Study on the Acrylamide Content Analysis in the Heat-Treated Mooncake by HPLC

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Abstract

Since 2002, high levels of acrylamide (AA) have been found in some starch-rich foods, such as fried, baked, grilled or toasted foods. AA has been found in the heat treated food at the high temperature (>120 ºC) processes such as cooking, frying, toasting, roasting or baking of high carbohydrate foods, It happens when amino acid asparagine react with reducing sugars (especially glucose and fructose) as a result of the Maillard reaction. Many countries and international societies have conducted a risk assessment of acrylamide in foodstuffs and conclude that much effort is required to reduce the content of this substance as much as possible. In this study, analytical procedure of acrylamide, which can be found in heat-treated mooncake, using high performance liquid chromatography- Diode Array Detector (HPLC-DAD) has been investigated. The selectivity of the method was validated, the RSD of repeatability was less than 6.1 %, the recovery of method was higher than 80%, and limit of detection (LOD) of the method was 29.24 ppb. The analytical method has been successfully applied for determination of acrylamide in the heat-treated mooncake samples, the acrylamide contents of the samples were from 200 to 900 ppb.

Keywords: Acrylamide, mooncake, high performance liquid chromatography (HPLC)

1. Introduction

Acrylamide (AA) is potential carcinogenic compound that possesses neurotoxicity activity and increases the risk of tumors of the mammary glands, central nervous system, thyroid gland-follicular epithelium, uterus, colon and clitoral gland in rats [1,2]. These potential risks may seriously affect to human health and it has been considered as ‘‘probably carcinogenic to humans’’ (group 2A) by the International Agency for Research on Cancer [3].

In 2002, Swedish National Food Administration added AA to the list of neo-formed contaminants (NFCs), when it was identified in several heat processing of carbohydrate-rich foods such as potato chips, crisps, coffee and bread [4]. Therefore, evaluation of AA content in foods is urgent issue for the government and researchers. In February 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) summarized the analysis of acrylamide in 6752 food samples in 24 countries (67.6% from Europe, 21.9% from North America, 8.9% from Asia and 1.6% from Pacific) [3]. The results showed that the daily intake of acrylamide was 3.0 - 4.3 μg of AA/kg body weight (average body weight is 60 kg). Particularly, French fries and potato snacks have been identified as major sources of acrylamide exposure with an average content of 477 μg/kg [5]. In 2007, the European Food Safety Authority reported that AA was detected in many kind of foods such as Friend fries, potato snack, bread, breakfast cereals, biscuit from hundred to few thousand μg/kg [6].

Moon cake is indispensable food on the festival day of the nation. Moon cakes are made from flour, sugar and other ingredients that are baked at high temperatures, generating the significant amounts of AA. However, the content of acrylamide depends on the ingredients, the baking temperature, and dwelling time of baking and other additives. Thus, the content of AA in moon cake needs determine to make recommendations for consumers. In this paper, we focused on developing analytical procedures to determine AA in mooncake by high performance liquid chromatography (HPLC). Subsequently, the optimal analytical procedure was use to examine and evaluate the content of AA in some mooncake samples on today’s market in Vietnam.

2. Experimental

2.1. Chemicals

The standard solution of AA > 99.0% was purchased from Sigma Aldrich. Acetone and methanol were also obtained from Merck. Distilled

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water used in all experiments processing and sample analysis are 18.2 MΩ quality; TOC <10 mg/L).

20 mg/mL AA was prepared and stored at the dark at a temperature of 5 °C. Other standard solutions were prepared from this standard solution.

2.2. Equipment and tools

Acrylamide has been analyzed by HPLC (Agilent 1260 Inifity II (USA) equipped with high-pressure 1260 Quat Pump (G7111B) and detector DAD 1260 HS (G7117C).

Other common equipment have been used for performing the experiments include analytical balance CPA 224S (Germany), grinding machine (PAXD-MX-AC400WRA, Panasonic), centrifuge (22331 Hamburg Germany), ultrasonic (GT-1620QTS, GTSonic), machine vortex (MS3D IKA USA).

2.3 Sample preparation

Based on methods [7], we made some changes for extraction to fit the pattern of mooncake matrix. Because the sample contains fat components those could create problems in chromatographic analysis by giving peaks overlapping with the target analyses or block the polar column. The sample should be removed by n-hexane, a non-polar solvent during the sample treatment. The acrylamide is then extracted with a well soluble solvent mixture and impurities are extracted minimized.

