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Biogenic Amines in Low- and Reduced-Fat Dry Fermented Sausages Formulated with Konjac Gel

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ABSTRACT: Biogenic amines in low- and reduced-fat dry fermented sausages made with konjac gel (KG) as pork backfat replacer were studied. An increase ($P < 0.05$) was observed in the microbial count during the fermentation process, reaching levels of over 8 Log cfu/g of total viable microorganisms and lactic acid bacteria. However, no significant differences were observed in the microbiota evolution as a function of the reformulation process (fat and konjac gel content). High levels of physiological amines (spermidine, spermine, and agmatine) were observed in the raw material. From day 2 of the fermentation process an increase ($P < 0.05$) was observed in tyramine and putrescine, which were the predominant amines at the end of the storage period. The increase in these amines was proportional to the presence of KG and fat reduction. This can also be seen for spermine, with agmatine showing the inverse. The biogenic amine levels in these products reformulated with KG are not considered to pose a health risk to consumers.

KEYWORDS: *konjac gel, dry fermented chorizo sausages, microbiology, biogenic amines*

INTRODUCTION

Biogenic amines are formed widely in fermented products (including fermented meat products), mainly by decarboxylation of free amino acids by the action of enzymes of microbiological origin. The biogenic amine content depends on a number of interrelated factors such as the raw material (meat composition, pH, handling and hygienic conditions, etc.), additives (salt, sugar, nitrites, etc.) affecting free amino acid availability, microbiological aspects (bacterial species and strain, bacterial growth, etc.), technical processing of the meat or meat products (e.g., steaks, roasts and hams, and ground, restructured, comminuted, fresh, cooked, smoked, and fermented meats, etc.), and storage conditions (time/temperature, packaging, temperature abuse, etc.). The combined action of all these factors will determine the final biogenic amine profile and concentrations by directly or indirectly determining substrate and enzyme presence and activity.[1−3] Fermented products are among the meat derivates which generally have the highest levels of biogenic amines,[4−7] since the production process is led by microorganisms which may contain amino acid decarboxylase.[8,9] The leading bacterial group during the sausage ripening process is lactic acid bacteria, which constitute the predominant microbiota during most of the process, and lactic acid bacteria are the main producer of biogenic amines.[8−12] The presence of biogenic amines in fermented products is a health and quality concern; therefore, there is evidently interest in the control and reduction of the amounts of biogenic amines in meat derivates and foodstuffs in general.

Like other agrifood sectors, the meat industry is undergoing major transformations, driven among other things by changes in consumer demands, leading to development of healthier meat products. In this context, lipids are among the components that have received the most attention in relation to development of these kinds of meat products.[12] This is especially important in products like dry fermented sausages, popular traditional meat products, which have some negative health concerns because of their high-fat (25−45%) and energetic content (300−450 kcal/100 g), and animal fat fatty acid profiles.[13] This is why fat reduction is generally considered as an important strategy to improve fat content, leading to new or modified traditional formulations.

Different studies have explored the possibilities of fat reduction in dry fermented sausages using fat replacement by lean meat as a formulation strategy, often linked to addition of nonmeat ingredients.[13−16] However, these reformulation processes often increase product toughness due to higher water losses during fermentation. In a previous work,[17] our research group studied a fat reduction strategy in dry fermented sausages based on replacing animal fat by konjac gel (without altering the proportion of lean meat). This study, through an evaluation of the processing and quality characteristics of reduced- and low-fat fermented dry sausage, demonstrated the viability of this reformulation strategy, depending on fat reduction through replacement of animal fat, showing that the reformulated products had acceptable sensory characteristics. With this reformulation strategy, as well as the health effects associated with fat reduction, there are other effects of the presence of konjac gel, including numerous physiological effects and therapeutic applications.[18−20] It has been suggested that fiber-like KGM exert chemoprotective effects in the colonocytes which may prevent the occurrence of colorectal tumors.[21] Although konjac gel has been used in reformulation of various meat products such as frankfurter, bologna, fresh sausages, chicken nuggets, or pâté,[22−28] to our knowledge, no

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other studies have been reported of its application to dry fermented sausage, apart from the one focusing on the technological and sensorial properties carried out by this research group.[17]

Changes in formulation, processing, and preservation conditions can obviously alter various factors (e.g., presence of free amino acids, decarboxylase enzyme content, and suitable environmental conditions), which may potentially affect formation of biogenic amines in quantitative and qualitative terms.[1] This effect has been described in meat product reformulation processes based on incorporation of ingredients such as seaweed or walnut.[29,30] As a result, it would not be appropriate to approach development of healthier products without taking into account these considerations, which may compromise the safety of these products. This is particularly important in dry fermented sausages where, as mentioned above, the presence of biogenic amines is particularly relevant because of their composition and processing.

On this basis, the aim of this paper was to evaluate how compositional changes, associated with the reformulation processes for a new, healthier formulation of dry fermented sausage, can affect the quantitative and qualitative levels of biogenic amines. To do this, a study was carried out on the effect of replacing animal fat (0%, 50%, and 80% of pork backfat) by an equal proportion of konjac gel on formation of biogenic amines and microbial growth during the fermentation and ripening stages in the processing of reduced- and low-fat fermented dry sausage.

