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Stability of heterocyclic amines during heating

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Abstract

The stability of 15 Heterocyclic Amine (HA) standards was kinetically studied during heating at 100, 150 and 200°C for varied lengths of time. Parameters used to evaluate the stability of each HA standard include the degradation rate constant (h^{-1}), the activation energy (kJ/mol) and the A value. Results showed that the HA loss increased both with increasing temperature and heating time, and the degradation rate of each HA during heating fits a first-order model. 2-Amino-3-dimethylimidazo[4,5-f]quinoxaline (IQx) was found to possess the highest stability during heating, followed by 2-aminodipyrido[1,2-a:3′,2′-d]imidazole (Glu-p-2), 9H-pyrido[4,3-b]indole (Norharman), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-p-2), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (7,8-DiMeIQx), 1-methyl-9H-pyrido-[4,3-b]indole (Harman), 2-amino-6-methyldipyrido-[1,2-a:3',2'-d]imidazole (Glu-p-1), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-p-1), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-9H-pyrido[2,3-b]indole (AαC), 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAαC), and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP).

1. Introduction

Heterocyclic amines (HAs) are an important class of food mutagens and carcinogens, which can be found in fried or broiled meat and fish products (Abdulkarim & Smith, 1998; Chiu, Yang & Chen, 1998; Knize et al., 1998). Major precursors have been shown to be single amino acids or amino acids together with reducing sugar, creatine or creatinine (Arvidsson, Van Boekel, Skog & Jagerstad, 1997; Jagerstad, Skog, Grives & Olsson, 1991; Nagao, Sato & Sugimura, 1983). Epidemiological studies have shown that the consumption of HAs-containing foods, in excess, might induce different types of cancer such as pancreatic, colorectal and urothelial cancer (Gerhardsson de Verdier, 1995). Thus, it is important to learn more about the formation and stability of HAs in foods during heating. However, the stability of HAs during heating has seldom been studied.

Several parameters, such as temperature, heating time and method of cooking have been reported to affect the variety and amounts of HAs formed during heating (Barrington, Baker, Truswell, Bonin, Ryan & Paulin, 1990; Berg, Overvick & Gustafson, 1990; Chiu et al., 1998; Knize, Dolbeare, Carroll, Moore & Felton, 1994; Skog, Steineck, Augustson & Jagerstad, 1995). For instance, microwave cooking has been found to cause the formation of carboline types of HAs, while frying caused the formation of both IQ and carboline types of HAs (Chiu et al.). Of the various parameters, temperature alone is the most important (Sugimura & Sato, 1983). It has been well established that both the varieties and amounts of HAs increased along with increasing temperature (Chiu et al., 1998; Knize et al., 1994; Skog et al., 1995). Due to the complexicity of food itself, many authors have used a model system to study the formation and degradation of HAs during heating (Arvidsson et al., 1997; Chen & Meng, 1999; Nagao et al., 1983). Some authors reported that the formation and degradation of HAs may proceed simultaneously during heating of a model system containing glucose, amino acid, creatine or creatinine (Arvidsson et al., 1997; Jackson & Hargraves, 1995). Arvidsson et al. (1997) studied the stability of pure HA standards at 225°C and found that PhIP is the most susceptible to degradation, followed by 7,8-DiMeIQx, MeIQx, 4,8-DiMeIQx and IQx. Since only polar HAs were collected for studying the stability by Arvidsson et al. (1997) the degradation of apolar HAs during heating remains unknown. The objectives of this study were to examine the stability of both polar and apolar HAs under various heating treatments.

