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Ways to Reduce the Amount of Heterocyclic Aromatic Amines Formed in Meat Products. The Review

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Keywords: *heterocyclic aromatic amines, meat products, inhibitors, precursors, catalysts, heat treatment, extracts, antioxidant activity.*

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Ways to Reduce the Amount of Heterocyclic Aromatic Amines Formed in Meat Products the Review

Dmitry A. Utyanov ^α, Andrey V. Kulikovskii ^σ, Alexandra S. Knyazeva ^ρ & Anastasia A. Kurzova ^ω

Abstract- This review presents the results of studies on the issue of reducing the amount of heterocyclic aromatic amines formed in meat products. The analyzed works have shown that it is possible to influence the amount of heterocyclic aromatic amines through their precursors, heat treatment of meat products, and the introduction of non-meat ingredients into the recipe. An analysis of works devoted to the effect of temperature and duration of heat treatment on the amount of heterocyclic aromatic amines formed is presented. As a result of the analysis, it was found that free amino acids, moisture, and the water-holding capacity of the raw material play an essential role in the formation of heterocyclic aromatic amines. Many analyzed works proved the inhibitory effect of vitamin E in the formation of heterocyclic aromatic amines. Inhibitory effects of pomegranate seed extract, artichoke extract are described. The impact of replacing animal fat with vegetable oils on the amount of heterocyclic aromatic amines formed is described.

Keywords: heterocyclic aromatic amines, meat products, inhibitors, precursors, catalysts, heat treatment, extracts, antioxidant activity.

Abbreviations

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

DiMeIQx - 2-amino-3,4,8-trimethyl-imidazo[4,5-f]-quinoxaline

PhIP - 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine

n/d - not detected

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I. INTRODUCTION

During the heat treatment of meat products, due to its complex chemical composition, a large number of new chemical compounds are formed. Often, these compounds have carcinogenic and mutagenic activity. These carcinogens include HAA, which are formed in meat products during its high-temperature heat treatment as products of the Maillard reaction. The carcinogenic and mutagenic potential of HAA has been proven by many works on laboratory animals and protozoa [1-3]. Consumption of products with HAA is associated with the occurrence of cancer in humans, precisely with cancer of the gastrointestinal tract [4-6].

Considering the potential danger of HAA, it is necessary to find ways to reduce their formation in meat products during heat treatment. This paper is the review of articles that present studies on reducing the amount of HAA. Based on the analysis, it was found that a decrease in the amount of HAA formed in the product is possible in the following ways:

- impact on HAA precursors;
- changing cooking modes;
- changing recipes.

II. MAIN PART

a) Precursors

Since the amino-imidazoarenes group is formed during the Maillard reaction, the most obvious way to influence their quantity is to impact on amino acids, creatine/creatinine and carbohydrates in the raw material.

It has been established that sugars are one of the main precursors in the formation of HAA. Sugars have been established to be inhibitors in the reaction of HAA formation [7]. In [8], the impact of mono- and disaccharides on the amount of HAA formed was studied. Catalytic effect of mono- and disaccharides have been found in cases when the amount of sugars involved in the reaction are less than creatine/creatinine, but in cases when there are more sugars than creatine/creatinine, they have a strong inhibitory effect. This is due to the fact that during the Maillard reaction from sugars, with their excess, 5-hydroxymethyl-2-

furfural is formed, which simultaneously reduces the amount of HAA formed, because of its reaction with creatine/creatinine. Thus, in [9], honey, a rich source of glucose and fructose, have been found as the most effective among all sources of low molecular weight carbohydrates in terms of reducing the amount of HAA.

Amino acids, especially unbound amino acids, play a significant role in the formation of HAA since they more easily enter into the reaction of formation of HAA than the bounded in protein amino acids. In [10], a summary of the effect of unbound amino acids on the formation of aminoimidazoarenes is given.

The moisture content and water holding capacity of the raw material also affect the amount of HAA formed since moisture acts as a carrier of precursors in the HAA formation reaction, delivering them from the middle of the product to the surface [7].