MATERIALS AND METHODS

Materials, Konjac Gel, and Dry Fermented Sausage Preparation (Chorizo). Raw meat materials and additives as well as konjac gel (KG) and dry fermented preparation have been reported by Ruiz-Capillas et al.[17] Briefly, fresh postrigor pork meat and pork backfat were obtained from a local market. The KG was prepared in triplicate as follows: 5% konjac flour (glucomannan 83%, 120 mesh) from Trades S.A. (Barcelona, Spain) was homogenized (Stephan Universal Machine UM5, Stephan Machinery GmbH and Co., Hameln, Germany) with water (64.8%) for 3 min, left to rest for 5 min, and then homogenized for a further 3 min, i-carrageenan (1%) (Hispanagar S.A, Burgos, Spain) was then added, and the mixture was homogenized again for 3 min. Pregelled cornstarch powder (3%) (Amigel, Julio Criado, S.L. Madrid, Spain) was dispersed in 16.2% of water and homogenized with a mixture of konjac flour and i-carrageenan, left to rest for 5 min, and then homogenized for a further 3 min. The mixture was cooled to 10 °C, and then 10% of a $Ca(OH)_2$ solution (1%) was added with gentle stirring at room temperature. Konjac gel was placed in suitable containers (so as to form blocks resembling native pork backfat) and stored at 2 ± 2 °C until used (within 24 h of preparation).

Three different formulations of dry fermented sausage (chorizo) were produced: a control sample, prepared with normal fat content (NF), using 74% meat and 18.5% pork backfat; a reduced-fat sample (RF) prepared with 74% meat and replacing 50% of added pork backfat by the same proportion of konjac gel; and, finally, another low-fat sample (LF) formulated with 74% meat and replacing 80% pork backfat by the same proportion of konjac gel. All samples also contained 5.5%, 0.18%, and 1.85% of the two commercial curing salts "choravi", "curavi" (ANVISA, Arganda del Rey, Spain), and NaCl, respectively. In these formulation conditions the pork backfat proportions were 18.5%, 9.2%, and 3.7%, and the konjac gel proportions were 0%, 9.2%, and 14.8% in NF, RF, and LF, respectively. No starter culture was used. Preparation of this chorizo-type dry fermented sausage was reported in detail by Ruiz-Capillas et al.[17] Briefly, the konjac gel and backfat were minced at 15 mm (Vam.Dall. Srl. Modelo FTSIII, Treviglio, Italy) and then mixed manually with the meat (1 min) and minced at 15 mm again. This batter was homogenized with the additives ("choravi", "curavi", and NaCl). In all cases the final temperature was less than 11 °C. The prepared sausage mixture was stuffed into collagen casing (Fibran S. A. Sant Joan de les Abadesses, Gerona, Spain) using a 4 cm diameter stuffer (MAINCA, Granollers, Barcelona, Spain), and the sausages were placed in a ripening cabinet (BINDER model KBF 240 Tuttlingen, Germany) programmed to operate under following conditions: one fermentation step for 48 h at 23 °C and a relative humidity (RH) of 90% followed by another ripening process step at 13 °C, 70−80% RH, until the end of the experiment (17 days). To monitor the ripening process, samples from each formulation were taken periodically for analysis.

Proximate Analysis of Dry Fermented Sausages. Sample moisture and ash contents were determined[31] in triplicate in all samples. Protein content was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corp., St. Joseph, MI). Fat content was evaluated in triplicate according to Bligh and Dyer.[32] Carbohydrates were calculated taking into account ingredient composition (konjac flour, i-carrageenan, and cornstarch plus the curing salts "choravi") and amount used.[17]

Microbiological Analysis. Microbiological analysis of sausages samples during processing (fermented and ripening steps) was carried out as follows: 10 g of each sample (from 2 sausages) was taken and placed in a sterile plastic bag with 90 mL of peptone water (0.1%) with 0.85% NaCl. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, U.K.), appropriate decimal dilutions were pour plated (1 mL) on the following media: Plate Count Agar (PCA) (Merck, Germany) for the total viable count (TVC) (30 °C for 72 h); De Man, Rogosa, Sharp Agar (MRS) (Merck, Germany) for lactic acid bacteria (30 °C for 3−5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37 °C for 24 h). All microbial counts were converted to logarithms of colony-forming units per gram (Log cfu/g).

Analysis of Biogenic Amines by Ion-Exchange Chromatography. Tyramine, phenylethylamine, histamine, putrescine, cadaverine, agmatine, triptamine, spermidine, and spermine were determined in an extract prepared by blending 25 g of each sample (two sausages were taken from sample) with 50 mL of 7.5% trichloroacetic acid in an omnimixer (Omni Internacional, Waterbury, CT) (20000 rpm, 3 min) and centrifuged at 5000*g* for 15 min at 4 °C in a desktop centrifuge (Sorvall RTB6000B, DuPont, USA). Supernatants were filtered through a Whatman no. 1 filter and passed back through a 0.22 μm Nylon filter (Millipore, Ireland). This filtrate was injected into an HPLC model 1022 with a Pickering PCX 3100 postcolumn system (Pickering Laboratories, Mountain View, CA) following the methodology described by Triki et al.[33] The results are the mean of at least 3 determinations.

