# THE BACTERIOLOGY OF MEAT AND FISH PASTES, INCLUDING A NEW METHOD OF DETECTION OF CERTAIN ANAEROBIC BACTERIA

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### INTRODUCTION

MEAT and fish pastes packed in small glass containers have been popular in England for many years, and it was thought that the methods adopted in controlling the output of a large factory would be of interest. Preliminary notice of this work has been given in a previous communication (Crossley, 1936).

The ingredients used vary somewhat in different factories, but in general it may be stated that meat pastes are mixtures of veal, bacon, beef, and fat, with an addition in some cases of chicken or turkey (often tinned).

Similarly fish pastes may contain tinned salmon, herrings, pilchards, bloaters, small proportions of anchovies, and beef fat, lard or butter; in addition, particular pastes may contain shrimps or tinned shellfish. Finally, salt and various seasonings and colouring materials are frequently added, together with a small amount of starchy material not usually exceeding 5%.

The ingredients are subjected to preliminary cooking where necessary, and then mixed to a paste by passage several times through mincing and milling machinery. The prepared paste is mechanically filled into small glass jars, which are then hermetically sealed by means of a metal cap fitted with an internal rubber ring. The sealed jars are heated in large retorts by means of pressure steam; the heat treatment employed must be sufficient to kill all bacteria capable of causing subsequent decomposition, but overcooking of the paste must be avoided. Providing the heat processing has been sufficient, and the closure remains airtight, the paste is safe from bacterial decomposition for an indefinite period.

### The causes of failure

Complaints of decomposition of these pastes are rare and frequently unfounded, but genuine cases of spoilage can usually be ascribed to (1) imperfect sealing of the jar, or (2) faulty heat processing, resulting in the survival and subsequent development of resistant bacterial spores. It will be shown that certain putrefactive spore-forming anaerobes of the *Cl. putrificum* and *Cl. sporogenes* types are the main causes of decomposition.

The results of extensive bacteriological examinations of the raw materials, manufacturing processes, and the finished products will now be given.

### Experimental methods

Standard bacteriological media were employed, viz. nutrient agar, nutrient broth, Maconkey broth, skim milk, and Rettger's egg and meat medium. Solid materials were inoculated direct into the appropriate media, whilst for enumeration purposes suspensions in sterile distilled water were used. Anaerobic cultivation was carried out in Macintosh and Fildes' anaerobic jars. During the course of this work a simplified method (to be described later) was evolved for the cultivation of anaerobes.

### The bacterial flora of the pastes before processing

It is well known that the rate of thermal destruction of bacteria is proportional to their initial number. In addition, it is also known that putrefactive . anaerobes do not readily attack complex proteins. It follows, therefore, that a high bacterial content before processing is undesirable for two reasons:

(a) sterility is difficult to achieve, and

(b) extensive bacterial growth before processing may result in some protein decomposition, which would encourage the growth of any anaerobes which survive processing.

The bacterial flora of pastes was found to be derived from the raw materials, handling operations during manufacture, machinery and equipment, the atmosphere, and the glass jars.

### Raw materials

The main bacteriological features of good quality materials in general commercial use were studied; summarized results of large numbers of examinations are given in Table I.

As would be expected, tinned products were frequently sterile, or contained only very small numbers of bacteria. These were mainly aerobic spore-forming species, but anaerobic species appeared in a small proportion of the samples.

The various cooked meats were rarely sterile, but usually contained only small numbers of harmless bacteria. Chicken meat appeared to require the most careful attention, since putrefactive anaerobes were found therein the most frequently.

Of the fish products, shrimps were the greatest source of danger. In addition to an extensive bacterial flora, putrefactive anaerobes were of common occurrence. Shrimp extracts were still worse, and in the opinion of the author should be avoided.

A prolific source of bacteria, and the main source of anaerobic species, was found to be seasoning and colouring materials. (This is no doubt due to the soil origin of seasoning materials.) In spite of the very small quantities of these materials incorporated, they usually introduced more putrefactive anaerobes than the actual meat or fish ingredients. Attempts to sterilize seasonings without loss of aromatic properties were unsuccessful. To summarize, the sum

total of bacteria actually derived from the ingredients should not be large, but may include small numbers of objectionable putrefactive anaerobes. Acidfermenting anaerobes, e.g. *Cl. butyricum* and *Cl. welchii*, were of more frequent occurrence.

