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Bioactive amines in chicken breast and thigh after slaughter and during storage at 4±1 °C and in chicken-based meat products

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Abstract

The levels of bioactive amines in chicken meat were determined. Immediately after slaughter, spermine and spermidine were detected in red and white meats. Spermine was the prevalent amine—70% of total. Low levels of histamine were also detected in thighs. During storage at 4±1 °C, there was a decrease in spermine, spermidine levels remained constant, and putrescine, cadaverine, histamine and tyramine were formed. At 15 days, higher levels of amines were found in breast compared to thigh. An index based on ratio of the polyamines spermidine/spermine levels was considered appropriate for the evaluation of chicken meat quality. Chicken-based meat products (mortadella, frankfurters, sausage, meatballs, hamburger and nuggets) were analyzed for bioactive amines for the first time. Nuggets were the only products with amine profiles similar to fresh chicken meat. There was a prevalence of spermidine over spermine for most of the products, suggesting the incorporation of significant amounts of vegetable protein in the formulations. Sausage contained higher biogenic amine levels than the other products. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Bioactive amines; Chicken meat; Meat products; Quality; Tyramine; Histamine

1. Introduction

The production and consumption of chicken meat has increased significantly throughout the world. The largest producers are the United States of America, China and Brazil. In 2000, Brazil produced 4.2 million tons, exporting 22% of its production. The per capita consumption of chicken meat in Brazil has increased from 2.3 kg in 1970 to 18.0 kg in 1994, reaching 29.1 kg in 2001. An increased percentage of the chicken meat produced has also been used as raw material for the processing of chicken-based meat products, among them cooked emulsions, comminuted and frozen products. These products are becoming more popular due to their sensory characteristics, easiness of preparation and improved shelf-life compared to fresh meat (ABEF, 2001; Silva, 1995).

Meat is very susceptible to chemical and physical changes. It is also susceptible to biological agents, among them, microorganisms and endogenous or microbial enzymes, which can make the meat unsuitable for consumption. The most common, fastest and most accepted method to detect quality of fresh meat has been by organoleptic means. However, this method is subjective (Mietz & Karmas, 1977). Furthermore, it can only detect late stages of deterioration (Botta, 1995; Graner, 1984). It would be desirable to identify the status of meat with respect to spoilage before its condition becomes evident to the senses. Accurate assessment of meat's shelf life is important to prevent meat loss and to provide a basis for regulations controlling meat quality.

Several indicators have been proposed for the evaluation of meat quality—volatile bases, nucleotides breakdown, volatile acidity, comet DNA—but all are limited (Mietz & Karmas, 1977; Nakamura, Wada, Sawaya, & Kawabata, 1979; Veciana-Nogués, Mariné-Font & Vidal-Carou., 1997; Yano, Kataho, Watanabe, Nakamura, & Asano, 1995). Furthermore, it is generally recognized that a ratio of catabolites, rather than the measurement of a

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single compound, is much less prone to variability (Veciana-Nogués et al., 1997).

Bioactive amines are organic bases, of low molecular weight, which participate in normal metabolic processes in living tissues (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Lima & Glória, 1999). They can be classified in polyamines and biogenic amines (Lima & Glória, 1999). The polyamines, spermine and spermidine, are naturally occurring amines in fresh pork and beef meat (Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997a). They are involved in nearly every step of DNA, RNA and protein synthesis, being essential for growth and cell proliferation (Halász et al., 1994; Lima & Glória, 1999). The biogenic amines, histamine, putrescine, tyramine, tryptamine, 2-phenylethylamine and cadaverine, can be formed during storage of fresh meat or during processing of meat products. Biogenic amines can be formed by bacterial enzymatic decarboxylation of free amino acids (Hernández-Jover et al., 1997a; Vidal-Carou, Izquierdo-Pulido, Martin-Morro, & Mariné-Font, 1990).