Statistical Analysis. In the processing determinations two-way analyses of variance (ANOVA) as a function of type of fermented sausage (NF, RF, LF) and processing time were performed. Tukey's HSD test was used to identify significant ($P < 0.05$) differences between type of fermented sausage and time and least-squares differences for comparison of mean values between different sausages. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL). The experiment was replicated twice.

RESULTS AND DISCUSSION

Proximate Analysis. The proximate composition of dry fermented sausages (Figure 1) was affected ($P < 0.05$) by formulation (partial replacement of pork backfat with KG) as described previously by Ruiz-Capillas et al.[17] In the reduced-fat (RF) and low-fat (LF) sausages, the fat levels were 19.69% and 13.79%, respectively, compared with 29.96% of the batch with normal fat (NF, control). Under these experimental conditions a general trend was observed that as the fat content is reduced the proportion of moisture, protein, ash, and carbohydrates increases (Figure 1).

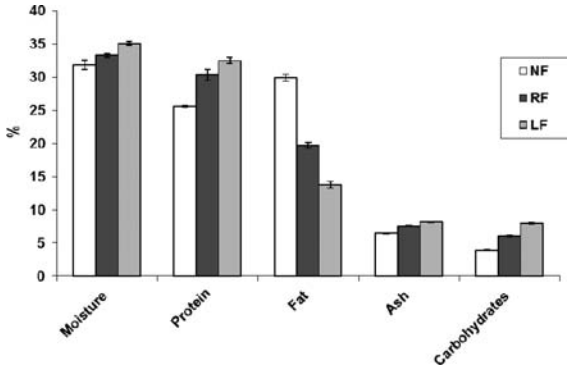

Figure 1. Proximate analysis (%) of dry fermented sausage chorizo samples with different levels of fat (normal fat (NF), reduced fat (RF), and low fat (LF)). Tool bars indicate the standard error of the individual data.

Microbiology Analysis. The results of microbiology counts performed on the different products during the manufacturing process are shown in Table 1. The initial levels (in raw material) of total viable micro-organisms and lactic acid bacteria were 6.34−6.72 and 4.54−4.69 Log cfu/g, respectively, being lower in sausages with added konjac gel. After day 2 of processing, during the fermentation period, a sharp and significant increase was observed (two logarithmic units) in the microbial population, reaching levels higher ($P < 0.05$) than 8 and 6 Log cfu/g in the total viable and lactic acid bacteria, respectively (Table 1). This increase was produced mainly by the fermentation process conditions (48 h at 23 °C RH 90%, see Materials and Methods), which favor growth of the micro-organisms, mainly lactic acid bacteria, with a corresponding rapid decrease in pH,[17] due to the metabolic activity of these bacteria. A similar behavior of lactic acid bacteria has been described in other studies on the processing of chorizo.[34]

On day 7 of the process, during ripening, an increase in the level of micro-organisms was observed, significant in the case of the lactic acid bacteria which became (quantitatively) the predominant flora. These levels were maintained without significant changes until the end of processing. The lower growth rate of the microbiota in this second phase of the chorizo processing may be attributed to the ripening process conditions (13 °C, RH 70−80), as indicated in a previous study.[17] This evolution has also been reported in other studies of dry chorizo sausages.[34,35] Other authors have pointed out that the temperature at which fermentation takes place (usually between 7 and 28 °C) is a factor influencing the growth of micro-organisms, thus ensuring favorable conditions for starter growth.[4,6,36] The influence of the processing temperature is that the higher fermentation temperature gives the starter culture the opportunity to outgrow nonstarter lactic acid bacteria.[6,36]

Initial enterobacteria counts were 2.11−2.30 Log cfu/g, with little change in quantitative terms during processing time. Similar values to those described in this experiment have been reported by other authors in dry fermented chorizo sausage[34] and salami sausage.[4]

Significant differences were not observed in the microbiota evolution in terms of the fat and konjac gel content used in the chorizo reformulation, which suggests that the processing conditions are the main factor affecting the micro-organism growth, rather than the product composition (fat levels and presence of konjac). In agreement with the results of this experiment, various authors[15,37] did not observe variations in microbial growth in fermented sausage as a function of substitution of pork backfat by an oil-in-water emulsion. No differences in lactic acid bacteria and *Enterobacteriaceae* counts were observed between the high-fat and the low-fat control fermented sausages, although in this case fat reduction was achieved through an increase in lean meat.[14,38] Similarly, no differences were observed in microbial growth from the effect of incorporating fiber into dry fermented sausages with different fat levels.[39,40]

Biogenic Amines. During the production processes (fermentation and ripening) of fermented meat products such as chorizo a series of factors concur (including the nature of the matrix and the environmental conditions), which favor growth of the micro-organisms responsible for biogenic amine formation.[1,8,36,41]

Evolution of the biogenic amine content throughout the manufacturing process of reformulated chorizo is shown in Table 2. In the raw material, at the start of the process, spermidine, agmatine, and spermine had the highest levels of biogenic amines. The presence of these amines is found naturally in meat, which is a very important source of spermine.[5,7] The concentration of these amines varies with the meat source; pork meat contains lower concentrations of

