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Study on formation of acrylamide in asparagine–sugar microwave heating systems using UPLC-MS/MS analytical method

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Abstract

Microwave heating can be regarded as a possible way to produce a considerable amount of acrylamide. The present study investigated the formation of acrylamide in asparagine–glucose, asparagine–fructose and asparagine–sucrose microwave heating systems by the response surface methodology (RSM) and the orthogonal array methodology (OAM). The acrylamide content was rapidly quantified by a validated ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method. Results of RSM study indicated that in the asparagine–glucose system, the acrylamide content increased in the combined condition of high temperature accompanying with short heating time (>190 °C, <20 min) or low temperature accompanying with long heating time (<180 °C, >30 min). In the asparagine–fructose system, the similar conclusion was made in the combined condition of high temperature accompanying with short heating time (>175 °C, <20 min) or low temperature accompanying with long heating time (<170 °C, >25 min). In the asparagine–sucrose system, the amount of acrylamide enhanced with the increase of both heating temperature and heating time. The fitted mathematic models were successfully applied to the quantification of acrylamide formation when the heating temperature and heating time fell into the ranges of 120–240 °C and 5–35 min simultaneously. OAM study showed that acrylamide is readily formed via heating binary precursors 5 min at 180 °C in the asparagine–glucose and asparagine–fructose systems. However, acrylamide is readily generated when the binary precursors are heated 15 min at 180 °C in the asparagine–sucrose system.

Keywords: Acrylamide; Microwave heating; Asparagine; Sugar; UPLC-MS/MS

1. Introduction

Acrylamide, which is formed during the heat processing (frying, roasting, baking or microwave heating) of carbohydrate-rich foods under high temperatures and low moisture situations, has been regarded as a potential carcinogen and neurotoxin. Such contaminant evoked an international health alarm throughout the world. Researchers from academic schools, industries and official laboratories focused their attentions on the possible sources and relative formation mechanisms of acrylamide in foods (Stadler & Scholz, 2004). During these years, the formation mechanism of acrylamide in asparagine–carbohydrate model systems has been clearly elucidated (Becalski, Lau, Lewis, & Seaman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Yaylayan, Wnorowski, & Locas, 2003). The link of acrylamide to asparagine, which directly provides the backbone chain of acrylamide molecule, was approved by labeling experiments. Mass spectral studies showed that the three carbon atoms and the nitrogen atom of acrylamide were all derived from asparagine (Zyzak et al., 2003). Subsequently, studies on acrylamide formation were further carried out based on many food matrixes (Taeymans et al., 2004). Many researchers made their efforts to find the factors contributing to the formation of acrylamide in certain matrixes, such as potatoes (Gökmen, Palazoğlu,

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& Şenyuva, 2006), cereals (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005), bread (Mustafa, Andersson, Rosén, Kamal-Eldin, & Åman, 2005), almonds (Amrein et al., 2005), coffee (Andrzejewski, Roach, Gay, & Musser, 2004), etc. Various factors affected the reaction yields of acrylamide, such as the heating temperature, heating time, concentrations, types of sugar compounds and water presence (Ciesarová, Kiss, & Kolek, 2006; Mestdagh, de Meulenaer, Cucu, & van Peteghem, 2006; Taeymans et al., 2004). The interaction between heating temperature and heating time of the reaction has also been considered as an important factor (Taubert, Harlfinger, Henkes, Berkels, & Schömig, 2004). However, some factors such as oil types did not significantly affect the formation of acrylamide (Mestdagh et al., 2005). Furthermore, previous studies also demonstrated the formation and elimination of acrylamide in the asparagine–sugar model system on a kinetic basis (Claeys, de Vleeschouwer, & Hendrickx, 2005; Corradini & Peleg, 2006; Gökmen & Şenyuva, 2006).

