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Effect of the Chlorinated Washing of Minimally Processed Vegetables on the Generation of Haloacetic Acids

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ABSTRACT: Chlorine solutions are usually used to sanitize fruit and vegetables in the fresh-cut industry due to their efficacy, low cost, and simple use. However, disinfection byproducts such as haloacetic acids (HAAs) can be formed during this process, which can remain on minimally processed vegetables (MPVs). These compounds are toxic and/or carcinogenic and have been associated with human health risks; therefore, the U.S. Environmental Protection Agency has set a maximum contaminant level for five HAAs at 60 μg/L in drinking water. This paper describes the first method to determine the nine HAAs that can be present in MPV samples, with static headspace coupled with gas chromatography−mass spectrometry where the leaching and derivatization of the HAAs are carried out in a single step. The proposed method is sensitive, with limits of detection between 0.1 and 2.4 μg/kg and an average relative standard deviation of ∼8%. From the samples analyzed, we can conclude that about 23% of them contain at least two HAAs (<0.4−24 μg/kg), which showed that these compounds are formed during washing and then remain on the final product.

KEYWORDS: *minimally processed vegetables, chlorine solutions as sanitizer, haloacetic acids, headspace gas chromatography−mass spectrometry*

INTRODUCTION

Food contaminants cover a diverse range of compounds from a number of potential origins. They can be present at trace and ultratrace levels; therefore, identification and quantitation in foods can present a challenge for the analytical chemist. An emergent contaminant group is that of disinfection byproducts (DBPs) formed during the disinfection of the water, which has been one of the major public health advances of the 20th century, since it is effective in destroying microorganisms that cause disease in humans.[1] Ingestion of DBPs can occur as direct beverages of treated water or as a result of a simple inclusion or more complex interactions of treated water with foods. Haloacetic acids (HAAs) are the second most prevalent class of DBPs (after trihalomethanes) formed during the disinfection of water with disinfectants mainly with chlorination.[2] Toxicological studies on laboratory animals have found that some HAAs exhibit toxicity and carcinogenic activities[3−5] and may have adverse reproductive outcomes.[6] Five HAAs have been evaluated by the World Health Organization (WHO) IARC as probable (group A2) or possible carcinogens (group B2) to humans.[7] Because of their potentially harmful effects on human health, HAAs have been receiving a lot of attention in recent years, and many countries or international organizations have promulgated regulations to control these compounds in drinking water. The U.S. Environmental Protection Agency (EPA) has set the maximum contaminant level of the sum of five HAAs at 60 μg/L;[8] the WHO set the guidelines of monochloroacetic (MCAA), dichloroacetic (DCAA), and trichloroacetic acids (TCAA) at 20, 50, and 200 μg/L, respectively;[9] and the European Union is considering regulating the nine HAAs at 80 μg/L.[10] In spite of this, the EPA only has established a safety margin for three (of the nine species) HAAs in drinking water; the maximum contaminant level goals are 0.07, 0, and 0.02 mg/L for MCAA, DCAA, and TCAA, respectively.[11] However, there is no regulation for food. Drinking water is the first source of ingestion of HAAs for humans, but there are other potential contamination sources such as direct exposition of foods to disinfected water (e.g., juices, coffee, teas, etc.). Thus, a possible source of exposure that has become important over the past few years are minimally processed vegetables (MPV),[12] also called ready-to-use fruit and vegetables. The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruit or vegetables that have been trimmed and/or peeled and/or cut into 100% usable products that are bagged or prepackaged to offer consumers high nutrition, convenience, and flavor while still maintaining their freshness.[13] Both microbial and biochemical activities, enhanced by the peeling and cutting operations, contribute to MPV instability during its shelf life.[14] Washing after peeling and cutting removes microbes and tissue fluids and thus reduces microbial growth and enzymatic oxidation during storage, which significantly increases the shelf life of MTV.[15] Techniques to extend the quality of MPV include chemical-based washing treatments (viz. chlorine, organic acids, hydrogen peroxide, calcium salts, ozone, etc.); the advantages and shortcomings of each of them have been reviewed.[16] Chlorine-based chemicals, particularly liquid chlorine, chlorine dioxide, and hypochlorite, are the most widely used sanitizers for decontaminating fresh produce. In some European countries including Germany, The Netherlands, Switzerland, and Belgium, the use of chlorine is prohibited. The levels of free chlorine in the washing tanks range from 50 to 200 mg/L, whereas in drinking water, their

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values are lower (~0.4 mg/L).[16] The ionic nature and lower volatility of HAAs at typical food pH (5−6.5) can help explain their presence in them. However, although vegetables can become contaminated with residues of HAAs and other DBPs formed during the washing step with chlorinated water, a scan of the available literature revealed the lack of a specific method and studies about these compounds in MPV.

