# Biogenic Amines and Polyamines and Total Aerobic Count During Storage of Vacuum-Packaged Porcine Kidney, Liver and Spleen

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# Biogenic Amines and Polyamines and Total Aerobic Count During Storage of Vacuum-Packaged Porcine Kidney, Liver and Spleen

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Biogenic amines and polyamines (cadaverine, histamine, 2-phenylethylamine, putrescine, spermidine, spermine, tryptamine and tyramine) were analysed in vacuum-packaged porcine livers, kidneys and spleens stored at 3 °C and 7 °C (for up to 6 days) or 0 °C (for up to 21 days). Total aerobic count, pH and sensory assessment were done in parallel. While histamine, 2-phenylethylamine and tryptamine concentrations were nearly constant, spermidine and spermine showed a moderately declining trend, irrespective of the storage temperature. Cadaverine, putrescine and tyramine concentrations increased with storage time and temperature. Maximum concentrations at day 21 at 0 °C were: 122 mg/kg for cadaverine, 207.35 mg/kg for putrescine and 63.19 mg/kg for tyramine. The correlation of concentrations of the latter three amines and the total aerobic count was ranging from r = 0.54 to 0.89. A significant rise in amine concentrations was observed only when the total aerobic count exceeded 6 $\log^{10}$ cfu/g. Concentrations of cadaverine, putrescine and tyramine may be useful to confirm spoilage of vacuum-packaged inner organs. The fraction of the potential food-borne pathogen *Aeromonas* in high-pH organs (spleen, kidney) during storage was significantly higher than in liver, with low pH. It was observed that the spermine : spermidine ratio of spleen (3 : 2; weight base) was significantly different from that of liver and kidney (4 : 1).

*Key Words:* vacuum-package, storage, liver, spleen, kidney, pork, *Aeromonas*, biogenic amines

## INTRODUCTION

The hygienic condition and 'freshness' of meat are essential quality issues to the food processing industry as well as to the consumer. Numerous studies have been conducted on muscle tissue, but only a few on edible inner organs. Among the metabolites formed during ageing of meat and other proteinaceous foods, amino acid degradation products, such as biogenic amines (Silla Santos, 1996) have significance as spoilage indicators (Dainty, 1996; Vinci and Antonelli, 2002). The mode of formation is mostly by decarboxylases originating from tissue or contaminant bacteria (Halasz et al., 1994). Therefore, the quantity of biogenic amines is also considered to be a marker of the level of bacterial contamination in food. For vacuum-packaged meat stored at low temperatures, Enterobacteriaceae, especially *Hafnia alvei* and *Serratia liquefaciens*, have been identified as playing a major role in the accumulation of diamines (Dainty et al., 1986). The situation is complicated by the fact that so-called polyamines constitute essential compounds of living cells (Kremmer et al., 1984), while other amines cause adverse health effects (Bardocz, 1995) such as histamine (reviewed by Lehane and Olley, 2000) and tyramine (Beutling, 1996). Other amines potentiate such effects or are associated with tumorous growth (spermine, spermidine and putrescine; Kaminski et al., 1987; Seiler, 2003). During the EU COST 917 action a lack of data on amine contents in various edible tissues was identified, and the collection of such data was

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emphasised within the framework of EU COST 922 action 'Health Implications of Dietary Amines', with respect to a risk-assessment based approach (Maijala, 2001). This study contributes to EU COST 922 action by generating data on the concentrations of biogenic amines and polyamines in porcine offals during vacuum-packaged storage; further, a comparison of those values to conventional microbiological and sensory shelf-life assessment is made. Consumer-relevant storage conditions are studied as well as unusually long storage periods. We chose three temperatures: 3 °C (maximum temperature for slaughter by-products, Council Directive 64/433/EEC, 1964: Chapter IX), 0 °C (recommended storage temperature for vacuum-packaged meats, Wirth et al., 1990) and 7 °C (temperature for household refrigerators, as recommended by various environmental protection and consumer organisations, see e.g. www.greenpeace.de or www.meat-n-more.info). The data generated from long-term storage are needed for comparison when the use of spoiled organs for the production of heat-processed food (e.g. liver-pate) or petfood (Paulsen et al., 2000; Hagen, 2001) is suspected.

