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A FIELD AND LABORATORY STUDY OF THE STORAGE AND PRESERVATION OF BREADFRUIT AS PRACTICED IN THE SOUTH PACIFIC ISLANDS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

INTRODUCTION

General Background

Breadfruit has been used by people of the South Pacific Islands as an important source of food since prehistoric times. Today, it is found growing on many of the volcanic and coral islands scattered throughout the tropical and semi-tropical South Pacific areas of Micronesia, Polynesia, and Melanesia.

In general, Micronesia includes the Caroline, Marshall, Mariana, Palau, and Yap Groups of islands. Polynesia encompasses Tahiti, American and Western Samoa, New Zealand, the Hawaiian, Marquesas, Ellice, Society, Tuamotu, Cook, Tonga, and Austral islands plus a great number of other scattered islands. Melanesia, as the term is used in general, overlays the territory of Papua and New Guinea, Fiji, New Caledonia, New Hebrides, New Ireland, New Britain, the Solomon, Loyalty, and Admiralty Islands.

The breadfruit tree is said to be a strictly tropical plant and, in general, attains its greatest development in a warm to hot, humid climate, with plenty of rain, moisture, fertile soil, and adequate drainage (1) . Some breadfruit trees give rise to seedless and others to seeded varieties of breadfruit during certain fruiting periods of the year which vary from one place to another.

Botanically, the breadfruit is Artocarpus incisa. Artocarpus communis or Artocarpus altilis are commonly used synonyms.

"According to Raoul, the French botanist, the species from which the cultivated varieties have come is Artocarpus incisa, "bread tree", a wild tree of oriental Malaysia" (2) . The breadfruit tree belongs to the Moraceae or Fig Family.

During the latter part of the eighteenth century, the British, among other foreigners developed a special interest in breadfruit as a supplemental source of food for laborers of British West Indies planters. In 1787, Britain's King George III sent a Naval Lieutenant William Bligh to Tahiti in quest of breadfruit trees. As history tells us, a mutiny prevented Bligh from succeeding in his mission and goal the first time. In 1792, now a Captain, William Bligh was again sent to Tahiti. On his second mission, he was successful in delivering breadfruit plants to the West Indies in 1793. Ten years before Bligh's second mission, the French had already introduced a seeded variety of breadfruit of lesser importance into the West Indies (1) .

The introduction of breadfruit, a native of the Malay Archipelago, into the South Pacific area is vaguely known. The Polynesian, Micronesian, and Melanesian peoples in general have held breadfruit in high esteem as a reserve and staple food for many years, so much so, that it has become one of the essential items or an important basic element in their diet to this very day.

Breadfruit can be prepared in one of several ways. It

can be baked, roasted, boiled, steamed, or fried and made into a variety of food dishes. Breadfruit is most often mixed with coconut or coconut milk but has also been combined with coconut oil, cassava starch, flour, manioc, taro, bananas, butter, lemon juice, salt, water, and eaten with fish or fowl.

On many of the South Pacific Islands, fresh breadfruit, in the mature to ripe stages, is usually baked by means of heated stones in an underground oven, the construction of which varies from one place to another. The fresh breadfruit may: 1.) be baked in the native oven after which the rind and core are removed and it is then eaten with or without addition or mixture of other ingredients such as coconut milk, taro, and other cooked fruit; 2.) once cooked it may be baked again, after being mixed with other ingredients; and 3.) be pounded and mixed with an ingredient such as coconut milk or the core taken out and the space once occupied by it filled with coconut cream before being baked and consumed.

Fresh breadfruit may be roasted. This is usually done by placing the whole breadfruit on a bed of hot coals, stones, or on a wood fire in a shallow pit. The rind and core are normally taken off after the breadfruit has been roasted but, there are a few exceptions when the rind is scraped lightly and the stalk of the fruit removed before roasting. When the breadfruit is roasted, it is ready for

consumption with or without the addition of other ingredients.

Fresh breadfruit may be boiled. This is usually done by taking a whole breadfruit, peeling, coring, dicing, and then placing it into boiling water. When it is soft and tender, it may be seasoned or mashed and mixed with other ingredients to give it flavor.

Fresh, steamed, or baked breadfruit may be fried, plain or rolled in flour, seasoned or mixed with other ingredients, and eaten.

Buck (3) saw ripe breadfruit eaten raw on the island of Hari in Polynesia after the core and seeds had been removed and the breadfruit was scraped lightly, mashed, and mixed with some water.

Over the years, the people of the South Pacific area have developed various practices, some of which are still in existence, of storing and preserving breadfruit. These practices have arisen mainly: 1.) because of the possibility of famine during the periods when breadfruit trees are not in the fruiting season, prominent especially during earlier times; and 2.) because breadfruit, being an important staple in their diet and a seasonal fruit, at times results in supplies being in excess of the quantity used for normal human consumption. This latter fact is true even to this very day.

Among the various practices of preservation of bread-

fruit developed by the South Pacific Islanders, the practices of preservation of breadfruit by fermentation in pits in the ground are perhaps the oldest and certainly the most interesting to note and study. It is doubtful that any studies of the fermentation process of the breadfruit during the period of preservation in the ground have been done. This study is especially concerned with various aspects of one of the older methods of storage and preservation of breadfruit by fermentation in pits in the ground as still practiced in certain areas of the North and South Pacific (4) . Essentially, the process involves: 1.) peeling of the rind of the breadfruit; 2.) removal of the core; 3.) splitting of the breadfruit into halves or parts; and 4.) washing with fresh water. The breadfruit is then put into pits in the ground which have been lined with stones, and ti, banana, breadfruit, or coconut leaves. The dimensions of the pits are determined by the quantity of breadfruit to be stored and preserved. Massal and Barrau (18) state: "layers of fresh fruit are alternated with layers of old fermented preparation in order to facilitate fermentation and to obtain a more homogeneous product." When filled the pits are covered over with more leaves, rocks, and earth. The breadfruit pulp ferments or turns sour after several days (5) . Fermentation is accompanied by an increase in acidity which maintains the breadfruit, stored in this manner, for months. When the pits are opened after

sometime, several months, the breadfruit is a homogeneous fermented mass with a very strong odor which may induce reactions in individuals not adapted to the smell.

This breadfruit preservation practice was once extensively used in the Marquesas Islands (4) . It was also common in such South Pacific locations as Tahiti, Samoa, and Ponape in the Carolines.

Several authors have referred to the fermented breadfruit prepared, in the above manner, as ma in the Marquesas (4,5,6) , mahi in Tahiti (4) , mahr in Ponape (7) , namandai in New Hebrides (4) , and masi in Samoa (8) .

Although new foods are being introduced into the South Pacific area, many native islanders will continue to eat breadfruit prepared in a manner suitable to their taste and liking.

Through various personal interviews, correspondence, and contact with East-West Center students who live on some of the islands in the South Pacific area via oral communication and a study questionnaire, it was learned that the practice of preservation of breadfruit by fermentation in pits in the ground still occurs in some places in the North and South Pacific area today.

Another practice of preservation of breadfruit by fermentation in pits in the ground is described by Murai et al (9) and occurs in the Marshall Islands. It also occurs on the atoll island of Kapingamarangi and possibly other

islands in the South Pacific area. It is called Bwiru and differs from the above mentioned preservation practice in several respects, the main one being immersion of the cored and peeled breadfruit in salt water for 24 hours or more before being placed in the earth pit. The preserved breadfruit paste formed by this method will also last for several months to a year or more.

Buck (3) describes another practice of preservation of breadfruit using heat and drying. This practice is called Jankwin (7) in the Marshalls and paku kuru on Kapingamarangi atoll. It differs from the above mentioned preservation practice, in one respect, that is the use of heat. The preserved breadfruit formed by this method is normally flattened into sheets, dried in the sun, rolled into cylinders, and wrapped with pandanus leaves. It will, as most other preserved breadfruit, keep for long periods of time and serve as reserve food for emergencies or for long sea voyages.

A native islander from Bougainville, in the Northern Solomons (40) related another practice of preservation of breadfruit using heat and drying which his people practiced. It differed from the above just mentioned practice, in that, the peeled and cored breadfruit, after being cooked and dried, were not flattened and rolled into tubes but usually were placed directly into insect-proof bamboo safes exposed to the air. The breadfruit when they were well

preserved would become hard and firm in texture, taste like Navy or Hartman's biscuits, and keep for several years or until used up.

On those islands, in the South Pacific area, where preserved breadfruit is still made, it is customarily mixed with one or more ingredients to add flavor to it. In the Marshall Islands, preserved breadfruit (Bwiru) is mixed with coconut milk, coconut syrup, or mashed pumpkin, and cooked before being eaten. It is also boiled in water and sugar or fried in hot grease. In the Caroline Islands, preserved breadfruit (termed Apot by Murai et al) is mixed with water and coconut cream and possibly with a number of other ingredients already mentioned. Preserved breadfruit has also been mixed with grated coconut, grated pumpkin, manioc, fresh breadfruit, taro or poi, ripe bananas, lemon juice, flour, and sweetened condensed milk in the preparation of various food dishes.

In general, preserved breadfruit can be cooked, dried till hard, and kept for long periods of time. When it is to be used, it can be soaked in water, mashed, and mixed with one or more other ingredients.

Problem of Preservation in a Primitive Economy

Fruits have been preserved, during past and present times, in a number of ways such as drying, storing at low temperatures, and use of chemical preservatives. In a modern up-to-date economy, where people have ready access

to a refrigerator and other modern conveniences, preservation of fruits, let alone other foods, is easy, efficient, and quite safe. In a more primitive economy, however, many of the modern conveniences such as electric refrigerators, which we take for granted, are not present. The people living in a primitive economy without modern conveniences have had to develop their own methods of preservation. The preservation of breadfruit by fermentation in pits in the ground is one example of a method of preservation which had to be developed because of lack of other adequate means of preservation. Primitive preservation methods, although efficient, may not be safe in terms of Public Health.

Presently, there are more than 2.6 million people living in the areas of Micronesia, Melanesia, and Polynesia, which in general, make up the South Pacific realm. Some of the people in these areas still hold to that practice of preservation of breadfruit by fermentation in pits in the ground, with which we are concerned in this study.

Problem of Illness Due to this Practice of Preservation

Recent reports from the field indicate that there may be a relationship between consumption of fermented breadfruit taken from pits in the ground and the occurrence of reactions or disorders of the gastrointestinal type among islanders in the South Pacific area.

William R. Norwood (41), High Commissioner of the Trust Territory of the Pacific Islands, and Micronesian

staff informants on Saipan, in the Marianas, conveyed in a letter that: "Some people do get sick from eating preserved breadfruit prepared when very little cleaning is done before cooking. This is preferred by the older generation especially in Truk. The fermented breadfruit pulp is removed from the pit and is kneaded on a piece of flat board until it is the consistency of thick paste. This paste is then placed in a wooden bowl, more water is added to soften the paste to more or less a liquid consistency and then poured by cups into breadfruit leaves, tied, cooked by boiling and consumed. The offensive odor is great and it is greatly esteemed by the older folks. Symptoms experienced are usually mild-abdominal cramps, headaches, occasionally chills and aches in the joints. There is no diarrhea, nor nausea or vomiting, no itching, no bloody stools. These are the symptoms as experienced by one young Trukese who is presently on Saipan who once got sick from eating cooked preserved breadfruit prepared in this way some years back. There is very little record known of this reaction because those who became ill did not suffer serious ill-effects and the condition usually passes away within a few hours without medication or notice to the medical people."

Another informant (38) on Truk, in the Caroline Islands, reported: "The people in Truk preserve their breadfruit by peeling it, fermenting in open air for 3-4 days till it is soft and sweet (like ripe mango), then they bury

it in a banana leaf-lined pit. This pit is usually located in moist ground. The breadfruit is turned from time to time (usually every 6 months) and the banana leaves changed. The preserved breadfruit is high in acid content. Most people of Micronesia prefer acid-breadfruit. The preserved breadfruit is removed from the pit, mixed with water, and consumed. Others place the preserved breadfruit in a burlap sack and wash the acid out in ocean water. This breadfruit is then mixed with coconut oil and sugar and baked. The doctors in Truk informed me (38) that most reactions from the breadfruit are due to consumption of the acid variety. People who show reactions are given anti-histamine as treatment. This generally cures them."

A native islander of the Truk District (37) , in the Eastern Caroline Islands, related that he experienced fever, chills, nausea, headache, and generalized pain, especially of the joints, and generalized lymphadenopathy after consuming cooked fermented breadfruit obtained from pits in the ground. He further mentioned, "that when the sour juice had been squeezed out and the taste became tasteless, the above reaction would not occur. The reaction usually lasted 24 hours. Some highly reactive persons may even react to the smell. There has never been any known death case due to this reaction."

Another native islander of the Truk District (45), in the Eastern Caroline Islands, related that he experienced

fever, chills, and headache after consuming cooked fermented breadfruit from pits in the ground. It was further mentioned that the most common reaction in some people is fever and chills and that other people are without any symptoms after eating the fermented breadfruit.

Food-borne diseases may be caused by a number of agents. Foremost, among these agents are bacteria. Some food-borne diseases have been investigated as to the causative agent and the causative agent determined, others have not been investigated as to the causative agent, and there are some in which the causative agent could not be identified (10) . If the above mentioned relationship exists, it is not known whether or not bacteria are responsible for the occurrence of the above reactions or disorders among those South Pacific Islanders who have consumed fermented breadfruit from pits in the ground.

These facts gave stimulus to the design of this study, a study of the storage and preservation of breadfruit as practiced in the South Pacific Islands.

PURPOSE AND SCOPE

The purpose of this study was to carry out a field and laboratory investigation into one of the older methods of storage and preservation of breadfruit by fermentation in pits in the ground as still utilized on some of the islands in the South Pacific area. The study was designed with a view toward learning more about the fermentation process by which the breadfruit is preserved and understanding more about a possible causal relationship between consumption of fermented breadfruit and likely bacterial infection giving rise to gastrointestinal disorders or reactions which have been reported in many unpublished reports. Emphasis was placed on the laboratory investigation of raw fermenting breadfruit because of natural limitations upon observations in the field.

The results of this study may aid in determining: 1.) better sanitation methods of storage in the pits, for safer food consumption and future field and laboratory studies; 2.) the type of fermentation occurring; 3.) the bacteria responsible; and 4.) the causal relationship between the consumption of fermented breadfruit and possible bacterial infections giving rise to gastrointestinal disorders or reactions.

The purpose of the investigation is to shed more light on the process and to gather information to add to the limited scientific knowledge of the fermentation process of

breadfruit during the period of preservation in the ground in the South Pacific area. It is also the purpose of this study to contribute to limited Public Health knowledge of the reported occurrence of reactions or disorders attributed to consumption of fermented breadfruit taken from earth pits in the ground and to stimulate further study in the field of food-borne diseases in which the causative agent has not been fully delineated.

The scope of this investigation has been limited to study of the storage and preservation by fermentation of a seedless variety of breadfruit obtained on the island of Oahu and includes: 1.) general observation of change in the breadfruit during fermentation; 2.) consideration of possible soil contamination, type of fermentation occurring and conditions under which the breadfruit ferments, namely, temperature, pH, and moisture; 3.) colorimetric test for sugar determination; 4.) estimation of bacterial growth; 5.) quantitative determination of total volatile acids produced during the fermentation; 6.) qualitative determination of ethyl alcohol formation; 7.) determination of gross heat of combustion values of the breadfruit during the fermentation; 8.) gas analysis to determine those gases evolved during fermentation; and 9.) isolation and tentative identification of the predominant bacteria responsible for the fermentation.

REVIEW OF LITERATURE

The literature concerning breadfruit describes its role in South Pacific folklore, history, geographical distribution, botany, composition and nutritive value, climatic requirements, and uses. The methods of preparation and preservation of breadfruit are found in the literature in fair abundance. The literature is sufficient to acquaint those unfamiliar with or interested in this tropical fruit.

Scientific studies on breadfruit and the breadfruit tree, however, are not prevalent. Several nutrition and composition studies on breadfruit have been done (4, 9, 11, 12, 13) . A survey of the Journal of Food Science (Food Research), Volume 1, 1936 to Volume 31, 1966 revealed no studies or research on the fermentation process of preservation of breadfruit during its period of preservation in pits in the ground in the South Pacific area. Mention was made by Massal and Barrau (4) of the occurrence of an intense acidification stage during the period of fermentation of breadfruit in pits in the ground. This acid preserved the breadfruit stored in this manner for long periods of time usually several months to a year or more.

Various references Buck (14) , Murai et al (9) , Gladwin (15) , Massal and Barrau (4) , Handy (5) , Linton (6) , and MacKenzie et al (7) described the practice of preservation of breadfruit by fermentation in pits in the ground.

No mention of reactions or disorders of the gastro-

intestinal type attributed to the consumption of fermented breadfruit taken from pits in the ground was uncovered in the literature.

Recent field reports, in the form of personal communications and correspondence, indicate that development of reactions or disorders of the gastrointestinal type may be attributed to consumption of fermented breadfruit taken from pits in the ground.

Ehlers and Steel (10) write: "the unsuccessful examinations of foods and stomach contents indicate that more research of food poisonings and infections is needed."

METHODS AND PROCEDURES

A field and laboratory study of the storage and preservation of breadfruit by fermentation in pits in the ground as practiced on some of the islands, in the South Pacific area was conducted with emphasis placed on the laboratory phase of research because of certain limitations evident in the field phase. The breadfruit, Artocarpus incisa, used in this study was obtained from the Kaneohe area on the windward side of the island of Oahu. It was a seedless variety with abortive ovules around the core.

Field Phase

The field phase was carried out in Palolo Valley, an area not far from the University of Hawaii, having climatic and other conditions, such as, warm to hot, humid weather, plenty of rain, moisture, and fertile soil, which seemed suitable for development of a breadfruit tree. In this area: 1.) several rectangular-like earth pits, measuring approximately 24" x 18" x 2', were dug and lined with rocks on the floor and part way up the earth walls; 2.) 3' x 26" regular window screens of 16 mesh were pushed into the pits to form flexible basket-like receptacles which were then carefully lined on the floor and sides with freshly picked ti (Cordyline terminalis) leaves in several layers; 3.) about 3 to 10 peeled and cored breadfruit, Artocarpus incisa, split into halves and washed with fresh water from the tap, were placed on top of the ti leaves; 4.) the mound of breadfruit

was then carefully covered with more ti leaves in several layers; 5.) large, heavy rocks, which acted as weights, were then placed on top of the ti leaf covered fruit as close to each other as possible; and 6.) the remainder of the pit was then filled in with earth up to the ground level and flattened. The breadfruit in the pits was then left to ferment for approximately 2, 4, and 6 month periods after which samples of breadfruit were dug up and tested for the following: 1.) pH; 2.) approximate amount of sugar in terms of sucrose; 3.) gross heat of combustion value; 4.) temperature; and 5.) bacterial growth. In transferring the breadfruit paste samples from the pits to the laboratory for analysis, care was taken to avoid any undue further contamination.

