FORMATION AND CONTENT OF BIOGENIC AMINES IN CZECH RAW SAUSAGES

STANDARA S., POL P., KAMEN J., BUDIG J.

Veterinary Research Institute, Brno, Czech Republic, University of Veterinary sciences and Pharmacy, Palack, Brno, Czech Republic, DERA FOOD TECHNOLOGY CZ, Brno, Czech Republic.

S-VIA.45

Fermented sausages form 2/3 of all meat products with histamine levels higher than the limit for meat products (200 mg.kg-1) valid in the Czech Republic. The aim of the study was to determine profile of biogenic amines (BA) in fermented sausages wit h respect to the process of ripening, BA content and selected bacteria strains. Analysed samples were several kinds of raw sausages fermented under various conditions during 4 - 6 weeks of ripening. Analyses of BA were performed by ion-exchange liquid chrom atography. Other measurements like pH, aw, content of ammonia, NaCl, dry matter, ash were performed besides microbial analyses. Raw batter only contained negligible amounts of BA (10 mg.kg-1). Putrescine (PU), Tyramine (TA) and Agmatine (AG) content increased significantly next day after sampling. The highest increase of BA amount was recorded during the first week of fermentation. Even after two weeks of BA production the process has not been finished. PU (approx. 190 mg. kg-1) a nd TA (approx. 170 mg.kg-1), in lesser extent Histamine (HA)(approx. 70 mg.kg-1) besides significantly low amount of cadaverine (CD)(approx. 15 mg.kg-1) and Tryptamine(TR) (approx. 15 mg.kg-1) are typical for these fermented meat products. AG has not been determined later then. Spermine (SM) and spermidine (SD) amount varies in finished products and SM amount continually decreases during ripening. Fermented raw sausages contain about 600 mg.kg-1 of BA total which is rou ghly 10 times higher level than found in cooked sausages. Agmatin (AG) can be considered as a marker for beginning of decarboxylase activities.

INTRODUCTION

The content of biogenic amines (BA) in various foodstuffs is being studied from the point of view of their possible toxicity. HA, TA and phenylethylamine (PEA) are the most important ones from the toxicological point of view. PU and CD, which have lower ph armacological activity than HA and TA, enter the interactions with aminooxidases and inhibit HA and TA detoxication. Some medicines, taken as psychopharmacological agents, antihistamines and tuberculostatic agents (Stratton, 1991), have an inhibitory effect , too. Consumption of food with high BA content can significantly negatively influence the health of patients taking these medicines. Other parts of population can be endangered, too, e. g. people with gastric ulcers, high blood pressure, asthmatics, and small children etc.

Besides natural occurrence and spontaneous formation of BA in foodstuffs due to enzymatic decarboxylation of corresponding amino acids during storage, BA also appear during technological processes in the production of various foodstuffs, namely fermented d airy and meat products, sauerkraut, beer and wine. Fermented meat products are 2/3 of products, in which the hygienic limit for HA (200 mg.kg-1), used in the Czech Republic for meat and meat products (Nahodilov, 1988), is often exceeded. However , this limit does not take into account the presence of other amines in foods, or amine content in eatables consumed at the same time. For the determination of toxic levels of BA in food, it is necessary also to consider the amount of consumed food, alcohol and medicines, which is often omitted. Hygienic limits for other amines have not yet been determined in the Czech Republic. In the Netherlands e. g., the Netherlands Institute for Dairy Research (NIZO) proposed the limit 100 mg.kg-1 for HA, 100 - 8 00 mg.kg-1 for TA, and 30 mg.kg-1 for PEA in food (Brink ten, 1990).

It must be noted that BA, as well as PU, CD, SD ans SM can react with nitrites forming carcinogenic nitrosamines (Nakamura, 1979). For hygienic-toxicologic evaluation of an eatable it is therefore necessary to know the whole profile of hygienically importa nt BA and to evaluate toxicologically the most significant amines against the background of the whole profile.