# MATERIAL AND METHODS

## Samples

Porcine livers, kidneys and spleens were collected after slaughtering in two occasions from an abattoir and separately vacuum-packaged (Cryovac BB6 foil). Two experiments were then conducted to evaluate the changes in amine content, pH and microflora during storage.

### *Experiment 1*

For each type of organ, three batches were formed and stored at 0 °C, 3 °C and 7 °C, respectively. From each batch, three samples were taken immediately and after 2, 3, 4, 5 and 6 days.

### *Experiment 2*

Batches were formed as in experiment 1, and six samples were taken immediately and after 7, 14 and 21 days of storage at 0 °C, and 7 days storage at 3 °C and 7 °C.

## Methods

### *Microbiological Counts*

Total aerobic count (Plate count agar, Merck 5463, Merck, Darmstadt, Germany, incubation 72 h at 30 °C) and *Aeromonas* count (GSP agar, Merck 10230, incubation 72 h at 20 °C) were determined in duplicate.

### *Measurement of the pH Value*

The pH was measured using a SenTix Sp (WTW, Germany) electrode on a pH-Meter (CG822, Schott, Hofheim, Germany).

### *Aspect and Odour Evaluation*

Samples were allowed to reach room temperature and then two analysts assessed colour, odour and slime formation (ASU, 1983; Poetzelberger et al., 1998).

### *Determination of Biogenic Amines and Polyamines*

Extraction and analysis of biogenic amines and polyamines was done according to Paulsen et al. (1997). In brief, amines were extracted with 10% (w/w) trichloroacetic acid and, after pre column derivatisation with dansyl chloride, separated on a C18 RP column according to Mietz and Karmas (1977). Dansylated amines were then detected by UV absorption at 254 nm (histamine) and fluorescence (excitation at 330 nm, emission at 500 nm; amines other than histamine).

### *Statistical Analysis*

Statistical calculations were done with Statgraphics for Windows 2 plus (Statistical Graphics corp., USA) software package. Unless indicated otherwise, data are displayed as mean values ± standard deviation.

# RESULTS

## Storage of Vacuum-Packaged Porcine Liver, Spleen and Kidney for Six Days

### *Biogenic Amines and Polyamines*

Initial concentrations were in the range of 5.6–10.2 mg/kg for cadaverine and 3.3–5.2 mg/kg for tyramine. After 72 h of storage at 3 °C and 7 °C, and after 96 h of storage at 0 °C, contents increased. Final concentrations at day six of storage were in the range of 20–25 mg/kg for cadaverine and 30–60 mg/kg for tyramine. Initial concentrations of putrescine were 2.1–8.3 mg/kg. For liver in general, and for spleen and kidney stored at 0 °C, the final putrescine concentrations were below 10 mg/kg. Six days of storage of kidney and spleen at 3 °C and 7 °C effected putrescine concentrations >30 mg/kg (Table 1). The concentrations of histamine, 2-phenylethylamine and tryptamine were constant or showed only a moderately increasing tendency. Spermidine and spermine concentrations moderately declined during the storage. For the latter five amines, the storage temperature had no significant effect on the amine concentrations. For these amines, data for different storage temperatures were pooled

**Table 1.** Total aerobic counts and concentrations of putrescine, cadaverine and tyramine in vacuum-packaged porcine kidney, liver and spleen stored at 0 °C, 3 °C and 7 °C for six days (mean ± SD, n = 3).

