ELSEVIER

PII: S0308-8146(97)00021-6

Food Chemistry, Vol. 60, No. 4, pp. 521–526, 1997

0308-8146/97 $17.00+0.00

Formation of biogenic amines during the maturity process of raw meat products, for example of cervelat sausage

E. Treviño, D. Beil & H. Steinhart

Institute of Biochemistry and Food Chemistry, Department of Food Chemistry, University of Hamburg, Germany

(Received 18 April 1996; revised version received 26 November 1996; accepted 5 December 1996)

The raw meat product cervelat sausage with a diameter of 90 mm was investigated during maturation and storage over a period of about 12 weeks. The examination was carried out by ion exchange chromatography (amino acid analyzer) and included the amines ethylamine, propylamine, butylamine, putrescine, histamine, cadaverine, tyramine, β-phenylethylamine, spermidine and spermine. In addition, the starter bacteria cultures varied according to manufacturer. Over the duration of investigation, five different production batches were observed in parallel. During the maturity process and storage, after 87 days, tyramine (58.9 mg kg^{-1}) showed a clear dominance, followed by spermine (34.1 mg kg^{-1}), putrescine (26.1 mg kg^{-1}) and spermidine (8.7 mg kg^{-1}), if starter bacteria culture 1 was used. Other amines were not detected. The use of starter bacteria culture 2 led, after 87 days, to the highest content of putrescine (244.1 mg kg^{-1}), followed by tyramine (119.2 mg kg^{-1}), histamine (52.9 mg kg^{-1}), spermine (37.5 mg kg^{-1}) and spermidine (9.2 mg kg^{-1}). With the use of starter bacteria culture 3, for spermine and spermidine a comparable behavior was shown to that with culture 1 or 2. After 87 days, putrescine reached the highest value of 484.2 mg kg^{-1}, followed by tyramine (119.4 mg kg^{-1}) and histamine (111.0 mg kg^{-1}). Cadaverine (36.9 mg kg^{-1}) could also be determined. As an accompanying investigation, the parameters pH, water activity (a_w) and microbial count (lactobacilli, enterobacteria, staphylococci and yeast) were determined. An investigation of the sections center-middle-edge showed that the content of amines increases to its highest values in the middle. The edge showed the lowest content. This behavior correlates with the a_w distribution of a raw sausage. © 1997 Elsevier Science Ltd

INTRODUCTION

Biogenic amines are organic bases of low molecular weight with the structure of aliphatic, aromatic or heterocyclic compounds which will be formed and metabolized in the normal metabolism of human beings, animals, plants and microorganisms, and they are natural components of many foods. Amines occur in high concentrations, particularly in fermented products with high levels of protein. Because of their possible toxic effects, which become clinically manifest as reddening of the skin, itching, dizziness, stomach trouble and migraine (Lüthy & Schlatter, 1983), the occurrence of biogenic amines as a criterion in the quality control of foods has been discussed (Treptow & Askar, 1987). In this context, the problem of the formation of carcinogenic nitroso amines has been mentioned (Patterson & Mottram, 1974). However, it must be taken into account that biogenic amines shape the typical and characteristic odor and taste of mature foods and that they are precursors of certain aroma substances (Askar & Treptow, 1989). In the last few years attention has turned to the formation of biogenic amines during production, maturation, storage and decay by biochemical and microbiological shaping of food with high levels of protein (Binder & Brandl, 1983). Compared with the amounts in fish and cheese, there are only low biogenic amine contents in meat and meat products (Németh-Szerdahelyi *et al.*, 1993; Petridis & Steinhart, 1996*a*,*b*).

Cervelat sausage is a typical raw meat product in Germany. The target of this investigation was to examine the formation of biogenic amines and the change of the parameters pH, water activity and microbial count (lactobacilli, enterobacteria, staphylococci and yeast) in the fat reduced raw meat product cervelat sausage dependent on time over a duration of about 12 weeks (maturation/storage). In addition, the starter bacteria

Table 1. Sampling of cervelat sausage (storage at + 7°C)

Sample no.	Days of maturity process/storage	Step of production/storage
1	2	1st (production)
2	3	2nd (production)
3	5	3rd (production)
4	7	4th (production)
5	14	5th (production)
6	17	6th (production, day of packaging)
7	45	1st (storage)
8	59	2nd (storage)
9	73	3rd (step of storage)
10	87	4th (storage)

cultures varied according to manufacturer. An investigation of the sections (center-middle-edge) was also carried out. The analysis of the biogenic amines occurred by ion exchange chromatography (amino acid analyzer) as described previously (Treviño *et al.*, 1996).

