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Contents of biologically active polyamines in chicken meat, liver, heart and skin after slaughter and their changes during meat storage and cooking

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ABSTRACT

Dietary polyamines, putrescine, spermidine (SPD) and spermine (SPM), participate in an array of important physiological roles, including tumour growth. Thus, reliable information on polyamine content in foods has been needed. We therefore determined polyamine contents in chilled chicken meat and giblets ($n = 20$) and skin ($n = 10$) 24 h after slaughter. The polyamines were determined, after extraction with perchloric acid, as dansyl derivatives, using an HPLC method. Mean SPD values were 4.8, 10.2, 11.4, 48.7 and 12.1 mg kg^{-1} and SPM values were 36.8, 38.0, 24.3, 133 and 82.7 mg kg^{-1} in breast, thigh, skin, liver and heart, respectively. Significant statistical correlations between SPD and SPM contents were observed in breast, thigh, skin and liver, whereas correlations were insignificant in heart. An increase of SPD and SPM was apparent in breasts and thighs stored at −18 °C for 6 months; however, it was significant only for SPM in thighs. The losses of both SPD and SPM were statistically insignificant during storage of aerobically packaged breasts up to 9 days at +2 °C. A significant decrease of SPM to about 60% of the initial contents was observed in both vacuum-packaged and in modified atmosphere (20% CO_2 and 80% O_2)-stored breasts on day 21 at +2 °C. For both SPD and SPM, roasting, grilling and frying of fresh breasts caused losses of about 40–60% of the initial contents (higher than boiling and stewing). Similarly, losses of SPM, due to roasting of breasts frozen for 3 or 6 months, were higher than those caused by stewing. Putrescine was detected only sporadically and at levels close to the detection limit of 1.0 mg kg^{-1} (fresh matter).

1. Introduction

Polyamines putrescine [PUT, butane-1,4-diamine], spermidine [SPD, *N*-(3-aminopropyl)-butane-1,4-diamine] and spermine [SPM, *N*,*N*′-bis(3-aminopropyl)-butane-1,4-diamine] 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 (Kusano, Berberich, Tateda, & Takahashi, 2008). Putrescine, though structurally a diamine, is also classified as a polyamine due to its role as the precursor of both physiological ("true") polyamines (PUT → SPD → SPM). The polycationic polyamines participate in cell proliferation and differentiation and are thus of great interest in research on tumour growth. Recent studies have suggested that reducing the level of polyamines in cells may help to slow the cancer process. The polyamine body pool is maintained by three primary sources: (i) endogenous (*de novo*) biosynthesis, (ii) production by intestinal microorganisms, and (iii) dietary intake. Thus, polyamines in the diet are among the determinants of their total body pool (for a review see Kalač and Krausová (2005)).

One direction in cancer therapy research is to limit the intake of dietary polyamines (Cipolla, Havouis, & Moulinoux, 2007). 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). The main roles of polyamines in health and disease were recently reviewed (Larqué et al., 2007; Moinard, Cynober, & de Bandt, 2005).

Diet provides a larger daily quantity of polyamines than does endogenous biosynthesis (Bardócz, 1995). 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, 18.7, 12.6 and 11.0 mg of PUT, SPD and SPM, respectively, were reported for the United Kingdom, Italy, Spain, Finland, Sweden and the Netherlands (Ralph, Englyst, & Bardócz, 1999). The values adopted for Japan were 9.9, 12.0 and 7.9 mg (Nishibori, Fujihara, & Akatuki, 2007) and for a USA convenient sample diet 14.0, 7.9 and 7.2 mg were selected (Zoumas-Morse et al., 2007). Unfortunately, daily cellular requirements for the polyamines have not yet been determined.

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Table 1
Literature data on mean polyamine contents (mg kg^{-1}) in fresh and cooked chicken meat and liver.