| | | | Storage time (h) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Organ | Parameter | T (°C) | 0 | 44 | 72 | 96 | 120 | 144 |
| Kidney | Total aerobic counts ($log_{10}$ cfu/g) | 0 | 4.9 ± 0.4 | 5.3 ± 0.6 | 5.0 ± 0.6 | 5.4 ± 0.4 | 6.3 ± 0.6 | 6.3 ± 0.6 |
| | | 3 | | 5.6 ± 2.4 | 6.0 ± 2.5 | 6.5 ± 1.9 | 6.7 ± 1.9 | 7.2 ± 1.6 |
| | | 7 | | 6.5 ± 2.2 | 6.4 ± 2.1 | 7.0 ± 1.8 | 8.0 ± 2.1 | 8.5 ± 1.9 |
| | Putrescine (mg/kg f.w.) | 0 | 2.1 ± 1.1 | 1.2 ± 0.9 | 6.1 ± 2.6 | 1.3 ± 0.7 | 2.2 ± 0.6 | 5.7 ± 2.0 |
| | | 3 | | 2.5 ± 1.1 | 2.9 ± 1.8 | 1.6 ± 0.4 | 19.1 ± 2.0 | 34.3 ± 3.9 |
| | | 7 | | 0.9 ± 0.6 | 1.1 ± 0.4 | 5.6 ± 1.0 | 32.0 ± 5.1 | 47.4 ± 5.2 |
| | Cadaverine (mg/kg f.w.) | 0 | 7.6 ± 1.5 | 4.5 ± 1.2 | 2.6 ± 1.1 | 1.1 ± 0.5 | 6.0 ± 1.2 | 15.0 ± 2.0 |
| | | 3 | | 9.0 ± 1.4 | 21.8 ± 4.8 | 20.0 ± 3.0 | 15.4 ± 4.0 | 19.5 ± 3.8 |
| | | 7 | | 12.1 ± 2.1 | 21.9 ± 2.9 | 23.0 ± 3.7 | 46.3 ± 3.9 | 55.3 ± 5.1 |
| | Tyramine (mg/kg f.w.) | 0 | 3.2 ± 1.4 | 7.6 ± 1.7 | 7.6 ± 2.1 | 2.8 ± 0.3 | 8.0 ± 1.5 | 32.9 ± 3.7 |
| | | 3 | | 4.3 ± 0.8 | 3.8 ± 0.5 | 6 ± 1.1 | 24.6 ± 4.5 | 34.0 ± 4.0 |
| | | 7 | | 5 ± 1.3 | 1.9 ± 0.8 | 12.6 ± 2.1 | 32 ± 3.7 | 51.1 ± 4.7 |
| Spleen | Total aerobic counts ($log_{10}$ cfu/g) | 0 | 4.7 ± 0.6 | 4.9 ± 0.5 | 5.2 ± 0.6 | 5.3 ± 0.4 | 6.0 ± 0.5 | 6.2 ± 1.6 |
| | | 3 | | 5.5 ± 1.3 | 6.0 ± 2.1 | 6.8 ± 2.5 | 6.8 ± 2.3 | 7.0 ± 1.9 |
| | | 7 | | 5.6 ± 2.1 | 6.0 ± 2.2 | 6.9 ± 2.0 | 7.5 ± 3.1 | 8.5 ± 2.2 |
| | Putrescine (mg/kg f.w.) | 0 | 8.3 ± 1.9 | 3.6 ± 1.4 | 4.6 ± 1.3 | 3.2 ± 1.1 | 4.0 ± 0.9 | 4.1 ± 3.1 |
| | | 3 | | 3.3 ± 1.2 | 4.1 ± 1.2 | 9.2 ± 2.6 | 8.8 ± 2.5 | 32.1 ± 5.1 |
| | | 7 | | 3.1 ± 1.5 | 2.8 ± 0.9 | 6.5 ± 1.7 | 14.5 ± 3.9 | 37 ± 3.2 |
| | Cadaverine (mg/kg f.w.) | 0 | 10.2 ± 1.0 | 7.1 ± 1.8 | 9.8 ± 2.3 | 10.6 ± 3.2 | 12.0 ± 2.7 | 17.6 ± 2.0 |
| | | 3 | | 9.0 ± 2.5 | 9.0 ± 1.9 | 13.7 ± 3.6 | 15.7 ± 3.1 | 25.8 ± 2.9 |
| | | 7 | | 17.8 ± 4.1 | 18.2 ± 3.5 | 27.7 ± 5.0 | 22.9 ± 3.6 | 32.7 ± 2.9 |
| | Tyramine (mg/kg f.w.) | 0 | 5.6 ± 1.0 | 4.3 ± 1.4 | 6.9 ± 1.9 | 8.3 ± 1.9 | 12.8 ± 4.0 | 27.1 ± 3.8 |
| | | 3 | | 9.3 ± 2.0 | 9.3 ± 3.0 | 12.1 ± 2.7 | 35.4 ± 5.1 | 55.9 ± 6.7 |
| | | 7 | | 7.2 ± 1.9 | 12.2 ± 3.4 | 20.1 ± 3.4 | 63.8 ± 5.5 | 72.4 ± 6.5 |
| Liver | Total aerobic counts ($log_{10}$ cfu/g) | 0 | 10.2 ± 1.0 | 5.0 ± 0.8 | 5.4 ± 0.6 | 5.6 ± 0.6 | 6.3 ± 0.6 | 6.8 ± 0.4 |
| | | 3 | | 6.1 ± 2.1 | 5.9 ± 2.0 | 5.9 ± 1.4 | 7.6 ± 3.1 | 7.2 ± 1.9 |
| | | 7 | | 5.7 ± 1.9 | 6.3 ± 1.6 | 7.0 ± 2.1 | 7.9 ± 2.7 | 8.6 ± 2.0 |
| | Putrescine (mg/kg f.w.) | 0 | 5.6 ± 1.0 | 1.1 ± 0.9 | 1.9 ± 1.0 | 2.8 ± 0.7 | 3.4 ± 1.8 | 5.7 ± 1.3 |
| | | 3 | | 2.3 ± 1.0 | 2.2 ± 0.6 | 2.4 ± 1.1 | 1.4 ± 0.7 | 7.9 ± 2.1 |
| | | 7 | | 2.6 ± 1.0 | 2.4 ± 1.4 | 3.4 ± 2.1 | 5.1 ± 2.0 | 12.4 ± 3.4 |
| | Cadaverine (mg/kg f.w.) | 0 | 4.1 ± 0.9 | 5.1 ± 2.1 | 4.3 ± 1.5 | 5.2 ± 2.3 | 17.4 ± 1.8 | 23.5 ± 2.9 |
| | | 3 | | 2.0 ± 1.1 | 6.7 ± 1.7 | 9.6 ± 2.6 | 9.0 ± 2.7 | 29.3 ± 4.0 |
| | | 7 | | 4.2 ± 1.7 | 8.4 ± 2.3 | 10.9 ± 3.1 | 15.3 ± 3.1 | 32.3 ± 5.0 |
| | Tyramine (mg/kg f.w.) | 0 | 5.0 ± 1.3 | 4.3 ± 1.8 | 3.5 ± 1.1 | 9.1 ± 3.0 | 16.7 ± 3.8 | 31.4 ± 4.2 |
| | | 3 | | 6.5 ± 1.9 | 2.3 ± 1.0 | 15.8 ± 4.5 | 55.2 ± 6.0 | 65.3 ± 5.4 |
| | | 7 | | 7.5 ± 2.3 | 12.1 ± 2.6 | 29.2 ± 3.9 | 48.7 ± 5.4 | 78.3 ± 6.3 |

