Analytical Methods

Ultrasonic-assisted extraction and dispersive liquid-liquid microextraction combined with gas chromatography-mass spectrometry as an efficient and sensitive method for determining of acrylamide in potato chips samples

Maryam Zokaei, Abdul-Samad Abedi, Marzieh Kamankesh, Saeedeh Shojaee-Aliababadi, Abdorreza Mohammadi

Department of Food Science and Technology, Faculty of Nutrition Science, Food Science and Technology/National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Spectroscopy, Micro and Nano-extraction Laboratory, Department of Chemistry, Iran University of Science and Technology, Tehran, Iran

Abstract

In this research, for the first time, we successfully developed ultrasonic-assisted extraction and dispersive liquid-liquid microextraction combined with gas chromatography-mass spectrometry as a new, fast and highly sensitive method for determining acrylamide in potato chips samples. Xanthydrol was used as a derivatization reagent and parameters affecting in the derivatization and microextraction steps were studied and optimized. Under optimum conditions, the calibration curves showed high levels of linearity ($R^2 > 0.9993$) for acrylamide in the range of 2–500 ng mL$^{-1}$. The relative standard deviation (RSD) for the seven analyses was 6.8%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.6 ng g$^{-1}$ and 2 ng g$^{-1}$, respectively. The UAE-DLLME-GC-MS method demonstrated high sensitivity, good linearity, recovery, and enrichment factor. The performance of the new proposed method was evaluated for the determination of acrylamide in various types of chips samples and satisfactory results were obtained.

Keywords:
- Acrylamide
- Potato chips
- Ultrasonic-assisted extraction
- Dispersive liquid-liquid microextraction
- Gas chromatography-mass spectrometry

1. Introduction

Acrylamide is a hydrophilic unsaturated amide with properties of white odorless, crystalline solid, low molecular weight, polar and low volatile. This compound is considered as the most actively investigated compound among heat-induced food contaminants (Krska et al., 2012) and forms as a by-product of cooking process in carbohydrate-rich foods at high temperatures and low moist conditions (Franek, Rubio, Diblikova, & Rubio, 2014). Maillard reaction of reducing sugars with asparagine at temperature higher than 120 °C is the most probable route to acrylamide formation during the browning process (Özer, Kola, Altan, Duran, & Zorlugenc, 2012). Acrylamide is a neurotoxic compound identified as a probable human carcinogen (group 2A) and genotoxicant (Liu, Zhao, Yuan, Chen, & Hu, 2008). Since November 2013, the European Commission (EC) proposed indicative value of acrylamide between 50 and 4000 μg kg$^{-1}$ in various foodstuff (Boushey, Beresford, Omenn, & Motulsky, 1995).

Many method have been used for measuring acrylamide in different samples, including planer chromatography (Alpmann & Morlock, 2008), high-performance liquid chromatography (HPLC) (Kaplan, Kaya, Ozcan, Ince, & Yaman, 2009; Wenzl et al., 2006), gas chromatography-mass spectrometry (GC–MS) (Fernandes & Soares, 2007; Hoenicke & Gatermann, 2005; Oracz, Nebesny, & Żyżeliewicz, 2011; Zhang, Zhang, & Zhang, 2005), and bioanalytical methods, such as immune enzymatic tests and biosensors (Tekkeli, Önal, & Önal, 2012). Among this method instrumental analysis method especially gas chromatography-mass spectrometry (GC–MS) was used as a powerful technique for the determination of acrylamide in various food samples. Prior to GC–MS analysis, derivatization of acrylamide is necessary to increase of vapor pressure and decrease of interaction with GC column. Recently, some studies have been employed xanthydrol as a novel acrylamide-derivatization reagent. This method was applied to aqueous sample and it is claimed to be more environmentally friendly, requires mild reaction conditions at low temperature, and proceeds in aqueous solution (Tsukakoshi et al., 2012; Yamazaki, Isagawa, Kibune, & Urushiyama, 2012).
The isolation of acrylamide from carbohydrate-rich samples such as potato needs primary extraction procedures and multistep sample-preparation techniques (Gökmen, Şenyuva, Acar, & Saranoğlu, 2005; Twaddle et al., 2004). Ultrasonic assisted extraction (UAE) due to its high extraction efficiency and rapid sample preparation has attracted much attention in recent years as a successful and well-developed method (Sharma et al., 2006). UAE is fast and easy to operate with high enrichment factor. (Jia et al., 2010). Therefore this method can be applied as an effectively sample preparation technique for the release of acrylamide from sample matrix prior to GC–MS analysis.

