This article was published in Meat Science, 96 (1), 82-87, 2014
http://dx.doi.org/10.1016/j.meatsci.2013.05.042

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). Bio- genic 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 re- liable 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). Further- more, 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; 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, Hierro, Bruna, & Hoz, 1999) and eventual extensive conversion to biogenic amines by the surviving microflora: remember that this was the only meat type subjected to smoking, which may account for the disparate behaviour observed. As will be discussed below in further detail, the largest increase in total biogenic amines occurred also in this type of sausage. According to Ruiz et al. (1999) and Ventanas et al. (1992), free amino acids may decrease or increase in concentration depending on the prevailing chemical and enzymatic reactions, in particular amino acid decarboxylase activity of the surviving microorganisms (Virgili, Saccani, Gabba, Tanzi, & Bordini, 2007), which is in turn dependent on the processing technique (Martin et al., 1998). A decrease in free amino acids was likewise reported during extended ripening of dry-cured ham (Alfaia et al., 2004; Buscailhon et al., 1994).

Inspection of Table 1, one realises that the main amino acids in horse sausage were methionine (0.15–23.20 mg/kg), glycine (1.54–20.96 mg/kg), proline (0.70–10.15 mg/kg), arginine (0.58–9.07 mg/kg), glutamic acid (0.47–8.18 mg/kg), lysine (1.15–7.24 mg/kg) and tyrosine (0.14–7.09 mg/kg); altogether, they accounted for 55% of the total amino acids by 28 d. It is interesting that the concentrations of some free amino acids were consistently lower by 21 d – yet no apparent rationale could be found for this realisation.

The predominant amino acids in beef sausage (Table 2) were alanine (7.63-63.41 mg/kg), aspartic acid (0.61-44.39 mg/kg), glycine (2.98-20.16 mg/kg), asparagine (0.08-7.67 mg/kg), leucine (3.75-7.19 mg/kg), lysine (2.70-4.22 mg/kg) and glutamine (3.76- 4.11 mg/kg); as a whole, these seven amino acids represented 93 % of the total amino acids by the end of storage.

Finally, the chief amino acids in turkey sausage were alanine (35.60- 4.35 mg/kg), glycine (2.23-2.33 mg/kg), lysine (1.65-1.75 mg/kg), serine (1.22-1.55 mg/kg), proline (1.20-1.31 mg/kg), leucine (1.04-1.22 mg/kg) and valine (0.75-0.94 mg/kg), as depicted in Table 3; they summed up 75% of the total amino acids by 28 d.

3.2. *Biogenic amines*

The evolution of the independent concentrations of 6 biogenic amines is depicted in Fig. 1, for the three meat types; the total concentrations are, for convenience, tabulated in Tables 1-3.

The highest concentration of total biogenic amines (i.e. 730.3 mg/kg) was found in turkey sausage, followed by beef (i.e. 496.0 mg/kg), whereas a total of only 126.9 mg/kg was observed in horse sausage by 28 d ($P < 5\%$); note that a maximum threshold of 1000 mg/kg of total bio- genic amines has been considered as heuristic rule, in terms of danger for human health (Silla-Santos, 1996). This dominance of turkey meat correlated well with the lower levels of free amino acids observed; this was expected in view of the microbial-mediated decarboxylation pathways. Note that lactic acid bacteria (among other food-borne adventitious microorganisms) possess decarboxylase activity – chiefly tyrosine-, histidine-, lysine- and arginine-decarboxylases, so availability of Tyr, His, Lys and Arg is a must, but their decreasing levels are associated with increasing levels of the corresponding biogenic amines. Rabie et al. (2010) reported total biogenic amine contents in Egyptian fermented sausage ranging from 277 to 5815 mg/kg, within 30 d of storage, which is well above our large sample of European sausages manufactured from less conventional meats. The aforementioned distinct contents may correlate with different conditions of manufacture, besides the type of source meat and the associated variability of adventitious (or added) microflora; lack of quality control during manufacture has been claimed by Bodmer, Irmak, and Kneubühl (1999) to also play a role.