The 0.5 cm thick of samples was grounded with grinding machine. The 4.00 (±0.01g) of sample has been added into the 50 mL centrifuge tube. Add 10 ml of acetone containing 0.01% water, shake vigorously. Add 10 mL of n-hexane saturated acetone 0.01% water, 5 minutes vortex, 4000 rpm for 5 minutes, cool to 0-5 °C for 30 minutes. The upper hexane layer was removed with a Pasteur pipette. Transfer the extraction to another falcon tube. The procedure has been repeated three times. 15 mL extraction was used to dry, diluted with 1 mL of water. It has been filtered by using the membrane 0.25 µm before injecting into the chromatography system

.3. Results and disscution

3.1. Survey condition HPLC-DAD

3.1.1. Wavelength selection

AA is sensitive to wavelengths from 200 - 226 nm [9]. Wavelengths 202, 210, 226 nm have been chosen to study. We conducted the above three wavelengths and obtained the following results

The wavelength 202 nm has been chosen because of the symmetrical shape and the largest peak area. The results are shown in Table 1.

Table 1. The peak area at different wavelengths

Wavelength (nm)	Area (mAU*s)
202	**2530,667**
210	1492,335
216	471,117

3.1.2. Mobile phase selection

An isocratic elution pattern was adopted for the separation of the target analyte. Water was used throughout the analysis. Alternatively, 4 % methanol was used to shorten the analysis time but methanol elution unwanted substances confounding baseline. As shown in Fig.1, water is more suitable mobile phase in a reasonable time 10 minutes. In all cases the column temperature was set at 30 °C, flow rate was maintained at 0.6mL.min^{-1} while detection was performed at 202nm.

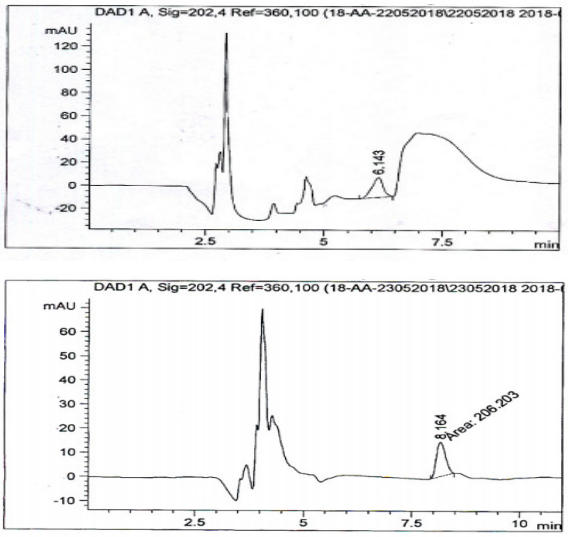

Fig. 1. a. Mobile phase: H_2O:MeOH = 96:4;

b. Mobile phase: H_2O nanorods

3.1.3. Chromatographic column selection

Based on the actual conditions of the laboratory we conducted the survey on 2 columns: C8 InertSustain and Eclips Plus C18. The blank has been spiked a 1000 ppm standard solution.

As shown in Fig.2, the more symmetric peak and its larger area were obtained with Eclipse Plus C18 column. The area peak obtaining from Eclipse Plus C18 column was 194.759 mAU*s which is larger than the one obtaining from the C8 InertSustain

column (157.562 mAU*s). Column C18 has been used to conduct on the next survey.

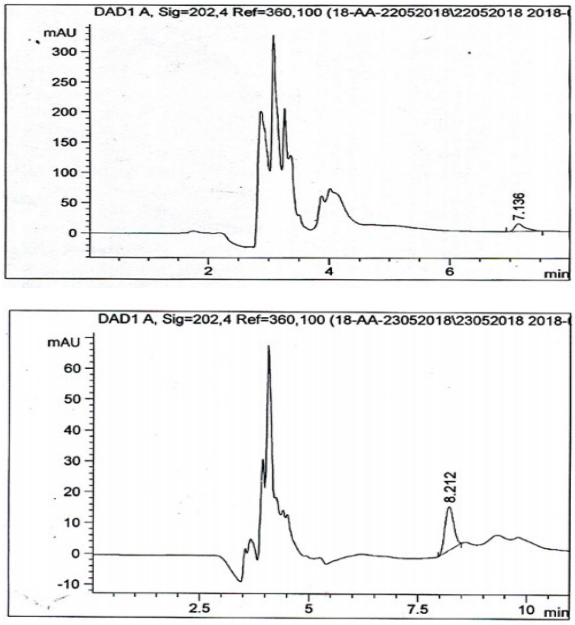

Fig. 2. Chromatography using C8 InertSustain column (a) and Eclips Plu C18 Agilent (b)