Since biogenic amines are metabolites of microbial activity and bioactive amines are resistant to heat treatment (Glória, Daeschel, Craven, & Hilderbrand, 1999; Halász et al., 1994; Lima & Glória, 1999), they have been considered to be useful indices of quality of fresh and processed meat, reflecting the quality of the raw material used and of hygienic conditions prevalent during its processing. The estimation of biogenic amines in foods is also important from the sensory and toxicological point of view (Halász et al., 1994). Amines can impart putrid odours and off-flavours that can affect food acceptance (Geornaras, Dykes, & Holy, 1995). The ingestion of high levels of histamine and tyramine can cause histamine poisoning and migraine headache, respectively (Lima & Glória, 1999; Taylor, 1985). Biogenic amines constitute a potential health risk, especially when coupled with additional factors, such as monoamine oxidase inhibitor drugs, alcohol and gastrointestinal diseases (Stratton, Hutkins, & Taylor, 1991).

Mietz and Karmas (1977) followed the decomposition of tuna fish and observed increase in putrescine, cadaverine and histamine levels, decreases in spermine levels and an apparent decrease in spermidine levels. Based on these results, they proposed a chemical quality index (CQI) calculated from the levels of putrescine + cadaverine + histamine divided by spermine + spermidine + 1. A CQI between 0 and 1 indicates good quality tuna, between 1 and 10, borderline, and, higher than 10, decomposed. This index was observed to be adequate for the evaluation of the quality of fish and seafood (Yamanaka, Shimakura, Shiomi, & Kikuchi, 1987). Veciana-Nogués et al. (1997) suggested that the levels of tyramine should also be included in the Mietz and Karmas index. These authors proposed a biogenic amine index (BAI), based on the sum of histamine, cadaverine, putrescine and tyramine, to describe the freshness of tuna. According to these authors, BAI values below 50 mg/kg would indicate meat of high hygienic quality.

Very little information is available on the levels of biologically active amines in chicken meat. Therefore, this study was undertaken to: (1) determine the profile and levels of bioactive amines in chicken meat (breast and thigh), immediately after slaughter and during storage at 4.0±1.0 °C, (2) correlate the levels of amines with quality control parameters such as pH, total volatile bases, sulfhydric gas and moisture content, (3) investigate the possibility of using bioactive amines as an index of quality for chicken meat, and (4) determine the levels of bioactive amines in chicken-based meat products.

2. Materials and methods

2.1. Samples

White breast muscle and dark muscle of the thighs of chicken (*Gallus domesticus*) were obtained from a slaughterhouse located in Belo Horizonte, state of Minas Gerais, Brazil. The samples, 16 of each, were packaged individually after slaughter, placed in isothermal boxes and transported to the laboratory. Upon arrival, four samples were taken for analysis (control—time 0) and the remainder was stored at 4.0±1.0 °C. Samples were taken at 4, 10 and 15 days of storage for analysis.

Chicken-based meat products (10 of each) were purchased at local retail stores. They included cooked emulsions (mortadella and frankfurters), fresh sausage and frozen products (meatballs, hamburgers and nuggets). The samples were kept under refrigerated storage (4 or −18 °C) and analyzed prior to expiration date.

The samples were ground in a food processor (Kitchen Machine, Arno, SP, Brazil), homogenized and analyzed for bioactive amines, moisture content, pH, sulfhydric gas and total volatile bases. All of the analyses were performed in duplicate.

2.2. Chemicals

Bioactive amine standards were purchased from Sigma Chemical Co. (St. Louis, MO, EUA). They included putrescine (PUT) dihydrochloride, spermidine (SPD) trihydrochloride, spermine (SPM) tetrahydrochloride, agmatine (AGM) sulfate, cadaverine (CAD) dihydrochloride, serotonin (SRT) hydrochloride, histamine (HIM) dihydrochloride, tyramine (TYM), tryptamine (TRM) and 2-phenylethylamine (PHM) dihydrochloride.

All reagents were of analytical grade, except HPLC reagents, which were LC grade. Ultrapure water was obtained from a Milli-Q System. The mobile phases were filtered through HAWP and HVWP membranes, used, respectively, for aqueous and organic solvents (47 mm diameter and 0.45 μm pore size, Millipore Corp., Milford, MA, EUA).