Table 1. Microbiological Counts (Log cfu/g) of Raw Material and Dry Fermented Sausage (Chorizo) Samples with Different Levels of Fat [Normal Fat (NF), Reduced Fat (RF), and Low Fat (LF)] during Fermentation and Ripening Processes[a]

		days of processing				
microorganism	sample	raw material	2	7	13	17
total viable count	NF	6.72 ± 0.12^{a1}	8.51 ± 0.23^{b1}	8.98 ± 0.42^{b1}	8.92 ± 0.06^{b1}	8.71 ± 0.05^{b1}
	RF	6.57 ± 0.15^{a1}	8.59 ± 0.05^{b1}	9.06 ± 0.03^{c1}	8.83 ± 0.02^{bc1}	8.78 ± 0.00^{bc1}
	LF	6.34 ± 0.08^{a1}	8.56 ± 0.00^{b1}	9.10 ± 0.02^{b1}	8.97 ± 0.02^{b1}	8.94 ± 0.14^{b1}
lactic acid bacteria	NF	4.65 ± 0.04^{a1}	6.48 ± 0.00^{b1}	9.09 ± 0.12^{c1}	8.95 ± 0.07^{c1}	9.00 ± 0.00^{c1}
	RF	4.54 ± 0.18^{a1}	6.48 ± 0.00^{b1}	9.18 ± 0.04^{c1}	8.82 ± 0.12^{c1}	8.91 ± 0.12^{c1}
	LF	4.69 ± 0.09^{a1}	6.48 ± 0.00^{b1}	9.18 ± 0.10^{c1}	9.06 ± 0.03^{c1}	9.05 ± 0.14^{c1}
Enterobacteriaceae	NF	2.30 ± 0.26^{a1}	2.08 ± 0.67^{a1}	2.24 ± 0.65^{a1}	2.28 ± 0.00^{a1}	2.37 ± 0.41^{a1}
	RF	2.25 ± 0.03^{a1}	1.95 ± 0.49^{a1}	2.72 ± 0.09^{b1}	3.16 ± 0.59^{c2}	2.84 ± 0.56^{bc1}
	LF	2.11 ± 0.10^{a1}	3.10 ± 1.44^{b2}	2.76 ± 0.12^{ab1}	2.13 ± 0.02^{a1}	2.30 ± 0.86^{a1}

[a]Means ± standard deviation. Different letters (a, b, c) in the same row and different numbers (1, 2, 3) in the same column indicate significant differences ($P < 0.05$).

Table 2. Biogenic Amine Contents (mg/kg) of Raw Material and Dry Fermented Sausage (Chorizo) Samples with Different Levels of Fat [Normal Fat (NF), Reduced Fat (RF), and Low Fat (LF)] during Fermentation and Ripening Processes[a]

		days of processing				
biogenic amine	sample	raw material	2	7	13	17
tyramine	NF	0.00 ± 0.00^{a1}	34.41 ± 2.65^{b1}	57.29 ± 1.83^{c1}	37.73 ± 0.25^{d1}	41.67 ± 0.12^{e1}
	RF	0.00 ± 0.00^{a1}	57.08 ± 0.10^{b2}	47.6 ± 0.18^{c2}	59.78 ± 0.21^{d2}	68.89 ± 0.15^{e2}
	LF	0.00 ± 0.00^{a1}	62.00 ± 0.20^{b3}	49.24 ± 0.19^{c3}	67.38 ± 0.16^{d3}	183.44 ± 1.20^{e3}
putrescine	NF	0.00 ± 0.00^{a1}	1.36 ± 0.26^{ab1}	6.78 ± 0.18^{b1}	17.51 ± 0.65^{c1}	18.88 ± 1.4^{c1}
	RF	0.00 ± 0.00^{a1}	7.79 ± 0.41^{b2}	31 ± 0.39^{c2}	73.87 ± 1.99^{d2}	85.59 ± 6.15^{e2}
	LF	0.00 ± 0.00^{a1}	13.12 ± 0.30^{b2}	44.84 ± 1.74^{c3}	101.99 ± 2.71^{d3}	115.92 ± 5.00^{e3}
tryptamine	NF	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.55 ± 0.29^{b1}	1.10 ± 0.30^{c1}
	RF	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	2.35 ± 0.35^{b2}	2.7 ± 0.38^{b3}
	LF	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	2.60 ± 0.16^{c2}	1.66 ± 0.10^{b2}
agmatine	NF	8.64 ± 2.40^{a1}	21.56 ± 1.40^{c2}	20.59 ± 0.14^{bc2}	21.49 ± 0.75^{c3}	18.95 ± 1.39^{b3}
	RF	7.89 ± 0.11^{a1}	26.59 ± 0.01^{e3}	21.54 ± 0.26^{d2}	15.48 ± 0.04^{c2}	13.41 ± 0.31^{b2}
	LF	12.37 ± 0.25^{b2}	17.58 ± 0.26^{c1}	6.74 ± 0.70^{a1}	6.34 ± 0.02^{a1}	6.78 ± 0.60^{a1}
spermidine	NF	1.76 ± 0.42^{b2}	1.53 ± 0.35^{ab2}	1.33 ± 0.01^{a2}	2.29 ± 0.11^{c2}	1.69 ± 0.05^{ab1}
	RF	0.61 ± 0.03^{a1}	0.82 ± 0.10^{a1}	0.95 ± 0.03^{a1}	1.82 ± 0.24^{b1}	1.46 ± 0.04^{b1}
	LF	1.41 ± 0.01^{ab2}	1.07 ± 0.15^{a1}	1.62 ± 0.18^{b2}	2.86 ± 0.16^{d3}	2.22 ± 0.09^{c2}
spermine	NF	21.47 ± 4.89^{a2}	21.92 ± 1.68^{a1}	23.25 ± 0.57^{a1}	29.33 ± 1.25^{b1}	29 ± 2.70^{b1}
	RF	12.96 ± 0.02^{a1}	21.5 ± 0.07^{b1}	25.35 ± 0.25^{b1}	36.03 ± 0.25^{c2}	40.83 ± 1.95^{d2}
	LF	19.46 ± 0.88^{a2}	20.2 ± 0.22^{ab1}	25.12 ± 1.38^{b1}	42.31 ± 0.93^{c3}	53.16 ± 2.56^{d3}

