Meat Science 94 (2013) 369–375

Contents lists available at SciVerse ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Changes in biogenic amine levels during storage of Mexican-style soft and Spanish-style dry-ripened sausages with different a_w values under modified atmosphere

Roberto González-Tenorio [a], Beatriz Fonseca [b], Irma Caro [b], Ana Fernández-Diez [b], Victor Kuri [c], Sergio Soto [a], Javier Mateo [b,*]

[a] Institute of Agricultural and Livestock Sciences, Universidad Autónoma del Estado de Hidalgo, Av. Universidad s/n Km 1 Rancho Universitario CP. 43600. Tulancingo, Hidalgo, Mexico
[b] Department of Food Hygiene and Technology, University of León, Campus Vegazana s/n, 24007 León, Spain
[c] Food, Health and Nutrition, School of Biomedical and Biological Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, United Kingdom

ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form 13 March 2013
Accepted 17 March 2013

Keywords:
Tyramine
Fermented meat
Sausage storage
Chorizo
Sausage packaging

ABSTRACT

Two raw sausages were prepared: a soft and a dry-ripened one, both by local traditional and industrial manufacturing practices. Sausages were packaged under a CO_2/N_2 atmosphere at different targeted activity water (a_w) values: 0.96 and 0.92 (soft sausages) and 0.88 and 0.82 (dry-ripened sausages). Sausages were then stored at 5 °C for 42 days or at 12 °C for 240 days (soft and a dry-ripened sausages, respectively). The time-related changes in dominant microbiota, pH and biogenic amine contents during storage were determined. Tyramine was the most abundant biogenic amine in all the sausages. Biogenic amine levels were higher in dry-ripened sausages than in soft sausages at packaging. However, during refrigerated storage soft sausages were fermented and the levels of biogenic amines increased ($P < 0.05$). At the end of storage, traditional soft sausages with 0.96 a_w presented comparable levels of biogenic amines to traditional dry-ripened sausages.

1. Introduction

Among the amines found in food, monoamines (histamine, tyramine, phenylethylamine, serotonin, tryptamine) and diamines (cadaverine, putrescine) are mainly formed by microbial enzymatic decarboxylation of free amino acids during food processing and storage, and are considered as biogenic amines. Furthermore, polyamines and also diamines can originate from de novo synthesis carried out in plant and animal tissues, where they play physiological roles (Ruíz-Capillas & Jiménez-Colmenero, 2004; Silla-Santos, 1996). The consumption of food with high contents of biogenic amines constitutes a health hazard (Shalaby, 1996). The most important toxic effects are shown by histamine and tyramine (Stratton, Hutkins, & Taylor, 1991). Moreover, putrescine and cadaverine, not considered toxic per se, can enhance the toxic effect of histamine and tyramine (Sattler, Häfner, Klotter, Lorenz, & Wagner, 1988). Additionally, spermidine and spermine have been associated with the development of food allergies (Lux, Marton, & Baylin, 1980). It is difficult to establish limits of toxicity for the amines in a given product. Amine toxicity not only depends on their presence, but also on dietary factors and consumers' susceptibility (EFSA Panel on Biological Hazards, 2011; Ruíz-Capillas & Jiménez-Colmenero, 2004).

Fermented sausages tend to have high levels of biogenic amines (Ruíz-Capillas & Jiménez-Colmenero, 2004), with tyramine, cadaverine and putrescine being the most prevalent (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000a; Hernández-Jover, Izquierdo-Pulido, Veciana-Nogues, Marine-Font, & Vidal-Carou, 1997). Lactic acid bacteria (LAB) seem to be the main producers of biogenic amines in fermented meat (Masson, Talon, & Montel, 1996). *Enterobacteriaceae* can also be involved in the production of biogenic amines, mainly putrescine and cadaverine (Bover-Cid & Holzapfel, 1999; Durlu-Ozkaya, Ayhan, & Vural, 2001). The presence of this microbial group experiences a continuous and drastic decline in sausages during ripening (Lücke, 1985). However, decarboxylases released from *Enterobacteriaceae* in earlier steps of sausage production could be responsible for continued biogenic amine generation (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000b, 2001a).