Laboratory Phase

The apparatus proposed for the laboratory study of the breadfruit fermentation process consisted of a series of plywood boxes fronted with transparent lucite panels and provided with various sampling points for analysis. Fig. 1 shows a cross sectional view of one of the breadfruit fermenters. The fermenters, 4 in all, were filled with: 1.) first, rocks which allowed for drainage of the breadfruit fermenter juice; 2.) second, several layers of ti (Cordyline terminalis) leaves; 3.) third, peeled, cored, and washed breadfruit halves; 4.) fourth, several more layers of ti leaves; 5.) fifth, more rocks; and 6.) sixth, soil taken

from Palolo Valley. The manner of filling the box fermenters followed very closely the manner of filling the pits in the ground as practiced on some of the South Pacific Islands. The fermenters were simply designed to give a cross-sectional view of the breadfruit fermentation process as it might occur underground. The breadfruit was left to ferment in the fermenters from two to three or more months. No water was poured into the breadfruit fermenters to simulate rain water.

The methods and procedures used for the examination of fermented breadfruit samples drawn from the laboratory fermenters and taken from the field pits were the same. The tests performed included determinations of temperature, pH, approximate sugar content, moisture content, total volatile acids, gases evolved, bacterial growth, gross heat of combustion values, and isolation and tentative identification of the predominant bacteria responsible for the fermentation. Observations included the changes of the breadfruit during the period of fermentation, taste, and odor.

Temperature Changes

Temperature fluctuations were determined with standard 100°C thermometers. Soil, fermented breadfruit, and room temperatures were observed and recorded, especially with regard to the laboratory fermenters. The temperature of the soil and fermented breadfruit in the pits was also observed and recorded.

pH Determination

Fermented breadfruit samples were taken through various sampling points from the laboratory fermenters and from the field pits with the help of a No. 3 cork borer. In order to prevent gross external contamination, aseptic technique was utilized. The cork borer was immersed into a graduated cylinder containing 95% ethanol, flamed, cooled approximately 30 seconds, and then used to obtain enough of the fermented breadfruit to raise the level of distilled water, in a 50 ml plastic graduated cylinder, from 8 to 10 milliliters. When this was done, the distilled water and fruit sample were poured into a small beaker and mixed thoroughly with a stirring rod to obtain as homogeneous a mixture as possible. The pH of this mixture was then determined with a Beckman pH meter. Standard Buffer solutions of pH 4 and 7 were used to standardize the pH meter before determining pH values. pH readings were taken every two days for about 3 months from the laboratory fermenters and for every field pit sample taken. The above mixture of fermented breadfruit in distilled water was then poured carefully into 75 x 125 mm test tubes, stoppered, and put into a refrigerator for later analysis of sugar content.

Sugar Content in Terms of Sucrose

Because of the transfer of the fermented breadfruit mixture from beaker to test tube, only an approximate estimation of the amount of sugar, in terms of mg/l sucrose

could be determined in the fermented breadfruit samples taken from the laboratory fermenters and field pits over the periods of analyses. The method for determining the approximate amount of sugar present was adapted from that described by Dubois *et al* (16) . In these determinations, the fermented breadfruit samples which had been stored in test tubes and placed in a refrigerator were used. The approximate dilution of these samples was an estimated 1:5 ratio. Each of the samples was then taken separately and poured directly from the test tubes into a Waring Blender. The test tube was then rinsed with 10 ml of distilled water and the contents also poured into the Waring Blender. The blender was turned on high speed for 5 minutes, stopped, and the contents poured into a plastic 10 x 4 cm centrifuge tube. The Centrifuge, International Clinical, Model Cl was turned to maximum speed for 5 minutes, stopped, and the supernatant solution was filtered with a monoject 12 ml plastic syringe through 0.45 µ metricel gridded filter paper into clean test tubes. The approximate dilution of these samples was an estimated 1:10 ratio. These samples were then diluted to appropriate volumes with distilled water and the amount of sugar present, in them, determined by a colorimetric method described by Dubois *et al* (16) . This method is delineated in the Appendix.

The colorimetric method for the determination of sugar developed by Dubois *et al* (16) was rapid and simple. It can

be used to determine submicro amounts of simple sugars, oligosaccharides, polysaccharides, and related substances. The method involves treating samples, containing sugars and related substances, with phenol and concentrated sulfuric acid (sp. gr. 1.84) . Upon the addition of concentrated sulfuric acid to a mixture of phenol and sugars or related substances, a yellow-orange color results which is stable for a period of time. If the yellow-orange color is within a proper range of concentration of sugars or related substances at a wave length of 480 or 490 mμ, it can with the help of a Bausch and Lomb Spectronic 20 colorimeter be used to determine the amount of sugar present.

In the determination of sugar all samples were prepared in triplicate. Blanks were made by substituting for the fermented breadfruit sugar solutions of appropriate strength with distilled water. Results were recorded as sugar in terms of mg/l of sucrose and were calculated by multiplying the final dilution factor times the amount of sucrose (mg/l) corresponding to the particular per cent transmittance value at wave length 480 mμ of each sample derived from previously determined sucrose standards.

Change in Moisture Content

Fermented breadfruit samples were taken from the laboratory fermenters every week for two and one-half months and the per cent moisture determined. Aseptic technique was used when sampling to avoid undue contamination of the contents.

A No. 3 cork borer was immersed in 95% ethanol, flamed, cooled about 30 seconds, and used to obtain a sample through one of the fermenter's sampling points. 2 to 4 gram samples were usually obtained in this manner. Moisture content was determined by drying in 100°C oven for 24 hours.

The change in per cent moisture content of the fermented breadfruit samples taken from the earth pits was determined in the same manner as those samples taken from the laboratory fermenters.

After the moisture content was determined, the fermented breadfruit samples from the fermenters and field pits were saved for later analysis of their gross heat of combustion values.

Bacterial Growth

Approximately 1 gram of the fermented breadfruit sample was obtained aseptically from the laboratory fermenters and field pits by means of a No. 3 cork borer for estimation of the amount of bacterial growth occurring during the breadfruit fermentation process. These 1 gram samples were then aseptically dispensed into 99 ml sterile water bottles and after being shaken thoroughly to form as homogeneous a mixture as possible a series of dilutions for each sample, ranging from 1/100 to 1/1,000,000,000 dilution, was made. Each dilution bottle was shaken thoroughly to insure good mixing and dispersion of the fermented breadfruit sample. Known amounts of each dilution were then transferred with

sterile pipettes to appropriately labeled petri dishes. Triplicate plates were made for all dilutions. To the petri dishes warm, sterile Stock Culture Agar was added and the contents thoroughly mixed and uniformly spread over the bottom of the petri dishes. The media was allowed to solidify and the petri dishes were then stored in an incubator for one week at 26° to 28°C. Readings were made every day on those triplicate plates showing not less than 20 and not more than 200 colonies of bacterial growth. A Darkfield Quebec colony counter was used for counting of colonies. The average of countable triplicate plates was taken and the results recorded as number of colonies per gram of fermented breadfruit.

Total Volatile Acids

Breadfruit juice samples collected every two days from sampling points at the base of the laboratory fermenters for a period of about two months were used for determining, quantitatively the total volatile acids occurring during the fermentation process. A Column Partition Chromatography Method, found in Standard Methods (17) , was used for determining total volatile acids.

The procedure involved filtering through Whatman No. 1 filter paper into test tubes enough of each sample to obtain approximately 20 ml . In some cases, where there was not enough of a sample or where the total volatile acid content of a sample was too high, portions of such samples were di-

luted to appropriate volumes and the dilution factors recorded. After filtering and/or dilution, 15 to 20 ml of each sample was put into a small beaker and three or more drops of thymol blue indicator solution (80 mg thymol blue in 100 ml of absolute methanol) added. 10N H_2SO_4 was then added, dropwise, until the first appearance of a red color was indicated. Ten grams of silicic acid, 100 mesh, slurried in distilled water and dried in an oven for 24 hours at approximately 100°C, was packed into a Buchner pyrex fritted-disc porosity F funnel by suction to form a column. Five milliliters of the acidified sample was then distributed uniformly over the surface of the silicic acid column with a pipette. Suction was applied until all of the sample entered into the column. Immediately, 50 ml of chloroform-butanol reagent was added to the column and suction applied at the bottom until nearly all of the reagent entered into the column. The 500 to 1000 ml filter flask was removed and a few drops of phenolphthalein indicator solution added to the filtrate in the flask. The filtrate was then titrated with 0.10N NaOH in absolute methanol dispensed. Aeration of the samples, thus contact with atmospheric CO_2, during titration of the sample was kept to a minimum. When titrating, the flask containing the filtrate was placed on a Mag-mix mixer at a speed just enough to slowly spin a magnetic stirring rod put into the flask. This prevented excessive swirling of the contents of the flask, by hand, to mix the titrant with

the filtrate. As an added and recommended precaution, nitrogen gas was delivered into the filtrate sample through a small glass tube throughout the titration process. Distilled water blanks were run in a similar manner, 5 ml samples were acidified, extracted with 50 ml of chloroform-butanol reagent, and titrated. Samples were done in triplicate and the average of the results recorded as organic acids in mg/l as acetic acid.

Gases Evolved During Fermentation

Gas chromatography utilizing a Fisher Gas Partitioner, model 227-PL and a Heathkit Servo Recorder was the method used for detecting gases evolved during fermentation of the fruit in the laboratory fermenters. Gas samples were obtained from one of the laboratory fermenters through a sampling point equipped with a rubber diaphragm with a 1.0 ml glass syringe. The samples (0.5 ml) were then injected into the inlet syringe port of the Fisher Gas Partitioner set at 100% sensitivity with a direct current of 7 milliamperes and under a pressure of 18 psi of helium flowing through the unit. The gases evolved were recorded as timed peaks on graph paper with a Servo recorder. Atmospheric gas and representative samples of pure or nearly pure gases were taken from a gas sampling tube and injected into the Fisher Gas Partitioner for the purpose of setting standards to determine where on the Servo Recorder graph paper and at what time intervals various gases would appear. Atmospheric, car-

bon dioxide, nitrogen, and oxygen gases were used to determine standards.

Gross Heat of Combustion

The dried fermented breadfruit samples from the laboratory fermenters and field pits upon which per cent moisture was determined were used to determine gross heat of combustion values of the fermented breadfruit samples. After the dried fermented breadfruit samples were formed into cylindrical pellets with the help of a pellitzer and weighed, their gross heat of combustion values were determined with a Parr oxygen bomb colorimeter (18) and assembly. Sample weights ranged from 0.8 to 1.8 grams. Results were calculated and recorded as the number of calories per gram of fermented breadfruit.

Isolation and Tentative Identification of the Predominant, Pigmented, and Other Bacteria Responsible for Fermentation

An attempt was made to isolate and tentatively identify some of the predominant bacteria responsible for the fermentation of breadfruit in the laboratory fermenters and field pits with special emphasis on the detection, and possible isolation and identification of types of microorganisms causing food-poisoning.

Microscopic examinations of Gram stain smears of distinctly different microbial colonies appearing on the Stock Culture Agar plate cultures prepared from a series of dilutions of fermented breadfruit samples taken from the labor-

atory fermenters and field pits, were made to determine the morphological types of bacteria occurring in the fermenting breadfruit during the fermentation process.

The predominant, pigmented, and other striking types of bacterial colonies found on the Stock Culture Agar plates and other plating media were selected and isolated on Nutrient, Stock Culture, and Asparagine-modified Beef Malt Agar plates until pure cultures were obtained. These microorganisms were then aseptically inoculated and stocked on tubed agar slants of the same media and saved for tentative identification.

Methods recommended by the American Public Health Association (19) for the microbiological examination of foods were used as a means for the detection, isolation, and/or identification of food-poisoning types of microorganisms, specifically, Clostridia, Staphylococci, Streptococci, Shigella, and Salmonella microorganisms in the fermented breadfruit samples taken from the laboratory fermenters and field pits.

For the detection of viable Clostridia microorganisms, two techniques for maintenance of anaerobiasis during culturing were used. The first technique, a simplified technique for anaerobic culture (20) , was quick and simple. It involved inoculating directly some of the fermented breadfruit samples taken from the laboratory fermenters and field pits onto plain or Tryptose Blood Agar plates using outdated

human blood, inverting the blood agar plates on top of their respective covers upon which had been previously placed a Whatman No. 1 filter paper and 1 part of sodium carbonate, reagent grade, mixed with 4 parts of pyrogallic acid. The inverted blood agar plates were immediately sealed to their covers with melted paraffin. Anaerobic conditions were indicated by the reaction of sodium carbonate and pyrogallic acid with oxygen present as evidenced by a darkening of the chemicals. The second technique used for maintenance of anaerobic conditions was more time consuming and involved the use of an inflatable polyethylene Glove Bag, Model X-37-27, into which all essential equipment was placed before purging and inflating of the glove bag with an inert atmosphere of nitrogen gas, delivered from a cylinder, was undertaken. The nitrogen gas was pumped in and taken out by suction about 5 times before actual culturing was initiated. The inoculating needles used for streaking and transferring of cultures were sterilized either beforehand in an autoclave or with a Sensaur Bacti-Cinerator placed within the glove bag. Fermented breadfruit samples were inoculated either directly or from previously inoculated cooked meat media tubes maintained under anaerobic conditions by rubber connections to comparable tubes containing 10% NaOH and pyrogallic acid. Triplicate cultures were transferred to specially made anaerobic petri dishes containing Brewer's Anaerobic Agar, with 10% NaOH and pyrogal-

lic acid as a scavenger, sealed with paraffin or into triplicate regular petri dishes filled with Brewer's Anaerobic Agar or Blood Agar. The common petri dishes were then put into an anaerobic bell jar within the glove bag. The bell jar was purged with nitrogen gas for approximately 30 minutes, and clamped tight. Incubation of the above culture media, maintained under anaerobic conditions, was carried out for approximately 1 week at 25° to 30°C. Anaerobic conditions, within the glove bag, were indicated by the reaction of a 10% NaOH solution and pyrogallic acid with oxygen as evidenced by a darkening of the mixture or by observation of a change in the color of Brewer's anaerobic media due to the sensitivity of an indicator, incorporated within the media, to the presence of oxygen.

For the detection of Staphylococci, fermented breadfruit samples were inoculated onto Mannitol Salt, Blood, or Staphylococcus Medium No. 110 Agar plates either directly or with 0.1 ml of 1/10, 1/30, 1/50, 1/70, and 1/100 dilutions of the sample and incubated at 35° to 37°C for 3 days. Duplicate to triplicate plates were inoculated. Gram stain smears were made of the different colonies found in checking for Staphylococcal organisms.

For the detection of Streptococci, fermented breadfruit samples were streaked directly onto the surfaces of plain or Tryptose Blood Agar plates and incubated at 35° to 37°C for 48 hours. Duplicate to triplicate plates were made

for each sample. Gram stain smears were made of the different colonies found.

For the detection of Salmonella organisms, an enrichment technique utilizing Tetrathionate Broth was used. Approximately 1 gram of fermented breadfruit sample was put, aseptically, into 10 ml of Tetrathionate Broth prepared the same day of use. Triplicate tubes of the broth were made, shaken to expose as much of the sample, to the broth medium and incubated at 35° to 37°C for 24 hours. At the end of the period of incubation, inoculum from each of the Tetrathionate Broth tubes was streaked onto triplicate plates of Salmonella-Shigella (SS) Agar and Eosin Methylene Blue (EMB) Agar. All plate cultures were then incubated at 35° to 37°C for 24 to 72 hours. Gram stain smears were made of the colonies growing.

From the above cultures, an attempt to identify the principal, pigmented, and other microbial colonies found, was carried out after isolation of pure cultures.

Information was gathered on the morphological, cultural, and biochemical characteristics of each microorganism. The tests for Coliform bacteria and those tests necessary for the identification of other bacteria were employed utilizing all available equipment, reagents, and standard or differential media necessary. All reagents and media that were used are listed in the Appendix.

Detection of Alcohol

A 250 ml portion of combined samples of breadfruit fermentation juice collected from the laboratory fermenter over a two month period, was distilled by direct simple distillation after the addition of salt and a watery-like, turbid distillate obtained. The temperature remained at 103°C throughout the distillation process.

Approximately 50 ml of the distillate was treated once with activated charcoal and filtered through a 0.45 μ metricel gridded filter paper.

Two qualitative tests, Iodoform and Ethyl Benzoate tests (21) , were carried out to determine the presence of ethyl alcohol.

In the Iodoform test, 10 drops of a solution of 10% NaOH was added to a 5 ml portion of the distillate. The mixture was swirled and a solution of iodine in potassium iodide added dropwise until a slight permanent yellow color was produced. The solution was left to stand a few minutes, shaken, and the formation of iodoform, a yellowish crystalline precipitate with a characteristic odor, noted. Two trials and one control using 95% ethanol were run.

In the Ethyl Benzoate test, 3 drops of benzoyl chloride and 3 ml of a solution of 10% NaOH were added to 2 ml of the distillate, shaken together and warmed gently. Formation of the ethyl benzoic ester denotes the presence of ethyl alcohol and may be identified by its odor or boiling point. Two trials and one control using 95% ethanol were run.

RESULTS AND DISCUSSION OF RESULTS

During the period of this study, general observations were made of the breadfruit as it fermented in the laboratory box fermenters and when it was brought into the laboratory from the field pits.

The laboratory box fermenters, hereafter called fermenters, made possible observation of a cross-sectional view of the breadfruit fermentation process as it would occur underground. Figures 1 through 9 reveal the progress and some of the changes made in the breadfruit as it fermented in the fermenters.

During the first 2 days of fermentation of the breadfruit, in the fermenters, the following general observations were made: 1.) a drop in the level of the soil in the fermenter indicating a softening of the breadfruit under the weight of the overlying rocks; 2.) the appearance of liquid condensation on the inside of the lucite panel fronting the fermenting breadfruit inside the fermenter indicating the presence of moisture; 3.) the rapid accumulation of breadfruit juice at the base of the fermenter due to pressing of the fermenting breadfruit by the weight of the overlying rocks; 4.) a sweet and pungent, passion fruit-like odor emitted by the fermenting breadfruit; 5.) no noticeable change in color of the surfaces or the interior of the fermenting breadfruit; 6.) the intrusion of overlying soil onto the fermenting breadfruit occurred in 3 of the 4 fermenters

Figure 1. Cross sectional view of breadfruit in laboratory fermenter after 0 days.

Figure 2. Cross sectional view of breadfruit in laboratory fermenter after 3 weeks.

Figure 3. Cross sectional view of breadfruit in laboratory fermenter after 6 weeks.

