FORMATION OF BIOGENIC AMINES IN CHICKEN MEAT STORED UNDER MODIFIED ATMOSPHERE

L. Gallas[1*], I. Steinhauserova[1], L. Steinhauser[1], I. Borkovcova[2] and L. Vorlova[2]

[1]Department of Meat Hygiene and Technology, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho 1-3, 612 42 Brno, Czech Republic

[2]Department of Milk Hygiene and Technology, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho 1-3, 612 42 Brno, Czech Republic

[*]Corresponding author (phone: +420-541-562-750; fax: +420-541-321-230; e-mail: lgallas@vfu.cz)

Abstract

The aim of the study was to investigate the effects of two modified atmospheres on selected groups of microorganisms and on concentrations of biogenic amines (BAs) in samples of chicken 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. 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 parameters, concentrations of biogenic amines (putrescine, cadaverine, histamine, tyramine, spermine and spermidine) 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 2 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).

Index Terms — **biogenic amines, food microbiology, HPLC, poultry meat, shelf – life.**

I. INTRODUCTION

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. Modified atmosphere packaging (MAP) is used to increase the shelf life of fresh chicken meat and chicken products. 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 a strict cold chain compliance. 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). Biogenic amines (especially histamine, tryptamine and 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. It has been reported that 5 – 10 mg of histamine can be considered potentially hazardous for some sensitive people. Ten mg is considered as a tolerable limit, 100 mg may induce a medium toxicity and a dose of 1 000 mg histamine is highly toxic.

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 % O_2 and 75 % CO_2) during cold storage.

II. MATERIALS AND METHODS

Preparation of Chicken Meat Samples and Storage Conditions

Fresh chicken breast meat was obtained from a local poultry slaughterhouse. 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 EVA sealant layer. Meat samples were individually packaged on a Vac-Star S 223 GX (Frimark CZ Ltd., Czech Republic). 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 of modified atmospheres A (75 % O_2 a 25 % CO_2) and B (75 % N_2 and 25 % CO_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. 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.

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 hours 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 hours 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 hours at 37 ± 1 °C. All analyses were performed in duplicate. The number of formed colonies was counted and reported as $\log_{10}$ of cfu.g^{-1} for every sample.

Biogenic Amines Measurement

Biogenic amines (putrescine (PUT), cadaverine (CAD), histamine (HIS), 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 x 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

Statistical data analyses were conducted using the statistical programme STATISTICA Cz (Statsoft, Czech Republic). Microbiological counts ($\log_{10}$ cfu.g^{-1}), storage time and content of biogenic amines were analysed. Student's test was applied to determine the differences between individual storage days. The 0.05 level of significance was used.

III. RESULTS AND DISCUSSION

Microbiological Parameters

Microorganism counts of meat samples packaged under the two types of modified atmosphere (MA) are given in Tab. 1. The initial **psychrotrophic bacteria** counts were 3.1 $\log_{10}$ cfu.g^{-1} in both sets of samples. 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. Initial levels 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, 2006). The initial **lactic acid bacteria** (LAB) count was 1.3 $\log_{10}$ cfu.g^{-1} in the MA A and 1.6 $\log_{10}$ cfu.g^{-1} in MA B respectively. The numbers of LAB on the end of storage were 1.7 $\log_{10}$ cfu.g^{-1} (MA A) and 0.6 $\log_{10}$ cfu.g^{-1} (MA B), respectively. 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}. Jiménez 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 that our results probably because initial levels 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. In their study of various types of atmosphere, 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}; simple aerobic packaging (control) – the last relevant value after 9 days of storage was 7.23 ± 0.47 $\log_{10}$ cfu.g^{-1}. The initial *B. thermosphacta* counts in that experiment were 3.04 ± 0.21 $\log_{10}$ cfu.g^{-1}. Our counts are lower, particularly thanks to lower initial *B. thermosphacta* counts and a lower temperature of storage (3.2 ± 0.7 °C). No **coliform microorganisms** were found in none 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. Jiménez 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). 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}. The initial levels in this experiment were 3.04 ± 0.21 $\log_{10}$ cfu.g^{-1}.

