Letters in Applied Microbiology 2000, **30**, 61–66

Microbial populations and volatile compounds in the 'bone taint' spoilage of dry cured ham

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2330/99: received 6 September 1999 and accepted 28 September 1999

C. GARCÍA, A. MARTÍN, M.L. TIMÓN AND J.J. CÓRDOBA. 2000. 'Bone taint' is one of the most important causes of spoilage of dry cured ham. This alteration is characterized by a foul-smelling odour. The microbial population and volatile compounds associated with incipient 'bone taint' were evaluated. Enterobacteriaceae species were found at levels of $7{\cdot}94 \times 10^5$ cfu g^{-1} in spoiled hams and were not detected in unspoiled hams. *Serratia* sp. and *Proteus* sp. are the main organisms characterized. The volatile compounds from the spoiled hams give rise to higher levels of ketones, alcohols and esters than unspoiled hams, that could be originated by microbial metabolism of the above bacteria. Thus, volatile and Enterobacteriaceae analyses should be used to determine this incipient spoilage in the meat industry.

INTRODUCTION

One of the most important causes of spoilage of dry cured ham is called 'bone taint' or 'deep spoilage' (Blanco *et al.* 1997). This alteration occurs most commonly in the large muscle masses adjacent to the bone structures and is characterized by a foul-smelling odour. The 'bone taint' alteration accounts for annual loses of about 5% of the total production, estimated at approximately 30 million spoiled pieces per year (Blanco *et al.* 1997). In many cases this alteration could be small and overlooked in the piece, being undetected until consumption.

'Bone taint' has been associated with proteolytic activity of the enzymes of the meat (Blanco *et al.* 1997), but most researches consider the microbial population as being mainly responsible for this alteration (Giolitti *et al.* 1971; Cornejo *et al.* 1990).

Since, in most of the cases, the alteration is detected in fully ripened hams when water activity is around 0·85 (Córdoba *et al.* 1994), the micro-organisms growing in the finished product could not be those that cause the alteration. Thus, it would be more appropriate to investigate micro-organisms responsible for the alteration when the hams are starting to spoil, which usually takes place in the first stages of the processing (Bersani *et al.* 1984). Furthermore, since the main defect is off-odour, evaluation of volatile compounds when the 'bone taint' is developing could be reliable information to detected this alteration.

The aim of this work was to characterize incipient 'bone taint' spoilage by detection of associated micro-organisms and volatile compounds released.

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MATERIALS AND METHODS

The study involved 14 Iberian hams from two different meat manufacturers. The hams were taken from the post-salting stage at 5 °C. Ten were selected showing incipient internal off-odour detected after internal puncture with a thin bone. Four hams from this stage, without internal off-odour, were sampled as controls.

For sampling hams were sterilized on the surface by searing to avoid external contamination. Then, an internal cylinder of 15 cm depth and 10 cm of diameter from the middle of the piece, near to the coxofemoral joint, was taken. The most internal part of the cylinder constituted with biceps femoris as the basis muscle was selected for analysis.

Microbial analysis

Ten g of the internal sample of ham were homogenized in 90 ml sterile 0·1% peptone in a Stomacher (Lab Blender, Model 4001, Seward Medical, London, UK) for 30 s. Appropriate dilutions were made with 0·1% peptone broth and 1 ml

were plated in each of the culture media listed below. For microbial examination the following criteria were assessed: (a) plate count agar (PCA) (Oxoid, Basingstoke, UK) at 30 °C, 48 h for total aerobic count; (b) violet red bile dextrose agar (VRBD) (Merck, Darmstadt, Germany) at 30 °C, 48 h for Enterobacteriaceae; (c) mannitol salt agar (MSA) (Oxoid) at 30 °C, 48 h to enumerate Micrococcaceae; (d) De Man, Rogosa, Sharphe broth (MRS) (Oxoid) at 30 °C, 48 h for lactic acid bacteria; (e) sulphite, polymyxin, sulphadiazine agar (SPS) (Oxoid) at 45 °C, 48 h in anaerobic conditions for *Clostridium* sp. and (f) dichloran 18% glycerol agar (DG18) (Oxoid) at 25 °C, 7 d to enumerate moulds and yeasts.