Figure 4. Cross sectional view of breadfruit in laboratory fermenter after 10 weeks.

Figure 5. Cross sectional view of breadfruit in laboratory fermenter after 14 weeks.

Figure 6. Cross sectional view of breadfruit in laboratory fermenter after 17 weeks.

Figure 7. Cross sectional view of breadfruit in laboratory fermenter after 21 weeks.

Figure 8. Cross sectional view of breadfruit in laboratory fermenter after 23 weeks.

Figure 9. Cross sectional view of breadfruit in laboratory fermenter after 27 weeks.

prepared indicating a need for careful covering of the fruit with ti leaves when storing; and 7.) the gradual appearance of small, segmented worms or larvae on the surface of the fermenting breadfruit was noted in, at least, 3 of the 4 fermenters indicating inoculation presumably by fruit flies of the breadfruit prior to picking from the tree.

The visual appearance of the breadfruit in the fermenters remained the same after 1 week, with the exception of a few changes. One exception was that the water condensed on the inside of the lucite panel fronting the fermenting breadfruit began to disappear. Two other changes in appearance were noted: 1.) the appearance of a bubbly, milky, white latex-like substance over the surfaces of the fermenting breadfruit; and 2.) a foaming and formation of gas bubbles in the breadfruit juice collecting over the rocks at the base of the fermenter, indicating gas evolution.

After 2 weeks, everything was essentially the same with the exception that the odor, while still being pungent, was no longer sweet but sour. The fermenting breadfruit appeared to be much softer, at least on the surfaces, and gave the impression of melting, like butter, and running down the inside of the lucite panel inside the fermenter. The surfaces of the fermenting breadfruit began to take on a yellowish-brown color while the interior remained essentially yellow or yellowish-white. Aside from the worms or fly larvae which were still moving over the surface of the fermen-

ting breadfruit small, black bugs, fruit flies, and other small insects were noted on or near the surfaces of the ti leaves and fermenting breadfruit within the fermenter.

Within 1 month, the amount of breadfruit juice collecting at the base of the fermenter gradually diminished. The appearance of a dark mold and a white scum-like growth, possibly yeasts, was noted on portions of the ti leaves and/or fermenting breadfruit. However, such growth was not extensive and did not increase to any great degree during the 6 month period of study.

After 2-3 months in the fermenter the fermenting breadfruit appeared much the same as it did after 1 month. The surface color was a light to dark yellowish-brown while the interior of the fruit remained yellow to yellowish-white in color. It was soft and paste-like when handled and had developed a rather strong and very unpleasant odor.

Observations of the breadfruit fermenting in the field pits were limited to periods when fermenting breadfruit samples were brought out of the field pits after 2, 4, and 6 month intervals. Figures 10 and 11 show fermenting breadfruit as it appeared when removed from the field pits after 2 and 6 months of storage. The following general observations were made of the breadfruit fermenting in the field pits: 1.) a drop in the level of the soil above the fermenting breadfruit, in the field pits, occurred indicating a softening of the breadfruit under the weight of the over-

Figure 10. Breadfruit from field pit after 2 months storage.

Figure 11. Breadfruit from field pit after 6 months storage.

lying rocks as mentioned in the fermenter; 2.) soil contamination on certain portions of the breadfruit occurred indicating the need for careful covering of the fruit with ti or other leaves when storing as mentioned in the fermenter. Over-all, the layer upon layer of ti leaves shielding the fermenting breadfruit can and did act as an efficient cover in both protecting and aiding preservation of the major portions of the stored breadfruit; 3.) no visible mold or white scum-like growth, as seen in the fermenter, was noted on the surfaces of the fermenting breadfruit; 4.) small, segmented, whitish worms or larvae, black bugs, and various other small insects were seen crawling over the ti leaves and surfaces of the fermenting breadfruit. The interior portions of the fermenting breadfruit appeared free of such insects; 5.) upon peeling away the criss-crossed mat of ti leaves covering the fermenting breadfruit a very strong and rather unpleasant odor, difficult to describe, was emitted; 6.) on the surface, the fermenting breadfruit was a light to medium yellowish-brown in color. The surfaces of the fermenting breadfruit appeared to be spoiled especially by the worms or fly larvae and various other insects crawling over them; and 7.) the interior and major portions of the fermenting breadfruit were yellow to yellowish-white in color, soft to firm in texture, and appeared as fresh as the day they were put into the field pits.

A small portion of fermented breadfruit taken from the

field pit after 2 months was tasted. The portion sampled was a very pale yellow in color. It was neither sweet nor disagreeable but rather soft, almost tasteless, and had a strong odor. A native islander from the Truk District, in the Eastern Caroline Islands (45) , related, "the fermented breadfruit tastes sour and has an awful smell. The best way some people prefer it, is when they squeezed off the juice and it doesn't taste sour."

Temperature Conditions

Table I together with Figure 12 shows the temperature relationship observed between laboratory and soil and breadfruit stored in the fermenters over a period of 2 months. The range of temperatures recorded for laboratory, soil and fermenting breadfruit were 22.0° to 28.1°C, 22.5° to 27.0°C, and 24.0° to 27.3°C, respectively. The temperature of the fermenting breadfruit was found to vary closely with and was always greater than that of the overlying soil. The temperatures of the soil and breadfruit in the fermenters were independent of the laboratory temperatures.

In the field pits, the temperature of the fermenting breadfruit at the pits was approximately 22.0°C. The fermenting breadfruit sample taken out of the field pit after a 2 month period registered a temperature of approximately 27.0°C. The soil, in the field pits, after the fermented breadfruit was removed also registered a temperature of approximately 22.0°C.

TABLE I. TEMPERATURE RELATIONSHIP BETWEEN LABORATORY AND SOIL AND BREADFRUIT IN LABORATORY FERMENTER

Number of Days	Temperature, °C*		
	Laboratory	Soil	Breadfruit
2	22.0	22.5	24.0
6	25.5	22.5	24.5
10	23.5	22.5	24.5
14	25.8	25.0	26.8
18	26.5	24.5	26.5
22	26.0	24.0	26.0
26	26.5	25.5	26.5
30	26.9	25.8	27.2
34	27.8	25.0	26.5
38	28.1	24.5	26.0
42	27.8	25.7	27.0
46	25.7	24.5	25.5
50	28.0	25.8	26.5
54	27.2	27.0	27.3
58	26.8	25.8	26.8
62	24.7	24.7	25.5

*Approximate temperatures

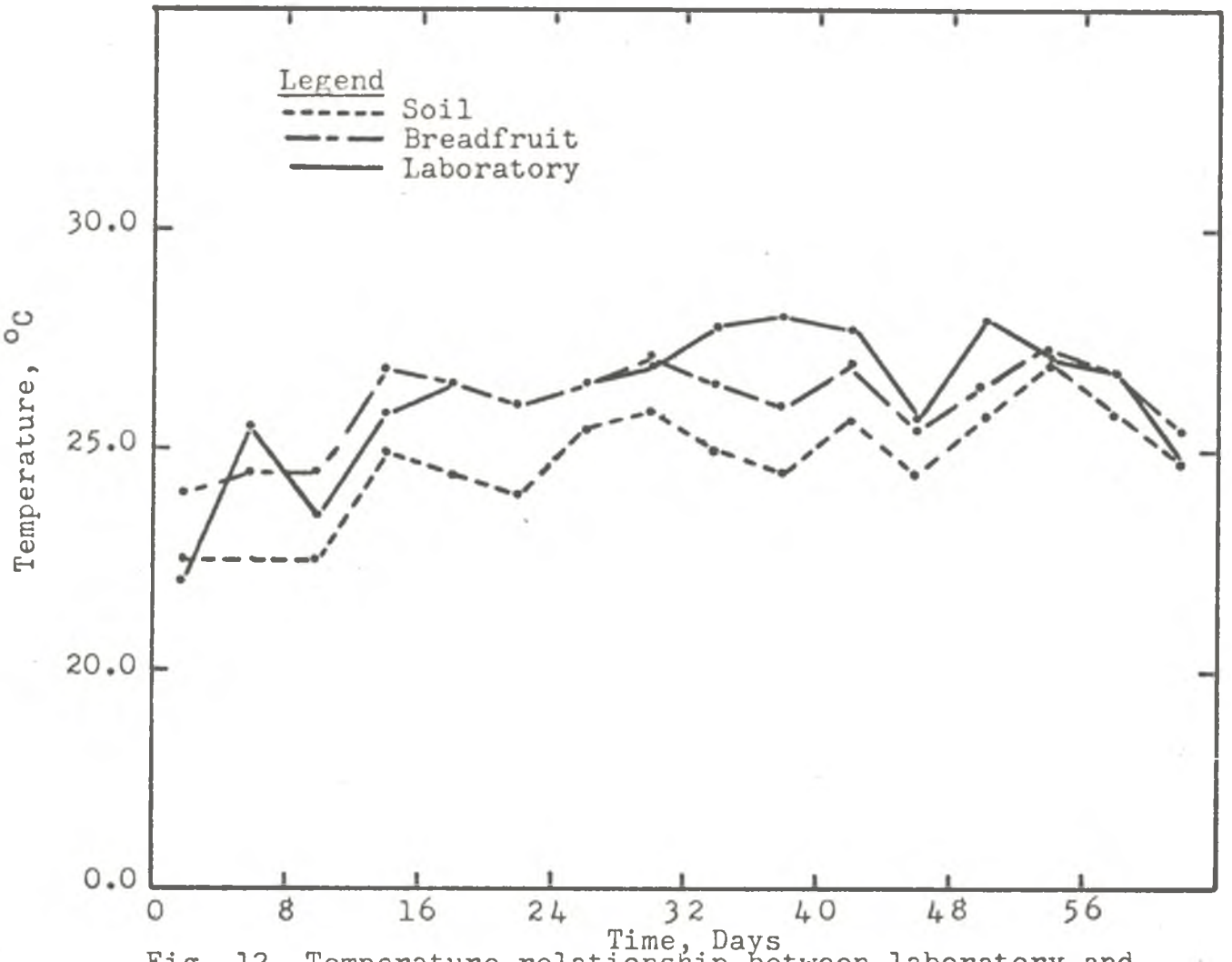


Fig. 12. Temperature relationship between laboratory and fermenting breadfruit and soil in laboratory fermenter over a period of time.

Three major groups of bacteria classified according to their growth temperatures are the Psychrophiles, Mesophiles, and Thermophiles. The Mesophilic group is perhaps the most important of the three groups because it is within this group that pathogenic bacteria are included. In general, the Mesophilic bacteria are limited to an optimum growth temperature range of from 20° to 45°C (22) .

Temporarily ignoring pH conditions, the temperature range of approximately 22° to 27°C observed in the fermenting breadfruit indicates the possibility of pathogenic bacteria occurring.

Moisture Content

Data gathered on the per cent moisture content of breadfruit fermenting in the fermenters and field pits is recorded in Tables II and III. Figure 13 shows graphically the per cent moisture trend in one of the fermenters with time.

The average per cent moisture content of a fresh, semi-ripe to ripe breadfruit of the type used, in this study, was approximately 66.4%. Murai et al (9) found the Batakdak, Bukdrol, and Mijiwan varieties of fresh breadfruit, in the Marshall Islands, to have approximate per cent moisture contents of 68.2%, 70.8%, and 68.3%, respectively and the Atchapar, Meichon, Meikoch, Napar, Meisoso, and Sawan varieties of fresh breadfruit in the Caroline Islands, to have

TABLE II. APPROXIMATE PER CENT MOISTURE OF BREADFRUIT STORED IN LABORATORY FERMENTER

Number of Weeks*	Per Cent Moisture**
0	66.4
1	63.9
2	66.2
3	65.7
4	65.0
5	64.7
6	70.2
7	72.2
8	73.8
9	75.7
10	75.6

*0 = Semi-ripe breadfruit from tree
**Average of 2 moisture determinations

TABLE III. APPROXIMATE PER CENT MOISTURE OF BREADFRUIT STORED IN FIELD PITS

Number of Months*	Per Cent Moisture***
0**	66.4
2	77.3
4	82.5
6	69.7

*Approximate number of months
**0 = Semi-ripe to ripe breadfruit from tree
***Average of 2 moisture determinations except for the 4 and 6 month values

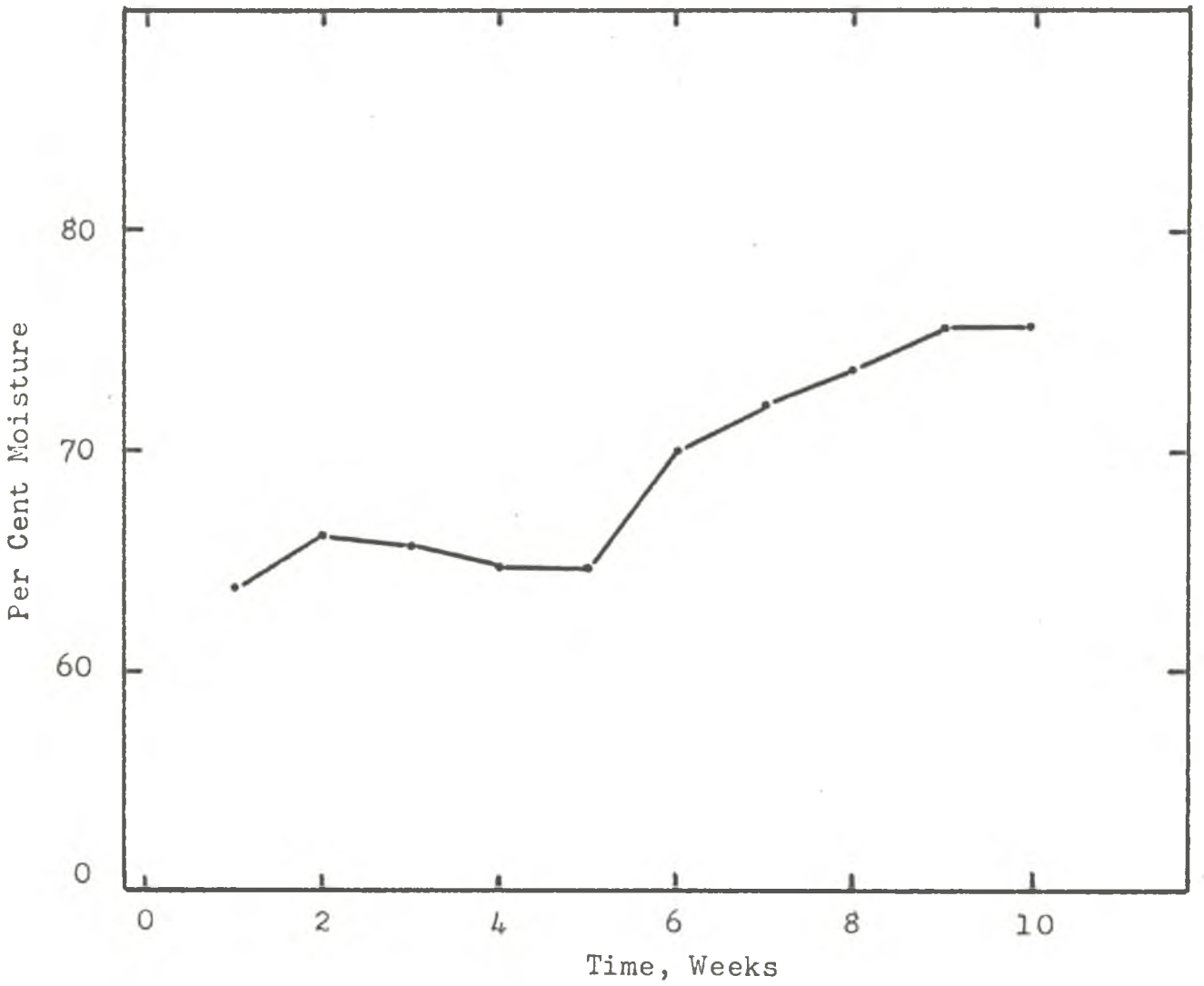


Fig. 13. Change in per cent moisture content of breadfruit stored in laboratory fermenter over a period of time.

approximate per cent moisture contents of 69.5%, 67.6%, 74.3%, 63.8%, 72.9%, and 66.9%, respectively. Peters et al (13) found a fresh breadfruit from Western Samoa to have an approximate per cent moisture content of 80.0%.

The approximate per cent moisture content of the breadfruit fermenting in the fermenters, ranged from 63.9% to 75.6% as shown in Table II. During the first 5 weeks, fermenting breadfruit was obtained through the same sampling port of one of the fermenters and no appreciable change in the per cent moisture content of the fermenting breadfruit was noted. The following 5 weeks, fermented breadfruit was obtained through another sampling port of the same fermenter situated directly below the first sampling port because it was no longer possible to obtain fermented breadfruit through the first sampling port with the No. 3 cork borer used. A slight rise of approximately 2%, in per cent moisture, was recognized in the fermenting breadfruit during the 7th, 8th, and 9th weeks. No rise was noted during the 10th week. Over-all, there does not seem to be an appreciable change in per cent moisture content. The degree of ripeness of the one type of fresh breadfruit used in this study may have had a bearing on the per cent moisture content of the fermenting breadfruit.

The approximate per cent moisture content of the breadfruit fermenting in the field pits for approximately 2, 4, and 6 months is shown in Table III. The per cent moisture

content appears, on the average, to be much higher in the fermenting breadfruit in the field pits than in the fermenter. Here, aside from the degree of ripeness of the breadfruit, the amount of rainfall or precipitation passing through the soil in the field pits may also have been an influencing factor. No rainfall or water was allowed to percolate through the soil in the "laboratory" fermenter. This was one of the limiting factors mentioned earlier.

Change in pH

The pH value of a fresh, semi-ripe to ripe breadfruit of the type used in this study was approximately 6.02. Murai et al (9) found the pH values of the Atchapar, Meichon, Meikoch, Napar, Neisoso, and Sawan varieties of fresh breadfruit pulp, in the Caroline Islands, to be 6.1, 5.7, 6.1, 5.9, 6.1, and 6.0, respectively.

