ELSEVIER

PII: S0308-8146(96)00246-4

Food Chemistry, Vol. 58, No. 4, pp. 385–390, 1997

0308-8146/97 $17.00+0.00

Analytical, Nutritional and Clinical Methods Section

Determination of biogenic amines in mini-salami during long-term storage

E. Treviño, D. Beil & H. Steinhart

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

In order to collect data on the formation of biogenic amines in raw meat products during maturity process and storage, a method for their determination by an amino acid analyzer was developed. In this case, the amines ethylamine, propylamine, butylamine, putrescine, histamine, tyramine, cadaverine, β-phenylethylamine, spermine and spermidine in the exemplary selected product mini-salami were investigated. The duration of maturation and storage was 8 months. In this period, 13 samplings were performed. In addition, the starter bacteria cultures varied according to manufacturer. Over the duration of investigation, five different production batches were observed in parallel. The examination showed 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. As an accompanying investigation, the parameters water activity, pH and microbial count (lactobacilli, staphylococci, enterobacteria and yeast) were determined. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

One of the essential criteria of food quality is its hygiene status, which will be determined decisively by the presence or activity of microbes. In fermented foods, the effect of certain microbes also is welcomed. In addition to many flavor and odor substances, biogenic amines may be formed by microbes. On the one hand, the properties of flavor and odor also may be attributed to biogenic amines; on the other hand, biogenic amines are classified as unhealthy (Lüthy & Schlatter, 1983). They can develop distinctive pharmacological, physiological and toxic effects in the organism. The role of histamine as a cause of poisoning in fish and cheese is well known. Biogenic amines are, moreover, precursors of carcinogenic nitroso-amines (Patterson & Mottram, 1974). Therefore, the establishment of a so-called 'biogenic amine index' (BAI) as a criterion in the quality control of foods was proposed (Treptow & Askar, 1987). This index by Karmas (1981) was devised to determine the freshness status of fish and seafood. Sayem-el-Daher *et al.* (1984) later found that the BAI also is suitable for beef (Askar & Treptow, 1989). There have been only a few investigations of biogenic amines in meat and meat products, in contrast to fish and cheese, hence the object of this examination is to help to close this information gap (Németh-Szerdahelyi *et al.*, 1993).

Within the framework of this investigation, a method for the quantitative determination of biogenic amines in raw sausage (mini-salami) with an amino acid analyzer was established; their formation during the maturity process and storage for 8 months was observed. On occasions, the starter bacteria cultures varied according to the manufacturer. The parameters pH, water activity and microbial count (lactobacilli, staphylococci, enterobacteria and yeast) were additionally determined.

MATERIALS AND METHODS

Sample material

The raw meat product mini-salami was investigated during maturation and storage for 8 months. The ingredients of the product were beef, pork, bacon fat, nitrite pickle salt, sugar, spice, ascorbic acid, and beech wood 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 starter bacteria cultures (SAGA, Microlife Technics) varied between the production batches according to manufacturer. The following three different groups were formed:

First and third production batches	19 g *Pediococcus pentosaceus* (SAGA 75), 19 g *Staphylococcus carnosus* (SAGA 448) for 270 kg meat
	= Starter bacteria culture I

Second and fourth production batches	Dilutions of the starter bacteria culture of the fifth production batch = Starter bacteria culture II
Fifth production batch	4 g *P. pentosaceus* (SAGA 75), 19 g *Staph. carnosus* (SAGA 448), 19 g *P. acidilactici*, *Micrococcus varians* I (SAGA III) and 350 g water for 270 kg meat = Starter bacteria culture III

Extraction and clean-up

To determine the contents of biogenic amines, the samples were extracted according to the modified method of Stockemer and Stede (1979) (Fig. 1). In this case, the unpackaged frozen samples of the first five production steps and the packaged samples, stored at 25°C, of the following steps were homogenized with a moulinex mixer. For each sampling, the sausage mixture from five packings (5×25 g) was taken. Then, 5 g of the homogeneous substance were mixed with 45 g of trichloroacetic acid (10%). After subsequent suspension with an ultra turrax mixer (1–2 min, speed 8000 rpm), filtering was performed.