Despite the toxic effects and potential human exposure to DBPs through food, there is scarce information about the levels of DBPs (and what exists mainly refers to chloroform) in foods and beverages prepared using chlorinated drinking water. Thus, Huang and Batterman have measured trihalomethanes in 11 foods and 17 beverages prepared in chlorinated water. Tea formed the highest chloroform levels (up to 67 μg/L), followed by coffee, rice, soups, and vegetables (51 μg/L).[17]

For the determination of HAAs by gas chromatography (GC), a prior derivatization step is necessary due to their low volatility and high polarity. This step is usually carried out by esterification of the carboxylic group (typically methylation with acidic methanol) after a leaching of the solid food and liquid−liquid extraction to an organic phase for chromatographic separation.[18,19] An adapted EPA Method 552.2 (for the determination of HAAs in water) has been proposed to determine HAAs and other DBPs in spiked foods and beverages prepared with relatively large amounts of tap water.[18] The derivatization of the HAAs to their respective methyl esters was carried out with acidic methanol (65 °C, 3 h), and their determination by GC with electron capture detection (ECD). The method not only is labor-intensive and time-consuming but also results in poor precision [relative standard deviation (RSD), ~30%] and inadequate sensitivity. To our knowledge, there is only one method to determine the nine HAAs in spiked vegetable samples, which is focused on the possible presence of HAAs in these samples as a result of agricultural irrigation with chlorinated water containing HAAs, which is unlikely to be used as it is more expensive than nondrinking water.[19] The method allows a simultaneous leaching and derivatization of HAAs from vegetable samples with acidic methanol as both an extractant and a derivatization reagent. After leaching, several steps including solvent change-over and cleanup prior to analysis by GC-ECD were required.

In a previous work, we developed a method for the determination of the nine HAAs by headspace (HS)-GC-MS in water samples in which the derivatization of the HAAs was carried out in an aqueous medium with tetrabutylammonium hydrogen sulfate (TBA-HSO_4) as the ion-pairing agent and DMS as the methylation agent.[20] In this study, we demonstrated that the addition of aliquots of *n*-pentane increases the derivatization yields of HAAs as well as minimizes their decomposition to trihalomethanes. A static HS technique is being considered as an interesting alternative in the analysis of foods due to the absence of matrix effects, in detriment to liquid−liquid extraction alternatives. The aims of the present work were to (i) develop a sensitive/selective method for the determination of the nine HAAs in MPV samples by using static HS-GC-MS; (ii) detect the HAAs that can be formed by the direct interaction of vegetables with disinfectants during industrial washing, with typical contact times of less than 5 min; and (iii) establish if these contaminants remain after packaging and storage/distribution taking into account their nonvolatile nature. In accordance with this and taking into account the toxicity of these HAAs, it would be desirable to include these compounds on the list of emerging contaminants in ready-to-use vegetables and fruits.

MATERIALS AND METHODS

Reagents. MCAA, monobromoacetic (MBAA), DCAA, TCAA, bromochloroacetic (BCAA), dibromoacetic (DBAA), bromodichloroacetic (BDCAA), dibromochloroacetic (DBCAA), and tribromoacetic (TBAA) acids, 1,2-dibromopropane (internal standard, IS), and 2,3-dibromopropionic acid (surrogate standard) were supplied by Sigma-Aldrich (Madrid, Spain). The derivatization reagent, dimethylsulfate (DMS), the ion-pairing agent, TBA-HSO_4, and anhydrous sodium sulfate were purchased by Fluka (Madrid, Spain). Sulfuric acid, *n*-pentane, methyl *tert*-butyl ether, and ethanol were supplied by Merck (Darmstadt, Germany). Stock standard solutions containing 1 g/L of each HAA were prepared in methyl *tert*-butyl ether and stored in amber glass vials at −20 °C. More dilute cumulative solutions were prepared daily in ethanol at the microgram-per-liter level and used to spike uncontaminated whole vegetables. Milli-Q water was discarded since it contains a few μg/L of DBPs; thus, mineral water (the only untreated water and therefore free of HAAs) was used in the experiments.

