JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ACS Editors' Choice

Article

pubs.acs.org/JAFC

How the Inclusion of Treated Water in Beverages Influences the Appearance of Halogenated Volatile Organic Compounds

Isabel Montesinos and Mercedes Gallego*

Department of Analytical Chemistry, University of Córdoba, Campus of Rabanales, E-14071 Córdoba, Spain

ABSTRACT: A simple, robust, and reliable headspace gas chromatography method has been developed for the determination of 14 halogenated volatile organic compounds, including iodinated trihalomethanes (THMs), at nanogram per liter levels in beverages. The main source of the presence of THMs in reconstituted fruit juices, nectars, and soft drinks is the treated water included as an ingredient; the concentration and speciation depend on the volume and disinfection process of the treated water either from the distribution network or from water directly disinfected by the food factory. Chloroform appears at concentrations below 1 μg/L in natural juices and soft drinks prepared with mineral water due to contamination from the chlorinated sanitizers usually employed in the food industry. However, the beverages manufactured with treated water contain, in addition to chloroform, brominated THMs and dichloroiodomethane (detected in beverages for the first time), which can be used as indicators of the presence of treated water.

KEYWORDS: *halogenated volatile organic compounds, trihalomethanes, soft drinks and fruit juices, static headspace gas chromatography−mass spectrometry*

INTRODUCTION

The water used as drinking water or in a wide variety of industrial applications is frequently disinfected before use.[1] The employment of treated water in the food industry produces chemical contaminants in food and beverages, such as disinfection byproducts (DBPs) and volatile organic compounds (VOCs).[2−4] DBPs are formed during the disinfection of water, whereas VOCs can be present in untreated groundwater as a result of industrial pollution. These compounds are considered to be potentially carcinogenic and mutagenic, representing a real health risk for humans.[5−7] Because of that, Aggazzotti et al. have evaluated the exposure of pregnant women to DBPs through different media, one of them being bottled water-based beverages (i.e., juices, sodas, etc.).[8] The most common DBPs in drinking water are four trihalomethanes (THMs), which are regulated by the U.S. Environmental Protection Agency (EPA)[9] or European Union (EU)[10] at 80 or 100 μg/L, respectively. With regard to VOCs, dichloromethane, 1,2-dichloroethane, and carbon tetrachloride are also regulated at 5 μg/L.[9] Furthermore, the World Health Organization has published a provisional guideline value of 0.4 μg/L for 1,2-dibromoethane.[11] To comply with current regulations, a large number of water utilities in the United States have changed chlorination for chloramination because the latter forms lower amounts of regulated THMs. Unfortunately, NH_2Cl forms its own suite of DBPs, including iodinated THMs.[12−14] These species have enhanced mammalian cell cytotoxicity and genotoxicity as compared to their brominated and chlorinated analogues,[6] but no similar regulation for these compounds has been established to date. Thus, iodo-organic compounds should be considered when drinking-water exposure is evaluated.

The most significant pathway of exposure to DBPs and VOCs is the ingestion of drinking water. This can occur as direct ingestion or as a result of its inclusion in beverages and food. Consumption surveys indicate that approximately two-thirds of drinking water is ingested through other sources, for example, juices, soft drinks, coffee, soups, and infusions.[15] The types and levels of DBPs in beverages will depend on the disinfection process used to produce the treated water and the chemical constituents of the source water. In addition, VOCs can appear in food from other sources such as the water used for production, wrapping materials, polluted air, and retained solvents used for the extraction of natural components.[3] Thus, international organizations FAO/WHO recommend the development of methods to determine DBPs in beverages and foods.[16] Despite toxic effects and potential human exposure to DBPs through food, information is scarce concerning their levels and they are mainly referred to as chloroform. The formation of THMs has been investigated using static headspace−gas chromatography (SHS-GC) during the preparation of 17 beverages (teas, coffees, concentrated juices, and chocolates) and 11 solid foods (vegetables, baby foods, starchy foods, and soups) using chlorinated drinking water.[17] However, these experiments are unrealistic because they use ultrapure solutions of water with high concentrations of chlorine instead of drinking water. By way of example, tea formed the highest chloroform levels (up to 67 μg/L), followed by coffee, rice, soups, vegetables, and baby food. Different methodologies have been used for the determination of THMs and VOCs in beverages and foods. Generally, EPA methods based on liquid−liquid extraction and GC have been used for this approach,[18,19] although this technique is time-consuming and environmentally unfriendly. Headspace combined with GC has also been applied for the determination of THMs and VOCs in beverages and foods,[4] but the method is laborious and requires drastic conditions

Received: July 18, 2014
Revised: September 23, 2014
Accepted: September 25, 2014
Published: September 25, 2014

dx.doi.org/10.1021/jf503431q | J. Agric. Food Chem. 2014, 62, 10240−10247

Table 1. Analytical Characteristics for the HSH-GC-MS Determination of Halogenated VOCs and Mass Values Used for MS Detection

compound	LOD (ng/L)	linear range (μg/L)	precision RSD (%)[a] intraday	precision RSD (%)[a] interday	m/z[b]
dichloromethane (DCM)	50	0.17−100	5.1	5.9	**49**, 84, 86
1,2-dichloroethane (1,2-DCE)	50	0.17−100	5.2	6.2	49, **62**, 64
carbon tetrachloride (CTC)	8	0.03−50	4.6	5.4	82, **117**, 119
1,2-dibromoethane (1,2-DBE)	35	0.12−100	4.8	5.6	27, **107**, 109
trichloromethane (TCM)	10	0.03−50	4.7	5.5	47, **83**, 85
bromodichloromethane (BDCM)	10	0.03−50	4.9	5.7	**83**, 85, 129
dibromochloromethane (DBCM)	15	0.05−50	5.1	6.1	91, 127, **129**
dichloroiodomethane (DCIM)	20	0.07−50	5.2	6.0	**83**, 85, 175
tribromomethane (TBM)	20	0.07−50	4.9	5.7	171, **173**, 252
bromochloroiodomethane (BCIM)	35	0.12−100	5.0	6.1	**127**, 129, 131
dibromoiodomethane (DBIM)	50	0.17−100	5.2	6.1	**173**, 175, 127
chlorodiiodomethane (CDIM)	50	0.17−100	5.0	6.0	127, **175**, 177
bromodiiodomethane (BDIM)	100	0.30−100	5.2	6.0	127, **219**, 221
triiodomethane (TIM)	100	0.30−100	5.3	6.2	127, 140, **267**

[a]RSD, relative standard deviation (n = 11) for 5 μg/L. [b]m/z values (base peaks for quantification are boldfaced).

(1 h in a 90 °C water bath). Other HS−solid phase microextraction (SPME)[20] or purge-and-trap[21] GC methods require the dilution or centrifugation of beverages, which produces a loss of the species through volatilization. More recently, an HS-SPME method has been applied to determine four THMs in soft drinks[22] and beer.[23] In general, these methods require manual sample manipulation with low sensitivity because they are adapted from water methodologies.

