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BIOGENIC AMINES FORMATION INFLUENCED BY TISSUE ENZYMES AND pH VALUE IN THE MIXTURE PREPARED FOR RAW SAUSAGES

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The purpose of this paper was to clarify the influence of tissue enzymes and pH value in the mixture prepared for the production of raw sausages on the formation of biogenic amines (histamine and tyramine).

A meat mixture very similar in composition to the typical fermented sausage (40% of beef, 40% of pork and 20% of pork fat tissue) was prepared, and divided in to three parts. The pH value of these portions was adjusted to 5,5; 6,0 and 7.0 through the addition of acetic acid, water and sodium hydrocarbonate solution, respectively. The samples were then radiodecontaminated (5 kGy) to exclude the possible influence of native microflora and stored at 18-22^0 C. The histamine and tyramine contents were determined immidiately and on the 0th, 5th, 10th and 15th days of storage.

The obtained results showed that the initial contents of biogenic amines in the fresh meat mixtures were low: 1,05-1,16 μg of histamine and 1,82-2,04 μg of tyramine per gram of mixture. The final contents of these two biogenic amines, created after storing under conditions similar to the normal processing conditions in the production of this type of fermented sausages (two weeks at 18-22^0 C) also were low and unimportant for the hygienic safety of sausages (1,64-1,88 μg of histamine and 2,26-2.94 μg of tyramine per gram of the mixture).

From the results presented the general conclusion could be drawn that enzymatic activity of the meat itself does not contribute very much to the total biogenic amines formed in the fermented sausages. However, comparison of the relative biogenic amine contents in the three groups of samples revealed that pH value of the meat exerts great influence on the intensity of amino acids decarboxylation and on the activity of enzymes which catalyse the formation of biogenic amines.

Key words: fermented sausages, biogenic amines, histamine, tyramine, tissue enzymes

INTRODUCTION

Fermentation and drying processes represent the most common food preserving techniques, well known since ancient times. The observation that some foods gained prolonged storage capability when let to spontaneously change in texture, sensory and other properties encouraged the first application of fermentation processes in history. These processes have been improved in time through the application of heat, by carefully choosing the conditions of stoage, and finally through the use of some additives which could enhance these processes. All these fermentation processes result from the activity of the native microflora, among which lactobacilli prevail.

Fermentation is mostly used in the production of raw (dry or fermented) sausages. However, this process can cause some unwanted compounds to appear, among which are two biogenic amines tyramine and histamine (Taylor and Speckhard, 1984; Teodorović, 1991; Bunčić et al., 1992). Under certain circumstances, these two compounds can trigger alimentary intoxications in humans, so their presence in high concentrations should be considered as a factor which can endanger the safety of meat products (Gunn et al., 1980; Bean et al., 1990). The potential hazard imposed by the presence of biogenic amines in fermented sausages has been recognized only in recent times and the scientific approach to this problem is still in the preliminary stage. The published data about the alimentary intoxications are some what confusing because fermented sausages have never been accused of causing human intoxications through biogenic amines in spite of some newer investigations which showed that fermented sausages could contain extremely high concentrations of histamine, and particularly of tyramine (Taylor et al., 1978; Wortberg and Zieprath, 1981; Wortberg and Woller 1982; Vuković et al. 1988). The amounts of these two amines in fermented sausages sometimes even surpassed the amounts found in fish that have been positively incriminated as the cause of human intoxication. This situation indicates that the formation of biogenic amines in fermented sausages is governed by several factors still not clearly defined and explained.

Hence, the purpose of our present investigation was to elucidate the influence of tissue enzymes and pH value in the mixture prepared for the production of raw sausages on the formation of biogenic amines. It is important to know which part of the totally produced biogenic amines are due to the activity of tissue enzymes and which part is the result of microorganismic activity.

MATERIALS AND METHODS

A mixture was prepared, of composition very similar to typical fermented sausages: 1700 g (40%) of beef round, 1700 g (40%) of pork neck and 850 g (20%) of pork fat tissue. All components were ground in a cutter (Alexanderwerk, DDR) according to the scheme: 40 seconds of low speed grinding; then 20 seconds of high speed grinding. The plate and the knives of the cutter were previously washed and disinfected with a mixture of ethanol and diethyl ether (1:1), and let to dry in the air.

The ground mixture was divided into three equal (1450 g) portions to which equal volumes (225 ml) of the following solutions were admixed: 0,1 mol/l of acetic acid, 5% of sodium hydrocarbonate and distilled water, respectively. The resulting pH values of the final mixtures were 5.5, 7.0 and 6.0, respectively.

Each portion was mixed with necessary additives in a separate plastic container, manually homogenized for 3 minutes and subdivided in smaller portions (50 g). These were packed in plastic bags; the air was removed and the bags welded shut. All samples packed in that way were subjected to ionizing (gamma) radiation (5 kGy) to remove all viable bacterial cells and finally stored at 18-22^0 C.

