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ORIGINAL RESEARCH PAPER

Determination of acrylamide in foods by automatic accelerated solvent extraction and gas chromatography-mass spectrometry

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ABSTRACT

The aim of the experiment is to establish a method for the determination of acrylamide in food by automatic accelerated solvent extraction-gas chromatography-mass spectrometry. D₃-acrylamide was used as isotope internal standard, crushed samples were extracted and purified by automatic accelerated solvent, acrylamide was derivatized into 2,3-dibromopropanamide by potassium bromide and potassium bromate under acidic conditions, and then the derivative was extracted by ethyl acetate and detected by gas chromatography-mass spectrometry. The method had a good linear relationship in the concentration range of 10–2000 ng/mL, and the coefficient of determination (R^2) was 0.9997. The detection limit of the method was 3 µg/kg. The quantification limit of the method was 10 µg/kg. The standard addition recovery of acrylamide was between 105 and 120%, and the relative standard deviation of the recovery of acrylamide was less than 3.0%. The experimental result showed that the method was simple, sensitive, efficient and accurate, and could be used for the determination of acrylamide in food.

KEYWORDS

acrylamide, automatic accelerated solvent extraction, gas chromatography-mass spectrometry, baked and fried foods

INTRODUCTION

Acrylamide, abbreviated as AA, is a white, odorless, crystalline chemical with strong polarity, and soluble in water, methanol, ethanol, acetone, ether, but insoluble in n-hexane and benzene. In 1994, acrylamide was listed as potential carcinogen under the Group 2A by the International Agency for Research on Cancer (IARC) [1]. In April 2002 the Swedish National Food Administration (NFA) and researchers from Stockholm University announced their findings that acrylamide is formed in many baked and fried foods with high concentrations, and subsequently researchers in Norway, China, Japan, Sweden, the United States and the United Kingdom also published similar reports [2–8]. International organizations and governments of different countries have paid close attention to acrylamide as its contamination of food cooked at high temperatures. A number of studies have shown that acrylamide is generated from food during heat treatment (>120 °C) as a result of Maillard reaction between reducing sugars and free amino acids, for instance, asparagine, and the levels of acrylamide increase with prolonging of heating time [9–12]. In February 2005 risk assessment of acrylamide in food have been reviewed by the Food and Agriculture Organization (FAO), World Health Organization (WHO) and Joint Expert Committee on Food Additives (JECFA), they report indicated that acrylamide has potential neurotoxicity, reproductive/developmental toxicity, genotoxicity and carcinogenicity [13]. Baked and fried foods are the main food of people, so in order to strength the monitoring and control of acrylamide in food, it is of great significance to establish a convenient, reliable, efficient and safe method for the determination of acrylamide in starch foods.

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At present, the determination methods of acrylamide in food mainly include gas chromatography (GC) [14–16], gas chromatography-mass spectrometry (GC-MS) [17–19], high performance liquid chromatography (HPLC) [20–22] and high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [23–25]. GC and HPLC are relatively inexpensive and ubiquitous in research laboratory and testing institution, however, the sensitivity and accuracy of these two methods are relatively low, the results of this experiment are susceptible to impurities and matrix effects, and stability and repeatability are relatively poor. The operation method of HPLC-MS/MS is convenient and does not need derivation, in addition, it has high sensitivity, precision and accuracy, which is fit for many samples detection. However, HPLC-MS/MS is very expensive, and the measurement cost is very high, so it is only used in some large-scale testing institution, which is not conducive to popularization and application. Thus it can be seen, HPLC-MS/MS is inconvenient to real-time monitoring of acrylamide in food. The sample pretreatment method for determination of acrylamide in foods is complicated by GC-MS. On the one hand, the derivatization reagent usually use highly toxic saturated bromine water, and it takes a long time to complete the preparation of saturated bromine water, which seriously affects the experiment efficiency; On the other hand, the procedure of sample preparation is complicated, which can easily cause a loss of the target substance, and then the accuracy of the experimental method is reduced. However, GC-MS method has high sensitivity, high precision and low detection limit, and the measurement cost is inexpensive, it is used in many research laboratories and testing institutions. Therefore, we only need to optimize the pretreatment method, which can greatly improve the experimental efficiency and the accuracy of the determination. In this experiment, by taking biscuits, potato chips and fried dough twists as the research object, and automatic accelerated solvent extraction-gas chromatography-mass spectrometry (AASE-GC-MS) method was established for the determination of acrylamide in food. On the one hand, APLE is used to extract target substances and purify samples, which greatly improves the extraction efficiency, and avoids man-made operation errors. On the other hand, instead of using saturated bromine water, the derivatization reagent was replaced by potassium bromide and potassium bromate, which greatly shortened the sample pretreatment time and improves the safety of the experiment. The method is simple, rapid, accurate and safe, and is suitable for the determination of acrylamide in food.