MATERIALS AND METHODS

The sample material of fatreduced cervelat sausage with a diameter of 90 mm was used over the duration of maturation and storage for about 12 weeks. The ingredients of the product were beef, pork, bacon, nitrite pickle salt, sugar, spice, ascorbic acid and smoke. The sampling occurred at different points during this period. The details of sampling are shown in Table 1. Samples of five different production batches were taken. The sausage mixture of one packing (100 g) for each sampling was used. The extraction of amines with trichloroacetic acid and the clean up with a cation exchange column were carried out according to the method which is described by Treviño *et al.* (1996). The starter bacteria cultures (SAGA, Microlife Technics) varied between the production batches according to manufacturer. The following three different groups were formed:

1st production batch	19 g *Pediococcus pentosaceus* (SAGA 75), 19 g *Staphylococcus carnosus* (SAGA 448) for 270 kg meat = starter bacteria culture 1
2nd production batch	dilutions of the starter bacteria culture of the 3rd, 4th and 5th production batches = starter bacteria culture 2
3rd, 4th and 5th production batches	4 g *Pediococcus pentosaceus* (SAGA 75), 19 g *Staphylococcus carnosus* (SAGA 448), 19 g *Pediococcus acidilactici*, *Micrococcus varians* I (SAGA III) and 250 g water for 270 kg meat = starter bacteria culture 3

Parallel to the examination of the total product, the amine contents in relation to the distance between center and edge of the sausage were determined (investigation of the sections). In this case the cervelat sausage was divided into three sections (Fig. 1). The analysis was carried out by an amino acid analyzer (Beckman model 119 BL). The precise description of the method with details of retention time, determination level, precision, linearity and recovery is given by Treviño *et al.* (1996). The sample analysis occurred with five-fold determinations (measurements) of each sample extract.

Other more sensitive methods for the determination of amines such as RP-HPLC after automated pre-column derivatisation with *o*-phthaldialdehyde (Petridis & Steinhart, 1995) were practicable in principle. However, because of its sufficient sensitivity and the possible inclusion of the amines spermine and spermidine, ion exchange chromatography with ninhydrine post-column derivatisation was preferred.

The following investigations were carried out only on the total product and not on the sections:

- The pH of the homogeneous, warmed sample, at room temperature was determined with a pH-electrode (WTW).
- The water activity was determined by a special apparatus (Lufft), which works on the principle of a hair hygrometer.
- The microbiological examination proved the presence of lactobacilli (growth on ROGOSA-agar, Merck); staphylococci (growth on BAIRD-PARKER-agar, Merck); enterobacteria (growth on violet-red-bile-agar, Merck), and yeast (growth on malt-agar, Merck).
- In the process, the steps 2 (3rd day), 6 (17th day), 7 (45th day), and 9 (73rd day) were used for the investigation. The choice of microbes followed from already completed examinations (Tschabrun *et al.*, 1990).

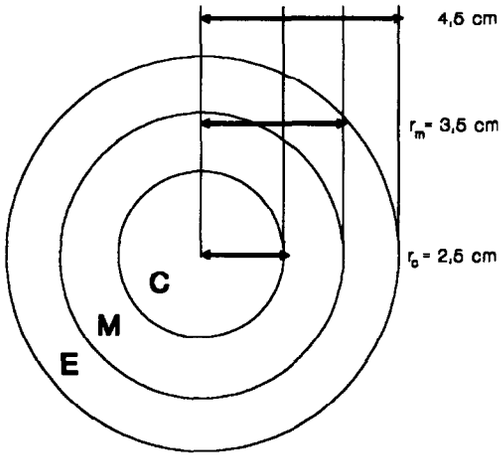

Fig. 1. Diameter of the sausage. Center (C): radius r_c = 2.5 cm, middle (M): sausage mixture between the radius of r_m = 3.5 cm and r_c = 2.5 cm edge (E): sausage mixture outside the radius r_m = 3.5 cm.

