BIOCHEMICAL STUDIES ON A TRADITIONALLY PREPARED FOOD OP NORTHERN NIGERIA. (FURA - NONO)

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LIST OF DEFINITIONS

DIET:-

A diet is used here to mean what a person or animal usually eats or drinks; 'daily fare' Mode of living with special reference to food.

FOOD:-

What one feeds on. It has been used in certain places in this text to mean meal.

MALNUTRITION:-

Imperfect or faulty nutrition. In manutrition, there is consumption of sufficient amount of food (or calories) but with limiting supply of certain nutrients resulting in a spectrum of disease.

MEAL:-

The food taken at one time, as breakfast, dinner or supper.

PROTEIN CALORIE MALNUTRITION:-

This is regarded as a spectrum of disease arising from inadequate diet, - usually with insufficient fat

and carbohydrate to meet energy requirement of the animal, so that protein is then used to supply energy.

PROXIMATE COMPOSITION:-

This is used to mean the following fractions of a food - moisture, ash, crude protein, ether extract, crude fibre and carbohydrate (Nitrogen-free Extract).

SUPPLEMENT:-

A supplement is a food or food mixture used with another to improve the nutritive balance or performance of the total.

UNDERNUTRITION:-

A state resulting from insufficient consumption of food and nutrients.

STAPLE:-

Main element; Leading commodity.

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Chapter One

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

1.1 Nutrition in Nigeria:

In every nation, there is need for well established data on chemical composition and nutritive value of foods available to her people. This provides a first step towards identifying and solving nutritional problems. In developed countries, the ingredients and nutrient composition of diets consumed have been well established. In Nigeria, as in most developing African countries, the ills of poor nutrition have been recognized, but research into the chemical composition and nutritive value of the traditional foods has been slow. It is therefore not surprising that poor nutrition remains a major health problem of the world, and undernutrition and malnutrition occur widely in this country as in most other developing African countries.

The first nutritionist was appointed in Nigeria only about 1947 (Adadevoh 1972) and that date marks the beginning of scientific awareness in this country of nutritional problems. Although, recent years have

witnessed a series of biochemical studies (see Basis of Investigation Page 8) on local foods, aimed at the improvement of the nutrition of the Nigerian, much work is still left to be done. Programme in systematic nutritional work with a view to the improvement of nutritional level of the Nigerian is hindered by a lack of adequate information on the chemical constituents of the locally available traditionally prepared foods. Information obtained by calculation from "food tables" can only serve temporarily as a guide to foods and feeding, since such tables do not take into account variations likely to arise from different species or varieties, from soil conditions and from differences in cooking procedures. It is also necessary to know the composition and amount of representative meals.

1.2 Some traditionally prepared foods in Northern Nigeria:

There are over 50 staple food crops in Nigeria, some of which have been introduced into this country from Western world by Europeans, table 1 from Okiy 1960). Some are indigenous, others have mountain origin, table 2 from Okiy 1960).

The traditionally prepared foods are based on the available food crops and the staple food varies immensely from one area to another owing to differences in climatic and soil conditions as well as eating habits. For example,

Table 1

[illegible] group introduced by [illegible]
from Eastern Zaire (Only [illegible])

Common Name	Botanical [illegible]
[illegible]	Mygihar [illegible]ma
[illegible]	Amachic [illegible]
[illegible]	Ipomoea batatas
[illegible]	Zea mays
[illegible] lemons	Citrus Sp.
[illegible] Peas	Jacobsenga [illegible]
[illegible]	Amoran [illegible]
[illegible]	Carica papaya
[illegible]	Psidium guajava
[illegible] fruit	Anacardium [illegible]
Tomato	Lycopersicon esculentum
Onion	Allium cepa
Avocado pear	Persea gratissima
Spinach	Tetra [illegible]

Table 2

Some food crops either indigenous to Nigeria or of uncertain origin
(OKIY 1960)

Common Name	Botanical Name
Rice	Oryza Sp.
Bulrush millet	Pennisetum typhoideum
Oil Palm	Elaeis guineensis
Water melon	Citrullus Vulgaris
Oil bean tree	Pentaclethra macrophylla
Yam bean	Sphenostylis Stenocarpa
Cowpea	Vigna unguiculata
Yam species	Dioscorea Species
Guinea corn	Sorghum Sp.

cassava forms the staple food in the South, millet and guinea corn form the staple foods in the Northern part of this country.

Some traditionally prepared foods in Northern Nigeria and the ingredients used for their preparations are shown in table 3. (This table continues in the appendix). The major ingredients are marked with asterisks

Table 3[1]

Food	Ingredients	
	Local Name	Common Name
Fura	Dawa*	Guinea corn
	Gero*	Millet
	Citta	Ginger
	Masoro	Black pepper
	Kaninferi	Cloves
	Barkono	Red pepper
Kono	Dawa*	Guinea corn
	Gero*	Millet
	Barkono	Red pepper
	Kaninferi	Cloves
	Citta	Ginger
Kosai	Albasa	Onion
	Tumatir	Tomatoes
	Wake*	Cowpeas
	Gishiri	Salt
	Man Gyada	Groundnut oil
	Barkono	Red pepper
Kunu	Gero*	Millet
	Citta	Ginger
	Barkono	Red pepper
	Kaninferi	Cloves
	Masoro	Black pepper
	Tsamiya	Tamarind
	Suga	Sugar

1. This table continues at the appendix

Table 3

Food	Ingrediients	
	Local Name	Common Name
Nono	Madara	Fresh milk
	Kuka or Bahe	Cream of tartar
Shinkafa da Miya (Rice and Stew)	Shinkafa*	Rice
	Tumatir	Tomatoes
	Albasa	Onions
	Barkono	Red pepper
	Tattasai	Red pepper (more sweet)
	Man ja	Palm oil
	Kuka	Baobab leaves
	Kubewa	Okra
	Kabewa	Squash, Pumpkin
Tuwo da Miya	Dawa*	Guinea corn
	Manja	Palm oil
	Man gyada	Groundnut oil
	Albasa	Onions
	Barkono	Red pepper
	Tattasai	Red pepper (more sweet)
	Kuka, Kubewa	Vegetables
	Goji	Pumpkin
	Gishiri	Salt

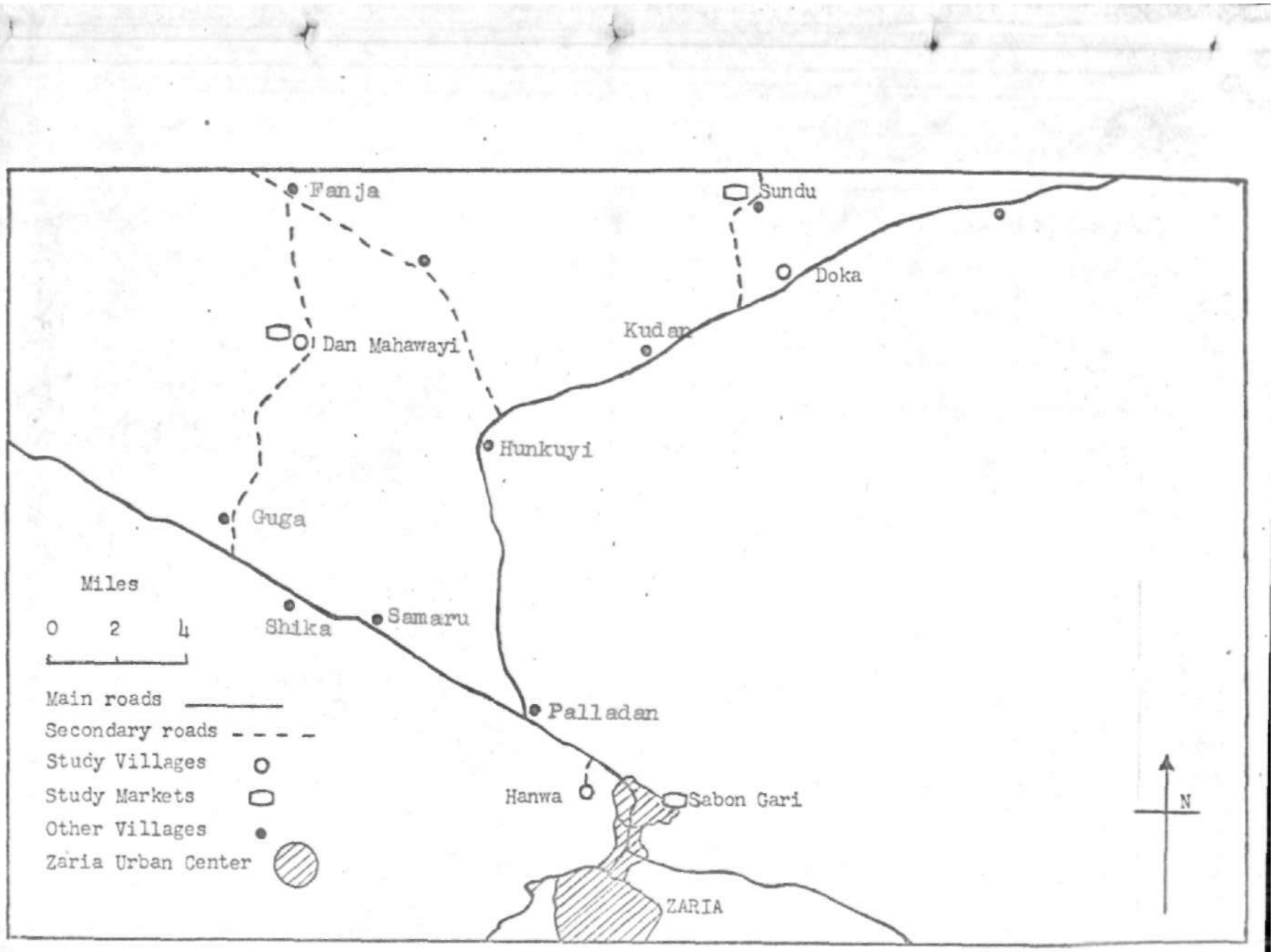


Figure 2. A map showing the selected study villages and markets in the Zaria area.

From Hays, H. M. (1973)

1.3 Pattern of consumption of some foods common in Northern Nigeria:

The different foods commonly consumed in the Northern parts of Nigeria have been shown in Table 3. The consumption of some of these foods is restricted to that part of the country, while some like Shinkafa da Miya (Rice and Stew) and Kosai (bean cake) are also widely consumed in the Southern parts. The restriction of some of these foods to a particular region of the country is probably due to ready availability of major ingredients used in their preparation in that part. No doubt, such foods could be introduced to those parts of the country where they are at present unknown, when improved methods of preparation, storage and preservation are developed.

From survey work carried out by Mrs. Simmons (Simmons, E.B. 1971) of the Institute of Agricultural Research Samaru, the pattern of consumption of several foods in three villages around Zaria (see Map on opposite page) was established (Table 4). Tuwo is taken largely as the evening meal while fura-nono forms the major meal consumed as Lunch in all the villages. Koko or Kunu and leftovers are taken to a large extent as breakfast. Leftovers are usually Tuwo and the percentage of days in which this was consumed is high in the most isolated rural village (Dan - Mahawayi). In Doka (along Kano road),

Table 4

Meal analysis in three Zaria Villages,
May, 1970 - Jan. 1971

	Hanwa (Near Zaria)	Dan Mahawayi (Past Shika)	Doka (Kano Rd)	Total
1. Number of House Holds	37	39	43	119
2. No. of "Meals" eaten	928	1008	1090	3026
Breakfast	253	270	307	830
Lunch	236	269	227	732
Snacks	190	203	200	643
Dinner	249	266	306	821
Pattern of Consumption: % of days in which specified items were eaten				
BREAKFAST				
Left overs	24.5%	82.2%	48.5%	
*Some prepared foods	48.6	44.0	48.5	
Koko/Kunu	39.1	42.6	48.2	
Kosai	27.7	0.0	9.8	
Danwaka	5.5	11.9	8.5	
Rice and Soup	5.5	neg.	0.7	
New Tuwo	56.5	14.4	34.2	

Table 4

	Hanwa (Near Zaria)	Dan Mahawayi (Past Shika)	Doka (Kano Rd)	Total
Lunch				
Some *prepared foods	72.5%	79.1%	84.1%	
Fura and Nono	68.2	67.7	48.0	
Alele	7.6	0.0	7.0	
Kulikuli	3.0	11.2	11.0	
Rogo	6.8	5.6	14.5	
Rama/Zogala	neg.	7.1	2.2	
SNACKS				
Kolanuts	85.3%	9.9%	98.8%	
*Prepared food items	23.2	85.2	4.4	
Rogo	4.7	6.4	neg.	
Kosai	3.2	50.7	4.0	
Kulikuli	0.0	9.9	neg.	
Gyada	1.0	9.9	0.0	
Rake	11.1	9.4	neg.	
DINNER				
Tuwo	68.3%	86.4%	75.8%	
*Prepared food items	6.0	3.4	10.5	
Kosai	3.6	0.0	neg.	
Starchy roots - (Yam and Rogo)	6.0	4.9	6.2	

SOURCE: Simmons, E.B. Calorie and Protein intakes in three villages in Northern Zaria Province.

May 1970 - July 1971 (forthcoming)

* "Prepared foods" refers to Purchased ready to eat foods not prepared or cooked in the households. These are usually Koko, Kosai, Danwake, gauda, fura, nono, and rogo.

the percentage of days in which fura-nono was consumed was found to be fairly low compared to the other two villages. This is due to the fact that *madara* (fresh milk) is less available in this area. This clearly illustrates how the staple meal of any locality could be influenced by the availability of the raw materials (or ingredients) used in its preparation. Although rice is cultivated extensively in the North, the quantity of this food crop consumed in the three villages around Zaria is remarkably low. This is because rice is not grown in the vicinity of these particular villages.

1.4 Basis of investigation:

It is now clear that a number of different foods are consumed in this country. Little however, is known about their chemical composition and nutritive value. Several of investigators have carried out some biochemical studies on certain Nigerian foods (Oyenuga and Amazigo 1957, Bassir 1964, 1968, Oke 1965, Malik 1965, Akinrele 1966, 1967, Oke and Tella 1968, Akinrele et al 1971 and Umoh 1973). Some of these workers (Malik 1965, Akinrele 1966, Umoh 1973) have studied the losses that may be incurred in nutrients as a result of preparation methods. Their studies have been almost entirely restricted to foods consumed in Southern parts of Nigeria. If the prevention

and eradication of malnutrition and undernutrition is to be achieved at a National level, then there is the necessity to extend such biochemical studies to foods consumed in the Northern parts of this country.

A drastic change from our traditional diets and food habits to a more modern European diet may not provide the right solution to our nutritional problems in Nigeria. As a result of change in food habits accompanying increasing urbanization and socio-economic awareness of a group of Nigerians, either improved nutrition may result or because of too rapid change in traditional eating habit, disease may ensue (Adadevoh 1972).

Lack of adaptation is suggested as one of causes for diseases that may arise, i.e. defects in the absorption, the digestion and metabolism of the newly presented food may be responsible. For example, it has recently been shown that the absorption of lactose (the sugar of milk and milk products) is impaired in Nigerians especially those from the non-dairy areas and whose intake of milk and milk products cease after weaning (Olatunbosun and Adadevoh 1972). Milk intake as a therapentic diet in protein deficiency syndrome may because of its lactose content, give rise to symptoms of intolerance with abdominal pain and diarrhoea. Thus milk may not be the best protein

replacement or supplementation diet in our environment. It follows that local food products must be relied upon in the main for providing adequate nutrition. This therefore calls for extensive analyses of local agricultural products especially as regards their essential nutrients.

