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Assessment of biogenic amines content in fresh cattle livers during chilling storage and pan-roasting

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Abstract

The levels of biogenic amines in addition to microbial and chemical parameters of fresh cattle livers were determined. The influence of chilling at 0 ± 1 °C, 4 ± 1 °C and pan-roasting on beef liver quality and safety was investigated. The obtained results revealed that the mean values of pH, Enterobacteriaceae count, total volatile nitrogen (TVN) and thiobarbituric acid (TBA) were 6.86 ± 0.03, 3.08 ± 0.8 log10 CFU /g, 16.09 ± 0.08 mg N/100 g and 0.18 ± 0.02 mg malonaldehyde /kg, respectively. Liver samples were contained high levels of spermidine (up to 5.1±0.99 mg/kg), and low levels of histamine and cadaverine. Therefore, beef liver constitutes one of the richest dietary sources of spermidine. During refrigerated storage, there were significant bacterial and chemical changes: the pH decreased, TVN increased, and the levels of most of the naturally occurring amines increased at rates which were faster at higher storage temperature. During the pan-roasting at 180°C for 5 min, the levels of the biogenic amines increased significantly. A shelf life of up to 6 and 4 days during storage at 0 ± 1 °C and 4± 1 °C, respectively, is recommended.

Keywords: Biogenic amines, pH, cattle liver, Total Volatile Nitrogen, and thiobarbituric acid.

Introduction

Varieties of meat offal produced in Egypt are mainly used for domestic consumption, and commercial distribution is normally made under refrigeration conditions. Offal are usually of poor hygienic quality, exposed to rapid spoilage and with a high incidence of pathogenic organisms[9)].

Liver of freshly slaughtered cattle deteriorates 4-6 days after slaughter, regardless of storage conditions. The major causes of spoilage are microbial growth and autolytic activities. Due to the high protein content and to the proteolytic activity of contaminating microorganisms, liver is also susceptible to biogenic amines formation and accumulation. Therefore, reliable means to evaluate the quality and to maintain the nutritional value of liver as well as warrant its safety are needed[9,11,13)]. Biogenic amines (BA) are basic nitrogenous compound formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones. They are generated in course of microbial, vegetable, and animal metabolisms[23)]. Biogenic amines are involved in natural biological processes such as synaptic transmission, blood pressure

control, allergic response and cellular growth control. Nonetheless, biogenic amines may be hazardous to human health if their levels reaches a critical threshold. Biogenic amines are potential precursors for the formation of carcinogenic N-nitroso compounds[18,19]. In Egypt, little information is available on the levels of biogenic amines in beef liver and on the changes which occur during refrigerated storage and cooking. Furthermore, no information was found regarding the use of biogenic amine indices to evaluate the quality of liver. Therefore, the objective of this study was to investigate the levels of biogenic amines in beef liver immediately after slaughter as well as during chilling storage and pan-roasting.

Material and Methods

Samples:

Ten cattle liver samples were randomly collected from apparent healthy slaughtered cattle in slaughterhouse located in Zagazig city, Sharkia governorate, Egypt, directly after post-mortem examination and approved for human consumption under the supervision of veterinary inspectors. The animals were 36 months old or less (Hybrid cattle from local and foreign breeds). The liver samples (1500 g. of each) were packed individually in clean polyethylene bags, well identified then, they were transported in insulated, iced containers to meat hygiene laboratory, Faculty of Veterinary Medicine, Zagazig University under hygienic condition where they were immediately treated and analyzed.

The organic and aqueous solvents were filtered through filter paper Whatman No. 1. Standards of six biogenic amines included Spermidine (SPD), putrescine (PUT), tyramine (TYM), histamine (HIM), cadaverine (CAD) and β-phenyl ethylamine (PHM) were from Sigma Chemical Co. (St. Louis, MO, USA).