Besides, the heat processing method contributing to the formation of acrylamide should also be taken into consideration. Microwave heating as a fast and convenient heat processing method is widely applied all over the world. Microwaves offer fast temperature rise in the foods owing to their capacity to generate heat energy inside the food, without requiring any medium as vehicle for heat transfer. A low thermal conductivity product may quickly reach high temperatures, and this does not occur in conventional heating (Campañone & Zaritzky, 2005). Microwave heating provides a favourable medium for the occurrence of acrylamide and probably affects the formation and kinetics of acrylamide distinguishingly due to its extraordinary heating style. Although a large amount of acrylamide may be formed in carbohydrate-rich foods during the microwave heating, few studies have been concerned about the formation of acrylamide in microwave heating products so far. Tareke, Rydberg, Karlsson, Eriksson, and Törnqvist, 2002 quantified a large amount of acrylamide (551 μg/kg) in microwave-heated grated potatoes, which was even higher than the same potato samples under a frying treatment (447 μg/kg). Moreover, Takatsuki, Nemoto, Sasaki, and Maitani, 2004 demonstrated that concentrations of acrylamide in potato, asparagus and green gram sprouts baked after being precooked by the microwave pretreatment were higher than those in the products baked without being precooked. These results indicated microwave heating might induce a high amount of acrylamide contaminant in foods. Under a further consideration, acrylamide is readily generated in a relatively short heating time at high heating temperature in the microwave-heated potato products (Zyzak et al., 2003). The characteristics of high heating temperature and short processing time are in good agreement with the routine use conditions of microwave heating. Based on the above considerations, it is important to study the effect of key heat processing parameters on the formation of acrylamide in the microwave heating system.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals and other reagents used in this study were listed as follows: Acrylamide (purity > 99%), D-(−)-fructose, D-(+)-glucose monohydrate, sucrose (Sigma–Aldrich, St. Louis, MO, USA), D_3-labeled acrylamide (isotopic purity 99%, Cambridge Isotope Laboratories, Andover, MA, USA), formic acid (96%), ethyl acetate (Tedia, Fairfield, OH, USA), methanol (Merck, Whitehouse Station, NJ, USA) and L-asparagine monohydrate (Biocity Science and Technology Inc., Beijing, China). Ultra-purified water was used throughout the study (Milli-Q Technology, Millipore, Bedford, MA, USA). All of other solvents and chemicals were of analytical grade. The stock solution of acrylamide standard (1 mg/mL) was prepared by dissolving in ultra-purified water. The working standard solutions for the linear calibration were prepared by diluting the stock solution to a concentration sequence of 1, 5, 10, 50, 100, 150 and 200 ng/mL and spiked with the internal standard (100 ng/mL). Both the stock and working solutions were kept at 4 °C for a month. The substrate solutions, i.e. asparagine monohydrate (0.2 mol/L), glucose monohydrate (1 mol/L), fructose (1 mol/L) and sucrose (0.5 mol/L) were prepared by dissolving calculated chemicals to the phosphate buffer (NaH_2PO_4 and Na_2HPO_4, 0.1 mol/L, pH 6.80).

2.2. Study on formation of acrylamide via response surface methodology (RSM)

The formation study of acrylamide was performed by RSM according to Bråthen and Knutsen, 2005 with some improvements. The experimental design was achieved by considering three replicates of the central point in order to evaluate the experimental error. The independent variables were microwave heating temperature, heating time, asparagine monohydrate content and sugar (fructose, glucose monohydrate or sucrose) content, and their interactions between each other were also taken into consideration. Corresponding coefficients of both variables and interaction variables were estimated by SAS ver. 8.2 (SAS Institute Inc., Beijing, China) while their response surface graphs were drawn by Minitab ver. 14.0 (Minitab Inc. State College, PA, USA). Statistical analysis on the significance of coefficient estimations was performed via Student's *t*-test.

2.3. Study on formation of acrylamide via orthogonal array methodology (OAM)

Based on the results of RSM, this formation study was also performed by a three-factor and three-level ($L_9(3)^4$) OAM with triplicate repeats in each test ($n = 3$). The conditions of three important factors including heating temperature, heating time and the molar quantity of asparagine monohydrate, which significantly contribute to the generation of acrylamide, were optimized by the

results of OAM range analysis and analysis of variance (ANOVA) also calculated by SAS 8.2. Statistical analysis on the significance of ANOVA was performed via *F*-test.

2.4. Use of microwave digestion labstation

The microwave heating reaction between two substrates was performed via an Ethos D microwave digestion labstation from Milestone Inc. (Shelton, CT, USA). In this labstation, there are 9 microwave sample vessels in the carousel, which allows 9 groups of reactions between substrates simultaneously under identical reaction conditions. The ATC-400CE automatic temperature control system (mainly an advanced fiber-optic temperature sensor) and the APC-55 automatic pressure control system within the standard reference vessel allow continuous monitoring and control of internal temperature (±1 °C) and internal vapor pressure (±1 bar). In addition, a focused and high-sensitivity IR sensor is used for monitoring the surface temperature of all sample vessels inside the cavity. The reaction temperature and time and their limits can be modulated via a digital intelligent control panel connected with all of the above control systems. In the preliminary test of our study, the maximum pressure of the asparagine–sugar reactions was determined as 9.5 bar (950 kPa), which was safe enough (maximum safe pressure: 3500 kPa) to perform the reaction in this labstation.