With regard to histamine specifically, the lowest level (2.96 mg/kg) was observed in beef sausage by 0 d, and the highest (263.45 mg/kg) in turkey sausage by 28 d ($P < 5\%$); however, the content of this biogenic amine in beef sausage by 28 d (31.24 mg/kg) was 3-fold its horse counterpart (P b 5%). This biogenic amine is directly influenced by the level of histidine, which must be in excess of normal microbial growth requirements

(Eitenmiller & de Souza, 1984); in all cases (except horse sausage by 28 d), the level of histidine remained essentially constant, so it appears that the excess release of that amino acid was used up in full to synthesize histamine. Taylor, Lieber, and Leatherwood (1978) reported similar results for histamine levels in various dry sausages; in Turkish sausages, histamine concentrations were 6.72-362.22 mg/kg (Şenöz et al., 2000) or 0.85-378.29 mg/kg (Bozkurt & Erkmen, 2002), but values as low as 50 (Stratton, Hutkins, & Taylor, 1991) and 100 (Eerola, Xavier, Lilleberg, & Aalto, 1997), and as high as 768 (Rabie et al., 2010) and 1000 mg/kg (Erginkaya & Varlik, 1989) have also been reported. The aforementioned levels will likely pose a risk for public health in the case of turkey sausages: recall that allowable limits for histamine are 40-100 mg/kg, and levels above 100 mg/kg may already cause some degree of poisoning (Maijala, Eerola, Aho, & Hirn, 1993). However, the actual toxicological level is difficult to establish a priori because it depends on the physiological responses of each individual, coupled with presence (or not) of other biogenic amines (Halász et al., 1994; ten Brink, Damink, Joosten, & Huis in't Veld, 1990).

Putrescine was the chief biogenic amine found in turkey and beef sausages, and its content increased 3.4- and 7.1-fold, respectively ($P < 5\%$), as storage time elapsed. The level of this biogenic amine is supposed to correlate with its precursor free amino acid, ornithine, via a synergistic deiminase-decarboxylase mechanism (Virgili et al., 2007); however, ornithine was not detected in our sausages.

The content of tyramine remained also high in beef and turkey sausages, and a high relative increase (2.9-fold) within 28 d occurred in horse sausages (P b 5%). This biogenic amine increases in content as its precursor tyrosine is released throughout ageing; this can be realised by the almost constant levels of tyrosine in beef and turkey sausages, despite the fact that this amino acid is being continuously released by proteolysis. Recall that total intakes of 10-80 mg/d of tyramine has proven toxic to sensitive people, and levels above 100 mg/kg may cause migraine (Maijala et al., 1993). These ranges are consistent with those encountered before in several Southern European sausages, e.g. Spanish *chori- zo*, *salsichón* and *fuet* (Bover-Cid & Holzapfel, 1999; Hernandez-Jover et al., 1996), Austrian salami (Pechanek, Woidich, & Pfannhauser, 1983), Italian *salsiccia* and *soppressata* (Parente et al., 2001), and Belgian fermented sausages (Ansorena et al., 2002). Shalaby (1996) suggested that tyramine levels within 100-800 mg/kg (as well as histamine levels within 50-100 mg/kg, for that matter) are still compatible with Good Manufacturing Practices.

The highest content of cadaverine (9.18 mg/kg) was found in tur- key sausage by 7 d, whereas the lowest (1.36 mg/kg) was detected in horse sausage by 0 d ($P < 5\%$); its content in beef sausage was essentially constant as storage time elapsed, but an increasing tendency in horse and a decreasing one in turkey were observed. Cadaverine originates via decarboxylation of lysine, yet the concentration of the latter increased in all types of sausage tested as time elapsed. A relatively high content of cadaverine (as well as putrescine) enhanced the toxicity of histamine in turkey meats (Jung & Bjeldanes, 1979), likely due to inhibition of detoxifying enzymes.