It is to be realized that new foods could be evolved as a result of extensive analyses of our local food ingredients and diets. For example, three sources of high quality protein foods have now been recommended for Nigerians from nutritional studies. One of these, "Soy Ogi" a corn and soya bean protein-enriched food has been developed over the last six years by the Federal Institute of Industrial Research, Oshodi (Akinrele et al 1970, Akinrele and Edwards 1971). The other, Gari fortified with Soya has also been studied (Bassir 1964, Bassir et al 1968, and Akinrele 1967).

They have been shown to be suitable on chemical evaluation for their nutrient compositions, biological protein quality and in some cases clinically for their therapentic qualities, (Table 5a, b, c). A third source, - leaf proteins is obtained from green leaves and compares favourably with commercial protein supplements and "Soy Ogi" (Oke 1969, Fafunso 1971). It is a high quality protein source and has recently been shown to have therapeutic efficacy in the management of protein calorie

Table 5a

Percentage composition of Soy-Ogi as Compared with other Protein foods.

Akinrele and Edwards (1971)

Constituent	Soy-Ogi (Nigeria)	Lactogen (Nestle)	Corn-Soya meal (America)
Protein (N x 6.25)	20.3	16.1	20.9
Moisture	4.7	3.0	9.5
Fat	6.3	24.2	1.2
Ash	3.0	3.6	2.1
Calcium	0.47	-	0.40
Phosphorus	0.44	-	0.44
Carbohydrate	63.7	52.4	61.0
Energy (K-cal/g)	4.0	4.95	3.58

(-) usually means not specified.

Table 5b

Amino Acid pattern (mg/g)

Akinrele and Edwards (1971)

Amino acid	Soy-Ogi	Lactogen	Corn-Soya meal	F.A.O.(1957) reference Protein
Tryptophan	2.10	2.18	2.40	2.88
Threinine	7.63	7.15	-*	5.76
Isoleucine	8.85	9.89	-	8.64
Leucine	17.69	15.21	-	9.78
Lysine	9.46	12.05	9.60	8.64
Methionine	2.75	3.79	3.61	8.64
Cystine	3.05	1.39	2.80	
Phenylalanine	10.68	7.51	-	5.76
Valine	9.76	10.64	-	8.64
Histidine	4.88	4.08	-	-
Serine	9.76	9.14	-	-
Protein Score	93	78	89	100

Usually (-) means not specified.

Table 5c

Comparative Biological Evaluation of
Some food Proteins

(Mean values for diets containing 10% Protein)
Akinrele and Edwards (1971)

Analysis	A Casein	B Soy-Ogi	C Lactogen
Protein efficiency ratio:			
Experimental*	2.6±0.37	2.3±0.21	2.2±0.62
Corrected (using Std. Value for Casein)	2.5	2.2	2.1
Net Prot. Utilization: Experimental	85.5	83.6(Std) 69.5±3.79 (Operative)*	86.6
Corrected (using Std. Value for Casein)	72.0+	70.4 (Std) 58.5 (Operative)	72.9
Nitrogen dig. Coef.: Experimental*	96.6±0.61	91.6±2.15	93.1±2.59
Biological Value: Experimental*	88.5±3.63	91.3±2.86	93.0±2.45

* Mean values with their standard errors

\+ Value of WHO (1965)

malnutrition (Olatunbosun and Oke 1972). These three protein sources especially, Soy Ogi and lef protein compare favourably with known commercial protein supplements and hold greater advantage since they are potentially much cheaper, (Akinrele and Edwards 1971). According to Adadevoh (1972), "The objectives of a public health nutrition programme should place priority on the effective utilization of the locally available Agricultural resources to provide a cheap and adequate local protein feed or a supplementation feed".

1.5 Scope of work in Nutritional studies:-

1.5:1 Chemical analyses:

Chemical analyses of nutrient composition are the starting point in biochemical evaluation of a diet. Such chemical analyses will embrace proximate (carbohydrate, fat, protein, Ash and Crude fibre), mineral, vitamin and amino acid compositions of the food or diet.

Chemical analyses when carried out on raw mixed materials of the food containing all the ingredients in the same proportion as they are in the prepared food, will offer good comparative data for deducing the losses in nutrients that may occur as a result of preparation methods.

1.5:2 Effect of Cooking and Preparation:

Nutrients may be destroyed by cooking. On the other hand, toxic materials may be got rid off in the process of food preparation. This is the case with cassava preparation where a cyanogenetic glycoside is excluded to a large extent (Oke 1965). Mineral elements are usually lost in certain Nigerian prepared foods due to leaching, excessive washing and mincing of food materials (Malik 1965). Nicotinic acid is stable, but there may be some loss in processing, or from the throwing away of cooking water. Riboflavin is labile when heated in alkaline solution. Bright sunlight causes some loss of this vitamin from milk, and it may also be lost when cooking water is thrown away after vegetables are boiled. In the process of cooking and processing, losses of vitamin C may be considerable. Vegetables covered with water may lose 80% of their Ascorbic acid content. Bruising and wilting of fruits and vegetables allow ascorbic acid oxidase to destroy the vitamin. The method of peeling of potatoes has a significant effect on their content, and so called fruit drinks may contain little or no Ascorbic acid. In Hospital kitchens, frozen peas have shown a fall in ascorbic acid content from 20.5mg/100g to 1.1mg/100g in a 2.5 hr which elapsed between starting to cook them and serving them to patients, (Girdwood R.H. 1971).

1.5:3 Calorific Value of foods:

Maximum utilization of nutrients is only possible when the intake of energy does not fall below a certain level. This energy level must be satisfied by fat and carbohydrate. Below this level, the efficiency of protein utilization falls because protein is used primarily for energy purposes. For any food of interest or chosen for study, there is the need for evaluation of the calorific value of the prepared meal and as well as those of their ingredients. Since suggestions are accumulating that most protein malnutrition in the world is due to calorie insufficiency, (Miller and Payne 1969) the calorific values of major diets in this country call for extensive investigation.

In the United States of America, metabolizable energy is expressed in terms of physiological fuel values in which the energy value of foods and diets are calculated from table of food analysis. These tables give figures for the carbohydrate, protein and fat content of each food as determined by chemical analyses. The calorific value is obtained by multiplying these figures by the Atwater factors (4 for carbohydrate, 4 for protein and 9 for fat) or some varients of them. Full account of the various factors in use today with full references to the classical

papers has been given by Widdowson (1955). However, it is generally suggested that physiological fuel value entails some defects and is therefore not a very good method of establishing accurately the calorific value of diets especially in this country. The Atwater factors are based on work done over 60 years ago, on diets that may differ considerably from present day European diet and more so the African or Nigerian diets. Direct calorimetric method is therefore necessary in order to ascertain accurately the calorific value of our diets.

1.5.4 The importance of Biological Studies:

It is not sufficient however, to deduce the nutritive value of a diet from mere chemical analyses of the nutrients present. This is because the presence of a particular nutrient does not mean that it is fully available to the animal for body use. To establish fully therefore, the nutritive value of any diet, a feeding experiment using rats need be incorporated. For example, the value of a diet as a source of protein is determined by the concentration of protein in it, the digestibility of the protein and the availability of its amino acids for body protein synthesis and the content and proportions of essential amino acids. Digestibility of protein and availability of its amino acids are best determined by biological methods. In human nutrition, rats are commonly used for biological evaluation of diets. Human volunteers can also be employed.

1.5:5 Mixed diets:

Although in Nigeria and most other developing countries, what may be referred to as mixed diets fall very short of European recommended standard, improvement could be made when chemical composition of available diet ingredients are known. From data obtained from such analyses, a well balanced food with high biological value could be evolved, by increasing or adding a particular ingredient to supply a particular nutrient deficient in others. This is the supplementary relationships between foods, which has led to a world wide advocacy for a well mixed diet, with hope that what may be lacking in one food is provided by another.

Work has been done to elucidate a number of examples of the supplementary relationship between protein foods. For example, proteins were divided into those of first class and those of second class by early nutritionists. The former being of animal, and the latter of vegetable origin, proteins of animal origin being richer in essential amino acids than those of plant origin. In a mixed diet with its different proteins, supplementary relationship becomes apparent, whereby amino acid deficiencies in one food are made good by a relative surplus in another. For example, experimental evidence in support of supplementary relationship between protein foods have been obtained

in growth experiment with rats (see table 6). The experimental rats grew little or failed to grow when beef tea and bread or wheat gluten, blood protein or yeast were fed individually, but combining these foods in pairs without increasing the total protein caused appreciable growth (Kathleen and Kon 1958). Geiger (Kathleen and Kon 1958) however, observed that when one component of a pair of foods was eaten 12 hours before the other, (Table 7) the supplementary effect was lost. Thus proteins of mixed diets show high biological values rare in individual proteins. Biological values of 80 - 90 have been reported for the proteins of mixed, non-vegetarian human diets, while values of 73 - 84 have been reported for cereal and pulse mixtures (Kathleen and Kon 1958).

It must be noted that supplementary effect is not only with proteins and amino acids but also exists with vitamins and minerals. Vitamin B_{12} increases the biological value of casein in the presence of homocysteine and methionine, (Kathleen et al 1956). The presence of adequate amount of vitamin D has been found to minimize the deleterious effect on growth of a gross imbalance between dietary Ca and P (Harrand and Hartles 1968). Several reports have suggested that the action of vitamin E on blood formation may also be involved with the amount

Table 6

Supplementary relationship between Protein foods:

Growth exp. with Young Rats.

Protein Source	Prot. in diet %	Main Amino acid defficiency	Gain/week (g)	Reference
A 1. Beef-tea	12.5	All except lysine	0	Chick & Slack
2. White flour	12.5	Lysine	13	(1945)
3. 25% beef-tea nitrogen plus 75% flour nitrogen	12.5	-	19	
B 1. Wheat gluten	9.0	Lysine	0	
2. Blood protein	9.0	Isoleucine	0	
3. Yeast	9.0	Methionine	0	
4. 50% Wheat gluten N. + 50% blood N.	9.0	-	9	
5. 50% yeast N.+ 50% blood N.	9.0	-	8	Geiger (1948)
6. 50% wheat gluten N. + 50% yeast N.	9.0	-	11	

From: Kathleen and Kon (1958)

Table 7

Effect on growth of rats of giving them pairs of Protein together or separated by a 12 hr. interval.

Protein Source	Wt. gain in 16 days (gm)	
	Given Together	Given with 12hr. interval
1. Wheat gluten + blood protein	22	- 0.25
2. Yeast + Blood protein	18	- 4.0
3. What gluten + yeast	26	+ 2.0

From: Kathleen and Kon (1958)

and nature of dietary protein. Anaemia of Thai children suffering from protein calorie malnutrition was cured by a combination of vitamin E and protein and not by vitamin E alone (Whitaker et al 1967).

The "Soy Ogi" now recommended for Nigerians (Akinrele and Charity 1971) is a well mixed protein food and its evolution must have been prompted by a knowledge of nutrient composition of the ingredients. No doubt, similar work in the same direction could be achieved with other traditional diets.

1.5:6 Fermented foods:

In Nigeria, some staple foods of the people are fermented plant and animal products. Some Nigerian foods which are fermented at one stage or the other in their preparation are: Nono (Hausa) Soured milk, "Ogi" (Yoruba) prepared from fermented maize, "Ogilli-ugba" (Ibo) or daddawa (Hausa) prepared from seeds of locust bean, "Foofoo" prepared from fermented whole cassava, "giya" produced from guinea corn and "burukutu" produced from millet (Nduka 1966). Although useful metabolites such as vitamins and amino acids can arise from fermentation by micro organisms, the fact remains that dangerous compounds may be produced. For example, some higher alcohols may be formed in certain drinks. (The drink 'Port' is high in amylalcohol which is toxic). Thus the microbiology of our traditional foods especially those produced by fermentation or stored for a considerable length of time after cooking need very serious study.

Chapter Two

THE PROJECT

2.1 Fura-non as a meal in Northern Nigeria:

Nono is a food prepared from fermented or soured milk. It is usually taken along with fura (prepared from millet). Fura-nono forms part of the staple foods taken in Northern parts of this country, and is popularly consumed by people in both urban and rural areas, and by infants, children, adults alike. In terms of cost, fura-nono is fairly cheap (one kobo for 128 - 142 gm of nono and one kobo for 71 - 85 gm of fura)[1]. (Simmons, E.B. 1971). A serving or lunch time dish of fura-nono might cost two to five kobo[2] for each food item. The simple method of preparation and availability enhances the popularity. It is commonly hawked about in urban market places and forms the common ready prepared lunch

1. At 1970/71 Prices. Fura generally, increases or decreases in price because of increase or decrease in cost of millet.
2. This also depends on the cost of millet in a particular season or year.

of the petty traders and business men. Major ingredients in fura-nono have been shown in table 2. In view of its popularity, it has been considered necessary to assess its nutritive value.

Both fura and nono can be stored for up to a maximum period of three days. Changes in nutrient composition, nutritive value may occur as a result of growth of certain microorganisms. Millet flour is usually sprinkled over the fura balls if they are to be kept any length of time. Presumably, this millet powder provides a dry coat, thus helping both to keep the balls separate from one another, as well as preventing lost of moisture from surface of balls. However, the effect of this flour on the keeping quality of fura is at present obscure and needs to be clarified by some biochemical studies.

Finally, some ingredients used in the preparation of fura and nono are unique, with uncertain nutritive values. The soaked seeds of baobab tree (optional ingredient in nono) is used as an adulterant by sellers to make nono slightly thicker while increasing the liquid volume of nono available for sale. This may indeed be affecting the nutritive value of nono. The minor ingredients in fura (largely spices) are usually added in negligible quantities and their contributions may therefore be small.

2.2 Plan of Project:

A plan of project which when completely investigated would offer a full knowledge of the nutritive value of nono comprises six phases:

1. Chemical analyses to be carried out on the food ingredients. Such chemical analyses will embrace proximate composition, assay of amino acids and vitamins (especially vitamins A, B_1, B_2 and C that are easily lost by most food preparation methods).

2. The above chemical analyses to be extended to the 'Standard' foods of known ingredient composition. The standard foods consisting separately of two types: (a) the cooked foods and (b) the raw foods (containing the prepared ingredients in the same proportion as in the cooked foods). Only then can losses in nutrients due to preparation methods be ascertained. To make easy comparative work, the standard foods have been divided into the various parts:-

(a)	Nono without 'Kuka*'	(1) Raw
		(2) **Cooked
(b)	Nono with 'Kuka'	(1) Raw
		(2) Cooked

---

* Cream of tartar from soaked baobab seeds.

** Nono is not cooked, but cooked nono is used in this text to represent the prepared (soured) milk.

(c) Fura
- (1) Raw
- (2) Cooked

(d) Fura-nono without 'Kuka' (Mixed foods)
- (1) Raw
- (2) Cooked

(e) Fura-nono with 'Kuka' (Mixed foods)
- (1) Raw
- (2) Cooked

3. Since our standard foods may not represent average ones (in composition), chemical analyses may be extended to randomly collected batches of the prepared foods (fura, fura-nono, and nono). This will illustrate the magnitude of the variations that are likely to result from variations in quality and quantity of ingredients used, and in handling and preparation methods.

4. The energy supply of the foods and their ingredients to be assessed in the following ways:-

   (i) Gross energy:- As determined by the Calorimeter.

   (ii) Physiological fuel value (P.F.V.):- This is calculated from the fat, carbohydrate and protein content by multiplying by the factors 9, 4, 4, respectively.

(iii) Metabolizable energy:- This can be obtained from the gross energy by calculation using an established equation:

$$M_{E/g} = (G_{E/g} \times 0.95) - (N\% \times 0.075)$$

(iv) Protein calorie percent (Pcals %):- Energy contribution from protein. This can calculated from the equation:-

$$\text{Pcals \%} = \frac{\text{Protein \%} \times 4}{M_{E'}}$$

5. Biological assay of food proteins:- By a (biological) feeding experiment using rats and the standard foods (2 c, d, e) the following aspects of protein quality could be investigated:-

(1) True digestibility (T.D.)
(2) Biological Value (B.V.)
(3) Protein efficiency ratio (P.E.R.)
(4) Net protein utilization (N.P.U.)
(5) Net dietary protein calories percent (N.D.Pcals %).

