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Formation of heterocyclic aromatic amines in model systems of sucrose, lactose, amino acids and creatine

Dmitry Utyanov[1*], *Ekaterina* Polishchuk[1], *Anastasiya* Kibitkina[1], and *Maksim* Reshchikov[1]

[1]V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, 26, Talalikhina st., Moscow, Russia

Abstract. The article presents experimental data on the study of heterocyclic aromatic amines formation in model matrices consisting of amino acids (8 essential, glutamic acid and aspartic acid), carbohydrates (sucrose, lactose) and creatine. The results of the studies showed that carbohydrates in the reaction of MeIQx and PhIP formation can exhibit both inhibitory and catalytic properties in the reaction of their formation. Of all the studied matrices, in the samples with phenylalanine noted the highest amount of PhIP, its content reached 27837.79 ng/g, based on which it is fair to assume that of all the amino acids, phenylalanine is the main precursor in the reaction of PhIP formation. High concentrations of PhIP were also noted in the samples with tryptophan up to 4891.36 ng/g.

1 Introduction

Thermal processing of products is necessary to improve the nutritional qualities of the product and increase digestibility. Thermal processing increases the bioavailability of the product's nutrients, increases its shelf life, allows for better flavor and aroma properties, and also helps to destroy most of the microorganisms that may be present in the product. At the same time, thermal processing, especially high-temperature processing, leads to the formation of various types of chemical compounds in the product, which are the result of chemical reactions of the components included in the product. Such compounds include heterocyclic aromatic amines. Currently, HAA are classified as 2a and 2b compounds, i.e. probable and possible carcinogens [1-2].

Research on the issue of HAA formation in foods began with the works of T. Sugimura in the 1970s. Since then, a lot of work has been devoted to the issue of HAA content in food products. The overwhelming majority of these works are devoted to the issues of reducing HAA content in meat products by adding non-meat ingredients or changing heat treatment modes, such as duration and temperature of treatment. But there are also a large number of scientific works devoted to the chemistry of the formation of HAA [3].

For example, in the work [4] the reaction of PhIP formation, its precursors and intermediate products of its formation reaction were studied. In this work, it was

* Corresponding author: d.utyanov@fncps.ru

established that phenylalanine is the main precursor in the reaction of PhIP formation. As a result, such products of phenylalanine thermal degradation as phenylethylene, phenylacetaldehyde, 2-phenylethylamine, 2-phenylethanol and phenylacetic acid were identified as intermediate compounds in the reaction of PhIP formation from phenylalanine. Also, in this work it was established that PhIP is formed only from the products of phenylalanine thermal degradation, since the experiment with model matrices in which creatinine and phenylalanine were left at room temperature for 2 weeks showed that PhIP is not formed in this case. The reactions of HAA formation are described in detail in the work [5], where a review of works from the late 1990s - early 2000s devoted to this issue is given. In general, the reactions of HAA formation occur as a result of the pyrolysis of amino acids and proteins. It is the products of amino acid pyrolysis that form mutagenic compounds, while the pyrolysis products of nucleic acids, starch or oils do not form compounds with mutagenic properties. In addition, it is important to note that formation of HAA is possible as a result of simple pyrolysis of amino acids only, i.e. not even as a result of the reaction of such products with other components included in the food product. In general, this kind of HAA are designated as non-polar. But the majority of polar HAA are the result of the reaction of amino acids, creatine or creatinine and carbohydrates during the Maillard reaction. The Maillard reaction does not mean a single way of forming chemical compounds, but a complex of various chemical reactions. Over time, the study of the Maillard reaction showed that pyrazines, quinoxalines and pyridoimidazoles are formed during it, which then participate in the reactions of HAA formation [6].

An influence of the content of different amino acids was shown in study [7] in which grilled beef steaks and cutlets were examined. The results showed that this method of heat treatment resulted in the formation of both aminoimidazoarenes-related HAAs and pyrolytic HAAs. Among the aminoimidazoarenes, 4,8-DiMeIQx was detected in the highest quantity in the range from 4.50 to 7.55 ng/g, followed by MeIQx in the range from 1.10 to 7.20 ng/g, MeIQ – from 1.70 to 5.30 ng/g and IQ – from 0.38 to 2.00 ng/g. At the same time, 7,8-DiMeIQx, IQx and 4,7,8-TriMeIQx were not detected. In this case, beef patties contained more HAA than steaks. The authors of the work suggest that the difference in HAA content can be explained due to the fact that patties contain more amino acids and more glucose than steaks. However, it should be noted that this assumption may be incorrect due to the fact that the heat transfer process in chopped products is better than in lumpy products, which is why in patties higher content of HAA might be formed. The authors of the article also provided a correlation matrix for the dependence of the content of a specific HAA on amino acids, creatinine, and carbohydrates. According to the matrix, the content of almost all HAA they studied correlates with the content of creatinine, glutamic acid, and glucose in the product.