RESULTS AND DISCUSSION

All production batches showed a decrease of the pH values below pH = 5.0 (Fig. 2) until a slight increase was observed which is traceable to the breakdown of lactic acid and the almost complete metabolism of the added sugar (glucose as substratum for the bacteria) in the sausage (Klettner & List, 1978; Klettner & Rödel, 1979; Rödel & Stiebing, 1987).

Figure 3 shows that the a_w values in all batches decreased from about 0.97 in the beginning to about 0.92 after 87 days as a result of the increased dry-up during storage. The behavior of this parameter corresponds to the course which is described by Rödel *et al.* (1980) and Rödel & Stiebing (1987).

The microbial counts of lactobacilli, staphylococci, enterobacteria and yeast were combined as means, due to their great similarity between the three groups (Fig. 4).

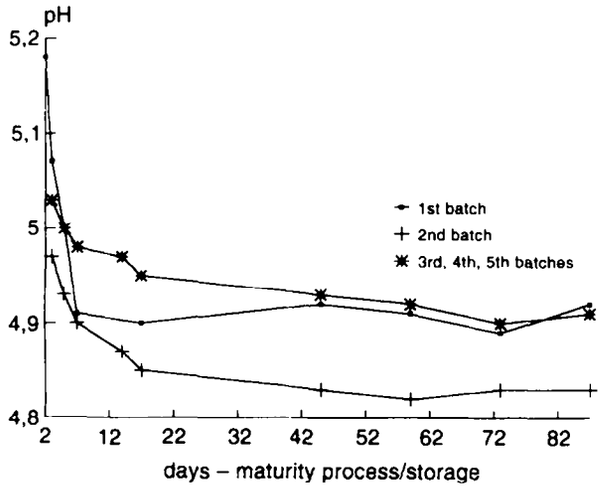

Fig. 2. Development of pH during maturity process and storage of cervelat sausage. (The values of the 3rd, 4th and 5th production batches were combined as means, due to their great similarity).

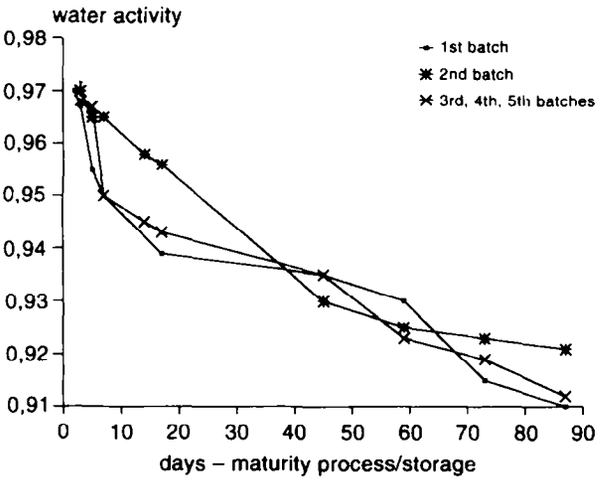

Fig. 3. Development of water activity during maturity process and storage of cervelat sausage. (The values of the 3rd, 4th and 5th production batches were combined as means, due to their great similarity).

The microbial count of lactobacilli showed a decreasing tendency as a result of the increasing consumption of substratum; yeast, an increasing tendency until the third day. Clear zone forming black staphylococci were at first detectable after 45 days, which was possibly caused by an unsterile packaging method. The microbial count of clear zone forming black staphylococci increased continuously in the successive steps. Enterobacteria were no longer detectable after 17 days.

The previously discussed results refer in their entirety to all production batches. The dependence of the basic parameters on the kind of starter bacteria culture is to be viewed as insignificant. In the case of the biogenic amines, the investigation shows a clear connection between the quantitative relation of biogenic amines to each other, to the dimension of their concentrations and to the starter bacteria culture used. Common to all amines is a continuous increase in concentration observed over the whole duration of the investigation.

In the first production batch, tyramine (58.9 mg kg^{-1}) predominated after 87 days, followed by spermine (34.1 mg kg^{-1}) and putrescine (26.1 mg kg^{-1}) (Fig. 5).

