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Research Article

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Relationship between Biogenic Amine Content and Hygienic Quality of Raw Meet in Fresh Fermented Sausage

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Abstract

The effect of the hygienic quality of raw materials due to differences in the storage conditions on biogenic amine production during fermentation and cold storage at 4°C ± 1 was studied. Sample (A) was processed into sausage immediately; sample (B) was kept at 4°C for 48 hours, while sample (C) was kept at 25°C for 6 hours before processing. A great variability was observed in biogenic amines content of samples from different treatments. Biogenic amine production as it existed in treatment B and C at zero time when substantial amount of tyramine, cadaverine, putrescine were produced. At the same time the amount of biogenic amine in treatment (A) was lower than the other batches as indigenous bacteria was inhibited by starter culture and may be due to the treatment (A) had a lower number of *Enterbacteriaceae* and *Pseudomonas* sp. compared to other treatments. The data also showed that tyramine was detected in all batches with maximum in sample (C) and minimum in sample (A). Tyramine, cadaverine, putrescine and histamine were the most biogenic amines commonly associated with sausage production, with the lower levels in sausage produced from more hygienic raw meat. The initial values of total volatile nitrogen in the different three investigated treatments were ranged between 5.51 to 20.10 mg/100 g. During fermentation and cold storage a significant increase in total volatile nitrogen for all treatments occurred with different rates depending on the initial treatments. The sample (C) showed the highest incremental rate compared to other treatments. The data showed that during storage; sample A and B had high numbers of lactic acid bacterial (10^8 CFU/g) and low numbers of *Pseudomonas* and *Enterobacteriaceae* compared to sample C.

Keywords

Critical control point; Hygienic quality; Sausage; Biogenic amine; Total volatile nitrogen

Introduction

Biogenic amines, such as Histamine (HIS) and Tyramine (TYR) have been of concern in relation to potential toxicological effects due to their vasoactive and psychoactive properties. Biogenic amines in food are the result of microbial transformation of amino acids through decarboxylation. The origin of these and other biogenic amines (such as the diamines putrescine and cadaverine) makes them suitable as chemical indicators of the hygienic quality of some foods [1-4].

Formation of biogenic amines in foods is important not only because of the unfavourable effects on flavour, but also as regards health. Biogenic amines affect blood pressure, and excessive quantities in food can trigger migraines, gastric and intestinal problems and allergic responses in sensitive people [5-7]. These substances are especially dangerous in people being treated with monoaminooxidase enzyme inhibitors [6,8].

Activity of present and developed microflora can cause the formation of biogenic amines that are organic bases produced by decarboxylation of amino acids. This requires the availability of amino acids, the presence of microorganisms capable of amino acids decarboxylation and favorable conditions for their growth and development of their decarboxylase activity [9-14].

Food quality and safety are of paramount importance to health and research organizations worldwide. The improvement of food products in relation to quality attributes arises from the requirement of good manufacturing practices and the need for minimizing the risks, while ensuring the desired sensory traits of food products. Biogenic amines have been classically regarded as potentially hazardous microcomponents of food that may cause disorders to consumers, although the toxic doses and the mechanisms of such effects are not well established. Besides the toxicological implications, biogenic amines are of concern in relation to food hygiene [15,16].

Biogenic amine formation in fermented meat products is subject to several technological factors, such as formulation, sausages characteristics and processing parameters [17]. These factors may be influenced through several phenomena associated with aminogenesis, including microbial growth and interaction among microbial communities, acidification, redox potential (anaerobiosis), proteolysis which can, eventually, affect decarboxylase enzyme production and the activity of aminogenic microorganisms. To ensure fast growth of fermentative bacteria manufacturers of fermented meat products often accelerate the initial fermentation by applying higher processing temperatures and relative humidities in comparison with the traditional practices [18].