Instruments. Static HS-GC analyses were carried out using an HP 6890 N gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with an HP 7694 HS autosampler and an HP 5973 N mass selective detector. The autosampler consisted of an oven, to heat up to six vials, a carousel (with capacity for 44 vials) equipped with a robotic arm, and a six-port injection valve with a 3 mL loop. A sample HS was collected through the 3 mL sample loop and automatically transferred to the GC via a heated transfer line. The preliminary HS autosampler conditions used for the HS-GC-MS operation were as follows: oven temperature, 70 °C; vial equilibration time, 20 min with high-speed shaking; vial pressurization time, 30 s; venting time, 12 s; and loop fill time, 3 s. The sample valve loop and transfer line temperatures were set at 90 and 100 °C, respectively. Helium (6.0 grade purity, Air Liquid, Seville, Spain) regulated with a digital pressure and flow controller was used both to pressurize vials and to drive the HS formed to the injection port of the gas chromatograph. Injection was done in the split mode (split ratio 1:20) for 1 min with an inlet temperature of 200 °C. The gas chromatographic separation was achieved on a 30 m × 0.25 mm i.d., 0.25 μm film SLB-5MS capillary column coated with a stationary phase consisting of 5% phenyl−95% methylpolysiloxane supplied by Supelco (Madrid, Spain). The chromatographic oven temperature program was as follows: 40 °C (3 min), raised to 60 at 20 °C/min (3 min), then increased to 100 at 5 °C/min, and finally to 250 at 25 °C/min (3 min); the chromatographic run was complete in 24 min. Helium carrier gas was passed at a constant rate of 1 mL/min. The mass spectrometer was operated in the electron ionization mode at 70 eV and ion source and quadrupole temperatures of 230 and 150 °C, respectively. A solvent delay of 4 min was set to protect the filament from oxidation. Optimization experiments were conducted in total ion chromatography (TIC) mode at 3.5 scans/s. Quantitation of HAA methyl esters was performed in selected ion monitoring (SIM) mode, and five different acquisition windows were defined taking into account the retention times and suitable fragments of HAA methyl esters (the base peaks used for quantitation are boldfaced): **59**, 79, 108 (MCAA); **59**, 93, 95 (MBAA); **59**, 83 85 (DCAA); 59, **117**, 119 (TCAA); 59, 127, **129** (BCAA); 59, 171, **173** (DBAA); **59**, 161, 163 (BDCAA); 59, **207**, 209 (DBCAA); **59**, 251, 253 (TBAA); 42, 123, **121** (1,2-dibromopropane, the IS); and 59, **165**, 167 (2,3-dibromopropionic acid, surrogate standard). All scans were performed in high-resolution mode and with a dwell time of 100 ms. The system operation, as well as data acquisition, collection, and evaluation, were accomplished using an Agilent MSD ChemStation software version G1701DA D.01.02 (Agilent Technologies).

Sampling. MPVs and whole vegetables were purchased at local markets in Spain from November, 2011, until February, 2012. The MPV samples analyzed included the following : 40 single ingredients [three of chopped onion, four of grated carrot, three of green pepper,

10 of chicory, 10 of lettuce (iceberg, batavia, and romaine), and 10 of spinach] and 60 ready-to-eat salads, containing from three to five ingredients such as lettuce (different varieties), chicory, carrots, spinach, and red cabbage. All of the salad plastic bags were kept refrigerated and within the use-by date before their analyses. Samples were analyzed unwashed since the HAAs are expected to be found on the vegetable surface. Thus, a global sample consisting of 15−20 units of each whole vegetable or 20−50 packs of each MPV (125−300 g) was initially selected and subsequently reduced by quartering to 2−4 packs (0.5−1 kg, laboratory sample). In the case of whole vegetables (lettuce and spinach), the laboratory sample was cut in pieces or slices of ∼5 g and then reduced by quartering to ∼50 g (sample test). The packs of MPV were opened, and the content was also quartered to ∼50 g (sample test). The sample test was then chopped into smaller pieces (40−80 mg) to obtain the 3 g fractions required by the proposed method and immediately analyzed.

Stability of HAAs in Vegetables. A thorough study was performed concerning the stability of the nine HAAs in iceberg lettuce samples spiked with 20 μg/kg of each compound. Spiked lettuce samples were stored in amber glass containers at 4 °C and analyzed at 1 h intervals the first day and of 4−8 h subsequently up to 5 days. The results obtained show that the concentrations of five HAAs (DCAA, TCAA, BCAA, DBAA, and BDCAA) remained constant up to 3 days, after which they decreased slightly. There were significant differences for the other compounds (MCAA, MBAA, CDBAA, and TBAA) since they only remained constant up to 36 h. Therefore, the samples were fortified overnight (24 h) to ensure the stability of the nine HAAs.

Fortification Process. The vast majority of fresh minimally processed manufacturers use chlorine-based washing as a sanitizer. Therefore, DBPs including HAAs can be formed and retained on vegetable surface. To ensure reliable simulation on the real contamination, the fortification process was carried out as follows: 3 g of chopped whole vegetable was spiked with 1 mL of ethanol containing variable concentrations of each HAAs (between 1.2 and 450 ng; 0.4 and 150 μg/kg) and 60 ng (20 μg/kg) of 2,3-dichloropropanoic acid (surrogate standard). The spiked sample was then allowed to stand at room temperature in a closed fume hood during 1 h to evaporate the ethanol and then overnight at 4 °C to simulate potential analyte interaction with the sample matrix (weathering process). Blanks and standard calibration graphs were run by using uncontaminated vegetables.

Analytical Method. An accurately weighed amount (∼3 g) of vegetables (HAA concentrations, 0.4−8 to 150 μg/kg) containing 20 μg/kg of 2,3-dichloropropanoic acid (surrogate standard) was added to a 20 mL glass vial and supplied with 10 mL of 0.5 g/mL Na_2SO_4 solution at pH ∼4 (containing 5 μg/L of 1,2-dichloropropane, IS). Then, 100 μL of 0.05 mol/L of the ion-pairing agent (TBA-H_2SO_4), 100 μL of derivatization reagent (DMS), and 150 μL of *n*-pentane were added sequentially, after which the vial was sealed and vortexed for 3 min to homogenize it. Finally, the samples were analyzed by HS-GC-MS using the operating conditions mentioned above. The HAA concentrations were calculated by relating to previously created calibration curves, where the peak area ratios (sample/IS) were plotted as a function of the sample concentration.

