Z Lebensm Unters Forsch A (1997) 205: 351–355

ORIGINAL PAPER

Susanna Eerola · Artur-Xavier Roig Sagués
Leena Lilleberg · Heljä Aalto

Biogenic amines in dry sausages during shelf-life storage

Received: 4 April 1997

Abstract The formation of biogenic amines in dry sausages after ripening was studied. Four batches of dry sausages were provided by a manufacturer after slicing and vacuum-packaging and were stored at +4 °C and +10 °C. Biogenic amines (tyramine, histamine, tryptamine, phenylethylamine, putrescine, cadaverine, spermine and spermidine) were analysed 4 times during the 58-day storage time. Dry sausages were also evaluated according to their sensory acceptability, pH values and contents of thiobarbituric acid and volatile nitrosamines. Tyramine and putrescine were formed during the storage period and differences between batches were detected. The sensory quality remained acceptable throughout the trial. The slight decrease in sensory scores during the storage time could not been explained either by increased tyramine and putrescine levels or by other chemical parameters. The results of this study demonstrate that contamination by amine-producing bacteria and/or amine formation can also occur after the fermentation process of dry sausages.

Key words Biogenic amines · Dry sausages · Shelf-life · Quality control

S. Eerola (✉)
National Veterinary and Food Research Institute,
Department of Chemistry, P.O. Box 368 (Hämeentie 57),
FIN-00231 Helsinki, Finland

A.-X. Roig Sagués
U.D. Higiene, Inspecció i Control dels Aliments,
Facultat de Veterinária, Universitat Autònoma de Barcelona, Spain

L. Lilleberg
National Veterinary and Food Research Institute,
Department of Milk Hygiene, P.O. Box 368 (Sirrikuja 1),
FIN-00231 Helsinki, Finland

H. Aalto
HK-Ruokatalo, PL 49, FIN-01511 Vantaa, Finland

Introduction

Small quantities of biogenic amines (BA) are commonly found in various foods [1]. The manufacture of dry sausages may give rise to the formation of BA, mainly due to microbes present in the sausages. High levels of BA in dry sausages have been reported in surveys since the 1970s [2–5]. The main concern is related to their toxic properties. Histamine is the amine most studied with regard to its toxicological effects and on the basis of data from food intoxication outbreaks a legal upper limit of 100 mg histamine/kg food has been proposed [6]. Less is known about the toxic doses of other amines, and toxicity threshold values as varied as 100–800 mg/kg have been reported for tyramine. The diamines putrescine and cadaverine do not have adverse health effects similar to those of the vasoactive amines (tyramine, histamine, tryptamine, phenylethylamine), but they are known to potentiate the effects of histamine by inhibiting the detoxifying enzymes diamine oxidase and hydroxymethyl transferase [7]. Furthermore, their accumulation is related to the microbial deterioration of quality [8]. An additional health risk is the possible role of BA as precursors of nitroso compounds. Secondary amines are known to form carcinogenic *N*-nitrosamines by reaction with nitrosating compounds. However, as primary amines BA can convert to secondary amines, not only on heating but also during storage at room temperature, and further reaction with nitrite can occur [9].

Prerequisites for BA formation are the availability of free amino acids, the presence of decarboxylating microorganisms, either derived from environmental contamination or from an added starter culture, and conditions supporting the growth and activity of amine-producing bacteria [1]. The major protease activity is derived from endogenous meat enzymes [10], which provide free amino acids as substrates for amine accumulation.

Methods for preventing the formation of BA aim at eliminating and/or deactivating the decarboxylating microbes in dry sausages, i.e. the use of high-quality raw materials, amine-negative starter cultures and processing

conditions which favour the growth of the starter strains [11, 12].

It is common practice in the sausage manufacturing industry to distribute and store dry sausages sliced in vacuum-packages. Current vacuum-packaging technology enables sliced products to remain acceptable for consumption for approximately 2 months at temperatures < 15 °C. The effect of vacuum-packaging on amine accumulation has been studied using stored fresh meat [8, 13, 14]. Changes in amine concentration during the storage of vacuum-packed meat at chill temperatures are restricted to increases in putrescine, cadaverine and tyramine, which are proposed as objective indicators of spoilage.