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2. Materials and methods

2.1. Materials

The HPLC-grade acetonitrile was from Merck Co. (Darmstadt, Germany), and was degassed under vacuum and filtered through a 0.2 μm membrane filter prior to use. The ammonium acetate solution was also treated in the same way for HPLC use. Deionized water was obtained from a Milli-Q water purification system (Millipore Co, Bedford, MA). A TSK-GEL ODS C18 column (250×4.6 mm I.D.) (Tosoh Co., Tokyo, Japan), containing 5-μm particles, was used to separate the various HAs. Sixteen HA standards, including 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3-dimethylimidazo[4,5-f]quinoxaline(IQx), 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4-8-trimethylimidazo[4,5-f] quinoxaline (4,8-DiMeIQx), 2-amino-3,7,8-trimethylimidazo [4,5-f]quinoxaline (7,8-DiMeIQx), 2-amino-1-methy-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-6-methyl-di-pyrido-[1,2-a;3',2'-d]imidazole (Glu-p-1), 2-amino-dipyrido-[1,2-a:3',2'-d]imidazole (Glu-p-2), 2-amino-9H-pyrido-[2,3-b]indole (AαC), 2-amino-3-methyl-9H-pyrido-[2, 3-b]indole (MeAαC) and internal standard 2-amino-3,4,7,8-tetramethylimidazo[4,7,8-f]quinoxaline (4,7,8-TriMeIQx), were purchased from Toronto Research Chemical Wako Co. (Osaka, Japan), and 1-methyl-9H-pyrido-[4,3-b]indole (Harman) and 9H-pyrido-[4,3-b]indole (Norharman) were from Aldrich Co. (Steinheim, Germany). All the HAs were 100% pure as determined by a Jasco MD-915 photodiode-array detector (Tokyo, Japan).

2.2. Instrumentation

The HPLC instrument consisted of a Jasco MD-915 photodiode-array detector (Tokyo, Japan), a Jasco 970/975 UV/VIS detector (Tokyo, Japan), a Jasco 821-FP fluorescence detector (Tokyo, Japan), two Jasco PU-980 pumps (Tokyo, Japan), and a BORWIN computer software system for processing data. The oil bath (B503) was from I-SENG Co. (Taipei, Taiwan).

2.3. Model system

Ten mg each of HA standards (Glu-p-2, IQx, IQ, Glu-p-1, MeIQx, MeIQ, 7,8-DiMeIQx, 4,8-DiMeIQx, Norharman, Harman, Trp-p-2, PhIP, Trp-p-1, AαC and MeAαC) was dissolved in 10 ml deionized water, after which 150 μl was collected and diluted to volume (25 ml) for a final concentration of 6 ppm of each standard solution. A five hundred μl sample was collected from each standard solution and placed in an ampoule (80×6 mm I.D., 1.0 mm wall thickness), which was then sealed with a flame produced from oxygen and gas. The ampoules were heated in a thermostat-controlled oil bath (40×30×20 cm) containing about 12 ℓ oil, with temperature at 100, 150 or 200°C for 1, 2, 3, 6, 12, 18 or 24 h, after which each ampoule was inserted into an ice bath to terminate the reaction. A one hundred μl sample was then collected and mixed with 100 μl internal standard 4,7,8-TriMeIQx at a concentration of 2 ng μl^{-1} for HPLC analysis. All experiments were performed in duplicate and a total of 630 ampoules were used.

2.4. HPLC Analysis of HAs

Quantitation was carried out using an internal standard method. After HPLC analysis, the HAs in each sample were quantified using a method as described by Chen and Yang (1998). A binary gradient solvent system, consisting of acetonitrile as solvent A and 0.05 M ammonium acetate solution (pH 3.6) as solvent B, was employed: a mixture of 9% A and 91% B, was used initially; then the mixing was linearly programmed to 15% A within 8 min, 27% A within 18 min, 55% A within 28 min, and 100% A within 30 min. The flow rate was 1.0 ml/min with sensitivity at 0.005 absorbance units full scale. Of the 15 HAs, IQx, IQ, MeIQx, 7,8-DiMeIQx, and 4,8-DiMeIQx, were detected by UV (258 nm) while the other HAs were detected by employing programmable fluorescence. The following settings (excitation wavelength/emission wavelength) were used: $\lambda_1 = 360$ mm/450 nm (Glu-p-2, Glu-p-1), $\lambda_2 = 300$ nm/400 nm (Norharman, Harman), $\lambda_3 = 315$ nm/390 nm (PhIP, AαC, MeAαC), $\lambda_4 = 263$ nm/410 nm (Trp-P-1, Trp-P-2). Duplicate analyses were conducted, and mean values were determined. The data were also subjected to analysis of variance and Duncan's multiple range test (SAS/SAT, 1985).

