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Determination of Acrylamide in Foods by Solid Phase Microextraction-Gas Chromatography

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Abstract A new approach for the determination of acrylamide (AM) in foods by solid phase microextraction-gas chromatography (SPME-GC) was established. AM was bromized and transformed to 2-bromoacrylamide (2-BAM). 2-BAM was then extracted by a commercial SPME fiber, 75-μm Car/PDMS fiber, for GC detection. The influence of extraction and desorption parameters such as extraction temperature and time, stirring rate, desorption temperature, and time were studied and optimized. The mass concentration was proportional to the peak area of 2-BPA from 1.0 to 8,000 μg/L. The detection limit of the SPME-GC for 2-BAM was found to be 0.1 μg/L, and the recoveries and relative standard deviations for different food samples were 74.5 to 102.0%, and 4.2 to 9.1%, respectively. The presented method was applied to the determination of AM in fried foods.

Keywords: solid phase microextraction, gas chromatography, acrylamide, 2-bromoacrylamide, food

Introduction

Nowadays, the conventional methods for the determination of acrylamide (AM) including gas chromatography (GC) (1), gas chromatography-mass spectrometry (GC-MS) (2), high performance liquid chromatography (HPLC) (3), liquid chromatography-mass spectrometry (LC-MS) (4), and LC-MS/MS (5) are reported. However, these methods have some drawbacks, such as complicated process, expensive mass spectrograph, high cost, and time-consuming, which limit their applications. Solid phase microextraction (SPME) combining simultaneous extraction and preconcentration of analytes from different matrices into one step was developed by Martos and Pawliszyn (6). It is a kind of rapid, sensitive, convenient technology for pretreatment of samples not containing any solvent. From then on, it has been used for the extraction of various compounds from environmental (7,8), pharmaceutical (9), biological (10,11), and food samples (12,13), and several kinds of commercial SPME fibers have currently been successfully applied in many fields.

In this study, we established an approach for the determination of AM using SPME coupled to GC. The analysis for AM through bromination (14,15) and GC determination was relatively advanced even before AM was discovered in heated foods. In the analysis, the bromination of AM affords a product, 2-bromoacrylamide (2-BAM), that could be analyzed more easily at a trace level than that of AM, and the analytical time could be controlled in 12 min. After the optimization of the SPME-GC conditions, a determination limit with 0.1 μg/L of 2-BAM could be achieved. The method was applied to the analysis of AM in food samples presenting simple operation, rapid analysis, less reagent, low cost, extensive application, high sensitivity, and selectivity to the selected samples.

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Materials and Methods

Reagents and solutions Acrylamide was purchased from Sigma-Aldrich (Oakville, Canada). The stock solution of AM was prepared by diluting the pure standard (99.9%) to 1,000 μg/L, stored at 4°C and diluted with water before use. Potassium bromide (KBr), potassium bromate ($KBrO_3$), sodium chloride (NaCl), sodium thiosulfate ($Na_2S_2O_3$), and *n*-hexane were all of analytical reagent grade, and their solutions were prepared in deionized distilled water. Potato chips, French fries, toast, fried chicken, Chinese twist cruller, potatoes, and flour were all purchased from a local supermarket of Xiamen.

Instrumentation In order to compare results, commercial manual sampling SPME devices with 75-μm carboxen/polydimethylsiloxane (Car/PDMS), 65-μm carbowax/divinylbenzene (CW/DVB), 85-μm polyacrylate (PA), and 100-μm polydimethylsiloxane (PDMS) fibers were obtained from Supelco Company (Bellefonte, PA, USA). A Shimadazu GC-2010 GC (Tokyo, Japan) system equipped with an electron capture detector (ECD) system was employed for the SPME-GC experiments and a 30 m (L)×0.32 mm (i.d.), 0.25 μm Rtx-Wax polar capillary column (Shimadazu Co. Ltd.) was applied to analyze the extracted analytes. Nitrogen (99.999%) was used as the carrier gas and kept at a flow rate of 2.3 mL/min. Temperature program to be applied in the GC-ECD was as follows: held at 110°C for 1 min, then at the temperature increased by 10°C/min to 140°C and held for 8 min and finally at the temperature increased by

