BACTERIOLOGICAL QUALITY ASSESSMENT OF MOIN-MOIN

BY

SULE ENYISI ISAH

DECLARATION

I hereby solemnly declare that the work reported in this thesis was carried out by me in the Department of Microbiology Faculty of Science, Ahmadu Bello University Zaria, under the Supervision of Drs. L.J. Ogbadu (Mrs) and V.J . Umoh (Mrs).

The work of other Investigators in this area is duly acknowledged and referred to appropriately. No part of this thesis has previously been submitted for a degree or diploma.

	5/04/95
SULE ENYISI. I	DATE.

CERTIFICATION

This theisis titled: Bacteriological Quality assessement of Moin - Moin by SULE ENYISI. I. meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University,Zaria and is approved for its Scientific knowledge and Literary Presentation.

Dr. (Mrs) I.J. Ogbadu — 5/4/95
Chairman: Supervisory Committee. — Date.

Dr. (Mrs) V.J. Umoh — 19-04-95
Member: Supervisory Committee. — Date

Prof. N. Uriah — 09-02-95
External Examiner. — Date

Dr. (Mrs) L.E. Odama — 20/4/95
Head of Department. — Date

16/2/99
Dean of Post Graduate School. — Date

DEDICATION

Through the Grace of God.

To My Father Mr. SULE ALIKALI .B. And My Mum For Her Love And Moral Support.

To My Wife: Hafusat, Our Children: Hamza, Hameed And Nafisatu, All for their Love.

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SULE ENYISI .I.

ABSTRACT

A total of 30 moin-moin samples wore collected from three food sales outlets namely, hawkers, cafeteria and restaurant were analysed for total aerobic plate count, *staphylococcus aureus* and *B a c i 11us cereus.* Moin-moin samples obtained from the restuarant showed slightly lower values for total aerobic plate count (1.3 x 10 - 1.6 x 10^6) as compared to counts obtained from cafetaria and hawkers (2.7 x 10^4 - 2.6 x 10^6 and 4.6 x 10^4 - 3.2 x 10^6) respectively. *Staphylococcus a**ureas*** count from moin-moin samples obtained from hawkers varied between 1.2 x 10^3 - 2.0 x 10^5 while that of cafetaria and restaurant showed count varying between (1 .3 x 10^3 - 1.0 x10^5 and 2.2 x 10^3 - 1.1 x 10^5). *Baci1lus cereus* count from moin-moin samples obtained from hawkers varied between (1.0 x 10^3 - 1.3 x 10^4) and that of cafeteria and restaurant varied between (1.0 x 10^{22}- 2.1 x 10^4 and 1.0 x 10^3 - 1.0 x10^4 respectively. The enterotoxin producing ability of the 13 randomly selected isolates of *s. aureus* using cellophane over agar method. Seven (53%) of the isolated produced enterotoxin A while none produced enteroxin C and D. Fifteen isolates *Bacillus cereus* were tested.for toxin production using rabbit ileal loop test. Ten 66% of the strain wore mild enterotoxin producers and 5 (33%) were moderate enterptoxin producer. Fifteen other 'moin-moin samples's observed for shelf stability showed that moin-moin could remain wholesome **for** 18hrs, 30hrs, and 42hrs at a storage temperature of 25^{oc} , (ambient temperature) 30^{oc} and 20^{oc}.

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CHAPTER 1

INTRODUCTION:

Moin - Moin is a steamed savoury cowpea meal popular in Nigeria and other West African Countries . The importance of moin - moin in African diet is due to its high protein contents and therefore furnishes part of the protein need of the people.

Apart from Moin - Moin appearing regularly in home diets, it features commonly in Commercial Catering as well as ceremonies and special occasions as the major accompaniment to jollof rice, white rice and stew. It is also commonly served or sold as fast food and street food. In what ever category of meal it is placed, the importance of the Moin - Moin as a meal cannot be over emphasised given its popularity among the few indigenous foods that are now being projected at public eating places.

Microbial contamination of street foods is an indication of poor sanitary practice in the preparation and storage of the food. Bacteria may be introduced into food along the line from the raw materials,environments and poor handling of the food to the finished products. Such contaminated microorganisms can proliferate in the food resuting in a potential health hazard. The adherence by food handlers to good personal hygiene and to hygienic food handling practices is essential, if microbiological contamination is to be prevented. (Dawson et al;1991).

Common mesophilic microorganisms usually regarded as harmeless have been known to cause illness when present in excessive number. from the various studies so far conducted, it is obvious that there is high potential for serious health problem related to the preparation and handling of street foods. Studies have confirmed both microbial and chemical contaminations of food being sold by vendors (FAO/FTDC, 1984).
It is therefore imperative to ensure that there are standards for our indigenous foods which show dearth of scientific information on the guildines for microbiological analysis.

In 1981, 43% of the outbreak of food borne diseases reported to the centre for disease control in America were caused by food eaten in restaurants (CDC, 183). It was noted that the sites most frequently involved in the outbreak are the food service establishments and homes (Angelotti, 1971). Food quality is the degree of excellence which a food possesses. Maintenance of high food quality is therefore an essential ingredient for successful promotion of foods, consequently quality assurance in the food industry or in the place of manufacture is the key to increasing the demands for indigenous food. Most elites shy away from indigenous foods at public and commercial outlets becuase they cannot be sure of their quality.
There is a serious dearth of information on guidelines for traditionally produced foods in the country. lack of guidelines for microbiological analysis is the major set back encoutered with setting standard for indigenous foods.
Since the food and drug decree of 1975 and subsequent regulations went into effect there has been no apparent guidelines for indigenous foods. However, the importance of having standard for foods in developing countries is viewed more seriously now than ever before (FAO, 1987).

Refrence has been made to pathogens isolated from street foods analysed in different countries; observation shows that waters collected from water container of vendors in pune (India) was contaminated with Salmonella Newport while samples of locally produced ice cream and chicken all of which are vended in a similar manner to moin-moin in Ibadan (Nigeria) also revealed Samonella species as contakinants (FAO, 1987).

In view of the afore mentioned observations enumerated, the present work is aimed at;

(1). Assessing the bacteriological quality of moin-moin served at selected food selling establishments.

(2). Isolation and identifying organisms of food borne hazard associated with the moin-moin samples.

(3). Assaying the pathogens isolated from moin-moin for enterotoxigenicity.

(4). Monitoring the keeping quality of moin-moin.

CHAPTER (TWO) 2

LITERATURE REVIEW

MOIN-MOIN PRODUCTION

2.2.1 **Moin-Moin** is one of the numerous Nigerian Cowpea meals which is very popular in West Africa Countries. The preparation of Moin-Moin is similar among the different group of peoople that eat it. The little differences occur not in the method, but in the ingredients that are incorporated and in the packaging method before steaming (Anthonio and Isoun, 1982).

Moin-Moin is made mainly from cowpea (Vigna unguiculata). The required amount of the cowpea is soaked in water for about one hour and the seed coat is then removed either by pounding lightly in a mortar or by rubbing between palms. The seed coat is then washed away and the cotyledons are ground with pepper and onions to form an aerated paste. The paste is mixed to taste with salt, palm oil or groundnut oil. Optional ingredients which enhance and enrich the meal include deboned fish, diced liver, diced boiled egg and condiments (Anthonio and Isoun, 1982).

The seasoned mixture is then packaged by wrapping in either clean leaves, Aluminium Kitchen foil, Polythene bags or poured in small tin cans. The moulds are then placed in a pot which has been lined with sticks to prevent wrapper from sticking to the pot and also to maintain the water for adequate steaming.

The pot is covered and allowed to steam for 45 to 60 minutes depending on the quantity being made and amount of heat applied. Moin-Moin is proteinaceous and is commonly eaten with carbohydrate based foods such as 'Ogi' and 'Kafa' (Oyenuga, 1968).

2.2.2 NUTRITIVE VALUE OF COWPEA

Cowpea (Vigna : unguiculata) from which Moin-Moin is made is said to be a prominant source of plant protein in the diet of Nigerians (Oyenuga, 1968). Cowpea is known to be rich in protein and has 22.7g of protein per 100g of edible portion (Aykrod and Daughty, 1982). Moin-Moin also will retain most, if not all of protein of Cowpea since the cooking does not seem to reduce the amount of protein in the true diet.

The type of nutrient composition of any food will determine the type of microorganism that will grow on the food (Lynch et. al, 1979).

Table 1 shows the nutritional compostition of Cowpea seed while table (2) shows the mineral and vitamin composition of cowpeas.

TABLE 1 NUTRITIONAL COMPOSITION OF COWPEA SEEDS

Composition	CH_2O	Protein	Moisture (%)	Fat	Crude Fibre(g)	Ash
Cowpea	61.0	22.7	11.5	1.6	4.2	3.2

TABLE 2. THE MINERAL AND VITAMIN COMPOSITION OF COWPEA

Minerals	Iron (mg)	Thiamin	Vit.A.	Riboflavin	Nicotic[illegible] Acid	Cal.	Phosphorous (ML)
Cowpea	0.89	50	0.14	0.12	2.3	4.51	90

sources: Oyenuga, 1968, Aykroyd and Daughty, 1982.

2.2.3 QUALITY ASESSMENT OF FOOD.

The Microbiological quality of any food item is determined by a series of microbiological examination which reveal whether the food or food ingredients meet the required standard or microbiological limit. To determine the microbilogical quality, sample is drawn in such a way that at least a fairly large number of food from different food establishments are examined for a series of organisms. The microbiologist is faced with two major problems when analysing food. First is the problem of obtaining a truly representative sample of a batch and secondly only a few hundred grams of the product are taken as a sample of production batch of several tonnes. (Gibbs, 1986).

