10th European Meeting of Meat Research Workers
Roskilde, Denmark, 10th-15th August, 1964

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Problems encountered in the bacteriological examination of canned meat

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Introduction

In 1954, during the Symposium of 'Bactériologie des semi-conservers de viandes', many suggestions were made for the examination of canned meats. However, no specified conclusion was drawn; even no common opinion appeared on the pre-incubation of the products. Since 1954 the discussions are still going on (cf. 6, 17, 18) and still more new methods are developed.

There are several reasons for these discussions. Not only a great variety of organisms play an important role in the studies, but also differences of products, transport and storage conditions complicate the problems. It would be useful if in each paper a sharp description of the circumstances used, was given. There are, of course, differences in results when products are incubated for 2 days at 41 °C or 7 days at 32 °C, but these differences are also determined by the temperature of the product at the beginning of the incubation.

It is necessary to collect all available data on canned meats in the right way and to make propositions for standards of these products and their bacteriological status. The application of realistic and sound standards for canned meats gives a valuable contribution to the safety and the quality of the products.

In making up our mind as to the problems concerning the examination of canned meats, we can distinguish three points of discussion:

1. Technological designs
2. Bacteriological analysis
3. Statistical approaches.

1. Technological designs

All canned meat products have to be manufactured from meat and meat by-products of approved animals. The meat is sometimes cured in large pieces (hams), but often comminuted and mixed in a grinder or chopper with a great variety of additives. The products are heated to internal temperatures between 65 °C and 125 °C.

All these operations affect the safety and the quality of the products. Some of these effects are discussed now in detail.

1.1. Pre-slaughter conditions

There is a definite influence of pre-slaughter infections of animals on the quality of the meat. There is an increase of Salmonellae contaminations to pigs if they were transported from farm to stockyard or killing floor (9, 11). There is no evidence to believe that only these organisms give a contribution to the contamination level.

Besides, also the physiological conditions of animals influence the quality of the products. Resting and/or sugar feeding influence the glycolytic breakdown and the lactic acid content of the tissues. Meat with a low pH has a better bacteriological quality than meat of a high pH, see table 1.

Table 1. Influence of sugar and rest before slaughter on the bacteriological count of canned hams

bacteria	number of bacteria per g	number of hams: pigs with sugar and with rest	number of hams: pigs without sugar and without rest
aerobes	$< 10^4$	12	6
aerobes	10^4-10^6	0	4
anaerobes	$< 10^4$	12	5
anaerobes	10^4-10^6	0	5

Low bacterial counts correspond with sugar feeding and rest before slaughter.

1.2. Post-slaughter conditions

Quite a lot of work has been done by Jensen (7) and others, who demonstrated a close correlation between hygienic slaughtering conditions and the shelf life of the products. Mol (12) published the results of hams incubated at 35 °C which were manufactured under normal and under strictly hygienic conditions, see table 2.

Table 2. Relation between hygiene and keepability of canned hams at 35 °C

hygienic condition	number of hams: blown	flipper	normal
normal	29	7	15
strictly high	4	-	46

In these experiments only the effect of gas producing organisms has been recorded, but other kinds of bacteria present in the product may give an outgrowth without visual consequences. Therefore animals have to be slaughtered under hygienic conditions and the carcasses or parts of them have to be stored at low temperatures to prevent multiplication of micro-organisms.

1.3. Influence of additives

Salt, nitrite, nitrate, phosphate, cereals and spices have a notable influence on the safety and the quality of the products. It is not only their quantity but also the distribution of these additives which affects the shelf life of canned meats. It is well known that the salt and nitrite content varies considerably in products as canned hams. In chopped or minced products the distribution of ingredients is much better. However, in these products the cereals and spices used are mostly infected with a great number of organisms (13).

1.4. Influence of heat treatment

By heating meat products bacteria are brought into an unmultiplicable status depending on the temperature used and the ingredients added.

For a good interpretation of the results of heating it is necessary to measure the exact temperatures during processing with thermocouples. However, most of them conduct the heat from the heating medium along the couple to the centre of the can. In our Institute a couple was developed (1) without a notable conduction, see table 3.