Several authors highlighted the relationship between hygienic quality of raw material and aliphatic amines accumulation: changes in concentration of Putrescine (PUT) and Cadaverine (CAD) are known to correlate with the microbial spoilage, storage temperature and storage time of meat [19], while Tyramine (TYR) is usually the most common amine found in fermented sausage and dry cured meat products because it is mainly related to the activity of fermentative lactic acid bacteria [20].

The aim of the present work was to study the influence of the hygienic quality of raw materials on biogenic amine accumulation (as Critical Control Point) during fermentation and storage of fresh fermented sausage. Other parameters (pH value, water content and microbial counts) that might provide further information on the product under study were also determined.

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Materials and Methods

The materials used in this research included raw meat, fat (from round cuts of hindquarter of cow carcass), spices and mutton casing obtained from the local market were directly transferred to Food Technology Department, Faculty of Agriculture Suez Canal University.

Mutton casing was prepared by removing it carefully from the slaughtered animal without punctures to avoid contaminate carcass as well as to provide the minimum length requirements. Three essential operations were performed prior to curing: fat was removed as completely as possible the intestinal contents were stripped under a spray of water to keep the exterior clean, then slime were removed. Natural casing was salt cured and packed in barrels with salt. Before using the casings were soaked and well washed with water and were kept wet at all times once the salt was removed prior filling according to the method described by El-Deep [21].

Chemicals

All reagents were analytical grade. Biogenic amine standards were purchased from sigma chemical Co. A11 media used in microbiological analysis (Man, Rogosa and Sharpe (MRS) agar, violet red bile dextrose agar and pseudomonas agar were obtained from Merck (Darmstadt, Germany). Starter cultures (*Lactobacillus plantarum*, *Bifidobacterium lactis Bb-12* and *Bifidobacterium bifidum RBL 71*) were obtained from DVS, Chr Hansen's lab. Denmark.

Sample preparation

The meat and fat used in sausage preparation were minced to a 6 mm particle size in a meat mincer and divided into three portions A, B and C to study the variability of meat quality due to differences in the storage conditions of meat. Sample (A) was processed into sausage immediately after mixing it with the other ingredients and additives, [2% sodium chloride; 6% starch; 13.0 % water (as ice); 0.16% sodium glutamate; 0.5% garlic; 0.50% onion; 0.03% potassium nitrate; 0.01% sodium nitrite and 0.56% spices mixture (red pepper 11.51%; all spices 10.0%; black pepper 45.0%; coriander 12%; nutmeg 2.5%; cumin 15.0% and clove 4.5%)].

Sample (B) was kept at 4°C for 48 hrs before sausage processing while sample (C) was stored for 6 hrs at 25°C before processing. Fermentation of the three samples was carried out by using cultures *L. plantarum* plus *B. lactis* and *B. bifidum*.

Each mixture was homogenized and stuffed into sheep casing according to the method described by El-Deep [21]. After that, sausage samples received a one minute dip in 0.001% sorbic acid solution to prevent the growth of fungal culture on the surface of sausage sample during the fermentation. The sausage was fermented for 72 hr at 20 to 22°C [93 to 98% relative humidity] according to Bover-Cid et al. [22]. The end products were stored at 4°C for 14 days.

Biogenic amines (BAs) contents, total volatile nitrogen (TVN), water content, pH and microbial counts (LAB, Pseudomonas bacteria and Enterobacteriaceae) were determined in duplicates at zero, 12, 24, 48 and 72 hrs during fermentation and during cold storage for 14 days.

Chemical analysis

Moisture contents were determined according to the method described by AOAC [23] method No. 24003. The pH value was measured directly in 3:1 (water: meat) homogenate according to method described by Zaika et al. [24]. Total Volatile Nitrogen (TVN) was determined according to the method described by Pearsons [25]. As the following: about 100 ± 0.5 g was weighed from prepared sample and placed into a blender with 300 ml trichloracetic acid (TCA) (5%) until obtaining uniform slurry. The resulting slurry was centrifuged at 5000 rpm to obtain a clear extract. Exactly 5 ml of the extract was pipetted into a semi micro distillation apparatus (UDK, 130A) and 5 ml of NaOH (2 N) was added. The final steamed distillate was collected in 15ml of standard hydrochloric acid (0.01 N). Rosolic acid was added to the solution as an indicator (1percent rosalic acid in 10 percent v/v ethanol). Titrate to a pale pink end point with sodium hydroxide. The amount of TVBN was expressed as mg/100 g.