Methods:

Effect of chilling storage temperature on liver quality and formation of biogenic amines:

Each one of the ten liver samples was divided into ten parts of about 150 g each according to Krausova, *et al.*,[17]. Five parts was analyzed immediately (zero time) and the others were placed in polyethylene bags and stored under two different refrigeration temperatures for up to eight days. The chosen temperatures were 0±1°C (recommended storage temperature for meat) and 4±1°C (temperature of household refrigerators). At 2-days intervals samples were taken and analyzed for pH, *Enterobacteriacae* count, TVN, TBA and biogenic amines.

Effect of heat treatment on biogenic amines levels:

The effect of heat treatment was investigated using five samples of fresh livers. The influence of pan-roasting, which is the most commonly used cooking procedure for cattle liver in Egypt, was evaluated. The samples were cut into 1-cm thickness slices, which were pan-roasted without oil in a preheated Teflon-coated pan, at 180 °C for 5 min each side. Before and after pan-roasting, the samples were analyzed for moisture and existed biogenic amines contents.

Bacterial and chemical analysis:

The samples were analyzed for determination of pH, *Enterobacteriaceae* count, TVN, TBA, and biogenic amines. Prior to analysis, the samples were ground in a food processor and homogenized thoroughly. The measurements of pH were carried out using a digital pH meter. *Enterobacteriacae* count was carried out according to ICMSF[14]. TVN was estimated according to FAO[8] and TBA with the method recommended by Kirk and Sawyers[15].

Determination of biogenic amines:

Five BAs included Spermidine (SPD), putrescine (PUT), tyramine (TYM), histamine (HIM) and cadaverine (CAD) were extracted and determined in all examined samples using HPLC (Agilent 1100 HPLC system, Agilent Technologies, Waldbronn, Germany, model G 1311A) equipped with UV detector (Model G 1314A) set at 254nm wavelength, auto sampler (model G 1329A VP-ODS) and Shimpack (150× 4.6 mm) column

(Shimadzu, Kyoto, Japan) was used for biogenic amines separation according to Eerola et al. [3].

Statistical analysis:

Data were analyzed using Pearson correlation test, one way ANOVA at a significance of $P<0.05$ 22).

Results and discussion

Effect of chilling storage on the Bacterial, chemical characteristics of cattle liver:

The pH of fresh cattle livers ranged from 6.26 to 6.91 with a mean of 6.76 ±0.03, total *Enterobacteriacae* count ranged from 2.2 to 3.74 with a mean value of 3.08 ±0.8 log10 CFU/g, TVN from 10.56 to 24.82 mg N/100 g with a mean value of 16.09±0.08 mg N/100 g and TBA from 0.12 to 0.72 mean of 0.18 ± 0.02 mg malonaldehyde/kg at zero time. (Fig. 1 and 2). Hernandez-Herrero, *et al.*[14] reported nearly the same pH values for fresh cattle liver 6.46±0.15 from a commercial slaughterhouse in Spain. pH mean values in the examined samples within the safe permissible limit stipulated by EOS[5] for pH in edible offal (6 - 6.8). The difference on pH values may be associated with inherent and exogenous factors including genetics, age, sex, diet, pre/post-slaughter handling and microbial flora[20].

The obtained results of total *Enterobacteriaceae* in the examined beef liver samples were according to EC[6] which stated that the maximum permissible limit for *Enterobacteriaceae* count in meat and edible offal should not exceed 3.17×102 cfu/g.