MATERIALS AND METHODS

Materials

Biscuits, potato chips and fried dough twists were purchased from supermarket (Shanghai, China).

Reagents

Acrylamide at a concentration of 1,000 mg/L was used as external standard, and the methanol is the solvent, D₃-acrylamide was used as isotope internal standard; Methanol, n-hexane and ethyl acetate were chromatography grade; Concentrated sulfuric acid, potassium bromide, potassium bromate and sodium thiosulfate were analytically pure. All the reagents were purchased from ANPEL Laboratory Technologies (Shanghai) Inc. (Shanghai, China).

The preparation of standard solution

0.05, 0.10 and 0.20 mL of acrylamide (1,000 mg/L) was respectively absorbed in 10 mL volumetric flask, diluted and volumetrized with methanol, and made into 5, 10 and 20 mg/L standard working solutions. 0.10, 0.50 and 1.0 mL of acrylamide (10 mg/L) was respectively absorbed in 10 mL volumetric flask, diluted and volumetrized with methanol, and made into 0.1, 0.5 and 1.0 mg/L standard working solutions. All standard solutions were stored at –10 °C.

0.100 g of D₃-acrylamide was accurately weighed in 100 mL volumetric flask, dissolved and volumetrized with methanol, and made into 1,000 mg/L reserve solution. 0.10 mL of D₃-acrylamide (1,000 mg/L) was accurately absorbed in 10 mL volumetric flask, diluted and volumetrized with methanol, and made into 10 mg/L standard working solution. All standard solutions were stored at –10 °C.

Preparation of samples

Use a grinder to crush 50 g of the sample, 1,000 g of the crushed sample was accurately weighed in 50 mL centrifuge tube, added 0.1 mL of D₃-acrylamide (10 mg/L) standard solution, 5 mL of ultra-pure water, 6.5 g of diatomite and stir the mixture, and then put into 34 mL of extraction tank for sample extraction and purification by APLE (APLE-3500, Titan Instruments Co., Ltd, Beijing, China). the extracted solution was transferred to the concentration bottle, and then concentrated to about 3 mL in 45 °C water bath by rotary evaporator (Value digital G3, Heidolph Instruments GmbH & CO. KG, Germany). the concentrated liquid was transferred to 15 mL centrifugal tube which had added 1 mL of ultra-pure water, and washed the residue of the concentration bottle with 3 mL of ethyl acetate, and the washing liquid and the concentrated liquid were merged into the same centrifugal tube and then vortex oscillated. the upper ethyl acetate of the concentrated solution was blown off by nitrogen blows instrument (EFAA-DC24-RT, ANPEL Laboratory Technologies (Shanghai) Inc., Shanghai, China), and then added 5 mL of n-hexane to degreased, and vortex oscillated. After standing for 2 min, the upper n-hexane was removed from 15 mL centrifuge tube, and 5 mL of n-hexane was added repeatedly, the mixed liquor was centrifuged at 4,000 r/min for 10 min by centrifuge (TDL-40C, Shanghai Anting Scientific Instrument Factory, Shanghai, China). The lower aqueous phase was taken for derivatization.