Anatomical part/cooking	Number of samples	Putrescine	Spermidine	Spermine	References
Fresh					
Breast	4	<0.8	7.3	17.9	Silva and Glória (2002)
	3	–	9.8–14	75–82	Rokka et al. (2004)
	6	53.2	10.6	55.0	Balamatsia et al. (2006)
	30	0.4[a]/1.0[b]	6.3[a]/27.4[b]	29.5[a]/38.7[b]	Moreira et al. (2008)
Thigh	4	<0.8	7.2	16.2	Silva and Glória (2002)
	10	1.1	13.4	1.6	Cipolla et al. (2007)
Wing	10	0.7	9.3	23.2	Cipolla et al. (2007)
Non-specified	2	2.9	9.3	59.2	Bardócz, Grant, Brown, Ralph, and Pustai (1993)
	2	<0.4	2.9	62.6	Okamoto, Sugi, Koizumi, Yanadiga, and Udaka (1997)
	1	1.5	6.7	69.1	Nishimura et al. (2006)
	4	0.7	6.5	45.6	Nishibori et al. (2007)
Cooked					
Grilled	5	2.0	17.3	44.4	Eliassen, Reistad, Risøen, and Rønning (2002)
Roasted breast	10	<2.1–5.5	25.5	54.1	Kalač, Křížek, Pelikánová, Langová, and Veškrna (2005)
Fried breast	6	0.7	170	15.7	Patsias, Chouliara, Paleologos, Savvaidis, and Kontominas (2006)
Giblets					
Raw liver	38	<2.1–4.4	56.9	119.6	Krausová et al. (2006a)

[a] Immediately after slaughter.
[b] After ageing at 1–5.7 °C for 8 h.

As previously reviewed (Kalač, 2006; Kalač & Krausová, 2005), higher SPM contents, as 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 raw materials as the intimate cell content, and their production by bacterial activity in foods, is limited (if any). Polyamine levels are high in young and metabolically active tissues and organs (Nishimura, Shiina, Kashiwagi, & Igarashi, 2006).

Meat was determined to be the main source of SPM in all the above-cited intake studies and reviews. Due to the high consumption of chicken meat and giblets, it is necessary to have credible information on their polyamine contents. Literature data are given in Table 1. The information seems to be sufficient for raw chicken meat, regardless of the differences among the results of different laboratories. However, information on polyamine content in giblets and on changes during chicken meat storage and different cooking treatments has been scarce.

The objective of this study was therefore to determine polyamine contents in raw chicken meat, liver, heart and skin and polyamine changes in meat as the most important food item from chicken under different storage conditions and cooking treatments.

Table 2
Sampling scheme.

Experiment	Number of sampled carcasses	Number of analysed samples	Sample Sources
I. Initial PA content 24 h after slaughter	20	Breast (20) Thigh (20) Liver (20) Heart (20) Skin (10)	Processing facility
II. Changes of PA content during frozen-storage	8	Breast (8) Thigh (8)	Processing facility
III. Changes of PA content during cold-storage	9	Breast (9)	Processing facility
IV. Changes of PA content during cooking			
(A) Chilled meat	4	Breast (4)	Supermarket Experiment II
(B) Frozen meat			
(a) After 3 months of storage		Breast (4)	
(b) After 6 months of storage		Breast (4)	

PA, polyamines.

2. Materials and methods

2.1. Sampling

Sampled carcasses were taken several minutes after the slaughter of chickens in a commercial poultry processing facility of Jihočeská drůbež, a.s. in Vodňany, between October 2007 and February 2008. The chicken broilers were of ROSS 308 and COOB 500 varieties, mean live-weight of 1.85 kg and age of 35–38 days. Chilled or frozen samples (see below) were transported from the facility to the laboratory in a cool box during about 45 min. An overall sampling scheme is given in Table 2.

In Experiment I, 20 chicken carcasses, together with their livers and hearts, were sampled on four sampling days (4 × 5 chickens) for the determination of initial polyamine contents 24 h after slaughter. The carcasses and giblets were chilled, transported to the laboratory and then stored at 2–3 °C until 24 h after slaughter. Meat of the right breast and thigh (n = 20) was then separated from the carcasses and analysed, together with the giblets. Moreover, skin was removed from breasts and thighs of ten chickens and analysed together as one sample for each of the chickens.

Another set of eight chicken carcasses for the determination of PA content changes during frozen-storage (Experiment II) was taken from the slaughter equipment several minutes after the slaughter. The carcasses were cut into halves. The right half was chilled and used in the fresh state, the left one was packaged in a polyethylene bag (HDPE, foil thickness of 0.017 mm) and immediately frozen in a freezing tunnel at −30 °C until the inner temperature of −18 °C was acquired. The samples were then transported to the laboratory. The analyses of the initial PA content of breasts and thighs of all eight right halves were immediately started, i.e., about 3–4 h after the slaughter.

Similarly, nine carcasses were used for cold-storage experiments (Experiment III). The chilled samples were transported to the laboratory and breasts were separated. The following operations are described in Section 2.2.

In Experiment IV A, four carcasses for the fresh-meat cooking experiments were purchased from a supermarket on the third day after the slaughter and packaging. The chickens were handled after being refrigerated to about +3 °C. Both parts of the breast were separated and used for different cooking treatments. Moreover, breasts, which passed frozen-storage, were used for reduced cooking experiments (Experiment IV B). Four breasts from

Experiment II were cooked after 3 months and four breasts after 6 months of frozen-storage.