Table 3. Determination of F_o values in liverpaste at 110 and 128 °C

	F_o-110 °C	F_o-128 °C
TNO-couple (a)	0.63	1.02
Normal couple (b)	0.58	0.53
% difference[x)]	8	48

x) calculated from: $\frac{a-b}{a} \times 100\ \%$

Not only the heat penetration is important for reporting comparable results, also the conditions in the can such as the vacuum (3), the use of fat emulsions (8), the fat content (2) and even the direction of the meat fibres (5).

1.5. Storage conditions

Rather few data are available on the relation of storage conditions to the shelf life of canned meats. In general pasteurized products have to be stored at low temperatures to suppress an outgrowth of sporeforming bacteria.

In sterile products a more or less chemical decrease in quality occurs after a storage period at higher temperatures. However, no exact data are available to discuss this point in detail.

2. Bacteriological analysis

Before discussing the problems concerning the bacteriological analysis of canned meats, it is desirable to make the following statement.

Bacteria found in heated canned meats passed through the meat as heating medium and are still present in the meat as survival medium. There is a big difference between both media.

During heating physico-chemical changes take place and there is a breakdown of vitamins and proteins (10). The nitrite content decreases during heating, so that 30 - 80 % is lost (that depends on pH, temperature and heating time) and therefore also the inhibiting capacity of the medium. Salt and phosphates seem to have a protective influence on spores during heating while at a neutral pH the spores have their highest resistance to heating. If bacteria and spores have survived heat processing, it depends on storage conditions and the composition of the medium whether there is an outgrowth or not. It is well known that some enzymes are regenerated to a great extent after subsequent storage.

It would be valuable not only to compare bacteriological data, but to make always a comparison between these data, the chemical composition and the history of the product.

The following classification of canned meat products may be made.

Table 4. Classification of canned meat products 160

Conserves	Destination	Type of organism		Storage test		Canned meat products
		non-spore-forming	spore-forming	days	°C	
sterile	temperate area	absent	absent or no out-growth	14	30-32	luncheon meat liver paste corned beef hams (2-3lbs) a.s.o.
	tropical and sub-tropical area	absent	absent or no out-growth	14 + 7	30-32 + 50	
semi-sterile	all areas	absent or no growth	present	21	15	corned beef franks hams
				14	10	hams

In accordance with this classification the canned meat products have to be examined for bacteriological analysis. It is useful to distinguish between the two following methods of analysis:

a. qualitative analysis

b. quantitative analysis

2.1. Qualitative analysis

This method of analysis has to be carried out to get an impression of the stability of the products under specified conditions. For that reason it is necessary to examine the cans during incubation at different temperatures and to look for visual alterations of the can as swelling or for chemical changes as gelatin liquefaction or pH-changes. Sometimes it will be useful to draw - aseptically - a sample of the content and to inoculate this sample in different media. Incubating these media at several temperatures may give an opportunity to activate the dormant organisms out of the survival medium into an optimal growth medium.

In sterile products no growth or visual changes may take place; for semi-sterile products growth may only be observed at abnormal temperatures - mostly elevated temperatures - after a certain period of incubation.

2.2. Quantitative analysis

2.2.1. Microscopical methods

For several reasons it is useful to examine canned products microscopically. Direct smears with and without staining indicate

whether surviving bacteria are present or not. Sometimes it is possible to distinguish between motile and non-motile, between sporeforming and non-sporeforming organisms. All this information may indicate if underprocessing or leakage has occurred in the can. By making Gram stained smears the results can be completed with the results of other smears. Application of these techniques is also possible to trace any "pre-process" spoilage that may be present.

For interpretation of the results it is necessary to take into account the history of the product. Moreover there is a difference between comminuted products as luncheon meat and other products than hams.

The methods mentioned under 2.2.1. are not useful in all cases, because only bacteria numbers higher than 10^6 per gramme can be determined (15). Therefore other methods have to be used in cases where low bacteria counts are expected.

2.2.2. Cultural methods

The principle of these methods is to count all bacteria or the bacteria belonging to a special group. The purpose is to predict if unexpected organisms have possibilities to deteriorate the product. Mostly a sample is taken aseptically and diluted in suitable media. The chemical composition of the dilution fluid should be the same as the survival medium; this is impractical, however, and unnecessary for most of the ingredients! An aliquot of the diluted sample is seeded into or dropped upon solid media. Even these media should have the same composition as the survival medium or have to be used as selective media which have another composition. Unfortunately, a great variety of meats is used.