6. Microbiological quality of foods:- This will involve identification of micro-organisms especially those accounting for sourness in the nono and elucidation of likely microbial activity during the keeping period of the foods.

Although, all the above outlined aspects of nutritional studies are important in assessing fully the nutritive value of a food or diet, priority was given in this investigation to aspects 1 to 4. The results obtained on the four aspects did infact provide sufficient information for assessing to a reasonable measure, the nutritive value of the foods studied.

2.3 Ingredients used in preparation of 'fura-nono':

Fura:- The major ingredient used in the preparation of fura is millet (gero). In some instances however, people employ guinea corn (dawa) in place of millet. Ginger (citta), Black pepper (masoro), Cloves (kaninferi) and Pepper (barkono) are commonly used as spices. The quantity and number of these spices employed depend on the discretion of the individual. In general however, the quantity of these spices added is usually small relative to the quantity of grain flour used.

Nono:- Nono is prepared from fresh milk (madara). Cream of tartar (kuka, baha, or bapa) is commonly an optional ingredient.

1.4 Traditional Methods of Preparation of fura and nono:

Fura:-

1. Millet is pounded into flour by hand in the morning and left without drying until used later in the day.

2. Water is boiled over a guinea corn stalk or wood fire in a kerosine tin.

3. Flour is compressed into balls (no water or liquid is added), and dropped into the boiling water. A little of the flour is reserved for sprinkling on top of the finished balls (see step 9).

4. Balls are boiled for 20 to 30 minutes.

5. Some of the cooking water is drained off into a bucket or calabash; some is left in the kerosine tin with the cooked balls. The balls and remaining water are then poured into a wooden mortar.

6. The cooked balls are broken with the pestle (some flour may still be dry in the centres of the balls) and mixed with the water. All or some of the reserved cooking water may be added while pounding. Spices (barkono-pepper, citta - ginger, masoro - black pepper, and kaninferi - cloves) are ground together and added at this point if they were not already placed in the mortar before the cooked balls were poured in.

7. The mixture is then pounded vigorously until a cohesive and slightly sticky lump of dough is formed (about 20 minutes). More pounding achieves a smoother and more elastic product. Additional amounts of the reserved cooking water may have to be added during the pounding process in order to achieve the right consistency.

8. The pounded lump of dough is removed to a calabash. Small pieces of the dough are broken off and rolled into smooth balls between the interior curve of the calabash and the palm of the hand. These curi(or balls) may weigh about 71 - 85 gm each[1].

9. If all the balls are not to be eaten immediately, the reserved flour is sprinkled over the tops of them as they are arranged in symmetrical layers in a large container - a calabash or a large enamel bowl. The flour makes them 'look nice' but it apparently also gives them better keeping qualities if they are to be kept any length of time. The maximum keeping time seems to be about three days.

10. The fura balls are then ready for consumption. If nono (soured skimmed milk) is available, this is the preferred accompaniment, but water can also be mixed with the fura for drinking. The balls of fura are broken or crushed slightly in the hand before being placed into a drinking bowl where they are mixed with the nono or water (with a spoon or small calabash) until a fairly thin gruel is formed. Some sugar may be added to the mixture if the fura is mixed with water.

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1. Weight of the fura balls, however depends on the cost of millet in any particular year, the cheaper the millet, the larger the balls.

Nono:

1. Fresh milk is brought back to the house and divided amoung the women of the house. Each woman keeps her milk in a calabash in her room.

2. The calabash is usually kept covered in the cold season (with woven cover or faifai) but in the hot season it is left uncovered so that it remains cooler. The debris that blows into it is skimmed out the next morning before making the nono.

3. After the nono has been allowed to sit at least 24 hours, (usually 1 - 2 days depending on temperature) the thickened cream is skimmed off the top with a spoon or by hand and placed in another calabash,

4. The skimmed milk remaining in the original calabash is the nono used for sale. It is beaten with a "maburgi" to make it smooth. Sometimes, water in which an amount of baobab seed (cream of tartar) has been soaked and dissolved is added to the nono. This not only increases the liquid volume of the nono available for sale, but also makes the nono slightly more sour and thicker.

5. The cream in the second calabash is also beaten with the 'maburgi' (wooden stirrer) to make it smooth. It is then poured into a long-necked gourd (gora) whose neck can be stoppered with a maize cob or piece of wood.

The gora is then shakened vigorously until the butter is separated from the liquid butter milk. This may take up to an hour depending on the butter fat content of the cream and the amount of cream in the gora. A little warm water is sometimes added to the cream to "make the butter come out faster". The liquid butter milk in the gora is also called nono and is kept, if possible, for family consumption as it is said to be superior in quality. The butter is formed into patties (curi), these are usually thoroughly washed in cold water before use. The butter is not eaten along with either fura or nono, but is sold separately.

2.5 Collection and treatment of Samples:

2.5:1 Food Ingredients:

The fura ingredients and 'kuka' for this investigation were obtained from the Samaru market. Samples were bought between the end of August and early September 1972. These ingredients were in the forms that they were normally preserved and used by the people. They have therefore low water content since they were sun-dried. No ingredient was obtained fresh.

Enquiries at the time of purchase showed that most of the foodstuffs were cultivated or grown widely, around Zaria, but no seller seemed to know the time of harvesting

of the foodstuffs. All the food ingredients analyzed however, may be said to have been grown in parts of Northern Nigeria, around Zaria.

Most of the milk samples analyzed were obtained from Shiká farm of the Institute of Agricultural Research (A.B.U.). Two milk samples of Fulani breeds, however, were obtained from the North Central State Government farm Shika, and from a local Fulani man.

2.5:2 Prepared foods:

Some samples of the foods as prepared and taken by the people were analyzed. They were obtained through Mrs. Simmons from a Supplier who bought the food samples randomly from local sellers in a village near Zaria. The age of each food sample analyzed was not known.

Analyses were also carried out on the standard cooked foods, raw foods and 'mixed foods'* of known ingredient compositions. The standard cooked foods were prepared at Hanwa (a village near Zaria) under the supervision of Mrs. Simmons and myself. The weighings were made by us and the quantity and choice of ingredients were left to the discretion of the local woman who prepared the foods and also supplied all the food materials or ingredients.

The raw foods were prepared in the laboratory, from part of the raw ingredients used for the standard cooked

* 'Mixed food' refers to a mixture of fura and nono whether cooked or raw.

foods. The ingredients were mixed in the same proportion as they were in the standard cooked foods.

2.5:3 The 'mixed foods' (Fura-nono mixture)

One of the 'mixed foods' was made at Hanwa by allowing the local woman to mix the foods at her own discretion. The weighings were also made by us. With this as standard, other mixed foods (fura + nono) were made in the laboratory in exactly the same proportion as that mixed at Hanwa.

The description of the various foods with their ingredient compositions and proportion of mixture in the case of the 'mixed foods' are shown in Tables 8, 9 and 10.

2.5:4 Treatment of Samples:

Samples when conveyed to the laboratory, were dried almost immediately in hot air circulating oven at about 60°C depending on sample type. Drying was usually completed within 24 hours. In the case of the food ingredients, they were ground in a mill before drying. Where samples formed lumps on drying (especially the prepared foods) these were broken up in mortars, to very fine powder that passed through 40 - 45 mesh sieve, before storing in air-tight screw-capped bottles. The dried samples formed the stock for further chemical analyses.

The drying temporature, 60°C might not be too drastic to destroy the vitamins assayed, since no over-heating occured (Oyenuga - Nigeria's foods and feeding-stuffs 1968 ed.). The fluid foods or food ingredients were however not dried but stored in a deep freezer.

Table 8

Composition of Standard Fura Food[1]

Food Ingredient	W e i g h t	
	Cooked*	Raw
Millet flour (Gero)	4.083 kg	100.000 gm
Pepper (Barkono)	5.8 gm	0.142 gm
Black Pepper (Masoro)	3.10 gm	0.076 gm
Ginger (Citta)	-	-
Cloves (Kaninferi)	-	-
Water (By difference)	3.276 kg	80.230 gm
Total Weight	7.368 kg	180.448 gm

* Prepared at Hanwa.

1 Raw and Cooked foods contain same proportion of ingredients.

Table 9

Composition of Standard Nono Foods[1]

Food Ingredient	Nono without 'Kuka'		Nono with 'Kuka'	
	Raw	Cooked*	Raw	Cooked*
Fresh milk	50.0 gm	-	50.0 gm	-
Soured milk	-	1.702 kg	-	1.985 kg
Butter removed	-	99.5 gm	-	107.0 gm
Water added (By difference)	42.0 gm	1.432 kg	77.0 gm	3.059 kg
Kuka added	-	-	1.35 gm	53.5 gm
Total Wt. of Nono	92.0 gm	3.034 kg	128.35 gm	4.99 kg

* Prepared at Hanwa.

1. Raw and Cooked foods contain same proportion of ingredients.

Table 10

Proportion of Mixed Foods[1]

Food Description	Fura-Nono without 'Kuka'		Fura-Nono with 'Kuka'	
	Raw	Cooked*	Raw	Cooked
Fura	20.0 gm	212.0 gm	20.0 gm	72.0 gm
Nono	18.7 gm	198.0 gm	18.7 gm	67.5 gm
Water added	5.34 gm	56.8 gm	5.34 gm	19.3 gm
Total Wt. of mixture	44.04 gm	466.8 gm	44.04 gm	158.83 gm

* Prepared at Hanwa.

1. Fura and Nono in equal proportions in both raw and cooked 'mixed foods'.

Chapter Three

EXPERIMENTAL METHODS

The samples were analyzed by the standard A. O. A. C. methods (1965), for Moisture content, crude and true proteins, ether extract, crude fibre and total ash. Figures for available carbohydrate were arrived at by difference, deducting the sum of the crude proteins, crude fibre, ether extract and total ash (each expressed as gm per 100g dry sample) from a hundred.

The minerals: Iron, Phosphorus, Calcium and Sulphur were determined by standard chemical A.O.A.C. methods (1965). Sodium and Potassium were determined by flame photometric method. The atomic absorption spectroscopic method was used to determine magnesium and in some samples also for calcium and potassium.

Oxalate was determined by the method of Rees but as modified by Oke (1965).

Milk fat was determined by the Gerber test (Ling, E.R. 1947).

Vitamins B_1 and B_2 were estimated by the standard "Association of Vitamin Chemists" methods (3rd ed. 1966)

while Vitamin C was estimated by the method of Evered, D. F. (1960).

Amino acid analysis of only a single sample of Raw fura was carried out by direct acid hydrolysis followed by analysis with the Beckman model 120C Automatic amino acid analyzer.

The set-backs and likely sources of error in each of the methods employed for the analysis of various nutrients are given under "Discussion of Methods".

3.1 Moisture Content:- (A.O.A.C. Page 191)

Procedure: About 2g of the ground food sample was accurately weighed in a small crucible (10 - 15 ml) with lid. The crucible containing the weighed sample was then put in a hot air circulating oven maintained at about 110°C and the food material dried to constant weight (usually left over-night). The crucible was covered while in the oven, transferred to a desiccator with calcium oxide as drying agent, and weighed again when it reached room temperature. The loss in weight is equal to the moisture content.

3.2 Ash Content:- A.O.A.C. Page 192

Procedure: About 5g of well mixed powdered or ground food material was weighed in a 10 - 15 ml porcelain crucible (with lid), that has been ignited, cooled to room

temperature in a desiccator (with calcium oxide as drying agent) and weighed. The sample was then ignited at 450 - 500°C till material was completely white. This usually took 6 - 18 hours depending on sample type. The crucible with ash was then cooled in a calcium oxide desiccator and weighed soon after it reached room temperature. The weight of ash was obtained by substracting the weight of crucible.

3.3 Lipid Content (Ether Extract):- (A.O.A.C. Page 331)

Procedure: About 5g of the dried food sample was weighed accurately in a thimble and covered with a piece of glass wool. The thimble was then put into it's chamber. About 250 ml of petroleum ether (B.P.60 - 80°C) was put into the flask and the apparatus assembled for distillation. The temperature of the heating mantle was slowly raised until the ether just boiled (Boiling chips were employed to achieve uniform boiling). As the ether evaporates, it is condensed and drops on the thimble. The extraction process was continued for about 12 - 16 hours (usually over-night). The solvent was then distilled till only about 50 ml was left in the flask. The flask content was washed into a weighed beaker with some ether and the later evaporated to dryness on a hot water bath, so that the lipid extract was left in the beaker. The beaker was finally dried in hot air circulating oven at about 40°C to a constant weight, (usually for about 30 minutes),

cooled in a calcium oxide desiccator and weighed. The difference between this weight and the original weight of the beaker gave the lipid content. The extract is best called ether extract, rather than lipid extract since other substances like carotene, certain essential oils that are non-lipid products consisting primarily of aromatic aldehydes and esters are also extracted. Some volatiles however, may be lost in hot air circulating oven.

3.4 Total Solids in Milk and Nono Samples:
(A.O.A.C. Page 222)

Procedure: About 5 - 10g of sample was introduced into a weighed flat bottom porcelain crucible of about 100 ml capacity (which may if desired contain 15 - 20g of pure dry sand spread over the bottom) and the weight quickly ascertained. The major part of the water was expelled by heating the open crucible on a water bath and heating continued in a hot air circulating oven at 97 - 100°C until weight was constant. The difference between this weight and that of the crucible gave the total solids in the sample.

3.5 Ash in Milk and Nono Samples:-(A.O.A.C. Page 192)

The residue from total solid determination was used in the determination of ash content according to the method already described(page 40).

3.6 Lipid Content of Fresh Milk and Nono:

The Gerber method was employed to estimate fat content of fresh milk and nono samples (Ling, E.R. 1947). In this method, milk casein is dissolved by means of sulphuric acid of specified specific gravity (1.82), thus leaving the milk fat suspended in a heavy acid solution from which it is separated by centrifugal force. The addition of small amount of amyl alcohol greatly facilitates the fat separation.

Apparatus:

1. Gerber tubes
2. Gerber pipettes - 11 ml, 10 ml and 1 ml.
3. Wooden stands to hold Gerber tubes and pipettes
4. A water bath maintained at 68°C
5. A Gerber centrifuge of diameter 18 - 20 inches, holding 16 tubes and capable of rotation at 1,100 revolutions per minute.

Reagents:

1. Concentrated sulphuric acid (S.G. 1.82)
2. Amyl alcohol.

Procedure:

10 ml of the Gerber sulphuric acid (S.G. 1.82) was measured into the Gerber tube. The milk sample bottle was inverted twice or thrice and 11 ml of milk (actually

the pipette transfers only 10.94 ml) transferred into the Gerber tubes such that the milk forms a separate layer above the acid. Then, 1 ml. of amyl alcohol was added. The Gerber tube was then stoppered, shakened until all solid particles have been dissolved, and placed in water bath at 68°C for about 5 minutes (when the temperature of the sample must have reached equilibrium). It was then centrifuged for 4 - 5 minutes at 1100 R.P.M. in a Gerber centrifuge of diameter 18 - 20 inches. The tube with stem upwards was again placed in water bath (at 68°C) with the water level touching the upper limit of the graduations. After about 2 - 3 minutes it was removed from the water bath and the percentage fat read directly from the graduated portion to the nearest 0.05 percent.

3.7 Crude Fibre Content of Samples:-(A.O.A.C. Page 332)

The dried fat free samples were used for the estimation of crude fibre content and the method was based on the separate extraction of substances soluble in boiling sulphuric acid and boiling sodium hydroxide solutions.

Apparatus:

1. A rubber - tipped glass rod.
2. 400 ml beakers
3. Buchner funnels
4. Furnace.

Reagents:

1. 5% Sodium hydroxide solution
2. 5% Sulphuric acid solution
3. 1% Hydrochloric acid solution
4. 95% Alcohol
5. Celite filter aid (acid washed).

