Biogenic Amine Sources in Cooked Cured Shoulder Pork

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Changes in biogenic amines were followed during pork meat storage, the manufacturing process, and the storage of cooked cured pork shoulder to establish the sources of these amines in this type of product. Microbial counts were also followed. During storage of pork meat at 6−8 °C, spermidine remained constant, spermine slightly decreased, and amounts of histamine, tyramine, putrescine, and cadaverine appeared. Significant correlation coefficients were obtained between aerobic total counts and tyramine, putrescine, and cadaverine. A biogenic amine index calculated from the sum of cadaverine, putrescine, tyramine, and histamine seemed to be useful as an indicator of the meat freshness. Spermidine and spermine initially present in meat used as raw material remained constant during the processing and the storage of the cooked pork shoulder. No additional biogenic amine formation was observed. Results of our work confirmed that only spermine and spermidine should be present in cooked pork shoulder, if fresh pork meat is used and a correct processing is followed. High levels of cadaverine, putrescine, tyramine, and histamine in cooked pork shoulder would be an indicator of the use of poor hygienic quality meat.

Keywords: *Biogenic amines; cooked meat products; meat spoilage*

INTRODUCTION

Most of the biogenic amines in foods are produced by the breakdown of amino acids due to the action of decarboxylases of microbial origin (Chander et al., 1989). The presence of high levels of biogenic amines in foods could be of great public health significance because of their possible involvement in various disorders such as migraine, headache, gastric and intestinal ulcers, and pseudoallergic responses (Taylor, 1986; Brink et al., 1990).

The estimation of biogenic amines is important not only because of their toxicity but also because of their use as spoilage indicators (Halász et al., 1994; Yano et al., 1995). Mietz and Karmas (1977) proposed the biogenic amine index (BAI = histamine + putrescine + cadaverine/1 + spermidine + spermine) to evaluate the quality of raw and processed seafood. A BAI value between 0 and 1 would indicate fresh fish, a value between 1 and 10 initial spoilage, and a value exceeding 10 advanced spoilage.

Meat and meat products have been reported to contain considerable levels of biogenic amines (Vidal-Carou et al., 1990; Brink et al., 1990; Shalaby, 1993; Bauer et al., 1994). However, very little data are available on biogenic amines in cooked meat products. Meat is the raw material which contributes to the final biogenic amine content of cooked meat products. On the basis of our previous works and those of other authors (Szerdahelyi et al., 1993; Hernández-Jover et al., 1996), the only amines found at significant levels in fresh meat are spermine and spermidine. Then, if fresh meat is used and no additional formation occurs during manufacture, only spermine and spermidine should be present in cooked meat products. However, tyramine, cadaverine, putrescine, and histamine, biogenic amines frequently related with food spoilage, have also been detected by Vidal-Carou et al. (1990) and Hernández-Jover et al. (1996) in some cooked meat products at relatively high levels. The presence in cooked meat products of those amines could be a result of the use of poor hygienic quality meat.

The aim of this work was to identify the sources of biogenic amine in a cooked meat product such as cooked cured pork shoulder. For this purpose, we studied the changes of biogenic amine levels during meat storage, manufacturing process, and storage of cooked pork shoulder. Particular emphasis has been placed on obtaining data on the amine evolution during storage of meat, to know whether an improper or a prolonged storage could explain the presence of biogenic amines related with spoilage. Therefore, if no biogenic amine changes happen during cooked ham manufacture, the levels of biogenic amines related to food spoilage in cooked ham would indicate the degree of freshness of the meat used as raw material. In addition to biogenic amines, microbial analyses were also carried out.

MATERIALS AND METHODS

Samples. *Storage of Pork Meat.* A piece of meat (pork shoulder) of ca. 1 kg was divided in two portions: One portion was ground with a domestic grinder and divided into small portions; the other one was cut into thick steaks (ca. 2−3 cm). Meat was ground because many meat products are manufactured using minced meat. Samples were covered with aluminum foil and split into three lots: (a) minced pork, (b) sliced pork, and (c) minced pork. Samples from lots a and b were kept at 6−8 °C for 8 days, while samples from lot c were stored at −18 °C for 12 days. Chemical and microbial determinations were performed at zero time and every 24 h in lots a and b and every 48 h in lot c.