The aims of this work were (i) to develop a solventless SHS-GC−mass spectrometric method with enough sensitivity to determine target analytes at nanogram per liter levels in beverages; (ii) to include six emerging iodinated THMs for the first time, in addition to the four common THMs and four VOCs regulated in treated water; and (iii) to discriminate the source of these species through treated water employed for beverage preparation or by contamination through the industrial process (i.e., washing, bottling line, etc.).

MATERIALS AND METHODS

Chemicals and Standards. The 14 species included in this study with their corresponding acronyms are indicated in Table 1. The majority of standards and the internal standard, 1,2-dibromopropane, were supplied from Sigma-Aldrich (Madrid, Spain). Iodinated THM (DCIM, BCIM, DBIM, CDIM, and BDIM) standards were supplied by Cansyn (Toronto, Canada). Methanol, sulfuric acid, and salts were supplied from Merck (Darmstadt, Germany). Stock standard solutions containing 1 g/L of each compound were prepared in methanol and stored in amber glass vials at −20 °C. Working-standard solutions were prepared on a daily basis by dilution at the microgram per liter level in mineral water (untreated water and free of DBPs).

Static Headspace and Gas Chromatographic Conditions. The experimental setup for the SHS-GC-MS determination of VOCs and THMs consisted of an SHS autosampler G1888 and an HP 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP 5973N mass selective detector. The operating conditions for the HS autosampler were as follows: vial equilibration time, 15 min; oven temperature, 80 °C; vial pressurization time, 30 s; loop fill time, 3 s; valve/loop temperature, 100 °C. Helium (6.0 grade purity, Air Liquid, Seville, Spain) was used both to pressurize vials and to drive the headspace formed to the injection port of the chromatograph via a transfer line at 110 °C. Injection was done in the split mode (split ratio 1:20) for 1 min with an inlet temperature of 250 °C; flow rate of carrier gas (He) was fixed at 1 mL/min. The gas chromatographic separation was achieved on an HP-5MS UI fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness) coated with a stationary phase consisting of 5% phenyl−95% methylpolysiloxane supplied by Agilent. Oven temperature was programmed as follows: 40 °C for 5 min, raised to 100 °C at 25 °C/min, held for 2 min, ramped at 30 °C/min to 250 °C, and finally held for 3 min. The MS was operated in the electron impact ionization mode at 70 eV, and ion source and quadrupole temperatures were set at 250 and 150 °C, respectively. Optimization experiments were conducted in total ion chromatography mode between m/z 25 and 400 at 3.5 scans/s. The ions selected for identification and quantification of VOCs and THMs (SIM mode) are listed in Table 1; m/z values for 1,2-dibromopropane (IS) were 42, 121 (base peak), 123.

Sample Preparation. The commercial beverages (soft drinks and juices) used in this study were purchased at local markets in Spain (one of the largest producers of fruits and derivatives in Europe). Several brands with different packaging types and different flavors were selected. In the laboratory, samples were stored refrigerated or at room temperature, depending on the manufacturer's instructions, and the seal of each bottle was broken right before its analysis. Freshly squeezed orange juice (blank) was used to optimize the analytical parameters of the method. For the preparation of the blank, fresh oranges were hand-peeled and homogenized with a laboratory squeezer. Food utensils were always washed with mineral water to ensure the prevention of DBP contamination.

Analytical Procedure. Beverages were mixed in their own container for 1 min by manual shaking. Ten milliliters of beverage or standard solution containing between 0.03 and 100 μg/L of each target analytes and 5 μg/L of 1,2-dibromopropane (IS) was placed in a 20 mL glass vial containing 4 g of NaCl. For carbonated soft drink analyses, 300 μL of a 6 mol/L NaOH solution was also added to eliminate the carbonic acid present. Then, the vial was immediately sealed and vortexed for 1 min for mixing purposes and placed in the autosampler carousel. Samples were analyzed by SHS-GC-MS, using the operating conditions mentioned above.

RESULTS AND DISCUSSION

Evaluation of Chemical Parameters. First, the SHS variables were studied using 10 mL of freshly squeezed orange juice containing 10 μg/L of each VOC and THM, 5 μg/L of the IS, and 3 g of NaCl in a 20 mL glass vial. The most relevant parameters were oven temperature (60−80 °C) and vial equilibration time (5−20 min), providing the best results at 80 °C and 15 min, respectively. Other instrumental parameters did not present significant changes in the abundance signals for the

compounds, and a pressurization time of 30 s and a venting time of 12 s were selected as the working values.

Salting-out increases the ionic strength of the aqueous solution; this can decrease the solubility of organic species, improving their distribution from the aqueous solution to the headspace. Hence, the addition of NaCl was studied between 0 and 5 g. The analytical signal of the compounds increased with increases in the amounts of salt up to 4 g; therefore, this amount was selected per 10 mL of sample. The effect of the sample pH on the extraction of the 14 species was studied in the 2.5−9.0 range by adjusting the fortified juice with diluted H_2SO_4 or NaOH as required. As the peak area ratio of all species remained constant throughout this range, beverage samples were analyzed without pH adjustment. Fruit juices did not present any problem for their analyses by SHS, but carbonated soft drinks can have certain problems due to the presence of CO_2. When a soft drink bottle is opened, the pressure is reduced to atmospheric pressure, causing decomposition of the carbonic acid, releasing CO_2. This loss of CO_2 originates a sweep of the most volatile compounds into the atmosphere. Moreover, the CO_2 could be competing with the analytes for the headspace in the vial. To solve this problem, different procedures have been proposed, such as nitrogen bubbling, agitation, or ultrasonication.[24] However, these decarbonation methods can remove the volatile compounds from the matrix, so the best alternative can be the addition of NaOH to eliminate carbonic acid, which does not involve the volatilization of the analytes. With the wide pH range of the proposed method (2.5−9.0) taken into account, the addition of NaOH up to pH 8−9 is possible. For this purpose, the recovery of the 14 species was studied by adding various volumes (0−350 μL) of a 6 mol/L NaOH solution to 10 mL of a representative carbonated soda. As can be seen in Figure 1 (for nine representative analytes),

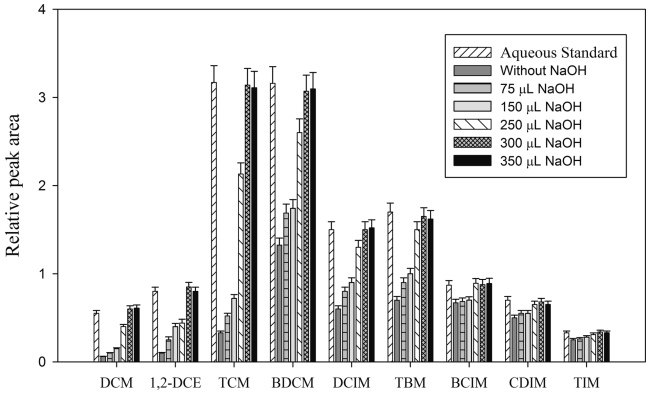

Figure 1. Influence of the addition of variable volumes of a 6 mol/L NaOH solution to 10 mL of fortified natural orange juice on the recoveries of nine representative analytes versus an aqueous standard. Concentration: 10 μg/L of each compound. Error bars are the standard deviation for five measurements.

