Formation of Biogenic Amines in Chicken Meat Stored under Modified Atmosphere

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Abstract

The aim of the study was to investigate the effects of two modified atmospheres with a different combination of gases on selected groups of microorganisms and on concentrations of biogenic amines (BAs) in samples of poultry breast muscle. The samples were packaged under modified atmosphere A (75% O_2 a 25% CO_2) or B (75% N_2 and 25% CO_2) and stored at temperatures from +2 to +4 °C for 14 days. During the storage period, O_2 concentrations in modified atmosphere A (MA A) decreased from the initial 74.8 ± 0.3% to 55.9 ± 6.6% at the end of the storage period. In all samples, counts of psychrotrophic bacteria counts, *Brochothrix thermosphacta*, lactic acid bacteria and coliform microorganism were determined. The tests were made on the packaging day, and then after three, nine and fourteen days of storage. At the end of the storage period, higher numbers of psychrotrophic bacteria (6.5 ± 0.7 $\log_{10}$ cfu·g^{-1}), *Brochothrix thermosphacta* (4.8 ± 0.3 $\log_{10}$ cfu·g^{-1}) and lactic acid bacteria (1.7 ± 0.4 $\log_{10}$ cfu·g^{-1}) were found on samples packaged under MA A. Samples packaged under modified atmosphere B on the other hand contained higher numbers of coliform bacteria (4.1 ± 0.6 $\log_{10}$ cfu·g^{-1}) at the end of the storage period. In addition to microbiological indicators, concentrations of biogenic amines (putrescine, cadaverine, histamine, tyramine, spermine, spermidine and *β*-phenylethylamine) were also determined. In fresh samples and after three days of storage, only spermine and spermidine were found. After 9 and 14 days, also other BAs were detected. The biogenic amine totals at the end of the storage period was 60.0 ± 13.2 mg·kg^{-1} in samples packaged under MA A and 129.0 ± 41.3 mg·kg^{-1} in samples packaged under MA B. The most abundantly represented biogenic amines in samples packaged under MA A were putrescine and spermine (49.7 and 24.8%, respectively, at the end of the storage period), and putrescine and cadaverine in samples packaged under MA B (47.0 and 32.9 %, respectively, at the end of the storage period).

Poultry meat, *shelf-life*, *microbiological quality, amines, HPLC*

Poultry is a highly perishable food and the time it takes to deteriorate varies from 4 to 10 days after slaughtering, in spite of having been stored under chill systems (Phillips 1996). Modified atmosphere packaging (MAP) has become widespread (Susiluoto et al. 2003) in recent years to increase the shelf life of fresh chicken meat and chicken products. Various combinations of gases are used, and combinations of O_2, CO_2 and N_2 are among the most frequent ones. Most of the gases used for food packaging exhibit various degrees of bacteriostatic or bactericidal effects. Shelf life of meat packaged under modified atmosphere is decisively influenced by its initial microbial contamination, the appropriateness of gas mixture used and strict cold chain compliance (Jeremiah and Gibson 2001; Farber 1991). Other gases may also be used for MAP, for instance argon (Ar) or carbon monoxide (CO), which act to stabilize the red colouring of meat and to extend its shelf-life (Sørheim et al. 1997; Sivertsvik et al. 2002). Microorganisms present in packages significantly influence sensory properties of the packaged meat, such as its colour, smell and shelf life (Balamatsia et al. 2006). In some cases, biogenic amines may be produced when certain types of microorganisms decarboxylate free amino acids (Min et al. 2004; Halász et al. 1994). Biogenic amines (especially histamine, tryptamine, *β*-fenylethylamine and

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tyramine) that penetrate from food to blood circulation can cause health problems of psychoactive or vasoactive nature to people. These may include increased blood pressure, onset of migraines, increased heart and respiratory rates, etc. In addition, biogenic amines (BA) are potential precursors of carcinogenic N-nitroso compounds. It has been reported that 5–10 mg of histamine can be considered potentially hazardous for some sensitive people. Ten mg are considered as a tolerable limit, 100 mg may induce a medium toxicity and a dose of 1 000 mg histamine is highly toxic (Shalaby 1996; Karovičová and Kohajdová 2005).

The aim of our study was to make a quantitative and qualitative comparison of microflora and the formation of biogenic amines in chicken breast muscle packaged under two types of modified atmospheres (A - 25% CO_2 and 75% O_2, B - 25% CO_2 and 75% N_2) during cold storage.

