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Study on formation of acrylamide under low-moisture asparagine–sugar reaction system

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Abstract

The formation of acrylamide under three asparagine–sugar low-moisture systems were analyzed by the response surface methodology (RSM) and orthogonal array methodology (OAM) in the present work. The acrylamide content was quantified by a novel validated ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS). Results of RSM study showed that in the asparagine–glucose system, the acrylamide content achieved a maximum level (442.7 μmol/mol Asn) when the system was heated 18 min at about 199 °C; in the asparagine–fructose system, the acrylamide content increases in the combined condition of higher temperature and shorter heating time (>200 °C, <15 min) or lower temperature and longer heating time (<150 °C, >35 min); in the asparagine–sucrose system, the amount of acrylamide enhanced with the increase of temperature and the decrease of time, but declined with the decrease of temperature and the increase of heating time. The coefficients of determination (R^2) of the model indicated that all the three models adequately represented the real relationship among the parameters chosen. OAM study indicated 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 binary precursors 15 min at 180 °C in the glucose and fructose system while acrylamide was readily generated when the binary precursors were heated 15 min at 210 °C in the sucrose system.

Keywords: Acrylamide; Low-moisture system; Asparagine; Sugar; UPLC–MS/MS

1. Introduction

Since undesirable acrylamide content in heat-treated foods was initially observed by Swedish scientists in 2002 (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002), numerous research groups in academic schools, industry, and official laboratories commenced studies into the possible sources and corresponding mechanisms for the study of acrylamide formation. During these years, the formation mechanism of acrylamide in asparagine–carbohydrate model systems has been systematically demonstrated (Becalski, Lau, Lewis, & Seaman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Stadler et al., 2004; Yaylayan, Wnorowski, & Locas, 2003; Zyzak et al., 2003). Besides this, the formation of acrylamide is also affected by food matrixes and some important effect parameters on generating acrylamide were investigated based on the asparagine–carbohydrate model studies. Many researchers involved such food matrix studies to find the factors affecting acrylamide formed in certain matrixes including potatoes (Amrein et al., 2003; Gökmen, Palazoğlu, & Şenyuva, 2006), cereals (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005), bread (Bråthen & Knutsen, 2005), almonds (Amrein et al., 2005), and coffee (Andrzejewski, Roach, Gay, & Musser, 2004). Various parameters affect the reaction yields of acrylamide from asparagine, such as the concentration of precursors, type of sugar compounds and the reaction conditions. The interaction

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between reaction temperature and time has been also suggested as an important factor (Taubert, Harlfinger, Henkes, Berkels, & Schömig, 2004).

Actually, the effect of moisture content on acrylamide generation should be especially taken into consideration. However, few studies have been carried out on the relationship investigation between the moisture content and acrylamide formation. Leung, Lin, Tsang, and Yeung (2003) measured water loss in a fritter made from wheat flour, which was deeply fried at 170, 190, or 210 °C. The original moisture content of the fritter was ~48% and fell into ~10% after 15 min at 170 °C; that is, over one-fifth of the original water in the fritter remained, at which time the acrylamide content was ~200 μg/kg. Similar researches demonstrated that the acrylamide formation did not occur to a large degree until the moisture contents of potato flake fell below 5% (Elmore et al., 2005). In other words, acrylamide was easily generated under the low-moisture reaction system so that the fundamental formation study and the heat processing parameter optimization of acrylamide should be a prerequisite research.

As for the acrylamide research under low-moisture conditions, previous studies (Robert et al., 2004, 2005) investigated the interplay between chemical reactivity and physical changes during the acrylamide formation, and compared the acrylamide yield generated from a model system in both crystalline and amorphous states. Since considerable acrylamide contents occurred under the low-moisture system, it is important to study the effect of key heat processing parameters on the acrylamide formation under such conditions. The response surface methodology (RSM) and orthogonal array methodology (OAM) are both able to evaluate the effective factors and establish mathematical models in order to study interaction and select optimum conditions of variables for a desirable response (Haaland, 1989). Compared to other methodologies such as artificial neural network (ANN), response surfaces plotted by 3D plots can provide a better way to visualize the parameter interaction and also be in favor of observing both growth and decline tendencies in the present study. The aim of present study is to use ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) to fast quantify the acrylamide level in model reaction products after necessary pretreatment procedures and investigate the acrylamide formation with the change of important factors (heating time, heating temperature and molar quantity of precursors) under the low-moisture asparagine and glucose/fructose/sucrose reaction system using RSM and OAM.

