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Bioactive amines in fresh beef liver and influence of refrigerated storage and pan-roasting

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Histamine (PubChem CID: 5818)
Serotonin (PubChem CID: 160436)

ABSTRACT

The profile and levels of ten bioactive amines in fresh beef liver was determined and associated with physico-chemical parameters of quality. Furthermore, the influence of refrigerated storage at 0 ± 1 °C and 7 ± 1 °C and of pan-roasting on beef liver quality and safety was investigated. Fresh beef liver was characterized by pH of 6.71–6.92, TVB-N of 98.58–154.72 mg N/100 g and negative H_2S. It contained high levels of spermine (up to 119 mg/kg), and low levels of spermidine, putrescine, tyramine and histamine. Therefore, beef liver constitutes one of the richest dietary source of spermine. During refrigerated storage, there were significant physico-chemical changes: the pH decreased, TVB-N increased, and hydrogen sulfide was moderate. The levels of most of the naturally occurring amines increased at rates which were faster at higher storage temperature. Two amines which were not initially detected, reached detectable levels – tryptamine and cadaverine. The proposed bioactive amines based indices of quality to access liver quality were not appropriate to follow gradual quality changes. A shelf life of up to 6 and 4 days during storage at 0 ± 1 °C and 7 ± 1 °C, respectively, is recommended. During pan-roasting at 180 °C for 10 min, the levels of the polyamines increased significantly.

1. Introduction

Brazil is the world's largest beef exporter and the second largest beef consumer and producer. Beef production and exports are forecast to increase in 2015 by three and ten percent, respectively, mostly due to the increased international demand and domestic consumption (Beef2live, 2015). Meat wasted by-products constitute nearly 60–70% of the slaughtered carcass, of which nearly 70% is edible (Mirabella, Castellani, & Sala, 2014). A major challenge facing industries is to add value to by-products, increasing their market value and, therefore, industry profitability. Furthermore, it can allow an environmentally sustainable production and the availability of innovative and nutritious products.

Edible by-products generally include offals, also called organ or variety meat, among them, head or head meat, tongue, brains, heart, liver, spleen, stomach or tripe. In some countries, other parts such as feet, throat and lungs are also used for human consumption. Offals are usually a dense, rich and economical source of essential nutrients that are more readily available to humans. Among edible offals, liver is valued as it is an important source of nutrients: high quality protein, vitamins, minerals, and polyamines (Abdullah, 2008; Devatkal & Mendiratta, 2007; Paulsen, Dicakova, & Bauer, 2008). However, liver and other edible offals are highly perishable because of the high content of readily available nutrients for microbial growth. Furthermore, being treated as waste, poor product handling, undesirable hygienic conditions and poor temperature control may prevail, which can favor microbial contamination and growth. They are also prone to autolytic activities.

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According to Hernández-Herrero, Roig-Sagués, López-Sabater, Rodríguez-Jerez, and Mora-Ventura (1999), liver deteriorates 4–6 days after slaughter, regardless of storage conditions. The major causes of spoilage are microbial growth (mainly *Pseudomonas* spp. and *Enterobacteriaceae*) 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.

Traditionally, shelf-life studies of perishable meat and meat products have been carried out by means of sensory and microbiological quality of the product, which are subjective and time consuming, respectively (Balamatsia, Patsias, Kontominas, & Savvaidis, 2007; Devatkal & Mendiratta, 2007; Hernández-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1996). Alternative methods, involving chemical changes have been suggested as quality indicators of meat, such as pH, total volatile bases, hydrogen sulfide production and biogenic amines (Hernández-Herrero et al., 1999; Galgano, Favati, Bonadio, Lorusso, & Romano, 2009). Biogenic amines and polyamines have been considered reliable indices of quality and safety of foods as their formation is primarily a consequence of the decarboxylation of specific amino acids due to microbial and autolytic enzyme activity (Li et al., 2014; Vinci & Antonelli, 2002). The formation and build up of putrescine and cadaverine can affect sensorial acceptance of the product, whereas accumulation of histamine, tyramine, phenylethylamine and tryptamine can cause adverse effects to human health, such as redness, headache, migraine and hypertensive crisis. Furthermore, polyamines can also be relevant as they are naturally present in tissues and are known to exert antioxidant activity due to their polycationic structure. Therefore they can play important role in the protection of the tissue against oxidation, increasing shelf life (Jastrzebska, 2012; Kalac, 2014; Li et al., 2014). Biogenic amines indices have been proposed as a useful indicator of spoilage in several foods (Galgano et al., 2009; Hernández-Jover et al., 1996; Mietz & Karmas, 1977; Vinci & Antonelli, 2002). The analysis of bioactive amines in liver could be used to warrant the nutritional quality and safety of the product.

