BIOGENIC AMINES CONTENT OF POULTRY BREAST STORED UNDER DIFFERENT CONDITIONS AT + 4°C FROM TWO DIFFERENT INDUSTRIAL GENOTYPE CHICKENS

Giuseppe Martino, Sonia Marchetti, Lisa Grotta, Valentina Ponzielli

Faculty of BioSciences and Technologies for Agriculture Food and Environment, University of Teramo, v. C. Lerici 1, 64023 Mosciano Sant'Angelo (TE), Italy

Abstract

In this experimental work, forty breast chickens of two different industrial genotype, were analysed at 4, 8, 11 and 15 days from slaughtering. All breast chickens were divided in two groups. A group was stored at 4°C, and the other was stored at 4°C in sterilised water added with antibiotic (400 mg/l Enrofloxacin). BAs quantitative determination was carried out by means of HPLC, with spectrophotometric-UV detection. The amines were extracted in $HClO_4$ and derivatised by dansylchloride. In the samples stored in sterile water added with antibiotic, no bacterial growth was observed until the end of the experimental period. Significant differences in BAs accumulation were observed between the two samples groups, while BAs content was different during time. In fact, in samples stored in sterilised water added with the antibiotic, the presence of some BAs, such as spermine and spermidine, was revealed, probably due to the action of tissue enzymes.

Key words: *biogenic amines, meat, quality, chicken, genotype*

1. INTRODUCTION

Biogenic amines (BA) are organic bases with aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structures that can be found in several foods, in which they are mainly produced by microbial decarboxylation of amino acids, with the exception of physiological polyamines (Silla Santos, 1996; Ten Brink et al, 1990). Polyamines putrescine (PUT), spermidine (SPD) and spermine (SPM) were separated from their traditional classification within biogenic amines during the 1990s due to their mode of formation and biological roles. Putrescine, though structurally a diamine, is also classified as a polyamine, being the precursor of both physiological ("true") polyamines (PUT→SPD→SPM). Because the polyamines are fully protonated under physiological conditions, they can interact with numerous cell constituents, such as nucleic acids, ATP, specific proteins and phospholipids. Thus, they are essential for cell growth (Agostinelli *et al.*, 2010; Igarashi *et.al.*, 2010; Kusano *et al*., 2008).

The decarboxylation process can proceed through two biochemical pathways which are activity of endogenous decarboxylase enzymes naturally occurring in food or activity of exogenous enzymes released by various microorganisms. So, in virtually all foods that contains proteins or free amino acids and subjected to conditions enabling microbial or biochemical activity Bas formation can be expected. However, the total amount of the different amines is strongly variable depending on the nature of food and the microorganisms involved (Halasz et al., 1994; Ruiz-Capillas *et al*., 2004; Standnik J. *et al.*, 2010).Meat is very susceptible to chemical and physical changes and to biological agents; among them, microorganisms and endogenous or microbial enzymes can make the meat unsuitable for consumption. The surface of the chicken breast offers optimal conditions for the growth of a broad spectrum of microorganisms. The selective metabolic activities of microorganisms play a key role in their propagation. The degree of deterioration of the meat depends on the number and conditions for their growth (source of nutrients, temperature, pH, composition of the atmosphere and water activity) (Ruiz-Capillas *et al*.,2004; ICMSF-International Commission on Microbiological Specification for Foods, 2005).

Knowledge of the biogenic amine levels in food is important for assessing health hazards, for example, they can cause some neurotransmission disorders due to their action as false

