Biogenic Amine and Polyamine Contents in Meat and Meat Products

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Biogenic amines and polyamines in meat and meat products were determined by HPLC. Spermine and spermidine were the only amines always detected in meat and meat products, ranging from 6.4 to 62.1 mg/kg for spermine and from 0.7 to 13.8 mg/kg for spermidine. Tyramine, histamine, putrescine, and cadaverine contents varied greatly, especially in ripened products and even among samples from the same commercial brand. Biogenic amines in cooked products were, in general, lower than 10 mg/kg, whereas 40% of ripened products reached levels above 300 mg/kg. Amine content in dry-cured ham was similar to those found in cooked products. High amounts of biogenic amines in some cooked products could be related to the use of low hygienic quality meat. Besides the contribution of the raw materials, amine formation can occur during the fermentative-ripening process. Adverse reactions due to interaction between monoamine oxidase inhibitors drugs and tyramine are expected in ripened products.

Keywords: *Biogenic amines; meat; meat products; tyramine; histamine; polyamines*

INTRODUCTION

Biogenic amines and polyamines are organic bases with an aliphatic, aromatic, or heterocyclic structure which have been found in many foods, such as fishery products, cheese, wine, beer, and other fermented foods (Stratton et al., 1991; Hálasz et al., 1994; Izquierdo-Pulido et al., 1994; Veciana-Nogués et al., 1996). Meat and meat products have also been reported to contain amines (Wortberg and Woller, 1982; Brink et al., 1990; Shalaby, 1993). Although the contents reported on certain amines in ripened sausages are, occasionally, higher than those reported on fish products, there is still a lack of information on the incidence of these compounds in these widely consumed products.

Polyamines play important roles in many human and animal physiological functions (Brink et al., 1990). Spermidine and spermine, both polyamines, are involved in signal transduction and in nearly every step of DNA, RNA, and protein synthesis, being essential for growth and cell proliferation (Tabor and Tabor, 1984; Bardocz et al., 1993). On the other hand, biogenic amines are usually produced during the final processes of protein breakdown by bacterial enzymatic decarboxylation of free amino acids. Consumption of food containing high amounts of some biogenic amines, such as histamine and tyramine, can have toxicological effects. Exogenous biogenic amines constitute a potential health risk especially when coupled with additional risk factors such as monoamine oxidase inhibitors (MAOI), alcohol, and gastrointestinal diseases (Stratton et al., 1991). Legal limits have been established only for histamine in certain fish products (Food and Drug Administration, 1995). However, toxicological levels have been proposed, such as 10−100 mg of histamine/100 g of food and 100−800 mg of tyramine/kg of food (Taylor, 1986; Brink et al., 1990; Stratton et al., 1991). Putrescine and cadaverine have been recognized as potentiators of histamine or tyramine toxicity, but no recommended about levels have been suggested.

Finally, biogenic amines are also of concern in relation to food spoilage (Halász et al., 1994). These amines can occur as a result of bacterial growth and metabolism in meat and are thus indicators of the extent of spoilage (Eerola et al., 1993; Hernández-Jover et al., 1996a). Moreover, biogenic amines can be found as a consequence of microbial activity attributable to the fermentation process involved in the manufacturing of certain sausages (Maijala et al., 1995; Hernández-Jover et al., 1996a).

Since little information is available on the biologically active amine content of most meat products, the following study was performed to provided data on the presence of biogenic amines in meat and in a variety of Spanish meat products, both cooked and ripened. Emphasis was placed on ripened meat products since amine build-up generally occurs in products undergoing fermentation or long-term aging. Furthermore information has been obtained on intersample variability on amine contents of different production batches.

MATERIALS AND METHODS

Samples. *Meat and Meat Products.* All samples were purchased from Spanish retail stores and kept at −18 °C prior to analysis. We analyzed (a) 13 samples of fresh pork and six samples of fresh beef, (b) 48 samples of cooked meat products (20 samples of cooked ham, 20 samples of mortadella, and eight samples of "botifarra catalana", a cooked sausage which has undergone a short ripening process at low temperature before the heat treatment), (c) 23 samples of Spanish dry-cured ham, (d) 60 samples of ripened meat products (22 samples of "salchichón", 20 samples of "chorizo", 11 samples of "fuet", and seven samples of "sobrasada"). These ripened meat products consisted of mixtures of pork and beef meat, seasoned with salt, curing agents, and spices, packed in natural and/or artificial casings, and ripened. The differences between these products are the proportion of fat and meat, the spices used, the diameter of the sausage, and/or the length of ripening process (from 15 days to 2 months).