Presently, the methods employed depend on the nature of the food item. These include the examination of food for aerobic plate count, coliform and feacal coliform estimation, Staphylococcus aureus count, Bacillus cereus count, and intestinal pathogens like salmonella (FAO), 1979). Homogenate of the food item to be examined must be prepared and diluted accordingly. Preliminary treatment of food is required to release into a fluid medium those organisms which may be embedded within the food. Excessive speed of the cutting blades and undully prolonged use of the mixer may cause injury to microbial cell while insuffficent mixing on the other hand may neither release embedded bacteria or provide a heterogenous distribution with suspension.

A 'Stomacher' may be used for proper mixing of the food. The enumeration of mesophilic aerobes gives an index of the quality of food except where fermentation or a natural ripening process gives rise to a very large number of bacteria. It has a limited validity for assessing safety, but is usually used to indicate the adequacy of sanitation and temperature control during processing, transport and storage. When a total of five samples are observed marginal acceptability is 10^4 cfu/gm or ml while maximum acceptability's 10^6 cfu/gm or ml (FAO, 1979).

2.2.4. STREET FOODS (BENEFIT, QUALITY AND PROBLEMS)

Street foods are ready to eat food and beverages prepared and or sold by vendors especially in the streets and other similar places (FAO, 1989); FAO/FTDC, 1986).

Street vended foods have a long tradition in most Countries of the World, but because of socio-economic changes many countries have also experienced the rapid growth of this sector. While street foods are often attractive for their own unique taste and convenience, street vended foods are essential to some communities, especially in developing countries, as they provide ready to eat meals which are accessible and affordable for even the poorest (FAO, 1989).

Street foods include a large variety of items with different degree of potential for causing diseases.

Research has shown that parched grains, dry bakery product, sufficently sugard, salted or aciduated foods and many fermented items have a much lower diseases producing potential than certain foods which readily support bacteria growth . Similarly, foods throughly fried or cooked and consumed on the spot are inherently safer than pre-cooked foods such as rice, moin-moin especially when they are held at ambient temperature for more than four to five hours. Consequently consideration of the varying potential of different food items to cause diseases can be used in classifying local street foods for application of appropriate control measure.

FAO (1988a) expert consultation on street food examined the problem of food hygine and sanitation and reported that there exists a high potential for serious health problem related to preparation and handling of street foods. Studies of data from Indonesia, Nigeria, Pune, Bombay and Colombia confirmed both Microbial and Chemical contamination of foods being sold by vendors (FAO, 1988a) .

One of the major contributors of bacterial contamination is the cooking foods which are supposed to be served hot, but are made up inadvance and stored for long period of time before being served. When served they are often not reheated to a sufficiently high temperature to kill the harmful bacteria. This can lead to a very high plate count, as well as elevate the coliform.

and faecal coliform counts, if exposed to these bacteria, Such foods represent a potential public health hazard to the consumers.

Studies have shown also a number of unpermitted food additives in street foods.

The Pune city report demonstrated the use of Non-permitted coaltar colours and the artificial Non-nutritive sweeteners saccarine being used in place of sugar in soft drinks manufactured by local vendors. Also Non-permitted green colour had been added to soaked full grain Jawar to make it appear to consist of young tender Jawar grains which are naturally green in colour. In Indonesia, study also confirmed the presence of heavy metal (copper, lead and iron) contamination in samples of hot dogs, Bakoni/Bihun Bakso, a wheat/rice noddle soup with meat balls, steamed rice with mild and spices. The lead contamination of these foods being displayed in an open air table exposed road dusts which may contain lead from auto and bus exhaust fumes (FAO, 1989). These has been now epidemiological studies to suggest that street foods contribute to a significant number of food poisoning.

FAO/SPHL,(1986) in 1981 found that Cholera epidemic in Pune city, India was caused by consumption of contaminated sugar cane juice with added ice. In this case the ice was found to be contaminated with Vibro cholerae.

Handling practices of street foods vary greatly and are identified as a major contribution to contamination. These include the lack of sufficent heat source for food preparation or for re-heating of food using proper time temperature condition in the processing of street foods. Delays between food preparation and consumption were also reported. The lack of refrigeration leads to improper storage and contributed to food contamination problem. The use of raw materials from uncertain sources which can be contaminated and unsafe for consumption, was also idenfitied as a contamination factor.

Among the most critical problem shown by the studies in Latin America, Asia and Africa in relation to Microbioloical contamination was lack of adequate supply of potable water for cooling, cleaning of mixing and eating utensils.
Water is essential in food preparation, for washing before and after cooling and in most food drinks. It was found that because of the difficulties in obtaining clean potable water, many vendors simply re-use their water especially for cleaning utensils, equipment and dishes. Disposal of waste water and refuse including garbage was shown to be an alsmost universal problem.
In India, Bombay, it was reported (FAO and FIDC, 1986a) that there was no system of garbage disposal. if there is no facility for liquid drainage, waste water and garbage remain in the street encouraging rodents and breeding of mosquitoes and flies. The linkage between house flies and diarrhoeal diseases has been well documented (Smith, 1986).

Important, as these foods are in the food supply, however, street vended foods also are recognised as a possible health hazard. In their report, the Joint FAO/WHO Expert Committee on food safety which was convened at WHO Head Quarters in Geneva in 1983, identified both the important and potential hazard of street vended foods because of the chemical and microbiological contamination which conceivably could occur under street condition. The committee concluded that efforts must be made to :-

(a). To educate the personel involved.

(b). To improve the environmental conditions in which the trade is practised.

(c). To provide the essential service to assist food vendors in assuring the safety of their wares.

2.2.5. MICROORGANISMS OF HEALTH HAZARD IN FOODS.

There are different kinds of organisms that are of health hazards in foods, among the most common are Staphylococcus , cereus , Bacillus cereus , Salmonella spp, shigella SPP The involvement of these organisms in food borne diseases, is well documented (Frazier, 1988).

2.2.6. STAPHYLOCOCCUS AUREUS AS HEALTH HAZARD.

Staphylococcal food poisoning is a food intoxication in which Staphylococcus aureus grow in the food and produces toxin in it. It is associated with mishandled foods. Their presence in large number in food can be hazadous to consumers (Uriah and Nkanga, 1980). Stanphylococci are enumerated in food for three reasons.:-

(1) To examine food suspected to have caused food poisoning for which presence of large number of coagulase positive staphylococci would be suggestive.

(2). To demonstrate the risk that the test food may be capable of inducing food poisoining when stored under conditions conducive to toxin formation or if used as an ingredient in some other food.

(3). To establish the occurence of objectionable food processing contamination (Odumosu, 1983).

The symptom of staphylococcal food poisoning are nausea, vomiting, abdominal pain, Prostration and diarrhoae. These symptoms involve a period of only few hours.

2.2.7. Foods Associated with Staphylococcus aureus food poisoning

A number of food items sold locally have been shown to be highly contaminated with staphylococci, possibly due to poor sanitary practice, (Nkanga and Uriah, 1981).

There is a need for an extensive study on the growth of enterotoxigenic strain of Staphylococcus aureus in local food to avoid the risk of intoxication following their consumption. The food usually involved in otbreak or which the potential exists include raw milk and its products. (Adesiyun and Kwaga, 1983 and Robinson, 1981). Meat and its products (Adesiyun, 1984a, 1984b). Poultry and egg products (Fraizer 1988). others are prepared salds, baked goods sandswiche filling and prepared dishes(Board, 1983, fraizer 1988).

Dry cured hams are frequently implicated in outbreak of staphylococcal poisoning (Bryan 1988, Hechelman et al, 1988 and Halpin et al 1989). Staphylococcus aureus has been the second most frequently reported micro organism larging outbreak of food poisoning (Anonymous 1990).

Meat product have been the second most frequently reported food involved in such outbreak. (Anonymous 1990). Human beings are the most important source of Staphylococci. Food handlers may contaminate raw materials equipment and finished products via cut in the hand and throat infection. (Katsman et al., 1985)

A study was conducted from 1982 - 1986 to assess the microbiological quality of 2 classes of fermented sausages manufactured under good hygienic condition. Dry and semi dry sausages have had a good, though not perfect safety record as they have been infrequently implicated in food borne diseases. Staphylococcus aureus was associated with gasteroenterities caused by consumption of Genova salami (Anon, 1971) and Italian dry Salami (Anon, 1975). Pepperoni has also been implicated in Staphylococcal food poisoning (Anon, 1978, CDC, 1972).

In 1984 - 1985, another study conducted in the province of ontario canada assessed the bacteriological quality of three types of non dairy substitute including creamers, filling and toopings. The dry non dairy toppings are frequently reconstitued with milk and egg before use to increase the nutritional value of flavour. These reconstitued powders as well as thawed frozen products are reputed to support microbial growth to the same extent as their dairy conterparts (Siliker, 1969).

In Nigeria, a study was conducted on six different types of ready to eat foods in Ekpoma market, namely, cooked rice, vegetable Stew, Moin-Moin, Akara, Agidi, and Agidi Jolof. All the foods were found to be highly contaminated with - Staphylococcus aureus although there has been no documented Staphylococcal poisoning outbreaks related to these foods, but outbreak is imminent if care is not taken (Owhe - Ureghe et al, 1993).

2.2.8. Pathogenicity of Staphylococcus aureus contaminating in foods

In 1984, in the United Kingdom, an outbreak of Staphylococcal food poisoning was traced to an imported dried paste product containing egg (Lasagna) in which a high S. aureus ($10^4 - 10^8$ cfu/gm and presence of enterotoxin A were demonstrated (Gibbs, 1986)

In Canada between 1973 and 1978 there have been at least 238 incidents of food borne diseases with sausage comprising of of a total of at least 73 cases; out of these most were caused by fermented sausages and involved Staphylococcus aureus, Salmonella spp, enteropathogenic E. coli HPB , 1976 , 1978.