Material and Methods

Preparation of chicken meat samples and storage conditions

Fresh chicken breast meat was obtained from a local poultry slaughterhouse. A total of 80 samples from 40 broilers (ROSS 308, 40 days old) were analyzed. Samples were individually packaged into AMILEN PA/PE (Verpackungen GmbH, Germany) bags with 60 μm coat of polyamide and 20 μm coat of polyethylene with ethylene-vinyl acetate (EVA) oxygen barrier layer and a gas transmission rate declared by the manufacturer for O_2, N_2 and CO_2 permeability 50, 10 and 150 $cm^3 \cdot m^{-2}\ d^{-1}$, respectively, at 23 °C, 0% relative humidity. Water vapour transmission rate was 3.0 $g \cdot m^{-2}\ d^{-1}$ at 23 °C, 85% relative humidity. Weight per area was 80 $g \cdot m^{-2}$, tensile strength at break 45% longitudinally and 35% transversally.

Meat samples were individually packaged on a Vac-Star S 223 GX (Frimark CZ Ltd., Czech Republic). Half of the samples were packed in modified atmosphere A (25% CO_2 and 75% O_2) and second half in modified atmosphere B (25% CO_2 and 75% N_2). The air was first evacuated from the packages (99% vacuum) which were then flushed once prior to the final treatment with the gas mixture. Food grade CO_2 - O_2 and CO_2 – N_2 (Linde Gas, Brno, Czech Republic) were used. All the plastic bags were heat-sealed. Packages were placed in isothermal boxes and transported from the poultry slaughterhouse to cold storage at our institute. All the packages containing poultry meat samples were stored chilled at +2–4 °C. The temperature was recorded six times an hour using the LOGGER S3120 digital thermometer (Comet System Ltd., Rožnov pod Radhoštěm, Czech Republic).

Sampling was carried out at predetermined time intervals, i.e. on day 0 (control - day of packaging), and on days 3, 9 and 14 after packaging.

Head space gas analysis

The head space gas composition (% O_2) was assessed in triplicate by using an oxygen analyzer (OxiMETR, VEIT Electronics, Brno, Czech Republic). An aliquot (20 ml) of the head space gas was collected with a syringe inserted through the cover film. Before the syringe was inserted, a foam rubber septum (VEIT Electronics, Brno, Czech Republic) was added to the cover film to avoid introduction of false atmosphere into the gas analyzer. Mean values were used for the statistical data analysis.

Microbiological analyses

Microbial contamination of chicken breast muscle was evaluated by determining the psychrotrophic bacteria count, *Brochothrix thermosphacta* count, lactic acid bacteria count and total coliform bacteria count.

The psychrotrophic bacteria count was determined on Plate Count Agar (CM0463, Oxoid Ltd., Basingstoke, Hampshire, UK), aerobically, 10 days at 6.5 ± 1 °C, in accordance with the ISO 17410:2001 guidelines. *Brochothrix thermosphacta* was cultivated on STAA Agar Base (CM0881, Oxoid) aerobically for 48 ± 4 h at 23 ± 1 °C in accordance with the ISO 13722:1998 guidelines. The quantification of lactic acid bacteria (LAB) was performed on de Man, Rogosa, Sharpe agar (MRS Agar, CM0361, Oxoid) anaerobically for 72 ± 3 h at 30 ± 1 °C, in accordance with the ISO 13721:1995 guidelines. The total coliform bacteria count was determined by detection on Oxoid Brilliance *E. coli*/coliform Selective Agar (CM1046, Oxoid) aerobically for 24 ± 2 h at 37 ± 1 °C. All analyses were performed in duplicate. The number of formed colonies was counted and reported as $\log_{10}$ of $cfu \cdot g^{-1}$ for every sample.

Measurement of biogenic amines

Biogenic amines (putrescine (PUT), cadaverine (CAD), histamine (HIS), *β*-phenylethylamine (PHE), tyramine (TYR), spermidine (SPD) and spermine (SPN)) were determined by pre-column dansylchloride derivatization HPLC as described by Paulsen et al. (1997). Concentrations of biogenic amines were determined by HPLC using an Alliance 2695 liquid chromatofigure (Waters, USA) with a 2475 fluorescence detector and a PDA 2996 detector. The separation was performed using a Polaris C18 column (Varian, USA) with reversion phase 4.6 × 150 mm, stationary phase grain size 3 μm and the column temperature of 35 °C. Amine dansylderivates were quantified by the external standard method using Empower software (Waters, USA).

