A BIOCHEMICAL STUDY OF THE TRADITIONAL METHOD OF PREPARATION OF 'OGI' AND ITS EFFECTS ON THE NUTRITIVE VALUE OF CORN.

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S Y N O P S I S

Corn (Zea mays) is an indigenous crop of Africa, widely eaten in the form of a sour meal. Amongst the ten million Yorubas in the Western region of Nigeria, it is the traditional staple cereal and the first native food given to babies as 'Ogi'. It is therefore important that a food such as this should be studied in order to elucidate the microbiological and biochemical changes that take place during its preparation, and to determine to what extent its nutritive value is affected.

Traditionally, the seeds of corn are soaked in luke-warm water for one to two days, after which they are wet milled and sieved with much water to remove the fibre, hulls and much of the germ. The filtrate is then allowed to sediment and become sour. Usually, the sediment may be diluted to 8 to 10 per cent total solids in water and boiled into a porridge or pap, or may be turned into a stiff gel for a meal when it is known as 'oko'.

The microflora of the fermentation was determined using the plate dilution method on a peptone, glucose, yeast extract, inorganic salts, agar medium. The important micro-organisms were enumerated, isolated and identified by the procedure recommended by Pederson and Albury (1955, 1962).

The moulds isolated consist of Cephalosporium, Fusarium, Aspergillus and Penicillium. Of the aerobic bacteria, only Corynebacterium and Aerobacter could be identified. Lactic acid bacteria were also isolated and found to be Leuconostoc mesenteroides, and Lactobacillus plantarum. There were also yeasts of which Candida mycoderma, Saccharomyces cerevisiae and Rhodotorula were identified. During the soaking period, the predominant organism was Aerobacter cloacae whilst at souring, it was Lactobacillus plantarum.

The dominant organisms, selected on the basis of their relative populations were then used to ferment samples of corn mash singly and combined, in order to determine their roles. The various products were then analysed for nutrient composition including vitamins and organic acids. The effects of steeping and of fermentation on the sugars of the corn were investigated using paper chromatographic methods (Bond and Glass 1963, Dubois, Gilles, Hamilton, Rebers and Smith 1956), and on the starch by viscometric measurements.

There was indication that Corynebacterium michiganense excreted alpha amylase enzymes into the mash which hydrolysed the starch of corn into dextrins. The soaked seeds secreted invertase which converted their sucrose to glucose and fructose, and raffinose to mellibiose and fructose. Aerobacter cloacae was shown to be

capable of synthesising riboflavin and niacin, while Candida mycoderma which predominated at the end of souring period (pH 3.6) increased slightly the calcium, thiamine, niacin and pyridoxine (vit.B_6) contents of the corn mash. Both Saccharomyces cerevisiae and Candida mycoderma were found to contribute to the improved flavour of ogi.

A biological evaluation of the protein quality of whole corn, fermented and unfermented corn flour (Ogi) and soya fortified ogi at 30 to 70 mixture was carried out using 5 female rats per diet of 8 to 10 per cent protein (Goyco 1947). They were compared with a standard casein diet of 10 per cent protein while an egg diet of 5 per cent protein was used to determine endogonous and metabolic nitrogen (Mitchell and Carman 1926).

The fermentation did not affect significantly the protein quality of corn, but the biological value, the net protein utilisation and the protein efficiency ratio of ogi were inferior to corn because milling and sieving during processing, removed a considerable portion of the hulls, aleurone layer and germ. When ogi was fortified with full fat soya at 30 per cent level, the protein efficiency ratio was improved three folds giving a value higher than the theoretical and so proving that a high supplemental relationship exists between their proteins.

The traditional fermentation of corn therefore brings about its nutrient enrichment through the synthesis of some B-vitamins, the dextrinisation of the starch, and the development of flavour. The flavour of ogi is related to the pH of the mash and it is usually not acceptable above 3.7.

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C O N T E N T S

I N T R O D U C T I O N

Within the last decade, the world has been made conscious of the alarming extent of hunger and malnutrition, particularly in the developing countries of Africa, Asia, the Middle East and Latin America. Wright (1962) estimated that a staggering figure of about 400 million people were actually hungry, by not having adequate calorie intake. Another 1,500 million, about half of the world's population, suffered from varying degrees of malnutrition. The ambient conditions in the tropics where most of the affected people live are such that quick deteriorative changes in the food take place resulting in as much as 50 per cent loss of the total production. In Nigeria, for example, Akinrele (1964) estimated that not less than £10 million is lost in cash value per annum from food spoilage. In the technologically more advanced countries, on the other hand, food loss due to spoilage was estimated at about 10 per cent by the Food and Agriculture Organisation (Wright 1962). This contrast is further aggravated by the general dearth of basic knowledge of nutrition among those poorly nourished, while their food habits and culinary practices still require study and development. The food scientist or technologist is therefore faced with an enormous challenge.

The role of corn in human diets, particularly in West Africa.

Corn occurs in the diets of human populations in one

form or another throughout the world, but it is used primarily as a staple food in a few countries. Extensive consumption of corn is made by the peasants of Mexico, Venezuela, Guatemala, Romania, South Africa, Kenya, Tanzania, Malawi, Zambia, Southern Rhodesia, Western Nigeria and Ghana. In some of these areas, between 80 to 90 per cent of the total calorie intake for the greater part of the year is derived from corn according to the F.A.O. publication on "Maize and maize diets" (1953). Because of their low economic level and faulty food habit largely due to ignorance, these consumers usually suffer from tremendous nutritional disorders.

One of the earliest studies on corn diets was recorded in the name of Gaspar Casal in 1762 (F.A.O.1953). He noted an occurence of the pellagra disease among Asturian peasants living largely on corn, with very little meat. This has since been followed up by a considerable amount of research into the nutritional problems arising from diets almost wholly based on corn. Robinson, Payne and Calvo (1944), Anderson, Calvo, Robinson, Serrano and Payne (1948), Calvo, Serrano, Millan, Miranda, de P. and Anderson (1946) carried out nutrition appraisals among the corn eating natives in Mexico. Aykroyd, Alexa and Nitzulescu (1936) in Moldavia, Romania, Cluver (1929 and 1939), Haylett and Smit (1951) in South Africa and Raymond (1942). These studies reviewed in F.A.O. nutritional studies No.9, revealed that

the populations were usually short of calories, although the total protein intake, mainly of cereal origin, was fairly adequate. The most remarkable deficiencies were in calcium, except in Mexico where extra calcium was provided by the lime water used in the preparation of tortillas, vitamin A except where yellow corn was used, riboflavin, niacin and one or more essential amino acids. These were reflected in reduced capacity for work, impairment of growth and physical development, diminished resistance to infection and a high mortality among infants and children. Woolley (1946) again reached a conclusion that there was a pellagragenic agent in corn.

In West Africa, Williams (1933) first reported the incidence of kwashiorkor, a protein deficiency syndrome, among children between the ages of 1 to 4 years in the Gold Coast, now known as Ghana. In all the cases examined, there was a history of deficient breast-feeding, and the only supplementary food used consisted of preparations of maize, namely, akasa and kenkey; products which are similar to ogi and eko respectively in Western Nigeria. The dietary surveys carried out recently in 2 typical villages in Western Nigeria, namely Igbo Ora and Ilora by U N I C E F fellows (1964 and 1965) revealed that corn provided about 30 and 35 per cent of the total protein and

calorie intake respectively, particularly among children of the age group in which Williams noted the incidence of kwashiorkor. Morley (1958) also observed that one out of every five children brought into the Wesley Guild Hospital, Ilesha, was in this condition, and he was seeing up to 500 cases of kwashiorkor in one year according to Collis, Dema and Omololu (1962). Brock (1961) went further to suggest that for every one case of kwashiorkor, there were 100 cases of protein malnutrition in the pre-kwashiorkor condition. Among adults, corn has a versatile use as a food. It is eaten either in the form of pap or porridge and called ogi or agidi, or in the form of a solid meal known as eko. In either form it is usually preferred to many other indigenous foods during ill health. Bassir (1962) mentioned that certain Yoruba people in Nigeria believed that agidi or ogi was a galactogogue. Since, however, there was no good quantitative evidence for the milk producing effects of carbohydrate drinks, he concluded, therefore that the belief stemmed from the fact that mothers often felt thirsty when the draught reflex was operated.

<u>Corn as an indigenous food crop and its preparation into a meal</u>.

The origin of the West African corn has been intensively investigated in recent years. The classical view was that the cultivation of corn (<u>Zea mays</u>) originated in the New World - the Americas, and was introduced into Europe only

at the end of the 15th century after the discovery of the Americas by Christopher Columbus. Another theory held that corn was introduced to Europe from Asia by the Arabs in the 13th century. Porteres and later Willett have also suggested according to Jeffreys (1963) that corn was introduced to West Africa by the Portuguese through the Guinea Coast. Jeffreys however, using evidences from local names, the fact that the term "milho de Guine" was used by the Portuguese themselves, and the existence of native names for corn in Senegal before the Portuguese arrived in 1446 has concluded that the West African corn was of an indigenous origin. In his own postulate, corn first appeared at the mouth of Senegal and moved into the valley of Niger, and at Timbuktu, it met the West to East caravan route which took it through Benue, Lake Chad to Darfur and the Nile. Corn then crossed the Red Sea into Arabia Felix or Hadramant, a popular place for the usual interchange of food plants of Africa and Asia. Jeffreys suggested that it then proceeded up the Persian Gulf to enter Asia Minor from where it spread West wards into the Mediterranean, and into Europe, and eastwards into Persia and thence to China.

The rapid spread of corn has been attributed to a number of factors. First, it is a plant which possesses remarkable adaptability and versatility, because of its great diversity of forms. Unlike wheat and rice, the cultivation

of which is limited by climate, corn flourishes under the most varied conditions. Another important factor is that it gives the highest yields of food energy per acre among the cereals, the least labour demand and a relatively small expense of seed, while on the whole it is resistant to diseases. In the Western provinces of Nigeria, where corn is the traditional staple cereal, agricultural statistics of 1958-59 showed that it was planted on 36.5 per cent of the total acreage of the main farm crops, that is, on 795,000 acres of land yielding 220,000 tons per annum. Other main crops occupied the following percentages:- Yam 25, Cassava 16.1, Cocoyam 4.9, melon 10.7 and beans 8.0. The rate of growth in yield of corn has since been maintained at about 2 per cent per annum. The predominant variety used for human consumption is the "flour" (Zea mays L. erythrolepsis) usually referred to as "Lagos white". An attempt was made recently to introduce a yellow Mexican variety because of its better nutritive quality. It met with considerable consumer resistance, and it is now being used exclusively for livestock feeds.

Corn is eaten mainly in the form of a fermented product in Africa. The meal is known by various names in the different parts of the continent. It is called mahewu (mealie meal) by the Bantus of South Africa, uji and chenga in East Africa, ogi, akamu and eko in Nigeria and akasa

and kenkey in Ghana. Although there are minor variations in the method of preparation of this food from place to place, yet the basic practice of soaking, milling, sieving and souring of the corn is common to all.

The traditional method of preparation employed by the Yorubas of Western Nigeria consists of washing dried corn with water, followed by steeping in lukewarm clean water for a day or two. The grains are then wet-milled, diluted considerably with water, and sieved through a wire mesh of approximately B.S.S. No. 52 (aperture 300 microns). The filtrate is then allowed to stand for another day or two at room temperature (30°-32°c), when it sediments and becomes sour. The supernatant is poured away and the sediment used as ogi. It is usually boiled at 8 to 10 percent total solids into a porridge or pap, and eaten with a bean cake (akara or moinmoin). When further diluted, it is used in the rural areas as the first native food for weaning babies. In a concentrated form, it is boiled into a thick gel and then allowed to set stiff in leaf moulds. This last meal is known as eko.

Previous studies on the nutrient content of corn meals.

Research on corn in the last sixty years has been largely devoted to its genetics, nutrient composition and industrial utilisation. A considerable amount of data has been given on the chemical composition of corn by Greaves

and Hirst (1929), Praps and Kemmerer (1941) Earle, Curtis and Hubbard (1946), Chatfield (1949), Aurand and Miller (1949), and Rodriguez, Hunt and Bethke (1950). The vitamins were also estimated by Aykroyd and Swaminathan (1940), Cabell and Ellis (1942), Burkholder, McVeigh and Moyer (1947), Goldberg and Thorp (1945), Hunt, Ditzler and Bethke (1947), Ditzler, Hunt, and Bethke (1948) and Heathcote, Hinton and Shaw (1952). These investigations showed that the different parts of the corn kernel differed in chemical composition. The endosperm which constitutes about 80 per cent of the grain contained over 80 per cent of its starch and about 75 per cent of its protein. The germ which accounts for about 10 per cent of the grain, contained nearly all the oil and most of the minerals; while the outermost portion or horny gluten which contains the aleurone cells was found to be one of the richest fractions of the grain with a protein content of about 20 per cent. A great variation in the protein and fat contents was also observed among the different strains of corn. The vitamins were found located chiefly in the germ and in the outermost layer of the endosperm, which includes the aleurone cells. The thiamine and riboflavin contents were high compared with other cereals like wheat and rice, but the main value was relatively very low. Vitamin A was only found in yellow maize, vitamin E or tocopherol was found in

the embryo in a range of 15 to 36 mg. per gram. The amounts of vitamins C and D in corn were very small and thought to be insignificant.

The proteins of corn and their nutritive quality were also exhaustively studied by Osborne and Mendel (1914), Showalter and Carr (1922), Boas-Fixxen, Hutchinson and Jackson (1934), Block and Mitchell (1946), Sure and House (1948), Schulz and Thomas (1949), Block and Bolling (1951), Frey (1951), Chaudhuri and Kodicek (1951), Mitchell, Hamilton and Beadles (1952) and Wolfe and Fevden (1957). Corn was found to contain three types of protein: prolamines chiefly as zein, globulins and glutelins. Zein accounted for about half of the total protein in the whole grain and for about half of that in the endosperm. In the germ, zein was present only in a small amount, the chief protein in this organ being glutelin. Zein was found to be an imperfect protein, devoid of the important amino acid, lysine, and very deficient in tryptophan. This defect is known to have an important bearing on the relation of maize to pellagra. On the basis of experiments with rats, the biological values and protein efficiency ratio ascribed to corn were inferior to those of rice and whole wheat. Tests with pure zein showed that it could not support any growth at all in rats, but when supplemented with corn glutelin, some degree of weight gain was observed (Osborne and Mendel 1914).

Storage and a number of processing methods have been found to change the nutritive quality of corn. Willits and Kokoski (1935), Jones, Divine and Gersdoff (1942), Gilman and Semeniuk (1948) and Zeleny (1948) examined the changes in the chemical and nutritive composition of corn under storage and upon infection with moulds. While serious losses may be caused by insects and rodents, chemical changes which are occuring all the time during storage were found to be enzymatic in nature, resulting from the action of the enzymes of the grain itself, or of the fungi or bacteria present in it. The factors which determined the extent of change under storage were moisture content, storage temperature, the access of oxygen to the stored material, and the degree of soundness of the grain. Low moisture levels, low temperature and lack of oxygen decreased enzymatic and respiratory activities and consequently helped to prevent deterioration. The change affecting carbohydrates was essentially the decomposition of starch under the influence of amylases. Proteolytic enzymes in the grain or in parasitic organisms associated with it hydrolysed the proteins into amino acids or denatured them. Although the hydrolytic phase was found to be usually slow, yet the free amino acid content of certain samples of spoiled corn rose to more than three times during defective storage.

The reduction in nutritive value of the proteins during storage was demonstrated in a series of metabolic trials with rats by Jones, Divine and Gersdorff (1942) During a 42-day period, groups of young rats were fed corn freshly ground, and after storage for 6, 12 and 24 months at 76°F. The groups showed weight gains of 84, 63, 56 and 57 grams respectively. The grain and meal used in these experiments were sound and free from infection with insects and moulds. Fat hydrolysis also took place during storage. The development of rancidity is strongly influenced by the oxygen content and temperature of the surrounding atmosphere. When white corn of 14 per cent moisture was stored for 12 months at temperatures below 13°C, no significant changes in fat content were found to occur, whereas the sample stored at higher temperatures had their fat content reduced by 45 per cent and over (Willits and Kokoski 1935). With drier meal, of 8 per cent moisture content the temperature of storage could be raised to 37°C without loss of fat. In all the cases, however, large increases in acidity was observed and this bore no relation to the fat content. The mineral content of corn was not greatly influenced by storage, and of the vitamins, only vitamin A (Carotenoids) contained in yellow corn was seriously affected (Fraps and Kemmerer 1938 and 1941).

Platt (1943), Massieu, Guzman, Cravioto and Calvo (1949), Mitchell, Hamilton and Beadles (1949), Laguna and Carpenter (1951) and Schweigart and Pellingham (1963) examined the various methods of preparation of corn for human consumption and their influences on the intrinsic food quality. The main processes which have been known to affect the nutrient content of corn when employed are soaking or steeping, milling, sieving or bolting, cooking and fermentation. In Latin America, for example, where whole grains are usually soaked in lime water to soften the hull before grinding, the process has been found to increase the calcium content of the grain, while it is also believed that it may be important in rendering the niacin in the corn more available to digestion. Grinding was found to improve the digestibility and to make possible the preparation of a greater variety of dishes which would be pleasing to the eye and palate. The influence of milling on the nutritive quality of corn is dependent on what portions of the grain are removed during the process. The traditional practice of using stone milling in various parts of Latin America and Africa has the advantage of ensuring that the meal obtained includes all parts of the whole grain. Modern milling methods with machines result in the separation of the bran and germ from corn, and so bring about profound changes in the nutrient content. Cooking was observed to improve the palatability, the digestibility

and the availability of certain nutrients. A considerable proportion of the thiamine (about 30 per cent) and riboflavin was found lost (Mitchell, Hamilton and Beadles 1949). When corn was fermented with Lactobacillus delbruckii to make mahewu, the concentrations of the thiamine, riboflavin and niacin, and also the proteins remained unaffected (Schweigert and Pellingham, 1963).

The basis of the investigations carried out.

Schweigert and de Wit (1960) studied the preparation, drying and nutritive value of mahewu, a South African equivalent of ogi, and found that the bacteria that brought about its souring were introduced by the added wheat flour. The bacteria propagated rapidly in the corn porridge converting the sugars derived from the starch by wheat amylotic enzymes, into lactic acid. Also Schweigert and Pellingham (1963) noted that the traditional spontaneous souring process was not suitable for the large scale industrial production of mahewu, because it took too long and proceeded too irregularly with the risk of contamination with undesirable bacteria. This could produce secondary fermentations, the products of which (for example, acetic and butyric acids) influenced the taste detrimentally. They, therefore, employed Lactobacillus delbruckii at an optimum temperature of 45°C, and in the presence of

enzyme rich materials such as wheat bran and calcium phosphate salts, were able to reduce the fermentation time from 36 hours to 3 hours. They found that the concentrations of the vitamins, particularly thiamine, riboflavin and niacin were not affected by the fermentation. Dreyer and Schweigart (1961) too, observed no appreciable deterioration of the proteins from the controlled fermentation with Lactobacillus delbruckii. In West Africa, particularly in the Western provinces of Nigeria, the traditional souring of corn takes place spontaneously during the preparation of ogi, but without the addition of inoculants or enzymes. It is therefore important that the organisms involved in this unaided fermentation should be identified and their effects on the nutritive value of the meal investigated. Also the flavour characteristics of the product need to be correlated with the roles of the fermenting organisms so that an integrated recommendation for the industrial manufacture of the ogi could be made.