In another study in 1982, six incidents occured, involving 226 people who ate processed turkey. One major incident involved 55 people who ate Turkey contaminated with Staphylococcus aureus (HPB, 1986). Although , there is the potential for problems with these products,over all they have enjoyed a fairly good safety record, good hygenic practice during slaughtering, deboning and processing can keep bacterial cross contamination to a minimum (Todd, 1980). However, the processing of poultry especially defeathering and evisceration gives the opportunity for these organism to spread from birds to birds once contaminated, hands and gloves of the processing plant workers can also contribute to the disemination of micro organisms (Tomkin, 1983).

2.2.9. **Enterotoxigenicity of Staphylococcus aureus.**

Staphylococcus aureus contains and produces varous toxic substances that contribute to the pathology of the infection. Out of these, the most studied are the enterotoxins which are protein are more responsible for the pattern of acute food poisoning. The production of Staphylococaal entrotoxin is influenced by the environment.

Enterotoxins may be generated in the PH range of 5.0 - 9.0 (Daly et al. 1973). This therefore makes food the most suitable media for the growth of enterotoxigenic staphylococci and production of enterotoxin.

The enterotoxins have been shown to be mainly Neurotoxic, reducing body temperature and blood pressure and stimulating the vomiting reflex (Dubos and Hirsch, 1975). There is salivation and sudden Nausea followed by cramp, Diarrhoea and severe prostration.

The cattle are another source of enterotoxin producing staphylococci organisms (Todd 1978). Bovines milk and milk products have often been implicated as vehicle for Staphylococcal food poisoining (Todd, 1978). The Enterotoxigenic Staphylococci have also been isolated from Horses, Sheep, Swine, Rats and Dogs, emphasizing their potential as reserviour for human intoxication (Hajak and Marsalek , 1973).

In 1984, Enterotoxigenic Staphylococci was also isolated from milk of nursing mothers in Kaduna in Nigeria (Adekeye et al, 1984) and it was found that Eterotoxin A predominated. Data on the enterotoxigencity of Staphylococcus aureus from human being and foods generally (Mossel et al., 1990) indicates that more than 50% of strains were Enterotoxigenic. The Enterotoxigencity of Staphylococcus aureus is ussually correlated with coagulase production (Adesiyun, 1984b).

2.3.0. **ENTEROTOXIN ASSAY:** There are many ways of detecting Staphylococcal enterotoxin in food. these include Microslide technique, Optimum sensitivity plate technique, soft agar method among others.

Recent advances in Immunological technique have resulted in fast and sensitive methods for detecting Staphylococcal enterotoxin, such as reversed passive latex agglutination (RPLA) method (Wieneke, 1988).

2.3.1. The Genus Bacillus cereus

Bacillus cereus is another micro organism which is widely distributed in the air, water and dust . Contamination of foods by this organism occurs mainly during processing and handling. It has been isolated from foods such as rice, spicies, flour, meat and diary products and dried infants milk (Orakwue and Uriah, 1981).

2.3.2 Bacillus cereus food poisoning

Over the last 30 years, Bacillus cereus has been recognised as the agent of two disticnt type of food poisoning. The emetic and diarrheal. The rapid onset of symptoms and afebrile nature and short duration of the illness tends to suggest that Bacillus cereus food poisoning is toxin mediated (Spira and Goepfert, 1972; Gilbert, 1979).

Hange (1955) reported the first Bacillus cereus food poisoning in Norway. The implicated food was vanilla sauce prepared and stored at room temperature for one day before being served. Samples contained $25 - 110 \times 10^6$ cell/ML. Corn starch contained up to 10^4 Bacillus cell in an outbreak with incubation period of 12 hours .
Outbreak of Bacillus cereus food poisoning have been reported from several European countries including Denmark, Netherlands, Italy, Swenden, Hungrary, Romania, Germany and USSR. In Hungrary Bacillus cereus formed the third most common bacterial food poisoning during the period of 1960 - 1968. It was responsible for 8% of reported out break and 15% of cases (Ormay and Novotny,1970). In a review by Gilbert and Taylor, 1976, several unconfirmed out breaks of Bacillus cereus food poisoning occured in United Kingdom before 1971 . The first documented outbreak occured in Great Britain in 1971 (Anon, 1972b). In Nigeria, Bacillus cereus has been isolated from dried food condiments (Obuekwe and Ogbimi, 1989). There is lack of information on food poisoning outbreaks linked to agents in the country, making it difficult to know how much flour based foods contribute to this type of food poisoning.

2.3.3. FOOD ASSOCIATED WITH B. CEREUS POISONING.

In Netherland (Mossel et al. 1967) reported that mashed potatoes and vegetables, minced meat, Liver Sausages, rich dishes and soup were the major vehicle of Bacillus cereus with 10^7 Cell/gm and 10^5 Cell/gm required for Adult and Children respecitively. In United Kingdom, boiled and fried rice usually from Chinese restaurants and take away shops have been reported as commonly incriminated in Bacillus cereus food poisoning (Gilbert et. al 1981

The practice gives rise to selection and proliferation of heat resistant strain Bacillus cereus originally present in the raw rice, which later grow in cooked rice stored at room temperature. Other foods increminated include paste, pasteurized cream and infant food (Gilbert and Kramer, 1986), Mashed potatoes, and vegetables, minced meat, liver, sausages, pudding and soup (Mossel et al, 1967).

2.3.4. Pathogencity of Bacillus cereus in food.

The Pathogencity is usually an intoxication (Bonventre and Johnson, 1967). The extracellular metalolites produces an exotoxin, enterotoxin, phospholipases and beta-lactamase. The part played by any of this in pathogencity of the organism is unknown. (Logan, 1981). Nigeria 1962, suggested that the symptom of B. cereus were due to liberation of phospherylcholine from lecithine by the enzyme phosphclipase. Mortimer and Mccanni (1974) reported 6 incidences of different form of gastero-intestinal illness due to B. cereus after the consumption of contaminated fried rice in 1979. In another review 34 incidences of food poisoning attributed to B. cereus were reported by the centre for disease control between 1971 and 1973, 32 of the thirty four incidences were associated with Chinese restaurant(PHLS,1972).
Homes et al. (1981) reported another out break involving eight persons in 1980 after eating macronni and cheese meal developed symptoms nausa, vomiting, abdominal cramp and morderate diarrhoea.

2.3.5. Bacillus cereus Enterotoxins

Geopfert (1972) has shown that B. cereus produce an Enterotoxin substance which causes fluid to accumulate in ligated ileal Segments in Rabbit and increased motility and overt diarrhoea upon adminstration to rhesus Monkeys. Isolation depends on the use of Media containing Egg Yolk (Kim and Geopfert, 1971) as their detection depends on the Phospholipase activity in culture of Bacillus cereus. Polymixin B. Suphate has been used to keep other contaminant out (Donovan, 1958, Kim and Geopfert 1971, Gilbert and Taylor, 1976)

CHAPTER THREE (3)

3.1.1 MATERIALS AND METHODS

SAMPLING: A total of 36 samples of moin-moin were purchased from three different food serving outlets (establishment) distributed as follows: Hawkers (12), Cafetaria (12) and Restaurant (12). During sampling, the sellers were interviewd for information such as the storage time of the Moin-Moin and an on the spot measurement of the temperature of the sample was taken. Samples colected were wrapped aseptically in a clean Polythene bag and carried to the Laboratory immediately for anlysis.

Freshly prepared Mon-Moin under special arrangement numbering a total of 15 bathes were analysed for their storage stability.

FLOW DIAGRAM FOR PRODUCING MOIN-MOIN.

DRY. GRAIN – COWPEA

↓

STEEPING IN WATER

↓

HAND RUBBING TO SEPARATE TESTA FROM COTYLEDON.

↓

REMOVAL OF SEED COAT (TESTA) BY FLOATATION AND DECANTING.

↓

ADDITION OF PEPPER, OINIONS. ⟶ DEHULLED OR DECOATED SEEDS.

↓

WET MILLING

↓

PASTE (THICK)

↓

ADDITION OF WARM WATER AND OIL

↓

WHIPPING

↓

ADDITION OF OTHER INGREDIENTS

↓

PACKING IN CANS OR LEAVES

↓

STEAMING

↓

MOIN- MOIN

SECTION A. DETERMINATION OF MICROBIAL COUNTS OF MOIN-MOIN.

3.1.2 PREPARATION OF FOOD HOMOGENATE.

Twenty five grams of each of the samples was weighed and transfered aseptically into a conical flask containing 225ml of sterile peptone water. Samples were homogenised separately in sterile polythene bags for one minute in a stomacher 80 Model NO. (B.A. 6020). The food homogenate was throughly shaken after which ten fold serial dilutions were prepared from the homogenate.

3.1.3. INITIAL PRELIMINARY ISOLATIONS AND IDENTIFICATIONS

The preliminary isolation and identification of organisms from moin-moin samples obtained from three food establishment, Cafetaria, Restaurant and Hawkers were carried out.

The isolates obtained were subjected to basic procedures for identification of bacteria such as Gram staining technique and biochemical characterisation. Subsequent isolations and enumeration were later narrowed down to Staphylococcus aureus and B. cereus for more detailed study as these two organisms were detected in all the samples and in much higher number.

3.1.4. ENUMERATION OF MESOPHILIC AEROBIC BACTERIA (TOTAL AEROBIC PLATE COUNT).

One millitre each of appropriate dilution(1:100, 1:1000 1, 10,000) were pipetted into seperate appropriately marked duplicate molten plate count agar (Oxoid) was poured into each petri-dish and thoroughly mixed with sample and allowed to solidify. The set plates were incubated at 35^{oC} for 48 hours, after which plates containing between 30 and 300 colonies were counted.