2.5. Preparation of microwave heating system and pretreatment of reaction products

For the RSM evaluation, the prepared asparagine and sugar solution were sequentially added into microwave digestion vessels according to the experimental design of RSM (Table 1). Then, the volume of reaction solutions (10 mL) was fixed with the phosphate buffer (pH 6.80). All the microwave digestion vessels in each batch were carefully sealed. For the OAM analysis, the asparagine–glucose and asparagine–fructose microwave heating systems were similarly prepared by adding equimolar levels of asparagine and sugar solution while the asparagine–sucrose system was prepared by adding two products with a molar ratio of 2:1. Three kinds of mixed reaction solutions in sealed digestion vessels were all microwave-heated according to the experimental design of RSM and OAM after a prepared temperature programming of the microwave digestion labstation. Details on the temperature programming under different conditions of RSM and OAM are shown in Table 2. The fluctuation range of set temperature in the microwave system was less than ±1 °C. At the end of heating, the microwave digestion vessels filled with the final reaction products were taken out from the labstation and immediately cooled in prepared ice water to stop any further reaction. The whole cooling process was performed in a special room with stable air temperature and

Table 1
Acrylamide content generated from microwave heating systems by 5-level and 4-variable response surface methodology (RSM)

Test no.	X_1, Set heating temperature (°C)	X_2, Set heating time (min)	X_3, Added asparagine (mmol)	X_4, Added sugar (mmol)			Acrylamide (μmol)		
				Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose
1	120 (−2)	15 (0)	1 (0)	1 (0)	1 (0)	0.5 (0)	0.60	9.04	n.d.[a]
2	150 (−1)	5 (−1)	1.4 (1)	1.4 (1)	1.4 (1)	0.7 (1)	6.03	9.15	0.12
3	150	5	1.4	0.6 (−1)	0.6 (−1)	0.3 (−1)	0.67	2.05	0.03
4	150	5	0.6 (−1)	1.4	1.4	0.7	0.57	6.16	0.04
5	150	5	0.6	0.6	0.6	0.3	0.16	2.37	0.05
6	150	25 (1)	1.4	1.4	1.4	0.7	11.23	8.46	3.32
7	150	25	1.4	0.6	0.6	0.3	4.24	4.70	2.38
8	150	25	0.6	1.4	1.4	0.7	5.19	7.55	1.59
9	150	25	0.6	0.6	0.6	0.3	2.33	3.14	2.17
10	180 (0)	15	1.8 (2)	1	1	0.5	13.85	15.25	2.62
11	180	15	1	1	1	0.5	10.16	8.32	0.81
12	180	15	1	1	1	0.5	10.08	10.21	0.50
13	180	15	1	1	1	0.5	9.78	7.90	0.46
14	180	15	1	0.2 (−2)	0.2 (−2)	0.1 (−2)	2.92	2.11	0.18
15	180	15	1	1.8 (2)	1.8 (2)	0.9 (2)	9.30	12.71	2.55
16	180	15	0.2 (−2)	1	1	0.5	3.70	4.18	0.89
17	210 (1)	25	1.4	1.4	1.4	0.7	10.48	10.46	6.20
18	210	25	1.4	0.6	0.6	0.3	9.28	8.25	3.88
19	210	25	0.6	1.4	1.4	0.7	8.85	6.08	2.43
20	210	25	0.6	0.6	0.6	0.3	6.30	5.56	1.55
21	210	5	1.4	1.4	1.4	0.7	20.85	20.63	5.24
22	210	5	1.4	0.6	0.6	0.3	12.10	9.52	2.09
23	210	5	0.6	1.4	1.4	0.7	12.18	11.78	1.07
24	210	5	0.6	0.6	0.6	0.3	10.75	7.73	2.09
25	180	0 (−2)	1	1	1	0.5	17.99	17.06	3.38
26	180	35 (2)	1	1	1	0.5	11.11	7.89	3.29
27	240 (2)	15	1	1	1	0.5	2.31	1.37	0.96

[a] n.d., not detected.

Table 2
Temperature programming of the microwave digestion labstation at different set heating temperature in the experimental design of RSM and OAM

Set temperature (°C)	Temperature programming and working power[a]
120	RT → 120 °C (200 W, 10 min)
150	RT → 120 °C (200 W, 5 min); 120 °C → 150 °C (400 W, 5 min)
180	RT → 120 °C (200 W, 5 min); 120 °C → 180 °C (500 W, 5 min)
210	RT → 120 °C (200 W, 5 min); 120 °C → 210 °C (600 W, 5 min)
240	RT → 120 °C (200 W, 5 min); 120 °C → 240 °C (700 W, 5 min)

[a] RT, room temperature.

relative humidity adjusted by air-condition. For the sample pretreatment, an aliquot of the final reaction products (0.2 mL) was sampled and then diluted to 10 mL by the phosphate buffer. Then, an aliquot (2 mL) of the diluted sample solution was homogeneously spiked with 500 μL of D_3-labeled acrylamide internal standard (IS) solution (2 μg/mL). The IS-spiked solution was subsequently extracted with 2 mL of ethyl acetate for three times. The organic phase was combined and dried by nitrogen gas. The residue was re-dissolved by water and further purified by the solid phase extraction (SPE) method, which was performed according to our previous study (Zhang, Jiao, Ren, Wu, & Zhang, 2005). Finally, the pretreated analyte was submitted to UPLC-MS/MS analysis.