Pure (1960) following the biochemical changes in the carbohydrates of corn during steeping and germination postulated that corn contained only beta amylase in the resting seed and up to the third day of germination. Thereafter, alpha amylase was secreted and became predominantly active. On the contrary, Bond and Glass (1963) working on yellow dent corn steeped in water for

48 hours at 16°C before germination in air at 100 per cent relative humidity, observed no alpha and beta amylase activity during the 2-day steeping period. Alpha amylase was detected on the sixth day, whilst beta amylase did not appear throughout the test period lasting 14 days. There is, again, need to clear the ambiguity in order to correctly appraise the role of steeping in the traditionnal preparation of ogi from corn.

Gilbert, Gillman, Mandelstan, Gillman and Goldberg (1943) reported that rats of 50 g. to 60 g. weight, when fed on mealie meal or mahewu and water, became dwarfs showing very little increase in weight in 10 weeks. If they were, however, transferred to a full diet, they grew almost as rapidly as normal animals, showing that the potentiality for growth was not destroyed by the previous stunting. Chick (1951) observed that the proteins of soyabeans, probably, because of their high lysine content, had a special value in supplementing those of cereals. Sure (1948) in experiments with growing rats fed on corn diets supplemented with 2.6% of soyabean meal, the protein level having been adjusted to 8%, found that the protein efficiency ratio (gain in weight per gram of protein eaten) of corn was raised by over 30%, from 1.23 to 1.66. Figures at higher levels of fortification would need to be known for ogi, if it is to be developed into a food adequate for

the use of infants and children, using soyabean meal as the main protein supplement.

The investigations which form the basis of this thesis were planned as follows: (a) A preliminary survey of the methods of preparation of ogi by various commercial producers in Lagos and Ibadan was conducted so as to determine the one most widely employed. (b) The micro-organisms present in the corn mash during the period of steeping and souring were then enumerated, isolated and identified. The roles of those appearing to be dominant by sheer numbers at any significant period were examined in terms of the biochemical and nutritive changes brought about in ogi. (c) The sugar composition of the Nigerian corn, Lagos white variety, was determined and the changes with steeping and souring followed. (d) The biological evaluation of the effect of the traditional preparation of ogi on the proteins of corn was carried out using albino female rats. The nutritional adequacy of a mixed protein made by the addition of 30 parts of full fat soya flour to 70 parts of ogi was investigated and compared with casein.

EXPERIMENTAL

All the chemicals used in these experiments were of analytical reagent grade obtained from Hopkins and Williams. The corn was of the Lagos white variety obtained from the local market at Ibadan.

1. Pattern of the Microflora

(a) Using the various traditional methods of preparation of ogi.

Preparation of Samples. 100 ml. of sterile water were transferred into each of a number of beakers labelled A,B,C, and D respectively. About 20 g. of wholesome grains of corn were added to each beaker and covered with filter papers which had been perforated with needle points. Sample A had its water retained throughout and its corn wet-milled and sieved after 2 days.

Sample B had its water changed every day and its corn wet-milled and sieved after 2 days.

Sample C had its water changed every day and its corn wet-milled and sieved after 3 days.

Sample D had its corn dry-milled before being soaked in water.

Procedure.

All the samples were kept for a period of 4 days at room temperature (30°-32°C), 1 ml. being taken each day for bacteriological analysis, while 5 ml. aliquots were used for chemical assays.

Bacteriological analysis.

The method used is according to Pederson, Niketic and Albury (1962) and based on the plate dilution technique. 400 ml. of nutrient agar were prepared to contain:-

Peptone	2.5 g.
Glucose	10 g.
Bacto-Agar	10 g.
Difco Yeast extract	1.25 g.
Salt A	2.5 ml.
Salt B	2.5 ml.
Distilled water	400 ml.

Composition of Salts

Salt A		Salt B	
K_2HPO_4	100 g.	$MgSO_4.7H_2O$	40 g.
KH_2PO_4	100 g.	NaCl	2 g.
Distilled water	1000 ml.	$FeSO_4.7H_2O$	2 g.
		Distilled water	1000 ml.

4 ml. aliquots of the melted and mixed nutrient medium were transferred into each of 100 Bijou bottles and sterilised at 10 lb. pressure for 10 minutes. 1 ml. from each of the samples under test was adequately diluted in turn with sterile water, and 1 ml. of such dilute used to inoculate each bottle of melted nutrient agar at about 35°C. The contents were then mixed and poured aseptically into sterile petri dishes for incubation at room temperature (30°-32°C). The number of colonies growing in each plate was counted after 48 hours of incubation and classified on the basis of colonial morphology.

Chemical analysis. The pH of each sample was measured everyday with a Cambridge direct reading pH meter, while the total titrable acidity was determined on 5 ml. aliquot of the sample against 0.1N NaOH solution in a micro-burette using phenol red as the indicator (pH 6.8-8.4).

(b) Using a chosen method of preparation of Ogi.

40 g. of wholesome grains of corn were steeped in 200 ml. tap water for 1 day in a closed brown jar. Then the grains were taken out and milled wet in a laboratory hand mill which had been previously washed clean and steeped in boiling water. The milled corn was sieved through B.S.S. No.44 (aperture 355 microns) and transferred quantitatively with the steeping liquor back into the jar, and then allowed to continue fermenting for a further period of 2 days. The bacteriological and physico-chemical changes occuring during the process were followed by taking samples at frequent intervals for bacto-chemical analyses as described previously.

(c) Results. The variations in the pattern of the microflora arising from the different methods used during the traditional preparationof ogi from corn are shown in Table 1. The changes in the populations of the micro-organisms and the development of acidity can be followed in Table 2, using a process selected to give the best result under traditional practice.

Development of acidity and microbial populations during 'Ogi' preparation

Time	Samples	Population = No. x 10^7 colonies/g. Corn				pH of medium	Equivalent vol. 0.1N NaOH required (ml.)
		Mould species	Yeast species	Aerobacter cloacae	Lactobacillus plantarum		
0 hr.	A	0.005	0.002	0.001	0.003	5.60	0.00
	B	0.002	0.000	0.003	0.002	5.60	0.00
	C	0.022	0.000	0.001	0.000	5.60	0.00
	D	0.004	0.000	0.007	0.003	5.70	0.00
At 24 hrs.	A	0.000	0.040	30.000	46.000	5.00	0.06
	B	1.000	0.040	12.000	29.600	5.03	0.06
	C	2.000	0.000	19.200	42.400	5.05	0.06
	D	12.400	2.000	68.000	304.800	4.85	0.32
At 48 hrs.	A	0.000	0.560	435.000	500.000	4.45	0.64
	B	0.000	0.100	125.000	352.400	4.90	0.24
	C	0.000	0.005	232.200	712.500	4.90	0.16
	D	0.000	10.000	960.000	1150.000	3.77	3.16
At 72 hrs.	A	0.000	44.000	1060.000	2252.000	3.79	2.04
	B	0.000	21.300	848.000	800.000	4.25	1.20
	C	0.000	0.500	940.000	1160.000	4.70	0.36
	D	0.000	96.000	330.000	2100.000	3.68	4.92
At 96 hrs.	A	0.000	1010.000	200.000	300.000	3.75	3.48
	B	0.000	998.000	1675.000	9060.000	3.79	1.68
	C	0.000	24.000	1120.000	1025.000	3.85	1.08
	D	0.000	1505.000	15.000	752.000	3.50	10.8

A = Milled after 48 hours steeping
B = " " 48 " "
C = " " 72 " "
D = " dry at 0 hour.

Table 1

Bacteriological and physico-chemical changes during 'Ogi' preparation

No. of viable cells per g. of dry corn

Time in Hours	Acid equiv. in .01N NaOH (ml.)	Fungal Colonies	Coryne-bacterium	Aerobacter cloacae	Lactobacillus plantarum	Saccharomyces cerevisiae	Candida mycoderma	Factor for popu-lation
0a	0.3	49.0	10.5	16.5	66.0	0.5	0.2	$x10^3$
0b	0.2	19.0	5.6	9.0	37.0	0.1	0.0	$x10^3$
6	0.5	0.0	18.0	332.0	60.0	0.0	0.0	$x10^6$
24	2.3	0.0	24,700.0	42,000.0	70,000.0	0.0	0.0	$x10^6$
				Milling, Sieving and Souring in same liquor				
31	9.4	0.0	8.0	29.7	19.8	1.6	0.0	$x10^{10}$
37	12.4	0.0	0.0	13.2	61.2	8.6	0.6	$x10^{10}$
44	17.7	0.0	0.0	126.0	4,000.0	3.0	2.0	$x10^{10}$
50	18.4	0.0	0.0	47.0	562.0	0.0	4.0	$x10^{10}$
56	24.1	0.0	0.0	7,400.0	18,500.0	1.6	160.0	$x10^{10}$
70	34.2	0.0	0.0	2,820.0	9,100.0	0.0	2,060.0	$x10^{10}$

Table 2

a = Surface microflora of unwashed corn.

b = Surface microflora of corn washed once (40g. corn with 200ml. water)

MOISTURE ABSORPTION OF CORN DURING STEEPING

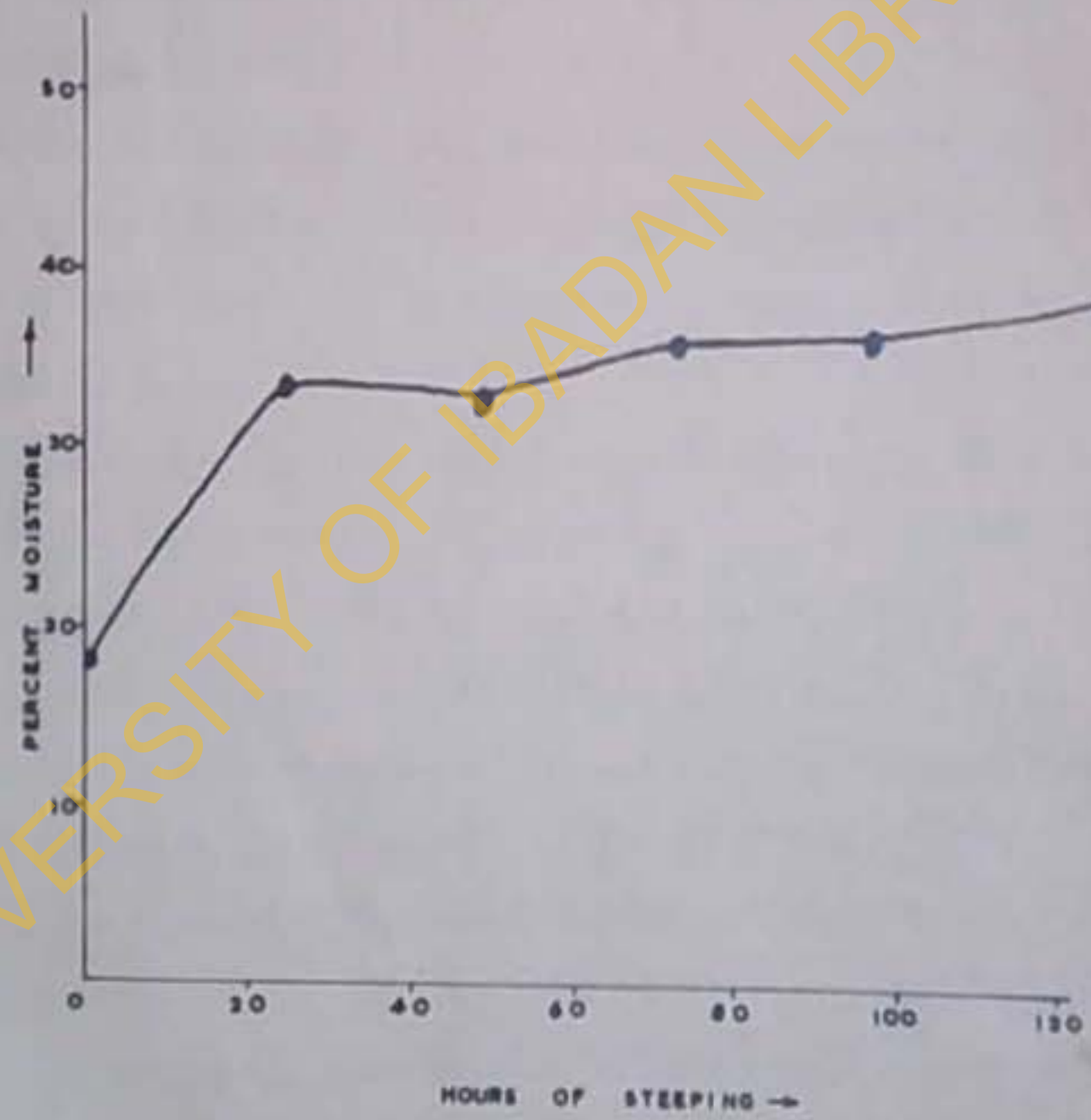


FIG. 1

2. Identification of predominant organisms

(a) Microscopical examination. A representative colony of each of the dominant organisms was isolated and transferred aseptically on the nutrient agar slants in replicates. All of the slants were then allowed to grow at room temperature (30°-32°C) for about 48 hours. Each pure culture was sampled with a sterile platinum wire loop and examined under the oil immersion lens of the microscope for morphological characteristics. The fungal colonies in the petri dishes were sliced vertically and examined under a low power objective of the microscope.

(b) Biochemical tests

(i) Fermentation tests. The method used is according to Christensen et al (1958). A standard basic medium of nutrient broth containing 0.5% peptone, 0.25% yeast extract and 0.5% each of salts A and B was used. 10 ml. of the broth were transferred to a number of fermentation tubes, followed by 0.1 g. of the specific sugar. The tubes were plugged with non-absorbent cotton wool and sterilised at 10 lb. pressure for 10 minutes. A total number of 18 sugars were tested. The ability to ferment a sugar was measured by the titration of the acid produced against 0.1N NaOH solution using phenol red as the indicator (pH 6.8-8.4).

(ii) Voges-Proskauer test. O'Meara's modification.

A knife point of creatine and 5 ml. of a 40% solution of caustic soda were added to the culture of micro-organism in glucose-phosphate-peptone broth. The tube was corked and shaken vigorously. The appearance of a pink colour within 10 minutes was considered as a positive reaction for acetylmethyl carbinol.

(iii) Nitrate reduction test. Two solutions of alpha naphthylamine and sulphanilic acid in acetic acid respectively were mixed in equal proportions. To 1 ml. of the mixture in a clean test tube was added 1 loopful of the culture of the organism grown in a nutrient broth containing 1.0% (W/V) KNO_3, 1.0% (W/V) peptone and 0.5% (W/V) NaCl. The development of a rose pink colour within 5 minutes at room temperature was taken as a positive test for nitrite.

(iv) Indole test. 5 drops of a saturated aqueous solution of potassium persulphate followed by 5 drops of para-dimethylamino benzaldehyde were put on the tip of a small piece of absorbent cotton wool which had been formed into a plug. The cotton wool was used to plug a tube of culture fluid and pushed downwards until the tip was about an inch from the fluid. The tube was stood in a bath of boiling water. A red colour formed at the tip of the plug was considered as giving a positive test.

(v) Starch hydrolyis test. A loopful of the culture was inoculated on to 0.2% soluble starch in nutrient agar and allowed to grow for about 2 days at room temperature. Then the starch agar plate was flooded with a saturated solution of iodine in 50% alcohol. It was allowed to remain on for 2 minutes and then drained from the plate. The presence of a clear zone around the growth in which there was no blue colour was considered an indication of starch destruction.

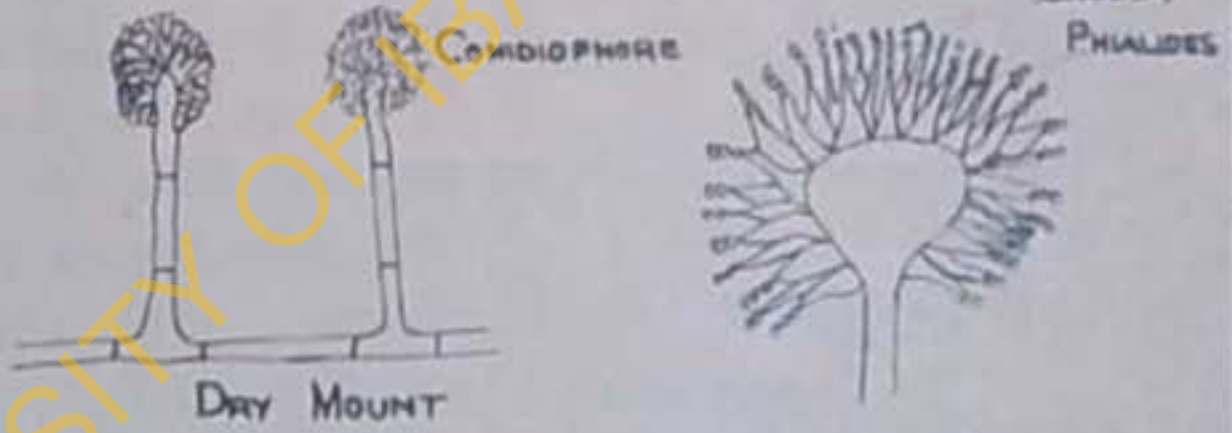
(vi) Gram's Iodine test. A thin film of the culture was made on a clean slide and fixed with the minimum amount of heat. It was then flooded with a mixture of 30 parts of 2% aqueous crystal violet and 8 parts of 1% aqueous $NaHCO_3$ and allowed to remain for 5 minutes. It was washed to remove excess stain and treated with fresh iodine for 2 minutes. The smear was then decolourised by adding acetone to it drop by drop. The slide was again washed in tap water and counter-stained for 20 seconds with 0.1% aqueous solution of safranine. It was finally washed in tap water, blotted dry and examined under the microscope. Bacterial cells stained violet were regarded as gram positive, and those stained red as gram negative.

(c) Results. The photo micrographs of the organisms isolated are shown in figures 2 to 10.