2. Materials and methods

2.1. Chemicals and reagents

Acrylamide (99%) and D_3-labelled acrylamide (isotopic purity 99%) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Ethyl acetate and formic acid (96%) were obtained from Tedia (Fairfield, OH) while methanol (HPLC-grade) was supplied by Merck (Whitehouse Station, NJ). L-Asparagine monohydrate was purchased from Biocity Science & Technology Inc. (Beijing, China). All of other solvents and chemicals including D-(−)-fructose, D-(+)-glucose monohydrate and sucrose were of analytical grade and were used without further purification.

2.2. Study on acrylamide formation using RSM

The formation study of acrylamide was performed via RSM according to Bråthen and Knutsen (2005) with some modifications. The experimental design was done with three repetitions of the central point in order to evaluate the experimental error. The independent variables were heating temperature, heating time, asparagine monohydrate and sugar (fructose, glucose monohydrate or sucrose), and their interactions between each other were also taken into consideration. Corresponding coefficients of both variables and interaction variables were estimated by SAS Institute software, version 8.2 (SAS Institute Inc., Beijing, China). Statistical analysis on the significance of coefficient estimations was performed via Student's *t*-test.

2.3. Study on acrylamide formation using OAM

Based on the results of RSM, this formation study was also done 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. The analysis of variance (ANOVA) was also calculated by the SAS Institute software. Statistical analysis on the significance of ANOVA was performed via *F*-test.

2.4. Preparation of low-moisture system

For the RSM analysis, three systems were prepared by weighing and mixing different levels of asparagine monohydrate and sugar powders according to the real weights recommended by the experimental design of RSM (Table 1). For the OAM analysis, the asparagine–fructose and asparagine–glucose low-moisture systems were prepared by weighing equimolar quantity of asparagine monohydrate and alternative sugar powder while the asparagine–sucrose system was prepared by weighing two products with a molar ratio of 2:1. The real weights for the OAM analysis were shown in Table 4. The asparagine–sugar reactant powders used in each test were carefully grounded together to ensure enough surface areas for the reaction. Three kinds of mixed powders were all oven-heated under the selected heating temperature and heating time according to the requests of RSM and OAM. At the end of heating,

Table 1
Acrylamide content in samples by 5-level and 4-variable RSM[a]

Test number	X_1, Heating temperature (°C)	X_2, Heating time (min)	X_3, Added asparagine (mmol)	X_4, Added sugar (mmol)			Acrylamide (nmol)		
				Glucose	Fructose	Sucrose	Glucose	Fructose	Sucrose
1	120 (−2)[a]	25 (0)	1 (0)	1 (0)	1 (0)	0.5 (0)	1.64	13.46	n.d.[b]
2	150 (−1)	15 (−1)	1.4 (1)	1.4 (1)	1.4 (1)	0.7 (1)	137.68	158.09	22.30
3	150	15	1.4	0.6 (−1)	0.6 (−1)	0.3 (−1)	37.86	33.72	48.72
4	150	15	0.6 (−1)	1.4	1.4	0.7	66.56	27.78	24.89
5	150	15	0.6	0.6	0.6	0.3	27.56	32.66	29.02
6	150	35 (1)	1.4	1.4	1.4	0.7	317.03	797.75	256.12
7	150	35	1.4	0.6	0.6	0.3	162.50	744.10	235.02
8	150	35	0.6	1.4	1.4	0.7	225.14	553.25	83.59
9	150	35	0.6	0.6	0.6	0.3	89.07	325.06	81.17
10	180 (0)	25	1.8 (2)	1	1	0.5	717.48	715.75	501.68
11	180	25	1	1	1	0.5	400.06	429.20	269.40
12	180	25	1	1	1	0.5	425.72	442.27	258.32
13	180	25	1	1	1	0.5	418.98	464.04	225.83
14	180	25	1	0.2 (−2)	0.2 (−2)	0.1 (−2)	153.10	279.57	160.35
15	180	25	1	1.8 (2)	1.8 (2)	0.9 (2)	556.22	590.57	390.26
16	180	25	0.2 (−2)	1	1	0.5	117.40	86.12	76.91
17	210 (1)	35	1.4	1.4	1.4	0.7	131.76	114.73	n.d.
18	210	35	1.4	0.6	0.6	0.3	n.d.	n.d.	259.98
19	210	35	0.6	1.4	1.4	0.7	58.90	69.45	64.67
20	210	35	0.6	0.6	0.6	0.3	40.79	49.35	95.57
21	210	15	1.4	1.4	1.4	0.7	677.36	1045.65	599.79
22	210	15	1.4	0.6	0.6	0.3	490.00	849.86	565.98
23	210	15	0.6	1.4	1.4	0.7	404.26	316.48	324.91
24	210	15	0.6	0.6	0.6	0.3	250.47	315.57	216.37
25	180	5 (−2)	1	1	1	0.5	40.42	n.d.	n.d.
26	180	45 (2)	1	1	1	0.5	92.71	213.70	n.d.
27	240 (2)	25	1	1	1	0.5	17.75	49.49	108.01

