Physico-chemical Characterization of “Bone Taint” in Spanish Dry-cured Hams

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ABSTRACT

Samples of Spanish dry-cured hams were analyzed using several physico-chemical parameters (moisture content, chlorides, water activity, nitrate, nitrite, total volatile basic nitrogen [TVBN], pH, and oxidation-reduction potential [Eh]). The samples (n = 76) were taken from three basic types of dry-cured hams produced in Spain: slow-cured hams from white pigs (n = 39), fast-cured hams from white pigs (n = 15), and hams from black-skinned Iberian pigs (n = 22). Overall, 56 samples (73.7%) showed the “bone taint” condition, and the remaining 20 hams (26.3%) were normal, and therefore considered as a control group. The objective of this research was to establish the possible circumstances that determine the alteration by means of the differences found in the values of the analyzed measurements in both groups of samples (altered versus normal ones). The hams with “bone taint” were, in general terms, those with a higher TVBN content, a greater pH, and a lower Eh, attributable to an anomalous development of the proteolytic phenomena. The conjunction of a lower concentration of chlorides, greater moisture content, and a higher aw in the affected hams may have created the conditions favorable for tissue enzyme and/or microbial activity.

Key words: “Bone taint,” ham souring, deep spoilage, deep putrefaction, Spanish dry-cured hams, physico-chemical analysis

Spanish ham is an uncooked, cured and dried meat product of intermediate moisture content from the hind leg of a pork carcass. It requires up to 24 months for its production (16), which makes for a greater susceptibility, both internal and external, to frequent alteration and spoilage by chemical, physical, and biological agents. One of the most important types of spoilage is a disagreeable and, at times, repugnant foul-smelling odor of variable intensity which occurs most commonly in the large muscle masses adjacent to the bone structures (15, 27). This alteration was first described in incorrectly cured hams and beef carcasses in the USA (31). This type of alteration is called (amongst many other names) “bone taint,” “ham souring,” “deep spoilage” (23), or “deep putrefaction” (13). The commonly used terminology associated with this type of spoilage lacks a scientific rationale. Also, the standard terms used are subject to a wide range of interpretations that can be confusing (36).

Most researchers who have studied this phenomenon consider the cause of the spoilage to be bacterial (23, 27), naming a great number of species as the ones responsible, while others point out the importance of the meat’s own enzymes (32). This deep form of decomposition is unassociated with any surface change so that is only detected after the ham is ready for sale causing the condemnation of the piece. Thus, in the final quality control process, finished hams are checked for wholesomeness by means of the “calal—a term used to refer to the insertion of a long bone needle into the ham at certain predetermined points to check for any anomalous odors (13).

Bone taint is a periodic and costly problem for the meat industry. Figures obtained from a review of the literature along with a survey carried out by the authors established that the percentage of affected hams in Spain ranged from 1 to 5% of the total production, estimated at approximately 30 million units per year, which means an annual cost to the industry of 7,500 million pesetas (=$60 million).

The objective of the present research was to use physico-chemical methods to elucidate the degradative processes leading to the “bone taint” condition of spoiled hams, comparing them with normal hams, and to ascertain the main differences between the two groups of samples.

MATERIALS AND METHODS

Samples

Seventy-six hams (56 spoiled and 20 control) belonging to the three basic types of Spanish dry-cured hams were selected at three commercial dry-cured ham plants: (i) 15 fast-cured hams from white pigs (6 to 7 months preparation) from the province of Barcelona, (ii) 39 slow-cured hams from white pigs (9 to 12 months) produced in the province of Teruel, and (iii) 22 hams from Iberian pigs (18 to 24 months) from Guijuelo (Salamanca). Of the 76 hams analyzed, 54 were from white pig (Sus vitatus) and 22 were from Iberian pig (Sus mediterraneus), generally black-

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skinned with a higher level of fat infiltration in the muscle tissues than the former (6).

Traditionally, the processing of Spanish dry-cured hams consists of three basic phases: (i) the salting phase, in which salt is applied to the surface of the pork hind leg and the temperature is maintained below 5°C for a period of 1 to 1.5 days per kg of ham, (ii) the post-salting phase, in which salt is distributed uniformly throughout the ham and temperature continues to be maintained at a low level for 30 to 45 days, and (iii) the drying phase, in which a gradual increase of temperature brings about a lowering of the moisture content of the ham allowing for maturation. The duration of the third and final phase is highly variable (between 3 and 20 months) and determines the desired type of ham, whose sensorial characteristics are influenced by both tissue enzyme—the meat’s own enzymes—and microbial activity.