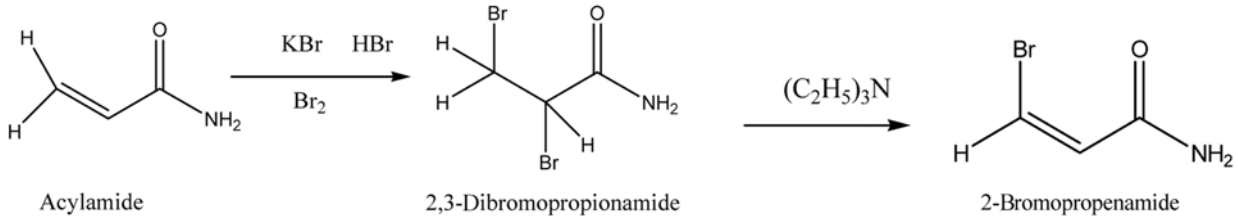

Fig. 1. Derivation procedure of AM transformed into 2-BAM.

25°C/min to 240°C and held for 5 min. The temperature of the injector and ECD system were held at 225 and 240°C, respectively.

In order to obtain the comparative results of GC-ECD for the AM analysis, an Agilent GC-MS (6890-5973) system with a column of HP 50C (30 m×0.25 mm i.d., 0.25 mm film thickness) capillary column from Agilent (Santa Clara, CA, USA) was employed, and the selected equipment conditions were the same as those described in Olmez *et al.* (2).

Sample preparation All samples were purchased from a local market and analyzed within the same day. These samples were cut up and accurately weighed 1.000 g in a beaker. After that, 10 mL re-distilled *n*-hexane was added, and the mixture was degreased in an ultrasonicator for 10 min. Then 7 mL of 2 mol/L NaCl solution was added and mixed ultrasonically for 20 min. After extraction, the extract was transferred into a volumetric flask and diluted to 15 mL using deionized distilled water. The recovery of the approach was estimated by adding 50, 150, 10, 250, or 100 mL AM working solution (10 μg/mL) to potato chips, French fries, toast fried chicken, or Chinese twist cruller sample, correspondingly.

Derivation procedure Before GC-ECD detection, AM was bromized and transformed to 2-BAM as shown in Fig. 1 according to the method of Castle *et al.* (15). One mL of 10% sulfuric acid stored at 4°C, 1 mL of 0.1 mol/L $KBrO_3$, and 1.5 g solid KBr were added into 5 mL extract liquor. The mixture was oscillated to mix homogeneously and reacted at 4°C for 90 min. Then 0.4 mL of 0.1 mol/L $Na_2S_2O_3$ was added to remove residual bromine. Lastly, the addition of 400 μL 10% triethylamine made the main products transform into 2-BAM.

Solid phase microextraction (SPME) In the SPME-GC analysis of 2-BAM, a commercial fiber, 75-μm Car/PDMS, was employed kept. In the extraction, a 15-mL glass vial was used as a sample container, and 10 mL of sample solution was placed into the sample vial with a 1-cm spin bar. A magnetic stirrer (IKA-Werke, Saufen, Germany) with speed of 750 rpm was used to obtain the optimal stirring rates. The syringe holding the commercial SPME fiber was placed into the sample vial at the proper height so that the fiber was completely immersed into the sample solution. The fiber was exposed to the solution after the septum was pierced with the stainless steel needle housing the fiber. Extraction solutions were performed at room temperature (30±0.5°C) for 40 min. After extraction, the fiber was withdrawn into a sample injection needle, and then introduced into the GC inlet.

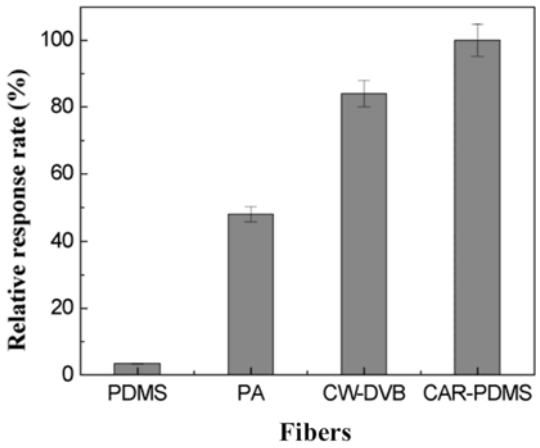

Fig. 2. Comparison of extraction efficiency using different commercial fibers.

