

Investigations on the Formation of Histamine in Raw Sausages

P. KRANNER⁺, F. BAUER^{*} and E. HELLWIG⁺

⁺ Bundesanstalt für Lebensmitteluntersuchung und -forschung, Kinderspitalgasse 15, A-1090 Wien

^{*} Institut für Fleischhygiene, Fleischtechnologie und Lebensmittelkunde, Vet. Med. Univ., Linke Bahngasse 11, A-1030 Wien

SUMMARY: To find the causes of an excessive formation of histamine raw sausages (Mettwurst) produced using meat stored under different conditions with and without addition histamine producing lactobacteria were investigated in respect to the content of histamine, histidine, total count of microorganism and the number of histamine producing bacteria. The results show that the formation of undesired high amounts of histamine depends on a sufficient number of histamine producing microorganisms and the presence of free histidine as substrate and is influenced by the storage temperature and the freshness of the meat used for production.

INTRODUCTION: High contents of histamine in fish are an indicator for a beginning or proceeding spoilage (PECHANEK et al. 1980a; SCHMIDTLEIN 1979; SCHULZE and ZIMMERMANN 1980). In foods, which underlie a microbiological ripening, such as cheese, wine or fermented sausages biogenic amines could be detected in amounts higher than the original concentration in the corresponding raw material (PECHANEK et al. 1980b, PECHANEK et al. 1983). Whereas fresh meat contains 1 to 7 mg histamine/kg (ROGOWSKY and DÖHLA 1984, SLEMR 1981) and spoiled meat up to 26 mg/kg (ROGOWSKY and DÖHLA 1984). Different contents of histamine between 1 and over 600 mg/kg dry matter were found independent on the type of the sausage but dependent on the producer (BAUER et al. 1989). The main share of histamine is formed within the first two weeks of ripening. When meat stored only for a short period is used to produce raw sausages the amount of histamine could be reduced (TSCHABRUN et al. 1990).

The objective of our work was to investigate the influence of the age of the raw material in connection with the amount of free histidine and the number of histidine decarboxylating microorganisms on the formation of histamine in raw sausages.

MATERIALS AND METHODS:

Materials under investigation: Raw sausages were produced according to the recipe of Austrian Food Codex (ÖLMB 1987) "Feine Mettwurst" was made without starter culture using spices commercially available and 18 g curing agent/kg sausage (sodium chloride + 0.5% to 0.6% sodium nitrite). Pork stored 5 days (batch I), 7 days (batch II), and 10 days (batch III) at 7°C was used for the production. To one half of each batch 10^5 CFU (colony forming units) *Lactobacillus hilgardii* /g sausage were added. The sausages are stored 3 days at 18°C and 15 days at 7°C or 18°C.

Determination of Histamine: Histamine was measured fluorimetrically as o-phthalaldehyde-derivate (LERKE and BELL, 1976)

Determination of Histidine: Histidine content was analysed by an aminoacidanalyser LKB-ALPHA PLUS with 6 Buffer lithium systems and ninhydrin reaction according to the instructions of the producer.

Bacteriological investigations: Isolation and detection of histamine producing microorganisms was carried out by the MPN - technique using:

HDM by YAMANI (1982): 2 g peptone (Oxoid L34), 1 g Lab Lemco (Oxoid L29), 5 g NaCl, 10 g L-histidine·HCl (USB Art. No.63324), 10 ml bromocresol green solution and 10 ml chlorophenol red solution/1000 ml water; pH 5.3

L-HDM by YAMANI (1982): 8 g peptone (Oxoid L34), 8 g Lab Lemco (Oxoid L29), 5 g yeast extract (Oxoid L21), 1 g Tween 80 (Merck Art. No. 822187), 5 g sodium acetate anhydrous, 1 g sodium citrate dihydrate, 2 g K_2HPO_4 , 0.2 g $MgSO_4 \cdot 7 H_2O$, 0.05 g $MnSO_4 \cdot 4 H_2O$, 0.2 g glucose (Oxoid L71), 25 g L-histidine·HCl (USB Art. Nr. 63324), 0.4 g agar (Oxoid L11), 10 ml bromocresol green solution, 10 ml chlorophenol red solution / 1000 ml water; pH 5.5

Bromocresol green solution: 0.1 g bromocresolgreen in 100 ml ethanol/water 50/50

Chlorophenol red solution: 0.2 g chlorophenol red and 9.4 ml NaOH (0.05 mol/l) / 100 ml water

Histidine decarboxylating microorganism (*Lactobacillus hilgardii*, *Morganella morganii*, *Klebsiella oxytoca*) used for addition to the test sausages were isolated from meat or raw sausage.