2.5. Determination of the rate constant and activation energy of HAs degradation

The degradation of each HA standard during heating was kinetically studied (Chen, Chen & Chien, 1994; Chen & Huang, 1998). The correlation coefficient (r^2) was calculated from the plot of the logarithm of the concentration of each HAs standard versus time. The degradation rate constant (h^{-1}) was obtained from the following formula: $K = -\ln(CA/CA_o)|t$ where CA = the concentration of each HA standard after heating, CA_o = the initial concentration of each HA standard, T = heating time.

The activation energy for degradation of each HA standard was calculated according to the Arrhenius equation: $\ln(k) = -(E_a/R)(1/T) + \ln(A)$ where E_a is activation energy (J/mol) and R is the ideal gas constant (8.3148 $J/kmol^{-1}$).

3. Results and discussion

Table 1 shows the concentration change of HAs during heating at 100°C for 1, 2, 3, 6, 12, 18 and 24 h. In

Table 1
Concentration change of residual HAs (ng/μl) during heating at 100°C for 1,2,3,6,12,18 and 24 h[a,b]

Compound	Heating time (h)[c]							
	0	1	2	3	6	12	18	24
Glu-P-2	3.00a	2.95b	2.92bc	2.90cd	2.87d	2.83e	2.78f	2.74g
	(0.0)	(1.8)	(2.6)	(3.5)	(4.2)	(5.7)	(7.2)	(8.8)
IQx	3.00a	2.99a	2.93b	2.90bc	2.87cd	2.84d	2.79e	2.74f
	(0.0)	(0.5)	(2.2)	(3.4)	(4.3)	(5.5)	(6.9)	(8.7)
IQ	3.00a	2.99a	2.90b	2.84bc	2.79c	2.68d	2.63d	2.50e
	(0.0)	(0.3)	(3.5)	(5.2)	(7.0)	(10.6)	(12.2)	(16.6)
Glu-P-1	3.00a	2.86b	2.78b	2.66c	2.56d	2.39e	2.26f	2.14g
	(0.0)	(4.8)	(7.4)	(11.4)	(14.8)	(20.4)	(24.8)	(28.7)
MeIQx	3.00a	2.87b	2.66c	2.50d	2.39e	2.24f	2.09g	1.97h
	(0.0)	(4.4)	(11.5)	(16.8)	(20.2)	(25.4)	(30.5)	(34.2)
MeIQ	3.00a	2.60b	2.42c	2.30d	2.21d	2.10e	2.00f	1.84g
	(0.0)	(13.5)	(19.2)	(23.5)	(26.5)	(29.9)	(33.3)	(38.8)
7,8-DiMeIQx	3.00a	2.97a	2.81b	2.69c	2.54d	2.33e	2.15f	2.00g
	(0.0)	(1.1)	(6.5)	(10.5)	(15.4)	(22.2)	(28.5)	(33.2)
4,8-DiMeIQx	3.00a	2.86b	2.82bc	2.76c	2.68d	2.60e	2.53e	2.41f
	(0.0)	(4.8)	(6.0)	(8.0)	(10.6)	(13.5)	(15.7)	(19.6)
Norharman	3.00a	2.99a	2.98ab	2.97ab	2.91bc	2.86c	2.78d	2.69e
	(0.0)	(0.4)	(0.7)	(1.1)	(2.9)	(4.7)	(7.2)	(10.4)
Harman	3.00a	2.98a	2.81b	2.72c	2.60d	2.45e	2.26f	2.12g
	(0.0)	(0.9)	(6.3)	(9.3)	(13.5)	(18.4)	(24.7)	(29.5)
Trp-P-2	3.00a	2.92ab	2.90bc	2.87bc	2.81cd	2.74de	2.68e	2.58f
	(0.0)	(2.8)	(3.5)	(4.4)	(6.3)	(8.7)	(10.6)	(13.9)
PhIP	3.00a	2.36b	2.26b	2.12c	1.96d	1.70e	1.51f	1.28g
	(0.0)	(21.5)	(24.6)	(29.2)	(34.6)	(43.2)	(49.6)	(57.5)
Trp-P-1	3.00a	2.78b	2.55c	2.43d	2.24e	2.02f	1.82g	1.58h
	(0.0)	(7.2)	(15.1)	(19.1)	(25.5)	(32.7)	(39.2)	(47.2)
AαC	3.00a	2.66b	2.56c	2.51c	2.36d	2.20e	2.02f	1.82g
	(0.0)	(11.2)	(14.8)	(16.5)	(21.5)	(26.8)	(32.7)	(39.5)
MeAαC	3.00a	2.63b	2.50c	2.46c	2.36d	2.15e	1.96f	1.77g
	(0.0)	(12.5)	(16.8)	(17.9)	(21.5)	(28.2)	(34.6)	(41.1)