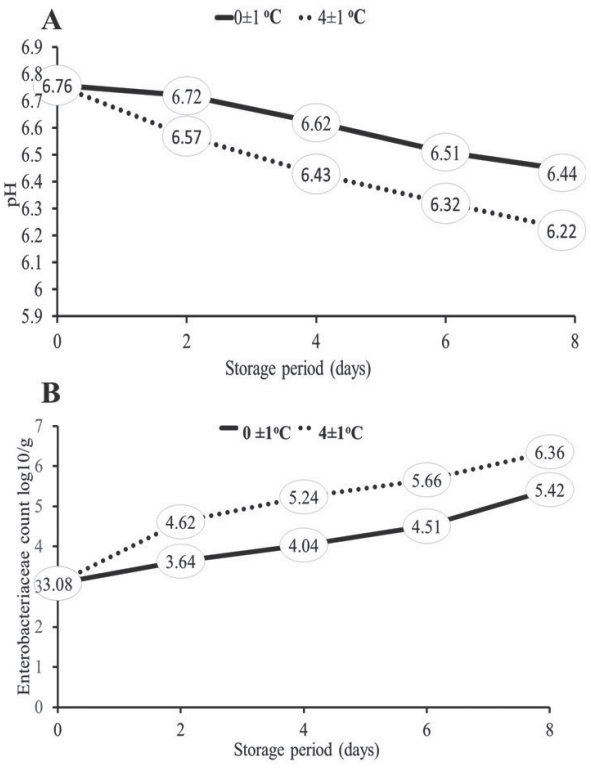


Figure (1) (A). Effect of storage temperature on pH of cattle liver at 0±1°C and 4±1°C. (B) Effect of storage temperature on *Enterobacteriaceae* count log10 cfu/g. of cattle liver.

All the examined samples of fresh beef liver were accepted according to the safe permissible limit recommended by EOS [5] for TVN in edible offal (should not exceed 30 mg/100 gm). TVN value was more useful for assessing the degree of meat deterioration than for evaluating the changes occurring during the first storage stages [4], meanwhile, TBA mean values (mg malonaldehyde / kg of sample) in the examined fresh beef liver were accepted based on their TBA content according to EOS [5] which stated that the maximum permissible limit for TBA in edible offal should not exceed 0.9 mg malonaldehyde/kg. TBA is a good indicator for the quality of meat. TBA value is a widely used indicator for the assessment of degree of lipid oxidation[21]. During refrigerated storage of the liver samples, the pH values decreased (Fig. 1.A)

whereas the *Enterobacteriaceae* count (Fig.1.B), TVN figure (Fig.2.A) and TBA figure (Fig.2.B), increased significantly. The decrease on pH values fitted linear regression and were affected by storage temperature with higher rates observed at 4 ± 1°C compared to 0 ±1°C. Similar decreases on pH values were observed in beef liver stored under refrigeration 0-3 °C[13]. The decrease on pH values was associated with bacterial growth, mainly *Pseudomonas, Enterobacteriacae* and lactic acid bacteria.

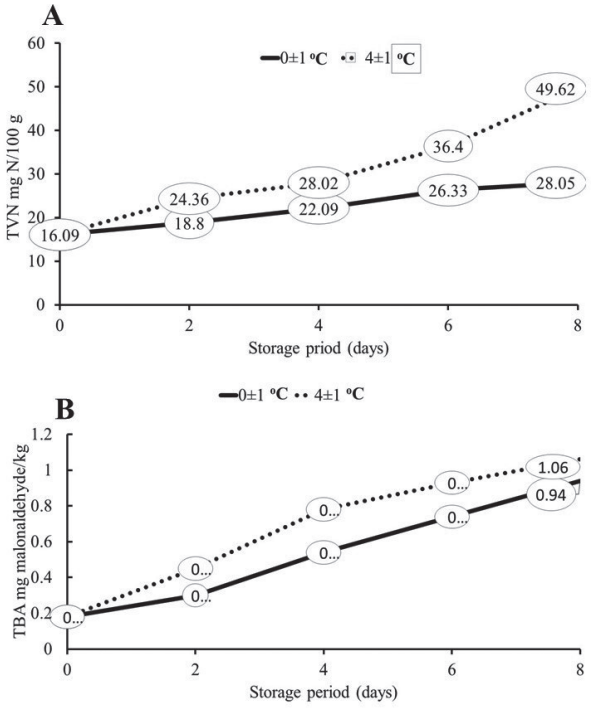


Figure (2) (A) Changes on TVN mg N/100 g value during storage of Cattle liver at 0±1°C and 4±1 °C. (B) Changes on TBA mg malonaldehyde / kg value during storage of fresh cattle liver at 0±1 °Cand 4±1°C.