The 5 µL of concentrated sulfuric acid, 238 mg of potassium bromide and 8.35 mg of potassium bromate were

sequentially added to the sample which was waiting for derivatization. After 45 min of derivatization at 4 °C, sodium thiosulfate (0.1 mol/L) was added to terminate the derivative reaction, drop by drop, until the solution became colorless, now acrylamide had become 2,3-dibromopropanamide, D₃-acrylamide had become D₃-2,3-dibromopropanamide. The 6 mL of ethyl acetate was added to the solution to extract target substance, and vortex oscillated for 1 min, centrifuged at 4,000 r/min for 5 min, and then extracted supernatant to taken through anhydrous sodium sulfate column. The extract was concentrated up to nearly dryness by nitrogen blows instrument, the residue was dissolved in ethyl acetate, vortex oscillated and filtered by 0.22 µm organic membrane, the filtrate was determined by GC-MS (TRACE 1300-ISQ, Thermo Fisher Scientific Inc., USA).

The drawing of acrylamide standard curve

The concentrations of acrylamide in standard series solutions were 0.1, 0.5, 1.0, 5, 10 and 20 mg/L, respectively. The 0.1 mL of acrylamide standard solution of each concentration was added separately to the centrifugal tube which had been added with 1 mL ultra-pure water and 0.1 mL of D₃-acrylamide (10 mg/L) standard solution, and then the standard series solutions were derived, the method of derivation was the same as that of sample derivation in 2.3. The peak area of the 2,3-dibromopropanamide and the D₃-2,3-dibromopropanamide was determined by GC-MS. The standard curve was drawn by taking the concentration of acrylamide in standard series solutions as abscissa and the ratio of the peak area of the 2,3-dibromopropanamide to the D₃-2,3-dibromopropanamide as ordinate.

The extraction conditions of automatic accelerated solvent extraction (AASE)

The volume of extraction tank was 34 mL, the extraction solution was ethyl acetate, the preheating time was 60 s, the thermal equilibrium time was 60 s, the purge time was 60 s, the cleaning time was 15 s, the elution volume was 100%, the extraction pressure was 10 MPa, the extraction temperature was 80 °C, the extraction time was 20 min, and the extraction times was once.

Gas chromatography and mass spectrometry (GC-MS) analysis

The GC chromatographic column was CD-5MS capillary column (30 m × 0.25 mm × 0.25 µm, ANPEL Laboratory Technologies (Shanghai) Inc., Shanghai, China). The carrier gas was helium with a flow rate of 1.0 mL/min. The

temperature of injector is 220 °C, the injection mode was splitless, the injection volume is 1 µL. The initial oven temperature was at 65 °C, holding for 1 min, then ramped to 200 °C at the rate of 15 °C/min, and finally increased to 240 °C at the rate of 40 °C/min, holding for 5 min. The MS was run in the electron impact positive ion (EI+) mode with an electron energy at 70 eV. The ion source temperature is 280 °C, the MS transfer line temperature is 280 °C. The scan mode is selective ion monitoring, the specific parameters were shown in Table 1.

The compounds were identified based on their retention times in the GC column. The retention time and corresponding monitoring ion abundance ratio of compounds in the sample and the standard should be consistent. The concentration of acrylamide in the sample was determined by the standard curve, and the content of acrylamide in the sample was calculated by internal standard method.

RESULTS AND DISCUSSION

Optimization of AASE conditions

Automatic accelerated solvent extraction is a sample pre-treatment method for the automatic accelerated extraction of solid or semi-solid samples at high temperature (25–200 °C) and high pressure (3–20 MPa). Compared with traditional solvent extraction and ultrasonic extraction, on the one hand, AASE can extract many samples automatically at the same time, which shortens the extraction time, and many impurities have been removed while completing the target extraction. This greatly improves the extraction efficiency, and avoids the artificial errors. On the other hand, by using AASE can greatly reduce the amount of extraction solvent, save the cost, and improve the experimental safety. In order to obtain the optimal AASE conditions, it is necessary to select the extraction time, the extraction temperature and the extraction pressure.