THE AIM OF THE STUDY

The aim of the study was: (1) to gather knowledge on the content of BA profile in a typical product of the group of fermented sausages produced in the Czech Republic, (2) to observe the dynamics of the formation of hygienically important BA during the ripe ning of the product, (3) to evaluate the relations between the results of chemical analyses and the microbial picture of the product, (4) to estimate possible influence of undesirable variations of production regime on the risk of formation of hygienically significant BA, (5) to compare the resulting values with imported products of similar character and with data in the literature.

MATERIAL AND METHODS

The object of the analyses was fermented sausage "Herkules" produced with the use of a starter culture. The sausage is produced from lean beef and pork, pork back fat, typical spices, the addition of glucose and saccharose, adding Lactobacillus pen tosus as a starter culture. The production cycle lasts 21 days.

In 4 series there were analysed 56 samples of sausage batter after stuffing into casing, as well as the final product - fermented sausage "Herkules" produced with AUTOTHERM LASKA equipment. In 2 other series the dynamics of BA formation during ripening w as observed.

Basic chemical analyses were performed according to standard reference methods, aw was calculated according to Mirna (1970). pH values were measured with combined stabbing electrode Crytur (R). Dry matter content was determined gravimetrically, NaCl content in ash after digestion at 600o C by titration of AgNO3 to K2Cr2O7 and NH3 with the method by Conway.

ÁBA were extracted with 5% TCA from the samples, and the supernatant was purified by ultrafiltration. By means of liquid chromatography (AAA Mikrotechna Praha, T 339) on the ion exchanger OSTION LGANB, 8 biogenic amines (HA, TA, PU, CD, TR, AG, SD and SM) w ere isolated in the system of sodium-potassium citrate buffers, and they were determined with ninhydrin after postcolumn derivatization. Detection limit of the method was 1 - 2 mg . kg-1 food. Recovery ranged from 86 to 108 %.

In order to observe of the correlation of microbiological data with chemical analyses. Plate Count Agar was used for the determination of total psychrotrophic bacteria count, (room temperature, 48 hours).

Lactobacilli were quantified by decimal dilution and inoculation on MRS agar. Each colony was biochemically identified with STREPTO test for Arginine degradation and carbohydrate fermentation.

RESULTS

Main physico-chemical parameters and BA contents in sausage batter are given in Table 1. Measured values meet the standard requirements for reaching the optimum conditions for product ripening. Fresh sausage batter contains only a small amount of B A. Except for AM, which is common ingredient of the fresh meat too, the BA values fluctuated about 15 mg.kg-1, with the exception of TA and AG. The highest AG value 30 mg.kg-1 was measured in the 3rd series of samples. This series al so differs in the other results of chemical analyses. The cause could be the changed or in another way distorted character of the input raw material.

The greatest increase of BA content was observed during the 1st week of ripening (1). TA and PU content show the fastest increase. During the 1st week TA reaches about 70% of its final value, and PU about 50%. The increase in HA and CD cont ent is slower indicatively. SD and SM remain in a ratio of about 1 : 10 during the whole ripening period, SM decreases moderately. The decrease in AG content is significant, which has not been proved any more in any sample the 2nd week of ripening. Its relatively high content in the sausage batter and absence in the product can be explained by the change into PU (Wortberg, 1982). Probably the biosynthesis of PU from AG is an important way of PU formation in the initial phase of ripening of fermented s ausages. Agmatine can be proposed as a marker for beginning of decarboxylases activities at the ripening of fermented sausages. Apparently, AG detection is as important in meat products as in seafood (Squid) AG level is used as an indication of the index of freshness (Suzuki, 1990).