3.1.5. ENUMERATION AND IDENTIFICATION OF Staphylococcus aureus.

On to sterile plates of mannitol salt agar were transfered 0.1ml of the dilutions (1:10, 1:100, 1:1,000) on to the surface of the dried agar plates. The inoculum was then spread with an alcohol flamed bent glass rods.

The plates were incubated at 37^{oC} for 24-48 hours, yellow colonies appearing after 24 hours with evidence of mannitol fermentation were counted. Representiative colonies of Staphylococcus aureus from mannitol salt were Gram stained and Gram positive cocci in clustered forms were purified by streaking on Nutrient agar plate and incubated for another 24 hours at 37^{oC}. The isolates were preservedon nutrient agar slants for further identification by Biochemical test which included Catalase test, Carbonhydrate fermentation test, Coagulase test, Dnase production, Gelatinase production, B - Haemolysis on blood agar, Lecithinase activity, Nitrate reduction test and Voges-proskayer reaction.

3.1.6 ENUMERATION AND IDENTIFICATION OF Bacillus cereus

Egg yolk medium with polymyxin B.sulphate was prepared and used to culture B. Cereus. Appropriate dilutions (1:10, 1:100) were selected and 0.1ml each was transfered using sterile pipette on to the surface of dried Egg Yolk medium . The inoculum was spread using sterile bent glass rods. Plates were incubated at 30^{oC} for 24 hours . Plate containing counts between 30 and 300 colonies with a dense halo of precipitate (Lecithinase activity) were counted. Representative colonies of Bacillus species were Gram stained and the Gram positive rods were purified by streaking on nutrient Agar Plates, incubated at 32^{oC} for another 24 hours and stored on Nutrient Agar slant for further identification by Biochemical tests which included gelatin liquefaction, motility test, nitrate reduction test, starch hydrolysis, indole production, urea hydrolysis and carbohydrate fermentation.

3.1.7. PHYSICO-CHEMICAL ANALYSIS

(a). TEMPERATUTRE DETEMINATION: The temperature of the Moin-Moin samples was determined by inserting the thermometer (SILBER BRAND) inside the Moin-Moin mould and allowed to stand for five minutes after which the reading was taken.

(b). PH DETERMINATION: Ten Grams of each of the Moin-Moin was blended with 40ml of distilled waster using a sterile blender after which the PH readings were determined using a Caliberated pH meter (-UNICON MODEL 29 MKS).

3.1.8. BIOCHEMICAL TESTS USED IN THE CHARACTERISATION AND CONFIRMATION OF THE ISOLATES .

The Biochemical identification of the isolates was carried out as descirbed by Gordon et al. (1973) for Bacillus cereus (Harmon (1979) , Gilbert et al (1981). for S. aureus .

While the confirmation tests for S. aureus were growth on MSA, mannitol fermantation, lecithinase activity, catalase test, nitrate reduction, V.P reaction, Nitrate Gelatin liquefaction, B-Haemolysis on blood Agar, Dnase production coagulase test and acid-gas from Mannitol, those for B. cereus were Voges-proskauer test, Citrate Utilization,Growth on Blood Agar, B-haemolysis on blood agar, indole production, motility test, starch hydrolysis, catalase activity, gelatin liquefaction, urease activity and Lecithinase activity.

(a). CATALASE TEST

Catalase production tested on the Nutrient plate culture with 0.5ml of 10% hydrogen peroxide (H_2O_2). An immediate evolution of abundant bubbles indicate that the culture is catalase positive. Absence of bubble shows the culture is catalase negative.

(b). HAEMOLYSIS ON SHEEP REDBLOOD CELLS:

Ability of the isolate for haemolytic action on sheep redblood cells was tested by inoculating Blood Agar base (Oxiod) containing 7% defibrinated sheep blood and incubating at 32^{oc} for 24 hours. The presence of halo-zone of blood lysis around the colonies was recorded as a positive reaction while absence of halos showed negative reaction.

(c). COAGULASE TEST (TUBE)

About 2 or 3 colonies were emulsified in 0.5ml of saline contained in a clean serological tube. One Millitre of citrated human plasma was added and incubated at 35^{oc} sign of increased viscosity or complete clotting of the plasma after 24 hours showed that the culture was coagulase positive. Absence of viscosity or clotting indicated a negative coagulase reaction.

(d). UTILIZATION OF CITRATE:

Simmon Citrate Agar (Oxiod) slant were inoculated and incubated at 32^{oc} and the utilization of Citrate was shown by the appearance of an Alkaline colour of Branothymol Blue from Pale Green after 14 days incubation.

(e). VOGES-PROSKAUER (V.P) TEST.

A volume of 5.0ml of V.P broth (Difco) was inoculated with the culture in triplicate and tested for Acetyl methyl Carbinol (Acetoin) production after 3, 5 and 7 days.

Incubation at 32^{oc} by mixing 3ml of 40% NAOH solution with the culture and adding 0.5 – 1.0g of creative from knife point. The development of red colour after 30 – 60 Minutes at room temperature indicated that the culture produced Acetyl-Carbinol and thus Voges-proskauer positive. The absence of red colour after 7 days indicated V.P. negative reaction.

(f). INDOLE PRODUCTION:

The test Organism was grown in 5ml Peptone water for 24 hours . After incubation, Kovacs indole reagent was added and gently shake. Development of red colour in the reagent layer above the broth within 1 Minute indicated positive reaction.

Retention of the yellow colour of the indole reagent indicated negative indole reaction.

(g). MOTILITY TEST

Test tubes of Motility medium (Oxiod) were inoculated by stabbing the culture on a wire 5mm deep from the surface and incubating at 32^{oc} for 2 – 3 days. Development of growth at the bottom of the tube and spreading of the growth throughout the tube indicated that the organism is motile (Motility Positive) while growth at only the stabbing line and length is motility Negative (Non-Motil).

(h). STARCH HYDROLYSIS

One gram of Potato Starch was suspended in 10ml of cold distilled water mixed with 100ml Nutrient Agar, Autoclaved, cooled to 45^{oc} and throughly shakened to mix before dispensing into sterile petri dishes. The plates were then inoculated with loopful culture and incubated at 32^{oc} for 48 hours . The plates were flooded with Iodine solution after 48 hours. A Zone of clearance underneath the growth indicate Starch Hydrolysis.

(i). DNASE (DEOXYRIBONUCLEASE TEST)

A sector of DNase agar plate was inoculated with the culture medium. Incubated at 37^{oC} for 24 hours to 48 hours. After 48 hours the plates were flooded with 10% HCL. A positive reaction was indicated by a distinct Zone of clearing around the streak.

(j). LECITHINASE TEST:

Sterile Nutrient agar cooled to $49^{oC} \pm 1^{oC}$ in water bath was added 100ml of concentrated egg yolk emulsion (Oxiod) and the flask thoroughly shaken to mix and dispensed into petri dishes. The plates were inoculated and incubated at 32^{oC} for hours. the Egg yolk reaction indicating Lecithinase production was shown by the appearance of dense - Halo Zone around the colonies. Those without it were recorded as Lecithinase Negative.

(k). UREASE TEST:

Urea agar slants in Bijou bottles were inoculated with the test isolates and incubated at 37^{oC} for 24 - 72 hours . Development of bright pink or red colour indicated a positive reaction.

(l). GELATIN - LIQUEFACTION TEST:

Culture were inoculated into tubes of plain Gelatine (Oxiod) incubated at 32^{oC} and tested for Gelatine liquefaction at 3 - 4 days interval for two weeks. Before each testing cultures were kept at 20^{oC} for 4 hours to allowed unchanged Gelatin to harden. Liquefied Gelatin remained liquid even after 4 hours given a positive reaction for the test while negative reactions were observed by hardened Gelatin.

SECTION B

3.2.1. *Staphylococcus aureus*: Enterotoxin Production

The Cellophane over agar method of Robbin et al (1974) was used, using a 5cm diameter filter papers, Cellophane discs were cut from $3\frac{1}{4}$ inch dialysis tubing and placed alternatively with filter papers were moistned with distilled water to eliminate wrinkling of the Cellophane discs. The Petri dish containing the discs was Sterilized at 121^{oC} for 20 Minutes.

BHI agar (15 - 20ml) in plates and BHI broth (5ml in screw capped tubes) were prepared. Sterile Cellophane was aseptically placed into the BHI agar plate and about 0.1m of BHI broth culture of the test strain was transfered on to the surface of thin Cellophane disc and spread evenly with a sterile bent glass rod. After 24 hours incubation the resulting growth was harvested using 2.5ml of 0.C1m disodium hydrogen phosphate (Na_2HPO4) and was centrifuged at 2000 rpm. The supernatant fluid was then decanted in to clean sterile Bijou bottles and analysed for Enterotoxin. Where analysis was delayed, they were frozen until time for the analysis.

3.2.2 ENTEROTOXIN ASSAY.

The Supernatant was tested for Entrotoxin A, C and D by the double gel diffusion (Microslide) technique of Casman and Benneth (1965) using standard Antisera and Enterotoxin.

3.2.3. TAPING AND PRECOATING

Electrician insulating tape was wrapped twice around each end of clean dust free slides. Two Centimeter space was left between the two ends. The surface between the tapes were precoated with 0.2% Bacteriological Agar.

3.2.4 PREPARATION OF MICROSLIDE AND ADDITION OF REAGENTS AND TEST MATERIAL

About 2ml of 1.2% melted Agarose was placed on the precoated slide. The lubricated template were then immediately layered on the agar. Sterile pasteur Pippette was used to drop the Antiserum into the centre well of the template. The standard Enterotoxin was placed on the top wells while the unknown samples were placed in the other wells. The tests were carried out in triplicate and the slides were incubated at 25^{oc} for 48 hours in a humid Chambers. After the incubation period, the slides were then immersed directly into Thiazine Red Solution for 5 Minutes and then transfered to 1% Trichloro Acetic Acid (to enhance the precipitated line) for easy readings.