Microextraction techniques have been characterized as a promising basis for a new generation of sample preparation techniques and have recently received a great deal of attention (Abedi, Mohammadi, Azadniya, Mortazavian, & Khaksar, 2014; Asadi, Dadfarnia, Shabani, & Abbasi, 2015; Ghobadi, Yamini, & Ebrahimpour, 2015; Kamankesh, Mohammadi, Hosseini, & Tehrani, 2015; Kamankesh, Mohammadi, Tehrani, Ferdowsi, & Hosseini, 2013; Kim, Hwang, & Lee, 2007; Madani-Tonekaboni, Kamankesh, & Mohammadi, 2015; Mollahosseini, Toghroli, & Kamankesh, 2015; Rebroedo-Rodríguez et al., 2014; Song, Shi, & Chen, 2013; Wu et al., 2013).

One of the techniques attracting special attention is the dispersive liquid-liquid microextraction (DLLME), which was introduced in 2006 by Rezaee and co-workers (Rezaee et al., 2006). DLLME, which is based on the use of a ternary mixture of solvent systems, involves the rapid injection of an appropriate mixture of an extraction solvent and a disperser solvent into the sample solution. The disperser solvent must be fully miscible with both the aqueous sample and the extraction phase. But the extraction solvent must be miscible only with the dispersing phase, and must be insoluble in water. The main advantages of DLLME include its simplicity, requirement of very small volumes of extraction solvents, and the presence of very large surface area between the extraction solvent and the aqueous sample which rapidly reaches a state of equilibrium between the organic and aqueous phases. High enrichment factor, high speed and high recovery are other advantage of this technique. In addition, this microextraction method has been successfully employed for the determination of many compounds in various food samples (Aeenehvand et al., 2015; Bashiry et al., 2016; Mohammadi, Ghasemzadeh-Mohammadi, Haratian, Khaksar, & Chaichi, 2013; Mohammadi et al., 2013; Nojavan, Kamankesh, Shahraz, Hashemi, & Mohammadi, 2015; Pirsaheb & Fattahi, 2015; Ramezani, Hosseini, Kamankesh, Ghasemzadeh-Mohammadi, & Mohammadi, 2014).

In this work, for the first attempt, UAE-DLLME-GC-MS after derivatization with xanthydrol was applied as a fast, sensitive and accurate method for the determination of acrylamide in potato chips samples. Experimental variables of derivatization and microextraction process, such as type and volume of extraction and disperser solvents, derivatization reagent amount, sample pH, time and temperature of derivatization were optimized. The merit figures of the new proposed method compare with other previous methods. Finally, the newly developed method was used in the determination of acrylamide in potato chips samples and suitable results were obtained.

2. Experimental

2.1. Chemicals and reagent

Chemicals standards of acrylamide (99%) and acetamide were purchased from Merck (Darmstadt, Germany). Hydrochloric acid, sodium chloride, ethanol, methanol, acetone, acetonitrile tetra-chloroethylene, chloroform, carbon tetrachloride, dichloro-methane, hydroxide potassium, xanthydrol, di-potassium hydrogen phosphate (K2HPO4), potassium hexacyanoferrate (II) and zinc acetate were obtained from Merck (Darmstadt, Germany).

The derivatization reagent was prepared by dissolving 5 g of xanthhydril in 100 mL methanol. For preparation of carrez solution I, 10.6 g of potassiumhexacyanoferrate (II) was dissolved in 100 mL distilled water. Carrez solution II was prepared by mixing 21.9 g of zinc acetate with 3 mL of acetic acid, then adjusting the volume to 100 mL with distilled water. All solvents were analytical reagent grade or HPLC grade.

2.2. Standard

Stock standard solution (2000 μg mL–1) was prepared in methanol. To obtain a working solution, the upper standard solution was diluted with methanol; this working solution was applied to evaluate extraction performance under different conditions (2–500 ng g–1). Acetamide was used as an internal standard, and prepared in methanol at a concentration of 1000 μg mL–1. Stock and working solutions were stored at 4 °C in a refrigerator and were used daily in proper concentrations or directly.