MICROSCOPICAL STRUCTURE OF FUNGAL COLONIES

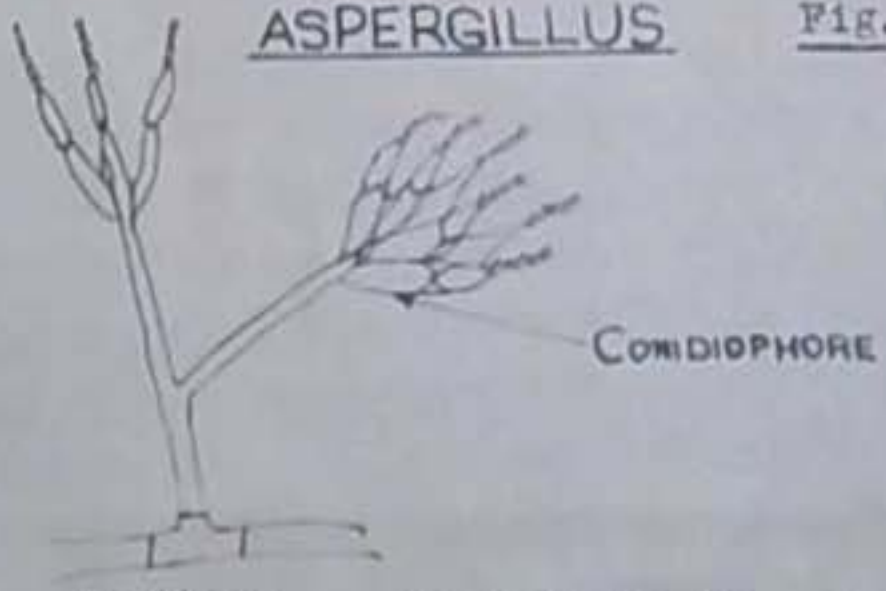
DRY MOUNT SHOWING CONIDIOPHORES WATER MOUNT

CEPHALOSPORIUM Fig. 2



DRY MOUNT

ASPERGILLUS Fig. 3



DRY MOUNT

PENICILLIUM Fig. 4

A B

A = Agar plate showing colonies grown from the surface microflora of corn.
The large semi transparent colonies are those of Aerobacter aliquens.
The creamy white opaque colonies on the surface are those of Corynebacterium michiganense.
The small white opaque colonies submerged are those of Lactobacillus plantarum.
The rough surfaced colony at the top left hand corner is that of Candida mycoderma.
The white wooly colonies are those of Aspergillus and Penicillium and the pink ones those of Cephalosporium and Fusaruim.

B = Agar plate showing colonies grown from a 24 hours old corn steep culture. This is marked by predominance of Aerobacter colonies and complete elimination of the moulds.

Figure 5.

A photomicrograph of the cells of Corynebacterium michiganense showing stained granules under an oil immersion lens.

Figure 6.

A photomicrograph of the cells of Aerobacter cloacae stained with Gram's iodine and put under an oil immersion lens.

Figure 7.

A photomicrograph of the cells of Corynebacterium michiganense showing stained granules under an oil immersion lens.

Figure 6.

A photomicrograph of the cells of Aerobacter cloacae stained with Gram's iodine and put under an oil immersion lens.

A photomicrograph of the cells of Lactobacillus plantarum stained with Gram's iodine and put under an oil immersion lens.

Figure 8.

A photomicrograph of the cells of Saccharomyces cerevisiae, stained and examined under an oil immersion lens.

Figure 9.

A photomicrograph of the cells of Candida mycoderma, stained and examined under an oil immersion lens.

Figure 10.

A photomicrograph of the cells of Saccharomyces cerevisiae, stained and examined under an oil immersion lens.

Figure 9.

A photomicrograph of the cells of Candida mycoderma, stained and examined under an oil immersion lens.

Figure 10.

Fermentation tests with microorganisms isolated from 'Ogi'

Carbon Source	Aerobacter cloacae		Lactobacillus plantarum		Corynebacterium michiganense		Saccharomyces cerevisiae		Candida mycoderma	
	Gas	N/10 NaOH	Gas	N/10 NaOH	Gas	N/10 NaOH	Growth	Ferm.	Growth	Ferm.
Arabinose	+	1.08ml	-	0.95ml	-	0.0ml	-	-	+	-
Dextrin	+	0.7ml	-	1.3ml	-	0.0ml	-	-	+	-
Glucose	++	1.8ml	-	4.3ml	-	1.2ml	+	+	++	+
Sucrose	++	1.8ml	-	5.9ml	-	0.4ml	+	+	++	-
Lactose	+	1.0ml	-	1.15ml	-	0.0ml	-	-	+	-
Fructose	++	1.9ml	-	3.3ml	-	0.4ml	+	+	++	+
Maltose	++	1.9ml	-	3.4ml	-	0.3ml	+	+	++	-
Galactose	++	1.9ml	-	2.6ml	-	0.2ml	+	+	++	-
Raffinose	-	0.0ml	-	2.2ml	-	0.0ml	+	+	+	-
Sorbitol	+	1.8ml	-	1.05ml	-	0.3ml	+	+	+	-
Starch	-	0.5ml	-	0.8ml	-	0.4ml	-	-	+	-
Mannitol	++	1.8ml	-	1.8ml	-	1.0ml	-	-	+	-
Xylose	++	1.8ml	-	1.4ml	-	0.0ml	+	+	+	-
No Carbon	-	0.0ml	-	0.5ml	-	0.0ml	-	-	+	-
5% salt + Glucose	-	0.4ml	-	2.8ml	-	0.0ml	-	-	-	-
Ethyl Alcohol	-	-	-	-	-	-	+	-	++	-
Lactic Acid	-	-	-	-	-	-	-	-	++	-
Acetic Acid	-	-	-	-	-	-	-	-	++	-
Pyruvic Acid	-	-	-	-	-	-	-	-	-	-
Biochemical tests										
Gram's Iodine stain	-		+		+		n.a.		n.a.	
Voges Prausknuer test	+		+		-		n.a.		n.a.	
Nitrate reduction test	+		+		-		n.a.		n.a.	
Indole test	-		-		-		n.a.		n.a.	
Motility	+		-		+		-		-	
Starch hydrolysis	-		-		+		-		-	

+ Positive ++ Very positive - Negative Ferm. Fermentation n.a. not applicable.

Table 3

Their reactions to the various biochemical tests are given in Table 3. The following morphological and colonial characteristics were observed in the micro-organisms.

MOULDS

Cephalosporium. The colonies possessed white tufts of fibre over pink bases. The conidiophore was swollen encapsulating numerous conidia which were one celled, cylindrical and of uniform size.

Fusarium. The colonial morphology was similar to cephalosporium. Two sizes of conidia were, however, observed, namely micro and macro conidia.

Aspergillus. The colonies were black. The conidiophores were upright, simple, terminating in a globose to elliptical swelling, bearing phialides radiating from the entire surface. The conidia were one celled and spherical.

Penicillium. The colonies were circular with green velvet surface. The conidiophores arose from mycelia in synnemata, branching near the apex to form brush like conidial bearing apparatus. The conidia were one celled and spherical.

BACTERIA

Corynebacterium michiganense. The colonies were cream and sometimes yellow in colour, convex, smooth and translucent on the surface of nutrient agar.

They were relatively large, about 1 to 2 mm. in diameter. The cells were rods which were sometimes elongated into curved bars with stained granular bands along their length. The cells appeared in a palisade arrangement with occasional snapping form. The cells showed a very weak diastatic action on starch agar, produced much acid from glucose and mannitol and slight acid from sucrose, fructose, maltose, galactose, sorbitol and starch. The organism was gram positive, motile and did not reduce nitrates nor produce indole.

Aerobacter cloacae The colonies were large, mucoid, circular, and sometimes spreading on hutrient agar. They were convex, height about 1 mm. and diameter about 3 to 4 mm., smooth and semi-transparent. The cells were spherical to oval in shape occuring in large clusters, but mostly in pairs and few chains. The organism was gram negative fermenting arabinose, glucose, sucrose, fructose, maltose, galactose, sorbitol, mannitol and xylose to produce acid and gas. Its growth was arrested in 6% brine solution and was able to reduce nitrates but not produce indole.

Lactobacillus plantarum. The colonies were small, circular, orientated in an oblique angle but submerged in nutrient agar. The cells were rods forming into chains but showed no granular stain along their length.

The organism produced more acid than Aerobacter cloacae but no gas from the following sugars: glucose, arabinose, sucrose, fructose, maltose, galactose, raffinose, mannitol, xylose, dextrin and sorbitol. It was not motile, but nitrate reducing, gram positive and showed salt tolerance at 5% brine solution.

Yeasts

Saccharomyces cerevisiae. The colonies on nutrient agar were white, large, thick and dense, growing into a mushroom shape. Those submerged grew towards the surface, and also into dichotomous projections. The surface colonies had their tops dry, slightly convex and sometimes raised apices. The cells were spherical to oval showing multilateral budding, and fermented glucose, sucrose, fructose, maltose, galactose, raffinose, sorbitol and xylose. Ethyl alcohol was used for growth.

Rhodotorula. The colonies were small, pink in colour and moist on the surface of nutrient agar. The cells were very similar to those of Saccharomyces cerevisiae except that they occurred mostly as single cells.

Candida mycoderma. The colonies were large, white, opaque and dry on the surface, with raised centres and a crater at the peak. The cells were ellipsoidal and formed long chains which constitute a pseudomycelium. No sporulation or ascus was observed.

The cells formed a dry wrinkled pellicle on the surface of nutrient broth, and were able to utilise arabinose, dextrin, glucose, sucrose, lactose, fructose, maltose, galactose, raffinose, sorbitol, starch, rhamnose, mannitol, xylose, ethyl alcohol, lactic acid and acetic acid for growth; while only able to ferment glucose and fructose.

3. The role of the dominant organisms

(a) Fermentation characteristics. 100 g. of corn were soaked in 5% $Na_2S_2O_5$ solution for one day to prevent microbial infection. The corn grains were then drained and blotted dry. They were then resoaked in 1.6 litres of distilled water and almost immediately milled wet and sieved through a wire mesh B.S.S. No.44 (aperture 355 microns). The portion that passed through the sieve was thoroughly mixed and 100 ml. aliquots transferred into each of a number of bottles, sterilised at 10 lb. pressure for 15 minutes and labelled as follows:-

Sample SC was inoculated with 2 ml. of a 2 day old steep liquor of corn

Sample Cory was inoculated with 2 ml. of isolated Corynebacterium culture solution.

Sample AC was inoculated with 2 ml. of isolated Aerobacter culture solution.

Sample LP was inoculated with 2 ml. of isolated Lactobacillus culture solution.

Sample Cd was inoculated with 2 ml. of isolated Candida culture solution.

Sample AC x LP was inoculated with 2 ml. mixture (1:1 v/v) of Aerobacter and Lactobacillus culture solutions.

Sample AC x CM was inoculated with 2 ml. mixture (1:1v/v) of Aerobacter and Candida culture solutions.

Sample LP x CM was inoculated with 2 ml. mixture(1:1v/v) of Lactobacillus and Candida culture solution

Sample AC x LP x CM was inoculated with 2 ml.(1:1:1v/v) mixture of Lactobacillus, Aerobacter and Candida culture solutions.

Sample AC x LP x Sacch. was inoculated with 2 ml.(1:1:1v/v) mixture of Lactobacillus, Aerobacter and Saccharomyces culture solutions.

A control sample was set up which was not inoculated. Two other samples inoculated with Aerobacter and Lactobacillus culture solutions respectively were allowed to ferment at 45°C in a thermostatic bath for 2 days. The labelled samples, on the other hand, were allowed to ferment for 2 days also but at room temperature (30°-32°C). 5 ml. quantities were taken from each sample every 4 hours for titration against 0.1N NaOH using phenolphthlein as the indicator (pH 8.4-10.0). All these operations were carried out in a sterile room under ultra violet light. The light was put out during the time of inoculations, and a different pipette was used for each sample.

(b) Identification and estimation of organic acids produced.

(i) Separation of the volatile and non volatile acids. 5 ml. aliquots from samples SC, LM, IP and CM were transferred into a Markham's microkjeldahl apparatus and distilled in turn in the presence of 5 ml. of 2.5% (V/V) H_2SO_4 in saturated $MgSO_4$ solution. About 50 ml. were distilled and then titrated against 0.1N NaOH solution with phenolphthalein as indicator (pH 8.4-10.0), in order to estimate the volatile acids concentration. A control distillation with 5 ml. of distilled water and 5 ml. of 2.5% (V/V) H_2SO_4 in sat. $MgSO_4$ solution was also carried out and the distillate used to estimate the blank correction value. The concentration of the non volatile acid was deduced by difference from the total titrable acidity value of a 5 ml. portion of the same sample.

(ii) Paper chromatography of the volatile acids. The method used is according to Reid and Lederer (1951). The ammonium salts of volatile acids were prepared and separated on chromatographic paper. The acids spot out as yellow spots in the presence of bromocresol purple indicator (pH 5.2-6.8).

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Reagents. 1. 0.05N $Ba(OH)_2$

2. 0.04% (W/V) phenol red (pH. 6.8-8.4)

3. Saturated solution of ammonium oxalate.

4. 0.880 Ammonia

5. Solvent system: n-butanol was redistilled and then shaken with an equal volume of 1.5 N NH_3. The two phases were separated before use.

6. Indicator solution: 0.04% (W/V) bromocresol purple in a 1:5 (V/V) dilution of B.P. formalin (40% solution of formaldehyde in water) in ethanol, the pH of the solution was adjusted to approximately 5.0 by addition of 0.1N NaOH.

7. Whatman No. 1 special chromatography paper.

Procedure. The distillates of the samples used in 3b (1) were separately neutralised to phenol red (pH 6.8-8.4) with 0.05 N $Ba(OH)_2$ solution and evaporated to small volumes. They were then transferred in turn to small centrifuge tubes and evaporated to dryness. A slight excess of a saturated solution of ammonium oxalate was added to each tube to generate the ammonium salts of the acids.

The barium oxalate was centrifuged off leaving a supernatant suitable for spotting on to chromatographic paper. The sheets of Whatman No.1 paper were exposed to an atmosphere of concentrated aqueous NH_3 in a closed vessel for 3 to 4 hours immediately before use. Approximately 0.005 ml. of the ammonium salts of the fatty acids prepared as above was spotted on the papers with a micropipette. The starting line was 3 cm. from the bottom and the spots were placed 3 cm. apart. Ammonium salts of the following fatty acids were prepared:- acetic, formic, propionic, butyric, caprylic and lactic, and were spotted on the chromatography papers as well.

The sheets were clipped on to some glass rods in the chromtank and made to stand in the layer of the butanol phase of the solvent system, and the close fitting lid of the vessel put into position. The aqueous phase of the solvent systems was put in beakers which were stood in the butanol. 24 hours were allowed for the development of the chromatograms by ascending technique after which the papers were taken out and dried at room temperature.

The papers were then sprayed as evenly as possible with the indicator solution and held for 2 to 3 minutes in an atmosphere saturated with dilute ammonia, removing at brief intervals to watch the progress. Bright yellow spots appearing on a stable purple background identified the location of the constituent acids. The duplicates of the chromatograms were sprayed with 0.1N $AgNO_3$ solution containing one fifth of its volume of concentrated NH_3. They were heated at 105°C for a period of up to 30 minutes, when the presence of formic acid should produce a dark spot unlike other acids. In this way, formic acid could be detected in the presence of acetic and lactic acids.

(iii) Paper chromatography of the non-volatile acids.

The method is also according to Akinrele (1964) 20 ml. from each of the fermented samples under test were passed through, in turn, a column of Amberlite resin IRA-400 in the carbonate form in order to absorb the organic acids. They were subsequently eluted with 0.1N $(NH_4)_2CO_3$ solution and concentrated to about 2 ml., the excess of $(NH_4)_2CO_3$ having decomposed; 0.005 ml. from each of the prepared samples was spotted with a micropipette on to Whatman No.1 paper. Reference spots were also made with the following

aliphatic acids:- lactic, succinic, malic, tartaric, oxalic and citric. The paper was developed with phenol-water-formic acid reagent (75:25:1) using the descending technique. The chromatogram was dried, and sprayed with bromocresol green indicator solution (0.04 g. bromocresol green in 15 ml. of ethanol and 5 ml. of water, its pH adjusted to 5.5).

(iv) Column chromatography of the acids of Ogi. The method used is according to Frohmann et.al. (1951) and based on the gradient elution principle.

Preparation of acid mixture. 20 g. of the wet mash of ogi sold commercially and equivalent to about 10 g. dry material were extracted with about 200 ml. of distilled water and then allowed to settle before the supernatant was decanted off. The extracted acids were neutralised with 0.1N NaOH solution to a slight excess, and then evaporated to less than 5 ml. before transferring to a test tube. The evaporation was continued to dryness in a current of air. A few glass beads were then added to the cooled residue and just enough conc. H_2SO_4 water (1:1) to convert all the sodium salts to free acids. The glass beads were rotated to make sure that all the sodium salts had been wetted, then enough anhydrous Na_2SO_4 was added to make

the contents semi solid. The acids were finally extracted from the semi solid with 1 ml. of 4% (V/V) tert-amyl alcohol in chloroform.

Procedure. 3 g. of silica gel were mixed thoroughly with 1 ml. of water and 0.15 ml. of conc. H_2SO_4. The mixture was slurried with 50 ml. of chloroform, poured into a glass chromatographic tube (0.8 cm. diameter x 75 cm.), and packed into the column by the passage of a further 200 ml. of chloroform. The acid mixture dissolved in 1 ml. of 4% (V/V) tert-amyl alcohol in chloroform was applied to the column. The chromatogram was developed by the successive addition of 50 ml. chloroform, 50 ml. of 4% tert-amyl alcohol in chloroform 50 ml. of 10% and 50 ml. of 12% tert-amyl alcohol in chloroform respectively. 3 ml. fractions of the eluate were collected in a number of test tubes, and to each tube was added another 3 ml. of water before titrating against 0.05N NaOH solution with phenolpthalein as the indicator (pH 8.4-10.0).

(c) Results. The rates and amounts of total acid production during the fermentation of ogi by selected micro-organisms are shown in Table 4.

The acids produced also by the organisms are compared graphically in Figure 11. The relationship of pH to the volume of alkali used to neutralise ogi acids is depicted in Figure 12.

The nature and amounts of the acids produced by the micro organisms are laid out in Tables 5 and 6. The composition of the aliphatic acids of commercial ogi determined by column chromatographic technique is shown in Figure 13.

4. Changes in the nutrient composition of corn during the preparation of ogi

(a) Carbohydrates

(1) Quantitative estimation of the sugars. The method is according to Dubois et. al. (1956). This involves the separation of the component sugars on chromatographic paper using the descending technique; then eluting the spots and assaying the concentration of the eluted sugar colorimetrically.

Preparation of the samples.