# Handling operations

Manual contact is practically unavoidable in many operations involved in the preparation of pastes, such as the cutting, trimming, and weighing of ingredients. Excessive handling not only increased the total bacteria, but occasionally added putrefactive anaerobes which were originally absent. The presence of a large proportion of cocci in the flora was taken as an indication of faulty handling. Some contamination must follow manual handling under the best conditions; an indication of the results to be expected is shown by the comparison obtained between meats immediately after cooking and after preparation for use (Table I). Mechanical handling was introduced wherever possible, and the use of a chlorine disinfectant insisted upon for the washing of hands.

The washing of ingredients, which is sometimes desirable apart from bacteriological considerations, may considerably increase the bacterial population, owing to contamination of the wash water by accumulated bacteria from ingredients and by the operatives' hands (e.g. on one occasion washing a batch of shrimps increased the bacterial count four times).

On the other hand, careful washing in running water greatly reduced bacterial contamination.

### Machinery

Plant control samples showed that properly cleaned machinery only contributed small numbers of bacteria, and that any contamination was almost directly proportional to the amount of handling involved in the operation of the machine. Under these conditions the mincing process required the greatest care.

Machinery selected was constructed of suitable non-corroding materials and of accessible design for cleaning purposes, whilst efficient cleaning of tables was achieved by substituting stainless steel tables for wooden ones.

### The atmosphere

As judged by the number of organisms falling into 95 mm. Petri dishes exposed for 10-20 min., the organisms present in the atmosphere varied widely within a short space of time in a given room, and were affected by the system of ventilation, presence of air currents, the materials handled, the number of people working in the room, and the season of the year. On the whole, atmospheric contamination was not of major importance.

### The glass jars

Glass jars as received from the makers may contain dust which harbours bacteria, although putrefactive anaerobes were not found. Hand washing was found unreliable, but unfortunately some automatic washing machines were

Other organisms	Cocci found	Cocci found	Cocci found. Occasion-	ally traces coliform organisms	1	Cocci found		I	I	]	Proteolytic cocci com-		Cocci found in 3% of samples	Cocci usually found	Mixture of varied	Mixture of varied		Cocci found Mixture of varied species including coli- form trans	Mixture of varied species including coli- form types	res of these.
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Cl. butyricum and Cl. welchii	Not uncommon Not uncommon	Not uncommon Not uncommon	đ		Found in	d	d		Found in	Found in	Not found	Found in		Found in	a	ч	Found in	70 0	Found in samples	data are giver
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Aerobic spore-forming bacteria	Main species present Main species present Occasional traces	Main species present Main species present	Main species present Main species present	-	Main species present	Main species present	Main species present Main species present	Only 7 samples examined	Main species present	Main species present	Main species present	Main species present	Only 15 samples examined 10 Found in 30% of samples	Common	Common	Common	Common	Main species present Common	Соттоп	Seasoning materials include salt, mustard, pepper, nutmeg, mace, and coriander. For brevity data are given for mixtures of these.
Samples sterile %	5 Nil	5 Nil	01 Nil		ũ	IIN	60 10	Only	70	50	70	65	Only 1 10	liN	IIN	liN	10	20 Nil	Nil .	nustard, j
mbers ria	60 6000 Mainly sterile		460 10.000		200	2300	Traces Traces	650	Traces	Traces	Traces	Traces	All sterile Traces	20	125,000	Varied from	Traces	250 140,000	150,000	ərials include salt, ı
, Ingredients	Bacon, cooked Bacon, prepared for use Veal. tinned	Veal, cooked Veal, prepared for use	Beef, cooked Beef, prepared for use		Corned beef, tinned	Corned beef, prepared for use	Tongue, tinned Chicken and turkey, tinned	Chicken and turkey, prepared	tor use Tinned salmon	Tinned tomalley	Tinned sardines	Tinned shellfish	Tinned pilchards Tinned anchovies in salt	Bloaters, packed in salt	Shrimps	Shrimp extract	Tomato purée	Fat, various sources, cooked Mixed seasoning materials*	Colouring materials	* Seasoning mate

# Table I. Summary of the main bacteriological features of the ingredients of meat and fish pastes

also open to criticism. Owing to rapid loss of strength detergent rinses showed no reliable bactericidal action, and the provision of a final treatment with steam and hot air was necessary. Chemical and bacteriological tests of the detergent and rinse solutions, and of the finished jars, were very valuable. This work indicated that contamination from the glass jars was relatively small.