3.2.5. Bacillus cereus' Toxin Production and Extraction

The B. cereus isolates tested for enterotoxin production were cultured in Brain - heart infusion broth (Difco) with 0.1% (W/V) Glucose supplement (BHIG) following the procedure of Kramer et al (1982) and Turubull and Kramer (1983).

The medium was filter sterilized using 0.45mm pore membrane paper (Millipore) and dispensed in 20ml volume in 250ml sterile Erlenmeyer flask using sterile 20ml syringes.
The medium was then inoculated with 0.5ml BHIG culture grown at 32^{oC} for 16 – 18 hours and incubated at 36^{oC} for 6½ hours on rotatory action shaker (Lab-line) set at 200 Rev/Mins. During incubation, 1ml of 0.1 NaoH was added to the Toxin production medium at the interval of 3½ and 4½ hours of the commencement of incubation.
The addition of 0.1N NaoH into each flask helps to make the medium alkaline there by conteracting the PH fall in the fermentation process towards Acidic condition which does not favour toxin production.
After the 6½ hours incubation, cultures were then centrifuged, filter sterilzed through 0.45mm Milipore membrane and the filtrates stored in the freezer until needed .

3.2.6 RABBIT ILEAL LOOP TEST FOR THE SCREENING OF ENTEROTOXIGENIC *Bacillus cereus*

The test was carried out using the procedure of Duncan et al (1969) and spira and Geofert (1972). Three white Rabbits were used. The ventral side of the Rabbits were shaven clean. They were then weighed and fasted for 40 – 48 hours prior to externalising the Ileum . The Rabbits were injected with 0.5ml Ketamine Hydrochloride for general Anaesthesia. The mid ventral side of the Animal were opened up and the Ileum externalised and tied to form six test loops each approximately 10cm long separated from each other by a 5cm blank loop. Intra luminal injection of 2cm each of the extract prepared above (3.2.5) were made into each of the six test loops. In each Rabbit, one loop at the distal end of the Ileum was injected with sterile Brain heart infusion broth + 0.1% glucose as the control.

The Ileum was then returned to the abdominal cavity and the incision closed . Following recovery, the Animals were provided with water but no food and allowed to live for 6 - 7 hours.

The animals were killed and opened up immediately for examination. The gross apperance of the loop were noted. Where either the control or any of the blank loops contained fluid, all the tests in the Rabbits were considered invalid. The length and fluid volume of each valid test was measured and the ratio of the fluid volume (ml) to loop length (cm) was determined and recorded.

3.2.7. STATISTICAL ANALYSIS

Result were statistically analysed using T. test analysis, and Linear graphs.

CHAPTER 4

RESULT

All the 36 samples of Moin-Moin collected from the three different food sales establishments: Hawkers, Cafetaria and Restaurant and 15 others prepared under special arrangement for storage stability test showed the presennce of Staphylococcus aureus Bacillus cereus Bacillus subtilis, Staphylococcus epidimidis, Escherichia coli but none of the sample was positive for either Closridium or Salmonella species. Aerobic plate counts of the Moin-Moin samples from the three out lets showed a general increase with time (Table 4.1 -4.3)

Table 4.1 showed the Microbial counts of moin-moin - obtained from : Hawkers. The Aerobic plate count varied from 4.6×10^4 to 3.2×10^6 with temperature range of 26^{oC} to 31^{oC} and PH of 4.8 - 5.8. Staphyloccus aureus and Bacillus cereus were isolated from the samples. The Staphylococcus aureus count varied between 1.2×10^3 and 2.0×10^5 while that of Bacillus cereus was 1.0×10^2 to 1.3×10^4. It was observed that moin-mo obtained from hawkers had a slightly higher aerobic plate than Moin-Moin obtained from other food establishments (... Cafetaria and Restaurant). The level of contamination of Moin-Moin obtained from cafetaria is shown in Table 4.2. A total aerobic plate count (TAPC) of 2.7 $\times 10^4$ to 2.6×10^6 was recorded. The Staphylococcus aureus count varied from 1.0×10^3 to 1.0×10^5 and Bacillus cereus from 1.0×10^2 to 2.1×10^4. The temperature of the samples ranged from 26^{oC} to 32^{oC}.

Table 4.3 shows the total aerobic plate count of Moin-Moin samples obtained from restaurant The aerobic plate count of the sample range from 1.3×10^4 to 1.6×10^6 . All were positive for Staphylococcus aureus with a count of 2.0×10^3 to 1.0×10^5 and most of the samples contained Bacillus cereus with a count varying between 1.0×10^2 and $1.0\ 10^4$. Temperature of the samples varied between 26^{oC} to 31^{oC}. With "Student T Test" analysis, the aerobic plate count obtained from each of the three food establishments are not significantly different ($P<0.05$).

Results of the Biochemical tests to which the isolates were subjected are shown in appendix I and II. The Staphylococcus aureus isolates were coagulase positive and it exhibited B - Haemolysis on Blood agar. All the isolates were Dnase positive, fermented Mannitol and also Liguefied Gelatin while the Bacillus cereus isolates were all gram positive spore forming Rods, Urease and Lecithinase positive and Liguefied Gelatin. All the isolates were Catalse positive and Motile. Table 4.4 shows the Enterotoxin production ability of 13 randomly picked isolates out of 24 Staphylococcus aureus isolates screened using Cellophane over agar method. A total of 7(53%) of the isolates tested produced Enterotoxin A while none of the isolates produced Enterotoxin C and D. Enterotoxin B and E were not tested for due to non availability of the specific Antisera.

Table 4.5 shows the Enterotoxin production ability of 15 out of the 22 isolates screened for Bacillus cereus enterotoxin using rabbit ileal loop ten (66%) of the isolates were mild Enterotoxin producers while 5(33.4%) were moderate and none of the isolates was a severe toxin producer based on the range of fluid/ length ratio. The volume of the fluid produced ranged 2.0ml to 7.0ml, all of which were straw coloured except one that produced bloody coloured fluid (Appendix 3).

Table 4.6, 4.7 and 4.8 shows the shelf stability of the Moin-Moin samples stored at 30^{oC}, 20^{oC} and at ambient temperature. At this temperature of storage, Moin-Moin could remain whole some and devoid of odour until after 24 hours, 42hrs and [illegible] hours respectively, as counts obtained after this time period had exceeded the threshold value (10^6).

The Aerobic plate counts obtained at this different temperature were statiscally analysed using Student - T - test. which showed a significant difference between storage at a temperature of 20^{oC} and at ambient temperature ($P<0.05$) but there was no significant difference between storage temperatuare of 30^{oC} and at ambient temperature. ($P>0.05$).

TABLE 4.1

CHANGES IN THE BACTERIAL COUNTS, PH AND TEMPERATURE WITH TIME OF 'MOIN - MOIN ON SALE BY HAWKERS

NO. of moin-moin samples	AEROBIC PLATE COUNT.	S. aureus COUNT	B. cereus COUNT	STORAGE TIME (HRS)	PH.	TEMPERATURE 0^C
1.	4.6×10^4	1.2×10^3	–	3	5.8	31^{oC}
2.	5.2×10^4	1.9×10^3	–	4	5.8	30.5
3.	5.6×10^4	1.4×10^4	1.0×10^2	5	5.7	30.5
4.	6.1×10^4	4.1×10^3	3.9×10^2	7	5.6	30
5.	6.8×10^4	1.7×10^4	3.4×10^3	8	5.4	30
6.	3.1×10^4	2.6×10^4	2.7×10^3	10	5.2	30
7.	4.1×10^5	2.8×10^4	2.6×10^3	14	5.0	29
8.	5.6×10^5	5.0×10^4	2.7×10^3	15	5.0	28
9.	8.1×10^5	6.4×10^4	3.2×10^3	17	4.9	28
10.	1.5×10^6	1.1×10^5	1.0×10^4	19	4.9	27.5
11.	2.3×10^6	1.2×10^5	2.0×10^4	20	4.8	27
12.	3.2×10^6	2.0×10^5	1.3×10^4	22	4.8	26.5

TABLE 4.2

CHANGES IN THE BACTERIAL COUNTS, PH AND TEMPERATURE WITH TIME OF "MOIN - MOIN" ON SALES AT THE CAFETERIA

NO. of Moin-moin samples	AEROBIC PLATE COUNT	S. aureus COUNT	B. cereus COUNT	STORAGE TIME (HRS)	P.H	TEMPERATURE 0^{C}
1.	2.7×10^4	1.3×10^3	1.0×10^2	3	6.2	32^{oC}
2.	3.2×10^4	2.1×10^3	1.4×10^2	5	5.9	30.5
3.	2.9×10^4	2.6×10^3	1.7×10^2	6	5.8	30
4.	3.1×10^4	3.2×10^3	-	7	5.5	29.5
5.	3.6×10^4	3.4×10^3	2.1×10^3	9	5.4	29
6.	3.9×10^4	2.1×10^4	1.0×10^3	10	5.4	29
7.	3.0×10^5	2.5×10^4	1.5×10^3	14	5.3	28
8.	3.5×10^5	2.6×10^4	1.8×10^2	15	5.3	27.5
9.	4.7×10^5	3.9×10^4	2.0×10^3	17	5.0	27.3
10.	6.1×10^5	4.1×10^4	3.6×10^3	19	5.0	27
11.	2.4×10^5	2.0×10^5	2.0×10^4	20	5.0	27
12.	2.6×10^6	1.0×10^5	2.1×10^4	22	4.9	26.5