The concentrations of histamine and tyramine in the samples were determined by thin-layer chromatography (Langner and Kellinghusen 1979) on 350 mm silicagel plates (Kieselgel 60H; Merck, Germany) followed by densitometric measurements using a TLC Scanner (Camag, Wilmington, U.S.A.).

The concentrations of histamine and tyramine were determined immediataly and on the 5th, 10th and 15th day of storage. To check the sterility of samples the total bacterial count was also determined. The results are shown as mean values of three parallel analyses.

RESULTS AND DISCUSSION

The properties of raw meat used in the production of fermented sausages may be critical for the hygienic status and quality of the product. Among other properties, two of them are the most important: the freshness of meat or the degree of meat ripeness (proteolysis) at the moment of processing, and its pH value. These characteristics are responsible for the route and the duration of the fermentation process, proper binding of the mixture components, color formating, attaining proper consistency and other sensory properties of the sausage, but in this investigation, they are considered only as possible factors of influence on the formation of biogenic amines.

The initial content of free amino acids in raw meat prepared for the production of sausages depends on the degree of meat ripeness. During ripening, the proteins of meat can be partially hydrolyzed through the action of the native enzyme system (cathepsins) or through the action of enzymes originating from the contaminant microflora. The free amino acids that appear in the course of these proteolytic processes form a good substrate for the action of microorganismic decarboxylases, representing actually the precursors of biogenic amines. Therefore, very fresh meat with a low content of free amino acids represents raw material with only trace quantities of biogenic amine precursors. On the other hand, meat that has been considerably ripened or meat in the first stage of decay represents a perfect substrate for the activity of amino acid decarboxylases.

As previously stated, the route and the intensity of all enzymatic reactions in meat depend considerably on its pH value. The formation of biogenic amines

in meat is influenced by pH in two ways. A low pH value promotes the activity of meat catepsins (Vuković, 1998) creating favorable conditions for the hydrolysis of meat proteins. Also, the decarboxylation of free amino acids proceeds more intensively in the vicinity of a pH value optimal for the activity of decarboxylases.

The initial content of biogenic amines in the basic mixture (which was prepared from fresh meat and fat tissues as previously explained) was very low: 1.05-1.16 mg of histamine and 1.82-2.04 mg of tyramine per gram of mixture (Table 1).

Table 1. The influence of pH on biogenic amine formation in radiodecontaminated minced meat stored at 18-22^0 C

pH	Days of storage	Content of histamine		Content of tyramine	
		Absolute (μg/g)	Relative* increase (%)	Absolute (μg/g)	Relative* increase (%)
5.5	0	1.16	0.00	2.04	0.00
	5	1.70	46.55	2.41	18.13
	10	1.70	46.55	2.73	33.82
	15	1.88	62.06	2.94	44.12
6.0	0	1.07	0.00	1.82	0.00
	5	1.44	34.58	2.62	43.96
	10	1.50	40.19	2.52	38.46
	15	1.64	53.27	2.60	42.86
7.0	0	1.05	0.00	1.99	0.00
	5	1.25	19.05	2.12	6.53
	10	1.88	79.04	2.08	4.52
	15	1.31	24.76	2.26	13.57

*/ To the initial content

These values coincide with the results of other authors (Terplan, 1971; Slemr 1981; Rogowski and Dohla, 1983; Teodorović et al., 1994) obtained for fresh beef and pork: 1-3 mg of histamine and 1-1,5 mg of tyramine per gram of meat. The final contents of these two biogenic amines, created after storing under conditions similar to the normal processing conditions in the production of this type of fermented sausages (two weeks at 18-22^0 C) remained low and unimportant for the hygienic safety of sausages (1,64-1,88 mg of histamine and 2,26-2.94 mg of tyramine per gram of the mixture). There are no published data on the formation of biogenic amines in sterile meat under the conditions which exist in the production of fermented sausages. Our results, however, clearly show that enzymatic activity of the meat itself does not contribute much to the total biogenic amines formed in the fermented sausages.

On the other hand, this part of our investigation revealed that pH value of meat exerts great influence on the intensity of the amino acid decarboxylation and

on the activity of enzymes which catalyse the formation of biogenic amines. While the overall amount of biogenic amines formed was low, its relative increase during storage was greater in an acid environment (Figure 1).

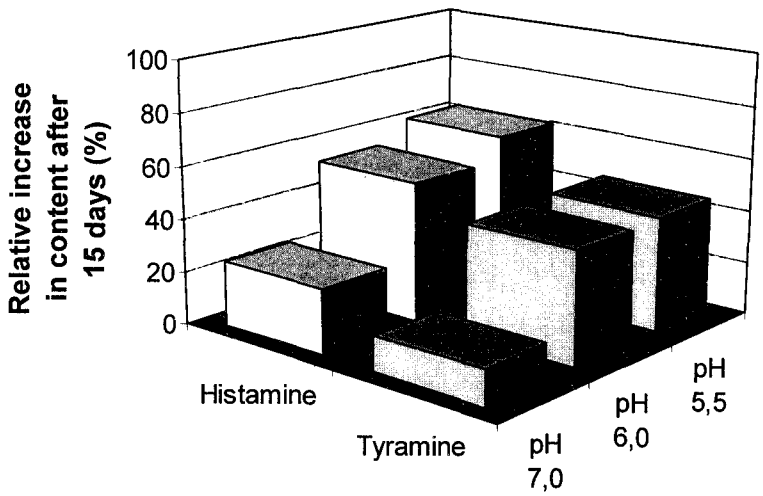

Figure 1. The influence of pH on biogenic amine formation in radiodecontaminated minced meat stored at 18-22^0 C