Biogenic amines were determined in chicken breast muscle. All analyses were performed in duplicate. Mean values were used for the statistical data analysis.

Statistical analysis

Results of microbiological and chemical analyses are reported as mean values ± standard deviation (s.d.). Microbiological counts ($\log_{10}$ cfu·g^{-1}), % O_2 and content of biogenic amines were analysed. Student's *t*-test was applied to determine the differences between individual storage days. The 0.05 level of significance was used. Correlation coefficients of microbiological counts and biogenic amines amount were generated using the Pearson's correlation coefficient. Statistical data analyses were conducted using the statistical programme STATISTICA Cz (Statsoft, Czech Republic).

Results

Temperature and O_2 concentration in modified atmosphere

The mean temperature throughout the meat sample storage period was 3.2 ± 0.7 °C. The O_2 levels in modified atmosphere A at the time of packaging and at the end of the 14-day period were 74.8 ± 0.3% and 55.9 ± 6.6%, respectively. In modified atmosphere B, the O_2 levels at the beginning and at the end of the storage period were 0.4 ± 0.2% and 0.7 ± 0.3%, respectively (Table 1).

Table 1. Changes in O_2 concentrations [%] during storage time in modified atmospheres A and B (mean ± s.d.)

	Day 0	Day 3	Day 9	Day 14
A	74.8 ± 0.3	65.9 ± 9.3	58.2 ± 8.9	55.9 ± 6.6
B	0.4 ± 0.2	0.6 ± 0.2	0.6 ± 0.4	0.7 ± 0.3

Microbiological indicators

Microorganism counts of meat samples packaged under the two types of modified atmosphere (MA) are given in Table 2. The initial psychrotrophic bacteria counts were 3.1 $\log_{10}$ cfu·g^{-1} in both sets of samples. After three days of storage, their counts decreased slightly to 2.7 and 2.8 $\log_{10}$ cfu·g^{-1} in MA A (with O_2) and MA B, respectively. From day 3 until the end of the storage period, the numbers of psychrotrophic bacteria grew continuously to reach 6.5 and 5.8 $\log_{10}$ cfu·g^{-1} in MA A (with O_2) and MA B, respectively. Neither at the beginning nor during the storage period were there any statistical differences in psychrotrophic bacteria counts between samples in MA A and MA B.

Table 2. Changes in microbiological indicators of chicken breast during storage under modified atmospheres A and B [$\log_{10}$ cfu·g^{-1}] (mean ± s.d.)

Microorganism	Modified atmosphere	Day 0	Day 3	Day 9	Day 14
Psychrotrophic bacteria	A	3.1 ± 0.2	2.7 ± 0.4	4.4 ± 0.5	6.5 ± 0.7
	B	3.1 ± 0.6	2.8 ± 0.5	4.8 ± 0.6	5.8 ± 0.8
Brochothrix thermosphacta	A	2.2 ± 0.3	3.2 ± 0.2	3.5 ± 0.2	4.8 ± 0.3
	B	1.2 ± 0.4	1.1 ± 0.5	1.4 ± 0.3	2.5 ± 0.2
Lactic acid bacteria	A	1.3 ± 0.3	1.0 ± 0.1	0.7 ± 0.2	1.7 ± 0.4
	B	1.6 ± 0.2	2.1 ± 0.7	1.0 ± 0.3	0.6 ± 0.2
Coliform microorganisms	A	n.d.	1.1 ± 0.3	1.1 ± 0.6	1.9 ± 0.4
	B	n.d.	0.5 ± 0.2	2.7 ± 0.3	4.1 ± 0.6

n.d. not detected

The initial lactic acid bacteria (LAB) count was 1.3 $\log_{10}$ cfu·g^{-1} in the MA A set of samples. The LAB counts in MA A set of samples decreased over the period of storage. The minimum count of 0.7 $\log_{10}$ cfu·g^{-1} was recorded on day 9. Towards the end of the storage period, LAB counts of the MA A set of samples increased to 1.7 $\log_{10}$ cfu·g^{-1}. The

initial LAB count in the MA B set of samples was 1.6 $\log_{10}$ cfu·g^{-1}. After three days of storage, their numbers peaked at 2.1 $\log_{10}$ cfu·g^{-1} and from then continued to decrease to reach the end-of-storage level of 0.6 $\log_{10}$ cfu·g^{-1}. In spite of the variations in LAB counts, no significant differences between samples in MA A and MA B sets were found at the beginning of or during the storage period.