neurotransmitters. Nevertheless, their presence can cause several problems for susceptible consumers, such as nausea, respiratory distress, hot flushes, sweating, heart palpitation, headache, bright red rash, oral burning, hyper- or hypotension, whose intensity is dependent on quantitative and qualitative differences (Ladero *et al*., 2010a), especially if some monoamine oxidase (MAO) inhibitors are also ingested as drugs or alcohols. Amines were also investigated as a possible mutagenic precursor, since some amines may be nitrosated or act as precursors for other compounds capable of forming nitrosamines which are carcinogenic to various species of animals and pose a potential health hazard to humans (Bouchereau *et al*., 2000, Kalac *et al*., 2005; Latorre- Moratalla *et al*., 2008; Moret *et al*., 2005).BAs content may potentially be considered as a freshness marker or as an indicator of quality deterioration in meat, since only small concentration of BAs are found in fresh food, while the concentration of most BAs usually increases during storage (Rokka *et al.,* 2004). Great fluctuations of amines content are reported in the same type of product. These differences depend on many variables: the quali-quantitative composition of microbial microflora, the chemico-physical variables, the hygienic procedure adopted during production, and the availability of precursors (Suzzi *et al.*, 2003).Very little information is available on the levels of biologically active amines in poultry meat and specifically in fresh and cooked chicken meat. Recently, Silva and Gloria (2002) reported levels of biogenic amines in chicken meat and chicken-based meat products. The presence of putrescine, cadaverine, histamine, and tyramine was observed after 15 days of refrigerated storage (4 °C), and was higher in breast meat as compared to thigh meat. Chicken-based meat products also contained spermine and spermidine (Silva *et.al*, 2002).

The objectives of the present study were to determine the formation of BAs in a chicken meat, immediately after slaughter and during storage at 4 °C in sterilised conditions to investigate the possible role of tissue enzymes on the formation of BAs and the content of BAs chicken breast of two different industrial genotypes.

2. MATERIALS AND METHODS

2.1. Preparation of the chicken samples and storage conditions

In this experimental work, forty chicken breasts, obtained from two different industrial genotype, Ross 708 Broiler and ISA Warren o ISA Brown and fed with the same formulation of feed, were analysed. All breast chickens were taken within 25-30 minutes after slaughter, avoiding any possible contamination, and transported to the laboratory on ice, and divided in two groups. A group was stored at 4 °C and the other was burned 45 sec. in boiling water and immediately immersed in a glass bottle, previously sterilized, containing sterilized water added with antibiotic (400 mg/l Enrofloxacin), and stored at 4 ° C.

Chicken breasts of Ross 708 Broiler were analysed at 0, 4, 8, 11 and 15 days from slaughtering to investigate the role of tissue enzymes on the formation of BAs during storage in different conditions. To investigate the effect of the genotype on the formation of BAs, samples were analysed at 0, 4 and 8 days from slaughtering. Before being analyzed, the chicken breast was cut off the outside so that we can go on to analyze the inner most steril.

2.2. Biogenic amines analysis

Biogenic amines were determined by HPLC according to Eerola *et al.* (1993). 2,5 grams of the samples were homogenized in 15 ml of 0.4M perchloric acid with Ultra-Turrax IKA T-25. The samples were centrifuged for 15 min at 4000 rpm and the supernatant rinsed into a test-tube through filter paper. The extraction was repeated with 15 ml of 0.4M perchloric acid. Make 1 ml sample extract alkaline by adding 200 µl 2N sodium hydroxide solution, and buffer sample by adding 300 µl saturated sodium bicarbonate. Add 1 ml dansylchloride solution and transfer reaction mixture to 45°C incubator for 45 min. Remove residual dansylchloride by adding 150 µl 30% ammonia. After 1 hour at dark, adjust to 2 ml with acetonitrile, mix with vortex mixer, centrifuge 15 min at 3500 rpm, and filter supernatant through 0.45 µm filter.

2.3. Chromatographic separation

Column temperature (T_{col}) = 40°C; flow rate = 1.0 ml/min; injection = 20 µl sample; detection wavelength = 254 nm were used. Use gradient elution program with mixture of 0.1M ammonium acetate as solvent A and acetonitrile as solvent B. Gradient begins at 50% and ends at 90% acetonitrile in 19 min. Equilibrate system 10 min before next analysis.

The biogenic amines content was expressed as mg Kg^{-1} of sample.

2.4. Microbiological analysis: samples preparation

The preparation of the samples has provided following steps. After addition of samples in the company, these were sterilized in an autoclave at 121 ° C for 15 minutes in the glass jars filled to half of water and then cooled and added of a sterile solution of water and antibiotic (400 mg /l Enrofloxacin). In the solution were immersed one half of chicken breast previously blanched in boiling water for about 45 seconds and stored at 4 °C.