the relative peak areas were extremely low for the most volatile compounds (DCM, 1,2-DCE, CTC, and TCM) without NaOH, whereas the addition of NaOH solution to the carbonated sample reduced the loss of these compounds. The recovery (versus an aqueous standard solution) for all analytes improved as the volume of NaOH increased to 300 μL (pH ∼8.4). This improvement was more noticeable for the most volatile analytes, which underwent an increase of ∼90% as compared to the aqueous standard. For 1,2-DBE, BDCM, DBCM, DCIM, and TBM, recoveries increased ∼40−50%, whereas for the majority of iodinated THMs (BCIM, CDIM, DBIM, BDIM, and TIM), this increase was only ∼10−20%. The final volume selected was 300 μL of a 6 mol/L NaOH solution added to 10 mL of carbonated soft drink because it provided a sample pH of ∼8.4 (the prevalent species is HCO_3^-; pK_a = 6.1).

Finally, the efficiency percentage of the whole process for the 14 compounds in a fresh orange juice (blank) was calculated in quintuplicate by a second SHS extraction of the remaining liquid phase to check the absence of any compound. For this purpose, 10 mL of the blank was fortified with 10 μg/L of each compound and salt according to the procedure. The average yield of the whole analytical process was ≥95% for all analytes, showing that the method is adequate for the determination of these species.

Sensitivity and Validation of the Method. Linear range, analyte detectability, and precision of the proposed method were studied under optimal experimental conditions (see Table 1). Calibration curves were constructed by using fresh orange juice fortified with 0.03−100 μg/L of each compound and processed as described under Analytical Procedure. The equations for the standard curves were obtained by plotting the analyte to internal standard peak area ratios against the amount of the analytes. Regression coefficients were >0.995 in all cases. Limits of detection (LODs) were determined as the analyte concentration that provides a chromatographic peak equal to 3 times the regression standard deviation, $S_{y/x}$, divided by the slope of each calibration graph. The lower limit of the linear range corresponds to the limit of quantification, which is 3.3 × LOD.[25] The precision was calculated by measuring 11 fresh orange juices on the same day and different days. The results obtained were satisfactory, with average RSD values ranging from 5.0 ± 0.2 to 5.9 ± 0.3% for intra- and interdays, respectively, which indicates that the method is absolutely repeatable and reproducible.

In the bibliography, different solventless techniques have been employed for the determination of several VOCs and THMs in beverages and foods. All of these methodologies have been proposed only for the four common THMs and do not reach the sensitivity of the proposed method.[4,17−23] In addition, some of these methodologies involve the manipulation of the beverages by dilution[20]/centrifugation[21]/heating in water baths,[4] etc., with the consequent loss of volatile compounds. More recently, the four THMs have been determined in soft drinks[21] and beers,[22] but the method is ca. 10 times less sensitive than the present alternative.

To validate the proposed method, a recovery study was conducted by analyzing several representative soft drinks and fruit juices fortified with 5 and 20 μg/L of each compound (n = 5). The analytical responses (taking into account the genuine concentration of the analytes) from all samples were compared to similar standard additions to mineral water. Good recoveries (93−95% for apple, 94−96% for pineapple, 92−96% for orange, and 90−92% for peach juices; 94−96% for tonic; and 95−97% for soda) were obtained for all compounds.

Analysis of Beverage Samples. VOCs and THMs can occur in beverages through multiple pathways, namely, (i) inclusion of drinking water as an ingredient in the production of the beverages; (ii) accumulation and sorption from retained water in beverage packaging and wrapping that had been disinfected/washed with chlorinated sanitizers; (iii) contamination by contact with cleaners/disinfectants used in beverage processing equipment, rinses with water in beverage processing, storage, and/or marketing; and (iv) formation during beverage preparation due to reactions between residual chlorine and

