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Determination of Acrylamide in Starchy Foods by RP-HPLC

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Abstract

In order to establish a simple method for detecting acrylamide in food, this article optimizes the determination conditions, sample and processing conditions using high-performance liquid chromatography ultraviolet detector. The research results show that, the samples defatted by hexane, ultrasonic extraction in chloroform. The analytes were isolated by reversed phase high performance liquid chromatography on a C18(4.6×250mm，5μm) column and eluted with 5% methanol aqueous solution at a flow rate of 0.8mL/min. The detection wavelength was 210 nm, Quantitative analysis method was external standard method. The experimental results indicated that the linearity of the calibration curve was good in the range of 0.2μg/ml~2.5μg/ml (r=0.9991), and the recoveries of acrylamide from spiked samples by this method were within 87.6%~97.8% (n=5). The method was simple, accurate, reliable and suitable for the determination of acrylamide in potato chips, instant noodles, biscuits, bread and other starchy foods. Compared with traditional methods, this method does not rely on expensive Mass spectrometry detection equipment and is easily adopted by most laboratories, which is conducive to its promotion and application in the food testing industry.

Keywords: Acrylamide, ultrasonic extraction, extraction purification, HPLC, starchy foods.

1. Introduction

Acrylamide is a moderately toxic neurotoxin with neurotoxicity and cumulative effects. It can be absorbed into the human body through intact skin, mucous membranes, lungs, and the digestive tract. In 2017, the International Agency for Research on Cancer of the World Health Organization classified acrylamide as a Group 2 carcinogen [1]. Both humans and animals exposed to large doses of acrylamide can experience changes in the central nervous system. Further research has revealed that acrylamide affects brain energy metabolism, causing partial damage to brain tissue function. It is believed that the impact on brain energy metabolism is the biochemical basis for acrylamide-induced neuronal damage.

The industrial application of acrylamide is the synthesis of polyacrylamide. This polymer can be used as a water-soluble thickener and water treatment flocculant and is widely used in wastewater treatment, gel electrophoresis, papermaking, and wrinkle-free treatments for fabrics. Therefore, products related to polyacrylamide often retain some monomers, posing potential hazards to human health in daily life. Continuous research has found that foods processed at high temperatures, such as French fries, potato chips, cereals, and bread, may contain acrylamide, which has potential carcinogenicity [2-5]. This has attracted significant attention from the international community and governments around the world. Currently, acrylamide is recognized as a neurotoxin and a probable carcinogen and is classified as a hazardous chemical by various countries. The Ministry of Health of China has issued a notice advising consumers to avoid consuming fried potato products [6] and called for measures to reduce the health hazards that acrylamide in food may cause, advocating for rational nutrition and balanced diets. On April 13, 2005, China included the detection of acrylamide in food as a major scientific and technological project in the "Food Safety Key Technologies" program during the 10th Five-Year Plan and established the detection methods for acrylamide in food as an important part of the food safety plan.

Since the amount of acrylamide produced in food during processing is minimal and the food matrix is complex, general chemical analysis methods are insufficient for food analysis. Therefore, instrumental analysis is the primary method for determining acrylamide content. The common detection methods include gas chromatography (GC), high-performance liquid chromatography (HPLC), and mass spectrometry (MS). Gas chromatography-mass spectrometry (GC-MS) [7,8] is highly sensitive and specific but involves complicated sample separation, extraction, purification, and derivatization steps. Liquid chromatography-mass spectrometry (LC-MS) [9-11] is also highly sensitive and does not require derivatization, making it simpler for separation. However, during the sample extraction process, there is significant interference from impurities, especially in complex matrices. To achieve good sensitivity and accuracy, extensive and time-consuming sample pretreatment is necessary, often involving multi-step solid-phase extraction (SPE) to maximize acrylamide recovery [8,11]., which is inefficient Additionally, the high cost and technical requirements of GC-MS and LC-MS equipment limit their widespread use. This paper establishes a method based on extensive experimental comparison, using n-hexane defatting, ultrasound-assisted extraction, chloroform extraction, and purification to handle samples. We employ high-performance liquid chromatography (HPLC) with a UV detector to determine the acrylamide content in starchy foods. This method is convenient, uses simple equipment, provides reliable results for actual sample testing, and is suitable for routine daily detection.