Finally, the maximum contents of spermidine and spermine in horse sausage were 6.86 for spermidine and 18.92 mg/kg for spermine, by 0 and 28 d; 9.85 for spermidine and 24.54 mg/kg for spermine in beef sausage, by 28 d; and 10.72 for spermidine and 11.30 mg/kg for spermine in turkey sausage, by 0 and 21 d (P b 5%). There is in general a decreasing pattern of the concentration of spermidine (especially in the case of turkey sausage) and an increasing pattern of the concentration of spermine in all types of sausage. This realisation is consistent with the synthesis of spermine from spermidine, and of spermidine from putres- cine (Bodmer et al., 1999), although the rate of formation of putrescine is far higher, chiefly in the case of beef and turkey sausages.

4. Conclusions

The concentration of total free amino acids increased significantly ($P < 5\%$) during refrigerated storage up to 28 d in the case of horse and beef sausages; however, it decreased for turkey sausage, along with notorious increases of histamine, tyramine and putrescine levels. In particular, histamine in turkey sausage attained a level that is twice the maximum recommended (and enforced) by EC regulations; hence, special care should be exercised regarding consumption of this type of sausage, especially by health compromised or otherwise susceptible individuals.

Acknowledgements

Author M. A. Rabie received a postdoctoral fellowship (ref. SFRH/BPD/ 67089/2009) from Fundação para a Ciência e a Tecnologia (Portugal) and Fundo Social Europeu (III Quadro Comunitário de Apoio), supervised by author F. X. Malcata.