In another work devoted to the study of HAA formation in model systems [8], the issue of the influence of different concentrations of amino acids on the amount of HAA formed in a mixture of glucose, phenylalanine and creatine (theoretically the main precursors in the reaction of PhIP formation) was studied. Solutions of different concentrations of tryptophan, lysine, proline, leucine, methionine, valine, isoleucine, threonine, phenylalanine and asparagine were selected for the experiment. According to the results obtained, with an increase in the concentration of tryptophan introduced into the model system, the amount of PhIP formed decreased to values below the detection limit of the method. A similar picture was observed in samples with lysine. Addition of all other amino acid solutions except asparagine to the model matrices also showed a directly proportional relationship: the higher the concentration of the added amino acid, the smaller the amount of PhIP formed in the model matrix, but in these samples, it was not possible to achieve PhIP concentrations below the detection limit, as was the case in the samples with tryptophan and lysine. In the samples with asparagine, different concentrations of this amino acid led to both a decrease and an increase in the amount of PhIP formed.

The aim of this work was to investigate the formation of PhIP and MeIQx in model systems of amino acids, sucrose, lactose and creatine.

2 Materials and research methods

For the experiment, 10 amino acids were selected (Reachim, Russia): essential amino acids – valine, phenylalanine, methionine, threonine, lysine, leucine, isoleucine, tryptophan – and asparagine and glutamic acid were selected because they are usually present in meat raw materials in quantities greater than other amino acids (especially glutamic acid). For the experiment, mixtures of each amino acid with sucrose and lactose (PanReac AppliChem, Germany) with the addition of creatine monohydrate (Merck, USA) and water (obtained on the MilliQDirect 8 system, France) and one control group without carbohydrates were prepared. The masses of the ingredients and the composition of the prepared matrices are given in Tables 1-3. The corresponding numbers for the amino acids are:

- Valine.
- Phenylalanine.
- Methionine.
- Threonine.
- Lysine.
- Leucine.
- Tryptophan.
- Glutamic acid.
- Asparagine.
- Isoleucine.

The experimental matrices were prepared in porcelain crucibles, which were first brought to constant mass in a drying cabinet at a temperature of 105ºC for 30 minutes, after which they were weighed. The dried crucibles were stored in a desiccator. The crucibles with samples were placed in a drying cabinet, where they were kept for 20 minutes at a temperature of 200ºC for heat treatment. After heat treatment, the porcelain crucibles were placed in a desiccator, where they cooled to room temperature. After cooling, the crucibles with samples were weighed for further calculations.

Tables 1-3 show the composition of the obtained matrices and their mass before and after heat treatment (HT).

Table 1. Composition of experimental control samples.

Sample code	m AA, mg	m creatine, mg	m carbohydrate, mg	m H_2O, mg	m samples before HT, mg	m samples after HT, mg
C1	96.4	5.0	-	558.6	660.0	100.0
C2	96.0	5.5	-	428.5	530.0	100.0
C3	99.0	5.0	-	466.0	570.0	100.0
C4	98.8	4.5	-	276.7	380.0	90.0
C5	103.4	4.5	-	322.4	430.3	110.0
C6	98.8	4.5	-	1387.0	1490.3	104.0
C7	101.2	5.3	-	1399.5	1506.0	100.0
C8	100.0	5.5	-	201.3	306.8	90.0
C9	96.5	4.6	-	124.9	226.0	100.0
C10	103.1	5.4	-	984.5	1093.0	110.0

Table 2. Composition of experimental samples with sucrose.

Sample code	m AA, mg	m creatine, mg	m carbohydrate, mg	m H_2O, mg	m samples before HT, mg	m samples after HT, mg
S1	105.1	4.5	4.5	548.5	662.6	100.0
S2	102.0	4.8	5.1	420.1	532.0	80.0
S3	104.5	4.5	5.5	1002.8	1117.3	100.0
S4	105.3	4.5	5.0	746.5	861.3	100.0
S5	98.0	4.7	5.0	519.3	627.0	110.0
S6	95.2	4.5	4.9	9076	1012.2	94.0
S7	99.1	5.2	5.1	1930.6	2040.0	80.0
S8	95.3	4.5	4.7	539.9	644.3	100.0
S9	97.5	4.5	5.0	419.7	526.7	100.0
S10	99.9	4.9	5.1	520.8	630.7	102.2

Table 3. Composition of experimental samples with lactose.