Results and Discussion

Extraction ability of commercial SPME fibers The coating characteristic of a SPME fiber is the most important factor for the extraction selection and the determination sensitivity (16). In this study, bromized product of AM, 2-BAM, was used to evaluate the extraction performance of the commercial SPME fibers. The extraction ability of the commercial SPME fibers was evaluated by comparing extraction efficiencies under the same condition. As can be seen in Fig. 2, 75-μm Car/PDMS fiber exhibited the highest extraction efficiency for 2-BPA. This could be attributed mainly to similar polarity between Car/PDMS and 2-BPA. In the following experiment, 75-μm Car/PDMS fiber was applied.

Optimization of SPME conditions Although the associated effect of SPME extraction and GC determination conditions on the final analytical results should be taken into account, generally, as the many reports, the single optimal parameter for each condition can be achieved when the other conditions are kept at constant. In 2-BAM analysis, to obtain the maximum SPME extraction efficiency of 75-μm Car/PDMS fiber, several experimental parameters, such as extraction time, extraction temperature, stirring rate, desorption time, and temperature were studied and optimized.

Optimization of the extraction time Typically, SPME

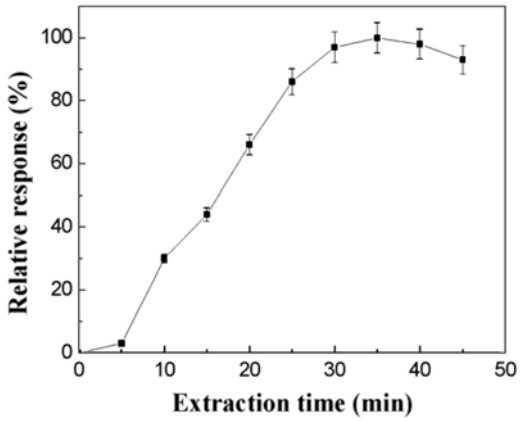

Fig. 3. Effect of extraction time on GC peak area.

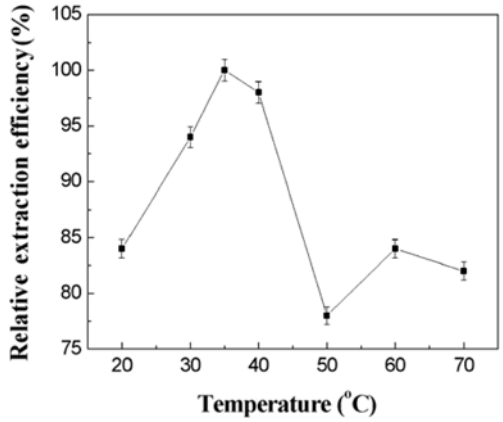

Fig. 4. Effect of extraction temperature on extraction efficiency.

extraction is considered to reach equilibrium when the analytes adsorbed by fiber coating is independent to further increase of extraction time (17). The extraction time profile for 2-BAM was established by plotting the peak area versus the extraction time. As shown in Fig. 3, the adsorption for 2-BAM from water samples to the Car/PDMS fiber was a time consuming process. The results showed that about 40 min was needed to reach equilibrium. Therefore, 40 min was chosen as the optimal extraction time.

Optimization of the extraction temperature Extraction temperature generally increases the extraction efficiency (18). Thus, the effect of extraction temperature on the amounts of analytes absorbed was considered and studied by exposing the fiber to the sample for 40 min at temperatures, such as 20, 30, 40, 50, 60, and 70°C. As shown in Fig. 4, the extraction amount of 2-BAM reached the maximum at 30°C. Thus, the extraction temperature was chosen at 30°C.

Optimization of stirring rate Higher stirring rate would enhance the distribution velocity of the analyte from a sample matrix to the SPME fiber and thus improve the extraction efficiency (18). Based on the obtained results, at the selected extraction time and temperature, the chromatographic peak areas of the analytes were increased

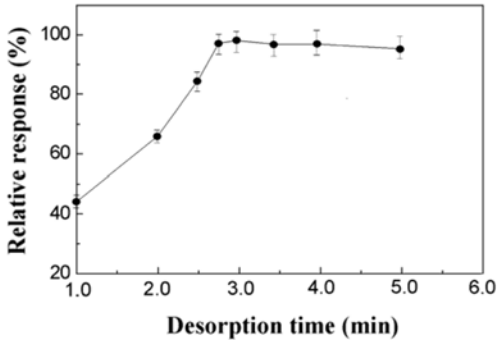

Fig. 5. Effect of desorption time on GC peak area.

when the agitation speed was increased from 500 to 1,500 rpm. From the experimental result, in the range of 500 to 1,200 rpm, about 5% increase of the chromatographic peak area could be found for each 100 rpm increase of the stirring rate. When the agitation speed was more than 800 rpm, a deep whirlpool formed, which caused the fiber could not be immersed in the solution completely. Therefore, a suitable stirring rate was selected at 750 rpm for the subsequent experiments after considering both extraction efficiency and stability.