The selection of spoiled and control hams was carried out by experienced technical personnel working at the commercial dry-cured ham plants during the quality control process. Thus, ready-for-sale cured pork hams were checked for putrid or sour odors by means of the "cuela"—a technical term referring to the insertion of a long bone needle into the finished ham at certain predetermined points and then withdrawal to check for any anomalous odors. Spoiled hams showed a sour to pungently foul-smelling odor around the hip joint, and they were subsequently inspected by official veterinarians and condemned. This process of inspection was merely based on organoleptic characteristics (pronounced disagreeable odor)—a subjective evaluation of ham quality that is traditionally accepted and relied on by meat inspectors.

Then, when samples were received in our laboratory, each ham was given four transverse cuts down to the bone structure (Fig. 1) to look for the cut sections where the spoilage (color and odor changes) was most intense (2, 38). This allowed to clearly distinguish between spoiled and control hams. The cuts were as follows: (i) transverse cut to the coxa at the level of the ilium body, (ii) transverse cut to the femur close to the coxofemoral joint or the head of the femur (hip joint), giving access to the marrow of the femur, (iii) transverse cut to the femorotibial joint (knee joint), and (iv) transverse cut in the middle of the tibia diaphysis area and fibula, leaving the marrow of the tibia visible.

After choosing the cut section where spoilage was found with greatest intensity and frequency (cut no. 2, hip joint), a series of samples were taken from the muscle tissue adjacent to the bone structure. The samples were analyzed within 2 h or maintained at -20°C until analysis. In order to make the comparative analysis meaningful, samples were taken from the same regions of the control hams.

**Physico-chemical analyses**

All physico-chemical analyses were performed in duplicate on the spoiled and control hams (except for pH and Eh, which were made at 10 different points) so as to reduce the possibility of error to a minimum and obtain a check on the reliability of the method.

Moisture content (expressed in %) was determined by drying following the international method ISO R-1442 (20). Chlorides (as NaCl expressed as % relative to fresh material) were determined by the Carpentier-Volhard method (precipitation of the proteins with 0.1 N silver nitrate and evaluation of the excess with ammonium thiocyanate) according to ISO R-1841 procedures (20). Water activity (aw) was determined by the method of Palmia (33), which evaluates parameters derived from moisture and sodium chloride content in cured hams using the following formulas: (i) if the %NaCl/%H2O ratio is less than 0.1, aw = -0.535(%NaCl/%H2O) + 0.959; (ii) if the %NaCl/%H2O ratio is greater than 0.1, aw = -0.631(%NaCl/%H2O) + 0.969.

**Nitrates and nitrites**

Nitrate and nitrite (expressed in ppm) were analyzed spectrophotometrically in accordance with the ISO/DIS 2918 standard (20), using a Kontron-Uvikon 810 apparatus. Standard curves for nitrate and nitrite were obtained daily for each group analyzed. Total volatile basic nitrogen (TVBN) was measured following the

![FIGURE 1. Diagram of the four cuts made on the analyzed hams.](image)

![FIGURE 2. Diagram of the resulting section from cut no. 2 showing the locations of the 10 points where pH and Eh were measured. Muscle tissue: (1) rectus femoris, (2) vastus medialis, (3) pectineus, (4) biceps femoris, (5) semimembranosus, (6) adductor, (7) semitendinosus; covering fat tissue: (8) and (9); marrow of the femur: (10).](image)
RESULTS AND DISCUSSION

No external differences could be noted between the hams spoiled by “bone taint” and control hams since this deep-seated form of decomposition is unassociated with any surface change. The cuts made indicated that the area where the spoilage manifested itself with greatest intensity was around the hip joint. This occurred in 42 of the 56 spoiled hams (75%). Therefore, this zone was chosen as the area for taking samples for physico-chemical analyses, from both spoiled and control hams. Muscles from spoiled hams were grayish to dark brown-black near the bone, and offensive foul-smelling odor was apparent from the affected tissue and disseminated throughout the internal topography of the ham.

