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Evolution of amino acids and biogenic amines throughout storage in sausages made of horse, beef and turkey meats

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ABSTRACT

The changes in concentration of free amino acids and biogenic amines, along 28 d of storage at 4 °C, were monitored in a wide range of European ripened sausages manufactured from horse, beef and turkey meats. Generally speaking, both chemical families became more concentrated with elapsing time – but rather distinct patterns were followed in each meat type: total free amino acids increased by 13-fold in the case of horse sausages, and 5-fold in the case of beef sausages, but decreased to one third in the case of turkey sausages; and total biogenic amines attained 730 mg/kg in turkey sausages, 500 mg/kg in beef sausages and 130 mg/kg in horse sausages by 28 d of refrigerated storage. For putrescine, maximum levels of 285 mg/kg were attained in turkey and 278 mg/kg in beef sausages; for cadaverine, maximum levels of 6 mg/kg in turkey and 9 mg/kg in beef; and for histamine, maximum levels of 263 mg/kg in turkey and 26 mg/kg in beef. Hence, public safety concerns may be raised in the case of turkey sausages.

1. Introduction

Dry fermented sausages are popular delicacies in various parts of the world; their quality in terms of slicing ability, firmness, color and taste hinges critically on the levels of proteolysis attained during ripening. However, adventitious (or otherwise added) microflora will uptake free amino acids as nutrients and may bring about decarboxylation thereof for energetic purposes, given the nutritionally poor environment and the relatively long storage period (Konings et al., 1997), to yield biogenic amines. Common examples of the latter are histamine produced from histidine, putrescine from ornithine, cadaverine from lysine, and tyramine from tyrosine; spermidine and spermine are sequentially produced from putrescine (Eitenmiller & de Souza, 1984). Microorganisms possessing decarboxylating capacity encompass pathogens belonging to the *Bacillus*, *Pseudomonas*, *Escherichia* and *Salmonella* genera, as well as such food borne starters as *Lactobacillus*, *Enterococcus*, *Lactococcus* and *Leuconostoc* spp. (Edwards, Sandine, & Public, 1981). Biogenic amines may raise safety issues due to their toxicity (Luthy & Schlatter, 1983), associated with e.g. hypertension, headaches, fever, nausea, urticaria, and gastric and intestinal ulcers; they can even be precursors of carcinogenic nitrosamines (Patterson & Mottram, 1974). The Food and Drug Administration has established a maximum tolerance level of 100 mg/kg for histamine in flesh (FDA, 1990); EFSA has recently established a daily maximum intake of 50 mg of histamine, and 600 mg of tyramine can be considered safe for healthy individuals, although such limits may be drastically reduced in case of reported intolerance or use of monoamine oxidase inhibitor drugs (EFSA, 2011).

High levels of putrescine and cadaverine appear to potentiate the toxicity of histamine, as well as tyramine (Taylor, 1985). Although reliable dose-response data are not available pertaining to human consumption, the limited number of animal studies published so far have suggested an oral toxicity of 180 mg/kg body weight/day in Wistar rats (EFSA, 2011).

Spermidine and spermine are found in almost all sausages manufactured from fresh beef and pork; spermidine averages at 3.0 mg/kg, whereas spermine ranks in 33.5–39.8 mg/kg (Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1997). Furthermore, several authors working with such Spanish dry sausages as *chorizo*, *fuet*, *sobrasada* and *salsichón* (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000; Bover-Cid, Miguélez-Arrizado, & Vidal-Carou, 2001; Lorenzo, Michinel, López, & Carballo, 2000; Roig-Sagués, Hernández-Herrero, López-Sabater, Rodriguez-Jerez, & Mora-Ventura, 1999; Ruiz-Capillas & Jiménez-Colmenero, 2004; Santos, Jalon, & Marine, 1985), as well as French (Buscailhon, Monin, Cornet, & Bousset, 1994) and Iberian (Alfaia et al., 2004; Hernandez-Jover, Izquierdo-Pulido, Veciana-Nogués, & Vidal-Carou, 1996; Martin, Antequera, Ventanas, Menitez-Donoso, & Cordoba, 2001) dry cured hams found that tyramine and cadaverine could reach 600 mg/kg, whereas putrescine was present up to 450 mg/kg, and 2-phenylethylamine and tryptamine up to 50 mg/kg;

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specifically, histamine reached 330 mg/kg in *chorizo* and *fuet*, which are thresholds that pose a health concern. On the other hand, tyramine and putrescine were the most abundant biogenic amines in dry Finnish sausages (Eerola, Roig-Sagués, & Hirvi, 1998), and high levels of cadaverine were detected in Danish *pepperoni* (Hernandez-Jover et al., 1997). Histamine was also detected in Russian sausages, but usually not above 100 mg/kg (Hernandez-Jover et al., 1997). In the case of Turkish sausages, contents of 1,100 mg/kg of tyramine and 350 mg/kg of histamine were found in *sucuks* (Şenöz, Işıklı, & Çoksöyler, 2000), and 400 mg/kg of putrescine and 250 mg/kg of tyramine in *soudjoucks* (Ayhan, Kolsarici, & Özkan, 1999).