2.3. Determination of bioactive amines

Amines were extracted from samples (5 g) with 7 ml 5% trichloroacetic acid (TCA). After agitation for 5 min in a Vortex mixer, the slurry was centrifuged at 10,000×g at 4 °C, and the supernatant was collected. The solid residue was extracted two more times with volumes of 7 and 6 ml of TCA. Supernatants were combined and filtered in 0.45 μm membrane. The amines were separated by ion-pair reverse phase HPLC and quantified fluorimetrically after post-column derivatization with *o*-phthalaldehyde (OPA) as described by Vale and Glória (1997).

Liquid chromatography was performed with a LC-10AD system connected to a RF-551 spectro-fluorimetric detector at 340 and 445 nm for excitation and emission, respectively, and to a CBM-10AD controller (Shimadzu, Kyoto, Japan). A reversed-phase μBondapak C18 column, 300×3.9 mm i.d., 10 μm, was used with a μBondapak C18 guard-pak insert (Waters, Milford, MA). The mobile phases were: A, solution of 0.2 M sodium acetate and 10 mM 1-octanesulfonic acid sodium salt, adjusted to pH 5.0 with acetic acid, and B, methanol, acetonitrile, 10 mM 1-octanesulfonic acid sodium salt (1+9+1, v/v/v). The flow rate was set at 0.6 ml/min and the gradient was: 20 min at 12% B, 22 min at 13%, 35 min at 13%, 43 min at 26%, 66 min at 26%, 71 min at 12% and 80 min at 12%. The post-column derivatization reagent was delivered at 0.4 ml/min. It consisted of 1.5 ml Brij-35, 1.5 ml mercaptoethanol and 0.2 g OPA, dissolved in 500 ml solution of 25 g boric acid, and 22 g KOH (pH adjusted to 10.5 with 3% KOH). The column and the post-column reaction apparatus were at room temperature (23±1 °C). The identification of amines was performed by comparison of retention times of amines in sample to standard solutions and also by addition of the suspected amine to the sample. Amine levels were calculated by direct interpolation in the standard curve.

2.4. Determination of pH, moisture content, sulfhydric gas and total volatile bases

Total volatile bases, pH, moisture content and sulfhydric gas were determined according to IAL (1985). The pH was determined potentiometrically in 10 g of sample homogenized in 100 ml distilled water for 5 min. Total volatile bases were determined by Kjeldahl distillation of 5 g samples in the presence of MgO for 25 min. Sulfuric acid (15 ml, 0.1 N) and methyl red were added to the distillate, and the excess sulfuric acid was titrated with 0.1 N NaOH. Moisture content was determined by weight loss on drying at 105 °C. Sulfhydric gas was determined by the lead acetate procedure.

2.5. Statistical analysis

The data were submitted to analysis of variance and the means were compared by the Tukey test at 5% of probability. Linear regression analysis was used to determine relationships between storage time, amine levels and physicochemical characteristics.

3. Results and discussion

3.1. Bioactive amines and physicochemical quality of fresh chicken meat

The types and levels of bioactive amines found in chicken white breast muscle and dark muscle of the thigh, immediately after slaughter (time 0), are indicated on Table 1. Spermidine and spermine were detected in both types of meat. In the thighs, the presence of histamine was also detected. According to Halász et al. (1994), Yano et al. (1995), Veciana-Nogués et al. (1997) and Bover-Cid, Schoppen, Izquierdo-Pulido, and Vidal-Carou (1999), under normal physiological conditions, it is expected to find spermine and spermidine in meat, as these amines play an important role in growth. Histamine can also be present in tissues stored in mastcells or basophils (Michels, 1963; Taylor, 1985).

Total amine levels in breast and thigh were similar (25.5 and 25.0 mg/kg, respectively; $P \leqslant 0.05$, Tukey test). In both types of tissue, spermine was the predominant amine, followed by spermidine. Spermine contributed 70 and 65% of the total amine content in breast and thigh, respectively, and spermidine 29% in both tissues. Spermine and spermidine are also the prevalent amines in pork and beef (Bardocz, Grant, Brown, Ralph, & Pusztai, 1993; Hernández-Jover, Izquiredo-Pulido, Veciana-Nogues, & Vidal-Caron, 1996; Maijala, Nurmi, & Fischer, 1995).