Table 2. Concentrations[a] of THMs (Micrograms per Liter) Found in Soft Drinks

sample	flavor	TCM	BDCM	DBCM	DCIM	TBM	TTHMs[b]
tea 1	lemon	1.1 ± 0.1	0.51 ± 0.03	0.23 ± 0.01	ND[c]	<0.07[d]	1.8 ± 0.1
tea 2	peach	27 ± 2	3.1 ± 0.2	0.24 ± 0.01	ND	ND	30 ± 2
tea 3	passion fruit	14 ± 1	3.5 ± 0.2	0.73 ± 0.04	ND	<0.07	18 ± 1
isotonic 1	lemon	23 ± 1	4.6 ± 0.3	0.76 ± 0.04	ND	ND	28 ± 2
isotonic 2	lemon	1.8 ± 0.1	0.18 ± 0.01	0.08 ± 0.01	ND	<0.07	2.1 ± 0.1
isotonic 3		27 ± 2	3.4 ± 0.2	0.27 ± 0.02	ND	<0.07	31 ± 2
isotonic 4		31 ± 2	3.4 ± 0.2	0.26 ± 0.02	<0.07	<0.07	35 ± 2
fruit beverage 1	lemon	7.1 ± 0.4	0.70 ± 0.04	0.11 ± 0.01	ND	ND	7.9 ± 0.5
fruit beverage 2	apple	21 ± 1	1.3 ± 0.1	0.10 ± 0.01	ND	ND	22 ± 1
fruit beverage 3	lemon	5.1 ± 0.3	0.44 ± 0.03	<0.05	ND	ND	5.5 ± 0.3
fruit beverage 4	orange	6.8 ± 0.4	1.1 ± 0.1	0.11 ± 0.01	ND	<0.07	8.0 ± 0.5
fruit beverage 5	orange	14 ± 1	1.2 ± 0.1	0.13 ± 0.01	ND	ND	15 ± 1
fruit beverage 6	pineapple	23 ± 1	1.4 ± 0.1	0.20 ± 0.01	ND	ND	25 ± 1
tonic 1		20 ± 1	1.2 ± 0.1	0.18 ± 0.01	ND	ND	21 ± 1
tonic 2		3.8 ± 0.2	0.39 ± 0.02	<0.05	ND	ND	4.2 ± 0.2
tonic 3		16 ± 1	2.1 ± 0.1	0.32 ± 0.02	ND	ND	18 ± 1
soda 1		35 ± 2	2.0 ± 0.1	0.25 ± 0.01	ND	ND	37 ± 2
soda 2	lemon	20 ± 1	4.5 ± 0.3	0.82 ± 0.05	0.11 ± 0.01	<0.07	25 ± 1
soda 3	lemon	7.6 ± 0.4	0.62 ± 0.04	0.07 ± 0.01	ND	ND	8.3 ± 0.5
soda 4	lemon	9.6 ± 0.6	0.70 ± 0.04	<0.05	ND	ND	10 ± 1
soda 5	lemon	31 ± 2	1.5 ± 0.1	0.12 ± 0.01	ND	ND	33 ± 2
soda 6	lemon	28 ± 2	1.4 ± 0.1	0.19 ± 0.01	ND	ND	30 ± 2
soda 7	orange	1.0 ± 0.1	0.22 ± 0.01	0.13 ± 0.01	ND	<0.07	1.4 ± 0.1
soda 8	orange	22 ± 1	3.4 ± 0.2	0.55 ± 0.03	ND	<0.07	26 ± 2
soda 9	orange	3.9 ± 0.2	0.31 ± 0.02	<0.05	ND	<0.07	4.2 ± 0.2
soda 10	orange	31 ± 2	1.5 ± 0.1	0.12 ± 0.01	ND	ND	33 ± 2
soda 11	orange	24 ± 1	1.3 ± 0.1	0.10 ± 0.01	ND	ND	25 ± 1
soda 12	cola	6.5 ± 0.4	0.68 ± 0.04	0.08 ± 0.01	ND	ND	7.3 ± 0.4
soda 13	cola	5.4 ± 0.3	0.45 ± 0.03	0.06 ± 0.01	ND	ND	5.9 ± 0.3
soda 14	cola	5.7 ± 0.3	0.44 ± 0.03	<0.05	ND	ND	6.1 ± 0.4
soda 15	cola	27 ± 2	4.5 ± 0.3	0.80 ± 0.05	0.10 ± 0.01	<0.07	32 ± 2
soda 16	cola	7.4 ± 0.4	0.63 ± 0.04	0.07 ± 0.01	ND	<0.07	8.1 ± 0.5
soda 17	cola	28 ± 2	4.9 ± 0.3	0.92 ± 0.05	<0.07	<0.07	34 ± 2
soda 18	cola	43 ± 3	7.8 ± 0.5	1.3 ± 0.1	0.13 ± 0.01	0.17 ± 0.01	52 ± 3
soda 19	cola	16 ± 1	1.0 ± 0.1	0.08 ± 0.01	ND	ND	17 ± 1
soda 20	cola	28 ± 2	6.3 ± 0.4	1.3 ± 0.1	0.13 ± 0.01	<0.07	36 ± 2
soda 21	cola	1.6 ± 0.1	0.09 ± 0.01	0.06 ± 0.01	ND	ND	1.8 ± 0.1
soda 22	cola	31 ± 2	6.4 ± 0.4	1.2 ± 0.1	<0.07	<0.07	39 ± 2
soda 23	cola	14 ± 1	0.90 ± 0.05	0.08 ± 0.01	ND	<0.07	15 ± 1
soda 24	cola	34 ± 2	7.1 ± 0.4	0.52 ± 0.03	<0.07	<0.07	42 ± 2

[a] ± standard deviation, $n = 5$. [b] Total THMs. [c] Not detected. [d] <LOQ.

precursors present in food, for example, carbohydrates, lipids, and proteins. The samples selected for analysis, soft drinks and fruit juices, are products that contain water as an ingredient and that are frequently consumed every day.

The proposed SHS-GC-MS method was applied to determine 4 VOCs and 10 THMs in 100 types of samples: 40 soft drinks (teas, isotonics, fruit beverages, tonics, and sodas) and 60 fruit juices (natural juices 100%, reconstituted juices, and nectars). All samples gave positive results containing up to five THMs including DCIM, in different distributions and levels; none of the four VOCs studied were present in these beverages. Tables 2 and 3 list the concentrations of the THMs found at detectable concentrations in soft drinks (ca. 90% water) and fruit juices (up to 70% water), respectively. The analytes not shown were either not contained or present at levels below their LODs. The species TCM, BDCM, and DBCM were present in practically all samples and at higher concentrations, whereas TBM and DCIM were found in lower proportions. This could be explained because the water used in food applications is frequently disinfected with chlorine due to its economic impact and simple use.

Table 2 lists the levels of each THM found in soft drinks as well as the total concentration of trihalomethanes (TTHMs). There are two levels of TTHM concentrations: soft drinks A for concentrations <15 μg/L ($n = 15$; average value of 5.5 ± 2.8 μg/L) and soft drinks B containing ≥15 μg/L ($n = 25$; average value of 28.8 ± 9.1 μg/L). From Table 2 it can be concluded that (i) there were no significant differences in either TTHM concentrations in each kind of soft drink (tea, tonic, soda, etc.) or in the species of THMs; (ii) there were no differences between flavors (lemon, orange, cola, etc.) in the same kind of soft drinks; and (iii) the presence of THMs in these beverages should be ascribed to the type of treated water employed in their preparation. One factor that influenced the TTHM concentrations was the factory that bottled the samples. Each factory has a different source of water, and the levels of