The initial *Brochothrix thermosphacta* counts in the MA A and B sets of samples were 2.2 and 1.2 $\log_{10}$ cfu·g^{-1}, respectively. The numbers of *Brochothrix thermosphacta* gradually increased in both MA A and MA B sets to reach the final 4.8 and 2.5 $\log_{10}$ cfu·g^{-1}, respectively, *Brochothrix thermosphacta* counts on storage days 3, 9 and 14 were significantly higher in samples stored under MA A ($p < 0.05$).

No coliform microorganisms were found in any of the samples at the beginning of storage. After three days of storage, however, some coliform microorganisms were found under both MA A and MA B, and their numbers grew from then onwards until reaching the final level of 1.9 and 4.1 $\log_{10}$ cfu·g^{-1}, respectively. On days 9 and 14, fewer coliform microorganisms were found under MA A, and the difference was significant ($p < 0.05$).

Biogenic amine content

Contents of biogenic amines (BAs) found in poultry meat samples packaged under the two types of modified atmosphere (MA) are given in Table 3.

Table 3. Changes in biogenic amines concentrations on chicken breast during storage under modified atmospheres A and B [mg·kg^{-1}] (mean ± s.d.)

Day	modified atmosphere	PUT	CAD	HIM	TYM	SPM	SPD	Total
0	A	n.d.	n.d.	n.d.	n.d.	17.8 ± 0.6	7.5 ± 0.7	25.3 ± 0.7
	B	n.d.	n.d.	n.d.	n.d.	17.7 ± 0.6	7.6 ± 0.8	25.2 ± 0.8
3	A	n.d.	n.d.	n.d.	n.d.	17.3 ± 0.6	7.7 ± 0.9	25.0 ± 0.7
	B	n.d.	n.d.	n.d.	n.d.	17.9 ± 0.4	7.3 ± 1.3	25.2 ± 1.5
9	A	26.4 ± 4.8	8.5 ± 8.4	n.d.	n.d.	16.0 ± 1.0	6.1 ± 0.9	57.1 ± 13.1
	B	72.5 ± 63.8	21.7 ± 16.6	1.8 ± 1.4	1.9 ± 1.5	16.5 ± 0.7	6.3 ± 0.8	119.8 ± 75.6
14	A	29.8 ± 10.9	9.5 ± 4.1	n.d.	n.d.	14.9 ± 1.4	5.9 ± 1.6	60.0 ± 13.2
	B	60.6 ± 30.7	42.4 ± 30.6	1.6 ± 1.2	3.2 ± 3.1	15.3 ± 0.8	6.4 ± 1.4	129.0 ± 41.3

n.d. not detected

At the beginning of storage and on day 3, no putrescine was detected in any of the samples. After nine days of storage, 26.4 mg·kg^{-1} putrescine was detected in samples stored under MA A, and that level slightly increased by the end of the storage period to 29.8 mg·kg^{-1}. After nine days of storage, putrescine was also detected in MA B samples at 72.5 mg·kg^{-1}, but that level decreased by the end of the storage period to 60.6 mg·kg^{-1}. After days 9 and 14 of storage, samples stored under MA B contained significantly more putrescine than MA A samples ($p < 0.05$).

At the beginning of storage and on day 3, no cadaverine was detected in any of the samples. After nine days of storage, 8.5 mg·kg^{-1} cadaverine was detected in samples stored under MA A, and that level remained practically unchanged (9.5 mg·kg^{-1}) until the end of the storage period. After nine days of storage, cadaverine was also detected in MA B samples at 21.7 mg·kg^{-1}, and that level increased by the end of the storage period to 42.4 mg·kg^{-1}. After days 9 and 14 of storage, cadaverine contents in samples stored under MA B were significantly higher than those in MA A samples ($p < 0.05$).

