Journal of Chromatography A, 1509 (2017) 9–15

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Simultaneous determination of 14 disinfection by-products in meat products using microwave-assisted extraction and static headspace coupled to gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 15 February 2017
Received in revised form 6 June 2017
Accepted 11 June 2017
Available online 12 June 2017

Keywords:
Meat products
Disinfection by-products
Microwave-assisted extraction
Static headspace
Gas chromatography–mass spectrometry

ABSTRACT

This paper described the first analytical method to simultaneously determine 14 disinfection by-products (DBPs) in meat products using microwave-assisted extraction (MAE) and static headspace (SHS) followed by gas chromatography–mass spectrometry (GC–MS). The DBPs included were 4 trihalomethanes, 7 haloacetic acids, 2 haloacetonitriles and trichloronitromethane, which are commonly formed as a consequence of the disinfection process of water. The combination of the MAE and SHS techniques allows meat samples to be analysed in two sequential steps into the same HS vial in spite of the sample's complexity. Detection limits were obtained within the range of 0.06–0.70 ng/g, and the average relative standard deviation was 7.4%. Recoveries throughout the whole process were between 86 and 95%. The SHS–GC–MS method was applied to determine DBPs in meat products with different industrial processing which could be contaminated through contact with disinfectants and/or treated water employed in the factory either for washing or for the cooking of meat. Up to 5 DBPs were found at ng/g levels in about 36% of the samples analysed, cooked ham being the most contaminated meat product because of the brine solutions employed in its manufacturing process.

1. Introduction

Meat processing requires continuous cleaning and disinfection of the plant, utensils and surfaces to accomplish with the Good Manufacturing Practices [1]. Moreover, after animals are quartered, the carcasses should be rapidly rinsed and chilled (4.4 °C or less) to minimise microbial growth and preserve their quality [2,3]. The water used in the factory should be disinfected to control the microbial population of the final products and for this purpose several sanitisers (chlorine based-disinfectants, hydrogen peroxide, ozone, etc.) are permitted by US and EU regulations [4,5]. Chlorine based-chemicals are the most used disinfectants because they represent a safe, cheap, convenient and effective treatment for food and water [6]. These chlorine-based disinfectants react with organic matter in water or precursors from foods –such as proteins and amino acids– which leads to the formation of disinfection by-products (DBPs), including trihalomethanes (THMs) as the most common volatile species, and haloacetic acids (HAAs) as the major non-volatile DBPs. Apart from this, the Food and Agriculture Organisation of the United Nations and the World Health Organisation (WHO) also warn about the possible formation of nitrogen-containing DBPs in foods –such as N-nitrosamines, haloacetonitriles (HANs), or chloropicrin (trichloronitromethane, TCNM), among others– due to the high content in nitrogenous precursors. Because of this, current guidelines of the WHO and Codex Alimentarius allow a maximum concentration of 50 mg/L of free chlorine in the water in chillers. Utensils and surfaces in the meat industry are an ideal breeding ground for bacteria and, therefore, a more concentrated chlorine solution is allowed for their disinfection (~100 mg/L of free chlorine) [6]. Several studies confirm the presence of chloroform (trichloromethane, TCM) at a wide range concentrations (0.7–46 μg/kg) in poultry tissues that were immersed in chlorinated water (free chlorine levels of 50–200 mg/L) [7–10]. These studies also establish that TCM concentrations were higher in fat tissues (where TCM accumulates) than that found in poultry skin and breast. To our knowledge, there is no information on the occurrence of other DBPs in meat, such as HAAs and nitrogen-containing DBPs.

The determination of micropollutants in meat constitutes a difficult task because of the complexity of the matrix and the normally very low concentrations of target analytes. Common analytical procedures for the analysis of meat samples include several sample

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http://dx.doi.org/10.1016/j.chroma.2017.06.028
0021-9673/

preparation steps, such as extraction, filtration, purification, and/or evaporation. Moreover, in the case of non-volatile compounds such us HAAs, a derivatisation step to their respective methyl esters is also necessary to make them suitable for gas chromatography (GC). Common methods for the isolation and preconcentration of target compounds from a meat matrix include distillation [11] and solvent extraction in relatively low polarity organic solvents [12], as well as the combination of the two techniques [13,14], the Soxhlet extraction technique [15], supercritical fluid extraction [16], and pressurised liquid extraction [17,18]. All these methods have the disadvantages of a high consumption of hazardous organic solvents, an increased risk of analyte losses and prolonged analysis time. In this sense, microwave-assisted extraction (MAE) has become an appropriate alternative to conventional techniques, because it reduces extraction time, simplifies operations and increases recovery yield [19,20]. Static headspace has shown to be an affective technique for the simultaneous extraction and derivatisation of the HAAs in food matrices, without affecting the volatilisation of other volatile target analytes such as THMs [21,22].

The aim of this study was to develop the first method to determine DBPs in meat products, which includes the most relevant species formed in the process of water disinfection (4 THMs, 7 HAAs, 2 HANs and TCNM). The advantages of MAE and SHS techniques were taken into consideration in order to simplify the method and reduce analysis time. The method was applied to determine the possible presence of DBPs in a wide range of meat products with different industrial processing to establish if the manufacturing conditions increased the levels of these species.

