CADAVERINE DETERMINATION IN MEAT AND THE POSSIBILITY OF USING THIS INDICATOR IN MEAT FRESHNESS ASSESSMENT

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Background

During inspection one of the most important indication of meat safety and quality is its freshness, which characterizes the state of protein and lipid fractions in food products.

Undesirable changes can take place during the storage of meat raw material, especially when the temperature and humidity parameters are abused and the storage duration is above norm.

In Russia meat is classified as fresh, doubtfully fresh, and not fresh.

Only fresh meat is used on common basis (without restriction). If meat is classified as doubtfully fresh, the decision about the possibility and conditions of its using is taken after the set of laboratory tests. The meat raw material, which is not fresh, is not used for food purposes.

Today, sensory tests, tests with copper sulfate, volatile fatty acids detection, the microscopy of impression-preparations of testing samples, microstructural investigation are used to control freshness of meat raw material in Russia.

It is known, that stated laboratory methods can give controversial results (1), since the conditions of animal feeding have changed during last decades and for these reasons meat quality characteristics have undergone changes and sometimes do not correspond to the formed conceptions. The assessment of microstructure is unilateral and can be used only in complex investigation as its component and not always can be used in practical application.

In this regard new methods of meat freshness assessment are required to be developed.

Meat freshness is directly linked with its microbial contamination, because during their vital activity microorganisms excrete into the substrate the ferments causing protein degradation. Some substances formed due to the meat biodegradation can act as the diagnostic substances, which presence and quantity can serve as a basis for testing freshness (2,3). Among these substances are biogenic amines (the substances produced during decarboxylation of amino acids), namely cadaverine, putrescine and histamine. In our investigation it was found that most appropriate is to detect meat (beef and pork) freshness using cadaverine. Histamine is used mainly for fish freshness and safety determination (3), and its concentration during spoilage can even decrease. Putrescine concentration increases slowly, causing difficulties with differentiation of various degrees of freshness.

Objectives

The purpose of this study was to develop a simple and reliable method of determination of cadaverine content in meat, and to assess the possibility of using this method for determination of meat raw material freshness.

Methods

Several methods are used to detect biogenic amines: liquid chromatography (4,5), gas chromatography (2) and thin-layer chromatography (6,7). However, most of the methods are developed to detect biogenic amines in fish. The method of histamine determination in tuna was taken as a basic and improved (6). Our improvement was related to some stages of cadaverine content detection, including chromatographic fractionation of meat extract. Concentration assessment was carried by data processing software for thin-layer chromatography type Scion Image.

As a result the method of cadaverine determination in meat consisting of the following stages was developed:

Extraction

50 cm^3 of methanol are added to 10 g of minced sample and homogenized 3 min. The obtained homogenizate is held in water bath 15 min at 80^0C . The homogenizate is centrifuged 10 min. at 2000-3000 rpm, the top layer is removed. The obtained after the centrifugation extract is evaporated in water bath at 80^0C and cooled to the room temperature.

Preparation of standards

Standards: 102.9 mg of cadaverine dihydrochloride are dissolved in 4 cm^3 distilled water and brought to the volume of 20 cm^3 with methanol. The result is a standard solution with concentration of pure cadaverine 3 mg/cm^3. The solution is held in refrigerator. Before using it is diluted with mixture of methanol and distilled water (8:2) and as a result the working standards are obtained with cadaverine content of 0.2, 0.1, 0.05 and 0.005 mg/cm^3.

Running of standards and extracts

10 mcl of the obtained extract and working standards are each put on the plate for thin-layer chromatography. The plate with extracts and working standards is put in chromatographic chamber filled with mixture of acetone and concentrated ammonia and held 2 h. The plate is dried until the disappearance of ammonia odour.

Visualization of biogenic amines

The plate is sprayed with the solution of ninhydrin (300 mg ninhydrin are dissolved in 100 cm^3 butanol adding 3 cm^3 ice acetic acid). The plate is dried and held 5 min at 100^0C.

Assessment of cadaverine concentration

Then the plate with spots is scanned immediately, and spots are assessed according to their cadaverine content using data processing software for thin-layer chromatography type Scion Image (obtained data for pork is divided by 2).

Results and discussion

We studied cadaverine content and changes in beef and pork freshness during storage, and in addition, all samples were assessed using methods adopted in Russia. Four samples of beef and pork were each purchased in retail stores and used as objects, samples were stored at 4^0C. The results of the study are presented in table 1.

It follows from the data in this table that during the storage of meat the cadaverine content increased depending on the state of the product. We also came to the conclusion that the determination of meat freshness by cadaverine was possible and in contrast to the sensory assessment the precise numerical data were obtained as a result.

Sensory assessment of meat confirmed the gradation of its state by its cadaverine content.

The study of meat freshness by tests with copper sulfate, volatile fatty acid detection, the microscopy of impression-preparations of testing samples, pH, acid and peroxide numbers, amino nitrogen, CFU reflected impossibility of obtaining the precise assessment of meat state, using investigated methods.

On the basis of the conducted study, the margins of meat freshness degrees were established (table 2).

Conclusions

The results of our study showed that the most appropriate biogenic amine for meat freshness determination was cadaverine; the simple and reliable method of its content determination and the assessment of meat raw material freshness was developed.

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Table 1 Changes in meat freshness and cadaverine content during storage

Sam-ple no	Storage at 4^0C				
	Storage duration				
	0 h	6 d	8 d	12 d	15 d
Beef					
1	fresh– 9,56	d.fresh*- 39,77	d.fresh – 48,92	not fresh – 108,86	not fresh – 114,47
2	fresh –5,37	fresh 23,36-	d. fresh –42,71	not fresh -77,41	not fresh –92,14
3	fresh –8,44	d. fresh–28,93	d. fresh –39,13	not fresh –54,44	not fresh –84,91
4	fresh–10,95	d. fresh -33,04	d. fresh –47,54	not fresh –93,63	not fresh –98,05
Pork					
1	fresh –2,12	fresh – 4,15	d. fresh – 9,77	d. fresh – 11,56	d. fresh – 14,07
2	fresh –2,98	fresh –5,64	d. fresh –8,15	d. fresh –14,34	not fresh –15,61
3	fresh –3,87	fresh –4,92	d. fresh –7,35	d. fresh –10,18	d. fresh –12,19
4	fresh –5,01	fresh –4,89	d. fresh –6,99	d. fresh –11,27	d. fresh –13,78

* d. fresh – doubtfully fresh meat

Table 2 Meat freshness assessment by cadaverine content

Meat type	Meat freshness, mg/kg		
	fresh	doubtfully fresh	not fresh
beef	<25	25-50	>50
pork	<6	6-14,5	>14,5