Extraction time is one of the important factors affecting the extraction effect [26, 27]. If the extraction time is too short, the target substance cannot be extracted completely. The extraction rate of the target substance can be guaranteed in the case of long extraction time, but the efficiency of experiment will be reduced. Therefore, it is necessary to find an optimal extraction time which can ensure the extraction rate without affecting the experimental efficiency. The effect of different extraction time on the peak area of 2,3-dibromopropanamide was investigated under the conditions of extraction pressure 10 MPa and extraction temperature 80 °C, as shown in Fig. 1. With the prolongation of

Table 1. Mass spectrometric parameters of standards

Compound	CAS NO	Retention time/min	Quantitative ion (<i>m/z</i>)	Qualitative ion (<i>m/z</i>)
2,3-dibromopropanamide	15102-42-8	8.99	150	152,106,108
D ₃ -2,3-dibromopropanamide	/	9.01	153	155,109,111

Fig. 1. Effect of the extraction time on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

extraction time, the peak area of 2,3-dibromopropanamide increased gradually, and the increase was rapid in the early stage and slow in the late stage. The peak area of 2,3-dibromopropanamide reached a maximum at 20 min and then tended to be stable, indicating that the extraction rate of 2,3-dibromopropanamide had reached the highest. From the viewpoint of experimental efficiency, the optimum extraction time was 20 min.

Extraction pressure is one of the main factors affecting extraction efficiency [28, 29]. The effect of different extraction pressure on the peak area of 2,3-dibromopropanamide was investigated under the conditions of extraction time 20 min and extraction temperature 80 °C, as shown in Fig. 2. When the extraction pressure changed from 3 to 5 MPa, the peak area of 2,3-dibromopropanamide increased rapidly, which indicated that the extraction rate of target substance increased rapidly in this pressure section. The peak area of 2,3-dibromopropanamide increases slowly in the process of extracting pressure from 5 to 10 MPa. The peak area of 2,3-dibromopropanamide decreased slowly when the extraction pressure reached 10 MPa, this was most probably because the decomposition of acrylamide caused by excessive pressure. Therefore, the optimal extraction pressure is 10 MPa.

Fig. 2. Effect of the extraction pressure on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

Extraction temperature is the key factor affecting extraction efficiency [29, 30]. The effect of different extraction pressure on the peak area of 2,3-dibromopropanamide was investigated under the conditions of extraction time 20 min and extraction pressure 10 MPa, as shown in Fig. 3. When the extraction temperature was lower than 80 °C, the peak area of 2,3-dibromopropanamide increased with the increase of extraction temperature. When the extraction temperature was higher than 80 °C, the peak area of 2,3-dibromopropanamide decreased with the increase of extraction temperature. This was because acrylamide was decomposed easily at high temperature [31], and the decomposition was more serious with the increase of temperature, so the optimal extraction temperature is 80 °C.

Through the analysis and discussion of extraction time, extraction pressure and extraction temperature, we determined that the optimal extraction conditions of AASE was 20 min, 10 MPa and 80 °C.

Optimization of derivatization conditions

GC-MS is an analytical instrument for the analysis of volatile and semi-volatile substances. It requires that the detected objects had thermal stability, but acrylamide have small molecular weight, strong polarity, and it is easily decomposed at high temperature. Therefore, it is not suitable for direct detection by GC-MS, need to be derivatized. Acrylamide contains unsaturated compound with C=C double bond, so the addition reaction of acrylamide with potassium bromide and potassium bromate could produce 2,3-dibromopropanamide under acidic conditions, the chemical reaction process is as follows.

Fig. 3. Effect of the extraction temperature on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

Fig. 4. Effect of amount of concentrated sulfuric acid on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

The 2,3-dibromopropanamide has large molecular weight and volatility, it is relatively difficult to decompose at high temperature. In addition, the instrument has a high response to it, which greatly improves the sensitivity of detection. In order to improve the derivatization efficiency, it is necessary to select the amount of concentrated sulfuric acid, potassium bromide, potassium bromate and the derivatization time, along with the amount of ethyl acetate used in the extract after derivatization.

