THERMOBACTERIOLOGY OF SOME GHANAIAN CANNED FOODS

A THESIS PRESENTED TO
THE BOARD OF GRADUATE STUDIES
OF THE UNIVERSITY OF GHANA

BY

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IN FULFILMENT OF REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

DECEMBER 1976

ACKNOWLEDGEMENT

I extend my sincere gratitude to Dr. Mark L. Woolfe, my chief supervisor through whose guidance and constant supervision this work came to a conclusion. I give him a special note of thanks for his ever readiness to give advice and the free atmosphere he created for the discussion and exchange of ideas concerning the work.

I am also very grateful to the other members of the supervising committee Dr. R.J. Priestley and Dr. Susan Grondin for their suggestions and criticisms at various stages in the work.

I finally record my appreciation of the laboratory facilities provided by the Ghana Standards Board and the encouragement given me by staff and students of the Department of Nutrition and Food Science, University of Ghana.

TO MY WIFE - Ivy

This souvenir of one of the most challenging years of my life which by her loving care and constant attention has turned out to be one of the most successful.

QUOTATION

"The demolition of hypothesis instead of testifying to the futility of research is the method and condition of progress"

Gilbert.

ABSTRACT

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The microbiological content and the thermal processes in use for six locally canned vegetables, Bambara Beans in sauce, Garden eggs in Brine, Okro in brine, Whole Tomato in tomato juice and Pepper puree were studied to establish causes of spoilage in the canned products.

Preliminary can examination and microbiological analysis of the canned products showed that both leakage and underprocessing occurred widely in the products. Leakage occurred chiefly as a result of improper double seaming operations. It was shown that the seaming processes in use at the factories tended to produce cans with loose double seams (i.e. large Free Space and small % Overlaps). Side seam defects (due mainly to inadequate soldering) were also considered significant as a cause of leakage.

A procedure for predicting when leakage was likely to occur in the products was evolved based on Free Space measurements; and it was considered that Free Space measurements could be used in the factories to prevent the occurrence of leakage.

Heat processes in use at the Factories were evaluated and compared with recognized processing standards for low acid foods. It was found that the heating processes in use were not adequate to prevent a residual population of thermophilic spores occurring in the end products and also to prevent the occurrence of Clostridium botulinium spores in the products.

While this was shown to contribute to the high incidence of spoilage due to underprocessing the products were also a potential health hazard to consumers.

Some of the thermophilic spores isolated from the products included Bacillus polymyxa, Bacillus stearothermophilus and Clostridium thermosaccharolyticum.

The isolation of relatively low heat resistant spores of Bacillus cereus and Clostridium histolyticum from the products suggested a likely occurrence of high preprocessing bacterial contamination. Investigation conducted on the raw vegetable products prior to heat processing showed that delays which occurred between the cleaning of the vegetables and the heating process could result in a 10 - 100 fold increase of the initial bacterial load in the raw vegetables.

While this could lead to pre-processing spoilage, a residual spore population could also remain in the end product after the heat processing.

Based on the results obtained from the microbiological analysis and heat process evaluation new heating processes were defined for use in the factories to prevent the occurrence of underprocessing.

Further work is however needed on the relative heat resistance of thermophilic spores isolated from tropical products. This information could be used to define minimum processing standards for tropical based products.

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1 LITERATURE REVIEW

1.1 THE DEVELOPMENT OF THE CANNING INDUSTRY IN GHANA

1.1 1 HISTORY

Canned foods were introduced when Ghana was a British Colony. With the increase in their consumption the Government was prompted to establish three Fruit and Vegetable processing factories to produce canned substitutes for these imported products.(7) The Canneries were located in different areas of the country and were intended to process excess fruits and vegetables produced in these regions. (i.e. Nsawam Cannery in the South-Eastern region, Wenchi Cannery in the Brong Ahafo region and Pawligu in the Upper Region.) (1).

Subsequent to these factories other Canneries were established through both Governmental as well as private initiatives. New Canning Factories were constructed which dealt in vegetable and citrus juices, Fish, Meat and Diary products.

The primary rationale behind the establishment of these Canning Industries was import-substitution; i.e. to produce locally some of the products imported into the country (7).

K. Amoah (2) has shown that when this forms the basis of any industrialization programme, the development of industries becomes slow and stunted. Industrial products are usually low quality and production efficiency is also very low.

It is postulated that these characteristics of import substitution industries are the result of the need to import technology (2). Unlike classical Canning and Food processing in Europe, which developed from kitchen processes into modern sophisticated technological processes, the canning industry in Ghana and other postcolonial countries acquired this technical knowledge through importation (2).

From the outset therefore, the Canning Industry and other processing industries were beset by a number of very peculiar problems. The processing methods in use were not well understood and industrialists became rigidly stuck to set processes. A highly advanced processing technology became established over a relatively primitive agricultural technology. Such imbalance caused raw material problems (1), and consequently poor quality products (8). Because processing technology was imported auxilliary industries and services were usually absent; this also introduced problems of inability to maintain and repair equipment.

Import-substitution industries also encounter personnel problems due to inadequate facilities to train the requisite type of processors (8). These conditions lead to general underdevelopment of the processing industries.

1.1. 2 LEVEL OF PRODUCTION, CANNED FRUITS AND VEGETABLES

The annual output of canned fruits and vegetables has been estimated at 700,000 kg for fruit products and 80,000 kg for vegetable products (8), at the present average output of less than 35% of the maximum output capacity.

An effect of importation of technology is high equipment maintenance and operating cost (1); while this affects output capacity (10), improper application of technology also contributes to low production.

With respect to vegetable processing, high spoilage rates of between 10 - 15% (10) have been recorded. Such, losses in production have generally induced changes in production emphasis from canned vegetables to high acid products like citrus and pineapple juices which, apart from the acidity being naturally preservative, requires less stringent processing supervision (10).

Figures for the period from 1972-1974 illustrate the lack of development in canned vegetable production and the progress in the production of high acid products. Citrus juice production increased from 324,000 kg. to 500,000 kg. between the period of 1972-74 and pineapple products from 15,512 kg. to 26,404 kg. within the same period.

In contrast, Garden Eggs production stood at 2064.2 kg. in 1972, 3396 kg. in 1973 and 3946.5 kg. in 1974. The production of whole tomato started off at 9,326 kg. in 1973 dropped to 3,842 kg by 1974 and finally, to 108.96 kg by 1975. The figures for Okro and Papper products show similar drops in production.

Export orders have recently been received for some of these vegetable products (especially Garden Eggs)(10); a market for these vegetable products also exists in institutional catering. The fall in production is more due to production problems than to inavailability of markets.

1.1.3 METHODS IN PRODUCTION VEGETABLE PROCESSING

A consideration of the methods in use in the vegetable processing factories would reveal some of the problems that face these processing industries.

1.1.3 1 RAW MATERIAL SUPPLY

The raw materials used in these factories are supplied from a wide variety of farms differing in their level of organization and farming methods. In most cases farmers are not able to meet the specifications of the factories(10). This situation illustrates the classical case of a high processing technology imposed on a near medieval agricultural technology.

In addition to this variability in raw materials supply, most factories do not possess adequate Quality Control facilities to deal with incoming raw materials and make requisite processing adjustments.

A recent survey of Industries in Ghana(8) revealed that none of the fruit and vegetable industries undertook microbiological examination of materials. Processing methods are therefore not tailored to suit the particular incoming raw material. Large spoilage rates and products of poor quality thus result.

1.1.3 2 PROCESSING

Table (1.1) summarizes the production processes in use for five Canned Vegetables examined in the present work; Bambara Beans in tomato sauce, Garden Eggs in brine, Pepper Puree, Okro in sauce and Whole Tomato in tomato juice.

A comparison of the methods in use in these industries with conventional processes of European and American manufacturers (13, 17) show general similarities in processes. However, when unit by unit processes are considered, the defects and differences in the Ghanaian methods become apparent.

CLEANING AND SORTING

Female workers sort the piles of raw materials into buckets of water. The water used is from nearby tap water sources. Duckworth (13) suggests the use of clean running water, preferably in large quantities for cleaning. Many other authors have suggested chemical treatment of wash water (6, 13, 17, 29) with an appropriate chemical. To prevent recontamination, the wash water must be changed regularily.

The present method in use at the factories encourages recontamination of the raw materials; although regular changes in wash water are advised this is rarely the case in practice, due to lack of adequate supervision.

BLANCHING AND HOT FILLING

The methods for blanching vary from product to product; from hot water scalding to steam blanching. With respect to soft vegetables like tomato only hot water scalding are undertaken, while for Garden Eggs and Bambara beans steam blanching is used.

As a result of low level production in comparison with the capacity of the factories, large breaks and bottlenecks appear in the process. Thus products are held in holding containers awaiting filling into cans or in cans for 4-7 hours before retorting. In between processes, workers may leave for lunch breaks while the products are left and the filling or pulping stage. These delays will result in high initial microbiological load (26, 29, 59) and pre-processing spoilage might occur.

HEAT PROCESSING

Heat processing of foods is usually undertaken with special reference to micro-organisms in the food that are either spoilage agents or food poisoning agents (59). When the pH is greater than 4.5, heat processes are often designed to destroy the spores of Clostridium botulinum; and where the food is prone to contamination from flat-sour thermophiles more stringent heat processes might be applied (26).

In the processing of the vegetable products these points are not considered. Table (1.1) shows the various heat processes given to the vegetable products. The heat processes follow a general pattern; products in 8z cans (Bambara, Pepper puree) are retorted at 120^{o}C for 30 mins, while products in A2½ and A1 cans are retorted at 110^{o}C for 45 mins and 30 mins respectively. The general pattern suggests that processes are not defined to suit specific products. (e.g. Pepper puree is a highly viscous product compared to Bambara beans in tomato sauce :- these two products are retorted under similar conditions) under the circumstances some of the former will probably be underprocessed.

Heat processes are also defined by trial and error (10) and depending upon the rate of spoilage in the previous

Table 1.1 PROCESSING SPECIFICATIONS

	Bambara in Tomato Sauce	Okro in Sauce	Garden Eggs in Brine	Pepper Puree	Tomato in Tomato Juice
Blanching	Steam 15 - 20 (Mins)	Steam 15-20 (mins)	Steam 15-20 (mins)	Steam 15-20 (mins)	Hot Water Scalding
Can Type & Dimension	8z (211 x 304) A2½(401 x 411)	A1(211 x.400)	A1(211 x 400) A2½ (401 x 411)	8z (211 x 304) A2½ (401 x 411)	A2½ (401 x 411)
Filling (Filling Medium)	Hot Filling 2½% Salt + 2% Sugar in Tomato Juice	Hot Filling (Same as for Bambara	Hot Filling 2% Brine	Hot Filling -	Hot Filling Tomato Juice
Processing Temperatere	8z(cans) - 120°C A2½(cans)-110°C	A1 can - 110°C	A1 cans - 110°C A2½ " - 110°C	8z cans - 120°C A2½ " - 110°C	A2½ cans - 110°C
Processing Times	8z cans -30 mins A2½ " - 45 mins	A1 cans - 30 mins	A1 cans - 30 mins A2½ " - 45 mins	8z cans - 30 mins A2½ " - 45 mins	A2½ cans - 30 mins

season's products the heating times and temperatures may be increased or maintained.

The present processing methods are therefore not based on any scientific investigation; large spoilage rates occur (8) while products also present a health hazard to consumers.

COOLING

After retorting, the steam is shut off and cold tap water run into the retort (10). The cooling process is thus begun and after sometime the water is drained off and the cans dried.

The cans are usually left overnight for the internal residual heat to dry the cans (10).

Two major points are usually of interest when can cooling is considered; the nature of the cooling water and the rate of cooling (17, 26, 29, 96).

Due to expansion during heating, the double seam components are usually loose (17, 59). With initial cooling and steam condensation inside the can a partial vacuum develops inside the can and a suction effect may be produced. Many workers have suggested a continuous spray of water instead of a water bath as used in the present situation (17, 26, 59). There is less opportunity for suction of cooling water into the cans when a spray is used.

Where the cooling water bath is used a chemically treated water is suggested. The object is to reduce the microbiological load in the water and therefore prevent post-processing spoilage from leakage (26). The present Ghanaian cooling processes use the water bath cooling system and an untreated cooling water. This combination would increase leaker spoilage in the final products.

A continuous running water is suggested for cooling (26, 59) because the cooling process must be rapid and effective. A prolonged cooling process could provide enough heat lag for the development of flat souring Bacilli spores that might remain after the processing.

The cooling process is not adequate because the temperature of the cooling water soon rises in the retort and the whole cooling process takes up a much longer time.

STORAGE TEST

After the drying and the labelling process the canned products are stored for periods between two weeks to one month. Cans that develop swells are discarded while those that do not develop swell are released for marketing.

Though this method screens out most of the leakers, spoilage through underprocessing or flat souring remain undetectable.

1.1.4 CONCLUSION AND OBJECTIVE OF PRESENT WORK

This review has attempted to highlight some of the processing technological problems that face the vegetable canning industry. The industry was imported into the country and did not arise from the existing basic technical knowledge. This has led to a number of defects in the general operation of the canning industry (8).

Apart from lack of proper understanding and application of the principles of canning and food processing, other auxilliary industries like agriculture are not well developed to supply the requisite quality of raw vegetables. The industry therefore incurs large spoilage rates in their products with resulting stunting of growth of the industry.

The object of the present study under the theme "Thermobacteriology of some Ghanaian Canned Foods" is to find solutions to some of the technical problems that face the vegetable processing industry, and also highlights the following :

a) Causes and Sources of Spoilage

b) Organisms that are of major importance as spoilage agents in the vegetable products.

c) The study also evaluates the existing heat processes and attempts to define new processes for the products investigated.

The present work should provide a practical guide for vegetable processors and other food processors and give information about some of the implications of the methods they are using.

It is hoped that the study would help promote better growth and development of the food industry in Ghana.

1.2 ORGANISMS OF GREATEST IMPORTANCE IN CANNED FOOD

1.2.1 INTRODUCTION

It is now well recognized that micro-organisms are the major causes of spoilage in canned foods (6, 29, 59). This was not the case prior to the publications of Russel (38) and Prescott and Underwood (4, 50), when canning was considered more of an art than a scientifically controllable process. The work of Russel on "Gaseous fermentations in the Canning Industry" and that of Prescott and Underwood on "Micro-organisms and sterilization Processes in the Canning Industry" started inquiries into the microbiological basis of canned food spoilage.

Vaillard (18) examined many preserved meats and canned vegetables and found viable micro-organisms in 70-80% of the cans. The organisms he isolated are presently considered to be mainly members of the Bacillaceae family. McClung (52) reported that the importance of thermophilic bacteria in the spoilage of canned foods was established in 1912 and many other workers including Dickson (30), Esty and Meyer (28) worked on Clostridium Botulinum and other associated anaerobic Clostridia (5, 40, 52).

By the end of the 1st World War micro-organisms had been fully implicated in canned food spoilage.

The work of Esty and Meyer (28) showed that the spores of Clostridium Botulinum Type A were the most heat resistant food poisoning organisms. Minimum heat processing standards were therefore defined based on the heat resistance of this organism (26).

Several other workers (37, 52, 54, 55, 56) undertook further investigation into the microbiology of canned foods and isolated micro-organisms with heat resistances far greater than Cl. botulinum (Table 1.2). Most of these organisms identified were members of the genera Bacillus and Clostridium and included Cl. thermosaccharolyticum and B. stearothermophilus.

Cameron and Esty (58) suggested a classification of foods based on their different acidity ranges. They classified food into 4 groups.

1. Low acid foods pH $>$ 5.0
2. Medium or semi-acid foods pH 5.0 - 4.5
3. Acid foods pH 4.5 - 3.7
4. High acid foods pH $<$ 3.7

Later workers including Berry (32) and Faville (33) recognized that below pH 4.5 the food poisoning organism Cl. botulinum could not grow, while below pH 4.0 the growth of most thermophilic flat sour micro-organisms except Bacillus coagulans was inhibited. They suggested a new classification of food based on three acidity ranges.

a) Low acid food $pH > 4.5$

b) Acid food pH 4.0 - 4.5

c) High acid food $pH < 4.0$

This classification is used in this work.

(Table 1.2)

Table 1.2
Comparative Heat resistance of Bacteria (26)

Bacteria Groups	Approximate Heat resistance
Low Acid Foods (pH > 4.5)	
Thermophiles:-	
	D_{250^oF}
Flat Sour (B. stearothermophilus)	4.0 - 5.0
Gaseous-spoilage group (Cl. thermosaccharolyticum)	3.0 - 4.0
Sulphide stinkers (Cl. nigrificans)	2.0 - 3.0
Mesophiles:	
Putrefactive anaerobes	
Cl. botulinum Types A and B	0.10 - 0.20
Cl. sporogenes (including P.A 3679)	0.10 - 1.5
Acid Foods (pH 4.0 - 4.5)	
Thermophiles:-	
B. coagulans (facultative mesophile)	0.01 - 0.07
Mesophiles:-	
	D_{212^oF}
B. polymyxa and B. macerans	0.10 - 0.50
Butyric anaerobes (Cl. pasteurianum)	0.10 - 0.50
High Acid Foods (pH < 4.00)	
Mesophilic non-sporeforming bacteria	D_{150^oF}
Lactobacillus sp. Leuconostoc sp. and yeasts and moulds	0.50 - 1.00

1.2.2 CLASSIFICATION OF SPORE FORMING BACTERIA

It is observable from table (1.2) that the non-sporulating bacteria have the lowest heat resistances, with D_{250} between 0.50 and 1.0, while the sporulating forms have high D values (D_{250} between 0.1 and 5.0). The ability of bacterial spores to withstand extremes of temperature make them the major spoilage organisms in heat processed foods.

Stumbo (26) has divided the spore-forming bacteria into 3 main groups.

a) Obligate aerobes

b) Facultative anaerobes

c) Obligate anaerobes

1.2.2.1 OBLIGATE AEROBES

A classification of aerobic spore-forming bacteria presented by Gorden and co-workers showed that these organisms were members of the Bacillus genus. They include <u>B. subtilis</u>, <u>B. megaterium</u>, <u>B. badius</u> and <u>B. firmus</u>. The role of these organisms in the spoilage of canned food is limited, since modern day canning practice ensures an adequate vacuum in the top of cans (17). However, when they occur in large numbers in the food before retorting, they might cause pre-processing spoilage (29, 139).

The heat resistances of these bacteria are relatively less than those of the other spore-formers and in low acid and acid vegetables preserved exclusively by heat, the spores of these organisms are rapidly destroyed. The aerobic spore-formers are however important in canned cured meat and other such products that are given relatively mild heat processes (59).

Owing to their oxygen requirements and low heat resistances, when these sporulating types occur in the end products of heat processes, a high preprocessing contamination and gross under-processing are suspected (22, 26, 29).

1.2.2.2 FACULTATIVE ANAEROBES

The spore-forming facultative anaerobes implicated in the spoilage of canned foods are members of the genus Bacillus. These organisms are widely distributed in soils (6) and they readily contaminate vegetable products.

Cameron and Bigelow (61) reported that the obligate thermophiles, B. stearothermophilus and B. coagulans were more heat resistant than the other facultative anaerobes. The high heat resistance of the spores and their ability to propagate in low oxygen tensions make them ideally suited for growth in both pre-processed and post-processed food material.

Work undertaken on South American low-acid vegetable products preserved by high temperature (60) found B. stearothermophilus and B. coagulans as the most important spoilage microorganisms. Similar work done by Patterno and Gopes (141) in the Phillipines on canned vegetables identified these facultatively anaerobic sporeformers as the chief spoilage organisms. Kim and Goepfert (40) isolated B. cereus from some mildly processed vegetable products.

These high heat resistant facultative anaerobes occur in underprocessed canned products and their presence has been used in the diagnosis of underprocessing (31, 42).

1.2.2.3 OBLIGATE ANAEROBES

The spore-forming obligate anaerobes are made up of two groups, thermophilic Clostridia and mesophilic

Clostridia (6, 26, 77). Colin and Lyne (6) have tabulated some of these food spoilage Clostridia as

1) Thermophiles
    a) Clostridium nigrificans
    b) Clostridium thermosaccharolyticum
    c) Clostridium putrefaciens
2) Mesophiles
    a) Clostridium botulinum
    b) Clostridium welchii

Stumbo (26) also includes Clostridium putrificium, Clostridium histolyticum, Clostridium sporogenes as proteolytic or putrefactive mesophiles that have also been found in canned foods.

McClung (52) worked with Clostridium thermosaccharolyticum and found it incapable of growth at 30°C, but it had an optimum growth temperature around 55°C. Clostridium nigrificans is also considered another food spoilage obligate thermophile (105).

Among the mesophilic Clostridium species the toxin-producing Clostridium botulinum is the most important food spoilage organism from the public health point of view. This pathogen produces the botulinum exotoxin (69). It is however only capable of growth in food with pH greater than 4.6 (26, 6) and in the absence of competition from other micro-organisms (42, 144).

The Clostridium species are widely distributed in soils (29, 68, 81) and animal intestines (6), they readily contaminate animal and vegetable matter and have been associated with the spoilage of canned meat (77) and vegetable products (68).

The spoilage of canned foods by Cl. thermosaccharolyticum is accompanied by the production of large quantities of gas which produces swells on the ends of the cans (22). McClung (52) named this Clostridium species Cl. thermosaccharolyticum because it was able to ferment carbohydrates to produce large quantities of gas. The other forms of thermophilic Clostridia, Cl. putrefaciens and Cl. nigrificans are feebly saccharolytic but produce hydrogen sulphide. They have been termed sulphur stinkers (59). Campbell (51) isolated a type species from canned food which he termed Spirovibrio desulfuricans. This organism has been found to be identical with the original Cl. nigrificans isolated by Werkman and Weaver (144).

The hydrogen sulphide produced is often soluble in the food product and the can may remain flat (31), the contents might however become black due to the interaction of H_2S with the iron of the can.

Hersom and Hulland (59) state that such spoilage types are comparatively rare.
Work done in Norway isolated 46 strains of putrefactive bacteria from canned fish and meat and 80% of the isolates were found to be Cl. sporogenes.

With regard to heat resistance, the anaerobic Clostridia have quite high heat resistance (table 2); especially the thermophilic types. Clostridium thermosaccharolyticum has a D_{250}°F value of 3.0 to 4.0.

Beevens et al (71) and Esty and Meyer (28) determined the heat resistances of the variants of Clostridium botulinum types A and B. The present minimum heat processes have been based on their values of the heat resistance for this organism. Gillespie (137) undertook extensive work on the heat resistances of thermophilic anaerobes and found their heat resistance higher than most facultative anaerobes except

B. stearothermophilus.

When these organisms occur in canned foods they usually develop after processing when the cans are in anaerobic conditions (22). Their presence in cans is usually taken to indicate underprocessing (6, 22).

1.2.3 IDENTIFICATION OF THE BACILLUS AND CLOSTRIDIUM SPECIES

In the Bergey's Manual (105), the spore-forming bacteria, Bacillus and Clostridium are classified in the family Bacillaceae. Keys to the identification of the members of these genera have also been provided and, based upon these, other workers have recommended simplified models for the identification of these organisms.