The high glucose contents and high pH prevalent in liver favor the growth of bacteria with the accompanying production of acid and reduction on pH values [13]. *Enterobacteriaceae* count, TVN and TBA increased with storage time at a faster at 4 ±1°C compared to 0±1°C until reached to unacceptable limits at the 8th storage day at each of 0.0±1.0 °C and 4.0±1°C. However, it is known that when glucose is exhausted, spoilage micro-organisms use amino acids as substrate with the release of volatile nitrogenous and sulphur compounds [13].

Changes in biogenic amines of cattle liver during chilling:

The presence of spermidine was expected as it is the predominant amine in animal tissues, and considered as essential factors for cell proliferation and differentiation and other relevant functions of normal cells [20].

The presence of low levels of putrescine was also expected as it is an obligate intermediate in the formation of the polyamines. The presence of spermidine and the diamine putrescine was also reported in beef liver[24] and in liver from other animals,[1.2,24].The occurrence of histamine and tyramine in fresh liver was described for the first time by Villanueva-Valero *et al*.[24].

However, tyramine was reported in fresh pork and hare liver[20]. The levels of spermidine ranged from 4.38 to 8.24 mg/kg with a mean value 5.1±0.99 mg/kg and the levels of putrescine varied from 1.45 to 5.05 mg/kg with a mean 1.54± 0. 73 mg/kg (Table 1). These levels are low compared to those reported by Krausova, *et al*.[17] for pig liver; however putrescine levels have been reported to vary widely among liver samples of many species. Krausova, *et al*.[16] investigating polyamines in beef liver from the Czech Republic, found higher mean levels of spermidine 121.5 mg/kg for bulls and cows. The differences on polyamines levels could reflect peculiarities in the metabolism of ruminant compared to non-ruminant species.

Table (1): Levels of biogenic amines in beef liver during chilling storage at 0±1 ℃ and 4 ±1 ℃ for eight days.

Storage temp.	Time (days)	Biogenic amines (mg/kg) ± standard deviation					
		Spermidine	Putrescine	Tyramine	Histamine	Cadaverine	Total
at 0±1°C	0	5.1±0.99^{b}	1.54±0. 73^{a}	4.84±2.47^{b}	0.16±0.12^{a}	N.D.	11.64±4.31^{c}
	2	4.98±1.02^{b}	1.46± 0.92^{a}	4.63±2.89^{b}	0.07±0.05^{a}	N.D.	11.14±4.88^{c}
	4	5.42±1.45^{b}	1.63±1.23^{a}	5.98±3.02^{b}	0.09±0.6^{a}	0.05±0.03^{b}	13.17±6.33b^{c}
	6	7.88±2.63^{a}	3.62±1.24^{a}	10.42±3.08^{a}	0.26±0.19^{a}	0.12±0.09^{b}	22.3±7.30^{b}
	8	6.09±1.43^{ab}	3.61± 2.45^{a}	12.53±3.25^{a}	4.86±2.8^{a}	1.88±0.9^{a}	28.97±10.83^{a}
4 ±1°C	0	5.1±0.99^{a}	1.54±0.07^{b}	4.84±2.47^{b}	0.16±0.12^{b}	N.D.	11.64±4.31^{c}
	2	5.76±1.24 ^{a}	2.23±0.85^{b}	5.36±1.96^{b}	0.24±0.08^{b}	0.04±0.01^{c}	13.63±4.14^{bc}
	4	6.41±1.12^{a}	3.21±0.89^{b}	6.42±1.86^{b}	0.65±0.40^{b}	0.08±0.06^{c}	16.77±4.33^{bc}
	6	8.24±1.64^{b}	6.36±2.11^{b}	9.96±2.78^{a}	16.44±4.45^{b}	8.74±2.36^{b}	49.74±13.34^{b}
	8	10.36±2.09^{a}	26.08±9.44^{a}	12.32±3.14^{a}	136.68±64.6^{a}	42.14±16.11^{a}	227.58±95.38^{a}

Different superscripts (a-c) during storage time for each amine at each storage temperature are significantly different ($p<0.05$).
N.D: not detected (below the detection limit)

Anyway, fresh liver is a very rich source of polyamines; in fact it is one of the foods with the highest content of polyamines. With respect to the biogenic amines tyramine and histamine, they were found at low levels in the fresh liver with a mean values of 4.84±2.47 and 0.18±0.13 mg/kg, respectively. The contribution of these amines to total levels was very small.