The fat content of the product also plays a role in the formation of HAA. In [11], it was found that a lower fat content in raw materials leads to a larger amount of HAAs formed. This may be due to the fact that fat, being a good heat carrier, leads to faster cooking of the product, which reduces the amount of HAA in the finished product.

b) Heat treatment mode

Perhaps the greatest number of works on the issue of HAA formation is devoted to the influence of the heat treatment method. The main provisions established in these works are that the processing temperature should be above 150 °C, and the longer the heat treatment process lasts, the greater the amount of HAA is formed. Accordingly, during heat treatment such as frying, grilling/barbecuing, and baking, the tremendous amount of HAA is formed. In [12], it was found that an increase in the duration of heat treatment of a meat lump semi-finished product by 2.5 minutes on each side leads to an increase in the amount of HAA by 1.5 times. It was found in [13] that an increase in the duration of

heat treatment of chopped semi-finished products by 4 minutes on each side at temperatures from 175°C to 225°C increases the amount of HAA by 2-5 times. The most obvious way to reduce the amount of HAA, in this case, is to reduce the temperature and duration of heat treatment of the product. Still, in this case, the organoleptic properties of the product deteriorate.

The prethermal processing by microwave radiation has been noted as the way to reduce the amount of HAA formed during further heat treatment. Studies [14] found that the processing of raw materials in a microwave oven before heat treatment for two minutes leads to a decrease in the amount of PhIP up to 86%.

Another method that can reduce the amount of HAA formed is breading the product. However, the breading must be thick enough to do this. Currently, there are not so many works in this area, which indicates the need for research in this direction, given that breading is a relatively common method of technological processing of raw materials.

c) Recipe changes

Recipes changes are perhaps the simplest and most effective way to influence the amount of HAA produced. Prethermal processing of meat raw materials with ingredients with an established inhibitory effect in the formation of HAA can lead to a significant decrease in their amount. The greatest reduction in the amount of HAA can be achieved by adding antioxidants to the product.

Thus, in [13], the effect of adding vitamin E and rosemary extract to chopped semi-finished beef products was studied. Putative inhibitors in the reaction of HAA formation were added to beef cutlets with a fat content of ≈15% in the form of 1% and 10% solutions in 1 cm³ of corn oil. Cutlets with added corn oil were used as a control sample. The results are presented in Table 1.

Table 1: Effect of vitamin E and rosemary extract on the amount of HAA formed in beef cutlets [13]

Analyte	Concentration of vitamin E and rosemary extract added into beef cutlets				
	Control sample	1% vitamin E	10% vitamin E	1% rosemary extract	10% rosemary extract
IQ					
Concentration, ng/g	5,3±3,5	0,7±0,2	0,6±0,2	1,5±0,6	1,5±1,3
Decreasing, %		85,7	88,1	72,4	71,9
MeIQ					
Concentration, ng/g	3,5±1,2	0,8±0,4	1,3±0,6	0,7±0,3	1,8±0,9
Decreasing, %		78,6	64,3	87,0	47,9
MeIQx					
Concentration, ng/g	5,7±1,7	2,9±1,9	4,1±0,8	3,8±0,7	5,1±0,3
Decreasing, %		48,0	26,0	30,1	12,1
DiMeIQx					
Concentration, ng/g	4,7±4,4	1,0±0,3	1,4±0,2	1,1±0,6	1,4±0,1
Decreasing, %		79,2	70,5	77,0	68,3
PhIP					
Concentration, ng/g	31,3±13,5	9,6±5,3	8,6±3,9	17,4±2,9	17,3±12,1
Decreasing, %		69,0	72,5	44,0	44,6

Studies have shown that both vitamin E and rosemary extract have a strong inhibitory effect on the formation of HAA in meat products during heat treatment. Moreover, in the reaction of MeIQx and PhIP formation, most significant impact was achieved by adding vitamin E to the product.