RESULTS AND DISCUSION

pH Impact. Although this work is focused on MPVs, special attention has been paid to fresh lettuce and spinach since they are the most popular ready-to-eat vegetables. Initially, the optimization of the method was done with raw lettuce (iceberg variety), without the washing and freeing of HAAs, as the model for its use in salads. To optimize the chemical variables, 2 g of iceberg lettuce spiked with 20 μg/kg of each HAA and surrogate standard (2,3-dibromopropionic acid), 10 mL of 0.5 g/mL Na_2SO_4 solution containing a 5 μg/L concentration of 1,2-dichloropropane (IS), 125 μL of a 0.05 M TBA-HSO_4 solution and 100 μL of pure DMS (derivatization reagents), and 150 μL of *n*-pentane were added in 20 mL glass vials.

The leaching of the HAAs from the vegetable samples along with the generation of the HS are markedly affected by the sample pH due to the strong acidic and the hydrophilic character of these compounds. As a result, the first chemical variable studied was the influence of the pH on the leaching of the nine HAAs in the Na_2SO_4 solution, whose pH was made to range from 1 to 6 (adjusted with diluted sulfuric acid). As can be seen in Figure 1, all HAAs were effectively extracted in the

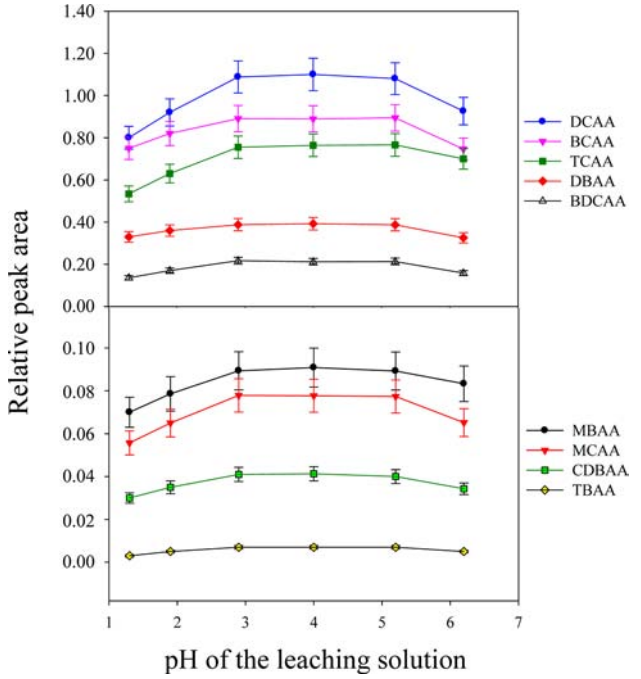

Figure 1. Influence of the pH of the leaching solution on the extraction of the nine HAAs. Error bars are the standard deviation for three measurements.

pH range 3−5. It is known that the addition of a soluble salt increases the ionic strength of the aqueous solution. This makes organic compounds less soluble, so the analyte partitioning coefficients, between the sample and the HS, can increase several times. The abundance signal increased for the nine HAAs as the Na_2SO_4 concentration increased up to 0.5 g/mL. Therefore, a 0.5 g/mL Na_2SO_4 solution at pH ∼4 was selected as the aqueous extractant.

Derivatization Agents. The effect of the concentration of the derivatizing reagent was studied using amounts of pure DMS between 50 and 200 μL. The reaction yield for the nine HAAs increased as the volume rose to 90 μL, above which it remained constant. Taking into account that above 120 μL the excess of DMS was extracted in *n*-pentane and volatilized, appearing in the chromatogram, 100 μL was chosen as the optimal volume. As mentioned above, the addition of TBA-HSO_4 as an ion-pairing agent increased the derivatization yields of the HAAs; volumes between 50 and 200 μL of a 0.05 mol/L TBA-HSO_4 solution were assayed in the reaction. The optimal relative peak areas were obtained above 80 μL, remaining constant from this value. Therefore, 100 μL was finally chosen as the optimal value. The addition of *n*-pentane into the vial as an organic modifier increases the derivatization yields of the HAAs; thus, volumes of the *n*-pentane in the interval 50−200

μL were assayed. The signal abundance increased for all HAAs on the *n*-pentane volume up to 150 μL, above which it decreased, probably due to the fact that the volatilization of the organic phase was incomplete, leaving some amounts of analytes in the organic phase.