Physicochemical analyses are shown in Table 2. The pH of breast is in accordance with values reported by Mano, Pardi, Queiroz, and Smith (1993). The pH of thigh was significantly higher than breast meat ($P \leqslant 0.05$, Tukey test). No significant differences were observed in total volatile bases or sulfhydric gas test values between tissues.

3.2. Influence of storage time at 4±1 °C on bioactive amine levels and on the physicochemical characteristics of chicken meat

The types and levels of bioactive amines detected in chicken meat during storage at 4±1 °C are described in

Table 1
Bioactive amines in chicken breast and thigh muscle during storage at 4.0±1.0 °C

Muscle/time (days)	Bioactive amines (mg/kg)[a]						
	PUT	CAD	SPD	SPM	HIM	TYM	Total
Breast							
0	nd by	nd by	7.3±0.8ax	17.9±1.3ax	nd by	nd by	25.5±2.0by
4	nd by	nd by	7.7±0.6ax	17.2±0.9ax	nd by	nd by	24.9±1.6by
10	nd by	nd by	6.0±1.0ax	12.5±1.6bx	nd by	nd by	19.2±2.7by
15	20.4±1.8ax	4.3±1.5ax	8.7±1.0ax	11.2±1.2bx	10.3±4.5ax	17.4±4.0ax	72.3±1.8ax
Thigh							
0	nd cy	nd by	7.2±1.8ax	16.2±O.9ax	0.7±0.2 bx	nd by	25.0±1.4ay
4	nd cy	nd by	9.0±1.9ax	15.9±0.6ax	0.5±0.2bx	2.1±0.3ax	28.0±1.2ay
10	0.5±0.1bx	nd by	6.1±0.4ax	10.8±0.9bx	nd cy	nd by	17.4±1.3by
15	3.8±5.3ay	1.3±0.4ay	6.4±0.6ax	8.9±0.6bx	5.4±1.3ay	1.4±0.6ay	27.1±2.5ay

[a] Mean values±standard deviation ($n=4$) were calculated using zero for nd values (PUT, CAD, SPD, SPM and HIM$\leqslant$0.80; TYM and TRM$\leqslant$1.40 mg/kg). Mean values with a common letter (abc for the same type of meat and xy for the same storage time) in the same column do not differ significantly ($P\leqslant0.05$, Tukey test).

Table 2
Physicochemical characteristics of chicken breast and thigh meat during storage at 4.0±1.0 °C

Meat type/time (days)	Physicochemical characteristics[a]		
	pH	TVB[b] (mg N/100 g)	H_2S[c]
Breast			
0	5.72±0.09by	14.32±6.23by	0.0
4	5.89±0.12by	13.16±1.30by	1.0
10	5.89±0.13by	17.63±2.63by	3.0
15	6.30±0.17ay	34.39±9.40ay	5.0
Thigh			
0	6.30±0.15cx	16.80±3.63by	0.0
4	6.63±0.14abx	16.80±3.63by	1.0
10	6.41±0.11bcx	34.02±12.91ax	3.0
15	6.65±0.09ax	46.46±10.54ay	5.0

[a] Mean values±standard deviation ($n=4$) with the same letter (abc for the same tissue and xy for the same storage time) in the same column do not differ significantly ($P\leqslant0.05$, Tukey test).
[b] TVB, total volatile base.
[c] The sizes of spots in the filter paper impregnated with lead acetate were classified as 0 = no spot, 1 = light, 3 = moderate, and 5 = heavy.

Table 1. For both types of tissue, the level of spermine decreased with storage time and the levels of spermidine remained constant. These results are similar to those reported for fish (Mietz & Karmas, 1977; Veciana-Nogués et al., 1997) and pork (Hernández-Jover et al., 1996; Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, Mariné-Font, & Vidal-Carou, 1997b; Maijala, Eerola, Aho, & Hirn, 1993; Nakamura et al., 1979). According to Hamana, Akiba, Uchino, and Matsuzaki (1989) and Hernández-Jover et al. (1997b), the decrease in spermine content may occur because this polyamine is taken up from the media as a nitrogen source by microorganisms.

