Investigation on the Contents of Acrylamide in Baked and Fried foods

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Abstract: The current work aims to develop a method for the determination of acrylamide in baked and fried foods using high performance liquid chromatography-mass spectrometry (UPLC-MS/MS). In this study, the extraction of acrylamide was using water and then cleaned up on a HLB and MCX SPE cartridge, followed by separating on a [Waters ACQUITY UPLC BEH C18 (100 mm×2.1 mm, 1.7 μm)] column using acetonitrile and 0.1% formic acid as mobile phase. Thereafter, the concentration of acrylamide was determined in multiple reaction monitoring mode, usingD₃-acrylamide as internal standard. It was found that the proposed calibration curve was linear in the range of 0.1 to 100 μg/L with a correlation coefficient greater than 0.99 indicating a very good linearity. The limit of detection (LOD) was 5.0 μg/kg and the quantitative limit (LOQ) was 10.0 μg/kg. The average recovery at three spiked levels (5, 20 and 100 μg/kg) of roasted coffee and deep-fried dough sticks was ranged from 88.0 % to 106 % with RSD smaller than 6%. The method was also tested by analysing acrylamide in baked and fried food samples (biscuits, roasted coffee, cake, fried potato, potato chips and deep-fried dough sticks). The acrylamide concentrations were found between 28 μg/kg and 635 μg/kg in those samples. Taken together, the developed method has superior for determination of acrylamide in baked and fried foods samples, which is of simplicity, high accuracy and good stability.

1. INTRODUCTION

Acrylamide (NH₂-C(=O)-CH-CH₂) has been found in a variety of heated carbohydrate-rich food and is now regarded as probably carcinogenic to humans [1,2]. Acrylamide is formed during heating sugar and protein containing foods above 120 °C [3]. The formation of acrylamide is largely due to the Maillard reactions in a wide range of foods [4]. Baked and Fried foods are the most important sources of acrylamide [5,6].

Currently, mass spectrometric (MS) and non-MS based techniques are the two major strategies for detection of acrylamide.. Recently, liquid chromatography (LC) coupled with pulsed electrochemical detection, time-of-flight MS, and capillary zone electrophoresis arisen as new analytical techniques. Foreexample, ion-exclusion and ion-exchange LC have been used for determination of acrylamide in food [7]. Other methods including gas chromatography (GC) [8-10], liquid chromatography [11], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [12-14] and enzyme-linked immunosorbent assay (ELISA) [15,16] have been also applied for detection of acrylamide. The ELISA determination is
quick and sensitive, but always give false positive results. Additionally, the GC-MS detection with transformation or derivatization is rather tedious and time-consuming. Nevertheless, the application of LC-MS greatly improved the ability of detection of acrylamide at regulated levels. With the developing of this technology, UPLC-MS/MS has emerged as an important strategy for determination and confirmation[17,18].

The present paper investigates the Contents of Acrylamide in Baked and Fried foods using UPLC-MS/MS. This study aims to develop an analytical strategy with high sensitivity, selectivity, easy to handle and fast to perform for detection of acrylamide in baked and fried foods.

2. MATERIALS AND METHODS

2.1 Reagents and materials
Acetonitrile (chromatographic grade) was purchased from Spectrum. Methanol (chromatographic grade) was purchased from Spectrum. HLB SPE cartridge (6cc, 200 mg) And MCX SPE cartridge (3cc, 60 mg) were obtained from waters. Acrylamide (≥98 %) and D3-acrylamide (≥98 %) were purchased from Dr. Ehrenstorfer company. Baked and Fried foods were obtained from local markets of Zhanjiang, Guangdong Province, China.

2.2 Instruments
High performance liquid chromatography-mass spectrometry (ACQUITY UPLC™-TQ) were purchased from waters. MS3 Vortex mixer and T25 homogenize were purchased from IKA. The Milli-QA1 water purification system was purchased from Millipore with a resistivity higher than 18 MΩ·cm for purification of water. CR22GIII centrifuge were purchased from HITACHI. EE120H Ultrasoundoscope were purchased from Elma.