In this study the effects of slicing and vacuum-packaging of dry sausages on the formation of BA were studied. Sausages were also analysed by their sensory acceptability and pH values. Thiobarbituric acid values (TBA) were determined in order to evaluate the possible change in quality associated with lipid oxidation. The determination of volatile nitrosamines was included in this study with the aim of evaluating the role of BA in their formation.

Materials and methods

Sausages. The dry sausage samples (salami) were provided from a local meat plant from four successive batches (A–D) of the same product (same recipe and ripening process, different raw material batches, different day of manufactur). A reference sample was taken from each batch of ready sausage before the slicing procedure. The sausages were sliced and vacuum packaged in the meat plant and a 150-g package of sausage was always taken as a sample for chemical and sensorial analyses. The storage temperatures were +4 °C and +10 °C.

Sampling. Sampling times were 1, 7, 30 and 58 days (expiration date) after slicing. The samples were homogenized and frozen at –22 °C on the sampling date. The TBA and pH values were determined and sensory analyses were carried out on the sampling date.

Chemicals. All chemicals and solvents were of analytical or HPLC grade. Water was obtained from a Milli-Q UF water purification system (Millipore).

Biogenic amines. BA were extracted from 2.0 g of sample with 0.4 M perchloric acid (Merck) and detected as their dansyl derivatives by HPLC [15]. The reproducibilities of the method (relative standard deviations) when the same sample was analysed six times were: tyramine 3.8%, histamine 1.3%, phenylethylamine 5.3%, putrescine 0.65%, cadaverine 2.0%, spermidine 2.3% and spermidine 2.7%. The limits of determination were 1 mg/kg for tyramine, histamine, phenylethylamine, putrescine, cadaverine, spermidine and spermine and 10 mg/kg for tryptamine. Samples were analysed in duplicate.

TBA values. TBA numbers were determined from 10.00 g of sample with a modification of the method of Raharjo et al. [16]. The sample was homogenized twice with cold 5% trichloroacetic acid (Merck, 30+20 ml) and the supernatants after centrifugation (10 min at 2730 g) were filtered through filter paper. The volume after filtration was adjusted to 50 ml with 5% trichloroacetic acid. A 5-ml sample of this extract was allowed to react with 5 ml of 80 mM TBA solution (BDH Laboratory) at 94 °C for 5 min. The reaction mixture was cooled and extracted with 3 ml 1-butanol (Merck) and the phases were separated by centrifugation (5 min at 3120 g). The 1-butanol phase was measured spectrophotometrically at 534 nm against a reagent blank prepared simultaneously. TBA numbers were calculated by multiplying the absorbance values by the constant coefficient as described by Witte et al. [17]. The mean recovery of the method was 71% and the linear working area was determined for malonaldehyde (Fluka) over the range of 0.5–10 μM.

Nitrosamines. Volatile nitrosamines: *N*-nitrosodimethylamine (NMEA, Supelco), *N*-nitrosopyrrolidine, NPYR, Sigma), *N*-nitrosodiethylamine (NDEA, Sigma) and *N*-nitrosopiperidine (NPIP, Sigma) were determined with our previously described method [18]. Briefly, 10.00 g of sample was weighed together with 300 mg propyl gallate (Sigma) and spiked with 0.1 μg internal standard *N*-nitrosodipropylamine (NDPRA). Then, 15 g Celite (Fluka) and 15 g Na_2SO_4 (Merck) were added and the sample was extracted with 50 ml dichloromethane (DCM, J. T. Baker). The sample was concentrated in a rotavapor and dissolved in n-pentane and a clean-up step was performed using a silica column with pentane-DCM and analytes were eluted with DCM-ether. Effluent was evaporated in a rotavapor and the sample residue was dissolved in 50% methanol (J. T. Baker). The sample was filtered before liquid chromatographic-atmospheric pressure chemical ionization mass spectrometric analyses. The reproducibilities of the quantification (relative standard deviation) for five replicate analyses of 5 ng injected were 3.2% for NMEA, 6.6% for NPYR, 8.9% for NDEA, 4.7% for NPIP and 2.7% for NDPRA. The limits of determination were 0.5 μg/kg (NMEA), 1.0 μg/kg (NPYR) and 0.2 μg/kg (NDEA, NPIP).

pH. pH was measured directly from homogenized samples using a WTW pH 537 meter equipped with an Ingold DXK-S7/25 electrode.