2.5. Microbiological Analysis: Count Mesophilic Total (CMT)

In the evaluation of the content of biogenic amines in the samples of chicken, microbiological analysis was performed on a part of them, performing the Count of Mesophilic Total. The analysis allows to evaluate the number of microorganisms including bacteria, yeasts and molds, can develop at 30 °C. The analysis was performed on the samples after 8 days of storage at 4 °C, with drawing the aqueous solution containing the antibiotic, to confirm the sterility of the sample or evaluate a possible microbial growth. The microbiological investigation has been carried out previously preparing plates containing medium PCA (Plate Count Agar, Oxoid, Basingtoke, UK) suitable for the growth of microorganisms mesophilic (whose optimum temperature is between 20 °C and 40 °C), and sterile tubes containing saline solution (0.9 g NaCl in 1 liter of distilled water). The microbiological sampling was performed by setting up a suitable number of serial dilutions (usually 3 dilutions) and then spatulating 0.1 ml of sample on plates of PCA. The plates were incubated at 30 °C for 48 hours, then was counted the number of colonies which may be developed.

2.6. Statistical analysis

Results were analysed using SPSS 9.0 for windows and the mean values compared by T student test.

3. RESULTS AND DISCUSSION

In the samples stored in sterile water added with antibiotic, no bacterial growth was observed until the end of the experimental period. In the present study, six biogenic amines were recorded namely, putrescine, cadaverine, tryptamine, tyramine, spermine and spermidine.

Table 1 shows amines content in Ross 708 Broiler breasts in different conditions of storage at 0, 4, 8, 11, 15 days from slaughtering. Significant differences in BAs accumulation were observed between the two different conditions of storage. BAs content was different during storage time.

The type and levels of biogenic amines found in chicken breast of Ross 708 Broiler immediately after slaughter (time 0) are indicated on Table 1. The types and levels of biogenic amines detected in chicken meat during storage at 4°C and at 4°C in sterilised water added with antibiotic, are described in Table 1. Putrescine and cadaverine are detected only in the samples refrigerated without antibiotic while tryptamine, tyramine, spermine and spermidine were detected in both type of samples. Tryptamine was only detected at 4 days from slaughtering both in presence and in absence of antibiotic. Putrescine and cadaverine were detected in this kind of samples stored in normal conditions of refrigeration at 4, 8, 11 and 15 days from slaughtering while putrescine was not detected in sterilisation conditions. Cadaverine was present in samples stored at 4°C in antibiotic solution at 8, 11

and 15 but not at 4 days form slaughtering. For samples refrigerated with antibiotic, the level of spermine decreased with storage time and the levels of spermidine remained constant.

These results are similar to those reported for fish and pork. Levels of the diamines, putrescine and cadaverine increased from initial (day 0) values of 0,55 and 2,47 ppm attaining final values of 25,54 and 79,37 ppm, respectively (day 15). Significantly higher concentrations of cadaverine, spermidine and spermine were recorded in Ross 708 Broiler refrigerated without antibiotic at 8, 11 and 15 days.

TIME (Days)									
Biogenic amines (ppm)	**0**	**4**		**8**		**11**		**15**	
	4°C	4°C	4°C antib.	4°C	4°C antib.	4°C	4°C antib.	4°C	4°C antib.
Tryptamine	n.d.	0.27± 0.02	0.15± 0.01	n.d	n.d.	n.d.	n.d.	n.d.	n.d.
Putrescine	0.55± 0.13	1.58± 0.20	n.d.	2.85± 0.55	n.d.	17.08± 3.25	n.d.	25.54± 1.17	n.d.
Cadaverine	2.47± 0.48	12.27± 3.29	n.d.	45.25± 2.35	0.50± 0.12	47.81± 4.35	0.20± 0.02	79.37± 3.66	0.70± 0.12
Tyramine	n.d.	0.38± 0.04	0.30± 0.07	30.30± 4.15	n.d.	36.35± 3.45	0.06± 0.01	52.35± 4.05	0.05± 0.01
Spermidine	n.d.	0.74± 0.12	0.63± 0.13	0.90± 0.08	0.64± 0.11	0.63± 0.09	0.55± 0.01	0.58± 0.11	0.27± 0.04
Spermine	n.d.	46.31± 3.24	36.80± 2.56	52.88± 4.56	22.02± 2.32	42.35± 3.22	27.92± 4.55	43.36± 4.55	7.21± 0.73