Cowan and Steel (150), however, criticize these classificatory schemes which divide higher ranks of organisms into two or more kinds of a lower rank. They recognize that the different kinds of bacteria are not separated by sharp divisions but by slight and subtle differences, and suggest that a pragmatic classification be built from the basal units (species) (150). Tables (1.3 and 1.4) are diagnostic tables which contain tests they have recommended for the identification of the genera Bacillus and Clostridium respectively.

Other workers have also described schemes for identifying the members of the Bacillaceae family. Wolf and Baker (125) have provided sets of tests arranged in series

Table 1.3 — 2nd Diagnostic Table for Bacillus (ref: Cowan & Steel) 105

Tests	B. anthracis	B. cereus	B. badius	B. firmus	B. lentus	B. licheniformis	B. megaterium	B. pumilus	B. subtilis	B. coagulans	B. pulvifaciens	B. pantothenticus	B. alvei	B. brevis	B. circulans	B. laterosporus	B. macerans	B. polymyxa	B. stearothermophilus	B. sphaericus
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+	v	v	v	v	v	v	v	v
Morphological group	1	1	1	1	1	1	1	1	1	1or2	2	2	3	2	2	2	2	2	2	3
Motility	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 65°C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Anaerobic growth in glucose broth	+	+	–	–	–	+	–	–	–	+	+	–	+	–	d	+	+	+	+	–
Citrate utilization	+	+	–	–	–	+	+	+	+	–	–	d	–	d	–	–	–	–	–	–
Gelatin hydrolysis	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	+	+	+	+	–	+	+	+	+	–	+	+	+	+	–	+	–	+	d	d
Starch hydrolysis	+	+	–	+	+	+	+	–	±	+	–	+	+	–	+	–	+	+	+	–
Glucose (acid)	+	+	–	–	–	+	+	+	+	+	ng	+	+	+	+	+	+	+	+	–
Arabinose (acid)	–	–	–	–	–	+	+	+	+	d	ng	d	–	–	+	–	+	+	d	–
Mannitol (acid)	–	–	–	–	–	+	+	+	+	d	ng	–	–	+	d	+	+	+	–	–
Indole	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	d	–	–	–	–
V-P	+	+	–	–	–	+	–	+	+	d	–	–	+	–	–	–	–	+	–	–
Nitrate reduction	+	+	–	+	–	+	–	–	+	d	+	d	–	d	d	+	+	+	+	–
Urease	–	d	–	–	+	d	d	–	d	–	–	–	–	–	–	–	–	–	d	d
LV	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	d	–

Key to Table

+ :– 80% – 100% strains positive

d := 79 – 21% strains positive

– := 0–20% strains positive

NG :– no growth

V := variable

Table 1.4 — 2nd Diagnostic Table for Identification of Clostridium Species — Ref: Cowan & Steel (150)

	Cl. Welchii	Cl. butyricum	Cl. tertium	Cl. carnis	Cl. septicum	Cl. chauvoei	Cl. oedematiens	Cl. tetanomorphum	Cl. botulinum (non protealytic)	Cl. botulinum (proteolytic)	Cl. bifermentans	Cl. sordellii	Cl. sporogenes	Cl. difficile	Cl. innocuum	Cl. histolyticum	C. putreficiens	Cl. tetani	Cl. fallax
Motility	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+
Spore	co	co	To	so	so	so	so	Tr	Co	Co	c/so	c/so	so	To	To	so	Tr	Tr	so
Microoerophilic	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Gelatin hydrolysis	+	−	−	−	+	+	+	−	−	+	+	+	+	−	−	+	−	+	−
Meat digestion	−	−	−	−	−	−	−	−	−	+	+	+	+		−	+	−	−	−
Serum "	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	+	−	d	−
Milk	AGC	agc	ac	ac	a(c)	agc	gc	−	A	D	CD	CD	D	−	Z	D	−	Z	a(c)
Glucose (acid)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
Lactose (acid)	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+
Sucrose (acid)	+	+	+	+	−	+	−	−	−	−	−	−	−	−	+	−	−	−	+
Salicin (acid)	−	+	+	+	+	−	−	−	−	−	+	−	−	+	+	−	−	−	+
Indole	−	−	−	−	−	−	d	+	−	−	+	+	−	−	−	−	−	+	−
Nitrate reduction	+	+	+	−	+	+	+	+	−	−	+	+	+	−	−	−	−	+	+
H_2S	+	−	−	−	+	+	+	+	+	+	+	+	+	+		+	+	−	−
LV	+	−	−	−	−	−	d	−	+	+	+	+	+	−		−	−	−	−
Haemolysis	+	−	−	+	+	+	+	d	+	+	+	+	−	−	−	+	−	+	+
Ureese	−	−	−	..	−	..	−	−	−	d	−	+	−			+		d	

KEY:- (O = oval) r = round c = central, S = subterminal

A = acid, C = clot D = digestion Z = some strains give soft clot which lead to the identification of the species of the Bacillus genus. They have also suggested the application of serological technique in the identification of this genus.

1.3 PRINCIPLES OF HEAT PROCESSING

1.3.1 CAUSE OF DEATH

The lethal effect of heat on micro-organisms is only partially understood, however some useful conclusions on this subject have been made, and upon these have been based the principles of heat processing.

A bacterium is considered to be dead when it fails to reproduce under suitable conditions for reproduction (155). This definition for the death of a bacterium has envinced some pertinent objections; (e.g. Nelson (153 and 154) found out that the survival of heat treated micro-organisms depended on the recovery medium) but it has persisted in usage because no other definition has been suggested (26).

Bacterial spores and vegetative cells show striking differences in heat resistance. Vegetative cells of bacteria, yeasts and moulds are destroyed almost instantaneously at 100°C (212°F) (14, 60, 168) but the spores of some bacteria require prolonged exposure to heat before they are killed. (86, 136).

It has been suggested by Virtanen (156) that the heat resistance of bacterial spores could be linked with enzyme stability. This postulate was supported by Friedman and Henry (157) who ascribed the relatively high heat resistance of bacterial spores to the fact that a large proportion of the water content of the bacterial spores was not available for protein coagulation. Further work by Murrel and Scott (158, 159) also showed that heat resistance depended upon the water activity of the spores and that when the water activity of bacterial spores were equilibrated to between 0.2 to 0.4, species to species differences in heat resistance disappeared.

Other workers have implicated different mechanisms in the death of bacterial cells; these include the decrease of R.N.A. and D.N.A. in the cells. (161, 162, 163, 164).

In spite of the intensive work undertaken in this field the specific mechanism for the cause of death of a bacterial cell still cannot be adequately explained. (166, 168).

1.3.2. <u>ORDER OF DEATH</u>

The death of bacteria, when exposed to heat, follows a logarithmic order, (i.e. equal percentages of the surviving cells die in each successive unit of time). This conclusion was reached by Bigelow (24) after he had plotted thermal death curves on semi-logarithmic paper.

Various workers supported Bigelow's claim. Watkins and Winstow (168) made extensive investigations of the heat resistances of both vegetative cells and spores of bacteria and concluded that the order of death of both vegetative cells and spores were logarithmic. Yesar and Cameron (169) demonstrated that, when it was possible to fractionate a given cell suspension with varying specific gravity, the different fractions exhibited varying thermal death times. This led to the conclusion that the non-logarithmic order of death reported by Anderson (172), Cameron and Bohrer (173) could have been due more to non homogeneity of the suspensions they used in their determinations.

Rahn (170) explains the log-order of death with the postulation that the loss of reproduction is due to the denaturation of a single gene responsible for reproduction. The significance of this explanation is that the order of death could be considered a unimolecular or a first-order bimolecular chemical reaction.

Expressed mathematically

$$\frac{-dc}{dt} = KC \quad \text{.........}(1)$$

The integration of (1) gives

$$t = \frac{2.303}{k} \log \frac{Co}{c} \quad \text{.........}(2)$$ where Co is the initial bacteria population and C is the number of surviving cells after time t.

When the log of number of surviving cells is plotted against time a survival curve (Fig. 1.1) is obtained, which can be represented by $t = \frac{2.303}{k} (\log a - \log b) \ldots (3)$ where a, represents the initial number of cells and b, represents the number of cells surviving after time t of heating.

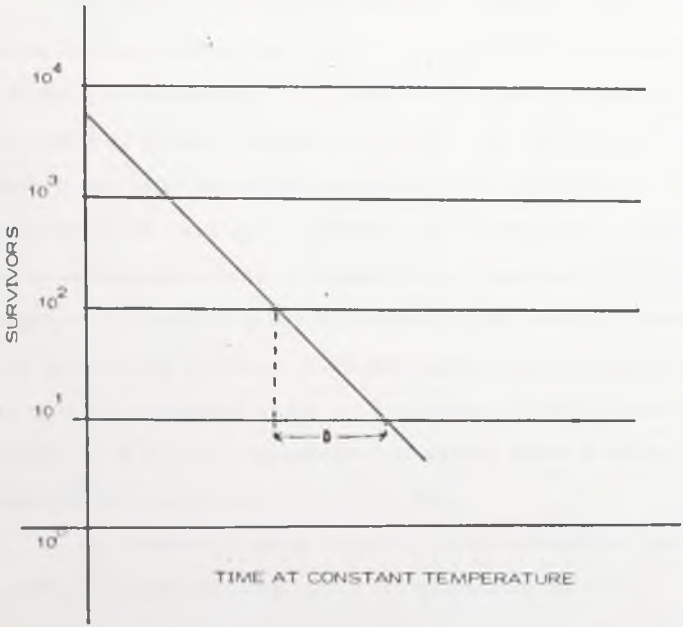
The concept of 'D value' (Decimal reduction time) was introduced by Katzin and co-workers (171) who defined "D" as the time required to destroy 90% of the cells (Fig. 1.1). When "D" is substituted into equation (3) it yields

$$t = D (\log a - \log b) \quad \ldots\ldots\ldots (4)$$

The above equation has been used in calculating processing times (26) for various canned foods.

Fig. 1.1

SURVIVOR CURVE



1.3.3. HEAT PENETRATION

When food in cans is subjected to heat processing there is always a heating lag between the centre of the can and the heating medium (42), which has to be considered in process evaluation (59).

Von Ecklund (188) has described various methods using thermocouples and thermistor probes for heat penetration measurements. The probes are usually placed at the point of slowest heating lag in the can. This point has been found to be dependent upon the mode of heating of the contents of the can (42). Jackson and Olson (189) employed varying concentrations of bentonite and simulated the consistency of foods in order to determine the point of slowest heating lag in cans. A 5% bentonite solution was found to heat by conduction while a 1% solution heated by convection. It is now recognized that canned foods heat by one of the following modes of heating (42).

(a) Conduction pack - heating is by conduction and the point of slowest heating lag is the geometric centre.

(b) Convection pack - heating is by convection and the appropriate position for the probe is ¾ ins. above the bottom of the can on the longitudinal axis for small cans and $1\frac{1}{2}$ ins. above the bottom for large cans (larger than a No. 10 can).

(c) Broken Curves (heating is by convection followed by conduction): - the appropriate position of the probe is approximately midway between the geometric centre and that for the slowest heating point for a convection pack (42).

1.3.4 HEAT PROCESS EVALUATION

Bigelow and co-workers (191) in 1920 described a "General Method" for evaluating the lethality of heat processes. Their method essentially integrated the lethal effect of the time - temperature relations taken from the point in the container with the greatest temperature lag during heating and cooling (192).

Figure 1.1a shows heat penetration data illustrating a typical temperature/time relation at the geometric centre of a canned product. According to the General Method (191) each temperature point on the heat penetration curve (Fig. 1.1a) possessed a sterilizing value or a lethal rate equal to the reciprocal of the number of minutes required to destroy some given percentage of spores of a micro-organism at this temperature (191 and 192). Fig.1.2 shows a thermal

Fig. 1.1a

A HEAT PENETRATION CURVE (Ref. Stumbo, 26)

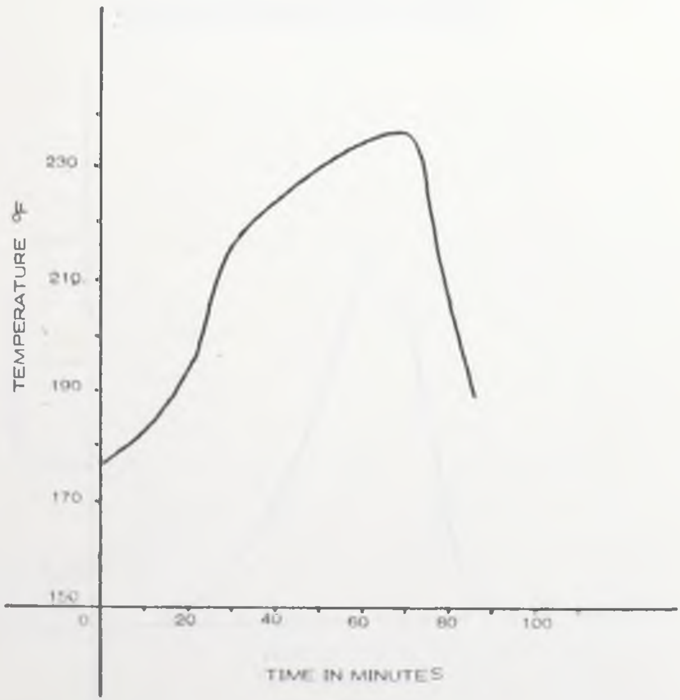
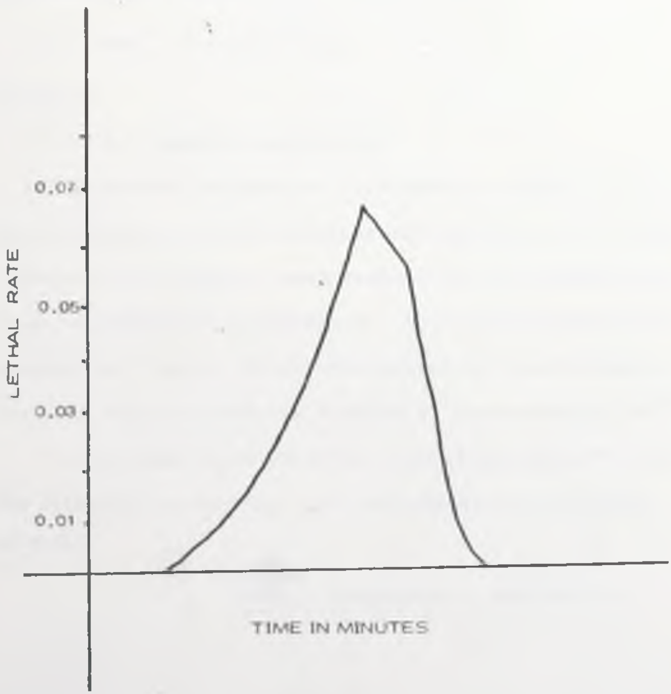


Fig 1.2

A LETHALITY CURVE (Ref. Stumbo 26)



death curve (TD curve) from which the lethal rate values can be estimated (185). A plot of lethal rate values against time gives a lethality curve (Fig. 2). The area under such a curve is used in estimating the processing value (185).

Olin Ball (181) provided an improvement of the "General Method" by introducing a formula for determining the lethal rate value.

$$L = \log^{-1} \frac{T - 250}{Z} \qquad \text{...........} \quad (5)$$

in which

T = any lethal temperature

Z = number of degrees Fahrenheit required for the thermal death curve to traverse one log cycle. "Z" characterizes an organism with respect to its relative resistance to different temperatures. Ball also introduced the concept of F value, which represented the equivalent of the entire heat process in minutes of processing at 250°F.

The F value is obtained by determining the area under the lethality curve (Fig. 1.2) and substituting into the formula

$$F = \frac{m^A}{10^{nd}}$$

suggested by Stumbo (26)

Stumbo (185) also introduced mathematical methods for evaluating heat processes in canned foods. These methods were found to be more accurate and also evaluated the effect of the heat process in the whole can instead of the point of the slowest heating lag.

2. MATERIALS AND METHODS

2.1 SELECTION AND EXAMINATION OF CANS

2.1.1 Sampling and Selection of Cans

Industrial Canning practice usually aims at high levels of production coupled with minimum spoilage rates; in order to study defects in can populations there is the need to devise adequate sampling procedures that would bring out the various abnormalities in the cans.

Tables 2.1 and 2.2 show model sampling schemes drawn up by the Indian Standards Institution (11) and the National Canner'sAssociation (59) respectively. In the Indian Standards the number of cans to be selected is based on the retort size while for the National Canner's Association it is based on the rate of spoilage.

Table 2.1 SCALES OF SAMPLING (Indian Standards Institution (7))

No. of Cans in Retort Lot	No. of Packing Cases To be Selected	No. of Cans to be selected
Up to 200	3	6
201 - 300	4	8
301 - 500	5	10
501 - 800	6	12
801 - 1300	7	14
1301 - 3200	8	16
3200 and above	10	20

Table 2.2 NATIONAL ASSOCIATION OF CANNERS SCALE OF SAMPLING (59)

% Spoilage	Number of Cans Required	
	*Probability 0.95	*Probability 0.99
0.5	597	919
1	298	458
2	148	228
3	99	151
4	74	113
5	58	90
6	48	74
7	41	64
8	36	55
9	32	49
10	28	44
11	26	40
12	24	36
13	22	33
14	20	31
15	18	28
16	17	26
17	16	25
18	15	23
19	14	22
20	13	21
25	10	16

* Probability that at least one can in the incubated sample will show spoilage.

Procedure :

Preliminary investigations made at the factories revealed that the retorts in use would hold between 300 - 500 cans (10) and also that the percentage spoilage rate in the vegetable products was about 10%. Based on figures given in the Indian Standards Institution table, the number of cartons to be picked were five (table 1) from which 28 cans were to be selected (National Canner's Association table).

The five cartons were selected by arranging them in a systematic order and removing each 15th packing case. Then, 6 cans were drawn out randomly from each packing case.

This selection process was continued for 3 months after which 350 cans were selected for examination.

2.1.2 Incubation of Cans

The object of incubation of canned samples before analysis is to increase the number of spoilage organisms in the can so that detection of microorganisms becomes easier.

It has been reported that sublethal exposures to heat may cause dormancy of spores and also that at such temperatures thermophilic bacteria tend to undergo rapid auto-sterilization (59). The time and temperature of incubation hence become critical.

Half the cans in the early stages of spoilage (i.e. flat - soft swell: refer to section 2.1.3) were incubated at 37°C for 7 days. The other half were incubated at 55°C for 4 days.

Before incubation the cans were examined and classified as flat, flipper, springer, soft swell or hard swell (refer to section 2.1.3), and examined again after incubation for changes in can-end conformation.

2.1.3 External Examination of Cans

Procedure :

The following general information was recorded :

a) Name and nature of product

b) Container size

c) Date produced.

2.1.3.1 Leaked Contents

A laboratory lens (x 5 magnification) was used in examining leakage of the can contents by investigating the presence of black rotten food material around the seams. The cans were then washed with water and rubbed with fine abrasive before further examination.

2.1.3.2 Condition of Can Ends

The ends of the cans were examined visually and tactually with the thumb for the following conditions :

a) Flat: no evidence of a swell, can ends appear concave (down).

b) Flipper: appears flat but when it is brought down sharply on a flat surface one end will flip out; when lightly pressed, this end will flip back in.

c) Springer: one end flat and the other permanently bulged; when pressure is applied to the bulged end it will flip in, and the other end will flip out.

d) Soft swell: both ends are bulged but not tightly: they yield to thumb pressure.

e) Hard swell: both ends are bulged tightly and permanently; they will not yield readily to thumb pressure.

These conditions were observed and recorded as external conditions of the cans.

Using the laboratory lens rusts on the body of the cans were examined; when rust was extensive an attempt was made to find the pres■ence of pinholes. This was checked by finding whether the rust appeared both on the inside as well as outside of the can.

Condition of the Double Seam

The double seam was visually and tactually inspected for the following gross defects :

1) Skidders: this is a form of imcomplete seam formation, resulting from a chuck slip during the second seaming operation.

Fig 2.1 (A SKIDDER)

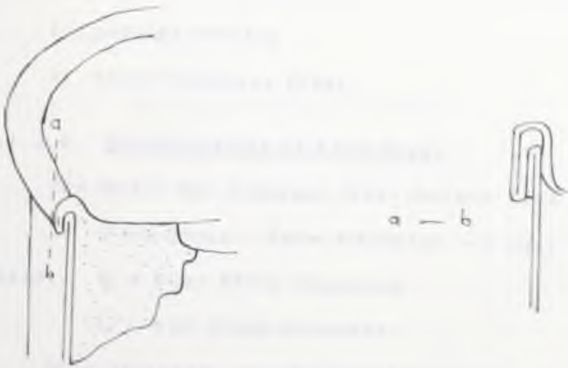
2) <u>Knocked down Flange</u>: this is another gross defect of a double seam, a mislocked seam resulting from either a damaged body flange or a mushroomed body flange.

This condition is shown in a diagram (Fig.2.2)

<u>(Fig.2.2) (KNOCKED DOWN FLANGE)</u>

(3) False seam: this is another form of mislocked seam which can be detected by touch. This form of false seam can be seen much more easily after cutting the double seam at that point with a pair of cutters.

(Fig. 2.3) FALSE SEAM



Other Defects

Other forms of defects which are important operative sites of leakage like Fractured or Perforated plates (other than at the droop) were looked for and recorded.

2.1.4 Seam Examination

The double seam was examined for Compactness which encompasses.

1) percent Overlap

2) Seam Tightness (194).

2.1.4.1 Determination of Free Space

The Metal Box Company (194) defines Free Space as

$$\text{Free Space} = \text{Seam thickness} - 2\,(t_b + 3\,te)$$

where t_b = Body Plate thickness

te = End Plate thickness.

Seam thickness was measured using the Seam thickness dial guage (Metal Box Company), which minimizes human error compared to similar measurements by a hand micrometer. The readings were taken in 6 places on both ends of the can and the average used as representative seam thickness for the can.

The end plate and body plate thickness were determined by cutting triangular pieces (sides about 1 inch) from the end plates and body plates of the cans, and measuring the thickness at the apex of the triangular piece with a special dial guage (Metal Box Company). Free Space was then calculated from these results; or alternatively by operating a slide rule provided for calculating Free Space (Metal Box Company).

2.1.4.2 Determination of % Overlap

The official method of the Metal Box Company (194) which employs a Seam Examination Kit was used in determining the % Overlap. The double seam was systematically torn down into its various components and the Seam Length, Hook Lengths and Plate thicknesses determined with a hand micrometer. These dimensions were substituted into the recommended % Overlap formula (194).

$$\text{Percentage formula} = \frac{X + Y + 1.1\ te - L \times 100}{L - (2.2\ te + 1.1\ t_b)}$$

where x = body hook length

Y = End hook length

L = Seam length

te = End plate thickness

t_b = Body plate thickness

Alternatively a table provided by the Metal Box Company (194) was used in determining the % Overlap.

2.1.4.3 <u>Internal Droop</u>

The droop is the double seam formation at the point of the side seam (59). Fig. 2.5 illustrates the internal droop; this was measured on the End hook using a hand micrometer. It is recommended that the internal droop should not dip below half way down the End hook length (22).