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Table 1. Polyamine Contents (mg/kg) in Pork and Beef

	pork ($n = 13$)		beef ($n = 6$)	
amines	mean (SD)[a]	range	mean (SD)	range
spermidine	3.0 (1.0)[b]	0.8−4.5	3.1 (0.8)[b]	1.9−4.2
spermine	33.5 (4.4)[c]	27.3−40.6	39.8 (5.8)[c]	28.7−44.6

[a] Mean (standard deviation). Values in the same row bearing a common superscript letter are not different ($p \geq 0.05$).

Biogenic Amine and Polyamine Analysis. Tyramine, histamine, tryptamine, β-phenylethylamine, serotonin, octopamine, dopamine, putrescine, cadaverine, agmatine, spermidine, and spermine were determined following the high-performance liquid chromatographic (HPLC) method described by Hernández-Jover et al. (1996b). The method is based on ion-pair chromatography partition and involves a postcolumn reaction with *o*-phthalaldehyde (OPT) to form fluorescent derivatives with amines. Biogenic amines were extracted from the samples with 0.6 N perchloric acid.

Statistical Analysis. When there were wide variations in the biogenic amine contents, results have been indicated as median and the deviation quartile was used to describe variability (Altman, 1991). Nonparametric tests were applied since data do not follow a normal distribution. Kruskall−Wallis and Dunn's tests were performed using the Statistical Software Package for Windows 6.0.1 (SPSS Inc., Chicago, IL, 1994).

RESULTS AND DISCUSSION

Biogenic Amines and Polyamines in Fresh Meat. Among the amines determined, only spermidine and spermine (both polyamines) were found in all the samples (Table 1). Spermidine and spermine levels were similar in both types of meat. Rogowski and Döhla (1984) and Maijala and Eerola (1993) also reported similar levels of both polyamines in fresh meat. Therefore, it seems that spermine and spermidine are naturally occurring amines in fresh pork and beef meat, and their formation is not due to food spoilage or fermentation processes (Szerdahelyi et al., 1993; Hernández-Jover et al., 1996a,c). Tryptamine, β-phenylethylamine, dopamine, octopamine, and agmatine were not detected in any sample, while tyramine, putrescine, and cadaverine were found only in one sample of pork meat (<3.5 mg/kg) and putrescine, histamine, and serotonin were found in two beef samples (<2.0 mg/kg).

While spermine and spermidine always occur in fresh meat at relatively constant levels, biogenic amines are not usually found in meat. However, some of these amines such as tyramine, putrescine, and cadaverine can be formed during the storage of fresh meat (Maijala et al. 1993, Szerdahelyi et al. 1993, Bauer et al. 1994). In a previous study (Hernández-Jover et al., 1996a), it was found that histamine, tyramine, putrescine, and cadaverine formation occurred during the storage of pork, while spermidine remained constant and spermine slightly decreased. The sum of cadaverine, putrescine, histamine, and tyramine led to an useful biogenic amine index to evaluate meat freshness. Thus, values below 5 mg/kg would indicate meat of high hygienic quality. Therefore, according to the amine contents found here, these meat samples were well below the limit of 5 mg/kg.

Polyamines in Cooked and Cured Meat Products. Spermidine and spermine were the only amines found in all the samples tested, and spermine levels were always higher than those of spermidine, in agreement with the results obtained in fresh meat (Table 2). Levels of polyamines in cooked and ripened meat products were lower than those of fresh meat due to the dilution of meat with fat and other ingredients used in the manufacturing process. In contrast, in dry cured ham, levels of polyamines were slightly higher than in fresh meat due to the drying process involved in the manufacture of this product. Few data are available about contents of spermidine and spermine in foods and particularly in meat products. This is the first time that levels of polyamines in Spanish sausages have been reported. According to our results, and to those previously reported, it can be considered that spermine is the major polyamine in foodstuffs of animal origin, such as meat and fish, while spermidine may be the prevailing amine in vegetable food (Bardocz et al., 1993; Izquierdo-Pulido et al., 1996; Veciana-Nogués et al., 1996).

As pointed out above, both polyamines occur naturally amines in fresh meat. Therefore, their presence in meat products may be due to the use of meat as raw material. Differences in polyamine contents could be attributed to the differences in the manufacture of meat products and could be related to the amount of meat used. Studies in our laboratory with both cooked and cured products showed that the levels of polyamines remained constant throughout the manufacture of these meat products (Hernández-Jover et al., 1996a, 1997).

