Comparison of two derivatization methods of acrylamide between bromination and xanthydrol reaction for gas chromatography-flame ionization detection

Anuwat Ratsamisomsi, Patanasak Rodphai, Lalitphat Suppraphakorn, Warawut Tiyapongpattana*
Department of Chemistry, Faculty of Science and Technology, Thammasat University, Pathumthani 12121, Thailand
*e-mail: twarawut@tu.ac.th

Abstract:

Acrylamide (AA), which is toxic and probably carcinogenic for humans, can be formed from food products containing carbohydrates and asparagine during high-temperature cooking. The objective of this work was to study bromination and xanthydrol reactions for derivatization of AA. The first method was based on derivatization of AA with potassium bromide to 2,3-dibromopropionamide (2,3-DBPA) under an acidic condition. 2,3-DBPA was then converted to 2-bromopropenamide (2-BPA) by dehydrobromination with triethylamine for more stable derivative prior to inject to gas chromatography coupled with flame ionization detector (GC-FID). For xanthydrol derivatization, AA was derivatized with xanthydrol to xanthyl-AA under an acidic condition with heating for 20 minutes. The solution was extracted by ethyl acetate and analyzed by GC-FID. The effects of some physical and chemical parameters on sensitivity of both methods have been studied such as reaction time, reaction temperature, extraction solvent and extraction time. Under the optimum conditions, the calibration curves ($R^2 < 0.99$) and less than 3% relative standard deviation (%RSD) were obtained from both derivatization methods. Furthermore, the sensitivity of xanthydrol derivatization is higher than bromination method. However, an additional preconcentration step will be further employed and developed for improving the analytical performance.

1. Introduction

Acrylamide (AA), is considered as a potential genetic and reproductive toxin with mutagenic and carcinogenic properties in humans.[1] AA attained from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of reducing sugars such as glucose during baking and frying.[2]

Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) approximated an average daily food intake of AA) in the range of 0.3 – 0.8 μg/kg bw/day.[3] Permissible levels were established for drinking water by the WHO at 0.5 μg/L,[4] by the European Union (EU) at 1 μg/L,[5] and by the US Environmental Protection Agency (EPA), AA is not presented in drinking water.[6]

Many analytical methods such as ion-exclusion chromatography coupled with mass spectrometry (IC-MS),[7] high performance liquid chromatography (HPLC) coupled with MS[8] or MS/MS,[9] gas chromatography (GC) coupled with flame ionization detection (FID),[10] electron capture detection (ECD),[11] MS[12] and MS/MS[13] have been also used for the analysis of AA. IC–MS, HPLC-MS and GC-MS appeared to be the most widely used methods due to its good resolution, sensitivity and selectivity.[2,14,15] However, they are highly expensive, and the issues of the maintenance and management of them are mostly a major problem.[16] Thus, GC-FID is an alternative instrument.

Although, GC–FID method is simple, rapid and low-cost instrument, a derivatization procedure is required for

enhancing the sensitivity of AA detection. Bromination is extensive reaction that utilized to determine the AA content in water or foods. The advantage of AA bromination is a production of relative more volatile compound, which can lead to improved GC characteristics (less polar) and high sensitivity.[2] In 2011, Yamazaki et al.[17] developed and validated an analytical method for derivatizing AA with xanthydrol in foods by GC–MS. This reaction requires mild reaction conditions at low temperature and favorable for sensitive detection.[18]

The objective of this work is to compare bromination and xanthydrol reactions of AA for GC-FID analysis. The effects of some physical and chemical parameters on sensitivity of both methods have been studied. The high sensitivity reaction was selected for applying to determine AA in fried potato samples.

2. Materials and Methods

2.1 Sample

Fried potato samples were obtained from local stores in Pathumthani, Thailand.

2.2 Chemicals

All solvents and chemicals used for the analysis of AA were GC and analytical grade.

Acrylamide (99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

For bromination reaction, tri ethylamine, hydrobromic acid (48%), potassium bromide (100%) and sodium thiosulfate pentahydrate (99.5%) were purchased from Fisher (Leics, Le, UK). Bromine (99%) was purchased from Panreac Quimaca SA (Castellar del Vallès, Barcelona, Spain). and sodium sulfate anhydrous (99%) was purchased from Lab Scan (Pathumwan, Bangkok, Thailand).

For xanthydrol reaction, xanthydrol (98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (37%) and sodium chloride (99%) were purchased from Lab Scan (Pathumwan, Bangkok, Thailand)

Methanol (99.9%), n-hexane (99%), methyl tert-buthyl ether (99.9%), ethyl acetate (99.9%), and chloroform (99.8%), were purchased from Lab Scan (Pathumwan, Bangkok, Thailand).