2.2. Storage conditions

In Experiment II, the left halves of eight carcasses were stored in a freezer at −18 ± 0.5 °C. Breasts and thighs of four halves were analysed after 3 months of storage; the remaining four halves were analysed after a 6 month storage period. Prior to analyses, the samples were thawed at the laboratory temperature of 21–22 °C for 2 h.

The effects of cold-storage (Experiment III) were tested in triplicate, using breasts from three carcasses in each of the experiments. Each half of breast was cut into three parts (2 × 3) of about 50 g. One part was used for the PA determination 3–4 h after slaughter; five parts of one chicken were packaged in one of three different ways and then stored at 2 ± 0.5 °C:

- in the polyethylene bags (the same as in frozen-storage), simulating aerobic packaging and storage in households. The analyses were carried out on days 0, 1, 2, 5 and 9,
- vacuum-packaged in the processing facility using an equipment Tiromat 320 (Germany) according to standard industrial practices. The meat was packaged using a polyamide/polyethylene foil of thickness 0.22 mm. The samples were analysed on days 0, 2, 5, 9, 15 and 21,
- in a modified atmosphere of 20% CO_2 and 80% O_2 (v/v), using a Mondini E350 apparatus (Italy). The cup was formed from polypropylene, and the cover foil of polypropylene/polyethylene. The meat was analysed on the same days as the vacuum-packaged samples.

Thus, in each of the three experiments, one sixth of the breast of each of three chickens was analysed on days given above.

2.3. Cooking treatments

In each of four parallel experiments (Experiment IV A), both parts of chilled breast from one carcass were divided into six parts. One part was used for the initial PA contents determination; remaining parts were used for five cooking treatments, simulating common chicken meat processing in central European cuisine:

- *boiling:* 50–60 g of meat was cut to cubes of about 1 × 1 × 1 cm, the same weight of distilled water was added and the mixture was sealed in a polyethylene bag. The bag was immersed in the boiling water bath for 30 min. The inner temperature was measured by a puncture thermometer (accuracy ±0.5 °C, Amarell Electronic, Germany) and it reached its maximum of 97 °C after 20 min and then remained stable for 10 min. The bag was then cooled to air temperature and both the cubes and broth were used for polyamine and dry matter determinations,
- *stewing:* this was carried out under conditions similar to boiling. A lower distilled water volume was added, only one fourth of the meat weight,
- *roasting:* a cut of about 50–60 g and thickness of about 4 cm was roasted in an oven at 180–190 °C for 90 min in a pan covered with an aluminium foil. No fat was added, while distilled water was added to prevent burning. All grease was evaporated,
- *grilling:* a similar cut was grilled using an ELOMA ELG 5 grill at 215 °C for 60 min,
- *frying:* a similar cut was mechanically tenderised and the slice was then covered successively with wheat flour, whisked egg and breadcrumbs (“Wiener schnitzel style”) and then fried in sunflower oil in a teflon pan at 180 °C for about 10 min for each side. The covering layer was removed after cooling and only meat was analysed.

Thawed breasts (at 21–22 °C for 2 h) from frozen-storage experiments (Experiment II) were only boiled and roasted (Experiment IV B).

2.4. Analytical methods

Concentrations of the polyamines were calculated and expressed in the experiments described in Sections 2.2 and 2.3 in milligram per kilogram of dry matter due to the changes of dry matter content during the storage and the cooking treatments. All chemicals used were of analytical grade.

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

Acid extracts for the polyamine determination were prepared by homogenisation of 40 ± 1 g of a sample in 0.6 M perchloric acid by the procedure described in our previous paper (Krausová, Kalač, Křížek, & Pelikánová, 2006a). The polyamines were determined after derivatisation with dansyl chloride, using an HPLC method modified in our laboratory. Derivatisation procedures for the solid samples and for the broth were described in our current paper (Krausová, Kalač, Křížek, & Pelikánová, 2008).

Briefly, a high-performance liquid chromatography instrument SpectraSYSTEM (ThermoSeparation Products, USA) was used for the determination of the polyamine dansyl derivatives. Water solutions of acetonitrile (A: 50%, v/v; B: 95%, v/v) were used as a mobile phase. Gradient elution on a RP-C_{18} column was performed with an elution time of 41 min. Volume of the injected sample was 10 μl. A UV-detector (225 nm) was used. The analysed material, mainly skin, clogged the column more extensively than with the other analysed samples (beef, pork, pork liver and kidney) and a more effective elution of the column was thus necessary.

