FORMATION OF BIOGENIC AMINES AS AN INDICATOR OF ANIMAL PROTEIN OXIDATION PROCESS

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Abstract – The paper describes the methods for mass-spectrometric detection of biogenic amines formed in parallel with the process of animal protein oxidation. The factors influencing their quantitative content were determined and the dependency of the biochemical status of meat products on duration of product storage at different temperatures was established by the quantity of the benchmark biogenic amines (cadaverine, histidin). The role of several additives stabilizing the process of protein oxidation, in particular, sodium nitrite and ascorbic acid was discussed.

Key Words – LC-MS/MS, biogenic amines, meat products, proteins.

I. INTRODUCTION

At present, ecology of nutrition is considered the most important part of human life. Safety and quality of food to a large extent depend on the level of content of microimpurities of various substances, which are initially constituents of a product coming with raw material, or are formed in it during storage.

Nowadays, the problem of assessing food product quality is regarded as fairly important for maintaining population health due to an extension of using different additives in food technology, which in the breakdown form substances with low benefits or even unsafe. These substances include the products of oxidative breakdown of proteins – biogenic amines [1].

A significant share of products that reaches the human table belongs to meat products – the protein components of animal origin, as well as meat-plant compositions. The processes of biochemical breakdown of proteins contained in meat and meat products lead to deterioration of product consumer properties, worsening ecology of human nutrition.

For example, toxic cadaverine is formed in the breakdown of amino acid lysine, histamine is formed from histidine, serotonin from hydroxytryptophan, putrescine from ornithine, tyramine from tyrosine etc. A range of biogenic amines formed in the breakdown and oxidation of proteins has pronounced toxicity; therefore, it is desirable to exclude their presence in food products.

Substances formed as a result of protein biodegradation can serve as benchmark substances. The presence and quantity of such substances can serve for testing food safety [2]. These substances include biogenic amines, primarily, histamine, serotonin, tyramine and cadaverine. The quantity of these substances in food products usually differs and varies from < 1 to 2500 mg/kg. It is believed that the content of biogenic amines of >100 mg/kg of a product is harmful for human health [3].

The aim of this work was to study the biochemical status of meat products, which composition includes beef, mutton and pork and which had different duration of storage at different temperatures. The raw material and products were used for the following identification of biogenic amines formed during storage, in particular, the typical diamine – cadaverine by chromatographic method.

II. MATERIALS AND METHODS

An analysis was carried out by chromato-mass-spectrometry. To this end, a mixture of 10 g of minced sample and 50 ml of methanol was homogenized, held at 80°C for 15 min., evaporated to a volume of 10 ml and centrifuged at 10000g. 2 µl of the obtained extract were introduced into an analyzer. The composition of the biogenic compounds was studied on the gas chromatograph AT7890 with 5975 VL Triple-Axis MSD Detector Agilent (Germany). The peaks of the substances with a concentration of more than 0.01 mg/kg with a probability of identification

more than 65% were analyzed using the automatic database Nist 08. Microbiological analysis and determination of the physico-chemical parameters were carried out by the traditional methods [3].

III. RESULTS AND DISCUSSION

The biogenic amine content is easily determined chromatographically. There is information that the quantity of cadaverine and putrescine in fresh meat from different species is approximately at the same level of <10 mg/kg; while in meat with signs of spoilage it is 140 and more than 50 mg/kg, respectively. In particular, in the freshly prepared small sausages, which were produced based on the recipe mixture of pork and beef according to the Russian technology, the quantitative values of putrescine, cadaverine and histamine were 1.95, 2.85 and 2.65 μg/g.

The decisive fact that influences formation of biogenic amines in food products is the presence of the specific microorganisms that are seldom met in fresh products.

Table 1. Coefficients of correlation of the quality indicators with the content of microorganisms (CFU) in beef and mutton during storage.
Abbreviations: Put – putrescine, His – histamine, Cad – cadaverine, Tyr – tyramine, VN – volatile nitrogen (ammonia, alkylamines) , TBA – thyobarbituric acid, Cond – conductivity, Glu – D-glucose , Lac – lactose

Indi-cators	Coefficient of correspondence of microbial contamination assessment			
	beef		pork	
	storage temperature, °C			
	0	4	0	4
Put	0.90	0.93	0.85	0.95
His	0.91	0.92	0.90	0.91
Cad	0.84	0.95	0.93	0.94
Tyr	0.87	0.94	0.85	0.93
VN	0.96	0.97	0.95	0.95
TBA	0.91	0.91	0.94	0.95
Cond	0.71	0.88	0.91	0.89
pH	0.43	0.48	0.46	0.40
Glu	0.95	0.97	0.96	0.95
Lac	0.82	0.85	0.87	0.95

Table 1 gives a comparative presentation of the main moments, which allow assessing a biochemical status of food products on the basis of meat raw material. It can be seen from the data in Table 1 that the frequency of the correspondence of product microbial contamination with the level of the biogenic amine content is fairly pronounced. An analysis of the residue content of these substances can allow making a firm conclusion about the status and possibilities of product spoilage.

