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# Concentration of biologically active polyamines in meat and liver of sheep and lambs after slaughter and their changes in mutton during storage and cooking

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ABSTRACT

Putrescine (PUT), spermidine (SPD) and spermine (SPM) concentrations using a UPLC method, in chilled mutton, lamb and livers 24 h after slaughter were determined. PUT concentrations were quantifiable only in some samples. Mean SPD concentrations were 4–6, 13.5 and 16.8 mg $kg^{-1}$ in the meats, sheep and lamb livers, respectively. The respective SPM concentrations were 17–25, 128 and 79 mg $kg^{-1}$. SPD and SPM losses of about one fifth and half of the initial level, respectively, were apparent in mutton loins stored at −18 °C for 6 months. Significant losses of SPD and SPM were found in mutton loins stored aerobically, vacuum-packaged or in a modified atmosphere at +2 °C. Boiling and stewing of mutton legs caused SPD and SPM losses of about 40% and roasting of about 60% of the initial content.



## 1. Introduction

Polyamines putrescine [PUT; $H_2N(CH_2)_4NH_2$], spermidine [SPD; $H_2N(CH_2)_3NH(CH_2)_4NH_2$] and spermine [SPM; $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] were separated from their traditional classification within biogenic amines during the 1990s due to their mode of formation and biological roles. Putrescine, though structurally a diamine, is also classified as a polyamine, being the precursor of both physiological ("true") polyamines (PUT → SPD → SPM). Because the polyamines are fully protonated under physiological conditions, they can interact with numerous cell constituents, such as nucleic acids, ATP, specific proteins and phospholipids. Thus, they are essential for cell growth (Agostinelli et al., 2010; Igarashi & Kashiwagi, 2010; Kusano, Berberich, Tateda & Takahashi, 2008).

The polyamine body pool is maintained by three primary sources: i) endogenous (*de novo*) biosynthesis, ii) production by intestinal bacteria, and iii) dietary intake. According to Bardócz (1995), diet provides more of the polyamines than does endogenous biosynthesis. SPD and SPM are not enzymatically degraded in the alimentary tract and both polyamines taken orally are quickly absorbed from intestinal lumen and distributed to organs and tissues (Uda, Tsujikawa, Fujiyama & Bamba, 2003). Thus polyamines in the diet are among the determinants of their total body pool (for a review see Kalač & Krausová, 2005).

Recent studies have suggested that reducing the level of polyamines in cells may help to slow cancer development. One direction in cancer therapy research is to limit the intake of dietary polyamines (Cipolla, Guille & Moulinoux, 2003; Cipolla, Havouis & Moulinoux, 2007, 2010). However, dietary polyamines may be required in wound healing and for growth, maturation and regeneration of the intestinal mucosa. The role of dietary polyamine intake increases in elderly people with limited ability to biosynthesise them (Larqué, Sabater-Molina & Zamora, 2007; Soda, Dobashi, Kano, Tsujinaka & Konishi, 2009). The main roles of polyamines in health and disease have been reviewed (Larqué et al., 2007; Moinard, Cynober & de Bandt, 2005). Various physiological roles of polyamines were recently described (Dandrifosse, 2009).

Information on the content of polyamines in foods and beverages would thus be of great interest for assessing their dietary intake (Zoumas-Morse et al., 2007). Mean daily intakes of 18.7, 12.6 and 11.0 mg of PUT, SPD and SPM, respectively, have been reported for the United Kingdom, Italy, Spain, Finland, Sweden and the Netherlands (Ralph, Englyst & Bardócz, 1999). The values in Japan were 9.9, 12.0 and 7.9 mg (Nishibori, Fujihara & Akatuki, 2007) and for the USA sample diets contained 14.0, 7.9 and 7.2 mg (Zoumas-Morse et al., 2007). Unfortunately, daily cellular requirements for the polyamines have not yet been determined.

As previously reviewed (Kalač, 2006; Kalač & Krausová, 2005), higher SPM compared to SPD contents, are usual in foods of animal origin, mainly in muscle, while the opposite is observed in foods of plant origin. Unlike PUT, dietary SPD and SPM originate from the raw

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materials as their production by microbial activity in foods is minimal. Polyamine levels are high in young and metabolically active tissues and organs (Nishimura, Shiina, Kashiwagi & Igarashi, 2006).

Meat has been identified as the main source of SPM in intake studies and reviews.

Surprisingly, limited data on polyamine contents is available for lamb meat (Cipolla et al., 2007; Nishimura et al., 2006) and ovine liver (Krausová, Kalač, Křížek & Pelikánová, 2006b) and to the best of our knowledge, information on mutton is lacking.

The objective of this study was to determine polyamine contents in raw lamb, mutton and ovine liver and the changes in mutton after different storage and cooking conditions.

## 2. Materials and methods

The methods used were the same as those used for polyamine determination in fresh, stored and cooked beef (Krausová et al., 2006b; Kozová, Kalač & Pelikánová, 2009a), pork (Krausová et al., 2006b; 2008), chicken (Kozová et al., 2009b) and livers (Krausová et al., 2006a) enabling comparison with results obtained with meats and livers of different origin.

### 2.1. Sampling

Meat and liver samples were taken from sheep and lamb carcasses 20 h after slaughter in an abattoir between March 2009 and April 2010. The carcasses were chilled rapidly to 3–4 °C and were kept in a cool store. Leg and loin samples weighing 200–250 g were taken from each carcass as were liver lobes weighing about 150 g. The animals were hybrids of several breeds and originated from numerous small-scale sheep breeders. Mean sheep age was 70.9 ± 31.0 months, carcass weight 32.3 ± 11.4 kg. In lambs, the ages were 5.9 ± 1.1 months and the weights 12.6 ± 2.7 kg. In total, 22 ewes, 4 rams and 31 lambs were sampled.