TABLE 4.3

CHANGES IN THE BACTERIAL COUNTS, PH AND TEMPERATURE WITH TIME OF MOIN - MOIN ON SALE AT THE RESTAURANT

NO. of Moin-Moin samples.	AEROBIC PLATE COUNT	*S. aureus* COUNT	*B. cereus* COUNT	STORAGE TIME (HRS)	P H	TEMPERATURE 0^{C}
1.	1.3×10^4	2.2×10^2	1.0×10^2	4	6.0	31
2.	1.5×10^4	$2.2. \times 10^2$	1.3×10^2	5	5.7	31
3.	1.8×10^4	2.3×10^2	–	6	5.8	29
4.	4.2×10^4	2.5×10^2	–	7	5.5	29
5.	2.8×10^4	1.6×10^2	1.1×10^2	8	5.4	29
6.	4.3×10^4	2.7×10^2	1.7×10^3	10	5.4	29.5
7.	2.6×10^4	3.5×10^2	2.0×10^2	14	5.2	28
8.	3.7×10^4	3.2×10^2	2.1×10^2	15	5.1	28
9.	4.9×10^4	1.7×10^4	1.6×10^3	17	5.0	27.5
10.	5.5×10^5	3.3×10^4	1.8×10^3	19	4.9	27
11.	6.2×10^5	4.9×10^4	2.1×10^3	20	5.0	27
12.	1.6×10^6	$1.1. \times 10^5$	1.0×10^4	22	4.9	26.8

TABLE 4.4

ENTEROTOXIN PRODUCTION PATTERN BY 13 ISOLATES

OF Staphylococcus aureus FROM MOIN-MOIN

ENTEROTOXIN TYPE	NO. OF ISOLATES TESTED FOR ENTEROTOXIN TYPE	NO. & PERCENTAGE OF ISOLATES POSITIVE FOR ENTEROTOXIN TYPE.
A	13	7 (53%)
C	13	– (0%)
D	13	– (0%)

TABLE 4.5

ENTEROXIN PRODUCTION BY B. cereus
ISOLATES FROM "MOIN-MOIN SAMPLES
OBTAINED FROM DIFFERENT FOOD ESTABLISHMENT

SAMPLE SOURCE	NO OF ISOLATES	NO AND PERCENTAGE SHOWING DEGREE OF TOXIGENCITY. (RANGE OF FLUID LENGTH RATIO)			TOTAL NO.& PERCENTAGE OF TOXIGENIC ISOLATES.
		0.2 - 0.4 ↔ 0.5 - 0.7 ↔ 0.8 - 1.5			
		Mild	Moderate	Severe	
Hawkers	5	3(60)	2(40)	-	5(100)
Cafeteria	5	4(80)	1(20)	-	5(100)
Restaurant	5	3(60)	2(40)	-	5(100)
TOTAL	15	10(66)	5(33)	-	15(100)

TABLE 4.6

CHANGES IN THE BACTERIAL COUNT
pH TEMPERATURE AND ODOUR WITH TIME OF
EXPERIMENTAL MOIN-MOIN STORED AT 30^{oC}

Physico-chemical attribute of moin-moin samples				Bacterial counts (cfu/g)		
Storage time of Moin-moin	Nature of odour	Temperature Moin-moin (0^{C})	pH	Aerobic plate count	*S. aureus*	*B. cereus*
6	Non-offensive odour	30	6.02	7.8×10^{3}	6.3×10^{2}	9.1×10^{1}
12	"	"	6.0	2.4×10^{4}	2.0×10^{3}	2.1×10^{2}
18	"	"	5.82	3.6×10^{4}	3.1×10^{3}	2.6×10^{2}
24	Offensive odour	"	5.74	4.8×10^{4}	4.0×10^{3}	3.1×10^{2}
30	"	"	5.51	2.1×10^{5}	1.2×10^{4}	1.0×10^{3}
36	"	"	5.33	3.9×10^{5}	3.4×10^{4}	2.7×10^{3}
42	"	"	5.01	1.0×10^{6}	5.7×10^{4}	3.5×10^{3}

TABLE 4.7

CHANGES IN THE BACTERIAL COUNT
pH TEMPERATURE AND ODOUR WITH TIME OF
EXPERIMENTAL MOIN-MOIN STORED AT 20°C

Physico-chemical attribute of moin-moin samples				Bacterial counts (cfu/g)		
Storage time of Moin-moin	Nature of odour	Temperature Moin-moin (0^C)	pH	Aerobic plate count	S. aureus	B. cereus
6	Non- offensive odour	20	6.1	6.8×10^3	8.4×10^2	7.0×10^1
12	"	"	6.04	2.5×10^4	2.1×10^3	2.0×10^2
18	"	"	6.0	3.4×10^4	3.1×10^3	2.9×10^2
24	"	"	5.83	4.0×10^4	3.9×10^3	3.2×10^2
30	"	"	5.76	5.6×10^4	4.4×10^3	4.1×10^2
36	"	"	5.80	5.9×10^4	4.7×10^3	4.6×10^2
42	Offensive odour	"	5.60	1.7×10^5	2.0×10^4	1.1×10^3
48	"	"	5.55	3.0×10^5	2.6×10^4	2.4×10^3
54	"	"	5.53	4.9×10^5	3.8×10^4	3.0×10^3
60	"	"	5.50	1.4×10^6	6.0×10^4	4.1×10^3

TABLE 4.8

CHANGES IN THE BACTERIAL COUNT
pH TEMPERATURE AND ODOUR WITH TIME OF
EXPERIMENTAL MOIN-MOIN STORED AT AMBIENT TEMPERATURE

Physico-chemical attribute of moin-moin samples				Bacterial counts (cfu/g)		
Storage time of Moin-moin	Nature of odour	Temperature Moin-moin (0^C)	pH	Aerobic plate count	*S. aureus*	*B. cereus*
5	Non-offensive odour	29.5	5.98	1.9×10^4	8.1×10^2	1.5×10^2
12	"	28	5.89	4.3×10^5	3.6×10^3	2.9×10^2
18	Offensive odour	27	5.56	8.4×10^5	1.6×10^4	8.0×10^3
24	"	26.5	5.38	1.5×10^6	1.0×10^4	1.3×10^4

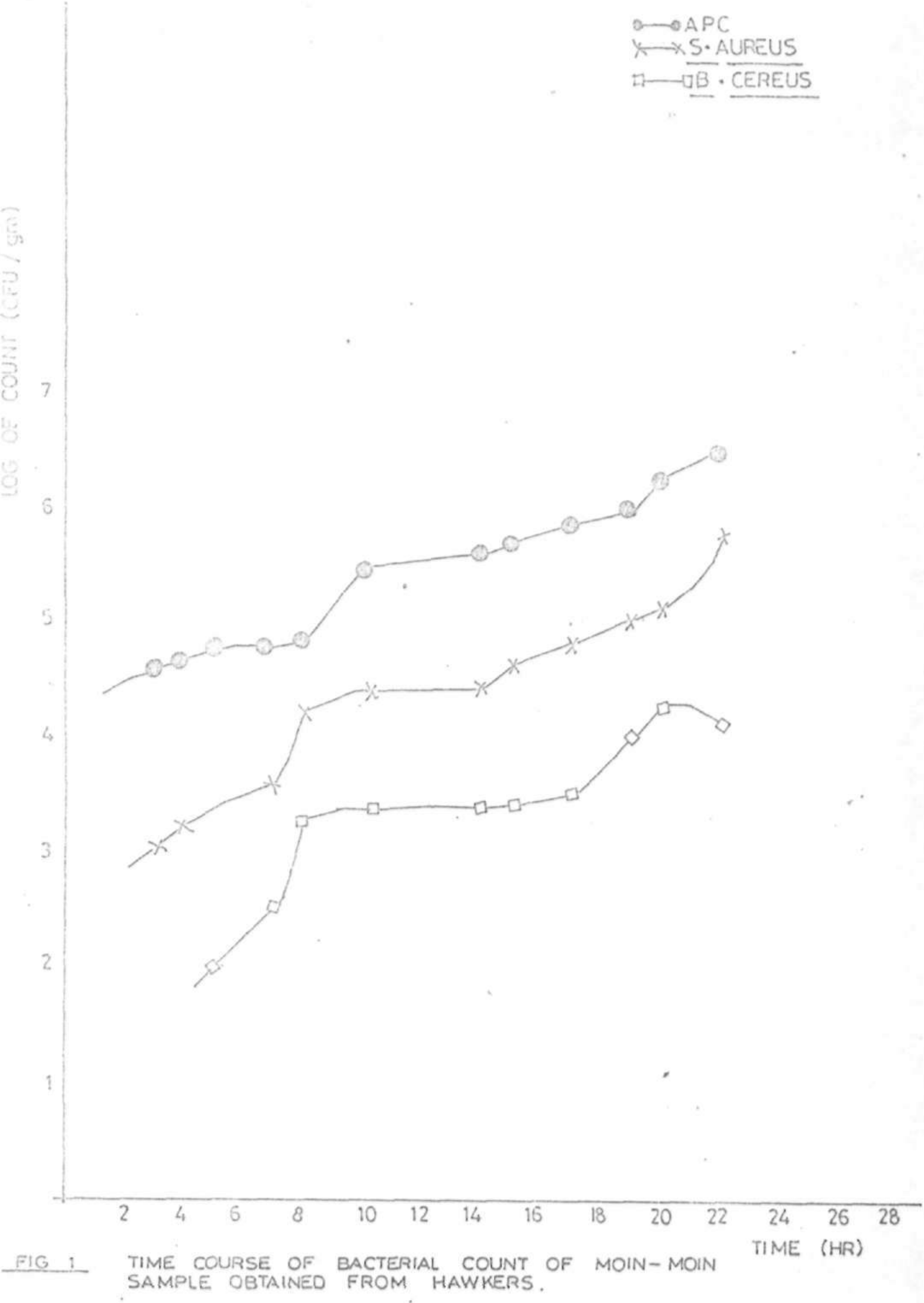

FIG 1 TIME COURSE OF BACTERIAL COUNT OF MOIN-MOIN SAMPLE OBTAINED FROM HAWKERS.