Sample W - 10 g. of corn grains were dry milled and then extracted with 100 ml. boiling 70% aqueous ethanol for about 15 minutes. The supernatant was filtered,

Total acid production by microorganisms isolated from Ogi

Day	Time	Equivalent ml. of N/10 NaOH at 30°-32°C.											45°C.	
		SC1	Cory 2	AC 3	LP 4	CM 5	AC LP 6	AC CM 7	LP CM 8	AC LP 9 CM	Control 10	LP x AC x Saccch. 11	AC 12	LP 13
10/3/66	5 pm	0.30	0.30	0.34	0.40	0.36	0.34	0.30	0.30	0.34	0.30	0.32	0.30	0.32
	8 pm	0.40	0.34	0.34	0.32	0.34	0.40	0.34	0.32	0.36	0.30	0.32	0.34	0.32
11/3/66	3 am	0.34	0.48	0.46	0.34	0.34	0.34	0.36	0.34	0.28	0.34	0.36	0.34	0.36
	7 am	0.42	0.52	0.44	0.64	0.42	0.42	0.42	0.40	0.40	0.40	0.44	0.30	0.34
	1.30pm	0.90	0.80	0.60	0.74	0.68	0.60	0.76	0.64	0.66	0.44	0.70	0.36	0.38
	6.15pm	1.60	1.20	0.82	0.84	0.96	0.90	0.86	0.94	0.88	0.48	1.42	0.32	0.42
	10.40pm	2.06	1.30	0.78	0.94	1.00	1.10	1.28	1.20	1.34	0.54	1.62	0.40	0.62
12/3/66	4.5am	2.76	1.50	1.56	1.64	1.22	1.66	1.50	1.30	1.74	0.60	2.44	0.48	0.84
	8.30am	3.00	1.20	2.08	2.26	1.64	1.92	1.76	1.50	1.98	0.68	2.82	0.62	0.82
	1 pm	3.20	1.80	2.14	2.51	1.50	2.48	1.88	1.76	2.08	0.64	3.04	0.58	1.04
	6 pm	3.42	1.90	2.24	2.62	1.44	2.66	2.02	2.00	2.20	0.80	3.28	0.66	0.98
Flavour test		Good	Poor	Poor	V.Fair	Fair	Fair	V.Fair	V.Fair	V.Fair	Poor	V.Good	Poor	Poor

SC = Steep culture obtained by steeping corn grains in water for 2 days.
Cory = Culture of Corynebacterium michiganense
AC = " " Aerobacter cloacae
LP = " " Lactobacillus plantarum
CM = " " Candida mycoderma
Saccch = " " Saccharomyces cerevisiae
Samples 1 - 11 = fermented at 30° - 32°C.
Sample 12 - 13 = fermented at 45°C.
V.F. = Very Fair
V.G. = Very Good
Flavour test = Six people who were familiar with the flavour of 'Ogi were asked to smell the various samples, and their reactions pooled.

Table 4

ACID PRODUCTION BY THE MICROORGANISMS OF OGI

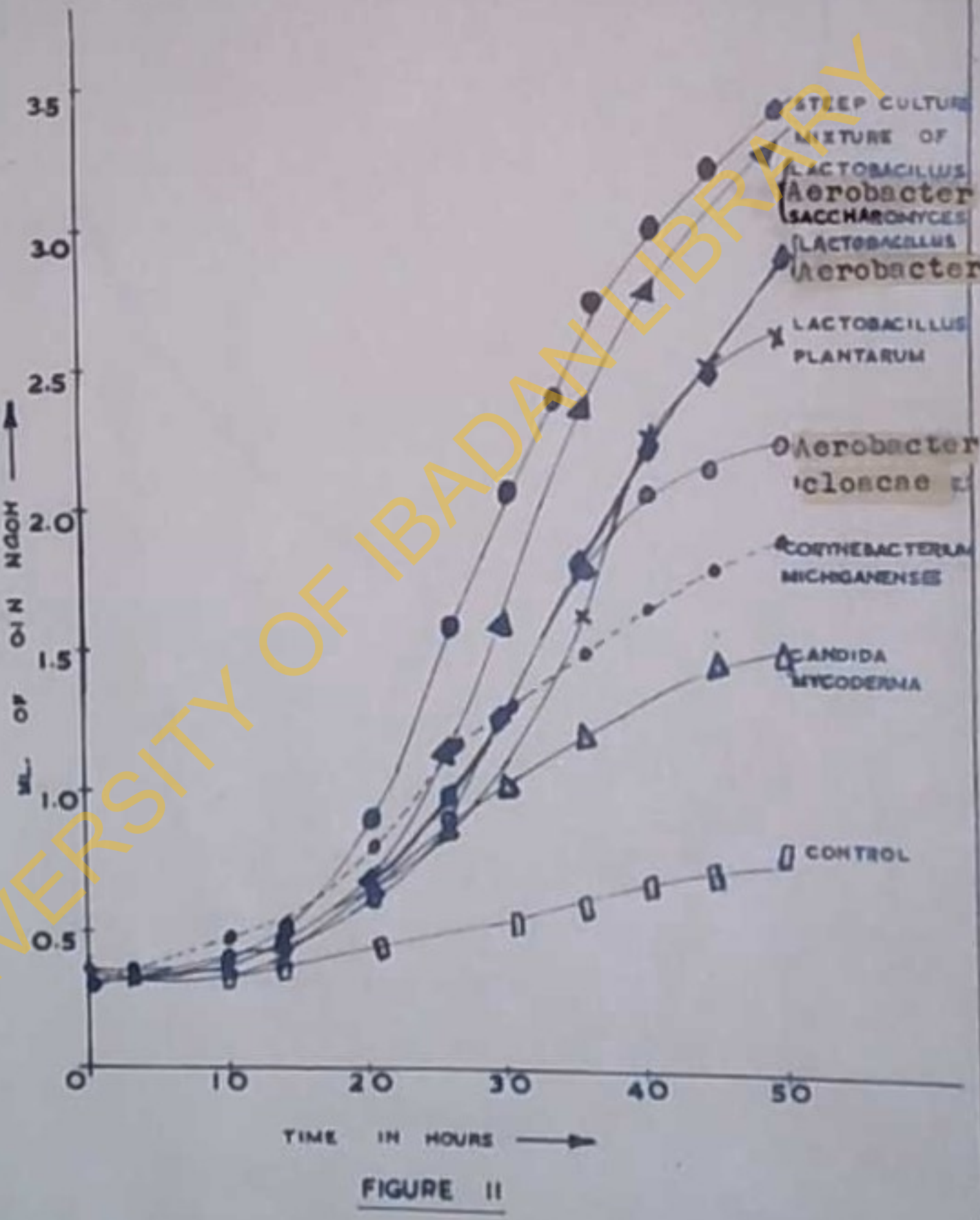

FIGURE II

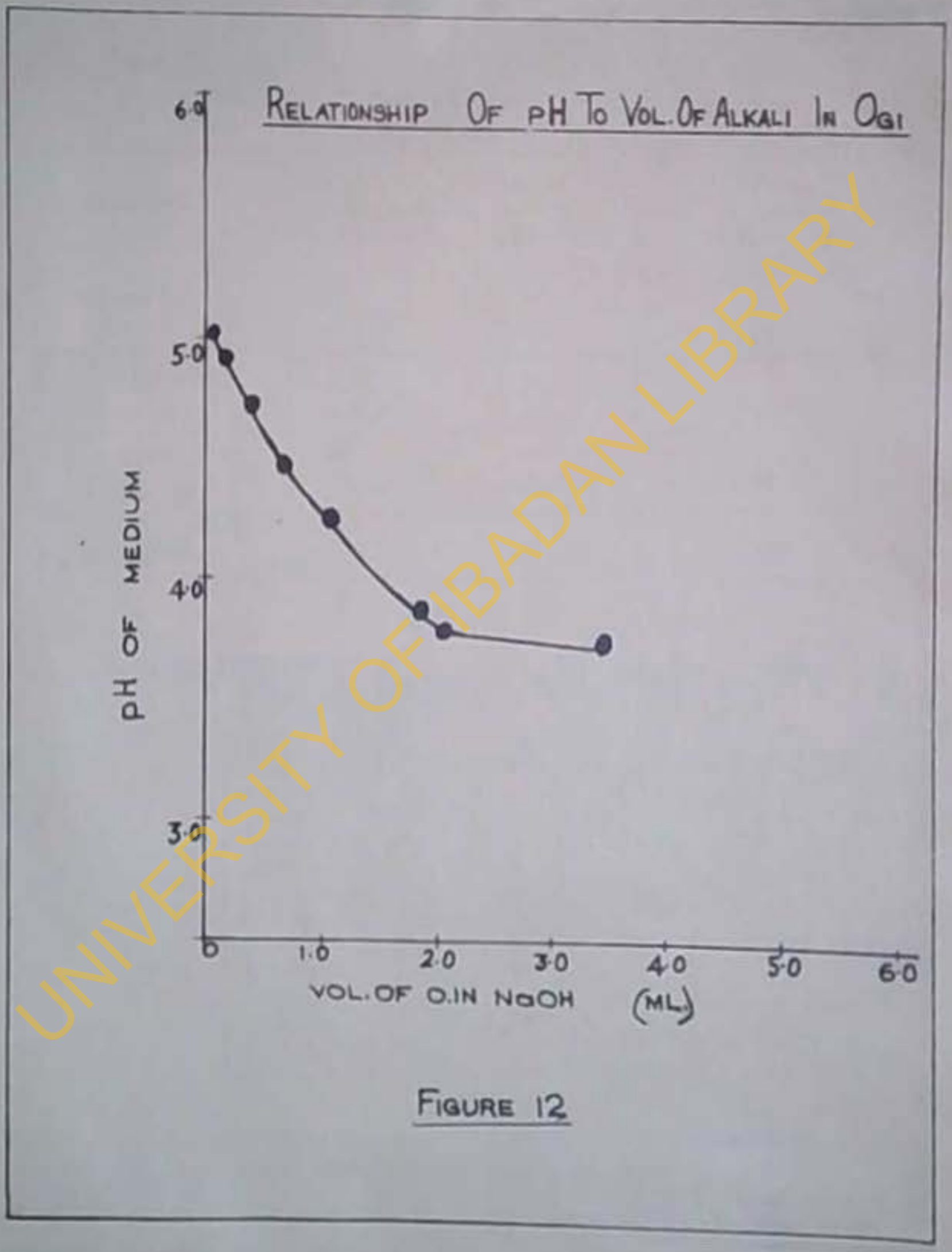

FIGURE 12

Distribution of the volatile and non volatile acids in different Samples of Ogi

Samples	Equivalent vol. of 0.1N NaOH (ml.)		
	Total Acids	Volatile Acids	Non-Volatile Acids
Laboratory Ogi F1	3.42	1.03	2.39
Aerobacter fermented F3	2.24	0.81	1.43
Lactobacillus fermented F4	2.62	0.26	2.36
Yeast(Candida) fermented F5	1.44	0.80	0.64

Table 5

Organic Acids found in different samples of Ogi by paper chromatography

Std. Acids	F3	F5	F4	F1
Formic	-	-	-	-
Butyric	-	-	-	tr.
Acetic	+	+	+	+
Propionic	-	-	-	-
Octanoic	-	-	-	-
Oxalic	-	-	-	-
Lactic	+	+	+	+
Succinic	-	-	-	-
Citric	-	-	-	-
Tartaric	-	-	-	-

Table 6

F1 Laboratory ogi prepared by natural fermentation.
F3 Ogi fermented by *Aerobacter cloacae* only.
F4 " " " *Lactobacillus plantarum* only.
F5 " " " *Candida mycoderma* only
\+ Present - Absent tr. Present in trace amount.

COLUMN CHROMATOGRAPHIC SEPARATION OF THE ORGANIC ACIDS OF 'OGI'

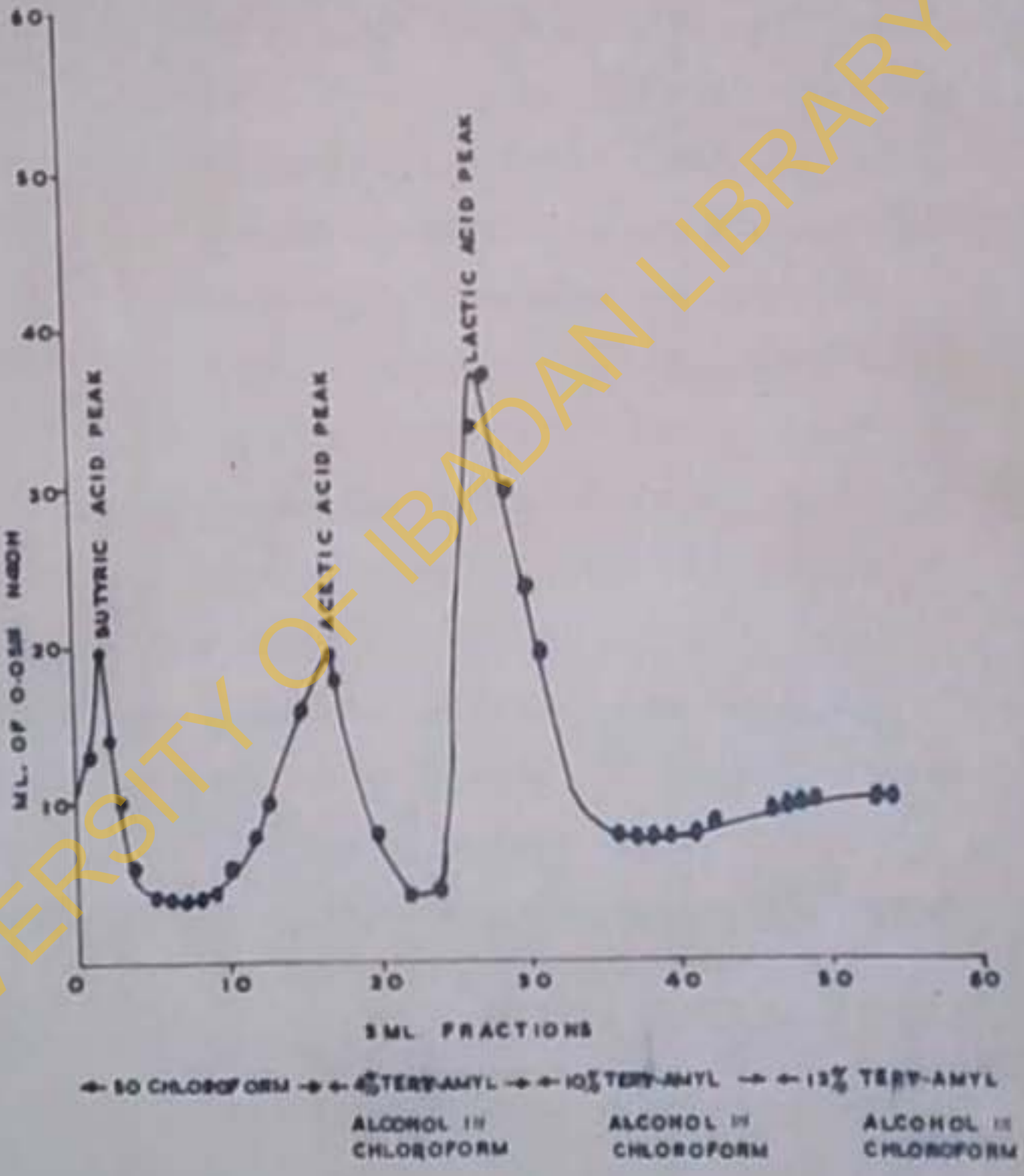

FIG. 13

evaporated to about 5 ml. and then made up to 10 ml. with more 70% ethanol.

Sample C - 10 g. of corn grains were soaked in 50 ml. water and 5 ml. of toluene added, for 2 days. The toluene stopped the growth of microorganisms. At the end of the period, the corn was wet milled and returned quantitatively to its soaking water which was made to 100 ml. with ethanol. The whole contents were boiled for 15 minutes and filtered. The filtrate was evaporated to about 5 ml., and made to 10 ml. on cooling, with 70% aqueous ethanol.

Sample F - 10 g. of corn grains were similarly treated as in sample C, but without the addition of toluene.

Sample LO - 10 g. of corn grains were treated as in sample F but after wet milling, it was sieved to remove coarse materials, then left in its soaking water for another 24 hours to sour. The treatment with ethanol followed by boiling, filtration and evaporation were subsequently carried out.

Sample CO - 20 g. of commercial wet ogi (approximately equivalent to what should be derived from 10 g. dry corn) were extracted with 100 ml. of 70% aqueous ethanol, filtered and the filtrate evaporated to 10 ml.

Procedure. Each sheet of Whatman No.2 paper used was divided into two sections, one carrying duplicate spots of the other. 0.02 ml. of each sample was spotted on each half of the paper at about 2 inches from one another. A solution of mixed standard sugars at 0.1% (W/V) each, in 70% aqueous ethanol was prepared with xylose, arabinose, fructose, glucose, galactose, sucrose, maltose, mellibiose, raffinose and glucuronic acid. The standard sugar solution was spotted at 0.005 ml. and 0.01 ml. levels for comparison on the same paper that carried the spots of samples under test. The chromatographic papers were first equilibrated in a tank containing some of the ethylacetate-pyridine-water solvent (2:1:2:) for about 4 hours. Then the solvent was poured into the top troughs of the chromtank and the papers dipped into them for irrigation for 22 hours. They were afterwards removed and dried at room temperature (30^{o}-32^{o}C).

The papers were each cut into duplicate sections. One section was kept for use in the colorimetric determination of the sugar spots, while the other was first sprayed with $AgNO_3$ reagent (0.1 ml. sat. $AgNO_3$ solution in 20 ml. acetone), dried at room temperature, and then with 0.5N NaOH solution in ethanol, the colour being allowed to develop in 15 minutes. This paper section was washed in 6N.NH_3 solution to remove the background colour and followed by dipping in tap water. It was finally dried at room temperature when the sugar spots were revealed as black spots against a white background.

The spots equivalent to the black spots in the duplicate section were cut and soaked in 5 ml. of distilled water in labelled test tubes separately. After 18 hours, the tubes and contents were shaken and given another hour to settle. 1 ml. of the supernatant from each test tube was then mixed cautiously with 1 ml. of 5% phenol solution and 5 ml. of conc. H_2SO_4 to develop the colour. The tubes were allowed to cool and the brown colour measured for optical density at 490 mu in a unicam SP600 colorimeter.

A blank was prepared by extracting an equivalent portion of clean paper from the same chromatogram with water and reacting with phenol and H_2SO_4 as in the test spots. The determinations were carried out in triplicates, the average value for each sugar being deduced from straight line graphs produced from the standard sugar spots.

(ii) Changes in viscosity accompanying steeping and souring.

Preparation of samples.

Sample C - Corn grains were soaked in a 5% (W/V) $Na_2S_2O_5$ solution for 2 days to stop microbial infection. They were then milled, sieved through a wire screen (B.S.S. No.44 apperture 355 microns) to remove coarse particles, and then centrifuged. The sediment was collected and dried at 75°C in an air oven, and milled to a flour.

Sample F - Corn grains were soaked in plain water for 2 days, and then treated as in sample C.

Sample LO - Laboratory prepared ogi, using 2 days of steeping followed by 1 day of souring, was dried at 75°C and milled to a flour.

Procedure. A 5% solution (W/V) of each sample was made in water. 20 ml. aliquot from each solution, after thorough mixing, was transferred into an Oswald viscometer tube BS IPU 71/58 No.1951 and immersed in a large beaker of water heated with a bunsen flame. The temperature of the water was maintained for 10 minutes prior to the time of taking a reading. The time of flow in seconds of the sample solution through two marked menisci on the tube was determined at various temperatures.

(b) Results. The composition of the sugars of the Nigerian corn, 'Lagos White' variety, and the changes in their concentrations during steeping and souring in the process of making ogi are recorded in Table 7. The effects of these operations on the viscosity of the cornflour in water are shown in Figure 14. A photographic record of the separation of the various sugars on Whatman chromatographic paper No.2 is presented in Figure 15.