The derivative reaction needs to be carried out under strong acidic conditions, so the amount of sulfuric acid is one of the important factors affecting the derivative reaction [31–32]. The effect of different sulfuric acid consumption on the peak area of 2,3-dibromopropanamide was investigated under the conditions of 238 mg of potassium bromide, 8.35 mg of potassium bromate, 45 min of derivation time and 6 mL of ethyl acetate, as shown in Fig. 4. The peak area of 2,3-dibromopropanamide increased with the increase of sulfuric acid consumption when the consumption was below 5 µL, and reached the maximum at 5 µL. The peak area of 2,3-dibromopropanamide decreased with increasing consumption when the sulfuric acid consumption exceeded 5 µL. This was due to excessive acidity would affect the derivative

reaction and reduced the derivatization efficiency. Therefore, the optimal amount of sulfuric acid was 5 µL.

The dosage of potassium bromide is one of the key factors affecting the derivative reaction [33, 34]. The effect of different potassium bromide consumption on the peak area of 2,3-dibromopropanamide was investigated under the conditions of 5 µL of concentrated sulfuric acid, 8.35 mg of potassium bromate, 45 min of derivation time and 6 mL of ethyl acetate, as shown in Fig. 5. With the increasing amount of potassium bromide, the peak area of 2,3-dibromopropanamide increased first and then tended to be stable, when the amount of potassium bromide is 238 mg, the peak area of 2,3-dibromopropanamide had reached a large value. It is indicated that 238 mg of potassium bromide could guarantee the completion of derivative reaction, so the optimal dosage of potassium bromide was 238 mg.

The dosage of potassium bromate is another key factor affecting the derivative reaction [35, 36]. The effect of different potassium bromate consumption on the peak area of 2,3-dibromopropanamide was investigated under the conditions of 5 µL of concentrated sulfuric acid, 238 mg of potassium bromide, 45 min of derivation time and 6 mL of ethyl acetate, as shown in Fig. 6. The peak area of 2,3-dibromopropanamide increased first and then stabilized with the increase dosage of potassium bromate. When the amount of potassium bromate was 8.35 mg, the peak area of 2,3-dibromopropanamide reached the maximum value. Therefore, the optimal amount of potassium bromate was 8.35 mg.

The duration of derivatization directly affects the degree of derivatization reaction [33, 37]. Insufficient derivation time will reduce the rate of derivation, while excessive derivative time can guarantee the derivation rate, but it will reduce the experimental efficiency. The effect of different derivation time on the peak area of 2,3-dibromopropanamide was investigated under the conditions of 5 µL of concentrated sulfuric acid, 8.35 mg of potassium bromate, 238 mg of potassium bromide, and 6 mL of ethyl acetate, as shown in Fig. 7. With the extension of derivation time, the peak area of 2,3-dibromopropanamide increased first and then tended to be stable. When the derivation time was 45

Fig. 5. Effect of the dosage of potassium bromide on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

Fig. 6. Effect of the dosage of potassium bromate on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

Fig. 7. Effect of the derivatization time on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

min, the peak area of 2,3-dibromopropanamide had reached the maximum value, indicating that the derivatization reaction had been completed at this time.

The dosage of ethyl acetate extract determines whether or not the derivative product is completely extracted [35, 36]. The effect of different ethyl acetate consumption on the peak area of 2,3-dibromopropanamide was investigated under the conditions of 5 μ L of concentrated sulfuric acid, 8.35 mg of potassium bromate, and 238 mg of potassium bromide, as shown in Fig. 8. The peak area of 2,3-dibromopropanamide increased first and then stabilized with the increase dosage of ethyl acetate. When the dosage of ethyl acetate is 6 mL, the peak area of 2,3-dibromopropanamide had reached a large value. The peak area of the target substance did not increase after increasing the volume of extracting liquid, which indicated that 6 mL ethyl acetate could completely extract the derivative product. Considering the cost of the experiment, the optimum dosage of ethyl acetate is 6 mL.

Through the analysis and discussion of the derivatization time and the dosage of sulfuric acid, potassium bromide and potassium bromate, we determined that the optimal derivatization condition was 5 μ L of concentrated sulfuric acid, 238 mg of potassium bromide, 8.35 mg of potassium bromate and derivatization for 40 min. After derivatization, the target substance is extracted from the aqueous solution by 6 mL of ethyl acetate.