Agmatine, another polyamine, was detected in only 30% of meat products and always at low levels, ranging from 0.3 to 7.9 mg/kg. No differences in agmatine contents were found among meat products. The presence of this polyamine in foodstuffs is not well documented and information is scarce on its origin and significance. Agmatine production was observed during the storage of fresh anchovies (Veciana-Nogués et al. 1996), but no agmatine formation occurred either during meat spoilage or during the manufacture of meat (Hernández-Jover et al., 1996a, 1997).

Biogenic Amines in Cooked Products and Dry-Cured Ham. In contrast to the relative uniformity of polyamine contents among meat products, the levels of the other amines showed wide fluctuations, especially in cured meat products. Dopamine was not found in any of the samples tested, octopamine was found only in some samples of dry-cured ham (always below 7.0 mg/kg), and serotonin was detected only in a few samples of mortadella (always below 3.0 mg/kg). No previous data are available about the contents of these amines in meat or cooked products. Only Tiecco et al. (1985) reported the presence of dopamine and serotonin in a few samples of Italian salami. Table 3 summarizes the range as well as the median (and the quartile deviation) of tyramine, histamine, putrescine, and cadaverine in cooked meat products. In general, low levels of those amines were found in the different cooked meat products, even though in some cases high levels were detected. No differences in tyramine and histamine were observed among the different types of cooked products, while some differences were found on cadaverine and putrescine in "botifarra catalana" and the other cooked products. Differences in cadaverine and putrescine levels could be related to the fact that "botifarra catalana" has a short ripening step before cooking, which can cause the eventual formation of cadaverine and putrescine. Likewise, the use of low hygienic ground meat could be related to higher amounts of those two amines. Finally, tryptamine and β-phenylethylamine were found only in three samples of mortadella, and always below 5.0 mg/kg.

Table 2. Polyamine Contents (mg/kg) in Spanish Meat Products

meat products	n[a]	spermidine mean (SD)[b]	spermidine range	spermine mean (SD)	spermine range
cooked					
cooked ham	20	2.1 (0.6)[c]	1.4−3.5	21.4 (8.4)[c]	6.4−35.7
mortadella	20	4.0 (2.3)[cd]	1.0−8.9	17.2 (7.5)[c]	7.6−32.2
"botifarra catalana"	8	2.9 (1.5)[c]	1.2−5.3	17.9 (4.8)[c]	12.5−25.8
dry-cured ham	23	5.6 (0.9)[d]	4.4−7.3	35.7 (8.2)[d]	24.9−62.1
ripened					
"chorizo"	20	4.1 (2.5)[d]	1.9−10.0	26.1 (8.1)[c]	13.8−43.5
"salchichón"	22	4.5 (3.1)[d]	0.7−13.8	14.8 (7.6)[c]	6.9−42.5
"fuet"	11	5.1 (3.3)[d]	0.9−11.0	16.8 (6.9)[c]	9.4−30.1
"sobrasada"	7	3.2 (1.8)[cd]	2.1−7.0	13.5 (7.2)[c]	10.3−17.8

[a] Sample size. [b] Mean (standard deviation). Values in the same column bearing a common superscript letter are not different ($p \geq 0.05$).

Table 3. Biogenic Amine Contents[a] (mg/kg) in Spanish Cooked Meat Products

amines	cooked ham ($n = 20$)	mortadella ($n = 20$)	"botifarra catalana" ($n = 8$)
tyramine	nd−78.1	nd−67.0	4.0−22.0
	1.0 (4.2)[b]	2.5 (6.6)[b]	5.1 (5.8)[b]
histamine	nd−0.9	nd−4.8	nd−1.8
	0 (0)[b]	0 (0.4)[b]	0 (0)[b]
putrescine	nd−12. 4	nd−5.7	nd−3.7
	0 (0.4)[b]	1.3 (0.6)[c]	0.7 (0.8)[bc]
cadaverine	nd−9.5	4.3−28.8	nd−40.0
	0.4 (0.5)[b]	1.7 (1.4)[b]	9.6 (6.0)[c]

[a] Range and median (deviation quartile). Values in the same row bearing a common superscript letter are not different ($p \geq 0.05$).

Table 4. Biogenic Amine Contents (mg/kg) in Spanish Dry-Cured Ham

amines	range	median (DQ)[a]
tyramine	nd−46.5	0.7 (3.3)
histamine	nd−150.0	0.8 (0.2)
putrescine	nd−17.4	2.3 (1.6)
cadaverine	nd−305.0	2.1 (8.3)

[a] Mean (deviation quartile).