[a]Means ± standard deviation. Different letters (a, b, c) in the same row and different numbers (1, 2, 3) in the same column indicate significant differences ($P < 0.05$).

these physiological amines than beef or chicken.[24,30] Similar levels of spermidine and spermine to those observed in this study were showed by other authors in fresh dry salami and chorizo sausages[4,9,42] and frankfurter sausage-type products made with pork meat.[24]

Other biogenic amines such as histamine, phenylethylamine, cadaverine, tryptamine, tyramine, and putrescine were not detected initially. Low initial levels of these amines have also been observed by other authors in fermented meat products and related to the high quality of the ingredients, especially of the fresh meat used in the product manufacture.[4,7,36] Tyramine and putrescine along with cadaverine (in some cases) are the biogenic amines generally found in the greatest amounts in this type of product during processing.[4−6,34,43] In this study, at the end of the fermentation process (after day 2 of processing) a significant increase ($P < 0.05$) in tyramine and putrescine levels was observed depending on the KG substitution levels. The highest level of pork backfat replacement by konjac gel showed higher values ($P < 0.05$) of these biogenic amines (Table 2). In the case of putrescine this trend was maintained throughout the ripening process, as also in the case of tyramine, except on day 7 of ripening. These biogenic amines were those which presented the highest levels at the end of the storage period, mainly in the batches with KG. The increase in these biogenic amines is linked to an increased level of micro-organisms (Table 1), mainly of lactic acid bacteria and *Staphylococci*, as also reported by other authors.[4,8−12,34,43] These two main groups of bacteria are considered technologically important in the fermentation and ripening of chorizo, with proteolytic and lipolytic activity.[44] This proteolysis leads to formation of the free amino acid precursors of the biogenic amines. However, although biogenic amines are produced by decarboxylation of the free amino acids, the presence of these amino acids does not always indicate formation of the corresponding biogenic amine, since it is affected by numerous factors.[1−3] Amine formation depends on the presence of the amino acid decarboxylase enzyme of the micro-organisms and its activity in the medium.[9,11] In fact, correlations have not always been found between these FAAs and biogenic amines.[1,2] Tyrosine, histidine, ornithine, and serine seem to be better substrates for microbial metabolism.[9]

In the case of agmatine, an increase ($P < 0.05$) was also observed in the fermentation phase (Table 2), but this increment was lower in the batches which contained higher levels of konjac gel (LF), in contrast to what was observed in the case of putrescine and tyramine. A clear trend was only observed in agmatine levels as a function of the KG levels after 7 days ripening. These initial changes in the agmatine may possibly be due to formation metabolism of this biogenic amine which is formed by arginine decarboxylase activity, mainly of microbial origin. Arginine may also lead to the formation of putrescine, which in turn may lead to spermidine and spermine, as formation of these three amines is interrelated.[2] Increased levels of spermidine and spermine were also observed in a study carried out on Spanish dry-cured chorizo sausage treated with high pressure and kept in chilled storage.[43] Martuscelli et al.[45] also observed production of tyramine, spermine, and spermidine from strains of *S. xylosus* from homemade fermented sausages. Perhaps this may explain that after days 13−17 of ripening a slight decrease was observed in agmatine levels, a slight increase observed in spermidine, and a significant increase in spermine, all related to the high levels of micro-organisms detected in these batches (Table 1). Curiel et al.[10] also confirmed the presence of strains of lactic acid bacteria and

enterobacteria producing agmatine as well as those producing tyramine and putrescine associated with fresh pork sausages.

The batches which presented higher levels of spermine from day 7 onward were those reformulated with KG, with proportional increase to KG levels similar to that observed in the case of putrescine and tyramine and not so clearly in the case of spermidine. This may be due to the fact that the batches with a higher KG content have a higher humidity and protein content and lower fat content (Figure 1). It is widely recognized that humidity along with high temperatures favors microbial growth. Various studies have also shown that the fat content influences formation of biogenic amines.[4,5,46] This type of analysis has been studied more often in cheeses, observing that the concentration of biogenic amines decreases along with fat content,[47] similarly to what was observed in this study. It must also be taken into account that not only are quantitative aspects affected (microbial growth) but also growth of certain strains with greater amino acid decarboxylase capacity. Within the same species, the presence, activity, and specificity of decarboxylases are strain specific.[4,43,48] The histamine and tryptamine levels were lower than 3 mg/kg during the whole process, and phenylethylamine was not detected in any of the samples.