2.3 Standard solutions
One milligram of acrylamide was dissolved in 10.0 mL of methanol to prepare a standard acrylamide stock solution (0.10 mg/mL). The stock solutions were further diluted with methanol to obtain a series of standards (0.1, 1.0, 2.0, 10.0, 50.0, 100 μg/L). D3-acrylamide at a concentration of 10 mg/L was used as the internal standard (IS). All standard solutions were stored at 4 ℃ until further use.

2.4 Sample preparation
All baked and fried foods were purchased from a local market of Zhanjiang, Guangdong province, China. All samples of around 200 g of each were stored at 4 ℃ immediately. The analysis was performed in triplicate for each type of sample. Prior to use, all the samples were homogeneously grounded to less than 2 mm of mesh size.

Baked and fried food powders of 1.0 g, 10 mg/ L D3-Acrylamide of 20 μL, and water of 10 mL was sequentially added into a 50 mL centrifuge tube. The mixture was periodically shaken for 5 min and then centrifuged at 8000 r/min for homogenization. Thereafter, the mixture was extracted with ultrasonic bath for 2 min and followed by centrifugation at 8000 r/min for 6 min. The collected supernatant was transferred into a new 50 mL centrifuge tube, and 5 mL of hexane was added in. After periodically shaken for 5 min, the mixture was centrifuged at 8000 r/min for 6 mins to remove the hexane.

2.5 SPE clean-up for samples
For sample cleaning-up, 3 mL methanol, 3 mL water and then 1.5 mL extraction solution were used for washing the HLB SPE cartridge. Thereafter, the SPE column was eluted by 4 mL methanol (80%). The MCX SPE cartridge was sequentially rinsed by 2 mL methanol, 2 mL water and then 4.0 mL eluted solution. All the solutions were collected and heated in a water bath at 40℃, and the products were rotoevaporated to dryness under reduced pressure condition. The obtained products were dissolved in 1.0 mL formic acid (0.1 %) and then centrifugated at 15000 r/min for 6 min. The solution
was filtered with a 0.22 μm filter and then collected for UPLC-MS/MS determination and confirmation.

2.6 UPLC-MS/MS method
A gradient UPLC system (Table 1) with acetonitrile and 0.1 % formic acid was used to separate acrylamide at a rate of 0.30 mL/min on a Waters ACQUITY UPLC BEH C18 (100 mm×2.1 mm, 1.7 μm). The temperature of the column was 40 ℃, while the injection volume was 10.0 μL. The analysis was carried out using positive-ion electrospray interface (ESI⁺) under multiple reaction monitoring mode. The interface conditions were as follows: source temperature of 110 ℃, capillary voltage of 3.0 kV, desolvation temperature of 350℃, collision gas of argon, the cone and desolvation gas (nitrogen) were with a flow rate of 50 L/h and 700 L/h, respectively. The parameters of MS/MS were presented in Table 2.

| Table 1. The gradient profile of UPLC for detection of acrylamide |
|---|---|---|---|
| Time (min) | Flow rate (mL/min) | Acetonitrile (%) | 0.1% formic acid (%) |
| 0 | 0.30 | 5 | 95 |
| 0.5 | 0.30 | 5 | 95 |
| 2.0 | 0.30 | 90 | 10 |
| 3.0 | 0.30 | 50 | 50 |
| 5.0 | 0.30 | 5 | 95 |

| Table 2. Parameters of MS/MS for detection of acrylamide |
|---|---|---|---|
| Compound | Precursor ions(m/z) | Product ions(m/z) | Cone voltage(V) | Collision (eV) |
| Acrylamide | 72.2 | 43.7 | 25.0 | 10.0 |
| D3-acrylamide | 75.2 | 57.7* | 20.0 | 10.0 |

*Ions for quantification

3. RESULTS AND DISCUSSION

3.1 Optimisation of the chromatographic conditions
The kinds of mobile phase have a great effect on UPLC analysis. Therefore, test was performed to investigate the analysis effect when different mobile phase (acetonitrile-0.1% formic acid, acetonitrile-5mmol/L ammonium acetate, methanol-water) were used. A better separation effect, higher signal/noise and better chromatographic peak shape were found when acetonitrile-0.1% formic acid was used as mobile phase to separate acrylamide in Waters ACQUITY UPLC BEH C18.