Procedure:

The fat free residue (from ether extract already described in page 41) was transferred to a 400 ml beaker which had been previously marked at 200 ml level. 50 ml 5% sulphuric acid was added and mixture made up to 200 ml mark with distilled water. The contents of the beaker were heated to boil, and kept boiling for exactly 30 minutes. During the boiling, the mixture was stirred with a rubber-tipped glass rod, removing all particles from the side, and keeping the volume constant by addition of hot water from time to time. The mixture was then filtered immediately through acid washed celite on a buchner funnel attached to a filter pump. The residue was transferred to the funnel with a jet of hot water and washed with hot water until filtrate was acid free (tested with methyl orange indicator). The residue was scraped off from the celite with a spatula and replaced in the same beaker

removing the last traces with a jet of hot water. 50 ml 5% sodium hydroxide was added and then distilled water to make up to 200 ml mark. Again, the mixture was brought to boil and kept boiling for exactly 30 minutes, while stirring from time to time with a rubber tipped glass rod and keeping the volume constant by the addition of hot water at intervals. The content was filtered immediately through the same celite of the same buchner and residue transferred to the filter by means of a jet of hot water. Residue was washed few times with hot water, then with a solution of 1% hydrochloric acid and again with boiling water until free from acid (checked with methyl orange indicator). Finally, the residue was washed twice with a little 95% alcohol and transferred to a 100 ml porcelain crucible, removing traces with a jet of alcohol. After drying in an oven at 100^{o}C to constant weight, the crucible (with contents) was cooled in a calcium oxide desiccator, weighed and ignited in a furnace. Then it was cooled again in the desiccator and weighed. The crude fibre content was determined from the loss in weight of crucible after ignition.

3.8 Determination of Crude Protein by Kjeldahl Method:
A.O.A.C. Page 744.

Apparatus:

1. Digestion rack with electric heater.
2. Kjeldahl digestion apparatus
3. Digestion flasks.

Reagents:

1. Sulphuric acid
2. Mercuric oxide
3. Potassium sulphate
4. Sodium hydroxide - sodium thiosulphate solution: 60g of sodium hydroxide and 5g of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) was dissolved in water and diluted to 100 ml.
5. 0.02N Hydrochloric acid.
6. Methyl red-methylene blue indicator:- 200 ml. of 0.1% ethanolic solution of methyl red and 50 ml of 0.1% ethanolic methylene blue were mixed and stored in dark bottle, (keeps indefinitely).
7. Boric acid - Indicator mixture: 20g Boric acid was dissolved in 400 ml ethanol, 10 ml methyl red-methylene blue indicator (6) was added and mixture made up to 1 litre with distilled water (stored in dark bottle).

Procedure:

About 0.1g of the dried food sample accurately weighed (or 2 - 5 ml of the milk or nono), 1.9g potassium sulphate, 40mg mercuric oxide and 3ml concentrated sulphuric acid were put in a digestion tube. Two or three boiling chips were added to the tube. The digestion tubes were then set up in the digestion rack with electric heater and the temperature slowly increased till the acid came to true boiling. The tubes were heated for 6 - 12 hours till mixture or solution was clear, and digestion carried on for about 2 - 3 hours longer. After digestion, the tubes were cooled and minute quantity of water added to dissolve solids in each tube. A thin film of vaseline was put on the rim of the flask and the digest transferred quantitatively to distillation apparatus. The flask was rinsed about six times with 2 ml. portions of distilled water, (1 or 2 drops of methyl organge indicator was usually added so that complete transfer of digest could be ascertained). A 50 ml flask containing 5 ml Boric acid-indicator solution was put under the condenser of the Kjeldahl distillation unit with tip extending below the surface of the solution. 8 ml of the sodium hydroxide-sodium thiosulphate mixture was added to the digest and the mixture distilled. Approximately 20 ml of the distillate was collected, transferred to 250 ml flask and diluted to 50 ml.

This was then titrated with the 0.02N HCl to a pink end-point. A number of digestions, using tryptophan, and distillations using ammonium chloride, were carried out to check recoveries in the Kjeldahl method (see discussion of methods Page 90). A blank determination was also carried out, and two or three separate digests, distillation and titrations were made for each sample.

Mg N = (ml HCl - ml blank) x Normality of acid x 14.007.

Protein content was obtained by multiplying percentage nitrogen with the following factors:

Milk and Nono	- 6.38
Millet	- 5.90
Prepared food and other food ingredients	- 6.25

(A.O.A.C. 1965)

3.9 'True Protein' Determination:

Apparatus:

1. As in Kjeldahl crude protein determination
2. 50 ml beakers
3. Water bath
4. Buchner funnel
5. Filter papers.

Reagents:

1. As in Kjeldahl crude protein determination
2. 3% (w/v) trichloroacetic acid.

Procedure:

About 0.1g of the food sample (accurately weighed) was transferred to 20 ml of water in a 50 ml beaker, and the mixture heated on a water bath. After boiling for 10 minutes, 5 ml of 3% (w/v) trichloroacetic acid solution was added and boiling continued for about one minute longer. The precipitate was allowed to settle, filtered using a buchner funnel and suction pump, and thoroughly washed with hot water. (The precipitate contains the 'true protein' of the sample). The precipitate was transferred to a Kjeldahl flask and nitrogen determined as in crude protein.

'True protein' is defined as the trichloroacetic acid insoluble nitrogen.

3.10 Energy Value of Foods:

This was determined for the prepared food samples, and food ingredients using the Adiabatic bomb calorimeter, produced by Gallenkamp and Co. Limited. The gross energy obtained from the calorimeter was converted into metabolizable energy using the equation:

$$M_{E/g} = (G_{E/g} \times 0.95) - (N\% \times 0.075)$$

(Miller and Payne 1959)

Apparatus:

1. Adiabatic bomb calorimeter and the accessories provided by the manufacturers.

Reagents:

1. Oxygen - free from combustible matter and available at a pressure of not less than 30 atm.
2. Firing wire (usually provided with instrument).
3. Cotton: White cellulose cotton.
4. Benzoic acid: Thermochemical standard. The energy of combustion certified by National Physical Laboratory (N. P. L.)

Procedure:

The appropriate crucible was accurately weighed and the sample to be burnt introduced (amount sufficient to cause a rise in temperature of 2 - 3°).

To prevent spattering, the sample was soaked with water, molded into a compact mass, dried to constant weight in an oven, and cooled in calcium oxide desiccator before weighing.

A piece of firing wire was connected tautly across the terminals of the bomb and a known weight of cotton tied round it. The crucible was then placed in position and the ends of the cotton were arranged so that they touched the sample.

1 ml of distilled water was put in the bomb and assembled according to the manufacturers instructions. The bomb was then connected to the oxygen supply and charged slowly with oxygen to a pressure of 30 atm. without

displacing the original air.

Sufficient water was put in the calorimeter vessel to cover the flat upper face of the bomb cap to such a depth that it did not break the surface of the water during stirring, (the oxygen inlet and the terminal need not be covered). This quantity of water was constant in all the determinations to within 1g, as that used to determine effective heat capacity. The temperature of the water was such that the final temperature reached in the determination was approximately within a degree of the standard reference temperature, 25°.

The calorimeter vessel was transferred to the water jacket, and the bomb lowered carefully into the calorimeter vessel. It was then checked to be gas tight. (If gas escaped from the bomb, the test was discarded and the cause of leakage eliminated). The stirrer, the thermometer, the electric leads and the cover were assembled according to the manufacturers instructions. The depth of immersion of the thermometer was also kept constant throughout the determinations and as far as possible all determinations involved the same part of the thermometer scale.

The stirrers, and pump were started and kept in continuous operation throughout the determination. The thermometer was tapped lightly and then with the aid of a viewer was read to 0.001 deg. and the charge fired, (No part of the body should extend to the calorimeter during

firing nor for 20 seconds after). After an interval of 5 minutes when the temperature stabilized, the thermometer was tapped again and reading taken. The difference between the second and the first temperature readings gave the rise in temperature.

Using the standard benzoic acid, the heat capacity of the apparatus was determined in a similar manner, (calorific value of benzoic acid is 6324). The benzoic acid was heated in oven at 121° for 1 hour, cooled in a desiccator before weighing.

$$\text{Heat Capacity} = \frac{6324 \times \text{Wt of benzoic acid}}{\text{Rise in temperature}}$$

3.11 Mineral Elements:

3.11:1 Atomic Absorption Spectrophotometer:

The sample digest of appropriate dilutions were run, using the Perkin-Elmer Atomic Absorption Spectrophotometer (Model 290B) and the appropriate lamp for each element. Standards were also run and concentration of elements in samples determined from the readings of the standards.

Digestion (Sample Preparation):

Sodium and Potassium:

Apparatus:

1. Digestion rack with electric heater
2. Digestion flasks (100 ml).
3. 100 ml Volumetric flasks.

Reagents:

1. Concentrated Nitric acid
2. 72% perchloric acid

Procedure:

About 0.5g (or 5 ml of sample in case of fluids) of sample (accurately weighed) was digested in a mixture of 17 ml of concentrated nitric acid and 3 ml of 72% perchloric acid. Care was taken to ensure that the final solution after digestion was colourless. After the solution had cleared, heating was continued till volume was reduced to about 2 ml.

The solution was then filtered into 100 ml volumetric flask and the digestion flask and filter paper washed thoroughly with distilled water; before diluting the filtrate to volume. The filtrate was analyzed for sodium and potassium diluting further when it was necessary.

Comments on number of digestions and determinations carried out for each sample are given under discussion of methods (Page 90).

Digestion for Calcium and Magnesium in Atomic Absorption Spectrophotometry:

Apparatus:

1. Concentrated Nitric acid
2. 72% perchloric acid.
3. Conc. Sulphuric acid
4. 5% Lanthanum oxide solution.

Procedure:

About 2g of the dry food material (or 5 ml of fluid sample) was accurately weighed into a 100 ml Kjeldahl digestion flask. 4 ml of 7:1 $^{v}/_{v}$ perchloric acid-sulphuric acid mixture, and 15 ml of conc. nitric acid were added. The sample was then heated on electric heaters (extra nitric acid was added when the solution was not clear) and digestion continued until only sulphuric acid and inorganic constituents of the plant material remained, (solution became clear). The sample was cooled, about 5 ml of distilled water added and mixed thoroughly before filtering through a filter paper into a 100 ml volumetric flask. The flask and filter paper were washed several times with about 5 ml portions of distilled water and solution diluted to volume with distilled water. From this digest solution aliquots were taken for dilution to put Ca concentration within a range of 1 to 10µg/ml Ca or the magnesium concentration 0.2 - 2.0µg/ml Mg. 5% Lanthanum oxide solution was added to the aliquots taken for dilution in a volume to make La 1% in the final solution, (The lanthanum releases the Ca and Mg present in the insoluble oxalate forms). This dilution waswanalyzed for calcium and magnesium using the appropriate lamp and wavelength provided in the instrument manual.

Comments on number of digestions and determinations are given under discussion of methods.

3.11:2 Determination of Sodium and Potassium by Flame Photometry:

Apparatus:

1. The flame photometer (EEL)
2. Na and K filters
3. Air pump
4. 50 and 100 ml volumetric flasks
5. Sample tubes.

Reagents:

1. Digestion:- Same as those used for atomic absorption spectrophotometry.
2. Potassium stock solution: 1000 ppm K 1.907g dry KCl was dissolved in water and diluted to I L.
3. Sodium stock solution: 1000 ppm Na. 2.542g dry NaCl was dissolved in water and diluted to I L.
4. Working standards: From the stock solutions, dilutions were made of the following concentrations: (K 20 ppm, 10 ppm, 5 ppm, and 1.0 ppm) (Na 10 ppm, 5 ppm, 2.5 ppm and 1.0 ppm).

Procedure:

Suitable dilutions were made from the perchloric acid digest (used in atomic absorption spectrophotometry) and ran in instrument according to the instructions in the manual. Sodium and Potassium concentrations were determined from a calibration curve obtained from the standards.

Comments on number of determinations carried out for each sample are given under discussion on methods.

3.11:3 Determination of Calcium (Chemical Method):
(Titrimetric Calcium oxalate method, A.O.A.C. P.193)

The food material is ashed and the ash (containing the Ca salts) is dissolved in HCl solution. Calcium is precipitated as the oxalate at controlled pH. The calcium oxalate is dissolved in dilute sulphuric acid and the liberated oxalic acid titrated with standard $KMnO_4$.

Apparatus:

1. Burette
2. Crucible
3. Funnel
4. Filter paper
5. Ashing oven

Reagents:

1. Conc. Hydrochloric acid
2. Bromocresol green indicator (14.3 ml 0.1N NaOH is 0.1g indicator) diluted to 250 ml for 0.04% reagent.
3. 20% NaOAc solution
4. 3% oxalic acid solution
5. Ammonium hydroxide (1 vol of Ammonia solution + 50 vols of water)

6. Sulphuric acid (5 vols conc. H_2SO_4 + 125 vols water)

7. 0.05N Potassium permanganate solution.

Procedure:

About 2 - 10g dry sample accurately weighed in a porcelain crucible was ashed in a furnace until carbon free. The residue was boiled in 40 ml. HCl (1 vol HCl + 3 vols distilled water) and few drops of HNO_3. The solution was filtered into 250 ml volumetric flask and the filter paper washed several times with distilled water before diluting to volume. 100 ml of this filtrate was transferred to a 400 ml beaker. 8 - 10 drops of bromocresol green indicator were added, and enough 20% NaOAc solution to change pH to 4.8 - 5.0. This was indicated by the blue colour of the indicator. The beaker was covered with a watch glass and mixture heated to boiling. The calcium was then precipitated slowly by adding 3% oxalic acid solution, dropwise until pH was 4.4 - 4.6, (optimum for calcium oxalate precipitation) as indicated by distinct green shade. (Yellow tints indicate undesirable displacement of pH. After boiling for 1 - 2 minutes, mixture was left over-night. Precipitate was filtered on quantitative filter paper, and beaker and precipitate washed with approximately 50 ml NH_4OH solution. (1 vol Ammonia + 50 vols distilled water) in small portions. Point of filter paper was broken and precipitate washed into a beaker with

30 ml dilute sulphuric acid (125 vols H_2SO_4 + 5 vol water) at 80 - 90°C. The washing was titrated with 0.502N $KMnO_4$ at 70 - 90°C until slight pink was obtained. The filter paper was added and titration continued. A blank titration was also carried out.

1 ml 0.05N $KMnO_4$ ≡ 1 mg Ca.

3.11:4 Determination of Iron:- (Chemical Method)
(O-phenathroline method, A.O.A.C. Page 192)

Ferrous ion reacts with O-phenanthroline to form an organge - red complex $\left[(C_{12}H_8N_2)_3Fe\right]^{2+}$. The colour intensity is independent of the acidity in the pH range 2 - 9, and is stable for long periods. Ferric ion is reduced with hydroquinone.

Apparatus:

1. Crucibles (porcelain)
2. Furnace
3. 100 ml Volumetric flask
4. Colorimeter - spectronic 20
5. 25 ml Volumetric flasks.

Reagents:

1. Orthophenanthroline solution: 0.1g orthophenanthroline was dissolved in approximately 80 ml of water at 80°C. The solution was cooled and diluted to 100 ml. This reagent remains stable for several weeks if kept in cool dark place.

2. Hydroxylamine hydrochloride solution:-
10g $NH_2OH.HCl$ was dissolved in water and diluted to 100 ml.

3. Acetate buffer solution: 8.3g anhydrous sodium acetate (previously dried to 100°) was dissolved in water, 12 ml HOAc was added and solution diluted to 100 ml.