The pH values of the breadfruit fermenting in the fermenters and field pits are summarized in Tables IV and V. A sharp decrease and leveling off in pH of the breadfruit fermenting in the fermenters, as shown in Table IV and Figure 14, was observed. Over a 2 month period of fermentation, the pH value of the fermenting breadfruit dropped from approximately 6.02, pH of a fresh semi-ripe to ripe breadfruit, to 3.78. Massal and Barrau (4) report that fermentation causes an intense acidification which will preserve the fresh breadfruit stored in pits in the ground for months and perhaps years. The rapid decline in pH and consequent increase in

TABLE IV. pH VALUE OF MIXTURE OF DISTILLED WATER AND BREADFRUIT TAKEN FROM LABORATORY FERMENTER

Number of Days*	pH Value
0	6.02
2	5.11
4	4.32
6	4.13
8	4.08
10	4.11
12	4.08
14	4.07
16	4.00
18	3.93
20	3.88
22	3.84
24	3.89
26	3.84
28	3.80
30	3.81
34	3.79
38	3.79
42	3.79
46	3.87
50	3.74
54	3.80
58	3.80
62	3.78

*0 = Semi-ripe to ripe breadfruit from tree

TABLE V. pH VALUE OF MIXTURE OF DISTILLED WATER AND BREADFRUIT TAKEN FROM FIELD PITS

Number of Months*	pH Value
0**	6.02
2	4.28
4	4.60
6	4.21

*Approximate number of months
**0 = Semi-ripe to ripe breadfruit from tree

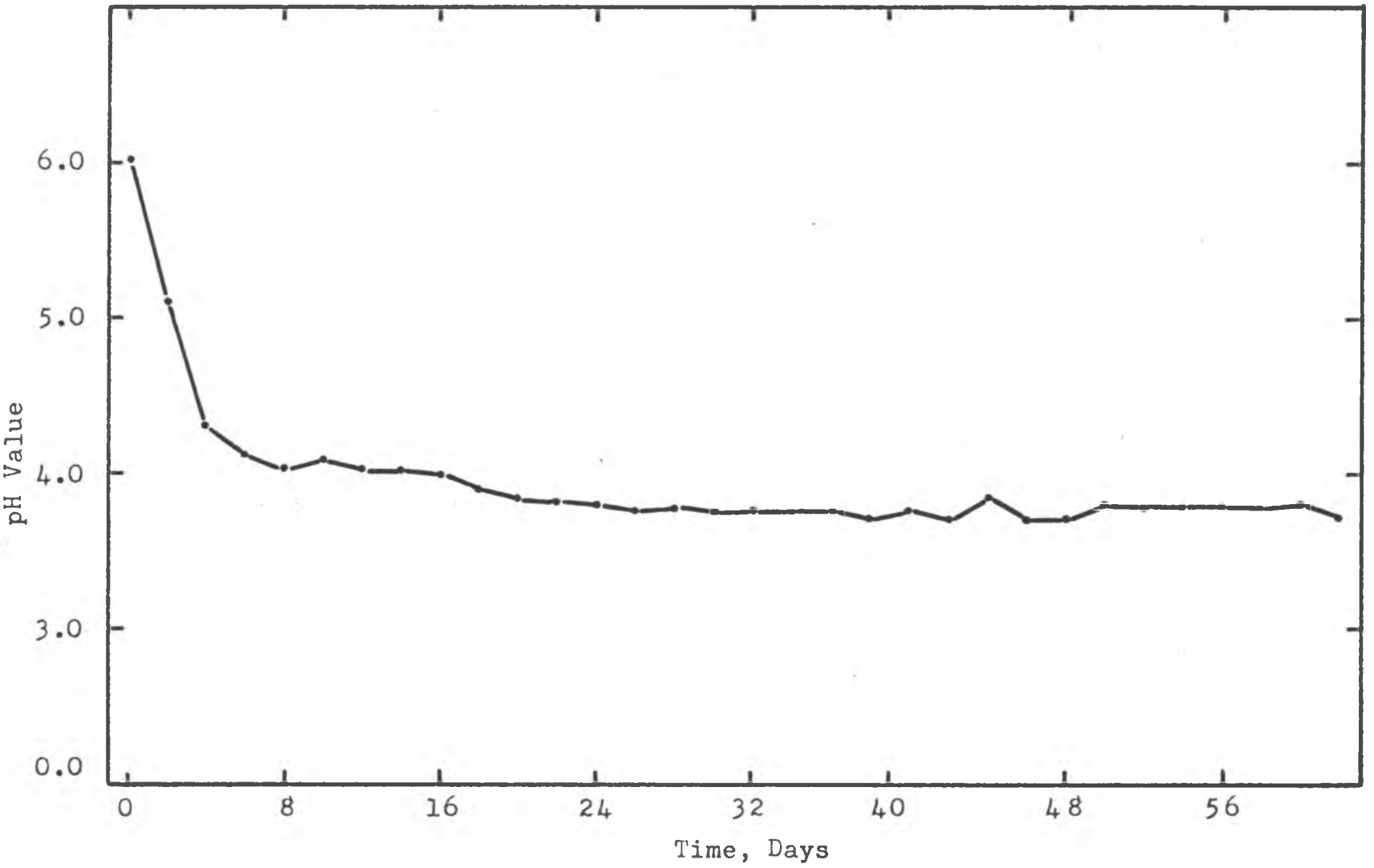


Fig. 14. Change in pH value of breadfruit stored in laboratory fermenter over a period of time.

acidity of the fermenting breadfruit in the fermenter would indicate the occurrence of an acid type of fermentation. An acid fermentation is one of the common methods of food preservation.

The pH values of the breadfruit fermenting in the field pits for nearly 2, 4, and 6 months, as shown in Table V, were 4.28, 4.60, and 4.21, respectively. The pH value of 4.28 of the 2 month old fermented breadfruit from the field pit is much higher than the pH value of 3.78 of the 2 month old breadfruit from the fermenter. It appears from these results that under natural environmental conditions, the pH values of the breadfruit fermenting in the field pits may be higher than those of the breadfruit fermenting in the fermenter. There is some doubt as to the validity of the pH value of 4.60 of the 4 month old fermented breadfruit obtained from the field pit. The 4 month old fermented breadfruit appeared abnormal, in regard to color, odor, and texture, when compared with the other better preserved breadfruit samples taken from the field pits.

Total Volatile Acids in Fermented Breadfruit Juice

The Column Partition Chromatography Method used for analysis of total volatile acids present in fermented breadfruit juice obtained from the fermenter over a period of 2 months was reported in the literature to give, "average recovery efficiencies of about 95% for organic acid concen-

trations in excess of 200 mg/l as acetic acid" (17) .

Table VI and Figure 15 present the results of the quantitative determination of the total volatile acids present in the fermented breadfruit juice obtained from the fermenter.

The results indicate a sharp rise in the total volatile acid content of the fermented breadfruit juice from approximately 13,464.4 mg/l in 2 days to 18,205.6 mg/l in 10 days, and to a maximum of 23,108.5 mg/l in 22 days. Thereafter, a gradual leveling off and decline in total volatile acids present were observed. An increase in the total volatile acids present in the fermented breadfruit juice is most likely due to greater microbial breakdown of breadfruit carbohydrate. The increase in total volatile acids, in the fermented breadfruit juice, further supports, at least in part, the rise in acidity occurring in the breadfruit fermenting in the fermenter.

The test for volatile acids served to indicate the presence of low-molecular weight acids occurring during the fermentation process and as an index of fermentative breakdown.

Gases Evolved During Fermentation

With the aid of a Fisher Gas Partitioner and Heathkit Servo Recorder, an analysis to determine those gases produced during the fermentation of breadfruit carbohydrate and protein, in one of the fermenters, was carried out. The

TABLE VI. TOTAL VOLATILE ACIDS PRESENT IN BREADFRUIT JUICE OBTAINED FROM LABORATORY FERMENTER

Number of Days	Organic Acids in Mg/l as Acetic Acid*
2	13464.4
6	13626.2
10	18205.6
14	19313.1
17	23009.0
22	23108.5
26	18595.1
30	19535.4
34	18681.7
38	16702.2
40	17647.2
43	18204.5
46	18885.6
48	16161.1
50	16408.8
52	11702.9
54	10216.8
56	8359.2
58	4644.0
60	6291.1
62	5510.9

*Average of 3 sample determinations

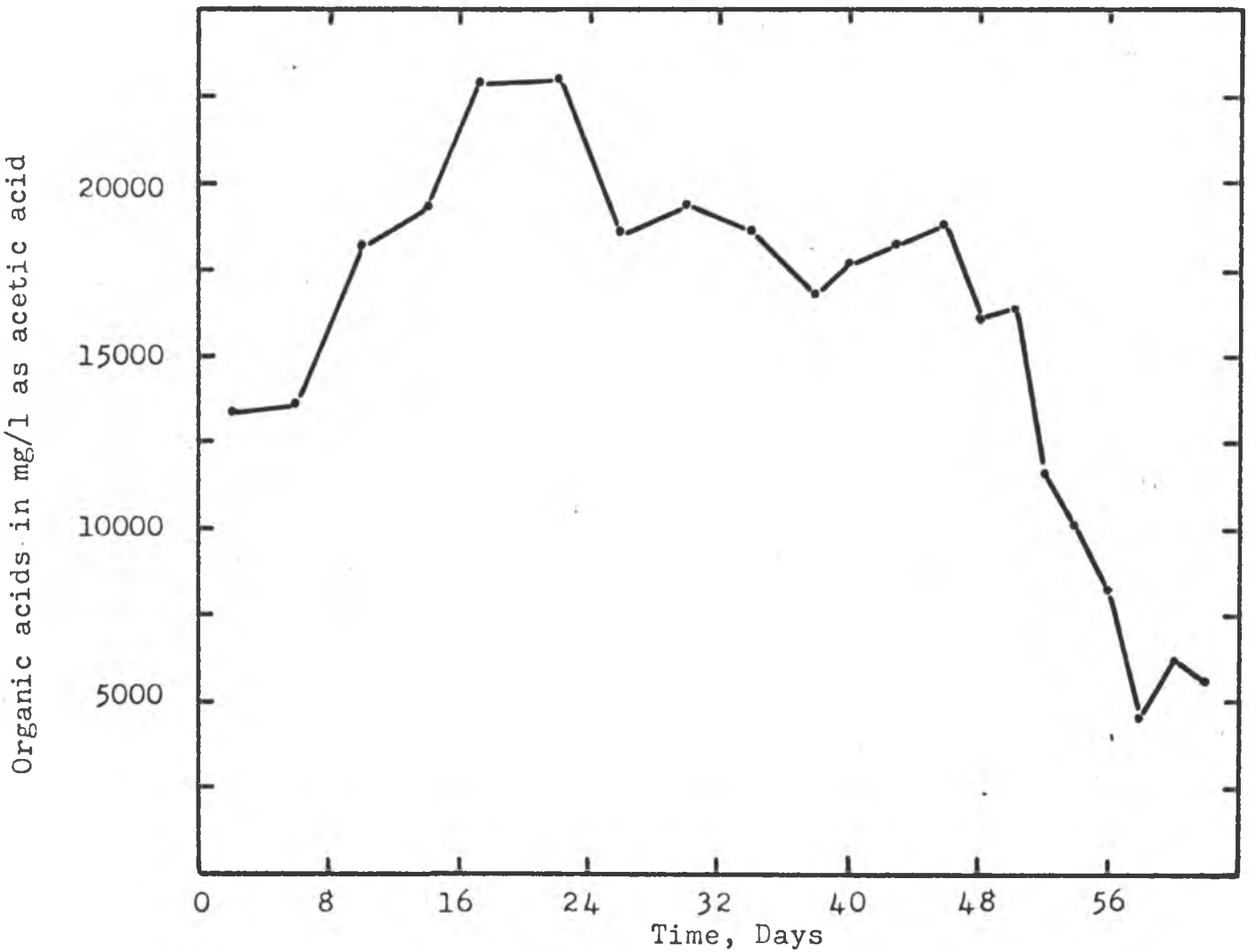


Fig. 15. Total volatile acids occurring in breadfruit juice obtained from breadfruit stored in laboratory fermenter over a period of time.

fermenter had been set up and prepared approximately 1 month prior to the first gas analysis and a fermentation of the stored breadfruit contents was assumed to be well in progress. However, before a gas analysis could be carried out, one of the sampling ports of the fermenter had to be replaced with a cork stopper through which a small piece of glass tubing equipped with a rubber diaphragm for the purpose of gas sampling was inserted. Atmospheric oxygen may have been introduced in this or some other way because oxygen was among the gases detected in the first gas analysis.

The gases detected from the breadfruit fermenting in the fermenter over a 2 month period following 1 month of previous storage and fermentation were carbon dioxide, nitrogen, and oxygen as determined by comparison with pure or nearly pure samples. Oxygen was detected in very small quantities during the 14 days following the first gas analysis with the exception of the 6th day in which it was not detected. After the 14th day, oxygen was no longer detected, except again on the 30th day, an isolated sample, for the remainder of the 2 month period following the month when the fermenter was set in operation. Carbon dioxide and nitrogen were the only two gases detected consistently throughout the 2 month period of analysis.

The absence of oxygen indicated the presence of anaerobic conditions throughout most of the fermentation process. The production of carbon dioxide and nitrogen indicated the

fermentative breakdown of carbohydrate and protein in the breadfruit stored in the fermenter.

Gross Heat of Combustion Values

The gross heat of combustion values represent the amount of heat given up when the dried fermented breadfruit samples were completely oxidized plus the amount of heat given up by the water vapor formed within the oxygen bomb as it was condensed and cooled to the temperature of the oxygen bomb (18) . The gross heat of combustion values determined for the breadfruit fermenting in the fermenter and field pits are given in Tables VII and VIII.

From the results, as shown in Table VII and graphically presented in Figure 16, there appears to be no appreciable change in the gross heat of combustion values of breadfruit fermenting in the fermenter. After 1 week of fermentation, the gross heat of combustion value of the breadfruit stored in the fermenter was approximately 4,157.6 calories per gram. After 5, 9, and 13 weeks, it was approximately 4,137.6, 4,198.1, and 4,133.7 calories per gram, respectively. In 15 weeks, it was approximately 4,189.1 calories per gram. For a fresh, semi-ripe to ripe breadfruit picked from the tree and of the type used, in this study, the gross heat of combustion value was approximately 4,070.7 calories per gram. There is no significant difference between this value and those obtained from breadfruit fermenting, in the fermenter, over a period of 15 weeks.

TABLE VII. GROSS HEAT OF COMBUSTION VALUES FOR BREADFRUIT STORED IN LABORATORY FERMENTER

Number of Weeks*	calories Per Gram of Breadfruit**
0	4070.7
1	4157.6
2	4182.3
3	4198.8
4	4194.3
5	4137.6
6	4112.4
7	4239.7
8	4161.2
9	4198.1
10	4078.9
11	4108.4
12	4109.2
13	4133.7
14	4151.9
15	4189.1

*0 = Semi-ripe to ripe breadfruit from tree
**One determination made for each sample

TABLE VIII. GROSS HEAT OF COMBUSTION VALUES FOR BREADFRUIT STORED IN FIELD PITS

Number of Months*	calories Per Gram of Breadfruit***
0**	4070.7
2	4186.7
4	3920.2
6	3844.7

*Approximate number of months
**0 = Semi-ripe to ripe breadfruit from tree
***One determination made for each sample

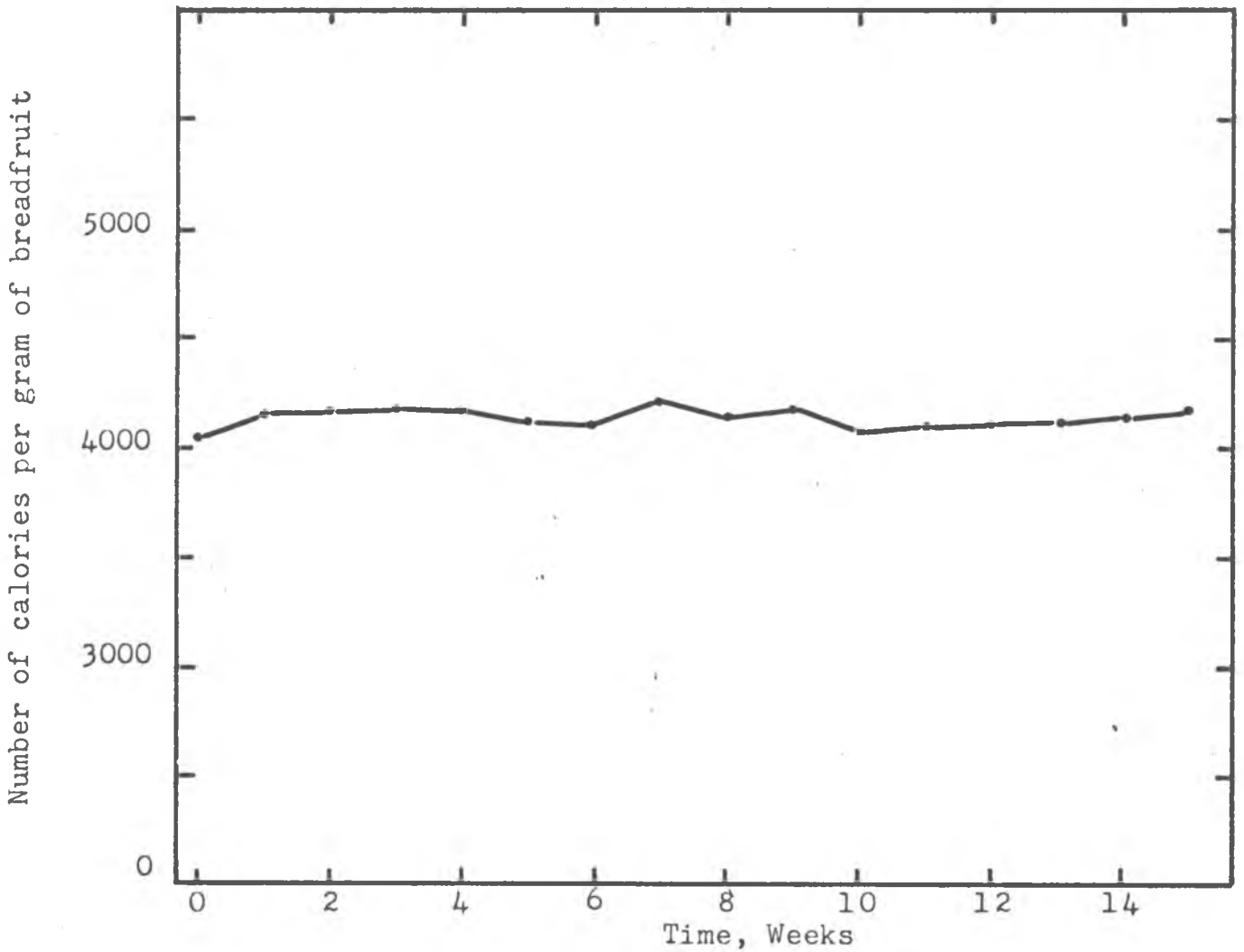


Fig. 16. Change in gross heat of combustion value for breadfruit stored in laboratory fermenter over a period of time.

The gross heat of combustion values determined for the breadfruit fermenting in the field pits, as shown in Table VIII, indicate that under natural environmental conditions, there may be a slight decrease in the gross heat of combustion value over a period of time. More trials per fermented breadfruit sample should be run and fermented breadfruit samples, taken out of the field pits at 1, 3, and 5 month intervals, at least, should be tested to better interpret the results already obtained.