Table 3. Concentrations[a] of THMs (Micrograms per Liter) Found in Fruit Juices

sample	fruit	factory	TCM	BDCM	DBCM	TBM	TTHMs[b]
natural juice 1	apple	A	0.20 ± 0.01	ND[c]	ND	ND	0.20 ± 0.01
natural juice 2	pineapple	A	0.39 ± 0.02	0.22 ± 0.01	ND	ND	0.61 ± 0.03
natural juice 3	orange	A	0.27 ± 0.02	ND	ND	ND	0.27 ± 0.02
natural juice 4	orange	B	0.48 ± 0.03	0.11 ± 0.01	ND	ND	0.59 ± 0.03
natural juice 5	orange	C	0.60 ± 0.03	0.23 ± 0.01	ND	ND	0.83 ± 0.05
natural juice 6	orange	D	0.33 ± 0.02	ND	ND	ND	0.33 ± 0.02
natural juice 7	orange	E	0.52 ± 0.03	ND	ND	ND	0.52 ± 0.03
reconstituted juice 1	apple	F	0.36 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	<0.07[d]	0.48 ± 0.03
reconstituted juice 2	apple	G	0.92 ± 0.05	1.6 ± 0.1	1.4 ± 0.1	0.26 ± 0.02	4.2 ± 0.2
reconstituted juice 3	pineapple	H	3.6 ± 0.2	3.3 ± 0.2	2.9 ± 0.2	0.40 ± 0.02	10 ± 1
reconstituted juice 4	orange	D	ND	ND	0.07 ± 0.01	0.12 ± 0.01	0.19 ± 0.01
reconstituted juice 5	orange	H	3.0 ± 0.2	3.7 ± 0.2	3.3 ± 0.2	0.50 ± 0.03	11 ± 1
reconstituted juice 6	orange	I	0.45 ± 0.03	ND	0.08 ± 0.01	0.13 ± 0.01	0.66 ± 0.04
reconstituted juice 7	peach	H	1.8 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	0.22 ± 0.01	5.1 ± 0.3
reconstituted juice 8	peach	J	3.1 ± 0.2	0.27 ± 0.02	ND	ND	3.4 ± 0.2
reconstituted juice 9	grape	D	0.56 ± 0.03	0.18 ± 0.01	<0.05	ND	0.74 ± 0.04
reconstituted juice 10	cranberry	A	0.32 ± 0.02	0.25 ± 0.01	0.21 ± 0.01	ND	0.78 ± 0.04
reconstituted juice 11	mix	A	0.38 ± 0.02	0.05 ± 0.01	<0.05	ND	0.43 ± 0.03
reconstituted juice 12	mix	A	0.37 ± 0.02	ND	0.16 ± 0.01	0.29 ± 0.02	0.82 ± 0.05
reconstituted juice 13	mix	A	0.72 ± 0.04	3.7 ± 0.2	ND	ND	4.4 ± 0.3
reconstituted juice 14	mix	C	0.39 ± 0.02	0.08 ± 0.01	0.05 ± 0.01	<0.07	0.52 ± 0.03
reconstituted juice 15	mix	C	0.27 ± 0.02	<0.03	<0.05	<0.07	0.27 ± 0.02
reconstituted juice 16	mix	D	10 ± 1	2.2 ± 0.1	0.62 ± 0.04	ND	13 ± 1
reconstituted juice 17	mix	D	0.23 ± 0.01	ND	<0.05	ND	0.23 ± 0.01
reconstituted juice 18	mix	D	2.2 ± 0.1	ND	ND	ND	2.2 ± 0.1
reconstituted juice 19	mix	H	2.0 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	0.10 ± 0.01	4.9 ± 0.3
reconstituted juice 20	mix	J	6.0 ± 0.3	0.92 ± 0.05	0.16 ± 0.01	ND	7.1 ± 0.4
reconstituted juice 21	mix	K	1.9 ± 0.1	0.30 ± 0.02	0.15 ± 0.01	0.11 ± 0.01	2.5 ± 0.1
nectar 1	apple	A	0.93 ± 0.05	0.30 ± 0.02	0.16 ± 0.01	0.10 ± 0.01	1.5 ± 0.1
nectar 2	apple	C	0.35 ± 0.02	0.24 ± 0.01	0.18 ± 0.01	0.13 ± 0.01	0.90 ± 0.05
nectar 3	apple	C	6.3 ± 0.4	2.1 ± 0.1	0.52 ± 0.03	0.18 ± 0.01	9.1 ± 0.5
nectar 4	apple	J	4.5 ± 0.3	1.1 ± 0.1	0.19 ± 0.01	0.10 ± 0.01	5.9 ± 0.3
nectar 5	pineapple	A	3.2 ± 0.2	2.6 ± 0.2	2.8 ± 0.2	0.51 ± 0.03	9.1 ± 0.5
nectar 6	pineapple	B	2.2 ± 0.1	0.21 ± 0.01	ND	ND	2.4 ± 0.1
nectar 7	pineapple	F	0.38 ± 0.02	ND	ND	ND	0.38 ± 0.02
nectar 8	pineapple	J	12 ± 1	1.6 ± 0.1	0.24 ± 0.01	ND	14 ± 1
nectar 9	pineapple	J	8.5 ± 0.5	1.0 ± 0.1	0.12 ± 0.01	ND	9.6 ± 0.6
nectar 10	pineapple	L	32 ± 2	5.3 ± 0.3	0.96 ± 0.06	ND	38 ± 2
nectar 11	pineapple	L	16 ± 1	3.3 ± 0.2	0.60 ± 0.03	ND	20 ± 1
nectar 12	orange	B	5.8 ± 0.3	0.86 ± 0.05	0.12 ± 0.01	ND	6.8 ± 0.4
nectar 13	orange	B	2.1 ± 0.1	0.43 ± 0.03	0.11 ± 0.01	ND	2.6 ± 0.2
nectar 14	orange	C	0.26 ± 0.02	0.12 ± 0.01	0.17 ± 0.01	0.39 ± 0.02	0.94 ± 0.05
nectar 15	orange	C	18 ± 1	4.8 ± 0.3	0.86 ± 0.05	ND	24 ± 1
nectar 16	orange	D	0.22 ± 0.01	0.19 ± 0.01	0.25 ± 0.01	0.36 ± 0.02	1.0 ± 0.1
nectar 17	orange	G	5.9 ± 0.3	4.6 ± 0.3	2.9 ± 0.2	0.31 ± 0.02	14 ± 1
nectar 18	orange	I	1.3 ± 0.1	0.82 ± 0.05	0.89 ± 0.05	0.46 ± 0.03	3.5 ± 0.2
nectar 19	orange	J	4.0 ± 0.2	0.93 ± 0.05	0.17 ± 0.01	0.12 ± 0.01	5.2 ± 0.3
nectar 20	orange	K	3.3 ± 0.2	0.41 ± 0.02	ND	ND	3.7 ± 0.2
nectar 21	orange	L	17 ± 1	3.8 ± 0.2	0.78 ± 0.04	ND	22 ± 1
nectar 22	peach	G	1.5 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	<0.07	3.9 ± 0.2
nectar 23	peach	L	16 ± 1	2.9 ± 0.2	0.61 ± 0.03	ND	20 ± 1
nectar 24	peach	L	9.6 ± 0.6	2.0 ± 0.1	0.45 ± 0.03	ND	12 ± 1
nectar 25	pear	G	2.6 ± 0.2	2.5 ± 0.1	1.9 ± 0.1	0.27 ± 0.02	7.3 ± 0.4
nectar 26	grapefruit	G	2.4 ± 0.1	2.2 ± 0.1	1.7 ± 0.1	0.20 ± 0.01	6.5 ± 0.4
nectar 27	grapefruit	J	3.9 ± 0.2	0.64 ± 0.04	0.19 ± 0.01	ND	4.7 ± 0.3
nectar 28	guava	A	2.9 ± 0.2	1.5 ± 0.1	0.97 ± 0.06	0.14 ± 0.01	5.5 ± 0.3
nectar 29	mango	C	0.67 ± 0.04	0.25 ± 0.01	0.24 ± 0.01	0.20 ± 0.01	1.4 ± 0.1
nectar 30	passion fruit	A	4.8 ± 0.3	2.3 ± 0.1	1.1 ± 0.1	0.16 ± 0.01	8.4 ± 0.5
nectar 31	mix	C	0.12 ± 0.01	0.17 ± 0.01	0.25 ± 0.01	0.38 ± 0.02	0.92 ± 0.05
nectar 32	mix	D	ND	ND	0.16 ± 0.01	0.29 ± 0.02	0.45 ± 0.03

[a] ± standard deviation, $n = 5$. [b] Total THMs. [c] Not detected. [d] <LOQ.