2.1.4.4 <u>Hook Defects and Side Seam Defects</u>

After tearing down the double Seam (2.1.4.2) the Body, and End Hooks (Fig. 2.4) were examined for wrinkles, pleats and puckers and graded accordingly. The system of grading was to give a numerical value to the defects as to the depth of extension (dip) of the Hook. Figures (2.6 and 2.7) are illustrations of wrinkles, pleats and puckers and the ratings given to them.

The side seam was also examined for weakness and defective soldering. Visual examination was undertaken on the lap joint for signs of leakage; this was usually

Fig 2.5

THE DROOP

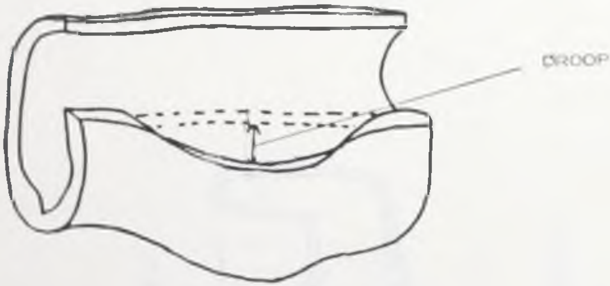
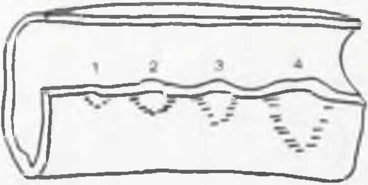


Fig 2.6

WRINKLES ON END HOOK

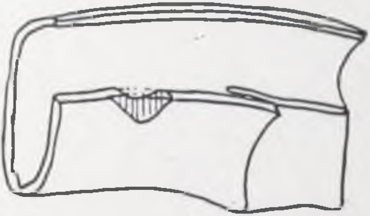


GRADE

Smooth to		12%	-	0
12%	"	25%	-	1
25%	"	37½%	-	2
37½%	"	50%	-	3
Greater than 50%			-	4

Fig 2.7

PLEAT AND PUCKER ON END HOOK



PUCKER PLEAT

indicated by the presence of sulphite staining at the point of leakage. After the double seam had been stripped apart, the lap joint was then separated to reveal the solder. This was done by levering straight the body hook with a screw driver, and by gripping the body close to the edge with pliers, the lap was pulled apart. The ease of separation was an indication of deficiency in soldering. A 3 inch (5 x magnification) laboratory Hand lens was used to examine the solder for channels and pores.

2. MATERIALS AND METHOD

2.2 PRELIMINARY MICROBIOLOGICAL EXAMINATION

The aim of this section was to screen the can contents for any viable microorganism and to make preliminary presumptive attempts to identify the isolated organisms. The media employed in the isolation were non-selective and the methods were orientated towards isolating heat resistant sporeforming bacteria.

2.2.1 Method of Opening Can

Procedure:

After the preliminary examination (Section 2.1.3) the external parts of the can were scrubbed with soap and water, then immersed in 1.5% (W/V) phenol solution for 1 hour. After removal from the antiseptic bath the exterior of the can was scrubbed with sterile cotton wool dipped in 95% ethanol. The top of the can was covered with a sterile petri dish, until opened. Prior to opening, the top of the can was sterilized with 95% ethanol and flamed. The pressure or vacuum in the can was determined using a pressure gauge with a detachable piercing unit (American Can

Company Vacuum Gauge, RAO - 2607A). The piercing unit had been kept sterile by immersing in 1.5% phenol solution for 1 hour and storing in 95% ethanol.

The pressure was determined by piercing the top of the can with the gauge. The puncture was covered with a sterile petri dish. Using a sterile can opener a triangular cut with base approximately 2.5 cm and sides 3.4 cm was opened on top of the can.

The enlarged opening was covered with a sterile petri dish until samples were taken from the can. The can number and the pressure were recorded.

2.2.2 Sampling of Can Contents

Large samples were taken at a time in order to increase the chances of recovery of organisms. Wernzirl (5) in his "Bacteriological examination of canned foods" took out samples of about 50 gm at a time.

Procedure: (a) Pepper Puree

Six portions of 225 ml of each broth medium (Section 2.2.5) were prepared in 500 ml conical flasks and sterilised in a steam retort (120°C for 15 mins.). About 20 - 25 gm of the puree were withdrawn aseptically from the can and innoculated into the tubes of broth media. The tubes were incubated at 37°C, 45°C and 55°C, both aerobically and anaerobically for 48 hours.

(b) Garden Eggs in Brine, Okro in Sauce, Bambara Beans in Sauce and Whole Tomatoes in Tomato Juice

These canned products were shaken for 2 - 3 minutes to distribute the vegetable pieces in the solutions. The procedure for taking out samples was as described for pepper puree.

2.2.3 Further Isolation

For all the broths used in the preliminary isolation process, two corresponding agar plates were prepared by adding plain agar to the broths. After 48 hours incubation, 1 ml samples were taken from the broths and spread on the agar plates. The plates were incubated for 24 hours and the number of colonies were described and counted.

2.2.4 Incubation Procedures

Aerobic incubation was undertaken in thermostatically controlled incubators (Baird and Tatlock) and waterbaths (Griffin 200 series) at 37°C, 45°C and 55°C. Agar surfaces were found to dry out in the dry air incubators especially at high temperatures. This was prevented by enclosing the petri dishes or slants in closed containers containing some water.

Anaerobic incubation was undertaken at the various temperatures (as above) in anaerobic jars (Baird and Tatlock Ltd. London). The use of the anaerobic jar has been described by Willis (78).

2.2.5 Medium Preparation and Usage

Six media were tested for suitability for use in the preliminary isolation of microorganisms from the canned products.

These were

a) Nutrient Agar/Broth

b) Nutrient Agar/broth + 1% yeast extract

c) Dextrose - Tryptone yeast extract broth/Agar

d) Cooked meat broth/Agar

e) Thioglycollate medium broth/Agar

f) Malt extract broth/Agar

a) Nutrient broth/Agar: - The Merck brand was used.

b) Dextrose - Tryptone yeast extract broth/Agar : - The formula described by Hersom and Hulland (59) and the Indian Standards Institution was used; this has been presented in Appendix (1.1).

c) Cooked Meat medium: – This medium has been recommended by the Society of American bacteriologists (117) for isolating Clostridium spores. The "Oxoid" brand of the medium was used.

d) Thioglycollate medium broth/Agar: – The "Oxoid brand" of this medium was used.

2.2.5.1 Procedure for Selecting Suitable Medium

Broths were prepared for all the six media and were inoculated with appropriate samples taken from the products under study.

a) Garden Eggs in Brine

b) Bambara beans in sauce

c) Okro in sauce

d) Pepper puree

e) Tomato (Whole)

After 2 days of growth in the broths 1 ml samples were taken from the broths and inoculated on to agar plates prepared from the six media. The amount of growth was described as scant, moderately profuse and very profuse; and the number of organisms on each agar plate was recorded. The medium that showed the largest number of organisms was the most suitable. Suitability was further tested by observing the growth of known Clostridium and Bacillus species on the selected medium.

2.3 IDENTIFICATION OF MICRO-ORGANISMS

Micro-organisms isolated from cans suspected of under-processing were purified and identified to the species level. Fig. 2.8 shows the format used for recording the various characteristics of the organisms.

One difficulty associated with the identification of organisms especially the Bacillus group has been changes in morphological characteristics with the aging of the cultures (118). As much as possible, all studies were conducted under similar conditions. Young cultures were used for most of the studies and greater emphasis was placed on biochemical tests (150) than on morphological tests. When not in use the cultures were stored at 4°C on agar slopes.

2.3.1 Microscopy

For the microscopical studies a Leitz Weltzar (model 5705044) microscope was used. All the observations were made under oil immersion lens using X20 eyepiece lenses.

Fig. 28

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED

Culture No. ....   Source

Descriptions	Sketches
CELL MORPHOLOGY Medium: Form & Arrangement Motility in broth Flagella: Size: Sporangia: Endospores: Shape: Position	

AGAR STROKE Age: at °C Amount of growth: Form: Consistency: Chromogenesis: Medium: Opacity:	AGAR COLONIES Age: at °C Form: Elevation: Surface: Margin: Chromogenesis: Opacity:
NUTRIENT BROTH Age: at °C	GELATIN STAB. Age: at °C

2.3.2 Method of Staining

The recommended procedures for the Simple Stain and the Gram Stain (117, 150, 196) were used in all cases.

For the Spore stain the Schaeffer and Fulton (24) method for staining the endospore was used. The formula and method has been shown in Appendix 1.2.

2.3.3. Morphological Studies

The following characteristics of the bacterial vegetative cells and spores were observed under the microscope:

a) Form and arrangement of cells

b) Motility

c) Size of vegetative cells and spores

d) Condition of sporangia

e) Endospore shape and position.

Motility: - The "hanging drop" method was used to study motility and observation was made as rapidly as possible under reduced illumination from broth media.

2.3.3.1 Condition of Sporangia and Position of Endospore

These characteristics were used in differentiating the Bacillus genus into groups 1, 2 and 3 (150). The shape of the spores was described as cylinderical, oval or ellipsoidal or spherical, while the position of the spores was described as central or terminal. Following the suggestion of Cowan and Steel (150) no distinction was made between terminal and subterminal positions of the spores. The condition of the bacillary body bearing the spores was also observed and recorded as swollen or not swollen.

2.3.4 Biochemical Tests

The sets of biochemical tests used in differentiating the isolated Bacillaceae organisms were mainly those suggested by Cowan and Steel (150). These authors have provided standardized procedures for preparing the media and also claim reproducibility of these biochemical tests.

Results were recorded as positive (+ve), partially positive (d) and negative (-ve). When there was doubt as to a partially positive result, the experiment was repeated. From the results of the four tubes it was decided to assign +ve, d or -ve to the reaction.

2.3.4.1 Methyl Red and Voges - Proskauer Test (MR - VP)

These tests were conducted in parallel using the same culture tube, detecting the production of acid and acetylmethylcarbinol.

The MR-VP medium (150) was inoculated with the test organism and incubated at 37°C for 2 days. 2 drops of the methylred solution were added and the mixture swirled round. A red colour was recorded as positive for acid production, orange colour for partial acid production (d) and yellow as negative. For the Voges - Proskauer test (VP) 0.6 ml α-naphthol solution and 0.2 ml KOH were added to the tube after the methyl-red test. A positive reaction was indicated by a deep red colour.

2.3.4.2 Indole Production

The test culture was grown in Nutrient broth containing 1% yeast extract and Dextrose - Tryptone yeast extract broth (Appendix 1.1) for 48 hours. 0.5 ml of Kovac's reagent (109, 117) was added to the culture and shaken well, then observed for development of red colour, indicating indole production.

A culture of E. coli was used as a positive standard for comparison.

2.3.4.3 Nitrate Reduction

The reduction of nitrate is usually shown by the disappearance of nitrate from the medium or the appearance of nitrate breakdown products (nitrite, hyponitrite, hydroxylamine). The experiment is in two parts. The first is for nitrite, if the test is negative then there is a test for nitrate content.

Procedure:

The nitrate broth (150) was inoculated with the test organism and incubated for 5 days after which I ml of nitrate test reagent A (Appendix 1.3) followed by test reagent B (Appendix 1.3) were added. The development of red colour indicated that nitrate had been reduced. To tubes that did not show red, zinc powder (5g/ml of culture) was added; the absence of red colour after the addition of zinc was taken to indicate that there was no nitrate reduction, while the presence of red colour showed that the nitrate produced had been further broken down.

2.3.4.4 Starch Hydrolysis

Starch agar (Appendix 1.4) was inoculated with the test organism and incubated at 30°C for 2-5 days according to the rate of growth of the test organism. (Some organisms spread very fast on the medium. When the whole medium is covered by the growing organism it becomes difficult to discern between the blue black medium and the hydrolysed medium).

The culture was then flooded with Lugol's iodine (150). Where the starch had not been hydrolysed the medium appeared blue; hydrolysis was indicated by a clear colourless zone (sometimes light brownish).

2.3.4.5 Casein Hydrolysis

Casein agar (milk agar) plates (150) were inoculated with the test organism, incubated at 37°C and observed daily up to the fourteenth day for a clear zone (hydrolysis) around the bacterial colonies. The tests were conducted in duplicate.

2.3.4.6 Urease Activity

Urease activity was tested in Christensen's medium (117). Steel (150) has suggested that Nessler's reagent may be added to show the presence of ammonia.

Procedure:

Slopes of Christensen's medium (117) were inoculated heavily and incubated at 30°C for 5 days. Observation for appearance of red colour were made after the 4th hour and daily for five days. Positive and negative controls were prepared from Proteus Vulgaris and E. Coli. The tests were conducted in duplicates.

2.3.4.7 Gas From Carbohydrates

Standardized methods with medium containing ammonia as sole nitrogen source (117, 104, 196) were used to test for Gas production from carbohydrates. Gas production was shown by gas collection in inverted Durham tubes and acid production from changes in colour of Bromocresol dye.

2.3.4.8 Citrate Utilization

Koser's citrate medium (109) was used in this test. Vaughn et al (198) showed that the presence of agar in the medium invalidated the test. Special care was taken to exclude media from the loops when inoculum was being taken from a light suspension in saline buffer.

Procedure:

3 loopfuls of bacterial culture were taken from slopes and dispersed into 0.5 ml saline solution. Koser's medium was inoculated with the bacterial saline suspension. The

culture was incubated at 37°C and observed daily for 7 days. Turbidity indicated a positive reaction. The positives were again confirmed by subculture into Koser's medium.

2.3.4.9 Gelatin Liquefaction

The gelatin agar (Appendix 1.5) was inoculated and incubated at 37°C for 14 days. After every 2-3 days the culture was cooled for two hours in a refrigerator and observed for liquefaction. An uninoculated tube served as a negative control for comparison.

2.3.4.10 Lecithovitellin Agar (LV Agar)

The L.V. agar (Appendix 1.6) was inoculated and observed for growth and opalescence within the medium and pearly layer formation around the colonies. The plates were then flooded with $CuSO_4$(ag). The reagent was allowed to dry in an incubator for 20 mins. The appearance of greenish-blue hues meant free fatty acid had been formed in those areas. The development of opalescence and a pearly layer constituted positive reactions.

2. MATERIALS AND METHODS

2.4 Estimation of Bacterial Numbers

The bacterial numbers in the raw material (vegetables) prior to heat processing and the growth rate of some microorganisms in the vegetable products were studied.

The problem was approached in two parts.

a) Obtaining the total aerobic counts in the raw vegetables and investigating the effect of blanching on the microorganism population.

b) Investigation of delays in the processes before retorting to find out their effect on the bacterial population prior to heat processing.

In the Literature Review (Section 1.1.3.1) it was observed that the raw materials that came to the factories varied widely according to season of production, locality and farming methods. To obtain representative data of the bacterial numbers would mean many seasons of visits to the factories. The task was beyond the scope and time of the present work.

It is conceded that the present data for bacterial numbers are insufficient on which to base broad generalisations. However they would furnish indications as to the kind of bacterial populations to be dealt with at these factories.

2.4.1 Sampling of Raw Materials

Samples were taken from the raw material (vegetable) piles at the factory premises and at the purchasing depot on route to the factory. At the factory the vegetables were stacked in wooden boxes, where they were stored overnight, in open air, before the next day's processing.

Five of the crates were selected randomly from the packs and about a kilogram of vegetables were taken from each of the five selected crates. The five one kilogram samples were then pooled together to make one sample. Three of such samples were selected from each vegetable pile on three different occasions of visits to the factories.

The samples were stored in clean polythene bags under refrigerated conditions until examined.

2.4.2 Total Aerobic Count (Preblanching)

Materials: Sterile blender

0.1% peptone water (for dilution)

Dextrose - Tryptone Yeast Extract Agar

(Appendix 1.1)

Procedure:

50gms. of each test sample was ground in the sterile blender with 450 ml of 0.1% peptone solution to make a 1 : 10 dilution. Further dilutions up to 10^{-5} were made and the total aerobic count in the original sample was determined by pour plating.

2.4.3. Total Aerobic Count (Post - Blanching)

The vegetables were blanched in a steam blancher at atmospheric pressure for 15 mins and the total aerobic counts determined on 50 gm samples as described above (2.4.2).

2.4.4 Bacterial Growth in the Vegetables

Products:

An observation made at the factories was that due to the batch nature of the methods in use at the factories, there was a delay of between 4-7 hours in-between blanching and retorting during the processing of the vegetables. The effect of this delay on the raw material (vegetables) was investigated.

2.4.4.1 Preparation of Vegetable Growth Media

For Pepper and Garden Eggs, canned purees were opened and 100 gm samples taken in 250 ml wide-mouthed conical flasks.

The flasks were plugged with cotton-wool and then sterilized at 121°C for 30 mins.

For the Okro and Bambara, the canned products (Okro in sauce and Bambara in sauce) were mashed in blenders, and 100 gm of the mash were put into wide mouthed 250 ml. conical flasks and sterilized at 121°C for 30 mins.

Extracts of the above vegetables were also prepared by diluting the vegetable purees with twice the volume of water and straining with a cheese cloth. The extracts were then added to base nutrient broth at 10% and 20% levels. The rate of sporulation and growth of the two organisms Bacillus polymyxa and Clostridium histolyticum were then studied in these prepared media.

2.4.4.2 Preparation of Inoculum and Determination of Growth Rate on Vegetable Extracts

Cultures of the test organisms (B. polymyxa and Clostridium histolyticum) were grown in Dextrose-Tryptone Yeast extract broth for 24 hours, and then transferred into the experimental medium (vegetable extracts). A 14-18 hours old culture resulting from the transfer was used as inoculum.

200ml. of the vegetable extracts were taken in 250 ml. Erlenmeyer flasks and inoculated with 2 ml. of a 12 - 18 hours old culture prepared as above.

At the time of inoculation (0 hr.) and at selected time intervals extending over a 16 to 24 hour period two samples of the growth medium were aseptically withdrawn from the flask and used for estimated growth.

Growth was estimated from viable cellcounts using Dextrose - Tryptone - Yeast extract agar. Duplicate plates were incubated up to 48 hours at the optimum temperature of the organisms. The log of viable cell counts per millilitre of medium was plotted against time in hours. Slopes of the curves at the exponential phase were calculated. Generation times were determined from such slope values (15).

2.4.4.3 <u>Preparation of Inoculum and Growth Rate Determination: Vegetable Medium</u>

Cultures of the test organisms (B. polymxya, Clostridium Histolyticum were grown in broths for 24 hours after which serial dilutions were made up to 10^{-6} with sterile 0.1% peptone water.

The number of organism in the 10^{-6} dilution was determined. This was done by pour plating 1 ml of the dilution. The determination was undertaken on three different days to establish the average number of organisms in the 10^{-6} dilution of 24 hour growth of the test organisms.

1 ml of this dilution was taken and used to inoculate the sterile vegetable medium (2.4.4.1).

Using a sterile stirring rod, the inoculum was well distributed in the vegetable medium.

The cultures were then incubated at $30^{o}C$.

After 4 hours of incubation about 1 gm of the culture was aseptically removed from the culture and diluted in 9 mls of 0.1% peptone water. Further dilutions were made up to 10^{-4} and 1 ml of each dilution was pour plated on Dextrose Tryptone yeast extract agar. Similar 1 gm samples were taken after the 8th and 24th hours, diluted and pour plated in a similar way.

Counts were taken for the dilutions which had between 30-300 colonies.

For each vegetable medium the process was repeated.

2. MATERIALS AND METHODS

2.5 Heat Process Evaluation

The heat processes given to the canned vegetables were evaluated by determining the rate of heat penetration into the products, under the retorting conditions as employed at the factories.

In section I (Literature Review) the retorting conditions at the factories were tabulated and the heat processes were given as time of heating at given retort temperatures. These retorting conditions were examined in this section.

2.5.1 Equipment Used for the Heat Penetration Test

The determination of heat penetration was carried out in a laboratory since it was not possible to conduct the experiment in the factory retorts, where the true retorting conditions would have been obtained. The use of the laboratory retorts would however introduce some unavoidable errors in the heat penetration results.

A manually operated retort with a side outlet for cable leads from thermistor probes was used in heating the canned products.

The temperature changes at the centre of the canned products with heating, were recorded using a Grants Miniature recorder. The recorder is basically a Wheatstone Bridge which monitors changes in the resistance of a thermistor probe inserted at the centre of the canned products. The recorder has a timed moving chart on which is recorded the temperature changes at one minute intervals, and is powered by an external battery.

2.5.2 Heat Penetration Test

A 6 mm (diameter) hole was drilled on the side of each of three cans at points recommended for conduction, convection and "broken curve" packs. For each vegetable product three such cans were prepared. A suitable thermistor probe was then inserted into each hole (when inserted the tip of the probe must fall in line with the central axis of the can). To prevent leakage the probes were secured by nuts and rubber gaskets.

The vegetables were cleaned in water, steam blanched and then filled into the cans with the appropriate filling medium (Table 1.1). The probe end was further embedded in the vegetables in order to monitor the rate of heat penetration into the vegetable. A head space of $\frac{1}{4}$ ins. was left on the top of the cans. The cans were seamed and then placed in the centre of the retort.

For each of the products examined an experimental test run was conducted using three cans, each with thermistor probe in one of the three positions recommended (25) for conduction, convection and "broken curve" packs in order to determine the mode of heating of the vegetable product.

Retorting was undertaken at the specified retort temperature (Section I Literature Review) and an attempt was made to attain the retort temperature as quickly as possible. The retort temperature was maintained within $\pm$ 1^{o}C range.

When the product attained the maximum temperature the cooling process was started. This was accomplished by simultaneously stopping the steam supply and opening the cold water tap.

The following data were recorded :

(a) Product Type

(b) Container size

(c) Position of thermistor probe

(d) Time steam was turned on

(e) Time retort temperature reached the processing temperature

(f) Time the cooling process was started

(g) Temperature of cooling water at the start of the cooling process.

3. RESULTS AND DISCUSSION

3.1 PRELIMINARY MICROBIOLOGICAL EXAMINATION

3.1.1 Introduction

The normal spoilage micro-organisms found in heat processed canned foods are those types whose heat resistant spores are present in the food material in sufficient numbers to resist the heat process. In underprocessed canned products, the spores of the Bacillaceae family, the Bacillus and Clostridium groups are usually found (26, 28, 59). Whenever these organisms have been isolated from cans whose physical conditions and seam characteristics excluded the likely occurrence of leakage, underprocessing has been suspected (22, 26). In contrast, cans are purported to have leaked when low heat resistant, non-sporulating forms are isolated from the food contents (6, 22).

This criterion was used for detecting when underprocessing or leakage had occurred after both the physical conditions and the contents of the cans had been examined.

The microbiological examination was orientated towards isolating Gram positive, spore-forming members of the

Bacillaceae family, while physical examination of the cans concentrated on gross defects i.e. seam compactness (Free Space and % Overlap), side seam defects, and End hook and body hook defects (194). The methods for examining and grading these defects have been dealt with in Section 2.1 in this work. The media selected for this preliminary microbiological examination were also orientated towards isolating sporulating Bacillaceae forms.