Fig. 8. Effect of the amount of ethyl acetate on peak area of 2,3-dibromopropanamide. Bars represent the standard deviation ($n = 4$)

Fig. 9. Standard curve of acrylamide standard solution

Linear relationship, limit of detection (LOD) and limit of quantification (LOQ)

As seen in Fig. 9, when the concentration of acrylamide was in the range of 10–2000 ng/mL, the linear regression equation of standard curve was $y = 0.0008x - 0.0004$, and the coefficient of determination (R^2) was 0.9997, indicating that the linear relationship is good in this range of concentration. The detection limit of the method was calculated by 3 times the signal-to-noise ratio of chromatographic peak, and the result was 3 μ g/kg. The quantification limit of the method was calculated by 10 times the signal-to-noise ratio of chromatographic peak, and the result was 10 μ g/kg. The chromatogram was shown in Fig. 10.

Accuracy and precision

In order to verify the accuracy and precision of the method, 1,000 g of biscuits, potato chips or fried dough twists were accurately weighed, added respectively 100,500 and 1,000 μ g/kg acrylamide standard solution, 1,000 μ g/kg D_3 -acrylamide standard solution. Each concentration point of each

Fig. 10. Mass chromatogram of 2,3-dibromopropanamide and isotope internal standard D_3 -2,3-dibromopropanamide. That was total ion chromatogram (TIC), selected ion chromatograms of D_3 -2,3-dibromopropanamide (m/z 153) and selected ion chromatograms of 2,3-dibromopropanamide (m/z 150) from top to bottom

Table 2. Recovery rate and relative standard deviation of acrylamide in samples at different standard addition levels ($n = 6$)

Sample	Added concentration ($\mu\text{g/kg}$)					
	100		500		1,000	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Biscuit 1	119.1	0.80	109.3	0.67	109.4	0.23
Biscuit 2	119.5	0.57	109.3	0.30	109.6	0.21
Potato chip 1	118.9	0.76	106.3	1.42	109.3	0.06
Potato chip 2	116.8	2.94	109.1	0.38	109.4	0.31
Fried dough twist	115.5	0.91	108.8	0.10	109.9	0.90

samples were determined six times in parallel, and the recovery rate and relative standard deviation (RSD) were calculated, as shown in Table 2. The standard addition recovery of acrylamide was between 115 and 120% when the added concentration was 100 $\mu\text{g/kg}$. The standard addition recovery of acrylamide was between 105 and 110% when the added concentration was 500 and 1,000 $\mu\text{g/kg}$. It showed that this method had good recovery effect and high accuracy. The relative standard deviation of the recovery of acrylamide in each sample was between 0.05 and 3.0%, which was very low, indicating that the method had good precision.

Detection and analysis of actual samples

Acrylamide was detected by this method in 16 batches of biscuits, potato chips and fried dough twists, as shown in Table 3, and the total ion chromatograms of the three kinds of samples are shown in Fig. 11. The content of acrylamide in

seven biscuit samples was between 0.05 and 0.55 mg/kg, with an average value of 0.29 mg/kg and a maximum value of 0.55 mg/kg. The content of acrylamide in six potato chips was between 0.24 and 2.64 mg/kg, with an average value of 1.33 mg/kg and a maximum value of 2.64 mg/kg. The content of acrylamide in three fried dough twist was between 0.21 and 0.31 mg/kg, with an average value of 0.26 mg/kg. It can be seen that the average content of acrylamide in potato chips was significantly higher than that in biscuits and fried dough twists. All the content of acrylamide in biscuits, potato chips and fried dough twists was within the scope of literature reports, which was further explained the accuracy and rationality of

Table 3. Content of acrylamide in 16 practical samples ($n = 4$)

Categories	Samples	Content of acrylamide (mg/kg)
Biscuits	biscuit 1	0.05
	biscuit 2	0.15
	biscuit 3	0.22
	biscuit 4	0.24
	biscuit 5	0.30
	biscuit 6	0.54
	biscuit 7	0.55
Average value		0.29
Potato chips	potato chip 1	0.24
	potato chip 2	0.56
	potato chip 3	0.58
	potato chip 4	1.82
	potato chip 5	2.12
	potato chip 6	2.64
Average value		1.33
Fried dough twists	fried dough twist 1	0.21
	fried dough twist 2	0.27
	fried dough twist 3	0.31
Average value		0.26

Fig. 11. The total ion chromatograms of the three kinds of samples

the experimental method [38–40]. The content of acrylamide in food is closely related to its processing methods and conditions, such as frying, processing temperature, processing time, etc. It is suggested that relevant food enterprises improve food processing technology to reduce the content of acrylamide in products.