[a] Figures in brackets indicate the level number of RSM.
[b] n.d., not detected.

the final reaction products were taken out from the oven and quickly cooled to approximately 20 °C via venting the cold air from the air-condition. The cooled reaction products were ready for sampling at the beginning of the pretreatment.

2.5. Pretreatment of low-moisture reaction products

Ten milliliter of PBS buffer (0.1 mol/l, pH 6.80) was added into the cooled low-moisture system to dissolve the final reaction products. Then, 500 μl of D_3-labelled acrylamide internal standard solution (2 μg/ml) was homogeneously spiked and the mixed solution was shaken in KQ3200E ultrasonic shaker (Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, Jiangsu, China). After dissolving, 2 ml of the mixed solution was sampled and subsequently extracted with 2 ml of ethyl acetate for three times. The organic phase was combined and dried by nitrogen gas. The residue was redissolved by water and removed for clean-up. Oasis HLB SPE cartridges (6 ml, 200 mg) purchased from Waters Technology were conditioned with 3.5 ml of methanol followed by 3.5 ml of water, and the methanol and water portions were discarded. Each cartridge was loaded with 1.5 ml of redissolved sample. The sample solution was allowed to pass through the sorbent material and discarded. Then the cartridge was eluted with 3 ml of water and the eluant was collected. Finally, the analyte after clean-up was submitted for UPLC–MS/MS analysis.

2.6. UPLC–MS/MS analysis of acrylamide

Quantification of acrylamide in pretreated reaction products was performed on a UPLC–MS/MS with the electrospray positive ionization (ESI+). In detail, an ACQUITY UPLC quaternary pump system equipped with the micro vacuum degasser, thermostated autosampler and thermostated column compartment (Waters, Milford, MA) was coupled with a Micromass Quattro Ultima triple-quadrupole mass spectrometer from Micromass Company Inc. (Manchester, UK). Chromatographic separation was carried out on a UPLC BEH C_{18} column (50-mm length, 2.1-mm i.d., 1.7-μm particle size; Waters, Milford, MA, USA) maintained at 25 °C. The mobile phase was 10% methanol/0.1% formic acid in water with a flow speed of 0.2 ml/min. The conditions used for the electrospray source were as follows: capillary voltage, 3.5 kV; cone voltage, 50 V; source temperature, 100 °C; desolvation gas temperature, 350 °C; desolvation gas flow, 400 l/h nitrogen; cone gas flow, 45 l/h nitrogen; and argon collision gas pressure to 3×10^{-3} mbar for MS/MS, which gave a highest acrylamide response in this study. The collision energy (CE) was

optimized for each multiple reaction monitored (MRM) transition. The HPLC–ESI-MS/MS run time was 3 min per sample. The collision energy for each monitored transition was optimized in MRM mode. The transitions monitored according to Rosén and Hellenäs (2002) for acrylamide were 72 > 72 at 1 eV, 72 > 55 at 6 eV, 72 > 44 at 9 eV and 72 > 27 at 15 eV. The transitions monitored for D_3-labelled acrylamide were 75 > 75 at 1 eV, 75 > 58 at 6 eV, and 72 > 30 at 15 eV.