(BAs) were determined by the high-performance liquid chromatography (HPLC) according to method described by Chiu-Chu et al. [26] as a following: Finely ground sausage sample (5 g) was transferred to 50-ml centrifuge tubes and homogenised (Ultra Turrax homogenizer, IKA-Werke, Janke, & Kunkel, Staufen, Germany) with 20 ml of 5% TCA solution for about 2 min. The supernatant was collected by centrifugation (10,000 g, 4°C, 10 min) and the residue was extracted again with an equal volume of TCA solution.

Both supernatants were combined and filtered through a filter paper (Whatman No. 4). The filtrate was made up to 50 ml with 5% TCA solution and stored at 0–4°C for high-performance liquid chromatography (Agilent 1100 Series; Agilent, Santa Clara, CA) analysis within a week.

The derivatisation reagent was prepared by transferring 100 mg o-phthalaldehyde (OPA), 1 ml acetonitrile and 130 µl 2-mercaptoethanol to a 10 ml volumetric flask, and then diluting with 0.4 M borate buffer (pH 10.2) to 10 ml. The resulting solution was mixed well, and stored refrigerated and used within 24 hrs.

Pre-column derivatisation with OPA was performed automatically.

A reverse-phase Hypersil ODS C18 (125×4.60 mm, particle size 5 mm) column was used for separation. The column temperature and flow rate were set at 40°C and 1.0 ml/min, respectively. The mobile phase consisted of solvent A (pH 7.2), 7.35 mM sodium acetate solution: triethylamine:tetrahydrofuran (500:0.12:2.5 v/v), and solvent B (pH 7.2), 7.35 mM sodium acetate solution:methanol:acetonitrile (1:2:2 v/v). Fluorescence was monitored at an emission wavelength of 450 nm using an excitation wavelength of 340 nm.

Biogenic amine standards, tyramine hydrochloride, tryptamine hydrochloride, putrescine dihydrochloride, cadaverine dihydrochloride, 2- phenylethylamine hydrochloride, spermidine trihydrocholoride, spermine tetrahydrochloride and histamine dihydrochloride were purchased from Sigma (St. Louis, Missouri, USA). Double-distilled and deionised water was used for dilution and chromatographic separation.

Bacteriological analysis

For bacterial enumeration 10 g of sausage samples without casing were cut into small pieces and homogenized in 90 ml 0.1% peptone water using homogenizer, serial dilutions up to 10^6 were prepared from the original dilution. Lactic Acid Bacteria (LAB) were enumerated on Man, Rogosa and Sharpe (MRS) agar (Merck) incubated at 30°C for 48 hr anaerobically (anaerobic Jar System BBL, Cockeysville

Md.); Enterobacteriaceae on violet red bile dextrose agar (Merck, Darmstadt, Germany) incubated at 30°C for 24 hrs anaerobically and Pseudomonas on pseudomonas agar (Difco Laboratories, Detroit, MI, USA) at 25°C for 48 hr.

Statistical analysis

Statistical analyses were performed using general linear models of SPSS 9.1 package (Tulsa, Oklahoma, USA, 2009). One way analyses of variance together with the least significant difference test were applied to examine the statistical significance of the changes throughout the fermentation and cold storage as well as of the differences between treatments ($p<0.05$) values are reported at the mean (and standard error) of the duplicate analysis.

Results and Discussion

Biogenic amines (BAs) develop and accumulate in food and beverages as a consequence of enzymatic amino acids decarboxylation due to microbial enzymes and – to a lesser extent – to tissue activity [13]. The determination of biogenic amines in fresh and processed food is getting of great interest not only for their potential risk for human health, but also because they could have a role as chemical indicators of unwanted microbial contamination and processing conditions [27].