2.6. UPLC-MS/MS analysis of acrylamide

Quantification of acrylamide in pretreated reaction products was performed by a UPLC-MS/MS with the electrospray positive ionization (ESI+) method according to our previous study (Zhang, Jiao, Cai, Zhang, & Ren, 2007). Furthermore, the analysis was integrated within the scope of an authorized proficiency test controlled by the official Food Analysis Performance Assessment Scheme (FAPAS) for accreditation. The UPLC-MS/MS method used in the present study had been successfully validated in-house with repeated ($n = 6$) analysis of a crispbread sample used in a FAPAS ring trial (Central Science Laboratory, UK; Series 30 Round 11, T3011, robust mean value 1404 μg/kg). The result from our laboratory (No. 021) regarding the acrylamide content (1381 μg/kg) in dispatched test material is satisfactory (Z-score = −0.1) and fulfills requirements from the organization (FAPAS, 2005).

3. Results and discussion

3.1. RSM study on formation of acrylamide in the microwave heating system

In the present study, the ethyl acetate extraction procedure is very necessary for the pretreatment of reaction products. Although it was a reaction between pure chemicals, too many impurities were present in the final products, such as sugar residue, asparagine residue and salts from buffer, which could be easily removed by the ethyl acetate extraction. Meanwhile, our previous publication demonstrated that both high recovery and effective time consuming were achieved when employing ethyl acetate to extract acrylamide for three times (Zhang, Ren, Zhao, & Zhang, 2007). Such extraction method was used in this study in order to remove most of impurities and obtain a high acrylamide response during the UPLC-MS/MS analysis. The actual content of acrylamide generated from the microwave heating systems in each test item according to RSM is shown in Table 1. Results demonstrated that the reaction between asparagine monohydrate and sugar under the microwave heating situation was dramatically strenuous and consequently releases a large amount of acrylamide especially in the combination of high heating temperature and short heating time. Based on the experimental results of RSM, coefficients of both variables and interaction variables estimated by SAS were calculated (Table 3). Since the equimolar asparagine–sugar model systems were investigated throughout the study, the unit of acrylamide content was presented as mmol/mol asparagine. Fig. 1 shows the response surface graphs of acrylamide content (mmol/mol Asn) with the increase of heating temperature and heating time in three kinds of equimolar asparagine–sugar (molar quantity ratio of asparagine:sucrose = 2:1) model systems, which can be fitted as the following equations:

Asparagine–glucose system:

$$Y = -0.0026x_1^2 + 0.0104x_2^2 + 1.1275x_1 + 0.9657x_2 - 0.0076x_1x_2 - 105.4558 \quad (1)$$

Asparagine–fructose system:

$$Y = -0.0013x_1^2 + 0.0060x_2^2 + 0.5879x_1 + 0.5518x_2 - 0.0049x_1x_2 - 49.1826 \quad (2)$$

Asparagine–sucrose system:

$$Y = -0.0029 \times 10^{-2}x_1^2 + 0.0073x_2^2 + 0.0512x_1 + 0.0403x_2 - 0.0012x_1x_2 - 6.5364 \quad (3)$$

(Y, acrylamide content, mmol/mol Asn; x_1, heating temperature, °C; x_2, heating time, min).

Compared to the linear and intercept items estimated by SAS (Table 3), there were some differences in the same items of the above equations because several items were merged when considering the equimolar model reaction system. Interestingly, the response surface graphs presented two different tendencies regarding the formation of acrylamide within the selected ranges of heating temperature and heating time. For the asparagine–glucose system, the acrylamide content elevated in the combined condition of high temperature and short heating time (>190°C, <20 min) or low temperature and long heating time (<180 °C, >30 min). We also found and calculated an

Table 3
Coefficients of the fitted response surface equations and significant estimations for three asparagine–sugar microwave heating systems[a]