3.1.2 Selection of Media

Hersom and Hulland (59), the Indian Standards Institution (11) and other workers (6, 117) have all suggested the suitability of Dextrose - Tryptone yeast extract broth/Agar for isolating and studying organisms from canned foods. Nutrient agar with 1% Yeast extract has also been recommended for routine microbiological isolation and identification work (117), and is purported to be useful even for fastidious organisms. These two media were tested with four others to select the most suitable media for this work. These media have been listed in 2.2.5.

The results for this preliminary test have been shown in tables 3.1 and 3.2. From the results it can be seen that

Table 3.1

MEDIUM SELECTION

MEDIUM		No. OF TYPES OF ORGANISM			PRODUCT TYPE
		37°C	45°C	55°C	
Nutrient b/A plain	Pr	2	1	0	
Nutrient + 1% yeast Extract	V.P	3	3	2	BAMBARA
Dextrose-Tryptone yeast Extr.	V.P	3	2	2	BEANS
Cooked Meat Medium	Sc	1	1	1	IN
Thioglycollate b/A	No	2	1	0	SAUCE
Malt Extract b/A	No growth	0	0	0	
Nutrient b/A plain	Pr	1	2	0	
Nutrient b/A + 1% yeast	Pr	3	2	1	OKRO IN
Dextrose-Tryptone yeast Extr.	V.P	3	3	0	
Cooked meat b/A	No	2	2	1	TOMATO
Thioglycollic acid b/A	Sc	1	1	0	
Malt Extract b/A	Sc	1	0	0	SAUCE

Sc = Scanty, Pr = Profuse, V = very B/A = broth and agar.

Table 3.2

MEDIUM SELECTION

MEDIUM		No. OF TYPES OF ORGANISM			PRODUCT TYPE
		37°C	45°C	55°C	
Nutrient broth/Agar	Sc	2	1	0	GARDEN EGGS
Nutrient agar + 1% yeast Extr.	Pr	3	2	1	
Dextrose-Tryptone yeast "	Pr	3	2	1	IN
Cooked meat broth/Agar	V.Pr	3	2	0	
Thioglycollate broth/Agar	Sc	1	0	0	BRINE
Malt Extract b/A	Sc	1	1	0	
Nutrient b/A (plain)	No growth	0	0	0	
Nutrient b/A + 1% yeast	Pr	3	3	1	
Dextrose-Tryptone Yeast Extr.	V.P.	3	2	1	PEPPER
Cooked meat medium	Pr	2	2	1	
Thioglycollic acid b/A	Sc	2	1	0	PUREE
Malt extract b/A	No growth	0	0	0	

Sc = Scanty, Pr = Profuse, V = very B/A = broth and agar, Mo = moderate the Dextrose-Tryptone yeast Extract medium, the Nutrient broth/Agar with 1% yeast extract and the Cooked meat media were most suited for isolating organisms from the canned vegetables.

The first two media Dextrose-Tryptone yeast Extract medium (D.T.Y.E.) and Nutrient broth/Agar with 1% yeast extract were used in all the procedures because of the simplicity in their preparations.

3.1.3 <u>Isolation of Micro-organisms</u>

Tables (3.3 - 3.10) are detailed summaries of the results of the preliminary microbiological investigations conducted on the various canned vegetable products examined in this work; (Bambara Beans in tomato sauce, 25 cans, Garden Eggs in brine, 25 cans, Pepper puree, 27 cans and Okro in sauce, 27 cans. The results of the physical examination (can seam evaluation) have also been included in the tables.

Below is shown the various abbreviations used in the tables :

Seam Characteristics

Flat	-	FL
Hard Swell	-	H.S.
Soft Swell	-	S.S.
Flipper	-	Fr
Springer	-	Sp
Intact	-	IN
Weak	-	W
Channels	-	CH
Normal	-	N

Internal Content Examination

Objectionable	-	Ob
Normal	-	N
Stale	-	S.T.
1 coccitype, 2 cocci Types	-	IC, 2C
1 rod type, 2 rod types	-	IR, 2R
Leaker	-	L
Sound	-	S
Underprocessed	-	U

3.1.3.1 Bambara in Sauce Tables (3.3, 3.4)

7 out of the 25 cans examined (28%) were not sterile (Cans 1,5,7,9,12,18,21) and out of the seven cans, leakage was considered to have occurred in five of them

Table 3.3 BAMBARA IN TOMATO SAUCE

TYPE OF ORGANISM

Can No.	Odour	Taste	Pressure Ins. Hg	Isolated Under Aerobic Conditions	Gram	Catalase	Isolated Under Anaerobic Conditions	Gram	Catalase	Suspected Spoilage Type
1	ob	ob	5.7	2C, IR			IR			L
2	N	N	-1.3							S
3	N	N	-2.6							S
4	N	N	0		+	+				S
5	ST	ST	0.5	2R	+	+				U
6	N	N	-3.6							S
7	N	ST	4.1	2R, 2C	+					L
8	N	N	0							S
9	ST	ST	3.4	1C, 1R			1R, 1C			L
10	N	N	0							S
11	N	N	0.7							S
12	N	ST	0	2R	+	+	1R	+	+	U
13	N	N	-4.9							S
14	N	N	-1.6							S
15	N	N	-2.5							S
16	N	N	-3.7							S
17	N	N	-2.5							S
18	ST	ob	5.3							L
19	N	N	0							S
20	N	N	-1.0							S
21	N	ST	-1.0	2C, 1R	+	+	IR	+	+	S
22	N	N	-1.5							S
23	N	N	-4.3							S
24	N	N	-2.0							S
25	N	N	-1.5							S

Table 3.4

PHYSICAL EXAMINATION

BAMBARA IN TOMATO SAUCE

Can No.	End Plate Swell	Hook Defects (Grade)	Pressure (Ins.Hg)	Free Space	% Over-lap	Side Seam Defects
1	SS	N	5.7	15.6	31	W,CH
2	FL	I	-1.3	7.1	62	IN
3	FL	N	-2.6	6.3	63	IN
4	FL	N	0	5.8	66	IN
5	FL	N	0.5	4.0	66	IN
6	FL	I	-3.6	9.4	53	IN
7	HS	I	4.1	14.9	34	IN
8	FL	N	0	8.2	63	P
9	SS	N	3.4	13.5	44	IN
10	FL	N	-0.5	2.9	70	CH
11	FR	N	0.7	10.1	54	IN
12	FL	2	0	9.4	53	IN
13	HS	N	4.9	13.9	33	IN
14	FL	N	-1.6	10.1	54	IN
15	FL		-2.5	8.0	56	IN
16	FL	I	-3.7	7.8	58	W
17	FL	N	-2.5	5.2	71	IN
18	HS	N	5.3	15.9	39	WP
19	FL	N	0	10.2	50	P
20	FL	N	-1.0	11.5	45	IN
21	SP	3	1.0	13.5	38	IN
22	FL	N	-1.5	12.9	42	IN
23	FL	I	-4.3	10.6	53	IN
24	FL	N	-2.0	12.4	40	W
25	FL	N	-1.6	11.3	32	IN

(Cans 1,7,9,18,21). When spoilage occurred through leakage the content usually had objectionable odours and tastes, and high internal pressures (1,7,9,18) which showed visibly as swells on the ends of the cans (Table 3.4). The internal pressures of cans suspected of leakage ranged between 0-5.7 ins. Hg. With the exception of can 21 which had flat ends all the other cans had internal pressures greater than 3.4 ins. Hg, and the ends were either Soft swells or Hard swells. This observation suggests that cans with pronounced swells are usually leakers. The microbiological examination of these leakers showed heterogenous flora of cocci as well as rod forms (tables 3.3). The presence of such heterogenous mixtures of cocci and rod forms was considered to be indicative of leakage. The conditions of the seams often confirmed these observations (table 3.4). The major causes of leakage in this product were seam looseness (large free space and small % overlap) Cans 1,7,18 and 21, and weak side seams; cans 1, 7, 18,9, 21). Hook defects in the cans examined were low grade defects (table 3.4) and except in can 21, which was graded 3, hook defects were generally considered to have negligible influence on leakage.

The presence of viable organisms in 2 cans (cans 5 and 12) was suspected of occurring through underprocessing. The seams of these cans remained hermetic (table 3.4) and did not possess any gross defect; while the internal microbiological flora were practically homogenous.

The internal pressure of these cans were 0.5 and 0 ins. Hg respectively.

One of the organisms isolated from can 12 was suspected of being a Clostridium species on the basis of its morphology, positive Gram reaction and negative catalase reaction.

3.1.3.2 Garden Eggs in Brine (T able 3.5 and 3.6)

A higher number of underprocessed cans were recorded with this product than for Bambara Beans; 16% of the total number of cans examined (4 out of 25 cans) were suspected of underprocessing. These were cans 2,6,17,20 (tables 3.5).

The major criterion used in deciding on whether underprocessing had occurred was the homogeneity of microbial flora (Bacillaceae group) and hermetic and intact double and side seams. All the organisms found in these cans (Tables 3.5) were flat-souring Bacillus group of organisms (positive Gram reaction, positive catalase reaction and capability

Table 3.5

GARDEN EGGS IN BRINE

TYPE OF ORGANISM

Can No.	Odour	Taste	Pressure ins.Hg	Isolation Under Aerobic Conditions	Gram	Catalase	Isolation Under Anaerobic Conditions	Gram	Catalase	Suspended Spoilate Type
1	N	N	-3.2							S
2	N	N	-1.0	1R	+ve	+ve				U
3	ob	ST	4.1	2R,2C			IR	+ve	+ve	L
4	N	N	-2.0							S
5	N	N	-1.5							S
6	N	ST	0	2R	+v	+ve	IR	+ve	+ve	U
7	N	N	-4.1							S
8	N	N	-3.2							S
9	N	N	-2.6							S
10	ob	ob	5.6	2C			IR,1C			L
11	ST	ST	0	1R,1C			1R	+ve	+ve	L
12	N	N	-3.9							S
13	N	N	-2.5							S
14	ST	ST	1.1	1C						L
15	N	N	-1.5							S
16	N	N	-2.4							S
17	N	N	0.6	2R	+ve	+ve	1R	+ve	+ve	U
18	N	N	-1.4							S
19	N	N	-2.6							S
20	N	ST	1.0	1R	+ve	+ve	1R	+ve	+ve	U
21	N	N	-4.1							S
22	N	N	-5.2							S
23	N	N	-2.7							S
24	N	N	-1.3							S
25	N	N	-1.7							S

Table 3.6 PHYSICAL EXAMINATION

GARDEN EGGS IN BRINE (A1)

Can No.	End Plate Swell	Hook Defects (Grade)	Pressure Ins. Hg	Free Space	% Over-lap	Side Seam Defects
1	FL	N	-3.2	10.3	54	IN
2	FL	N	-1.0	4.6	63	IN
3	SS	N	4.1	12.0	31	IN
44	FL	N	-2.0	8.3	48	IN
5	FL	2	-1.5	8.4	57	W
6	FL	N	0	7.3	59	P
7	FL	N	-4.1	7.7	56	IN
8	FL	1	-3.2	7.5	52	IN
9	FL	1	-2.6	9.2	49	IN
10	HS	N	5.6	10.8	32	W,CH
11	FL	4	0	15.1	40	IN
12	FL	1	-3.9	5.9	48	IN
13	FL	N	-2.5	12.1	51	IN
14	FR	N	1.1	12.4	27	W
15	FL	N	-1.5	8.7	60	IN
16	FL	N	-2.4	9.8	56	IN
17	FR	1	0.6	6.5	59	IN
18	FL	1	-1.4	6.1	55	IN
19	FL	N	-2.6	10.6	47	IN
20	FR	1	1.0	7.4	55	IN
21	FL	N	-4.1	9.3	46	W
22	FL	2	-5.2	7.2	49	IN
23	FL	N	-2.7	5.4	52	IN
24	FL	N	-1.3	8.1	57	IN
25	FL	7	-1.7	10.5	44	CH

to form spores), and the internal pressures of the cans ranged between -1.0 to 1.0 ins Hg. The cans therefore showed flat ends and spoilage was evident only after opening the cans.

Cans 3, 10, 11, 14 (Tables 3.5) were suspected of being leakers; Cans 3 and 10 had high internal pressures, soft and hard swells respectively. The internal contents were characterised by objectionable odours and taste.

Microbiological examination revealed a heterogenous mixture of non-sporulating forms with sporulating forms. Again, the major causes of leakage were seam defects. Cans 3, 10, 11 and 14 (tables 3.6) had loose seams (large Free Space and small % Overlap) and cans 3, 5, 10, 14, 21, 25 were found with faulty side seams, suggesting the importance of side seam defects as a cause of leakage.

Hook defects were not of any real significance as a cause of leakage. However, the hook defect of grade 4 in can 11 could have contributed to leakage; all others had low grade hook defects.

3.1.3.3 Okro in Tomato Sauce Tables(3.7 and 3.8)

9% of the total number of cans examined were suspected of underprocessing (can 17, 19 and 20). Sporulating

Table 3.7 OKRO IN SAUCE

TYPE OF ORGANISM

Can No	Odour	Taste	Pressure Ins. Hg.	Isolated Under Aerobic Condition	Gram	Catalase	Isolated Under Anaerobic Condition	Gram	Catalase	Suspected Spoilage Type
1	N	N	O							S
2	ob	ob	6.4	2C,1R			1R	+ve	+ve	L
3	N	N	-1.4							S
4	N	ST	0							S
5	N	N	-2.6							S
6	N	N	-1.3							S
7	N	N	-1.8							S
8	N	N	-3.2							S
9	N	N	-4.2							S
10	ob	ob	+5.7	3C,1R			1R	+ve	+ve	L
11	ST	N	0	2C						L
12	N	N	-5.2							S
13	N	N	-4.1							S
14	N	N	-1.6							S
15	N	N	-2.3							S
16	N	N	-2.0							S
17	ob	ST	+3.2	1R	+ve	+ve	1R	+ve	-ve	U
18	N	N	-2.5							S
19	N	ST	1.1	2R	+ve	+ve				U
20	N	ST	1.4	2R			2R	+ve	+ve	U
21	N	21	3.2	1R,1C			1C			L
22	N	N	0							S
23	N	N	-3.1							S
24	N	N	-4.5							S
25	N	N	-0.5							S
26	N	N	-5.0							S
27	N	N	-4.2							S

Table 3.8 PHYSICAL EXAMINATION

OKRO IN BRINE (A1)

Can No.	End Plate Swell	Hook Defects (Grade)	Pressure Ins. Hg.	% Overlap	Free Space	Side Seam Defects
1	FL	N	0	53	6.6	IN
2	HS	4	6.4	27	12.5	CH
3	FL	N	-1.4	58	5.4	IN
4	FL	1	0	56	4.6	IN
5	FL	1	-2.6	62	4.4	IN
6	FL	N	-1.3	49	8.4	W
7	FL	N	-1.8	54	6.2	IN
8	FL	2	-3.2	56	4.8	IN
9	FL	N	-4.1	42	9.1	IN
10	SS	4	5.7	27	12.5	IN
11	FL	N	0	58	5.3	W,P
12	FL	N	-5.2	50	4.2	IN
13	FL		-4.1	68	2.4	IN
14	FL	N	-1.6	49	7.7	IN
15	FL	1	-2.3	52	8.3	CH
16	FL		-2.0	50	7.3	IN
17	SP	S	+3.2	58	5.4	IN
18	FL	N	-2.5	43	10.2	W,CH
19	FL	N	1.1	51	7.9	IN
20	FR	N	1.4	55	6.4	P
21	SS	5	3.2	28	14.0	IN
22	FL	1	0	49	5.6	IN
23	FL	1	-3.2	60	8.8	IN
24	FL	N	-4.5	59	3.2	IN
25	FL	2	-0.5	51	7.5	IN
26	FL	N	-5.0	59	6.9	IN
27	FL	N	-4.2	47	8.3	IN

rod forms which also showed a positive Gram reaction (Bacillaceae family) were isolated from these cans. The presence of hermetic and intact seams (tables 3.8) confirmed underprocessing.

All the underprocessed cans had low internal pressure except can 17 which had an internal pressure of 3.2 ins Hg; the organism isolated from this can was a Gram positive, catalase-negative spore-forming rod; suggesting a gas producing Clostridium species as the spoilage organism. Apart from this can whose ends were flippers, all the other cans suspected of underprocessing had flat ends, hinting again that the major spoilage organisms in the underprocessed cans were the flat-souring non-gas producing Bacillaceae forms.

14.8% of the 27 cans examined were suspected of being leakers; these were cans 2, 10, 11 and 21. All the cans except can 11 had high internal pressure ranging between 3.2 - 6.4 ins. Hg, and which manifested as obvious end swells. This confirms the observation that in a population of cans, leakers could be readily selected from sound and underprocessed cans by the appearance of the swollen can ends.

The major cause of leakage was loose double seams, cans 2, 10, 21 (table 3.8). A high percentage of the cans, 22.2%, also had weak side seams; and of this lot 66.6% were suspected leakers. This observation again shows that side seam defects are important sources of leakage in these vegetable products.

Hook defects were more significant in this product than in Bambara or Garden Eggs. Cans 2 and 10 had defects with grade 4 and Can 21 had a grade 5 hook defect. Defects at such grades could cause leakage (194).

3.1.3.4 Pepper Puree (Tables 3.9 and 3.10).

For pepper 9 out of the 27 cans examined (33.3%) were found to contain viable organisms. 6 out of the 9 unsterile cans were diagnosed as leakers and the remaining three as underprocessed cans. The leakers, cans 6, 7, 11, 19, 20 and 23 (table 3.9) had higher internal pressures, from 0-5.3 ins. Hg; which showed as soft swells or hard swells. Organisms isolated from these leakers were a mixture of cocci and rod forms and their activity in the cans produced objectionable odours and tastes.

The major source of leakage was loose double seams (high Free Space and small % overlap), cans 7, 11, 19 and 23 (table 3.10). 9 cans were observed with defective side seams and in cans 20 and 6 this defect appears to be the sole cause of leakage. This observation emphasizes the importance of this defect as a major cause of leakage.

Hook defects were relatively insignificant and except for can 6 which had a hook defect of grade 4, the grades obtained on the remaining cans were considered low and insignificant as sources of leakage.

Cans 2, 10 and 15 were suspected of underprocessing.

The microorganisms isolated from these cans were Gram

Table 3.9 PEPPER PUREE

				TYPE OF ORGANISM						
Can No.	Odour	Taste	Pressure Ins. Hg	Isolated Under Aerobic Conditions	Gram	Catalase	Isolated Under Anaerobic Conditions	Gram	Catalase	Suspected Spoilage Type
1	N	N	-1.2							S
2	N	ST	0	1R	+ve	+ve		+ve	+ve	U
3	N	N	-1.5							S
4	N	N	-3.2							S
5	N	N	-2.7							S
6	ob	ob	4.3	1R,2C			1R			L
7	ST	ob	5.2	1R,3C			2C			L
8	N	N	-4.2							S
9	N	N	-1.6							S
10	N	ST	0	1R	+ve	+ve	1R	+ve	+ve	U
11	ob	ob	5.3	3C	1C,		1C,1R			L
12	N	N	-2.0							S
13	N	N	-3.2							S
14	N	N	-1.0							S
15	N	ST	-4.1				2R			U
16	N	N	-4.5							S
17	N	N	-3.0							S
18	N	N	-2.5							S
19	ST	ST	2.3	1R	+ve	+ve	2C,1R			L
20	N	N	0	2R,1C			1R	+ve	+ve	L
21	N	N	-2.4							S
22	N	N	-2.1							S
23	N	N	1.5							L
24	N	N	0							S
25	N	N	-2.7							S
26	N	N	-3.7							S
27	N	N	-4.0							S

Table 3.10 PHYSICAL EXAMINATION

PEPPER PUREE IN CANS A2½

Can	End Plate Swell	Hook Defect (Grade)	Pressure Ins. Hg	% Overlap	Free Space	Side Seam Defect
1	FL	N	-1.2	58	4.6	W
2	FL	N	0	60	2.7	IN
3	FL	1	-1.5	49	9.2	IN
4	FL	N	-3.2	54	8.4	IN
5	FL	1	-2.7	63	3.0	IN
6	SS	4	4.3	62	8.1	IN
7	HS	3	5.2	33	17.1	W,P
8	FL	N	-4.2	72	4.6	IN
9	FL	N	-1.6	55	10.2	IN
10	FR	N	0	57	6.0	P
11	HS	2	5.3	34	15.0	P
12	FL	1	-2.0	45	9.3	IN
13	FL	N	-3.2	58	5.9	IN
14	FL	1	-1.0	69	5.1	IN
15	FL	2	-4.1	59	6.3	IN
16	FL	1	-4.5	67	1.9	IN
17	FL	N	-3.0	50	8.9	IN
18	FL	N	-2.5	54	9.8	P
19	SP	N	2.3	32	14.9	IN
20	FL	N	0	66	5.7	IN
21	FL	N	-2.4	58	7.9	W,CH
22	FL		-2.1	60	6.7	IN
23	FL	1	1.5	35	13.4	IN
24	FL	1	0	54	5.5	WP
25	FL	N	-2.7	47	9.7	IN
26	FL	2	-3.7	68	3.4	IN
27	FL	N	-4.0	51	6.2	IN

positive, catalase positive sporulating rod forms of the Bacillus genus. These organisms are usually flat-souring organisms which ferment carbohydrates without the production of gas.

The underprocessed cans had internal pressure between -4.1 to 0.7 ins Hg. These cans were flat at the ends.

3.2 FREE SPACE AND LEAKER SPOILAGE

3.2.1 Introduction:

An important quality criterion of a good and effective double seam is Seam Compactness (194). The Metal Box Co. (194) classifies seam looseness as a gross defect, and such defects, when they occur invariably lead to leakage. Seam compactness is estimated by measuring Free Space and Percent Overlap (194). The definitions for these two parameters and methods for their determination were discussed under Section 2.1 .

Based on results of Free Space and % Overlap in tables (3.3 to 3.10), plots of Free Space against % Overlap havebeen constructed for three types of can sizes, (8z can = 211 x 400)) (A1 cans = 211 x 400) and (A2½ cans = 401 x 411) to find out whether any correlation exist between the two parameters.