CONCLUSIONS

In this study, a method for the determination of acrylamide in food was established by automatic accelerated solvent extraction-gas chromatography-mass spectrometry (AASE-GC-MS). The method utilized AASE to extract and purify samples, which not only greatly improved the extraction efficiency, but also avoided the artificial error. The extraction efficiency was further improved by optimizing the conditions of AASE. The optimum extraction conditions were extraction temperature 80 °C, extraction pressure 10 MPa and extraction time 20 min. The use of potassium bromide and potassium bromate as the derivatization reagent not only improved the derivatization efficiency, but also ensured the safety of the experiment. The derivatization efficiency was further improved by optimizing the derivatization method. The optimum conditions for derivatization were 5 µL of concentrated sulfuric acid, 238 mg of potassium bromide, 8.35 mg of potassium bromate, 45 min of derivatization time and 6 mL of ethyl acetate. The method had a good linear relationship in the concentration range of 10–2000 ng/mL, and the coefficient of determination (R^2) was 0.9997. The detection limit of the method was 3 µg/kg. The quantification limit of the method was 10 µg/kg. The standard addition recovery of acrylamide was between 105 and 120% when the added concentration was 100, 500 and 1000 µg/kg. The relative standard deviation of the recovery of acrylamide was less than 3.0%. The method was simple, safe, sensitive, accurate, reproducible, and was suitable for risk monitoring and exposure assessment of acrylamide in food. It had a good promotion and application value.