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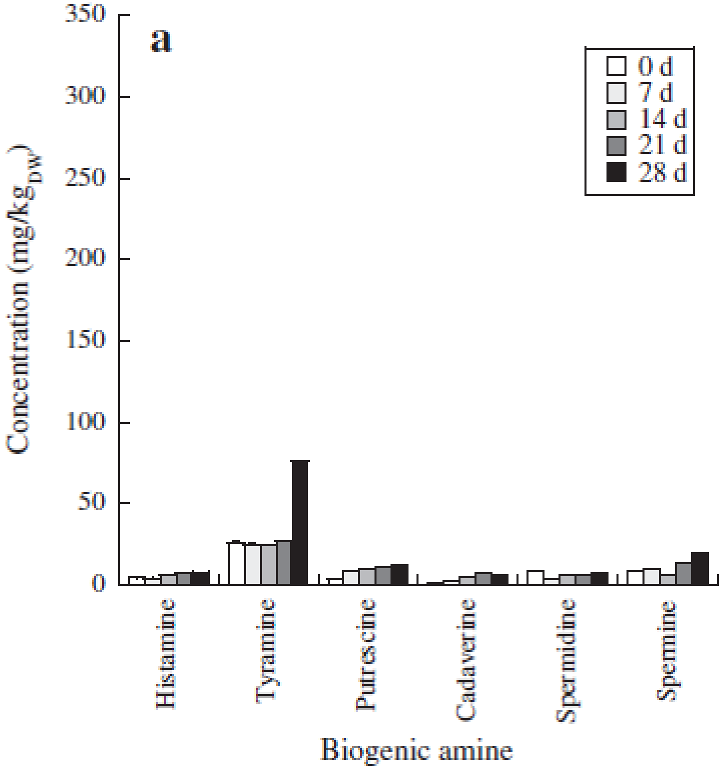
a
Concentration (mg/kg$_{DW}$)
350
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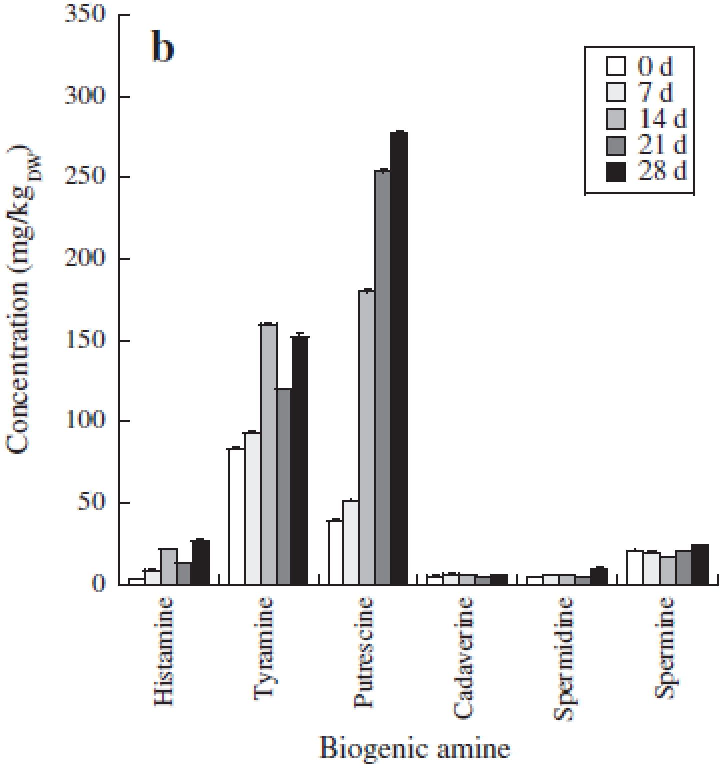
Histamine
Tyramine
Putrescine
Cadaverine
Spermidine
Spermine
Biogenic amine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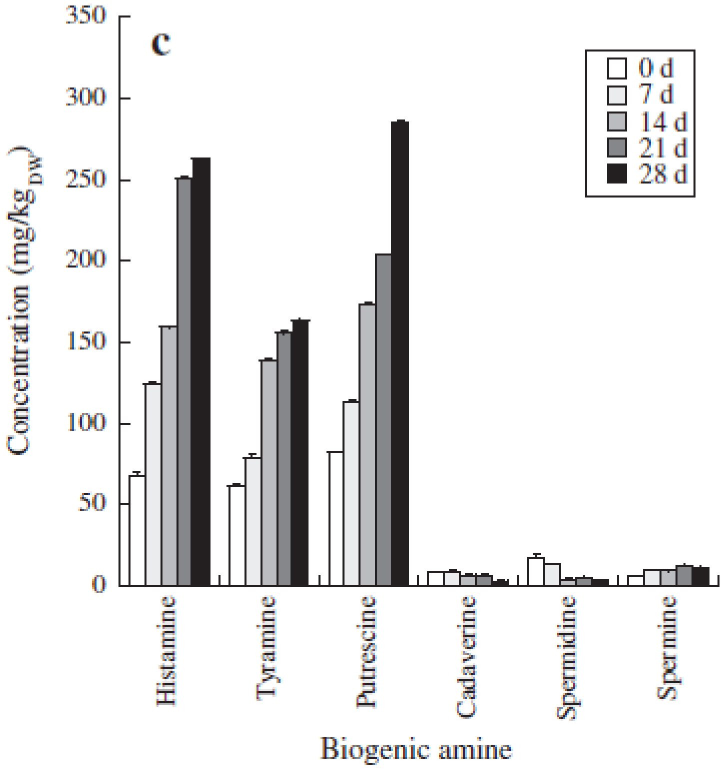


Fig. 1. Evolution, with storage time, of the concentration of selected biogenic amines in (a) horse, (b) beef and (c) turkey sausages (average ± standard deviation, $n = 3$)