Little information is available on the levels of bioactive 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 profile and levels of bioactive amines in beef liver immediately after slaughter as well as during refrigerated storage and pan-roasting. These values were compared to physico-chemical characteristics and to calculated bioactive amines indices to evaluate beef liver quality.

2. Material and methods

2.1. Material

Eleven beef livers were randomly collected from a commercial slaughterhouse located in Belo Horizonte, state of Minas Gerais, Brazil. The slaughterhouse operated under typical industry conditions at the auspices of federal inspection. The animals were 36–40 months' old Nellore cattle (*Bos primigenius indicus*). The liver samples (4.6–5.8 kg) were packaged individually and transported, under refrigerated conditions, to the laboratory where they were analyzed immediately.

The reagents used were of analytical grade, except HPLC solvents (acetonitrile and methanol) which were chromatographic grade. The organic and aqueous solvents were filtered through HAWP and HVWP 0.45 μm pore size membranes, respectively (Millipore Corp., Milford, MA, USA). The water used was obtained from Milli-Q Plus System (Millipore Corp., Milford, MA, USA).

Standards of spermine (SPM, tetrahydrochloride), spermidine (SPD, trihydrochoride), putrescine (PUT, dihydrochloride), cadaverine (CAD, dihydrochloride), tyramine (TYM, hydrochloride), histamine (HIM, dihydrochloride), agmatine (AGM, sulphate), serotonin (SRT, hydrochloride), 2-phenylethylamine (PHM, hydrochloride). and tryptamine (TRM, free base), as well as the derivatization reagent *o*-phthalaldehyde, were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Characterization of fresh beef liver

The fresh liver samples (n = 11) were analyzed, immediately after slaughtering, for bioactive amines, pH, hydrogen sulfide (H_2S), total volatile basic nitrogen (TVB-N) and moisture content.

2.2.2. Influence of refrigeration storage temperature on liver quality

Each one of the eleven liver samples was divided into nine parts of about 500 g each (according to Krausová, Kalac, Krizek, and Pelikánová (2007), there is negligible difference among amines levels in different liver parts). One part was analyzed immediately and the others were placed in polyethylene bags (aerobic conditions) 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 7 ± 1 °C (temperature of household refrigerators). At 2-days intervals samples were taken and analyzed for bioactive amines, pH, hydrogen sulfide and TVB-N.

2.2.3. Influence of heat treatment on bioactive amines levels

The influence 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 beef liver in Brazil, was evaluated. The samples were cut into 2-cm thickness slices, which were pan-roasted without oil in a preheated ungreased PTFE Teflon®-coated pan, at 180 °C for 5 min each side. Before and after pan-roasting, the samples were analyzed for moisture and bioactive amines contents. The results were reported on a dry weight basis to avoid interference from water loss during the heat treatment.

2.2.4. Methods of analysis

2.2.4.1. Physico-chemical characteristics. The samples were analyzed for moisture content, pH, total volatile basic nitrogen and hydrogen sulfide. Prior to analysis, the samples were ground in a food processor and homogenized thoroughly. The moisture content was determined according to AOAC (2000). The measurements of pH were carried out using a digital pH meter (AOAC, 2000). The total volatile basic nitrogen (TVB-N) was estimated by trichloroacetic acid extraction and steam distillation after alkalinization with MgO (Brasil, 1999). Hydrogen sulfide was determined by the lead acetate test. The response was given with respect to color intensity of the sample compared to standard solutions of H_2S and it was classified as negative (0), mild (1), moderate (2) or positive (3) (Brasil, 1999).