### Standards

The bacterial flora of the finished pastes before processing is summarized in Table II. In order to distinguish between bacteria inherent in the materials and those introduced during the processes of manufacture, small batches of pastes were occasionally prepared under aseptic conditions in the laboratory. Examinations of these pastes indicated the bacterial flora definitely contributed by ingredients; the results are incorporated in Table II. Standards were difficult to fix owing to variations of procedure required in the preparation of different varieties of pastes, but with very careful handling it was considered that the total bacteria present in the finished paste should not exceed twice the number present in the mixed ingredients. Putrefactive anaerobes should only be present in very small numbers. Tests were always made for the presence of coliform organisms, the numbers of which gave useful indications of good or bad handling.

Table II.	Summary	of the flor	a of meat	and fish	pastes before	processing
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Variety of paste	No. of samples	Average numbers of bacteria per gram	Cl. putrificum and Cl. sporogenes	Cl. butyricum and Cl. welchii	General flora				
Salmon and shrimp	30	93,000	Present in 3 samples	Present in 10 samples	Similar for all varieties. Aero- bic spore-forming bacilli				
Lobster	43	60,000	Present in 4 samples	Present in all samples	Large numbers of various common cocci, mainly sta- phylococci				
Anchovy	20	62,500	Not found	Present in all samples	Small numbers of coliform organisms, usually of the "aerogenes" type				
Sardine and tomato	40	38,000	Present in 2 samples	Present in all samples	Small numbers of spore- forming anaerobes				
Chicken and ham	52	185,000	Present in 10 samples	Present in 42 samples	· · · · ·				
ngredients mixed aseptically in laboratory:									
Lobster	12	12,900	Present in 1 sample	Present in all samples	—				
Chicken and ham	11	28,300	Present in 5 samples	Present in 5 samples	<u> </u>				

### THE RETORTING PROCESS

The retorting process is the most important manufacturing operation, since if it is inadequate serious failure may follow. The object of the work to be described was, first, to establish an adequate heat treatment, and secondly, to elucidate the errors which arose in practice.

It is known that heating at  $240^{\circ}$  F. for a period of 10 min. in laboratory media is sufficient to kill heat-resistant spores, including those of *Cl. botulinum*, whilst *Salmonella* toxins are destroyed in 30 min. at  $212^{\circ}$  F. It is also known that in practice this heat treatment must be extended in order to ensure penetration to the centre of the jars.

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### Experimental work

Two experimental methods were employed:

(1) Experimental processings were carried out in which the internal temperatures were measured and the time necessary to ensure the required heat penetration was obtained. Specially calibrated maximum thermometers of the clinical type can be used, but the only method of obtaining a continuous reading is to use a sealed-in thermocouple. Data obtained by this method for canned foods have been published by Black (1931) and Lancefield (1933). The method is very useful, but only approximate indications are obtained for two reasons:

(a) It is very difficult to carry out under practical factory conditions.

(b) It is based on the doubtful assumption that the thermal death-point of spores, i.e.  $240^{\circ}$  F. for 10 min., is the same in a paste as in laboratory media.

(2) A second series of experiments was carried out in which jars of paste heavily inoculated with heat-resistant spores were retorted in the factory along with ordinary batches of paste, and then tested for sterility, both at once and after 4 weeks' incubation at 37° C. In this way retorting processes were worked out which were satisfactory, under factory conditions, even with a large inoculation of organisms. The species used were pure cultures of Cl. putrificum, Cl. sporogenes, and Cl. welchii, and in addition cultures actually isolated from retorted pastes were employed. These included strains of aerobic bacilli identified as B. subtilis, B. megatherium, and B. vulgatus, but particular attention was given to an anaerobic species similar to Cl. flabelliferum (Sturges & Reddish) described by Bergey (1930). This organism was encountered during the summer of 1932 in tinned salmon, shrimps, and chicken meat, and caused very serious difficulty owing to its extremely pronounced heat resistance. A very similar heat-resistant strain of Cl. sporogenes has recently been described by Baumgartner & Wallace (1936). The experiments were made both with single cultures and with mixtures of anaerobic and aerobic species.