Optimization of desorption time and temperature The desorption temperature and time in GC application affect the desorption of analytes from the fiber. The higher temperature accelerates desorption, but decrease the long-life of coatings on the extraction fibers (19). Therefore, the optimization of the desorption temperature and time should be considered. In the experiments, the desorption temperature was selected from 150 to 240°C, and the results revealed that the chromatographic peak area of 2-BAM was increased when a higher desorption temperature was selected. The maximum desorption amount was found to be obtained at the temperature of 225°C. Similarly, the desorption time for the GC analysis was also investigated from 0.5 to 5 min at 225°C, and found that 2-BAM could be desorbed completely within 3 min (Fig. 5). Based on the result, desorption at 225°C for 3 min was selected as the optimum conditions. Under the conditions, there was no obvious residue effect after the fiber was desorbed.

Effect of co-existing axunges in foods Effect of co-existing axunges in real samples on the extraction of 2-BAM was also investigated. As shown in Fig. 6, the corresponding peak area of 2-BAM was obtained at 11.08 min. The result indicated that there was little perturbation. This could be attributed to the re-distilled *n*-hexane added to remove axunges in fried foods.

Analytical characteristics Standard concentrations of 2-BAM from 1.0 to 8,000 μg/L were investigated using external reference method. The results were showed as Fig. 7. The regression equation of the working curve could be expressed as $Y=3.305+0.304\ X$ ($n=11$, $r=0.9997$). The detection limit was found to be 0.1 μg/L. Compared to the liquid/liquid extraction reported (20,21), this method presented lower detection limit, wider linear response range and better precision.

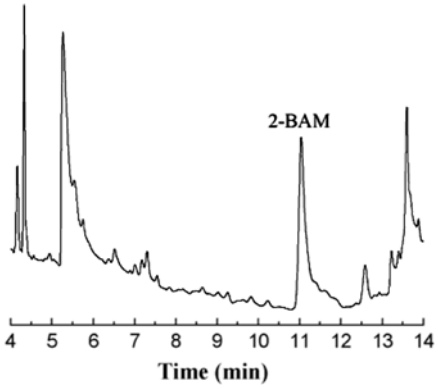

Fig. 6. Typical GC-ECD chromatogram of a food sample.

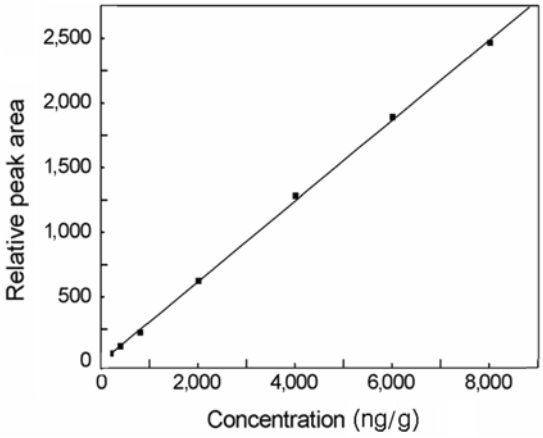

Fig. 7. Calibration curve for 2-BAM detection analysis.

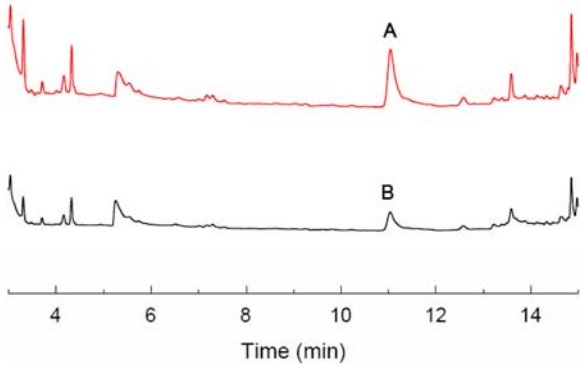

Fig. 8. Chromatograms of proposed SPME-GC-ECD analysis of chips spiked with 10 mg/L of AM (A) and potato chips (B).