Table 1

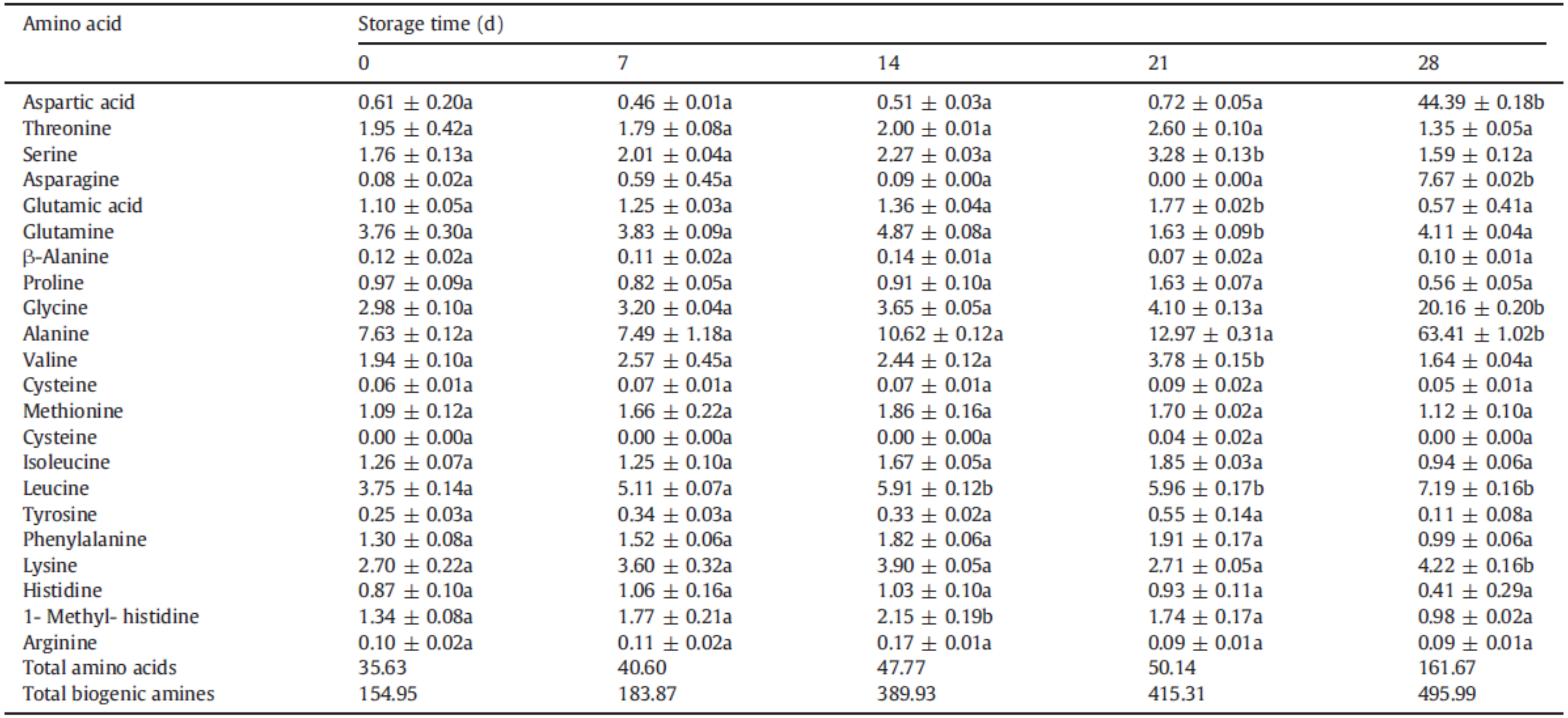
Evolution, with storage time, of the concentration (mg/kgDW) of each and the total amino acids, and the total biogenic amines in horse sausages (average ± standard deviation,

n = 3; figures within the same line followed by different letters are significantly different at *P* b 0.05).