Changes in the sugars of Corn during steeping

mg./100g. dry corn

Sugars	W	C	P	LO
Glucuronic Acid	17	33	45	0
Raffinose	63	0	71	0
Mellibiose	0	110	0	0
Sucrose	152	50	76	0
Galactose	0	25	0	0
Glucose	50	135	44	0
Fructose	17	59	5	0

Table 7

W = Dry whole corn grains.

C = Corn grains steeped in toluene treated water for 2 days.

P = Corn grains steeped in tap water for 2 days.

LO = Laboratory 'Ogi' prepared by milling soaked corn grains (e.g. Sample P), sieving and allowing the milky solution to sediment and become sour.

VISCOSITY CHANGES OF CORN FLOUR WITH STEEPING AND SOURING

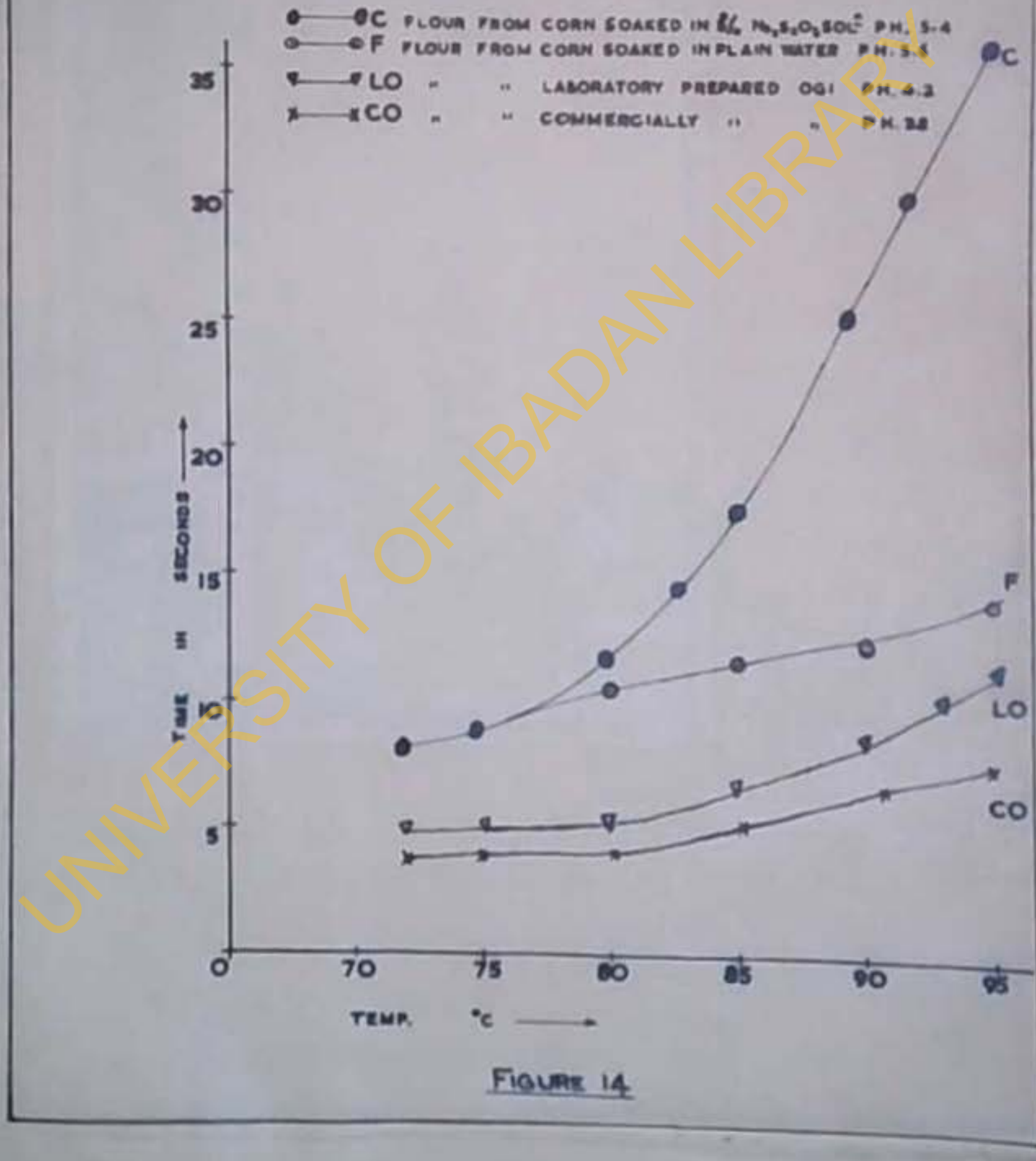

FIGURE 14

Chromatographic separation of the sugars of corn on paper.
Standard = Standard sugars 10ug. each
W = Whole corn
C = Whole corn steeped in 5% $\frac{W}{V}$ $Na_2S_2O_5$ solution to stop the growth of microorganisms.
F = Whole corn steeped in plain water.

Figure 15.

(b) Determination of the nutrient values of corn and ogi samples.

Preparation of samples

Sample W was whole corn grains milled to a fine flour.

Sample C was prepared by soaking corn grains in water containing toluene for 2 days, wet-milling, sieving and drying the sediment at 100°C to a constant weight. The sample was finally milled to a fine powder.

Sample Y was obtained by fermenting a heat sterilised 20% solution of sample C with Candida mycoderma inoculum for 2 days, then drying the mash and milling to a powder.

Sample L was obtained by fermenting a heat sterilised 20% solution of sample C with Aerobacter cloacae inoculum for 2 days, then drying the mash and milling to a powder.

Sample M was obtained by fermenting a heat sterilised 20% solution (W/V) of sample C with a mixed culture of Aerobacter and Candida for 2 days, then drying the mash and milling to a powder.

Sample P was obtained by fermenting a heat sterilised 20% solution of Sample C with Lactobacillus plantarum inoculum for 2 days, then drying the mash and milling to a powder.

Sample O was laboratory ogi prepared from corn.

Samples S was commercial ogi prepared from sorghum.

Chemical analyses of samples.

The official methods of the Association of Agricultural chemists (A.O.A.C.) were used.

(i) Moisture was determined by drying a given weight of the sample contained in a tared cover slip, in an air oven at 100°C until the weight was constant on cooling, in a dessicator.

(ii) Total Nitrogen by the semi-micro Kjeldahl method.

Reagents. Conc. H_2SO_4 Sp.gr.1.84, nitrogen free;

Mercuric oxide;

Potassium Sulphate nitrogen free;

Saturated Boric acid (about 4%);

0.01N Hydrochloric Acid;

Sodium Hydroxide-Sodium Thiosulphate solution - 500 g. of NaOH and 40 g. $Na_2S_2O_3.5H_2O$ were dissolved in water and made to 1000 ml.

Indicator solution - (Methyl red-bromo-cresol green solution) 1 part of 0.2% alcoholic methyl red solution was mixed with 5 parts of 0.2% alcoholic bromo-cresol green solution (pH 4.0-6.0).

Procedure. 1 g. of each sample was transferred into a kjeldahl digestion flask and to it was added 0.7 g. mercuric oxide and 15 g. powdered potassium sulphate followed by 25 ml. of conc. H_2SO_4. The flask was placed inclined on the digestion rack and heated gently until frothing ceased. The contents of the flask were then boiled briskly until it cleared and then for another 30 minutes longer. The flask was cooled and digest transferred with double distilled water into a 200 ml. flask (volumetric) and made to the mark, 10 ml. aliquot from the volumetric flask was put in the microkjeldahl distillation flask followed by the sodium hydroxide - sodium thiosuplhate solution until there was a precipitate of black mercuric sulphide and the solution becoming strongly alkaline. The contents were then steam distilled into a receiver containing 5 ml. saturated boric acid solution and 2-4 drops of the indicator solution, collecting about 40 ml. The distillate was titrated against 0.01N HCl until the first appearance of

violet colour.

(iii) Amino Nitrogen and Non-protein Nitrogen.

20 ml. solution of a 10% (W/V) of each sample were treated with 80 ml. of absolute ethanol to precipitate the proteins. 25 ml. portion of the supernatant were digested and distilled for its nitrogen content (a) as above. Another 25 ml. portion was passed through a column of Zeokarb 215 which had been put in the H^+ form by passing 0.1N HCl through it and washing with distilled water. The eluate was similarly digested and distilled for its nitrogen content (b) The value of (a-b) was taken for the amino nitrogen while (b) was taken for the non-protein nitrogen.

(iv) Crude Fat.

5 g. of each sample were weighed in a filter paper. This was inserted into a soxhlet apparatus and extracted with petroleum ether (40° - 60°C Analar) for about 6 hours. At the end of this, the tared receiving flask was heated to evaporate the solvent until a constant weight was obtained after cooling in a dessicator. The weight of the oil extracted from a given quantity of sample was thereby deduced.

(v) Acid Value.

25 ml. of ethyl alcohol were previously neutralised by adding 1 ml. phenolphthalein solution and enough 0.1N

NaOH to produce a faint permanent pink colour.

The neutralised alcohol was then added to the extracted oil in the flask and the contents were slightly warmed and titrated against 0.1N KOH solution with vigorous shaking until a permanent faint pink appeared and persisted for at least 1 minute.

(vi) Crude Fibre.

The residues from the ether extracts were used. 2 g. of the dry material were transferred to a boiling flask, 200 ml. of boiling 1.25% H_2SO_4 were added and brought to boiling within 1 minute in a reflux condensing system. The flask was rotated frequently to get the material thoroughly wetted. After 30 minutes of boiling, the contents were filtered through linen inserted in a Buchner funnel and washed with boiling water until the washings were no more acid. The charge was then washed back into the flask with 200 ml. of boiling 1.25% NaOH, connected with the reflux condensing system and made to boil within 3 minutes. After another 30 minutes of refluxing, the contents of the flask were filtered through a Buchner funnel with a Whatman ashless filter paper No.44. The filtered residue was then washed with boiling water followed by 15 ml. of ethanol. The filter paper was removed and dried at

110°C to constant weight. It was then cooled in a dessicator and weighed. The filter paper and residue were placed in a tared crucible and ignited in an electric muffle to an ash, cooled in a dessicator and weighed. The difference in the weight of the dry filter paper + residue and the dry filter paper + ash is the crude fibre.

(vii) Minerals. (Wet oxidation method) Preparation of solution. 2.5 g. of each sample were weighed into a 500 ml. kjeldahl flask and to it was added 25 ml. conc. HNO_3 and boiled gently for 30-40 minutes to oxidise all easily oxidisable matter. It was cooled and then 10 ml. of 70% $HClO_4$ were added. The contents were again boiled gently until the solution became nearly colourless with dense white fumes appearing. The flask was cooled slightly, and after the addition of 50 ml. H_2O, it was boiled to drive out any remaining NO_2 fumes. On cooling, it was diluted and filtered into a 250 ml. volumetric flask, made to the mark, and mixed thoroughly.

(viii) Calcium. (Official methods A.O.A.C. 1960)

25 ml. aliquot of the mineral solution was pipetted into a beaker, diluted to 100 ml. and 2 drops of methyl red added (pH 4.4-6.0). 0.88 NH_4OH-H_2O (1:1) was added dropwise to a pH 5.6 as shown by intermediate

brownish - orange colour. Then 2 drops of conc. $HCl-H_2O$ (1:3) were added when the solution turned pink indicating a pH 2.5-3. The solution was further diluted to about 150 ml. and brought to boil with the addition of 10 ml. hot saturated solution of ammonium oxalate and constant stirring. The contents of the beaker were left to stand overnight for the precipitate to settle. Then the supernatant was filtered through Whatman No.42 paper (quantitative) and then precipitate washed thoroughly with 0.88 NH_4OH-H_2O (1:50). The filter paper with precipitate was put back in the original beaker and to it was added a mixture of 125 ml. H_2O and 5 ml. conc. H_2SO_4. The contents were heated to about 70°C and titrated hot with 0.05N $KMnO_4$ solution to a first slight pink colour.

(ix) Phosphorus.

The method used is according to Gomori (1942) and is based on the measurement of colour produced by the reaction of phosphorus with metol.

Reagents. Ammonium molybdate solution was prepared to contain 7.5 g. salt in about 200 ml. water, then 100 ml. of 10N. H_2SO_4 were added and made up to 400ml. with water.

10% Trichloracetic Acid

Metol (p-dimethyl amino phenol sulphate) 2 g. in 200 ml. of 3% $NaHCO_3$ solution.

Standard Phosphorus solution was prepared by dissolving 0.22 g. of KH_2PO_4 in water and making up to a litre. A few drops of chloroform were added before the solution was stored. 100 ml. of solution contained 5 mg. Phosphorus.

Procedure. The solutions were mixed according to the following plan:

	Test	Standard	Blank
Solution of food sample	1 ml.	-	-
Standard Phosphorus solution	-	0.5 ml.	-
10% Trichloracetic acid solution	4 ml.	4.5 ml.	5 ml.
Ammonium Molybdate solution	1 ml.	1 ml.	1 ml.
Metol solution	1 ml.	1 ml.	1 ml.

The solutions were mixed in each test tube and allowed to stand for 30 minutes before being read for their optical densities in 2 mm. cuvettes at 680 mu in a Unicam SP 600 colorimeter.

(x) Iron.(Official methods A.O.A.C. 1960)

Reagents. Standard iron solution was prepared by dissolving 0.7 g. Ferrous Alum in a mixture of 20 ml. HCl and 50 ml. water, and then diluted to a litre. 100 ml. of the solution were transferred to a litre volumetric flask and made to the mark with water. Each ml. of the

latter solution contained 0.01 mg. iron.

Acetate Buffer solution was made by dissolving 8.3 g. of anhydrous sodium acetate in water and then adding 12 ml. of acetic acid, and diluting to 100 ml.

α,α-dipyridyl solution was prepared by dissolving 0.1 g. of the reagent in water and diluting to 100 ml.

10% Hydroxylamine hydrochloride.

Procedure. 10 ml. of the mineral solution prepared by the wet oxidation method, were transferred to a 25 ml. volumetric flask. In a similar manner, 2 ml., 5 ml, 10 ml, 15 ml. of the standard iron solution were transferred to a number of 25 ml. volumetric flasks respectively. To each flask was added 1 ml. of 10% Hydroxylamine hydrochloride solution and after 5 minutes, 5 ml. of buffer solution and 2 ml. of α,α-dipyridyl solution and then made to the mark with water. An aliquot was transferred from each solution to a 2 mm. cuvette and read for optical density at 510 mμ in a Unicam SP 600 colorimeter. The values for the unknown samples were read from the straight line graph obtained by plotting the readings of the standard solutions against concentration.

(c) Analyses of Vitamins

(i) Vitamin A.

The method used is according to Dann and Evelyn (1938) and is based on the measurement of the colour produced

by the reaction of vitamin A with antimony chloride. 10 g. of each sample were extracted for the oil in a continuous soxhlet apparatus with ether for 8 hours. The solvent was evaporated off, the flask dried and the weight of oil determined. 15 ml. of alcoholic KOH, prepared by dissolving 12 g. of KOH in 100 ml. of alcohol, were added to the oil containing flask, connected with a reflux condenser and heated until saponification was complete, about 25 minutes. The condensate was washed down with about 10 ml. of water. The flask was cooled, the contents diluted with about 100 ml. of water and transferred to a separatory funnel. The saponification flask was washed with another 50 ml. of ether and similarly transferred to the separatory funnel. The contents were shaken carefully to avoid the formation of emulsions and allowed to separate into layers. The aqueous layer was drawn off into another separatory funnel, whilst another 50 ml. of ether which had been used to wash the saponification flask the second time, was also used to extract the aqueous layer. This operation was repeated two other times, before the ether extracts were bulked in the first separatory funnel and again washed by pouring through 50 ml. of water without shaking. The aqueous layer was drawn off and discarded. The ether extract was again washed with

50 ml. of 0.5N NaOH solution, shaking gently, then allowed to separate and the aqueous layer drawn off and discarded. The ether extract was then washed with water repeatedly until the aqueous layer drawn off was alkali free on testing with phenolphthalein. The extract was filtered through anhydrous Na_2SO_4 placed on filter paper in a funnel into a 250 ml. flask. The separatory funnel was rinsed twice with two 25 ml. portions of ether and poured into the 250 ml. flask. Some glass beads were added to the ethereal solution and evaporated to dryness on a water bath. The residue was taken up in 10 ml. of chloroform. The following schedule was followed in developing the colour with antimony trichloride reagent.

	Test Tube 1	Test Tube 2	Test Tube 3
Pure chloroform	2 ml.	1 ml.	1 ml.
Antimony trichloride reagent	9 ml.	9 ml.	9 ml.
Unknown Sample extract	-	1ml.	-
Standard Vitamin A soln.	-	-	1 ml.

Other test tubes were made to hold 0.5 ml. and 1.5 ml. of standard vitamin A solution respectively with 9 ml. antimony chloride reagent and corresponding volumes of pure chloroform to make to 11 ml. each. The optical density of the mixed solutions in each test tube was quickly read at 620 mu in a Unicam SP 600 colorimeter and the values of the unknown

samples deduced from a straight line graph obtained from the results taken at the same time of the standard solutions of vitamin A.

B-Vitamins

The official methods of the Association of Vitamin Chemists were used.

(11) Preparation of sample by enzymatic digestion.

10 g. of each sample were transferred into a number of labelled reagent bottles with 60 ml. of 0.4N Sodium acetate buffer having a pH 4.5-4.7. Then 10 ml. of freshly prepared enzyme suspension containing 200 mg. of papain and 200 mg. of takadiastase were added. The enzyme suspension was prepared by mixing 200 mg. of papain with 10 drops of glycerine, then adding 200 mg. of takadiastase, and making to 10 ml. with water. The enzymes were mixed with the samples, a few drops of toluene added to the contents of each bottle, covered loosely, and incubated for 24 hours at 37°C. At the end of this period, the samples were autoclaved for 10 minutes at 10 lb. pressure. They were then shaken and filtered through Buchner funnel containing Whatman No. 1 paper into volumetric flasks (100 ml. capacity). The residues were washed with more water and the washings collected with the filtrate in the volumetric flasks which were then made to the mark. The extracts were transferred into amber bottles, a few drops of toluene added and

stored in a refrigerator until wanted for use.

(ii) Thiamine (Vitamin B_1) (Thiochrome method). The vitamin is oxidised to thiochrome and the fluorescence measured in a Coleman photo fluorometer.

Reagents. 15% NaOH in water,

0.2N sodium acetate buffer,

Alkaline potassium ferricyanide solution. 3 ml. of 1% $K_3Fe(CN)_6$ were diluted to 100 ml. with cool 15% NaOH solution and kept in a brown bottle.

Standard Thiamine Solution. 100 mg. of dry thiamine hydrochlorides were dissolved in 25% ethanol and diluted to one litre with the same reagent. 5 ml. of this solution were then diluted to 100 ml. with water. The final concentration was 0.2 ug. of thiamine/ml.

Working Quinine Sulphate solution. 100 mg. of quinine sulphate were dissolved in 0.1N H_2SO_4 and diluted to 1 litre with the same solvent. Then 3 ml. of the dilute solution were further diluted to 1 litre with 0.1N H_2SO_4 to give a final concentration of 0.3 mg./litre.