Ratio Sample: Extraction Volume. The effect of the ratio of the amount of the sample to extractant volume (w/v) was optimized. For this purpose, different samples of 1−4 g of chopped lettuce were fortified with the same amount (60 ng) of each HAA and allowed to stand overnight. Then, the samples were extracted with 10 mL of 0.5 g/mL Na_2SO_4 solution. Similar results were obtained in all cases, although for 4 g of vegetable, the precision (n = 3) was lower probably because the homogenization of the mixture is difficult, which then hinders the extraction of the compounds. So, the amount of sample can vary between 1 and 3 g with little difference in the efficiency of extraction; to increase the sensitivity of the method, 3 g was adopted as the optimal value. Finally, the optimal volume of the 0.5 g/mL Na_2SO_4 solution at pH ∼4 (extractant) was examined from 5 to 12 mL per 3 g of sample (in 20 mL vials). To ensure that the autosampler needle would not come into contact with the sample during the sampling time of the HS, 12 mL of extractant was taken as the highest value (3 g of lettuce sample and 12 mL of extractant solution occupy ∼3/4 of the vial volume). The signal abundance increased as the extractant volume did up to 8 mL. At lower volumes, the complete extraction of all compounds was not reached, probably because the sample was not suitably homogenized into the aqueous medium. Therefore, a portion of 3 g of vegetable and 10 mL of 0.5 g/mL Na_2SO_4 solution as extractant was adopted in the proposed method since it provided better results in terms of both sensitivity and reproducibility. To obtain replicate results, it is necessary to homogenize the solid and liquid phases. The best extraction efficiency and reproducibility of the method were obtained when the sample was vortexed for 3 min before its introduction into the HS unit.

Optimization of Instrumental HS Variables. There are many instrumental parameters of the HS autosampler that can affect analytical properties, such as sensitivity and precision of the method, among others. The instrumental parameters most closely related to the HAA concentration in the HS unit were oven temperature and the vial equilibration time; their effects were studied over the ranges 50−80 °C and for 10−40 min, respectively. The analytical signals significantly increased for seven HAAs (MCAA, MBAA, DCAA, TCAA, BCAA, DBAA, and BDCAA) as the oven temperature and equilibration time rose to 70 °C and 20 min, respectively. In this condition, some degradation (∼10%) of the CDBAA and TBAA to their respective trihalomethanes was observed in the chromatogram since the brominated trihaloacetic acids are the most unstable compounds.[20] To obtain the best adequate response for the seven HAAs and taking into account that the brominated HAAs are not prevalent in chlorinated water, an oven temperature of 70 °C and a vial equilibration time of 20 min were selected as a compromise for subsequent studies. The amount of HS sample to be analyzed by GC is related to the split ratio, vial pressurization, and the filling of the 3 mL loop of the injection valve by venting the vial. Different inlet split ratios (between 1:10 and 1:40) were studied to obtain the best sensitivity and resolution of chromatographic peaks of the HAAs. A split ratio of 1:20 was selected as the optimal value. Pressurization and venting times were finally assayed between 12 and 45 s. No significant changes in the abundance signals for the nine compounds were obtained for pressurization times above 15 s and a venting time above 12 s. Values of 30 and 12 s for the pressurization time and venting time, respectively, were chosen as optimal.

Efficiency of the Leaching/Derivatization Process. In the present study, the analytes required the extraction from vegetable samples and then a derivatization step to increase their volatility. So far, leaching and derivatization of the vegetable sample were simultaneously carried out into the HS unit, but it would be of interest to establish if this process is exhaustive. To check on this, two experiments in quintuplicate were conducted in parallel: (i) Three grams of iceberg lettuce (spiked with 20 μg/kg of each HAAs and 2,3-dibromopropionic acid) was extracted with variable volumes (5−10 mL) of extractant (0.5 g/mL Na_2SO_4 at pH ∼4). The mixture was vortexed for 3 min, after which it was centrifuged; the supernatant was added to a 20 mL vial together with the derivatizing reagents and finally introduced into the HS unit (sequential leaching and derivatization process, experiment A). (ii) A similar process to the previous one was performed by adding simultaneously 3 g of iceberg lettuce and the derivatizing reagents in a 20 mL vial; likewise, the volume of extractant was varied from 5 to 10 mL (simultaneous leaching/derivatization process, experiment B). The results of both experiments are shown in Figure 2, from which the following

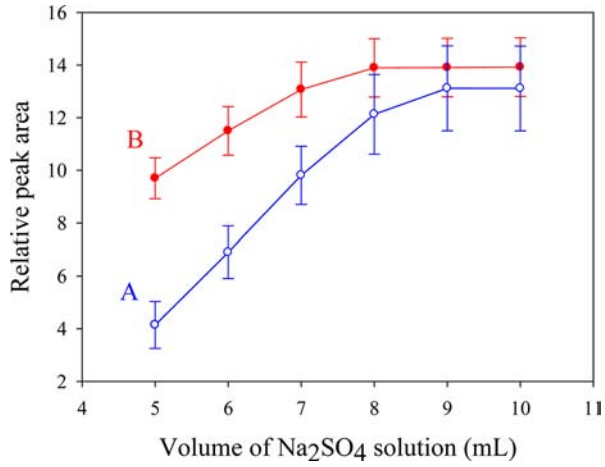

Figure 2. Efficiency of the leaching and derivatization process carried out sequentially (A) or simultaneously (B) by using variable volumes of extractant. The analytical signal is expressed as the sum of relative responses of the nine HAAs.