2.2.4.2. Determination of bioactive amines. The amines were extracted from the samples (5 g) with 7 mL of trichloroacetic acid (5% TCA). The samples were homogenized for 10 min in a shaker (TE Tecnal – 140, Piracicaba, SP, Brazil), centrifuged at 11,180 g at 4 °C for 21 min, and the supernatant was collected. The solid residue was submitted to two additional extractions with 7 mL TCA and the

supernatants were combined, filtered through qualitative filter paper and through a HAWP 0.45 μm pore size membrane (Millipore Corp. Milford, MA, USA) prior to HPLC analysis. The amines were determined by ion-pair reverse phase HPLC, post-column derivatization with *o*-phthalaldehyde (OPA) and fluorimetric detection (Silva & Gloria, 2002). Liquid chromatography was carried out in a Shimadzu, Model LC-10 AD HPLC connected to a RF-551 fluorimetric detector at 340 and 445 nm of excitation and emission, respectively. The amines were identified by comparison of the retention times of the amines in the sample with those of standard solutions and also by addition of the suspected amines to the sample. The amines were quantified by interpolation in external calibration curves ($r^2 \geq 0.9965$). In order to assure reproducibility throughout the day and between days, standards were run daily in between every four samples.

2.2.4.3. Quality indices based on bioactive amines. The levels of bioactive amines were used to calculate indices described in the literature for the evaluation of the quality of fish, pork and chicken meat. They were calculated according to the formulas, as follows (i) M&K = [(histamine + putrescine + cadaverine)/(1 + spermidine + spermine)] (Mietz & Karmas, 1977); (ii) H-J = [histamine + putrescine + cadaverine + tyramine] (Hernández-Jover et al., 1996); and (iii) S&G = (spermidine/spermine) (Silva & Gloria, 2002). The adequacy of these indices to evaluate the quality of liver was investigated.

2.3. Statistical analysis

Data were submitted to an analysis of variance test for normality (Shapiro Wilks) and for homogeneity of variances (F test). Data with normality deviations or heterogeneity of variances were transformed into $\log_{10}(x + 1)$. Then, the data were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% probability. Pearson's correlation ($p < 0.001$) was used to investigate correlations between the levels of amines and the physico-chemical characteristics of the beef livers.

3. Results and discussion

3.1. Physico-chemical characteristics and bioactive amines in fresh beef liver

3.1.1. Physico-chemical characteristics of fresh beef liver

The fresh beef livers were characterized by pH values ranging from 6.71 to 6.92 (mean of 6.84 ± 0.07), TVB-N from 98.58 to 154.72 mg N/100 g (mean of 123.84 ± 25.37 mg N/100 g) and moisture content varying from 72.67 to 76.02 g/100 g (mean of 74.12 ± 1.13 g/100 g). The results were negative for hydrogen sulfide. The variation among results for the liver samples was small for pH and moisture content (coefficient of variation – CV of 1.02 and 1.52%, respectively); however, there was a large variation for TVB-N values (CV of 20.5%).

No information on TVB-N and hydrogen sulfide was found for beef liver to the best of our knowledge. Hernández-Herrero et al. (1999) reported lower pH values for seven samples of fresh beef liver (mean of 6.46 ± 0.15) from a commercial slaughterhouse in Spain. 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 (Jastrzebska, 2012; Paulsen et al., 2008).

3.1.2. Profile and levels of bioactive amines in fresh beef liver

Among the ten amines investigated, five were detected in the fresh beef liver, among them, spermine, spermidine, putrescine, tyramine and histamine. These amines were present in every sample analyzed, except for histamine which was only present in six out of 11 samples, which represents 55% of the samples. The presence of spermine was expected as it is the predominant amine in animal tissues, followed by spermidine. These polyamines are essential factors for cell proliferation and differentiation and other relevant functions of normal cells (Gloria, 2005; Kalac, 2014; Paulsen et al., 2008). 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 spermine, spermidine and the diamine putrescine was also reported in beef liver (Krausová, Kalac, Krizek, & Pelikánová, 2006) and in liver from other animals (Dadáková, Pelikánová, & Kalac, 2011; Dadáková, Pelikánová, & Kalac, 2012; Krausová et al., 2006; Krausová et al., 2007; Kozová, Kalac, & Pelikánová, 2009; Paulsen et al., 2008; Villanueva-Valero et al., 2005).