Bacterial Growth

The viable or plate count method, one of the standard methods for estimating bacterial growth, was used in enumerating the number of bacterial colonies per gram of fermenting breadfruit in the fermenter and field pits. In using this method, Collins (23) reports that a maximum of care in technique should be taken and plate counts obtained should be interpreted freely with the idea that errors due to material analyzed or technique may result even if plate counts are done in triplicate.

Estimation of the amount of bacterial growth occurring in breadfruit fermenting in the fermenter and field pits is presented in Tables IX and X as the number of colonies and the log number of colonies per gram of fermenting breadfruit. From the data collected, it is evident that with a drop in pH there is a consequent reduction in the number of bacterial colonies per gram of fermenting breadfruit. This ap-

TABLE IX. BACTERIAL GROWTH IN BREADFRUIT STORED IN LABORATORY FERMENTER

Number of Days	Number of Colonies Per Gram of Breadfruit x 10^6*	Log. Number of Colonies Per Gram of Breadfruit
2	186.000	8.26951
6	180.000	8.25527
10	200.000	8.30103
14	171.000	8.23300
18	40.000	7.60206
22	125.000	8.09691
26	151.000	8.17898
30	56.000	7.74819
34	9.900	6.99564
38	14.200	7.15229
42	7.000	6.84510
46	91.000	7.95904
50	0.037	4.56820
54	8.800	6.94448
58	3.800	6.57978
66	5.800	6.76343
70	0.840	5.92428
74	0.770	5.88649
78	0.360	5.55630
82	2.600	6.41497
86	1.140	6.05690
90	0.930	5.96848

*Average of triplicate plate counts

TABLE X. BACTERIAL GROWTH IN BREADFRUIT STORED IN FIELD PITS

Number of Months*	Number of Colonies Per Gram of Breadfruit x 10^6**	Log. Number of Colonies Per Gram of Breadfruit
2	34.0	7.53148
4	4.2	6.62325
6	4.5	6.65321

*Approximate number of months
**Average of triplicate plate counts

pears to be the case, in the fermenter, as shown in Table IX and Figure 17. The rise in acidity, which develops during the breadfruit fermentation process, apparently has considerable effect on members of the initial bacterial population in the fermenter.

The results found in Table X suggest that there is also a marked reduction in the number of bacterial colonies per gram of breadfruit fermenting in the field pits especially when they are compared with the number of bacterial colonies per gram of breadfruit fermenting in the "laboratory" fermenter after 2 days of fermentation. The values obtained from the fermenter appear to be much lower than those in the field pits suggesting a greater acidification in the fermenter under laboratory conditions and lesser acidification in the field pits under natural environmental conditions.

Approximate Amount of Sugar in Terms of Sucrose

The organic matter of fruits is composed almost entirely of carbohydrates and this is mainly sugar (22) . During fermentation of fruits, a decrease in the amount of sugar present would be expected to occur due to microbial breakdown of carbohydrates and resultant production, in general, of acids, alcohols, gases, and other intermediate products. The colorimetric test for sugar determination served as an indicator in analyzing the approximate amount of sugar breakdown over a period of time.

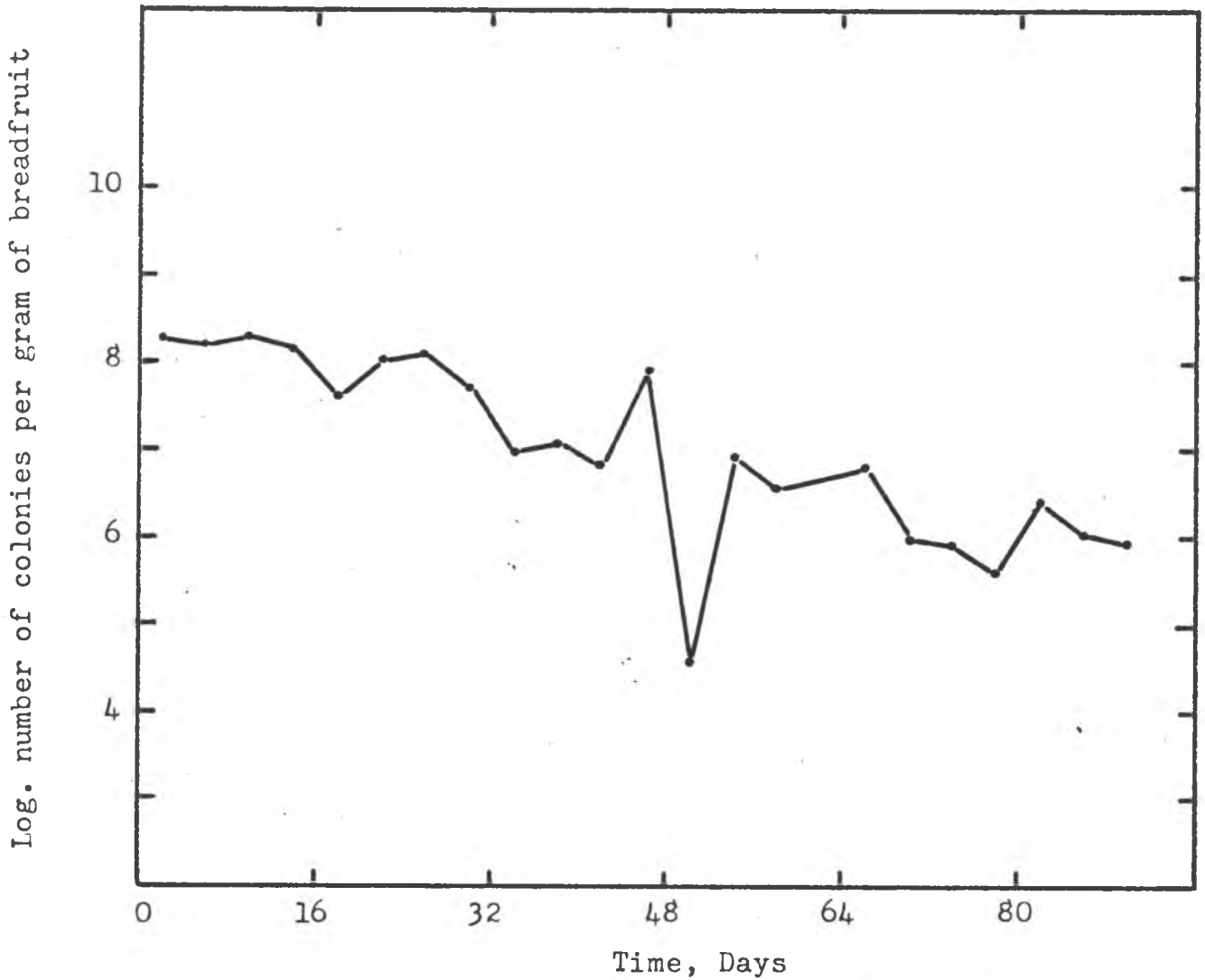


Fig. 17. Population curve of bacteria in breadfruit stored in laboratory fermenter over a period of time.

The results for the colorimetric determination of the approximate amount of sugar, in terms of sucrose, found in breadfruit fermenting in the fermenter and field pits over a period of time are summarized in Tables XI and XII.

A sharp decrease, as shown in Table XI and Figure 18, in the approximate amount of sugar in the breadfruit fermenting, in the fermenter, during the first month followed by a gradual fluctuating decline for another 2 months was observed.

The approximate amount of sugar in breadfruit fermenting, in the field pits, as seen in Table XII appears to be much lower after 2 months of fermentation than that in the fermenter after 2 months of fermentation. This suggests, along with the higher pH value and plate count found, that greater microbial action, under natural environmental conditions in the field pits may result in more efficient breakdown in sugar content of the fermenting breadfruit, at least after 2 months.

Identification of Some of the Predominant, Pigmented, and Other Bacteria Responsible for the Fermentation

The microscopic examinations of Gram stain smears of noticeably different microbial colonies appearing on the Stock Culture Agar plate cultures showed the predominant bacteria occurring in the 1/1,000,000 and higher dilutions of breadfruit samples. Bacteria in samples of breadfruit taken from the fermenter over a period of 4 months, consist-

TABLE XI. APPROXIMATE AMOUNT OF SUGAR IN TERMS OF SUCROSE IN BREADFRUIT STORED IN LABORATORY FERMENTER

Number of Days	Per Cent Transmittance in mg/l as Sucrose From Standards	Dilution Factor	Sugar in Terms of mg/l Sucrose
4	23.9	2250	53,775
8	84.1	450	37,845
12	97.9	450	44,055
16	80.0	300	24,000
20	98.2	450	44,190
24	42.0	300	12,600
28	38.0	400	15,200
32	43.0	400	16,120
36	43.8	400	17,520
40	35.2	400	14,080
44	34.6	400	13,840
48	25.7	400	10,280
52	28.0	400	11,200
56	38.9	400	15,560
60	43.5	400	17,400
64	34.0	400	13,600
68	31.0	400	12,400
72	27.3	400	10,920
76	35.0	400	14,000
80	30.0	400	12,000
84	23.7	400	9,480
88	14.6	400	5,840

TABLE XII. APPROXIMATE AMOUNT OF SUGAR IN TERMS OF SUCROSE IN BREADFRUIT STORED IN FIELD PITS

Number of Months*	Per Cent Transmittance in mg/l as Sucrose From Standards	Dilution Factor	Sugar in Terms of mg/l Sucrose
2	14.0	300	4,200
4	12.9	200	2,580
6	14.9	200	2,980

*Approximate number of months

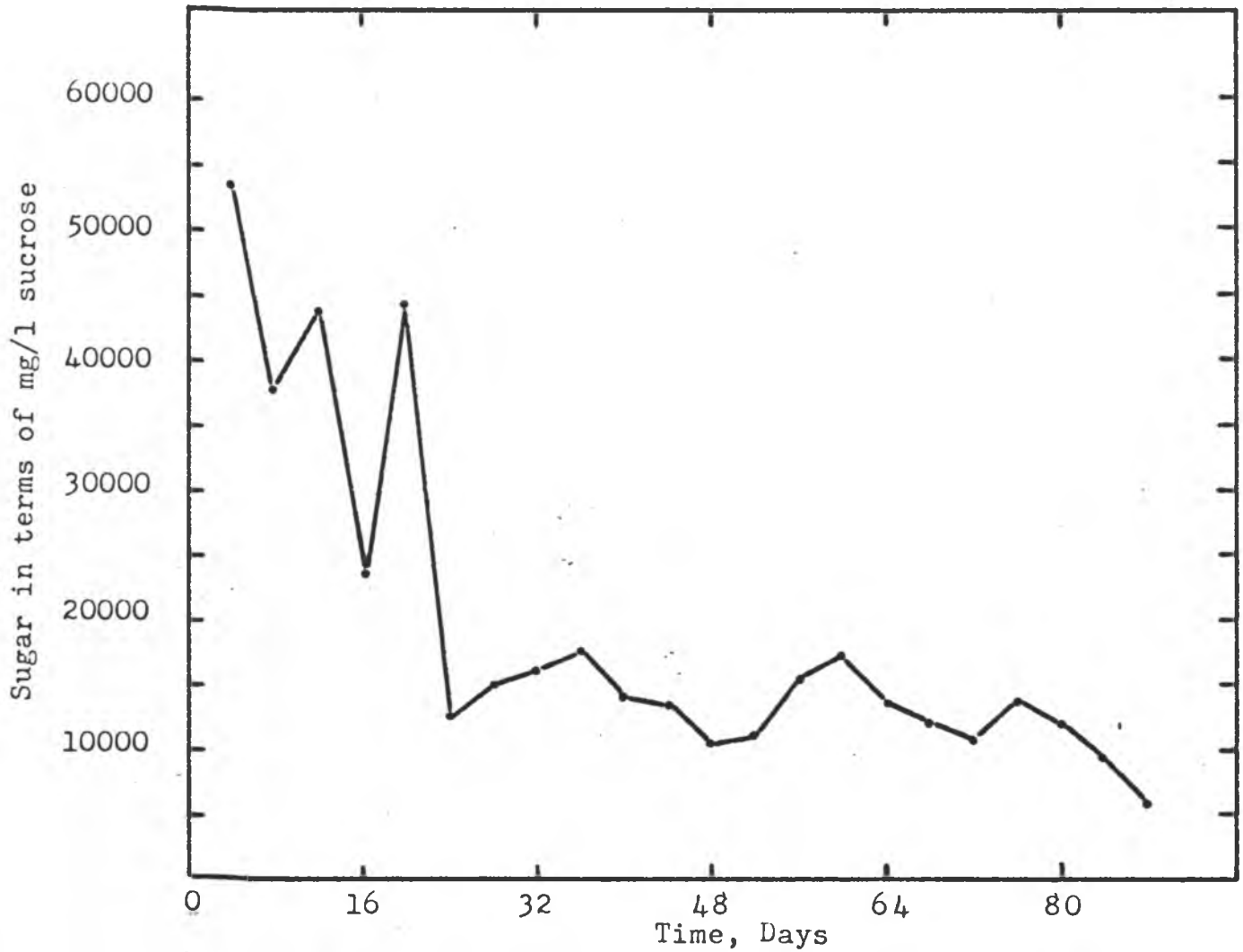


Fig. 18. Change in approximate amount of sugar in terms of sucrose in breadfruit stored in laboratory fermenter over a period of time.

ed principally of Gram positive and Gram negative rod-like organisms of varying shapes and sizes. An abundance of yeast-like cells was also recognized. The predominant bacteria occurring in the 1/1,000,000 and higher dilutions of breadfruit samples taken from the field pits over 2, 4, and 6 month periods also consisted essentially of Gram positive and Gram negative rod-like organisms of varying shapes and sizes. Yeast-like cells were also apparent.

The above preliminary data indicates the predominant morphological types of microorganisms occurring during fermentation of the breadfruit stored in the fermenters and field pits.

The task of identifying all of the predominant bacteria responsible for the breadfruit fermentation process was a much greater project than anticipated for the time period of this study. The prime objectives of this study led to the decision that emphasis be put on the detection, and possible isolation and tentative identification of food-poisoning types of microorganisms, specifically, Clostridia, Staphylococci, Salmonella, Shigella, and Streptococci. The methods used for isolation and identification were those recommended by the American Public Health Association (19) for the microbiological examination of foods using various standard and selective media.

Isolation and identification of other microorganisms occurring on the same media used to detect food-poisoning

types was emphasized to a greater degree in this study. If the more familiarly known food-poisoning types of microorganisms were not detected, it was the expressed hope to enumerate, if any, other possible food-poisoning types of microorganisms that may have been present.

Attempts were made to isolate and identify some of the predominant bacteria responsible for the fermentation though with lesser emphasis than was first proposed.

Examination of the fermenting breadfruit contents, in the fermenters, after 2 and 6 month periods utilizing recommended methods and media revealed no food-poisoning types of Salmonella, Shigella, Staphylococci, or Streptococci microorganisms. The microorganisms found consisted of Gram positive and Gram negative rod-like organisms. Attempts at isolating and identifying viable Clostridia from fermenting breadfruit taken out of the fermenter after 6 months were not successful. This may have been due to the fact that only the simplified technique for anaerobic culture (20) was employed in this particular case. The microorganisms isolated and identified morphologically consisted of facultative, Gram positive rod-like microorganisms, some of which were hemolytic on Blood Agar, and facultative Gram negative rod-like microorganisms. Clostridium botulinum is an anaerobic, spore-forming microorganism responsible for Botulism, a highly fatal bacterial food-poisoning caused by ingestion of a toxin produced by this microorganism. Townsend et al

(24) studied various products, including some fruits, and concluded that a pH of 4.7 appears to be near the lowest level germination and growth of Cl. botulinum spores may be expected. The pH of the breadfruit stored in the fermenters and field pits was below 4.7. No known deaths, however, due to the consumption of fermented breadfruit previously stored in pits in the ground as practiced in the South Pacific area have been recorded.

Examination of the fermenting breadfruit contents, in the field pits, after a 2 month period, utilizing recommended methods and media, revealed no food-poisoning types of Salmonella, Shigella, Staphylococci, or Streptococci microorganisms. The microorganisms found consisted of Gram positive and Gram negative rod-like microorganisms and a few yeast-like cells. Attempts at isolating and identifying viable Clostridia from breadfruit taken out of the field pits after 2 and 4 months were not successful. Both the simplified and glove bag techniques were employed. The microorganisms isolated and morphologically identified consisted essentially of facultative, Gram positive rod-like microorganisms, some of which were hemolytic on Blood Agar, and facultative, Gram negative rod-like microorganisms.

Results of the biochemical reactions of some of the strains of bacteria isolated and tentatively identified from the breadfruit fermenting in the laboratory fermenters and/or field pits are shown in Tables XIII, XIV, XV, and

TABLE XIII. RESULTS OF FERMENTATION TESTS

Strains of Bacteria	Available Substrates										
	Xylose	Glucose	Maltose	Sucrose	Lactose	Rhamnose	Mannitol	Dulcitol	Salicin	Arabinose	Raffinose
A	A2/2	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2	X	X
B	NC3/3	AG3/3	A3/3	A3/3	NC3/3	NC3/3	A3/3	NC3/3	A3/3	X	NC3/3
C	NC2/2	AG2/2	NC2/2	*A2/2	NC2/2	NC2/2	AG2/2	NC2/2	NC2/2	X	X
D	AG2/2	AG2/2	AG2/2	AG2/2	AG2/2	AG2/2	AG2/2	NC2/2	AG2/2	X	X
E	NC2/2	A2/2	A2/2	A2/2	NC2/2	NC2/2	NC2/2	NC2/2	A2/2	X	X
F	A2/2	A2/2	A2/2	A2/2	@A2/2	@A2/2	A2/2	NC2/2	A2/2	A2/2	NC2/2
G	A2/2	A2/2	A2/2	A2/2	@A2/2	NC2/2	A2/2	NC2/2	NC2/2	X	X
H	X	A2/2	A2/2	NC2/2	A2/2	A2/2	NC2/2	X	X	A2/2	X
I	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	X	X
J	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	X	X

Note: Fractions denote reactions to trials; * = late; @ = slight; A = acid reaction; AG = acid and gas formation; NC = no change; X = not tested.