All the plots, Fig. (3.1 to 3.3) indicated straight inverse relationships between the % Overlap and Free Space; i.e. large Free Space corresponded with small % Overlap. Kendal's coefficients (r) (134) were calculated for the plots, and for the 8z cans (r) = -0.84, for A1 cans

Fig 3.1

PERCENT OVERLAP VERSUS FREE SPACE (0.001") :8Z CANS

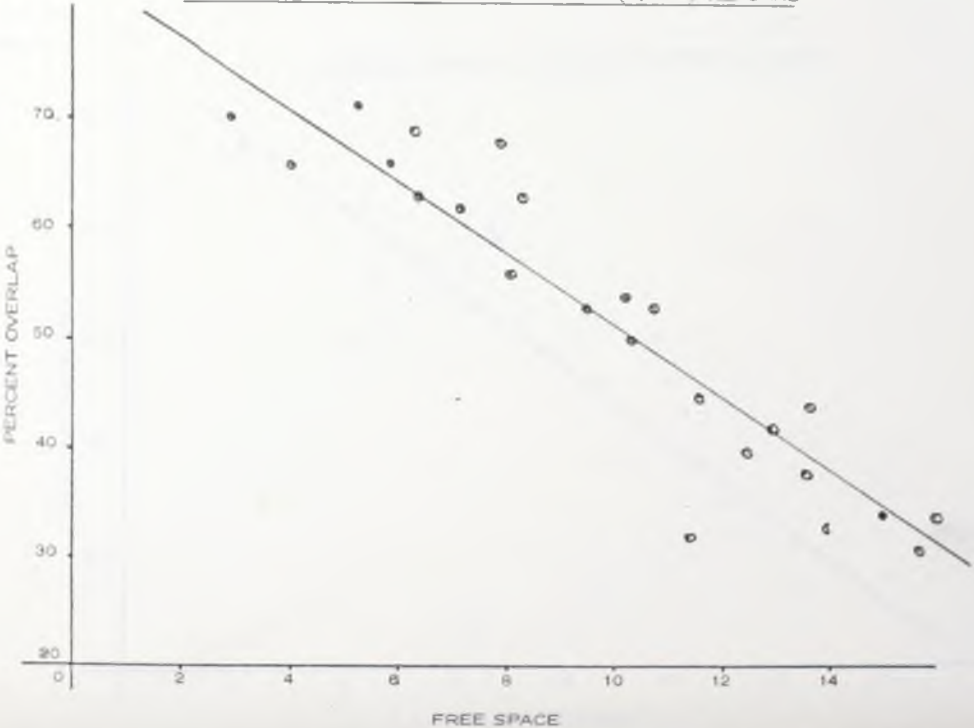


Fig 3.2

PERCENT OVERLAPT VRS. FREE SPACE :A1 CANS

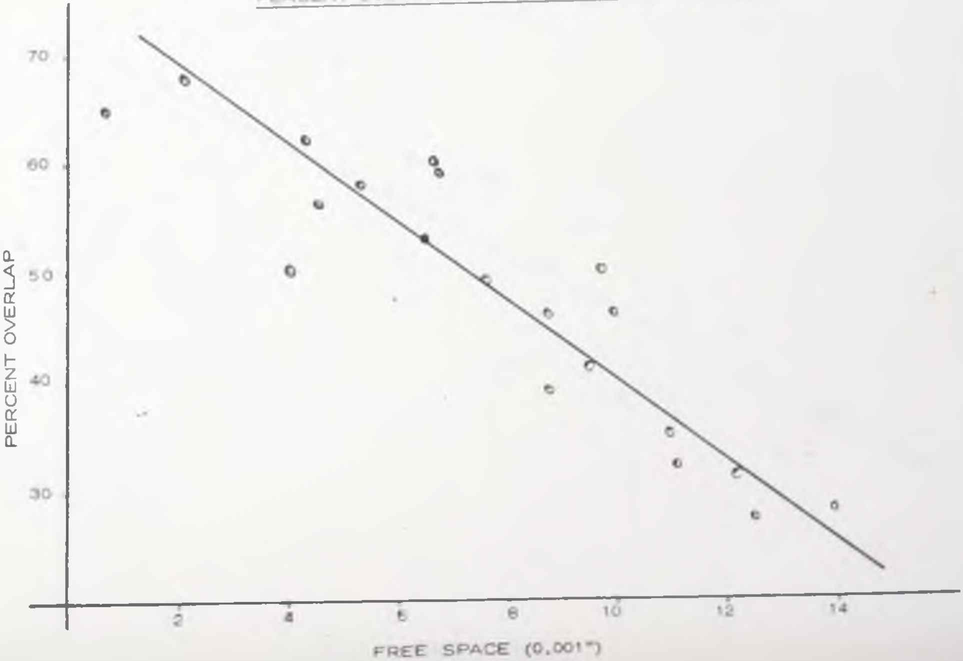


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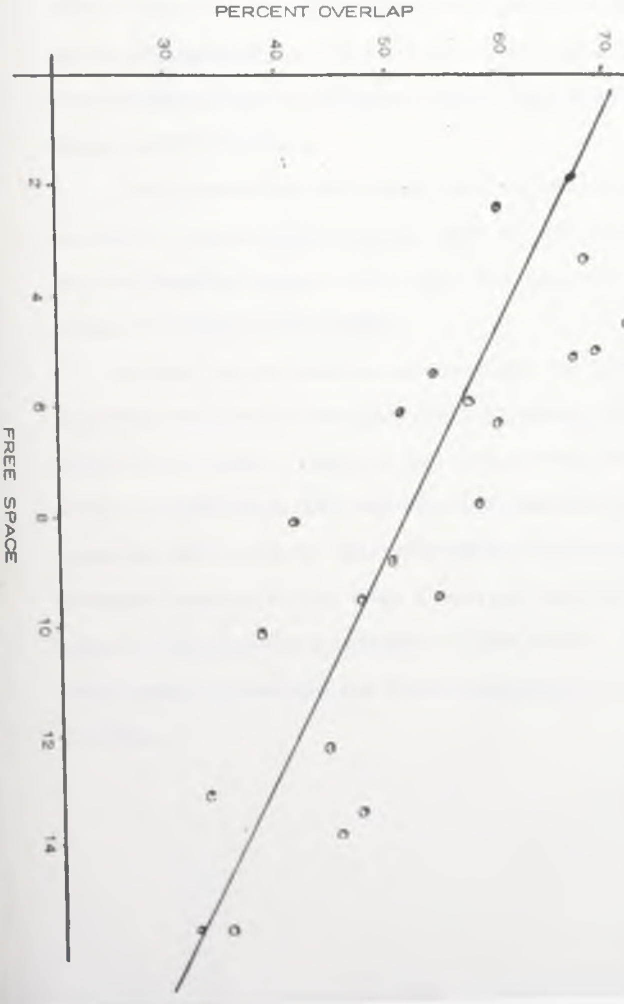
Fig. 3.3

PERCENT OVERLAP VRS. FREE SPACE (0.001") A2½ CANS



(r) = -0.80 and for A2$\frac{1}{2}$(r) = -0.73. Test of significance were conducted for the coefficients, which showed that at the 5% significance level, there were enough grounds for assuming a high correlation established between Free Space and % Overlap.

Free Space was therefore used in this study as a measure of seam compactness, both for its greater accuracy in limiting human errors and the ease and non destructiveness in its determinations.

A total of 329 cans were examined for Free Space, and this total was made up of 113, 8z cans, 114 A1 cans, and 102 A2$\frac{1}{2}$ cans. Table (3.11) summarizes the details of the number examined and also includes the number of each can type used for microbiological examination. The average figures for the Body Plate and End Plate thicknesses have also been included in this table. These figures were used to calculate the Free Space and the Percent Overlap.

Table 3.11 CAN EXAMINATION

Product Type		NUMBER OF CANS EXAMINED					Mean	Mean
		Free Space	% Over-lap	Micro-biological	Body Plate	End Plate	Body Plate Thickness (0.001)	End Plate Thickness (0.001
Bambara	8z	64	25	25	12	12	10.1	9.5
Beans	32z	15	10	10	5	5	9.3	9.1
Pepper	8z	50	27	27	15	10	10.1	9.5
Puree	32z	37	15	15	7	8	9.3	9.1
Okro in Sauce	Al	53	27	27	17	17	9.9	8.3
Garden	Al	60	25	25	12	14	9.9	8.3
Eggs	32z	46	20	20	9	9	9.3	9.5
Whole Tomato	32z	6	6	6	6	6	9.3	9.1
Total		329	155	155	83	81	-	-

Can sizes: 8z = 8 ounze cans Al = 16 ounze cans 32z (A2½) = 32 ounze cans

3.2.2 FREE SPACE AND LEAKER SPOILAGE

Figures (3.4 to 3.6) illustrate the frequency distributions of the Free Space measured for 8z, A1 and A2½ cans. The detailed results from which these polygons were ddrawn have been shown in Appendices 2-4. The Appendices also show the number of defectives (i.e. cans that showed swelled ends) recorded for each class interval, and those that showed flat ends.

These polygons Fig (1-3) were analysed, and their statistics, Mean ($\bar{x}$) and Standard Deviation (S) for the 8z cans distribution were, ($\bar{x}$ = 7.6, S. 3.9); for the A1 cans distribution ($\bar{x}$ = 6.7, S = 3.2), for the A2½ cans, ($\bar{x}$ = 8.2, S = 3.4).

A ready observation that can be made from these distributions is their close distribution around their means and the tendency to skew towards increasing Freespace. This observation becomes much clearer when a comparison is made between these distributions and the normal distribution.

For the normal distribution, the mean plus and minus one standard deviation, ($\bar{x}$ + S), accounts for 68.2% of the total area under the normal curve (143). In the case of the 8z distribution ($\bar{x}$ $\pm$ S) accounted for 74.4% of the total area while for A1, and A2½ distributions ($\bar{x}$ $\pm$ S) accounted for 79.4% and 70.5% respectively of the total area under the

Fig 3.4

FREQUENCY DISTRIBUTION OF FREE SPACE : 8Z CANS

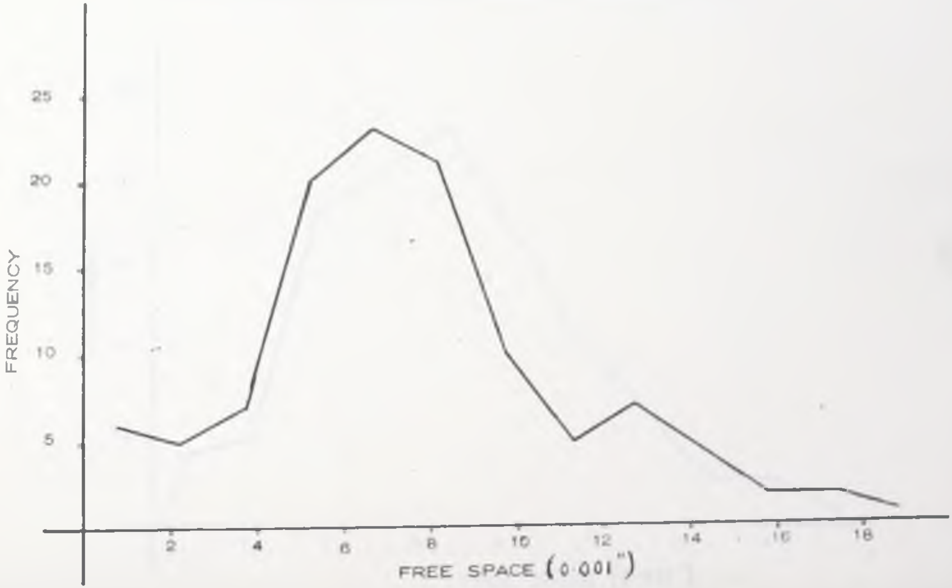


Fig 3.5

FREQUENCY DISTRIBUTION OF FREE SPACE : A1 CANS

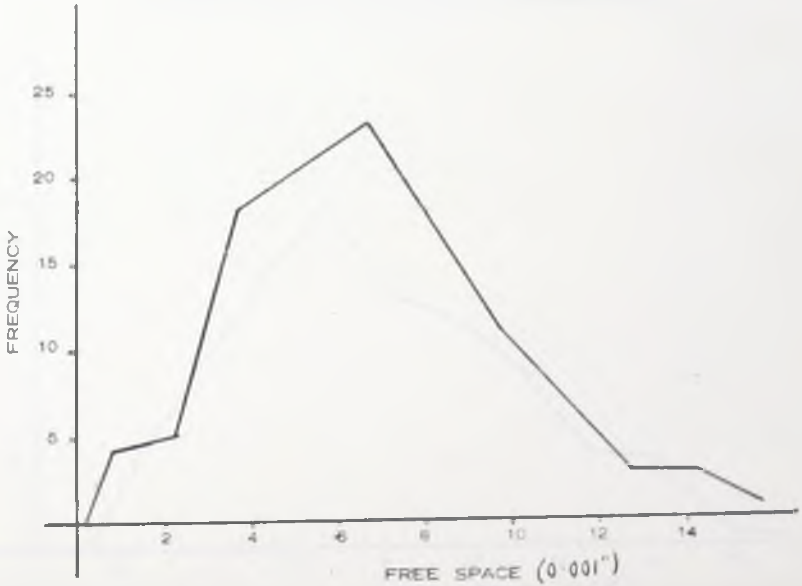
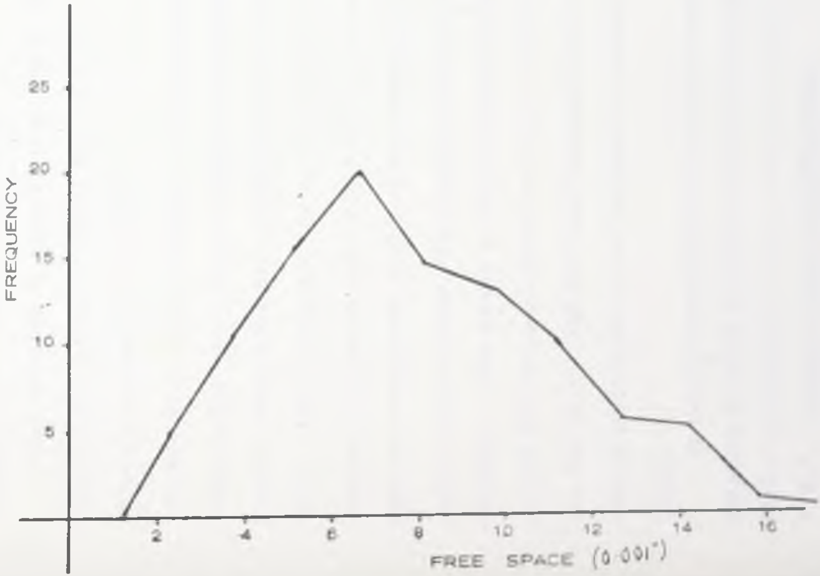


Fig 3.6

FREQUENCY DISTRIBUTION OF FREE SPACE : A2½ CANS



distributions. There is therefore greater concentration of the values of the Free space around their means than obtains in the normal distribution.

The tendency to skew is also illustrated by a comparison of the means obtained, with recommended Free Space values furnished by the Metal Box Company (194). The average Body plate thickness and End plate thickness (table 3.11) were used to obtain corresponding seam thicknesses from the tables provided by the Metal Box Company (194). These were for the 8z cans 54.5, for A1 can 48.5 and for A2½, 49.5, (given in 0.001"). Based on these, corresponding Free Space values were calculated and compared with the mean Free Space obtained from the frequency polygons. The means of the Free Space distributions exceeded the recommended Free Space by 1.9, 2.8 and 4.6 for the 8z, A1 and A2½ cans respectively, thus illustrating this general tendency of skewness towards increasing Free Space.

It can be concluded from the observations that the existing seaming operations tend to produce cans with a higher proportion of loose ends than normal.

3.2.3. <u>Use of Free Space To Predict Leakage</u>

An attempt was made to apply Free Space to predict when leakage would occur. This was done by using the proportion of defective cans recorded at the factories (defectives as indicated by the presence of swells on the can ends) of 11.2% for A1 cans, 9.7% for A2½ cans and 8.4% for 8z cans (10). In this discussion the Free Space distributions (Fig. 3.4 to 3.6) would be considered Normal based on the suggestion by Ratcliffe (134) that when large sample sizes (n) accompany large "p" (proportion of deffectives in the population) such distributions could be considered as approximating the Normal curve; (i.e. the product "np" must be greater than 6). In all the Free Space distributions "n" is greater than 100 and "p" greater than 0.08; for the 8z distribution n = 113 and p = 0.084, for the A1 distribution n =114 and p = 0.112, while for the A2½ distribution n = 104 and p = 0.097. The products "np" in these cases are greater than 6.

The distributions of Free Space for the various can types would therefore be considered Normal in this discussion.

The Binomial Theorem (134) was used to calculate the limits within which the proportion of defective cans in any randomly selected sample of cans could reasonably be expected to fall, based on the known proportion of defectives in the can population.

The limits in this respect are defined by a Standard Error of a Proportion of Defective in a Sample (S.E.) which is calculated by using the formula

$$S.E. = \sqrt{\frac{pq}{n}} \qquad (134)$$

where "p" and "q" are the proportion of defectives and proportion of sound cans respectively in the can population and "n" is the sample size.

Using the Free Space distribution of 8z cans as a working example (Fig 3.4, Appendix 2)

$n = 111$

$p = 8.4\% = 0.084$

$q = 91.6\% = 0.916$

the standard Error (S.E.) of sample proportion of defectives for this distribution is calculated as

$$\sqrt{\frac{8.4 \times 91.6}{113}} = \sqrt{6.8} \simeq 2 \pm 2.6\%$$

Since the distributions have been assumed Normal, the 2s (2 x Standard Deviation) could be used as marking the 95% Confidence limits (134). This means that in 95 out of 100 cases, when a sample of size 113 is taken out of the 8z can population, the proportion of defectives in the sample would lie within the limits of

$8.4 \pm 2.2.6\%$, i.e. 3.2% to 13.6%

In appendix (2) the proportion of defectives observed in the 8z distribution has been recorded, this is equal to 6.2%. This proportion (6.2%) falls within the limits calculated for the distribution.

Similar limits have been calculated for the A1 and A2½ can distributions (Table 3.12, rank 3). It can be seen that the sample defectives observed in these distributions (Appendices 2 to 4) fall well within the limits calculated for them.

Referring again to the 8z distribution, since the distribution is considered normal, a normal curve could be fitted over the distribution (139). Fig (3.7) illustrates such a fitting of the Normal curve over the 8z distribution. From the Normal Curve Tables "Z values" of 2.15 and 1.56 could be taken, which cut off an area 3.2% and 13.6% from the area under the normal curve. 3.2% and 13.6% are the limits calculated for the 8Z distribution (Table 3.12).

On the Free Space distribution these "Z values" are equivalent to 15.9 and 13.4. These values were obtained by using the formula.

$$Z = \frac{X \text{ (Free Space)} - \text{mean of distribution } (x)}{\text{Standard deviation of distribution } (S).}$$

Thus the "Z values" of 2.15 and 1.56 which cut of 3.2% and 13.6% off the Normal Curve (Fig.3.7) correspond to 15.9 and 13.4 on the Free Space axis (Fig.3.7).

From the Free Space distribution Appendix 2 the Free Space Values of 15.9 and 13.4 fall within the class intervals of (15.1 -16.5) and (12.1-13.5) respectively. The proportion of defective cans whose Free Space equalled or exceeded 15.9 was estimated; for the limit 15.9, all the

FIG 3.7

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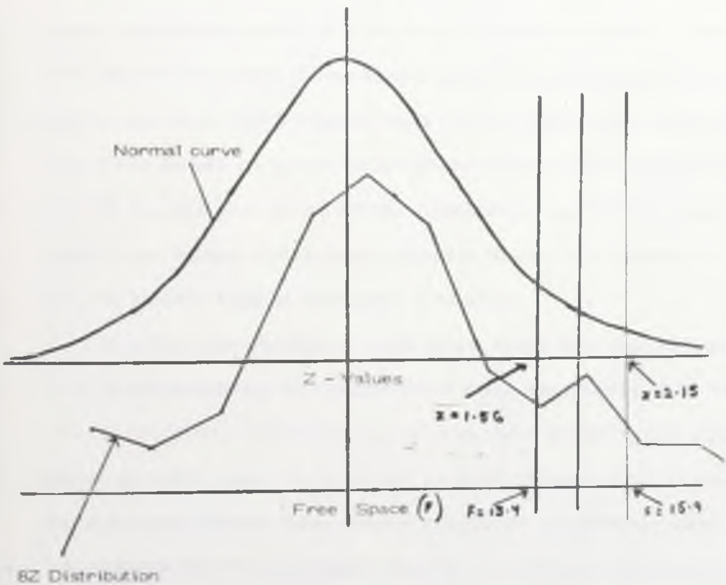
NORMAL CURVE FITTING OVER 8Z DISTRIBUTION



cans that equalled or exceeded this value were defective (100% defective). For the lower limit 13.4, 70.5% of the cans were defective. The mid-point of these two limits 13.4 and 15.9 is 14.3. At this mid-point 83.3% of the cans whose Free Space equalled or exceeded this value (14.3) were defective.

Using this procedure it becomes possible to use Free Space values to predict when leakage would occur. Thus for any 8Z can with Free Space equal to or greater than 15.9, there is 100% chance that such a can would leak. If the Free Space is equal to or greater than 14.3 there is an 83.3% chance that it would be defective, while for a can with Free Space equal to or greater than 13.6, there is 70.5% chance that it would be a leaker.

Similar computations have been made for the A1 and the A2½ distributions, and these have been summarized in table (3.12, rank 7). Thus for an A1 can with Free Space equal to or greater than 12.9, there is 100% chance that it would be a leaker; if the Free Space equals or is greater than 11.8 there is 87.7% chance that it would leak; while an A1 can with Free Space equal to or greater than 11.1 would

Table 3.12 FREE SPACE AND LEAKAGE

	CAN TYPE		
	8Z	A1	A2½
Defectives in Can Population %	8.4	11.2	9.7
Standard Error of Sample Proportion of Defectives (S.E.)	±2.6	±3.09	+2.9
95% Confidence Limits for Sample % Defectives	3,2 - 13.6	5.02 - 17.85	3.9 - 15.5
Defectives in Samples %	6.2	12.3	10.5
Z Values Corresponding To Limits (Confidence Limits)	2.15, 1.73, 1.56	1.96, 1.59, 1.36	2.06, 1.66, 13.6
X Variate (Free Space) Corresponding to Above Z Values	15.9, 14.3, 13.4	12.9, 11.8, 11.1	15.9, 14.3, 13.6
Defectives Accounted for B X Variate % (Free Space)	100 83.3 70.6	100 87.7 71.4	100 66.6 42.9

have a 71.4% chance of being a leaker.

In this manner a procedure can be evolved to monitor can seam suitability, and to prevent leakage caused by defective double seams.

In the above discussion it was assumed that the use of average figures for Body Plate thickness and End Plate thickness (table 3.11) does not introduce errors in the computation of Free Space. Free Space was computed from the formula

$$\text{Free Space} = \text{Seam thickness} - (2\ tb + 3\ te)$$

where tb = Body Plate thickness

te = End Plate thickness.

Table 3.13 shows the number of cans used for estimating the averages of Body Plate and End Plate thickness. The extent of dispersion of the individual values of the Plate thickness are also given. It can be seen that the plate thicknesses are narrowly distributed around their means (Standard deviation ranged between 0.54 - 1.04). It was assumed therefore that the variation in the plate thickness was not large enough to introduce serious deviations in the calculation of Free Space.

Table 3.13

DISTRIBUTION OF BODY PLATE AND END PLATE THICKNESS

Type of Can	Total No. of Cans Examined Body Plate	Total No. of Can Examined End Plate	Average Body Plate (Range) 0.001 and Range	Average End Plate Thick-ness 0.001 and Range	Standard Deviation Body Plate	Standard Deviation End Plate
8Z	25	22	10.1 Range (9.9-11.5)	9.5 Range (8.9-10.1)	0.81	0.73
A1	29	31	9.9 Range (8.8-10.6	8.3 Range (8.0-9.3	1.04	0.86
A2½	12	19	9.3 Range (8,9-10.2	9.1 Range (8.7-9.8	0.54	0.56

3.3 IDENTIFICATION OF ORGANISMS ISOLATED FROM UNDERPROCESSED CANS

3.3.1 Introduction:

Heat processes that are given to canned foods are always designed to destroy particular organisms in the food that are significant either as food poisoning agents or spoilage agents (26). For products with pH greater than 4.5 the heat resistant Clostridium botulinum Type A is the organism of greatest concern (138). Certain thermophilic spores of the Bacillaceae family are much more heat resistant than Clostridium Botulinum spores (26, 28, 52) and these may cause spoilage in canned foods when they occur as viable organisms in the finished product.