Then, 4 ml of the resulting extract were mixed with 50 ml clean-up buffer (0.1 mol/l sodium acetate trihydrate, with acetic acid pH 6) and afterwards the pH was adjusted to 6.0 ± 0.1 with sodium hydroxide (25%).

This solution was transferred to a column filled with the cation exchanger Amberlite CG 50 (Merck) and was then washed with 50 ml clean-up buffer. The elution with 0.2 mol/l hydrochloric acid in a 20 ml measuring flask was carried out, the flask being filled up to the measure with hydrochloric acid. Subsequently, analysis by the amino acid analyzer was carried out.

Separation of the amines

The use of the amino acid analyzer Beckman model 119 BL makes detection of the amines with ion exchange chromatography possible.

The separation took place on a DC 6a separation resin (Durrum) with various buffer systems; at this point, the amines ethylamine, propylamine, butylamine, putrescine, histamine, tyramine, cadaverine, β-phenylethylamine, spermine and spermidine (Serva) were separated chromatographically. Photometric detection at 570 nm after post-column derivatization with ninhydrine (Serva) was performed. The analysis followed the conditions shown in Tables 2 and 3. A chromatogram of a test solution is shown in Fig. 2. Details of retention time, determination level, precision, linearity and recovery are given in Table 4. 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 derivatization 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 ninhydrin post-column derivatization was preferred.

Additional investigations

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).

Table 1. Sampling of mini-salami (storage at −18°C with regard to steps 1–5 and storage at +25°C with regard to steps 6–13)

Sample No. (step of sampling)	After *x* days maturity process/storage	Step of production/storage
1	1	1st Step of production
2	2	2nd...
3	3	3rd...
4	5	4th...
5	7	5th... (Day of packaging)
6	37	1st Step of storage
7	67	2nd...
8	97	3rd...
9	127	4th...
10	157	5th...
11	187	6th...
12	217	7th...
13	247	8th...

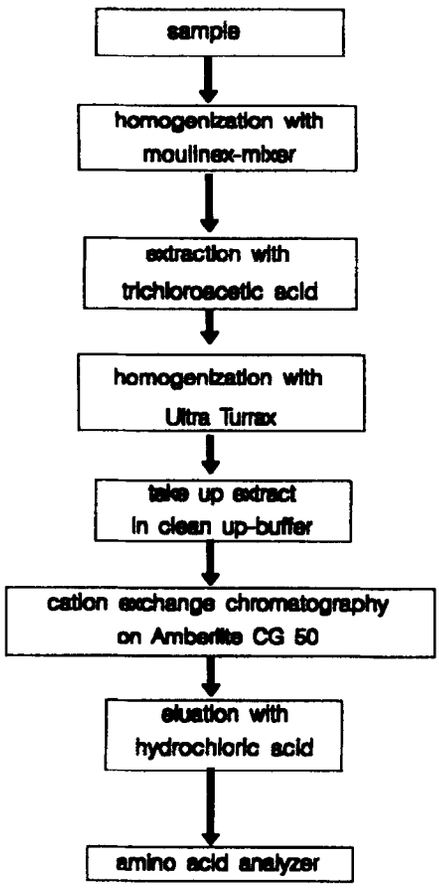

Fig. 1. Sample preparation and clean-up.

In the process, steps 3 (third day), 5 (seventh day), 8 (ninety-seventh day), 10 (one hundred and fifty-seventh day) and 13 (two hundred and forty-seventh day) were used for the investigation. The choice of microbes followed from already completed examinations (Tschabrun *et al.*, 1990).

RESULTS AND DISCUSSION

The pH is an important parameter in the investigation for the description of the maturation of raw sausage. The microbial fermentation of different kinds of sugar to acids causes a pH decrease, which affects microbial stability, pickle color and firmness.