Variable	Asparagine–glucose model		Asparagine–fructose model		Asparagine–sucrose model	
	Coefficient	P value[b]	Coefficient	P value[b]	Coefficient	P value[b]
Intercept	−121.5326	0.002**	−53.8927	0.173	10.1552	0.369
X_1	1.1326	0.001**	0.5285	0.126	−0.0173	0.857
X_2	1.0560	0.092	0.8474	0.237	−0.0403	0.843
X_3	5.3704	0.724	−9.5965	0.594	−26.9901	0.022*
X_4	15.0141	0.332	14.5259	0.423	−9.0748	0.102
$X_1 \times X_1$	−0.0026	0.003**	−0.0013	0.131	-0.0028×10^{-2}	0.909
$X_2 \times X_1$	−0.0076	0.010**	−0.0049	0.122	−0.0012	0.196
$X_2 \times X_2$	0.0104	0.196	0.0060	0.515	0.0073	0.017*
$X_3 \times X_1$	0.0037	0.954	0.0655	0.390	0.0861	0.068
$X_3 \times X_2$	−0.0536	0.779	−0.0589	0.794	0.1191	0.374
$X_3 \times X_3$	−3.0112	0.463	−0.5374	0.910	7.3266	0.206
$X_4 \times X_1$	−0.0088	0.890	−0.0061	0.935	0.0255	0.259
$X_4 \times X_2$	−0.0367	0.847	−0.2367	0.303	0.0211	0.749
$X_4 \times X_3$	5.8789	0.232	4.4570	0.434	5.6484	0.105
$X_4 \times X_4$	−7.1753	0.096	−4.1390	0.394	1.2223	0.390

[a] X_1, heating temperature (°C); X_2, heating time (min); X_3, added asparagine (mmol); X_4, added sugar (mmol). The unit of acrylamide content was μmol.
[b] $^{*}P < 0.05$; $^{**}P < 0.01$.

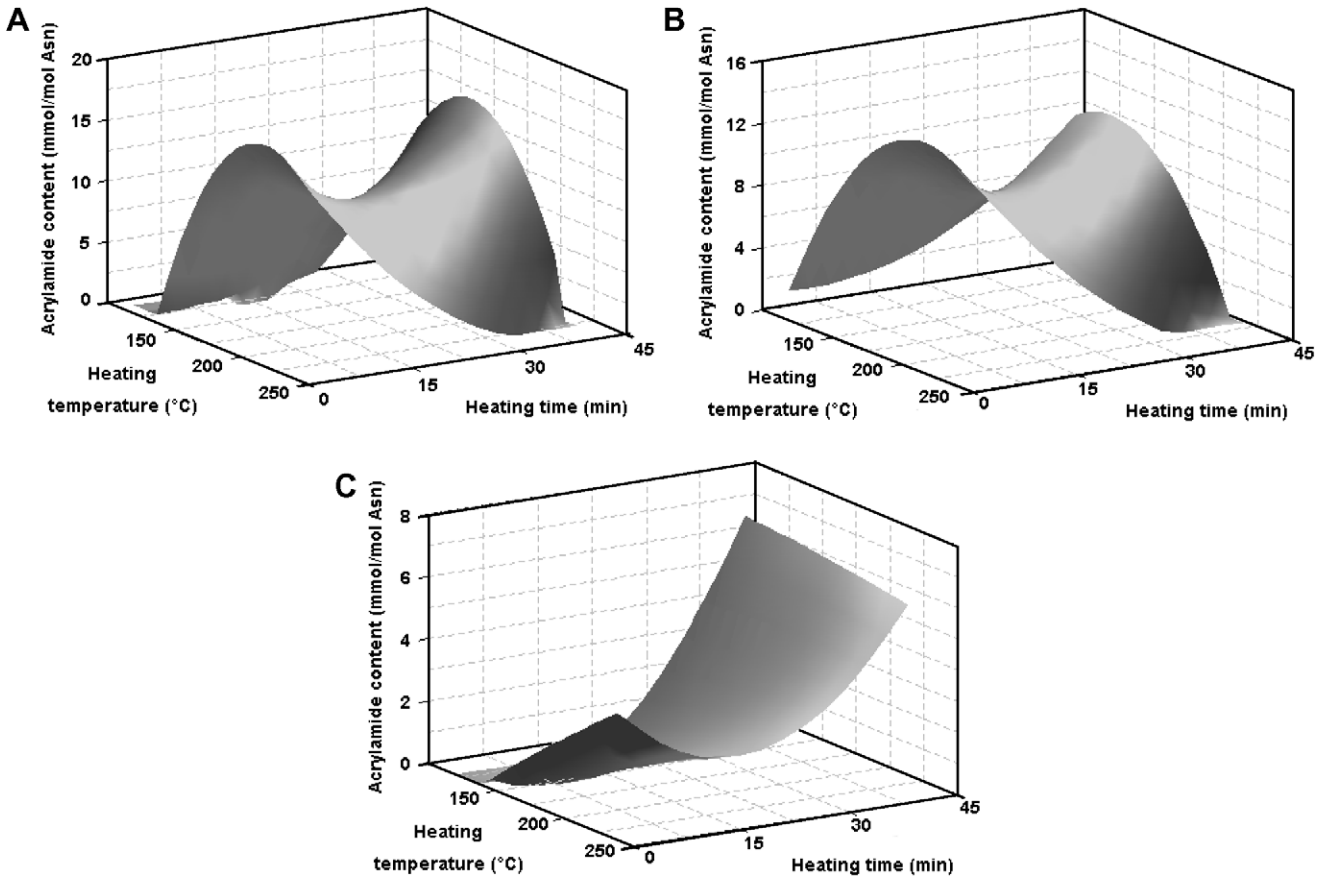

Fig. 1. Response surface graphs of acrylamide content with the variation of heating temperature and heating time in the (A) asparagine–glucose, (B) asparagine–fructose and (C) asparagine–sucrose microwave heating model systems.