Moisture content

The results obtained are shown in Table 1. Overall, the moisture content of the spoiled hams (54.25%) was significantly higher than that of the control (49.44%) (P < 0.001). This difference was more pronounced for slow-cured hams from white pigs and the hams from Iberian pigs. There was little or no noticeable difference in the moisture content between spoiled and control fast-cured hams from Barce-

Chlorides

The chloride content in control hams averaged 5.53%, and varied from 4 to 9% (Table 1). This was also noted by other authors (24–25), who found wide variations in NaCl concentrations, from 3.9 to 9.3%. The type of ham which showed the highest mean salt level (7.64%) was the fast-cured hams. The cause of this appears to be that, of all the three types studied, the fast-drying process includes the longest salting phase (21 days). The fast-cured hams also

Data analysis

Statistical analyses and significance were based on the Mann-Whitney nonparametric test. Thus, statistical significances were based on a two-tailed U statistic which was corrected for ties. The calculations were performed using StatView SE + Graphics (Abacus Concepts, Inc., 1988, Berkeley, CA, USA) for Macintosh personal computers.

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<th>Table 1. Mean values ± standard deviation of the physico-chemical parameters analysed in control batch and spoiled hams</th>
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Different letters in a row differ significantly: a(P < 0.05), b(P < 0.01), c(P < 0.001).
have the lowest moisture content due to the higher temperatures during the drying phase.

The average chloride content of the spoiled hams was significantly lower (4.81%) than that of the control hams (5.53%) \((P < 0.05)\). As expected, among the spoiled hams the fast-cured hams showed the highest salt content (5.85%), while chlorides in control hams amounted to 7.64%. The difference in salt concentration between spoiled and control slow-cured hams was practically nonexistent (5.21% and 5.22%, respectively).

It is likely that the low level of chlorides (plus the high \(a_w\)) may have played a role increasing the risk of development of “bone taint.” This may be explained by the role of chlorides in selecting a salt-tolerant flora as an impediment to ham-souring bacteria, along with the inhibitory effect of chlorides on both bacterial and tissue enzymes.

Various researchers have mentioned the relationship between “bone taint” and low salt concentrations in the affected area \((2, 18, 27)\). Sarraga et al. \((37)\) studied enzyme activity in the biceps femoris and semimembranosus muscles throughout the production of Spanish dry-cured ham, and reported that chloride contents of 5 to 6% totally inhibited tissue enzymes (calcium-activated factor and cathepsin D). Lower chloride concentrations imply, therefore, a higher tissue enzyme activity.

An added factor is that salt applied to DFD meats has greater difficulty in reaching the deeper parts of the ham in the desired quantities. It has been proved that the addition of potassium chloride \((KCl)\) to the curing salts aids in absorption, and this goes to explain the lower levels of deep spoilage when higher concentrations of KCl are used \((35)\).

Several researchers \((1, 26)\) consider that microbiological stabilization is achieved with concentrations of NaCl below 4.5 to 5%, while the \(a_w\) should also be less than 0.96 \((22)\). Only when these conditions are met can the ham be successfully passed from the salting phase, where the temperature is less than 5°C, to the drying and curing phases, where the temperature is gradually increased to 20 to 25°C. Thus, the initial stage of the drying phase is a critical factor in the onset of “bone taint” \((7, 28)\). In fact the highest microbial activity is to be found in the initial stages of the drying phase \((19)\). Therefore, if the spoilage flora is present, the alteration could occur if saline levels are not at the correct level in the deeper parts of the ham.

### Water activity

An average value of 0.90 in the control hams was obtained, although, as in the case of chlorides, there was a wide variation (Table 1). The hams from Barcelona, despite being fast-cured, showed a lower \(a_w\) level (0.87) than those of 0.90 and 0.89 encountered in slow-cured and Iberian hams, respectively. In the case of the latter this is understandable because of the higher level of intramuscular and external fat which impedes dehydration and delays salt penetration \((6)\).