Sensory analysis. A sensory panel of six trained panellists was used for evaluation of the acceptability of the sausage samples and of the differences between the two storage temperatures. The panellists were asked to give scores on a numerical scale with an accuracy of 0.5 from 1 to 5. Scores were defined as follows: 5 = excellent quality, 4 = good quality, 3 = acceptable quality, slight defect, 2 = distinct defect, the limit of trading quality, 1 = unacceptable quality. The differences between the two storage temperatures were tested with the duo-trio test [19]. The sausage samples were analysed in a randomized order using three-number coding and two samples (samples from the same batch but with different storage temperatures) were analysed at the same panel session.

Statistics. BA levels, statistical scores and pH were analysed by one-way analysis of variance and by the *t*-test. Calculations were performed using the Microsoft EXCEL 4.0 Analysis Tools.

Results

Histamine, phenylethylamine, spermidine and spermine were not formed during the storage of sliced vacuum-packaged dry sausages. The concentrations of these amines were in the following ranges in all the samples assayed: histamine < 1–2.9 mg/kg, phenylethylamine < 1–5.3 mg/kg, spermidine 3.8–6.9 mg/kg and spermine 34–44 mg/kg. Neither was cadaverine formed in batches A–C, which initially contained < 1–5.6 mg/kg cadaverine. Accumulation of cadaverine did occur, although modestly, in the samples of batch D, i.e. from 2.7 mg/kg (the reference sample) to 14 mg/kg (the expiration date samples). Tryptamine was detected at concentrations ranging up to 86 mg/kg, but its concentration varied within batches and the different storage temperatures could not explain the irregular tryptamine levels measured during the trial. The highest tryptamine contents were detected in samples taken before the expiration date, which indicates that some decomposition of tryptamine may have occurred.

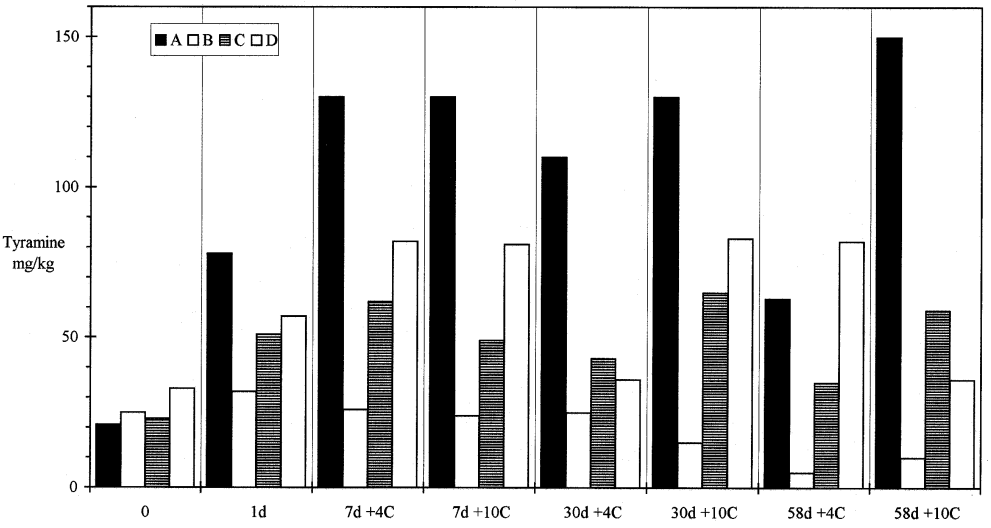

Fig. 1 Levels of tyramine (mg/kg) in dry sausages during the trial. (*A*–*D* are the different sausages batches)