Mexican-style chorizo is a red-coloured soft-sausage (2.5–4 cm diameter), prepared with minced pork and fat, to which salt, spices, and sometimes curing salts, carbohydrates and vinegar, are added (starter cultures are seldom used). This sausage is stored in manufacturing and retail establishments for periods ranging from hours to weeks after casing, under non-uniform conditions, i.e., refrigeration or room temperature (Hew, Hajmeer, Farver, Glover, & Cliver, 2005), where spontaneous lactic acid fermentation can take place (Kuri, Madden, & Collins, 1995). This type of chorizo is cooked before consumption. On the other hand, Spanish-style chorizo is a red-coloured dry-fermented sausage usually made from a mixture of minced pork and fat, with added salt, spices, additives (curing agents and antioxidants) and starter cultures (optional). Once the mix is stuffed into casings, Spanish chorizos, differently to Mexican chorizos, are dry-ripened, following the typical process in Mediterranean Europe, characterised by ripening periods of one to several months and slow acidification (Demeyer et al., 2000).

* Corresponding author. Tel.: +34 987291247; fax: +34 987291284.
E-mail address: jmato@unileon.es (J. Mateo).

0309-1740/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.03.017

Table 1
Drying and storage conditions of sausages.

Sausage batches[a]	Soft Mexican-style chorizo		Dry-ripened Spanish-style chorizo	
Processing type	Traditional	Industrial	Traditional	Industrial
First drying stage	20 ± 2 °C for 18 h		4 ± 2 °C 75% RH, 18 h	22 ± 2 °C 94% RH, 18 h
Second drying stage	8 ± 2 °C, 75% RH until targeted a_w		11 ± 1 °C, 77% RH until targeted a_w	
Targeted a_w levels (time required)	0.96 (1 day) and 0.92 (≈12 days)		0.86 (≈24 days) and 0.82 (≈30 days)	
Storage conditions	CO_2/N_2 (20%/80%), 5 ± 1 °C		CO_2/N_2 (20%/80%), 12 ± 1 °C	
Storage sampling days	1, 14, 28, 42		1, 60, 150, 240	

RH: Relative humidity; a_w: water activity.
[a] A total of two (soft sausages) or three (dry-ripened sausages) 24-kg batches were produced following traditional and industrial practices. After casing, batches were divided into two sub-batches, each dried in two stages for the time required to obtain two different targeted a_w levels, and sausages were then stored under modified atmosphere.

Levels of biogenic amines in fermented sausages have shown great variation. This variation has been attributed to several factors, namely, ingredients, microbial contamination of raw materials, microbial growth and amine decarboxylase activity during fermentation (Suzzi & Gardini, 2003). Despite the many studies reporting results on the biogenic amine content of fermented sausages the changes in biogenic amine content during storage of dry-ripened sausages has been little studied (Bover-Cid et al., 2000b, 2001a; Komprda et al., 2004). In addition, only one study has been found on the changes of biogenic amines during storage of soft sausages (Ruiz-Capillas & Jiménez-Colmenero, 2010). The aim of the present study is to evaluate the time-related changes of biogenic amine contents in two different sausages, a soft-fresh sausage and a dry-ripened sausage, each produced according to both traditional and industrial methods, during extended storage under a CO_2/N_2 modified atmosphere, at refrigeration- or 12 °C-temperatures (soft-fresh and a dry-ripened sausage, respectively). Furthermore, each group of sausages was packaged at two different a_w values in order to assess the effect of this factor on biogenic amine accumulation.