It is a feature of the second production batch that putrescine (244.1 mg kg^{-1}) and tyramine (119.2 mg kg^{-1})

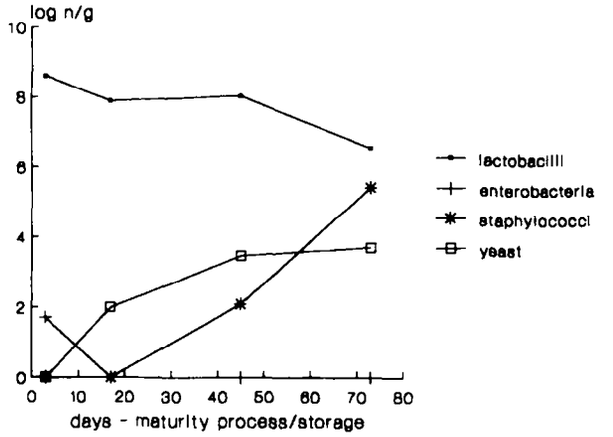

Fig. 4. Development of microbes during maturity process and storage of cervelat sausage (means of the three groups).

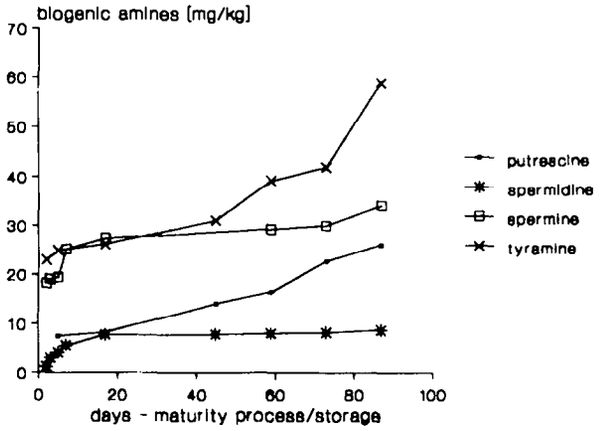

Fig. 5. Development of biogenic amines during maturity process and storage of cervelat sausage — 1st production batch.

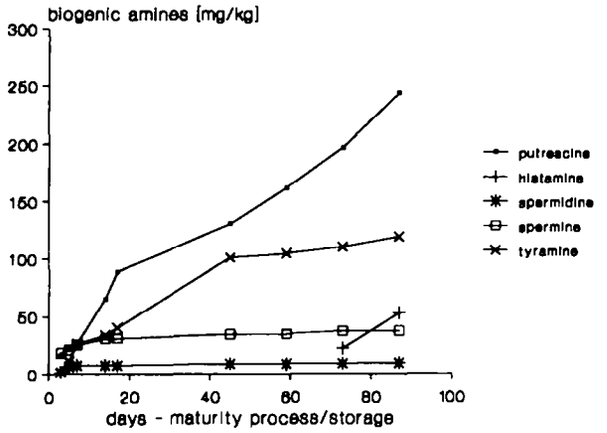

Fig. 6. Development of biogenic amines during maturity process and storage of cervelat sausage — 2nd production batch.

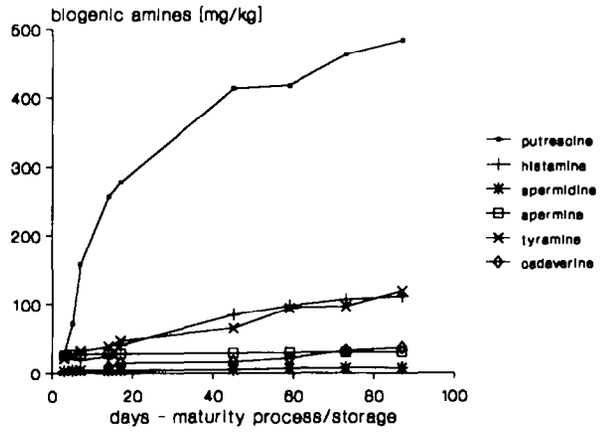

Fig. 7. Development of biogenic amines during maturity process and storage of cervelat sausage – 3rd, 4th and 5th production batches (means of the 3 production batches).

reached higher contents after 87 days. In addition to that, it was possible to determine histamine after 73 days. The histamine content increased after 87 days to 52.9 mg kg^{-1} (Fig. 6). The values of the third, fourth and fifth production batches were combined as means, due to their great similarity. The behavior of the third, fourth and fifth grouped production batches was similar to that of the second production batch in the case of tyramine. After 87 days, putrescine reached a value of 484.2 mg kg^{-1}, followed by tyramine (119.4 mg kg^{-1}) and histamine (111.0 mg kg^{-1}). Histamine was already detectable after seven days, compared with 73 days for the second production batch. In addition, it was possible to determine cadaverine after 14 days. After 87 days, cadaverine reached a content of 36.9 mg kg^{-1} (Fig. 7).