Procedure. 5 ml. of each enzyme digested sample were pipetted into each of two reaction vessels numbered 1 and 2. To vessel No. 1 was added 3 ml. of alkaline ferricyanide, mixed gently and then 15 ml. of

isobutyl alcohol. The vessel was shaken vigorously for 90 seconds. To vessel No. 2 was added 3 ml. of 15% NaOH solution followed by 15 ml. of isobutyl alcohol. It was also shaken vigorously for 90 seconds. Similar vessels were prepared using 5 ml. each of the standard Thiamine solution in place of the enzyme digested samples. The reaction vessels were all centrifuged for 3 minutes to separate them into two layers. The aqueous layers (lower) were run out using separatory funnels, about 2 g. of anhydrous Na_2SO_4 were added to each of the alcohol solutions and shaken for 30 seconds. They were allowed to stand until sparkling clear. At least 10 ml. of the clear, colourless isobutyl alcohol solutions from each vessel were decanted into separate matched cuvettes. The fluorescence of the isobutyl alcohol solutions were determined in terms of galvanometer deflections, operating the Coleman photofluorometer according to the manufacturer's directions. The photofluorometer was checked in between readings with the working quinine solution. Thiamine content of the sample in ug. per g.

$$= \frac{U - UB}{S - SB} \times \frac{1}{5} \times \frac{100}{Wt. \text{ of sample}}$$

where U = deflections of unknown.

UB = " " " blank.

S = " " standard

SB = " " " blank.

iv) Niacin Vitamin (Chemical Method)

Reagents. 10% Potassium dihydrogen phosphate,

0.5% Cyanogen bromide solution prepared by adding about 80 g. cold bromine to 1 litre-flask containing 500 ml. of cold water. This was cooled in a bath of ice water. Then a 10% solution of NaCN was added to the bromine solution from a burette until the reaction mixture became colourless with constant shaking. An excess of 10 drops of the NaCN solution was then added and the mixture made to one litre mark.

5% Metol prepared by dissolving 10 g. Metol in 200 ml.

0.5N HCl

Standard Niacin solution. 500 mg. of standard niacin were added to 5 ml. of 10 N H_2SO_4 and when the crystals were dissolved, the solution was made to 500 ml. with water. 5 ml. of this solution were diluted to 200 ml. with water so that the solution contained 25 ug. of niacin per ml.

Procedure. A number of test tubes were set up in a rack in series of 4 tubes for each sample. 4 tubes were also set for the instrument blank whilst another 4 set for the standard niacin. The tubes were arranged as shown in the

table (below) and carried the volumes of reagents specified for each tube.

Total Colour series	Amine Corr. Series	CNBr Corr. Series	Colour Corr. Series
Instrument blanks	(1)	(2)	(3)
7 ml. H_2O	9 ml.H_2O	7 ml.H_2O	9 ml.H_2O
1 ml.KH_2PO_4	1 ml.KH_2PO_4	1 ml.KH_2PO_4	1 ml.KH_2PO_4
2 ml.CNBr.	-	2 ml.CNBr	-
10 ml. Metol	10 ml. Metol	-	-
-	-	10 ml.0.5N HCl	10 ml.0.5N HCl
Standard			
1 ml.Niacin Soln.	1 ml.Niacin Soln.	1 ml.Niacin Soln.	1 ml.Niacin Soln.
6 ml.H_2O	8 ml.H_2O	6 ml.H_2O	8 ml.H_2O
1 ml.KH_2PO_4	1 ml.KH_2PO_4	1 ml.KH_2PO_4	1 ml.KH_2PO_4
2 ml.CNBr.	-	2 ml.CNBr.	-
10 ml.Metol	10 ml. Metol	-	-
-	-	10 ml.0.5N HCl	10ml.0.05N HCl
Total Colour Series	Amine Corr. Series	CNBr. Corr. Series	Colour Corr Series
Test Samples W.,C., etc.			
5 ml.extract	5 ml. extract	5 ml.sample extract	5 ml.extract
2 ml.H_2O	4 ml.H_2O	2 ml.H_2O	4 ml.H_2O
1 ml.KH_2PO_4	1 ml.KH_2PO_4	1 ml.KH_2PO_4	1 ml.KH_2PO_4
2 ml.CNBr.	-	2 ml.CNBr.	-
10 ml.Metol	10 ml.Metol	-	-
-	-	10 ml.05N HCl	10 ml.0.5N HCl

After setting up the tubes as shown, 5 ml. aliquots of sample

extracts were transferred to the proper tubes. The standard tubes carried 1 ml. of standard niacin solution each. To all tubes were added 1 ml. of 10% KH_2PO_4 each and water to amounts shown for each tube. All the tubes were placed for 5 minutes in water bath adjusted to 70°C. From a burette, 2 ml. CNBr were added to the tubes indicated, at intervals of 15 seconds and the contents mixed by swirling. Five minutes after the addition of CNBr, the tubes were transferred to water bath at room temperature (30-32°C). 10 ml. of freshly prepared acid Metol solution were added to those tubes and mixed . 10 ml. of 0.5N HCl were also added to tubes indicated to contain them and mixed. All the tubes were placed in the dark for one hour. The colour intensities of the solutions were read in turn in a Unicam SP600 colorimeter at 400 mu. Before reading a series, the instrument was set to read 100 with the instrument blank of that series and the optical densities of the other samples read directly.

Calculation.

Corrected density = Total density - [(1) + (2) - (3)]

$$\text{ug. of niacin per g.} = \frac{25}{\text{Corrected colour density of standard}} \times \frac{\text{corrected density of sample}}{\text{sample Wt.}} \times \text{dilution factor}$$

(v) Riboflavin, Pantothenic Acid and Folic Acid.

The method used is according to Landy and Dicken (1942) and is based on the fact that the growth and metabolism of certain microorganisms are influenced by the amounts of essential vitamins available in the culture media.

A complete medium for Lactobacillus casei was prepared as follows:-

Casein hydrolysate	10 g.,
Sodium acetate	12 g.,
Glucose	20 g.,
Asparagine	500 mg.,
Tryptophane	200 mg.,
Cystine	200 mg.,
Salt A Solution	10 ml.,
Salt B solution	10 ml.,
Xanthine	10 mg.,
Uracil	10 mg.,
Thiamine hydrochloride	200 ug.,
Biotin	10 ug.,
Folic acid	20 ug., *
Riboflavin	400 ug., *
Calcium pantothenate	400 ug., *
Nicotinic acid	400 ug.,
Pyridoxine hydrochloride	800 ug.,
Distilled water to	1000 ml.,
pH adjusted to	6.8

* omitted when being tested for.

Salt A solution

K_2HPO_4	5 g.
KH_2PO_4	5 g.
Water	50 ml.

Salt B solution

$MgSO_4.7H_2O$	10 g.
NaCl	0.5 g.
$FeSO_4.7H_2O$	0.5 g.
$MnSO_4.2H_2O$	0.337 g.
Water	250 ml.

Procedure. 1 ml., 3 ml., and 5 ml. of each sample extract were transferred into 3 test tubes respectively. Each tube was made to 5 ml. with distilled water. 5 ml. of the complete medium prepared above were added to each tube to make a total volume of 10 ml. Similar preparations were made using standard vitamins in place of the sample extract. For riboflavin, the standard solutions covered a range from 0.05 ug. to 0.5 ug. Pantothenic acid covered a range from 0.0 to 0.3 ug. whilst folic acid was from 0.0 to 0.2 ug. The tubes were plugged with cotton and sterilised at 10 lb. pressure for 15 minutes. After cooling, the tubes were inoculated with a suspension of L. casei and incubated at 30°C (room temperature for 72 hours). Following incubation, the amount of growth was determined by titration of the acid produced using 0.1N NaOH with bromothymol blue as the indicator.

Values obtained from the dilutions of the vitamin standard were used to construct a standard curve from which the vitamin content of any dilution of the sample was calculated. Only values falling within the linear portion of curves were accepted for assay values of the samples.

(vi) Pyridoxine. Vitamin B_6

The method used is according to Atkin et al (1943) and is based on the growth response of Saccharomyces cerevisiae to the nutrient availability of pyridoxine.

Reagents. Salt solution was prepared to contain 2.2 g. KH_2PO_4, 1.7 g.KCl, 0.5g. $CaCl_2$, 0.5g. $MgSO_4$, 0.01 g. $FeCl_3$ and 0.01 g. K_2SO_4 in one litre of water.

Potassium Citrate buffer was prepared by dissolving 100 g. of potassium citrate and 20 g. of citric acid in water and made to one litre.

Inositol solution was made at 0.1% in water.

Biotin solution. 25 mg. biotin dissolved in one litre of water, 40 ml. of this solution were diluted to 1 litre to make a concentration of 1 ug. per ml.

Calcium pantothenate solution was made at 200 ug. per ml.

Niacin solution was made at 1.5 mg. per ml. in water.

Standard pyridoxine solution was made by dissolving 100 mg. of anhydrous crystalline pyridoxine hydrochloride in one litre of 1N.HCl. 1 ml. of this solution was diluted to 100ml.

with water, and 1 ml. of this dilution further diluted to 100 ml. to give a concentration of 0.01 ug. per ml.

Basal medium stock was prepared to contain the following:- 250 ml. salt solution, 50 g. glucose, 50 ml. potassium citrate buffer solution, 50 ml. of 10% acid-hydrolysed casein, 2.5 ml. of thiamine solution (10 ug./ml.), 25 ml. inositol solution, 10 ml. biotin solution, 12.5 ml. calcium pantothenate solution and 1 ml. of niacin solution. This was mixed thoroughly in a beaker, the glucose was dissolved and the pH adjusted to 5.2 with 15% NaOH using a pH meter, and then made to 500 ml. with water in a volumetric flask.

Agar slants for stock culture 30 g. of Difco malt extract agar were dissolved in 1 litre of water by heating. 10 ml. aliquots of the hot solution were transferred to a number of test tubes, plugged with cotton and sterilised by autoclaving at 10 lb. pressure for 15 minutes. They were cooled in a slanted position.

Procedure. A pure culture of Saccharomyces cerevisiae was transferred aseptically into a number of agar slant tubes prepared as above and incubated for 24 hours at 30°C. On the dary prior to use, a fresh transfer was made from a stock culture to another agar slant. This was incubated for 24 hours at 30°C, and afterwards a wire loopful of the

yeast cells from the slant culture was transferred aseptically to tubes of sterile isotonic saline. Into a number of duplicate tubes were pipetted 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml. volumes respectively of the working standard pyridoxine solution. Water was added from a burette to make the volume in each tube 4 ml. To each tube was also added 5 ml. of the basal medium stock solution. Similar preparations were made in another lot of tubes with the sample extracts replacing the standard pyridoxine solutions at 1.0, 2.0 and 4.0 ml. levels. The tubes were all plugged with cotton and steamed at 100°C for 10 minutes. They were then cooled and aseptically inoculated with about 1 ml. of the yeast suspension each. The tubes were left to incubate at room temperature (30°C) for 18 hours with constant shaking. At the end of the period, the tubes were steamed for 5 minutes at 100°C to stop further growth of the microorganisms, and the density of the cell growth read in a Unicam SP600 spectrophotometer at 640mu, using water as the blank. A standard curve for the assay was drawn from which the values for the sample extracts were calculated.

vii) Vitamin C (Ascorbic Acid) Titrimetric Method

Reagents. 6% HPO_3, 3% HPO_3.

Standard Ascorbic Acid 100 mg. of ascorbic acid were dissolved in 3% HPO_3 solution and diluted to 500 ml. with the same solvent. This was used to standardise the dye-2, 6-dichlorophenol indophenol.

0.01% 2,6-dichlorophenolindophenol solution 20 mg. of the sodium salt of 2,6-dichlorophenolindophenol were dissolved in approximately 150 ml. of hot water containing 42 mg. $NaHCO_3$, cooled and diluted with water to 200 ml. This was standardised as follows:-

A 5-ml. aliquot of the standard ascorbic acid solution (containing 1 mg. ascorbic acid) was diluted with 5 ml. of 3% HPO_3. This was titrated with the dye solution to a pink colour which persisted for about 15 seconds since this volume of dye represented 1 mg. of ascorbic acid, the ascorbic acid equivalent (T) of 1 ml. of dye solution is equal to 1 divided by the volume in ml. of the dye solution used in the titration.

Procedure. 50 g. of each of the powdered samples were blended with an equal weight of 6% HPO_3 and mixed to give a homogeneous slurry. 50 g. of this slurry were weighed into a beaker and transferred to a 200 ml. flask (volumetric). The slurry was diluted to mark with 3% HPO_3. The solution was mixed and then centrifuged. The supernatant decanted and filtered. 25 ml. aliquot of the filtrate was pipetted into an Erlenmeyer flask and titrated immediately with the standardised solution of 2,6-dichlorophenol indophenol to a faint pink end point which persisted for 15 seconds.

0.01% 2,6-dichlorophenolindophenol solution 20 mg. of the sodium salt of 2,6-dichlorophenolindophenol were dissolved in approximately 150 ml. of hot water containing 42 mg. $NaHCO_3$, cooled and diluted with water to 200 ml. This was standardised as follows:-

A 5-ml. aliquot of the standard ascorbic acid solution (containing 1 mg. ascorbic acid) was diluted with 5 ml. of 3% HPO_3. This was titrated with the dye solution to a pink colour which persisted for about 15 seconds since this volume of dye represented 1 mg. of ascorbic acid, the ascorbic acid equivalent (T) of 1 ml. of dye solution is equal to 1 divided by the volume in ml. of the dye solution used in the titration.

Procedure. 50 g. of each of the powdered samples were blended with an equal weight of 6% HPO_3 and mixed to give a homogeneous slurry. 50 g. of this slurry were weighed into a beaker and transferred to a 200 ml. flask (volumetric). The slurry was diluted to mark with 3% HPO_3. The solution was mixed and then centrifuged. The supernatant decanted and filtered. 25 ml. aliquot of the filtrate was pipetted into an Erlenmeyer flask and titrated immediately with the standardised solution of 2,6-dichlorophenol indophenol to a faint pink end point which persisted for 15 seconds.

Calculation.

$\frac{V \times T}{W} \times 100$ = mg. ascorbic acid per 100 g. sample.

V = ml. dye used for titration of aliquot of diluted sample.

T = Ascorbic acid equivalent of dye solution expressed as mg. per ml. of dye.

W = g. of sample in aliquot titrated.

Results.

The figures obtained from these analyses are set in Table 8.

Changes in the nutrient composition of corn by fermenting organisms

Analyses on dry wt. basis	Whole Corn	Unfermented Ogi	Fermented Ogi flour	Candida fermented	Aerobacter fermented	Lactobacillus fermented	Sorghum Ogi
Total N%	2.08	1.38	1.49	1.56	1.63	1.51	1.67
Amino N%	0.09	0.04	0.05	0.05	0.03	0.02	0.07
Non-protein N%	0.45	0.10	0.08	0.06	0.07	0.07	0.06
Fat %	4.07	4.06	5.13	4.29	4.29	4.45	4.40
Acid Value	16.54	18.21	34.40	35.20	33.50	33.10	36.00
Crude Fibre %	1.75	0.74	0.72	0.72	0.75	0.73	0.94
Calcium mg./100g.	186.00	86.60	76.60	100.00	66.60	73.30	98.00
Phosphorus "	333.50	233.50	183.50	300.00	250.00	208.50	250.00
Iron "	27.10	15.70	16.70	19.20	20.80	20.80	33.30
Thiamine "	0.17	0.06	0.11	0.10	0.04	0.04	0.64
Riboflavin "	0.20	0.07	0.08	0.06	0.15	0.04	0.16
Niacin "	1.66	0.68	0.85	0.81	1.25	0.64	1.49
Pyridoxine "	0.01	0.00	0.01	0.01	0.00	0.00	0.00
Folic Acid mg./100g.	0.08	0.05	0.05	0.03	0.05	0.05	0.06
Pantothenic Acid mg./100g.	0.05	0.04	0.01	0.04	0.01	0.03	0.02

Vitamins A and C were not found in any of the samples.

Table 8.

5. Effect of fermentation and of supplementation with soya beans on the protein quality of corn.

Determination of Digestibility Coefficient, Biological Value and Protein Efficiency Ratio.

The method used is according to Coyco and Asenjo (1947).

Theory: Total nitrogen content of the diet is used as an index of protein measurement rather than the true protein content because (1) other nitrogenous constituents besides protein nitrogen contribute to the synthesis of nitrogenous constituents of the animal tissue and secretions, (2) losses of protein or more appropriately, amino acids, in digestion and metabolism, can only be measured in terms of nitrogen. The coefficients of digestibility of proteins and the biological values of proteins are necessarily based solely upon nitrogen determinations, and refer solely to the utilisation of food nitrogen in the animal body.

The biological values calculated for various rations of food represent the percentage of the absorbed nitrogen used by growing rats for both maintenance and growth. These calculations involve the assumption that the excretion of faecal nitrogen per g. of food consumed on the low nitrogen diet is the measure of metabolic nitrogen in the faeces. Also the excretion of nitrogen in the urine per 100 g. of body weight during low nitrogen feeding is a

measure of the endogonous nitrogen in the urine. If the estimated endogonous nitrogen in the faeces and urine exceeds the corresponding foecal and urinary nitrogen obtained from feeds, it is assumed that all of the food nitrogen is digestible and assimilated. Mitchell and Carman (1926) have established that 3 to 4 days are expedient for preliminary feeding in order to attain the endogonous level of nitrogen excretion when rats are fed at a low nitrogen diet. The endogonous nitrogen excreted per g. of food consumed and of urine nitrogen per 100 g. of body weight of rat were found to be practically the same on a ration containing from 0.6 to 0.75% of whole egg nitrogen. It was therefore recommended to substitute this low egg-nitrogen ration for a nitrogen-free ration in the standardisation of rats for endogonous nitrogen determinations during protein studies.

The metabolic cages used were made of wire screens 18" x 12" x 12" in dimensions, each cubicle carrying a specially designed perspex bottom to facilitate the collection of urine and faeces. Figure 16 is a picture of it.

Procedure. 30 female albino weanling rats were randomly selected into groups of five according to their weights ranging from 35 g. to 50 g. Each rat was caged and those in each group fed on a specified diet ad libitum. The tests were of 10 days duration divided into 2 periods:-

(1) a 3-day preliminary period for the adaptation of the

rats to their diets, during which no sample was collected, (2) 7-day experimental periods during which urine and faeces samples were collected daily and then bulked. The faeces collected daily were treated with dil. H_2SO_4 and dried in an oven to constant weight at 100°C. For the accurate collection of urine, the perspex bottoms of the wire cages were thoroughly washed daily with a hot solution of 0.6% H_2SO_4 into beakers, then filtered into reagent bottles and kept in a cool room with toluene as preservative. At the end of a 7-day period, the urine samples from each rat were well mixed and diluted to 200 ml. in a volumetric flask. 25 ml. portions were taken for total nitrogen determination by the kjeldahl digestion method. Each diet was made into a paste with a known quantity of water and kept in the fridgediare at about 0°C. Every morning, the food was thawed and accurately weighed quantities offered to the rats. After 24 hours, the residue left by each rat was again weighed; and from a knowledge of the moisture content, the dry weight of the food consumed was deduced. Water was also offered ad lib. in special bottles. The diets offered were prepared to contain from 7% to 10% protein, 5% fat, 2% salt mixture, 2% cellulose, 5 ml. of protovita drops and made to paste with water. The egg diet was prepared to contain about 5% protein and used for the determination of the endogenous urinary and faecal nitrogen.