Sample code	m AA, mg	m creatine, mg	m carbohydrate, mg	m H_2O, mg	m samples before HT, mg	m samples after HT, mg
L1	103.9	4.6	5.0	458.5	572.0	98.0
L2	98.3	5.2	5.3	445.5	554.3	84.0
L3	100.0	4.8	5.0	438.4	548.2	97.0
L4	102.0	5.4	4.6	761.5	873.5	76.0
L5	103.9	4.6	4.6	330.2	443.3	113.0
L6	96.2	5.4	5.0	770.6	877.2	103.3
L7	97.5	5.5	5.2	1162.8	1.271	100.0
L8	101.0	5.5	5.5	320.5	432.5	88.0
L9	97.1	5.3	5.0	328.7	436.1	99.0
L10	99.3	5.2	5.0	498.6	608.1	101.4

The content of HAA in the obtained samples was determined by [9].

The STATISTICA 10 was used for calculations, the results presented as "Mean±SD", statistical significance was calculated using variance analysis (ANOVA) with Tukey's criterion. The probability of 0.05 was chosen as a significant level.

3 Research results

The results of the HAA content in the obtained samples are given in Tables 4-5.

Table 4. Content of MeIQx in the studied samples, ng/g.

AA/carbohydrate	Control	Sucrose	Lactose
Valine	Less than 0.1	$16.95^{a*}\pm0.41$	$8.48^{d*}\pm0.07$
Phenylalanine	Less than 0.1	$2.05^{a}\pm0.05$	$1.76^{b}\sim\pm0.07$
Methionine	Less than 0.1	$4.00^{a}\pm0.11$	Less than 0.1
Threonine	$94.14^{a*}\pm0.93$	$138.39^{b\sim}\pm2.51$	$45.28^{e\wedge}\pm0.64$
Lysine	Less than 0.1	$13.56^{a\wedge}\pm0.35$	Less than 0.1
Leucine	Less than 0.1	$2.60^{a}\pm0.32$	Less than 0.1
Tryptophan	Less than 0.1	Less than 0.1	Less than 0.1
Glutamic acid	Less than 0.1	$1.56^{a}\pm0.02$	Less than 0.1
Asparagine	$6.78^{a}\pm0.35$	$14.15^{b\wedge}\pm0.94$	Less than 0.1
Isoleucine	Less than 0.1	Less than 0.1	Less than 0.1

The results are presented as "the average value of three parallel measurements ± standard deviation". The standard deviation shows the accuracy of the method.
The letters "a, b, d, e" show the significant difference between the results obtained in the rows.
The signs "*, ~, ^" show the significant difference between the results obtained in the columns.

Table 5. Content of PhIP in the studied samples, ng/g.

AA/carbohydrate	Control	Sucrose	Lactose
Valine	9.77^{a}±0.22	50.21^{b}	97.50^{e*}±0.52
Phenylalanine	20136.12^{a*} ± 613.35	13412.22^{b*} ± 521.69	8505.41$^{e\sim}$ ± 13.37
Methionine	20.72^{a}±0.74	27.72^{b}±0.26	24.78^{d}±0.31
Threonine	193.62^{a}±0.88	280.61^{b}±11.19	414.37$^{e\wedge}$±8.73
Lysine	19.62^{a}±0.09	Less than 0.1	119.22$^{d@}$±0.94
Leucine	0.68^{a}±0.04	8.46^{b}±0.48	12.06^{d}±0.25
Tryptophan	2593.66^{a}~ ± 85.82	4891.36$^{b\sim}$ ± 32.79	2064.00$^{d\&}$ ± 8.37
Glutamic acid	10.14^{a}±0.04	5.44^{b}±0.29	1.93^{e}±0.03
Asparagine	1.58^{a}±0.05	10.23^{b}±0.48	Less than 0.1
Isoleucine	10.42^{a}±0.55	13.48±0.39	8.26±0.31

The results are presented as "the average value of three parallel measurements ± standard deviation". The standard deviation shows the accuracy of the method.
The letters "a, b, d, e" show the significant difference between the results obtained in the rows.
The signs "*, ~, ^, @, &" show the significant difference between the results obtained in the columns.

4 Discussion

Table 4 shows that, in the control sample MeIQx was formed only in samples with threonine and asparagine. The largest amount of MeIQx was found in the sample with a mixture of threonine and sucrose. Also, the largest amount of MeIQx was found in samples with threonine relative to other samples. Considering the samples with threonine, it is noteworthy that only in the mixture with sucrose was the amount of MeIQx higher than in the control sample. In samples with lactose, its amount was approximately 3 times less. A similar picture is observed in samples with valine, lysine, methionine, leucine and glutamic acid, i.e. 6 out of 10 selected amino acids form the largest amount of MeIQx with sucrose rather than with lactose. For samples with threonine and asparagine, where MeIQx was formed in the control samples, it should be noted that sucrose acts as a catalyst and lactose as an inhibitor in the reaction of MeIQx formation.