3.2 Linearity of the methods
Increasing concentrations of acrylamide was used to study the range of linearity. The calibration curves for acrylamide were obtained by plotting the peak area (y) versus concentration (x) of each analyte with a correlation coefficient (r²) of 0.9994, indicating a good linearity. The calibration curves were generated from the peak area responses of standards with concentrations ranged from 0.1 to 100 μg/L.

3.3 LOD and LOQ of the methods
The acrylamide standard solution was added in to the samples, and then pre-treated and analysed based on the method described above. The limit of quantitation (LOQ) and limit of detection (LOT) of acrylamide were determined by calculating the signal-to-noise (S/N) ratio using the equipped software. The LOQ (S/N=10) and LOT (S/N=3) determined were 15 and 5μg/kg, respectively.
3.4 Accuracy of the methods
Samples for each matrix was spiked with 5.0-100.0 μg/kg of acrylamide. The recoveries were calculated for each sample (Table 3, Table 4).

| Spiked concentrations (μg/kg) | Mean measured concentrations (μg/kg) | Background content (μg/kg) | Mean recovery (%) | RSD (%) |
|-----------------------------|----------------------------------|--------------------------|------------------|---------|
| 5.0                         | 26.4                             | 22.0                     | 88.0             | 5.2     |
| 20.0                        | 40.4                             | 22.0                     | 92.0             | 4.1     |
| 100.0                       | 110.3                            | 22.0                     | 88.3             | 4.7     |

To estimate the detection accuracy, acrylamide standard solution was added into the samples. The analysis was performed at each of the three fortification levels with six replicate tests. The recovery of the method was investigated using deep-fried dough sticks and roasted coffee fortified at 5.0 μg/kg, 20.0 μg/kg and 100.0 μg/kg. The mean recovery of acrylamide detected in three separate assays were shown in Table 3 and Table 4, which was ranged from 88.0 % to 106 % with relative standard deviations (RSD) less than 6 %.

3.5 Food sample analysis
The method was used to analyse acrylamide in different baked and fried foods like biscuits, roasted coffee and cake. Samples of fried potato, potato chips and deep-fried dough sticks were also analysed. As shown in Table 5, the concentrations of acrylamide in Baked and Fried foods were 28 μg/kg to 635 μg/kg.

| Sample type     | Sample name          | Number of analysis | Detected Concentration (μg/kg) |
|-----------------|----------------------|--------------------|--------------------------------|
| Baked food      | Biscuit              | 10                 | 28-375                         |
|                 | Roasted coffee       | 10                 | 22-295                         |
|                 | Cake                 | 5                  | 311-491                        |
|                 | Fried potato         | 3                  | 345-394                        |
| Fried food      | Potato chips         | 4                  | 480-635                        |
|                 | Deep-fried dough sticks | 3               | 120-156                        |

4. CONCLUSIONS
In the current study, a UPLC-MS/MS method was developed for detection of acrylamide in baked and fried foods. The RSD, accuracy, LOQ and LOD suggest that the method provides a reliable approach to determination of acrylamide in different baked and fried foods. Results show that this method is of high sensitivity and accuracy, and has the advantages of easy to handle and fast to perform, which is suitable for determination of acrylamide in baked and fried foods. Moreover, it was confirmed that significant amounts of acrylamide were formed in baked and fried foods upon thermal processes.
ACKNOWLEDGEMENTS
This work was funded by Central Public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences (No. 1630122017020) and the National Program for Quality and Safety Risk Assessment of Agricultural Products of China (GJFP2019019; GJFP2019038).

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