inflection point (187 °C, 22 min) which presented the minimum acrylamide level (10.5 mmol/mol Asn) between the above two combined conditions (Fig. 1A). In general, such phenomenon might be ascribed to the kinetics of acrylamide formation and elimination. The initial rate of formation of acrylamide increased with heating temperature. In the range of 140–160 °C, the increase of acrylamide concentration was followed by a steady state in which the formation of acrylamide was probably in equilibrium with its degradation. Alternatively, the heating time could also greatly

affect the formation of acrylamide. After prolonged heating, a decline of the acrylamide content was observed, which could be ascribed to the elimination of acrylamide becoming predominant over the formation of acrylamide (Claeys et al., 2005). When the temperature falls into the scope of 180–200 °C, the elimination of acrylamide performs dominantly compared to the generation process (Knol et al., 2005). The loss of acrylamide after prolonged heating at 180 °C may due to the reactivity of acrylamide with food components (Wedzicha, Mottram, Elmore, Koutsidis, & Dodson, 2005). Acrylamide is mainly reactive through its double bond and react as an electrophile by 1,4-addition with nucleophiles such as SH- or NH_2-groups in biomolecules. Acrylamide can thus readily react with various other components formed in the food or model system. Claeys et al., 2005 found that a high amount of acrylamide could also be detected even though the asparagine–sugar model systems were heated at relatively low temperature (140 °C). The elimination of acrylamide is ascribed to acrylamide degradation or polymerization (Kolek, Šimko, Šimon, & Gatial, 2007a; Kolek, Šimon, & Šimko, 2007b).

For the asparagine–fructose system, similar results about the formation of acrylamide could be found (Fig. 1B). The acrylamide content increased in the combined condition of high temperature and short heating time (>175 °C, <20 min) or low temperature and long heating time (<170 °C, >25 min). Likewise, we also found an inflection point (174 °C, 25 min) which showed the minimum acrylamide content (8.6 mmol/mol Asn) between the above two conditions. Interestingly, in the microwave heating system, much higher concentration of acrylamide was generated from the asparagine–fructose system compared to the asparagine–glucose system only at the initiate stage of RSM study (Table 1, Test no. 1–5). The opposite results were concluded when the remaining tests of RSM were performed. Actually, different observations were demonstrated concerning the relative contributions of the sugars for generating acrylamide. Stadler et al., 2002 investigated the role of different carbohydrates in the generation of acrylamide and observed that D-fructose, D-galactose, lactose and sucrose released acrylamide with comparable yields in model reactions with asparagine monohydrate heated at 180 °C. However, Biedermann, Noti, Biedermann-Brem, Mozzetti, and Grob, 2002 demonstrated fructose appeared to be twice as effective in promoting acrylamide formation as glucose when added to dry potato (5%) and heated at 150 °C for 30 min in the model experiments. Conversely, Claeys et al., 2005 found glucose presented more effectively in generating acrylamide than fructose when the heating temperature was higher than 140 °C also in the asparagine–sugar systems, the conclusion of which was in good agreement with our findings. Such discrepancies based on all of the above observations may be due to the effect of both temperature and time on the relative activities of sugars toward the generation of acrylamide. Under such consideration, the physical state of the reaction system may be regarded as one of the decisive factors. Robert et al., 2004 investigated the formation and elimination of acrylamide in the low-moisture model systems and indicated that at given molecular mobility the chemical reactivity of the sugar is the major driver in the process of acrylamide formation. Meanwhile, the melting behavior and the release of crystallization water from the reaction system should also be taken into consideration (Robert et al., 2005). In hydrous systems, the molecular mobility is no longer a limiting factor and the relative reactivity of the sugar will be determined by its chemical reactivity (Claeys et al., 2005). In addition, the relative rates of caramelization of glucose and fructose were reported to depend on the extent with whether the reaction mixture was buffered. In the unbuffered media, the caramelization rate of fructose with amino acids was reported to be greater than that of glucose, whereas in buffered media, fructose caramelized more slowly than glucose (Davies & Labuza, 1997). The caramelization rate partly reflected the reaction rate. Therefore, the reaction rate of asparagine–fructose system was faster than the asparagine–glucose system. A higher critical heating temperature is required in the asparagine–glucose system. In fact, we demonstrated the critical heating temperature (174 °C) in the asparagine–fructose system was certainly lower than the asparagine–glucose system (187 °C). Results of our study performed in the buffered reactions demonstrated this hypothesis.