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 previously 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 analytical result of acrylamide (1381 μg/kg) from our laboratory (No. 021) in dispatched test material with a Z-score of −0.1 seemed satisfactory, which fulfilled requirements from the organization (FAPAS, 2005).

3. Results and discussion

3.1. Advantage of UPLC–MS/MS for the quantification of acrylamide

A robust HPLC–MS/MS analytical method for the determination of acrylamide in foods was validated in our previous study (Zhang, Jiao, Ren, Wu, & Zhang, 2005). In the present work, we improved this method and obtained a higher sensitivity and chromatographic efficiency compared to the HPLC–MS/MS method. A representative chromatogram for the quantification of acrylamide under the low moisture system by UPLC–MS/MS was shown in Fig. 1. Overall, it appeared that the UPLC based method could offer significant improvements in sensitivity, speed and resolution, and was well applied in the acrylamide analysis. Considering the analyte numbers and complexity increased in this formation study (not only the routine determination of acrylamide), and considering the need to compel and push limits of quantification to be lower and lower levels in shorter and shorter time become more critical to the conduct of optimization investigations, the performance of both chromatographic and mass spectral components of LC–MS systems becomes more and more critical. The goal of increasing overall performance of these techniques can be further enhanced by reducing constraints imposed by the chromatographic separation (Churchwell, Twaddle, Meeker, & Doerge, 2005).

3.2. RSM study on acrylamide formation under the low-moisture system

The actual content of acrylamide in each test according to RSM was shown in Table 1 and Fig. 2. Results showed that acrylamide could also be generated from the asparagine–sucrose low-moisture system. The reaction with sucrose resulted in acrylamide levels similar to those recorded for glucose monohydrate and fructose. A possible explanation to acrylamide formation from sucrose is that the sucrose is hydrolyzed upon thermal treatment to the individual monosaccharide. It is considered that one