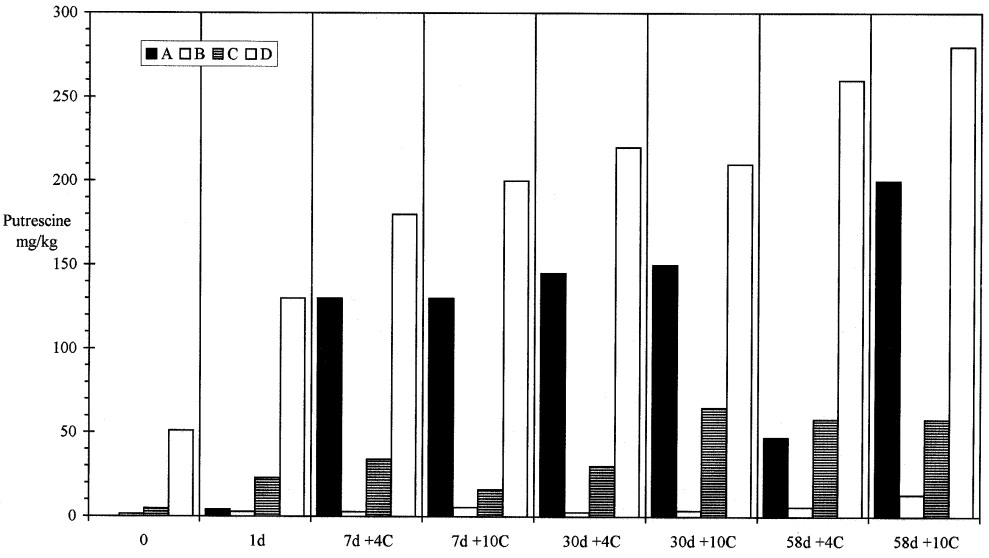

Fig. 2 Levels of putrescine (mg/kg) in dry sausages during the trial

Tyramine and putrescine were formed during the storage period (Fig. 1 and 2). Differences between different batches were significant ($P < 0.001$). The reference sausages from all the batches contained tyramine at the same level (21–33 mg/kg). Putrescine was determined in one batch at a concentration of 51 mg/kg but in all the others at less than 5 mg/kg. During the storage after slicing and vacuum-packaging the highest concentration of putrescine was 280 mg/kg and that of tyramine 150 mg/kg. There were no differences in the formation of these amines between the two storage temperatures.

In the determination of TBA numbers the absorbance readings remained below the linear range of quantitation and the calculated TBA numbers were < 0.2 for all samples.

The mean pH value of samples was 4.7 ± 0.12 (SD). There were no significant differences ($P > 0.05$) between batches.

The results of the sensory analyses supported those of the chemical determinations: the results of the duo-trio test showed no sensory differences ($p > 0.05$) between samples stored at +4 °C or +10 °C. The sensory scores given to the samples are presented in Fig. 3. Differences between storage temperatures and between batches were not significant ($P > 0.05$), with the exception of the 30-day samples, which had significant differences between batches ($P < 0.05$). When the scores of these samples were considered alongside the analysis results for putrescine and tyramine there was an apparent positive correlation between sensory scores and putrescine levels in sausages. However, when considering all samples, the significant decrease ($P < 0.05$) in sensory scores during the storage period cannot be explained by the formation of BA because the B-batch did not show an increase in BA levels.

Nitrosamines levels in the reference and expiration date samples were analysed, and were found to be below the limit of determination in the reference samples, except in the sample of C-batch which contained 3 µg/kg NPYR. Also, the expiration date samples did not contain NMEA, NDEA or NPIP, but NPYR was found at variable concen-