THMs depend on the disinfection process. However, in this case, no differences were found between TTHM concentrations, presumably because the samples manufactured came from only four different factories, so there was not great variability. By way of example, tea 2 and soda 7 contain 30 and 1.4 μg/L, respectively, and both were manufactured in the same factory. Thus, the differences between the two groups of soft drinks (A and B) could be associated with the type of treated water used in their manufacturing at each moment. No sample exceeded the limits established for the concentration of TTHMs, so the soft drinks were prepared with water from the distribution network or treated water prepared in the same factory following EU normatives.[10] This has been corroborated by analyzing five soft drinks elaborated with mineral water (free of DBPs), which was negative for the species studied or contained chloroform only at very low levels (<1 μg/L). Figure 2 shows the chromatogram for

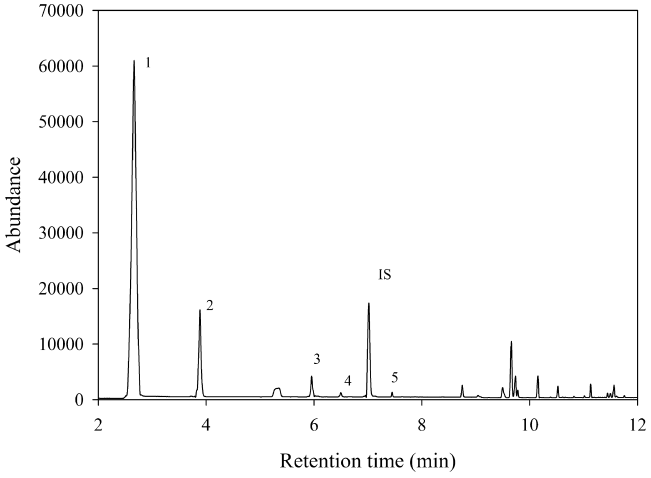

Figure 2. GC-MS chromatogram in SIM mode obtained in the analysis of soda 18. Peaks: (1) TCM; (2) BDCM; (3) DBCM; (4) DCIM; (5) TBM; (IS) internal standard. For compound abbreviations, see Table 1.

soda 18, which shows the peaks corresponding to the five species of THMs detected. After a retention time of 9 min, some peaks appeared in the chromatogram that correspond to flavors or other volatile compounds present in the beverage; these compounds did not interfere with the target analytes, so there was no matrix interference.

Table 3 shows the results obtained in the analysis of 60 fruit juices and nectars. In this case, only the four common THMs were found in these samples. DCIM was not detected because its level is associated with the amount of treated water in the beverage, and fruit juices contain a lower volume of water than soft drinks. The average TTHM concentrations in 100% natural juices were always <1 μg/L, and the predominant species was chloroform (TCM) followed by BDCM. As these juices do not contain treated water, the presence of these species at such a low level can be ascribed to contamination from the use of chlorinated sanitizers in the industry. In contrast, reconstituted juices can contain up to four THMs, and there are two distinctive groups as in soft drinks; reconstituted juices A with TTHM concentrations <1 μg/L and reconstituted juices B containing >2 μg/L. The average value for groups A and B were 0.5 ± 0.2 (n = 10) and 6.2 ± 3.6 (n = 11) μg/L, respectively. Moreover, the kind of fruit was not a relevant parameter because the same fruit was present in both groups. On the other hand, the average values of reconstituted juices A were similar to those of natural juice, but there was a significant difference because brominated species were present in reconstituted juices due only to the addition of treated water. With regard to nectar (ca. 50−75% of water), the average TTHM concentration was higher than that obtained in reconstituted juices, depending on the higher water volume.[26] Hence, there were noticeable variations in concentrations of TTHMs for natural juices, 100%, reconstituted juices, nectars, and soft drinks with average values of 0.47, 3.5, 8.3, and 20 μg/L, respectively, according to the volume of treated water included.

Figure 3 shows the distribution of the total trihalomethane concentrations as box plots in the beverages studied. The

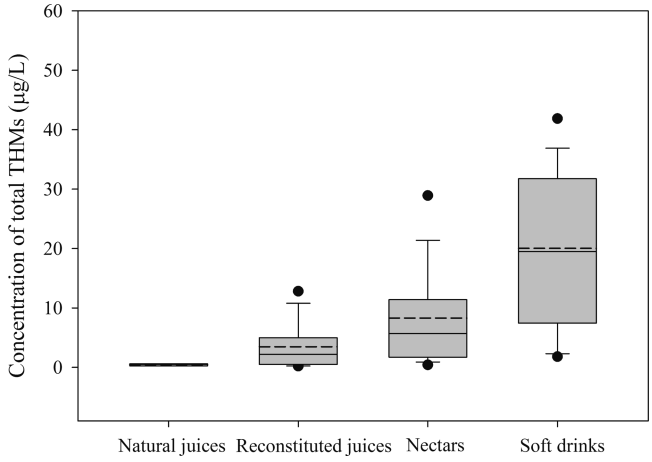

Figure 3. Representation of the total trihalomethane concentrations distributed as box plots in the beverages. The box plots indicate the mean concentration (dashed line) and the 10th, 25th, 50th, 75th, and 90th percentiles (points denote the 5th and 95th percentiles).

bottom and top of the box correspond to the 25th and 75th percentiles, whereas the band (median) inside the box is related to the 50th. The ends of the whiskers represent the lowest and highest observations, and the spacing between the different parts of the box give an indication of the degree of spread and skewedness in the data. There are again two different groups, nectars A with TTHM concentrations <6 μg/L (n = 17; average value of 2.6 ± 1.9 μg/L) and nectars B with concentrations >6 μg/L (n = 15; average value of 14.7 ± 8.7 μg/L). As occurred in the previous case, these variations cannot be attributed to the kind of fruit but rather to the difference between the qualities of the treated water used in their production. Moreover, this deduction was consolidated by comparison to the same product (nectar) manufactured in different bottling factories (n = 12) shown in Table 3 following a word code. Thus, the average TTHM concentrations in nectars produced by A (nectars 1, 5, 28, and 30) or L (nectars 10, 11, 21, 23, and 24) bottling factories were 6.1 or 22.2 μg/L, respectively. Thus, in our study, the juices from factory L always provided the highest concentrations of THMs. These variations in the concentration of THMs in the same product can be explained on the basis of the type of treated water employed at each moment. Figure 4 shows the chromatograms obtained from the analysis of natural juice 7 (A), reconstituted juice 2 (B), and nectar 21 (C). Finally, for comparison purposes, several reconstituted juices of various flavors were analyzed from different European countries. As can be seen in Table 4, no sample presented TTHM concentrations above the levels allowed by EPA or EU for drinking water (80 or 100 μg/L).[9,10] Once again, two differentiated groups existed: one