Quality of raw materials

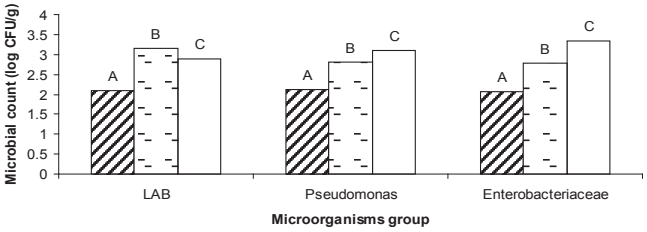
As predicted, the quality of the raw meat materials depended on the treatment. Fresh meat (Treatment A) maintained its quality, the microbial count was 2.08 ± 0.1013 log CFU/g for lactic acid bacteria (LAB), 2.11 ± 1.0021 CFU/g for *Pseudomonas* and 2.06 ± 0.0061 CFU/g for *Enterobacteriaceae*. However, storage under refrigerated conditions (Treatment B) allowed the growth of microorganisms, especially of LAB that rose to 3.15 ± 1.1511 log CFU/g. While *Pseudomanas* and *Enterobacteriaceae* counts were 2.81 ± 0.1131 and 2.78 ± 0.0021 log CFU/g respectively. Similar trend observed during storage meat at 25°C (Treatment C) as shown in Figure 1. The data also indicated that within 6 hrs at 25°C *Pseudomonas* and *Enterobacteriaceae* were higher than those of sample A and B (Figure 1) [28] reported that the low initial microbial counts suggests good hygienic manufacturing conditions.

Many authors support the belief that the microorganisms present in traditional sausages are derived from the raw materials or from the environment of manufacturing [3,29,30]. The quality of raw materials is the most critical factor affecting amine formation in fermented sausages. Indeed, high amounts of diamines, especially cadaverine, have been related to the low quality of raw materials with relatively high counts of enterobacteria [3]. In this respect, the selection of meat raw materials and the control of thawing, storage time and temperature are critical in avoiding or reducing biogenic amine accumulation [31]. The production of biogenic amines requires the presence of amino aciddecarboxylating microorganisms, the availability of the precursor amino acids and favourable conditions (such as slightly acid pH and anaerobiosis) for the synthesis and activity of microbial enzymes. Although these requirements usually converge during sausage fermentation, a wide quantitative range of several biogenic amines can be found in fermented sausages. Such variability has been attributed to the different bacteria present in raw materials and processing environments as well as to differences in formulation, the use or not of different types of starter culture, and other technological factors associated with sausage manufacture [32,33].

Microbiological changes throughout Fermentation and cold storage

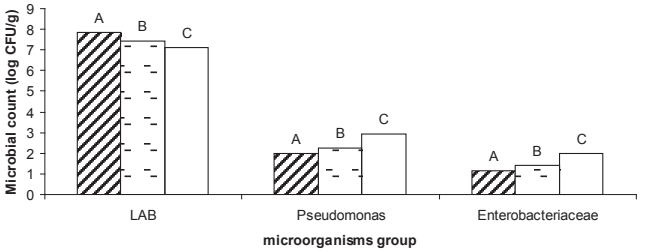
Microbial changes during fermentation are shown in Figure 2. Initial counts of *Enterobacteriaceae* (3.14 CFU/g) decreased during fermentation by more than logarithmic unit, ending up at less than 2 log CFU/g in all treatments. This is typical decrease due to the environmental conditions which make Gram–Negative bacterial growth difficult [34]. In contrast, LAB increased during the fermentation process and reaching to a maximum of 10^7 CFU/g after 14 day of cold storage (Figures 2 and 3).