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

		0. day	3. day	9. day	14. day
A (75 % O_2, 25 % CO_2)	psychrotrophic bacteria	3.1 ± 0.2	2.7 ± 0.4	4.4 ± 0.5	6.5 ± 0.7
	Brochothrix thermosphacta	2.2 ± 0.3	3.2 ± 0.2	3.5 ± 0.2	4.8 ± 0.3
	lactic acid bacteria	1.3 ± 0.3	1.0 ± 0.1	0.7 ± 0.2	1.7 ± 0.4
	coliform microorganisms	n.d.	1.1 ± 0.3	1.1 ± 0.6	1.9 ± 0.4
B (75 % N_2, 25 % CO_2)	psychrotrophic bacteria	3.1 ± 0.6	2,8 ± 0.5	4.8 ± 0.6	5.8 ± 0.8
	Brochothrix thermosphacta	1.2 ± 0.4	1.1 ± 0.5	1.4 ± 0.3	2.5 ± 0.2
	lactic acid bacteria	1.6 ± 0.2	2.1 ± 0.7	1.0 ± 0.3	0.6 ± 0.2
	coliform microorganisms	n.d.	0.5 ± 0.2	2.7 ± 0.3	4.1 ± 0.6

n.d. not detected

Biogenic Amines Content

Levels of biogenic amines (BAs) found in poultry meat samples packaged under the two types of modified atmosphere (MA) are given in Tab. 2. At the beginning of storage and on Day 3, no **putrescine** was detected in any of the samples. 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 statistically 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 levels in samples stored under MA B were statistically significantly higher than those in MA A samples ($p < 0.05$). 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. 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 ($p < 0.05$). 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 absolutely insignificant concentrations at the end of the storage period are indicative of good hygienic profile of input material and good inhibitory effects of MA. **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 not statistically significant. **Spermidine** levels 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. Their decrease on Days 9 and 14 of storage to levels between 5.9 and 6.4 mg.kg^{-1} was not, however, statistically significant. Silva and Glória (2002) reported practically the same initial levels of spermine (17.9 mg.kg^{-1}) and spermidine (7.3 mg.kg^{-1}) as those recorded in our study. Also the levels they found after 15 days of storage (SPM 11.2 mg.kg^{-1} and SPD 8.7 mg.kg^{-1}) correspond to our findings. In samples stored under MA A, no **tyramine** was 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. 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. 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 levels 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. Balamatsia et al. (2006) reported BA totals of about 500 $mg.kg^{-1}$ in the case of 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 ascertained in our study. 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 statistically significantly higher levels of biogenic amines than samples stored under MA A ($p < 0.05$ and $p < 0.001$, respectively).

Table 2: Changes in biogenic amines concentrations on chicken meat carcasses during storage under modified atmospheres A and B [$mg.kg^{-1}$] (mean ± s.d.)

	day	PUT	CAD	HIM	TYM	SPM	SPD	Total
A (75 % O_2, 25 % CO_2)	0	n.d.	n.d.	n.d.	n.d.	17.8 ± 0.6	7.5 ± 0.7	25.3 ± 0.7
	3	n.d.	n.d.	n.d.	n.d.	17.3 ± 0.6	7.7 ± 0.9	25.0 ± 0.7
	9	26.4 ± 4.8	8.5 ± 8.4	n.d.	n.d.	16.0 ± 1.0	6.1 ± 0.9	57.1 ± 13.1
	14	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 (75 % N_2, 25 % CO_2)	0	n.d.	n.d.	n.d.	n.d.	17.7 ± 0.6	7.6 ± 0.8	25.2 ± 0.8
	3	n.d.	n.d.	n.d.	n.d.	17.9 ± 0.4	7.3 ± 1.3	25.2 ± 1.5
	9	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	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

IV. CONCLUSION

This work deals with the significance of the modified atmosphere with different gases on present microflora and the formation of biogenic amines. We have demonstrated different effects of two types of modified atmospheres on microflora and the formation of biogenic amines in packaged chicken breast meat. These results may help to select a more appropriate form of packaging of raw chicken meat.

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