### Factory practice

During this work it became apparent that the actual factory processes were liable to errors which did not arise under laboratory conditions, in particular the following:

(1) The design of the majority of retorts at present available is not entirely satisfactory, resulting in uneven heat distribution in the retort, which is caused mainly by the arrangement of steam inlets. By the combined use of maximum thermometers and inoculated jars it was discovered that temperature variations could be quite considerable, and that in certain places in the retorts the jars were not receiving the heat treatment indicated by the external thermometer and pressure gauge; in these jars inoculated organisms were not killed. Great improvements followed a suitable rearrangement of steam inlets, but an entirely new type of retort is being designed to eliminate this defect.

(2) The actual temperature control required close attention. Hand operation of retorts could be satisfactory, but success depended entirely on the human element. Automatic temperature regulators combined with thermographs have obvious advantages in reducing the possibility of human error and in the provision of a written record. These instruments were found to be very satisfactory as a rule, but not infallible; they were somewhat susceptible to fluctuations due to various small causes, but with regular checking and supervision yielded good results when operated under suitable conditions.

The actual processing procedure must be worked out by individual investigations, since the heat treatment necessary varied with the size and shape of the glass jar, the size of the metal cap, and to a lesser extent the variety of paste. It appeared certain, however, that under practical conditions any temperature below  $235^{\circ}$  F. was unsafe. Furthermore, the extremely heat-resistant anaerobic species, *if present in large numbers*, could survive at  $235^{\circ}$  F. for 10 min. at the centre of the paste. The anaerobe isolated in 1932, and also an anaerobic species producing acidity and gas, common in tomato products, were unusually resistant both to factory retorting and in the laboratory autoclave. Apart from these strains the aerobic species. In doubtful cases the author found that a slight increase in temperature was more lethal than extension of the heating period. Most manufacturers employ temperatures from 235 to 240° F.

# THE BACTERIAL FLORA OF MEAT AND FISH PASTES

# (1) The flora of sound pastes

From August 1932 to January 1936 the author examined 14,365 jars of paste from a number of sources. The findings may be summarized as follows:

	Number	%
Sterile	12,624	87.9
Spores of "harmless" species, e.g. aerobic species identified as B. subtilis (Ehrenberg), B. cereus (Frankland), B. vulgatus (Migula),	1,726	12.0
B. mesentericus (Migula), B. mycoides (Flugge), B. megatherium		
(De Bary), and less common anaerobic species Cl. butyricum		
(Prazmowski) and <i>Cl. welchii</i> (Migula) Putrefactive anaerobes, e.g. <i>Cl. sporogenes</i> and <i>Cl. putrificum</i> types	15	0.1

It will be seen that the flora of non-sterile samples consisted mainly of common aerobic spore-forming bacilli; much less frequently putrefactive anaerobes were found. The organisms were almost entirely protein-decomposing types of soil origin.

### (2) The causes of spoilage

Jars of paste artificially inoculated with the above species were incubated in batches at 22, 32 and 41° C., for periods up to 3 months. At intervals jars were opened and the contents inspected for spoilage and examined bacteriologically. (a) The anaerobic bacilli.

The Cl. sporogenes and Cl. putrificum types were able to bring about very objectionable putrefaction, accompanied by more or less violent gas production. Nevertheless, spores of these anaerobes sometimes remained dormant in pastes for several months. It is well known that the germination of some spores is very irregular, and other factors must also be considered. Pastes containing less than 2% of salt were the most readily attacked, although slow growth could take place in pastes containing up to 3% salt. Anchovy paste, containing over 3% salt, was practically immune from attack. Other pastes, such as bloater paste, may have a very acid reaction (pH below  $6\cdot0$ ); in such pastes anaerobic species failed to develop. Other pastes containing Crustacea may have a neutral or slightly alkaline reaction; these were found very susceptible to anaerobic decomposition.

The acid-fermenting anaerobes, e.g. *Cl. welchii* and *Cl. butyricum*, have been found to cause "blown tins" of corned beef, and fermentation of meat and vegetable soups and of tomato products, but were never found to cause trouble in the meat or fish pastes.

### (b) The aerobic bacilli.

Contrary opinions have been expressed on the potential dangers of aerobic spore-forming bacilli. The work of Savage (1923) founded the general opinion that these types are not in themselves a cause of unsoundness. However, Lancefield (1934) has stated that B. subtilis is the chief cause of unsoundness in canned meats, and that some strains are not strictly aerobic. In this investigation it has been shown that aerobic spore-forming bacilli cannot unaided bring about decomposition within a sealed jar of meat or fish paste.

