Food Chemistry 274 (2019) 55–60

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

# Single-drop microextraction combined with gas chromatography-electron capture detection for the determination of acrylamide in food samples

Mohammad Saraji*, Salman Javadian

*Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, Iran*

ARTICLE INFO

*Keywords:*
Gas chromatography-electron capture detection
Single-drop microextraction
Derivatization
Food sample
Acrylamide

ABSTRACT

A single-drop microextraction method followed by gas chromatography-electron capture detection was developed to determine acrylamide in food samples. Acrylamide was extracted by water and derivatized by hydrobromic acid in the presence of ammonium peroxydisulfate. The derivatization was carried out at 45 °C in 15 min using 46 μL of hydrobromic acid and 98 mg of ammonium peroxydisulfate. A 3.0-mL volume of the derivatized analyte was extracted using a 1.0-μL *n*-octanol droplet hanging from the needle tip of a GC microsyringe. After extraction, the extract was injected into the gas chromatograph. The influence of experimental parameters effective on derivatization reaction yield and extraction performance was studied. The limit of detection and quantification, relative standard deviation and linearity of the method were 0.60 μg/L, 2.0 μg/L, < 6.0%, and 2.0–100.0 μg/L, respectively. The method was utilized to determine acrylamide in three food samples (i.e., bread, potato chips and cookie).

## 1. Introduction

Acrylamide is a low molecular weight compound with high solubility in water. It is very reactive in air and is rapidly polymerized. Acrylamide is commercially produced by the reaction between sulfuric acid and acrylonitrile, or the hydration of acrylonitrile using a copper catalyst (Asano, Yasuda, Tani, & Yamada, 1982; Smith & Oehme, 1991). Formation of acrylamide in carbohydrate-rich foods (e.g., bread, biscuit, potato chips, rice, and corn) at elevated temperatures was discovered by a group of Swedish researchers in 2002 (Zhang, Zhang, & Zhang, 2005). The main mechanism of the procedure is the Maillard reaction, in which asparagine amino acid is converted to acrylamide in the presence of reducing sugars such as glucose (Zhang et al., 2005). Acrylamide is distributed throughout the human body after entering blood (Lopachin & Gavin, 2008), damages the neuron system (Matthäus, Haase, & Vosmann, 2004), and causes infertility, eye infection and weakness of irritability (Brathen & Knutsen, 2005; Lignert et al., 2002). It is also known as a carcinogenic compound (Besaratinia & Pfeifer, 2007). The tolerable daily intake for the carcinogenicity and neurotoxicity of acrylamide is 2.6 and 40 μg per day for each Kg of body weight, respectively (Tardiff, Gargas, Kirman, Leigh Carson, & Sweeney, 2010).

Due to the daily consumption of carbohydrate-rich foods by human, determining the concentration level of acrylamide in these samples is very important. The analytical instruments used for determining acrylamide include gas chromatography-electron capture detection (GC-ECD) (Hashimoto, 1976; Zhang, Dong, Ren, & Zhang, 2006), GC-mass spectrometry (MS) (Weisshaar, 2004), liquid chromatography (LC) with ultraviolet (UV) (Geng, Jiang, & Chen, 2008; Shi, Zhang, & Zhao, 2009; Wang, Lee, Shuang, & Choi, 2008), or MS (Rufianhenares & Morales, 2006; Weisshaar, 2004) detection, and capillary electrophoresis (CE) with UV and MS (Bermudo, Nnuez, Puignou, & Galceran, 2006; Bermudo, Nnuez, Moyano, Puignou, & Galceran, 2007; Oracz, Nebesny, & Zyzelewicz, 2011). Because of the low sensitivity of LC-UV and CE-UV for the determination of acrylamide, it should be derivatized before detection (Bermudo et al., 2006; Shi et al., 2009; Oracz et al., 2011). Among the different instruments, GC has been widely applied for acrylamide determination. Measuring acrylamide concentration in food samples, without derivatization by GC, has been reported in a few articles (Kaykhaii & Abdi, 2013; Weisshaar, 2004). However, to improve the extraction capability of the analyte and increase the sensitivity of analysis, a derivatization step is necessary before GC analysis. For derivatization, acrylamide is generally brominated to 2,3-dibromopropanamide (2,3-DBPA) (Areke, Ydberg, Arlsson, & Riksson, 2002; Hashimoto, 1976; Pittet, Perisset, & Oberson, 2004). In almost all studies, a very toxic reagent ($Br_2$) has been used for brominating acrylamide, and the derivatization process was time-consuming. In the standard EPA method, the mixture of KBr, HBr and $Br_2$ (Hashimoto,

