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Changes in the content of biologically active polyamines during storage and cooking of pig liver

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Abstract

Dietary polyamines putrescine (PUT), spermidine (SPD) and spermine (SPM) participate in numerous human physiological processes, including tumour growth. Eight experiments with pig liver were carried out. In two, livers were stored at −18 °C for 168 days, in four, livers were stored aerobically (AE), vacuum-packaged (VP) and packaged in a modified atmosphere (MO; 70% N_2 and 30% CO_2, v/v) at +2 °C for 9, 21 and 21 days, respectively, and in two, the effects of four cooking treatments were tested. Polyamines were determined as dansyl derivatives using an HPLC method. Distribution of both SPD and SPM in the four main liver lobes was homogenous. The initial SPD and SPM contents in 14 livers 24 h after slaughter were 23.3 ± 6.7 and 94.5 ± 19.6 mg kg^{-1}, respectively. The putrescine content was below the limit of detection. The content of SPD and SPM decreased during frozen-storage to about 70% of the initial values. On day-9 of storage, mean SPD and SPM contents decreased to about 85% of the initial values in livers stored in MO and to about 75–80% in AE and VP at 2 °C. The decrease continued more extensively in VP than in MO. PUT was detected from day-15 of VP and MO storage. There was a significant decrease in SPD and SPM, to about 70–60% of the initial content during cooking.

Keywords: Dietary polyamines; Putrescine; Spermidine; Spermine; Pig liver; Storage; Cooking

1. Introduction

Polyamines putrescine [PUT, 1,4-diaminobutane], spermidine [SPD, *N*-(3-aminopropyl)-1,4-diaminobutane] and spermine [SPM, *N*,*N*′-*bis*(3-aminopropyl)-1,4-diaminobutane] have traditionally been classified within the group of biogenic amines. However, they started to be considered separately during the 1990s, because of their role in the growth and function of normal cells and due to their mode of formation. Putrescine, though structurally a diamine, is also classified as a polyamine due to its role as the precursor of both physiological polyamines (PUT → SPD → SPM). The polycationic polyamines participate in cell proliferation and are thus of great interest in research on tumour growth (e.g., Bachrach, 2004; Thomas & Thomas, 2003). One direction in cancer therapy research is to limit the intake of polyamines. However, polyamines may be effective in wound healing and for growth, maturation and regeneration of the intestinal mucosa (Deloyer, Peulen, & Dandrifosse, 2005; Peulen & Dandrifosse, 2004; Weiss et al., 2004). Both endogenous and dietary polyamines participate in such processes (Bardócz, 1995). The polyamines are derived both by *de novo* synthesis and by uptake from extracellular sources. The role of dietary polyamine intake, mainly of the most effective, SPM, increases in elderly people with limited ability to biosynthesise them (Larqué, Sabater-Molina, & Zamora, 2007). The main roles of polyamines in health and disease were recently reviewed (Larqué et al., 2007; Moinard, Cynober, & de Bandt, 2005; Teti, Visalli, & McNair, 2002; Wallace, Fraser, & Hughes, 2003).

Information on the content of polyamines in foods and beverages would thus be of great interest for dieticians and physicians. As previously demonstrated (Kalač, 2006; Kalač & Krausová, 2005), higher SPM contents, as

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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 mainly from raw materials and their production by bacterial activity in foods is limited (Durlu-Özkaya, Ayhan, & Vural, 2001; Martuscelli, Crudele, Gardini, & Suzzi, 2000; Min, Lee, Jang, Lee, & Kim, 2004). That polyamine levels are high in young and metabolically active tissues and organs was supported by a report describing a significant decrease of both SPD and SPM contents in muscles and numerous organs of mice on ageing (Nishimura, Shiina, Kashiwagi, & Igarashi, 2006).

Meat and meat products are the main dietary sources of SPM both in the UK (Bardócz, 1995) and Japan (Nishibori, Fujihara, & Akatuki, 2007). However, there is limited information on the polyamine content in offal. Very high SPD and SPM contents were reported in bovine, pig and chicken livers (Krausová, Kalač, Křížek, & Pelikánová, 2006). Information on changes in the polyamine contents during liver storage and cooking is lacking.

The objective was therefore to determine changes in polyamine contents in pig liver under different storage conditions and various cooking treatments.

2. Materials and methods

2.1. Sampling

Whole livers were taken in an abattoir in Týn nad Vltavou from pigs of live weight 115–120 kg. The pigs were hybrids of several meat breeds. The livers were chilled rapidly, immediately after slaughter to an inner temperature of 2–3 °C and were kept in a cold store for 20 h.

The livers were then transported to the laboratory in a cool box. Isolation of the polyamines started within 2–4 h of arrival. In total, analyses of the polyamine contents and experiments described below started 24 h after slaughter.

2.2. Testing of homogeneity in the polyamine content within liver lobes

It was necessary to know if the polyamines are distributed uniformly within all liver lobes. The pig liver has six lobes. Four of them, right and left lateral and medial lobes are large, while the two smaller ones are usually damaged during separation of the liver and their role in human nutrition is limited. Therefore, the four large lobes were cut from three different livers and analysed separately in triplicate.

2.3. Storage conditions

The effects of liver storage at -18 ± 1 °C for up to 168 days were observed in two experiments. Liver portions of about 350 g were packaged into bags made of high-density polyethylene (HDPE, foil thickness 0.017 mm), frozen and stored in a freezer. Prior to analyses, the samples were thawed in a refrigerator at +3 °C for 16 h. The analyses were carried out on days 0, 14, 28, 56, 84, 112 and 168.

The effect of cold-storage was tested in four experiments. In the initial experiment, the liver was cut into 15 slices of about 120 g, which were packaged in the three ways described below. In further three experiments, liver portions of about 350 g were packaged in the three different ways:

- into the HDPE bags as in the freezing experiments, simulating aerobic packaging and storage in households. The samples were stored and analysed on days 0, 1, 2, 5 and 9,
- vacuum-packaged, stored and analysed on days 0, 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.

For each treatment, the liver from one pig was used in the latter three experiments. In total, livers from 10 pigs (1 and 3×3) were examined in the four experiments.

The livers in the latter two treatments were packaged at the abattoir using a Vac-Star S240M (Busch, Germany). The polyethylene foil used was of thickness 0.080 mm, had a very low oxygen permeability (below 0.02 ml $m^{-2}\,d^{-1}$ at 0.1 MPa). The packaged samples were stored at $+2 \pm 0.5$ °C.

2.4. Cooking treatments

Two experiments were carried out. In each of them, the liver from the freshly slaughtered pig was divided into halves and chilled rapidly. One half was processed after storage at +2 °C for 24 h after slaughter, the second one was kept at +2 °C for six days and then processed. The liver was cut into slices of about 150 g. The following treatments, simulating liver processing typical of Central European cuisine, were tested:

- *Boiling*: 150 g of liver was cut into cubes of about $2 \times 2 \times 2$ cm, 150 ml of distilled water was added and the mixture was sealed in a polyethylene bag. The bag was immersed in a boiling water bath for 36 min. The inner temperature was measured by a puncture thermometer to ±0.5 °C (Amarell Electronic, Germany). The temperature reached 97 °C after 26–28 min,
- *Stewing*: This was carried out under the same conditions as for boiling except:
  A: no water was added and the liver cubes were stewed in their own grease, or
  B: 37 ml of water (about a quarter of the liver weight) was added,
- *Pan-roasting without oil*: A slice of about 150 g and thickness about 2 cm was heated in a steel pan with a five-layer bottom heated to 180 °C. No fat or water