TABLE XIV. RESULTS OF COLIFORM AND IMViC TESTS

Strains of Bacteria	Coliform Tests			IMViC Tests			
	Lauryl Tryptose Broth	Brilliant Green Lactose Bile Broth	Eosin Methylene Blue Agar	Indol	Methyl Red	Voges-Proskauer	Citrate
A	X	X	Gr 2/2	- 2/2	- 2/2	- 2/2	+ 2/2
B	X	X	Gr 2/2	- 3/3	- 3/3	+ 3/3	+ 3/3
C	X	X	Gr 2/2	+ 2/2	- 2/2	- 2/2	+ 2/2
D	+ 3/3	+ 3/3	Gr 3/3	+ 3/3	- 3/3	+ 3/3	+ 3/3
E	X	X	sGr 1/1	- 2/2	- 2/2	+ 2/2	- 2/2
F	X	X	X	- 3/3	+ 3/3	- 3/3	- 2/2
G	X	X	Gr 1/1	- 3/3	- 3/3	- 3/3	+ 3/3
H	X	X	X	- 3/3	- 3/3	- 3/3	- 3/3
I	- 3/3	- 3/3	Gr 2/2	- 3/3	- 3/3	- 3/3	- 3/3
J	- 3/3	- 3/3	sGr 2/2	- 3/3	- 3/3	- 3/3	+ 3/3

Note: Fractions denote reactions to trials; Gr = growth; s = scant; + = positive reaction; - = negative reaction; X = not tested.

TABLE XV. REACTIONS ON DIFFERENTIAL TUBE MEDIA

Strains of Bacteria	Test											
	Friewer Shaughnessy Medium			Triple Sugar Iron Agar		Russell Double Sugar Agar			Kligler Iron Agar		Krumwiede Triple Sugar Agar	
	Ferm.	Mot.	H_2S	Butt	Slant	H_2S	Butt	Slant	Butt	Slant	Butt	Slant
A	NC2/2	-2/2	-2/2	NC2/2	NC2/2	-2/2	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2
B	X	X	X	AG2/2	A2/2	-2/2	X	X	X	X	X	X
C	NC2/2	+2/2	-2/2	AG2/2	NC2/2	-2/2	A2/2	NC2/2	AG2/2	NC2/2	AG2/2	NC2/2
D	NC3/3	-3/3	-3/3	AG3/3	A3/3	-3/3	AG3/3	A3/3	AG3/3	sA3/3	AG3/3	A3/3
E	NC2/2	+2/2	-2/2	A2/2	sA2/2	-2/2	A2/2	NC2/2	A2/2	NC2/2	A2/2	NC2/2
F	X	X	X	A2/2	A2/2	-2/2	X	X	X	X	X	X
G	X	X	X	A3/3	A3/3	-3/3	X	X	X	X	X	X
H	X	X	X	X	X	X	X	X	X	X	X	X
I	NC3/3	-3/3	-3/3	NC3/3	NC3/3	-3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3	NC3/3
J	NC2/2	-2/2	-2/2	NC3/3	NC3/3	-3/3	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2	NC2/2

Note: Fractions denote reactions to trials; A = acid reaction; AG = acid and gas formation; s = slight; X = not tested; + = positive reaction; - = negative reaction; NC = no change or alkaline reaction.

TABLE XVI. RESULTS OF OTHER DIFFERENTIAL TESTS

Strains of Bacteria	Test						
	Nitrates to Nitrites	Gelatin	Starch	Litmus Milk	Urease	Catalase	Oxidase
A	*+2/2	+2/2	X	D or C2/2	-2/2	+1/1	+2/2
B	+3/3	+3/3	X	X	-2/2	X	X
C	+2/2	-2/2	X	NC2/2	+2/2	+1/1	X
D	+2/2	-2/2	X	A2/2	-3/3	+1/1	X
E	+2/2	+2/2	+2/2	D or C2/2	-2/2	+1/1	X
F	-3/3	-2/2	X	A3/3	X	-2/2	X
G	-3/3	+2/2	+2/2	D or C2/2	X	+1/1	X
H	+3/3	+2/2	+3/3	X	X	X	X
I	-3/3	-2/2	-2/2	NC2/2	-2/2	X	X
J	*+3/3	-2/2	-2/2	NC3/3	+2/2	+2/2	+2/2

Note: Fractions denote reactions to trials; C = coagulation; D = digestion; - = negative reaction; + = positive reaction; NC = no change or alkaline reaction; X = not tested; A = acid reaction; * = nitrates and nitrites reduced.

XVI.

The strains of bacteria found were tentatively identified with the aid of Bergey's Manual of Determinative Bacteriology (25) and Topley and Wilson's Principles of Bacteriology and Immunity (26) . On microscopic examination, strain A shown in Figure 19 was found to be a motile, Gram negative, non-sporing rod approximately 0.5 to 1.0 x 1.0 to 2.0 μ in size producing a greenish-yellow diffusible pigment on Stock Culture Agar, showing almost no fermentation of carbohydrates, complete liquefaction of gelatin, and coagulation and/or digestion of litmus milk, among other identifying reactions, shown in Tables XIII, XIV, XV, and XVI. The microorganism resembled a Pseudomonas species. Pseudomonas effusa, whose habitat is soil, was similar in many ways to strain A. Because of various similarities and because its habitat may have been soil, strain A was tentatively identified as Pseudomonas effusa.

Strain B shown in Figure 20 was a very small, motile, Gram negative, non-sporing rod approximately 0.5 x 1.0 μ in size showing definite rods and cocco-bacillary forms, producing a characteristic dark red chromogenesis with a slight metallic sheen at room temperature and almost no chromogenesis at 37°C. The microorganism closely resembled and was similar to Serratia marcescens. Serratia marcescens, according to Bergey's Manual (25) may be found in soil, water, and foods. Because of the very close similarities and

Fig. 19. Vegetative cells designated as Strain A.

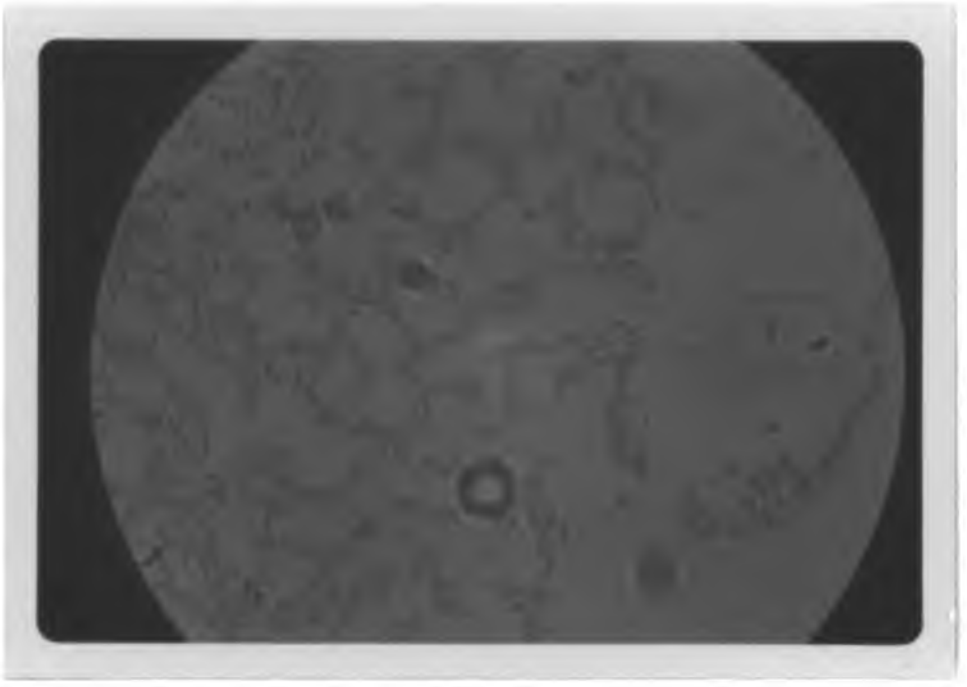


Fig. 20. Strain B - Serratia marcescens.

possible habitats, Strain B was tentatively identified as Serratia marcescens.

Strain C shown in Figure 21 was found to be a motile, Gram negative, non-sporing, non-acid-fast, rod approximately 0.5 to 1.0 x 1.5 to 2.0 μ in size producing acid and gas from mannitol, showing late acid production from sucrose, hydrolyzing urea, and showing a characteristic spreading on moist agar. Strain C closely resembled a Proteus species. The similarities of Strain C to Proteus rettgeri, in Bergey's Manual (25) and to the differential tube media reactions in Difco's Manual (27) , warranted tentatively identifying Strain C as Proteus rettgeri or a sub-species of it. According to Bergey's Manual (25), Proteus rettgeri has been isolated from sporadic and epidemic gastroenteritis patients and has been found in fecal matter. Strain C was obtained from breadfruit fermenting in one of the fermenters after a 6 month period.

Strain D shown in Figure 22 was found to be a non-motile, Gram negative, non-sporing rod approximately 0.5 x 2.5 μ in size closely resembling Aerobacter aerogenes, a member of the Coliform Group of bacteria. The close similarity of Strain D to Aerobacter aerogenes, as seen in Tables XIII, XIV, XV, and XVI, and because Aerobacter aerogenes is widely distributed in nature make it possible to tentatively identify Strain D as Aerobacter aerogenes. Standard Methods (17) classes Strain D as Aerobacter aero-

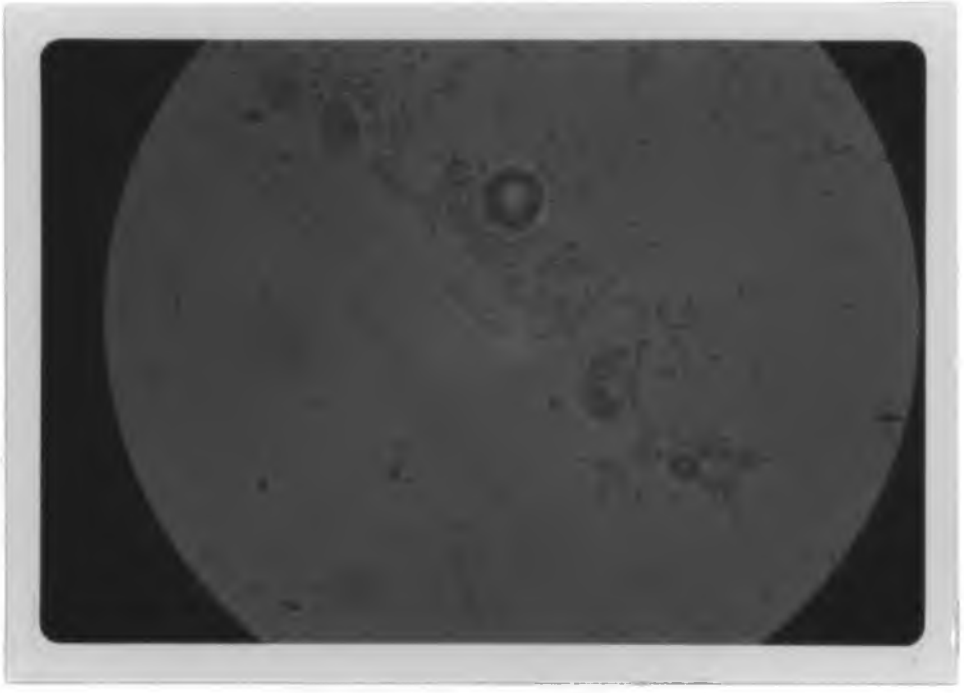


Fig. 21. Vegetative cells designated as Strain C.

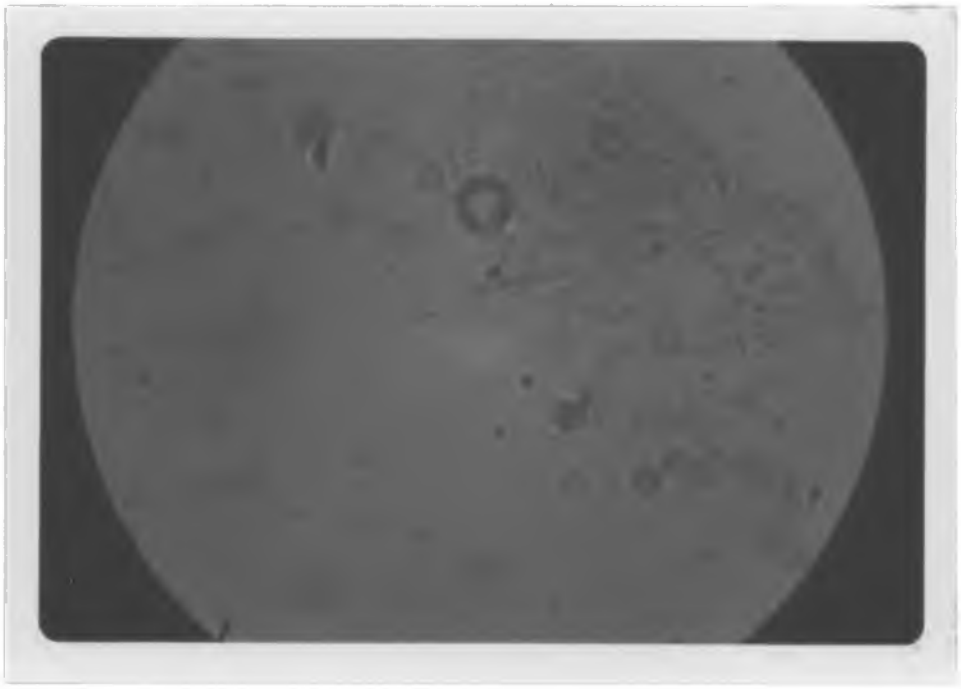


Fig. 22. Strain D - Aerobacter aerogenes variety II.

genes variety II based on its IMViC test results. According to Bergey's Manual (25), Aerobacter aerogenes is normally found on grains and plants.

Strain E shown in Figure 23 was found to be a motile, Gram positive, spore-forming bacillus approximately 2.0 x 6.0 μ in size showing hemolysis on Blood Agar, reducing nitrates to nitrites, hydrolyzing starch, liquefying gelatin, and coagulating and/or digesting litmus milk among other biochemical reactions shown in Tables XIII, XIV, XV, and XVI. Strain E was very similar to Bacillus cereus. Bergey's Manual (25) describes Bacillus cereus as a widely distributed microorganism in soil, dust, milk, and on plant surfaces. According to Ehlers and Steel (10), Bacillus cereus has been associated with outbreaks related to food. Topley and Wilson (26) describe it as non-pathogenic to man and animals.

Strain F shown in Figure 24, was found to be a non-motile, non-sporing, non-acid-fast, non-gas producing, Gram positive rod approximately 0.5 x 2.0 μ in size occurring for the greater part in long chains, catalase negative, and exhibiting a microaerophilic tendency on Nutrient and Stock Culture Agar. Strain F showed close morphological and biochemical similarities to bacteria belonging to the Genus Lactobacillus and was tentatively identified as a Lactobacillus species. The data obtained on the microorganism was not sufficient enough for satisfactory differentiation

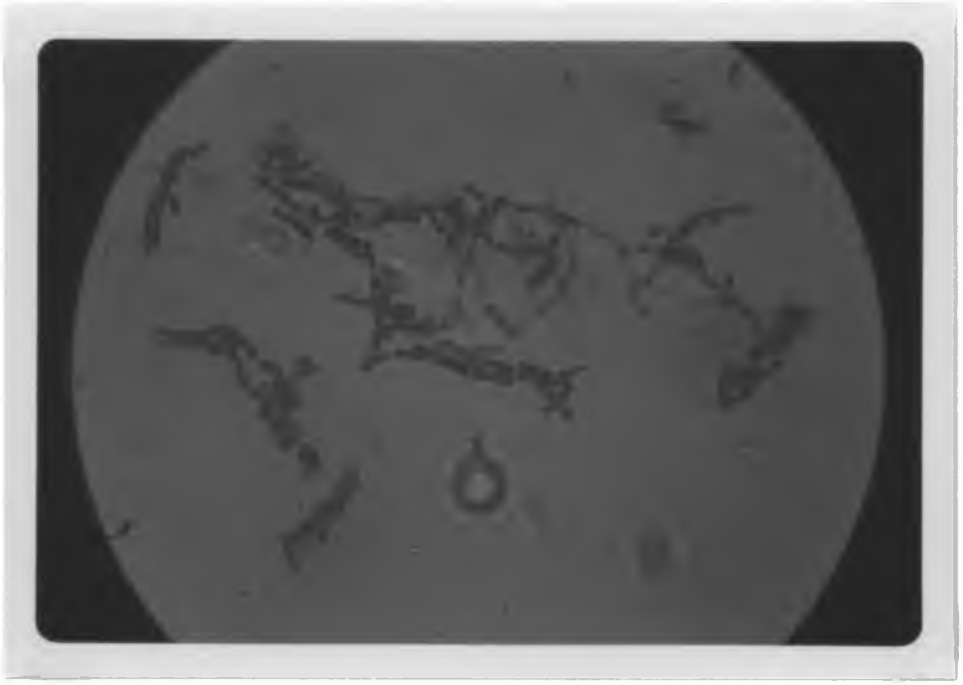


Fig. 23. Bacillus cells designated as Strain E.

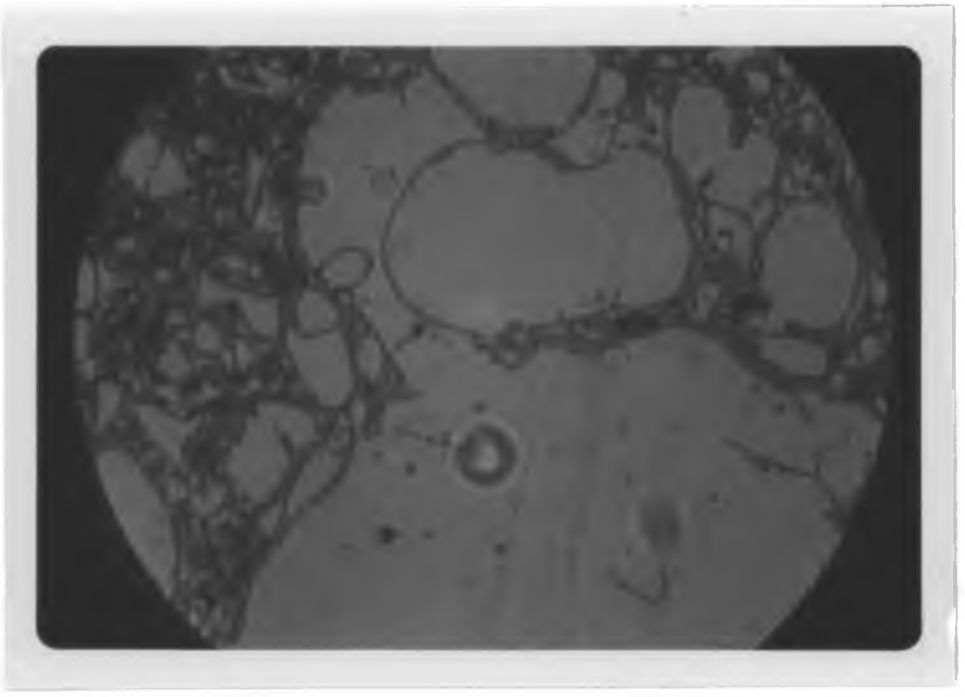


Fig. 24. Chain formation of Strain F.

into species type. According to Bergey's Manual (25) , Lactobacilli are found in fermenting animal and plant products. Pederson (28) writes, "that wherever a natural fermentation of plant materials is noted in which there is a reduced oxygen tension, it can be assumed that a lactic acid fermentation is being carried out by a Gram positive rod or coccus. It is difficult to say where these organisms originate, but they are ever present when opportunity is given for growth . . . Lactic acid rods of the non-gas producing type have been obtained from soured mashes made from various plant materials." Samish et al (29) report that lactic acid producing bacteria are found on crop surfaces.