In samples stored under MA A, no histamine was found either at the beginning of or during the storage period. In samples stored under MA B, 1.4 mg·kg^{-1} histamine

was detected after nine days of storage, and that level remained practically unchanged (1.2 mg·kg^{-1}) until the end of the storage period ($p < 0.05$).

Spermine was detected over the entire period of storage between 14.9 and 17.9 mg·kg^{-1} in both types of sample packaging. The slight decrease in spermine levels on days 9 and 14 of storage was non-significant.

Spermidine contents in samples developed very much like those of spermine. At the beginning and on day 3 of the storage period, levels between 7.3 and 7.7 mg·kg^{-1} were found in both of the atmospheres used, and a decrease on days 9 and 14 of storage to levels between 5.9 and 6.4 mg·kg^{-1}.

In samples stored under MA A, tyramine was not found either at the beginning of or during the storage period. In samples stored under MA B, 1.5 mg·kg^{-1} tyramine was detected after nine days of storage, and that level increased to 3.2 mg·kg^{-1} by the end of the storage period.

β-phenylethylamine was not found either at the beginning of or during the storage period in any of the samples.

At the beginning of storage and on day 3 of storage, total biogenic amines in all samples were around 25 mg·kg^{-1}, and they consisted only of spermine and spermidine. After three days of storage, BA contents increased to 57.1 mg·kg^{-1} and 119.8 mg·kg^{-1} in MA A and MA B, respectively. At the end of storage, BA levels were 60.0 mg·kg^{-1} and 129.0 mg·kg^{-1} in MA A and MA B, respectively. After 9 and 14 days of storage, the most abundantly represented biogenic amines in samples packaged under MA A were putrescine and spermine (49.7 and 24.8%, respectively, at the end of storage period), and putrescine and cadaverine in samples packaged under MA B (47.0 and 32.9%, respectively, at the end of storage period). After days 9 and 14 of storage, samples stored under MA B had significantly higher contents of biogenic amines than samples stored under MA A ($p < 0.05$ and $p < 0.001$, respectively).

Table 4 demonstrates correlation between microbiological indicators and biogenic amines contents.

Table 4. Correlation coefficients of microbiological counts and biogenic amines amount in chicken breast during storage under modified atmospheres A and B (only significant coefficients, $p < 0.05$)

Day	Modified atmosphere	PUT	CAD	HIM	TYM	SPM	SPD	Total
Psychrotrophic bacteria	A	0.7658	0.5050	-	-	-0.5979	-0.5170	0.7027
	B	0.5737	0.5488	0.3687	0.6022	-0.7980	-	0.6366
Brochothrix thermosphacta	A	0.3679	-	-	-	-	-	0.3694
	B	-	-	-	0.3663	-0.4608	-	-
Lactic acid bacteria	A	-	-	-	-	-	-	-
	B	-0.4353	-	-0.3148	-0.3508	-	-	-0.3989
Coliform microorganisms	A	0.3151	-	-	-	-	-0.3448	-
	B	0.6178	0.6020	0.4582	0.6153	-0.8118	-0.3585	0.6895

Discussion

Modified atmosphere packaging uses a combination of specific gases, which can enhance the shelf life of retail meat products (Jeremiah and Gibson 2001). Carbon dioxide is the major anti-microbial factor of MAP. The inhibitory effect is seen as an increase in the lag

phase and generation time during the logarithmic phase of growth of the microbes (Reddy et al. 1992). A minimum CO_2 concentration of 20–30% is necessary to exhibit the inhibitory effect (Stiles 1991). Carbon dioxide is most effective in foods where the normal spoilage organisms consist of aerobic, gram-negative psychrotrophic bacteria (Phillips 1996). Besides the direct inhibitory action of CO_2 in its gaseous form in modified atmosphere, CO_2 also exhibits inhibitory action in its dissolved form. Devlieghere and Debevere (2000) described the effect of dissolved CO_2 on various representatives of gram-positive and gram-negative spoilage microbial flora. The effectiveness of carbon dioxide as an antimicrobial agent is not universal and depends on the microbial flora present and the product characteristics. Oxygen affects the microbial flora on packaged meat. Generally, it stimulates the growth of aerobic bacteria and inhibits the growth of anaerobes (Phillips 1996). Nitrogen is used to replace oxygen in modified atmosphere packaged products to prevent rancidity and inhibit the growth of aerobic microbes (Farber 1991; Gill et al. 1990).