Note: f.w.: fresh weight

and changes in amine concentrations were related to storage time by linear regression (Table 2).

*Changes in pH*

The pH values of the organs at storage times up to six days and storage temperatures of 0 °C, 3 °C and 7 °C did not differ significantly. The pH values for the kidney were in the range of 6.3–6.51 (mean value: 6.38 ± 0.06), and 6.03–6.27 (6.15 ± 0.06) for the spleen and 5.74–6.01 (5.95 ± 0.14) for the liver, with statistically significant differences between these organs (Kruskal–Wallis test, $p < 0.05$).

*Microbiology and Sensory Evaluation*

The initial total aerobic counts were 4.1 $log_{10}$ cfu/g for the liver, 4.7 $log_{10}$ cfu/g for the spleen and 4.9 for the kidney. Sensory spoilage in terms of malodour, slime formation, and discoloration was detectable at days 3 and 4 of storage at 3 °C and 7 °C, and day 5 of storage at 0 °C. This corresponded with total aerobic counts >6 $log_{10}$ cfu/g.

## Storage of Vacuum-Packaged Porcine Liver, Spleen and Kidney for 21 Days

*Biogenic Amines and Polyamines*

Similarly to experiment 1 (short-time storage trial) significant increases by time and by temperature could be observed for the concentrations of cadaverine, tyramine and also putrescine (Table 3). The concentrations of histamine, 2-phenylethylamine and tryptamine were constant or showed only a moderately increasing tendency, with final concentrations not exceeding 10 mg/kg. Spermidine and spermine concentrations were practically constant during a 21 day storage at 0 °C, at the levels found in experiment 1 (data not shown).