4. Standard Fe solution: 3.512g Fe $(NH_4)_2SO_4.6H_2O$ was dissolved in water, 2 drops HCl were added and solution diluted to 500 ml. 10 ml of this solution was diluted to 1L so that 1 ml ≡ 0.01mg Fe. From this final solution, standards were prepared containing 2.0, 5.0, 10.0, 15.0, 20.0, 30.0, 35.0, 40.0, and 45.0 ml respectively, plus 2.0 ml HCl and each solution diluted to 100 ml. The resulting solutions therefore contained 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0 and 45.0 x 10^{-4}mg/ml Fe respectively. A blank containing only 2.0 ml HCl diluted to 100 ml was also made.

Procedure:

About 1-5g of the organic material (accurately weighed), was ashed. After ashing, the crucibles were cooled in CaO desiccators and 5 ml conc. HCl added to each sample, making sure that it rinsed the upper portion of the crucibles. The acid was evaporated to dryness on steam bath, and the residue dissolved again by adding 2.0ml HCl accurately measured. The solution was heated

for five minutes on steam bath while crucible was covered. The cover was rinsed with water, contents filtered into 100 ml volumetric flask and solution diluted to volume. 10 ml aliquots were taken separately into three 25 ml volumetric flasks, and 1 ml of the $NH_2OH.HCl$ solution added to each flask. 5 ml buffer solution and 1 ml of O-phenanthroline solution were added and mixture diluted to volume with distilled water. The intensity of the colour developed was read in the colorimeter (Spectronic 20) at 515nm.

The standard solutions were also treated as in sample solution, and the concentration of iron in sample was determined from a calibration curve with standards.

In some samples, the iron content was determined on the perchloric acid digest.

Comments on number of determinations carried out are given under discussion of methods.

3.11:5 Estimation of Phosphorus:-
(Molybdvanadate Method, A.O.A.C. Page 340)

Molybdovanadate reacts with inorganic phosphorus to form a pink-coloured complex - phosphomolybdovanadate which is measured at 400nm.

Apparatus:

1. Porcelain Crucibles
2. Colorimeter (Spectronic 20)
3. 100 ml Volumetric flasks
4. Furnace.

Reagents:

1. Molybdovanadate reagent: 40g Ammonium molybdate tetrahydrate was dissolved in 400 ml hot water and cooled. 2g Ammonium metavanadate was dissolved in 250 ml hot water, cooled and only 313 ml of 60% perchloric acid ($HClO_4$) added. Molybdate solution was gradually added to metavanadate solution with stirring and solution diluted to 2 litres.
2. Phosphorus standard solution:- Stock solution was prepared by dissolving 8.788g KH_2PO_4 accurately weighed in water and diluting to 1 litre. (This solution contains 2 mg P/ml).
3. Working standard: (0.1 mg P/ml), This was prepared by diluting 50 ml stock to 1 litre.

Procedure:

About 1.5 to 5g of the dry sample (accurately weighed) was ashed in a furnace till carbon free. The ash was allowed to cool to room temperature in a CaO desiccator,

and washed into a 250 ml beaker with 40 ml HCl (1 vol conc. HCl + 3 vol distilled water) in small portions. Several drops (10 - 15) of conc. Nitric acid were added, solution heated to boiling, cooled, transferred to 250 ml volumetric flask and diluted to volume with water. The solution was then filtered and 10 ml transferred to 100 ml volumetric flask. 20 ml of molybdovanatate reagent was added and mixture diluted to volume with distilled water. After thorough shaking, the solution was allowed to stand for 10 minutes and absorbance determined at 400nm against a blank.

Aliquots of working standard containing 0.5, 0.8, 1.0, and 1.5 mg P were transferred separately to 100 ml volumetric flasks. 20 ml molybdovanadate reagent was added to each flask and mixture diluted to volume with water. The absorbance of these standards were also determined as in sample solution. The concentration of P in sample was obtained from a calibration curve with standards.

In some samples, the perchloric acid digests were used in the determination of phosphorus content.

The number of determinations carried out on each food sample are stated under discussion on methods.

3.11:6 Estimation of Sulphur:
(By $Mg(NO_3)_2$ Method, A.O.A.C. Page 106)

The sulphur in samples is converted to the Magnesium sulphate by heating with $Mg(NO_3)_2$ before ashing. The sulphur in a solution of the ashed sample is then precipitated as Barium sulphate which is estimated gravimetrically.

Apparatus:

1. Large porcelain crucibles (100 ml)
2. Electric furnace
3. 400 ml beakers
4. Rubber - tipped glass rod.

Reagents:

1. 10% Barium chloride solution
2. Magnesium nitrate solution: 950g $(Mg(NO_3)_2.6H_2O$ was dissolved in water and diluted to 1 litre.

Procedure:

About 1.0g of the sample (or 10 ml in case of fluid samples) was weighed in a 100 ml porcelain crucible. 7.5 ml of $Mg(NO_3)_2$ solution was added. The mixture was heated on electric hot plate at a very high temperature to ensure boiling, and heating was continued till boiling subsided. (Complete evaporation of water seemed to have occured). The crucible was then transferred while hot to

electric furnace maintained at a temperature of about 400°C and allowed to remain till sample turned completely white (No black particles were allowed to remain). Crucible was removed from muffle and allowed to cool. Water was added to break up solids then concentrated HCl in excess till solids completely dissolved. Solution was heated to boil filtered and washed thoroughly with distilled water.

The entire filtered solution was diluted to 200 ml with water and further acidified with approximately 0.5 ml concentrated HCl. The solution was again heated to boil and 10 ml 10% $BaCl_2$ solution added dropwise with constant stirring. Boiling was continued for 5 minutes longer after which the solution was allowed to stand over-night in a warm place.

The precipitate was filtered, washed with 15-20 ml boiling water until filtrate from washing was chlorine free, (tested with $AgNO_3$ solution). The precipitate and filter were dried and ignited in a weighed porcelain crucible. Weight of $BaSO_4$ formed was obtained by difference.

S - content = wt of ppt x 0.1374

Triplicate determinations were carried out separately for each sample.

3.11:7 Estimation of Oxalate:
(By Titrimetric Calcium Oxalate Method)

Oxalate was determined by the method of Dye but as modified by Oke (1965).

Apparatus:

1. 250 ml Volumetric flask
2. 400 ml beakers
3. Burette
4. Filter papers
5. Funnel

Reagents:

1. 6N HCl
2. Conc. NH_3
3. Methyl red indicator
4. 5% $CaCl_2$ solution
5. 25% H_2SO_4 solution
6. 0.05N $KMnO_4$ solution.

Procedure:

A 2g sample of dry matter was digested with a mixture of 190 ml water and 10 ml 6N HCl, filtered and diluted to 250 ml. Three 50 ml aliquots of the extract were evaporated to about 25 ml and the brown precipitate which separated was filtered and washed with hot water. The combined solution and wash were titrated with conc.

ammonia until the salmon pink colour of the methyl red indicator was changed to a faint yellow. The solution was heated on a hot plate to about 90°C and the oxalate precipitated with 10 ml of 5% $CaCl_2$ solution. The solution was allowed to stand over-night, filtered, and the precipitate was washed into a beaker with 25% hot sulphuric acid, diluted to 125 ml and after warming to 90°, titrated with 0.0502N $KMnO_4$.

1 ml 0.05N $KMnO_4$ ≡ 2.2 mg Oxalate

$$CaC_2O_4 + H_2SO_4 = CaSO_4 + H_2C_2O_4$$

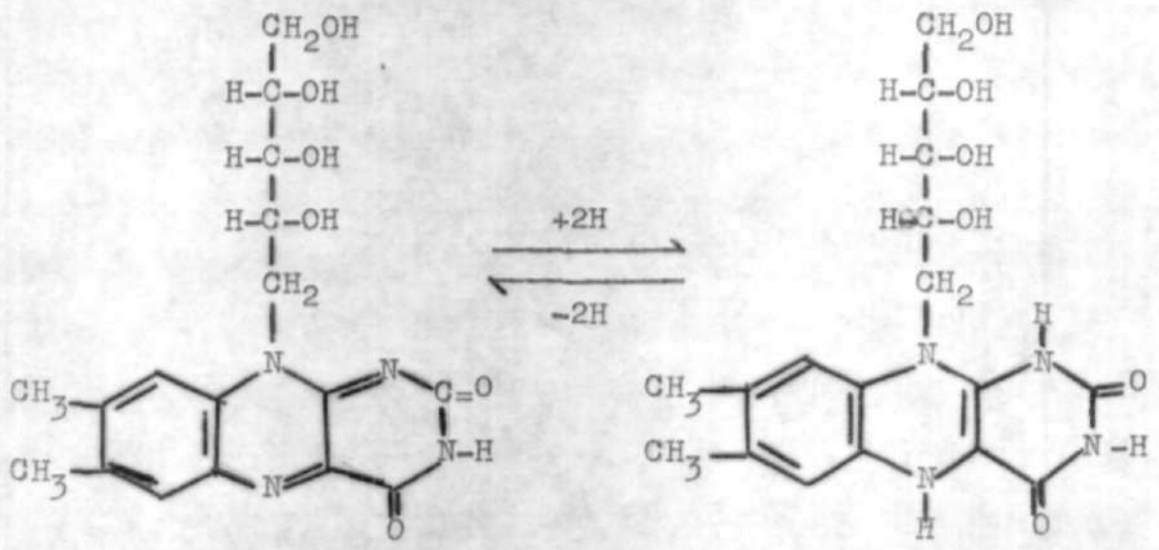
$$2MnO_4^- + 5H_2C_2O_4 + 6H^+ = 2Mn^{2+} + 10CO_2 + 8H_2O.$$

3.12 The Vitamins

2.12:1 Estimation of Riboflavin:

(Fluorometric method, A.O.V.C. Page 158)

Riboflavin fluoresces in light of wavelength 440 to 500nm. The intensity of the fluorescence is proportional to the concentration of riboflavin in dilute solutions. The riboflavin is measured in terms of the difference between the fluorescence before and after chemical reduction.



Riboflavin
(fluorescent form)

Leucoriboflavin
(reduced, non-fluorescent form)

Apparatus:

1. Autoclave
2. 125 ml conical flasks
3. Funnels
4. Filter paper
5. 100 ml conical flasks
6. Pipettes, assorted sizes.
7. Graduated Cylinders, 100 ml, 1000 ml
8. Volumetric flasks, 50 ml, 100 ml, 1000 ml, 2000 ml.
9. Test tubes 25 x 150mm
10. Test tube racks
11. Fluorophotometer - Hilger and Watts fluorophotometer, attached to UVISPEK, model H 700.
12. pH Meter.

Reagents:

1. 10N Hydrochloric acid
2. 1N Hydrochloric acid
3. 0.1N Hydrochloric acid
4. 10N Sodium hydroxide solution
5. 1N Sodium hydroxide solution: 100 ml of 10N NaOH diluted to 1 litre with water.
6. 0.1N Sodium hydroxide solution : 100 ml of 1N NaOH diluted to 1 litre with water.
7. Glacial acetic acid.
8. 3% Potassium permanganate solution: 3g $KMnO_4$ was dissolved in sufficient water to make 100 ml. Stable only for one week.
9. 3% Hydrogen peroxide: This was prepared at the time of use by diluting 30% H_2O_2 1:10 with water.
10. Stock Riboflavin solution: (25µg/ml) 50mg Riboflavin, which has been dried over concentrated sulphuric acid in an oven for 24 hours, was accurately weighed and transferred quantitatively to a 2L volumetric flask. About 1500 ml of water and 2.4 ml glacial acetic acid were added and mixture warmed to aid solution. After cooling to room temperature the solution was diluted to mark with water. This stock solution was preserved under toluene in a dark bottle.

11. Working Riboflavin standard solution: 20 ml stock riboflavin solution was diluted to 100 ml with distilled water. From this working standard, the following concentrations of riboflavin (0.05, 0.1, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50µg/ml) were made by diluting each of the following volumes (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml) to 100 ml with distilled water.
12. Solid sodium Hydrosulphite (Dithionite)

Procedure:

Extraction:- About 5g of solid sample (accurately weighed) or 10 ml of fluid sample was transferred into 150 ml flask. 50 ml of 0.1N HCl was added to the flask, and autoclaved for 30 min at 15lb pressure.

Precipitation of Interfering Impurities:

After autoclaving, the sample was cooled and adjusted to pH 6.0 with NaOH (using a pH meter). (Since riboflavin is unstable in alkaline solution, the extract was swirled constantly during the addition of alkali to prevent local areas of high pH). Immediately, 1N HCl was added in drops to bring the pH to 4.5. The solution was then diluted to 100 ml with water and filtered. 1N HCl was added dropwise to about 5 ml of the filtrate to see if further

precipitate forms. No further precipitate (impurities) formed in any of the samples. (If further precipitate occured, it should have been necessary to add 1N HCl dropwise to 50 ml aliquot of the filtrate followed by an approximately equal number of drops of 1N NaOH with constant shaking. After which, the aliquot could be diluted to 100 ml with water and filtered again if necessary).

Acidification of Extract:

20 ml of sample solution (extract) and 1 ml of water were added to each of two or three test tubes and mixed.

Internal Standard:

20 ml of sample solution and 1 ml of riboflavin solution (containing 0.5µg/ml) were added to one test tube (or each of two test tubes) and mixed. 1 ml of glacial acetic acid was then added to all four tubes and mixed.

Oxidation:

0.5 ml of 3% $KMnO_4$ was added to each tube, mixed and allowed to stand for exactly 2 min. After 2 min; 0.5 ml of 3% H_2O_2 (or 0.5 ml of 1% H_2O_2 in certain samples) were added and mixed thoroughly. The red colour disappeared within 10 - 15 seconds.

Fluorometry:-

The HIGLER AND WATTS UVISPEK with a fluorescent attachment was used. The instrument was operated according

to the manufacturers instruction, using the filter slid and circular filter marked 0.44 (indicating principal wavelength of λ 4400). The instrument was adjusted immediately before each reading to give a deflection of 100, on the galvanometer scale, using the riboflavin working standard of highest concentration.

Blank Reading:

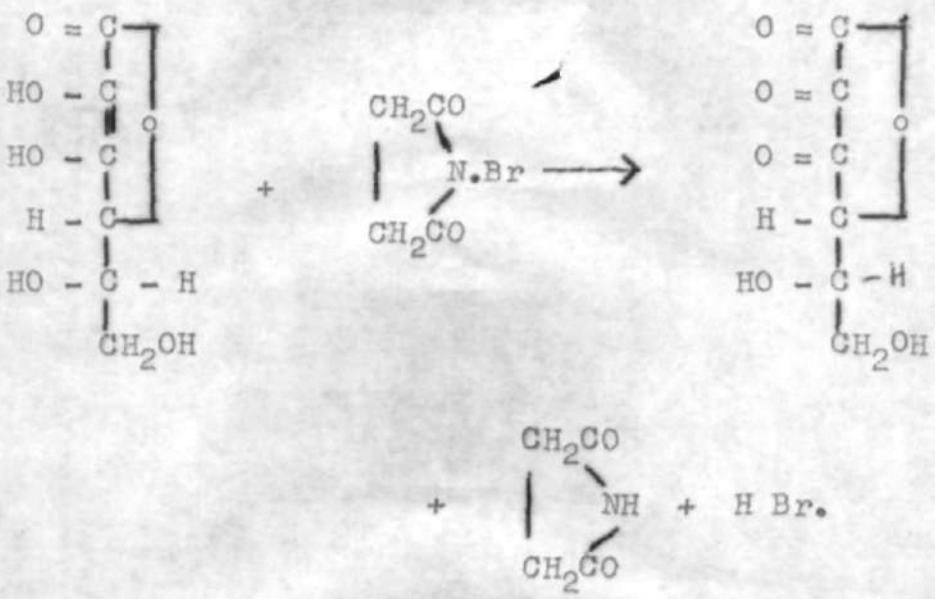
About 60mg of dithionite ($Na_2S_2O_4$) was added with mixing to the sample or standard solution in the cuvet, (after the sample reading had been taken) and fluorescence taken within 10 - 30 seconds. This reading corresponds to the blank for the riboflavin is converted into non-fluorescent form by the dithionite.