Table 1 – Biogenic amines content of Ross 708 Broiler breasts at different conditions of storage ($\mu \pm ds$). n.d.= not detected

The following is the performance of the individual amines in chicken breast of Ross 708 Broiler both in the absence and presence of antibiotic (Fig.1- Fig.2). it is possible to notice that the production of tryptamine was limited only to the fourth day of storage, both in terms of simple refrigeration, both in water and autoclaved with added antibiotic, even if - in the latter case - at lower concentrations (0.27 ± 0.02 vs. 0.15 ± 0.01), while for the putrescine, produced only in the control group, were detected increasing concentrations as a function of storage time, such as to describe a performance characteristic similar to the curve of microbial growth. Increasing more or less similar trends were obtained in the breast meat of Ross 708 Broiler of the control group also as regards cadaverine and tirammina for which, although at lower concentrations, was detected even in the experimental group, particularly in second week of storage.

The spermidine and spermine, however, both in control and in the flesh "treated", reported a decreasing trend. After reaching a maximum, their concentrations have decreased in both groups, although with different absolute values.

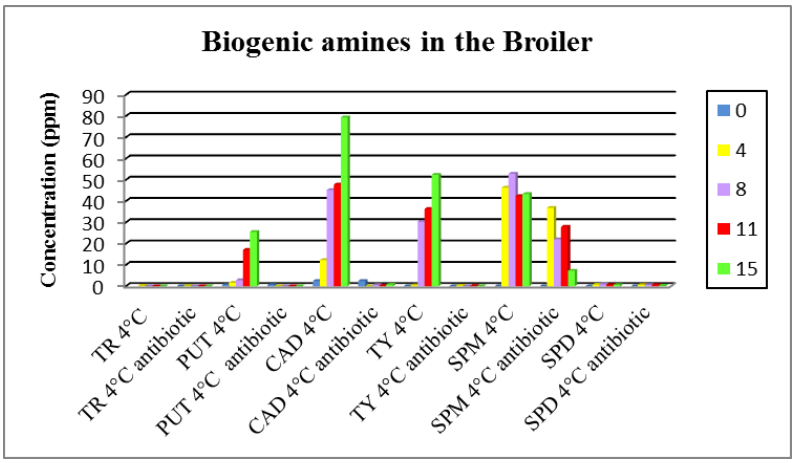


Fig.1. Concentration of biogenic amines in the Ross 708 Broiler in function of time (TR-Tryptamine; PUT-Putrescine; CAD-Cadaverine; TY-Tyramine; SPM-Spermina;SPD-Spermidina)