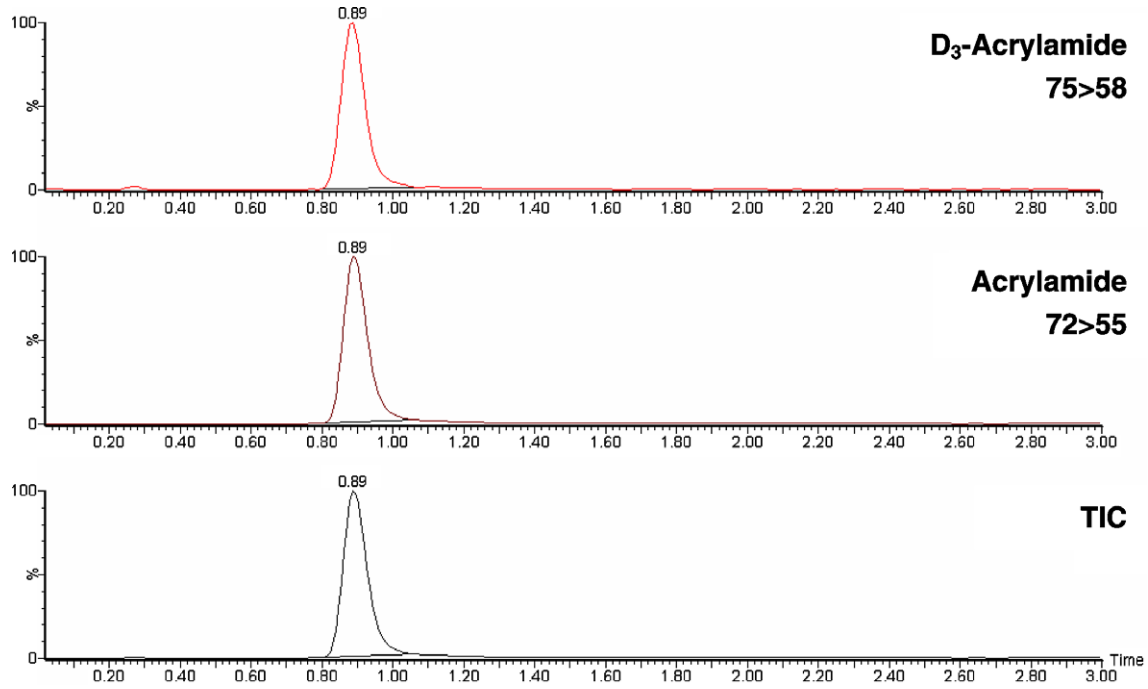

Fig. 1. A representative chromatogram for the quantification of acrylamide under the low moisture system by UPLC–MS/MS.

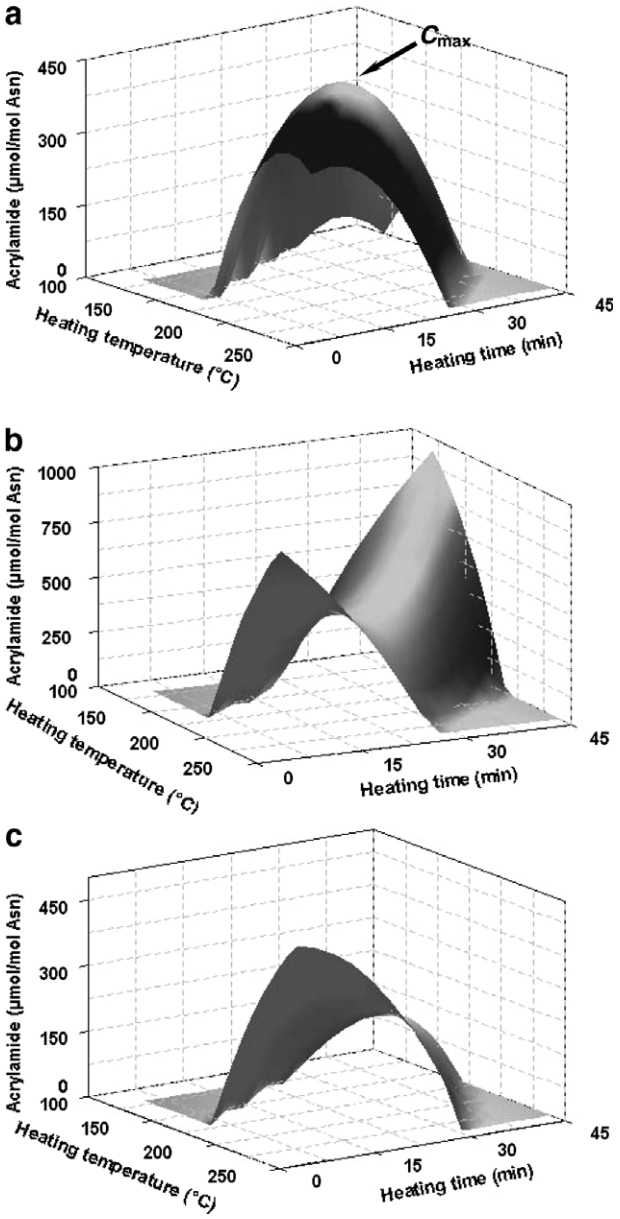

Fig. 2. Response surface graphs of acrylamide content with the variation of heating temperature and heating time under the: (a) asparagine–glucose; (b) asparagine–fructose and (c) asparagine–sucrose low-moisture systems.

sucrose molecule could 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, the added level of sucrose in each test was 50% less than the molar quantity of the added glucose monohydrate or fructose. We also found that fructose was more efficient in generating acrylamide from asparagine monohydrate compared to glucose monohydrate, the finding of which was in nice agreement with previous publications (Pollien, Lindinger, Yeretzian, & Blank, 2003). Fig. 2 showed the response surface graph of acrylamide content with the increase of heating temperature and heating time under three equimolar asparagine–sugar (molar quantity ratio of asparagine:sucrose = 2:1) systems, which could be calculated as the following equations:

Asparagine–glucose system:

$$Y = -0.1158x_1^2 - 0.8997x_2^2 + 54.1082x_1 + 120.2776x_2 - 0.4406x_1x_2 - 6035.9784 \quad (1)$$

Asparagine–fructose system:

$$Y = -0.0971x_1^2 - 0.6858x_2^2 + 58.4313x_1 + 202.8671x_2 - 0.9296x_1x_2 - 7385.2679 \quad (2)$$

Asparagine–sucrose system:

$$Y = -0.0498x_1^2 - 0.5828x_2^2 + 29.5486x_1 + 94.1572x_2 - 0.3787x_1x_2 - 3653.3145 \quad (3)$$

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

Interestingly, the response surface graphs presented three different tendencies of acrylamide formation. As for the asparagine–glucose system, results indicated that the acrylamide amount formed went through a maximum at around 180–210 °C as the heating temperature increases (Fig. 2a). After precise calculation, we found that the acrylamide content reached the maximum level (442.7 μmol/mol Asn) when the aparagine–glucose system was heated 18 min at about 199 °C. In the present work, the response surface graph of acrylamide amount generated in the asparagine–glucose system showed a decline tendency in the combined condition of elevated temperature and extended time. Such result was in good agreement with the findings by Rydberg et al. (2003), which reported the acrylamide formation in potato strips. Also, higher temperature (200 °C) combined with prolonged heating time produced reduced levels of acrylamide, due to elimination/degradation processes (Rydberg et al., 2003). Bråthen and Knutsen (2005) also found the amount of acrylamide went through a maximum at around 190–210 °C when the temperature increased in starch system and freeze-dried flat breads. As for the asparagine–fructose system, results demonstrated that heating binary mixtures of asparagine monohydrate and anhydrous reducing sugars led to higher acrylamide amounts in the presence of fructose compared to glucose monohydrate. However, the formation tendency of acrylamide seemed interesting (Fig. 2b). The acrylamide content increased dramatically in the combined condition of higher temperature and shorter heating time (>200 °C, <15 min) or lower temperature and longer heating time (<150 °C, >35 min). Meanwhile, we also found and calculated an inflection point (181 °C, 25 min) which presented the minimum acrylamide level (445.3 μmol/mol Asn) between the above two combined conditions. Robert et al. (2004) indicated that acrylamide was preferably formed by reacting glucose and asparagine at 120 °C for 60 min, whereas 160 °C was required at shorter reaction time (5 min) which would be not only expected from a simple kinetic theory but also generated with a special heating

treatment such as microwave heating, and these results suggested that, in addition to the chemical reactivity of ingredients as well as reaction temperature and time would influence the formation of acrylamide during food processing. Our investigation results were in good agreement with the above conclusions. Besides, the discrepancy of surface-to-volume ratio (SVR) in two low-moisture systems and different melting situations between two sugars also contributed to the different generation tendencies between glucose and fructose systems (Robert et al., 2004; Taubert et al., 2004). As for the asparagine–sucrose system, the amount of acrylamide enhanced with the increase of temperature and the decrease of time (Fig. 2c) and no inflection point was observed in this model system. However, it was different from the asparagine–fructose system that the acrylamide content declined with the decrease of temperature or the increase of heating time.

Coefficients of the fitted response surface equations and significant tests for the three low-moisture systems were shown in Table 2. The linear and quadratic terms in heating temperature and time under all of three low-moisture systems were significant ($P < 0.05$). In three cases of three low-moisture systems, the interaction term temperature × time was extremely significant ($P < 0.001$),

Table 2
Coefficients of the fitted response surface equations and significant tests for the three low-moisture systems[a]