The detection limits were 1.0, 1.7 and 2.2 mg kg^{-1} (fresh matter) for PUT, SPD and SPM, respectively. Repeatability of the analytical procedure was tested by five parallel analyses of a chicken breast stored for 3 days after slaughter at 2–3 °C. The repeatabilities were 7.5% and 6.7% for SPD and SPM at contents of 3.6 and 31.8 mg kg^{-1}, respectively. PUT levels were below the detection limit and repeatability was thus not calculated.

2.5. Statistical methods

Normal distribution of data was proved. Statistical significance of changes in the polyamine contents was then tested by Student’s test, analysis of variance ANOVA and regression analysis, using the software STATISTICA 6.0. Significance level $P < 0.05$ was used in all the statistical tests.

3. Results and discussion

3.1. Homogeneity of polyamines distribution

As it was necessary to use both thighs and both parts of breast for storage and cooking experiments, the homogeneity of the polyamine contents was initially tested. For five pairs of thighs, mean SPD contents of 10.9 ± 1.60 and 11.7 ± 1.40 mg kg^{-1} and SPM contents of 38.1 ± 4.11 and 39.8 ± 5.48 mg kg^{-1} in right and left thighs, respectively, were determined. For right and left halves of breasts of five chickens, mean SPD contents of 4.03 ± 1.08 and 4.60 ± 0.95 mg kg^{-1} and SPM contents 36.8 ± 4.35 and 37.0 ± 4.93 mg kg^{-1}, respectively, were observed. The differences of the contents were statistically insignificant. Thus, both parts of breast

and both thighs from a chicken were used as homogeneous materials.

3.2. Polyamines in meat, skin and giblets after slaughter

Spermidine and spermine contents in meat, skin and giblets 24 h after chicken slaughter are given in Table 3. Putrescine contents were observed only very sporadically, at levels near to the level of detection of 1.0 mg kg^{-1}. The determined values for meat and liver are comparable with the literature data collected in Table 1.

Significant statistical correlations ($P < 0.05$) between SPD and SPM contents were observed in breast, thighs, skin and liver, while they were insignificant in heart. The mutual correlations of SPD and SPM contents between pairs of the five analysed items were statistically insignificant with the only one exception of a significant correlation between SPD contents in breast and thigh.

Spermidine content in chicken meat (Table 3) was higher than values determined in pork and beef 24 h after slaughter (Krausová, Kalač, Křížek, & Pelikánová, 2006b). Spermidine was detectable in all chicken breast and thigh samples, but only in a limited number of pork and beef samples. The highest determined values were only 6.6 and 2.9 mg kg^{-1} in pork loin and sirloin, respectively. The determined contents of SPM in chicken meat (Table 3) seem to be somewhat higher or comparable with literature data for pork and beef collected by Krausová et al. (2006b).

Very high SPD and SPM contents have been reported in liver of different animals as a metabolically very active organ. Spermidine median values in chicken liver of 48.8 and 57.7 mg kg^{-1} were determined in this work (Table 3) and in our earlier paper (Krausová et al., 2006a), respectively. Values of about 108, 160, 29 and 16 mg kg^{-1} were observed in livers of young bulls, cows, pigs and lambs, respectively (Krausová et al., 2006a). Median values 34.8 and 8.3 mg kg^{-1} were reported for livers of roe deer and European brown hare, respectively (Paulsen, Dicakova, & Bauer, 2008).

Similarly, for SPM, median values of 130 and 118 mg kg^{-1} were observed in chicken liver in this and previous work (Krausová et al., 2006a), respectively. Values of about 39, 31, 92, 110 and 110 mg kg^{-1} were published for young bulls, cows, barrows, gilts and lambs, respectively (Krausová et al., 2006a), and 93 and 109 mg kg^{-1} in livers of roe deers and hares, respectively (Paulsen et al., 2008).

Thus, both chicken liver and meat can be classified among food items with high levels of both polyamines, but primarily of SPM. The variability of both SPD and SPM was observed to be lower than in bovine and porcine livers, beef and pork (Krausová et al., 2006a, 2006b).

The data for heart and skin were not reported previously. Spermidine contents in both heart and skin seem to be comparable with values in meat, while SPM levels are significantly lower in skin and higher in heart than those in meat, but lower than those in liver. Chicken heart should thus be ranked among food items with high SPM content.

Table 3
Polyamine contents (mg kg^{-1} fresh matter) in chicken meat ($n = 20$), giblets ($n = 20$) and skin ($n = 10$) 24 h after slaughter.