The effect of adding rosemary extract to chopped semi-finished beef products was also studied in another work [15]. In addition to rosemary extract, they have studied the inhibitory effect of grape seed

extract. Grape seed extract was added to the product by adding 1.5 g of a water-in-oil emulsion at concentrations of 0.2%, 0.4%, 0.6%, and 0.8%. Rosemary extract was added to the product by adding 1.5 g of sunflower oil with concentrations of 0.12%, 0.4%, 0.6%, 1.0%, and 1.5%. Meat products with the addition of 1.5 g of sunflower oil and 1.5 g of a water-in-oil emulsion were taken as control samples. The results obtained are shown in tables 2 and 3.

Table 2: The effect of adding grape seed extract on the amount of HAA formed in beef cutlets [15].

Analyte	Control sample	Grape seed extract added in water-in-oil emulsion at concentrations, %			
		0,2	0,4	0,6	0,8
MeIQ, ng/g	1,0±0,27	0,5±0,1	0,5±0,1	0,4±0,04	0,3±0,01
PhIP, ng/g	0,3±0,07	0,2±0,04	0,2±0,04	0,2±0,08	<0,02
Norharman, ng/g	0,5±0,02	0,5±0,01	0,5±0,03	0,7±0,03	0,6±0,01
Harman, ng/g	1,1±0,02	1,1±0,02	1,1±0,02	1,6±0,02	1,7±0,02

Table 3: The effect of adding rosemary extract on the amount of GAA formed [15].

Analyte	Control sample	Rosemary extract added in sunflower oil in concentrations, %				
		0,12	0,4	0,6	1,0	1,5
MeIQ, ng/g	0,7±0,05	0,6±0,07	0,6±0,07	0,6±0,04	0,5±0,05	0,3±0,03
PhIP, ng/g	0,2±0,02	0,2±0,02	0,1±0,04	0,1±0,03	0,06±0,01	0,02±0,01
Norharman, ng/g	0,2±0,02	0,3±0,03	0,3±0,01	0,3±0,01	0,2±0,01	0,3±0,02
Harman, ng/g	0,6±0,01	0,9±0,09	0,9±0,09	0,5±0,05	0,6±0,02	0,6±0,05

The results of the studies have shown that for such HAAs as MeIQx and PhIP, the most significant reduction was achieved when the water-in-oil emulsion with a grape seed extract concentration of 0.8% was added to the product. However, this method has led to an increase in the amount of HAA such as Harman and Norharman. The addition of rosemary extract to the product, in the same way as in [13], described earlier, led to a decrease in the amount of HAA formed, but in this case, the inhibitory effect was weaker. This may be due either to differences in the oils used to add rosemary extract to the meat product or to the heat treatment method since in [15], the concentrations of HAA were significantly lower than in the studies conducted in [13].

In [16], the inhibitory effect of pomegranate seed extract was studied in the preparation of minced poultry and beef products. Here, in addition to the meat component and a potential inhibitor were also added to

the minced meat fat to achieve 25% fatness, breadcrumbs in an amount of 20% by weight of the product, onion, salt, and spices. Then the pomegranate seed extract was added at a concentration of 0.5%. Next, the minced meat was formed into cutlets 1.5 cm thick and 5.0 cm in diameter, weighing approximately 30 g. The products were subjected to heat treatment in four ways:

1. Baking in the oven for 27 minutes at 180°C
 2. Frying in a pan at 180°C for 8 minutes on each side without using oil
 3. Charcoal barbecue. For this, a kilogram of coals was used. After the combustion of charcoal the product was cooked for 10 minutes on each side. The distance between the coals and the product was 8 cm. The surface temperature of the products, in this case, was about 280°C
 4. Deep frying at 150°C oil temperature for 5 minutes
- The results are shown in Table 4.

Table 4: Results of the study of the inhibitory effect of pomegranate seed extract [16].