Representative colonies were selected on the basis of size, pigmentation, elevation and consistency from those plates (PCA, VRBD and MSA) showing higher microbial counts. Strains were identified according to the identification criteria in *Bergey's Manual* (Brenner 1984). The following tests were employed: Gram-stain, motility, aerobic and anaerobic growth, indole production, Voges–Proskauer, oxidase activity (Faller and Schleifer 1981), glucose/lactose use, hydrogen sulphide, lysine decarboxilase and β-galactosidase activities (Cowan and Steel 1993), urease activity on urea agar (Christensen 1946) and acid production from lactose, sucrose, raffinose, L-rhamnose, maltose, threalose, cellobiose and melibiose (Devriese *et al.* 1985). In addition, for Gram-positive, catalase-positive cocci the following additional tests were performed: nitrate reduction in nitrite broth (Difco, Detroit, MI, USA), coagulase activity by the tube assay and sensitivity to novobiocin (Baird-Parker 1979); clumping factor using rabbit plasma plus EDTA (Devriese and Hájek 1980) and detection of DNAase on DNAse agar (Difco).

Physicochemical analysis

Moisture content in hams was measured according to the method described in ISO method 1442 (ISO 1973). To estimate the salt content, chlorides were extracted with water–ethanol (60 : 40 v/v) and quantified by the Carpentier Volhard method (AOAC 1984). The pH was measured with a pH meter Crison 2002.

For volatile compound analysis, the samples were frozen and then homogenized with a blade homogenizer (Sorvall Omnimixer) and 20 g were transferred into a flask for the extraction. Then, it was sparged with 1 ml/min of helium during 1 h to purge headspace volatiles onto an adsorbent trap of tenax. The volatiles concentrated in the adsorbent trap were subsequently released by thermal desorption and transferred into a gas chromatograph for analysis. For volatile compound identification a Hewlett-Packard quadrupole GC-MS 5971 A was used. Separation was performed with a HP5 fused silica capillary column (60 m × 0·32 mm) coated with phenyl silicone (film thickness 1 m). The carrier gas was helium and the oven temperature was programmed from 40 °C to 240 °C at a rate of 3 °C min^{-1}. Splitless injection was used and Kovats indices (Kovats 1965) were calculated and compared with available literature data. The mass spectra were measured by electron impact at 70 ev.

RESULTS

In spoiled hams total aerobic were at levels of $5{\cdot}01 \times 10^6$ cfu g^{-1} and Enterobacteriaceae and Micrococcaceae showed counts of $7{\cdot}94 \times 10^5$ cfu g^{-1} (Fig. 1). Unspoiled hams showed counts of $3{\cdot}98 \times 10^5$ cfu g^{-1} of total aerobic and $3{\cdot}16 \times 10^5$ cfu g^{-1} of Micrococcaceae, while Enterobacteriaceae were not detected. Lactic acid bacteria also showed higher levels in spoiled than unspoiled hams but always with counts below 10^3 cfu g^{-1}. Moulds and yeasts were at levels lower than 10^2 cfu g^{-1} and *Clostridia* were not detected in either spoiled and unspoiled hams.

Microbial identification from spoiled hams showed that *Serratia liquefaciens* and *Proteus mirabilis* were the Enterobacteriaceae most frequently isolated and *Staphylococcus xylosus* was the most predominant strain from the Micrococcaceae (Table 1). *S. plymuthica*, *P. vulgaris*, and *S. equorum* were also isolated several times.

Physicochemical analysis did not show statistical dif-

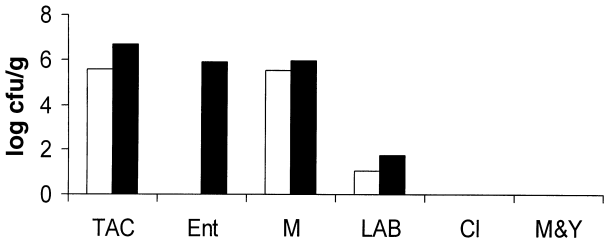

Fig. 1 Microbial population (shown as mean of log cfu g^{-1}) of unspoiled (□) and spoiled (■) hams: total aerobic counts (TAC), Enterobacteriaceae (Ent), Micrococaceae (M), lactic acid bacteria (LAB), *Clostridium* sp. (Cl), moulds and yeasts (M & Y)

Table 1 Incidence of Enterobacteriaceae and Micrococcaceae isolated from spoiled hams

Species	Number of isolates	%
Serratia liquefaciens	11	20·0
Serratia plymuthica	8	14·5
Serratia odourifera	1	1·8
Proteus mirabilis	12	21·8
Proteus vulgaris	7	12·7
Staphylococcus xylosus	11	20·0
Staphylococcus equorum	5	9·1

ferences between spoiled and unspoiled hams. The mean values for moisture were 62·54 for spoiled and 63·37 for unspoiled hams. Regarding salt content, spoiled hams showed a mean value of 1·79, slightly lower than 2·47 observed in unspoiled hams. The pH mean values were 6·28 in spoiled hams and 5·97 in unspoiled hams.

Volatile compounds of spoiled and unspoiled hams are showed in Table 3. Results are expressed as average of arbitrary area units of those volatile compounds observed at least in five of the 10 spoiled samples and in three of the four unspoiled hams.