Strain G shown in Figure 25 was found to be a motile, spore-forming, non-acid-fast, encapsulated, Gram positive bacillus approximately 2.0 x 4.0 to 8.0 μ in size producing acid from mannitol and not producing acetylmethylcarbinol. Strain G was similar to Bacillus megaterium as described in Bergey's Manual (25) and was tentatively identified as Bacillus megaterium which is widely distributed in soil, water, dust, and decomposing materials.

Strain H shown in Figure 26 was found to be a motile, encapsulated, spore-forming bacillus approximately 1.0 x 4.0 μ in size reducing nitrates to nitrites, hydrolyzing starch, and requiring accessory growth factors for fermentation of carbohydrates. The spores were ellipsoidal and approximately 0.5 x 2.0 μ in size. Strain H was identified as a Bacillus

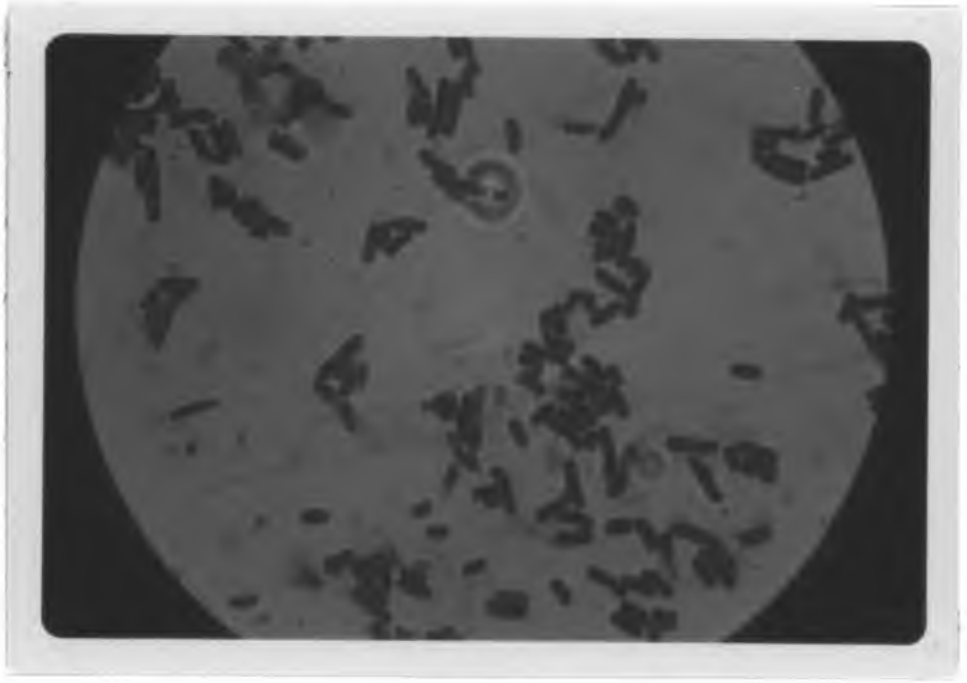


Fig. 25. Bacillus cells designated as Strain G.

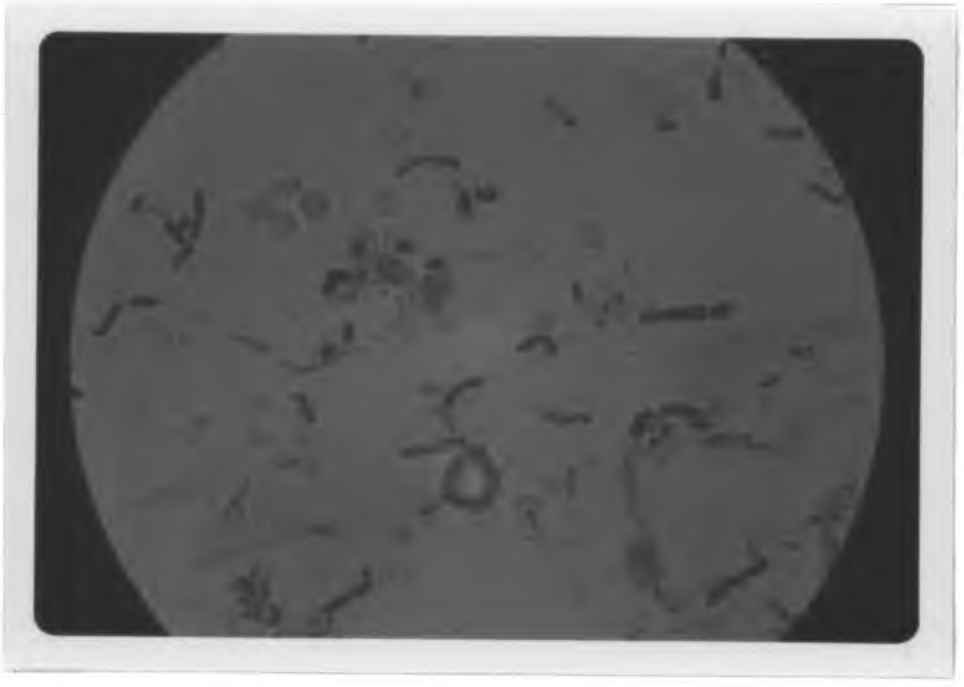


Fig. 26. Bacillus cells designated as Strain H.

species. The microorganism was one of the predominant types of bacteria found in both the fermenters and field pits. The data obtained on Strain H was not conclusive enough for satisfactory differentiation into species type. Tables XIII, XIV, XV, and XVI show acid production by this strain from several carbohydrate substrates, especially rapid acid production from arabinose and rhamnose.

Strain I shown in Figure 27 was found to be a non-motile, non-sporing, Gram negative rod approximately 0.5 to 1.0 x 1.0 to 2.0 μ in size showing no fermentative ability of carbohydrates but growth and producing no noticeable change in litmus milk. Strain I showed morphological and biochemical properties similar to bacteria of the Genus Achromobacter. Based on reactions shown in Tables XIII, XIV, XV, and XVI, and on other information gathered, Strain I was tentatively identified as an Achromobacter species as described in Bergey's Manual (25) . Topley and Wilson (26) recognize these microorganisms as having received very little systematic study to date and as being widespread in nature, commonly found in water, soil, and some foods.

Strain J was found to be a non-motile, non-sporing, Gram negative rod approximately 0.5 to 0.7 x 1.0 to 2.0 μ in size showing no fermentative ability of carbohydrates but growth, oxidase positive, and reducing nitrates to gaseous nitrogen or one of its products among the more important reactions shown in Tables XIII, XIV, XV, and XVI. The mor-

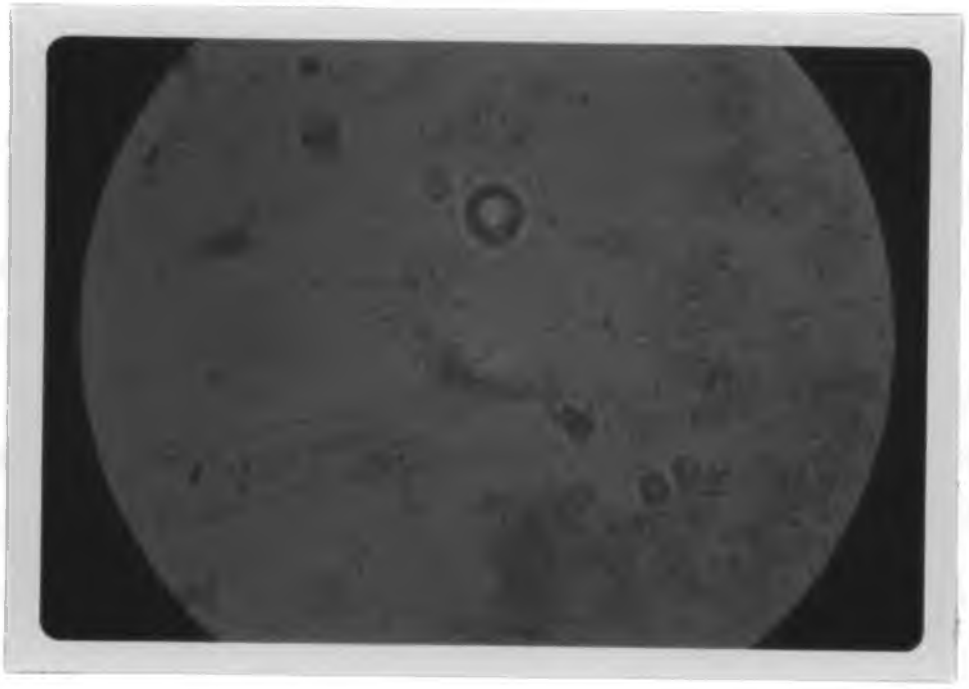


Fig. 27. Vegetative cells designated as Strain I.

phological and biochemical properties of Strain J were similar to those of bacteria belonging to the Genus Pseudomonas. Close similarity was also noticed toward bacteria of the Achromobacter-Alcaligenes Group. However, Strain J was tentatively identified, by virtue of certain reactions noted, as a non-pigmented Pseudomonas species. According to Bergey's Manual (25) , many Pseudomonads are common in soil, water, and are plant pathogens.

With exception of strains H and I which were predominant bacteria isolated from breadfruit fermenting in the field pits, the other strains tentatively identified were isolated from breadfruit fermenting in one of the fermenters.

The different bacteria isolated, described, and tentatively identified above were among the microflora observed during examination and study of the breadfruit as it fermented in the fermenters and/or field pits. Aside from the abundance of Gram positive and Gram negative rod-like microorganisms occurring, in the fermenters and field pits at various periods, other types of bacteria or organisms of lesser predominance were observed. These included various pigmented types, cocci, slightly curved and spiral-like filaments or rods, and several other unfamiliar morphological forms.

The sources from which the observed different types of bacteria may have originated are one or more of the follow-

ing: air, soil, tap and rain water, ti leaves, various insects, handling during processing and storage, and other external contaminants. Reasonable care was taken, in washing the hands, before handling the peeled and cored breadfruit and in taking care to avoid any undue external contamination during processing and storage of the breadfruit.

Castell and McDermott (30) have observed that the principal types of bacteria developing in water are Gram negative organisms resembling members of the genera Alcaligenes, Pseudomonas, and Achromobacter.

In their study of the microflora within the tissue of fruits and vegetables, Samish et al (29) concluded that the bacteria present in healthy fruit tissues consist mainly of motile Gram negative rods of the Families Pseudomonadaceae and Enterobacteriaceae.

Detection of Alcohol

An attempt was made to detect, qualitatively by means of the Iodoform and Ethyl Benzoate tests, the presence of ethyl alcohol in breadfruit juice obtained from one of the fermenters. In the Iodoform test, the gradual formation of a yellowish precipitate, in the cold, was observed along with a pungent odor. The results of the Ethyl Benzoate test were not conclusive enough to confirm or reject the presence of ethyl alcohol.

Two yeast-like organisms, shown in figures 28 and 29, were isolated from the fermenters and an attempt to identi-

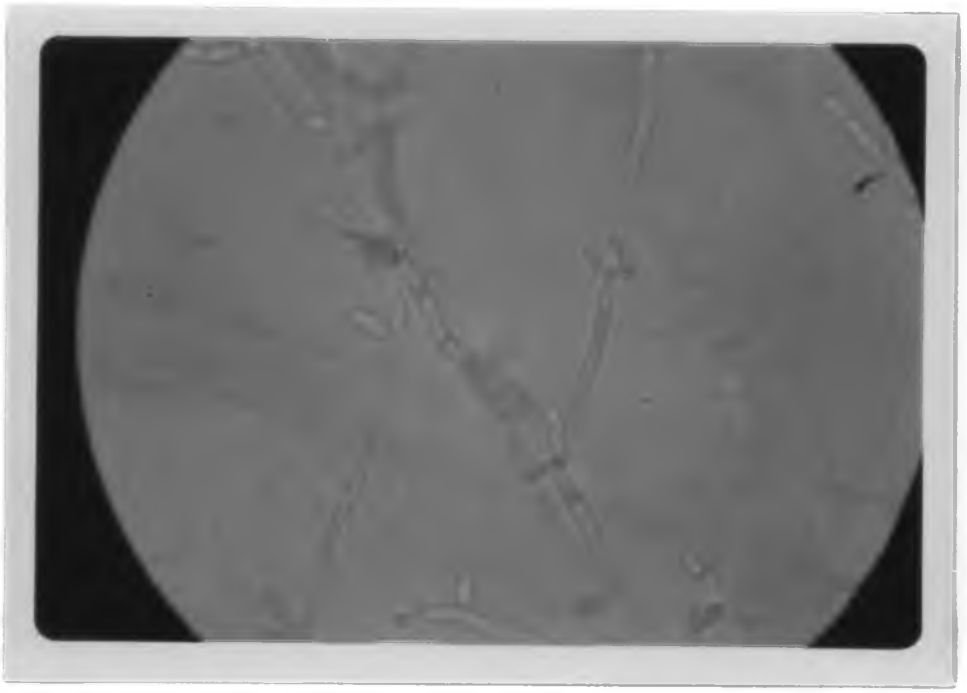


Fig. 28. Budding cells of yeast-like organism - Type I.

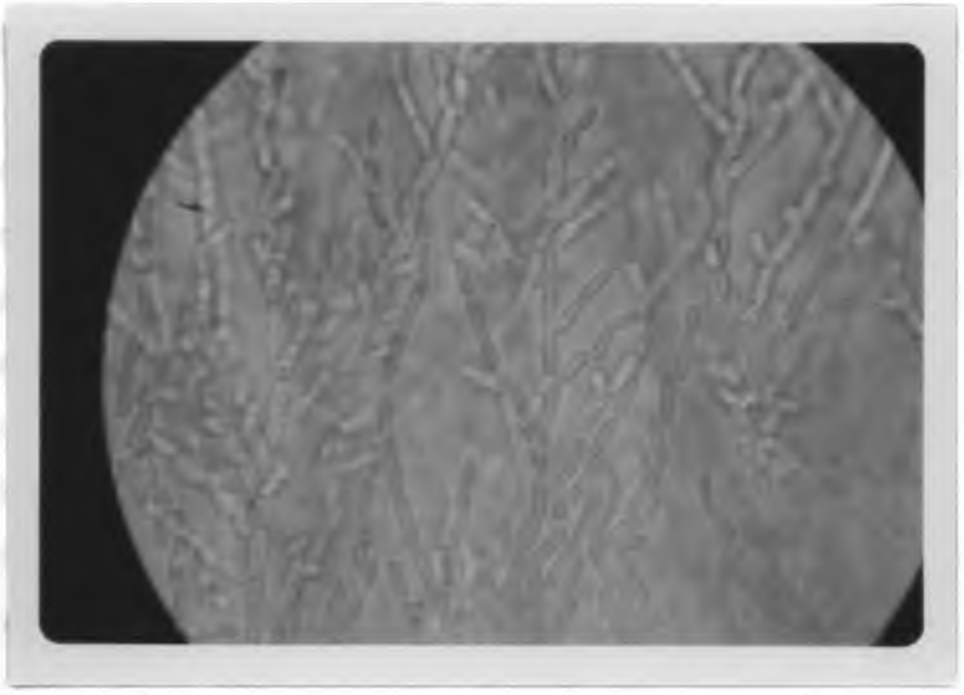


Fig. 29. Budding cells of yeast-like organism - Type II.

fy them was made without much success.

The results of this study are based almost entirely on analysis of raw breadfruit fermenting in laboratory fermenters which simulated the breadfruit fermentation process in pits in the ground. Limitations, in regard to the field phase of study, warrant that if field studies of this particular storage and preservation practice or nature are to be carried out, in the future, better methods of observation, especially with respect to changes in temperature of the fermenting breadfruit, should be developed. The results obtained do give an indication of the type of fermentation occurring, some of the bacteria responsible and a need for better sanitary methods of storage primarily because of soil contamination.

For improvement in results obtained on the colorimetric test for sugar and the estimation of microbial growth more accurate sampling methods should be utilized.

The causal relationship between the consumption of fermented breadfruit and the development of gastrointestinal disorders or reactions which may be attributed to bacteria living and multiplying within or on the fermented breadfruit is still open to question and requires further investigation and study. More work needs to be done, specifically, on the isolation and identification of bacteria in the fermenting breadfruit especially with regard to the Clostridia, and with greater emphasis on testing of suspicious bacteria,

like some of those found in this study, for evidence of pathogenicity.

SUMMARY AND CONCLUSIONS

Several methods for storing and preserving breadfruit, passed down from earlier times, are still being practiced to varying extents on certain islands in the South Pacific area. This study concerned a scientific investigation into one of the older and still utilized methods of storage and preservation of breadfruit by fermentation in pits in the ground as was found to be still practiced in Truk (37,38, 41,45) and to a lesser extent in Ponape (7,41) in the Caroline Islands of Micronesia and American Samoa (43) . From various personal communications and correspondence, it was reported as not currently occurring in Fiji (42,44) , New Caledonia (46) , the Gilbert and Marshall Islands (39,44) where another method of preserving breadfruit different from the above is practiced, Tonga (44) , Western Samoa (44), Niue (44) , Papua and New Guinea (44) , Palau (41) , Peliliu (41) , and the Yap district (41) with the possible exception of the outer islands of Ulithi.

Various observations and analyses were made on the fermented breadfruit and breadfruit juice obtained in the laboratory and/or field pits with a view toward understanding more about the fermentation process by which the breadfruit is preserved.

This study did not delve as deeply into the isolation and tentative identification of the predominant bacteria responsible for the fermentation process as was expected. Empha-

sis was, instead placed on the isolation and tentative identification of possible food-poisoning types of bacteria with the expressed hope of understanding more about a possible causal relationship between consumption of fermented breadfruit and likely bacterial infection giving rise to gastrointestinal disorders or reactions which have been reported through unpublished sources.

The results of this study including any observations or conclusions are summarized in the following general outline:

1. The surfaces of the breadfruit fermenting in the laboratory and field were subject to contamination by soil and various insects.

2. A definite softening in the breadfruit fermenting in the laboratory and field was observed.

3. Except for the surfaces of the breadfruit pulp fermenting in the laboratory and field, which became yellowish-brown, the initial yellowish-white to yellow color remained essentially the same.