[a] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).
[b] Values in parentheses represent the degradation percentage (%) of HAs.
[c] Average of duplicate analyses.

general, the HA concentrations decreased gradually wth increasing heating time. After 24 h, the degradation percentages of most HAs ranged between 10 and 40%. The highest degradation loss was observed for PhIP (57.5%), followed by Trp-P-1 (47.2%), MeAαC (41.1%), AαC (39.5%), MeIQ (38.8%) MeIQx (34.2%), 7,8-DiMeIQx (33.2%), Harman (29.5%), Glu-P-1 (28.7%), 4,8-DiMeIQx (19.6%), IQ (16.6%), Trp-P-2 (13.9%), Norharman (10.4%), Glu-P-2 (8.8%) and IQx (8.7%). A similar trend was observed for the concentration change of the 15 HAs during heating at 150°C (Table 2) or 200°C (Table 3) for 1, 2, 3, 6, 12, 18 and 24 h, i.e. the HA concentrations decreased along with increasing heating time. The degradation losses of most HAs were greater than 30% after 150°C heating for 24 h, and the greatest losses were observed for PhIP (67.2%) and MeAαC (59.3%). Likewise, at 200°C, the degradation losses were higher than 50% for most HAs after heating for 24 h, and the highest losses were found for PhIP (85.5%) and MeAαC (85.0%), while the lowest losses were found for IQx (18.8%) and Glu-P-2 (23.0%). From the preceding results it may be concluded that the higher the temperature and longer the heating time, the greater is the loss of HAs. Also, PhIP was found to have the highest loss while IQx had the lowest loss. The instability of PhIP during heating may be attributed to the following reasons: (1) PhIP contains more conjugated carbon–carbon double bonds, which make it more susceptible to chemical change than the other HAs, (2) PhIP contains a benzene ring attached to a single bond of a side chain, which makes it free to rotate.

Table 4 shows the degradation rate constants, activation energy and A value of HA during heating at 100, 150 and 200°C. The degradation rate constant of each HA was found to increase with increasing temperature, and the degradation rates of all the 15 HAs fit a first-order model because a linear correlation ($r^2 > 0.9$) was observed between heating time and ln (CA/CA_o), where CA is the concentration of each HA after heating, and CA_o is the initial concentration of each HA. At 100°C, PhIP was found to have the highest rate constant (h^{-1}),

Table 2
Concentration change of residual HAs (ng/μl) during heating at 150°C for 1,2,3,6,12,18 and 24 h[a,b]