Cooked Cured Pork Shoulder Manufacture. A whole pork shoulder without skin and bones was injected with a brine solution in a multineedle injector (Olgasa, model CH-30; Spain) and massaged in a massager (Scanio, model VT0, 30 L capacity; Denmark) for 24 h at 5 °C (massage was 3 h continuous and 21 h discontinuous, with 40 min working and

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20 min stopped). The proportion of the injection was 60 parts of brine for 100 parts of meat. The brine solution was composed of water (85%), sodium polyphosphate (4.2%), NaCl (4.2%), meat flavor (1.6%), preservatives (0.5% sodium sorbate, sodium nitrate, sodium nitrite), antioxidants (0.1% sodium ascorbate, sodium citrate), collagen protein (3.2%), lactose (0.5%), and carageenan type κ (0.7%). All the additives were from SBI (Systems Bio-Industries, France), and the NaCl was from Sal Costa S.A. (Spain). After massage, a cooking process at 75 °C was applied, until 68 °C was reached in the center of the piece. Samples were drawn before brine injection, after brine injection, and after massage. The last sample was the end product. The processing was carried out in a meat pilot plant, and the entire procedure was repeated on another pork shoulder. Spanish law regulations establish three categories of cooked pork products. The product obtained following the process above described is a grade III.

Cooked Cured Pork Shoulder Storage. A piece of cooked pork shoulder of ca. 2 kg was cut into slices (2−3 mm thickness), which were individually wrapped in aluminum foil and stored at 6−8 °C for 14 days. Samples were taken at zero time and after 0, 1, 2, 5, 6, 7, 8, 9, 12, 13, and 14 days of storage.

Biogenic Amine Determination. Biogenic amines [tyramine (TY), histamine (HI), serotonin (SE), octopamine (OC), dopamine (DO), β-phenylethylamine (PHE), tryptamine (TR), putrescine (PU), cadaverine (CA), agmatine (AG), spermidine (SD), and spermine (SM)] were determined by a liquid chromatographic method as described by Hernández-Jover et al. (1996). The method involves the separation of ion pairs formed between biogenic amines and octanesulfonic acid, a postcolumn derivatization with *o*-phthalaldehyde (OPT), and spectrofluorometric detection. All reagents were analytical grade except HPLC reagents that were LC grade. Biogenic amine standards were purchased from Sigma Chemical (St. Louis, MO).

pH Measurement. The pH was measured by direct reading with a pH meter (Madrid-Vicente, 1994). Two grams of sample from homogenized ground meat was taken and mixed with 10 mL of distilled water. The mixture was allowed to stand for 10 min, and the pH was measured in a pH meter (Crison, micropH 2001; Modena, Italy). Each determination was done in duplicate.

Microbial Analysis. A 20 g portion of sample was taken aseptically, placed in sterile Stomacher bags, and homogenized for 2 min in Ringer solution (ratio 1:9) using a Stomacher instrument (IUL Instruments). A series of decimal dilutions were prepared. The Ringer solution was composed of NaCl (2.25 g/L), KCl (0.10 g/L), $CaCl_2$ (0.12 g/L), and $NaHCO_3$ (0.05 g/L) at pH 7.2.

Aerobic mesophilic bacteria were determined on TSA (tryptone soy agar, Adsa Micro) incubated for 48−72 h at 30 °C, lactic acid bacteria on MRS agar (Man Rogosa and Sharp, Adsa Micro) incubated for 48−72 h at 30 °C, enterobacteria on MacConkey agar incubated for 48−72 h at 30 °C, staphylococci on mannitol agar, pseudomonads on *Pseudomonas* selective medium (Oxoid) incubated for 48−72 h at 30 °C, fungi and yeasts on Sabouraud agar (Oxoid) incubated for 48−72 h at 30 °C, and anaerobic bacteria on Wilkins Chalgren agar (Oxoid) incubated for 48−72 h at 30 °C.

Statistical Analysis. All statistical analyses were performed using the statistics package Statgraphics (Manugistics, Inc., 1994).