2. Materials and methods

2.1. Sausage manufacturing and sampling

Two different sausages were prepared and analysed: a soft-fresh Mexican chorizo and a dry-ripened Spanish chorizo, following both local traditional and industrial manufacturing practices. Two batches of soft sausages were produced on different days at the meat processing hall of the Institute of Agricultural and Livestock Sciences (Universidad Autónoma del Estado de Hidalgo, Mexico). For each batch, twenty-four kg of refrigerated pork shoulder meat and back fat purchased at a local slaughterhouse (within 48 h postmortem), in proportions of 70% and 30%, respectively, was simultaneously minced to a 6-mm particle size and then mixed, and the mixture was divided in two equal parts. One part, prepared using a traditional recipe (traditional sausages) consisted of a guajillo chili (*Capsicum annuum*) paste (a blend of equal amounts of dry chilies and water; 30 g/kg of meat and fat), NaCl (20 g/kg), apple vinegar (5 ml/kg), fresh garlic (3 g/kg), cumin powder (2 g/kg), black pepper powder (2 g/kg), clove powder (1 g/kg) and oregano (1 g/kg). The other part (industrial sausages) was mixed with water (100 ml/kg), a commercial mixture (MC-50; Corporación Fabpsa, Mexico D.F., Mexico; 62 g/kg) and curing salt (5 g/kg; with a nitrite proportion of 6%). The MC-50 mixture contained NaCl, sodium nitrite, glucose (5 g/100 g), maltodextrin (5 g/100 g), sodium erythorbate, monosodium glutamate, a powdered condiment mixture composed of chili, garlic, bay leaf, onion, clove, coriander and Spanish paprika, a combination of sorbate, benzoate and parabens as preservatives (1.5 g/100 g), and a mineral filler of calcium silicate (pH 10 in 5% aqueous solution). The most relevant differences between the traditional and industrial sausages consisted of the addition of sodium nitrite, carbohydrates, preservatives and a mineral filler with high pH in the latter sausages, and the use in these sausages of a more water to facilitate the mixing process.

Meat and non-meat ingredients were mixed for 6 min and the mixture kept at 4 °C for 20 h, was then stuffed into natural hog casings (35–38 mm diameter). Drying and storage conditions of the soft sausages are shown in Table 1. First, all sausages were hung and maintained at 20 °C for 18 h, and then half of the sausages were dried at 8 ± 2 °C for the time required to obtain a targeted water activity (a_w) of 0.96 ± 0.01 (1 day), and the other half until reaching a_w 0.92 ± 0.01 (for 12 days). Sausages were packaged under modified atmosphere with a gas mixture of 20% carbon dioxide and 80% nitrogen, using a 150-μm plastic film with oxygen permeability of 30 $cm^3\ m^{-2}\ bar^{-1}\ 24\ h^{-1}$ (at 23 °C and 0% RH) and stored refrigerated for up to 42 days.

Dry-ripened sausages were produced in the Food Processing Hall of the Hygiene and Food Technology Department (University of León) with methods that resemble local manufacturing practices. Three batches (24 kg per batch) of two types of sausages were manufactured, traditional and industrial sausages, each on different days. Sausages were made from pork shoulder (80%) and back fat (20%) purchased from a local slaughterhouse (within 48 h postmortem), which were minced together in a butcher's mincer using a 14-mm diameter sieve. The traditional formulation included Spanish paprika (25 g/kg), NaCl (20 g/kg), fresh garlic (5 g/kg) and oregano (0.5 g/kg) and sodium nitrite (0.15 g/kg). The industrial formulation consisted of the same ingredients and amounts used for the traditional chorizo plus glucose (6 g/kg), lactose (6 g/kg), sodium nitrite (0.15 g/kg) and a starter culture mixture (0.25 g/kg; previously suspended in 4 ml warm boiled water) with *Pediococcus acidilactici* and *Staphylococcus carnosus* strains (Microcampa, La Campana, Valencia, Spain). For each type of sausage, meat and non-meat components were mixed for 6 min at 12–14 °C. This mixture was manually compacted, kept for 18 h at 6 °C, and then stuffed into natural hog casings (34 mm diameter).

The drying and storage conditions for the two types of dry-ripened sausages are shown in Table 1. In the initial stage, traditional sausages were kept for 18 h in a chamber at 70–80% relative humidity (RH), whereas industrial sausages were kept in a warm chamber at high RH for the same time. Afterwards, all sausages were placed in a ripening chamber at 11 °C and 77 RH. For each sausage type, batches were divided into two sub-batches according to the length of their drying period (depending of the targeted final a_w: 0.86 ± 0.02 and 0.82 ± 0.02). Sausages were then packaged under modified atmosphere with the same gas mixture and plastic film characteristics as for the soft sausages and stored at 12 °C for up to 240 days.

A total of 100 g of the initial mixes (mix before casing), for each batch and sausage type, were sampled as baseline samples. In addition, a sausage of each batch, type and targeted a_w (approximately 150 g weight and 25 cm length per soft sausages, and 250 g weight and 50 cm length per dry-ripened sausages) were collected at different days of storage (Table 1; day 1 was the day of packaging). Twenty five g from each