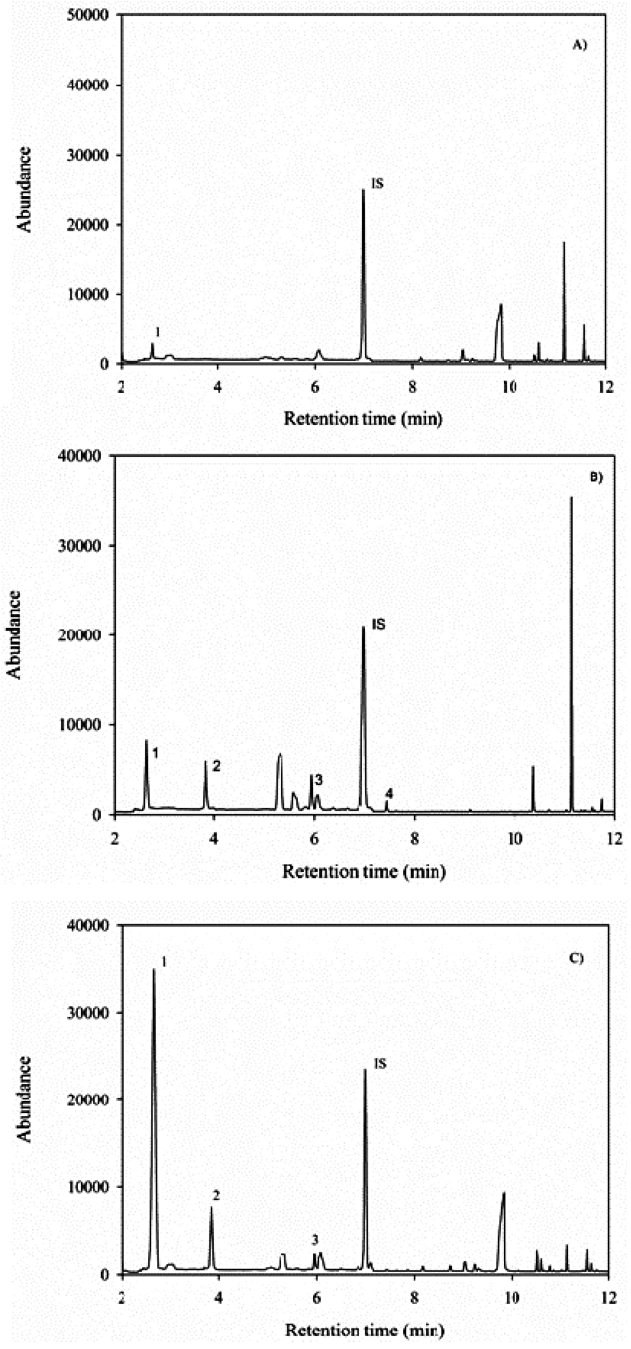

Figure 4. GC-MS chromatograms in SIM mode obtained in the analysis of natural juice 7 (A), reconstituted juice 2 (B), and nectar 21 (C) samples. Peaks: (1) TCM; (2) BDCM; (3) DBCM; (4) TBM; (IS) internal standard. For compound abbreviations, see Table 1.

for TTHM concentrations of 0.4 ± 0.2 μg/L (n = 8) and the other for 9.9 ± 8.2 μg/L (n = 6), similar to Spanish juices.

This study has revealed that chloroform can appear at concentrations <1 μg/L in beverages without treated water as a consequence of contamination due to the chlorinated sanitizers usually employed in the food industry. The treated water included as an ingredient is the main source of the presence of THMs, and the concentration and speciation of these compounds depend on the volume of treated water as well as the water-quality employed. Moreover, brominated THMs and one iodinated one (DCIM) appeared in these samples, which can be used as an indicator of the presence of treated water.

Table 4. THMs Found in Reconstituted Fruit Juices from Several European Countries (±SD, Micrograms per Liter, n = 5)

	country (flavor)													
compd	France (apple)	France (orange)	Poland (orange)	Poland (orange)	Belgium (orange)	Belgium (orange)	Belgium (orange)	Belgium (mix)	Belgium (mix)	Belgium (cranberry)	Portugal (apple)	Portugal (mix)	Portugal (mix)	Portugal (peach)
TCM	ND[a]	0.77 ± 0.04	0.36 ± 0.02	0.25 ± 0.01	0.35 ± 0.02	0.90 ± 0.05	18 ± 1	17 ± 1	0.61 ± 0.03	14 ± 1	3.6 ± 0.2	0.26 ± 0.02	1.8 ± 0.1	0.45 ± 0.03
DCBM	ND	ND	ND	ND	ND	0.21 ± 0.01	0.14 ± 0.01	1.3 ± 0.1	ND	1.1 ± 0.1	1.1 ± 0.1	0.12 ± 0.01	ND	0.18 ± 0.01
DBCM	0.13 ± 0.01	ND	ND	ND	ND	ND	0.08 ± 0.01	0.09 ± 0.01	ND	0.44 ± 0.03	ND	<0.05[b]	ND	ND
TBM	0.09 ± 0.01	ND	ND	ND	ND	ND	<0.07	ND	ND	<0.07	ND	ND	ND	ND
TTHMs[c]	0.22 ± 0.01	0.77 ± 0.04	0.36 ± 0.02	0.25 ± 0.02	0.35 ± 0.02	1.1 ± 0.1	18 ± 1	18 ± 1	0.61 ± 0.03	16 ± 1	4.7 ± 0.3	0.38 ± 0.02	1.8 ± 0.1	0.63 ± 0.04

[a]Not detected. [b]<LOQ. [c]Total THMs.

dx.doi.org/10.1021/jf503431q | J. Agric. Food Chem. 2014, 62, 10240−10247

The proposed method could be adopted by public laboratories to perform routine controls of VOCs and THMs in beverages.

On the other hand, if it is estimated that a person can consume 2 L per day of drinking water (this includes drinking water consumed in the form of juices and other beverages containing tap water)[27] and taking into account that the most contaminated sample (soda 18 in Table 2) contained 52 μg/L of total THMs, a person could ingest >100 μg/L of THMs each day (MCL 80−100 μg/L).[9,10] This problem is aggravated because the default assumption of 2 L per day is not always appropriate or conservative with respect to populations, climates, and physical activity; thus, variations between 3.8 and 4.8 L have been referenced.[28] On the other hand, although chlorinated THMs were the common THMs present in beverages, some samples contained high concentrations of brominated compounds (reconstituted juice 5 and nectar 5 in Table 3) and one iodinated compound (sodas 18 and 20 in Table 2), these compounds being more cytotoxic and genotoxic when compared to their chlorinated analogues. In our opinion, the contribution of human exposure through beverages such as fruit juices and soft drinks per day is significant, taking into account that there are other sources of exposure through foods during the day. Thus, it is deemed acceptable to include these compounds as emergent pollutants in beverages because they have been already established as such for drinking water in several countries.