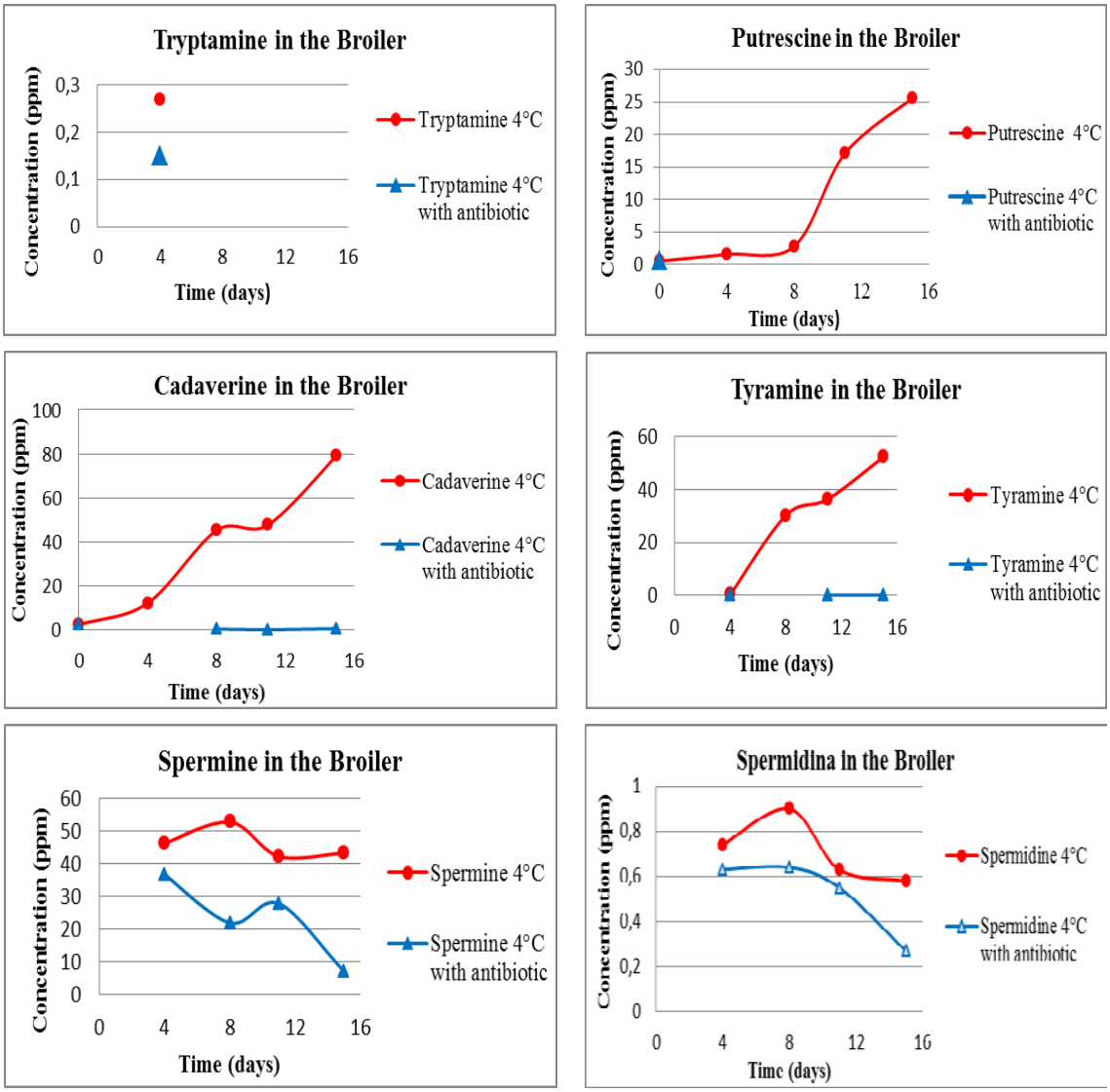


Fig.2 . Concentration of individual biogenic amines in the Ross 708 Broiler in function of time.

To investigate the effect of genetics, the types and levels of biogenic amines detected in chicken breast muscle of the two different industrial genotype, Ross 708 Broiler and ISA Warren o ISA Brown, are indicated on Tables 2 and 3. During storage, the presence of tryptamine, putrescine and cadaverine was detected in both types of meat while tyramine, spermine and spermidine were not detected in ISA Warren breast.

Tryptamine presence in Broiler breasts stored both in sterilised and not sterilised conditions of storage, was detected only at 4 days from slaughtering. This content was significantly higher in normal conditions of refrigeration than in sterilised ambient.

Putrescine was formed at 0 days from slaughtering both in Broiler samples and in ISA Warren breasts stored at 4°C. During storage (at 4 and 8 days) this amine was present in the Broiler samples only in not sterilised ambient while putrescine was detected in ISA Warren samples both in not sterilised and sterilised conditions of refrigeration. Cadaverine was present in sterilised conditions of storage in Broiler breast only at 8 days from slaughtering while in the ISA Warren breast was also detected at 4 and 8 days. The presence of BAS in samples stored in sterilised condition is probably due to the action of tissue enzymes.

During storage of Ross 708 Broiler, tyramine, spermine and spermidine contents increase from 4 to 8 days from slaughtering while decrease or remain constant in sterilised conditions of storage.

During storage tryptamine was formed at 4 days from slaughtering and disappeared at 8 days in Broiler breasts while was formed at 8 days and in ISA Warren stored at 4°C without antibiotic and at 4 days in sterilised ambient. Putrescine content increases during storage at 4°C in normal conditions of refrigeration in Broiler and decrease from 4 to 8 days both in ISA Warren stored in sterilised and not sterilised ambient, such as cadaverine.