2.3. Instrumentation

Chromatographic separations and detections of the target analytes were performed using a 7890A GC system from Agilent Technologies (Palo Alto, CA, USA) with a triple-axis detector fitted with a split/splitless injector and coupled with a 5975C inert MSD network mass selective detector. An HP-5 MS capillary column (5% phenyl siloxane/95% methyl polysiloxane: 30 m × 0.25 mm I.D., 0.25 μm film thickness) was used for the separation of chemical compounds. The oven temperature was programmed as follows: 100 °C held for 1 min, ramped to 300 °C at 20 °C min–1, held for 10 min. Helium was used as a carrier gas in a constant flow of 0.8 mL min–1. The injector temperature and auxiliary temperature were set at 290 °C and 280 °C, respectively. 2 μL of the sample was injected in a split mode with split ratio of 1:50. The selected ion monitoring (SIM) acquisition mode was used for the quantification of acrylamide, and the ions monitored were as follows: m/z 251 for acrylamide and m/z 239 for acetamide (IS). Also retention time for acetamide and acrylamide is 14.35 and 16.88 min, respectively.

An ultrasonic water bath, working at 50–60 kHz with maximum output power of 350 W (Euronda company, Vicenza, Italy) was used for ultrasonication of the samples.

2.4. Procedures

2.4.1. Preparation of real sample

Potato chips samples were purchased from main supermarkets and stored at a temperature of 4 °C. 1 g of potato chips sample was weighed, thoroughly ground and transferred to a conical flask. Then this sample was spiked with acetamide (internal standard) at a concentration of 50 ng g–1 and this mixture was thoroughly stirred to obtain a very homogeneous sample. 3 mL of hexane was added to remove the fat of sample. Then hexane was separated and the residual of solvent was evaporated. This sample was placed into the glass test tube and 5 mL deionized water was added. In order to accelerated extraction of acrylamide from sample matrix to aqueous phase the container of sample was immersed into an ultrasonic water bath for 5 min at 4 kHz of ultrasound frequency and 0.138 kW of power at 25 °C. After this stage, 0.5 mL carrez solution I and 0.5 mL carrez solution II were added to the sample solution to precipitate protein and soluble carbohydrate. This sample was thoroughly agitated and was centrifuged for 5 min in 4000 rpm. Then the supernatant was separated and filtered using
a syringe filter with cellulose acetate (0.45 μm). Finally, clean aqueous phase was transferred to another conical flask and 2 mL HCl (1 mol L⁻¹) and 40 μL xanthydrol (derivatization reagent) was added. This sample was kept in ambient temperature for 20 min for completely derivatization process. After this stage, 0.5 mL was added. This sample was kept in ambient temperature for 20 min for enrichment factor as a result of dilution. Therefore, 60 mL was added, the mixture was completely agitated for 2 min. Afterwards, the cloudy solution was centrifuged for 5 min at 4000 rpm. The dispersed fine droplets of the extraction solvent collected at the bottom of the test tube. Two μL of the sedimented phase was withdrawn using a 10 μL Hamilton microsyringe and injected directly into the GC–MS.

2.4.2. DLLME procedure
60 μL of tetrachloroethylene (extracting solvent) and 600 μL of ethanol (disperser solvent) were rapidly injected into the 10 mL sample solution, and then 1 g salt was added. The mixture was completely agitated for 2 min. Afterwards, the cloudy solution was centrifuged for 5 min at 4000 rpm. The dispersed fine droplets of the extraction solvent collected at the bottom of the test tube. Two μL of the sedimented phase was withdrawn using a 10 μL Hamilton microsyringe and injected directly into the GC–MS.

3. Result and discussion

3.1. Optimization of the UAE-DLLME method
Extraction and disperser solvents are two important parameters that can affect the extraction efficiency in the microextraction procedure. Therefore to achieve the optimal extraction conditions, extraction solvent and disperser solvents were screened. In the selection of extraction solvent, several factors such as low solubility in water, efficient extraction of the target compound, good chromatographic behavior and ability to formation of cloudy solution should be considered. Miscibility of dispersive solvent in sample solution and extraction solvent is the most important point for selecting a best disperser solvent. Carbon tetrachloride, chloroform and ethylene tetrachloride were tested as the extraction solvents. The results showed that ethylene tetrachloride had better recoveries for acrylamide extraction than the other solvents. This could be related to its higher density and lower water solubility. Based on these results, we chosen ethylene tetrachloride as an extraction solvent for the subsequent microextraction procedure. Methanol, acetonitrile, ethanol and acetone were evaluated to fine the appropriate dispersive solvent. Ethanol was found to be quite better disperser solvent, as it produced reasonable and repeatable sedimented phase volumes and stable cloudy solutions. Therefore, ethanol was selected as disperser solvent for the future experiments.