For the asparagine–sucrose microwave heating system, the amount of acrylamide increased with the increase of both heating temperature and heating time (Fig. 1C), which was a bit different from the occurrence of acrylamide in the other two asparagine–sugar systems. Nevertheless, we still found that the acrylamide content increased in the combined condition of heating temperature (T) and heating time (t), i.e. $T > 360$ °C and $t < 25$ min or $T < 350$ °C and $t > 30$ min based on a theoretical consideration. After precise calculation, an inflection point (358 °C, 26 min), which showed the minimum acrylamide content (3.2 mmol/mol Asn) between the above two conditions, was observed in the 3D profile of sucrose reaction system. A possible explanation to acrylamide formation from sucrose is that the sucrose is hydrolyzed upon thermal treatment to the individual monosaccharide. One sucrose molecule can then, in theory, provide two reducing hexoses, i.e., a molar ratio of sugar to amino acid of 2:1 (Taeymans et al., 2004). Therefore, considering the comparability among three systems in the present work, the added level of sucrose in each test was 50% less than the molar quantity of the added glucose or fructose.

Coefficients of the fitted response surface equations and significant tests for three kinds of asparagine–sugar systems are shown in Table 3. The linear and quadratic terms of heating temperature in the asparagine–glucose microwave heating system were significantly different ($P < 0.01$) while the discrepancy ($P < 0.05$) of the linear term of the added asparagine monohydrate level and the quadratic term of heating time in the sucrose system was observed. Besides, in the asparagine–glucose microwave heating system, the

difference of interaction term temperature × time was also significant ($P < 0.01$), indicating that the heating temperature brings about most of contribution to the formation of acrylamide in this model and also greatly depends on the heating time.

The analysis of variance (ANOVA) for the RSM model is shown in Table 4. Most of variances in glucose and fructose microwave heating system models had significant difference ($P < 0.05$, $P < 0.01$). The coefficients of determination (R^2) of the model indicated that all the three models adequately represent the real relationship among the parameters chosen.

3.2. OAM study on acrylamide formation in the microwave heating system

Becalski et al., 2003 investigated that when the molar ratio of asparagine to glucose was varied between 0.25 and 4, and the samples were heated at 175 °C for 10 min, the highest yield of acrylamide was obtained at molar ratios of 0.5 to 1:1. The equimolar asparagine–sugar system was usually considered as the default model for the formation of acrylamide. In the present study, we used OAM study to optimize the main parameters contributing to the formation of acrylamide and further understood the effects of temperature, heating time and molar quantities of precursors in the microwave heating system. The actual content of acrylamide in each test according to OAM and corresponding ANOVA analysis are shown in Tables 5 and 6. The optimal conditions obtained by OAM for the formation of acrylamide in three kinds of asparagine–sugar systems included the following parameters: (i) for both asparagine–glucose and asparagine–fructose systems: heating temperature (180 °C), heating time (5 min), precursor concentration (1.4 mmol); (ii) for the asparagine–sucrose system: heating temperature (180 °C), heating time (15 min), precursor concentration (1.4 mmol). Heating at excessively high temperature in a short heating time tends to reduce the amount of acrylamide in the asparagine–sugar systems. However, asparagine needs sucrose at relatively longer time to increase the amount of acrylamide. In the asparagine–fructose system, the heating temperature, time and added asparagine level showed significant difference, indicating that all of three factors play an important role during the generation of acrylamide.

Table 5
Acrylamide content generated from microwave heating systems by 3-level and 3-variable OAM[a]

Test no.	Heating temperature (°C)	Heating time (min)	Added asparagine (mmol)[a]	Acrylamide (μmol)		
				Glucose	Fructose	Sucrose
1	150 (1)	5 (1)	0.6 (1)	0.16	2.37	0.05
2	150	15 (2)	1 (2)	9.37	1.15	1.07
3	150	25 (3)	1.4 (3)	11.23	8.46	3.31
4	180 (2)	5	1	20.64	16.05	5.79
5	180	15	1.4	21.83	18.36	8.70
6	180	25	0.6	8.47	6.11	1.98
7	210 (3)	5	1.4	20.85	20.63	5.24
8	210	15	0.6	7.32	5.92	1.92
9	210	25	1	8.76	7.43	3.34

[a] Added asparagine/ glucose (fructose) ratio = 1:1, added asparagine/ sucrose ratio = 2:1.