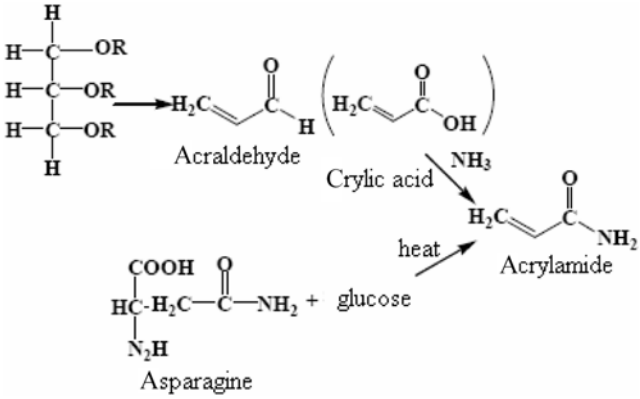

Fig. 9. Formation routes of AM in food.

Sample analysis To test the applicability of the proposed method to the analysis of real samples, the optimized approach was applied to different kinds of foods. As shown in Table 1, in this study, different amount of AM standard solution was added into the testing samples, then, the mixture was immediately treated following the procedures mentioned in 2.2 and 2.3. The recovery of the AM in foods ranged from 79.6 to 95.7%, and the relative standard deviation ranged from 3.4 to 9.0%. Typical chromatograms of potato chips and chips spiked with 10 mg/L of AM are presented in Fig. 8.

In order to investigate AM formation in foods by different cooking conditions, potato strip was taken as a typical tasting sample. One possible pathway to the formation of acrylamide in some foods is possibly via the Maillard reaction, which involves the reaction of a specific amino acid with a reducing sugar in the presence of heat (22,23). Another possible route involves the reaction of asparagine with octanal to form acrylamide (20). The detailed route of acrylamide formation was described as Fig. 9. In addition, the AM content in the sample was detected by the proposed approach. The experimental results indicated that the cooking time affected AM formation. The formation and elimination of AM reached equilibration on the surface of foods when the cooking time was between 15 and 25 min at 180°C. When the cooking time was over 25 min, the content of AM was increased. Furthermore, the cooking temperature is another factor affecting AM formation in foods. In the experiment,

Table 1. Concentration of AM in local foods and the detection recoveries using the proposed SPME-GC method under the optimal conditions (n=5)

Sample[1)]	Content (μg/kg)		RSD (%)	Added (μg/kg)	Found (μg/kg)	Recovery (%)
	SPME-GC	GC-MS				
Potato chips	480±20.0	512±23.0	4.1	500	398	79.6±5.1
French fries	1,369±42.0	1,283±58.0	3.1	1,500	1,433	95.5±9.1
Toast	135±10.0	130±8.0	7.4	100	96	96.0±6.3
Fried chicken	2,578±117.0	2,603±101.0	4.5	2,500	2,188	87.5±7.5
Chinese twist cruller	1,075±35.0	1,120±42.0	3.3	1,000	946	94.6±3.4

[1)]Samples were obtained from the local supermarkets in Xiamen.

the AM content in the potato strips sample reached the maximum when the cooking temperature was in the range of 170 to 190°C. A higher cooking temperature reversely caused less AM because of the disassociation of AM at higher temperature. Comparatively, less AM was found when potato strip sample was baked at 100°C for 25 min. The change of AM content in the fried sample with the stored time was also investigated, and the results showed that AM content kept unchanged even under a long time stored. This result indicated that AM presented high stability in the fried sample. Edible oil is not necessary condition for the formation of AM. However, the sort of edible oil used could affect the formation of AM to some extent. Due to the different boiling points of edible oil, the fried temperature and cooking time were different causing the concentration of AM forming different further.

In this study, we developed a new convenient SPME approach for the AM analysis in foods by GC-ECD. The selected commercial SPME fiber, 75-μm Car/PDMS, exhibited high extraction efficiency towards 2-BAM which is the bromination product of AM. The extraction and desorption parameters were optimized and the following were selected for SPME-GC: extraction temperature, 30°C; extraction time, 40 min; stirring rate, 750 rpm; desorption temperature, 225°C; desorption time, 3 min. In addition, the present approach was applied in the AM analysis of food samples including potato chips, French fries, toast fried chicken, or Chinese twist cruller. The results indicated that the method developed was suitable for the qualitative and semi-quantitative analysis of AM in various food samples.

Acknowledgments

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