**Table 2.** Concentrations of histamine, 2-phenylethylamine, spermidine, spermine and tryptamine in vacuum-packaged porcine kidney, liver and spleen stored at 0 °C, 3 °C and 7 °C for six days.

| Amine | Organ | Influence of temperature[1] | Concentration (mean ± SD, n = 9)[2] (mg/kg f.w.) Initial | Final | Development during storage[3] (mg/kg·h) |
|---|---|---|---|---|---|
| Histamine | | | | | |
| | Kidney | −($p = 0.544$) | 4 ± 0.5 | 3.1 ± 1.27 | (−0.011), r = −0.315 |
| | Liver | −($p = 0.813$) | 15.8 ± 5.5 | 12.4 ± 6.6 | ~ |
| | Spleen | −($p = 0.634$) | 7.2 ± 1 | 8 ± 3.7 | ~ |
| 2-phenylethylamine | Kidney | −($p = 0.863$) | 0.71 ± 0.1 | 3.2 ± 4.4 | (0.026), r = 0.357 |
| | Liver | −($p = 0.452$) | 0.25 ± 0.42 | 6.34 ± 3.06 | ~ |
| | Spleen | −($p = 0.375$) | 0.26 ± 0.30 | 4.4 ± 2.8 | (0.036), r = 0.584 |
| Spermidine | Kidney | −($p = 0.820$) | 18.6 ± 1.4 | 15.4 ± 2.6 | (−0.019), r = −0.180 |
| | Liver | −($p = 0.776$) | 29 ± 3.1 | 18.4 ± 8.1 | (−0.040), r = −0.298 |
| | Spleen | −($p = 0.934$) | 56 ± 2.7 | 44.5 ± 16.4 | (−0.067), r = −0.220 |
| Spermine | Kidney | −($p = 0.221$) | 72.2 ± 12.8 | 60.9 ± 7.4 | (−0.090), r = −0.348 |
| | Liver | −($p = 0.775$) | 103.5 ± 12.8 | 87.9 ± 19.2 | (−0.070), r = −0.188 |
| | Spleen | −($p = 0.876$) | 78 ± 3.4 | 69.1 ± 7.4 | (−0.040), r = −0.140 |
| Tryptamine | Kidney | −($p = 0.652$) | Nd | 4.8 ± 5.7 | ~ |
| | Liver | −($p = 0.128$) | Nd | 3 ± 3.3 | (0.022), r = 0.452 |
| | Spleen | −($p = 0.975$) | Nd | 7.2 ± 6.6 | (0.050), r = 0.554 |

Nd: not detected (<0.1 mg/kg).
[1]Estimated by ANOVA. (−): No significant difference.
[2]Mean of three temperature conditions determined in triplicate and pooled.
[3]Estimated as the slope of a linear regression (r: correlation coefficient), only significant regressions ($p < 0.1$) displayed (ANOVA). (~) No significant tendency.

*Changes in pH*

The pH values of the organs at storage times up to 14 days at 0 °C did not differ significantly. The initial pH values were statistically significant among organs: 6.48 ± 0.05 for the kidney, 6.33 ± 0.05 for the spleen and 6.19 ± 0.03 for the liver (ANOVA, $p < 0.05$). While no significant difference could be observed in the pH values of days 0, 7 and 14, pH at day 21 (0 °C) was significantly (ANOVA, $p < 0.05$) lower for the liver (final pH 5.48 ± 0.12) and higher for the kidney and the spleen (final pH 6.85 ± 0.03 and 6.53 ± 0.09, respectively).

*Total Aerobic Count and Aeromonas Count*

Similar results were obtained to those of the first trial, see Table 3. During the storage, *Aeromonas* sp. counts increased substantially, with final counts (21 days, 0 °C) of 5.35 ± 0.47 $\log_{10}$ cfu/g for the liver and significantly (Kruskal–Wallis test, $p < 0.05$) higher counts for the spleen and liver (6.75 ± 0.06 $\log_{10}$ cfu/g and 6.64 ± 0.18 $\log_{10}$ cfu/g, respectively).