For spermidine and spermine, in all three groups, similar values resulted after 87 days: the fluctuation for spermine was between 30.8 and 37.5 mg kg^{-1} and for spermidine between 7.9 and 9.2 mg kg^{-1}.

Table 2 shows in an exemplary manner the means and the precisions of the five-fold determined amines of the second production batch. The precisions range between 0.5 and 5.0%. Due to the similarity in the precisions of all production batches, the following descriptions omit their representation.

The investigation of the pure starter bacteria cultures in a dilution, comparable with the sample, shows that biogenic amines will not be carried over in the product in a detectable amount. However, if artificially high amounts of the starter bacteria cultures are used for examination according to the sample content, biogenic amines are detected with a total content of more than 100 mg kg^{-1}. The examination of the sections shows that the amine content of the middle is the highest,

Table 2. Results and precision of the determination of amines in cervelat sausage (2nd production batch), $n = 5$

Step	Day	Putrescine	Histamine	Tyramine	Spermidine	Spermine
		Mean ± s (mg kg^{-1}) Precision (%)	Mean ± s (mg kg^{-1}) Precision (%)	Mean ± s (mg kg^{-1}) Precision (%)	Mean ± s (mg kg^{-1}) Precision (%)	Mean ± s (mg kg^{-1}) Precision (%)
1	2					
2	3	16.0 ± 0.5 (3.1)			1.2 ± 0.03 (2.5)	17.6 ± 0.6 (3.4)
3	5	21.9 ± 0.9 (4.1)		10.7 ± 0.4 (3.7)	6.4 ± 0.2 (3.1)	20.5 ± 0.7 (3.4)
4	7	27.0 ± 1.2 (4.4)		25.8 ± 0.9 (3.5)	7.5 ± 0.1 (1.9)	25.0 ± 0.7 (2.8)
5	14	65.5 ± 1.8 (2.7)		33.3 ± 1.6 (4.8)	7.6 ± 0.1 (1.3)	30.4 ± 0.4 (1.3)
6	17	88.7 ± 3.3 (3.7)		39.5 ± 1,7 (4.3)	7.6 ± 0.4 (4.9)	30.8 ± 0.2 (0.5)
7	45	132.7 ± 4.8 (3.6)		102.0 ± 4.5 (4.4)	8.1 ± 0.3 (3.7)	34.0 ± 1.5 (4.4)
8	59	160.4 ± 6.4 (4.0)		104.6 ± 2.6 (2.5)	8.8 ± 0.4 (4.5)	34.8 ± 1.5 (4.3)
9	73	194.3 ± 9.7 (5.0)	21.7 ± 0.4 (1.8)	110.5 ± 4.1 (3.7)	8.8 ± 0.2 (2.3)	36.9 ± 1.1 (3.0)
10	87	241.8 ± 11.7 (4.8)	52.1 ± 1.4 (2.7)	120.9 ± 4.9 (4.1)	9.1 ± 0.3 (3.3)	37.8 ± 0.9 (2.4)

followed by the center, and this holds for all of the determined amines. The edge always shows the lowest amine content.