Main physico-chemical parameters and BA content for the final product are summarised in Tab. 2. The Cseries of the final product shows significantly lower dry matter value and higher ammonia content and higher aw, which corresponds with the res ults of BA content. For Czech fermented meat products, BA content ranging from 200 to 700 mg.kg-1 is typical. Analysed samples of the sausage "Herkules" contained about 300 mg.kg-1 total BA in 70% of all cases. For fermented sausages, PU con tent (up to 190 mg.kg-1) and TA content (up to 170 mg.kg-1) are typical, less often HA (up to 75 mg.kg-1) and CD (about 30 mg.kg-1). CD content in the samples of fermented sausages varies very much and can achieve values up t o 155 mg.kg-1. TR and SR contents are low (about 10 mg.kg-1). SM varies from 20 to 40 mg.kg-1 in finished products, and AG was not detected anymore at the end of the production process.

In order to evaluate relations between microbiological data and chemical analyses, the counts of lactobacilli and some psychrotrophic bacteria were observed during ripening. During ripening, the Lactobacilli count increased from 104 - 105 to 107 - 108 during the 1st week. Then a slow decrease followed. During the whole ripening process, lactobacilli that utilise Arginine were not identified. At the beginning of the ripening, contaminants of the Citrobacter, Klebsiella, Hafnia, E. coli, Enterobacter, Serratia, Proteus bacteria, etc. were identified in the batter. After a week there was no finding of the genus Proteus, and Enterobacteriaceae were detected in very small amounts only (10 . mL-1). After two week s of ripening no other microorganisms, except for Lactobacilli were identified. Thus the influence of the starter culture is manifested, as well as its ability to suppress growth of those microorganisms that are critical from the hygienic and or technologic al point of view.

CONCLUSIONS

From the results of the study it follows that in a quickly ripened sausage produced with addition of a starter culture, the increase of BA content during the ripening process is very important. Therefore consuming larger amounts of fermented sausages coul d cause health problems in selected groups of the population. Owing to the decarboxylases production by the contaminating microorganisms, the production technology and general sanitation level of production areas have significant influence on BA content. The addition of a starter culture has positive influence on the suppression of the contamining microorganisms in the most sensitive phase of ripening during the first week after stuffing. BA concentrations in Czech fermented sausages do not reach the limits t hat are dangerous for a common consumer. This is the result of achieved Good Manufacturing Practice (GMP) and proper attention paid by the producer to the quality of these products that belong to the category of products that are exacting as far as technol ogy and price are concerned.

Acknowledgements: The authors wish to thank LASKA PRAHA for financial support, Dr. P. Lny for technical cooperation at the lactobacilli determination, and Dr. E. Urbanov, for her help at other microbiological examinations.

REFERENCES

1. STRATON, J. A. - HUTKINS, R. W. - TAYLOR, S. L., 1991. Biogenic amines in cheese and other fermented foods. A review. J. Food Protect., 54, 460 - 470.

2. NAHODILOV<\A>, V. - LT, J., 1988. Hygienic signification of biogenic amines. Veterinstv., 38, 180 - 181.

3. BRINK ten, B. - DAMINK, C. - JOOSTEN, H. M. L. J. - HUIS in't VELD, J. H. J., 1990. Occurrence and formation of biologically active amines in foods. Intern. J. Food Microbiol., 11, 73 - 84.

4. NAKAMURA, M. - WADA, Y. - SAWA, H. - KAWABATA, T., 1979. Polyamine content in fresh and processed pork. J. Food Sci., 44, 515 - 523.

5. MIRNA, A. A., 1970. Versuche zur Berechnung der Wasseraktivitt von Fleischwaren aus analytischen werten. Fleischwirtschaft, 50, 831 - 833.

6. WORTBERG, B. - WOLLER, R., 1982. Zur Qualitt und Frische von Fleisch und Fleischwaren in Hinblick auf ihren Gehalt an biogenen Aminen. Fleischwirtschaft, 62, 1457 - 1463.

7. SUZUKI, S. - KOBAYASHI, K. - NODA, J. - SUZUKI, T. - TAKAMA, K., 1990. Simultaneous determination of biogenic amines by reversed - phase high - performance liquid chromatography. J. Chromatogr., 508, 1990, 225 - 228.