Table 4
Statistical analysis of variance of the asparagine–sugar microwave heating RSM model[a]

Type of model	Item	Degree of freedom	Sum of squares	Mean square	F value	P value
Asparagine and glucose	Linear	4	352.53	88.13	9.88	0.001**
	Quadratic	4	187.53	46.88	5.26	0.011*
	Crossproduct	6	98.85	16.47	1.85	0.172
	Total model	14	638.91	45.64	5.12	0.004**
	Lack of fit	10	106.92	10.69	266.42	0.004**
	Pure error	2	0.08	0.04		
	Total error	12	107.00	8.92		
Asparagine and fructose	Linear	4	281.75	70.44	5.68	0.008**
	Quadratic	4	56.08	14.02	1.13	0.388
	Crossproduct	6	67.64	11.27	0.91	0.520
	Total model	14	405.47	28.96	2.33	0.074
	Lack of fit	10	145.85	14.58	9.63	0.098
	Pure error	2	3.03	1.51		
	Total error	12	148.88	12.41		
Asparagine and sucrose	Linear	4	34.67	8.67	8.15	0.002**
	Quadratic	4	9.89	2.47	2.33	0.116
	Crossproduct	6	12.05	2.01	1.89	0.164
	Total model	14	56.62	4.04	3.80	0.013*
	Lack of fit	10	12.68	1.27	34.56	0.028*
	Pure error	2	0.07	0.04		
	Total error	12	12.76	1.06		

[a] Coefficient of variation (R^2): 0.856 (asparagine and glucose), 0.731 (asparagine and fructose), 0.816 (asparagine and sucrose).
* $P < 0.05$.
** $P < 0.01$.

Table 6
ANOVA analysis of variance of the asparagine–sugar microwave heating model by OAM[a]

Type of model	Variance	Degree of freedom	ANOVA sum of squares	Mean square	*F* value	*P* value
Asparagine and glucose	Temperature (°C)	2	152.03	76.01	10.19	0.089
	Time (min)	2	31.68	15.84	2.12	0.320
	Added Asn (mmol)	2	243.29	121.64	16.30	0.058
Asparagine and fructose	Temperature (°C)	2	149.03	74.52	114.86	0.009**
	Time (min)	2	54.22	27.11	41.79	0.023*
	Added Asn (mmol)	2	190.86	95.43	147.10	0.007**
Asparagine and sucrose	Temperature (°C)	2	24.16	12.08	9.44	0.096
	Time (min)	2	1.75	0.87	0.68	0.594
	Added Asn (mmol)	2	29.52	14.76	11.53	0.080

[a] Coefficient of variation (R^2): 0.966 (asparagine and glucose), 0.997 (asparagine and fructose), 0.956 (asparagine and sucrose).
* $P < 0.05$.
** $P < 0.01$.

3.3. Effect of reaction pressure on acrylamide formation

Pressure is like heating temperature and time that can affect the rate of chemical reactions. The vapor pressure in the microwave sample vessels was monitored via the pressure control system and shown in the digital intelligent control panel during the whole process of Maillard reaction. The average (460 kPa) and maximum (950 kPa) vapor pressures during the reaction were observed. RSM and OAM studies demonstrated that the reaction between asparagine and sugar produces larger amounts of acrylamide under high pressure condition of microwave heating system than under conventional pressure condition (101 kPa) because high pressure accelerated the rate of Maillard reaction. Study on the formation of acrylamide under high pressure situation is necessary for satisfying the requirements of high heating temperature. Such high pressure reaction model was successfully applied in previous study (Claeys et al., 2005). As a result, the reaction under high pressure condition can generate more levels of acrylamide, but it cannot change the reaction mechanism and types of final products.

4. Conclusions

The present study indicated that the formation of acrylamide presented a strong dependence on the heating temperature, heating time and their interactions in asparagine–sugar microwave heating systems. In the asparagine–glucose system, the acrylamide content increases in the combined condition of high temperature and short heating time (>190 °C, <20 min) or low temperature and long heating time (<180 °C, >30 min). In the asparagine–fructose system, the similar conclusion was made in the combined condition of high temperature and short heating time (>175 °C, <20 min) or low temperature and long heating time (<170 °C, >25 min). In the asparagine–sucrose system, the amount of acrylamide enhances with the increase of both heating temperature and time. The equimolar asparagine–sugar system is always considered as the research model for the formation study of acrylamide. OAM study showed that the molar quantity of asparagine monohydrate became the most important parameter contributing to the formation of acrylamide. Meanwhile, acrylamide was readily formed via heating 1.4 mmol of binary precursors 5 min at 180 °C in the glucose and fructose system. However, acrylamide is generated easier when the binary precursors are heated for 15 min in the sucrose system. The fitted mathematic models were successfully applied to the quantification of acrylamide formation within when the set heating temperature and set heating time fell into the ranges of 120–240 °C and 5–35 min simultaneously. This study could be considered as an important attempt to investigate the formation of acrylamide in microwave heating model systems. However, there are still some remaining issues, such as the kinetics of acrylamide generated and eliminated in this system, similar studies performed in food matrixes and possible mitigation ways of acrylamide, which will be conducted in due course.

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