The incubation experiments using inoculated jars showed that aerobic species remain alive in pastes for several months, but without increasing in numbers or causing any decomposition.

In addition, duplicates of the 14,365 samples reported above were incubated for 14 days at 37° C. Of this second group of 14,365 samples, only five showed signs of decomposition, and in each case this was due to anaerobes. Finally, many millions of these jars of paste are sold annually, so that spoilage caused by aerobic species would lead to numerous complaints if it did in fact take place.

The importance of aerobic species lies in the fact that the thermal deathpoint is about the same as, or only slightly higher than, that of the dangerous anaerobes. Survival of the aerobes indicates that the margin of safety is extremely small, and that a heavy infection of anaerobic species before processing might not be completely destroyed. It appears likely also that these aerobic species may commence to grow after the jars are opened by the consumer.

### (3) The examination of returned unsound pastes

Complaints of unsoundness are uncommon, so that the amount of material available has not been large. Faulty sealing has been recorded as the cause of a large proportion of the cases of decomposition encountered. Usually a displacement of the rubber ring was visible. The main bacteriological features were the presence of large numbers of atmospheric cocci and other non-sporeforming organisms, and concentration of the bacteria at the surface of the paste—the lower portions were frequently sterile. In the remaining cases, spoilage was associated with the presence of putrefactive anaerobes, or a combination of these with aerobic spore-forming bacilli, which had survived processing.

It seemed clear, therefore, that in order to ensure complete safety, some routine method of bacteriological examination of the finished pastes is an essential form of control.

### CONTROL OF THE FINISHED PRODUCTS

No batch was allowed to leave the factory until representative samples taken after processing were passed in the laboratory as satisfactory.

Owing to the large numbers of samples involved, difficulties arose in the laboratory due to the lack of a suitable test medium. The main requirements of a suitable medium were:

(1) It must be cheap, simple to prepare in large quantities and readily duplicated.

(2) It must detect rapidly small numbers of spores of the anaerobic species involved, and preferably certain aerobic species also.

(3) It should support growth without the necessity for elaborate methods of anaerobic cultivation, and in addition the use of the microscope should be minimized.

(4) Positive reactions must be striking and not of a transient nature.

Lengthy experiments were carried out, both in the presence and absence of sterile salmon and shrimp paste, using very dilute spore suspensions of pure cultures of *Cl. putrificum* (N.C.T.C. 4717), *Cl. sporogenes* (Bellette), *Cl. sphenoides* (Tholby), *Cl. oedematiens* (Cossard), *Cl. histolyticum* (Weinberg), *Cl. welchii* (Migula), *Cl. tertium* (Henry), *Cl. flabelliferum*<sup>1</sup> (Sturges & Reddish), and *B. vulgatus*<sup>1</sup> (Migula), inoculated into seven common media, viz. liver broth (Cameron & Williams, 1928), dried liver (Difco) broth, corn-liver medium (McClung & McCoy, 1934), cooked meat medium (Torrey, 1926), egg broth, egg and meat (Reddish & Rettger, 1923), and heart-liver-casein digest (Spray, 1933).

The data obtained are too lengthy for inclusion here, but it may be said that none of these media proved entirely suitable, for various reasons such as poor visibility or transient nature of the reactions, unreliability in the absence of

<sup>1</sup> Isolated from processed pastes.

# Meat and Fish Pastes

heavy inocula or of anaerobic conditions of cultivation, or the time and expense of preparation. The media were not improved in the presence of fish paste.

A new method was then devised, using milk as the basis of a routine test medium in place of meat or egg preparations. The method may be summarized as follows:

### (a) Medium

Skim milk containing 1% peptone and 0.01% bromo-cresol purple (1.0 g. indicator powder dissolved in 19 ml. decinormal sodium hydroxide, made up to 100 ml. with distilled water). Tubed in 10 ml. quantities and steamed for 20 min. on four successive days. Incubation of control tubes is desirable.

### (b) Inoculation

1.5-2.0 g. of paste taken from the centre of the jar by means of sterilized metal "section lifters" about 16–18 cm. long. In effect the whole central portion of the contents of the jar is inoculated; this heavy inoculation of paste is an essential requirement of the method.