In Fig. 3, the pH development of the five production batches is shown. For the mini-salami, the initial rapid decrease of the pH from about 6 to under 5.2 is common to all production batches, but then a slight increase is observed, which reaches numerical values near to 5.4 in the course of the investigation. This increase is traceable to the breakdown of lactic acid and the almost complete metabolism of the added sugar (glucose as substratum for the bacteria) (Klettner & List, 1978; Klettner & Rödel, 1979; Rödel & Stiebing, 1987).

The water activities (Fig. 4) of the five production batches follow a similar pattern to that of the pH values in the initial phase of the latter. Thus, as a result of the increasing dry-up during storage, water activities fall continuously from about 0.95 at first to a numerical

Table 2. Conditions of chromatographic separation

Apparatus	Amino acid analyzer (Beckman model 119 BL)		
Ion exchange material	DC 6a (Durrum)		
Column dimensions	0.9×4.0 cm		
Injection volume	10–1000 µl standard 1000 µl sample		
Flow — buffer	45 ml/h		
Flow — ninhydrin reagent	25 ml/h		
Detection — wavelength	570 nm		
Buffer (see Table 3)	A	B	C
Running time	42.5 min	30.0 min	35.0 min
Temperatures	62°C	79°C	79°C

Table 3. Buffer compounds

	Buffer A (with water add 1 liter)	Buffer B (with water add 1 liter)	Buffer C (with water add 1 liter)
Sodium citrate dihydrate	58.82 g	58.82 g	58.82 g
Sodium chloride	52.60 g	81.82 g	111.35 g
Brij 35	2.00 g	2.00 g	2.00 g
Phenol	0.50 g	0.50 g	0.50 g
Methanol	45.0 ml	55.0 ml	55.0 ml
Ethanol	35.0 ml	45.0 ml	45.0 ml
pH	5.4	5.9	6.8
Mol/l (Na ions)	1.5	2.0	2.5

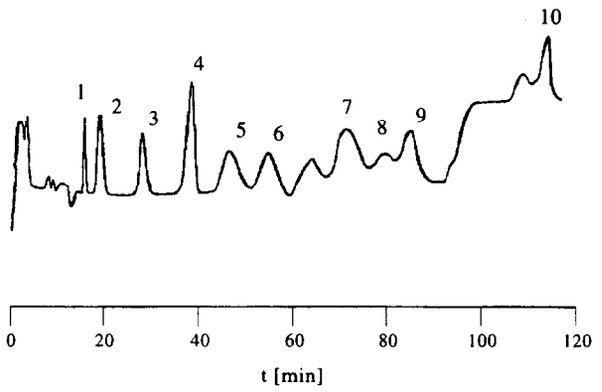

Fig. 2. Ion exchange chromatogram of a test solution. (1) Ethylamine; (2) propylamine; (3) butylamine; (4) putrescine; (5) histamine; (6) cadaverine; (7) tyramine; (8) β-phenylethylamine; (9) spermidine; (10) spermine. Injected vol.: 100 μl–*ca* 25 nmol of each amine (for conditions of chromatographic separation see Tables 2 and 3).

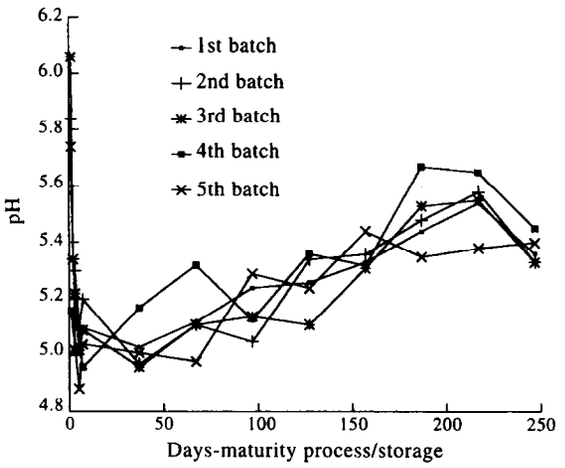

Fig. 3. Development of pH during maturity process and storage of mini-salami.

value of 0.85 after 247 days. The behavior of this parameter corresponds to the course which is described by Rödel *et al.* (1980) and Rödel & Stiebing (1987).

