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

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Putrescine (PubChem CID: 9532)
Cadaverine (PubChem CID: 80282)
Tyramine (PubChem CID: 66449)
Tryptamine (PubChem CID: 67652)
Phenylethylamine (PubChem CID: 9075)
Agmatine (PubChem CID: 2794990)
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