Steps 4, 6, 9 and 10 of the second production batch for the amines putrescine, tyramine and spermine are given as examples (Figs 8–10). This tendency is transferable to the findings of Karpati (1960) as well as of Rödel & Hofmann (1982). The dry-up of the sausage during maturation leads to a decrease of water activities, particularly in the edge-section, and thereby to different sodium chloride contents between edge and center. Because of this, a sodium chloride migration from outer (edge) to inner (center) area of the sausage will set in. As a result of this sodium chloride migration, the water activity will be reduced in the slowly drying center-section (preservative effect). This leads to a decrease in microbial activity and, furthermore, to a reduced amine synthesis. In the edge-section the water activity decreases as a result of stronger drying, with the same effect. Between the sections center and edge, no clear border line can be defined. The existing middle-section that has no definite delimitation shows a higher

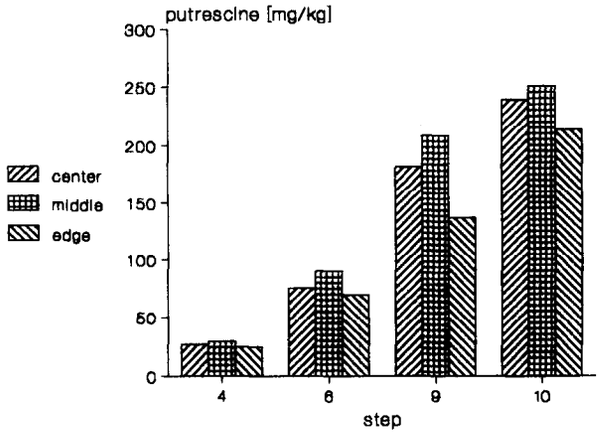

Fig. 8. Development of putrescine during maturity process and storage of cervelat sausage between the sections — 2nd production batch.

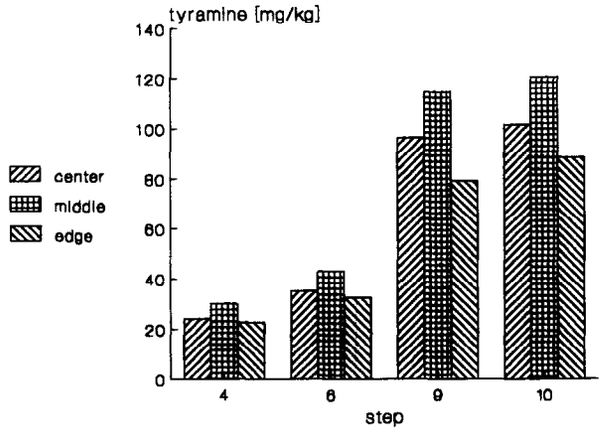

Fig. 9. Development of tyramine during maturity process and storage of cervelat sausage between the sections — 2nd production batch.

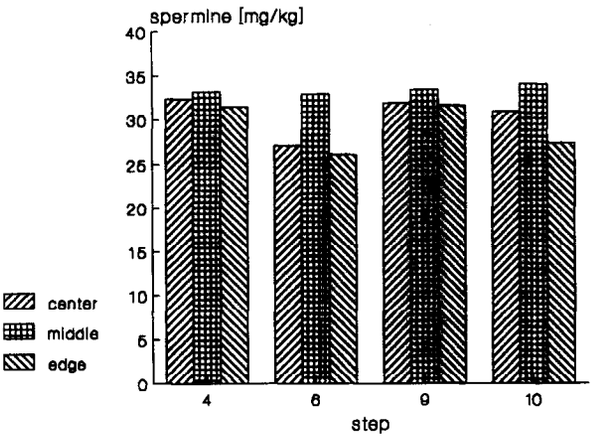

Fig. 10. Development of spermine during maturity process and storage of cervelat sausage between the sections — 2nd production batch.

a_w value because of its higher humidity than in the edge and shows a lower sodium chloride content than in the center. Therefore, the microbial activity and also the amine content is highest in the middle. In addition, the preservation effect by dry-up of the edge-section becomes higher than in the center because of the increasing sodium chloride content. These effects are reflected by the amine contents.

To support the results of the investigation of the sections, a differentiated examination over the diameter of the same sausage is imperative, particularly with regard to pH, water activity and microbial count.

Compared with mini-salami, in the cervelat sausage it was possible to determine higher amounts of biogenic amines (Treviño *et al.*, 1996). Furthermore, an increasing tendency of the respective amine contents is discernible in cervelat sausage. This effect is remarkable insofar as both products were produced with the same starter bacteria cultures. It may be assumed that other, unconsidered, parameters play an important role in the formation of amines. In contrast to fermented meat products, other fermented foods such as cheese show significantly higher amine contents. In Swiss cheese, the amine cadaverine occurs in highest amounts of more than 2000 mg kg^{-1}, where as cadaverine was only determined below 40 mg kg^{-1} in cervelat sausage. Putrescine, tyramine and histamine reach values of more than 500 mg kg^{-1} in Swiss cheese (Petridis & Steinhart, 1996*a*,*b*). The starter bacteria cultures used lead to the formation of different flavors and odors and this must be investigated with further sensory examinations.