3.1.4. Selection of flow rate

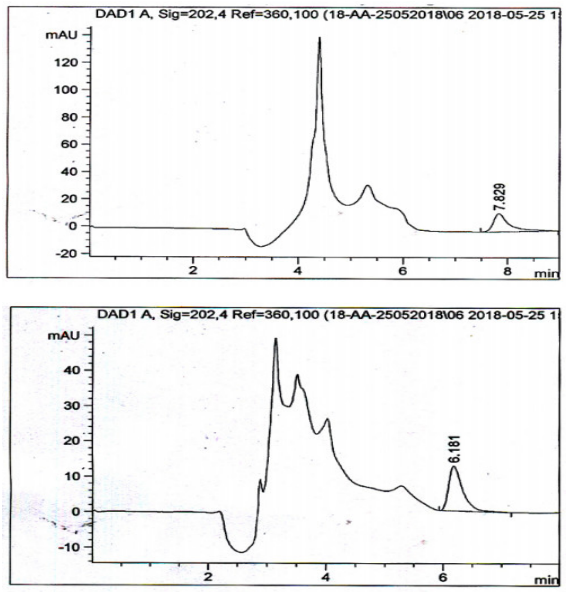

Fig. 3. Chromatograph at different flow rates 0.6, 0.8 and 1.0 mL.min^{-1}

The flow rate has been varied from 0.6 to 1.0 mL.min^{-1}. The results were shown in the Fig.3 and Table 2. When the flow rate increases, the peak of acrylamide was dissolved earlier, but it tends to seep into the peak of the sample matrix. The flow rate at 0.6 mL. min^{-1} has been chosen due to the highest area peak.

Table 2. The area of chromatography at the variation of flow rate

Flow rate (mL. min^{-1})	Retention time	Area (mAU*s)
0.6	**7.8**	**284.544**
0.8	6.1	211.759
1.0	4.7	186.697

3.2. Method of validation

3.2.1 Robustness of the system

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The injection has been repeated 6 times with the acrylamide 1000 ppb solution under the same chromatography conditions. The robustness of the system over the relative standard deviation of retention time and peak area has been evaluated as shown in Table 3.

Table 3. Results calculate the appropriate system

Number	Retention time (min)	Area peak (mAU*s)
1	8.164	206.2030
2	8.173	207.3630
3	8.179	207.0950
4	8.179	210.8470
5	8.175	207.8100
6	8.166	207.8830
Average	8.173	207.8668
SD	0.0064	1.4
RSD %	0.0784	0.6945

The calculated relative standard deviation of the retention time was 0.0784%, the peak area was 0.6945% are satisfactory (<2%). Because the acceptable results have been obtained, it can be stated that these variations are tolerable.

3.2.2 Specificity

The discrimination of a procedure has been confirmed by obtaining positive results. In the comparison with a known reference material from samples containing the analyte at 200 ppm, coupled with negative results from blank sample. There is no peak in the range from 6 to 9 minutes on the chromatogram. The blank samples have been spiked with 200 and 1000 ppm. The retention time of AA has been shown at 8.2 minutes in different concentrations. It can be stated that the procedure has a suitable identification.

3.2.3 Calibration curvet of AA

The results of calibration curvet were shown in Table 4. The calibration curve was y = 0.1956 *x* – 7.2852 with the square of the correlation coefficient, R^2, is very close to 1 (0.9998) to represent a linear fit.

Table 4. The results of calibration curvet

No	Concentration (ppb)	Area (mAU*s)
1	200	33.3500
2	400	71.9070
3	800	146.6580
4	1000	186.6740
5	1600	306.9540
6	2000	384.4750

3.2.4 LOD and LOQ determination

The limits of detection and quantitation have been carried as mentioned in the reference [8].

Table 5. Determination of LOD and LOQ

No	*Weight (gram)*	*Concentration (ppb)*
1	4.19	35.771
2	3.91	38.383
3	4.12	33.307
4	4.09	37.493
5	4.03	37.002
6	4.01	39.117
7	4.15	36.258
8	4.02	41.784
9	4.16	35.983
10	4.11	37.190
Average		37.229
SD		2.255
RSD		6.06%
LOD		29.24
LOQ		88.61

It can be seen in table 5, LOD is 29.24 ppb and LOQ is 88.61 ppb. Therefore, the method is suitable to use in the process of AA determination in mooncake samples.