Material	Spermidine			Spermine		
	Mean	Standard deviation	Median	Mean	Standard deviation	Median
Breast	4.84^{A}	1.70	4.56	36.8^{B}	5.89	37.4
Thigh	10.2^{B}	2.17	9.66	38.0^{B}	3.69	38.3
Skin	$11.4^{B,C}$	1.67	11.1	24.3^{A}	3.84	23.5
Liver	48.7^{D}	8.77	48.8	133^{D}	18.0	130
Heart	12.1^{C}	3.34	11.4	82.7^{C}	10.4	79.5

Values with the different superscript letters within the same column are statistically different ($P < 0.05$).

3.3. Polyamines in frozen meat

Changes in SPD and SPM contents during six-month storage of breast and thigh at −18 °C are given in Fig. 1. No PUT was detected. After the storage for 3 months, a decrease of polyamines was mostly observed, while a considerable increase was apparent after 6 months. However, the increase was significant only for SPM in thighs stored for 6 months, due to the commonly wide variation of determined contents. Wholly different changes of polyamines in aged chicken breasts ($n = 3$) stored at −18 °C for 89 days were recently reported by Moreira, Giombelli, Labanca, Nelson, and Glória (2008). Contents of SPD and SPM decreased from initial levels of 27.4 and 38.7 mg kg^{-1} to 8.0 and 7.4 mg kg^{-1}, respectively.

In pork, no changes were reported after 12 days (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1996), a considerable decrease during 15 days (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994) or a moderate increase during 6 months (Krausová et al., 2008). In beef loin, a slight increase was observed during the initial 3 months of frozen-storage and then a decrease

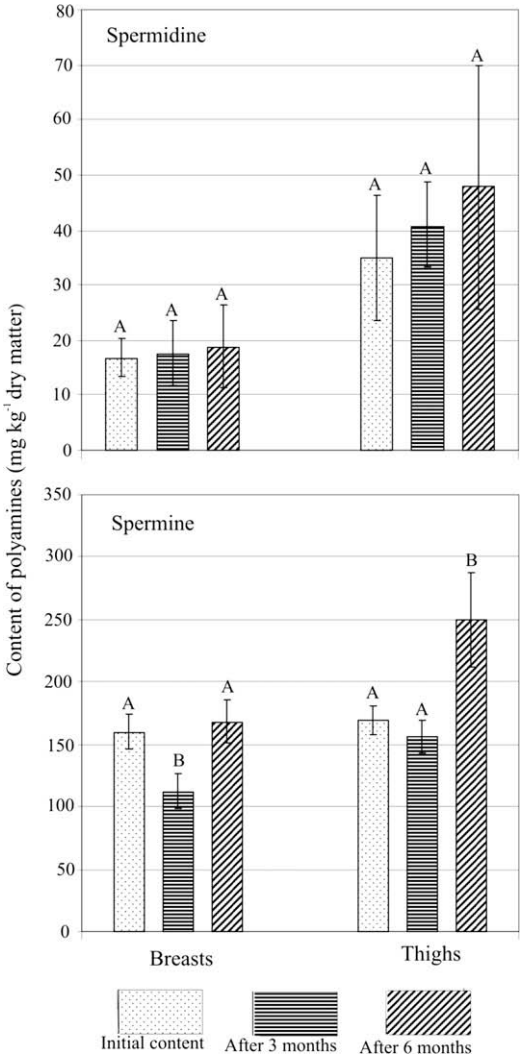

Fig. 1. Changes of polyamine contents in chicken breasts and thighs (mg kg^{-1} dry matter) after 3 and 6 months of frozen-storage at −18 °C. Means of four experiments. Different superscript letters between columns indicate statistical difference ($P < 0.05$) between storage periods.

to about 70% of the initial content after a half-year period (Kozová, Kalač, & Pelikánová, 2009).

The reasons for the observed increase of polyamines content during the frozen-storage of chicken meat remain unclear. It may be hypothesised that a disruption of cells by freezing caused a release of polyamines bound to proteins or some other cell components. Such bounds, mainly with phenolic acids, occur in plants (e.g., Righetti, Tassoni, & Bagni, 2008). However, to the best of our knowledge, information on the existence and stability of such complexes in meat and on the extractability of bound/released polyamines during their determination has been lacking.

3.4. Polyamines in cold-stored meat

Dry matter content changed little during storage in all three tested packaging systems. The relative changes in SPD and SPM contents are shown in Figs. 2 and 3. Putrescine levels remained below the detection limits until the end of the storage. Commonly, the variation of polyamine contents during the storage periods was more considerable in SPD, apparently due to its lower content, near the limit of quantification. Thus, the results of SPD content changes during storage and cooking experiments should be perceived as information of limited scientific value.