Contaminated raw materials and poor hygienic conditions prevailing during manufacture are likely contributors to the dangerous levels of histamine found (Komprda, Neznalovà, Standara, & Bover-Cid, 2001), and temperature abuses during storage contribute to accumulate tyramine, cadaverine and putrescine (Bover-Cid et al., 2000), namely because of contamination by Enterobacteriaceae (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Despite the above data, fermented sausages in general have been claimed as intrinsically safe for consumption owing to their reduced a_w and pH (Ferreira & Pinho, 2006). Hence, the effect of refrigerated storage upon the biogenic amine content of European sausages manufactured from meats other than pork or beef was not studied to date to sufficient depth, nor has its relationship with amino acid levels been established. Therefore, the aim of this work was to provide a consistent overview of the presence of biogenic amines in dry sausages, especially those manufactured from horse and turkey; and also search for public health risks derived from ingestion of those less usual sausages and associated with presence of biogenic amines.

2. Materials and methods

2.1. Collection of samples

A wide variety and a large number (210) of European ripened, dried sausages manufactured from plain horse, beef and (smoked) turkey meats, together with a small portion of pork but without nitrate/nitrite or any starter culture, were purchased from supermarkets at random right upon production, and kept refrigerated at 4 °C. Seventy sausages from each type with 200 g in weight, 26–36 mm in diameter and 13–25 cm in length were sampled (in duplicate) by 0, 7, 4, 21 and 28 d of storage (i.e. covering their expected shelf life). Samples (3 g each) were immediately homogenized and frozen upon collection, and kept as such in packages where oxygen had been excluded by back flushing with nitrogen, until analysis was in order (usually within 48 h).

2.2. Determination of dry weight

The reference drying method (MSZ EN ISO 1666:2000) was applied, using a WS 50 oven (MLW, Germany) for heating to 100–105 °C until constant weight.

2.3. Extraction of amino acids and biogenic amines

Amino acids and biogenic amines in the samples were extracted as originally described by Simon-Sarkadi and Holzapfel (1994), and later improved by Rabie, Siliha, el-Saidy, el-Badawy, and Malcata (2010) for meat matrices: 10 ml of 10 %(v/v) trichloroacetic acid was added to 3 g of sample, the mixture was shaken for 1 h using a Laboshake Ls 500i (Gerhardt, Germany), and the extract was finally filtered through Whatman No.1 filter paper. To remove fat, the extracts were kept at −20 °C for 1 d, and then subjected to centrifugation at 7000 *g* for 15 min using a T 24 apparatus (MLW). The supernatant was finally collected and filtered through 0.25 μm membrane filters (Nalgene, USA).

2.4. Quantitation of amino acids and biogenic amines

Analyses of free amino acids and biogenic amines were performed using an AAA 400 amino acid analyser (Ingos, Czech Republic) equipped with a Watrex Polymer 8 ion exchange column (20 cm long, 3.7 mm i.d.) for amino acids, and an Ostion LG ANB ion exchange column (6 cm long, 3.7 mm i.d.) for biogenic amines. Free amino acids and biogenic amines in 100 μL-aliquots were injected into said column, and separated by stepwise gradient elution at 0.30 mL/min (60 °C), using a Li^+ buffer for amino acids and a Na^+/K^+ buffer for biogenic amines – prepared as described in detail by Csomos and Simon-Sarkadi (2002); the total running times were 92 and 101 min, respectively. Colorimetric detection was accomplished at 570 and 440 nm, for amino acids and biogenic amines, after post column derivatization (121 °C) with ninhydrin, supplied at 0.20 mL/min (Csomos & Simon-Sarkadi, 2002). All analytical determinations were done in triplicate (free amino acids) and duplicate (biogenic amines).

Identification was by matching of retention times of aliquots of actual samples and chromatographic standards, whereas quantification was by peak area based on calibration curves previously prepared using chromatographic standards.

2.5. Statistical analyses

All experimental values pertaining to biogenic amine and amino acid concentrations were reported as average ± standard deviation of three replicates. Statistical significance of the differences found between data was ascertained via Student's *t*-tests; a probability value, *P*, of less than 5% was considered as statistically significant. The statistical software utilized was SPSS (from SPSS Inc., Chicago IL, USA).

3. Results and discussion

3.1. Free amino acids

The evolution in concentration of 22 amino acids is depicted in Tables 1–3 for the three meat types. Within a 28 d period, their total concentration increased significantly ($P < 5\%$) by 12.8-fold in horse sausage and 4.8-fold in beef sausage. Lysine, tyrosine and histidine are the chief precursors of biogenic amines in foods: all of them underwent considerable increases throughout storage as a result of microbial-mediated proteolysis, in the case of horse sausages; and lysine, in the case of beef sausages. It is well-known that the chief contributor to biogenic amine appearance is microbial pathways; this assumption also appears to hold in our case, in view of pH lowering as indirect evidence of microbial action (even though monitoring of viable numbers of the major microbial families was not pursued). On the other hand, the absence of nitrates/nitrites and the essentially constant levels of salt and water activity throughout storage rule out discrepancies in the resident microflora arising from such exogenous factors (Joosten, 1988).

A gradual release of amino acids throughout storage is typical in dry fermented sausages (Beriain, Lizaso, & Chasco, 2000a; Dainty & Blom, 1995; Hierro, Hoz, & Ordoñez, 1999); these compounds are important for correct taste development of the final product (Montel, Masson, & Talon, 1998). Such increasing trends were typically observed in our horse and beef sausages, and are consistent with reports by Hierro et al. (1999), Bolumar, Nieto, and Flores (2001) and Hughes et al. (2002); our data actually lie within ranges similar to those found by Beriain et al. (2000a,b); Beriain, Lizaso, and Chasco (2000b) and Bruna, Fernández, Hierro, Ordóñez, and Hoz (2000a,b).

Conversely, the total concentration of amino acids decreased 2.6-fold ($P < 5\%$) in turkey sausage within the 28 d period, thus suggesting microbial uptake (Bover-Cid et al., 2000; Ordóñez,