	Heat treatment method		Analyte				
			PhiP, ng/g	Norharman, ng/g	Harman, ng/g	IQ, ng/g	MeIQx, ng/g
Beef	1	Control sample	0,57±0,06	2,65±0,14	1,29±0,12	139,21±15,39	29,55±5,05
		Experiment sample	0,48±0,12	3,43±0,14	2,10±0,39	126,71±17,55	30,21±9,07
	2	Control sample	1,11±0,18	3,14±0,55	1,38±0,03	44,65±0,77	n/d

Poultry	3	Experiment sample	0,42±0,03	2,45±0,14	1,14±0,12	60,69±23,24	13,05±0,88
		Control sample	1,23±0,15	6,87±0,28	1,32±0,18	303,06±19,94	35,21±8,30
	4	Experiment sample	0,39±0,03	5,20±0,42	1,08±0,03	188,71±21,59	15,18±6,59
		Control sample	0,69±0,03	1,88±0,11	n/d	122,80±4,25	29,72±1,41
		Experiment sample	0,51±0,09	2,26±0,13	n/d	67,91±7,44	12,90±3,37
		Control sample	1,92±0,03	5,49±0,55	1,20±0,0	58,79±9,70	23,04±0,57
	1	Experiment sample	0,48±0,06	3,04±0,14	2,46±0,12	83,74±144	66,54±10,33
		Control sample	0,75±0,03	4,73±0,03	3,21±0,18	5,53±1,12	6,06±2,16
	2	Experiment sample	0,24±0,03	3,24±0,13	2,31±0,12	12,73±2,95	7,17±0,84
		Control sample	0,87±0,21	11,47±0,14	3,42±0,12	55,54±16,17	n/d
	3	Experiment sample	0,24±0,09	4,90±0,26	2,73±0,18	34,55±1,06	26,20±4,71
		Control sample	0,30±0,03	2,26±0,13	n/d	7,97±4,04	111,62±12,18
	4	Experiment sample	0,48±0,06	1,08±0,14	n/d	4,31±2,31	56,49±6,63
		Control sample					
		Experiment sample					
		Control sample					

As can be seen from Table 4, there was an increase in the amount of HAA formed in many experimental samples relative to control samples. This is most likely due to a much larger number of factors that can affect the amount of HAA formation since the product contained many more components besides meat and a potential inhibitor.

In [17], the inhibitory effect of hawthorn extract was studied. In this work, steaks 1 cm thick were

prepared from poultry and beef, which were treated by rubbing in solutions of hawthorn extract in distilled water with concentrations of 0.5% and 1.0%. Products were subjected to heat treatment by frying in a pan and baking in an oven at temperatures of 150 °C, 200 °C and 250 °C. The results are shown in tables 5 and 6.

Table 5: Effect of hawthorn extract on the formation of HAA in chicken [17].

Heat treatment method	Temperature of heat treatment, °C	Concentration of hawthorn extract, %	Analyte			
			IQ, ng/g	IQxng/g	MeIQng/g	MeIQxng/g
Pan frying	150	0	n/d	0,14	0,82	0,05
		0,5	0,18	0,12	0,15	0,43
		1,0	n/d	0,18	n/d	0,60
	200	0	0,17	0,17	n/d	0,45
		0,5	0,17	0,17	n/d	0,49
		1,0	n/d	n/d	n/d	0,38
	250	0	1,52	0,14	n/d	0,79
		0,5	n/d	n/d	0,46	0,41
		1,0	n/d	n/d	0,43	0,52
Baking	150	0	n/d	0,38	n/d	n/d
		0,5	n/d	n/d	n/d	n/d
		1,0	n/d	0,03	n/d	n/d
	200	0	n/d	0,15	n/d	0,14
		0,5	n/d	0,20	n/d	n/d
		1,0	n/d	0,14	n/d	n/d
	250	0	0,38	0,69	0,19	0,60
		0,5	n/d	0,64	0,29	0,60
		1,0	4,47	n/d	n/d	0,58

Table 6: Effect of hawthorn extract on the formation of HAA in beef [17].