2. Experimental

2.1. Chemicals and standard solutions

The 14 DBPs under study and their acronyms are indicated in Table 1. The 4 THMs, 7 HAAs (and their methyl esters), dichloroacetonitrile, trichloronitromethane and 1,2-dibromopropane, used as internal standard (IS), were purchased from Sigma–Aldrich (Madrid, Spain). Bromochloroacetonitrile was acquired from Dr. Ehrenstorfer (Augsburg, Germany). Reagents used in the derivatisation of the HAAs were dimethylsulphate (DMS) and tetrabutylammonium hydrogen sulphate (TBA-HSO_4) and were supplied by Fluka (Madrid, Spain). Methanol, methyl *tert*-butyl ether (MTBE), *n*-pentane, ethyl acetate, sulphuric acid, and anhydrous sodium sulphate were purchased from Merck (Darmstadt, Germany).

Stock standard solutions of each target analyte (1 g/L) and intermediate concentration mixed standard solution (100 mg/L) were prepared in methanol and stored in amber glass vials at −20 °C for a maximum period of 1 month. Working standard solutions were prepared daily in mineral water at the microgram per litre level, since in previous assays with distilled and ultra-pure water, chloroform was detected.

2.2. Instruments and operating conditions

The equipment used in the experiments consists of a 7890A/5975C gas chromatography/mass spectrometer equipped with a G1888 static headspace sampler (Agilent Technologies, Palo Alto, CA, USA). Headspace operating conditions were as follows: 20 min of strong shaking for sample equilibration at 70 °C; temperature of valve and sample loop (3 mL), 100 °C; pressurisation pressure, 18 psi; carrier gas pressure, 6.0 psi; vial pressurisation time, 30 s; loop fill time, 12 s. Sample injection was done in split mode (1:20 split ratio) for 1 min and the injector temperature was set at 180 °C. An HP-5MS column (30 m × 0.25 mm I.D. × 0.25 μm film thickness; Agilent Technologies) coated with a stationary phase of 5%-phenyl–95%-methylpolysiloxane was used with helium (6.0 grade purity, Air Liquide, Seville, Spain) as the carrier gas (1.0 mL/min). The oven temperature was initially set at 40 °C for 3 min, then increased at 20 °C/min to 60 °C (2 min), ramped to 100 °C at 5 °C/min, and finally increased at 40 °C/min to 180 °C and held for 3 min. The mass spectrometer was operated in electron impact mode with electron energy of 70 eV. The interface, ion source and quadrupole temperatures were all set at 200 °C. Solvent delay was set for 2 min. Quantitative analysis of DBPs was performed in the selected ion monitoring mode (SIM), and ions used in the SIM mode for quantification and confirmation of the analytes are listed in Table 1.

2.3. Meat product samples

The samples were selected according to 6 categories of FAO based on the processing technologies used, the treatment of raw materials and individual processing steps [23]. A total of 56 fresh-cut meats and meat products were purchased from local markets. Samples were stored as whole pieces at 4 °C until analysis. Then, the portions of meat or meat products were cut into pieces of approx-

Table 1
Analytical figures of merit of the proposed SHS–GC–MS method for the determination of 14 DBPs in meat products.

	m/z[a]	LOD (ng/g)	Linear range (ng/g)	RSD (%)	Recovery[b]	
					Low level	Medium level
Trichloromethane (TCM)	47, **83**, 85	0.06	0.2–200	5.8	86 ± 5	88 ± 5
Bromodichloromethane (BDCM)	**83**, 85, 129	0.06	0.2–200	5.8	87 ± 6	89 ± 5
Dibromochloromethane (DBCM)	91, 127, **129**	0.07	0.2–200	6.0	87 ± 6	88 ± 5
Tribromomethane (TBM)	171, **173**, 252	0.10	0.3–200	6.5	89 ± 6	87 ± 6
Monochloroacetic acid (MCAA)	**59**, 79, 108	0.70	2.0–200	10.5	92 ± 9	93 ± 9
Monobromoacetic acid (MBAA)	**59**, 93, 95	0.67	2.0–200	10.8	90 ± 10	93 ± 9
Dichloroacetic acid (DCAA)	**59**, 83, 85	0.15	0.5–200	6.8	94 ± 7	95 ± 7
Trichloroacetic acid (TCAA)	59, **117**, 119	0.16	0.5–200	6.9	94 ± 7	94 ± 7
Bromochloroacetic acid (BCAA)	59, 127, **129**	0.20	0.7–200	7.0	93 ± 7	95 ± 7
Dibromoacetic acid (DBAA)	59, 171, **173**	0.30	1.0–200	8.0	90 ± 8	91 ± 7
Bromodichloroacetic acid (BDCAA)	**59**, 161, 163	0.65	2.0–200	9.5	91 ± 8	93 ± 7
Dichloroacetonitrile (DCAN)	**74**, 82, 84	0.20	0.7–200	6.4	92 ± 8	95 ± 7
Bromochloroacetonitrile (BCAN)	**74**, 76, 155	0.30	1.0–200	6.9	94 ± 8	95 ± 7
Trichloronitromethane (TCNM)	46, **117**, 119	0.15	0.5–200	6.0	90 ± 6	92 ± 5

[a] *m/z* values for SIM mode (base peaks used for quantification are boldfaced); *m/z* for 1,2-dibromopropane (IS): 42, **121**, 123.
[b] Low level: 1 ng/g for 9 analytes; 3 ng/g for MCAA, MBAA, DBAA, BDCAA and BCAN. Medium level: 5 ng/g for each analyte.