Days	Genotype	Storage	Tyramine (ppm)	Spermine (ppm)	Spermidine (ppm)
0	Ross 708 Broiler	4°C	n.d.	n.d.	n.d.
		4°C antib.	n.d.	n.d.	n.d.
	Isa Warren	4°C	n.d.	n.d.	n.d.
		4°C antib.	n.d.	n.d.	n.d.
4	Ross 708 Broiler	4°C	0.38±0.04	46.31±3.24	0.74±0.12
		4°C antib.	.30±0.07	36.80±2.56	0.63±0.13
	Isa Warren	4°C	n.d.	n.d.	n.d.
		4°C antib.	n.d.	n.d.	n.d.
8	Ross 708 Broiler	4°C	30.30±4.25	52.88±4.56	0.90±0.08
		4°C antib.	n.d.	22.02±2.32	0.64±0.11
	Isa Warren	4°C	n.d.	n.d.	n.d.
		4°C antib.	n.d.	n.d.	n.d.

Table 2 – Tyramine, spermine and spermidine contents in Ross 708 Broiler and ISA Warren breasts at different conditions of storage (μ±ds). n.d.= not detected

Days	Genotype	Storage	Tryptamine (ppm)	Putrescina (ppm)	Cadaverine (ppm)
0	Ross 708 Broiler	4°C	n.d.	0.55±0.13	2.47±0.22
		4°C antib.	n.d.		
	Isa Warren	4°C	n.d.	0.26±0.11	2.04±0.32
		4°C antib.	n.d.		
4	Ross 708 Broiler	4°C	0.27±0.02	1.58±0.20	12.27±1.80
		4°C antib.	0.15±0.01	n.d.	n.d.
	Isa Warren	4°C	n.d.	6.04±0.98	6.35±1.15
		4°C antib.	0.20±0.03	3.95±0.75	2.76±0.75
8	Ross 708 Broiler	4°C	n.d.	2.85±0.55	45.25±2.35
		4°C antib.	n.d.	n.d.	0.50±0.08
	Isa Warren	4°C	3.66±0.36	0.27±0.04	4.12±0.21
		4°C antib.	0.13±0.02	0.51±0.05	4.75±0.14

Table 3 – Tryptamine, putrescine and cadaverine contents in Ross 708 Broiler and ISA Warren breasts at different conditions of storage (μ±ds). n.d.= not detected

Samples stored in sterilised water added with the antibiotic, show the presence of some BAs, such as spermine and spermidine, probably due to the action of tissue enzymes. In fact, in the samples stored in sterile water added with antibiotic, no bacterial growth was observed until the end of the experimental period.

CONCLUSION

In conclusion it's possible say that the raw chicken meat is a very complex ecosystem food. Its chemical and physical properties (pH, high availability of nutrients and water) can determine the colonization and development of a large number and a different types of microrganisms, which, in presence of proteins and free amino acids, are able to give rise to an entire activity decarboxylase. The meat has a microflora crucially dependent on its chemical composition, the environment in which it is produced, by the rearing and slaughter of those, by conservation, shipping and handling. From the obtained data, supported by microbiological analysis which showed, in most of the analysed samples, a total microbial load typically $\leq$ 10 ufc/ml and still too low to allow the production of biogenic amines. It's possible that the ammine produced in the meat object of study, also found in the experimental group from the fourth to the fifteenth day of storage, tissue origin have.

REFERENCES

Agostinelli, E., Marques, M. P. M., Calheiros, R., Gil, F. P. S. C., Tempera, G., Viceconte, N., *et al.*, 2010. Polyamines: Fundamental characters in chemistry and biology. *Amino Acids*, 38: 393−403.

Balamatsia, C.C., Paleologos, E.K., Kontominas, M.G., Savvaidis, I.N., 2006. Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4±1°C: possible role of biogenic amines as spoilage indicators. *Antonie van Leeuwenhoek* 89 (9):9-17.

Bouchereau, A, Guénot, P., Larher, F., 2000. Analysis of amines in plant materials. *Journal Chromatography B.*, 747, 49-67.

Cristiane M.G. Silvia, M. Beatriz A. Glòria, 2001. Bioactive amines in chicken breast and thing after slaughter and during storage at 4 ± 1°C and in chicken-based meat products. *Food Chemistry* 78: 241-248.