Amino acid	Storage time (d)				
	0	7	14	21	28
Aspartic acid	0.20 ± 0.01a	0.47 ± 0.01a	1.14 ± 0.15a	0.30 ± 0.02a	3.91 ± 0.19b
Threonine	0.43 ± 0.02a	0.78 ± 0.03a	1.30 ± 0.13a	0.54 ± 0.01a	6.26 ± 0.01b
Serine	0.65 ± 0.01a	1.34 ± 0.04a	1.91 ± 0.06a	0.89 ± 0.01a	5.52 ± 0.63b
Asparagine	0.00 ± 0.00a	0.02 ± 0.01a	1.62 ± 0.04b	0.06 ± 0.00a	3.00 ± 0.05c
Glutamic acid	0.47 ± 0.01a	0.91 ± 0.01a	1.71 ± 0.04a	0.70 ± 0.04a	8.18 ± 0.60b
Glutamine	0.31 ± 0.06a	1.36 ± 0.01a	0.09 ± 0.03a	1.16 ± 0.04a	4.07 ± 0.35b
β-Alanine	0.00 ± 0.00a	0.04 ± 0.00a	2.33 ± 0.07b	0.04 ± 0.01a	2.81 ± 0.14b
Proline	0.70 ± 0.07a	1.55 ± 0.05b	3.75 ± 0.03c	1.19 ± 0.02b	10.15 ± 0.08d
Glycine	1.54 ± 0.04a	3.07 ± 0.04b	11.86 ± 0.67c	17.36 ± 0.04d	20.96 ± 0.69d
Alanine	3.79 ± 0.02a	7.56 ± 0.03b	2.20 ± 0.05a	5.56 ± 0.31b	6.19 ± 0.03b
Valine	0.58 ± 0.01a	1.11 ± 0.03a	0.02 ± 0.01a	0.79 ± 0.02a	3.04 ± 0.62b
Cysteine	0.00 ± 0.00a	0.02 ± 0.00a	0.49 ± 0.01a	0.00 ± 0.00a	2.08 ± 0.20b
Methionine	0.15 ± 0.02a	0.35 ± 0.03a	0.06 ± 0.01a	0.18 ± 0.03a	23.20 ± 0.46b
Cysteine	0.00 ± 0.00a	0.02 ± 0.01a	1.32 ± 0.02a	0.06 ± 0.00a	1.58 ± 0.06b
Isoleucine	0.29 ± 0.02a	0.59 ± 0.02a	1.60 ± 0.03b	0.43 ± 0.02a	5.54 ± 0.62c
Leucine	0.33 ± 0.03a	0.82 ± 0.03a	0.25 ± 0.01a	0.51 ± 0.01a	6.84 ± 0.67b
Tyrosine	0.14 ± 0.02a	0.20 ± 0.01a	0.35 ± 0.02a	0.16 ± 0.01a	7.09 ± 0.67b
Phenylalanine	0.09 ± 0.01a	0.20 ± 0.02a	0.49 ± 0.01a	0.13 ± 0.00a	5.42 ± 0.55b
Lysine	1.15 ± 0.01a	2.02 ± 0.08a	2.94 ± 0.03a	1.52 ± 0.03a	7.24 ± 0.72b
Histidine	0.42 ± 0.02a	0.78 ± 0.02a	0.88 ± 0.04a	0.68 ± 0.12a	6.39 ± 0.60b
1- Methyl- histidine	0.35 ± 0.01a	0.90 ± 0.02a	1.33 ± 0.04a	9.31 ± 0.03b	7.39 ± 0.08b
Arginine	0.58 ± 0.01a	1.13 ± 0.07a	1.66 ± 0.03a	0.91 ± 0.01a	9.07 ± 0.38b
Total amino acids	12.15	25.21	39.31	42.49	155.95
Total biogenic amines	52.26	53.51	55.59	71.06	126.87

Table 2

Evolution, with storage time, of the concentration (mg/kgDW) of each and the total amino acids, and the total biogenic amines in beef sausages (average ± standard deviation, $n = 3$; figures within the same line followed by different letters are significantly different at *P* b 0.05).