conclusions can be drawn: (i) the recoveries were higher when the analytical process (leaching/derivatization) was carried out simultaneously; (ii) better results were obtained from 8 mL of extractant in the simultaneous process, whereas for the sequential one, at least 9 mL was required. This behavior can be ascribed to the fact that the leaching of the compounds was favored when the vial was heated in the HS unit; (iii) the precision was slightly better when the process was carried out simultaneously since the centrifugation increased the number of steps and consequently the associated errors. The efficiency of the simultaneous leaching/derivatization step was assessed with a second leaching/derivatization of the same sample with fresh derivatizing reagent solutions. The average relative process efficiency was calculated in quintuplicate, using a normalization method in which the combined analytical signal obtained in the two sequential extractions was assigned a value of 100%. The

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results obtained in the first run were 94 ± 5% for the nine analytes, whereas the second run provided negligible extraction values. From these results, the direct pretreatment of the vegetables in the HS unit was proposed as a simple technique that allows the simultaneous in situ extraction/derivatization of the HAAs, which greatly simplifies sample treatment.

Method Performance Characteristics. The performance and reliability of the proposed method were assessed by determining the calibration curves, linear range, analyte detectability, and standard deviation for the nine HAAs. For this purpose, individual amounts of 3 g of uncontaminated iceberg lettuce sample were spiked with standard solutions containing all HAAs at variable concentrations (in ethanol) and analyzed by using the analytical procedure described in the Materials and Methods. Calibration curves were obtained by plotting the analyte to the IS peak area against analyte concentrations. The limits of detection (LODs), the linear range, and the precision, expressed as RSD, are summarized in Table 1. LODs were calculated as the minimum concentrations providing chromatographic signals three times higher than background noise.[21] Limits of quantitation, which were set at the lower concentrations of the linear ranges, ranged between 0.4 and 8 μg/kg. The linearity of the HS-chromatographic method was satisfactory in the range of concentrations from 0.4−8 to 150 μg/kg with regression coefficients >0.995. Worth special note is the high sensitivity for TCAA and DCAA, which are typically found in chlorinated water. The within-day precision was evaluated by analyzing 11 samples containing 5 μg/kg of each HAA (15 μg/kg for TBAA). The precision obtained was acceptable for all of the compounds with an average value of 8 ± 2%. For comparison to previously reported methods, the LODs of the two alternatives for the determination of HAAs in spiked vegetables are also shown in Table 1. The proposed method provided average LODs (0.64 ± 0.73 μg/kg) lower than those obtained by the adapted EPA Method 552.2 for the determination of HAAs in spiked food samples (average LODs, 91 ± 54 μg/kg).[18] This value is better than those provided by the only method proposed to determine HAAs in spiked (at 0.8−4 mg/kg levels) spinach and chard (average LODs, 2.2 ± 3.2 μg/kg), which is also listed in Table 1.[19]

Table 1. LODs, Linearity, and Precision for the Determination of HAAs in Lettuce Samples by HS-GC-MS

	HS-GC-MS			LOD (μg/kg)	
compd	LOD (μg/kg)	linear range (μg/kg)	RSD (%)	method A[a]	method B[b]
MCAA	0.62	2−150	10.2	130	9.7
MBAA	0.61	2−150	10.4	170	4.9
DCAA	0.10	0.4−150	6.7	60	0.5
TCAA	0.12	0.4−150	6.4	20	1.0
BCAA	0.11	0.4−150	6.5	75	0.7
DBAA	0.22	0.8−150	7.0	40	0.5
BDCAA	0.58	2−150	6.9	155	1.1
CDBAA	1.00	3−150	7.5	125	0.4
TBAA	2.40	8−150	11.5	48	0.5

[a]Method A: adapted EPA Method 552.2 and analysis by GC-ECD (data taken from ref 16). [b]Method B: ultrasonic-assisted leaching with in situ derivatization and analysis by GC-ECD (data taken from ref 17).

The feasibility of the proposed method for the determination of HAAs in different models of vegetable (leafy and root) was evaluated by analyzing six uncontaminated samples spiked with 5 or 20 μg/kg of each HAA (15 or 30 μg/kg for TBAA). A wide variety of vegetables routinely employed for the production of MPV was considered, namely, carrot, green pepper, iceberg lettuce, onion, spinach, and tomato. Each sample of 3 g was spiked at the two concentrations in quintuplicate (n = 5) and analyzed by using the analytical procedure described in the Materials and Methods. The results were compared to those obtained with spiked aqueous extractant and reagent solutions analyzed under identical conditions. As can be seen in Table 2, there are no significant differences between the average recoveries of the nine HAAs in each type of vegetable. Lower precision was observed for spinach samples due to the higher volume of the sample, which hindered homogenization in the vortex mixer. Average recoveries (89−96%) are better than those provided by the two existing alternatives, the adapted EPA Method 552.2 (70−130%)[18] and ultrasonic-assisted leaching (80−115%).[19] The results obtained testify to the high selectivity of the proposed method since no interferences from the matrices studied were observed.