Eerola, S., Hinnkkainnen, R., Lindfors, E., Hirvi, T., 1993. Liquid chromatographic determination of biogenic amines in dry sausages. *Journal of the Association of Official Analytical Chemists* 76: 575-580.

Halàsz, A., Barath A., Simon-Sarkadi, L., Holzapfel, W., 1994. Biogenic amines and their production by micro-organisms in food. *Trends Food Science Tech.* 5: 42-49.

Icmsf – International Commission On Microbiological Specification For Foods, 2005. Microorganisms in foods 6 (2nd ed.). New York: Kluwer Academic/ Plenum Publishers.

Igarashi K., Ito K., Kashiwagi K, 2001. Polyamine uptake system in Escherichia coli. *Res. Microbial* 152: 271-278.

Jansen, S.C., van Dusseldorp, M., Bottema, K.C., Dubois, A.E.J., 2003. Intolerance to dietary biogenic amines: a review. *Annals of Allergy, Asthma & Immunology* 91: 233–241.

Kalac, P., Krausova, p., 2005. A review of dietary polyamines: formation, implications for growth and health and occurrence in foods. *Food Chemistry* 90: 219-230.

Kalac., P., Krizek, M., Pelikànovà, T., Langovà, M., Veskrna, O., 2005. Contents of polyamines in selected foods. *Food Chemistry* 90: 561-564.

Kusano, T., Berberich, T., Tateda, C., & Takahashi, Y., 2008. Polyamines: Essential factors for growth and survival. *Planta*, 228: 367−381.

Ladero, V., Calles-Enríquez, M., Fernández, M., Alvarez, M.A., 2010a. Toxicological effects of dietary biogenic amines. *Current Nutritional Food Science* 6, 145e156.

Latorre-Moratalla, M.L., Veciana-Noguès, T., Bover- Cid, S., Garriga, M., Aymerich, T., Zanardi, E., Ianieri, A., Frequenza, M.J., Patarata, L., Drosinos, E.H., Laukova, A., Talon, R., Vidal-Carou, M.C., 2008. Biogenic amines in traditional fermented sausages produced in selected European countries. *Food Chemistry* 107: 912-921.

McCabe-Sellers, B.J., Staggs, C.G., Bogle, M.L., 2006. Tyramine in foods and monoamine oxidase inhibitor drugs: a crossroad where medicine, nutrition, pharmacy, and food industry converge. *Journal of Food Composition and Analysis* 19: 58–65.

Moret, S., Smela, D., Populin, T., Conte, L.S., 2005. A survey on free biogenic amine content of fresh and preserved vegetables. *Food Chemistry* 89: 355-351.

Önal, A., 2007. A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry*, 103: 1475-1486.

Rokka, M., Eerola, S., Smolander, M., Alakomi, H-L., Ahvenainen, R., 2004. Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions B. Biogenic amines as quality indicating metabolites. *Food Control* 15: 601–607.

Ruiz-Capillans, C., Jiménez-Colmenero F., 2004. Biogenic amines in meat and meat products. *Critical Reiews in Food Science and Nutrition*, 44. 489- 499.

Shalaby, A.R., 1996. Significance of biogenic amines to food safety and human health. *Food Research International* 29: 675-690.

Silla Santos, M. H., 1996. Biogenic amines: their importance in foods. *International Journal Food Microbiology*, 29: 213–231.

Silva, C.M.G., Gloria, M.B.A., 2002. Bioactive amines in chicken breast and thigh after slaughter and during storage at 4±1°C and in chicken based meat products. *Food Chemistry* 78: 241–248.

Stadnik, J., Dolatowski, Z.J., 2010. Biogenic amines in meat and fermented meat products. *Acta Scientiarum Polonorum, Technologia Alimentaria*9(3) 2010: 251-263.

Suzzi, G. and Gardini, F., 2003. Biogenic amines in dry fermented sausages: a review. *International Journal of Food Microbiology* 88 (1): 41-54.

Ten Brink, B., Damink C., Joosten, H.M.L.J., and Huis in't Veld, J.H.J., 1990. Occurrence and formation of biologically active amines in foods. *International Journal Food Microbiological*, 11:73-84.