Lactose showed low reactivity in the reaction of HAA formation – MeIQx was formed in 3 samples out of 10 mixtures and in the sample with asparagine it is the carbohydrate that did not lead to the formation of MeIQx at all, considering that it was found in the control sample. And in the sample with threonine lactose reduced the amount of MeIQx by ≈52% relative to the control sample. But at the same time, the addition of lactose led to the formation of MeIQx in the samples with valine and phenylalanine. Of the selected amino acids, tryptophan turned out to be the least reactive. MeIQx was not formed in any sample.

It is possible to compare the results obtained only with the work [10], in which the issue of IQ, iQx and MeIQx education in model systems was studied. According to the results, a directly proportional dependence of the amount of HAA formed in the model mixture of creatine, threonine and glucose on their concentration was established. In our work, it was shown that sucrose, which consists of glucose and fructose, increases the amount of formed MeIQx.

Unlike MeIQx, PhIP was formed in almost every sample studied. PhIP was not detected only in mixtures of lysine with sucrose and asparagine with lactose. The highest levels of PhIP were in the phenylalanine samples. Colossal amounts indicate that this amino acid is the main precursor in the PhIP formation reaction, since its amounts are hundreds or even thousands of times higher than in other samples (except for samples with tryptophan). Sucrose and lactose have inhibitory properties in the reaction of its formation.

Large amounts of PhIP were also found in tryptophan samples. Its amount in each of the samples exceeds 2000 ng/g. In them, similarly to the formation of MeIQx in samples with threonine and asparagine, the catalyzing properties of sucrose and the inhibitory properties of lactose in the PhIP formation reaction are observed.

Comparing the results obtained with the work [8], in which amino acids were added to a mixture of creatine, phenylalanine and glucose and found that their introduction reduces the amount of PhIP formed, it is suggested why the introduction of other amino acids in the work [8] led to a decrease in the amount of PhIP formed. As the results of this work have shown, PhIP is formed in a mixture with each selected amino acid in the control samples, and in almost all mixtures of these amino acids with carbohydrates. Thus, it is fair to assume that other amino acids, reacting with creatine, also form other compounds, possibly other HAA, including PhIP, so there is less creatine left to react with phenylalanine, with which the formation of the largest amount of PhIP is possible.

The addition of sucrose to amino acids resulted in an increase in PhIP in 8 out of 10 samples. The increase was ≈513%, ≈133%, ≈144%, ≈1244%, ≈188% and ≈647% relative to the control sample for valine, methionine, threonine, leucine, tryptophan and asparagine, respectively. The addition of sucrose to phenylalanine, lysine and glutamic acid led to a decrease in the amount of PhIP ≈34%, 100%, ≈46%, accordingly. Of all the amino acids, the addition of lactose only to aspartic acid did not lead to the formation of PhIP. With other amino acids, lactose has shown both inhibitory and catalyzing properties. The addition of lactose increased the amount of PhIP in samples with valine, methionine, threonine, lysine and leucine by ≈898%, ≈20%, ≈213%, ≈508%, ≈1674% accordingly. In samples with tryptophan and glutamic acid, the amount of PhIP decreased by 20% and 80%, respectively

5 Conclusions

The conducted studies of model matrices have shown the important role of carbohydrates in the formation of PAA – carbohydrates in the reactions of formation of MeIQx and PhIP can be both inhibitors and catalysts. Lactose showed strong inhibitory properties. Based on this, it can be concluded that the use of sources rich in lactose in the preparation of meat products will lead to a decrease in the amount of HAA formed. The results obtained can be another step towards understanding the reactions of HAA formation, which in turn will allow us to look for more effective ways to reduce the amount of carcinogens formed in food products.

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Abbreviations used in article:

HAA – heterocyclic aromatic amines.
IQ – 2-amino-3-methyl-imidazo[4,5-f]-quinoline.
IQx – 2-amino-3-methyl-imidazo[4,5-f]-quinoxaline.
MeIQ – 2-amino -3,4-dimethyl-imidazo[4,5-b]-quinoline.
MeIQx – 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline.
PhIP – 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine.
DiMeIQx – 2-amino-3,4,8-trimethylimidazo [4,5-f]-quinoxaline.
7,8-DiMeIQx – 2-amino-3,7,8-trimethylimidazo[4,5-f]-quinoxaline.
4,7,8-TriMeIQx – 2-amino-3,4,7,8-tetramethyl-3H-imidazo[4,5- f]-quinoxaline.

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