Microflora composition of meat packaged under modified atmosphere with minimum levels of oxygen concentrations is considerably different. Major part of the specific microflora are psychrotrophic lactic acid bacteria, particularly members of the genera *Carnobacterium*, *Lactobacillus* and *Leuconostoc* (Gram et al. 2002; Björkroth et al. 2000; Borch et al. 1996). In chilled poultry meat packaged under MA, large populations of *Brochothrix thermosphacta* are also frequently found (Pin et al. 2002).

Initial concentrations of psychrotrophic aerobic microflora in our samples were around 3.1 $\log_{10}$ cfu·g^{-1}, which is near the lower limit of recently published values (2.8 to 4.7 $\log_{10}$ cfu·g^{-1}) (Chouliara et al. 2008; Charles et al. 2006). That means that our material was of good quality from the hygienic point of view. The absence of coliform microflora, too, is indicative of very good hygienic profile of our samples. The slight decrease in the psychrotrophic bacteria counts after three days of storage in samples from both of the two modified atmospheres can be explained by the synergy effect of a heat shock and modified atmosphere. The endpoint values correspond to those obtained in similar studies in other countries (Malicki et al. 2006; Charles et al. 2006).

Brochothrix thermosphacta (G+, facultatively anaerobic) is one of the most important spoilage microorganisms in poultry meat either stored in a simple anaerobic packaging or under MA packaging. Its spoilage-causing activity is most noticeable in an ambient with lower concentrations of oxygen and higher concentrations of CO_2. It can, however, grow very well in the vacuum and other types of environment. Jimenez et al. (1997) reported *B. thermosphacta* counts after a 14-day storage of poultry breast muscle at 4 °C at the level of 6.73 $\log_{10}$ cfu·g^{-1} (MA 70% N_2 and 30% CO_2) and 7.49 $\log_{10}$ cfu·g^{-1} (MA 70% CO_2 and 30% N_2). Their values were markedly higher than our results probably because initial concentrations of *B. thermosphacta* in their experimental material were also markedly higher (3.95 and 4.25 $\log_{10}$ cfu·g^{-1}) than those in our experiment. Chouliara et al. (2008) reported the following *B. thermosphacta* counts in poultry breast muscle after 15-day storage at the temperature of 4 °C: MA 1 (70% N_2 and 30% CO_2) 7.03 ± 0.43 $\log_{10}$ cfu·g^{-1}, MA 2 (70% CO_2 and 30% N_2) 7.21 ± 0.51 $\log_{10}$ cfu·g^{-1}. Our counts are lower, particularly thanks to lower initial *B. thermosphacta* counts (Table 2) and a lower temperature of storage (3.2 ± 0.7 °C).

In similarly designed experiments, some other authors (Jimenez et al. 1997; Chouliara et al. 2008; Vihavainen et al. 2007) reported lactacidogenic microflora counts higher than our results by an order of magnitude (about 10^6–10^8 cfu·g^{-1} after two-week storage), attributable especially to markedly higher initial levels of LAB in their experiment. In spite of different ratios between gases in the two MA, no significant differences in lactic acid bacteria counts were found during our experiment. The decline and subsequent increase in the number of LAB during storage may be due to different input numbers of LAB in

packaged meat. It is also possible that the decrease corresponds to the death of mesophilic LAB, with a consequent increase of psychrotrophic strains of LAB (Vihavajnen et al. 2007; Björkroth et al. 2000).

Coliform microflora counts depend largely on the conditions prevailing during slaughter. Jimenez et al. (2003) reported that 11.3% of carcasses showed faecal material and 5.2% showed bile on the surface after the evisceration step. The absence of *Escherichia coli* over the entire period of storage or of other coliform microflora at the beginning of storage is indicative of a good hygienic profile of samples (Zeitoun et al. 1994). The increase in coliform microflora abundance is due to the adaptation and development of its psychrotrophic members. The abundance of *E. coli* and of other coliform microflora may serve as a good indicator of cold storage chain disruption. The significantly lower numbers of coliform microorganisms in samples stored under MA A can be explained by a synergic inhibitory effect of low temperature and high O_2 and CO_2 concentrations. In their study of various types of atmosphere, Chouliara et al. (2008) reported the following *Enterobacteriaceae* counts in poultry breast muscle after 15-day storage at 4 °C: MA 1 (70% N_2 and 30% CO_2) 7.02 ± 0.52 $\log_{10}$ cfu·g^{-1}, MA 2 (70% CO_2 and 30% N_2) 6.71 ± 0.49 $\log_{10}$ cfu·g^{-1}; simple aerobic packaging (control) – the last relevant value after 9 days of storage was 7.48 ± 0.51 $\log_{10}$ cfu·g^{-1}.