Calibration and Calculation:

Using the various riboflavin working standards (0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50µg/ml, see reagent 11) prepared at the time of use, a calibration curve was prepared, by plotting the galvanometer deflections against the various concentrations. Within the given concentration range of standards, a straight line graph was obtained. The concentration of riboflavin in samples were determined from the calibration curve.

3.12:2 Vitamin C, N-Bromosuccinimide Method:
(Evered, D. F. 1960)

Vitamin C was assayed by the method of Evered, and this method depends on the oxidation of ascorbic acid by N-bromosuccinimide. Ascorbic acid solution is titrated with N-bromosuccinimide and excess of this reagent is detected by the libration of iodine into an upper layer of organic solvent from potassium iodide.



Apparatus:

1. 1. 100 ml Volumetric flasks
2. 25 x 150mm test tubes
3. Test tube racks
4. Pipettes assorted sizes
5. 10 ml micro-burette

Reagents:

1. N-Bromosuccinimide solution: A stock solution was prepared by dissolving 200mg of the reagent in warm water, cooling and diluting to 100 ml. This solution was only stored for two days in a refrigerator. Just before use, the stock solution was diluted (10 ml to 100 ml) with water. 1 ml of this solution is approximately equivalent to 0.2mg ascorbic acid.
2. Glacial acetic acid: analytical reagent grade.
3. KI solution: 4% w/v, aqueous solution.
4. Diethyl ether - analytical grade.
5. Standard Ascorbic acid solution: This solution (0.2 mg/ml ascorbic acid) was freshly prepared in 1% v/v acetic acid by dissolving 0.02g in 100 ml.

Procedure:

About 5 - 10g of the well-powdered solid sample (accurately weighed) was transferred to a 100 ml volumetric flask with 1% v/v aqueous acetic acid and diluting to mark with same reagent. The mixture was shakened vigorously for about 30 minutes to extract the ascorbic acid into solution, and then filtered through a filter paper.

In the case of fluid samples, 50 ml of 2% v/v acetic acid was added to 50 ml of the sample in a 100 ml

volumetric flask. The mixture was shakened vigorously and filtered through a filter paper.

5 ml of the extract was transferred to a 25 x 150mm test tube, 1 ml of the glacial acetic acid and 5 ml of potassium iodide solution were added with mixing after each addition. Then 3 ml of diethyl ether was added and the mixture titrated with N-bromosuccinimide solution added from a 10 ml semi-microburette. The test tube was shakened vigorously after each addition of titrant and the organic layer allowed to separate. The end-point was indicated by the first appearance of the brown colour of liberated iodine in the upper ether layer. Comparison against an untitrated "dummy" mixture was found to be necessary in the establishment of the end-point.

A blank titration was carried out using 5 ml of water plus the volume of water equivalent to the titre to replace the diluted sample. The blank titration gave the volume of N-bromosuccinimide solution necessary to impart a definite brown colour to the ether layer.

The N-bromosuccinimide was standardized using the same procedure but titrating against 5 ml aliquots of standard ascorbic acid.

Estimation of Thiamine:
(Thiochrome Method, A.O.V.C. Page 127)

Thiochrome procedure for the estimation of thiamine, depends upon the oxidation of thiamine to thiochrome, which fluoresces in U.V. light. The thiochrome is extracted into Isobutyl alcohol and the fluorescence measured.

Apparatus:

1. Volumetric flasks (1000 ml, 100 ml)
2. Funnels
3. Conical flasks (150 ml, 100 ml)
4. Base exchange tubes with 10 ml reservoir, 10mm diameter (inside) and 17cm long.
5. Pipettes - 3ml
6. Volumetric transfer pipets: 3 ml, 4 ml, 5 ml, 10 ml, 15 ml, 20 ml, and 25 ml.
7. Volumetric flasks - 25 ml.
8. Photofluorometer, HIGLER and WATTS fluorescent attachment to UVISPEK Model H 700.

Reagents:

1. Anhydrous sodium sulphate, granular.
2. 15% NaOH solution.
3. 1.0% Potassium ferricyanide solution (solution was kept in cool dark bottle).
4. Alkaline Potassium ferricyanide solution: 3 ml

of 1% Potassium ferricyanide was diluted to 100 ml with cool 15% NaOH solution. This solution was prepared fresh daily and kept out of sunlight in a dark bottle.

5. 0.1N HCl solution: 8.5 ml of conc. HCl (S.G. 1.18) was diluted to 1 litre with water.
6. 0.1N H_2SO_4 solution: 2.8 ml concentrated H_2SO_4 (S.G. 1.84) was diluted to 1 litre with water.
7. 2.5M sodium acetate solution: 205g of anhydrous sodium acetate or 345g $NaC_2H_3O_2.3H_2O$ was dissolved in water and diluted to 1 litre.
8. Isobutyl alcohol.
9. Enzyme: (Crude phosphatase) Alkaline washed acetone dried bakers' yeast was the enzyme source. (Westenbrink and Willebrands 1944). The yeast was treated in the following way:

Bakers' yeast (from Distillers Company) was stirred up with three times its weight of acetone. After 30 minutes, it was filtered off by suction, washed with acetone on the filter and dried in a thin layer of filter paper.

The yeast was washed three to five times with 50 ml of 0.1 mol. sec. Na phosphate per gram of yeast and finally once with 50 ml of water. Each time the yeast suspension was stirred for 5 minutes and then

centrifuged (H. Jurgens and Co. 1971 model centrifuge was used) at a speed of 1,900 - 2,400 R.P.M. The washed residue was suspended in 0.1 mol. phosphate solution, pH 3.7, and stored under little toluene. (This enzyme suspension, is stable for several weeks if stored in the refrigerator or even at room temperature without appreciable loss of phosphatase activity).

The resulting yeast enzyme was tested for thiamine activity before use so that correction factor could be applied if necessary.

10. 25% Potassium chloride solution: 250g potassium chloride was dissolved in water and diluted to 1 litre. This reagent is stable indefinitely.

11. Acid 25% KCl solution: 8.5 ml of conc. HCl (S.G. 1.18) was diluted to 1 litre with 25% KCl solution. This solution is stable indefinitely.

12. ACTIVATED CATION EXCHANGE RESIN:
(Weak acid exchanger mesh 14 - 52)

Since a finner mesh of resin (30-50 mixed with 60-80 mesh) required to provide greater absorbing capacity was not available, about 100g of the 14 - 52 mesh amberlite resin, IRC-50 (standard grade from B.D.H), was pounded vigorously in a motar, (with distilled water added) for about two

hours. It was then transferred to a buchner funnel, 200 - 250 ml of 0.1N HCl was added and allowed to remain in contact with the resin for about 10 minutes. Mild suction was then applied to drain off the acid. The acid wash was repeated twice using 250 ml of hot 3% acetic acid for each wash.

The resin was then washed with about 250 ml hot KOH solution (once) followed by a wash with hot 25% KCl solution and finally with hot acetic acid solution. After draining off the wash solution, the remaining KCl was flushed out by washing the resin with 500 - 600 ml portions of hot water, applying mild suction continually. The final wash solution was free from chlorides as indicated by failure to give a precipitate with 1% $AgNO_3$ solution. The resin was dried in an oven at about 80°C, and stored in a stoppered wide mouth bottle for daily use. (To retain the solutions in contact with resin in the buchner during the 10 - 15 minutes holding period, a pinch clamp was placed on the rubber tubing connected to the side arm of the filter flask. A slight pressure was built up within the flask and practically stopped passage of solution through the funnel).

13. Stock Thiamine solution: Thiamine hydrochloride was dried over P_2O_5 in a desiccator for not less than

24 hours. 100mg of the dry reagent accurately weighed was dissolved in 0.01N HCl, and stored in a refrigerator.

14. Intermediate Thiamine solution: 5.0 ml stock thiamine stolution was diluted to 100 ml with distilled water (1 ml = 5µg).

15. Working Thiamine solution: 4.0 ml of the intermediate thiamine solution was transferred to a 100 ml volumetric flask, 75 ml of 0.1N H_2SO_4 and 5 ml of sodium acetate mixed in a beaker (and pH adjusted to 4.5 when necessary) was added to the thiamine solution and diluted to mark with distilled water. The final concentration was equivalent to 0.2µg thiamine per ml and was prepared fresh daily.

16. Stock Quinine sulphate solution: 100mg of Quinine sulphate was dissolved in 0.1N H_2SO_4 and diluted to 1 litre with same reagent. This solution is stable indefinitely if stored in a dark brown bottle. (If solution becomes turbid, discard and prepare a new).

17. Working Quinine sulphate solution: 3 ml of the stock quinine sulphate solution w as diluted to 1 litre with 0.1N H_2SO_4 to give a final concentration of 0.3mg per litre.

18. 95% Ethanol

19. 3 - 5% Acetic acid solution: 30 ml of acetic acid was diluted to 1 litre with water.

Procedure:

1. Extraction:-

5 - 10g of the solid sample (accurately weighed) was transferred to 250 ml beaker and 75 ml of 0.1N HCl was added. (In case of the fluid samples, 6 ml NHCl was added to 50 ml of sample and sufficient water added to make a total volume of 75 ml, and conc. of acid 0.1N).

The mixture in each case, was heated for 30 minutes in a boiling water bath with occasional shaking.

T he extract was cooled to room temperature and pH adjusted to 3.7 by dropwise addition of 1N sodium hydroxide (using pH meter). 5 ml of enzyme suspension (reagent 9) was added, mixture stirred and allowed to stand just 30 minutes. (For milk and nono samples, extract was first incubated with 200mg of pepsin, (4490t Pepsin ex hog stomach mucosa, granular powder from KOCH-LIGHT LABORATORIES LTD.) at pH 2 and at 37^{o} for two hours, since the thiamine is bound in a protein complex).

After incubation, the extract was adjusted to pH 4.5 by dropwise addition of 2.5M sodium acetate solution, transferred quantitatively to a 100 ml volumetric flask, and diluted to mark with distilled water. This extract was

then mixed thoroughly before removing the yeast by centrifuging and determination continued in the centrifugate.

Purification:

(a) The adsorption tube was half-filled with water and about 1.0g of the activated resin (reagent 12) allowed to fall in place by gravity. The tube was allowed to drain to just the level of resin.

(b) 25 ml (5 ml portions) of the centrifugate from the sample extract was transferred into the reservoir of the prepared adsorption tube, and allowed to pass slowly through the resin column. The filtrate was discarded and the reservoir and column washed with three successive portion (about 10 ml each) of hot water discarding the washings.

(c) After washing, 10 ml of warm acid-KCl (reagent 1) was placed in the reservoir, and the effluent solution collected in a 25 ml volumetric flask. A second 10-15ml portion was added when all the first had entered the decalso, and the eluate collected in the same flask, to just 25 ml mark, (If just below the mark, contents was diluted to 25 ml with acid-KCl solution).

(d) Steps a, b, and c, were repeated with a new column and using 25 ml of working thiamine solution (reagent 15) containing 0.2µg of thiamine per ml, in place of the sample aliquot.

CONVERSION TO THIOCHROME:

(a) After proper mixing, of the acid-KCl eluate, 5 ml was transferred into a reaction vessel (100 ml flask)

(b) 3 ml of alkaline ferricyanide solution (reagent 4) was added to the reaction vessel, mixed gently, then 15 ml of isobutyl alcohol added. The mixture was shakened vo vigorously for not less than 90 seconds.

(c) Steps a, and b, were repeated using the working Thiamine eluate.

SEPARATION OF THIOCHROME SOLUTION:

(a) After separation of the phases, the aqueous layer (lower) was siphoned out and about 2-3g of anhydrous Na_2SO_4 added to the alcohol solution. The mixture was then shakened for about 30 seconds, and allowed to stand until sparkling clear.

MEASUREMENT OF THIOCHROME:

(a) The clear colourless isobutyl alcohol solution was decanted into separate matched cuvets.

(b) Using the working Quinine sulphate solution (reagent 17) the photofluorometer was adjusted to give a deflection of 100.0 on the galvanometer scale, and the fluorescence of the Isobutyl alcohol solution was determined also in terms of galvanometer deflection, operating the instrument (Apparatus No.8) according to the manufacturer's instructions.

The Quinine sulphate setting was checked between readings, and measurements were taken rapidly (within 30 seconds) since U.V. light destroys thiochrome.

PREPARATION OF BLANK:

5 ml aliquot of the acid-KCl eluate was transferred into a reaction vessel, and treated identically to the sample extract, except that 15% NaOH was added rather than alkaline ferricyanide. The sample and the blank were ran simultaneously.

Calculation:

Thiamine content of sample in µg per gram

$$= \frac{U - UB}{S - SB} \times \frac{1}{5} \times \frac{25}{V} \times \frac{100}{\text{w t of Sample.}}$$

Where U = deflections of unknown, UB = deflections of unknown blank, and S and SB are deflections for the standard and it's blank, and V = volume of solution used for adsorption on resin. The factor $\frac{1}{5}$ converts the reading to µg per ml instead of µg per 5 ml aliquot. Since the final volume of eluate is 25 ml, the factor $\frac{25}{V}$ corrects for volume changes during adsorption and eluation. Since 25 ml was adsorbed, this factor became unity.

Column Recovery:

After activation of the resin, the thiamine was tested for column recovery by applying 25 ml of the thiamine

working standard to the column, and treating as already described under Purification, Conversion to thiochrome, Separation of thiochrome and Measurement of thiochrome. Galvanometer deflections when compared to same standard thiamine treated in a similar manner except that it was not passed through the column, helped in the establishment of the column recovery. The range of recoveries obtained is given on table 27.

3.13 Amino Acid Analysis:

3.13:1 Automatic Amino Acid Analyzer:

The Beckman 120C automatic amino acid analyzer is based on that developed by Spackman in 1958. The sample containing the mixture of amino acids is introduced into the top of the column filled with ion-exchange resin. Two columns operated at 55^{o} are employed:

(1) a long column for the separation of acidic and neutral amino acids and (2) a short column for basic amino acids and tryptophan. Two buffer systems of the same ionic strength and differing in pH (one 3.25 and the other 4.25) are employed for the long column. The short column employs a citrate buffer of pH 5.25.

As the buffer is pumped down the column at a uniform flow rate, the resin retards the amino acids for characteristic lengths of time depending on the respective pK_a values, aromatic and aliphatic side chains. When the

individual amino acids emerge from the bottom of the column, they are therefore separated from each other and ready for determination. The buffer effluent (carrying the amino acids) is met by a regulated flow of ninhydrin reagent from a second pump. This mixture enters a boiling water reaction bath where the ninhydrin reacts with amino acids to form a blue colour. The optical density of each reaction product at 570nm is measured by a photoelectric cell colorimeter and the absorption curves are plotted on a recorder.. The yellow colour resulting from the reaction of proline (or hydroxyproline) with ninhydrin is plotted by an independent photometer at 440nm. A third trace also measured at 570nm is obtained from a cuvette of one-third the normal width. This permits the calculation of the concentration of amino acids which otherwise would be off the scale.

3.13:2 Preparation of Sample for Amino Acid Analysis
(Method for hydrolysis)

Direct acid hydrolysis:

The 'standard raw fura' sample was prepared for automatic amino acid analysis by direct acid hydrolysis using 6N HCl (analytical grade).

30mg of the dry and well ground (fine to pass through a 40 - 50 mesh sieve) food sample was weighed

into a clean dry pyrex ampoule. 5 ml of 6N HCl was then added, and the neck constricted slightly using a flame. (Nitrogen gas was bubbled into the 6N HCl before use, to exclude oxygen).

The tube was closed using a short rubber tubing and a pinch clamp, before placing in deep freeze.

