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OBSERVATIONS ON 'TAINT' IN GAMMONS

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INTRODUCTION

Bone-taint or the deep-seated spoilage of meat is of bacterial origin (Haines 1937, 1941: Ingram 1952: Nottingham 1960). Many different species have been isolated. For example Cl. oedematiens from the hip joint of beef (Haines and Scott, 1940): Aerobacter, Alcaligenes, Clostridium, Corynebacterium, Flavobacterium, Escherichia, Micrococcus, Proteus, Pseudomonas, Serratia, Streptococcus from chucks and rounds of beef and the prescapular lymph nodes by Lepovetsky et al (1953). Species of Streptococcus and Clostridium were recovered in lightly or uninjected hams and Micrococcus in gammons by Ingram (1952). Nottingham (1960) demonstrated the presence of Bacillus, coliform bacteria, Pseudomonas and Clostridium in the ischiatic lymph nodes of cattle.

An opportunity arose in this laboratory of examining 'taint' in gammons. These were part of a consignment for the Christmas trade which had been frozen as pork legs (-10°C) for 8 months, thawed at 10°C for 26 h, injected by multi-needle and immersion cured for 3 d. Within hours of reaching the customer, evidence of taint (strong 'off' odour in region of the femur bone and stifle joint) was noted in 10 - 15% of gammons. Two of the affected gammons were stored at +1°C for 18 h. One was examined within 24 h and the second was examined after 23 months.

MATERIALS AND METHODS

Samples

Gammon A had a sticky and moist outer surface (rind) with a distinctly strong odour in the bone region On cutting, the muscle colour was normal but the odour had permeated the whole tissue. Gammon (B) had similar characteristics. It was vacuum packed in a 'Cryovac' bag (polyvinylidene/

polyvinylchloride copolymer) and stored in a 'Novum' chest-type freezer (Novum Ltd., Bluebell, Dublin) at -10°C for 23 months.

Gammon A

Bacteriological

Muscle tissue was excised aseptically adjacent to the bone and 3.55 g weighed into 90 ml Ringer peptone (0.1%) diluent. This was homogenised in a Colworth stomacher 400 (A J Seward, Stamford St., London) and was designated 'muscle homogenate'. Serial dilutions in the same diluent were plated on Oxoid Plate Count Agar with 4% of added salt (4 PCA) for incubation at 25°C (3 d) and at 37°C (2 d). A Gram stain and a spore stain were made of the homogenate.

The femur was excised aseptically and rinsed with 200 ml Ringer/peptone water. A similar rinse of the deboned gammon was taken by shaking it in a plastic bag and recovering the rinse by filtration through a sterile funnel. Aliquots of each rinse were filtered through Oxoid membranes and these were incubated on m-Enterococcus medium (Difco) for a count of faecal streptococci by the method of Slanetz and Bartley (1957). Clostridium spp. were enumerated by filtering (a) heated (80°C/10 min) and (b) unheated portions of the rinses by the method described by Dempster (1973).

Classification of isolates

Twenty two isolates were picked from the 4 PCA plates incubated at 25°C and purified by successive streaking. Films were stained by Hucker's modification (1927) of the Gram stain. Motility was determined by the hanging-drop technique (Mackie and McCartney 1960) on 18 h nutrient broth (NB) cultures. Oxidase and catalase tests were made on 18 h nutrient agar (NA) streaks by the methods described by Harrigan and McCance (1966).

Chemical

Salt (NaCl) was determined on 10 g lean tissue surrounding the femur by the method of Schonhertz (1955); nitrate and nitrite by the methods of Follet and Ratcliff (1963) and Elliott and Porter (1971) using another 10 g of meat from the same area. The pH was measured electrometrically on 20 g tissue at room temperature.