Any attempt therefore to define meaningful heat processes for any canned product would have to consider the types of organism that are significant in the product either as spoilage agents or food poisoning agent.

For this work 18 pure cultures were isolated from underprocessed cans (Section 3.1) and an attempt was made to identify these organisms to the species level, using a combination of cell morphology, growth characteristics and biochemical tests. From the Preliminary microbiological investigation conducted earlier in this work (Section 3.1) it was shown that the Bacillus and Clostridium genera were the major groups of organism encountered in the underprocessed cans. The present identification procedures were therefore simplified to identify these species of organisms.

3.3.2 RESULTS

3.3.2.1 Bacillus polymyxa

Five of the organisms were identified as *Bacillus polymyxa*. They were cultures G_6 and G_{17} from Garden Eggs. P10 and P2 from Pepper Puree and B_5^2 from Bambara Beans. This organism is not a regular processed food contaminant, due to its low heat resistance, [D_{250} = 0.10 (26, 67)]. Its widespread occurence in soils and vegetable matter (6) could explain its presence in freshly harvested vegetables.

The organism was recognized by its peculiar colonial characteristics; spreading translucent colonies with lobate margins. The organism grew profusely on nutrient agar at both 31°C and at 37°C. In deep nutrient broth it exhibited heavy growth both on surface and sub-surface confirming its facultative anaerobic characteristics.

Most of the Bacillus species produce from carbohydrates acid without gas with the exception of B polymyxa and B macerans. In carbohydrate media (Glucose, mannitol, arabinose in ammonium base medium) some of the isolates produced gas, and this was used as a presumptive test for identifying B. polymyxa. This organism was further differentiated from the other gas producing Bacillus, B. macerans, by its ability to produce acetylmethylcarbinol (MR - VP test). Other tests used in confirming this organism were the ability to hydrolyse starch and gelatin, and the exhibition of urease and nitrate reduction activities. The results of other characterization tests used in confirming this organism has been summarized in (table 3.31 and 3.32).

The identification of this organism implies certain pertinent conclusions. The occurrence of this relatively low heat resistant Bacillus spore in the products, Garden Eggs, Bambara Beans, and Pepper Puree suggests that these products are grossly underprocessed. Furthermore, it could indicate that the original B. polymyxa load in the food product was high; bacterialspores die logarithmically when exposed to heat (137), a residual population could always remain when a given bacterial population is heated, especially when the initial load is large.

3.3.2.2 Bacillus Stearothermophilus

The occurrence of this organism in spoiled canned foods has been well documented (59, 85, 100), as a flat-souring organism, and its detection in foods usually implies an ineffective cooling process (6, 140).

Three cultures G_{25} from Garden Eggs P^2_{15} from Pepper Puree and B^1_5 from Bambara beans were identified as Bacillus stearothermophilus.

A major characteristic that was helpful in screening out this organism was its temperature relationships. Being an obligate thermophile; it grew only at 55°C in a water bath and failed to grow at 45°C and 37°C. It was differentiated from other thermophiles by its ability to grow at 65°C, by its pin-point non-spreading colonial characteristics and its ellipse-shaped spore.

The organism was a facultative anaerobe, growing well on agar slants incubated in anaerobic jars and under fully aerobic conditions; growth in deep glucose broth was uniform throughout the tube.

The organism gave a negative reaction to the Voges-Proskauer test and the lecithovitellin test.

The remaining biochemical tests used in confirming this organism have been summarized in tables 3.3.1 and 3.3.2. The significance of this organism with respect to food products is that its presence points to various heating lags in the processing, (i.e. points on the processing line where products are maintained at temperatures between 47°C to 65°C for a long time). In particular when cooling processes are not effective the product may remain in this temperature range for a long time.

3.3.2.3 Bacillus Coagulans

This is another flat-souring organism whose occurrence in spoiled canned foods has been well documented (29, 32, 66, 131). It was first isolated from sour milk and tomato products owing to its ability to grow in pH 5.0. This characteristic was used in making a presumptive differentiation of this thermophile from B. stearothermophilus. After demonstrating that the organism grew at incubation temperatures...

of 55 - 60°C an attempt was made at growing it in acid glucose broth, pH 5.0.

B coagulans is a facultative anaerobe and grows well in deep glucose broth, uniformly throughout the tube. The organism appeared translucent on agar and grew with difficulty on nutrient agar. It had small flat colonies which adhered strongly to the agar. Due to poor growth on nutrient agar all the cultural studies were made on Dextrose-Tryptone Yeast extract broth and agar.

Other tests used in further classifying this organism were the presence of starch-hydrolysing enzyme and its inability to reduce nitrate. The organism could not use citrate but produced acetylmethylcarbinol.

B coagulans was isolated from Okro in tomato sauce, culture 0_{19} and Bambara Beans in sauce, culture B^2_{12}.

3.3.2.4. Bacillus cereus

The importance of this organism in foods has been growing since it was shown to be a potential food poisoning organism (142). Work done in California (197) further confirmed its virulence and showed its wide occurrence in various foods. The organism is a saprophyte and is widely distributed in soils and on vegetable matter (6). It could therefore occur in vegetable products. Under sound manufacturing practice this organism would not be of any real importance as either a food poisoning agent or as a spoilage agent owing to its rather low heat resistance (26). However when low heat processes are given to the canned products a residual population of this organism could occur, especially, if its initial spore load is high enough.

This organism was isolated from all the products except Bambara Beans; culture G_2 from Garden Eggs, P^1_{15} from Pepper Puree and O_{20} from Okro.

A very distinct characteristic (6, 142) used in making an initial selection of this organism was the appearance of the colonies. The colonies had a tendency to spread over the agar plate and appeared dry and parched. There were white silvery streaks on the parched surface, and also the margins of the colonies were filamentous.

Further tests were used to confirm this organism as B cereus. These showed positive growth in Koser's medium and the ability to hydrolyse casein and gelatin and urea. The organism grew in anaerobic glucose broth without gas production at incubation temperatures of 37°C and 30°C. It grew sparingly, however, at temperature 45°C and not at all at 55°C.

The significance of the isolation of this organism from the canned foods is that it shows that the food products are grossly underprocessed. The occurrence of this organism also suggests that the products might have had a high initial bacteria population in the raw material before processing.

3.3.2.5. Clostridium histolyticum and Clostridium thermosaccharolyticum

Two cultures B^1_{12} and O_{17} were identified as Clostridium histolyticum and Clostridium thermosaccharolyticum respectively. These cultures were readily differentiated from the Bacillus species by their negative catalase reaction and the distinct swelling of their sporangia at the subterminal to terminal position. Spores of both organisms were spherical and this therefore presented initial difficulty in their identification.

However their range of growth temperature and oxygen requirements served to establish the differentiation. Cl. thermosaccharolyticum was definitely anaerobic, Cl. histolyticum grew at temperatures as low as 30°C.

Test with litmus milk showed strong coagulation with gas production for <u>Cl. thermosaccharolyticum</u> while no gas, and little coagulation was obtained with <u>Cl. histolyticum</u>.

Other tests used in confirming the identity of the organisms where hydrolysis of blood agar the indole test and carbohydrate fermentations. The results have been summarized in tables 3.3.1 and 3.3.2.

The occurrence of <u>Cl. thermosaccharolyticum</u> was not unexpected, being a regular food spoilage organism (52). Its spores are heat resistant, surpassed only by spores of <u>B. stearothermophilus</u> (26). Spoilage due to this organism produced objectionable odours and hard swells.

It is an obligate thermophile and its isolation indicates "heating lags" during processing.

<u>Cl. histolyticum</u> is a relatively low heat resistant organism (26); its presence in the canned foods suggests underprocessing.

3.3.2.6. <u>Bacillus macerans</u>

This organism is widely distributed in soils and is found on decomposing vegetable material (29, 139).

Two cultures O^1_{19} and G_{20} were identified as Bacillus macerans, and were isolated from Garden Eggs and Okro products.

The organism grew well on nutrient agar in thin near transparent spreading colonies. One major biochemical test used in discerning between this organism and others of the morphological group 2 Bacillus types was its ability to produce gas from the fermentation of arabinose in an ammonium nitrogen base medium. Further differentiation was then made between this organism and B. polymyxa by the test for acetylmethylcarbinol production; (MR -VP test). B. macerans unlike B. polymyxa was not able to produce acetylmethylcarbinol.

After this preliminary differentiation other tests were undertaken on this organism to confirm it as B. macerans. These tests included the indole test, test for the urease enzyme system and the ability to use citrate in Koser's medium.

The organism is a relatively low heat resistant organism D_{212} = 0.1 - 0.5 (26) . Its occurrence in the canned products again emphasizes the low heat process given to these vegetable products.

Table 3.3.2 CELL MORTHOLOGICAL CHARACTERISTICS

Product Type	Culture Number	Form	Arrange-ment	Motility	Size (U)	Sporangium Condition	Endospore Shape/Wall	Endospore Position	Morphological Group
Garden Eggs in Brine	G_2	Rods	Chains	-	0.9/4.0	N.Swollen	Oval/Thin	Central	1
	G^1_6	"	"	+	0.9/2.3	D. Swollen	Oval/Thick	Sub-Terminal	2
	G_{25}	"	Single	+	0.6/2.1	" 3 "	Racket/Thick	Central	2
	G_{27}	"	Chains	+	0.7/1.8	Swollen	Oval/Thick	"	2
	G_{20}		Single	+	0.5/4/0		Ellipse/Thick	Sub-Terminal	2
	G^2_6	"	"	+	0.4/4.9	N. Swollen	Cylinderical	Sub Technical	1
Pepper Puree	P_{10}	"	Chains	+	1.0/2.5	Swollen	Oval/Thick	Central	2
	P^1_{15}	"	Filamentous	-	0.9/4.0	N.Swollen	Oval/Thin	"	1
	P^2_{15}	"	Single	+	0.7/2.2	D. Swollen	Ellipse/Thick	"	2
	P_2	"	Chains	+	0.9/2.6		Oval/Thick	"	2
OKRO	O^1_{19}	"	Palisade	+	0.6/3.8	Swollen	Oval/Thick	Sub-Terminal	2
	O_{19}	"	Chains	-	0.8/4.2	S. Swollen	Oval/Thin	Sub-Terminal	1
	O_{20}	"	"	-	1.0/5.2	N. "	Oval/Thin	Central	1
Bambara Beans	B^1_5	"	Single	-	0.6/3.0	D. Swollen	Racket/Thin	Central	2
	B^2_{12}	"	"	+	0.8/4.9	N. Swollen	Oval/Thin	Sub-Terminal	1
	B^2_5	"	Chains	+	0.9/3.0	Swollen	Oval/Thick	"	2

Do Table 3.3.1 BIOCHEMICAL TESTS

Product Type		Culture N°	Gram	Catalase	Indole	Nitrate Red	Casein Hyd	Urease	Gas from Cho	Growth 65°C	I. V.	Mannitol (Acid)	Abrabinose (Acid)	Citrate	Glucose Broth	Anerobic Glucose Broth	
GARDEN EGGS IN BRINE	1	G_2	+	+	-	+	+	+	-	-	+		-	+	d	+	
	2	G_6^1	+	+	-	+	+	-	d	-	-	+		-	+	+	
	3	G_{25}	+	+			+		-	-			d			+	
	4	G_{17}	+	+	-	d	+	-	+	-	-	+		-	+	+	
	5	G_{20}	+	+	-			-	+	-	-			-	+	+	
	6	G_6^2	+	+	-	-	-	-	-		-			-	+		
PEPPER PUREE	1	P_{10}	+	+	-	d	+	-	+	-	-	+		-	a	+	
	2	P_{15}^1	+	+	-		+	+			+			+	d	+	
	3	P_{15}^2	+	+		+	+			+			-			+	
	4	P_2	+	+	-	+	+	-	d	-	-	+	+	±	+	+	
									+								
OKRO IN TOMATO SAUCE	1	O_{19}^1	+	+	-	+		-	+	-	-			-	+	+	
	2	O_{19}	+	+		d	-		-		-			-		+	c
	3	O_{20}	+	+	-		+		-	-	+				+	+	+
	4	O_{17}	+	-	-	-			+	d		-	+			+	
BAMBARA IN SAUCE	1	B_5^1	+	+			+			+		-				+	-
	2	B_{12}^2	+	+		--	-		-		-			-			-
	3	B_5^2	+	+	-	+	+	-	+	-	-	+		-	+	+	+
	4	B_{12}^1	+	-	-	-		+		-		-	-		-	-	+

while for Pepper a [illegible]

gram was obtained.

3.4 NUMBERS AND GROWTH OF BACTERIA

3.4.1 Introduction:

In section 3.3 some relatively low heat resistant microorganisms including B. polymyxa were shown to occur in the end products of the canned vegetables. The presence of such organisms in the products suggested

a) A high pre-retorting bacterial contamination

or b) Gross underprocessing.

In this section the pre-retorting bacterial contamination in the raw material products is studied, followed by a study of the growth characteristics of some of the microorganisms in vegetable extracts and products. The effect of pre-processing conditions on bacterial numbers is also considered in this section.

3.4.2 Total counts on Raw Materials

Table 3.16 shows the results of total aerobic counts conducted on two vegetables, Garden Eggs and Pepper. For Garden Eggs the total viable count for post-blanch samples ranged from 4.2×10^2 to 3.1×10^3 per gram while for Pepper a range of 8.4×10^2 to 4.4×10^3 per gram was obtained.

Table 3.16 <u>TOTAL VIABLE COUNT ON RAW MATERIALS</u>

VEGETABLE MATERIAL		TOTAL VIABLE COUNT PRE-BLANCH			TOTAL VIABLE COUNT POST-BLANCH		
		Dilution 10-4	Colony Count 10-5	No. of Organisms per/gm	Dilution 10-3	Colony Count 10-4	No. of Organisms per/gm
GARDEN EGGS	Sample 1	85	-	1.70×10^3	24	-	4.2×10^2
	Sample 2	172	21	3.82×10^4	91	-	2.22×10^3
	Sample 3	-	110	2.20×10^4	133	18	3.12×10^3
PEPPER	Sample 1	247	33	5.76×10^4	117	9	2.07×10^3
	Sample 2	70	-	1.40×10^4	42	-	8.4×10^2
	Sample 3	243	29	5.31×10^4	170	27	4.4×10^3

A comparison of these results with those reported by Geldrich and co-workers (35) on vegetables cultivated in a temperate climate suggests that while the total counts on the preblanch samples are comparable in bacterial load, the count on post-blanch samples or the spore count is higher than obtains on vegetables harvested in temperate climates. The results of work reported by Jay (29) Norman (175) and Johston (151) on the microbiology of temperate vegetation also lead to the above conclusion.

These results thus suggest that Ghana's vegetable processing industries might be dealing with raw materials that contain high levels of bacterial spores; and that higher heat processes might be needed to prevent the occurrence of underprocessing.

3.4.3 RATE OF GROWTH OF MICROORGANISMS IN VEGETABLE EXTRACTS

The growth rate constants and the generation times of *Clostridium histolyticum* and *B. polymyxa* in extracts of the vegetables were compared with the growth in nutrient medium, to ascertain whether the vegetable extracts are inhibitory to the growth of bacteria.

Table 3.17 is a summary of the growth rate constants and generation times of Cl. histolyticum and B. polymyxa in 10% and 20% extracts. The graphs from which these values were calculated have been shown in Appendices 5-11.

The presence of 10% extracts in nutrient broths reduced the generation time of B. polymyxa from 72.0 mins to 52.8, 57.0 and 39.0 mins for Garden Eggs, Pepper and Bambara respectively. At 20% levels, the generation times were 48.6, 63.0 and 16.8 for Garden Eggs, Pepper a d Bambara respectively. For Cl. histolyticum the generation times in nutrient broth containing vegetable extracts were likewise less than in plain nutrient agar.

The results suggest that the vegetable extracts are not inhibitory to the growth of bacteria.

Table 3.17 GROWTH RATE IN VEGETABLE EXTRACTS

Product	Medium Composition	Clostridium Histolyticum Growth Rate Constant	Clostridium Histolyticum Generation Time (Mins)	Bacillus Polymyxa Growth Rate Constant	Bacillus Polymyxa Generation Time (Min)
GREEN EGGS	Nutrient Broth	0.21	85.8	0.25	72.0
	10% Extract	0.24	73.2	0.34	52.8
	20% Extract	0.31	58.2	0.37	48.6
PEPPER	Nutrient Broth	0.20	90.0	0.26	71.4
	10% Extract	0.30	60.0	0.32	57.0
	20% Extract	0.32	57.0	0.29	63.0
BAMBARA	Nutrient Broth	0.20	90.0	0.24	73.2
	10% Extract	0.37	48.6	0.46	39.0
	20% Extract	0.42	42.6	1.05	16.8

RATES OF MULTIPLICATION OF BACTERIA IN VEGETABLE PRODUCTS

Samples of vegetable purees were sterilized and inoculated with prepared cultures of B. polymyxa and Cl. histolyticum. The samples were incubated at room temperature for 24 hrs. and the total count taken at the 4th, 6th, 8th and 20 hour. The object of the test was to find out the rate of multiplication of bacteria in the vegetable products.

A preliminary determination of the total number of bacteria in 1 ml of 10^{-6} dilutions of 24 hour cultures of the test organism showed that the number was between 20-50 organisms.

Table 3.18 is a summary of the results of the experiment. By the 6th hour the population of B. polymyxa had increased from log 1.7 to log 2.24, log. 2.73 and 2.78 in Garden egg, Pepper and Bambara purees. The largest in-increase in numbers (about 100 fold increase) was recorded in Bambara puree with Cl. histolyticum, log. 1.7 to 3.18.

In Section 1.1.3 (Literature Review), it was shown that delays of 3 to 7 hours occurred between blanching and

Table 3.18 RATE OF MULTIPLICATION IN VEGETABLE PRODUCTS

		LOGARITHM OF NUMBERS OF ORGANISM PER GRAM (INITIAL CONCENTRATION 1.3-1.7)			
PRODUCT TYPE	TIME HOURS	4	6	8	20
GARDEN EGGS	B. polymyxa	1.99	2.24	2.45	3.91
	Cl. histolyticum	1.87	2.53	2.92	4.21
PEPPER	B. polymyxa	2.01	2.73	3.12	5.18
	Cl. histolyticum	1.24	2.00	2.48	3.78
OKRO	B. polymyxa	1.56	1.85	2.48	3.94
	Cl. histolyticum	2.12	2.65	3.05	4.20
BAMBARA	B. polymyxa	2.21	2.78	2.94	4.72
	Cl. histolyticum	1.92	2.31	3.18	5.13

retorting during processing of the vegetables. The results of the above experiment indicate that about 10-100 fold increase in bacterial population would result from such delays. This would imply that a severer heating process might be needed to prevent underprocessing.

3.5 EVALUATION OF HEAT PROCESSES

3.5.1 Introduction

The results of the microbiological investigation discussed in Section 3.1 to 3.4 suggested that some of the canned vegetables studied in this work were spoiled as a result of underprocessing.

The present investigation sought to find further justification for the above conclusion by examining the existing heat processes in use at the factories. The heating times and temperatures are shown in Table 3.19 (for further information see Table 1.1 in the Literature Review)

Table 3.19 HEATING TIMES AND TEMPERATURES

PRODUCT	CAN TYPE	PROCESSING	
		TIME	TEMPERATURE
Okro and Garden Eggs	A1	30 mins	230°F
Pepper Puree and Garden Eggs	A2½	45 mins	230°F
Whole Tomatoes	A2½	30 mins	230°F
Pepper Puree Garden Eggs Puree Bambara Beans	8Z	30 mins	250°F

The rate of heat penetration into the canned products under these retorting conditions were investigated.

3.5.2 Results

Figures (3.8 - 3.14) depict temperature/time relationships at the point of slowest heating lag in the cans, during processing at the stated retort temperatures and times.

3.5.2.1. Garden Eggs - A1 Cans (Fig 3.8)

At the retort temperature of 230°F (110°C), 32.5 mins were required for the centre of the product to attain the maximum temperature of 109°C. Since this product is heated only for 30 mins there is virtually no effective heating at this maximum temperature. The graph shows that after the thirtieth minute the temperature of the slowest heating point would have barely reached 109°C. This result suggests that this product is inadequately processed.

3.5.2.2 Garden Eggs - A^1 Cans (Fig 3.9)

The come-up time was 42.5 mins and the maximum temperature attained was 109°C. Since the product is heated only for 45 mins at the factories, this product is heated effectively at the maximum temperature for only 2.5 mins.

Fig 3.8

HEAT PENETRATION : GARDEN EGGS IN A1 CAN

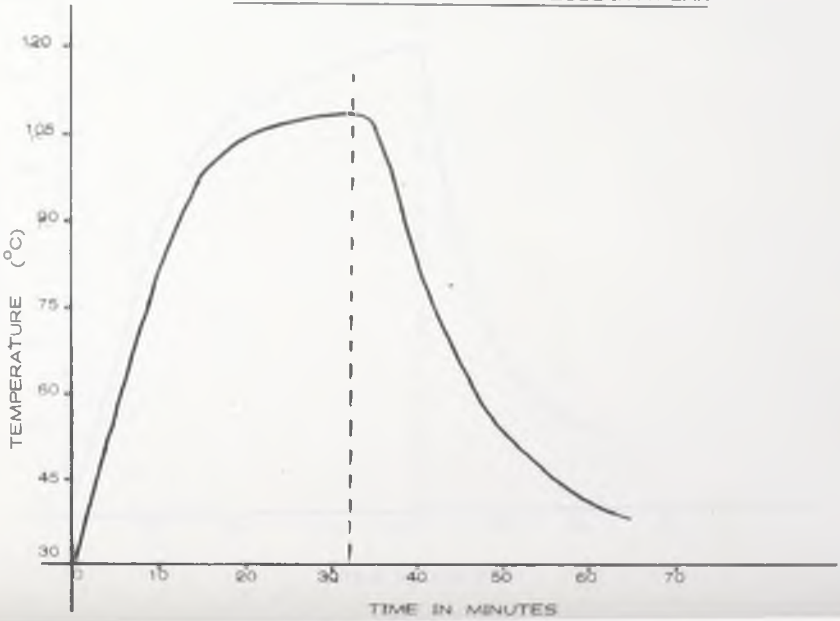
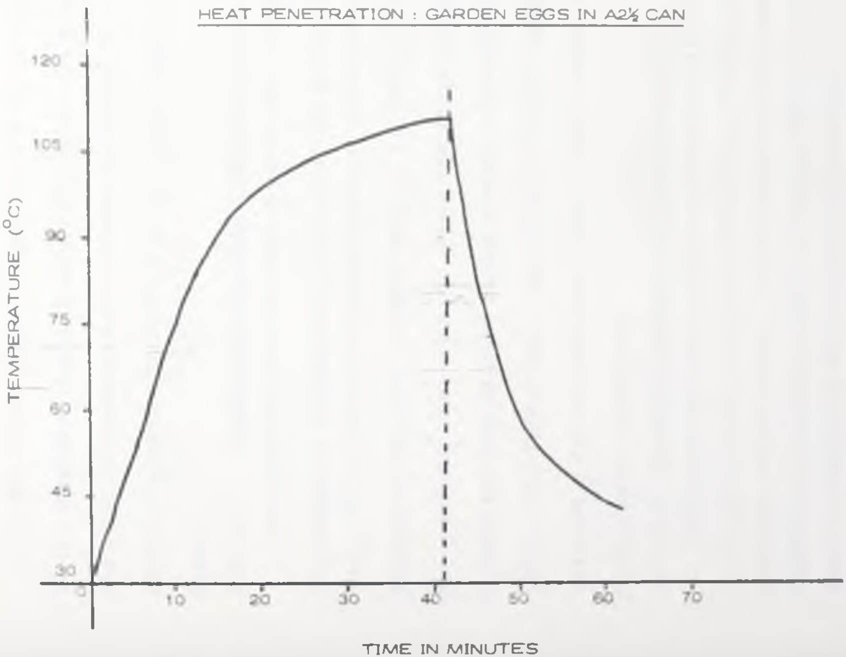


Fig 3.9


HEAT PENETRATION : GARDEN EGGS IN A2½ CAN
TEMPERATURE (°C)
120
105
90
75
60
45
30
0
10
20
30
40
50
60
70
TIME IN MINUTES

3.5.2.3 Okro - A1 Cans (Fig 3.10)

At the retort temperature of 110°C the come-up time to the maximum temperature of 109°C was 24 mins. Since the total processing time at the factories is 30 mins, the product is heated for only 6 mins at the maximum temperature.