At the beginning and during the first three days of storage, samples stored under both MA A and MA B contained only spermine and spermidine, which occur naturally in the organism. Concentrations of these biogenic amines decrease over the storage period because they serve as a source of nitrogen to the microorganisms present there. In their experiments with breast muscle tissue, Silva and Glória (2002) reported practically the same initial contents of spermine (17.9 mg·kg^{-1}) and spermidine (7.3 mg·kg^{-1}) as those recorded in our study. The concentrations they found after 15 days of storage (SPM 11.2 mg·kg^{-1} and SPD 8.7 mg·kg^{-1}) also correspond to our findings. Balamatsia et al. (2006), on the other hand, found the same initial content of spermidine (7.9 mg·kg^{-1}) in breast muscle tissue in simple (aerobic) packaging but higher initial contents (13.2 mg·kg^{-1}) in samples packaged under MA (30% CO_2 and 70% N_2). They also reported markedly higher initial contents of spermine (53.3 and 56.3 mg·kg^{-1}) than those found in our study. Spermine and spermidine were the only biogenic amines detected at the beginning of the storage period and after 3-day storage. This may suggest that the development of microflora with decarboxylase activity began only after that period, because other biogenic amines are produced by microbial action. In samples stored under MA A, no tyramine was found either at the beginning of or during the entire storage period. In samples stored under MA B, tyramine was found only after nine days of storage, and in very small quantities only. Silva and Glória (2002) also reported the first detection of tyramine (17.4 mg·kg^{-1}) in breast muscle only at the end of storage (after a 15-day storage period). Balamatsia et al. (2006), on the other hand, found low levels (tenths of milligrams) of tyramine already in the input material, and its concentrations increased over the entire period of storage to reach 4 mg·kg^{-1} (simple aerobic packaging) and 8.9 mg·kg^{-1} (MA of 30% CO_2 and 70% N_2) at the end of a 17-day period of storage. Because tyramine is produced mainly by coliform microflora and lactic acid bacteria (Min et al. 2004), the absence of tyramine is indicative of good initial microbiological conditions of our samples and good inhibitory effects of MA A on those types of bacteria.

In samples stored under MA A, no histamine was found either at the beginning of or during the entire storage period. In samples stored under MA B, histamine was found only after nine days of storage, and then in minimum quantities only. Silva and Glória (2002) also reported the first detection of histamine (10.3 mg·kg^{-1}) in breast muscle only at the end of storage (after a 15-day storage period). Balamatsia et al. (2006) first detected histamine (in units of milligrams per kg) after 11 days of storage. After 14 days of storage

in simple aerobic packaging, the authors reported 8.6 mg·kg^{-1} histamine; contrasting with it were surprisingly higher histamine concentrations (14.5 mg·kg^{-1}) after the same period of storage under MA (30% CO_2 a 70% N_2). Because histamine in significant quantities is produced by some members of *Enterobacteriaceae* and by lactic acid bacteria (Min et al. 2004), the absence of histamine during storage and its non-significant concentrations at the end of the storage period are indicative of a good hygienic profile of input material and good inhibitory effects of MA.

From storage day 9 onward, putrescine and cadaverine were detected in samples packaged under both MA A and MA B. Although literary sources differ somewhat from one another about what microorganisms are mainly responsible for the production of these BAs, members of *Enterobacteriaceae* and some other microorganisms seem to be responsible for the production of cadaverine, and pseudomonads mainly for putrescine. While Silva and Glória (2002) reported the first detection of putrescine (20.4 mg·kg^{-1}) and cadaverine (4.3 mg·kg^{-1}) at the time when it was also detected by the authors of the present study, i.e. only after a 15-day storage period, Balamatsia et al. (2006) detected the two BAs in initial samples already and their concentrations were at the levels of tens of milligrams per kg. After 14 days of storage, they reported putrescine and cadaverine concentrations of 250–300 mg·kg^{-1} and 120–160 mg·kg^{-1}, respectively.