In aerobically packaged breast, SPD content decreased slightly during the 9 day storage period (Fig. 2A). A similar SPD decrease was reported by Silva and Glória (2002) and Balamatsia, Paleologos, Kontominas, and Savvaidis (2006) in breasts stored at 4 °C. A very similar results were observed also in thighs (Silva & Glória, 2002). Mean SPM content decreased to about 85% of the initial value (Fig. 3A). The decrease was somewhat more extensive in both the cited papers (Balamatsia et al., 2006; Silva & Glória, 2002). Losses of both SPD and SPM were statistically insignificant in our experiments. Very similar results were observed in pork loin (Krausová et al., 2008) and beef loin (Kozová et al., 2009) stored under the same conditions.

The highest oscillation of SPD content during the cold-storage was observed in vacuum-packaged breasts (Fig. 2B). The decrease was insignificant. On the other hand, SPM loss (Fig. 3B) was significant ($P < 0.05$) and its content decreased to about 60% of the initial level. We were not able to find any data in the literature on polyamine changes during storage of vacuum-packaged chicken meat. Under the same experimental conditions, SPM decreased during the 21 day storage only by some 15% in pork loin (Krausová et al., 2008) and beef loin (Kozová et al., 2009).

Under the modified atmosphere, the mean contents of both polyamines (Figs. 2C and 3C) decreased to below 60% of the initial levels. The decrease was insignificant for SPD, but significant for SPM ($P < 0.05$). No literature data for the used modified atmosphere were found. Under the same experimental conditions, SPM decreased during the 21 day storage, insignificantly in pork loin (Krausová et al., 2008) and significantly, to about 80% of the initial content, in beef loin (Kozová et al., 2009). Under the atmosphere of 80% CO_2 + 20% N_2 and temperature ranging between 3 and 8 °C for 12 days, no decrease of either SPD or SPM was observed in chicken cuts (Rokka, Eerola, Smolander, Alakomi, & Ahvenainen, 2004). In another modified atmosphere (30% CO_2 + 70% N_2), very similar decreases of both SPD and SPM to about 60% of the initial contents on day 17 at 4 °C were reported for chicken breasts (Balamatsia et al., 2006).

Nevertheless, the tested storage of vacuum- and modified atmosphere-packaged breasts was prolonged as compared with the usual recommended time, being 10–11 days after slaughter. After this shorter storage period, the drop, of mainly SPM, was lower.

Information on the polyamine catabolic pathways in food matrices stored under low temperatures has been lacking. Enzy-