Amino acid	Storage time (d)				
	0	7	14	21	28
Aspartic acid	0.61 ± 0.20a	0.46 ± 0.01a	0.51 ± 0.03a	0.72 ± 0.05a	44.39 ± 0.18b
Threonine	1.95 ± 0.42a	1.79 ± 0.08a	2.00 ± 0.01a	2.60 ± 0.10a	1.35 ± 0.05a
Serine	1.76 ± 0.13a	2.01 ± 0.04a	2.27 ± 0.03a	3.28 ± 0.13b	1.59 ± 0.12a
Asparagine	0.08 ± 0.02a	0.59 ± 0.45a	0.09 ± 0.00a	0.00 ± 0.00a	7.67 ± 0.02b
Glutamic acid	1.10 ± 0.05a	1.25 ± 0.03a	1.36 ± 0.04a	1.77 ± 0.02b	0.57 ± 0.41a
Glutamine	3.76 ± 0.30a	3.83 ± 0.09a	4.87 ± 0.08a	1.63 ± 0.09b	4.11 ± 0.04a
β-Alanine	0.12 ± 0.02a	0.11 ± 0.02a	0.14 ± 0.01a	0.07 ± 0.02a	0.10 ± 0.01a
Proline	0.97 ± 0.09a	0.82 ± 0.05a	0.91 ± 0.10a	1.63 ± 0.07a	0.56 ± 0.05a
Glycine	2.98 ± 0.10a	3.20 ± 0.04a	3.65 ± 0.05a	4.10 ± 0.13a	20.16 ± 0.20b
Alanine	7.63 ± 0.12a	7.49 ± 1.18a	10.62 ± 0.12a	12.97 ± 0.31a	63.41 ± 1.02b
Valine	1.94 ± 0.10a	2.57 ± 0.45a	2.44 ± 0.12a	3.78 ± 0.15b	1.64 ± 0.04a
Cysteine	0.06 ± 0.01a	0.07 ± 0.01a	0.07 ± 0.01a	0.09 ± 0.02a	0.05 ± 0.01a
Methionine	1.09 ± 0.12a	1.66 ± 0.22a	1.86 ± 0.16a	1.70 ± 0.02a	1.12 ± 0.10a
Cysteine	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.04 ± 0.02a	0.00 ± 0.00a
Isoleucine	1.26 ± 0.07a	1.25 ± 0.10a	1.67 ± 0.05a	1.85 ± 0.03a	0.94 ± 0.06a
Leucine	3.75 ± 0.14a	5.11 ± 0.07a	5.91 ± 0.12b	5.96 ± 0.17b	7.19 ± 0.16b
Tyrosine	0.25 ± 0.03a	0.34 ± 0.03a	0.33 ± 0.02a	0.55 ± 0.14a	0.11 ± 0.08a
Phenylalanine	1.30 ± 0.08a	1.52 ± 0.06a	1.82 ± 0.06a	1.91 ± 0.17a	0.99 ± 0.06a
Lysine	2.70 ± 0.22a	3.60 ± 0.32a	3.90 ± 0.05a	2.71 ± 0.05a	4.22 ± 0.16b
Histidine	0.87 ± 0.10a	1.06 ± 0.16a	1.03 ± 0.10a	0.93 ± 0.11a	0.41 ± 0.29a
1- Methyl- histidine	1.34 ± 0.08a	1.77 ± 0.21a	2.15 ± 0.19b	1.74 ± 0.17a	0.98 ± 0.02a
Arginine	0.10 ± 0.02a	0.11 ± 0.02a	0.17 ± 0.01a	0.09 ± 0.01a	0.09 ± 0.01a
Total amino acids	35.63	40.60	47.77	50.14	161.67
Total biogenic amines	154.95	183.87	389.93	415.31	495.99

Table 3

Evolution, with storage time, of the concentration (mg/kgDW) of each and the total amino acids, and the total biogenic amines in turkey sausages (average ± standard deviation, *n* = 3; figures within the same line followed by different letters are significantly different at *P* b 0.05).