A little reduction was observed in *Pseudomonas* bacterial count during fermentation and cold storage especially in sample B and C, the data showed that during storage; sample A and B had high numbers of lactic acid bacterial (10^8 log CFU/g) and low numbers of *Pseudomonas*



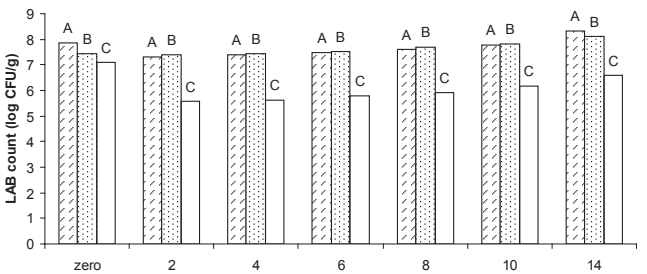
A=Fresh minced meat; B=Stored minced meat at 4°C for 48 hrs; C=Stored minced meat at 25°C for 6 hrs

Figure 1: Microbial counts of meat mixture at zero time.



A=Fresh meat; B=Stored meat at 4°C for 48 hrs; C=Stored meat at 25°C for 6 hrs

Figure 2: Microbial counts of sausage samples after 72 hrs of fermentation.



A=Fresh minced meat; B=Stored minced meat at 4°C for 48 hrs; C=Stored minced meat at 25°C for 6 hrs

Figure 3: Lactic acid bacterial counts of sausage samples during cold storage at 4°C ± 1.

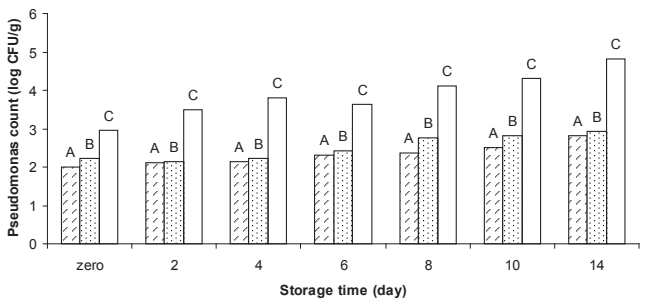
and *Enterobacteriaceae* compared to sample C (Figures 2-5). These data are in agreement with those reported by Latorre-Moratalla et al. [35] who reported that LAB and *Enterococci* increased in number through the manufacturing process, while *Enterobacteriaceae* counts decreased. Also Bover-Cid et al. [3] mentioned that a reduction of Enterobacteria development during sausage fermentation.

Moisture content and pH value

pH-values: The results of the main physico–chemical parameters (pH-values and moisture content) associated with the fermentation of sausage are shown in Table 1 and Figure 6. pH values showed a strong decrease during the first few days, coinciding with the growth of LAB, reaching values between 6.38 and 5.32 depending on the quality of meat. These results are good agreement with those reported by Latorre-Moratalla et al. [35] who reported that the inoculation of the starter resulted in a much stronger acidification during the first week of production.

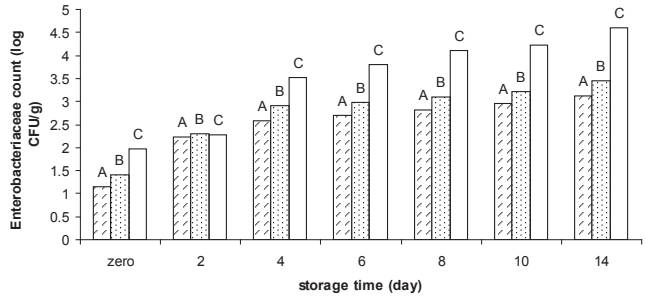
On the other hand, an increase in pH was observed at the end of the storage period (Figure 7) particularly in treatment (C) which may be elucidated to the production of volatile base compounds by bacterial activity [36].

Moisture content: There were little differences in the moisture content among the three treatments studied. The data showed that, the moisture content was decreased for all samples during fermentation and cold storage. After 72 hrs of fermentation the total loss in moisture content was 5.37, 6.64 and 6.96% for treatment A, B and C respectively, while it was 12.39, 14.13 and 14.48% for the same treatment respectively after 14 days of storage (Table 2).