The levels of biogenic amines are similar to those observed in chorizo by other authors[7] and higher than those observed by others at the end of ripening[34] except in the case of putrescine, which was lower. These differences may be due to the different raw materials and processing conditions (use of starter, temperature, relative humidity, sugars, etc.), which affect the growth of different microbiota with amino acid decarboxylase capacity.[4,6,8,12,48] Mayr and Schieberle[9] also showed that different amino acid decarboxylases are active in different foods depending on the microorganisms present. However, further studies are underway to clarify the influence of processing parameters in the microorganisms and their corresponding decarboxylase enzymes responsible for formation of amines from their amino acid precursors.

Histamine levels are very low as corresponds to this type of product (with levels lower than 1 mg/kg) and were only observed in the final days of ripening. Similar behavior was observed for tryptamine, where levels were only detected on days 13 and 17 of ripening, with higher levels (2.35−2.7 mg/kg) in the reformulated batches compared with the control batch. The cadaverine levels throughout ripening were <1 mg/kg.

In relation to the safety of these compounds it should be taken into account that consumption of foodstuffs with high levels of the tyramine (vasoactive substance) has been related to different toxic processes, reported as migraine, headache, and raised blood pressure.[1,3] Besides being toxic in itself, recent studies on tyramine have shown that it promotes adhesion to the gastric mucosa by pathogens like *Escherichia coli* O157:H7.[49] It should also be considered that the presence of putrescine enhances the toxicity of tyramine and is therefore also implicated in these processes.[1] However, this toxicity also depends on the detoxification system of the consumer. The human body can rapidly detoxify the histamine and tyramine absorbed from foods by means of the enzymes monoamine oxidase (MAO; EC 1.4.3.4), diamine oxidase (DAO; EC 1.4.3.6), and polyamine oxidase (PAO; EC 1.5.3.11), which play a major role in amine degradation in the human body.[1−3] However, these detoxification mechanisms may be altered by genetic factors, if large amounts are ingested, or if the individual consumer is undergoing treatment with oxidase enzymes (e.g., monoamine oxidase inhibitor, MAOI), which inhibit amino-oxidases or cause amino-oxidase deficiency.[2,3,44] Determination of the exact toxicity threshold of biogenic amines in individuals is extremely difficult. The toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of different individuals. The amount of tyramine considered toxic ranged from 125 to 800 mg/kg.[2] In contrast, 6 mg/kg of tyramine would be toxic if ingested with MAOIs.[1,2] This is particularly important at the present day because of the high consumption of MAOIs as antidepressants. Studies of biogenic amine concentrations in commercially processed Spanish meat products reported that 63% of salchichon sausage samples and 64% of chorizo sausage samples (both ripened meat products) contained enough tyramine to poison consumers taking MAOIs.[50] Other amines like spermidine and spermine have also been associated with food allergies.[3] In this study, these products reformulated with KG seem unlikely to pose any health risk to consumers due to the level of amines present in them.

In this context, the reformulation process of reduced- and low-fat dry fermented sausages with partial replacement of pork backfat by konjac gel modifies the biogenic amine profile without affecting microbial development to any relevant extent and without compromising product safety. Fat reduction linked to the presence of konjac gel favors production of certain biogenic amines (tyramine, putrescine, and spermine) and reduces production of agmatine in dry fermented sausage. Design of healthier meat products, reducing fat content and promoting inclusion of konjac gel, is a promising avenue of research, especially as the safety of these products in relation to the presence of biogenic amines is guaranteed.

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Notes

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REFERENCES

(1) Ruiz-Capillas, C.; Jiménez-Colmenero, F. Biogenic amines in meat and meat products. *Crit. Rev. Food Sci.* **2004**, *44*, 489−599.

(2) Halász, A.; Baráth, A.; Simon-Sarkadi, L.; Holzapfel, W. Biogenic amines and their production by microorganisms in food. *Trends Food Sci. Technol.* **1994**, *5*, 42−49.

(3) Bardócz, S. Polyamines in food and their consequences for food quality and human health. *Trends Food Sci. Technol.* **1995**, *6*, 341−346.

(4) Bover-Cid, S.; Torriani, S.; Gatto, V.; Tofalo, R.; Suzzi, G.; Belletti, N. Relationships between microbial population dynamics and putrescine and cadaverine accumulation during dry fermented sausage ripening. *J. Appl. Microbiol.* **2009**, *106*, 1397−1407.