Amino acid	Storage time (d)				
	0	7	14	21	28
Aspartic acid	0.53 ± 0.08a	0.99 ± 0.07a	0.38 ± 0.03a	0.62 ± 0.08a	0.56 ± 0.04a
Threonine	0.75 ± 0.05a	1.43 ± 0.09a	0.64 ± 0.00a	0.81 ± 0.02a	0.85 ± 0.04a
Serine	1.22 ± 0.06a	2.35 ± 0.03a	10.97 ± 0.40b	1.56 ± 0.04a	1.55 ± 0.22a
Asparagine	0.29 ± 0.00a	0.00 ± 0.00a	0.27 ± 0.02a	0.00 ± 0.00a	0.00 ± 0.00a
Glutamic acid	0.16 ± 0.01a	0.75 ± 0.05a	0.15 ± 0.03a	0.32 ± 0.04a	0.42 ± 0.02a
Glutamine	0.28 ± 0.02a	0.65 ± 0.08a	0.08 ± 0.02a	0.16 ± 0.07a	0.30 ± 0.02a
β-Alanine	0.00 ± 0.00a	0.03 ± 0.01a	0.02 ± 0.01a	0.25 ± 0.28a	0.02 ± 0.00a
Proline	1.20 ± 0.04a	2.03 ± 0.10a	0.95 ± 0.05a	1.47 ± 0.09a	1.31 ± 0.09a
Glycine	2.23 ± 0.08a	3.91 ± 0.13b	1.80 ± 0.04a	3.18 ± 0.05b	2.33 ± 0.02a
Alanine	35.60 ± 0.75a	6.95 ± 0.04b	3.29 ± 0.06b	6.46 ± 0.15b	4.35 ± 0.13b
Valine	0.75 ± 0.04a	1.66 ± 0.13a	0.63 ± 0.01a	1.12 ± 0.09a	0.94 ± 0.05a
Cysteine	0.00 ± 0.00a	0.02 ± 0.01a	0.11 ± 0.01a	0.02 ± 0.01a	0.00 ± 0.00a
Methionine	0.29 ± 0.02a	0.56 ± 0.02a	0.21 ± 0.02a	1.77 ± 0.04b	0.34 ± 0.01a
Cysteine	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Isoleucine	0.53 ± 0.07a	0.93 ± 0.05a	0.42 ± 0.07a	0.51 ± 0.07a	0.53 ± 0.02a
Leucine	1.04 ± 0.01a	1.99 ± 0.04b	0.88 ± 0.07a	0.99 ± 0.03a	1.22 ± 0.02a
Tyrosine	0.14 ± 0.06a	0.00 ± 0.00a	0.07 ± 0.00a	0.60 ± 0.62a	0.10 ± 0.00a
Phenylalanine	0.29 ± 0.02a	0.54 ± 0.08a	0.19 ± 0.00a	0.30 ± 0.05a	0.33 ± 0.01a
Lysine	1.65 ± 0.15a	2.89 ± 0.10b	1.25 ± 0.02a	0.03 ± 0.02a	1.75 ± 0.06a
Histidine	0.25 ± 0.02a	0.46 ± 0.02a	0.19 ± 0.02a	0.13 ± 0.02a	0.24 ± 0.03a
1- Methyl- histidine	0.63 ± 0.05a	1.22 ± 0.03a	0.47 ± 0.01a	0.46 ± 0.02a	0.65 ± 0.03a
Arginine	0.15 ± 0.02a	0.20 ± 0.03a	0.12 ± 0.03a	0.11 ± 0.01a	0.15 ± 0.03a
Total amino acids	48.01	29.51	23.09	20.87	17.93
Total biogenic amines	244.55	348.75	492.59	634.72	730.30