Compound	Heating time (h)[c]							
	0	1	2	3	6	12	18	24
Glu-P-2	3.00a	2.93b	2.90b	2.86bc	2.82c	2.80c	2.71d	2.63e
	(0.0)	(2.4)	(3.2)	(4.7)	(5.9)	(6.8)	(9.8)	(12.4)
Iqx	3.00a	2.98ab	2.94b	2.87c	2.84cd	2.78de	2.74ef	2.69f
	(0.0)	(0.8)	(2.0)	(4.2)	(5.5)	(7.2)	(8.8)	(10.3)
IQ	3.00a	2.98a	2.81b	2.75b	2.66c	2.53d	2.41e	2.29f
	(0.0)	(0.6)	(6.4)	(8.3)	(11.5)	(15.7)	(19.8)	(23.6)
Glu-P-1	3.00a	2.61b	2.60b	2.48c	2.36d	2.14e	1.96f	1.79g
	(0.0)	(12.9)	(13.5)	(17.5)	(21.4)	(28.8)	(34.6)	(40.4)
MeIQx	3.00a	2.67b	2.48c	2.33d	2.20e	2.03f	1.91g	1.79h
	(0.0)	(10.9)	(17.4)	(22.2)	(26.8)	(32.2)	(36.5)	(40.4)
MeIQ	3.00a	2.49b	2.18c	2.04d	1.86e	1.69f	1.57g	1.42h
	(0.0)	(17.1)	(24.4)	(32.0)	(37.9)	(43.7)	(47.6)	(52.8)
7,8-DiMeIQx	3.00a	2.90a	2.60b	2.49bc	2.38c	2.18d	2.01e	1.82f
	(0.00	(3.5)	(13.5)	(16.9)	(20.6)	(27.5)	(32.9)	(39.2)
4,8-DiMeIQx	3.00a	2.85b	2.81bc	2.69c	2.56d	2.44d	2.30e	2.16f
	(0.0)	(5.00)	(6.5)	(10.2)	(14.7)	(18.8)	(23.2)	(28.0)
Norharman	3.00a	2.95ab	2.93b	2.89bc	2.84c	2.75d	2.63e	2.50f
	(0.0)	(1.7)	(2.5)	(3.6)	(5.2)	(8.5)	(12.2)	(16.6)
Harman	3.00a	2.86b	2.70c	2.56d	2.39e	2.20f	2.00g	1.80h
	(0.0)	(4.6)	(10.1)	(14.8)	(20.4)	(26.6)	(33.5)	(39.9)
Trp-P-2	3.00a	2.87b	2.84bc	2.79bc	2.75c	2.59d	2.41e	2.25f
	(0.0)	(4.5)	(5.1)	(6.9)	(8.2)	(13.7)	(19.6)	(25.0)
PhIP	3.00a	2.18b	2.09b	1.86c	1.70d	1.46e	1.19f	0.98g
	(0.0)	(27.3)	(30.3)	(38.0)	(43.2)	(51.4)	(60.2)	(67.2)
	3.00a	2.57b	2.47c	2.27d	2.07e	1.80f	1.61g	1.40h
	(0.0)	(14.4)	(17.8)	(24.4)	(31.1)	(39.9)	(46.2)	(53.5)
AαC	3.00a	2.57b	2.44c	2.33d	2.14e	1.88f	1.66g	1.40h
	(0.0)	(14.4)	(18.8)	(22.5)	(28.7)	(37.2)	(44.6)	(53.2)
MeAαC	3.00a	2.54b	2.36c	2.21d	1.95e	1.75f	1.46g	1.22h
	(0.0)	(15.2)	(21.2)	(26.4)	(34.9)	(41.6)	(51.5)	(59.3)

[a] Values in parentheses represent the degradation percentage (%) of HAs.
[b] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).
[c] Average of duplicate analyses.

followed by Trp-P-1, MeAαC, AαC, 7,8-DiMeIQx, MeIQx, Harman, Glu-P-1, MeIQ, 4,8-DiMeIQx, IQ, Trp-P-2, Norharman, IQx and Glu-P-2. A similar trend was observed for the degradation rate constant of each HA heated at 150 or 200°C. However, IQx was found to have the lowest rate constant at 150 or 200°C. From this result it may be concluded that PhIP is the most susceptible to degradation while IQx is the least. The stability can also be assessed by activation energy and A value of the 15 HAs, Norharman possessed the highest activation energy and theoretically it is the most stable. In contrast, MeAαC had the highest A value and thus it is the most susceptible to chemical change. However, in practice both activation energy and A value should be carefully evaluated in terms of stability of HAs. For instance, IQx and IQ had similar activation energies (12.8 and 13.6 kJ/mol, respectively), and the stability of both should be similar. However, the A value of IQx (0.19) was smaller than that of IQ (0.54), implying that the former might be less susceptible to chemical change. Thus, IQx was assessed to be more stable than IQ. A similar phenomenon was observed for Glu-P-2 and Glu-P-1; the former might be more susceptible to chemical change than the latter because of a higher A value (0.55 vs 0.22). However, Glu-P-2 might be assessed to be more stable than Glu-P-1 because of the high activation energy difference (16.1 vs 9.0 kJ/mol). Likewise, 7,8-DiMeIQx was assessed to be more stable than 4,8-DiMeIQx, Harman more stable than Norharman, MeIQx more stable than MeIQ, and AαC more stable than MeAαC. Several HAs, such as IQx, 4,8-DiMeIQx, and PhIP, had similar activation energies, however, PhIP was assessed to be more reactive than the other two HAs because of its high A values; Likewise, both PhIP and AαC had similar A values; however, the former was assessed to be more unstable than the latter because of its lower activation energy.