For the mini-salami, the microbial counts of lactobacilli, staphylococci, enterobacteria and yeast were combined as means, due to their great similarity between the five production batches (Fig. 5). Lactobacilli appear first, with a microbial count of 7.6 log n/g, and are no longer detectable after 247 days because of the increasing consumption of substratum.

Staphylococci and enterobacteria, with significantly lower microbial counts in the beginning of the examination, are also no longer detectable after 247 days. The microbial counts of yeast at the 3rd day are still near 1.3 log n/g. After 7 days, yeast is no longer detected. The behavior of the microbial counts corresponds to the data in literature (Tschabrun *et al.*, 1990).

The development of biogenic amine formation in the five production batches is shown in Figs 6–10. The precisions of the five-fold determined amines of all production batches range between 0.4 and 18.2%.

The group comprised of the first and third production batches shows a dominant and constant behavior of spermine with an average value of 25 mg/kg until the end of investigation after 247 days. Spermidine also appears in this dimension in all other production batches. Similar behavior of spermidine, but with a decreasing tendency, is observed in all production batches with initially about 10 mg/kg until the end of investigation, where spermidine contents of less than 5 mg/kg are determinable. The temporal course of tyramine formation is very similar in all production batches. At the end of the investigation, tyramine is detectable in the range 3–12 mg/kg. Histamine is at first determinable in the group comprised of the first and third production batches, as well as the second one after 187 days, in the range 4–7 mg/kg, in contrast to the fourth production batch, where it was already measured after 127 days with a content of 16.2 mg/kg. In the fifth production batch, histamine is detectable with 12.7 mg/kg after 157 days. Until the end of the investigation, the histamine content remains in this dimension in all production batches. The group comprised of the second and fourth production batches shows, in comparison with the group comprised of the first and third batches, deviant behavior with regard to the putrescine formation. Here, putrescine reaches a dominant position and has, in the fourth production batch, nearly twice as high a value as spermine after 187 days (42.2 mg/kg). This behavior of putrescine is very clear in the fifth production batch, where a putrescine maximum of 139.1 mg/kg is determinable after 187 days.

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 (5 g), biogenic amines are detected with a total content of more than 100 mg/kg.

Table 4. Statistical data for the relevant determined amines

	Putrescine	Histamine	Tyramine	Spermidine	Spermine
Retention time (min)	37.3	46.1	68.3	80.1	110.0
Determination level (mg/kg)	0.7	1.7	2.3	1.0	3.0
Precision s (%); $n=8$	1.5	1.1	1.2	1.3	1.5
Linearity; $x=12$	0.999	0.997	0.999	0.999	0.999
Recovery (%)	105	97	85	96	104

Due to the identical manufacturing process of the five production batches, a variation in behavior of the water activities could not be found. The parameters pH and microbial count were, similarly, corresponding. These results are in accordance with the literature (Klettner & List, 1978; Klettner & Rödel, 1979; Rödel *et al.*, 1980; Rödel & Stiebing, 1987; Tschabrun *et al.*, 1990).

In this investigation, the influence of the used starter

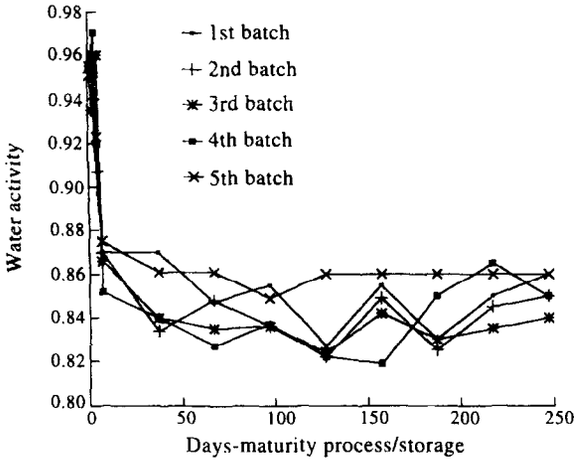

Fig. 4. Development of water activity during maturity process and storage of mini-salami.