A=Fresh meat; B=Stored meat at 4°C for 48 hrs; C=Stored meat at 25°C for 6 hrs

Figure 4: *Pseudomonas* bacterial counts of sausage samples during cold storage at 4°C ± 1.



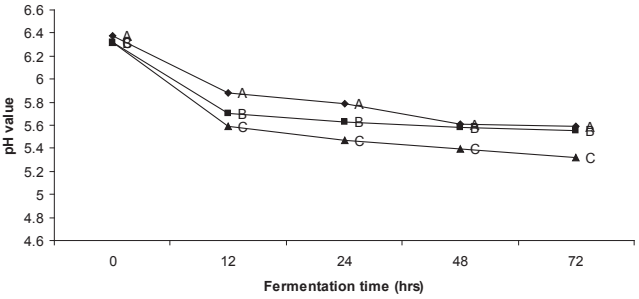
A=Fresh meat; B=Stored meat at 4°C for 48 hrs; C=Stored meat at 25°C for 6 hrs

Figure 5: *Enterobacteriaceae* bacterial counts of sausage samples during cold storage at 4°C ± 1.

Table 1: Changes in mean values ± standard error of moisture content during fermentation of sausage prepared from raw meat kept at different storage conditions.

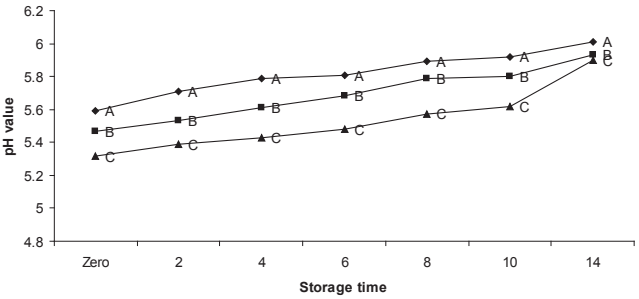
Time (hr) / Treatments	0	12	24	48	72
A	69.12^{a} ± 0.07	68.39^{a} ± 0.01	67.61^{a} ± 0.03	66.81^{a} ± 0.01	65.41^{a} ± 0.10
B	67.32^{b} ± 0.05	66.91^{b} ± 0.01	66.21^{b} ± 0.01	65.71^{b} ± 0.01	64.65^{b} ± 0.03
C	67.19^{b} ± 0.03	66.85^{b} ± 0.01	66.10^{b} ± 0.01	65.25^{b} ± 0.02	64.31^{b} ± 0.01

A= Sausage sample prepared from fresh meat
B= Sausage sample prepared from meat after storage at 4°C for 48 hrs
C= Sausage sample prepared from meat after storage at 25°C for 6 hrs
Mean in the same column with different superscripts are significantly different at p< 0.05



A=Fresh meat; B=Stored meat at 4°C for 48 hrs; C=Stored meat at 25°C for 6 hrs

Figure 6: Changes in pH value of sausage samples during fermentation.



A=Fresh meat; B=Stored meat at 4°C for 48 hrs; C=Stored meat at 25°C for 6 hrs

Figure 7: Changes in pH value of sausage samples during cold storage at 4°C ± 1.

Total volatile nitrogen (TVN)

The mean values of TVN are shown in Figure 8. From the data it could be noticed that the initial value of TVN in the different three investigated treatments were ranged between 8.51 to 12.42 mg/100g. During fermentation and cold storage a significant increase in TVN for all treatments occurred with different rates depending on the initial treatments. The sample (C) showed the highest incremental rate compared to other treatments. The progressive increase in TVN during cold storage may be attributed to the breakdown of nitrogenous substances as a result of microbial activity and any autolytic enzymes found naturally in tissue as reported by Abou-Taleb and Ibrahim [37].