Diet Composition.

Standard Casein Diet		Low Protein Egg Diet		Ogi/Soya Diet	
Casein	100 g.	7 eggs		Whole Corn Flour	325 g.
Corn Starch	710 g.	Corn Oil	52 g	Full fat Soya	140 g.
Corn Oil	40 g.	Butter	10 g	Corn Starch	465 g.
Butter	10 g.	Corn Starch	735 g.	Corn Oil	40 g
Vitamin Sol^{n}.	5 ml.	Vitamin Sol^{n}.	5 ml.	Butter	10 g.
Sucrose	100 g.	Cellulose	20 g.	Salt Mixture	20 g.
Salt Mixture	20 g.	Biotin	2 mg.	Vitamin Sol^{n}.	5ml
Cellulose	20 g.	Sucrose	79 g.	Water to paste	
Water to paste		Salt Mixture	20 g.		
		Water to paste			
Ogi Diet		Unfermented Ogi Diet		Whole Corn Diet	
Ogi flour	930 g.	Unfermented Ogi Flour	930 g.	Whole Corn Flour	930 g.
Corn Oil	40 g.	Corn Oil	40 g.	Corn Oil	40 g
Butter	10 g.	Butter	10 g.	Butter	10 g.
Vitamin Sol^{n}.	5 ml.	Vitamin Sol^{n}.	5 ml.	Vitamin Sol^{n}.	5ml.
Salt Mixture	20 g.	Salt Mixture	20 g.	Salt Mixture	20 g.
Water to paste		Water to paste		Water to paste	

The salt mixture used is that of Hubbell, Mendel and Wakeman (1937) and has the following composition:

$CaCO_3$	543 g.	$FePO_4.4H_2O$	20.5 g.
$MgCO_3$	25 g.	KI	0.4 g.
$NaSO_4$	16 g.	$MnSO_4$	0.35 g.
NaCl	69 g.	NaF	1.0 g.
KCl	112 g.	$Al_2(SO_4)_3.K_2SO_4.24H_2O$	0.17 g.
KH_2PO_4	212 g.	$CuSO_4.5H_2O$	0.9 g.

1 ml. of the vitamin solution contained the following:-

Vitamin A = 5000i.u.,
Vitamin B_1 = 2 mg.;
Vitamin B_2 (riboflavin) = 1 mg.;
Vitamin B (Nicotinic Acid) = 10 mg.;
Vitamin C = 50 mg.;
Vitamin D = 1000i.u.;
Vitamin E = 3 mg. This was also supplemented with 2 mg. pyridoxine, 2 g. Choline, 50 ug. B_{12} and 1 mg. Vit. K so as to satisfy the optimum requirements of the rats for vitamins according to Cuthbertson (1957).

Calculation.

(a) % Protein (Nx6.25) in food samples
(b) Total faecal nitrogen (mg. of Nitrogen per day average value)
(c) Metabolic nitrogen in faeces (mg. of Nitrogen per day average value)
(d) Food Nitrogen in faeces (b-c) "
(e) Total Nitrogen intake "
(f) Nitrogen absorbed (e-d) "
(g) Total Urinary Nitrogen "
(h) Endogenous Nitrogen in urine "
(i) Food nitrogen in urine (g-h) "
(j) Food nitrogen retained (f-i) "
(k) Biological value: $\frac{j}{f} \times 100$
(l) True digestibility coefficient: $\frac{f}{e} \times 100$
(m) Net protein utilisation $(k \times l)/100$

Protein Efficiency ratio = $\frac{\text{Gain in body wt. of rat (g)} \times 100}{\text{Protein intake (g)}}$

determined after 21 days of experimental feeding.

Balance of nitrogen, digestibility coefficient and biological value of proteins of different diets consumed by female rats (expt.-riod - 7 days)

Diet Description	Rat No.	% Nitrogen in Diet.	Total Faecal nitrogen (mg.)	Metabolic nitrogen in faeces (mg.)	Food nitrogen in faeces (mg.)	Total nitrogen intake (mg.)	Nitrogen absorbed (mg.)	Total urinary nitrogen (mg.)	Endogenous nitrogen in urine (mg.)	Food nitrogen in urine (mg.)	Food nitrogen retained (mg.)	Biological value	True Digestibility Coefficient	Net Protein Utilization	Protein Efficiency Ratio x	Initial Wt. of rats (g.)	Fl. gain of rats (g.)
Whole Corn	W1	1.52	61.4	50.8	10.6	375.6	365	169.9	10.9	159	206	56.47	97.17	54.[illegible]	2.24	36.6	13.3
	W2	1.52	83.1	55.6	27.5	430	382.5	200	11.5	188.5	194	50.71	93.59	47.33	1.14	35.4	10.7
	W3	1.52	61.4	49.7	11.7	366.8	355.1	167.3	11.9	155.4	199.7	56.23	[illegible]	[illegible].44	1.31	36.6	15.3
	W4	1.52	80.6	55.5	25.1	409.3	384.2	199.8	12.7	187.1	197.1	51.34	[illegible]	50.63	1.05	39.1	10.5
	W5	1.52	65.5	51.4	14.1	379.5	365.4	171	11.8	159.2	206.2	56.[illegible]	[illegible].28	54.33	1.20	33.5	9.5
'Ogi' flour	F1	1.26	73.9	62.6	11.3	382.7	371.4	226.3	12.2	214.1	157.3	42.35	97.04	42.10	0.70	37.2	8.7
	F2	1.26	71.8	56.2	15.6	343.5	327.9	233.3	13.3	200	127.9	39.00	95.45	37.23	0.66	35.6	8.4
	F3	1.26	64.4	55.4	9.0	339	330	191.9	11.5	180.4	149.6	45.33	97.34	44.12	0.80	42.6	8.8
	F4	1.26	85.7	69.2	16.5	423.5	407	260	14.7	245.3	181.7	44.64	96.10	42.90	0.76	41.0	9.6
	F5	1.26	84.0	73.4	10.6	448.7	438.1	248	14.8	234.2	205.9	46.54	97.63	45.44	0.93	45.3	8.7
Differentiated 'Ogi' flour	E1	1.52	105.2	87.59	17.7	560.6	543.1	305.6	16.1	290.5	252.6	46.51	96.87	45.05	0.88	45.6	9.0
	E2	1.52	80.3	66.20	16.1	424.9	408.1	268.6	13.1	255.5	180.6	44.75	96.20	43.57	0.80	40.3	10.8
	E3	1.52	101.9	85.4	16.5	547.5	531	300	16.1	283.9	247.1	46.53	96.98	45.12	1.02	48.6	12.0
	E4	1.52	114.6	92.6	22.0	593.5	571.5	340.9	16.1	324.1	247.4	43.28	96.29	41.67	0.93	49.6	8.4
	E5	1.52	97.3	79	18.3	506	487.7	273.9	15.0	256.9	230.8	47.32	96.38	45.61	0.98	46.1	10.7
Agi-amu flour (7:3)	F1	2.02	334.5	271.8	192.7	1[illegible].8	1007.1	279	18.3	260.7	743.4	71.99	83.87	69.06	2.00	47.5	11.6
	F2	2.02	243.1	109.8	133.3	1076.9	945.6	254.6	16.4	238.2	707.4	74.80	87.80	65.67	2.77	45.4	12.7
	F3	2.02	294.8	123	171.8	1[illegible].9	1034.1	256.8	17.2	239.6	794.5	76.83	85.75	65.88	2.53	43.0	15.7
	F4	2.02	387.3	140	247.3	1377.8	1130.5	418.8	18.8	400	730.5	64.61	82.34	53.20	1.50	49.3	10.3
	F5	2.02	326.6	130	196.6	1275.2	1078.6	290.4	19.2	271.2	807.4	74.89	84.54	63.31	2.27	50.2	14.3
Gari	G1	1.4	144.3	92.4	51.9	628.2	576.3	69.7	12.3	57.4	518.9	90.03	91.73	82.58	3.24	32.0	13.4
	G2	1.4	127.5	106.9	20.6	776.1	695.5	73.9	13.4	60.5	625	91.17	94.40	86.06	3.61	34.9	14.6
	G3	1.4	140.7	75.2	65.5	511.6	446.1	62.6	12.4	50.2	395.9	88.74	87.19	77.37	2.75	32.9	11.2
	G4	1.4	180.0	129.5	50.5	880.2	821.7	97.4	14.8	82.6	739.1	89.94	93.35	83.96	3.34	36.7	13.1
	G5	1.4	149.4	89.0	60.4	674.8	564.4	75.6	13.1	62.5	487.1	86.33	90.01	79.70	2.89	35.0	19.3

a determined over a period of 21 days.

TABLE 5

Group mean values for protein utilisation on the different diets (See Table 9)

(5 female rats per group diet)

Diet Description	Initial rat wt. (g.)	Wt. gain of rats (g./day) *	% Protein content of diet	N-Intake		N - Excretion (mg.)			N - Balance			True Digestibility	Biological Value	Net Protein Utilisation	Protein Efficiency Ratio *
				mg./day	mg./100g. rat wt.	Urinary (mg./day)	Faecal (mg./day)	Total	mg./day	mg./100g. rat wt.	% of Intake				
Whole Corn	36.3 ±1.34	0.692 ±0.02	9.50	55.5	153	25.9	10.1	36.0	19.5	53.7	35.2	95.48 ±1.73	54.75 ±2.24	52.30 ±2.86	1.19 ±.013
'Ogi' flour	40.2 ±4.88	0.416 ±0.03	7.88	55.3	138	31.0	10.9	41.9	13.4	33.3	24.2	96.71 ±1.00	43.57 ±2.83	42.13 ±2.90	0.77 ±.012
Unfermented 'Ogi' flour	46.2 ±3.16	0.51 ±0.06	8.15	75.2	163	41.7	14.3	56.0	19.2	41.6	25.5	96.54 ±0.24	45.58 ±1.73	44.0 ±1.73	0.86 ±.245
Ogi-Soya flour (7:3 mixture)	47.1 ±2.28	2.43 ±0.52	12.63	171	372	42.8	44.5	87.3	87.7	186.2	50.1	84.86 ±1.90	73.02 ±4.47	61.95 ±4.71	2.21 ±.242
Casein	34.3 ±1.90	1.89 ±0.38	8.16	95.7	279	10.8	22.0	32.8	62.9	183.4	65.8	91.34 ±2.37	89.69 ±1.00	81.93 ±3.07	3.17 ±.105
Whole Egg	49.4 ±2.61	1.68 ±0.20	5.50	77.3	126	2.5	18.2	20.7	56.6	114.6	73.2	100	100	100	3.48 ±.049

* Figures obtained after 21 days of test feeding.

Table 10

Statistical evaluation of the B.V., N.P.U. and P.E.R. figures.

Diets paired	't' values			
	Results obtained			
	Biological Value	Net Protein Utilisation	Protein Efficiency ratio	Theoretical levels of significance
1. Ogi and whole corn	5.54	4.43	42.41	2.31 at 5% probability
2. Ogi and unfermented Ogi	1.08	0.99	0.80	
3. Ogi and Ogi-Soya mixture	9.96	6.43	10.63	3.36 at 1% probability
4. Ogi and Casein	27.49	16.42	40.61	5.04 at 0.1% probability
5. Ogi-Soya mixture and Casein	6.51	6.33	6.51	

Table 11

Sets of wire cages used for metabolic studies on rats. The dimensions are 18" x 12" x 12". Under each cage is a triangular bottom, the lower angular sides of which are of perspex material allowing the easy flow of urine into the beakers. The upper hypotenuse side carries a fine screen mesh for the retention of the faeces.

Figure 16

D I S C U S S I O N

1. Variations in the methods of preparations of ogi.

A survey of the methods of preparation employed by commercial producers of ogi at Ibadan and in Lagos showed that the variations were mainly in the lengths of the steeping as well as the souring periods. These range from 1 to 3 days in either case. When warm water was used for steeping, the period was usually of one day duration but when cold water was used, the steeping was continued for 3 days with daily replacement of the old liquor with fresh water. The souring process was allowed to last until a good sour flavour was produced based on organoleptic tests.

The effects of these variations on the microflora, and consequently on the nature and rate of fermentation of corn were investigated. The results in Table 1 show that after 24 hours of steeping of whole grains of corn in tap water at about 30°C, the microflora of the steep liquor was dominated by the lactic acid bacteria. This continued for the next 48 hours, even with the daily changes of water. The same trend was also shown by the milled sample D. However, in the latter case, a high population of aerobic organisms still remained viable after 24 hours, which could have brought about secondary fermentations and the concomitant production of undesirable flavours. Schweigart and de Wit (1960) carrying out similar investigation in South Africa, observed that mahewu prepared by allowing

corn porridge to go sour naturally at 25°C developed poor flavour, and so employed a high temperature (45°C) to suppress the undesirable organisms. The practice of steeping whole grains instead of milled corn in West Africa could therefore be regarded as another means of cultivating the desirable microorganisms by natural selection in preparation for the souring process.

Another feature of corn steeping is the absorption of water by the grains. The moisture absorption pattern of corn during steeping in cold water (30°-32°C) is shown in figure 1. It can be seen that most of the absorption took place within the first 24 hours. Wagoner (1948) in his studies on the industrial corn steeping process found that it was during this period, which he described as the lactic acid phase, that the corn was softened. He noted that prolonged steeping into the sulphurous acid phase caused the disruption of the protein matrix and finally its washing away from the cell walls. The results in Table 1 have established that 24 hours of steeping were also sufficient for the natural selection of lactic acid bacteria, whereas further steeping only led to a greater depletion of the limited supply of fermentable carbohydrates of corn.

Again, Bond and Glass (1963) claimed that alpha and beta amylases were not present in the resting seed of corn but that it was only on the sixth day of steeping and growth initiation that alpha amylase was secreted, becoming predominantly active thereafter.

If this were true in all cases, then no significant starch conversion into simple sugars could be expected from a corn steep of less than six days duration. For ogi preparation, the steeping time is not normally extended beyond three days.

Therefore the main advantages to be derived from the steeping of corn in the preparation of ogi would be to soften the kernel and to build up a predominant lactic acid microflora. All this can be achieved within the first 24 hours of steeping in cold water.

2. <u>The microflora of the Ogi</u>.

Using a steeping period of 24 hours, followed by 48 hours of souring, the microbiological changes were investigated. The results given in Table 2 show that a wide variety of micro organisms were involved in the cycle of fermentation. First, the surface microflora of the corn was marked by the presence of a number of fungal spores. These germinated into large colonies when cultivated on nutrient agar medium. Those identified were <u>Cephalosporium</u>, <u>Fusarium</u>, <u>Aspergillus</u> and <u>Paniciillium</u>; but they were all eliminated early during the steeping period. All these fungi but <u>Cephalosporium</u> have been reported to cause ear rot in corn. Burtt-Davy (1914). <u>Cephalosporium acremonium</u> f major Penzig and <u>Cephalosporium maccheri</u> have also been described by Sprague (1950) as causing the black bundle disease in corn and that they are seed or seedling borne.

It is significant that in the survey of the surface microflora of over 20 samples of corn obtained from the markets and the stores of the Federal Agricultural Research station, Moorplantation, Ibadan, Cephalosporium colonies were preponderant in numbers among the moulds. They were definitely associated with the corn because they could not be found in the control cultivation of tap water alone nor at subsequent isolations during the course of fermentation.

Various bacteria were isolated from the fermenting mash and their identification was based on their morphology, colonial characteristics, fermentation and a number of specific biochemical tests which are recorded in Table 3. Those recognised were Corynebacterium michiganense, Aerobacter cloacae and Lactobacillus plantarum. Corynebacterium michiganense only, showed some diastatic action when grown on starch agar (Table 3), and would therefore seem to be responsible for the change in the paste viscosity of cornflour after steeping the grains for 2 days in water to ferment naturally. Collard and Levi (1959) in their studies of the fermentation of cassava also isolated a Corynebacterium strain they named 'manihot' which was reported to be actively connected with the hydrolysis of cassava starch to organic acids. It would seem, therefore, that Corynebacterium sp. could be associated with the natural fermentation of starchy vegetables.

The isolations of Leuconostoc mesenteroides and Lactobacillus plantarum from the Nigerian corn steep and mash again confirm the widely accepted view that they are the fermenting organisms of vegetable materials responsible for the production of biological acidity (Hucker & Pederson 1930, Pederson and Albury 1955 and 1962, Stamer, Albury and Pederson 1964, Rushing, Veldhuis and Senn 1956 etc.). Over 160 strains of Leuconostoc have been studied in detail by Hucker and Pederson (1930). Those strains fermenting pentoses and sucrose, and producing slime in sucrose solutions, were classified as Leuconostoc mesenteroides. Of the strains of Leuconostoc mesenteriodes tested by them only 14 per cent were found able to ferment sorbitol. The strain isolated from corn steep liquor in the current experiment would need to be further ascertained.

The following yeasts were also present in the microflora:- Saccharomyces cerevisiae, Rhodotorula spp. and Candida mycoderma. Their fermentation reactions and morphology as well as colonial characteristics were used to identify them. Their roles would seem to be associated with fermentation of the milled corn. The beginning of the souring period was marked by the rapid proliferation of Saccharomyces cerevisiae; while at the end, the film forming Candida mycoderma predominated the microflora (Table 2).

3. The products of fermentation.

The micro organisms enumerated in the preceding section were observed to have caused tremendous biochemical changes in the corn mash. The major effects arising from their fermentative activities were the formation of organic acids, leading to a change in flavour and taste. The organic acids formed during the fermentation of wet-milled corn into ogi were qualitatively and quantitatively estimated, and the results recorded in Tables 5 and 6 and Figure 13. Lactic and acetic acids mainly were produced. Butyric acid was found in trace amounts and it could have been derived from the rancidity of the corn fat during storage. The composition of the acids of ogi given in Table 5 does not correlate with those formed by the individual micro organisms primarily responsible for its acidification. When, however, the total acidity produced by each organism is followed with time, as shown in Figure 11, a general inference can be made that the acidification of the mash was initiated by Corynebacterium michiganense which was later replaced by Aerobacter cloacae. The highest amount of acidity was produced by Lactobacillus plantarum probably because it was able to utilise the dextrins of the corn after the depletion of the fermentable sugars. Lactic acid was the main acid formed by Lactobacillus plantarum.

This acid is a very good substrate for the growth of Candida mycoderma which can consume as well produce organic acids generally, Lafar (1910). The fermentative characteristics of Candida mycoderma shown in Table 3 clearly establish that while it can assimilate many carbon compounds, it is only able to ferment glucose and fructose. Lafar also reported that these fermentable sugars were found to be partly oxidised and partly converted into acids. Therefore, the role of Candida mycoderma in the preparation of ogi must be regarded as one involving the destruction of the organic acids formed by the bacteria.