RESULTS and DISCUSSION: Independent on the age of the meat the content of histamine was less than 10 mg/kg. In contrast to this the amount of histidine increased up to 190 mg/kg dry matter by increasing storage time of the meat. The total count of microorganisms were between $2 \cdot 10^8$ and $6 \cdot 10^8$ CFU/g meat stored for 5, 7 and 10 days after slaughtering, histamine producing bacteria (*Klebsiella oxytoca*, *Citrobacter freundii* and *Morganella morganii*) at least $5 \cdot 10^3$ CFU/g.

The determination of histamine was carried out at the day of production and 3, 12, and 18 days after the production (Fig. 1 and 2). The histidine content of sausages was analysed at the first and at the last day of storage (Fig. 3).

Figure 1 shows the formation of histamine in sausage stored at 18°C. During storage a concentration of histamine between 10 and 27 mg histamine/kg dry matter could be determined corresponding to the age of the raw material without addition of histamine producing microorganisms to the sausages. In contrast to this an addition of approximately 10^5 histamine producing bacteria effects an excessive formation of histamine up to 1140 mg histamine / kg dry matter at the last day of storage. The heights of the concentrations of histamine also depends on the age of the raw material. The formation of histamine in sausages stored 3 days at 18°C and 15 days at 7°C is depicted in Figure 2. In spite of the addition of a number of 10^5 histamine producing *Lactobacillus hilgardii*/g sausage no excessive increase of the concentration of histamine could be obtained. Approximately one tenth of the histamine content of raw sausages stored at 18°C was found.

Fig. 1: Formation of histamine at 18°C; with (+) and without (-) addition of histamine producing microorganism

Fig.2: Formation of histamine at 7°C; with (+) and without (-) addition of histamine producing microorganism

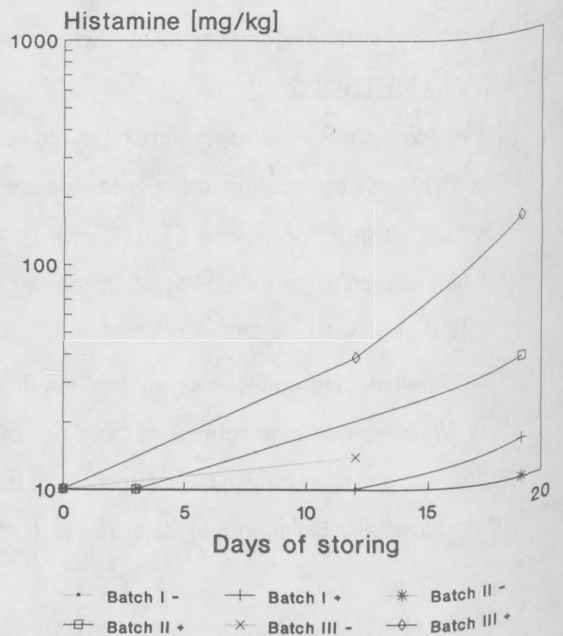
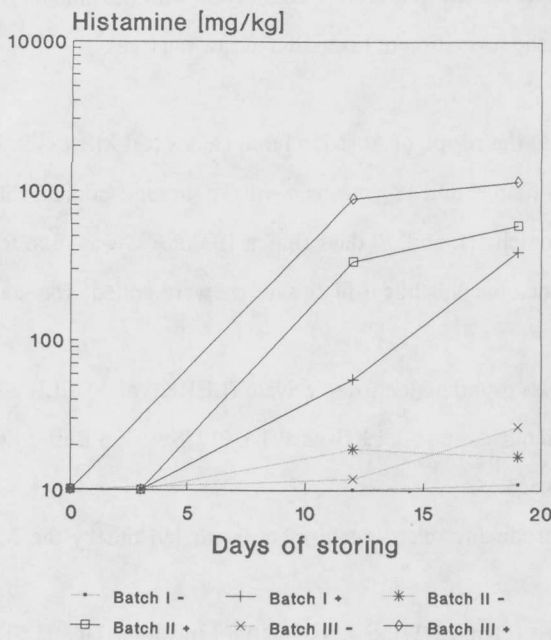
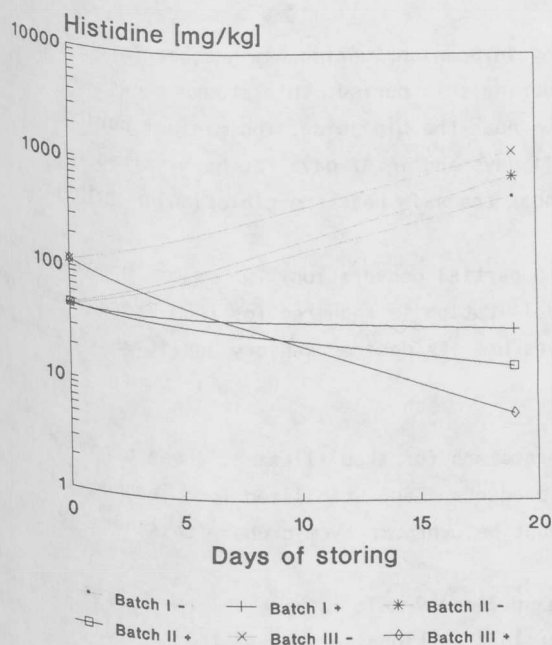