RESULTS AND DISCUSSION

Storage of Pork Meat at 6−8 °C. Fresh meat samples (time zero) only had CA (2.1 ± 0.1 mg/kg), SM (31.2 ± 2.5 mg/kg), and SD (4.7 ± 0.1 mg/kg). SD and SM levels are in agreement with values reported by other authors for fresh pork meat (Wortberg et al., 1982; Maijala et al., 1993; Hernández-Jover et al., 1996). Although CA was found in pork meat, its levels were lower than those reported by Maijala et al. (1993) and Halász et al. (1994); however, Szerdahelyi et al. (1993) and Yano et al. (1995) did not find CA in fresh meat.

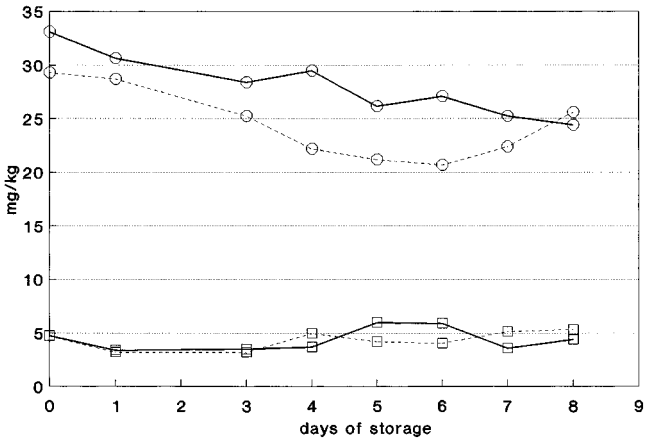

Figure 1. Changes in spermidine (□) and spermine (○) levels in minced (− − −) and sliced (—) pork meat during storage at 6−8 °C.

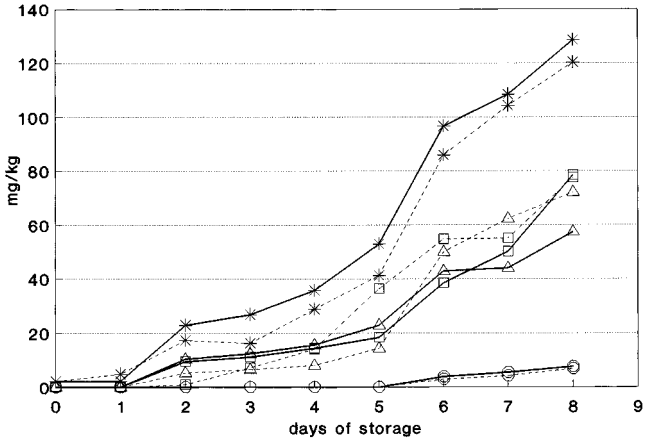

Figure 2. Changes in cadaverine (*), putrescine (□), tyramine (△), and histamine (○) levels in minced (− − −) and sliced (—) pork meat during storage at 6−8 °C.

Figure 1 shows that little differences were observed in SD and SM contents in both minced and sliced pork during storage at 6−8 °C. SD levels were constant during the 8 days of refrigerated storage, while SM showed a slight decrease. Bardócz et al. (1995) pointed out that SM contents often decreased during food spoilage since SM can be used as a nitrogen source by some microorganisms. Similar results have been reported by Bauer et al. (1994) and Yano et al. (1995) regarding SD and by Szerdahelyi et al. (1993) and Halásh et al. (1994) regarding SM. However, contradictory results have been also reported, with Szerdahelyi et al. (1993) and Halásh et al. (1994) observing changes in SD contents and Maijala et al. (1993), Bauer et al. (1994), and Yano et al. (1995) observing an increase or no change in SM during meat storage. Those contradictory results could be attributed to differences in the microflora present in meat.