(5) Hernández-Jover, T.; Izquierdo-Pulido, M.; Veciana-Nogués, M. T.; Vidal-Carou, M. C. Ion-pair high-performance liquid chromatographic determination of biogenic amines in meat and meat products. *J. Agric. Food Chem.* **1996**, *44*, 2710−2715.
(6) Maijala, R.; Nurmi, E.; Fischer, A. Influence of processing temperature on the formation of biogenic amines in dry sausages. *Meat Sci.* **1995**, *39*, 9−22.
(7) Ruiz-Capillas, C.; Jiménez-Colmenero, F. Biogenic amine content in Spanish retail market meat products treated with protective atmosphere and high pressure. *Eur. Food Res. Technol.* **2004**, *218*, 237−241.
(8) Bover-Cid, S.; Hugas, M.; Izquierdo-Pulido, M.; Vidal-Carou, M. C. Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. *Int. J. Food Microbiol.* **2001**, *66*, 185−189.
(9) Mayr, CH. M.; Schiberle, P. Development of stable isotope dilution assays for the simultaneous quantitation of biogenic amines and polyamines in foods by LC-MS/MS. *J. Agric. Food Chem.* **2012**, *60*, 3026−3032.
(10) Curiel, J. A.; Ruiz-Capillas, C.; de las Rivas, B.; Carrascosa, A. V.; Jiménez-Colmenero, F.; Muñoz, R. Production of biogenic amines by lactic acid bacteria and enterobacteria isolated from fresh pork sausages packaged in different atmospheres and kept under refrigeration. *Meat Sci.* **2011**, *88*, 368−373.
(11) de las Rivas, B.; Ruiz-Capillas, C.; Carrascosa, A. V.; Curiel, J. A.; Jiménez-Colmenero, F.; Muñoz, R. Biogenic amine production by Gram-positive bacteria isolated from Spanish dry-cured chorizo sausage treated with high pressure and kept in chilled storage. *Meat Sci.* **2008**, *80*, 272−277.
(12) Roig-Sagués, A. X.; Eerola, S. Biogenic amines in meat inoculated with Lactobacillus sake starter strains and an amine-positive lactic acid bacterium. *Z. Lebensm. Unters. F. A.* **1997**, *205*, 227−231.
(13) Jiménez-Colmenero, F. Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends Food Sci. Technol.* **2007**, *18*, 567−578.
(14) Liaros, N. G.; Katsanidis, E.; Bloukas, J. G. Effect of the ripening time under vacuum and packaging film permeability on processing and quality characteristics of low-fat fermented sausages. *Meat Sci.* **2009**, *83*, 589−598.
(15) Muguerza, E.; Fista, G.; Ansorena, D.; Astiasarán, I.; Bloukas, J. G. Effect of fat level and partial replacement of pork backfat with olive oil on processing and quality characteristics of fermented sausages. *Meat Sci.* **2002**, *61*, 397−404.
(16) Olivares, A.; Navarro, J. L.; Salvador, A.; Flores, M. Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Sci.* **2010**, *86*, 251−257.
(17) Ruiz-Capillas, C.; Triki, M.; Herrero, A. M.; Rodriguez-Salas, L.; Jiménez-Colmenero, F. Konjac gel as pork backfat replacer in dry fermented sausages: Processing and quality characteristics. *Meat Sci.* **2012**, *92*, 144−150.
(18) Al-Ghazzewi, F. H.; Khanna, S.; Tester, R. F.; Piggott, J. The potential use of hydrolysed Konjac glucomannan as a prebiotic. *J. Sci. Food Agric.* **2007**, *87*, 1758−1766.
(19) Tye, R. J. Konjac flour: properties and applications. *Food Technol.* **1991**, *45*, 82−92.
(20) Zhang, H.; Yoshimura, M.; Nishinari, K.; Williams, M. A. K.; Foster, T. J.; Norton, I. T. Gelation behaviour of konjac glucomannan with different molecular weights. *Biopolymers* **2001**, *59*, 38−50.
(21) Chen, H.-L.; Lin, Y.-M.; Wang, Y.-CH. Comparative effects of cellulose and soluble fibers (pectin, konjac glucomannan, inulin) on fecal water toxicity toward Caco-2 cells, fecal bacteria enzymes, bile acids and short chain fatty acids. *J. Agric. Food Chem.* **2010**, *58*, 10277−10281.
(22) Berry, B. W.; Bigner, M. E. Use of carrageenan and konjac flour gel in low-fat restructured pork nuggets. *Food Res. Int.* **1996**, *29*, 355−362.
(23) Chin, K. B.; Keeton, J. T.; Miller, R. K.; Longnecker, M. T.; Lamkey, J. W. Evaluation of konjac blends and soy protein isolate as fat replacements in low-fat bologna. *J. Food Sci.* **2000**, *65*, 756−763.
(24) Delgado-Pando, G.; Cofrades, S.; Ruiz-Capillas, C.; Solas, M. T.; Triki, M.; Jiménez-Colmenero, F. Low-fat frankfurters formulated with a healthier lipid combination as functional ingredient: Microstructure, lipid oxidation, nitrite content, microbiological changes and biogenic amine formation. *Meat Sci.* **2011**, *89*, 65−71.
(25) Jiménez-Colmenero, F.; Cofrades, S.; López-López, I.; Ruiz-Capillas, C.; Pintado, T.; Solas, M. T. Technological and sensory characteristics of reduced/low-fat, low-salt frankfurters as affected by the addition of konjac and seaweed. *Meat Sci.* **2010**, *84*, 356−363.
(26) Kao, W. T.; Lin, K. W. Quality of reduced-fat frankfurter modified by Konjac−Starch mixed gels. *J. Food Sci.* **2006**, *71*, 326−332.
(27) Osburn, W. N.; Keeton, J. T. Konjac flour gel as fat substitute in low-fat prerigor fresh pork sausage. *J. Food Sci.* **1994**, *59*, 484−489.
(28) Osburn, W. N.; Keeton, J. T. Evaluation of low-fat sausage containing desinewed lamb and konjac gel. *Meat Sci.* **2004**, *68*, 221−233.
(29) Ruiz-Capillas, C.; Cofrades, S.; Serrano, A.; Jiménez-Colmenero, F. Biogenic amines in restructured beef steaks as affected by added walnuts and chilling storage. *J. Food Protect.* **2004**, *67*, 607−609.
(30) Cofrades, S.; López-López, I.; Ruiz-Capillas, C.; Triki, M.; Jiménez-Colmenero, F. Quality characteristics of low-salt restructured poultry with microbial transglutaminase and seaweed. *Meat Sci.* **2011**, *87*, 373−380.
(31) Association of Official Analytical Chemistry (AOAC). *Official methods of analysis*, 18th ed.; AOAC: Maryland, 2005.
(32) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.
(33) Triki, M.; Jiménez-Colmenero, F.; Herrero, A. M.; Ruiz-Capillas, C. Optimization of a chromatographic procedure for determining biogenic amine concentrations in meat and meat products employing a cation-exchange column with a post-column system. *Food Chem.* **2012**, *130*, 1066−1073.
(34) González-Fernández, C.; Santos, E. M.; Jaime, I.; Rovira, J. Influence of starter cultures and sugar concentrations on biogenic amine contents in chorizo dry sausage. *Food Microbiol.* **2003**, *20*, 275−284.
(35) González, B.; Díez, V. The effect of nitrite and starter culture on microbiological quality of chorizo a Spanish dry cured sausage. *Meat Sci.* **2002**, *60*, 295−298.
(36) Latorre-Moratalla, L.; Bover-Cid, S.; Vidal-Carou, M. C. Technological conditions influence aminogenesis during spontaneous sausage fermentation. *Meat Sci.* **2010**, *85*, 537−541.
(37) Bloukas, J. G.; Paneras, E. D.; Fournitzis, G. C. Sodium lactate and protective culture effects on quality characteristics and shelf-life of low-fat frankfurters produced with olive oil. *Meat Sci.* **1997**, *45*, 223−238.
(38) Koutsopoulos, D. A.; Koutsimanis, G. E.; Bloukas, J. G. Effect of carrageenan level and packaging during ripening on processing and quality characteristics of low-fat fermented sausages produced with olive oil. *Meat Sci.* **2008**, *79*, 188−197.
(39) Fernández-López, J.; Sendra, E.; Sayas-Barberá, E.; Navarro, C.; Pérez-Alvarez, J. A. Physico-chemical and microbiological profiles of "salchichón" (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Sci.* **2008**, *80*, 410−417.
(40) Salazar, P.; García, M. L.; Selgas, M. D. Short-chain fructooligosaccharides as potencial functional ingredient in dry fermented sausages with different fat levels. *Int. J. Food Sci. Technol.* **2009**, *44*, 1100−1107.
(41) Parente, E.; Martuscelli, M.; Gardini, F.; Grieco, S.; Crudele, M. A.; Suzzi, G. Evolution of microbial populations and biogenic amine production in dry sausages produced in Southern Italy. *J. Appl. Microbiol.* **2001**, *90*, 882−891.
(42) Ruiz-Capillas, C.; Pintado, T.; Jiménez-Colmenero, F. Biogenic amine formation in refrigerated fresh sausage chorizo keeps in modified atmosphere. *J. Food Biochem.* **2011**, DOI: 10.1111/j.1745-4514.2011.00550.x.
(43) Ruiz-Capillas, C.; Jiménez-Colmenero, F.; Carrascosa, A. V.; Muñoz, R. Biogenic amines production in Spanish dry-cured chorizo