After 150 and 200°C heating for 24 h, the degradation rate constant (h^{-1}) of IQx was 0.0043 and 0.0083, respectively. This result showed that IQx is quite stable upon heating. In another experiment, Chen and Meng (1999) studied the formation of IQx in a model system of

Table 3
Concentration changes of residual HAs (ng/μl) during heating at 200°C for 1,2,3,6,12,18 and 24 h[a,b]

Compound	Heating time (h)[c]							
	0	1	2	3	6	12	18	24
Glu-P-2	3.00a	2.94a	2.83b	2.75c	2.69c	2.55d	2.44e	2.31f
	(0.0)	(2.0)	(5.6)	(8.5)	(10.5)	(14.9)	(18.8)	(23.09)
IQx	3.00a	2.96a	2.90b	2.83c	2.76d	2.64e	2.55f	2.44g
	(0.0)	(1.3)	(3.5)	(5.7)	(8.0)	(11.9)	(15.1)	(18.8)
IQ	3.00a	2.95a	2.75b	2.71b	2.62b	2.43c	2.21d	1.86e
	(0.0)	(1.7)	(8.3)	(9.7)	(12.6)	(19.0)	(26.4)	(38.0)
Glu-P-1	3.00a	2.59b	2.56b	2.32c	2.20d	1.93e	1.76f	1.54g
	(0.0)	(13.8)	(14.8)	(22.8)	(26.6)	(35.8)	(41.2)	(48.6)
MeIQx	3.00a	2.46b	2.22c	2.14cd	2.04d	1.86e	1.74f	1.60g
	(0.0)	(18.0)	(25.9)	(28.7)	(32.0)	(38.0)	(41.9)	(46.8)
MeIQ	3.00a	2.48b	2.11c	1.79d	1.52e	1.31f	1.11g	0.94h
	(0.0)	(17.3)	(29.8)	(40.2)	(49.5)	(56.4)	(62.9)	(68.7)
7,8-DiMeIQx	3.00a	2.83b	2.47c	2.37c	2.21d	2.04e	1.82f	1.57g
	(0.0)	(5.7)	(17.8)	(21.0)	(26.3)	(31.9)	(39.5)	(47.8)
4,8-DiMeIQx	3.00a	2.78b	2.72b	2.57c	2.39d	2.26e	2.04f	1.81g
	(0.0)	(7.3)	(9.5)	(14.3)	(20.5)	(24.8)	(31.9)	(39.6)
Norharman	3.00a	2.94ab	2.90ab	2.86b	2.72c	2.47d	2.20e	1.97f
	(0.0)	(2.1)	(3.2)	(4.8)	(9.5)	(17.7)	(26.8)	(34.2)
Harman	3.00a	2.71b	2.53c	2.42d	2.20e	2.00f	1.75g	1.55h
	(0.0)	(9.8)	(15.7)	(19.4)	(26.8)	(33.2)	(41.8)	(48.4)
Trp-P-2	3.00a	2.76b	2.71b	2.56c	2.39d	2.21e	2.00f	1.76g
	(0.0)	(7.9)	(9.8)	(14.8)	(20.5)	(26.2)	(33.5)	(41.5)
PhIP	3.00a	2.18b	1.97c	1.60d	1.31e	0.97f	0.71g	0.44h
	(0.0)	(27.4)	(34.2)	(46.6)	(56.5)	(67.6)	(76.5)	(85.5)
Trp-P-1	3.00a	2.30b	2.18c	2.05d	1.96d	1.69e	1.46f	1.16g
	(0.0)	(23.2)	(27.5)	(31.7)	(34.7)	(43.6)	(51.4)	(61.3)
Aαc	3.00a	2.39b	2.34b	1.96c	1.69d	1.49e	1.14f	0.83g
	(0.0)	(20.5)	(21.8)	(34.8)	(43.8)	(50.5)	(61.9)	(72.4)
MeAαC	3.00a	1.91b	1.73c	1.55d	1.27e	0.90f	0.70g	0.45h
	(0.0)	(36.3)	(42.3)	(48.3)	(57.7)	(69.9)	(76.7)	(85.0)