Formation of CA, PU, TY, and HI was observed in both minced and sliced meat during storage at 6−8 °C (Figure 2). A continuous increase in CA, PU, and TY after the second day of storage was observed. CA levels were linearly correlated with storage time ($r = 0.967$, $p < 0.001$), whereas PU and TY showed an exponential correlation with time ($r = 0.983$ and 0.982, respectively, $p < 0.001$). Average final levels of CA (124.4 ± 5.8 mg/kg), in both minced and sliced meat, were higher than those of PU (78.0 ± 0.7 mg/kg) and TY (64.7 ± 10.4 mg/

Table 1. Microbial Counts (log CFU/g) during Storage of Pork Meat at 6−8 °C

days	aerobic	enterobacteria	lactic acid bacteria	staphylococci	pseudomonads	anaerobic	fungi
0	4.90 ± 0.13[a]	3.60 ± 0.80	4.05 ± 0.15	3.80 ± 0.30	2.25 ± 0.34	4.00 ± 0.0	3.70 ± 0.28
1	6.25 ± 0.02	5.70 ± 0.55	5.20 ± 0.65	4.15 ± 0.15	4.03 ± 0.18	5.43 ± 0.04	5.30 ± 0.15
2	7.20 ± 0.20	6.65 ± 0.85	5.00 ± 0.30	4.40 ± 0.03	4.50 ± 0.20	6.40 ± 0.10	5.90 ± 0.08
3	8.25 ± 0.20	8.65 ± 0.25	5.50 ± 0.20	4.60 ± 0.25	5.00 ± 0.40	7.03 ± 0.67	7.13 ± 0.10
4	9.20 ± 0.11	8.90 ± 0.45	6.70 ± 0.20	5.55 ± 0.60	5.60 ± 0.35	7.95 ± 1.05	7.70 ± 0.23
5	9.00 ± 0.30	8.91 ± 0.40	6.98 ± 0.40	5.60 ± 0.50	3.15 ± 4.45	8.68 ± 0.75	8.10 ± 0.30
6	9.45 ± 0.43	8.90 ± 0.30	7.45 ± 0.50	5.80 ± 0.05	nd[b]	9.62 ± 0.97	8.38 ± 0.03
7	9.70 ± 0.45	9.45 ± 0.70	7.55 ± 0.25	6.10 ± 0.45	nd	9.80 ± 0.55	9.43 ± 0.95
8	10.00 ± 0.20	9.70 ± 0.04	7.30 ± 0.25	5.65 ± 0.07	nd	10.05 ± 0.42	8.85 ± 0.47

[a] Mean ± standard deviation. [b] Not detected.

Table 2. Amine Levels (mg/kg) during Elaboration of Cooked Cured Pork Shoulder

stages	SD	SM
meat pork	3.20 ± 0.30	32.05 ± 0.30
brine injection	1.50 ± 0.05	12.25 ± 0.07
	(3.65 ± 0.10)[a]	(30.60 ± 0.15)
maceration	1.40 ± 0.07	12.80 ± 0.50
	(3.50 ± 0.20)	(32.00 ± 1.20)
cooking	1.50 ± 0.07	12.40 ± 0.35
	(3.70 ± 0.20)	(31.05 ± 0.95)

[a] Amine levels expressed in pork meat after applying the dilution factor of the brine.

kg). Similar amine levels were found by Wortberg et al. (1982) and Slerm et al. (1985) in spoiled meat.

HI formation was only observed after the sixth day of storage, reaching up to 7.2 ± 1.5 mg/kg. An exponential correlation was established between HI and storage time ($r = 0.994$, $p < 0.05$). HI formation was lower than that reported by Vidal-Carou et al. (1990) during meat spoilage, who detected final levels of 25 mg/kg after 4 days of refrigerated storage. However, other authors observed that HI remained relatively constant during storage/spoilage of meat (Rogöwski et al., 1984; Sayem-el-Daher, 1984; Bauer et al., 1994). HI changes during meat spoilage seem to be very different from HI changes during the spoilage of certain types of fish, such as tuna, herring, and anchovy, where HI is the one of the main amines formed (Taylor, 1988; Veciana-Nogués et al., 1996).

The pH increased during storage, from 6.51 to 7.04, and no significant differences between minced and sliced meat were observed. Increases of pH values during meat storage/spoilage were also observed by Vidal-Carou et al. (1990) and Maijala et al. (1993). Significant correlations ($r > 0.9600$, $p < 0.001$) between CA, PU, and TY levels and pH were found. The accumulation of biogenic amines in meat could during storage contribute to pH increase which would explain the correlation mentioned above.