Fig. 3: Liberation of histidine during storage at 18°C; with (+) and without (-) addition of histamine producing microorganism



The liberation of histidine in sausages stored at 18°C is shown in figure 3. In the presence of an appropriate number of histamine producing microorganisms the liberated histidine is used as substrate for histamine production so that only 6 to 34 mg histidine /kg dry matter could be determined at the end of storage. On the other hand - without addition of histamine producing microorganisms - up to 1300 mg histidine /kg dry matter were liberated. This high amount of histidine could not stimulate the formation of histamine although up to $5 \cdot 10^3$ histamine producing bacteria/g meat were present.

The number of histamine producing bacteria increased in sausages stored at 18°C from 10^5 to 10^7 at the end of storage. At 7°C histamine producing Lactobacteria did not grow.

CONCLUSIONS: These investigations indicate that an excessive formation of histamine in raw sausages requires both - a sufficient number of histidine decarboxylating microorganisms and available histidine and is further increased by storage temperature and the age of the raw material.

REFERENCES

- BAUER F., TSCHABRUN R. and SICK K. (1989): Wien. Tierärztl. Mschr. **76**, 180
- LERKE P.A. and BELL L.D. (1976): A rapid fluorimetric method for the determination of histamine in canned tuna. J. Food. Sci. **48**, 1282
- PECHANEK U., BLAICHER G., PFANNHAUSER W. and WOJDICH H. (1980a): Beitrag zur Untersuchung biogener Amine in Käse und Fischen. Z. Lebensm. Unters. Forsch. **171**, 420
- PECHANEK U., WOJDICH H., PFANNHAUSER W. and BLAICHER G. (1980b): Untersuchungen über das Vorkommen biogener Amine in Lebensmitteln. Ernährung **4**, 58
- PECHANEK U., PFANNHAUSER W. and WOJDICH H. (1983): Untersuchungen über den Gehalt biogener Amine in vier Gruppen von Lebensmitteln des österreichischen Marktes. Z. Lebensm. Unters. Forsch. **176**, 335
- ÖLMB (1987): Österreichisches Lebensmittelbuch Kapitel B 14 Fleisch und Fleischwaren B/I Abs. 21. Verlag Gebrüder Hollinek Wien
- ROGOWSKY B. and DÖHLA I. (1984): Bestimmung und Gehalt biogener Amine in Fleisch und Fleischwaren. Lebensmittelchem. Gerichtl. Chem. **38**, 20
- SCHMIDTLEIN H. (1979) Bestimmung von biogenen Aminen mit Hilfe der Hochdruckflüssigkeitschromatographie. Lebensmittelchem. Gerichtl. Chem. **33**, 81
- SCHULZE K. and ZIMMERMANN T. (1980) Nachweis biogener Amine in Thunfisch- und Ölsardinenkonserven. Fleischwirtsch. **60**, 2236
- SLEMR J. (1981): Biogene Amine als potentieller Qualitätsindikator für Fleisch. Fleischwirtsch. **61**, 921
- TSCHABRUN, R., SICK, K., BAUER, F. and KRANNER, P. (1990): Bildung von Histamin in schnittfesten Rohwürsten. Fleischwirtsch. **70**, 448
- YAMANI, M.I. (1982): Ein Beitrag zur Ätiologie und Epidemiologie der Scombroidvergiftung. Dissertation Vet. Med. Univ. Berlin