Low levels of TVN in treatments A and B were due to either a reduced bacterial population [38] showed that low levels of TVN in

Table 2: Changes in mean values ± standard error of moisture content during cold storage at 4°C of fermented sausage prepared from raw meat kept at different storage conditions.

Time (hr) / Treatments	0	2	4	6	8	10	12	14
A	65.41^{a} ± 0.10	64.72^{a} ± 0.01	64.11^{a} ± 0.01	63.21^{a} ± 0.01	62.35^{a} ± 0.01	61.12^{a} ± 0.01	61.32^{a} ± 0.06	60.55^{a} ± 0.04
B	64.65^{b} ± 0.02	64.01^{b} ± 0.01	63.32^{b} ± 0.02	62.51^{a} ± 0.01	61.91^{a} ± 0.02	61.08^{a} ± 0.01	60.77^{a} ± 0.10	59.35^{a} ± 0.02
C	64.31^{b} ± 0.01	63.92^{b} ± 0.01	63.16^{b} ± 0.01	62.16^{b} ± 0.01	61.65^{a} ± 0.02	60.91^{b} ± 0.01	60.01^{a} ± 0.06	59.11^{a} ± 0.10

A= Sausage sample prepared from fresh meat
B= Sausage sample prepared from meat after storage at 4°C for 48 hrs
C= Sausage sample prepared from meat after storage at 25°C for 6 hrs
Mean in the same column with different superscripts are significantly different at p< 0.05

Table 3: Type and amount of biogenic amines (mg/kg) detected in sausage sample prepared from meat kept at different storage conditions.

		Treatments		
Amine	**Time**	**A**	**B**	**C**
Tyramine	zero time	6.52^{a} ± 0.0021	8.11^{a} ± 0.1132	21.61^{b} ± 0.0053
	after fermentation	11.13^{a} ± 0.0100	14.85^{b} ± 0.0311	18.02^{c} ± 0.0120
	after 14 days of storage	17.22^{a} ± 0.0036	19.01^{a} ± 0.0201	22.16^{b} ± 0.0314
Cadaverine	zero time	0.81^{a} ± 0.0016	1.22^{a} ± 0.0216	2.53^{b} ± 0.0235
	after fermentation	1.31^{a} ± 0.0024	1.56^{a} ± 0.0113	3.14^{b} ± 0.0126
	after 14 days of storage	1.85^{a} ± 0.0111	2.04^{a} ± 0.0031	4.36^{b} ± 0.0312
Putrescine	zero time	0.47^{a} ± 0.0080	1.02^{b} ± 0.0102	1.95^{c} ± 0.0035
	after fermentation	0.89^{a} ± 0.0021	1.49^{b} ± 0.0331	2.71^{c} ± 0.0001
	after 14 days of storage	1.20^{a} ± 0.0016	1.91^{b} ± 0.0251	3.79^{c} ± 0.0211
Histamine	zero time	0.31^{a} ± 0.0017	0.93^{a} ± 0.0025	1.32^{b} ± 0.0007
	after fermentation	0.56^{a} ± 0.0008	1.72^{b} ± 0.0103	1.96^{b} ± 0.1100
	after 14 days of storage	0.92^{a} ± 0.0001	1.82^{b} ± 0.0110	2.19^{c} ± 0.1030
Tryptamine	zero time	0.45^{a} ± 0.0031	0.82^{a} ± 0.0018	1.52^{b} ± 0.0031
	after fermentation	0.82^{a} ± 0.0120	1.35^{b} ± 0.0111	1.83^{b} ± 0.0012
	after 14 days of storage	1.03^{a} ± 0.0113	1.76^{b} ± 0.0019	2.66^{c} ± 0.0015

A= Sausage sample prepared from fresh meat.

B= Sausage sample prepared from meat after storage at 4°C for 48 hrs.

C= Sausage sample prepared from meat after storage at 25°C for 6 hrs.