**Relation of Bacterial Counts and Amine Formation**

Results from both experiments were pooled and correlations for $\log_{10}$ total aerobic count, putrescine, cadaverine and tyramine were calculated (Table 4). The sum of the latter three amines was related to the $\log_{10}$ total aerobic count (Table 5), with r = 0.70 ($p = 0.000$).

# DISCUSSION

**Amine Concentrations, pH, Total Aerobic Count and *Aeromonas* Count during Storage**

There were no significant differences between the storage temperatures of 0 °C, 3 °C and 7 °C for histamine, 2-phenylethylamine, spermine, spermidine and tryptamine, with only minor changes in the concentrations throughout the storage period of six days. Inner organs are commonly regarded as highly perishable, however, amine concentrations of stored inner organs corresponded well with findings for stored muscle tissue of various animal species, e.g. beef (Kaniou et al., 2001), pork (Nakamura et al., 1979) and rabbit (Guerrero-Legaretta and Chavez-Gallardo, 1991). Tryptophane- and phenylalanine-decarboxylating bacteria are not expected to be present in large numbers on raw meat (see Beutling, 1996; Suzzi and Gardini, 2003), so substantial tryptamine and 2-phenylethylamine should not be formed in muscle tissue and organs *post mortem*. In contrast to fermented food, histamine contents in fresh and spoiled meat are generally low (Ten Brink et al., 1990; Stratton et al., 1991; Bauer and Paulsen, 2001). Most probably, because temperatures for meat storage are considerably lower than mesophilic bacteria require for growth (15 °C) and also too low to allow substantial histidine-decarboxylase activity (Joosten and Van Boekel, 1988). Spermine and spermidine are generated by synthesis (Silla Santos, 1996), which is unlikely to happen *post mortem*. In contrast, a number of bacteria of

**Table 3.** Total aerobic counts and concentrations of putrescine, cadaverine and tyramine (mean ± SD, n = 6) in vacuum-packaged porcine inner organs during storage.

| | | | Storage | | | |
|---|---|---|---|---|---|---|
| Organ | Parameter | T (°C) | Day 0 | Day 7 | Day 14 | Day 21 |
| Liver | Total aerobic counts ($log_{10}$ cfu/g) | 0 | 4.35 ± 0.16 | 4.98 ± 0.47 | 7.66 ± 0.46 | 9.0 ± 0.14 |
| | | 3 | – | 7.35 ± 0.3 | – | – |
| | | 7 | – | 7.8 ± 0.18 | – | – |
| | Putrescine (mg/kg f.w.) | 0 | 1.03 ± 0.82 | 2.71 ± 2.2 | 48.62 ± 0.77 | 68.43 ± 3 |
| | | 3 | – | 33.69 ± 1.63 | – | – |
| | | 7 | – | 54.42 ± 2.35 | – | – |
| | Cadaverine (mg/kg f.w.) | 0 | 0.79 ± 0.68 | 4.95 ± 1.3 | 5.22 ± 0.5 | 47.45 ± 2.23 |
| | | 3 | – | 28.66 ± 1.05 | – | – |
| | | 7 | – | 47.9 ± 2.56 | – | – |
| | Tyramine (mg/kg f.w.) | 0 | 0.6 ± 0.51 | 5.34 ± 4.2 | 11.58 ± 1.04 | 41.46 ± 2.58 |
| | | 3 | | 49.22 ± 1.33 | – | – |
| | | 7 | | 59.7 ± 3.75 | – | – |
| Spleen | Total aerobic counts ($log_{10}$ cfu/g) | 0 | 4.44 ± 0.24 | 5.46 ± 0.36 | 7.78 ± 0.39 | 7.64 ± 0.17 |
| | | 3 | – | 7.48 ± 0.21 | – | – |
| | | 7 | – | 7.68 ± 0.14 | – | – |
| | Putrescine (mg/kg f.w.) | 0 | 4.2 ± 1.2 | 3.45 ± 0.9 | 50.82 ± 1.66 | 138.89 ± 1.78 |
| | | 3 | – | 58.76 ± 1.94 | – | – |
| | | 7 | – | 105.27 ± 10.98 | – | – |
| | Cadaverine (mg/kg f.w.) | 0 | 0.66 ± 0.64 | 2.3 ± 0.34 | 51.37 ± 1.6 | 122 ± 4.52 |
| | | 3 | – | 67.97 ± 3.22 | – | – |
| | | 7 | – | 104.75 ± 2.05 | – | – |
| | Tyramine (mg/kg f.w.) | 0 | 2.95 ± 1.9 | 2.65 ± 1.98 | 39.08 ± 0.92 | 63.19 ± 2.53 |
| | | 3 | – | 29.98 ± 1.46 | – | – |
| | | 7 | – | 58.02 ± 2.34 | – | – |
| Kidney | Total aerobic counts ($log_{10}$ cfu/g) | 0 | 4.25 ± 0.15 | 5.53 ± 0.25 | 8.00 ± 0.2 | 7.74 ± 0.17 |
| | | 3 | – | 7.48 ± 0.36 | – | – |
| | | 7 | – | 7.71 ± 0.19 | – | – |
| | Putrescine (mg/kg f.w.) | 0 | 0.76 ± 0.55 | 2.75 ± 0.2 | 60.01 ± 3.89 | 207.35 ± 5.01 |
| | | 3 | – | 15.23 ± 0.6 | – | – |
| | | 7 | – | 38.08 ± 2.47 | – | – |
| | Cadaverine (mg/kg f.w.) | 0 | 0.23 ± 0.23 | 2.72 ± 0.4 | 7.36 ± 3.3 | 21 ± 4.1 |
| | | 3 | – | 18.1 ± 0.82 | – | – |
| | | 7 | – | 47.57 ± 2.13 | – | – |
| | Tyramine (mg/kg f.w.) | 0 | 0.92 ± 0.31 | 3.92 ± 0.3 | 14.72 ± 0.59 | 34.19 ± 0.57 |
| | | 3 | – | 5.78 ± 0.18 | – | – |
| | | 7 | – | 13.5 ± 0.7 | – | – |