4. A pungent and very unpleasant odor was emitted by the breadfruit fermenting in the laboratory and field when taken out for analysis.

5. In regard to the breadfruit fermentation process in the laboratory and field:

   a. The temperature of the breadfruit fermenting in the laboratory and field ranged in general

from 22° to 27°C well within the optimum temperature growth range of some mesophilic bacteria and possibly pathogenic microorganisms.

b. There was no real appreciable change in moisture content of the breadfruit fermenting in the laboratory.

c. There was a marked decrease in pH of the breadfruit fermenting in the laboratory denoting an acid type of fermentation occurring.

d. An apparent sharp increase, in total volatile acids, at least during the first month was observed in the breadfruit juice collected from the breadfruit fermenting in the laboratory. The volatile acids are most likely derived from the fermentation of breadfruit sugar.

e. The chief gases given off from the breadfruit fermenting in the laboratory were CO_2 and N_2 indicating the development of anaerobic conditions.

f. No noticeable change in the gross heat of combustion values of breadfruit fermenting in the laboratory was observed.

g. A marked decline, in bacterial growth, was observed in breadfruit fermenting in the laboratory and field.

h. An apparent decline in the approximate amount of sugar in breadfruit fermenting in the laboratory and field was noted.

6. Morphologically, Gram positive and Gram negative rods of varying shapes and sizes were the predominant types of bacteria found in breadfruit fermenting in the laboratory and field.

a. No food-poisoning types of Staphylococci, Salmonella, Shigella, or Streptococci were detected, by the methods used in this study in the laboratory after 2 and 6 months and in the field after 2 months. Instead, morphologically Gram positive and Gram negative rod-like microorganisms were found to predominate on the media used.

b. The bacteria types that were isolated and tentatively identified from breadfruit fermenting in the laboratory and/or field consisted of members of the Families Pseudomonadaceae, Enterobacteriaceae, Bacillaceae, Achromobacteraceae, and Lactobacillaceae.

c. Two bacterial microorganisms, tentatively identified as Proteus rettgeri and Bacillus cereus which have been associated with gastroenteritic conditions or outbreaks were isolated from breadfruit fermenting in the laboratory.

7. An illness attributed to consumption of preserved

breadfruit does occur when very little cleaning of it has been done before cooking. There is very little record of it because of shortness of duration. It appears to be centered in the Truk District of the Caroline Islands in Micronesia.

Appendix I

Colorimetric Test Method For Sugar Determination

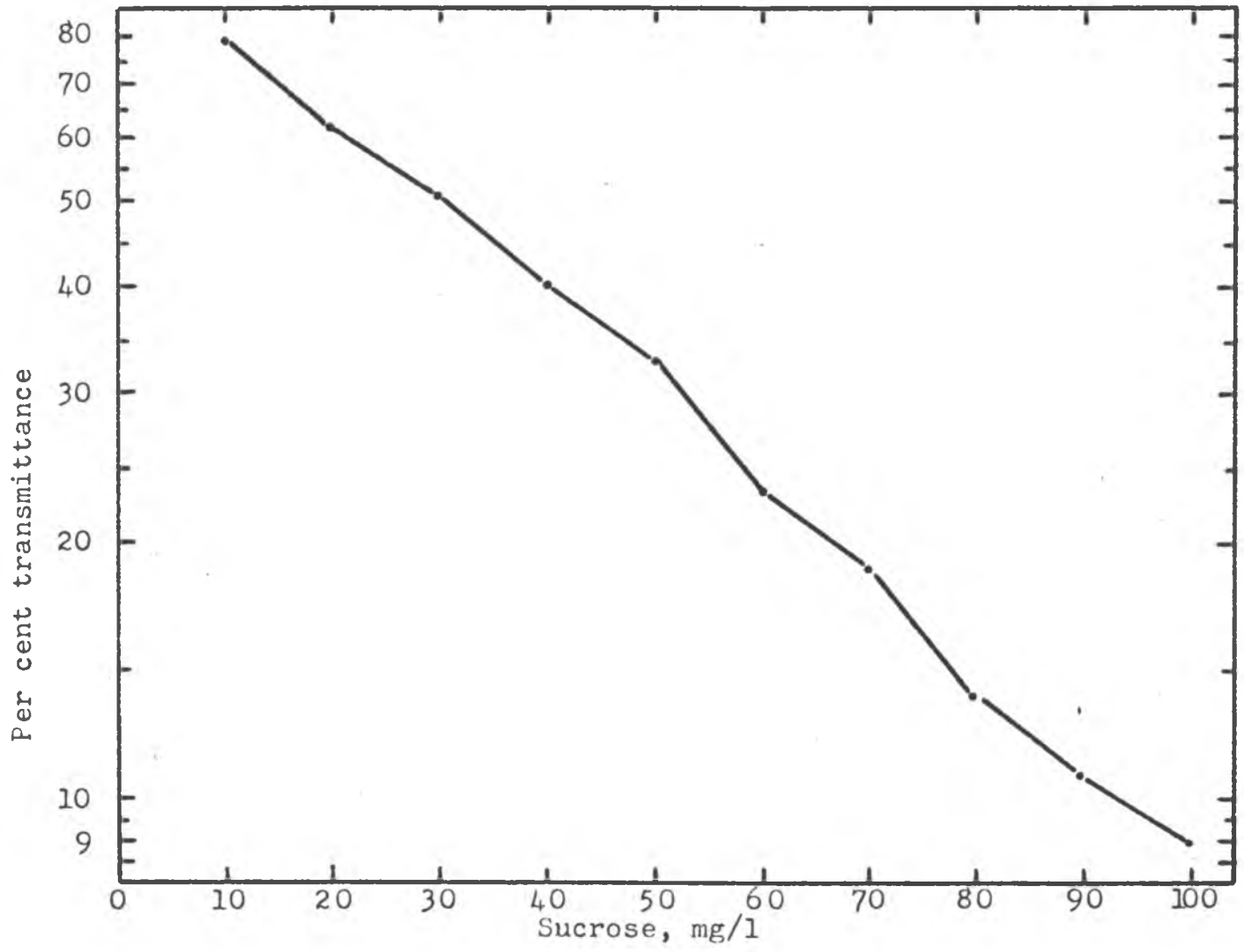
The method for determination of the approximate amount of sugar, adapted from Dubois et al (16) , involved pipetting 1 ml of the fermented breadfruit sugar solutions of appropriate strength and 1 ml of a 5% phenol solution into 2 x 20 cm pyrex screw cap test tubes and then immediately adding 5 ml of concentrated sulfuric acid (sp. gr. 1.84) with a fast delivering 5 ml pipette directing the flow of acid onto the liquid surface to obtain good mixing. The test tubes were left standing approximately 10 minutes, then swirled, and put into a water bath at 25° to 30°C for about 15-20 minutes. The contents of the tubes were then poured into standard size Bausch and Lomb colorimetric tubes and readings were taken at a wave length of 480 mμ on a Bausch and Lomb Spectronic 20 Colorimeter of the per cent transmittance of the yellow-orange color of the samples.

Appendix II

Sucrose Standards For Colorimetric Sugar Determination

Data

Sucrose, mg/l	% Transmittance at 480 mμ wave length
10	78.5
20	62.5
30	51.5
40	41.0
50	33.2
60	23.5
70	18.9
80	14.0
90	11.0
100	8.9



Appendix III

Standardization curve for colorimetric sugar determination

Appendix IV

Breadfruit Preservation Study Questionnaire

In this study, I am particularly concerned with one of the processes of the preservation of breadfruit by fermentation as practiced in the South Pacific area. Essentially, the process involves:

1. Peeling the breadfruit
2. Removal of the core
3. Splitting of the breadfruit into halves or parts
4. Washing with <u>fresh</u> <u>water</u>

The breadfruit is then put into pits in the ground which have been lined with stones, and ti, banana, or coconut leaves. When filled, the pits are covered over with more leaves, rocks, and earth. Under these conditions, the breadfruit pulp ferments and an intense acidification develops that maintains the breadfruit paste which is formed for several months to a year or more.

The extensiveness of this particular practice is not known to me as yet. I have also been told that consumption of the fermented breadfruit paste from the pits in the ground has resulted in reactions or disorders of the gastrointestinal type.

The following questions that I have asked you to answer in this questionnaire will help me to obtain a better understanding of the unconfirmed aspects of this study.

1. What is the name of the island, in the Pacific area, on which you live? _____.

   a. If your island belongs to a major group of islands, please specify major group by name _____.

2. Is such a process of preservation of breadfruit by fermentation, as mentioned above, practiced on your island? Check <u>one</u>: ( ) yes
   ( ) no

Appendix IV (Continued)

3. If the answer to question No. 2 is "yes", is the fermented breadfruit paste as taken from the pits:

   a. consumed raw? Check one: ( ) yes ( ) no

   b. consumed raw, but after being mixed with any other ingredient, such as coconut milk, etc? Check one: ( ) yes ( ) no

   c. consumed after being cooked? Check one: ( ) yes ( ) no

4. If you have or know of persons on your island who have consumed the fermented breadfruit paste raw, with or without mixing it with any other ingredients, have reactions or intestinal disorders occurred? Check one: ( ) yes ( ) no

5. If reactions or intestinal disorders did occur, which of the following symptoms were experienced or known to be experienced?
   Check only those that may apply:

( ) Fever	( ) Nausea	( ) Abdominal cramps
( ) Chills	( ) Vomiting	( ) Bloody stools
( ) Diarrhea	( ) Headache	( ) Others

Appendix IV (Continued)

Others, please specify _____

_____

_____

6. Any other comments or remarks:

_____

_____

_____

_____

_____

Name _____

Appendix V

Media and Reagents

Reagents Used For Colorimetric Determination of Sugar

Sulfuric acid, conc. (sp. gr. 1.84)

Phenol (5%). Dissolve 5 g phenol, reagent grade, in distilled water and dilute to 100 ml

Reagents Used For Determination of Total Volatile Acids (17).

Sulfuric acid, 10N. Approximately 278 ml of conc. sulfuric acid (36N) are diluted with 722 ml of distilled water.

Chloroform-butanol. Mix 300 ml chloroform, 100 ml n-butanol, and 80 ml 0.5N H_2SO_4 in a separatory funnel. Drain off organic layer through filter paper.

Sodium hydroxide, 0.10N, in absolute methanol. Dilute 6.7 ml 15N NaOH solution to 1 liter with absolute methanol.

Phenolphthalein indicator solution. Dissolve 80 mg phenolphthalein in 100 ml absolute methanol.

Thymol blue indicator solution. Dissolve 80 mg thymol blue in 100 ml of absolute methanol.

Silicic acid, solid, 100 mesh.

Reagents Used For Detection of Alcohol

Sodium hydroxide (10%). Dissolve 10 g of NaOH pellets in water and dilute to 100 ml.

Benzoyl chloride, liquid, Baker and Adamson, reagent grade.

Iodine solution (33) . Dissolve 6 g of iodine crystals and 5 g of KI crystals in 20 ml distilled water.

Alcohol (95%) .

Media Used For Isolation (27)

Nutrient Agar

Appendix V (Continued)

Beef Extract	3 g
Peptone	5 g
Agar	120 g
Distilled Water	1000 ml

Stock Culture Agar

Beef Heart Infusion	500 g
Proteose Peptone	10 g
Gelatin	10 g
Dextrose	0.5 g
Isoelectric Casein	5 g
Disodium Phosphate	4 g
Sodium Citrate	3 g
Agar	7.5 g
Distilled Water	1000 ml

Media Used For Fermentation Tests

Purple Broth Base (27)

Beef Extract	1 g
Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Brom Cresol Purple	0.015 g
Distilled Water	1000 ml

Brom Thymol Blue Broth Base (For Strain H bacteria)

Yeast Extract (0.02%)	0.20 g
Ammonium Sulfate	10 g
Brom Thymol Blue	0.015 g
Sodium Chloride	5 g
Distilled Water	1000 ml

The concentration of carbohydrate used for the fermentation tests was 1%.

Media and Reagents Used For Coliform and IMViC Tests (17,27)

Lauryl Tryptose Broth

Tryptose	20 g
Lactose	5 g
Dipotassium Phosphate	2.75 g
Monopotassium Phosphate	2.75 g
Sodium Chloride	5 g
Sodium Lauryl Sulfate	0.1 g
Distilled Water	1000 ml

Appendix V (Continued)

Brilliant Green Bile 2%

Peptone	10 g
Lactose	10 g
Oxgall	20 g
Brilliant Green	0.0133 g
Distilled Water	1000 ml

Levine E.M.B. Agar

Peptone	10 g
Lactose	10 g
Dipotassium Phosphate	2 g
Agar	15 g
Eosin Y	0.4 g
Methylene Blue	0.065 g
Distilled Water	1000 ml

Tryptophane Broth

Tryptone	10 g
Distilled Water	1000 ml

Simmon's Citrate Agar

Magnesium Sulfate	0.2 g
Monoammonium Phosphate	1 g
Dipotassium Phosphate	1 g
Sodium Citrate	2 g
Sodium Chloride	5 g
Agar	15 g
Brom Thymol Blue	0.08 g
Distilled Water	1000 ml

M.R. - V.P. Medium

Buffered Peptone	7 g
Dextrose	5 g
Dipotassium Phosphate	5 g
Distilled Water	1000 ml

Kovac's Reagent

Dissolve 5 g paradimethylaminobenzaldehyde in 75 ml normal amyl alcohol, ACS grade, and add 25 ml conc. HCl. The reagent should be yellow in color.

Methyl Red Indicator Solution

Dissolve 0.1 g methyl red in 300 ml 95% alcohol and di-

Appendix V (Continued)

lute to 500 ml .

Voges-Proskauer's Reagents

Naphthol solution. Dissolve 5 g purified α-naphthol in 100 ml absolute alcohol on the day to be used.

Potassium hydroxide solution. Dissolve 40 g KOH in 100 ml distilled water.

Media and Reagents Used For Differential Tests (27,33)

Friewer Shaughnessy Medium

Base No. 1

Beef Extract	3 g
Peptone	30 g
Agar	3 g
Brom Thymol Blue	0.01 g
Distilled Water	1000 ml

Base No. 2

Lactose	10 g
Lead Acetate	0.05 g
Distilled Water	1000 ml

Triple Sugar Iron Agar

Beef Extract	3 g
Yeast Extract	3 g
Peptone	15 g
Proteose Peptone	5 g
Lactose	10 g
Saccharose	10 g
Dextrose	1 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Agar	12 g
Phenol Red	0.024 g
Distilled Water	1000 ml

Krumwiede Triple Sugar Agar

Beef Extract	1 g
Proteose Peptone No. 3	12 g
Lactose	10 g
Saccharose	10 g

Appendix V (Continued)

Dextrose	1 g
Sodium Chloride	5 g
Agar	15 g
Phenol Red	0.025 g
Distilled Water	1000 ml

Russell Double Sugar Agar

Beef Extract	1 g
Proteose Peptone No. 3	12 g
Lactose	10 g
Dextrose	1 g
Sodium Chloride	5 g
Agar	15 g
Phenol Red	0.025 g
Distilled Water	1000 ml

Kligler Iron Agar

Beef Extract	3 g
Yeast Extract	3 g
Peptone	15 g
Proteose Peptone	5 g
Lactose	10 g
Dextrose	1 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Agar	12 g
Phenol Red	0.024 g
Distilled Water	1000 ml

Litmus Milk

Skim Milk	100 g
Litmus	0.75 g
Distilled Water	1000 ml

Nitrate Broth

Beef Extract	3 g
Peptone	5 g
Potassium Nitrate	1 g
Distilled Water	1000 ml

Christensen's Urea Agar

Urea Concentrate Mixture

Peptone	1 g

Appendix V (Continued)

Sodium Chloride	5 g
Glucose	1 g
Monobasic Potassium Phosphate	2 g
Phenol Red	0.012 g
Urea	20 g
Distilled Water	1000 ml

Agar Base

Agar	15 g
Distilled Water	900 ml

Oxidase Test Solution (1%)

Dissolve 1 g of P amino methylene oxalate in 100 ml of distilled water.

Catalase Test Reagent

Superoxol (Hydrogen Peroxide 30%, Merck), reagent grade.

Nutrient Gelatin

Beef Extract	3 g
Peptone	5 g
Gelatin	120 g
Distilled Water	1000 ml

Starch Agar

Beef Extract	3 g
Soluble Starch	10 g
Agar	12 g
Distilled Water	1000 ml

Media and Reagents Used For Enumerating Salmonella, Shigella, Staphylococci, Streptococci, and Clostridia (27,33)

Staphylococcus Medium No. 110

Yeast Extract	2.5 g
Tryptone	10 g
Gelatin	30 g
Lactose	2 g
d-Mannitol	10 g
Sodium Chloride	75 g
Dipotassium Phosphate	5 g
Agar	15 g
Distilled Water	1000 ml

Appendix V (Continued)

Mannitol Salt Agar

Beef Extract	1 g
Proteose Peptone No. 3	10 g
Sodium Chloride	75 g
d-Mannitol	10 g
Agar	15 g
Phenol Red	0.025 g
Distilled Water	1000 ml

Salmonella-Shigella Agar

Beef Extract	5 g
Proteose Peptone	5 g
Lactose	10 g
Bile Salts No. 3	8.5 g
Sodium Citrate	8.5 g
Sodium Thiosulfate	8.5 g
Ferric Citrate	1 g
Agar	13.5 g
Brilliant Green	0.00033 g
Neutral Red	0.025 g
Distilled Water	1000 ml

Tetrathionate Broth

Basal Medium

Proteose Peptone	5 g
Bile Salts	1 g
Calcium Carbonate	10 g
Sodium Thiosulfate	30 g
Distilled Water	100 ml

Iodine Solution

Iodine Crystals	6 g
Potassium Iodide Crystals	5 g
Distilled Water	20 ml

Sodium Carbonate, Anhydrous, Merck, reagent grade

Pyrogallol, Purified, J. T. Baker, Crystal

Brewer Anaerobic Agar

Yeast Extract	5 g
Tryptone	5 g
Proteose Peptone No. 3	10 g

Appendix V (Continued)

Dextrose	10 g
Sodium Chloride	5 g
Agar	20 g
Sodium Thioglycollate	2 g
Sodium Formaldehyde Sulfoxylate	1 g
Resazurin, Certified	0.002 g
Distilled Water	1000 ml

Tryptose Blood Agar Base

Beef Extract	3 g
Tryptose	10 g
Sodium Chloride	5 g
Agar	15 g
Distilled Water	1000 ml

Cooked Meat Medium

Beef Heart	454 g
Proteose Peptone	20 g
Dextrose	2 g
Sodium Chloride	5 g
Distilled Water	1000 ml

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