3.2.5 Precision and spike recovery

Precision is the reproducibility of a result. Instrument precision, also called injection precision, is the reproducibility observed when the same quantity of one sample is repeatedly introduced (≥ 10 times) into an instrument. Variability could arise from variation in the injected quantity and variation of instrument response. The comparison between the calculated values and the accepted value according to AOAC at three different concentrations (Table 6) were done [9].

Evaluation of the repeatability was performed through the analysis of three samples at the concentration of 400, 1000 and 2000 ppb, respectively. One sample was repeatedly introduced 6 times) into the instrument. The means, standard deviation, coefficient of variation was presented in Table 7.

Table 6. Repeatedly maximum accepted at different concentration according to AOAC

No	Content (%)	Ratio	Recovery, %
1	100	1.3	98-102
2	10	1.8	98-102
3	1	2.7	97-103
4	10^{-1}	3.7	95-105
5	10^{-2}	5.3	93-107
6	10^{-3}	7.3	80-110
7	10^{-4}	11	80-110
8	10^{-5}	15	80-110
9	10^{-6}	21	60-115
10	10^{-7}	30	40-120

Table 7. The results of average $\bar{x}$, standard deviation (SD), and relative standard deviation (RSD) of AA measurements

No	**Concentration**		
	Acrylamide		
	400 ppb	1000 ppb	2000 ppb
1	227.973	860.111	1802.115
2	236.307	866.115	1820.555
3	233.297	818.394	1896.201
4	233.172	854.621	1816.103
5	226.032	865.934	1893.195
6	227.590	841.274	1809.840
$\bar{x}$	230.727	848.960	1813.697
SD	4.080	29.298	72.219
RSD	**7.22**	**3.45**	**3.98**

Table 8. Recovery of AA in mooncake samples

Sample	400 (µg/kg)	1000 (µg/kg)	2000 (µg/kg)
1	56.99	86.01	86.44
2	59.08	86.61	86.90
3	58.32	81.84	94.81
4	58.29	85.46	90.81
5	56.51	80.88	94.65
6	56.89	86.13	90.49
Recovery $\bar{x}$ %	57.68	84.89	90.68

The results were compared to acceptable repeatability at three concentrations (400, 1000, 2000 ppb according to AOAC which is <11 %, the error in all three points are acceptable).

The spike recovery was also used to validate the method. The spike recovery was calculated for known samples. The results were shown in Table 8. As can be seen from the table, the method has a high precision and spike recovery at 1000 and 2000 ppb when spike 400, 1000 and 2000 ppb in sample matrix.

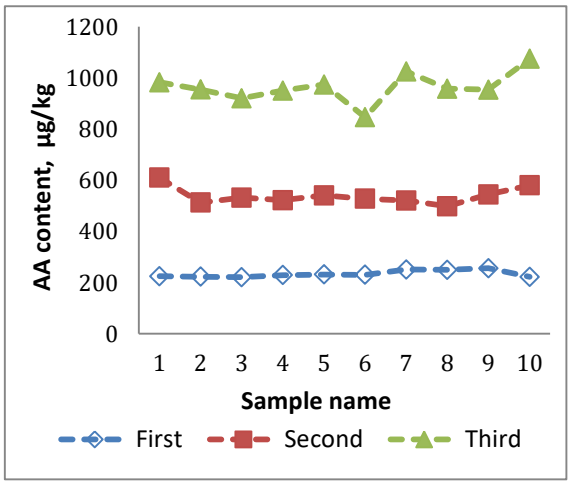

Fig. 4. The variation of AA content at different baking times of moon cake.

3.3 AA analysis of the mooncake

According to the analytical process that was built above, 30 mooncake samples were used to determine the AA content.

The process of moon cake production is based on the reference [10].

As shown in Fig.4, AA content was approximately 200, 500 and 900µg/kg after the first, second and the third baking times, respectively. AA increases significantly as baking times increase.

After the ingredients have been thoroughly mixed, knead carefully to form a crust. After then, bean-paste fillings were wrapped in the mooncake layer and shaped by mooncake mold. Finally, the mooncake were baked in an oven at 180 °C for 5 minutes three times.

4. Conclusion

The process of AA determination in the mooncake samples using HPLC-DAD has been developed with high sensitivity indicating with LOD value of 29.24 ppb and LOQ value of 88.61 ppb. Concentration range is from 400 to 2000 ppb. The spike recovery was obtained very high from 80.88 to 94.81% at very low concentration.

The method has been applied successfully to determine rapidly and precisely AA content of the mooncake samples used on today's market in Vietnam.

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