Flavour is another major product of fermentation. Its measurement has been based solely on organoleptic tests, although in recent years, numerous workers have tried to correlate it with a number of indices. Braverman (1963) reported that Harvey and Fulton tested 30 varieties of tomatoes the pH of which fell between 4.15 and 4.5 and found that their taste differed enormously, even though their pH differed only little. Harvey (1920) had earlier, used various buffers to prepare solutions of equal pH but widely different total titrable acidity, and came to the conclusion that the sour taste was a function of two variables, pH and total acidity. Bremond working on wines later, was reported (Braverman 1963) to have shown that the sour taste was determined more by the total titrable acidity than by pH.

The flavour acceptance at different levels of total titrable acidity of fermented corn is shown in Table 4. A good correlation was not established between the two factors, although there was a trend of good flavour in high acidity. This is because a third factor seemed to have been involved, the aroma or ester production potential of the fermenting organisms. The yeasts, Saccharomyces cerevisiae and Candida mycoderma were always able to produce an acceptable flavour even at relatively low acidity whenever they were present in the mash. Aerobacter cloacae , on the other hand, formed a relatively high acidity but with a poor flavour. Schweigart and Pellinghan (1963) believed that lactic acid was the main flavour source of mahewu, a sour corn beverage of the Bantus; the same could not, however be said of ogi because at a relatively lower content of lactic acid, Candida mycoderma produced a better flavour than Aerobacter cloacae . Lactic acid could, nonetheless, be considered as one of the factors responsible for the flavour of ogi. In Figure 12, the pH of ogi is related to the total titrable acidity. There was a good correlation between the two in the pH range 3.8-5.0 It is significant that the lower limit of this range corresponds with the pH value giving the minimum sour taste acceptable to the palate of Nigerians when eating fermented foods, Akinrele (1964). Whence pH may be used as a reliable test of acceptability of the sour taste of ogi. The flavour is usually best at pH 3.6.

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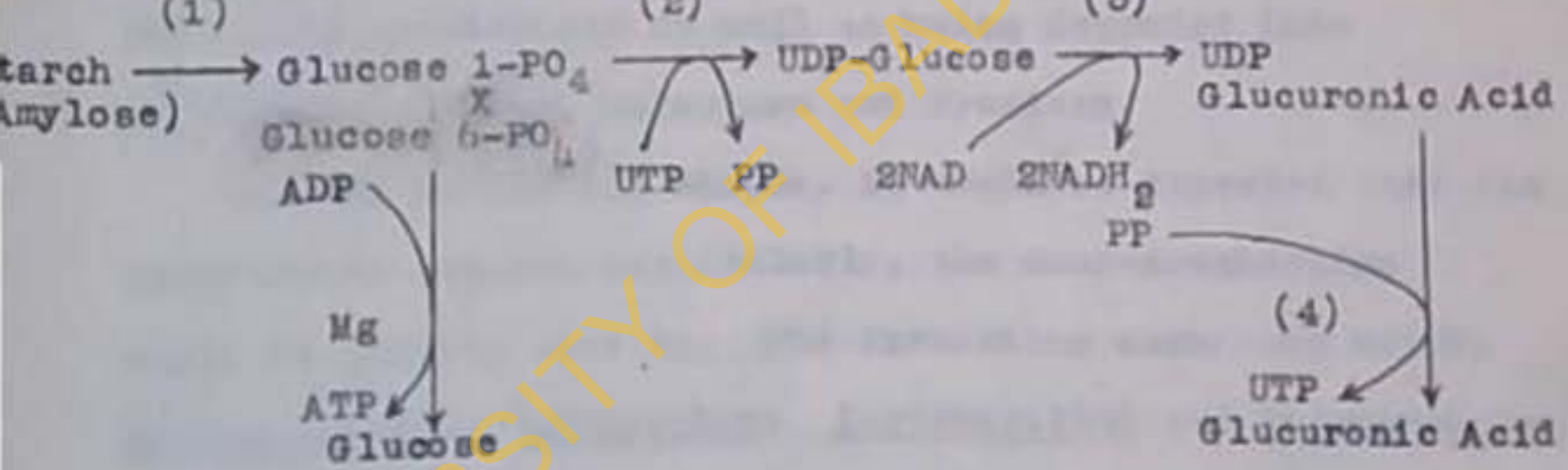
Samples 1 and 11 in Table 4 gave the best flavour and acidity and would therefore confirm that the most desirable and important micro-organisms in the souring process are Saccharomyces cerevisiae, Aerobacter cloacae and Lactobacillus plantarum.

4. Biochemical and physical changes in the corn carbohydrates during the preparation of Ogi.

The composition of the sugars of the Nigerian "white flour" corn and the changes arising from the effect of steeping in water are shown in Table 7.

An increase in the glucose, glucuronic acid and fructose contents of the corn was observed during steeping, and melibiose and galactose were formed. At the same time, the amounts of sucrose and raffinose decreased. Bond and Glass (1963) had similarly noted a progressive decrease in the sucrose and an increase in the glucose contents of dent corn on steeping in water for 2 days. Macleod and her co workers in their investigations of carbohydrate metabolism in the germinating barley were reported (Bond and Glass 1963) to have explained this observation by postulating that sucrose formed the principal respiratory substrate during the early stages of growth initiation. The experimental evidence obtained from the studies carried out on the steeping of Nigerian corn is that its sucrose was hydrolysed into glucose and fructose, by the enzyme invertase which also hydrolysed the raffinose into melibiose and fructose. The melibiose was further

hydrolysed into galactose and glucose by the enzyme melibiase. However, these interconversions could not be quantitatively accounted for on the basis of theoretical derivations. The quantities of melibiose and glucose formed during controlled steeping (Table 7) exceeded what could be expected from the hydrolyses of the raffinose and sucrose of unsteeped corn. Therefore, the large increases observed in the glucose and glucuronic acid during steeping could have arisen from a possible phosphorolytic degradation of the amylose portion of the corn starch according to the following scheme:-



Enzymes:

(1) Phosphorylase
(2) Glucose pyrophosphorylase
(3) Uridine diphosphate-glucose dehydrogenase
(4) Uridine diphosphate-glucuronic pyrophosphorylase.

Abbreviations:

UTP = Uridine triphosphate
PP = Pyrophosphate
NAD = Nicotinamide adenine dinucleotide
$NADH_2$ = " " " (reduced)
ADP = Adenosine diphosphate
ATP = Adenosine triphosphate
Mg = Magnesium
X = Phosphoglucomutase

Edelman et al (1959) have presented evidence that sucrose was synthesised in the scutellum of cereal seedlings by a mechanism involving uridine diphosphoglucose. It would therefore be expected that such a mechanism would lead to the formation of glucose and glucuronic acid in corn during growth initiation.

If the raffinose and melibiose estimations were considered together because a clear separation of the two was not achieved, an overall increase in the total amounts of the sugars could be said to have occurred during controlled steeping. This is also indicative that raffinose was being synthesised as well as being degraded into melibiose, glucose, galactose and fructose.

In the fermenting sample, it would be expected that the fermentable sugars, particularly, the mono-saccharides would be quickly used up. The fermenting organisms mainly Corynebacterium, Aerobacter, Lactobacillus and Saccharomyces have been shown to utilise nearly all the sugars mentioned in Table 7. Nevertheless, the sucrose and glucuronic acid values were higher than those of the unfermented sample. The increase in the sucrose value may have been the result of less dependence on it as a respiratory substrate, if enough energy was being made available through the microbial fermentation of the monosaccharides. Glucose may have been increasingly converted to glucuronic acid also by the microorganisms.

It is significant that no trace of maltose was detected either in the sample of dry whole grains of corn or in the samples steeped in water for 2 days. In Figure 15, faint spots could be observed in the cases of whole corn grains and the naturally steeped sample corresponding to maltose and mellibiose respectively. However, no sugar was detected on extracting the spots with water for quantitative determination. This finding would therefore support the conclusion of Bond and Glass (1963) that beta amylase was neither present in the resting corn nor secreted during growth initiation. The contrary view of Dure (1960) that beta amylase was present could not be accepted.

The effect of the natural steeping of corn in water on the viscosity characteristic of its paste is shown in Figure 14. In this experiment, it was assumed that the relative viscosity of the starch solution in the Oswald viscometer tube was directly related to the time of flow in seconds between the marked menisci, at a given temperature. Therefore, the time of flow in seconds was used to represent the relative viscosity values. A significant loss of viscosity could be observed in sample F when compared with sample C. This must have been the result of some diastatic activity on the starch of the corn arising from the secretions of the infecting micro-organisms. Of the microbes isolated in significant numbers from the steeping

liquor, only Corynebacterium michigenense showed a weak diastatic activity on starch agar. Since the formation of maltose could not be detected, it is believed that the starch was mainly converted to dextrins. This, therefore, could be the more important role of this organism during the traditional fermentation of corn rather than the initiation of acid production. The laboratory and commercial samples of ogi (LO and CO) showed still higher loss of viscosity. Their patterns were very similar and since no diastatic organism was found in the latter stages of souring, the loss of viscosity in the latter samples must have arisen from the effects of lower pH values.

5. Fermentation effects on the nutrient composition of Corn.

Changes caused by the microorganisms in the nutrient composition of the corn can be observed in Table 8. The effects of processing and fermentation, and the influence of the individual micro-organisms on these nutrient values are shown in the table. Whole meal corn had in general more nutrients than ogi, whether fermented or not. This would be expected in view of the fact that a major proportion of the germ was sieved out during processing. On the other hand, when the samples of fermented and unfermented ogi are compared, an indication is given that fermentation brought about an enrichment of the nutrients, notably in the gross nitrogen content and in some of the

B vitamins, but with a simultaneous decrease in the calcium and phosphorus values.

The predominant micro-organisms isolated during the souring period of ogi were also used to determine to what extent each could change the nutrient composition of the food. Employing a ratio of amino nitrogen to total nitrogen as a measure of the metabolic activity of the micro-organisms on the proteins of corn; there was very little degradation effected by any of the species; rather Aerobacter cloacae and Lactobacillus plantarum used up some of the free amino acids for growth. This dependence on environmental nutrients for growth was shown by the latter organism towards almost all of the minerals and vitamins. Aerobacter cloacae, on the other hand, while reducing the environmental thiamine and pantothenic acid, increased the riboflavin and niacin contents of the culture mash. Candida mycoderma too was able to increase slightly the level of total thiamine, niacin and pyridoxine of the unfermented ogi.

The figures presented in Table 8 denote that the Candida fermented sample was closest to the laboratory fermented ogi flour in nutrient composition, and could therefore lead to an erroneous impression that it provided best substitute for natural fermentation. The results shown in Table 4 have already established that such a

sample could not acquire sufficient acidity and a good flavour. Therefore the similarity in the nutrient composition of the two samples must be considered as reflecting the exacting influence of Candida mycoderma on the nutrients of ogi at the end of the souring period.

Apart from the losses brought about during the extraction of ogi from whole corn meal, the fat, crude fibre and iron contents were not significantly affected by the fermentation. Also, none of the microorganisms examined caused a striking increase in the acid value of the fat, thereby suggesting that no lipolytic enzyme was secreted. However, the difference in the acid values of the unfermented ogi and the other fermented samples could be ascribed to a hydrolytic action, possibly lipase, generated within the corn.

Vitamins A and C could not be detected in any of the samples. Since the effect of the biological acidity formed in the fermented samples would be to stabilise the vitamin C or ascorbic acid, it could be concluded that none of the microorganisms tested had the ability to synthesise this vitamin. Also, because glucuronic acid is an intermediary product in the conversion of glucose to ascorbic acid, the necessary enzymes for the completion of this change would appear to be lacking both in the corn and in the associated microorganisms.

6. <u>The protein qualities of corn and ogi</u>.

The qualities of the proteins of whole corn meal and ogi were evaluated in terms of the true digestibility coefficient, biological value, net protein utilisation and protein efficiency ratio. The figures presented in Table 9 are the daily averages for each rat while those in Table 10 represent the group averages per diet. Only the casein diet should be considered as a standard; the egg diet having been used at about 5 per cent protein to determine the endogonous and metabolic nitrogen in the urine and faeces respectively of the experimental rats.

A good correlation was obtained between the results of the biological values, net protein utilisation, protein efficiency ratio and the percentage of the total nitrogen intake retained in the body from all the diets. These figures indicate that the protein of ogi was inferior to that of whole corn mainly because a considerable portion of its germ had been sieved out during processing; and that fermentation per se, brought no significant change in the quality. The latter finding therefore supports the observation of Dreyer and Schweigart (1963), and further corroborates an earlier conclusion (page 104) that the micro-organisms found in the fermenting corn mash during the preparation of ogi effected little or no degradation of the proteins.

The protein efficiency ratios, net protein utilisation and biological values of the four experimental diets were paired and tested for significance level (Table 11) using the student's test and applying Bessel's correction because of the small number of samples (Moroney 1951). In nearly all of the cases except one, the probabilities that the differences between the diets compared would have arisen by chance are less than one in a thousand times, and so a high degree of significance has been established. When fermented and unfermented ogi are compared however, the probability is more than 10% and so significance is not shown.

The figures obtained for the true digestibility coefficients of the diets do not follow the same trend as the other indices of protein quality assessment. This is because of the different amounts of protein or nitrogen intake. Therefore, the rates of digestion and absorption of proteins must be considered as not having a direct relationship with quality alone but with quantity and physical nature or state. When, however, the nitrogen balance per 100 g. body weight of each rat group is divided by the corresponding nitrogen intake per 100 g. body weight of the rat group, the results expressed in percentages follow the trend given by the biological values.

Ogi is used extensively as the main food for babies immediately after weaning, particularly amongst the rural families in Western Nigeria. With a protein content of about 8%, biological value 43 and protein efficiency ratio 0.77, it cannot be considered suitable as an infant food. Since it has been established that the protein quality of corn is not significantly changed by natural fermentation per se, it can then be presumed that any enrichment of the basic protein will be carried over to the final product.

Studies carried out by the Food and Agriculture Organisation of the United Nations (1953) have indicated that the limiting amino acids of corn protein are tryptophan and lysine. Tryptophan is partially replaceable with nicotinic acid but the lysine deficiency can only be corrected by fortification. Taking note of the fact that the total protein level of ogi needs to be increased, and that most of the babies fed with this meal belong to a low income group, soya bean would appear to be the best supplement. It has a high protein content, about 50% in the Nigerian variety, and quite rich in lysine, but nonetheless, relatively cheap.

7. <u>The effect of soya supplement on the protein quality of ogi</u>.

When full fat soya flour which had been heated to destroy the anti-tryptic factor was added to ogi at 30 per cent level, the protein efficiency ratio of the mixture

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7. <u>The effect of soya supplement on the protein quality of ogi</u>.

When full fat soya flour which had been heated to destroy the anti-tryptic factor was added to ogi at 30 per cent level, the protein efficiency ratio of the mixture

was found to be 2.21, about three times the value for ogi (0.77). Jones and Divine (1944) found that the P.E.R. of full fat soya bean meal was equal to that of casein. Assuming this to be so, the P.E.R. of the experimental soya-ogi diet should theoretically be 1.49. Therefore, the observed value of 2.21 clearly confirms the observation of Chick (1951) that soya protein had a special supplemental value to cereals, probably because of its high lysine content. The biological value and net protein utilisation of ogi were similarly enhanced by the addition of soya, but the values were not as high as those of casein. When the weight gains of the rats fed on the different diets are examined however, the highest value was obtained in the case of the ogi-soya diet. This is directly dependent on the quantity of protein or nitrogen intake. Therefore the true assessment of the growth promoting potential of the different diets must be based on the protein efficiency ratio which is defined as the weight gain per unit protein intake. When this is done, casein scores a higher protein efficiency ratio (3.17) than ogi-soya (2.21); and so it must be concluded that while the use of full fat soya bean meal to fortify ogi at a 30:70 mixture provides a satisfactory diet, it is still not as good as the casein diet. On fermenting the soya-ogi mixture in the conventional manner, the flavour was still acceptable

C O N C L U S I O N

A biochemical study of the traditional methods of preparation of ogi has revealed that the steeping of corn grains in cold water prior to milling brings about the absorption of water as a result of which the kernel is softened. During this time also, a natural selection of the desirable microorganisms for the souring and flavour development of ogi takes place. Biochemical and physical changes in the carbohydrate moiety of the corn occur leading to increased formation of fermentable sugars and the dextrinisation of the starch by bacterial amylase. Alpha and beta amylase activities do not originate within the corn grain and so the increases in the glucose, fructose and glucuronic acid contents with steeping are believed to be due partly, to the hydrolytic actions of invertase and possibly melibiase on sucrose and raffinose, and partly to a phosphorolytic degradation of the amylose by a uridine diphosphate mechanism. A period of 24 hours is regarded adequate for the steeping.

The surface microflora of corn includes a number of moulds namely, Cephalosporium, Fusarium, Aspergillus, and Penicillium; the aerobic bacterium, Corynebacterium michiganense which hydrolyses the starch into dextrins and initiates the acidification of the mash; the heterofermentative Aerobacter cloacae which possesses the ability

to synthesise riboflavin and niacin; and the homofermentative Lactobacillus plantarum which is mainly responsible for the souring of ogi, utilising the dextrins to produce lactic acid, the major source of flavour. The yeasts, Saccharomyces cerevisiae and Candida mycoderma, invade and rapidly proliferate in the fermenting mash at the beginning and end respectively of the souring period, improving significantly the flavour acceptance of the final product. The latter organism, Candida mycoderma however, assimilates lactic acid and so can destroy the biological acidity produced during souring.

The processes of milling and sieving which removes a considerable portion of the hulls and germ reduces the nutrient value of the corn. The traditional fermentation per se, brings about a biological enrichment of the nutrients of ogi, notably in increasing the total thiamine and niacin contents; but causes no significant alteration of the protein quality.

A good supplemental relationship exists between ogi and soya proteins. The addition of 30 parts of heated full fat soyabean flour to 70 parts of ogi raises the protein efficiency ratio of the latter from 0.77 to 2.21 but the quality of the protein mixture is still not as good casein.

Major contribution to knowledge.

This thesis has thrown new light to the current knowledge and understanding of the traditional method of preparation of ogi from corn. The significant contributions are that:-

1. The biochemical characteristics of the Nigerian corn known as the 'Lagos white' variety are not remarkably different from those of the 'dent' and 'flour' varieties grown in other parts of the world.
2. The traditional practice of steeping whole grains in water before wet milling and souring brings about the natural selection of valuable microorganisms which enrich the nutrients of the corn. Aerobacter cloacae plays a major role in this wise and it is responsible for the synthesis of riboflavin and niacin.
3. The flavour of ogi is not derived solely from its lactic acid, but in combination with the aroma produced by the yeasts, Saccharomyces cerevisiae and Candida mycoderma.
4. The proteins of ogi are grossly deficient (P.E.R. 0.77) but when supplemented with soya protein at 30% level, they become adequate (P.E.R. 2.21) though not still as good as casein.

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