REFERENCES

1. Lyon, F. *Some Ind. Chem.* **1994**, 60, 389.
2. Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. *J. Agric. Food Chem.* **2002**, 50, 4998.
3. Dybing, E.; Sanner, T. *Toxicol. Sci.* **2003**, 75, 7.
4. Zhang, Y.; Zhang, G.; Zhang, Y. *J. Chromatogr. A* **2005**, 1075, 1.
5. Ono, H.; Chuda, Y.; Ohnishi-Kameyama, M.; Yada, H.; Ishizaka, M.; Kobayashi, H.; Yoshida, M. *Food Addit. Contam.* **2003**, 20, 215.
6. Svensson, K.; Abramsson, L.; Becker, W.; Glynn, A.; Hellenäs, K. E.; Lind, Y.; Rosen, J. *Food Chem. Toxicol.* **2003**, 41, 1581.
7. Rice, J. M. *Mutat. Res./Genet. Toxicol. Environ. Mutagen.* **2003**, 580, 30.
8. Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M. C.; Riediker, S. *Nature* **2002**, 419, 449.
9. Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. *Nature* **2002**, 419, 448.
10. Ahn, J. S.; Castle, L.; Clarke, D. B.; Lloyd, A. S.; Philo, M. R.; Speck, D. R. *Food Addit. Contam.* **2002**, 19, 1116.
11. Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K.; Villagran, M. D. *J. Agric. Food Chem.* **2003**, 51, 4782.
12. World Health Organization Health Implications of Acrylamide in Food: Report of a Joint FAO/WHO Consultation, WHO Headquarters, Geneva, Switzerland, 25–27 June **2002** (Food Safety Consultations). WHO.
13. JECFA Evaluation of Certain Food Additives and Contaminants. Sixty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives, **2006**.
14. Saraji, M.; Javadian, S. *Food Chem.* **2019**, 274, 55.
15. Ghiasvand, A. R.; Hajipour, S. *Talanta* **2016**, 146, 417.
16. Notardonato, I.; Avino, P.; Centola, A.; Cinelli, G.; Russo, M. V. *Anal. Bioanal. Chem.* **2013**, 405, 6137.
17. Elahi, M.; Kamankesh, M.; Mohammadi, A.; Jazaeri, S. *Food Anal. Methods* **2019**, 12, 1439.
18. Norouzi, E.; Kamankesh, M.; Mohammadi, A.; Attaran, A. *J. Cereal Sci.* **2018**, 79, 1.
19. Nematollahi, A.; Kamankesh, M.; Hosseini, H.; Ghasemi, J.; Hosseini-Esfahani, F.; Mohammadi, A. *Journal of Cereal Science* **2019**, 87, 157.
20. Faraji, M.; Hamdamali, M.; Aryanasab, F.; Shabaniyan, M. *J. Chromatogr. A* **2018**, 1558, 14.
21. Martínez, E.; Rodríguez, J. A.; Bautista, M.; Rangel-Vargas, E.; Santos, E. M. *Food Anal. Methods* **2018**, 11, 1636.
22. Samanipour, B.; Jalili, M.; Rezaei, K.; Faraji, R. *Qual. Assur. Saf. Crop. Foods* **2019**, 11, 411.
23. Kafouris, D.; Stavroulakis, G.; Christofidou, M.; Iakovou, X.; Christou, E.; Paikousis, L.; Yiannopoulos, S. *Food Addit. Contam.* **2018**, 35, 1928.
24. Qin, L.; Zhang, Y. Y.; Xu, X. B.; Wang, X. S.; Liu, H. W.; Zhou, D. Y.; Thornton, M. *Food Chem.* **2017**, 232, 633.
25. Ferrer-Aguirre, A.; Romero-González, R.; Vidal, J. L. M.; Frenich, A. G. *Food Anal. Methods* **2016**, 9, 1237.
26. Jiao, H.; Lu, S.; Hou, D.; Zhang, Q. *Se pu= Chin. J. Chromatogr.* **2019**, 37, 605.
27. Toubane, A.; Rezzoug, S. A.; Besombes, C.; Daoud, K. *Ind. Crop. Prod.* **2017**, 97, 620.
28. Sun, H. W.; Ge, X. S.; Lv, Y. K.; Wang, A. B. *J. Chromatogr. A* **2012**, 1237, 1.
29. Wang, B.; Xie, X.; Zhao, X.; Xie, K. Z.; Diao, Z. X.; Zhang, G. X.; Zhang, T.; Dai, G. J. *Molecules* **2019**, 24, 1830.
30. Ahmad, R.; Ahmad, N.; Shehzad, A. *Ind. Crop. Prod.* **2019**, 136, 37.
31. Zhang, Y.; Ren, Y.; Zhao, H.; Zhang, Y. *Anal. Chim. Acta* **2007**, 584, 322.
32. Tekkeli, S. E. K.; Önal, C.; Önal, A. *Food Anal. Methods* **2012**, 5, 29.
33. Nemoto, S.; Takarasuki, S.; Sakaki, K.; Maitani, T. *J. Food Hyg. Soc. Jpn.* **2002**, 43, 371.
34. Yang, S.; Zhang, H.; Wang, J.; Chen, F. *Se pu= Chin. J. Chromatogr.* **2011**, 29, 404.
35. Zhang, Y.; Dong, Y.; Ren, Y. R.; Zhang, Y. *J. Chromatogr. A* **2006**, 1116, 209.

36. Pittet, A.; Périsset, A.; Oberson, J. M. *J. Chromatogr. A*, **2004**, 1035, 123.
37. Geng, Z.; Wang, P.; Liu, A. *J. Chromatogr. Sci.* **2011**, 49, 818.
38. Zhou, P. P.; Zhao, Y. F.; Liu, H. L.; Jian, Y.; Li, X. W.; Xin, Y. A. N. G.; Wu, Y. N. *Biomed. Environ. Sci.* **2013**, 26, 421.
39. European Food Safety Authority. *EFSA J.* **2011**, 9, 2133.
40. Ajani, Z.; Fatunsin, O. T.; Oyeyiola, O. A.; Olayinka, K. O. Risk Assessment of Acrylamide for Some Commonly Eaten Fried Rice, **2019**.

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