[a] Values in parentheses represent the degradation percentage (%) of HAs.
[b] Symbols bearing different letters in the same row are significantly different ($P < 0.05$).
[c] Average of duplicate analyses.

phenylalanine, glucose and creatinine, and found that the rate constant (h^{-1}) was 4.788 and 6.313, respectively, after heating at 150 and 200°C for 10 min. However, only a minor change of HAs occurred thereafter. This result further demonstrated that the loss of IQx is minimal, even after prolonged heating at 150 and 200°C for 24 h. Likewise, the rate constant (h^{-1}) for MeAαC formation was found to be 8.55 and 11.2 after heating of the same model system at 150 and 200°C for 24 h, respectively (Chen and Meng, 1999), while the degradation rate constant (h^{-1}) was only found to be 0.0327 and 0.0664. This result implied that the formation rate of MeAαC is much greater than the degradation rate.

The results shown in this study are quite similar to that reported by Arvidsson et al. (1997), who examined the formation and stability of polar HAs by heating the precursors creatinine, glucose and amino acids at 150 and 225°C for 0.5–120 min. The formation of HAs was found to begin upon heating, and within 30 s high amounts had been formed. However, at 225°C, the plateau was only reached for 4,8-DiMeIQx, while the amounts of MeIQx

Table 4
Rate constant, activation energy and A value of HAs degradation during heating at 100, 150 and 200°C

Compound	Rate constant (h^{-1})[a]			Ea(kJmol)	A
	100°C	150°C	200°C		
Glu-P-2	0.0033	0.0047	0.0101	16.1	0.55
IQx	0.0034	0.0043	0.0083	12.8	0.19
IQ	0.0070	0.0106	0.0178	13.6	0.54
Glu-P-1	0.0130	0.0184	0.0240	9.0	0.22
MeIQx	0.0143	0.0156	0.0163	1.9	0.03
MeIQ	0.0126	0.0210	0.0375	15.9	2.05
7,8-DiMeIQx	0.0166	0.0187	0.0237	5.1	0.08
4,8-DiMeIQx	0.0077	0.0124	0.0187	13.0	0.51
Norharman	0.0044	0.0070	0.0173	19.7	2.34
Harman	0.0141	0.0196	0.0247	8.2	0.20
Trp-P-2	0.0054	0.0109	0.0200	19.2	2.59
PhIP	0.0291	0.0380	0.0703	12.7	1.62
Trp-P-1	0.0238	0.0279	0.0314	4.1	0.09
Aαc	0.0175	0.0272	0.0465	14.2	1.66
MeAαC	0.0182	0.0327	0.0664	18.8	7.57

[a] Average of duplicate analyses.

and 7,8-DiMeIQx decreased after peaking and no PhIP was detected. These findings indicate that both the formation and degradation of HAs could proceed simultaneously during heating. The stability study shows that PhIP was the most susceptible to degradation at 225°C, followed by 7,8-DiMeIQx, MeIQx, 4,8-DiMeIQx and IQx (Arvidsson et al., 1997). In this study IQx was found to possess the highest stability during heating, followed by Glu-P-2, Norharman, IQ, 4,8-DiMeIQx, Trp-P-2, MeIQx, 7,8-DiMeIQx, Harman, Glu-P-1, Trp-P-1, MeIQ, AαC, MeAαC and PhIP.

In conclusion, the degradation losses of all the 15 HA standards increased both with increasing temperature and heating time, and the degradation rate of each HA fits a first-order model. PhIP is the most unstable during heating, while IQx is the least.

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