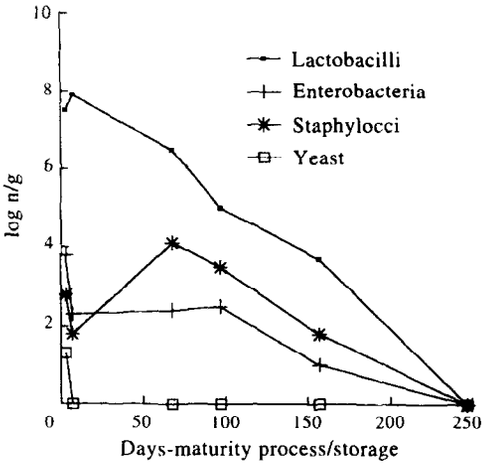

Fig. 5. Development of microbes during maturity process and storage of mini-salami (means of the three groups).

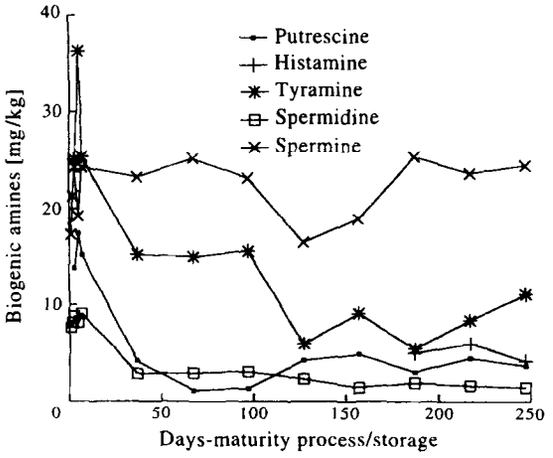

Fig. 6. Development of biogenic amines during maturity process and storage of mini-salami — first production batch.

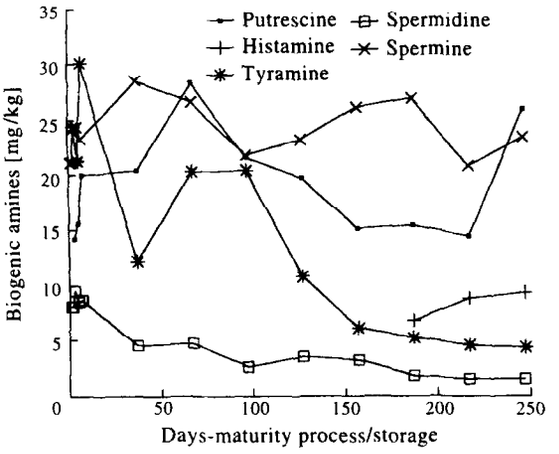

Fig. 7. Development of biogenic amines during maturity process and storage of mini-salami — second production batch.

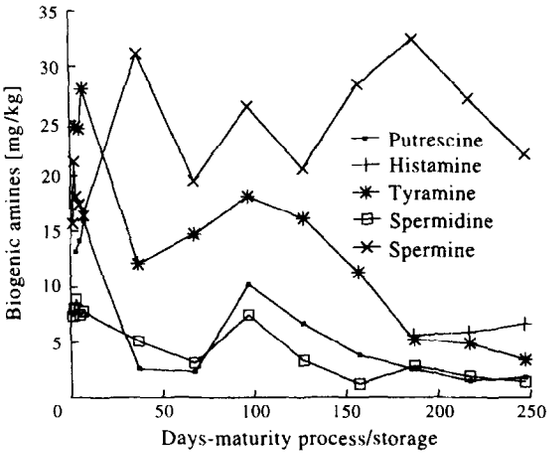

Fig. 8. Development of biogenic amines during maturity process and storage of mini-salami — third production batch.