3.5.2.4 Pepper Puree - A2½ Cans (Fig 3.11)

The come-up time for this product was 120 mins and the maximum temperature attained at the geometric centre was 106°C. Since the heating process in the factories is stopped at the 60th minute; there is no effective heating at the maximum temperature. From the graph, by the 60th minute the temperature of the geometric centre was only 88°C. The lethal rate equivalent of this temperature using (Z = 18) is less than 0.001. This product is evidently under processed.

3.5.2.5. Bambara Beans - 8Z Cans (Fig 3.12)

The come-up time for this product was 12 mins, and by the end of this time the product had attained the temperature of the retort. At the factories the product is heated for 30 mins at 120°C. This product is therefore heated effectively at the maximum temperature for 18 mins.

126

Fig 3.10

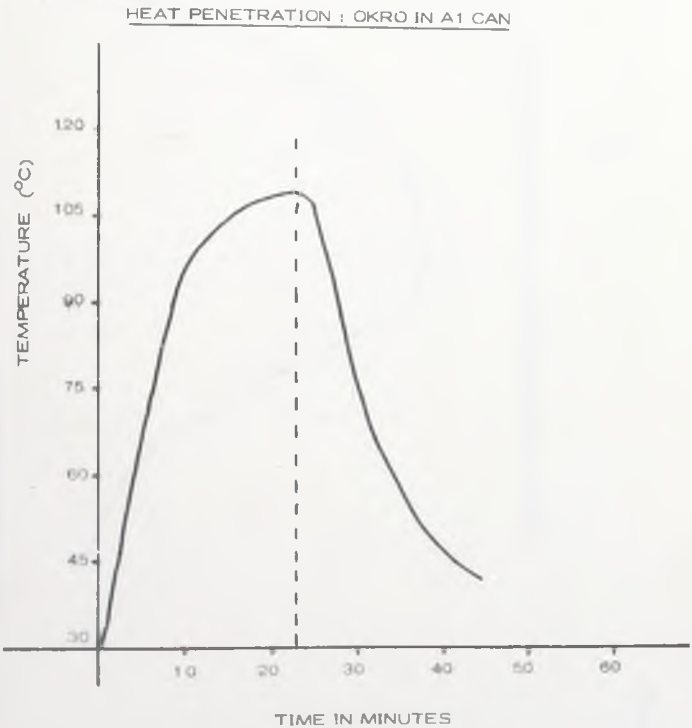

HEAT PENETRATION : OKRO IN A1 CAN
TEMPERATURE (°C)
120
105
90
75
60
45
30
10
20
30
40
50
60
TIME IN MINUTES

Fig 3.11

HEAT PENETRATION : PEPPER PUREE IN A2½ CAN

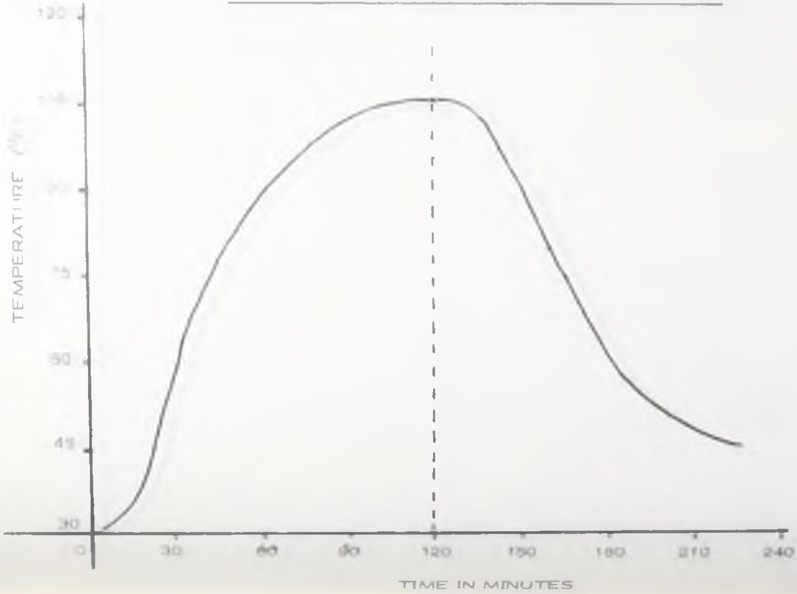


Fig 3.11

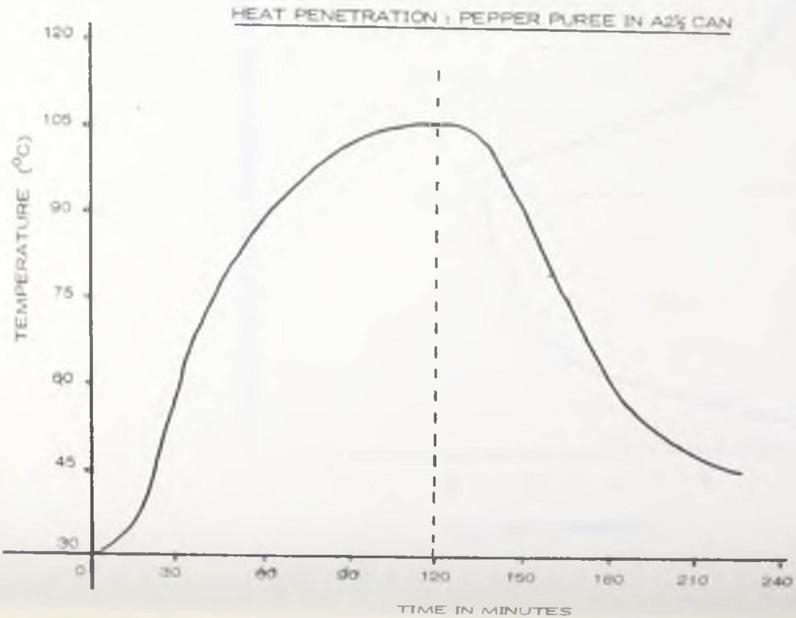

HEAT PENETRATION : PEPPER PUREE IN A2½ CAN
TEMPERATURE (°C)
120
105
90
75
60
45
30
0
30
60
90
120
150
180
210
240
TIME IN MINUTES

Fig 3.12

HEAT PENETRATION : BAMBARA IN 8Z CAN

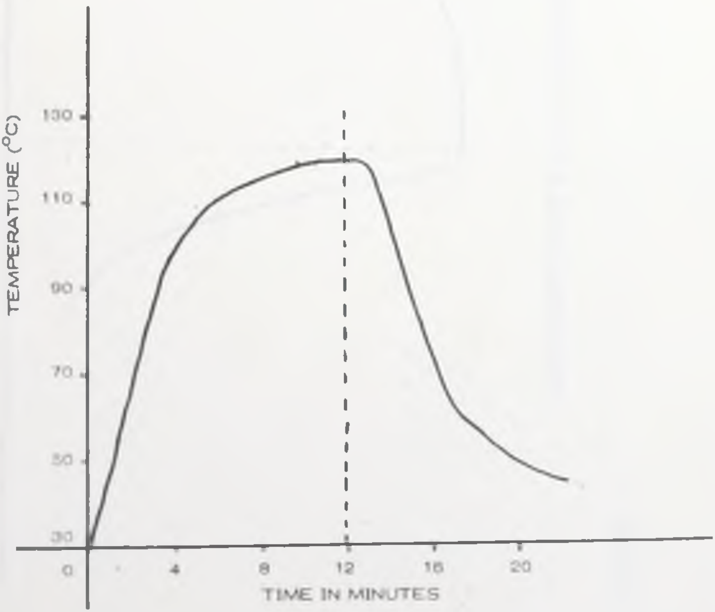


Fig 3.13

HEAT PENETRATION : WHOLE TOMATO IN A2½ CAN

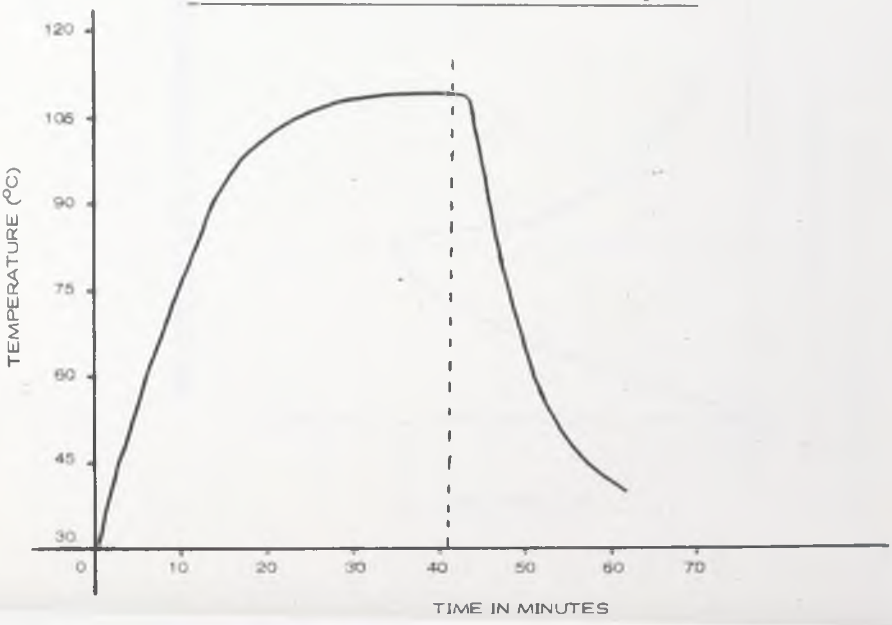
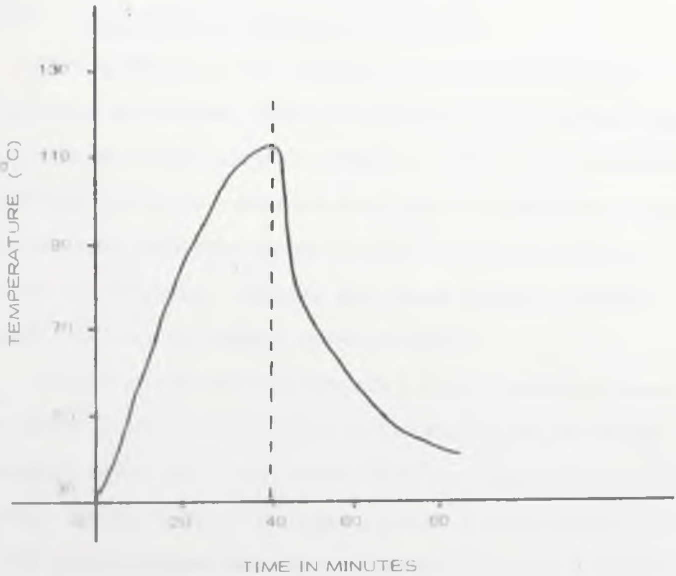


Fig 3.14

HEAT PENETRATION : PEPPER PUREE IN 8Z CAN



3.5.2.6 Whole Tomato - A2½ Cans; (Fig 3.13)

At the retort temperature of 230°C (110°C) the slowest heating point in the can took 34 mins to attain the maximum temperature of 109°C. The graph also shows that the come-up time exceeded the factories' processing time by 4 mins. This suggests that the product is not processed adequately at the factories.

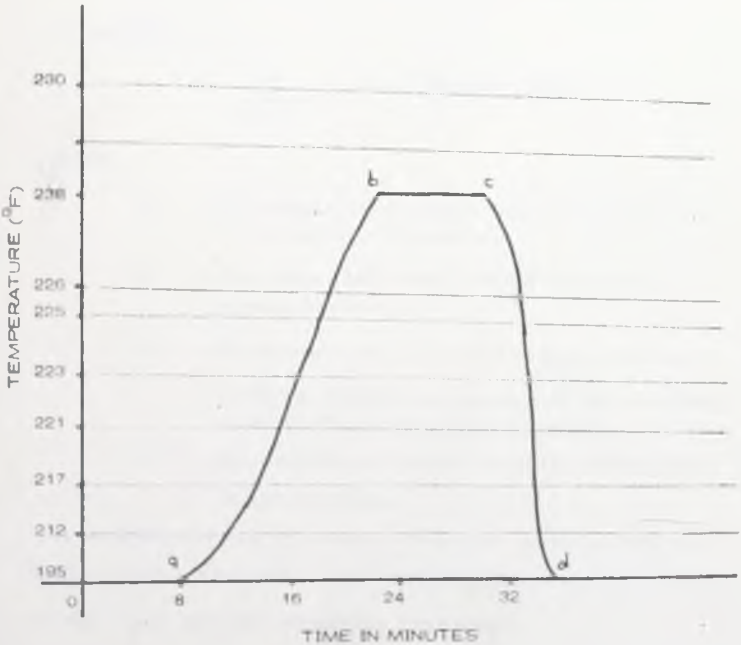
3.5.3 F_o Values of the Heat Processes

The F_o Values of the heating processes, Figs 3.8 to 3.14 were estimated. This was done by constructing lethality curves for the various products. Fig. 3.15 illustrates a lethality curve constructed with data from the heat penetration curve for Okro in an A1 can; Fig. 3.10. The procedure for drawing a Schultz and Olson (193) co ordinate paper has been described by Stumbo (26).

On this curve the come-up time to the maximum temperature of 109°C which is 22 mins (Fig. 3.10) is shown by a-b. From Fig 3.19, canned Okro is processed for 30 mins, therefore for 8 mins the product is maintained at 109°C ; this holding period is shown by bb-c. The portion c-d represents the cooling period.

Fig 3.15

LETHALITY CURVES : OKRO IN A1 CANS



Similar lethality curves were constructed for the other products; these have been shown in Appendices 12 - 14.

3.5.3.2 Calculation of F_o Valves

The areas (A) under the lethality curves were determined by Integration, using Simpson's Approximation rule (139). Table (3.17) shows the summary of areas calculated for all the lethality curves. These areas were substituted into the equation

$$F_o = \frac{m^A}{10^{n\ d}}$$ (Ref. Stumbo /26/)

in which

m = the number of minutes represented by 1 inch on the time scale;

A = the area under the Lethality curve in square inches;

n = the number of "Z unit" changes that were made to change the number of the top line from 250°F to the desired number;

d = the number of inches from the bottom line to the top line.

The results for the F_o values have been summarized in table 3.20). The table also contains the average figures of the pHs for the vegetable products.

Table 3.20 PROCESS EVALUATION

Product Type	Can Type	Average pH	Come-Up Time (mins)	Area Under Lethality Curve Sq. ins.	Processing Time (F/Min)
GARDEN	A1	5.6	32.5	5.78	0.78
EGGS	A2½		42.5	9.09	1.24
PEPPER	8_3	4.8	41	-	=0.0
PUREE	A2½		120.0		0.0
BAMBARA	A2½	-	-		
BEANS	8_3		4.0	15.76	18.9
OKRO IN	A1	5.1			
SAUCE			24.0	7.54	1.03
WHOLE TOMATO	A2½	3.7	34.0	1.71	0.26

From the pH results, the vegetable products can be considered low acid foods; the organism Clostridum botulinum can therefore grow and produce toxin in these products.

Stumbo (26) has calculated that for such low acid foods a minimum heat process of $F_o = 2.52$ is required to eliminate Clostridium botulinum spores.

A comparison of the F_o values obtained from the lethality curves (table 3.20) indicate that most of the vegetable products are inadequately processed. For Garden Eggs in A1 cans the F_o value was only 0.78 while the same product in A2½ and 8Z cans had no F_o value; Okro in A1 cans had an F_o of 1.03.

These results therefore support the results of the microbiological examination, (Sections 3.1, 3.2, 3.3) which showed that these vegetable products are underprocessed. The F_o values obtained for the products are less than the minimum required to prevent the occurrence of botulinum poisoning.

Some of the thermophilic microorganisms isolated from the canned products (Section 3.3) are much more heat resistant than Cl. botulinum. To prevent spoilage from such thermophilic organisms Stumbo (26) has calculated a severer heat process for them (F_o = 19). It can be seen that the F_o values obtained for these products are far less than required to prevent spoilage from thermophiles.

4. CONCLUSION

4.1 CONCLUSIONS FROM THE EXPERIMENTAL WORK

It was shown in the Literature Review that as a result of improper application of processing technology high spoilage rates occurred in the canned vegetable products and industries operated at low efficiency. The causes of spoilage in 5 canned vegetable products (Bambara in Tomato Sauce, Okro in Sauce, Garden Eggs in Brine, Pepper Puree and Whole Tomato in tomato juice) were therefore studied in this work.

Leaker spoilage was shown to be a major source of spoilage in the canned vegetables and it occurred as a result of defects in mainly the Double Seams and Side Seams. The defects in Double Seams were assessed by determining Seam Compactness; (i.e. Free Space and Percent Overlap). However, owing to an initially high negative correlation obtained between Free Space and Percent Overlap, Free Space was used as a sole measure of Seam Compactness, and for the ease and non-destructiveness in its determination.

It was concluded from the analysis of Free Space distributions that the Seaming Operation in use at the factories tended to produce cans with loose double seams (i.e. Large Free Space) which consequently led to leakage.

Free Space was also applied to predict when leakage would occur. In this respect Free Space limits were statistically defined based on the rate of spoilage recorded at the factories; and the probability that leakage would occur in cans with Free Space within these limits were calculated. It was demonstrated that, for example, an A1 (16 ounce) can with Free Space equal to or greater than 11.8 there was 87.7% chance of leaking; while there was 70.1% chance that it would leak of its Free Space was equal to or exceeded 11.1. It was considered that the use of Free Space in this manner would afford means for checking defective double seams and hence prevent leakage in the factories. Since the determination of Free Space was non-destructive, the Free Space of the cans could be monitored and kept within sound limits in order to prevent the occurrence of leaker spoilage in the products.

Defective side seams were also shown to be important as causes of leakage, and an average of 20% of the cans examined had defective side seams. The problems of the lap joints were mainly inadequate solder which resulted in channels and pores in the joints. The channels and pores consequently became operative sites for leakage. Hook defects were however demonstrated to be insignificant as causes of leakage.

A high initial bacterial load in the raw materials before retorting was considered to be one of the causes of under-processing. The results showed that vegetables produced in the tropics may contain a higher spore load compared with those produced in temperate countries. It was also shown that delays that occurred on the processing lines could result in a 10 - 100 fold increase in the initial bacterial population. The conclusions suggest that severer heat processes might be needed in order to prevent under-processing of the vegetable products.

The results of the heat processes evaluation confirmed the inadequacy of the heat processes in use at the factories. All the F_0 values were less than F = 2.52 which is the minimum heat process required to destroy Cl. botulinum spores in canned foods. For the prevention of spoilage from thermophilic spores, a higher heat process $F_o = 19.00$ has been suggested (26), it is apparent therefore that apart from the heat processes being inadequate to prevent spoilage, the vegetable products are a potential health hazard to consumers.

Microbiological examination of the canned vegetables revealed the presence of viable organisms in the products. A preliminary identification indicated that these spoilage organisms were mainly members of the spore forming Bacillaceae family.

Organisms identified included

a) Bacillus polymyxa

b) Bacillus stearothermophilus

c) Bacillus coagulans

d) Bacillus cereus

e) Bacillus macerans

f) Clostridium thermosaccharolyticum

g) Clostridium histolyticum

The presence of these spore-forming organisms in hermetically sealed cans was considered to suggest that the heat processes given to the vegetables were not adequate enough to destroy these spoilage micro-organisms. Gross under-processing was further confirmed by the occurrence of relative low heat resistant spores of Bacillus polymyxa, Bacillus cereus and Clostridium histolyticum.

The Bacillus species B. polymyxa, B. stearothermophilus and B. Coagulans were found to be the most common spoilage organisms in the vegetable products.

These conclusions suggest therefore an urgent need for the introduction of new heat processes to replace existing ones and the institution of measures to prevent the growth and multiplication of micro-organisms in the pre-retorting food materials in order to prevent losses due to spoilage of the canned products and to protect the health of consumers.

4.2 PROCESS IMPROVEMENTS

Following the above conclusions new heat processes have been defined based on results of this work, to help the vegetable processing industries improve their processing conditions.

Using the results of Section 3.4.2, the average maximum number of organisms in the raw vegetables was calculated as 2,000 spores/gram and since most of the spores identified were mesophilic spore-formers (Section 3.3.2) they were assigned an average D value of 2.

This information was then used to calculate the minimum heat process (F_o) required to prevent 10^{-5}(26) incidence of spoilage in the canned vegetable products. F_o in this respect was taken as $F_o = D_{250}$ (log a - log b) where a and b are the initial and final concentrations of bacterial spores in a given volume of food material (26). The minimum F_o value thus calculated equalled $F_o = 21$.

To attain this margin of safety (i.e. One contaminated can in 10^{-5} cans) the vegetable products must be processed at the following :

1. Garden Eggs with brine in A1 can;
   30 - 40 minutes at 250°F
2. Garden Eggs with brine in A2½ can;
   45-48 minutes at 250°F
3. Bambara Beans with Sauce in 8Z can;
   28 - 30 minute at 250°F
4. Whole Tomato with tomato juice in A2½ can;
   32 - 35 minutes at 250°F.

5. Okro with sauce in A1 can

42-44 minutes at 250°F

6. Pepper puree in 8Z can

52-55 minutes at 250°F

7. Pepper puree in 32Z can

85-90 minutes at 250°F.