(–) Condition not studied.

**Table 4.** Significant correlations (r) between total aerobic count (TAC, $log_{10}$ cfu/g) and selected amines (putrescine, cadaverine, tyramine (mg/kg f.w.).

| | Liver | Spleen | Kidney |
|---|---|---|---|
| TAC: putrescine | 0.77 ($p = 0.000$) | 0.57 ($p = 0.041$) | 0.54 ($p = 0.068$) |
| TAC: cadaverine | 0.78 ($p = 0.000$) | 0.62 ($p = 0.024$) | 0.66 ($p = 0.005$) |
| TAC: tyramine | 0.77 ($p = 0.000$) | 0.89 ($p = 0.000$) | 0.70 ($p = 0.001$) |

**Table 5.** Relation between total aerobic count (TAC) and the sum of selected amines (putrescine, cadaverine, tyramine).

| TAC ($log_{10}$ cfu/g) | Average (Minimum–Maximum) (mg/kg f.w.) |
|---|---|
| <6 | 13.8 (2.0–33.3) |
| 6–7 | 35.2 (10.8–60.6) |
| >7 | 110.9 (39.1–324.1) |

various genera producing putrescine, cadaverine and tyramine have been identified on meat (compiled by Beutling, 1996). As could be expected, in our study, the concentrations of these three amines increased with temperature and storage time. While for 0 °C, the amine levels did not change substantially during the first 7 days of storage, their levels increased significantly at 3 °C and 7 °C. This corresponded to bacterial levels of $>6 \log_{10}$ cfu/g (Table 5). This is in accordance with a number of other studies conducted with muscle tissue reporting increases in biogenic amines content at total aerobic counts ranging from 5–7 $\log_{10}$ cfu/g (Schmitt et al., 1988, for poultry; Poetzelberger et al., 1998, for beef and pork cuts; Paulsen et al., 2002, for minced beef and pork; Stonsaovapak et al., 2001, for ground beef, pork and chicken). A storage period of seven days did not affect the pH values, irrespective of the temperature (0 °C to 7 °C).