The media are incubated at several temperatures; this depends mostly on the kind of organism, but sometimes also on tradition. In the last few years a temperature range of 30 - 32 °C is accepted in many laboratories. It is easier to work with a single temperature. All mesophilic food spoilage strains recover quantitatively, just like most of the psychrophilic organisms. For special purposes, i.e. by the enrichment or the enumeration of obligate thermophilic and psychrophilic organisms, other temperatures have to be chosen.

Nowadays it is necessary to decide which medium and incubation temperature should be used, especially for total

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counts. The great variety of studies and their results obligate the workers to standardize the available methods. The FAO/WHO did already a good job in publishing "Meat Hygiene" (1957). In Annex 12 of this issue a good comprehensive survey is given of bacteriological examination of manufactured meat products. Since 1961 I.S.O. is also busy in this field in close co-operation with bacteriological societies. By exchanging reliable and comparable results between several institutes concerning various methods, it must be possible to make a choice between these methods.

In doing this work first, there are perhaps possibilities to make standards for bacteriological control of canned meats.

3. Statistical approaches

Canned meat products have to be considered as non-homogenous foods (cf. 1.3 and 2). It would be necessary therefore to examine the whole contents of the can. However, this is impractical and an adequate sample of the can has to be taken. This principle should also be used for the examination of a batch of canned meats or for a large quantity of goods. The problem is to sample at random a statistically sound number of cans to get a reliable and valid average judgment of the consignment.

The system of sampling chosen is determined by a criterion of rejection, that means that it should be clearly pointed out beforehand what results of the examination shall condemn the whole consignment. Mostly a compromise has to be accepted between requirements and possibilities.

The problems had to be solved mathematically. This is possible because there is a relationship between the probability (P) of accepting a batch and the percentage of defectives (d) in the batch. This relation can be demonstrated in a diagram called "operating characteristic curve" (14). Sampling based on calculation is possible now by using these curves or statistically developed tables. A part of such a table is given in table 5 (from Mossel and Drion, 14); other systems are reviewed by Riemann (16).

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Table 5. Reliability of various testing schemes

sample size (n)	number of allowed defective cans in the sample (d)	reliability %			
		percentage of defective cans accepted with P			
		99 %	95 %	5 %	1 %
3	0	0.3	1.7	63	78
10	0	0.1	0.5	26	37
350	0	0.003	0.01	0.9	1.3
3000	0	0.0003		0.1	

Fortunately there is a good agreement between the systems used in Denmark and The Netherlands, viz. sampling to 1 % if a batch contains at least 10000 cans. However, up till now no definition of "batch" is given.

It is necessary therefore to standardize the way of sampling and to make good descriptions of the definitions used for statistical approaches to the examination of canned meats.

SUMMARY AND CONCLUSIONS

To solve the problems encountered in the bacteriological examination of canned meats, it is necessary to pay attention to the technological and the statistical aspects concerning these problems. In doing this a real contribution is given to the judgment of the safety and the quality of the product.

The stability of the products has to be tested at several temperatures and - in special cases - in several optimal media. For the interpretation of growth of bacteria microscopical and cultural methods have to be used.

There is an urgent need for decisions with respect to generally accepted media and incubation temperatures; the work done by I.S.O. has to be supported. In doing this also a classification of canned meats van be made.

ZUSAMMENFASSUNG

Es ist notwendig die technologische und statistische Aspekte zu beachten zur Lösung von Fragen die sich aufdrängen bei der bakteriologische Untersuchung von Fleischkonserven. Dieses Verfahren gibt

einen reellen Beitrag zur Beurteilung von Sicherheit und Qualität des Produktes.

Die Stabilität der Produkte muss untersucht werden bei verschiedenen Temperaturen und (in Einzelfällen) in verschiedenen optimalen Media.

Es gibt ein dringendes Bedürfnis an einer Festsetzung von allgemein akzeptierten Media und Inkubationstemperaturen; die Arbeit vom I.S.O. muss unterstützt werden. Gleichzeitig soll es möglich sein eine Klassifikation von Fleischkonserven zusammenzustellen.

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