The maximum rate of amine formation in the mixture prepared for the production of raw sausages was registered at pH 5.5, typical for cooled beef. There are no published data about the pH value optimal for the activity of the native meat amino acid decarboxylases, but there is much evidence that the optimal pH values for the activity of bacterial decarboxylases fall in a narrow range between 4.0 and 5.5 for histidine-decarboxylases, and between 5.0 and 6.1 for tyrosine-decarboxylases, respectively. Considering these data together with our results, the conclusion could be drawn that a pH value in the vicinity of 5.0 should favour the formation of biogenic amines in meat, regardless of the source of decarboxylases. In other words, if the formation of biogenic amines in meat needs to be prevented, it would be desirable to use meat with higher pH values.

However, this conclusion opposes the recommended practice of using meat with lower pH value in this type of production to achieve other, very important goals. In fact, meat with a pH value higher than 5.8 does not contain enough carbohydrate material to ferment optimally and also it firmly binds water so interfering with the process of sausage drying. The lowering of pH which takes place during such fermentation is not enough to inhibit microorganisms which cause decay of meat or alimentary diseases (Lücke, 1994). The use of lower pH values in starting meat is the main reason for finding considerable quantities of biogenic amines in fermented sausages. Actually, these low pH values, along with the beneficial influence that they exert on the production of pathogen-free sau-

sages, also favour the production of biogenic amines histamine and tyramine, endangering the hygienic safety of the sausages.

From the preceding argument it follows that the formation of biogenic amines during the production of fermented sausages could be minimised through the fulfilment of two criteria the starting meat should not be too ripe (or too hydrolysed) and its pH value needs to be higher. The first criterion could be easily realised in practice. However, for the application of the second criterion probably there is no room the possible risks for the hygienic status of sausages would be too unpredictable and unacceptable. Therefore, it seems that the main scientific and professional efforts aimed to minimise the formation of biogenic amines in the fermented sausages must be focused on other factors which contribute to their formation: on microbiological status of raw material, and on microbiological, physicochemical and technological factors of the production process itself.

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STVARANJE BIOGENIH AMINA POD UTICAJEM TKIVNIH ENZIMA I pH SMEŠE PRIPREMLJENE ZA PROIZVODNJU SIROVIH KOBASICA

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SADRŽAJ

Glavni cilj preduzetog istrživanja bio je da se sagleda uticaj tkivnih enzima i pH vrednosti smeše pripremljene za proizvodnju sirovih kobasica na obrazovanje biogenih amina (histamina i tiramina).

Pripremljena je smeša, sirovinskog sastava tipičnog za nadev fermentisanih kobasica (40% govedine, 40% svinjetine i 20% svinjskog masnog tkiva) i podeljena u tri dela, a zatim je svakom delu posebno podešena pH vrednost dodatkom sirćetne kiseline (na pH 5,5), vode (na pH 6,0), odnosno rastvora natrijumbikarbonata (na pH 7,0). Sva tri dela smeše su zatim radiodekontaminirana jonizujućim zračenjem (5 kGy) kako bi se isključio uticaj postojeće mikroflore i uskladištena pri temperaturi od 18-22^0 C. Sadržaj histamina i tiramina u ovako uskladištenim uzorcima određivan je posle 5, 10 i 15 dana.

Dobijeni rezultati su pokazali da je početni sadržaj ispitivanih biogenih amina u svežem mesu bio nizak: 1,05-1,16 mg histamina i 1,82-2,04 mg tiramina po gramu smeše. Krajnji sadržaj ovih amina, nakon skladištenja u uslovima bliskim onim u uobičajenoj proizvodnji ovih kobasica (dve sedmice pri 18-22^0 C) takođe je bio nizak i sa gledišta higijenske ispravnosti bez većeg značaja (1,64-1,88 mg histamina i 2,26-2.94 mg tiramina po gramu smee).

Iz dobijenih rezultata može se izvesti opšti zaključak da enzimska aktivnost mesa ne doprinosi značajno obrazovanju biogenih amina u fermentisanim kobasicama. S druge strane, na osnovu poređenja relativnog sadržaja biogenih amina u tri grupe uzoraka različite pH vrednosti, može se zaključiti da pH ispoljava snažan uticaj na brzinu dekarboksilacije aminokiselina, odnosno na aktivnost enzima koji katalizuju obrazovanje biogenih amina.