THE FORMATION OF BIOGENIC AMINES IN MEAT AND MEAT PRODUCTS

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SUMMARY

Biogenic amines in fresh beef and pork stored under different temperatures and raw sausages ("Mettwurst" and Salami) produced from fresh and matured raw material with and without the presence of starter cultures and/or histamine producing microorganisms were investigated. The contents of biogenic amines were determined by HPLC as their dansyl derivatives.

During storage of beef and pork at 4°C an increase of tyramine, cadaverine and in some cases putrescine was observed. The amounts of these biogenic amines did not exceed 150 mg per kg sample at the 35th day of storage. In spoiled samples concentrations up to 1000 mg/kg were measured.

Using stored pork (10 days, 4°C) for the production of raw sausages first of all the concentration of tyramine increases up to the 10 to 100-fold of the initial concentration. An excessive formation of histamine could only be stimulated, if lactobacillus hilgardii was added to the sausages. Putrescine also increases distinctly, the formation of cadaverine is only influenced slightly by the age of the meat. The addition of different starter cultures could not reduce or prevent the formation of these biogenic amines.

These results indicate that tyramine represents a suitable indicator for long stored meat or that long stored or even spoiled meat has been used in raw fermented sausages.

Introduction

Biogenic amines can be formed during storage of fresh meat as well as during the ripening of raw sausages, where they are found as the undesired by-products of a desired bacterial activity (Pechanek et al., 1980, 1983). Next to their toxicological aspects biogenic amines have gained importance as quality indicators (Slemr, 1981; Guerrero-Legaretta and Chavez-Gallardo, 1991). So, it could be shown that an excessive formation of histamine in raw sausages depends both on the age of the raw material and the presence of histamine forming microorganisms (Tschabrun et al., 1990; Kranner and Bauer, 1991; Maijala et al., 1991; Maijala and Eerola, 1993).

The objective of this study was to investigate which biogenic amines will be formed during ripening of fresh meat and to obtain a survey which biogenic amines can occur in raw sausages. Furthermore, the influence of the age of the raw material, of the specific flora in a production site and the effect of starter cultures on the formation of biogenic amines should be investigated in order to find out the suitability of biogenic amines as indicators for spoilage or for the usage of spoiled meat for the production of raw sausages respectively.

Materials and methods

Investigated materials were fresh beef and pork stored under different conditions, commercially produced raw sausages which were investigated during the ripening period, raw sausages ("Mettwurst") produced from fresh and stored (10 days, 4°C) pork with and without addition of 10^3, 10^4, 10^5 Klebsiella oxytoca/g or 10^3, 10^4, 10^5 Lactobacillus hilgardii/g, "Mettwurst" and Hungarian-style salami made from fresh and stored pork with and without addition of starter cultures and 10^5 Lactobacillus hilgardii/g.

For the determination of biogenic amines, the samples were ground and mixed and extracted with trichloroacetic acid. To the filtered and alcalized extract dansylchloride solution was added and the mixture was allowed to react at 70°C for ten minutes. After evaporation, the residue was suspended in acetonitrile and after

sedimentation the supernatant was separated by HPLC using a RP-C_{18} column. Chromatographic conditions were as described by Mietz and Karmas (1979) with minor modifications.

Results and discussion

During storage of beef and pork at 4°C an increase of tyramine, cadaverine and in some cases also putrescine was obtained. The amounts of these biogenic amines did not exceed 150 mg/kg at day 35 of storage. In spoiled samples, concentrations up to 1000 mg/kg were found. Spermidine contents varied from 1-16 mg/kg and spermine was 20-50 mg/kg (Fig. 1 and 2) and did not change during storage as reported by Nakamura et al.(1979) and Rogowski and Döhla (1984). Thus tyramine appears to be a suitable indicator of spoilage.

In raw sausages from a market tyramine was found in all samples except one in concentrations of 100 mg/kg and above, whereas histamine and putrescine contents were quite varying from one producer to the other. Large amounts of cadaverine were found in only two samples and the concentrations of spermine and spermidine showed no remarkable differences between the different products (Fig. 3).

The addition of histamine-forming strains of Klebsiella oxytoca to sausage made of fresh as well as spoiled meat could not stimulate an excessive formation of histamine. The concentration of tyramine showed a drastic increase in the case of using stored meat although tyramine-producing microorganisms were not added. The addition of L. hilgardii led to a extreme increase of histamine as well as tyramine (Fig. 4). The use of stored pork results in threefold higher values and the formation depends on the number of microorganisms added (Fig. 5). The amounts of the other biogenic amines did not change during ripening in a way which would make them suitable for the detection of the usage of spoiled meat in sausage production. The addition of starter cultures according to the recommendations of the producer could not reduce or prevent the excessive formation of tyramine and histamine (Fig. 6). The growth of microorganisms showed that in spite of using different raw material the total count of microorganisms was around 10^9/g at the end of ripening and that during ripening the number of pseudomonas, enterobacteriaceae and brochothrix was reduced to a level which was also found in raw sausages made of fresh meat. Histamine forming microorganisms in amounts of 10 to 1000/g could also be found in samples without any artificial addition of L. hilgardii. Either these amounts were to small to stimulate the production of histamine or these bacteria were unable to form histamine under these ripening conditions. The results mentioned above were obtained in the case of self-made "Mettwurst" and Hungarian-style Salami produced under professional conditions.

Conclusions

Tyramine is a suitable indicator for the detection of the use of long-stored or spoiled meat for the production of raw sausages. High amounts of putrescine, and to some extent also cadaverine indicate the use of putrefactive raw material. An excessive formation of histamine could only be observed in the presence of specific microorganisms. The addition of starter cultures did not affect the formation of biogenic amines.

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Fig.1: Formation of biogenic amines in pork stored at 18°C
Fig.2: Formation of biogenic amines in pork stored at 4°C
Fig.3: Amounts of biogenic amines in different Austrian raw sausages produced by 4 different enterprises
Fig.4: Histamine and tyramine in "Mettwurst" with addition of 0, 10^3, 10^4, 10^5 Lactobacillus hilgardii per gram
Fig.5: The influence of the amount of added Lactobacillus hilgardii on the formation of histamine and tyramine in "Mettwurst"
Fig.6: The influence of starter cultures on the formation of biogenic amines in "Mettwurst".
0 - without starter cultures and L. hilgardii; FF, SP, BK ... different starter cultures;
L - addition of 10^5 L. hilgardii/g