The samples were transported to the laboratory in a cold box. Visible fat was removed prior to analysis. The analyses of the initial polyamine contents started 22–24 h after slaughter.

Mutton for the storage and cooking experiments was taken under similar conditions.

### 2.2. Mutton storage conditions

The effects in mutton loin chops during storage at −18 ± 1 °C on polyamine contents were observed in four experiments using loins from three ewes and one ram. In each experiment, individual ribs with meat and fat were separated and each packaged in a bag made of high-density polyethylene (HDPE; foil thickness 0.017 mm), frozen in a household freezer and then stored. Prior to analyses, the samples were thawed at 21–22 °C for 2 h and visible fat was removed. The analyses were carried out on day 0 (24 h after slaughter) and then monthly up to 6 months.

The effects of cold-storage were tested in four experiments using the chilled legs of four ewes 24 h after slaughter (day 0). Each leg was cut into 12 parts of about 150 g. One part was used for the determination of initial polyamine concentration; the other parts were packaged in one of three ways and then stored at 2 ± 0.5 °C:

- in polyethylene bags (the same as in frozen-storage), simulating aerobic packaging and storage. The analyses were carried out on days 2, 5 and 9,
- vacuum-packaged, stored and analysed on days 5, 9, 15 and 21,
- packaged in a modified atmosphere of 70% $N_2$ and 30% $CO_2$ (v/v), stored and analysed on the same days as the vacuum-packaged samples.

The samples in the latter two systems were packaged at the abattoir using a Vac-Star S240DK (Busch, Germany). The polyethylene foil was 0.080 mm thick and had very low oxygen permeability (below 0.02 ml $m^{-2}$ $d^{-1}$ at 0.1 MPa).

### 2.3. Cooking treatments

The experiments simulated usual mutton processing in Central Europe. Three experiments were carried out using three legs aged at + 2 ± 0.5 °C for six days after slaughter. The legs were packaged in polyethylene foil (thickness 0.017 mm) during storage. Three treatments were evaluated:

- boiling: About 150 g of leg, without visible fat was cut into cubes of about 2 × 2 × 2 cm, the same weight of water was added and the mixture was sealed in a polyethylene bag. The bag was immersed in boiling water for 60 min. The inner temperature was measured by a puncture thermometer (accuracy ± 0.5 °C, Amarell Electronic, Germany). The temperature reached its maximum of 97 °C after 16 ± 1 min. The bags were then cooled under tap water to air temperature and both the cubes and broth were used for polyamine and dry matter determinations,
- stewing was carried out under the same conditions as boiling, but water was added at only half the weight of meat,
- roasting: About 180 g of the cubes was roasted in an oven at 190 °C for 75 min in a porcelain bowl covered with an aluminium foil. About 25 ml water was added repeatedly to prevent burning. The gravy was evaporated.

### 2.4. Analytical methods

Changes of the polyamine concentrations were calculated and expressed as mg per kg of dry matter due to the changes in dry matter contents during storage and particularly during cooking.

All chemicals used were of analytical grade.

Dry matter content was determined by drying a homogenised sample in an oven at 105 °C until no difference in weight was observed.

Acid extracts for polyamine determination were prepared by homogenising 40 ± 1 g of sample in 0.6 M perchloric acid as described previously (Krausová et al., 2006a). The polyamines were determined after derivatisation with dansyl chloride, using an ultra-performance liquid chromatography (UPLC) method (Dadáková, Křížek & Pelikánová, 2009). Derivatisation procedures for the solid samples and the broth have been described (Krausová et al., 2008).

Briefly, the UPLC determination was carried out using an Agilent 1200 Series Rapid Resolution LC system (Agilent Technologies, Inc., Santa Clara, CA, USA). The system was equipped with binary pumps, a micro-vacuum degasser, high performance autosampler and DAD detector.

The separation was carried out on an Agilent Zorbax Eclipse XDB-C18 column (50 mm × 4,6 mm I.D., 1.8 μm particle size), equipped with a RRLC In-line filter, 0.2 μm (Agilent Technologies, Inc., Santa Clara, CA, USA). Chromatographic separation used a gradient elution of (A) acetonitrile (100%), (B) acetonitrile (50% water solution) as follows: 0–2 min, A 40%, B 60%, 2–3 min, A 40–80%, B 60–20%, 3–4 min, A 80–90%, B 20–10%, 4–6 min, A 90–95%, B 10–5%, 6–7 min, A 95–40%, B 5–60%, 7–12 min, A 40%, B 60%. Linear steps were performed in all cases. The flow rate was 1.0 ml $min^{-1}$ with a column temperature of 25 °C and injection of 5 μl; the detection wavelength was 225 nm.

Repeatability of the polyamine determinations was tested by ten analyses of a mutton leg aged for three days. The values were 4.0, 4.7 and 4.3% for PUT, SPD and SPM, respectively, at mean concentrations of 2.7, 3.2 and 15.7 mg $kg^{-1}$. The recoveries were 92, 90 and 98% for PUT, SPD and SPM, at concentrations of 5.3, 7.1 and 28.5 mg $kg^{-1}$ of added PUT, SPD and SPM, respectively. Limits of quantification (LOQ), calculated as amine concentration providing a signal equal to ten