ACKNOWLEDGEMENTS

We are grateful to Schafft Fleischwerke (Union Deutsche Lebensmittelwerke GmbH), Ansbach, in particular D. Bourquin, H. Horn and M. Röβler, for the provision of the sample material and for their support.

REFERENCES

Askar, A. & Treptow, H. (1989). Biogene Amine in Fleischprodukten. *Ernährung/Nutrition*, **13**, 425–429.

Binder, E. & Brandl, E. (1983). Über das Vorkommen und die Bedeutung biogener Amine in Lebensmitteln. *Österr. Milchwirtsch*, **38**, 257–259.

Karpati, G. (1960). Veränderungen im Salz- und Wassergehalt der Salami während des Reifens. *Husipar*, **9**, 77–83.

Klettner, P.-G. & List, D. (1978). Die Milchsäurebildung im Verlauf der Rohwurstreifung bei Starterkulturzusatz. *Fleischwirtschaft*, **58**, 136–139.

Klettner, P.-G. & Rödel, W. (1979). Die Bedeutung der Temperatur bei der Rohwurstreifung. *Die Fleischerei*, **30**, 907–909.

Lüthy, J. & Schlatter, Ch. (1983). Biogene Amine in Lebensmitteln: zur Wirkung von Histamin, Tyramin und β-Phenylethylamin auf den Menschen. *Z. Lebensm. Unters. Forsch*, **177**, 439–443.

Németh-Szerdahelyi, E., Freudenreich, P. & Fischer, F. (1993). Untersuchungen über den Gehalt biogener Amine in Schweinefleisch. *Fleischwirtschaft*, **73**, 789–790.

Patterson, R. L. & Mottram, D. S. (1974). The ocurrence of volatile amines in uncured and cured pork meat and their possible role in nitrosamine formation in bacon. *J. Sci. Fd. Agric.*, **25**, 1419–1425.

Petridis, K. & Steinhart, H. (1995). Automatische Vorsäulenderivatisierung mit o-Phthaldialdehyd (OPA). *Z. Lebensm. Unters. Forsch.*, **201**, 256–260.

Petridis, K. & Steinhart, H. (1996*a*). Biogene Amine in der Hartkäse-Produktion: I. Einfluß verschiedener Parameter auf den Amingehalt im Endprodukt am Beispiel von Emmentalerkäse. *Dtsch. Lebensm. Rundsch.*, **92**, 114–120.

Petridis, K. & Steinhart, H. (1996*b*). Biogene Amine in der Hartkäse-Produktion: II. Stufenkontroll-Studie einer standardisierten Emmentalerkäse-Produktion. *Dtsch. Lebensm. Rundsch.*, **92**, 142–146.

Rödel, W., Krispien, K. & Leistner, L. (1980). Die Wasseraktivität von Fetten tierischer Herkunft. *Fleischwirtschaft*, **60**, 642–650.

Rödel, W. & Hofmann, G. (1982). Beitrag zur Kochsalzdiffusion in der Rohwurst während der Reifung. *Fleischerei*, **33**, 24–26.

Rödel, W. & Stiebing, A. (1987). Kontinuierliche Messung des Reifungsverlaufs von Rohwurst. *Mitt. Bundesanstalt Fleischforsch., Kulmbach*, **97**, 7567–7573.

Treptow, H. & Askar, A. (1987). Biogene Amine erkennen und in Grenzen halten. *Gordian*, **9**, 163–166.

Treviño, E., Beil, D. & Steinhart, H. (1997). Determination of biogenic amines in mini-salami during long-term storage. *Food Chem.*, **58**, 385–390.

Tschabrun, R., Sick, K., Bauer, F. & Kranner, P. (1990). Bildung von Histamin in schnittfesten Rohwürsten. *Fleischwirtschaft*, **70**, 448–452.