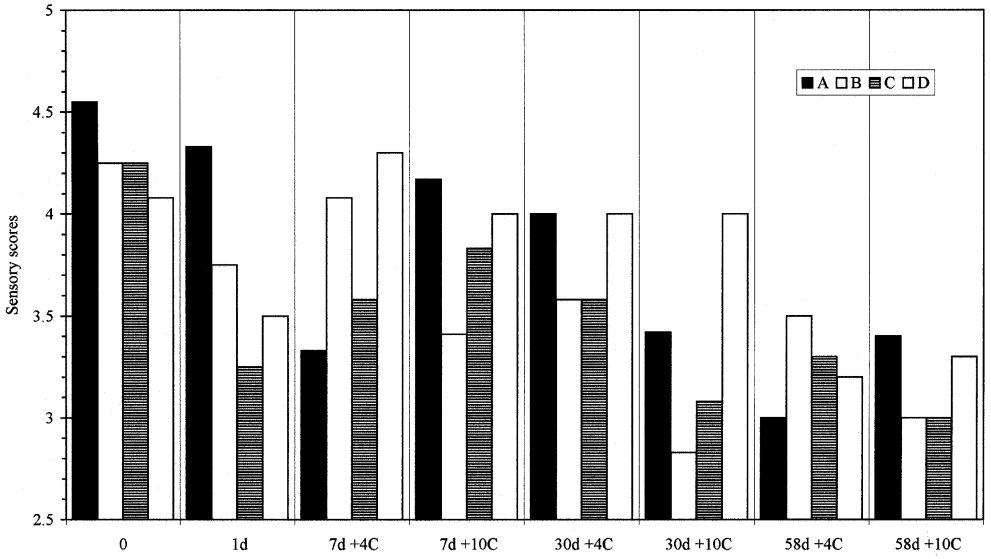

Fig. 3 Sensory scores for dry sausages during the trial

trations. The highest concentration, 8 μg/kg, was detected in the sample of D-batch stored at +10 °C, which also contained the highest amount of putrescine. Our recent study showed an apparent correlation between putrescine and NPYR levels in dry sausages [18]. Other positive samples were in the range of 1.6–2.9 μg/kg. B-batch samples contained NPYR below the limit of determination.

Discussion

The sensory quality of dry sausage samples remained acceptable throughout the storage trial. The slight decrease in sensory scores could not been explained by the chemical parameters studied. Only the 30-day samples showed significant differences between batches and the given scores correlated positively with the level of putrescine, indicating that higher concentrations of putrescine resulted in a more acceptable flavour. However, the correlation between the sensory quality and levels of BA in dry sausages were not observed in other samples. Furthermore, the observed correlation is contradictory to the suggested undesirable flavour of diamines [20]. However, it has also been proposed that BA may be a component of the characteristic flavour of dry sausages [5]. In the case of tyramine, vacuum-packaged beef is reported to be sensorily acceptable with tyramine levels up to 50–100 mg/kg [13]. Our study also showed that, at the detected levels, tyramine had no effect on the sensory quality. More research is needed to define sensorially acceptable maximum values of BA.

The formation of tyramine and putrescine, as measured in this study, showed that the factors affecting the sausage-ripening process are not the only parameters that must be controlled to avoid production of BA. There are two possible explanations for the formation of amines after the manufacture of sausages: highly probable is de novo contamination by decarboxylase-active microbes during the slicing procedure, but vacuum-packaging can also offer favourable anaerobic conditions for the activity of amine-producing bacteria originating from sausage manufacture.

Putrescine is associated with the deterioration of microbiological quality in vacuum-packaged stored meat, but the simultaneous occurrence of cadaverine at a higher concentration than putrescine is also reported in such cases [8, 21]. Levels of these diamines increase in stored meat before the maximum bacterial numbers are attained and before any off-odours are detected. In the present study only minor amounts of cadaverine were formed in one batch, indicating the absence and/or inactivity of Enterobacteriaceae, which are known cadaverine formers and common indicators of microbiological quality [21]. If these diamines are used as indicators of the freshness of meat and in the case of dry sausages as indicators of contamination during the whole manufacturing process, it can be suggested that both putrescine and cadaverine should be included in the estimation of quality.

Tyramine was observed to reach its maximum levels within 1 week of storage time after slicing and packaging, and there was also a slight decrease of the tyramine level during the trial. Putrescine showed a slow increase throughout the storage period. One reason for the different behaviours of tyramine and putrescine could be the availability of free amino acids as a substrate. Low levels of free tyrosine are reported to affect the further formation of tyramine in dry sausages [12], whereas arginine, a precursor of putrescine, is detected in higher concentrations in dry sausages.

Previous studies of meat samples showed that tyramine is not abundant in the samples until the bacterial numbers approach 10^6 colony-forming units (cfu) per gram [13, 21]. Our recent study of minced meat samples showed accelerated tyramine formation after lactic acid bacterial numbers reached 10^7 cfu/g [22]. Tyramine has been proposed as an indicator of the deteriorated quality of food because it is known to correlate with storage time and microbiological counts [22]. The role of tyramine as an indicator in fermented sausages cannot have been based only on micro-

biological counts because lactic acid bacteria, after starter addition, reach levels of 10^7-10^8 cfu/g. However, the formation of tyramine in dry sausages is a sign of undesirable microbial action, which could be avoided by good manufacturing practice.