2. Experimental Section

2.1 Experimental materials and reagents

Acrylamide standard (analytical reagent, recrystallized, HPLC purity >99.0%), methanol, and acetonitrile (for HPLC), ultrapure water, n-hexane, chloroform, and other reagents were all analytical reagent.

2.2 Sample sources

Potato chips, biscuits, instant noodles, fresh potatoes (from a supermarket). Packaged foods must be clearly labeled, easily identifiable, and within their shelf life. All foods were stored according to their requirements.

2.3 Major instruments and equipment

D-2000 Elite High-Performance Liquid Chromatograph (Hitachi, Japan) ; Analytical Balance (Sartorius Beijing); SK-1 Vortex Mixer (Shanghai Zhijin Instrument Equipment Co., Ltd.); High-Speed Centrifuge (Anhui Zhongke Zhongjia Scientific Instrument Co., Ltd.); Ultrasonic Cleaner (Zhangjiagang Gangsheng Ultrasonic Co., Ltd.)

2.4 Preparation of major solutions

Carrez I Reagent: Accurately weigh 15.00g $K_4[Fe(CN)_6]\cdot 3H_2O$ with an electronic balance, dissolve in a beaker, transfer and dilute to 100mL volumetric flask, mix well, and set aside.

Carrez II Reagent: Accurately weigh 30.00g $ZnSO_4\cdot 7H_2O$ with an electronic balance, dissolve in a beaker, transfer and dilute to 100mL volumetric flask, mix well, and set aside.

Sodium Chloride Solution: Accurately weigh 11.6880g NaCl, dissolve in a beaker, transfer and dilute to 100mL volumetric flask, mix well, and set aside to prepare a 2.00mol/L sodium chloride solution.

2.5 Chromatographic conditions

Column: Agilent RP-C_{18} (4.6×250mm, 5μm); Column Temperature: 25℃; Detection Wavelength: 210nm; Mobile Phase: Methanol-Water (5:95, v/v); Flow Rate: 0.8mL/min; Injection Volume: 10μL

2.6 Sample Determination Steps

Accurately weigh 5g of the ground and homogenized sample to 0.001g, place it in a 100mL flask, add 10mL of n-hexane, vortex mix for 5 minutes, discard the upper layer, and blow dry with an electric hair dryer. Then add 20.00mL of chloroform extraction solution, place it in an ultrasonic bath (water temperature 50℃), sonicate for 15 minutes, let it stand for a while, and filter through a Gooch crucible using a suction filtration bottle (150mL). Drain the clear solution as much as possible, leaving the insoluble material in the beaker. Repeat the extraction

and filtration with 20mL of chloroform each time, three times in total. Transfer the chloroform extract to a 100mL flask, rinse the filtration bottle three times with chloroform, and combine the filtrate and washing solution in the 100mL flask (this is the chloroform extract). Place the flask containing the chloroform extract in a hot water bath to evaporate the chloroform (chloroform recovery), let it cool to room temperature, add 10.00mL of pure water to dissolve thoroughly, and filter through a 0.45μm microporous membrane before immediate detection.

3. Results and Discussion

3.1 Optimization of pretreatment conditions for liquid chromatography

3.1.1 Selection of extraction solvent

Acrylamide has a high solubility in water (2155g/L), making water an ideal extraction solvent. Using water can effectively eliminate the interference of some non-water-soluble substances in fried foods and reduce experimental costs. Initially, water extracts were separated and analyzed in this experiment.

Experimental Method: Purchase a brand of potato chips from a supermarket, take about 10 chips, grind them thoroughly in a mortar to prepare a homogenized sample. Accurately weigh 5.00g of the ground sample into a beaker, add 10ml of n-hexane to defat, blow dry with an electric hair dryer, transfer to a 10ml centrifuge tube, add 10ml of pure water, ultrasonic extract for 15 minutes, centrifuge at 10000r/min for 10 minutes, take the middle layer filtrate, filter through a 0.45μm aqueous filter membrane, and detect using liquid chromatography. Separation was performed using methanol-water at 10:90 (v/v), methanol-water at 5:95 (v/v), and acetonitrile-water at 20:80 (v/v) as the mobile phase. Results showed numerous and complex absorption peaks, making acrylamide difficult to identify. Thus, water as an extraction solvent was found to be ineffective.