The occurrence of tyramine and histamine in fresh beef liver was described for the first time. Krausová et al. (2006) did not detect these amines in beef liver (LOD ≥ 1.2 mg/kg). However, tyramine was reported in fresh pork and hare liver by Villanueva-Valero et al. (2005) and Paulsen et al. (2008), respectively. Tyramine is a substrate for dopamine and, subsequently, norepinephrine and epinephrine formation using cytochrome P_{450} 2D6 (CYP2D6) or tyrosinase (Wassenberg et al., 2010), but no information was found about its role in the liver. The presence of histamine was reported in fresh pork and roe deer liver by Villanueva-Valero et al. (2005) and Paulsen et al. (2008), respectively. The sporadic presence of histamine in fresh liver could be related to its physiological status associated with some metabolic conditions, including malfunctions and diseases (immune system, wound healing and defense mechanism). According to Francis and Meininger (2010), hepatic mast cells consistently increase in number with the progression of various liver diseases. Upon activation, mast cells degranulate and secrete mediators, including histamine, into the surrounding tissue.

The total levels of bioactive amines in beef liver immediately after slaughter ranged from 66.7 to 136 mg/kg (mean – 98.4 mg/kg; median – 94.8 mg/kg). Spermine was the prevalent amine, with levels which contributed with 89% of the total amines content. Spermidine contributed with 4.3% of the total levels. Overall, the polyamines (spermine + spermidine) represented 93.3% of the total amines levels in liver. The CV for the levels of polyamines among the 11 samples analyzed was around 23%.

The levels of spermine ranged from 55.5 to 119 mg/kg (mean – 87.5 mg/kg; median – 89.8 mg/kg) and the levels of spermidine varied from 2.89 to 5.89 mg/kg (mean – 4.15 mg/kg; median – 4.18 mg/kg). However, Krausová et al. (2006), investigating polyamines in beef liver from the Czech Republic, found lower mean levels of spermine – 34.7 and 43.1 mg/kg and higher mean levels of spermidine – 121.5 and 160.5 mg/kg, respectively for bulls and cows. However, in the same study, the authors found higher spermine compared to spermidine levels for pork and chicken liver, which is the consensus in the literature regarding the profile of polyamines in liver from different animal species (Dadáková et al., 2011; Kozová et al., 2009; Paulsen et al., 2008; Villanueva-Valero et al., 2005). Krausová et al. (2006) assumed that the differences on polyamines levels could reflect peculiarities in the metabolism of ruminant compared to non ruminant species. They also observed higher spermidine contents in cow compared with young bull livers and negative correlation between age of bulls and spermidine levels, therefore confirming the influence of gender and age on the levels of polyamines. Anyway, fresh liver is a very rich source of polyamines; in fact it is one of the foods with the highest content of polyamines.

The levels of putrescine in the samples varied from 0.47 to 3.43 mg/kg (mean – 1.49 mg/kg; median – 0.8 mg/kg), which

represented 1.5% of the total levels of amines. These levels are low compared to those reported by Krausová et al. (2006) for beef liver; however putrescine levels have been reported to vary widely among liver samples of many species. The CV among samples for putrescine was high – 72%.

With respect to the biogenic amines tyramine and histamine, they were found at low levels in the fresh liver (≤9.37 and ≤0.76 mg/kg, respectively). The contribution of these amines to total levels was very small: tyramine contributed with 5% and histamine with 0.2%. The coefficients of variation among the levels of these amines were high (49 and 118%, respectively, for tyramine and histamine), suggesting that their levels are affected by inherent and exogenous factors. For example, Paulsen et al. (2008) observed a significant positive correlation between age and histamine levels in roe deer liver. 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 (EFSA, 2011). The presence of these amines at high levels in liver could be associated with spoilage and microbial growth; which was also the opinion of Villanueva-Valero et al. (2005), who detected tyramine, histamine, cadaverine and phenylethylamine 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 $\log^{10}$ cfu/g).

The profile and levels of bioactive amines encountered in beef liver indicate that polyamines are inherent to liver and differences on their levels could be associated with the diversity of species, breed types (dairy and meat), age, gender, and live weight of the animals. Some biogenic amines, like tyramine, are also inherent to liver at low levels. Histamine is associated with the physiological status (immune system, wound healing and defense mechanism) of the animal. Increased levels of cadaverine, putrescine, tyramine and histamine can result from microbial contamination and spoilage (Villanueva-Valero et al., 2005). Further studies are needed to better understand the factors affecting the presence of biogenic amines in fresh liver.