The spoiled hams, regardless of their origins and production time, showed a higher average \(a_w\) value than the controls (0.91 as opposed to 0.90), which marked a significant difference \((P < 0.01)\). However, as in the chloride results, this value is due mainly to the Iberian hams, which showed the most pronounced difference: 0.93 in the hams with “bone taint” as opposed to 0.89 in the control group. The range of variation in the spoiled hams was also high (0.85 to 0.94), and the scarce amount of material published on the subject \((2, 29)\) also showed a certain degree of variation (0.89 to 0.92).

Spoilage bacteria in meat and meat products do not multiply at \(a_w\) values below 0.91 \((39)\). Since most spoiled hams (89%) showed water activity values above 0.91, these products were clearly susceptible to microbial attack. Other researchers \((21)\) stated the necessity of maintaining low or intermediate moisture products \((a_w < 0.90)\) at refrigeration temperatures below 10°C to prevent from undesirable spoilage.

### Nitrate and nitrite

The average value of nitrate obtained from the control hams was 118.71 ppm (Table 1). Of all the parameters studied, this one showed the widest degree of variation (16 to 320 ppm). When the origin of the ham was used as a grouping factor, it was found that the Iberian hams had nitrite concentrations approximately 100% higher than the other hams studied. Nothing in the published literature gives any information to use as a contrast or to suggest any reasons for these higher values.

In the spoiled hams lower nitrate quantities were evident (74.51 ppm), although the difference between spoiled and control hams was only significant in the case of the hams from Salamanca \((P < 0.01)\). Other Spanish researchers \((29)\) also found lower nitrate levels in the spoiled hams that they studied. The nitrate levels in our spoiled hams ranged from 3.25 to 195 ppm, as was observed in Spanish \((29)\) and Italian \((38)\) spoiled hams where nitrate levels varied from 6.9 to 228 ppm. Two reasons might explain the lower nitrate level in the spoiled hams: the use of curing salts with a lower concentration of nitrate, and the existence of a nitrate-reducing microbial flora \((e.g., \text{strains of Enterobacteriaceae and Staphylococcus})\).

The average nitrite level in control hams was 8.27 ppm with a range from 0.75 to 29.5 ppm (Table 1). Nearly all the figures found by other researchers fall within this range. Logically, the level of nitrite runs parallel to that of nitrate, so it is unsurprising that the control Iberian hams showed the highest nitrite level (14.09 ppm).

Although the hams with “bone taint” showed an average nitrite level of 7.08 ppm, which is lower than the level found in the control hams, the difference was not significant. However, and as with the nitrate levels, the samples of spoiled Iberian hams showed nitrite levels significantly lower \((P < 0.01)\) than the control hams. The findings of the only published work in this regard to date coincide with our results in terms of finding lower nitrite levels in samples of spoiled material \((29)\).

### Total volatile basic nitrogen (TVBN)

The quantity of TVBN for a given product is directly proportional to the level of protein degradation \((30)\), which has led to its use in determining the level of spoilage in meat.
and meat products (2, 11). TVBN consists principally of ammonia and volatile amines, although small quantities of other, less volatile, amines such as cadaverine, putrescine, histamine, histidine, and spermine are also present.

Fermented or dried meat products in a good state of preservation must not exceed 100 mg NH₃ per 100 g (34). Above this figure the product is in an initial state of spoilage, and at twice this level the product is in an advanced state of putrefaction. With this figure in mind, practically all the control hams were in a fit state for consumption (101.24 mg NH₃ per 100 g). The spoiled hams varied from an early state of spoilage (Teruel and Salamanca with 149.26 and 169.81 mg NH₃ per 100 g, respectively) to a state of frank putrefaction (Barcelona hams with 246.87 mg NH₃ per 100 g). The difference between spoiled and control hams was clearly significant at the overall level \( (P < 0.001) \), and the differences were significant when considering the hams by origins with the exception of the fast-cured hams. These results contrast those found by researchers studying Italian dry-cured hams (10), where no appreciable differences could be found between spoiled and control samples in regard to TVBN. Other researchers studying spoiled Spanish slow-cured hams (2) reported appreciably lower TVBN levels than ours (84.6 to 126.6 mg NH₃ per 100 g).