Microbial counts were very similar in both minced and sliced pork meat during refrigerated storage. Therefore, the mean values (±standard deviation) including both types of samples are shown in Table 1. Microbial counts in samples from zero time are in agreement with the value of 5.00 log CFU/g reported for fresh meat (Pascual-Anderson, 1992). Counts of aerobic mesophilic bacteria, enterobacteria, anaerobic bacteria, and fungi increased markedly through storage. Lactic acid bacteria also increased during storage, but levels were always lower than those microorganisms mentioned above. Staphylococci counts had a moderate increase, and pseudomonad counts increased until the fifth day and afterward disappeared. All these bacteria have been related to biogenic amine production. After the second day of the study, aerobic mesophilic bacteria counts largely ex-ceeded the value suggested for fresh meat (Pascual-Anderson, 1992) coinciding with the initial increase in CA, TY, and PU levels. Significant correlation coefficients were obtained between aerobic total counts and TY, CA, and PU levels (0.868, 0.840, and 0.770, respectively, $p < 0.05$). Therefore, according to our results, the refrigeration did not avoid the formation of some biogenic amines, such as CA, PU, and TY. HI formation was also observed but later and lower than CA, PU, and TY formation. Therefore, pork meat used as raw material can be a source of those amines in cooked meat products.

Biogenic amines have been extensively used to evaluate the degree of spoilage in fish and fish products. This type of evaluation has been extended to meat and meat products by Sayem-el-Daher et al. (1984). In our study, all meat samples after the fourth day of refrigerated storage were within the interval of 13 and 20 mg/kg PU established by Sayem-el-Daher et al. (1984) for low-quality meat. However, only the samples corresponding to time zero and first day of storage were below the limit of acceptability for TY (10 mg/kg) reported by the same authors. To avoid this inconsistency and in agreement with many authors, meat freshness should be evaluated by considering an amine index, which includes all the biogenic amines related to meat spoilage. The Mietz and Karmas index (1977) employed regularly for fish and fish products does not include TY levels. Nevertheless, we feel that TY should also be included when evaluating meat freshness because important changes in the levels of this amine occurred during meat spoilage.

A reasonable biogenic amine index (BAI) could be the sum of CA + PU + TY + HI with the following limits: BAI <5 mg/kg for fresh meat, between 5 and 20 mg/kg for acceptable meat but with initial spoilage signs, between 20 and 50 mg/kg for low meat quality, and, finally, >50 mg/kg for spoiled meat. According to those values, the studied samples would be unacceptable after the third day of storage. Organoleptical changes in color and odors, assessed by six experienced laboratory workers, occurred after the fourth day of storage. It should be pointed out that the time periods found in our study can not be generalized because meat shelf life will depend onto the initial meat freshness and the conditions of storage.

Storage of Pork Meat at −18 °C. Halász et al. (1994) observed an increase in CA and PU and a decrease in HI, SD, and SM levels after 8 days of storage at −20 °C; however, CA, SD, and SM levels and pH values remained constant when we stored pork meat at −18 °C for 12 days. Amines were not formed because microbial counts remained constant during frozen storage. Our results agree with those of Chen et al. (1994) who followed biogenic amine evolution for 9 months at −18 °C.

Table 3. Microbial Counts (log CFU/g) during Manufacture of Cooked Cured Pork Shoulder

stages	aerobic	lactic acid bacteria	enterobacteria	staphylococci	pseudomonads	fungi	anaerobic
meat pork	5.60 ± 0.65	5.10 ± 0.65	4.40 ± 1.25	4.75 ± 1.20	3.98 ± 0.25	3.98 ± 1.40	5.55 ± 1.10
brine injection	6.15 ± 0.04	4.70 ± 0.55	5.22 ± 0.22	5.45 ± 0.23	4.60 ± 0.37	4.47 ± 0.55	5.60 ± 0.35
maceration	6.11 ± 0.27	5.33 ± 0.01	5.30 ± 0.30	5.15 ± 0.50	4.25 ± 0.05	4.22 ± 0.01	5.20 ± 0.54
cooking	2.10 ± 0.14	nd[a]	nd	nd	nd	nd	3.95 ± 0.50

[a] Not detected.