AUTHOR INFORMATION

Corresponding Author

*(M.G.) Phone/fax: +34-957-218-614. E-mail: mercedes.gallego@uco.es.

Funding

This work was funded within the frameworks of Projects CTQ2013-42701 (Spain's Ministry of Education) and P09-FQM-4732DGI (Andalusian Regional Government). FEDER provided additional funding. I.M. is grateful for the award of a predoctoral grant from Project P09-FQM-4732DGI.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Richardson, S. D.; Ternes, T. A. Water analysis: emerging contaminants and current issues. *Anal. Chem.* **2011**, *83*, 4614−4648.

(2) Fleming-Jones, M. E.; Smith, R. E. Volatile organic compounds in foods: a five year study. *J. Agric. Food Chem.* **2003**, *51*, 8120−8127.

(3) Heikes, D. L.; Jensen, S. R.; Fleming-Jones, M. E. Purge and trap extraction with GC−MS determination of volatile organic compounds in table-ready foods. *J. Agric. Food Chem.* **1995**, *43*, 2869−2875.

(4) Entz, R. C.; Thomas, K. W.; Diachenko, G. W. Residues of volatile halocarbons in foods using headspace gas chromatography. *J. Agric. Food Chem.* **1982**, *30*, 846−849.

(5) Hrudey, S. E; Charrois, J. W. *Dinsinfection By-products and Human Health*, 1st ed.; IWA Publishing: London, UK, 2012.

(6) Richardson, S. D.; Fasano, F.; Ellington, J. J.; Crumley, F. G.; Buettner, K. M.; Evans, J. J.; Blount, B. C.; Silva, L. K.; Waite, T. J.; Luther, G. W.; McKague, A. B.; Miltner, R. J.; Wagner, E. D.; Plewa, M. J. Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. *Environ. Sci. Technol.* **2008**, *42*, 8330−8338.

(7) Irwin, R. J.; VanMouwerik, M.; Stevens, L.; Seese, M. D.; Basham, W. *Environmental Contaminants Encyclopedia*; National Park Service, Water Resources Division: Fort Collins, CO, USA, 1998.

(8) Aggazzotti, G.; Righi, E.; Fantuzzi, G.; Biasotti, B.; Ravera, G.; Kanitz, S.; Barbone, F.; Sansebastiano, G.; Battaglia, M. A.; Leoni, V.; Fabiani, L.; Triassi, M.; Sciacca, S. Collaborative Group for the Study of Chlorinated Drinking Waters and Pregnancy. Chlorination by-products (CBPs) in drinking water and adverse pregnancy outcomes in Italy. *J. Water Health* **2004**, *2*, 233−247.

(9) U.S. Environmental Protection Agency: National Primary Drinking Water Standards, 2003; http://www.epa.gov/safewater/mcl (accessed May 15, 2014).

(10) Directive 98/83/EC of the Council of 3 November 1998. *Off. J. Eur. Communities* **1998**, *L330*, 32−54.

(11) *Guidelines for Drinking-Water Quality*, 4th ed.; World Health Organization: Geneva, Switzerland, 2011.

(12) Jones, D. B.; Song, H.; Karanfil, T. The effects of selected preoxidation strategies on I-THM formation and speciation. *Water Res.* **2012**, *46*, 5491−5498.

(13) Bichsel, Y.; Gunten, U. V. Formation of iodo-trihalomethanes during disinfection and oxidation of iodide-containing waters. *Environ. Sci. Technol.* **2000**, *34*, 2784−2791.

(14) Richardson, S. D.; Thruston, A. D., Jr.; Caughran, T. V.; Chen, P. H.; Collette, T. W.; Schenck, K. M.; Lykins, B. W., Jr.; Rav-Acha, C.; Glezer, V. Identification of new drinking water disinfection by-products from ozone, chlorine dioxide, chloramine, and chlorine. *Water, Air, Soil Pollut.* **2000**, *123*, 95−102.

(15) *Exposure Factors Handbook, General Factors, Vol. 1*; U.S. Environmental Protection Agency, Office of Research and Development: Washington, DC, USA, 1997; EPA /600/P-95/002Fa.

(16) Food and Agriculture Organization of the United Nations/World Health Organization. *Benefits and Risks of the Use of Chlorine-Containing Disinfectants in Food Production and Food Processing*; Rome, Italy, 2008.

(17) Huang, A. T.; Batterman, S. Formation of trihalomethanes in foods and beverages. *Food Addit. Contam.* **2009**, *26*, 947−957.

(18) Graham, R. C.; Robertson, J. K. Analysis of trihalomethanes in soft drinks. *J. Chem. Educ.* **1988**, *65*, 735−737.

(19) Miyahara, M.; Toyoda, M.; Ushijima, K.; Nose, N.; Saito, Y. Volatile halogenated hydrocarbons in foods. *J. Agric. Food Chem.* **1995**, *43*, 320−326.

(20) Page, B. D.; Lacroix, G. Application of solid-phase microextraction to the headspace gas chromatographic analysis of halogenated volatiles in selected foods. *J. Chromatogr.* **1993**, *648*, 199−211.

(21) Campillo, N.; Viñas, P.; López-García, I.; Aguinaga, N.; Hernández-Córdoba, M. Purgue-and-trap capillary gas chromatography with atomic emission detection for volatile halogenated organic compounds determination in waters and beverages. *J. Chromatogr., A* **2004**, *1035*, 1−8.

(22) Dos Santos, M. S.; Martendal, E.; Carasek, E. Determination of THMs in soft drink by solid-phase microextraction and gas chromatography. *Food Chem.* **2011**, *127*, 290−295.

(23) Dos Santos, M. S.; Carasek, E. Development of a simple analytical method for determining trihalomethanes in beer using a headspace solid-phase microextraction technique. *Quim. Nova* **2013**, *36*, 1052−1056.

(24) Pizarro, C.; Pérez-del-Notario, N.; González-Sáiz, J. M. Optimisation of a simple and reliable method based on headspace solid-phase microextraction for the determination of volatile phenols in beer. *J. Chromatogr., A* **2010**, *1217*, 6013−6021.

(25) Miller, N. J.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed.; Prentice Hall: Englewood Cliffs, NJ, USA, 2005.

(26) Directive 2001/112/CE of the Council of 20 December 2001. *Off. J. Eur. Communities* **2001**, *L10*, 58−66.

(27) World Health Organization. *Guidelines for Drinking-Water Quality, Vol. 1, Recommendation*, 3rd ed.; Geneva, Switzerland, 2008.

(28) World Health Organization. *Domestic Water Quantity, Service Level and Health*; Geneva, Switzerland, 2003.