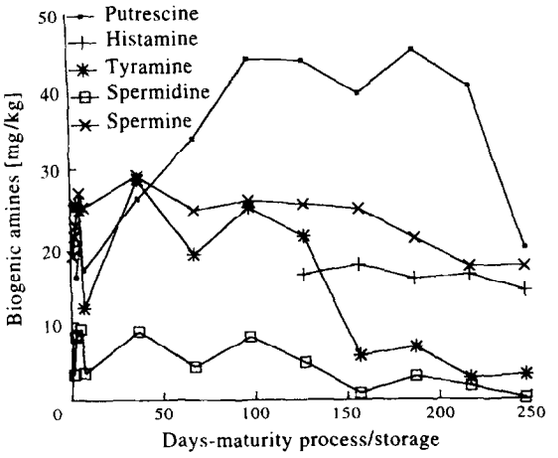

Fig. 9. Development of biogenic amines during maturity process and storage of mini-salami — fourth production batch.

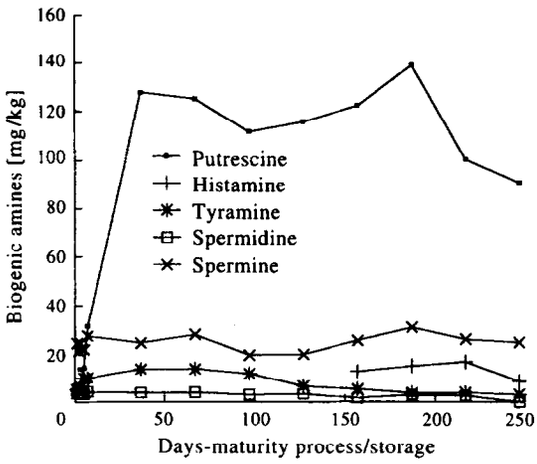

Fig. 10. Development of biogenic amines during maturity process and storage of mini-salami — fifth production batch.

bacteria cultures on the contents of biogenic amines during maturation and storage of raw sausage for the example mini-salami was observed. Spermine, spermidine and tyramine showed similar behavior over the duration of the examination, independent of the production batch and therefore of the used starter bacteria culture. However, putrescine was, interestingly enough, formed in clearly higher amounts using starter bacteria culture III, in comparison with the other cultures. Furthermore, histamine could be detected earlier if starter bacteria cultures II or III were used.

Compared with cervelat sausage, in the mini-salami only low amounts of biogenic amines could be determined (Treviño *et al.*, 1996). Furthermore, an increasing tendency of the respective amine contents is not discernible in mini-salami. This effect is remarkable in so far as both products were produced with the same starter bacteria cultures. It may 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. Cadaverine was not detected in mini-salami. Putrescine, tyramine and histamine reach values of more than 500 mg/kg in Swiss cheese (Petridis & Steinhart, 1996*a*,*b*).

In such a way, the used starter bacteria culture leads to the formation of different flavor and odor 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. Rößler, for the provision of the sample material and for their support.

REFERENCES

Askar, A. & Treptow, H. (1989). Biogene amine in fleischprodukten. *Ernährung/Nutr.*, **13**, 425–429.

Karmas, E. (1981). Biogenic amines as indicator of food freshness. *Lebensm.-Wiss. u. Technol.*, **14**, 273–275.

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 Fleisch.*, **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 occurrence 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. Unter. 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. & Stiebing, A. (1987). Kontinuierliche messung des reifungsverlaufs von rohwurst. *Mitt. Bundesanst. Fleisch., Kulmbach*, **97**, 7567–7573.

Sayem-el-Daher, N., Simard, E., Fillion, J. & Roberge, A. G. (1984). Extraction and determination of biogenic amines in ground beef and their relation to microbial quality. *Lebensm.-Wiss. u. Technol.*, **17**, 20–23.

Stockemer, J. & Stede, M. (1979). Quantitative histaminbestimmung durch fluorimetrie. *Arch. Lebensm.-Hyg.*, **30**, 59–61.

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

Treviño, E., Beil, D. & Steinhart, H. (1996). Formation of biogenic amines during maturity process of raw meat products for example of cervelat sausage. *Food Chem.* (submitted).

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