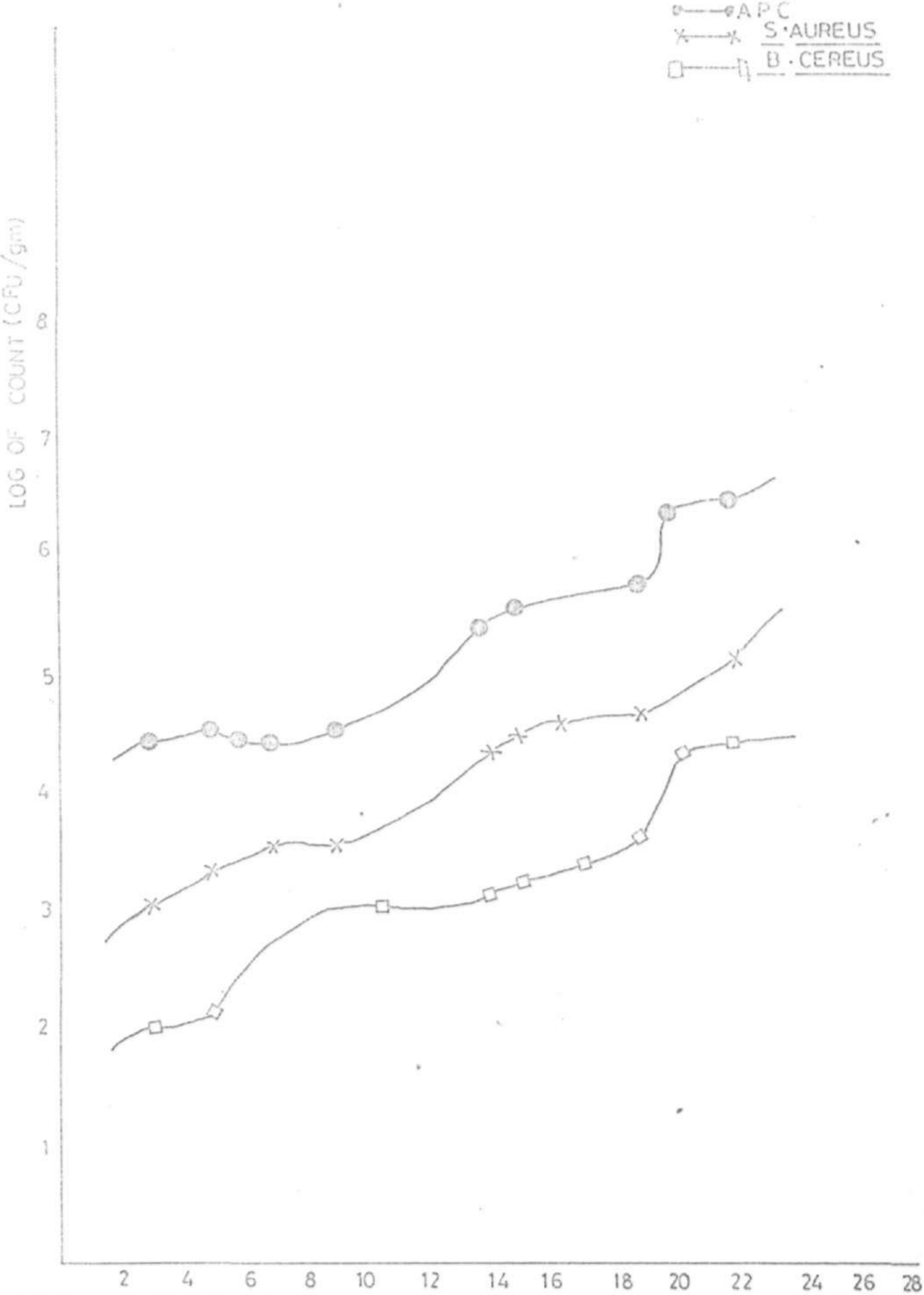

FIG 2 TIME COURSE OF BACTERIAL COUNT OF MOIN-MOIN OBTAINED FROM CAFTERIA.

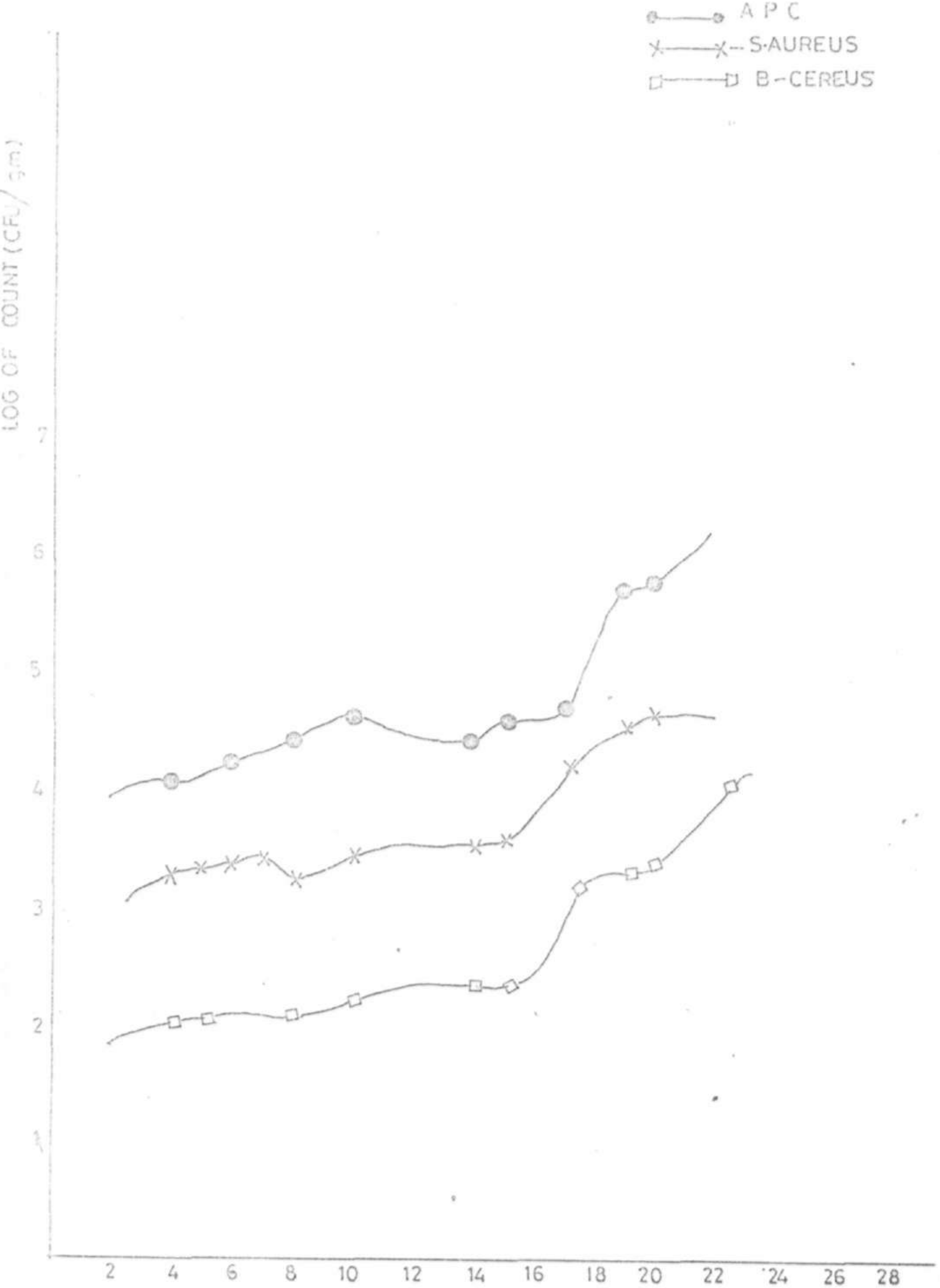

FIG . 3 TIME COURSE OF BACTERIAL COUNT OF MOIN MOIN SAPLE OBTAINED FROM REASTAURANT.

CHAPTER FIVE

DISCUSSION

Result of the aerobic microbial counts for the Moin-Moin samples obtained from the three food establishments demonstrate higher microbial count for samples obtained from the Hawkers compared to those of the samples from Cafetaria and Restaurant. The level of exposure to Microbial contaminants very often may be responsible for this observation. The result of the total Aerobic plate count, Staphylococcus aureus count and Bacillus cereus count obtained from this study showed that Moin-Moin samples obtained from hawkers have count above the acceptable limits set by the International Commission on Microbiological safety of food (ICMST) after 19 hours with a TAPC of 1.5×10^6, Staphylococcus aureus count of 1.1×10^5 and Bacillus cereus count of 1.0×10^4 (Table 4.1), From the fact that Moin-Moin obtained from Hawkers is most often exposed and frequently open to buyers, the extent of contamination is therefore expected to be higher and this observation was made by Owhe-Ureghe et al (1993) in a similar work, Although the TAPC obtained in their work was slightly lower than what was obtained in this study, it could be due to the fact that the Moin-Moin samples collected for their own analysis were only some few hours old compared to the samples used for this study . All the Moin-Moin samples are usally kept at ambient temperature at a length of time sufficient to encourage the proliferation of Pathogenic bacterial species which may lead to food intoxication. This is in agreement with the findings of Bryan, et al, (1981) concerning street foods in countries with higher ambient temperature condition.