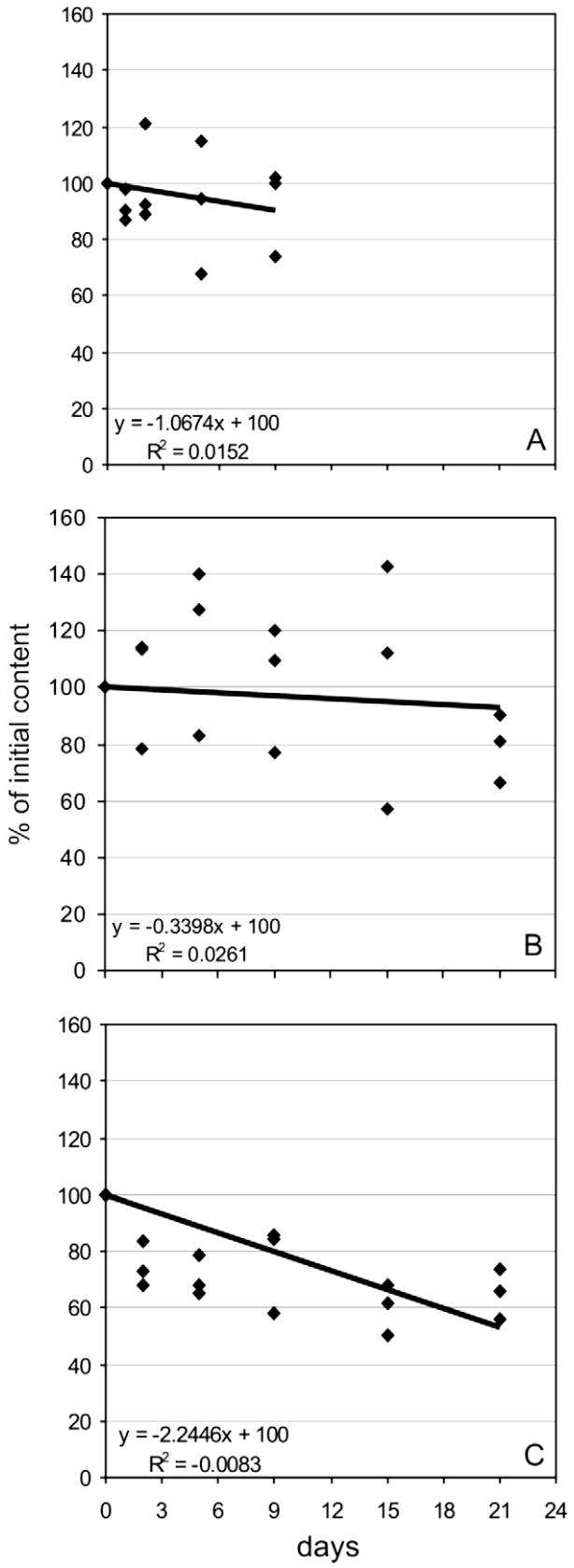

Fig. 2. Relative changes of spermidine during storage of chicken breast under aerobic conditions (A), vacuum-packaged (B) and under a modified atmosphere of 20% CO_2 and 80% O_2 (v/v) (C) at +2 ± 0.5 °C for 9, 21 and 21 days, respectively. The decrease was insignificant in all variants. Contents expressed on dry matter basis were used for the calculations.

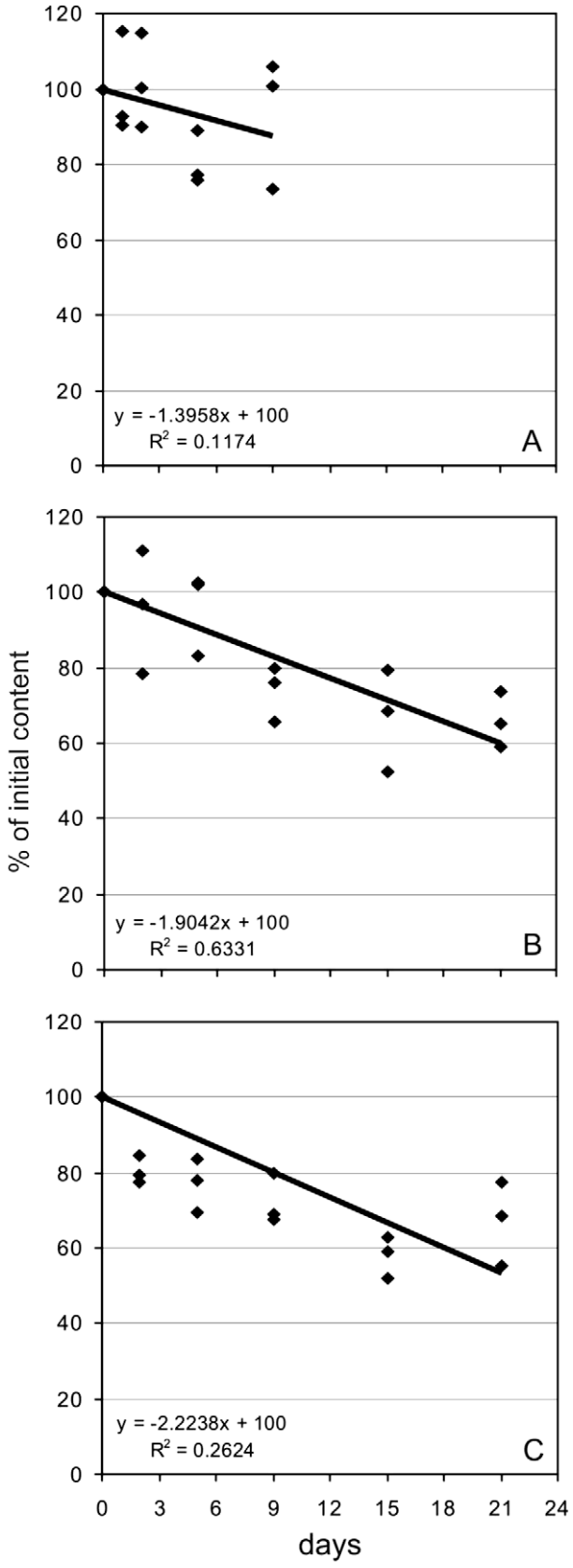

Fig. 3. Relative changes of spermine during storage of chicken breast under aerobic conditions (A), vacuum-packaged (B) and under a modified atmosphere of 20% CO_2 and 80% O_2 (v/v) (C) at +2 ± 0.5 °C for 9, 21 and 21 days, respectively. The decrease was insignificant in (A) and significant at $P < 0.05$ in (B) and (C). Contents expressed on dry matter basis were used for the calculations.