2.3 Standards and reagents

A stock solution of AA (100 mg/L) in deionized water was used to prepare working standard solutions. 5% w/v of xanthydrol was dissolved in methanol. All standard and reagent solutions were stored at 4 °C.

2.4 GC-FID operating condition

A gas chromatograph, Shimadzu Corporation, Model GC-2010 plus (Tokyo, Japan) was used for AA analysis.

For bromination reaction, a Rtx®-Wax analytical column was used (Restek, 0.32 mm I.D., 30 m length, 0.25 µm film thickness). The oven temperature program was started at 40 °C and ramped up to 160 °C after 2 min at 60°C/min. Then ramped up to 250 °C after 4 min at 60 °C/min. The temperature was maintained at 250 °C for 5 min. The carrier gas was helium which was maintained at a linear velocity of 55 cm/s. The temperatures of injector and detector were set at 250 °C.

For xanthydrol reaction, a Rtx®-5 analytical column was used (Restek, 0.32 mm I.D., 30 m length, 0.25 µm film thickness). The oven temperature program was started at 150°C and ramped up to 310 °C after 2 min at 35 °C/min. The temperature was maintained at 310 ˚C for 5 min. The carrier gas was helium which was maintained at a linear velocity of 35 cm/s. Injector and detector temperatures were set at 300 and 310 °C, respectively.

The sample injection volume was 3 µL in splitless injection mode for both reactions.

2.5 Sample preparation

A grinded sample (3.00xx g) was accurately weighed into a centrifugal tube. 30 mL of deionized water was added and

the mixture was homogenized for 30 min by shaker machine. After centrifugation of the extract at 5,000 rpm for 10 min, the supernatant 10 mL was transferred to a separatory funnel and washed with 10 mL of hexane.

2.6 Derivatization

For bromination reaction, the standard method (8032A) for determination AA in water was modified.[11] 10 mL of supernatant was transferred to a centrifugal tube. 3 g of potassium bromide (KBr) was added before adjust pH of the solution with hydrobromic acid (HBr) until pH was between 1 and 3. 3.0 mL of saturated bromine water was added and shaken. The mixture was then placed in ice bath in the dark and stand for one hour. The excess of bromine in solution was decomposed by adding 1 M of sodium thiosulfate solution until the solution becomes colorless. 1 g of sodium chloride (NaCl) was added in the solution before the analyte was extracted with 900 µL of ethyl acetate for 5 min by shaker machine. Finally, 100 µL of triethylamine was added after phase separation.

For xanthydrol reaction, 10 mL of supernatant was transferred to a centrifugal tube. 1 mL of 5% w/v xanthydrol solution and 1 mL of 0.6 M hydrochloric acid (HCl) were added in solution. The mixture was placed in a water bath at 50°C for 20 minute. 1 g of NaCl was added before analyte was extracted with 1 mL of ethyl acetate. The determination of acrylamide was performed using GC–FID.

3. Results & Discussion

3.1 Bromination reaction

AA was derivatized to 2,3-DBPA with KBr and bromine water under HBr condition as shown in Figure 1. 2,3-DBPA was further derivatized to 2-BPA by triethylamine after extracted to organic phase because 2,3-DBPA was unstable during GC analysis.

The experiments were optimized to determine how various factors affected extraction efficiency in terms of analyte enrichment. 10 mL aliquots of standard containing 1 mg/L of AA were used. For each parameter, the extraction method was performed at least three times.

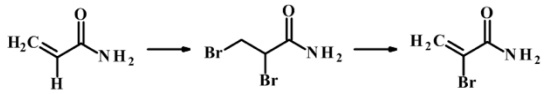

Figure 1. Bromination reaction of acrylamide**.**

3.1.1 Effect of extraction solvent

A proper organic extractant must be selected for an efficient extraction procedure. Their requirements of the organic extractant are as follows: water immiscibility, high affinity for the analytes of interest and good chromatographic behavior with GC.[19] Extraction performance was compared in chloroform, ethyl acetate, methyl tert-buthyl ether (MTBE) and n-hexane. Figure 2 showed that ethyl acetate gave the highest extraction efficiency because its polarity is suitable for extraction of AA-derivative. On the other hand, chloroform and n-hexane cannot extract 2,3-DBPA from water phase. In this study, ethyl acetate was adopted for extraction solvent.