Gammon B

Bacteriological

The gammon was thawed at room temperature (15°C) for 24 h. The bag was opened and the free liquor (drip) decanted (20 ml) into a sterile glass jar. The femur bone was excised aseptically and the adhering muscles (M semimembranosus; M quadriceps femoris; M Glutaeus medius) separated from the bone and each placed in a plastic bag. The bone was then rinsed in 200 ml Ringer-peptone solution. Ten g of the semimembranosus (freed of fat) were weighed into 90 ml Ringer-peptone solution and homogenised as described earlier. The drip, bone rinse and the muscle homogenate were diluted in the above diluent and plated on 4 PCA for incubation at 25°C and 37°C. Faecal streptococci and clostridia were enumerated as described above.

Classification of isolates

Seventy nine cultures were obtained from the 4 PCA plates (25°C/3 d), purified and tested as described above. The schemes devised by Gardner (1971:1973) for classifying rod-shaped bacteria from bacon curing brines were employed. In addition, the A.P.I. 20E (appareils procedes d'identification) system (API Lab Products Ltd., Rayleigh, Essex, England) for Enterobacteriaceae and other Gram negative bacteria was used in conjunction with the API data base.

Chemical

Salt (NaCl), nitrate, nitrite and pH determinations were made on the three samples as described previously.

RESULTS AND DISCUSSION

The results of examination of gammon (A) are shown in Table 1. The extremely high numbers of microorganisms in the muscle are consistent with the presence of a noxious odour and with the high pH value (6.52) of the material. Most rapid bacterial growth occurs on meat with pH values exceeding 6.0 (Ingram 1948; Kitchell and Ingram 1965; Dempster 1974: Rey et al, 1976). Ingram (1952) suggested that the addition of an appropriate acid sufficient to bring the pH of the muscle below 6.0

during curing might be beneficial. He considered that low acidity must be a predisposing cause of taint.

Rinses of the gammon and bone showed the presence of faecal streptococci and small numbers of clostridia. These were the major types found by Ingram (1952) in tainted hams but not in gammons and he also noted the presence of enormous numbers (10^5 to 10^8 g^{-1}) of bacteria in the spoiled regions of such material. The present results confirm Ingram's findings on numbers but are at variance on types. Ingram found micrococci to be the major component of the flora of spoiled gammons but no micrococci were found in this gammon. A Gram stain of the homogenate showed only Gram positive rods and cocco-bacilli. However, the cocco-bacilli may have been cold-damaged micrococci. There were no spores present, Tables 1 and 4.

The chemical results, presented in Table 1 indicate that the gammon was satisfactorily cured. This was confirmed by the 'normal' colour of the cut muscle when the gammon was dissected.

A more detailed examination was made of gammon B. Results are presented in Tables 2 and 3 and the composition of the microflora in both gammons is presented in Table 4. After 23 months storage at -10°C, viable bacteria were present in large numbers especially psychrotrophic types. Faecal streptococci and spore-forming bacteria were found in varying numbers. A microscopic count of the muscle homogenate (M. semimembranosus) was 1.3×10^9 g^{-1}. A similar count on the 'drip' was 3.2×10^8 ml^{-1}, Table 2, and a gram stained preparation of this showed Gram positive rods (1.0 - 4.0 x 0.5 µ), Table 4.

The chemical analyses of 3 muscles from this gammon are shown in Table 3. With the exception of pH, all other values were within the normal range and the gammon appeared to be satisfactorily cured. The pH values (6.15 - 6.25) were similar to that of the unstored gammon. Obviously these high values predisposed the meat to the formation of taint (cf. Ingram 1952).

The twenty two isolates from gammon A fell into 3 groups. Thirteen were Gram positive short rods (1.0 - 2.0 x 0.5 µ); 6 were Gram positive rods (1.0 - 4.0 x 0.5 µ) and 3 were Gram positive cocco-bacilli (0.25 - 1.0 x 0.5 - 1.25 µ). Eight were motile, nineteen were catalase positive and four were oxidase positive, see Table 4. No attempt was made at further identification of these.