During storage, the presence of putrescine, cadaverine, histamine and tyramine was detected in both types of meat. The amines were detected at a shorter period of storage in thigh compared to breast. However, at 15 days of storage, the levels of biogenic amines which accumulated in the tissues were significantly higher in breast than thigh.

According to the literature, biogenic amines are usually formed by bacterial enzyme activity (Halász et al., 1994). The types and levels of biogenic amines formed will depend on the microbial flora and count (Veciana-Nogués et al., 1997). According to Shalaby (1996), among microorganisms found in meat products, histamine was produced by *Hafnia alvei*, *Klebsiella oxytoca*, *Morganella morganii*, *Edwardsiella* spp. as well as lactic acid bacteria such as *Lactobacillus brevis*, *L. buchneri*, *L. divergens*, *L. carnis*, *L. curvatus* and *L. hilgardii*. Tyramine was produced by group D *Streptococci*, *Enterococcus faecalis*, coliforms and lactic acid bacteria, particularly *L. divergens* and *L. carnis*. Many strains of *Enterobacteriaceae* produce appreciable amounts of cadaverine, while *Pseudomonas* produce mainly putrescine. Based on this information and on the bacterial strains associated with poultry (Geornaras et al., 1995), putrescine, cadaverine, tyramine and histamine could be found in chicken meat.

Total amine levels did not differ significantly in the first 10 days of storage, but increased significantly on the 15th day, for both types of meat. Total levels were higher in breast than thigh. The shelf-life suggested by the Brazilian Federal Inspection Service for refrigerated chicken is 12 days. Therefore, the biogenic amines would not represent any harm, since their accumulation occurred on the 15th day of storage.

The physicochemical characteristics of chicken meat during storage are indicated on Table 2. Significantly lower pH values were observed for breast than thigh muscle, throughout the storage period. There was an increase in pH values during refrigerated storage, for both types of tissue. Only on the 15th day, were the values significantly different ($P \leqslant 0.05$, Tukey test) for breast. However, pH increased gradually with storage time for thigh. Similar results were observed during refrigerated storage of chicken and pork meat (Hernández-Jover et al., 1996; Mano et al., 1993). According to Maijala et al. (1993), Mano et al. (1993) and Hernández-Jover et al. (1996), the increase in pH could be related to the formation and accumulation of amines and ammonia.

Total volatile bases increased with storage time. Significantly higher levels were detected on the 10th day of storage for thigh and on the 15th day for breast. At the 10th day of storage, all of the samples were positive for sulfhydric gas. Positive values were also observed on the 15th day of storage, however at higher intensity. At this time, the product was inappropriate for consumption.

The results obtained for the physicochemical parameters of chicken meat with storage time are in agreement with Graner (1984) and Botta (1995). Sulfhydric gas proved to be subjective, depending on the expertise of the analyst. Total volatile bases and pH were significantly different from fresh meat after the expiration date of refrigerated chicken; therefore they could only detect late stages of deterioration.

3.3. Regression analysis of amines, physicochemical parameters and storage time

Significant linear correlation ($P \leqslant 0.05$) was observed between storage time and spermine levels for breast and thighs (Table 3). There was also significant correlation among amines. Similar results were observed by Hernández-Jover et al. (1996), Treviño, Beil, and Steinhart (1997) and Veciana-Nogués et al. (1997), for fish and pork. These results suggest that the formation of some amines in chicken breast or thigh meat is affected by similar factors.