The maximum value of tyramine in this study was 150 mg/kg. Ingestions of 10–100 mg can induce migraine in normal indivuals [23] and tyramine is reported to cause food toxicity in the concentration range of 22–150 mg/kg [24]. Although the toxicity of tyramine is beyond all doubt, it is very difficult to determine the exact toxicity threshold of this compound in foods. The toxic dose is strongly dependent on the efficiency of detoxification, which can be decreased by other amines, alcohol and monoamineoxidase-inhibiting drugs. The recommended maximum level of tyramine in foods has been proposed variously to be in the range of 100–800 mg/kg [6], which must be considered as too imprecise. The toxicities of diamines are much lower than those of tyramine or histamine; however, there is evidence to suggest their precursor role in the formation of carcinogenic nitrosamines by reaction with nitrite [9, 18].

It is concluded that formation of BA can also occur after the fermentation of dry sausages, either by microbial contamination during slicing and packaging and/or by activation of amine-producing bacteria under the anaerobic conditions of vacuum-packages. Thus, dry sausages without BA indicate hygienic conditions and good manufacturing practice throughout the process. For this reason the determination of BA should be included in the quality control of these products. Another equally important reason is to provide good-quality products to consumers which pose no health risk.

Acknowledgements This study was supported by the Academy of Finland and the European Commission, Agriculture and Fisheries Program (FAIRCT 965032).

References

1. Askar A, Treptow H (1986) Biogene Amine in Lebensmitteln: Vorkommen, Bedeutung und Bestimmung. Eugen Ulmer, Stuttgart
2. Vandekerckhove P (1977) J Food Sci 42: 283–285
3. Wortberg B, Woller R (1982) Fleischwirtschaft 62: 1457–1463
4. Vidal-Carou MC, Izquierdo-Pulido ML, Martín-Morro MC, Mariné-Font A (1990) Food Chem 37: 239–249
5. Scheuer R, Rödel W (1995) Fleischwirtschaft 75: 73–75
6. ten Brink B, Damink C, Joosten HMLJ, Huis in't Veld JHJ (1990) Int J Food Microbiol 11: 73–84
7. Chu CH, Bjeldanes LF (1981) J Food Sci 47: 79–80, 88
8. Edwards RA, Dainty RH, Hibbard CM (1985) J Appl Bacteriol 58: 13–19
9. Warthesen JJ, Scanlan RA, Bills DD, Libbey LM (1975) J Agric Food Chem 23: 898–902
10. Johansson G, Molly K, Geenen I, Demeyer D (1996) Optimisation of endogenous and bacterial metabolism for the improvement of safety and quality of fermented meat products. Proceedings of an European AIR Project AIR2-CT94-1517 Workshop at the 42nd ICoMST, pp 6–16
11. Maijala R, Nurmi E, Fischer A (1995) Meat Sci 39: 9–22
12. Eerola S, Maijala R, Roig Sagués AX, Salminen M, Hirvi T (1996) J Food Sci 61: 1243–1246
13. Smith JS, Kenney PB, Kastner CL, Moore MM (1993) J Food Prot 56: 497–500
14. Krizek AR, Smith JS, Phebus RK (1995) J Food Prot 58: 284–288
15. Eerola S, Hinkkanen R, Lindfors E, Hirvi T (1993) J Assoc Off Anal Chem Int 76: 575–577
16. Raharjo S, Sofos JN, Schmidt GR (1993) J Food Sci 58: 921–924, 932
17. Witte VC, Krause GF, Bailey ME (1970) J Food Sci 35: 582–585
18. Eerola S, Otequi-Aramburu I, Saari L, Rizzo A (1997) Food Addit Contamin (in press)
19. ISO-standard no. 10399 (1991) Duo-trio test.
20. Slemr J (1981) Fleischwirtschaft 61: 921–926
21. Edwards RA, Dainty RH, Hibbard CM, Ramantanis SV (1987) J Appl Bacteriol 63: 427–434
22. Roig Sagués AX, Eerola S (1997) Z Lebensm Unters Forsch (in press)
23. Edwards ST, Sandine WE (1981) J Dairy Sci 64: 2431–2438
24. Maijala R, Eerola S, Hirn J (1996) Suom Eläinlääkäril 102: 75–80