**, 502 (3-MCPD), m/z **308** (d_5-1,3-DCP) and m/z **486** (d_5-3-MCPD). To this end, the characteristic peak area ratio of the ions m/z 268, 306 and 308 to 304 for 1,3-DCP and m/z 446 and 502 to 482 for 3-MCPD were used as the acceptance criteria for confirmation of the presence of the two chloropropanols. Identification was also verified by the matching the retention time of the two analytes in samples against those in standards and the retention times were lie within ± 0.1 min.

Limit of Quantitation

The limit of detection was calculated from the measurement of the signal-to-noise (s/n) ratio for a sauce containing 1 μg/kg of each 1,3-DCP and 3-MCPD where the noise was selected from peaks adjacent to the 1,3-DCP and 3-MCPD respectively. For a s/n ratio of 3:1, the limit of detection in this laboratory was 0.3 μg/kg. A reporting limit of 1 μg/kg was used.

Quality assurance

All analytical batches met the quality criteria as set for the identification/quantitation of 1,3-DCP and 3-MCPD.

a) Calibration standard relative responses to exhibit a linear regression correlation coefficient > 0.995.
b) Reagent blanks of less than 0.3 μg/kg for both 1,3-DCP and 3-MCPD.
c) Recovery of spiked sample fell within 80 – 110%.
d) Acceptable limit of detection must be < 0.3μg/kg (s/n = 3) for both 1,3-DCP and 3-MCPD.
e) Relative response for repeat injections of calibration standards to be within ± 10% the relative response of calibration standards.
f) All the qualifier ion abundance ratios to be within ± 20% the mean of the ion abundance ratios of the calibration standards

Survey results

The results of the food survey are summarized in Table 1. Only three of the 84 samples (4%) were found to contain detectable 1,3-DCP at levels between 1 and 5 μg/kg. A considerable number, 25 of 84 (30%), of the samples contained quantifiable 3-MCPD. However, levels of 3-MCPD were below the Codex proposed limit of 400 μg/kg for all samples analysed. Furthermore, none of the samples contained 1,3-DCP or 3-MCPD above the FCC specification for 1,3-DCP or 3-MCPD respectively in acid-HVP.

The highest chloropropanol level found was 170 μg/kg 3-MCPD while 1,3-DCP was undetected in the product analysed. Since there was only three samples contain detectable 1,3-DCP, it is difficult to draw any relationship between 1,3-DCP and 3-MCPD.

The survey results showed that the daily intake of chloropropanols from sauces and/or condiments did not exceed the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives recommended maximum level.

Compared to the results obtained in 2001 – 2003, voluntary and regulatory compliance has been effective in reducing or eliminating chloropropanols in acid-HVP and related products, all tests sample conforms to the food safe requirements. Our department will continue monitor the chloropropanols level in sauces and condiments and international development in the regulatory limits.

Figure 1. Negative chemical ionization mass spectrum of HFBI derivatised 1,3-DCP.

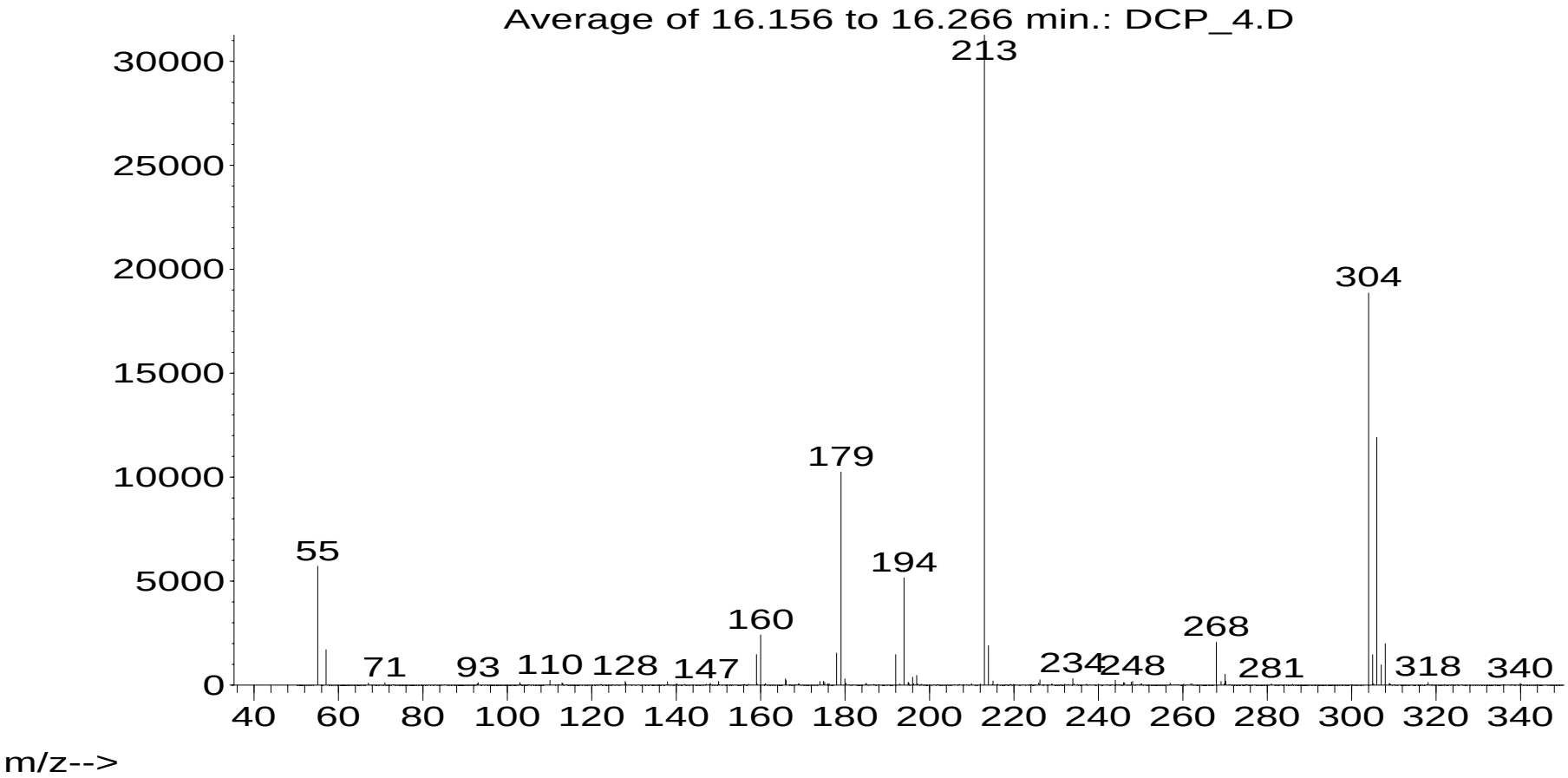

Table 1. Summary of 1,3-DCP and 3-MCPD in the food classes.

Food group	1,3-DCP (μg/kg)		3-MCPD (μg/kg)		
	Incidence no.	1 - 5	Incidence no.	1 - 20	20 - 200
Seasoning	1/38	1	9/38	8	1
Oyster sauce	0/14	0	3/14	2	1
Soup base powder	1/13	1	6/13	5	1
Miscellaneous	1/19	1	7/19	5	2

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