4.3 SUGGESTION FOR FUTURE INVESTIGATION

Further work on thermophilic spores isolated under tropical conditions may be needed to define more meaningful heat processes to suit tropical thermal processes. Information on the levels of Clostridium botulinum and allied thermophiles could be used in defining minimum heat process (e.g. $F_o = 2.52$, the present minimum heat process for low acid canned foods was established based on the levels of Cl. botulinum in temperate raw materials). The thermal resistance of these spores would also be required for similar purpose.

POSTCRIPT

It had not been possible to conduct a more comprehensive study of the microbial levels in all the raw vegetables investigated in the present work.

It was indicated in Section 1.1.4 of the Literature Review that the main objective of the work was to study the end products of the fruit and vegetable industries in the country and to find out the causes of spoilage in their products. As the work progressed, it became obvious that one of the causes of underprocessing was a high level pre-processing bacterial contamination in the raw vegetables.

It was however not possible to conduct a comprehensive study of the microbial levels in the raw vegetables because of three limitations to the present work.

(1) The three main fruit and vegetable factories whose products were investigated are situated in different areas of the country, far from each other.

(2) The raw vegetables which they use are purchased at depots which have been spread throughout the country. The vegetables are harvested seasonally and are therefore processed only during those short periods in the year.

(3) The practical aspects of the present work were supposed to be completed within one year.

Under these circumstances it was not possible to obtain representative samples of the raw vegetables for analysis, within the period of the present project.

Further work is however continuing at the Ghana Standards Board Laboratories to obtain the required information on the microbial levels in the raw vegetables.

Appendix I

MEDIA FORMULATIONS & COMPOSITION

1.1 Dextrose - Tryptone Yeast Extract Agar

Dextrose	-	5 gm.
Tryptone	-	10 gm.
Yeast Extract,	-	1 gm.
Dipotassium phosphate		1.25 gm.
Distilled water	-	1000 gm.

1.2 Nitrite Test Reagents

Solution A

0.8% sulphanilic acid in 5 N-acetic acid.

1.3 Starch Agar

Potato starch	-	10 gm.
Distilled water	-	50 gm.
Nutrient agar	-	1000 ml.

1.4 Lecithovitellin agar (LV agar)

Egg yolk (4 eggs)

NaCl 0.85% solution 1000 ml.

Beat the yolk in saline solution and filter with kieselguhr through a bacteria-proof filter. Add 100 ml. of this solution to 900 ml. of nutrient agar.

Appendix 2 8Z CANS FREQUENCY DISTRIBUTION OF FREE SPACE

Class Interval (Free Space)	Class Mid Points	Frequency	Cans With Flat Ends	Cans With Swelled End
0.1 - 1.5	0.75	6	6	-
1.6 - 3.0	2.25	5	5	-
3.1 - 4.5	3.75	7	7	-
4.6 - 6.0	5.25	20	20	-
6.1 - 7.5	6.75	23	23	-
7.6 - 9.0	8.25	21	21	-
9.1 - 10.5	9.75	10	10	-
10.6 - 12.0	11.25	5	5	-
12.1 - 13.5	12.75	7	6	1
13.6 - 15.0	14.25	4	3	1
15.1 - 16.5	15.75	2	-	2
16.5 - 18.0	17.25	2	-	2
18.1 - 19.5	18.75	1	-	1
Totals		113	106	7
Percentages		100%	93.8%	6.2%

Appendix 3:

Al CANS, FREQUENCY DISTRIBUTION

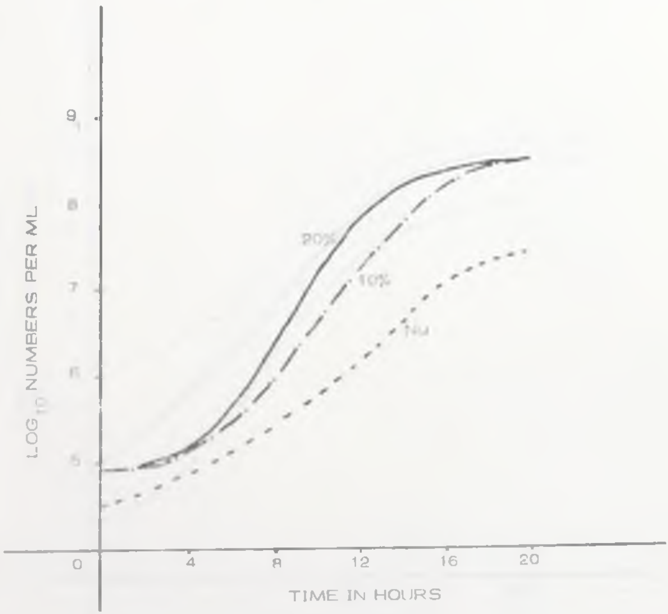
Class Intervals (Free Space)	Class Mid Points (x)	Frequency	Cans With Flat Ends	Cans With Swelled Ends
0 - 1.5	0.75	4	4	-
1.5 - 3.0	2.25	5	5	-
3.1 - 4.5	3.75	19	19	-
4.6= - 6.0	5.25	21	20	1
6.1 - 7.5	6.75	23	23	-
7.6 - 9.0	8.25	17	16	1
9.1 -10.5	9.75	11	9	2
10.6 -12.0	11.25	7	4	3
12.1 -13.5	12.75	3	-	3
13.6 -15.0	14.25	3	-	3
15.1 - 16.5	15.75	1	-	1
Total		114	100	14
Percentage		100%	87.7%	12.3%

Appendix 4: A2½ CANS, FREQUENCY DISTRIBUTION OF FREE SPACE

Class Interval (Free Space)	Class Mid Point (x)	Frequency	Cans With Flat End	Cans With Swelled Ends
0 - 1.5	0.75	0	0	-
1.6 - 3.0	2.25	5	5	-
3.1 - 4.5	3.75	11	11	-
4.6 - 6.0	5.25	16	16	-
6.1 - 7.5	6.75	20	20	-
7.6 - 9.0	8.25	14	13	1
9.1 -10.5	9.75	13	10	3
10.6 -12.0	11.25	10	8	2
12.1 -13.5	12.75	7	6	1
13.6 -15.0	14.25	5	3	2
15.1 -16.5	15.75	2	1	1
16.6 -18.0	17.25	1	-	1
Total		104	93	11
Percentage		100%	89.5%	10.5%

Appendix 5

RATE OF GROWTH BACILLUS POLYMYXA
IN GARDEN EGGS EXTRACT

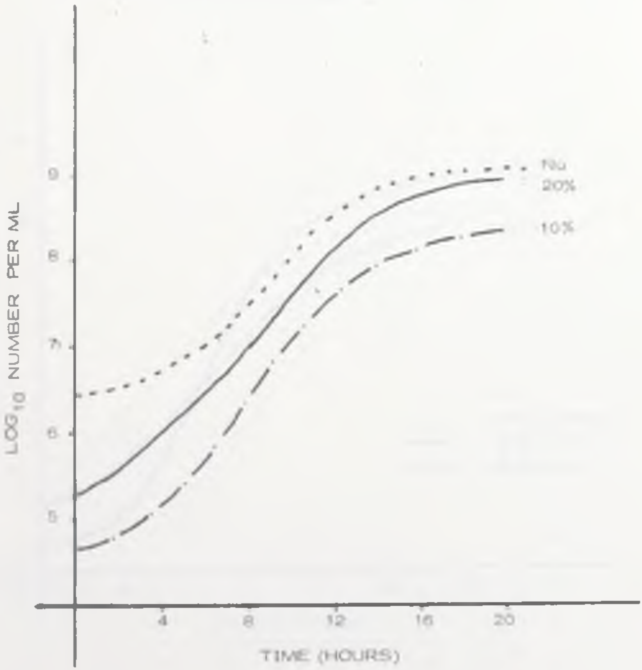


Key to Appendices 5 – 10

. . . .	Nutrient Broth
— · —	" " + 10% Extract
——	" " + 20% "

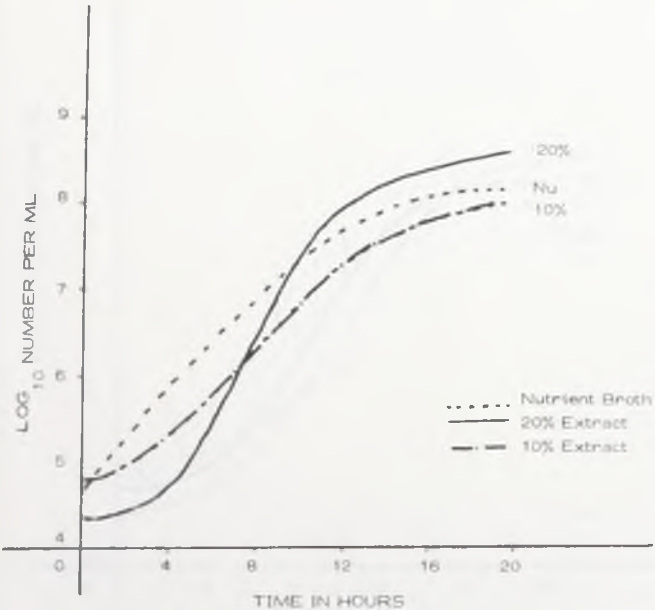
Appendix 6

RATE OF GROWTH BACILLUS POLYMYXA
IN PEPPER EXTRACT



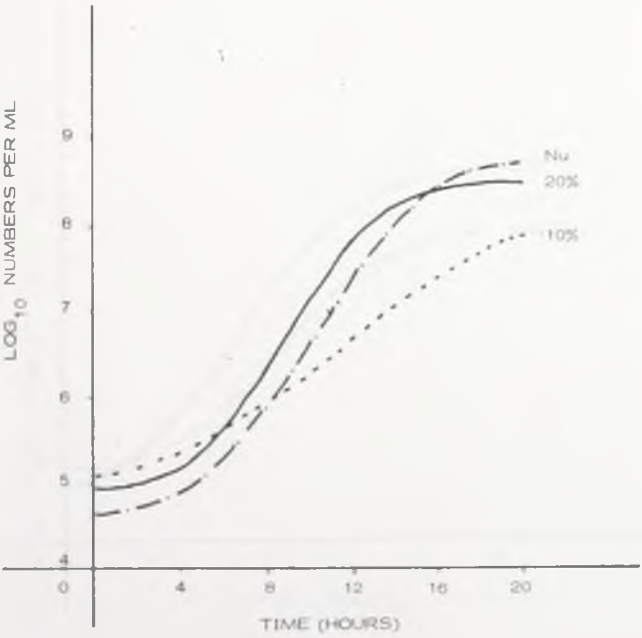
Appendix 7

RATE OF GROWTH BACILLUS POLYMYXA IN BAMBARA MILK



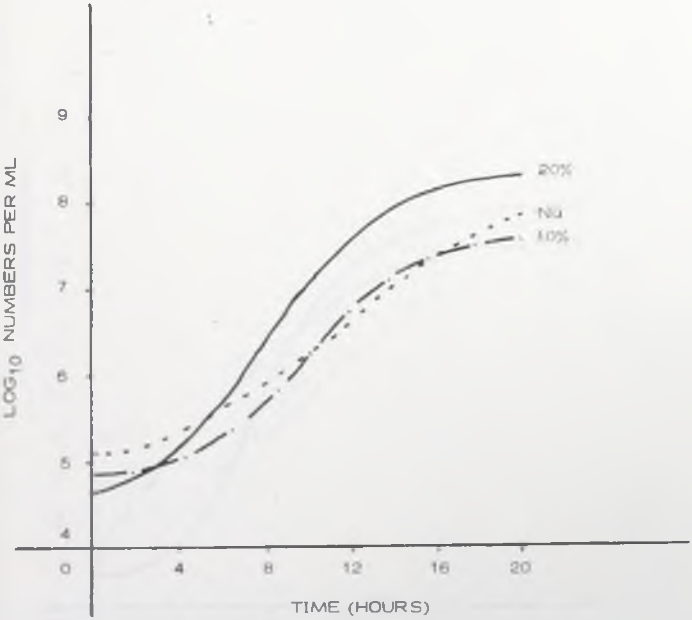
Appendix 8

RATE OF GROWTH CLOSTRIDIUM HISTOLYTICUM
IN BAMBARA MILK



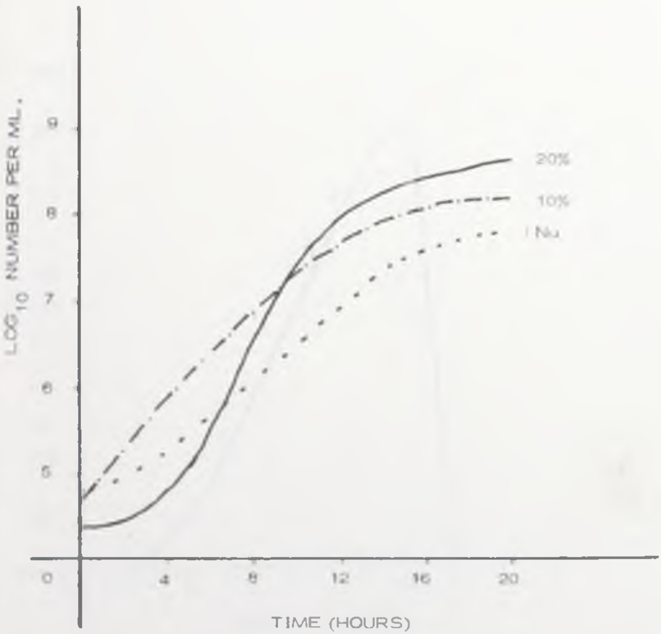
Appendix 9

RATE OF GROWTH CLOSTRIDIUM HISTOLYTICUM IN PEPPER EXTRACT



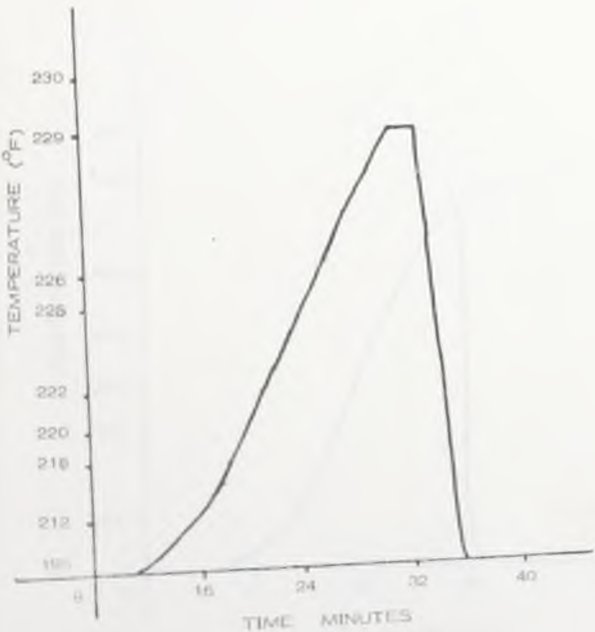
Appendix 10

RATE OF GROWTH CLOSTRIDIUM HISTOLYTICUM IN GARDEN EGGS



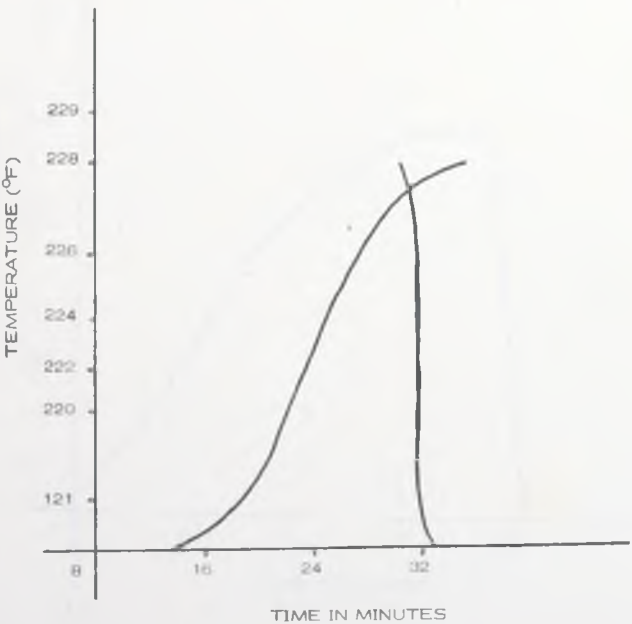
Appendix 12

LETHALITY CURVE : GARDEN EGGS IN A1 CAN



Appendix 13

LETHALITY CURVE : WHOLE TOMATO IN A2½ CAN



Appendix 14

LETHALITY CURVE : GARDEN EGGS IN A2½ CAN

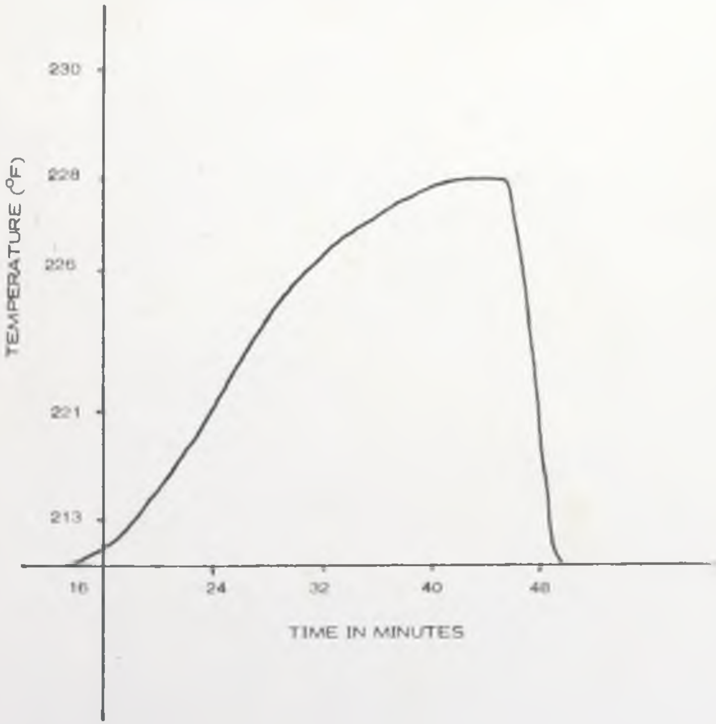


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Fig. 2.8

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED

Culture No. .... Source

Descriptions	Sketches
CELL MORPHOLOGY Medium: Form & Arrangement Motility in broth Flagella: Size: Sporangia: Endospores: Shape: Position	

AGAR STROKE Age: at °C Amount of growth: Form: Consistency: Chromogenesis: Medium: Opacity:	AGAR COLONIES Age: at °C Form: Elevation: Surface: Margin: Chromogenesis: Opacity:
NUTRIENT BROTH Age: at °C Surface growth: Sub surface growth: Amounth of growth: Deposit:	GELATIN STAB. Age: at °C Liquefaction: Rate:
STAINING CHARACTERISTICS Gram: Age: Method: Special stains:	OTHER MEDIA Age: at °C Glucose agar (acid) Glucose broth (acid) Anaerobic Growth in Glucose broth

BIOCHEMICAL TESTS

Catalase Test
Voges-Proskauer
Indole Prod.

Fig 2.8

CHARACTERISTICS OF MICRO-ORGANISMS ISOLATED

Culture No. .... Source

Descriptions	Sketches
CELL MORPHOLOGY Medium: Form & Arrangement Motility in broth Flagella: Size: Sporangia: Endospores: Shape: Position	

AGAR STROKE Age: at °C
Amount of growth:
Form:
Consistency:
Chromogenesis:
Medium:
Opacity:

AGAR COLONIES Age: at °C
Form:
Elevation:
Surface:
Margin:
Chromogenesis:
Opacity:

NUTRIENT BROTH Age: at °C
Surface growth:
Sub surface growth:
Amounth of growth:
Deposit:

STAINING CHARACTERISTICS
Gram: Age: Method:
Special stains:

GELATIN STAB. Age: at °C
Liquefaction:
Rate:

OTHER MEDIA Age: at °C
Glucose agar (acid)
Glucose broth (acid)
Anaerobic Growth
in Glucose broth

BIOCHEMICAL TESTS

Catalase Test
Voges-Proskauer
Indole Prod.
Nitrite from Nitrate
Starch hydrolysis
Casein hydrolysis
Urease test
Gas from carbohydrates
Growth at 65°C
L.V.
Mannitol (acid)
Arabinose (acid)
Citrate Utilization

Do Table 3.3.1 BIOCHEMICAL TESTS

Product Type		Culture N°	Gram	Catalase	Indole	Nitrate Red	Casein Hyd	Urease	Gas from Cho	Growth 65°C	I. V.	Mannitol (Acid)	Arabinose (Acid)	Citrate	Glucose Broth	Anerobic Glucose Broth	Gelatin Hyd.	V. P	Starch Hyd.	Growth 37°C	Growth 45°C	Growth 55°C	Blood Agar	Litmus Milk	Type of Organism
GARDEN EGGS IN BRINE	1	G_2	+	+	-	+	+	+	-	-	+		-	+	d	+	+	+	+	+	d	-			B. Cerevs
	2	G_6^1	+	+	-	+	+	-	d	-	-	+		-	+	+	+	+	d	+	-	-			B. Polymxa
	3	G_{25}	+	+			+		-	+			d			+	-		+	-	-	+			B. Stearothe- rmophilus
	4	G_{17}	+	+	-	d	+	-	-	-	-	+		-	+	+	+	+	+	+	-	-			B. Polymxa
	5	G_{20}	+	+	-			-	+	-	-			-	+	+	-	-		d	+	d			B. Macerans
	6	G_6^2	+	+	-	-	-	-	-		-			-	+		+		+		d	+			B. Coagulans
PEPPER PUREE	1	P_{10}	+	+	-	d	+	-	+	-	-	+		-	d	+	+	d	+	+	-	-			B. Polymxa
	2	P_{15}^1	+	+	-		+	+			+			+	d	+		+	d	+	-	-			B. Cereus
	3	P_{15}^2	+	+		+	+			+			-			+			+	-	-	+			B. Stearothe- rmophilus
	4	P_2	+	+	-	+	+	-	d	-	-	+	+	-	+	+	+	+	+	+	-	-			B. Polymxa
									+									-							
OKRO IN TOMATO SAUCE	1	O_{19}^1	+	+	-	+		-	+	-	-			-	+	+	-	-	+	+	+	d			B. Macerans
	2	O_{19}	+	+		d	-		-		-			-		+	d		d	d	+	+			B. Coagulans
	3	O_{20}	+	+	-		+		-	-	+				+	+	+	+		+	+	-			B. Cereus
	4	O_{17}	+	-	-	-			+	d		+	+			+	-			-	-	+	-	+	Cl. Thermos- accarolyti- cum
BAMBARA IN SAUCE	1	B_5^1	+	+			+			+		-				+	-	+	+		d	+			B. Stearothe- mophilus
	2	B_{12}^2	+	+		-	-		-		-			-			-		+	d	+	+			B. Coagulans
	3	B_5^2	+	+	-	+	+	-	+	-	-	+		-	+	+	+	+	+	+	-	-			B. Polymxa
	4	B_{12}^1	+	-	-	-		+		-		-	-		-	-	+		+				+	d	Cl. Histoly- ticum