3.2. Influence of refrigerated storage on the physico-chemical characteristics and bioactive amines levels of beef liver

3.2.1. Physico-chemical characteristics of beef liver during refrigerated storage

During refrigerated storage of the liver samples, the pH values decreased whereas the TVB-N increased significantly and hydrogen sulfide was detected with storage time (Fig. 1). The decrease on pH values fitted linear regression and were affected by storage temperature with higher rates observed at 7 ± 1 °C (inclination = −0.0563, R^2 = 0.7348) compared to 0 ± 1 °C (inclination = −0.0355, R^2 = 0.7110). Similar decreases on pH values were observed for beef liver stored under refrigeration 0–3 °C (Hernández-Herrero et al., 1999). According to these authors, the decrease on pH values was associated with bacterial growth, mainly *Pseudomonas*, enterobacteria and lactic acid bacteria. The high glucose contents and high pH prevalent in liver favor the growth of bacteria with the concomitant production of acid and reduction on pH values (Hasapidou & Savvaidis, 2011; Hernández-Herrero et al., 1999).

The TVB-N increased with storage time at a faster at 7 ± 1 °C compared to 0 ± 1 °C. The changes followed a polynomial regression with equations $y = 1.1953x^2 + 4.7295x + 122.73$, $R^2 = 0.6182$ at 7 ± 1 °C and $y = 1.0224x^2 + 0.151x + 123.81$, $R^2 = 0.4541$ at 0 ± 1 °C. Hydrogen sulfide was negative (0) until the second day of storage at both temperatures. In samples stored at 0 ± 1 °C, the results ranged from mild (1) response on the 4th day, to moderate (2) on the 6th day, becoming positive (3) on the 8th day (Fig. 1). For the samples

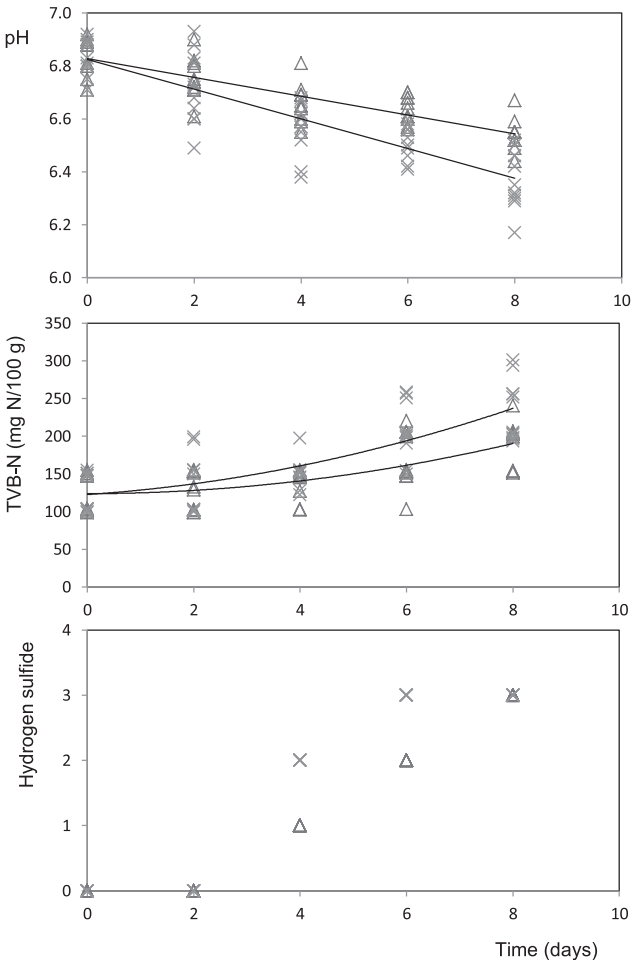

Fig. 1. Changes on pH values, total volatile basic nitrogen (TVB-N) and hydrogen sulfide during storage of beef liver at 0 ± 1 °C (Δ) and 7 ± 1 °C (×) (Hydrogen sulfide was evaluated as 0 – negative, 1 – mild, 2 – moderate, and 4 – positive).

stored at 7 ± 1 °C, the response was moderate (2) on the 4th day and positive (3) from the 6th day on. These results indicate that the liver became spoiled on the 8th storage day at 0 ± 1 °C and on the 6th day at 7 ± 1 °C. No data was found regarding TVB-N and hydrogen sulfide in beef and other liver samples. However, it is known that when glucose is exhausted, spoilage microorganisms use amino acids as substrate with the release of volatile nitrogenous and sulphurous compounds (Hernández-Herrero et al., 1999). Therefore they could be used as a screening quality index for liver due to their simplicity, rapidity and low cost.