Determination of the biogenic amine content in meat raw material at the various stages of raw material storage confirms that the quantity of these substances in raw material and finished products, as a rule, increases; however, this trend is inconclusive.

Table 2. Changes in the histamine concentration in animal raw material (mutton) dependent on duration and conditions of storage

Samples (storage at)	Histamine content (mg/kg) during storage				
	0 h	48 h	96 h	7 days	15 days
Mutton (20°C)					
No 1	5.87	7.86	11.35	>12	>12
No 2	6.07	8.60	10.77	–	–
No 3	6.21	8.93	11.52	–	–
Mutton (4°C)					
No 1	5.87	–	–	7.73	6.13
No 2	6.07	–	–	8.24	5.99
No 3	6.21	–	–	8.27	7.35

Table 2 presents the data on changes in the content of one of the biogenic amines, histamine, which concentration can change over time. The tendency is a practically monotonous increase. At manifestation of the first signs of microbiological spoilage of meat raw material, the samples (96 h at 20°C and 15 days at 4°C) were again analyzed for histamine content (Table 2). In the samples stored at 20°C, the level of histamine steadily increased up to 11.52 mg/kg in sample 3; later, this level insignificantly exceeded the limit of 12 mg/kg. In contrast to the non-fresh samples stored at 20°C, histamine decreased to the minimal level of 5.99 mg/kg in the samples stored at 4°C. Therefore, identification of histamine in the animal raw material does not show a stable relationship of the content of this amine with the biochemical changes in meat freshness.

An entirely different picture was observed for cadaverine, which content proportionally depended on the duration of raw material holding. For example, the initial samples of beef and mutton contained 10 to 14 mg of cadaverine

per kg of sample mass; while the quantity of this biogenic amine increased monotonically 3-10 times dependent on the conditions of storage and processing of products. This fact allows using cadaverine as a "signal" substance for identifying the biochemical status of meat and assessing quality of meat raw material and products on its basis.

The process of development of the biochemical changes in food raw material leads to parallel breakdown of raw material constituents, in particular, protein. As a result, not only the end products of this breakdown, biogenic amines, but also the intermediate substances of enzymatic degradation of proteins, amino acids, increase in products. It was interesting to assess this phenomenon on the quantitative level. As can be seen from the data in Table 3, the sum of free amino acids in beef increases during storage.

Table 3. Amino acid content in animal tissue (beef), fresh and after holding at 20°C, 24 h, mg/100 g of a sample

Amino acid	Meat raw material - beef	
	initial	after holding
TAU	14.9	17.8
ASP	11.2	17.6
THR	14.0	24.0
SER	16.5	18.9
GLU	2.4	68.1
PRO	6.9	54.5
GLY	12.0	12.9
ALA	52.1	55.6
CYS	2.3	2.6
VAL	17.9	38.3
MET	6.3	16.3
ILEY	13.7	25.2
LEY	27.9	53.1
TYR	12.8	20.3
PHE	14.9	29.7
HIS	106.2	133.4
OPN	5.2	15.1
LYS	19.5	55.9
ARG	11.7	15.9
Σ	368.4	675.2

The analysis of the amino acid composition shows that the level of the free amino acid content increases twice during the first day, which fully confirms the possibility of proteolytic process development, apparently, associated both with the activity of enzymes of the animal tissue per se, and the enzymatic systems of microorganisms contained in it. These processes are just the ones that symbatically promote an increase in the concentrations of the detected biogenic amines in samples.

IV. CONCLUSION

Therefore, the concentration intervals of the content of the main biogenic amines in the animal raw material, their quantity in dynamics and statics were determined. The study showed that the practically proportional dependence of the cadaverine content on the duration of sample holding was observed. The initial samples of meat raw material contained 10 to 14 mg of cadaverine per kg of sample mass. The quantity of this substance increased several times depending on the storage conditions.

The conducted investigations allow using a "signal" substance, cadaverine, for development of physico-chemical method for identification of meat biochemical status and assessment of quality of meat raw material and products on its basis.

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