Dry-cured ham showed, in general, similar levels of amines to those of cooked meat products (Table 4); however, noticeable histamine and cadaverine levels were detected in some samples. Intense proteolysis takes place during the successive stages of the dry-curing process (Aristoy and Toldrà, 1991), mainly attributed to muscle proteinases. Proteolysis yields free precursor amino acids, which can eventually be transformed into biogenic amines; however, no data are available concerning the evolution of biogenic amine during the manufacture of dry-cured ham.

Biogenic Amines in Ripened Meat Products. Amine contents found in ripened meat products were, in general, much higher than those found in cooked meat products or in dry-cured ham (Table 5) and the contents showed wide variations. When amine contents were compared among the different types of ripened meat products ("salchichón", "chorizo", "fuet", and "sobrasada"), no significant differences were found in the amine contents. Tyramine and putrescine were the major amines found, but their contents fluctuated greatly. Despite this variability (from 1.8 to 742.6 mg/kg), tyramine could be considered as the prevailing amine in products of this kind. Several authors (Brink et al., 1990; Vidal-Carou et al., 1990; Bauer et al., 1994) have also pointed out the variability of tyramine levels in ripened meat products. Putrescine is the second most abundant amine, also showing a noticeable variability. Tyramine and putrescine are usually present in fermented meat products as a consequence of the metabolism of fermentative bacteria. The use of "starters" seems to be a good way to reduce the amine production (Maijala et al. 1995; Hernández-Jover et al., 1997). Microorganisms used as "starters" shorten the fermentation process and also reduce the growth of deleterious microorganisms, such as *Enterobacteriaceae* commonly related to biogenic amines formation. However, the influence of low hygienic quality raw materials on the amine formation remains unclear.

Histamine and cadaverine were found, in general, at lower levels than those of tyramine and putrescine, even though some samples showed relatively high levels of histamine and cadaverine (above 100 mg/kg). Histamine was found in 70% of the samples, whereas cadaverine was found in the 85%. Similar or even higher levels of histamine were reported by other authors for different types of fermented sausage (Tiecco et al., 1985, Bauer et al., 1989). Histamine and cadaverine are extensively related to food spoilage in general and also in meat spoilage (Vidal-Carou et al., 1990; Hernández-Jover et al., 1996a). It is still not clear whether the fermentative flora has the ability to produce high amounts of amine. Levels of tryptamine and β-phenylethylamine were much lower than those amines found and cited above, but wide fluctuations were also observed. Unlike cooked meat products, the presence of tryptamine and β-phenylethylamine was found to be quite common in the majority of ripened meat products.

Biogenic Amine Variability in Different Batches of Meat Products. To study whether the variability among biogenic amine contents is related to the conditions of the manufacturing process, we followed the amine levels in different batches of the same meat product of the same commercial brand. One brand of a ripened product ("chorizo"), two brands of cooked products (cooked ham and mortadella), and one of dry-cured ham were studied.

Biogenic amine contents in "chorizo" and in cooked ham showed, in absolute terms, the greatest variability (Table 6). Wide fluctuations were observed in tyramine, histamine, putrescine, and cadaverine contents. Thus, it seems that the same system of manufacture can give products with very variable levels of amines. The relatively high levels of amines found in cooked ham could be related to the use of low hygienic quality pork meat, since the manufacture of this product did not involve amine formation as it has been reported (Hernández-Jover et al., 1996a). In the case of "chorizo", the potential amine formation during fermentation should be taken into consideration, but the differences observed among batches supported the role of the hygienic quality of the meat employed as a critical factor in amine