* Corresponding author.
*E-mail address:* saraji@cc.iut.ac.ir (M. Saraji).

https://doi.org/10.1016/j.foodchem.2018.08.108
Received 17 January 2018; Received in revised form 14 July 2018; Accepted 24 August 2018
Available online 25 August 2018

1976; US Environmental Protection Agency, 1996) is used for derivatization. Due to the use of very toxic reagents in this procedure, a mixture of KBr and $KBrO_3$ has also been employed for derivatizing acrylamide to avoid using bromine (Zhang et al., 2006; Zhang, Ren, Zhao, & Zhang, 2007). However, the reaction yield for producing 2,3-DBPA was < 5%. Hence, another reaction product (i.e., 2-bromopropenamide) with the reaction yield > 95% was used for quantification. As a disadvantage, the amount of the derivatization reagents should be carefully adjusted because it has a great effect on the derivatization reaction yield (Zhang et al., 2006; Zhang et al., 2007). In addition, the extract should be cleaned before instrumental analysis.

Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) have mainly been applied for extracting derivatized acrylamide (Areke et al., 2002; Hashimoto, 1976; US Environmental Protection Agency 1996; Zhang et al., 2005; Zhang et al., 2006; Zhang et al., 2007). LLE and SPE are tedious procedures that use large volumes of expensive and noxious solvents. Today, solvent microextraction techniques as simple, inexpensive and environmentally friendly methods have attracted researchers' attention for sample preparation. Only two studies have reported the analysis of acrylamide in food samples using microextraction techniques (Kaykhaii & Abdi, 2013; Qu, Liu, Luo, Qiu, & Chen, 2013). In the first report, the analyte in an aqueous solution was derivatized using a derivatization procedure by $KBr/KBrO_3$. The derivatized analyte (mono bromide propanamide) was extracted by a solid-phase microextraction fiber (Qu et al., 2013). In the second study, acrylamide was extracted from potato chips by an organic solvent (200 mL ethyl hexanoate), followed by single-drop microextraction (SDME) (Kaykhaii & Abdi, 2013). The procedure is not environmentally friendly due to using a large volume of ethyl hexanoate.

In the present study, SDME followed by GC-ECD detection was used for extracting and determining acrylamide in food samples. A novel and more environmentally friendly derivatization procedure was used to derivatize acrylamide to 2,3-DBPA. A mixture of HBr and $(NH_4)_2S_2O_8$ was used to perform the derivatization reaction at a relatively low temperature (45 °C). Extraction solvent and derivatization reagents were used at micro amounts. The parameters affecting derivatization yield and extraction reactions were studied and optimized. The analytical performance and feasibility of the method for analyzing food samples were also studied.

## 2. Experimental

### 2.1. Chemicals and reagents

Pure acrylamide and 2,3-DBPA were purchased from Merck (Darmstadt, Germany) and Alfa Aesar (Ward Hill, MA, USA), respectively. GC grade *n*-octanol was obtained from Fluka (Buchs, Switzerland). Methanol and HPLC grade hexane were purchased from Caledon Laboratories (Georgetown, Ont., Canada). Hydrobromic acid (48%) was obtained from Daejung Co. (Siheung, Korea). Ammonium peroxydisulfate, sodium chloride, and sodium hydroxide were also obtained from Merck. Pure water was prepared by a water purification system consisted of ion exchange and carbon cartridges (Overseas Equipment & Services, OK, USA).