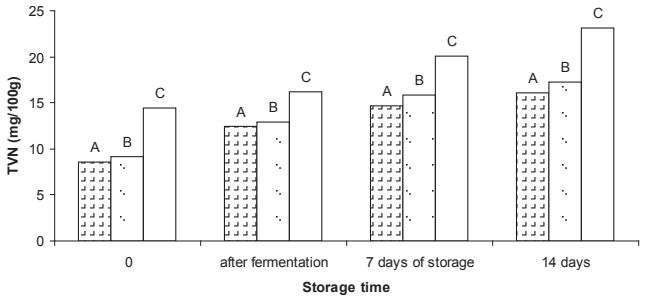
Mean in the same column with different superscripts are significantly different at p<0.05.

treated samples were due to either a reduced bacterial population or decreased capacity of bacteria for oxidative de-amination of non-protein nitrogen compounds or both of them. In raw-cured sausages, microorganisms of the Enterobacteriaceae family have been revealed to have a high capacity for producing biogenic amines, particularly putrescine and cadaverine [15].

Biogenic amines (BAs): The (BAs) values in samples are demonstrated in Table 3. A great variability was observed in the BAs content of samples from different treatments. As previous reported, TYR, CAD and PUT were the main amines found in meat [39-41]. In this work, TYR, CAD and PUT were the most abundant BAs in samples. The concentrations of TYR, CAD, PUT, HIS and TRY in the batch A at zero time were 6.52,0.81,0.47,0.31 and 0.45 mg/kg respectively while were 17.22, 1.85, 1.20, 0.92 and 1.03 mg/kg at the end of storage time, which were lower than the other two batches (Table 3) however the amount of HIS and TRY in all batches was lower than the others at 14 days of cold storage at 4°C ± 1 which probably starter culture can produce TRY oxidase reducing its accumulation similar results reported by Lu et al. [41].

Usually, indigenous *Enterobacteriaceae, Enterococcus, Pseudomonas* sp., *Micrococcus* sp. and *Lactobacillus*, are the producers of BAs [13].

So indigenous bacteria are responsible for BA production as it existed in treatment B and C at zero time when substantial amount of TYR, CAD and PUT were produced. At the same time the amount



A=Fresh minced meat; B=Stored minced meat at 4°C for 48 hrs; C=Stored minced meat at 25°C for 6 hrs

Figure 8: Changes in TVN content (mg/100g) of raw meat and sausage samples during fermentation and cold storage.

of BAs content in treatment (A) is lower than the other batches as indigenous bacteria was inhibited by starter culture and may be due to the treatment (A) had a lower number of *Enterbacteriaceae* and *Pseudomonas* sp. compared to other treatments.

Tyramine is the most common biogenic amine, capable of causing food poisoning symptoms, in fermented sausages [14]. Moreover, sausage reached lower pH values and higher free amino acid contents during fermentation. Both factors are known to favour biogenic amine production by microorganisms, since bacterial decarboxylase enzymes are induced by the presence of precursor amino acids at mild acid pH [34]. Nevertheless, the extremely low levels of biogenic amines in fuet sausages were surprising in comparison to the variable but higher levels (140 mg/kg on average with a relative standard deviation of 73%) usually reported for similar products [42].

In previous work [3], the aminogenesis in spontaneously fermented fuet (A kind of sausage) was much more important, even when the hygienic quality of raw materials was optimal in both cases. Who also mentioned that the temperature of fermentation was considerably higher (17°C) than in the present study (12°C), and this may suggest that, besides the hygiene of raw materials and formulation, temperature might be a technologically important parameter to control the aminogenic activity of spontaneous fermenting microorganisms.

On the other hand the data showed that the highest level of histamine was produced in sample (C). These phenomenon may be due to the temperature of 25°C was optimal for both microbial growth and histamine formation. These data are agreement with those reported by Bover-Cid et al. [3] who mentioned that at 25°C the strain produced the highest histamine level at 48 hrs of incubation in the late stationary phase. Who also reported that the histamine level produced at 37°C was much lower than at 25°C. During incubation at 15°C, the rate of microbial growth was significantly less than at 25°C and 37°C.

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