Variable	Asparagine–glucose system		Asparagine–fructose system		Asparagine–sucrose system	
	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
Intercept	−6294.344	0.000	−7359.026	0.002	−3055.859	0.015
X_1	52.236	0.000	56.376	0.003	25.272	0.014
X_2	127.093	0.000	212.787	<0.000	94.358	0.000
X_3	22.727	0.969	39.775	0.962	−615.016	0.535
X_4	273.387	0.639	−96.541	0.908	−590.322	0.244
$X_1 \times X_1$	−0.116	0.001	−0.097	0.026	−0.050	0.047
$X_2 \times X_1$	−0.441	0.000	−0.930	<0.000	−0.379	0.000
$X_2 \times X_2$	−0.900	0.003	−0.686	0.070	−0.583	0.014
$X_3 \times X_1$	1.552	0.512	2.419	0.480	3.966	0.329
$X_3 \times X_2$	−6.198	0.387	−11.490	0.272	−6.734	0.575
$X_3 \times X_3$	−14.065	0.926	30.894	0.888	351.135	0.501
$X_4 \times X_1$	0.321	0.891	−0.364	0.915	2.293	0.262
$X_4 \times X_2$	−0.617	0.930	1.570	0.878	3.166	0.598
$X_4 \times X_3$	88.477	0.617	95.398	0.708	117.766	0.694
$X_4 \times X_4$	−112.159	0.467	84.230	0.703	65.924	0.612

[a] X_1, heating temperature; X_2, heating time; X_3, added asparagine monohydrate; X_4, added sugar.

Table 3
Statistical analysis of variance of the asparagine–sugar low-moisture RSM model[a]

Type of model	Item	Degrees of freedom	Sum of squares	Mean square	*F*-value	*P*-value
Asparagine and glucose	Linear	4	371600	92900	7.63	0.0027
	Quadratic	4	371789	92947	7.63	0.0027
	Crossproduct	6	298424	49737	4.08	0.0183
	Total model	14	1041813	74415	6.11	0.0017
	Lack of fit	10	145838	14584	82.48	0.0120
	Pure error	2	354	177		
	Total error	12	146192	12183		
Asparagine and fructose	Linear	4	538831	134708	5.30	0.0108
	Quadratic	4	300701	75175	2.96	0.0650
	Crossproduct	6	1296251	216042	8.49	0.0009
	Total model	14	2135782	152556	6.00	0.0018
	Lack of fit	10	304579	30458	98.33	0.0101
	Pure error	2	620	310		
	Total error	12	305198	25433		
Asparagine and sucrose	Linear	4	311397	77849	8.90	0.0014
	Quadratic	4	149883	37471	4.28	0.0221
	Crossproduct	6	234587	39098	4.47	0.0132
	Total model	14	695866	49705	5.68	0.0023
	Lack of fit	10	103956	10396	20.27	0.0479
	Pure error	2	1026	513		
	Total error	12	104982	8748		

[a] Coefficient of variation (R^2): 0.877 (Asn & Glu); 0.875 (Asn & Fru); 0.869 (Asn & Suc).

indicating that the temperature which brought about the most acrylamide content greatly depended on the heating time.

The analysis of variance (ANOVA) for the RSM model was shown in Table 3. Except for the quadratic term in the asparagine–fructose system, all of other variances in three system models were significant ($P < 0.05$). The coefficients of determination (R^2) of the model indicated that all the three models adequately represented the real relationship among the parameters chosen.

3.3. OAM study on acrylamide formation under the low-moisture 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 is always considered as the research model for the formation study of acrylamide. In the present work, 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 under the low-moisture system. The actual content of acrylamide in each test according to OAM and corresponding ANOVA analytical results were shown in Tables 4 and 5. The optimal conditions obtained by OAM for the formation of acrylamide under three low-moisture systems included the following parameters: heating temperature 180 °C, heating time 15 min, precursor concentration 1.4 mmol (for both asparagine–glucose and asparagine–fructose systems); heating temperature 210 °C, heating time 15 min, precursor concentration 1.4 mmol (for the asparagine–sucrose system). Prolonged heating tended to reduce the amount of acrylamide in the asparagine–sugar systems. Asparagine needs sucrose at relatively higher temperature to increase the amount of acrylamide.