Significant positive correlation ($p < 0.001$) was found between TVB-N and hydrogen sulfide whereas significant negative correlation ($p < 0.001$) was found between pH and TVB-N and also between pH and hydrogen sulfide. These results suggest that the physico-chemical characteristics are affected by similar factors, such as microorganisms growth.

Based on these results, the shelf-lives of beef liver were suggested to be 6 days during storage at 0 ± 1 °C and 4 days during storage at 7 ± 1 °C. At this point, pH was ≥6.65, TVB-N was ≤170 mg N/100 g and hydrogen sulfide test provided a moderate response. Similar shelf-life, although a little less rigid, was established by Hernández-Herrero et al. (1999) for beef liver stored at 0–3 °C.

3.2.2. Bioactive amines of beef liver during refrigerated storage

The changes on the levels of amines during refrigerated storage

are indicated on Table 1. Total levels of amines remained 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. Similar results were observed for the polyamines spermine and spermidine – their levels remained unchanged until the 4th day at both temperatures, increasing afterwards. Regression studies indicated that the changes on polyamines and total amines with storage time did not follow a simple model. It is interesting to observe that during storage at 0 ± 1 °C, on the 8th day there was a significant decrease on the levels of polyamines. No information was found in the literature regarding the levels of polyamines during refrigerated storage of beef liver; however, increases on polyamines levels during storage can be caused by the release of polyamines bound to proteins or some other cell components due to autolytic activity (Kozová et al., 2009) or by synthesis from microorganisms (Gloria, 2005). Decreases on polyamines levels were reported by Krausová et al. (2007) during storage of pork liver. They hypothesized 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 7 ± 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 °C, 3 °C and 7 °C (Villanueva-Valero et al., 2005).

Some biogenic amines which were not detected in the fresh liver appeared during refrigerated storage, among them cadaverine and tryptamine. These amines were detected in some samples on the 4th and 2nd days during storage at 0 ± 1 and 7 ± 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 7 ± 1 °C compared to 0 ± 1 °C. This result suggests that storage at the higher temperature favored the production of both cadaverine and tryptamine.

Significant positive correlation ($p < 0.001$) was found between TVB-N and the levels of every one of the amines and also between hydrogen sulfide and the levels of every amine. However, significant negative correlation ($p < 0.001$) was found between pH and every one of the amines. These results suggest that the levels of amines are affected by similar factors such as the physico-chemical characteristics. The negative correlation between amines levels and pH are associated with the fact that amines are produced by microbial enzymes as a response to low pH values which can be detrimental to the survival of microorganisms (Gloria, 2005).

When considering the levels of some biogenic amines (histamine, tyramine and tryptamine) and their possible role in human health, the levels of histamine on the 8th storage day at 7 ± 1 °C reached levels above 100 mg/kg which could cause harmful effects to human health (EFSA, 2011; Gloria, 2005). Furthermore, the potentiating effect of some bioactive amines on histamine toxicity should be considered.

3.3. Quality index based on bioactive amines for quality assessment of beef liver

The application of bioactive amines indices to evaluate the quality of beef liver during refrigerated storage is indicated on Table 2. Among indices investigated, the one described by Silva and Gloria (2002) for chicken meat was not appropriate for beef liver since no significant difference was observed for the fresh and spoiled liver at both storage temperatures. The index proposed by Mietz and Karmas (1977) for tuna fish provided significantly different values ($p < 0.05$) after the 6th storage day at 7 ± 1 °C; however, no significant change was observed during storage at 0 ± 1 °C. The index proposed by Hernández-Jover et al. (1996) showed significant difference after the 6th storage day at both temperatures; however it was not possible to establish limits to define each class of beef liver quality due to the large variation among values. As indicated by Ruiz-Capillas and Jiménez-Colmenero (2004), it is a challenge to establish an index that

Table 2
Application of different indices of quality based on bioactive amines for beef liver during storage at 0 ± 1 °C and 7 ± 1 °C.