Heat treatment method	Temperature of heat treatment, °C	Concentration of hawthorn extract, %	Аналит			
			IQ, ng/g	IQx, ng/g	MelQ, ng/g	MelQx, ng/g
Pan frying	150	0	n/d	0,14	0,82	0,05
		0,5	0,18	0,12	0,15	0,43
		1,0	n/d	0,18	n/d	0,60
	200	0	n/d	0,17	n/d	0,45
		0,5	n/d	0,17	n/d	0,49
		1,0	n/d	n/d	n/d	0,38
	250	0	1,52	0,14	n/d	0,79
		0,5	n/d	n/d	0,46	0,41
		1,0	n/d	n/d	0,43	0,52
Baking	150	0	n/d	0,38	n/d	n/d
		0,5	n/d	n/d	n/d	n/d
		1,0	n/d	0,03	n/d	n/d
	200	0	n/d	0,15	n/d	0,14
		0,5	n/d	0,20	n/d	n/d
		1,0	n/d	0,14	n/d	n/d
	250	0	0,38	0,69	0,19	0,60
		0,5	n/d	0,64	0,29	0,60
		1,0	4,47	n/d	n/d	0,58

The results obtained are highly contradictory since were observed an increase and a decrease in the amount of HAA in the experimental samples relative to the control ones. Such results can be explained either by the method of adding the extract, which does not allow uniform distribution of the potential inhibitor in the product, in contrast to the cases when it is added to minced meat or by the absence of pronounced inhibitory properties in the hawthorn extract.

Similarly, the inhibitory properties of the artichoke extract were studied in [18]. As in [17], steaks 1 cm thick were prepared from poultry and beef meat, which were treated by rubbing artichoke extract solutions in distilled water with concentrations of 0.5% and 1.0%. Products were subjected to heat treatment by frying in a pan and baking in an oven at temperatures of 150°C, 200°C and 250°C. The results are shown in tables 7 and 8.

Table 7: Effect of artichoke extract on the formation of HAA in chicken [18].

Heat treatment method	Temperature of heat treatment, °C	Concentration of artichoke extract, %	PhIP, ng/g	IQxng/g	MelQng/g	MelQxng/g
Pan frying	150	0	n/d	0,07±0,02	0,85±0,12	0,29±0,04
		0,5	n/d	0,15±0,01	1,71±0,33	0,60±0,12
		1,0	n/d	0,16±0,02	1,58±0,13	0,60±0,20
	200	0	n/d	0,18±0,01	4,79±0,97	1,30±0,45
		0,5	n/d	0,49±0,02	3,36±0,42	1,16±0,08
		1,0	n/d	0,56±0,05	5,20±1,29	1,91±0,39
	250	0	4,86±0,27	1,57±0,04	3,69±0,17	1,40±0,30
		0,5	5,14±0,46	1,19±0,39	3,55±1,13	1,50±0,30
		1,0	7,00±1,10	1,14±0,16	2,77±0,29	1,21±0,15
Baking	150	0	0,35±0,07	0,08±0,01	n/d	n/d
		0,5	n/d	n/d	n/d	n/d
		1,0	n/d	n/d	n/d	n/d
	200	0	n/d	0,09±0,04	0,33±0,06	0,10±0,00
		0,5	n/d	n/d	0,22±0,02	0,08±0,02
		1,0	n/d	n/d	0,37±0,03	0,14±0,03
	250	0	n/d	0,75±0,07	12,05±1,38	1,66±0,37
		0,5	n/d	0,23±0,03	3,24±0,61	0,95±0,10
		1,0	n/d	0,26±0,03	3,99±0,36	0,95±0,11

Table 8: Effect of artichoke extract on the formation of HAA in beef [18].