Notably, on vacuum-packaged high-pH organs, bacterial flora is significantly shifted towards the potential food-borne pathogen *Aeromonas*. Thus, in the liver, *Aeromonas* constituted <1% of the total aerobic flora, while on the spleen and kidney, with a significantly higher pH, *Aeromonas* constituted 10–15% of the total aerobic flora. Similar observations have been made for high-pH muscle, for various meat species, e.g. lamb (Doherty et al., 1996), cattle (Gill and Reichel, 1989) and turkey (Mano et al., 2000), under various packaging regimes.

### Specific Amine Patterns

We observed consistent ratios of spermine to spermidine, which were 3:2 for the spleen and 4:1 for the liver and the kidney. This difference in amine ratios was statistically significant. Despite a decline of these amines during storage, the ratio was preserved throughout; which is in accordance with literature findings for muscle tissue (Wortberg and Woller, 1982; Rogowski and Döhla, 1984; Treviño-Treviño, 1993; Paulsen et al., 2002). As these amines are synthetised *ante mortem*, this ratio may reflect biochemical peculiarities of these organs. Further studies in this field may be useful. For a number of common edible tissues, specific amine patterns develop *post mortem*, as is the case for tuna, where both free histidine (up to 14,600 mg/kg, Lehane and Olley, 2000) and bacterial activity (which is influenced by time/temperature profiles, e.g. Klausen and Lund, 1986) may result in excessive histamine formation. In contrast, the tissue specific spermine:spermidine ratio described in our study is not bacteria-dependent.

### Significance of Selected Biogenic Amines as Indicators of Ageing Processes

Shelf life, freshness and spoilage of food are often assessed by sensory testing (Botta, 1995) and among the variety of chemical compounds, volatile nitrogenous substances, and ATP metabolites have some relevance (Dainty, 1996). While the diamine concentration has been proposed as a freshness indicator quite early (Dainty et al., 1986), tyramine has been identified to indicate ageing of beef (Hernandez-Jover et al., 1997; Vinci and Antonelli, 2002). To date, there is a general agreement that during storage of muscle tissue a rise in concentrations of cadaverine, putrescine and tyramine can be expected (Hernandez-Jover et al., 1997; Kaniou et al., 2001; Rokka et al., 2004). Consequently, the sum of these amines (in mg/kg) has been proposed as a freshness index for meat (Hernandez-Jover et al., 1997; Roig, 2002). Formation of putrescine and cadaverine is strongly related to the activity of contaminant bacteria, as in sterile meat, no significant rises in amine concentrations could be observed (Slemr, 1981). While in comminuted meat with average total aerobic counts $<5.5 \log_{10}$ cfu/g, mean putrescine and cadaverine concentrations are low (2–10 mg/kg; Poetzelberger et al., 1998; Paulsen et al., 2002), a substantial rise in the formation of these amines can be expected when the total aerobic bacterial count exceeds $6 \log_{10}$ cfu/g (Stonsaovapak et al., 2001), and sensory changes are perceived. We could prove that these data elaborated on muscle tissue apply also to porcine liver, spleen and kidney. Although specific biogenic amines are correlated to ageing and decomposition of proteinaceous foods, we could show that they are not very sensitive as indicators of early ageing conditions of vacuum-packaged porcine organs. Other authors come to the same conclusion for fish (Botta, 1995). A number of studies, however, presented evidence that biogenic amine contents in canned tuna (Mietz and Karmas, 1977; Veciana-Nogues et al., 1997), fermented dry sausage (Bover-Cid et al., 2001), cooked cured meat (Hernandez-Jover et al., 1996) and heat processed, canned restructured meat products (Paulsen et al., 2000) may serve as an indicator for the hygienic condition of the raw material, i.e. high amine levels in processed food indicate the use of poor quality raw materials. The estimation of this quality deficiency has to be based on experimental data on fresh as well as spoiled edible animal tissues.

### Health Significance for the Consumer

European Union has established limits only for histamine in certain fish and seafood species and due to individual variations (Luethy and Schlatter, 1993) it is complicated to elaborate valid dose-response models. The data presented in this study give an indication that potentially harmful levels of biogenic amines are not formed in porcine liver, spleen and kidney before sensory manifestation of spoilage. The data presented in this study, however, may be useful for exposure assessment estimations.

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