In unspoiled hams, 15 aldehydes were detected while in spoiled hams only seven aldehydes were found. Hexanal was the most predominant aldehyde in both spoiled and unspoiled hams.

Both batches of hams analysed showed similar numbers of others carbonyl compounds such as ketones, reaching higher mean values in spoiled than unspoiled hams (Table 3).

Spoiled and unspoiled hams showed similar numbers of alcohols, but it is worth noting that spoiled hams had higher amounts of most of the alcohols detected, especially 3-methyl butanol and ethanol.

Considering the hydrocarbon compounds, higher amounts of undecane in spoiled hams and heptane in those unspoiled hams were observed. Acids and esters were found in a superior number in spoiled than unspoiled hams, reaching higher mean values in the spoiled samples. From the remaining volatile compounds observed, it should be noted that higher amounts of dimetildisulphide were in spoiled than unspoiled hams.

DISCUSSION

Analysis of microbial population showed that Micrococcaceae were at similar levels in both batches of hams analysed. These micro-organisms have been reported, at these levels, as the predominant microbial population both on the surface and in the interior during ripening of different types of dry cured hams (Kemp *et al*. 1982; Huerta *et al*. 1988; Rodríguez *et al*. 1994). *S. xylosus* and *S. equorum* were found as the main Micrococaceae species by the biochemical characterization. These staphylococal species have been referred as the habitual micro-organisms in dry cured hams (Rodríguez *et al*. 1994).

Enterobacteriaceae were found at high levels in spoiled hams and they were not detected in unspoiled hams. These micro-organisms have been reported during traditional processing of dry cured ham at very low levels (Marín *et al*. 1996). The Enterobacteriaceae identified by biochemical characterization were *Serratia* sp. and *Proteus* sp., reported as being responsible for the alteration in several meat products (Hechelman *et al*. 1974, 1980; Bersani *et al*. 1984; Miranda *et al*. 1998). Therefore, it would seem reasonable to assume that these micro-organisms are responsible for the microbial spoilage detected in these hams.

The differences between Enterobacteriaeceae levels of spoiled and unspoiled hams can not be explained by the moisture content and pH values, since similar values were found for these parameters in both batches studied. Only the slightly lower values of salt content of the spoiled hams could favour the proliferation of Enterobacteriaceae. These micro-organisms have been reported as the most commonly present on working surfaces in the meat processing industry (Marín *et al*. 1996; Kuri *et al*. 1996), which favour the contamination of meat (Perez Chabela *et al*. 1999). The ability of these bacteria to grow in anaerobic conditions at low temperatures (Jones 1988) could justify their presence in the interior of the hams, causing 'bone taint' spoilage.

Off-odour is the first defect noted in spoiled hams, so the differences in the profile of volatile compounds could be indicative of this alteration. Regarding aldehydes, it is remarkable that they were found in lower numbers and in lower amounts in spoiled than unspoiled hams. These compounds have been referred to as the predominant volatile compounds in dry cured hams (García *et al*. 1991; Barbieri *et al*. 1994). However, other carbonyl compounds such as the ketones, related to microbial activity (Adamek *et al*. 1992), were found in higher amounts in spoiled hams. In this sense, 2-pentanone and metilketones have been considered that could be derived from acyl lipids through microbial degradation (Barbieri *et al*. 1994; Stahnke 1995). Numerous alcohols were found in both unspoiled and spoiled hams although those from spoiled hams had higher amounts overall of ethanol, 2-metilbutanol and 3-metilbutanol. These compounds have been found as a result of microbial growth in culture media and in meat products (Adamek *et al*. 1992; Stahnke 1995; Montel *et al*. 1996). A possible metabolic pathway to alcohol formation could be the oxidation of aldehyde to the corresponding acid and hereafter this is esterified with ethanol by microbial action (Stahnke 1994). That could explain the higher amount of alcohols and the lower levels of aldehydes in spoiled hams compared with unspoiled hams. The higher amounts of acids in spoiled than in unspoiled hams could be explained by the microbial fermentation of free amino acids via Stickland reactions in which the micro-organisms ferment amino acids by coupled oxidation reactions between pairs of amino acids (Gottschalk 1986). Amino acids could be released by micro-organisms with high proteolytic activity, such as the Enterobacteriaceae. Microbial fermentation of amino acids results in the production of acetic and butanoic acid, found in high amounts in spoiled hams. Also, spoiled hams showed a high amount of esters that could be related to microbial sterase activity (Stahnke 1995).

The higher amount of dimethyldisulphide, referred to as unpleasant odour (Ames and MacLeod 1985), found in spoiled hams could be related to off-flavours of this alteration.