A significant positive correlation between age and histamine levels in roe deer liver [20] based on these results, tyramine and histamine can be present in fresh liver at low amounts; and the levels found are not capable of causing adverse effects to human health [12]. The presence of these amines at high levels in liver could be associated with spoilage and microbial growth; which was also the opinion of [17], who detected tyramine, histamine, cadaverine and phenyl ethylamine in pork liver. According to these researchers, the contents of biogenic amines in livers can be considered a marker of the level of bacterial contamination ≥6 log10 CFU/g.The changes on the levels of amines during refrigerated storage are indicated in (Table 1). Total levels of amines remained nearly unchanged until the 4th day for both storage temperatures ($p>0.05$). However, from the 6th day on, at both storage temperatures, mean total amine levels reached values higher than 130 mg/kg at the 8th day of storage at 4±1°C. Similar results were observed for the spermidine where its level remained unchanged until the 4th day at both temperatures, increasing afterwards. On contrary decreases on polyamines levels[16] during storage of pork liver. They suppose that polyamines losses could be the result of autolytic and bacterial degradation by polyamine oxidases. Regarding the biogenic amines, during storage of the livers at 0±1°C, there was no significant change on the levels of putrescine and histamine. However, at 4±1°C there was a significant increase on the levels of these amines after the 6th day. With respect to tyramine, the levels increased on the 6th day for both storage temperatures ($p<0.05$). An increase on biogenic amines levels was also observed in pig liver during

storage at 0.0 °C, 3°C and 7°C[16]. Some biogenic amines which were not detected in the fresh liver appeared during refrigerated storage, among them cadaverine. These amines were detected in some samples on the 4th and 2nd days during storage at 0 ±1 and 4±1°C, respectively. After the 6th storage day there was a significant increase for both amines at both storage temperatures, but higher rates were observed at 4±1°C compared to 0±1°C. This result suggests that storage at the higher temperature favored the production of cadaverine.

Effect of cooking on levels of biogenic amines in beef liver:

The results recorded in (Fig.3) revealed that pan-roasting fresh liver at 180 °C/5 min each side only affected significantly the levels of biogenic amines. The mean levels of polyamines in pan-roasted liver were more than two times higher compared to mean levels in the fresh liver. Such result denotes an increase of biogenic amines levels, probably due to their release from conjugated forms[1,10]. On the other hand, decreasing levels of spermine and spermidine in fresh and stored pork livers processed by pan roasting without oil at 180 °C[17] however they heated the samples for a longer period of time (22 min) compared to this study (10 min).

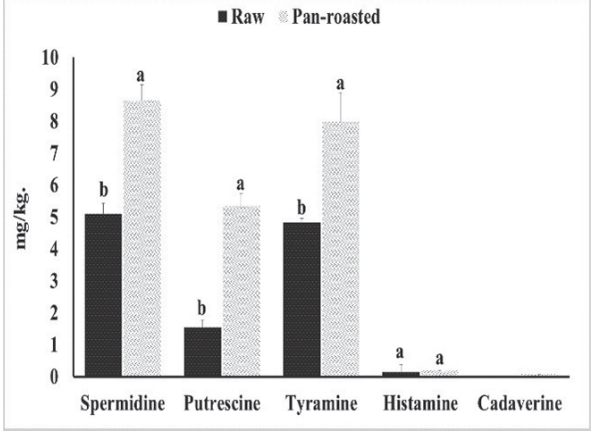


Figure (3) Biogenic amines ± SD for raw and pan-roasted samples (n=5).

Different superscripts on the same biogenic amine are significantly different ($p<0.05$).

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