The cultures (79) isolated from gammon B fell into two groups. Fifty nine (75%) were Gram positive rods; 18 out of the 59 were catalase positive and seven were motile. They were isolated from the semimembranosus muscle and the 'drip'. The remaining cultures (20) were Gram negative rods. They were found only in the bone rinse and were further examined, see Table 5. On the basis of the tests tabulated in this table and the A.P.I. 20 E diagnostic system for Enterobacteriaceae, three were assigned to the genus vibrio and the remaining seventeen as follows: Hafnia alvea (6): Enterobacter cloacae (2): Serratia marcesens (3): Pseudomonas (2) and unclassified (4).

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TABLE 1: Bacteriological and Chemical examination of spoiled gammon A

Sample	Total aerobic count ($x10^7$) at: 25°	37°	Faecal streptococcus per 5 ml	Clostridium spp. per 5 ml Total	Spores
Muscle homogenate[a]	3.75	1.825	NT*	NT	NT
Gammon rinse	NT	NT	279	2	Nil
Bone rinse	NT	NT	289	Nil	Nil

[a] Chemical analyses: NaCl 3.4% (w/v): $NaNO_3$, 110 ug/g: $NaNO_2$, 44 ug/g: pH 6.52. *NT - Not Tested.

TABLE 2: Bacteriological status of cold-stored (-1.0°C/23 months) gammon B

Sample	Total count at (x10^6) 25°C	37°C	Faecal streptococcus per ml (x.10^3)	Clostridium spp. per 5 ml Total (x10^3)	Spores (x10^3)
M semimembranosus[a]	93	0.159	25	-	19
Bone rinse	0.8	0.102	0.82	0.01	0.1
Drip[b]	0.8	0.0424	0.46	0.35	0.6

[a] Microscopic count, 131 x 10^7 g^{-1}. [b] Microscopic count, 320 x 10^6 ml^{-1}

TABLE 3: Chemical analyses of three muscles excised from gammon B

Muscle	% NaCl (M/v)	$NaNO_3$ ug/g	$NaNO_2$ ug/g	pH
M quadriceps femoris	4.03	152	22	6.25
M semimembranosus	4.68	186	15	6.15
M gluteus medius	5.06	234	24	6.25

TABLE 4: Composition of microflora of tainted gammons

Gammon	No. of isolates	Gram + ve short rod	Gram + ve rod	Gram + ve cocco-bacillus	Gram - ve rod
		1.0-2.0x0.5µ	1.0-4.0x0.5µ	(0.25-1.0x0.5-1.25µ)	(1.0-5.0x0.5µ)
A Muscle homogenate	22	13[a]	6[b]	3[c]	
B. M semimembranosus	25	25[d]			
Bone rinse	20				20[e]
Drip	34				34[f]

	Motility	Catalase	Oxidase
a	5/13+	11/13+	1/11+
b	3/6 +	5/6 +	3/6 +
c	0/3 +	1/3 +	0/3 +
d	0/25+	1/25+	1/25+
e	7/20+	16/20+	4/20+
f	0/34+	1/34+	1/34+

TABLE 5: Gram negative rods isolated from bone rinse of tainted gammons after storage at -18°C compared with vibrio spp. obtained from bacon (Dr G A Gardner) and reference strain V. costicolus

Test	Source of Cultures: Vibrio costicolus*	Bone rinse (20 isolates)	Bacon isolates (Gardner)
Motility	+	+ (7)**	-
Growth in broth containing 12% NaCl	+	-	-
Reduction of nitrate	+	+ (18)	+
Thornley's (1960) arginine test	+	+ (11)	-
Arginine decarboxylase Moller (1955)	+	-	-
Lysine decarboxylase	-	+ (13)	-
Penicillin sensitivity (2 i.u./ml)	+	+	+
Gelatinase	+	+ (4)	-
Fermentation of sucrose	+	+ (12)	+ (8)
Fermentation of glucose	+	+	+
Methyl red test	+	+ (10)	+
Voges-Proskauer test	+	+ (11)	+
Indole	-	-	-
Catalase	+	+ (15)	+
Oxidase	+	+ (3)	+
H_2S	-	+ (16)	-

* Reference strain NCMB 701 (see Gardner 1973)

** Number in brackets represents the number of culture giving a positive reaction

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