3.4. Quality index based on bioactive amines

The values obtained using the chemical quality index (CQI) proposed by Mietz and Karmas (1977) are shown in Table 4. Significant change of the CQI was only observed at the 15th day of storage for both types of meat, when it changed from good quality to borderline

Table 4
Application of different indices of quality based on bioactive amines for chicken meat stored at 4.0±1.0 °C

Meat type/time (days)	Quality indices[a]		
	Mietz and Karmas[b]	Veciana-Nogués[c] (mg/kg)	Spd/Spm
Breast			
0	0.01±0.01b	0.00±0.00b	0.40±0.03c
4	0.01±0.01b	0.00±0.00b	0.45±0.02c
10	0.01±0.01b	0.00±0.00b	0.55±0.04b
15	2.40±0.60a	52.4±0.70a	0.73±0.03a
Thigh			
0	0.06±0.01b	0.70±0.30b	0.45±0.02c
4	0.04±0.01b	2.60±0.20b	0.56±0.05b
10	0.03±0.01b	0.60±0.10b	0.58±0.02b
15	0.75±0.13a	11.8±0.25a	0.70±0.03a

[a] Mean values±standard deviation ($n=4$) with the same letter in the same column do not differ significantly ($P \leqslant 0.05$, Tukey test).
[b] (PUT+CAD+HIM)/(1+SPM+SPD). 0–1=fresh product, 1–10=early stage of deterioration, >10=advanced stage of deterioration.
[c] PUT+CAD+HIM+TYM. Values >50 indicate advanced stage of deterioration.

Table 3
Linear regression between storage time, amine levels and physicochemical parameters for chicken breast and thigh

Significance level	Correlation coefficient (*r*)	
	Breast	Thigh
$P \leqslant 0.001$	PUT—CAD (0.9999)	
	PUT—HIM (0.9999)	
	PUT—TYM (0.9999)	
	CAD—HIM (0.9999)	
	CAD—TYM (0.9999)	
	HIM—TYM (0.9999)	
$P \leqslant 0.005$	PUT—Total amine (0.9969)	
	CAD—Total amine (0.9965)	
	HIM—Total amine (0.9964)	
	TYM—Total amine (0.9962)	
$P < 0.01$		CAD—HIM (0.9940)
$P < 0.05$	PUT—TVB (0.9806)	PUT—CAD (0.9768)
	CAD—TVB (0.9814)	PUT—HIM (0.9899)
	HIM—TVB (0.9816)	SPM—TVB (−0.9889)
	TYM—TVB (0.9816)	SPM—Storage time (−0.9707)
	Total—TVB (0.9624)	Storage time—TVB (0.9684)
	SPM—Storage time (−0.9734)	

according to criteria established by these authors. However, at that time the meat would be unacceptable, organoleptically, for consumption. Therefore, the CQI is not adequate for evaluating the quality of chicken meat, since it could only detect late stages of deterioration.

The use of the biogenic amines index (BAI) proposed by Veciana-Nogués et al. (1997) provided results similar to those obtained with the CQI. Up to the 10th day of storage, the values were below 50 mg/kg, which means a product suitable for consumption. At the 15th day of storage the values were higher than 50 mg/kg for breast muscle, i.e. inappropriate for consumption. Therefore, this index would also be useful only in the detection of late stages of deterioration of white muscle.

Among amines investigated, spermine and spermidine were the ones with lower variability (lower coefficients of variation). The levels of spermine were the only ones to correlate significantly with storage time for both types of tissue, showing a linear decrease with time ($r \geqslant 0.9707$). The levels of spermidine remained constant during storage. The ratio between spermidine and spermine (Spd/Spm) for breast and thigh resulted in values which increased gradually with storage time (correlation coefficients, $P \leqslant 0.05$, of 0.9730 and 0.9551, respectively). Therefore, this ratio is proposed as a quality index for chicken meat. The limits suggested are: values below 0.50 would indicate a fresh product, between 0.50 and 0.70, a product for immediate consumption, and above 0.70, a product in an advanced stage of deterioration. However, more studies should be carried out to corroborate the limits proposed for the different variables that affect chicken meat quality.

The advantage of using this proposed index is that it depends only on the levels of polyamines which are affected by microbial growth, but independent of the type of flora (Hernández-Jover et al., 1996). The chemical quality index (CQI) and the biogenic amine index (BAI), proposed by Mietz and Karmas (1977) and by Veciana-Nogués et al. (1997), respectively, rely on biogenic amine levels. It is well known that different microorganisms have the ability to produce different amounts of specific types of biogenic amines (Shalaby, 1996).