The present work investigated the generation of acrylamide in three low-moisture systems and demonstrated three different response surface tendencies for the acrylamide formation and elimination, which indicated that not only the routine heating parameters but also some other factors including the melting points, physical states, chemical reactivity and exothermic reactions of precursors should be taken into consideration provided the mechanism of such formation and elimination rules needed to be elucidated. Based on a chemical point of view, glucose as an aldohexose sugar was expected to generate more acrylamide from asparagine monohydrate, due to its higher chemical reactivity provided by the more reactive aldehyde group compared to the ketohexose fructose (Robert et al.,

Table 4
Acrylamide content in samples by 3-level and 3-variable OAM[a]

Test number	Heating temperature (°C)	Heating time (min)	Added asparagine (mmol)[a]	Acrylamide (nmol)		
				Glucose	Fructose	Sucrose
1	150 (1)[b]	15 (1)	0.6 (1)	16.13	53.08	26.31
2	150	25 (2)	1 (2)	126.25	519.39	137.75
3	150	35 (3)	1.4 (3)	116.74	837.10	179.40
4	180 (2)	15	1	370.65	1309.28	262.80
5	180	25	1.4	570.62	771.13	348.90
6	180	35	0.6	133.96	187.20	114.09
7	210 (3)	15	1.4	756.56	1043.07	588.32
8	210	25	0.6	131.40	145.52	n.d.[c]
9	210	35	1	71.65	163.78	390.18

[a] Added asparagine/glucose (fructose) ratio = 1:1, added asparagine/sucrose ratio = 2:1.
[b] Figures in brackets indicate the level number of OAM.
[c] n.d., not detected.

Table 5
ANOVA analysis of variance of the asparagine–sugar low-moisture model by OAM[a]

Type of model	Variance	Degrees of freedom	ANOVA sum of squares	Mean square	*F*-value	*P*-value
Asparagine and glucose	Temperature (°C)	2	130010	65005	4.06	0.1977
	Time (min)	2	114361	57180	3.57	0.2189
	Added Asn (mmol)	2	244436	122218	7.63	0.1159
Asparagine and fructose	Temperature (°C)	2	175241	87620	0.61	0.6201
	Time (min)	2	275905	137952	0.96	0.5090
	Added Asn (mmol)	2	905322	452661	3.17	0.2401
Asparagine and sucrose	Temperature (°C)	2	68146	34073	2.28	0.3044
	Time (min)	2	25452	12726	0.85	0.5395
	Added Asn (mmol)	2	164682	82341	5.52	0.1533

[a] Coefficient of variation (R^2): 0.938 (Asn & Glu), 0.826 (Asn & Fru), 0.896 (Asn & Suc).

2004). However, results from our study and previous publications (Stadler & Scholz, 2004) demonstrated the opposite conclusion. The magnification of the initial phase from the study by Robert et al. (2004) showed that the mixture with fructose generated acrylamide earlier than that containing glucose. Interestingly, this finding correlated well with the respective melting points of the sugars, as far as fructose and the hexose sugars were concerned, which indicated that acrylamide was not released in detectable levels as long as the sugar has not started to melt. This phenomenon could partly explain the above opposite conclusion between the chemical point of view and actual results.

4. Conclusions

The present study statistically investigated the influencing factors for the acrylamide generation under asparagine–sugar low moisture reaction systems. In the asparagine–glucose system, the acrylamide content reached a maximum level (442.7 μmol/mol Asn) when the system was heated 18 min at about 199 °C. In the asparagine–fructose system, the acrylamide content increased in the combined condition of higher temperature and shorter heating time (>200 °C, <15 min) or lower temperature and longer heating time (<150 °C, >35 min). In the asparagine–sucrose system, the amount of acrylamide enhanced with the increase of temperature and the decrease of time, but declined with the decrease of temperature and the increase of heating time. 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 binary precursors 15 min at 180 °C in the glucose monohydrate and fructose system. However, acrylamide was readily generated when the binary precursors were heated 15 min at 210 °C in the sucrose system. Further studies will be focused on: (i) using other statistical methodologies such as ANN to evaluate the generation of acrylamide, and (ii) finding effective recipes to mitigate the formation of acrylamide under low moisture conditions.

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