Biogenic amine total is a sum of all detected BAs. The BA totals can therefore be viewed as a result of all the individual influences affecting the production of individual BAs, which is also the reason why the value varies so much from one input to another. At the beginning of the storage period and in its first three days, the BA total of 25.2 to 25.3 mg·kg^{-1} consisted of naturally occurring BAs, i.e. spermine and spermidine. From day 9 onwards, BAs of microbial origin (mainly putrescine and cadaverine) were produced in samples stored under both MAs, which corresponded with microflora development. While BA totals in samples stored under MA A at the end of the storage period were 60.0 ± 13.2 mg·kg^{-1}, concentrations twice as high were found in samples stored under MA B (129.0 ± 41.3 mg·kg^{-1}). Balamatsia et al. (2006) reported BA totals of about 500 mg·kg^{-1} for simple aerobic packaging and about 400 mg·kg^{-1} in samples packaged under MA (30% CO_2 and 70% N_2) after 14-day storage. Vinci and Antonelli (2002) found the BA total of 237.2 mg·kg^{-1} in poultry meat stored for 15 days at 4 ± 1 °C. In their study of breast muscle samples stored for 15 days, Silva and Glória (2002) on the other hand reported 72.3 mg·kg^{-1}, which corresponds to values found in our study.

With respect to the production of biogenic amines in our study, better results were obtained with modified atmosphere A (75% O_2 and 25% CO_2), where the mean biogenic amine concentration at the end of storage was 60 mg·kg^{-1}, whereas the mean biogenic amine concentration in samples packaged under modified atmosphere B (75% N_2 and 25% CO_2) was more than twice as high (129 mg·kg^{-1}). This marked difference may be connected with the development of coliform microflora in samples packaged under modified atmosphere B.

Tvorba biogenních aminů u kuřecího masa skladovaného v modifikované atmosféře

Cílem práce bylo sledování vlivu dvou modifikovaných atmosfér s rozdílným složením plynů na vybrané skupiny mikroorganismů a na obsah biogenních aminů (BAs) u vzorků kuřecí prsní svaloviny. Vzorky byly zabaleny do modifikované atmosféry A (75 % O_2 a 25 % CO_2) a B (75 % N_2 and 25 % CO_2) a byly skladovány při teplotě +2 až +4 °C po dobu čtrnácti dnů. V průběhu skladování se snižovala koncentrace O_2 v modifikované atmosféře A (MA A) z výchozí koncentrace 74,8 ± 0,3 % až na 55,9 ± 6,6 % na konci skladování. U všech vzorků se stanovovaly počty psychrotrofních bakterií, *Brochothrix thermosphacta*, bakterií mléčného kvašení a koliformních mikroorganismů. Analýza probíhala v den

zabalení a dále po třech, devíti a čtrnácti dnech skladování. Vyšší počty psychrotrofních bakterií (6,5 ± 0,7 $\log_{10}$ cfu·g^{-1}), *Brochothrix thermosphacta* (4,8 ± 0,3 $\log_{10}$ cfu·g^{-1}) a bakterií mléčného kvašení (1,7 ± 0,4 $\log_{10}$ cfu·g^{-1}) obsahovaly na konci skladování vzorky balené do MA A. Oproti tomu vzorky balené do modifikované atmosféry B (MA B) obsahovaly na konci skladování vyšší počty koliformních mikroorganismů (4,1 ± 0,6 $\log_{10}$ cfu·g^{-1}). Společně s mikrobiologickými parametry se stanovoval obsah biogenních aminů (putrescin, kadaverin, histamin, tyramin, spermin, spermidin, *β*-phenylethylamin). U čerstvých vzorků a po třech dnech skladování byly stanoveny pouze spermin a spermidin. Po devíti a čtrnácti dnech skladování byly detekovány i další BAs. Celkové množství biogenních aminů činilo na konci skladování 60,0 ± 13,2 mg·kg^{-1} u vzorků balených do MA A a 129,0 ± 41,3 mg·kg^{-1} u vzorků balených do MA B. Nejvíce zastoupenými biogenními aminy byly u vzorků skladovaných v MA A putrescin a spermin (49,7 a 24,8 % na konci skladování); zatím co u vzorků skladovaných v MA B to byli putrescin a kadaverin (47,0 a 32,9 % na konci skladování).

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