Table 2 Volatile compounds identified by GC/MS in unspoiled and spoiled hams

Compounds	Ik[a]	Unspoiled hams[b]	Spoiled hams[b]
Aldehides			
2-metyl propanal	553	1·86	2·27
3-methyl butanal	652	33·98	19·21
2-methyl butanal	662	15·58	4·57
pentanal	697	16·41	20·31
hexanal	800	580·85	145·32
2,4-octadienal	894	2·01	–
2-heptenal	959	5·44	–
2,4-nonadienal	995	13·63	–
octanal	1004	13·92	–
benzeneacetaldehide	1054	2·26	–
2-octenal (E)	1062	1·65	1·69
nonanal	1106	16·84	–
2-nonenal	1165	0·53	–
decanal	1209	2·78	–
2,4-decadienal (E,E)	1327	0·93	–
Ketones			
2-propanone		1·29	11·14
2,3-butanedione	586	1·44	8·27
2-butanone	600	2·88	30·75
2-pentanone	686	37·46	52·51
2–3 pentane dione	694	1·35	–
2-butanone 3-hydroxy	711	3·21	41·59
2-pentanone, 3-methyl	752	–	1·98
2-hexanone	789	13·34	33·52
2-heptanone	891	26·96	9·65
2,3 octanedione	982	2·11	2·17
6-methyl, 5 hepten-2-one	988	–	1·24
2-octanone	992	5·53	2·60
2-nonanone	1093	0·92	0·77
Alcohols			
Ethanol		4·89	61·66
2-propanol	500	3·73	12·14
propanol		–	17·51
butenol methyl	611	0·66	2·54
1-propanol, 2-methyl	626	4·21	3·52
1-penten-3-ol	682	4·45	3·23
1-butanol 3-methyl	734	26·87	1·52
1-butanol 2-methyl	738	10·03	35·25
1-pentanol	766	14·51	17·87
1–3 butanediol	815	1·57	12·00
1-hexanol	868	33·56	10·57
phenol 3-ethyl	923	1·24	–
1-octen-3-ol	980	6·82	2·89
1-hexanol-2-ethyl	1014	–	1·43
benzenemethanol	1042	4·82	–
benzeneethanol	1127	2·78	4·38
Hydrocarbons			
heptane	700	47·52	12·01
heptane 3-methylene	755	0·33	–
toluene	770	5·25	2·38
1-octene	799	5·80	
4-octene	806	2·9	1·36

Table 2 — *continued*

Compounds	Ik[a]	Unspoiled hams[b]	Spoiled hams[b]
2-octene (E)	810	–	2·86
2-octene	815	–	1·14
ethenyl benzene	897	2·83	–
nonane + O-xylene	902	28·98	12·45
2 pinene	944	3·51	–
benzene 1-ethyl 4-methyl	970	5·24	1·24
decane	1000	1·08	4·67
limonene	1040	1·46	–
undecane	1100	–	26·11
dodecane	1200	0·88	3·98
tridecane	1300	–	3·17
tetradecane		1·88	–
Acids and esters			
acetic acid		–	6·17
ac. acetic ethyl ester	614	–	2·52
propanedioic acid dimethyl	757	–	3·47
butanoic acid	784	3·08	24·45
3 methyl butanoic acid	838	1·31	3·17
2 methyl butanoic acid	848	0·78	1·25
butanoic acid, 2-methyl ethyl ester	850	0·56	1·76
butanoic acid, 3-methyl ethyl ester	853	0·99	3·30
pentanoic acid	875	5·67	1·53
hexanoic acid ethyl ester	997	4·18	4·01
octanoic acid ethyl ester	1195	1	–
nonanoic acid ethyl ester			
Sulphur compounds			
carbon disulphide	536	6·19	–
dimethyl disulphide	747	6·45	33·89
propanal methylthio	908	1·51	–
Other			
CO_2		2·88	13·17
furan-2-ethyl	703	2·12	–
2-methyl pyrazine	799	–	5·64
hexanenitrile	879	1·28	1·36

[a] Kovats indices calculated for DB-5 capillary column of the GC/MS system. [b] Expressed as arbitrary units of area $\times 10^{-6}$. Each value is the average from five spoiled and three unspoiled hams, respectively. (–) not detected.

Dimethyldisulphide derives from degradation of sulphur amino acids, probably by microbial desamination (Belitz and Grosch 1992).

The findings of this study showed that Enterobacteriaceae could be the main micro-organisms involved in 'bone taint' spoilage. The volatile compounds from the spoiled hams arise from higher amounts of ketones, alcohols and esters that could be originated by microbial metabolism of these bacteria. Thus, Enterobacteriaceae and volatile compound analyses should be considered in determination of incipient spoilage.

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