Temperature/time (days)	Quality indices – Mean ± standard deviation		
	Mietz & Karmas	Hernández-Jover	Silva & Gloria
0 ± 1 °C			
0	0.02 ± 0.02	6.58 ± 2.93	0.05 ± 0.02
2	0.01 ± 0.02	6.03 ± 3.88	0.05 ± 0.01
4	0.01 ± 0.01	7.77 ± 5.66	0.05 ± 0.01
6	0.01 ± 0.01	15.17 ± 12.46	0.04 ± 0.01
8	0.09 ± 0.21	17.11 ± 21.48	0.04 ± 0.01
7 ± 1 °C			
0	0.02 ± 0.02^{b}	6.66 ± 2.85^{b}	0.05 ± 0.02
2	0.01 ± 0.01^{b}	4.82 ± 2.01^{b}	0.05 ± 0.01
4	0.02 ± 0.02^{b}	7.00 ± 3.39^{b}	0.06 ± 0.03
6	0.20 ± 0.19^{b}	42.45 ± 35.30^{b}	0.04 ± 0.01
8	1.17 ± 0.70^{a}	237.9 ± 143.5^{a}	0.03 ± 0.01

Mean values ± standard deviation (n = 11) with different superscripts in each column for each temperature are significantly different (Tukey Test, $p < 0.05$).

Table 1
Levels of bioactive amines in beef liver during refrigerated storage at 0 ± 1 °C and 7 ± 1 °C for eight days.

Storage temperature/Time (days)	Bioactive amines (mg/kg)* ± standard deviation							
	Spermine	Spermidine	Putrescine	Tyramine	Histamine	Cadaverine	Tryptamine	Total
0 ± 1 °C								
0	87.5 ± 20.1^{c}	4.15 ± 0.99^{b}	1.49 ± 1.07	4.86 ± 2.37^{b}	0.22 ± 0.26	0.00	0.00^{c}	98.3 ± 20.3^{c}
2	95.5 ± 23.4^{bc}	4.15 ± 0.95^{b}	1.40 ± 1.21	4.59 ± 2.87^{b}	0.04 ± 0.14	0.00	0.00^{c}	106 ± 22.3^{c}
4	93.1 ± 19.1^{bc}	4.37 ± 1.55^{b}	1.61 ± 1.45	6.04 ± 3.91^{ab}	0.06 ± 0.14	0.06 ± 0.16	0.30 ± 0.54^{bc}	106 ± 22.2^{bc}
6	212 ± 81.5^{a}	8.08 ± 4.03^{a}	3.36 ± 3.28	11.5 ± 9.93^{a}	0.23 ± 0.31	0.11 ± 0.20	1.48 ± 1.63^{ab}	237 ± 93.0^{a}
8	141 ± 36.1^{b}	5.77 ± 1.81^{ab}	3.36 ± 4.51	6.53 ± 3.16^{ab}	5.11 ± 11.6	2.10 ± 5.53	2.14 ± 1.09^{a}	166 ± 34.3^{b}
7 ± 1 °C								
0	87.5 ± 20.1^{c}	4.15 ± 0.99^{a}	1.49 ± 1.07^{b}	4.86 ± 2.37^{a}	0.22 ± 0.26^{b}	0.00^{b}	0.00^{b}	98.3 ± 20.3^{b}
2	89.0 ± 20.6^{c}	3.89 ± 0.94^{b}	1.20 ± 0.91^{b}	3.52 ± 1.88^{b}	0.01 ± 0.05^{b}	0.03 ± 0.10^{b}	0.17 ± 0.55^{b}	97.9 ± 21.3^{bc}
4	86.5 ± 25.5^{c}	4.45 ± 1.28^{a}	1.50 ± 1.27^{b}	4.66 ± 2.75^{a}	0.09 ± 0.16^{b}	0.05 ± 0.16^{b}	0.69 ± 0.84^{b}	98.0 ± 26.0^{bc}
6	139 ± 46.8^{b}	6.38 ± 3.90^{a}	5.89 ± 7.80^{b}	9.17 ± 7.77^{a}	18.9 ± 16.8^{b}	9.24 ± 12.6^{b}	2.82 ± 1.48^{b}	192 ± 74.6^{b}
8	218 ± 105^{a}	7.36 ± 4.45^{a}	23.8 ± 20.3^{a}	10.5 ± 6.23^{a}	162 ± 143^{a}	42.4 ± 29.5^{a}	9.12 ± 8.69^{a}	476 ± 217^{a}

LOQ in mg/kg: Spermine = 0.20, Spermidine = 0.10, Putrescine = 0.04, Tyramine = 0.04, Histamine = 0.20, Cadaverine = 0.10, Tryptamine = 0.80.
Means (calculated considering non detected levels = zero) with different superscripts (a–c) during storage time for each amine at each storage temperature are significantly different (Tukey test, $p < 0.05$).

reliably predicts quality for this kind of product, especially because of the amplitude of biochemical changes occurring simultaneously in the liver.