The acceptable limit suggested by lcmsf for Bacillus cereus, Staphylococcus aureus and total Aerobic plate count are 10^3, 10^4 and 10^5 respecitively.

The tolerable level for Bacillus cereus, Staphylococcus aureus and total aerobic plate counts are 10^4, 10^5, and 10^6 respecitively for most foods.

Upper limit guidelines established by other agencies utilize the Aerobic plate count ranging from 1.0×10^5 to 1.0×10^6 cfu/gm while higher plate count in food ($\geq 10^6$) are strong indication of declining quality and potential health hazard (CDC, 1983). The results of the TAPC, Staphylococcus aureus count and Bacillus cereus counts showed that all the samples of Moin-Moin from the three outlets do not have high initial contamination level, as all the samples had counts below the acceptable limit for almost 18 hours of storage at ambient temperature.

The common occurence of Staphylococcus aureus and Bacillus cereus samples compare favourable with the findings of other researchers (Owhe-ureghe et al, 1973). The isolates of Staphylococcus aureus obtained from these samples were coagulase positive and most coagulase positive Staphylococci are responsible for food poisoning and their presence in Moin-Moin is indicative of poor handling after processing since the steaming process is long enough to kill off Non-heat resistant organism like Staphylococci. The Organism could gain access to the food through the food handlers as Staphylococci are normal flora of the nasal passage, hand, skin, wounds where they are commensal and harmless (Lajide, 1984, Talabi and Bassy 1984). Staphylococcus aureus has been isolated from various food stuffs and untensils (Onourah et al; 1987 Warburton and Weiss 1988). Staphylococcus aureus has also been repoted to grow and produce toxin in food at PH range of 4.0 - 9.0 (Daly, et al 1973). The observed PH range recorded in this study is within the PH range for toxin production and hence favours toxin production by the strains isolated in this study. Toxin production using Cellophane over agar method showed that 53% of the tested isolates produced Enterotoxin A; while none produced Enterotoxins C and D.

The fact that most 53% of the enterotoxigenic isolates produced entrotoxin A is not suprising as such strains are predominantly associated with humans. This might be indicative of contamination by handling the moin-moin after processing (Bergdoll, 1987). Moreover, enterotoxin A is most often implicated in the case of staphylococcal food poisoning. (Noteman et al, 1984)

The incidence of enterotoxigenic staphylococci in food items such as meat, and products, diary products, bakery products, custard and cream filled pasteries has been reported by several investigators (Onourah et al, 1987); Warburton and Weiss 1986; Nwosu, 1986, Adesiyun and Kwaga, 1983).

Staphylococal enterotoxins are unique among bacterial toxins in being heat resistants. This accounts for their ability to withstand heat warming given to moin-moin. Other enzymes produced by Staphylococcus aureus which correlate with the enterotoxigenicity are B- haemolysis, lecithinase and Dnase. The correlation between coagulase and enterotoxin production found in this study is in agreement with published works of Bergdoll (1972), Oteahajine and Mitra (1984) . It is of concern to note that most of the samples of moin-moin were contaminated with Bacillus cereus. The contamination of food by this organism could be attributed to the ubiquitous distribution of this organism . They are present in the soil, dust, water and air (Jay, 1978; Fraizer and Westhoff 1988). This organism could have got into the moin-moin samples during preparation from the enviroment. soil has been implicated as a source of bacterial contamination in related experiment carried out by Rocco and Wells (1988) where strains of Bacillus cereus have been associated with food poisoning (Hange 1955).

Moreover, the organisms being a spore former is capable of withstanding the heat treatment during the preparation. This can be hazardous since the spore may be forced to germinate and liberate toxin following heat shock and during reheating and cooling of the food. (Gilbert, 1979, Parry and Gilbert, 1980).

Bacillus cereus has also been reported to grow and produce toxin in food at a minimal PH range of 4.5 – 5.15 (Mikolajecik et al. 1969). The procedural handling of Moin-Moin could result in contamination and hence growth of the toxigenic strain in the infective diarrheal dose and this could be dangerous to consumers. The common practise of eating left over food stored at ambient temperature for up wards of 24 hours adds to the possibility of Bacillus cereus growth and toxin production in these food as the PH value recorded during this study was found to be minimal PH growth range. Toxin production using Ileal loop test showed that Enteortoxigenic strains are mild to moderate producers even though loop distention due to gas accumulation as recorded by Duncan et al (1968) was not observed in this study.

On the shelf stability study, the results obtained from this study had shown that storage of Moin-Moin at a temperature of 20^{oC} will keep longer than those stored at the other two storage temperature (30^{oC} and at ambient temperature). Generally, Microbial growth increase with storage time, but growth is rather slow at 20^{oC} compared to other two storage temperature. This is not suprising as low temperature tend to inhibit growth rate of the vegetative cells.

Statistical analysis using student T- test analysis showed that there was a significant difference in the results between storage temperature of 20^{oC} and other two temperature ($P>0.05$) but there was no significant difference between the storage temperature of 30^{oC} and at ambient temperature, this is not suprising as the two temperature are at a very close range.

A linear relationship also exist between the aerobic plate count and the storage time (fig. 1 - 3)

From the graph analysis, it was observed that count from Moin-Moin obtained from hawkers shows a steady increase in count compared to the other samples obtained from Cafetaria and Restaurant. This is not suprising as the Moin-Moin obtained from hawkers is most often more exposed to contamination hence increasing its level of contamination.

CONCLUSION AND RECOMMENDATION

From the results obtained in this study, it can be established that the Moin-Moin obtained from hawkers were highly contaminated when compared with the Moin-Moin obtained from the other two food establishments (cafetaria & Restaurant).

Isolation of food poisoning organisms such as Staphylococcus aureus, Bacillus cereus were made suggesting possible hazards associating with consumption of stored Moin-Moin . Certain measures such as intensive cooling, provision of clean showglass boxes to prevent excessive environmental contamination of cooked foods, using a clean wrapping material, serving the foods hot should be done to reduce the Microbial loads as much as possible.

The practice of basic sanitary rules in preparing foods and personal hygiene should be employed to improve the hygienic condition of this food. The ingredient and the spices added to the Moin-Moin should be of good quality. The hawking of Moin-Moin by children may expose the food due to carelessness, during the hawking for example some of food might drop off the tray and be picked only to continue the sale, this definitely would reduce the shelf life of the Moin-Moin due to contamination as well as the stress provided (such as loose texture) which will encourage Microbial proliferation.

Temperature regulation is another way of increasing the shelf life of the Moin-Moin. When Moin-Moin is not going to be eaten immediately, it could be kept in the refrigerator to decrease the temperature of the "Moin-Moin" rapidly, so that the development of vegetative forms will be inhibited. Moin-Moin should be heated properly so as not to allow for spore formation.

The environment in which Moin-Moin is made should be clean to achieve better storage. To encourage food sanitary condition, Public Health Campaigns on environmental Sanitation should be embarked upon by the government with greater vigor.

If the preventive measurs are not carried out, outbreak of food borne diseases is imminent, since this food, "Moin-MOin" are always bought as take away food by most of the consumers even at the close of their business in the evening. It is therefore necessary for food inspectors to monitor and prevent the sales of ready to eat food (Street Foods) by Hawkers in the Market or other Catering Establishments.

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APPENDICES

APPENDIX I

SUMMARY OF RESULTS OF BIOCHEMICAL IDENTIFICATION OF Staphylococcus aureus ISOLATES

TEST	RESULT	PERCENTAGE OF ISOLATES POSITIVE	NUMBER OF ISOLATE NEGATIVE
Gram Reaction	+	100	–
Growth on MSA	+	100	–
Mannitol fermentation in MSA	+	100	–
Lecithinase activity	+	72	4
Catalase Test	+	100	–
Nitrate Reduction	+	100	–
V.P. Reaction	+	100	–
Gelatin Liquefaction	+	100	–
B-haemohysis in Blood agar	+	100	–
DNase Production	+	100	–
Coagulase Test	+	100	–

Total number of Isolate tested 13.

APPENDIX 2

SUMMARY OF RESULTS OF BIOCHEMICAL IDENTIFICATION OF B. CEREUS ISOLATE.

TEST	RESULT	PERCENTAGE OF ISOLATE POSITIVE	NUMBER OF ISOLATE NEGATIVE
Gram reaction	+	100	–
V.P. reaction	+	100	–
Citrate Utilization	+	75	3
Acids from: Glucose	+	100	–
Mannitol	–	–	12
Xylose	+	12	10
growth on blood agar	+	100	–
-haemolysis in blood agar	+	42	7
Indole production	+	50	6
Motility Test	+	100	–
Starch hydrolysis	+	100	–
catalase Test	+	100	–
Gelatin Liquefaction	+	100	–
Urease activity	+	100	–
Lecithinase activity	+	100	–

Total numbers of Isolates tested 12

APPENDIX 3

ENTEROTOXIGENIC STRAINS AND AMOUNT OF FLUID ACCUMULATION

DURING ILEAL - LOOP TEST

ISOLATES	VOLUME (CM^3)	VOLUME/LENGTH RATIO	CHARACTERISTICS FLUID COLOUR.
BCH_1	3.5	0.35	Straw coloured
BCH_2	3.2	0.32	" "
BCH_3	5.0	0.50	Bloody coloured
BH_4	3.31	0.31	Straw coloured
BH_5	5.3	0.53	"
BCC_1	5.1	0.51	"
BCC_2	2.3	0.23	"
BCC_3	5.3	0.54	" "
BCC_4	2.0	0.20	"
BCC_5	3.1	0.31	"
BCH_1	2.3	0.23	"
BCH_2	2.1	0.21	"
BCH_3	3.0	0.30	"
BCH_4	5.1	0.51	"
BCH_5	2.5	0.25	"