3.1.1. Volume of extraction solvent
In order to evaluate the effect of extraction solvent volume, solutions containing 40, 60, 80, 100 and 120 μL at the same UAE-DLLME procedure were examined (Fig. 1). 40 μL ethylene tetrachloride was hardly collected and withdrawn with syringe. 60 μL ethylene tetrachloride was showed the higher efficiency of acrylamide extraction. The increased efficiency was due to the reduction in the volume of the extraction solvent to 60 μL leading to an increase in the concentration of the target analytes. At volumes greater than 60 μL, absorbance decreased due to a decrease in enrichment factor as a result of dilution. Therefore, 60 μL was chosen for subsequent experiments.

3.1.2. Volume of dispersive solvent
The disperser solvent volume directly affects the formation of cloudy solution. The experimental condition was fixed and included the use of different volumes of ethanol (300–1000 μL). According to the results shown in Fig. 2, by increasing of the volume of ethanol, the extraction recovery increases and then decreases. At lower volumes, ethanol cannot disperse extraction solvent properly and cloudy solution is not formed completely. At volumes higher than 600 μL of ethanol, the solubility of the extraction solvent in aqueous sample, partially increases, therefore, the extraction recovery decreases. Thus, 600 μL ethanol was selected as the optimum volume for disperser solvent.

3.1.3. Derivatization reagent amount
To achieve the best volume of derivatization reagent, 40 to 120 μL of xanthylor was tested. Maximum peak areas were obtained when 80 μL of xanthylor was added, and at higher volumes the responses decreased. It seems that at low volumes (<80 μL) the amount of xanthylor is not enough to derivatize all analytes. Therefore 80 μL of xanthylor was used for derivatization process.

3.1.4. Temperature of derivatization
The influence of the temperature of derivatization on the extraction efficiency of acrylamide was also examined. Different
temperature ranging from 25 to 60 °C was selected as previous study (Yang & Shin, 2013). The results showed that the increase the temperature from 25 to 60 °C, decreased response (Fig. 3). It was indicated that at a high temperature the stability of derivative format of acrylamide and as well as relative peak area was significantly decreased. Therefore in further experiments, derivatization was conducted at ambient temperature (25 °C).

3.1.5. Time of derivatization

Variation of derivatization time influences the extraction response. The effect of this parameter on the extraction efficiency in the range of 20–60 min was studied. For derivatization process, 40 min has better response because the complete reaction of acrylamide and xanthodrol occurred in this time and the relative peak area was not changed, thereafter. Therefore 40 min was selected for whole experiment.

3.1.6. Sample pH

Generally, sample pH determines the state of analytes in sample solution and plays a key role in extraction of acrylamide from chips samples. The effect of pH was studied in the range 2–12. Other experimental conditions were kept constant as described above. It was found that better extraction efficiencies were obtained at pH 7.0. The results indicated that in this pH, the elimination of background noise, respectively and were obtained 0.6 ng g⁻¹ and 2 ng g⁻¹, respectively. The figures merit of proposed method was compared with other methods (Gökmen et al., 2005; Soares & Fernandes, 2009; Tezcan & Erim, 2008; Zhang, Dong, Ren, & Zhang, 2006) in Table 1.

3.2. Quantitative analysis

To evaluate the UAE-DLLME-GC-MS method, dynamic linear range (DLR), repeatability (RSD), limit of detection (LOD), limit of quantification (LOQ), recovery and enrichment factors (EF) were investigated under optimal condition. Calibration curves of acrylamide (standard solution) were linear over the range of 2–500 ng mL⁻¹ with coefficient of determination (R²) higher than 0.9993. Relative standard deviation percent (RSD%) was evaluated by analyzing seven replicates of acrylamide and was obtained 6.8%. The recovery for acrylamide was determined by comparing the amount of analyte added to a chips sample with the concentration found after the procedure. The recovery value of the extraction of acrylamide from the chips sample was 97%. LOD and LOQ defined as the lowest concentration of the analyte in a sample that provides a chromatographic signal three and ten times higher than background noise, respectively and were obtained 0.6 ng g⁻¹ and 2 ng g⁻¹, respectively. The figures merit of proposed method was compared with other methods (Gökmen et al., 2005; Soares & Fernandes, 2009; Tezcan & Erim, 2008; Zhang, Dong, Ren, & Zhang, 2006) in Table 1.