3.4. Influence of heat treatment on bioactive amines levels

The influence of pan-roasting, which is a widely used way to prepare beef liver in Brazil, on the profile of bioactive amines in fresh beef liver was investigated. The moisture content of the liver samples indicated significant difference between values before (74.12 ± 1.13 g/100 g) and after heat treatment (65.06 ± 2.18 g/100 g). Therefore, the results were reported and compared on a dry weight basis.

Data showed normality deviations and heterogeneity of variances; therefore, they were transformed into $\log_{10}(x + 1)$ before comparison of means by the Tukey test ($p < 0.05$). According to Table 3, pan-roasting fresh liver at 180 °C/5 min each side only affected significantly the levels of polyamines. The mean levels of polyamines in pan-roasted liver were 2.2 times higher compared to mean levels in the fresh liver. This result suggests an increase of polyamines levels, probably due to their release from conjugated forms (Gloria, 2005; Kozová et al., 2009). The presence of conjugated spermine was reported by Haddox and Russell (1981) as a constitutive source of spermine in rat liver nuclei. However, no information was found for beef liver. On the other hand, studies by Krausová et al. (2007) indicated decreasing levels of spermine and spermidine in fresh and stored pork livers processed by pan-roasting without oil at 180 °C, however they heated the samples for a longer period of time (22 min) compared to this study (10 min).

In a similar way, the levels of the biogenic amines putrescine, tyramine and histamine were not affected by pan-roasting. No information was found regarding the influence of heat treatment on biogenic amine in beef and other types of livers. The studies available investigated only polyamines (Kozová et al., 2009; Krausová et al., 2007).

According to Kozová et al. (2009), amines can compete with the free amino groups of amino acids for glucose during Maillard reaction. Furthermore, amines can be converted into their *N*-nitroso derivatives by reaction with nitrous acid (Gloria, 2005). However, the conditions prevailing during heat treatment of beef liver did not seem to promote Maillard reaction and N-nitrosamine formation from bioactive amines.

4. Conclusions

Fresh liver was characterized by pH of 6.71–6.92, TVB-N of 98.58–154.72 mg N/100 g and negative hydrogen sulfide test. It was observed to be a good source of polyamines, mainly spermine, and a poor source of some biogenic amines, among them, putrescine, tyramine and histamine, which were present at low concentrations. Fresh liver is one of the foods with the highest content of polyamines.

During refrigerated storage at 0 ± 1 °C and 7 ± 1 °C, the pH decreased, TVB-N increased and the hydrogen sulfide test became positive at faster rates for the higher storage temperature. The levels of most of the amines increased and two amines, which were not detected in fresh liver, were formed, tryptamine and cadaverine. Histamine reached levels capable of causing adverse effects to human health on the 8th day of storage at 7 ± 1 °C. The investigated indices of quality based on bioactive amines were not efficient in following gradual quality and safety of beef liver.

Based on the levels of amines and also on the physico-chemical parameters pH, TVB-N and hydrogen sulfide test, refrigerated storage of liver can be undertaken at 0 ± 1 °C for up to 6 days and at 7 ± 1 °C for up to 4 days.

Pan-roasting fresh liver at 180 °C/5 min each side caused a significant increase on the levels of polyamines.

Table 3
Mean levels (±standard deviation) of bioactive amines (dry weight basis) in fresh beef liver before and after pan-roasting without oil at 180 °C for 5 min each side.

Amines	Bioactive amines (mg/kg dry matter)	
	Raw	Pan-roasted
Spermine	68.8 ± 8.36[b]	157 ± 90.5[a]
Spermidine	3.98 ± 0.73[b]	8.96 ± 5.51[a]
Putrescine	2.55 ± 0.54	5.79 ± 3.70
Tyramine	5.47 ± 1.92	7.29 ± 3.35
Histamine	0.08 ± 0.17	0.12 ± 0.17

nd was considered zero to calculate mean values.
Mean values ± standard deviation for raw and pan-roasted samples (n = 5) with different superscripts in each line are significantly different (Tukey Test, $p < 0.05$).

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