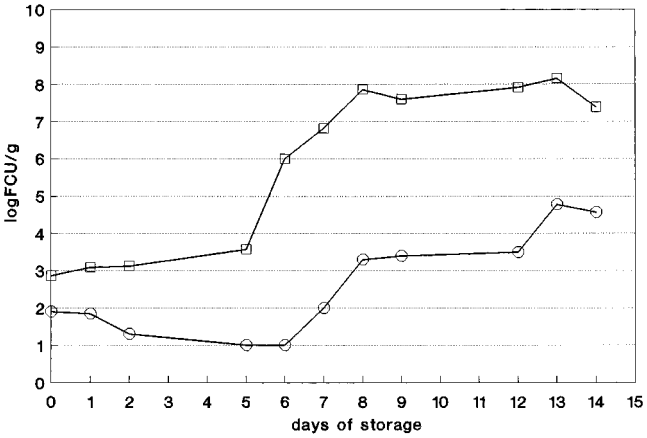

Figure 3. Changes in total aerobic count (□) and fungi (○) during storage of cooked cured pork shoulder.

Cooked Cured Pork Shoulder Study. Only SM and SD were found in the initial samples, corresponding to pork meat, and their levels were similar to those previously found in pork meat (Table 2). Results are the mean (±standard deviations) SD and SM values obtained from the two replicate studies, since no significant differences were found between them. Biogenic amines related to meat spoilage, such as CA, PU, TY, and HI, and other amines, such as DO, OC, TR, PHE, and AG, were not detected in any of the tested samples. SD and SM levels decreased in samples throughout cooked ham processing (Table 2). Lakritz et al. (1975) and Sayem-el-Daher et al. (1984) observed a decrease in SD and SM after the cooking of the meat; however, in our case the observed decrease should only be due to the dilution produced when incorporating the brine solution.

Evolution of microbial counts during cooked pork shoulder processing is summarized in Table 3. Aerobic mesophilic bacteria counts in initial samples were slightly higher than the value of 5.00 log CFU/g suggested for fresh meat (Pascual-Anderson, 1992). Aerobic and anaerobic bacteria counts strongly decreased during processing, and lactic acid bacteria, enterobacteria, staphylococci, pseudomonads, and fungi dissapeared after the cooking stage.

It seemed that cooked pork shoulder processing did not involve formation or degradation of any biogenic amine according to our results. Though microorganisms commonly associated with the amine formation were found in the raw material, the total aerobic counts were lower than those required ($>10^7$ CFU/g) for the formation of biogenic amines observed by us in this work and by other authors (Edwards et al., 1983). Also, the low temperature during the masssage (5 °C), the relatively speediness of the process, the presence of preservatives in the raw material, and the final cooking process applied tend to avoid or kill microbial population which could be able to form amines. Therefore, if a correct manufacturing is applied, biogenic amines found in cooked pork products only could come from the meat used as raw material.

Storage of Cooked Cured Pork Shoulder. SM and SD were the only amines found in cooked cured pork, and no changes were observed during the storage period. Average values were 24.4 ± 1.6 mg/kg for SM and 1.3 ± 0.2 mg/kg for SD throughout the 14 days of storage. No other biogenic amines were formed during the whole period of storage.

Microbial analysis showed that only aerobic and fungi were present. Counts of those microorganisms increased rapidly from the fifth to the sixth day of storage, reaching a maximum on the eighth day of storage, and remaining relatively constant until the end of the study (Figure 3). Gram-positive cocci were the prevailing aerobic microorganisms. Lactic acid bacteria, enterobacteria, and pseudomonads, microorganisms often related to biogenic amine formation, were not found in any of the tested samples. This could explain why TY, CA, PU, and HI, biogenic amines related to meat spoilage, were not formed.

On the basis of our results, only SM and SD should be present in cooked pork products, if fresh pork meat is used and a correct processing has been carried out. High levels of CA, PU, TY, and HI in cooked ham would be an indicator chiefly of the use of poor hygienic quality meat. The acceptable levels of SM and SD in cooked ham would depend on the proportion of meat and brine, and moreover those levels would be below those established for fresh meat because of the dilution when incorporating the brine. Thus, the maximum acceptable biogenic amine index for the cooked pork product studied would be below 10, taking into consideration the brine proportion employed.

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