Table 5. Biogenic Amine Contents[a] (mg/kg) in Spanish Ripened Meat Products

amines	"chorizo" ($n = 20$)	"salchichón" ($n = 22$)	"fuet" ($n = 11$)	"sobrasada" ($n = 7$)
tyramine	29.2−626.8	53.3−513.4	31.8−742.6	57.6−500.6
	282.3 (128.8)[b]	280.5 (108.5)[b]	190.7 (72.6)[b]	332.1 (130.9)[b]
putrescine	2.6−415.6	5.5−400.0	2.2−222.1	1.8−500.7
	60.4 (141.4)[b]	102.7 (75.9)[b]	71.6 (41.2)[b]	65.2 (50.1)[b]
histamine	nd−314.3	nd−150.9	nd−357.7	2.8−143.1
	17.5 (28.6)[b]	7.3 (13.8)[b]	2.2 (40.1)[b]	9.0 (16.5)[b]
cadaverine	nd−658.1	nd−342.3	5.4−51.3	3.0−41.6
	20.1 (16.1)[b]	11.7 (22.8)[b]	18.9 (17.5)[b]	12.6 (14.2)[b]
tryptamine	nd−87.8	nd−65.1	nd−67.8	nd−64.8
	15.9 (20.0)[b]	8.5 (10.7)[b]	8.7 (8.0)[b]	11.5 (22.8)[b]
β-phenylethylamine	nd−51.5	nd−34.7	nd−33.7	0.2−38.5
	1.2 (3.1)[b]	6.7 (6.4)[b]	1.9 (4.3)[b]	2.2 (5.7)[b]

[a] Range and median (deviation quartile). Values in the same row bearing a common superscript letter are not different ($p \geq 0.05$).

Table 6. Variability of Biogenic Amine Content[a] among Different Batches of Products from the Same Commercial Brand

amines	"chorizo" ($n = 5$)	cooked ham ($n = 5$)	dry-cured ham ($n = 5$)	mortadella ($n = 5$)
tyramine	297.6 (89.7)	64.6 (34.2)	1.3 (1.8)	0.4 (0.6)
histamine	85.9 (90.5)	3.9 (7.8)	1.0 (0.8)	0.5 (0.3)
tryptamine	22.5 (22.3)	1.4 (2.4)	nd[b]	nd
β-phenylethylamine	6.4 (11.2)	0.3 (0.3)	0.8 (0.8)	nd
putrescine	260.1 (148.2)	18.6 (27.6)	1.3 (0.5)	0.7 (0.1)
cadaverine	156.1 (280.0)	18.4 (13.6)	0.5 (0.4)	2.2 (2.2)

[a] Mean (standard deviation) of five samples. [b] Not detected.

formation. Unlike cooked ham and "chorizo", the variability, in absolute terms, found among the different batches of mortadella and dry-cured ham was low (Table 6). Dry-cured ham manufacture involves a careful traditional procedure, in which only NaCl is added as the main preservative (certain amounts of nitrites and nitrates can be used). Therefore, to obtain high-quality products, the meat must be of high quality. The manufacture of mortadella usually involves a small quantity of meat, high proportions of preservatives, and a cooking process; these conditions can hinder the development of the background flora present in meat and, therefore, the production of amines.

Sanitary Concerns. Levels of tyramine and histamine in cooked meat products and in dry-cured ham are, in general, too low to elicit direct adverse reactions. However, in some cases, ripened products showed histamine and tyramine contents above the limits reported as unsafe by some authors (Brink et al., 1990, Stratton et al., 1991). For example, following the wide range reported by Brink et al. (1990) for tyramine (100−800 mg/kg), 87% of samples would be within this range, while 13% of the samples showed higher levels than the suggested limit of 100 mg/kg (Stratton et al. 1991). However, no case of intoxication due to the consumption of those meat products has been reported. Therefore, it seems that there is a need to revise the limits for food products which are consumed in small quantities (i.e., meat products and some fish products).

Following the generally recommended guideline of 6 mg maximum allowable tyramine ingestion (McCabe, 1986) for patients under MAOI treatments, we calculated that 83% of ripened samples would be capable of provoking an increase in arterial pressure, after consumption of 50 g of product. Therefore, our data confirm that these products have to be included among the foods that are not to be consumed by patients under treatment with MAOI drugs. Cooked meat products and dry-cured ham may be ingested when consumption is moderate. Furthermore, it should be taken into consideration that levels of putrescine and cadaverine found in some samples, especially ripened, were high and those amines have been reported as a potentiators of the toxic effect of histamine and tyramine.

Conclusions. According to our results, spermine and spermidine appear to be the natural amines in meat and meat products, while tyramine, histamine, putrescine, cadaverine, tryptamine, and β-phenylethylamine levels in ripened meat products were higher than those found in meat, in cooked meat products, and in dry-cured ham. Formation of these amines has been reported during the manufacture of ripened products. However, it is not yet clear what microorganisms are responsible for the biogenic amine formation. Fermentative microflora and/or natural background flora of the meat used as raw material could be related to amine formation. The variability of biogenic amine contents is also observed in a same meat product from the same commercial brand but from different batch of production. Differences in the hygienic quality of the raw material can be responsible for the differences in the amine amounts present in the meat products.

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