The spoiled hams from Barcelona showed the highest TVBN levels, although they were the hams with the lowest \( a_w \) level and highest level of chlorides. These two factors, which normally act as a major impediment to microbial proliferation, forced us to revise our thinking and propose that in this case the spoilage must have occurred in the initial production stages or at the early stages of the drying phase. This is understandable when one bears in mind the higher temperatures used in the fast dry-curing ham process, which increase enzymic activity to the point where other factors such as the decrease in moisture content and \( a_w \) and the increase in salts no longer act as hurdles. It is worth noting that the control hams from Barcelona also showed a higher TVBN level.

\[ \text{pH} \]

In the muscle tissue, the pH values showed a statistically significant difference \( (P < 0.001) \) between spoiled and control samples (6.33 and 6.04, respectively) (Table 1). In the individual groups of hams the pH level was higher in the spoiled hams. Higher pH is one indicator of product putrefaction. Other researchers have also obtained higher pH levels in spoiled samples (28). The presence of actively proteolytic microorganisms or a greater and more prolonged activity on the part of the muscle tissue enzymes or a combination of the two might explain the higher pH values found in spoiled hams.

Seven pH measurements were taken from each sample in the muscle tissue (points 1 through 7, Fig. 2). Points 2, 3, 4, and 6 gave much higher pH levels in spoiled hams (frequently above 7.0) than in control samples. The other measurements made at points 1, 5, and 7 gave results which showed less difference between spoiled and control samples. The Iberian hams, which had the highest level of intramuscular fat, showed the lowest pH values, probably due to the release of free fatty acids by the lipolytic microflora, which would have counteracted pH increases. Italian researchers have reported significant differences in free fatty acids between spoiled and control hams (12).

Differences were also found in pH levels in the external fat (points 8 and 9, Fig. 2). The differences between spoiled and control hams were not as pronounced as those in muscle tissue, being just under 0.2 units (spoiled samples 5.93, control samples 5.74), but statistically significant \( (P < 0.05) \). There is a paucity of published data on the evaluation of pH in the fatty tissue of hams. In the case of Italian Parma hams, values between 5.74 and 5.90 have been reported (12), while in Spanish hams with “bone taint” much higher figures than ours (6.39 to 6.62) have been found (29).

In measurements on the marrow of the femur (point 10, Fig. 2) we found the situation to be the reverse of that in the muscle tissue and external fat. In the control samples the pH (6.44) was was higher than in the spoiled hams (6.25), although this difference was not significant \( (P > 0.05) \). Gardner (18) reported much higher pH values (6.8 to 7.2) in the marrow of the femur of control hams than those found in ours.

\[ E_h \text{ (oxidation-reduction potential)} \]

In foods an inverse correlation is often found between pH and \( E_h \) (9). Thus, those spoiled hams that had higher pH values were found to have correspondingly lower \( E_h \) values than the control (33 mV as opposed to 74 mV) (Table 1) \( (P < 0.001) \). In unspoiled Italian hams from different regions (11) values from 32 to 55 mV have been obtained, whereas negative \( E_h \) figures have been obtained for Parmastyle hams (5). In Spain, a previous study by Astiasaran et al. (3, 4) reported \( E_h \) values of 292 mV in fast-cured hams from white pigs, 108 mV in slow-cured hams from white pigs, and between 80 and 244 mV for Iberian hams.

In the external fat the inverse relationship between pH and \( E_h \) values is also clear. The lower \( E_h \) of spoiled hams (54 mV) as compared to control hams (91 mV) \( (P < 0.001) \) corresponds to the higher pH found in the spoiled samples. In the marrow, however, as was observed with pH values, no significant differences were observed in \( E_h \) values between control and spoiled hams.

CONCLUSIONS

In Spanish dry-cured hams the source of “bone taint” spoilage can be microbiological or enzymatic and is mainly due to the use of inadequate raw materials and a defective technology during the manufacturing and preservation. This is a product where the microbiological inhibition is essentially achieved by low moisture content (and low \( a_w \)), curing salts, and sodium chloride. Other factors like pH and \( E_h \) may also play a role in selecting against the microorganisms responsible for spoilage. It seems likely that when one or more of the spoilage inhibition factors is ineffective, and there is a concomitant increase of the temperature, bacteria proliferate and spread into the surrounding meat. Therefore, it is concluded that ham stability must be achieved during the salting and post-salting phases when temperatures are...
lower than 5°C so that the hams will not spoil when the temperature increases in subsequent stages of drying.

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