3.3. Analytical application to real samples

The practical applicability of the proposed method was evaluated under the optimum condition for the analysis of acrylamide in potato chips samples. Four potato chips samples were collected from different supermarket (Tehran, Iran). The analytical results are shown in Table 2. The concentrations of the acrylamide in the potato chips samples were determined by the standard addition method. Fig. 4 shows the chromatograms obtained by UAE-DLLME-GC-MS for a potato chips sample when (a) non-spiked and (b) spiked with acrylamide at a level of 50 ng g⁻¹. A clean separation and good chromatogram were readily achieved without the presence of sample matrix interferences.

**Table 1**

<table>
<thead>
<tr>
<th>Method</th>
<th>Compounds</th>
<th>Analyte</th>
<th>DLR (ng g⁻¹)</th>
<th>R²</th>
<th>RSD (%)</th>
<th>LOD (ng g⁻¹)</th>
<th>LOQ (ng g⁻¹)</th>
<th>Recovery (%)</th>
<th>EF</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE-DLLME-GC-MS²</td>
<td>Potato chips</td>
<td>Acrylamide</td>
<td>10–500</td>
<td>0.999</td>
<td>6.8</td>
<td>0.6</td>
<td>2</td>
<td>97</td>
<td>192</td>
<td>This study</td>
</tr>
<tr>
<td>GC-ECD³</td>
<td>Fried food</td>
<td>Acrylamide</td>
<td>1–200</td>
<td>&gt;0.998</td>
<td>7.5</td>
<td>0.1</td>
<td>3</td>
<td>87–97</td>
<td>192</td>
<td>Zhang et al. (2006)</td>
</tr>
<tr>
<td>MSPO-GC-MS³</td>
<td>Various foods</td>
<td>Acrylamide</td>
<td>100–1500</td>
<td>0.999</td>
<td>&lt;2.5</td>
<td>5.2</td>
<td>15.7</td>
<td>1–7</td>
<td>–</td>
<td>Soares &amp; Fernandes (2009)</td>
</tr>
<tr>
<td>HPLC-DAD²</td>
<td>Potato chips</td>
<td>Acrylamide</td>
<td>6–600</td>
<td>0.999</td>
<td>5.1–8.8</td>
<td>2</td>
<td>4</td>
<td>93–97</td>
<td>–</td>
<td>Golmen et al. (2005)</td>
</tr>
</tbody>
</table>

1: Capillary electrophoretic.
2: High-performance liquid chromatography-diode array detection.
3: Micro solid phase dispersion gas chromatography-mass spectrometry.
4: Gas chromatography-electron capture detector.
5: Proposed method.
Table 2
Acrylamide contents (ng g⁻¹) obtained in the analysis of potato chips samples by UAE-DLLME-GC-MS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acrylamide concentration</th>
<th>Added amount</th>
<th>Determined amount</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89.22 ± 6.06</td>
<td>50</td>
<td>136.43 ± 9.27</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>94.17 ± 6.40</td>
<td>50</td>
<td>139.84 ± 9.50</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>68.04 ± 4.62</td>
<td>50</td>
<td>116.85 ± 7.94</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>70.33 ± 4.78</td>
<td>50</td>
<td>117.92 ± 8.01</td>
<td>98</td>
</tr>
</tbody>
</table>

* Mean value ± standard deviation (n = 5).

![Fig. 4](image_url)

Fig. 4. The chromatogram obtained by UAE-DLLME-GC-MS for a real sample (sample 3) under optimum conditions. (a) non-spiked and (b) spiked with 50 ng g⁻¹ of acrylamide. 1) acetamide (internal standard); 2) acrylamide.
4. Conclusion

In this study, for the first attempt, we successfully developed a fast, efficient and reliable method to extract and analyze acrylamide in potato chips samples using UAE-DLLME-GC-MS. In the present study, xanthodrol as an efficient derivatization reagent was used. Effective variables of derivatization and microextraction process were studied and optimized. The results also show that the use of carrez solution to sediment proteins and carbohydrates can greatly decrease the interference of the real sample matrix. In comparison to other methods, the proposed method has significant advantages including low solvent volume, good precision, high enrichment factor, repeatability and recovery high. The developed method was applied for the trace determination of acrylamide in potato chips samples and satisfactory results were obtained.

Acknowledgment

The authors would like to thank the National Nutrition and Food Technology Research Institute of Iran for financial support of this work.

References