The ampoule containing the frozen sample was evacuated for 20 minutes through a stop cock. In-between the sample and the vacuum pump, a sodium hydroxide trap and a phosphorus pentoxide trap were introduced to absorb acidic vapours and prevent them from passing to the pump. During evacuation, the ampoule was tapped gently to exclude dissolved gases. The evacuated ampoule was sealed off at the constriction. The ampoule was then placed in a glycol bath at $110^{\circ} \pm 1^{\circ}$ for twenty hours, cooled, and opened carefully. The hydrolyzate was filtered into the flask of a rotary evaporator through sintered glass funnels of porosity 1 and then 2. Ampoule was rinsed 2-3 times with water along with the sintered funnels. The sample was evaporated under vacuum with a bath temperature of $45 - 50^{\circ}$ and excess acid was removed by repeated evaporation after the addition of water, (up to 4 times washing appear to be necessary to remove residual acid).

After final evaporation, the thin film of the hydrolyzate was taken up in 6 ml pH 2.2 citrate buffer. (Samples could be stored in a deep freeze at this time).

3.13:3 Running of Sample:

The Beckman 120C automatic amino acid analyzer was used to estimate the amino acid composition of hydroxylate.

Just before analysis, the hydrolyzate was diluted three times its own volume with pH 2.2 citrate buffer. 0.5 ml and 1.0 ml were applied to short and long columns respectively.

The recorder was operated at low speed, and the optical density of the column effluent after reaction with ninhydrin was read at 570nm and 440nm by the photoelectric colorimeters.

3.13:4 Calculations:

The concentrations of the various amino acids were obtained by integrating the peak areas by the height-width (HW) method.

In using the HW method, the height of the peak is measured in terms of time by counting the number of dots printed above the half-height of the peak. To facilitate the counting of the dots, every fourth dot of each of

the curves is black. The area of the peak is obtained by multiplying the net height (H) in absorbance, by the width (W) in dots.

3.13:5 The Standard:

The analyzer was standardized by running a calibration mixture containing 0.25 micro moles of each amino acid. 'HW' constants (C_{HW}) are calculated for each amino acid by dividing the area occupied by a peak (HW) by the concentration in micromoles, of the respective amino acid in the calibration mixture.

Three calibration runs were made for the initial standardization of the analyzer, and the average of the HW constants (standard HW constants) were obtained for the individual amino acids. The percentage recovery for calibration run was ± 2.

Concentration of unknown components in other samples were obtained by dividing their H x W values by the appropriate standard HW constants.

Chapter Four

DISCUSSION OF METHODS

All the methods employed to estimate the various nutrients are standard methods. In all cases (where possible) the methods were tested for percentage recoveries by incorporating internal and (or) external standards. The range of recoveries obtained for various analytical methods are given on table 27.

4.1 Kjeldahl Nitrogen Estimation:

To establish if digestion was complete within the time employed in the Kjeldahl digestion procedure, tryptophan which contains a ring nitrogen was digested. An average recovery value of 98% was obtained (for triplicate digests) and no loss of nitrogen was observed when digestion time was extended for even five hours longer than the digestion time adopted.

An external standard consisting of either standard ammonium sulphate or ammonium chloride solution, which was always included each time the Kjeldahl nitrogen

distillation was made, served as a check for precision during distillation. The range of recoveries for some of these external standards are given on table 27.

Two or three separate digests, distillations and titrations were made for each sample.

4.2 Minerals:

Difficulties were not encountered in most of the methods employed to estimate the various mineral elements.

Except for the standard foods where duplicate or triplicate determinations consisted of two or three separate aliquots taken from a single sample digest, duplicates or triplicates for food ingredients and randomly collected batches of the prepared foods, were carried out on separate digest or ash solutions. However, differences observed between separate digest solutions of a particular food sample were commonly less than 5%.

Recovery values for internal standards in mineral element determinations (given on table 27) do not take into account losses that might result from ashing process, since such standards were always added after ashing.

The end-point in the titrimetric determination of oxalate appeared to be difficult to ascertain, since the permanganate colour (at end point) did not persist for sufficiently long time. There appeared to be other

substances capable of decolourizing permanganate at a slow rate in the sample extract. However, by incorporation of internal standards, it was possible to know when other determinations agreed by comparison to internal standard.

4.3 The Vitamins:

Riboflavin:- High blank readings were obtained when the suggested 20 mg of dithionite was used to inactivate riboflavin in sample solutions (see riboflavin blank determination). It was therefore found necessary to add the dethionite in small portions (10mg) at a time, taking reading after each addition, till no further decrease in galvanometer deflection was observed with addition of more dethionite. About 60mg ditthionite was found to be adequate for samples assayed in the 20cc cuvette provided with the HILGER AND WATTS fluorophotometer. Another problem which was encountered in riboflavin estimation was that of avoiding much formation of bubbles which interferred with the reading of the fluorescence. The level of H_2O_2 just necessary to decolourize the $KMnO_4$ (see oxidation step in riboflavin estimation) without much bubble formation was therefore determined by trial. This concentration of H_2O_2 varied with sample type. Pouring the oxidized sample rapidly down the side of the cuvette

was also found to reduce formation of bubbles in the cell.

Vitamin C:- The Evered's method (1960) used to assay vitamin C is indeed a rapid procedure for estimating this vitamin. A great deal of skill however is needed in order to correctly ascertain the end-point of the titration. Inclusion of two or three tubes with known amount of ascorbic acid added to sample, (internal standard) was found to be almost always necessary in checking titration results.

Thiamine:- After activation of the resin used for purification of thiamine extracts, it was tested for percentage recovery using standard thiamine solution. This was necessary in order to ensure that there was complete activation of the resin, and that the quantity employed for absorption of the thiamine was neither too small or too large. An average column recovery of 93.3% was obtained for the resin employed. This recovery value is allowed for in the method used for calculation of thiamine content in samples. (See calculation equation Page 84).

4.4 Amino Acid Analysis:

The major problem in the ion-exchange chromatographic estimation of the amino acid composition of proteins, lies in the preparation of the sample. This involves hydrolysis

of the protein to a mixture of amino acids by cleavage of the peptide linkages. In practice, a variety of other reactions take place during the hydrolytic treatment, and amino acid analysis is influenced by the side reactions e.g. racemization and oxidation of some amino acids. The hydrolytic procedure is therefore of crucial importance in obtaining an accurate knowledge of protein composition.

Hydrolytic agents generally employed are of three kinds; acids, bases and enzymes. Each method has its own advantages and disadvantages and none can be employed singly to obtain the complete amino acid composition of a protein.

4.4:1 Acid Hydrolysis:

This method was employed to determine the amino acid composition of standard raw fura. It involves hydrolysis of the sample with 6N HCl in an evacuated ampoule at 110° for 20 hours.

Tryprophan is largely destroyed by acid hydrolysis. Asparagine and glutamine are completely converted to aspartic acid and glutamic acid respectively. Cysteine and cystine are partially destroyed during acid hydrolysis and better results are obtained by previous oxidizing them with performic acid to cysteic acid which is stable, (Tyrosine, histidine and tryptophan are destroyed by

performic acid oxidation). Serine and threonine are slowly destroyed during acidic hydrolysis, and their values are usually obtained by carrying out analysis on samples which have been hydrolyzed for different periods of time.

Finally, valine and Isoleucine are only slowly liberated and complete hydrolysis can be achieved between 70 - 90 hours.

4.4:2 Alkaline Hydrolysis:

This is of limited use; the destruction of arginine, serine, threonine, cysteine and cystine by alkali hydrolysis precludes its general application. It is now only commonly employed in determining tryptophan. The protein is hydrolysed for 24 hours using 5N $Ba(OH)_2$ in an evacuated tube at 120°.

4.4:3 Enzyme Hydrolysis:

This usually involves use of more than one enzyme to cleave the peptide bonds of protein. In addition to increased recovery of amino acids, enzyme hydrolysis makes possible the determination of glutamine and asparagine residues of proteins. The method however, is of limited use since the extent of digestion can only be judged after comparing values obtained with that from any acidic hydrolysis.

4.4:4 pH of Hydrolyzates:

The pH of the hydrolyzate applied to column was found to be very critical in the elution time of the amino acids. Repeated washing and evaporation of the sample hydrolyzate (see method of direct acid hydrolysis) should therefore be carried out not less than four times before residue is taken up with pH 2.2 citrate buffer. The acidic amino acids are more easily affected by pH. At low pH value (less than 2.2) they bind more firmly to the resin and their elution time increases. This creates some problems in the identification of the amino acids on the chromatograms.

Table 11

Proximate Composition of Randomly Collected Batches of Fura

Constituent g/100g dry Sample	Batches of Fura				
	1	2	3	4	5
*Water % (2)	49.24 49.00-49.48	56.61 56.02-57.20	61.89 62.0-61.78	56.57 56.56-56.58	58.69 58.68-58.69
Ash (2)	1.09 1.12-1.06	1.31 1.20-1.42	1.02 1.00-1.04	1.18 1.20-1.16	- -
Ether Extract (3)	1.72 (1)	4.05 4.00-4.10	3.84 3.76-3.96	3.12 2.95-3.24	- -
Crude Fibre (3)	1.22 0.99-1.41	1.97 1.96-1.98	1.71 1.70-1.72	1.63 1.20-2.06	- -
Crude Protein (4)	8.96 8.65-9.25	8.78 8.55-9.15	9.74 9.59-9.99	10.50 9.85-10.71	10.00 9.89-10.10
True Protein (3)	8.50 8.35-8.70	8.55 8.45-8.60	8.25 8.10-8.50	- -	- -
Carbohydrate	87.01	83.89	83.69	83.47	-

" - " usually means not specified throughout the tables

* Water expressed as % wet weight

Table 12

Mineral Composition of Randomly Collected Batches of Fura

Constituent	Batches of Fura				
mg/100g dry Sample	1	2	3	4	5
Sodium (3)	320.0	130.0 120-140	87.5 80-90	91.7 80-100	-
Potassium (3)	300.0	295 275-320	276.7 270-290	270.0 260-275	-
Calcium (4)	56.1	37.6 37.2-37.8	30.6 29.7-31.2	25.3 24.5-26.1	-
Magnesium (2)	48.0 47.5-48.5	53.0 52.0-54.0	50.0 49.7-50.2	-	
Iron (4)	8.33	6.83 6.8-6.90	9.05 8.65-8.90	15.58 15.2-15.9	-
Phosphorus (4)	234.0	203.7 195.6-208.1	197.5 195.6-203.1	218.7 218.7-218.7	-
Sulphur (3)	97.6	98.07 97.6-99.0	52.54 50.8-54.4	54.57 54.1-59.6	
Manganese	Detectable				
Oxalate (4)	365.3 357-374.0	416.3 415.0-420.0	335.3 320.0-345.0	- -	- -

Table 13

Proximate Composition of Randomly Collected Batches of Fura-Nono

Constituent g/100g dry Sample	Batches of Fura - Nono				
	1	2	3	4	5
*Water % (2)	84.14 84.00-84.28	92.00 92.50-91.50	82.74 82.44-83.00	86.10 85.50-86.80	83.47 83.40-83.54
Ash (2)	1.76 1.70-1.82	2.33 2.28-2.37	3.50 3.68-3.81	2.46 2.40-2.52	- -
Ether Extract (3)	4.76 (1)	6.00 5.96-6.04	3.57 3.47-3.66	4.92 4.43-5.20	- -
Crude Fibre (3)	1.65 1.36-2.24	2.21 2.20-2.23	2.03 2.00-2.05	1.40 0.78-2.02	- -
Crude Protein (3)	10.68	13.60 13.51-13.71	18.48 18.22-18.61	13.70 13.11-14.53	11.50
True Protein (3)	-	10.20 10.00-10.30	-	9.50 9.30-9.60	9.45 9.05-9.60
Carbohydrate	77.15	75.86	72.42	77.52	-

* As % wet weight.

Table 14

Mineral Composition of Randomly Collected Batches of Fura-Nono

Constituent Mg/100g dry Sample	Batches of Fura-Nono				
	1	2	3	4	5
Sodium (4)	313.0	226.3 220-235	274.0 272-275	160.0 140-170	- -
Potassium (4)	650.0	453.8 425-500	832.5 825-840	733.3 725-750	-
Calcium (4)	253.0	238.9 235.6-241.6	366.2 357.4-378.8	231.2 226.8-240.4	
Magnesium	60.0 59.5-60.4	85.0 80.0-90.1	64.5 64.0-65.0	-	-
Iron (4)	9.30	6.55 6.55-6.60	14.83 14.3-15.8	11.35 11.30-11.40	-
Phosphorus (4)	284.6	330.8 313-338.8	296.1 282-306.3	311.6 306.3-315	- -
Sulphur (3)	119.5	120.0 119.5-121.0	71.40 68.7-70.00	108.07 106.0-110.0	
Manganese	Detectable				
Oxalate (4)	470.0 470-480.0	341.3 320-358	385.0 375-395	- -	- -

Table 15

Proximate Composition of Randomly Collected Nono Batches

Constituent g/100g Sample	Batches of Nono				
	1	2	3	4	5
"Dry Matter (2)	4.32 3.94-4.70	5.12 5.00-6.24	5.09 5.01-5.17	5.38 4.98-5.78	4.81 3.68-5.94
Ash (2)	0.29 0.28-0.30	0.42 0.42-0.43	0.34 0.32-0.36	0.31 0.299-0.33	-
Ether Extract (3)	0.50 0.45-0.55	0.71 0.65-0.76	0.75 0.72-0.78	0.62 0.58-0.69	-
Crude Fibre (2or3)	0.23 0.226-0.228	0.14 0.14-0.14	0.29 0.27-0.35	-	-
Crude Protein (3 or 4)	1.05 1.02-1.07	1.96 1.85-2.08	1.24 1.23-1.28	1.06 1.02-1.09	1.20 1.19-1.22
True Protein (3)	-	-	1.10 1.10-1.10	0.88 0.87-0.89	1.01 1.005-1.02
Carbohydrate	2.25	1.84	2.76	3.39	-

* Dry matter in 100g fresh weight.