3.5. Bioactive amines in chicken based meat products

The types and levels of amines detected in chicken-based meat products are shown in Table 5. Among the 10 amines investigated, agmatine and 2-phenylethylamine

Table 5
Bioactive amines in chicken based frankfurter, mortadella, fresh sausage, meatball, hamburger and nuggets

Product	Bioactive amines (mg/kg)[a]								
	PUT	CAD	SPD	SPM	HIM	TYM	TRM	SRT	Total
Frankfurter									
% Positive	90	70	100	100	60	10	0	70	
Mean	0.6	0.4	15.8	10.8	0.9	0.1	0.0	0.3	28.9
Range	nd–1.4	nd–1.5	11.9–26.6	6.0–17.1	nd–1.2	nd–1.4	nd	nd–0.9	13.9–47.7
Mortadella									
% Positive	60	70	100	100	40	20	0	30	
Mean	2.6	0.6	10.8	10.1	1.5	0.2	0.0	0.3	26.1
Range	nd–19.2	nd–5.4	4.9–24.3	6.4–15.9	nd–7.2	nd–1.4	nd	nd–0.8	13.3–42.0
Sausage									
% Positive	100	50	100	100	20	50	50	50	
Mean	15.1	8.0	6.6	10.3	13.5	7.4	5.7	1.0	67.6
Range	0.8–82.0	nd–66.8	3.4–11.1	6.0–14.6	nd–46.6	nd–33.6	nd–20.8	nd–6.7	18.1–160.0
Meatball									
% Positive	90	80	100	100	0	40	0	40	
Mean	0.6	0.2	10.1	8.2	0.0	0.3	0.0	0.3	19.8
Range	nd–1.6	nd–1.0	3.5–18.6	5.1–11.0	nd	nd–1.4	nd	nd–1.3	9.0–28.6
Hamburger									
% Positive	90	60	100	100	60	40	0	80	
Mean	0.6	0.7	12.6	9.2	0.2	0.5	0.0	0.8	24.7
Range	nd–1.9	nd–4.1	4.2–24.4	4.5–15.6	nd–0.8	nd–2.7	nd	nd–2.2	9.3–38.2
Nugget									
% Positive	0	0	100	100	0	0	0	0	
Mean	0.0	0.0	5.7	8.0	0.0	0.0	0.0	0.0	14.0
Range	nd	nd	2.9–8.1	5.4–12.8	nd	nd	nd	nd	9.2–20.2

[a] Mean values ($n = 10$) were calculated using zero for nd values (PUT, CAD, SPD, SPM, HIM and SRT <0.80; TYM and TRM <1.40 mg/kg).

were not detected in any sample and tryptamine was only detected in sausage. Similar results have been found by Hernández-Jover et al. (1997a) for Spanish meat products.

Spermine and spermidine were detected in 100% of the samples analyzed. This is in agreement with the fact that these amines are inherent to any tissue (Halász et al., 1994; Hernández-Jover et al., 1997a). The biogenic amines putrescine, cadaverine, histamine and tyramine were detected sporadically in some of the samples analyzed. As pointed out, these amines were not detected in fresh meat.

The profile of amines in nuggets was the most similar to the profile observed for fresh chicken meat. The other products showed different amine profiles. Such a difference could be associated with the amount of meat used, types of ingredients added, quality of the raw material and also to hygienic conditions during manufacturing (Hernández-Jover et al., 1997a). Frankfurter and mortadella, as well as meatball and hamburger, presented similar amine profiles. This is probably due to the fact that these products are made with the same ingredients and follow similar production steps. The samples of fresh sausages analyzed showed a prevalence of biogenic amines, which suggests the use of low hygienic quality raw material or ingredients, or the use of inadequate manufacturing practices (Bover-Cid et al., 1999; Hernández-Jover et al., 1996; Vidal-Carou et al., 1990).

The prevalence of spermine over spermidine, as reported previously for chicken meat, was only observed for nugget and sausage. This could indicate that meat is the major ingredient in these products. The change in amine profiles in the other products is probably related to the presence and amounts of other ingredients included in the formulations (Bardócz et al., 1993). In fact, vegetable protein (soybean, wheat) can be added in generous amounts to some of these products. According to Bardócz et al. (1993) and Smith (1981), spermidine is the prevalent amine in vegetables.