sausage treated with high pressure and kept in chilled storage. *Meat Sci.* **2007**, *77*, 365−371.
(44) Silla, M. H. Biogenic amines: Their importance in foods. *Int. J. Food Microbiol.* **1996**, *29*, 213−231.
(45) Martuscelli, M.; Crudele, M. A.; Gardini, F.; Suzzi, G. Biogenic amine formation and oxidation by *Staphylococcus xylosus* strains from artisanal fermented sausages. *Lett. Appl. Microbiol.* **2000**, *31*, 228−232.
(46) Kebary, K. M. K.; El-Sonbaty, A. H.; Badawi, R. M. Effects of heating milk and accelerating ripening of low fat Ras cheese on biogenic amines and free amino acids development. *Food Chem.* **1999**, *64*, 67−75.
(47) Banwart, G. J. *Basic Food Microbiology*; AVI Publishing Co. Inc.:Westport, CT, 1981.
(48) Roig-Sagués, A. X., C. Ruiz-Capillas, Espinosa, D.; Hernández, M. The decarboxylating bacteria present in foodstuffs and the effect of emerging technologies on their formation. In *Biological Aspects of Biogenic Amines, Polyamines and Conjugates*; Dandrifosse, G., Ed.; Transworld Research Network: Trivandrum, India, 2009; pp 201−230.
(49) Lyte, M. Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol.* **2004**, *12*, 14−20.
(50) Vidal-Carou, M. C.; Izquierdo, M. L.; Matín-Morro, M. C.; Marine-Font, A. Histamine and tyramine in meat products: Relationship with meat spoilage. *Food Chem.* **1990**, *37*, 239−249.