Applications. The proposed method was applied to determine HAAs in 100 MPV samples. Samples were analyzed by using the analytical procedure described in the Materials and Methods. Table 3 lists the concentrations of the 1−5 HAAs found at detectable concentrations in the 23 positive samples (23% of the samples analyzed). The analytes not shown were

Table 2. Percent Recovery (±SD, n = 5) of the HAAs Spiked to Vegetables at Low[a] and Medium[b] Levels

	carrot		green pepper		iceberg lettuce		onion		spinach		tomato	
compd	low level	medium level	low level	medium level	low level	medium level	low level	medium level	low level	medium level	low level	medium level
MCAA	95 ± 10	97 ± 8	96 ± 10	99 ± 9	94 ± 11	95 ± 10	95 ± 10	99 ± 10	92 ± 12	94 ± 10	94 ± 10	95 ± 8
MBAA	94 ± 10	96 ± 9	94 ± 10	96 ± 10	92 ± 11	93 ± 10	93 ± 11	96 ± 10	91 ± 12	92 ± 11	92 ± 10	93 ± 9
DCAA	95 ± 7	97 ± 6	93 ± 7	97 ± 7	92 ± 8	94 ± 7	94 ± 7	96 ± 6	89 ± 8	92 ± 8	93 ± 7	95 ± 5
TCAA	96 ± 7	97 ± 6	95 ± 7	97 ± 6	91 ± 7	95 ± 7	93 ± 7	95 ± 7	88 ± 8	91 ± 7	92 ± 6	96 ± 5
BCAA	95 ± 8	95 ± 7	94 ± 7	96 ± 6	92 ± 7	93 ± 6	93 ± 7	95 ± 6	89 ± 9	90 ± 9	94 ± 7	97 ± 6
DBAA	92 ± 8	93 ± 8	90 ± 8	94 ± 7	91 ± 8	92 ± 8	91 ± 8	93 ± 7	90 ± 9	92 ± 8	94 ± 7	96 ± 6
BDCAA	91 ± 7	93 ± 7	93 ± 9	95 ± 8	90 ± 8	92 ± 7	92 ± 7	93 ± 7	90 ± 9	92 ± 9	95 ± 8	98 ± 7
CDBAA	90 ± 9	92 ± 7	92 ± 9	92 ± 8	89 ± 9	91 ± 8	90 ± 9	91 ± 8	88 ± 10	90 ± 9	93 ± 8	96 ± 7
TBAA	89 ± 10	92 ± 9	90 ± 10	93 ± 10	88 ± 11	90 ± 9	91 ± 10	91 ± 9	87 ± 12	89 ± 10	94 ± 10	96 ± 8
average	93 ± 8	94 ± 7	93 ± 8	95 ± 7	91 ± 9	93 ± 8	92 ± 8	94 ± 8	89 ± 10	91 ± 9	93 ± 8	96 ± 7

[a]5 μg/kg for each HAA (15 μg/kg for TBAA). [b]20 μg/kg for each HAA (30 μg/kg for TBAA).

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Table 3. HAA Concentrations[a] (μg/kg) Found in the MPV Samples Analyzed

	DCAA	TCAA	BCAA	DBAA	BDCAA
carrot	6.3 ± 0.5	13 ± 1	1.2 ± 0.1	0.9 ± 0.1	<2
green pepper	ND[b]	0.7 ± 0.1	ND	ND	ND
iceberg lettuce 1	15 ± 1	18 ± 1	3.9 ± 0.3	1.3 ± 0.1	3.2 ± 0.2
iceberg lettuce 2	3.1 ± 0.2	3.7 ± 0.3	<0.4	ND	ND
iceberg lettuce 3	1.4 ± 0.1	5.9 ± 0.4	0.6 ± 0.1	ND	<2
romaine lettuce 1	2.2 ± 0.2	1.9 ± 0.1	0.5 ± 0.1	ND	ND
romaine lettuce 2	3.5 ± 0.3	5.2 ± 0.4	ND	<0.8	<2
chicory	<0.4	1.6 ± 0.1	ND	ND	ND
spinach 1	ND	0.5 ± 0.1	ND	ND	ND
spinach 2	ND	1.8 ± 0.1	ND	ND	ND
spinach 3	1.5 ± 0.1	1.9 ± 0.1	0.6 ± 0.1	ND	ND
spinach 4	1.7 ± 0.1	5.3 ± 0.4	0.5 ± 0.1	ND	ND
spinach 5	16 ± 1	19 ± 1	4.1 ± 0.3	<0.8	ND
mixed salad 1	<0.4	0.6 ± 0.1	ND	ND	ND
mixed salad 2	0.5 ± 0.1	1.1 ± 0.1	ND	ND	ND
mixed salad 3	0.6 ± 0.1	1.4 ± 0.1	ND	ND	ND
mixed salad 4	0.8 ± 0.1	0.5 ± 0.1	ND	ND	ND
mixed salad 5	1.1 ± 0.1	4.3 ± 0.3	0.5 ± 0.1	ND	ND
mixed salad 6	1.2 ± 0.1	0.6 ± 0.1	<0.4	ND	ND
mixed salad 7	2.1 ± 0.2	1.6 ± 0.1	0.9 ± 0.1	ND	<2
mixed salad 8	2.8 ± 0.2	3.2 ± 0.2	0.5 ± 0.1	<0.8	<2
mixed salad 9	3.1 ± 0.2	4.5 ± 0.3	1.0 ± 0.1	ND	<2
mixed salad 10	22 ± 2	24 ± 2	3.2 ± 0.2	1.4 ± 0.1	4.8 ± 0.3