Total amine levels were lower in the frozen products (nuggets, meatball and hamburger), followed by mortadella and frankfurter. Sausage contained the highest total amine levels. In contrast to the relative uniformity of spermine and spermidine contents in the products analyzed, the levels of biogenic amines showed wide fluctuations, especially in sausage. Furthermore, the biogenic amines occurred sporadically, except for putrescine in sausage. Serotonin was detected at very low levels (below 1.0 mg/kg) in a few samples. Cadaverine, histamine and tyramine were detected at low levels (below 5.5, 7.2 and 2.7 mg/kg, respectively) in every type of product. However, in sausage the levels were high. Putrescine levels were very low (below 2.0 mg/kg) in every product, except in mortadella ($\leqslant$20.0 mg/kg) and in sausage ($\leqslant$82.0 mg/kg).

The levels of tyramine and histamine in chicken based meat products are too low to elicit direct adverse reactions in normal individuals. However, some sausage samples contained histamine levels of ca. 50 mg/kg and also high levels of amines that can potentiate the toxicity of histamine. Therefore, these samples could cause histamine intoxication in sensitive individuals (Taylor, 1985).

No information was found, in the literature, on the levels of bioactive amines in chicken meat products. This is probably due to the fact that these products are relatively new in the market. However, the levels detected for chicken-based meat products are lower than those reported for pork or beef (Hernández-Jover et al., 1997a; Nakamura et al., 1979; Smith, 1981; Vidal-Carou et al., 1990; Yano et al., 1995).

The moisture content of the products varied from 35 to 39% in sausage, hamburger, frankfurter and meatball, 41% in mortadella and 46% in nugget. The pH of the products ranged from 5.97 in sausage, 6.24 in nugget to 6.40 in mortadella, frankfurter and hamburger, respectively. The sausages had lower pH values and higher amine contents. According to Maijala et al. (1993), the acidic conditions, characteristic of sausages, are favourable to amino acid decarboxylation. It is possible that this activity is associated with microbial metabolism in order to maintain the environment appropriate for its growth.

The biogenic amine indices available in the literature, and the one proposed for chicken meat, were not adequate for the evaluation of the quality of the meat products analyzed. Studies are needed to investigate the role of ingredients in the bioactive amine content of meat products.

4. Conclusions

Spermine and spermidine are natural amines in chicken breast and thigh meat, with prevalence of spermine over spermidine. During storage of chicken meat at 4.0 ± 1.0 °C, there was a linear reduction in spermine levels while spermidine levels remained constant. The presence of putrescine, cadaverine, histamine and tyramine was observed on the 15th day of storage. On the 15th day of storage, breast meat contained higher putrescine, histamine, tyramine and total amine levels than thigh.

The quality index, based on the ratio of spermidine and spermine (Spd/Spm), was adequate for chicken meat. It showed advantages over the index proposed by Mietz and Karmas (1977) and Veciana-Nogués et al. (1997). It takes no account of biogenic amine levels which are susceptible to variation depending on the type of contaminating microbial flora. Furthermore, it allows detection of early stages of deterioration. Based on these results, the use of the index Spd/Spm is proposed during refrigerated storage of chicken meat.

Chicken-based meat products were analyzed for bioactive amines for the first time. Nugget was the only one with an amine profile similar to fresh meat. The polyamines spermine and spermidine were present in every sample. The prevalence of spermine over spermidine was only observed for nugget and sausage. In the other products (mortadella, frankfurters, sausage, meatballs and hamburger), there was a prevalence of spermidine over spermine, suggesting the incorporation of significant amounts of vegetable tissue in the formulation. The presence of biogenic amines varied widely among types and batches of products. Differences in the quality of raw material and hygienic conditions during processing may be responsible for the differences in the biogenic amines in the meat products. Overall, chicken-based meat products had lower biogenic amine levels than beef or pork. Histamine and tyramine levels found are low and probably unable to elicit direct adverse effects.

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