Using the same method, we compared methanol, acetonitrile, formic acid, chloroform, and sodium chloride solution as extraction solvents for acrylamide extraction. Experimental results showed that chloroform provided the best separation, regardless of extraction steps, solvent peak separation, peak shape, or number of impurity peaks. The results are shown in Figure 1.

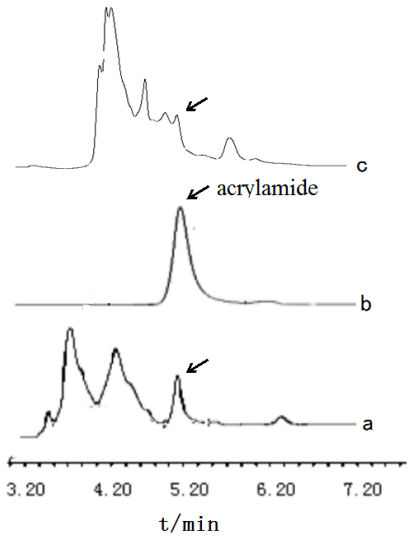

Figure 1 HPLC spectra of chloroform extract (a), sodium chloride extract (c), and acrylamide standard solution (b)

Chloroform provided better extraction results, possibly because easily water-soluble proteins, salts, high-molecular salts, and other substances in the food did not dissolve in chloroform, reducing interfering components in the extract. Moreover, chloroform extraction avoids the gelatinization phenomenon seen in water extraction, making separation easier. Due to the potential for chloroform to denature high-molecular substances, if the sample is low in fat (e.g., biscuits, instant noodles), CarrezI and CarrezII solutions may be omitted, simplifying the sample preparation process.

3.1.2 Selection of extraction time and temperature

Extraction time and temperature significantly affect acrylamide extraction. Using ultrasonic extraction, we compared the effects of different extraction times and temperatures on acrylamide content in the same sample. Results indicated that a short extraction time did not fully extract acrylamide, while a longer time reduced detection efficiency. Extraction stabilized after 15 minutes (Figure 2), so 15 minutes was selected as the optimal extraction time. Extraction temperature also had a significant effect on acrylamide extraction. As temperature increased, extraction rate improved, stabilizing above 50℃ (Figure 3). Detection values at 50℃ were 14.9% higher than at 25℃, with better phase separation observed. Thus, 50℃ was chosen as the optimal extraction temperature.