### (c) Incubation

Three to four days at 37° C. The method yielded excellent results with all the species tried, even when very small inocula were used, and was found to possess the following important advantages:

(1) Culture tubes are simply and cheaply prepared in large numbers.

(2) Anaerobic cultivation is not required.

Trials were made with all the media examined using simple methods of partial anaerobiosis, viz.:

(a) a surface layer of paraffin, and

(b) the addition of reduced iron, which was described during the course of this work by Hastings & McCoy (1932), and Scott & Brandly (1933).

In the presence of meat or fish pastes<sup>1</sup> these methods proved unnecessary and yielded no advantages; it was observed that anaerobic cultivation was very effective as a means of improving a poor medium, but that in general the nutritional composition of the medium was of primary importance.

(3) Only a small proportion of cultures require microscopical examination.

(4) Aerobic species commonly met with are also detected.

(5) Positive reactions are very striking and visible for several days; much superior in this respect to those obtained in any of the other media.

It is usually possible by rapid visual inspection to distinguish between reactions due to anaerobes or aerobes, and to record a rough grouping of bacterial types.

The types of reaction which may be met with are:

(a) Neutral or alkaline reaction (strong purple colour), gas production, soft curd followed by rapid digestion of the casein, development of a black sediment

<sup>1</sup> In the absence of paste reduced iron was of definite value.

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accompanied by a typical foul odour. Some species may later discharge the colour of the indicator. E.g. Cl. putrificum, Cl. flabelliferum, Cl. oedematiens, Cl. sporogenes, Cl. histolyticum.

(b) Slight acidity (slight yellow colour), soft curd, whey, slight gas. E.g. Cl. sphenoides.

(c) Acidity (bright yellow colour), firm clot, gas. E.g. Cl. butyricum.

(d) Acidity, "stormy" clot. E.g. Cl. welchii. Less gas and cloudy whey, usually Cl. tertium.

(e) Strong alkaline reaction, peptonization commencing at the surface and spreading downwards. Digestion not complete, no blackening, no odour, no gas production. E.g. B. subtilis, B. vulgatus.

(f) Acid, clot, no gas, or slight acidity only. Peptonization in some cases. E.g. B. cereus, B. coagulans, B. silvaticus.

The presence of peptone is not essential, but this simple enrichment of the milk medium was found to shorten the reaction time, often by as much as one day. The milk medium without peptone was used in the examinations already described of 14,365 samples of paste. Since that time a further series of 6110 samples has been examined using the new medium containing the addition of 1% peptone.

The results obtained were as follows:

	Number	%
Samples sterile	5262	86.1
Samples containing aerobic spore-forming bacteria	811	13.27
Samples containing Cl. welchii, Cl. tertium, or Cl. butyricum	33	0.54
Samples containing Cl. putrificum or Cl. sporogenes	4	0.065

7.1

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These figures agree closely with those obtained from the previous series of samples.

### Other applications

The method has yielded excellent results when used for the detection of anaerobes in other foodstuffs, such as raw or pasteurized milk, condensed milk, and tinned cream. For this purpose the medium is prepared by adding aseptically about 2 g. of autoclaved meat or fish paste to the milk, peptone, and bromo-cresol purple mixture. The tube is then inoculated and heated in the usual manner.

### SUMMARY

1. The bacteriological problems involved in the production of meat and fish pastes in sealed glass jars have been studied.

2. Data are presented showing the normal flora of the raw materials used. Particular attention is drawn to the anaerobic species present in seasoning materials.

3. Bacterial contamination due to manufacturing operations is discussed.

4. The requirements of a satisfactory heat treatment are outlined, together with experimental methods of study used to overcome possible causes of failure. 5. Bacteriological examinations of 14,365 samples of paste showed that 88% were sterile: the flora of the remainder was examined.

6. Experimental evidence is presented to show that certain anaerobic spore-forming bacilli are the main possible cause of spoilage, and the significance of aerobic spore-forming bacilli has been established. An extremely heat-resistant anaerobe was isolated.

7. Routine bacteriological control of the finished products is suggested.

8. A simple, inexpensive, and rapid method of detecting anaerobic species has been devised, which can also be applied to other foodstuffs. Data are presented from the examination of a further 6110 samples using this method.

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