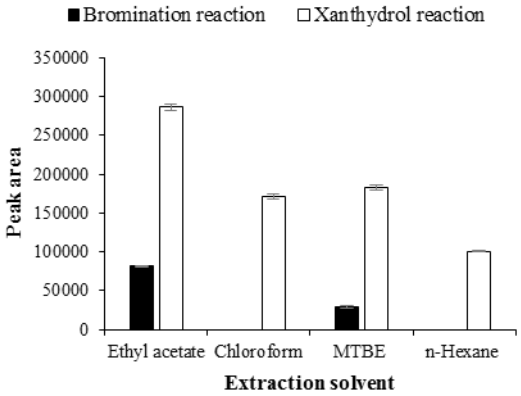

Figure 2. Effect of extraction solvent on extraction efficiency of AA-derivatives from bromination and xanthydrol reactions**.**

3.1.2 Effect of extraction time

The examination of extraction time in the range of 0.5–10 min by shaker machine was based on extraction efficiency. As results clearly shown in Figure 3, extraction time longer than 5 min did not show significant improvement of the extraction efficiency for the selected 2,3-DBPA. In this study, 5 min of extraction time was adopted.

3.2 Xanthydrol reaction

AA was derivatized with xanthydrol to xanthyl-AA under HCl condition as shown in Figure 4.

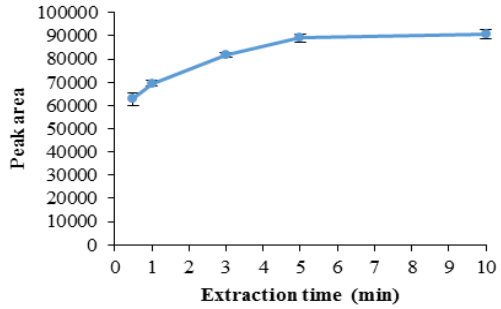

Figure 3. Effect of extraction time on the yield of 2,3-DBPA**.**

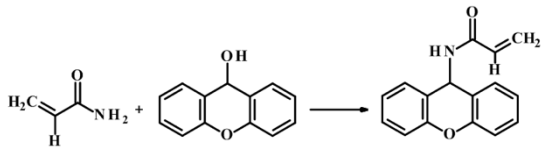

Figure 4. Derivative xanthydrol reaction formula of acrylamide**.**

3.2.1 Optimization of the derivatization condition

The optimal reaction condition for determination of AA in food were tested. For the first parameter, the concentration of HCl ranging from 0–100 mM for the derivatization was studied. Figure 5 showed that 50 mM of HCl concentration obtained the highest yield.

The next parameters were studied over derivatization temperature at 30 °C, 40 °C, 50 °C and 60 °C and derivatization time period in the range of 15–150 min. The results showed that reaction time was decreased with the increase of temperature. The highest yield with short reaction time was carried out using 50 °C at 20 min.

3.2.2 Effect of extraction solvent

The optimization of extraction solvent was based on the sensitivity of derivative in GC-FID analysis. Figure 2 showed that ethyl acetate gave the highest extraction efficiency and good chromatographic behavior among the tested solvents. Hence, ethyl acetate was considered as the suitable extractant.

3.2.3 Effect of extraction time

The effect of extraction time on the extraction efficiency by vortex was examined in the range of 15–300 sec. As can be seen in Figure 6, the peak area response of analyte increased when the extraction time was increased. However, slight decline of the signal intensities was observed when the extraction time prolonged longer than 60 sec. It may be attributed to the competing mass-transfer process of the analyte occurred at the interface of the organic phase immersed in the layer of water phase. In this study, 60 sec was adopted for extraction.

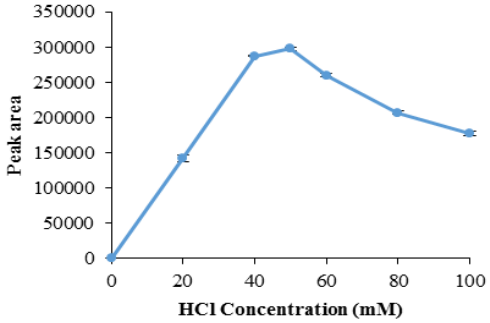

Figure 5. Effect of HCl concentration on xanthydrol reaction**.**

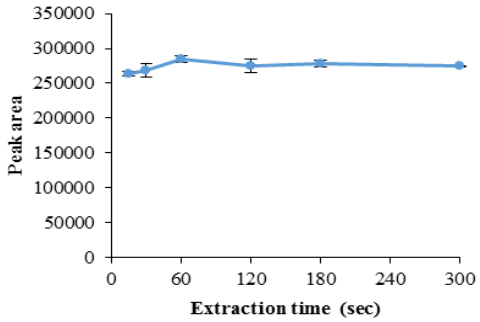

Figure 6. Effect of extraction time on the yield of xanthyl-AA**.**

3.3 Method validation

The analytical performance of two reaction methods under optimal conditions were validated through linearity (linear range and correlation coefficient), sensitivity (limits of quantification), and and precision (expressed as relative standard deviation). The results are summarized in Table 1. The linear range (LR) of bromination and xanthydrol reactions were from 0.1–5 mg/L and 0.005–5 mg/L with the coefficient of determination (R^2) were 0.9998 and 0.9996, respectively. The limit of quantification (LOQ) for two reaction methods were calculated by the signal-to-noise (S/N) ratio of ten, 0.1 mg/L and 0.005 mg/L, respectively. The relative standard deviation of repeatability (RSD, n =10) at 1 mg/L levels were 2.87% and 2.38%, respectively.