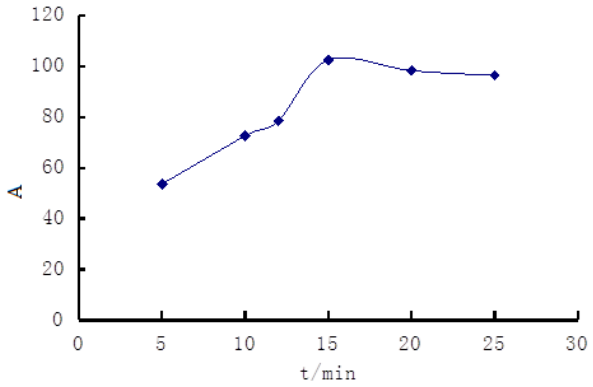

Figure 2 Relationship diagram between extraction time and acrylamide extraction

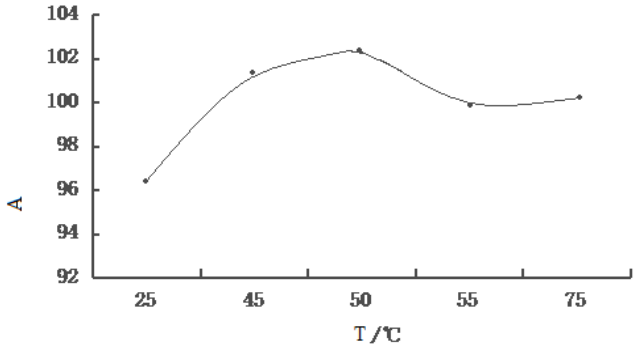

Figure 3 Relationship between extraction temperature and acrylamide extraction

Ultrasonic extraction leverages cavitation, acceleration, and mechanical effects of ultrasonic waves in liquid, dispersing and emulsifying samples for rapid extraction. Results showed stable extraction at 15 minutes and 50℃. Given that acrylamide undergoes significant polymerization only at high temperatures or in contact with initiators [12], these conditions should not affect acrylamide content and thus the results.

3.1.3 Optimization of liquid chromatography detection conditions

Based on the determined detection wavelength, HPLC conditions such as mobile phase composition and flow rate were optimized to establish a method for acrylamide determination in samples. Results are shown in Figure 1. Under experimental conditions, acrylamide standard eluted at 5.10 minutes. Chromatograms of samples showed an independent peak at the same retention time, well-separated from other components, with good peak shape and signal strength, meeting detection requirements.

3.2 Method precision

The same batch of potato chip samples was used to prepare six parallel samples for extraction and HPLC determination to evaluate the stability and precision of the method. The results are shown in Table 1. The relative standard deviation of acrylamide content in potato chip samples was found to be 3.7%, indicating that the method used for sample preparation and HPLC detection of acrylamide in fried potato chips has reliable reproducibility.

Table 1 Repeatability of the determination of potato chip samples.

Measurement Number	1	2	3	4	5	6	Mean value	RSD(%)
Peak Area	87	77	84	92	88	98	88	3.7

3.3 Recovery rate

Three 5.00g samples of ground and homogenized potato chips were accurately weighed into three 50mL beakers. Different amounts of acrylamide standard solution were added, and the samples were dried at low temperature. The extraction and determination were carried out according to the steps in section 2.5. The experimental results are shown in Table 2. The recovery rates of added standard were 87%, 94%, and 97%, respectively, indicating the method's accuracy for determining acrylamide content.

Table2 Results of recoveries(n=5).

Acrylamide content in sample (μg)	Spike amount (μg)	Detected acrylamide content (μg)	Recovery Rate (%)	RSD (%)
4.487	5	8.8655	87.57	0.51
4.487	10	13.909	94.22	0.31
4.487	15	19.163	97.84	0.70

3.4 Determination of acrylamide content in samples

Using the established method, randomly sampled potato chips, biscuits, and instant noodles from the market were measured. Each sample was measured three times. The acrylamide content in the tested samples is shown in Table 3.

Table 3 Contents of acrylamide in samples.

Sample Type	I (μg/g)	II (μg/g)	III (μg/g)	Mean Acrylamide Content (μg/g)
Potato Chips	0.8828	1.0385	0.9574	0.9574
Biscuits	0.5128	0.5476	0.5989	0.5532
Instant Noodles	0.04902	0.04221	0.04188	0.04437
Potato Starch	ND	ND	ND	ND

From Table 3, it can be seen that starchy processed foods contain acrylamide to varying degrees, with potato chips having higher acrylamide content than biscuits and instant noodles, while potato starch had no detectable acrylamide. The WHO recommended limit for acrylamide in drinking water is 1μg/L [13]. The detected values for potato chips, biscuits, and instant noodles far exceed this limit, indicating that acrylamide content in food is closely related to processing methods and conditions.

4. Conclusion

This paper established a high-performance liquid chromatography (HPLC) method for determining acrylamide in starchy foods. The method involves defatting with n-hexane and extracting with chloroform, greatly simplifying sample preparation. The acrylamide content in potato chips, biscuits, and instant noodles were found to be 0.95, 0.55, and 0.04 μg/g, respectively. The established method is simple, reproducible, and suitable for detecting acrylamide content in similar foods. Compared to traditional methods, this method does not rely on expensive mass spectrometry equipment, making it more accessible to most laboratories and promoting its application in the food testing industry.

Acknowledgments

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