matic catabolism of polyamines in mammalian organs and cells was thoroughly reviewed by Seiler (2004). In living cells, conversion of SPM to SPD and SPD to PUT is catalysed by a FAD-dependent polyamine oxidase. The SPD and PUT formed can be reutilised. These reactions participate in the so-called interconversion cycle. However, the application of biochemical pathways in living cells to *post-mortem* cells and tissues, e.g., chicken meat under non-physiological conditions, seems to be problematic.

3.5. Polyamines in cooked breasts

Dry matter content increased considerably in all the treatments and the changes were taken into consideration for the calculations of changes in polyamine contents. No detectable levels of polyamines were found in broth. Putrescine content in chicken breasts was below the detection limits.

For both SPD and SPM, roasting, grilling and frying of fresh breasts caused greater losses of about 40–60% of the initial contents, than did boiling and stewing (Table 4). A relative increase of SPM after boiling, calculated on dry matter, was surprising. Probably, a release of SPM from its possible complexes, discussed in the section dealing with polyamine changes during storage of frozen meat, may occur. Roasting was reported as a cooking treatment causing the highest SPM losses in pork loin (Krausová et al., 2008).

Similar trends were observed in breasts boiled or roasted after 3 or 6 months of frozen-storage (Table 5). Thus, cooking treatments at high-temperatures seem to cause higher polyamine losses than do boiling and stewing. It may be hypothesised that primary amino groups of polyamines react, under the conditions of high-temperature cooking treatments, with glucose, by the Maillard reaction. An ability of putrescine, cadaverine, spermidine and spermine to react with glucose (competitively), to free amino groups of amino acids, namely of L-lysine, under *in vitro* conditions at 37 °C for 90 days, was reported. Spermine was observed to be the most efficient amine (Méndez & Leal, 2004).

Table 4
Relative changes in spermidine and spermine contents in fresh chicken breasts (% of initial content, calculated per dry matter) caused by different cooking treatments. Means of four experiments.

Treatment	Spermidine	Spermine
Boiling	$78.5^{A} \pm 10.4$	$116^{B} \pm 24.6$
Stewing	$86.9^{B} \pm 15.8$	$94.3^{B} \pm 15.7$
Roasting	$46.8^{A} \pm 16.2$	$38.5^{A} \pm 5.3$
Grilling	$54.4^{A} \pm 19.5$	$47.4^{A} \pm 8.1$
Frying of breaded cuts	$67.6^{A} \pm 36.7$	$54.7^{A} \pm 20.9$

Different superscript letters within the same column indicate statistical difference ($P < 0.05$) between cooking treatments.

Table 5
Relative changes in spermidine and spermine contents (% of initial content, calculated per dry matter) in thawed chicken breasts caused by frozen-storage and cooking. Mean of four experiments. No statistical differences were found between cooking treatments of frozen breasts stored for 3 or 6 months.

Treatment	Frozen-stored for 3 months	Frozen-stored for 6 months
Spermidine		
Boiling	$51.9^{A} \pm 4.3$	$73.1^{A} \pm 8.0$
Roasting	$42.3^{A} \pm 5.7$	$44.2^{A} \pm 4.3$
Spermine		
Boiling	$93.5^{A} \pm 7.7$	$106^{B} \pm 10.7$
Roasting	$57.5^{A} \pm 3.0$	$55.4^{A} \pm 7.4$

Different superscript letters within the same column indicate significant difference ($P < 0.05$) between cooking treatments.

4. Conclusions

Intestinal absorption of dietary polyamines considerably influences their body pool. Knowledge on polyamines content in foods has thus been required, as they are necessary for cell growth and differentiation. Chicken meat and giblets rank among increasingly largely consumed food items.

Fresh chicken meat can be classified as a food with high levels of both polyamines, but primarily of SPM. Even higher contents are typical of heart and particularly of liver. Polyamine contents decrease moderately during cold-storage of meat under normal conditions, while an increase was observed after 6 months of frozen-storage. Losses of about one-half of the initial polyamine contents may be supposed during different cooking treatments of chicken meat. Cooking at higher temperatures (roasting, frying or grilling) seems to cause higher SPD and SPM losses than do boiling or stewing.

It has not been possible to give "tabular values" of SPD and SPM contents in different chicken meals due to the considerable variations observed in fresh-meat and giblets.

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