Heat treatment method	Temperature of heat treatment, °C	Concentration of artichoke extract, %	PhIP, ng/g	IQxng/g	MelQng/g	MelQxng/g
Pan frying	150	0	n/d	3,48±0,47	2,02±0,24	0,68±0,08
		0,5	n/d	2,47±0,24	3,20±0,20	1,19±0,18
		1,0	n/d	1,59±0,42	2,38±0,40	0,88±0,06
	200	0	n/d	2,13±0,13	4,39±0,48	1,44±0,33
		0,5	n/d	0,71±0,05	4,71±0,68	1,71±0,33
		1,0	n/d	0,45±0,07	4,98±0,23	1,80±0,60
	250	0	3,19±0,18	1,54±0,07	6,17±0,39	1,76±0,01
		0,5	3,57±0,07	1,31±0,01	5,51±0,13	1,90±0,20
		1,0	7,59±0,59	1,95±0,02	6,35±0,04	2,20±0,10
Baking	150	0	4,52±0,26	3,13±0,10	0,14±0,04	n/d
		0,5	2,34±0,03	1,92±0,11	0,08±0,01	n/d
		1,0	1,48±0,04	1,74±0,05	0,05±0,00	n/d
	200	0	0,50±0,0	0,37±0,10	0,19±0,02	0,06±0,3
		0,5	n/d	1,59±0,07	n/d	n/d
		1,0	n/d	1,08±0,01	0,19±0,04	0,05±0,03
	250	0	0,53±0,05	2,73±0,03	7,25±0,70	1,25±0,02
		0,5	n/d	0,91±0,01	5,93±0,05	1,29±0,45
		1,0	n/d	0,87±0,02	0,05±0,03	0,03±0,02

In contrast to hawthorn extract, treatment with artichoke extract solution resulted in a decrease in the amount of HAA formed in the meat product, except for PhIP in chicken and beef samples prepared by frying in a pan at a temperature of 250°C, where a catalytic effect was observed.

In [19], the effect of replacing animal fat with vegetable oils was studied. The essence of the experiment was that cutlets were made from defatted pork with the addition of pork fat, sunflower, olive, and pomegranate oils. Sample formulations are shown in Table 9.

Table 9: Recipe of research objects.

	Ingredients, g/kg				
	Defatted meat	Oil	Pork fat	Salt	Water
Control	700	0	100	20	180
Sample with sunflower oil	700	40	60	20	180
Sample with olive oil	700	40	60	20	180
Sample with pomegranate oil	700	40	60	20	180

From the prepared minced meat, cutlets were formed, weighing about 100 g, 9.0 cm in diameter, and 2.5 cm thick. These cutlets were baked in an oven at

temperatures of 180°C and 220°C until the temperature inside the product reached 73°C. The obtained results are shown in Table 10.

Table 10: Results of the effect of replacing animal fat with vegetable oils on the amount of HAA formed.

Sample	Температура обработки	IQ, ng/g	MelQ,ng/g	MelQx,ng/g	DiMelQx,ng/g	PhIP,ng/g
Control	180	n/d	18,26±14,46	8,34±1,78	25,66±1,51	11,43±6,33
	220	3,88±3,50	59,70±0,98	13,45±7,43	43,37±15,67	24,07±1,99
With olive oil	180	0,58±0,01	n/d	3,50±0,68	n/d	n/d
	220	1,30±0,42	n/d	2,52±0,36	1,31±0,22	14,78±1,49
With sunflower oil	180	n/d	n/d	4,32±0,50	1,02±0,50	n/d
	220	0,64±0,16	n/d	4,31±0,55	5,12±0,35	22,70±1,95
With pomegranate oil	180	n/d	n/d	n/d	n/d	n/d
	220	0,59±0,04	1,31±0,06	n/d	n/d	n/d

The results have shown that replacing of 40% of animal fat in a meat product with vegetable oil leads to a significant reduction in the amount of HAA formed

during heat treatment. The decrease in the total amount of HAA in products ranged from 83% to 100%. This effect can be explained by the high content of vitamin E

in vegetable oils As already described earlier, vitamin E has a strong inhibitory effect in the formation of HAA.

III. CONCLUSION

The analyzed works have shown that the risk of HAA formation can be controlled in various ways, the most accessible of which is to introduce ingredients with antioxidant activity into the meat product recipe. By such methods, it is possible to reduce the level of HAA up to 100%, which will reduce the carcinogenic load on the human body with endogenous xenobiotics and improve food safety. The analysis of the results of the studied works made it possible to determine the vector for further own research on this issue, namely, the introduction into the recipe of meat products ingredients that usually used to be added and ingredients that were not used, but have high antioxidant activity, such as sea buckthorn, blueberries, grapes, cranberries, mountain ash, chokeberry, currant, mangosteen.

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