Stock standard solutions of acrylamide and 2,3-DBPA were prepared at the concentration of 1000 mg/L in water and methanol, respectively. A solution at the concentration of 10.0 mg/L was prepared by diluting the standard solutions with water. Diluted working solutions were prepared daily from the above standard solution. To protect the standard solutions from light, the containers were covered with aluminum foil and kept in a refrigerator.

### 2.2. Instrumentation

A gas chromatograph (BFRL, Beijing, China), model 3420 equipped with ECD was utilized. Nitrogen gas with the purity of 99.999% at a head column pressure of 0.1 MPa was used as the carrier gas. A DB-1701 GC column (30 m, 0.25 mm I.D. and 0.15 μm thicknesses) from Agilent Technologies (Palo Alto, CA, USA) was used for separation. Injection port and detector temperatures were set at 240 and 280 °C, respectively. All injections were performed in split mode at the split ratio of 1/18. Column temperature programming was as follow: 60 °C for 1.0 min, then increased by 12 °C/min until 150 °C (2 min hold), and finally, increased to 170 °C (2 min hold) at the rate of 30 °C/min.

### 2.3. Derivatization procedure

A 3.0-mL standard aqueous solution of acrylamide (100.0 μg/L), 98 mg of ammonium peroxydisulfate and 46 μL of HBr were added to an 8-mL glass vial. The glass vial was sealed with a polyethylene cap and heated (at 45 °C) in a water bath on a magnetic stirrer for 15 min. The solution was then neutralized by adding NaOH solution (1.5 mol/L). To ensure complete neutralization process, the pH of the solution was tested by a pH paper. The solution was diluted with water in a 5.0 mL volumetric flask and used for SDME.

### 2.4. SDME procedure

A 10.0 μL GC syringe (Hamilton, Bonaduz, Switzerland) was employed to perform the SDME experiments. To perform SDME, 3.0 mL solution containing the derivatized analyte, 1.0 g NaCl, and a stir bar were added into a 5-mL extraction vial. The vial was placed on a magnetic stirrer (MR 3000D, Heidolph, Germany). A volume of 1.0 μL of *n*-octanol was withdrawn into the syringe and the syringe needle immersed into the solution. The plunger was depressed, and a 1.0-μL solvent drop was formed at the tip of the needle. The solution was stirred at the rate of 300 rpm for 5.0 min. After extraction, the organic solvent containing the extracted analyte was retracted into the syringe and analyzed by GC-ECD.

### 2.5. Real samples

To investigate the applicability of the present method in complex matrices, different food samples including bread, potato chips and cookie were analyzed by the method. The samples were provided from a local supermarket. They were dried in a heating oven (50 °C) for one night. After that, the samples were grinded by a metal mortar. An amount between 2 and 3 g of the grinded sample was transferred into a beaker. In the case of cookie and potato chips samples, a 3-mL volume of *n*-hexane was added, and the solution agitated on a shaker (Aria Teb Co., Tehran, Iran) for 5 min. After that, the sample was centrifuged, and the *n*-hexane phase was discarded. The fat extraction with *n*-hexane was repeated one more time. The sample was dried at room temperature. A volume of 100 mL of water was added to the sample and the solution ultrasonicated for 20 min. The clear solution was then appropriately diluted (10 times for cookie and bread samples and 5 times for potato chips) with water, and 3 mL of the diluted sample was used for performing the derivatization process.

For spiking the samples, a standard aqueous solution of 100 mg/L of acrylamide was used. The samples were spiked at two concentration levels. Bread sample (3.0 g) was spiked with 90 and 150 μL of the standard solution of acrylamide. For potato chips, 40 and 80 μL of the acrylamide solution was added to the samples. Cookie sample was spiked with 80 and 160 μL of the acrylamide solution.

Each experiment was replicated three times to calculate the relative standard deviation (RSD) of data. To assess the accuracy of the method, real samples were spiked with the different amounts of acrylamide and spiking recovery was calculated. The spiking recovery was defined as:

$$\text{spiking recovery } (\%) = \frac{C_{found} - C_{real}}{C_{added}} \times 100$$

where $C_{found}$, $C_{real}$, and $C_{added}$ are the concentration of analyte measured