[a] ±Standard deviation, n = 5. [b] Not detected.

either undetectable or present at levels below their LODs. As can be seen, two chlorinated HAAs (DCAA and TCAA) were found in practically all of the samples, whereas brominated HAAs (BCAA, DBAA, and BDCAA) were occasionally present and at lower concentrations (chlorinated HAAs were found at concentrations six times higher than brominated ones). This can be explained because, as mentioned above, a chlorine solution is generally employed in the washing step due to its economic impact and simple use. Most of the MPV contained HAAs at low levels, except four samples [one of grated carrot (from 4), lettuce 1 (from 10), spinach 5 (from 10), and mixed salad 10 (from 60)] that contained concentrations about 5−8 times higher than the other positive samples. For comparison, 25 samples of whole vegetables (15 of romaine lettuce and 10 of spinach) were also analyzed. These whole vegetables come directly from the field and had not experienced any industrial process; therefore, it was expected that these samples would be free of HAAs. No HAAs were detected in all of the 25 whole vegetables analyzed. Therefore, the HAA found in the MPV samples can be ascribed to the use of chlorine solutions, in the washing step, adopted by the fresh-cut industry due to their efficacy, cost-effectiveness ratio, and simple use. In our opinion, although HAAs can be present in MPV at low levels (mostly well below 10 μg/kg), it would be convenient to propose other washing alternatives (viz. organic acids, calcium lactate, γ-irradiation, etc.) to satisfy the consumers and maintain a balance between sensory, quality, and security of the MPV samples.

According to the literature, MPVs that do not contain any preservatives and have not gone through any heat or chemical treatment are becoming more and more popular in the market. The only "disadvantage" of these products is that refrigeration storage is essential, limiting its practice to "developed countries". However, this paper has demonstrated that minimally processed fresh food can be contaminated by DBPs in an additional preparation step, introduced by the fresh-cut industry, where they are washed with disinfectants. To avoid contamination with bacteria (e.g., coliforms), refrigerating the plastic bags is recommended as well as rinsing the vegetables, but not soaking them in water. However, what can be found in scientific literature about the removal of HAAs or other DBPs? In this sense, we have carried out a study on the cleaning MPV at home using home friendly products. So, four common alternatives for household vegetable washing were studied, namely, tap water, tap water containing common salt (~5 g/L of NaCl), and tap water with a ~10 drops of vinegar or five drops of 15% (v/v) sodium hypochlorite. Several portions of uncontaminated salads (~150 g) were spiked with 20 μg/kg of each HAA (overnight) and then rinsed, with the four alternatives above-mentioned, for 1 min (each rinse experiment was carried out in triplicate, n = 3). Following the rinsing process, the vegetable samples were subjected to centrifugation in a conventional salad colander to remove the remaining tap water. A parallel experiment was carried out using mineral water (this water does not contain HAAs) instead of tap water (that contains 15 μg/L of total HAAs) to discard possible contamination of MPV from the remaining tap water. Each rinsed MPV was analyzed by the proposed method in quintuplicate (using 3 g of chopped sample, n = 5). The rinse with salted tap water provided the best results since almost 70−80% (n = 15) of the total HAAs were removed from the MPV sample. The other three alternatives only removed 50−60% (tap water and tap water with vinegar) or 45−55% (tap water with sodium hypochlorite). No significant differences were found in the experiment carried out with mineral water instead of tap water. Therefore, the remaining HAA concentrations found in the rinsed MPV come from the original HAAs on the vegetables.

On the other hand, if it is estimated that a person can consume ~150 g of vegetables per day (portion) and that the

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most contaminated MPV (salad 10, in Table 3) contains 55 μg/kg of total HAAs, this person might ingest more than 8 μg of HAAs. Assuming a consumption of 2 L of tap water per day and an average total HAA concentration in tap water of 15−25 μg/L,[20,22−24] the total HAA intake from drinking water would be 30−50 μg per day. Therefore, the contribution of human exposure through just one portion of salad could be the fourth part of the amount established for ingested tap water, which is in our opinion significant. Thus, it should be acceptable to include these compounds as emergent pollutants in foods since they have been already established for drinking water in several countries.

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Notes

The authors declare no competing financial interest.

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