The analytical features showed that xanthydrol method gave lower LOQ and RSD than bromination method. In addition, the derivatization with xanthydrol can be achieved within 20 min. Therefore, xanthydrol method was preferred for derivatization of acrylamide in fried potato samples.

3.4 Analysis of real potato samples

The xanthydrol method was applied to the analysis of acrylamide in fried potato samples using GC-FID. The results are shown in Table 2. AA content was found in the range of 44.1–66.1 µg/kg.

In order to assess matrix effects, potato samples were spiked with 0.5 mg/L of AA for a recovery study. The recoveries of analytes from fried potatoes were in the range of 94.9-102.3%. It demonstrated that the xanthydrol reaction method was suitable for the determination of acrylamide at trace level concentrations in potato. Figure 7 presents the chromatograms of the standard, non-spiked and spiked sample obtained by GC-FID.

4. Conclusion

In this work, analytical procedure and analytical performance of two derivatizations (bromination and xanthydrol reactions) were compared in terms of linear range, sensitivity and precision. Notably, xanthydrol method was carried out with shorter analysis time and provided higher sensitivity with lower limit of quantification. Consequently, xanthydrol method was employed for determining acrylamide in fried potato samples. However, some potatoes samples cannot be identified and determined the presence of acrylamide. Additional pre-

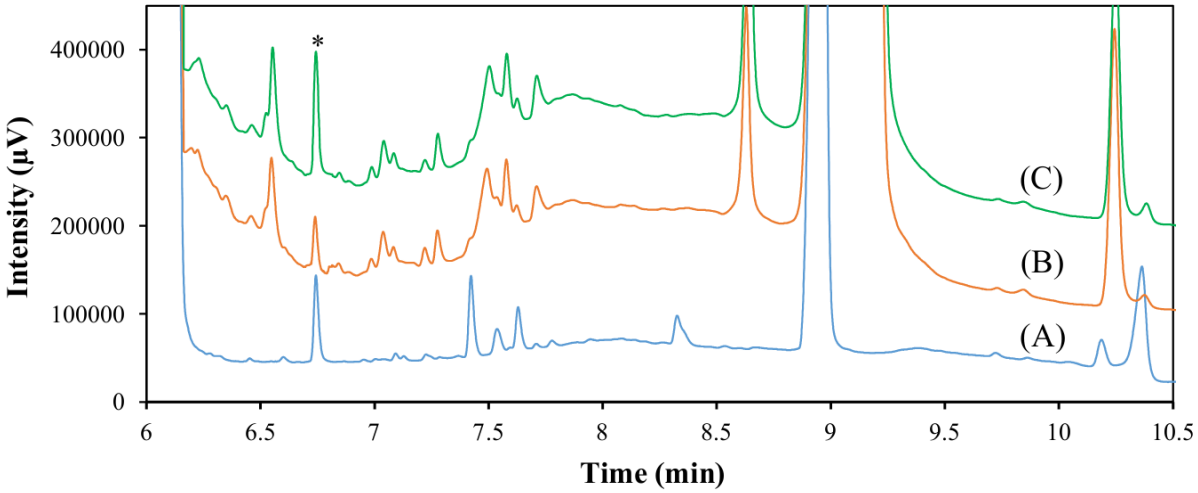

Figure 7. Chromatograms of (A) the standard acrylamide (0.5 mg/L), (B) non-spiked sample C and (C) spiked sample C (spiked 0.5 mg/L) by GC-FID. * represents xanthyl-AA.

Table 1. Analytical parameters for two reaction methods of acrylamide by GC–FID.

Reaction method	Calibration curve (mg/L)	R^2	Linear range (mg/L)	LOQ[a] (mg/L)	Repeatability[b] (%RSD)
Bromination	y = 90,641x-5,644.6	0.9998	0.1-5	0.1	2.87
Xanthydrol	y = 308,827x+2,937.3	0.9996	0.005-5	0.005	2.38

[a]LOQ = 10 S/N

[b]The concentration of acrylamide is 1 mg/L, n = 10.

Table 2. Acrylamide content in fried potatoes by GC–FID.

Sample	Acrylamide contents (Mean±SD[a]; µg/kg)
A	56.3 ± 0.7
B	ND[b]
C	54.6 ± 1.3
D	44.1 ± 1.3
E	ND[b]
F	66.1 ± 2.4

[a]Mean of three time of sample.

[b]Nondetectable, which concentration lower than LOQ.

concentration step, microextraction method will be further developed and optimized for improving the analytical performance.

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