Table 16

Mineral Composition of Randomly Collected
Nono Batches

Constituent Mg/100g Sample	Batches of Nono				
	1	2	3	4	5
Sodium (3 or 4)	30.2 29.8-30.6	29.9 28.6-31.2	32.6 31.8-33.6	24.2 23.2-25.6	21.8 20.4-22.8
Potassium (3 or 4)	101.0 99.8-102.0	105.0 104-105.5	62.2 54.0-66.8	102.0 101.5-104.0	97.3 97.0-97.5
Calcium (3 or 4)	48.5 47.0-49.8	43.0 42.1-43.8	65.0 62.7-68.4	48.0 46.0-48.6	40.0 39.8-41.4
Magnesium (2)	6.05 6.00-6.10	6.84 6.8 - 6.88	7.65 7.3-7.95	-	-
Iron (3 or 4)	0.40 0.39-0.41	0.49 0.47-0.51	0.53 0.52-0.56	0.99 0.975-0.995	-
Phosphorus (3 or 4)	42.5 41.5-43.0	31.4 30.1-32.6	40.5 38.0-41.5	48.0 48.0-48.0	-
Sulphur (3)	9.65 9.36-10.00	7.65 7.30-7.85	9.10 8.85-9.30	-	-
Manganese	Detectable				
Oxalate (4)	35.2 32.6-39.6	25.0 22.7-26.7	21.6 20.9-23.0	-	-

Table 17

Relation Between Protein and Lipid
Content with Milk Volumes of Some Samples

** Milk Source	*Milk Yield Lbs	Protein g/100g	% Fat
Friesian (2)	17½	3.05 2.90-3.20	6.80 6.80-6.60
Friesian (2)	19	3.38 3.20-3.40	5.90 5.90-5.90
Friesian (2)	17¾	3.05 3.05-3.05	6.50 6.50-6.50
Friesian (2)	10¼	3.60 3.58-3.62	7.10 7.00-7.20
½ Friesian (2)	30	3.26 3.20-3.32	5.70 5.70-5.70
½ Friesian (2)	14	3.05 2.96-3.14	6.20 6.20-6.20
½ Friesian (2)	28¾	3.26 3.26-3.26	6.00 5.90-6.10
½ Friesian (2)	39¼	3.60 3.52-3.68	5.45 4.90-6.00
Pure Fulani (2)	¾	4.10 4.00-4.20	7.65 7.50-7.80
Pure Fulani (2)	-	3.72 3.64-3.80	6.13 5.50-6.70

* Milk yield in one milking

** Milk Source or Cow breed from which milk was obtained.

Table 18

Proximate Composition of Food ingredients
(Values in GM/100g

Food Ingredients	Dry Matter	Total Ash	Crude Protein	True Protein	Fat	Crude Fibre	Carbohydrate
Millet (2 or 3)	88.10 (88.05-88.25)	2.02 (2.00-2.04)	9.50 (9.45-9.65)	830 (8.3-8.30)	4.03 (3.80-4.28)	2.06 (1.92-2.20)	82.39
Ginger (2 or 3)	89.15 (89.10-89.20)	7.10 (5.70-7.85)	4.43 (4.40-4.46)	297 (2.9-3.01)	7.18 (6.80-7.90)	8.47 (7.45-9.40)	72.82
Black Pepper (2 or 3)	86.25 (86.00-86.50)	8.42 (8.00-8.75)	10.45 (10.20-10.70)	900 (8.9-9.04)	10.83 (10.0-12.60)	7.40 (7.30-7.50)	62.90
Kaninferi (2 or 3)	87.10 (87.10-87.11)	8.08 (8.05-8.10)	6.85 (6.75-6.95)	550 (5.5-5.50)	4.20 (3.70-5.20)	12.00 (10.60-12.80)	68.87
Pepper (2 or 3)	86.25 (85.90-86.60)	4.83 (4.58-5.00)	10.00 (9.9-10.05)	910 (8.9-9.28)	13.43 (13.00-13.70)	13.37 (12.85-13.35)	58.37
Kuka (2 or 3)	85.30 (85.20-85.40)	7.10 (6.60-7.60)	2.31 (2.30-2.33)	180 (1.7-1.88)	0.19 (0.15-0.22)	9.18 (8.95-9.40)	81.22
Fresh Milk 1 (2 or 3)	16.40 (15.35-17.45)	0.67 (0.62-0.72)	3.72 (3.70-3.74)	-	6.13 (6.10-6.15)	-	5.88
Fresh Milk 2 (2 or 3)	16.10 (15.5-16.70)	0.85 (0.80-0.90)	3.30 (3.15-3.45)	-	5.84 (5.84-5.84)	-	6.11
Fresh Milk 3 (2 or 3)	22.45 (21.85-23.05)	0.73 (0.71-0.75)	4.10 (4.00-4.20)	-	7.65 (7.50-7.80)	-	9.97
Fresh Milk 4 (2 or 3)	15.25 (15.20-15.30)	0.74 (0.70-0.78)	3.27 (3.24-3.30)	-	6.58 (5.50-6.70)	-	4.66

Milk Sources

Milk 1 - White Fulani Breed
Milk 2 - ½ Friesien
Milk 3 - White Fulani Breed
Milk 4 - Friesien Breed

Table 19

Mineral Composition of Food Ingredients
(Value in Mg/100g)

Food Ingredients	Na	K	Ca	Mg	Fe	P	S	Mn
Millet (2)	40.0 (39.0-41.0)	390.0 (385.0-395)	42.5 (42.5-42.5)	112.0 (112.0-112.0)	8.0 (6.6-9.4)	300.0 (300-300)	180.0 (175-185)	
Ginger (2)	52.0 (50.0-53.0)	1,920.0 (1918-1922)	240.0 238.0-242.0	345.0 (344-346.0)	101.5 (99.8-102.7)	175.0 (174-176)	138.0 (136.5-139.5)	
Blaack Papper (2)	128.0 (127.5-128.4)	2,200.0 (2198-2202)	3,350.0 (3350-3350)	230.0 (229.0-231.0)	80.5 (80.2-80.8)	225.0 (220-229.0)	270.0 (260.0-280.1)	
Kaninferi (2)	590.0 (585-591.1)	1,500 1,497-1503	4,600 (4,600-4600)	395.0 (392.0-398.0)	74.0 (70.0-78.1)	175 (174.0-176.1)	269.7 (265.0-280.1)	
Pepper (2)	86.0 (85.5-87.4)	1,100 (1099-1101)	57.0 (57.7-57.7)	150.0 (148.0-152.0)	89.0 (85.0-93.0)	225.0 (223.0-227.0)	220.0 (218.0-222.0)	
'Kuka' (2)	46.0 (44.5-47.5)	2,350.0 (2345-2355)	2,700 (2695-2705)	220.0 (219.0-221.0)	0.0 (0.0-0.0)	75.0 (71.8-78.2)	157.0 (157.0-157.0)	
Fresh Milk 1 (2)	41.2 (40.4-42.0)	117.5 (117.5-117.5)	134.0 (132-136.0)	11.70 (11.5-11.9)	0.0 (0.0-0.0)	98.0 (96.2-99.8)	30.8 (30.6-30.8)	
Fresh Milk 2 (2)	48.0 (48.0-48.0)	137.5 (136.0-139.0)	119.0 (118-120.0)	11.70 (11.5-11.9)	0.0 (0.0-0.0)	44.0 (42.2-45.7)	32.2 (30.0-34.4)	
Fresh Milk 3 (2)	63.0 (59.0-67.1)	130.0 130.0-130.0	160 (160-160)	18.0 (17.5-18.5)	8.0 (6.0-10.0)	131.0 (130.1-131.8)	42.2 (38.0-46.4)	
Fresh Milk 4 (2)	48.8 (46.8-50.0)	160 (158.0-162.0)	122.0 (120-124.0)	11.70 (11.6-11.8)	0.0 (0.0-0.0)	112.0 (110.6-113.3)	38.4 36.0-40.4	

Table 20

Proximate Composition of Standard Foods.

Food Description	Dry Matter	Total Ash	Crude Protein	Fat	Crude Fibre	Carbohydrate
Nono Without Kuka: Raw (2)	8.20 (8.10-8.30)	0.42 (0.32-0.52)	2.20 (2.20-2.20)	3.52 (3.50-3.55)	-+	2.06
Cooked (2)	4.45 (4.25-4.65)	0.38 (0.34-0.42)	1.21 (1.20-1.22)	0.65 (0.60-0.70)	-	2.21
Nono With 'Kuka': Raw (2)	6.70 (6.68-6.72)	0.36 (0.35-0.37)	1.48 (1.48-1.48)	2.55 (2.50-2.60)	-	2.31
Cooked (2)	3.78 (3.48-4.08)	0.358 (0.346-0.370)	1.14 (1.10-1.18)	0.20 (0.20-0.20)		2.08
Fura:- Raw (2)	41.70 (41.6-41.8)	0.97 (0.95-0.98)	9.04 (8.78-9.31)	1.89 (1.7-2.02)	1.18 (1.08-1.28)	88.46
Cooked (2)	47.80 (47.80-47.81)	0.92 (0.88-0.96)	7.65 (7.60-7.70)	1.28 (1.25-1.30)	1.52 (1.50-1.54)	89.03
Fura-Nono Without 'Kuka': Raw (2)	29.60 (29.55-29.65)	1.31 (1.30-1.32)	10.70 (10.65-10.75)	5.70 (5.65-5.75)	0.99 (0.89-1.08)	81.30
Cooked (2)	28.70 (28.74-28.66)	1.77 (1.70-1.84)	12.30 (12.28-12.32)	3.37 (3.30-3.44)	1.35 (1.34-1.36)	81.21
Fura-Nono With 'Kuka:Raw (2)	21.60 (21.00-22.2)	1.20 (1.20-1.21)	9.20 (9.20-9.20)	4.66 (4.57-4.75)	1.25 (1.22-1.28)	83.69
Cooked (2)	21.20 (21.1-21.3)	1.49 (1.48-1.50)	9.20 (9.05-9.35)	1.47 (1.43-1.51)	1.25 (1.24-1.26)	86.59

Table 21

Mineral Composition of Standard Foods
(Values in Mg/100g)

Food Description	Na	K	Ca	Mg	Fe	P	S	Mn
Nono Without 'Kuka'								
Raw (2)	28.0 (25.0-31.0)	79.0 (78.5-79.5)	64.0 (63.5-64.5)	6.7 (6.6-6.8)	11.0 (9.0-13.0)	105.0 (100.0-110-0)	25.6 (25.0-26.2)	
Cooked (2)	25.0 (24.5-25.5)	79.6 (79.5-79.7)	66.0 (64.8-67.1)	7.0 (6.9-7.2)	11.5 (11.4-11.5)	45.0 (41.0-49.1)	16.6 (16.4-16.81)	
Nono With 'Kuka'								
Raw (2)	17.8 (16.0-19.6)	80.0 (80.0-80.0)	55.0 (54.0-56.0)	6.8 (6.8-6.8)	0.0 (0.0-0.0)	40.0 (33.0-47.0)	24.0 (22.0-26.0)	
Cooked (2)	16.0 (16.0-16.0)	73.0 (73.0-73.0)	48.5 (47.4-49.6)	5.8 (5.75-5.85)	9.5 (9.0-10.0)	35.0 (33.0-38.0)	19.0 (16.2-21.8)	
Fura: Raw (2)	48.0 (47.5-48.5)	200.0 (199.8-200.2)	53.0 (50.0-56.0)	48.0 (47.8-48.2)	11.5 (11.2-11.7)	125.0 (119.8-130.2)	167.0 (166.0-168.1)	
Cooked (2)	54.0 (53.5-54.5)	200.0 (199.0-201.0)	50.0 (48.0-52.0)	61.0 (59.5-62.4)	11.5 (11.5-11.5)	175.0 (169.9-181.0)	186.0 (176.0-190.0)	
Fura-Nono Without 'Kuka':Raw (2)	68.0 (68.0-68.0)	275.0 (275.0-275.0)	95.0 (94.0-96.0)	54.0 (53.8-54.0)	21.5 (20.5-22.4)	175.0 (170.0-180.0)	167.0 (160.8-173.2)	
Cooked (2)	84.0 (82.5-85.5)	355.0 (354.0-356.0)	172.5 (172.0-173.0)	66.0 (66.0-66.0)	11.5 (11.4-11.5)	250.0 (248.0-252.0)	187.0 (185.0-189.0)	
Fura-Nono With 'Kuka':Raw (2)	64.0 (62.3-65.6)	285.0 (285-285.0)	62.5 (61.0-64.0)	55.0 (54.6-55.4)	18.0 (18.0-18.0)	175.0 (174.0-176.0)	170.0 (168.7-172.10)	
Cooked (2)	66.0 (66.0-66.0)	355.0 (355.0-355.0)	90.0 (89.0-91.0)	65.5 (64.7-66.3)	11.5 (10.0-13.0)	150.0 (149.0-151.0)	169.0 (164.4-171.5)	

Table 22

Energy Value and Vitamin Composition of Randomly Collected Batches of Foods

Food Description		Gross Energy Cal/g	Metabolizable Energy K-Cal/100g	Physiological Fuel Value K-Cal/100g	Ascorbic Acid mg/100g	Thiamine mg/100g	Riboflavin mg/100g
Fura:	Batch 1 (3)	4157 (4150-4176)	394.89	405.1 (397.3-409.6)	0	0.284 (0.280-0.288)	0.106 (0.101-0.110)
	Batch 2 (3)	4218 (4161-4186)	399.89		0	0.320 (0.315-0.325)	0.132 (0.130-0.136)
	Batch 3 (3)	4171 (4142-4205)	396.19		0	0.298 (0.294-0.301)	0.125 (0.120-0.129)
Fura-Nono:	Batch 1 (3)	4252 (4200-4272)	403.88	420.5 (412.5-429.0)	0	0.435 (0.430-0.440)	0.148 (0.145-0.150)
	Batch 2 (3)	4265 (4243-4307)	405.18		0	0.343 (0.340-0.346)	0.131 (0.128-0.135)
	Batch 3 (3)	4295 (4213-4343)	408.08		0	0.322 (0.320-0.326)	0.134 (0.130-0.136)
Nono:	Batch 1 (3)	218.6 (218.0-218.9)	20.76	20.98 (20.67-21.35)	0	0.021 (0.020-0.021)	0.087 (0.085-0.090)
	Batch 2 (3)	216.0 (213.6-218.4)	20.52		0	0.015 (0.014-0.015)	0.061 (0.059-0.064)
	Batch 3 (3)	218.6 (217.7-218.9)	20.76		0	0.016 (0.016-0.017)	0.081 (0.079-0.085)

(0) Means not present or detectable by the assay method employed.

Table 23

Energy Value and Vitamin Composition
of Food Ingredients

Food Description	Gross Energy Cal/g	Metabolizable Energey K-Cal/100g	Physiological Fuel Value K-Cal/100g	Ascorbic Acid mg/100g	Thiamine mg/100g	Riboflavin mg/100g
Millet (3)	4380 (4370-4390)	416.09	403.3	0.0	0.421 (0.418-0.426)	0.199 (0.194-0.206)
Ginger (Root) (3)	4207 (4200-4214)	399.70	373.8	0.0	0.040 (0.039-0.041)	0.196 (0.186-0.205)
Black Pepper (3)	4480 (4470-4490)	422.59	391.30	1.53 (1.43-1.64)	0.730 (0.070-0.076)	0.122 (0.110-0.126)
Gloves (Kaninferi) (3)	4290 (4273-4307)	407.99	341.2	(5.14 (4.32-5.55)	0.111 (0.102-0.121)	-
Pepper (3)	5135 (5129-5140)	487.69	394.0	17.9 (16.5-19.2)	0.375 (0.366-0.390)	0.179 (0.178-0.180)
Cream of Tartar (Kuka) (3)	3352 (3346-3359)	318.4	335.9	26.7 (24.1-29.8)	0.412 (0.405-0.416)	-
Fresh Milk 1 (3)	-	-	-	0.67 (0.62-0.70)	0.023 (0.022-0.023)	0.193 (0.190-0.195)
Fresh Milk 2 (3)	-	-	-	1.058 (1.008-1.088)	0.036 (0.034-0.037)	0.180 (0.172-0.186)
Fresh Milk 3 (3)	-	-	-	0.75 (0.70-0.856)	0.033 (0.032-0.034)	0.133 (0.126-0.143)
Fresh Milk 4 (3)	-	-	-	1.20 (1.164-1.24)	0.038 (0.037-0.038)	0.205 (0.193-0.212)

(-) Usually means not specified.

Table 24

Energy Value and Vitamin Composition of Standard Foods

Food Description	Gross Energy Cal/g	Metabolizable Energy K-Cal/100g	Physiological Fuel Value K-Cal/100g	Ascorbic Acid mg/100g	Thiamine mg/100g	Riboflavin mg/100g
Nono Without 'Kuka': Raw (3)	-	-	-	Trace	0.015 (0.014-0.015)	0.101 (0.098-0.106)
Cooked (3)	194.9 (193.6-495.6)	18.50	19.53	0	0.017 (0.017-0.018)	0.088 (0.085-0.093)
Nono With 'Kuka':Raw (3)	-	-	-	Trace	0.013 (0.013-0.014)	0.078 (0.077-0.078)
Cooked (3)	162.4 (160.3-163.2)	15.45	14.67	0	0.016 (0.015-0.019)	0.079 (0.076-0.083)
Fura: Raw (3)	-	-	-	0	0.408 (0.400-0.417)	0.140 (0.127-0.147)
Cooked (3)	4228 (4198-4246)	401.59	408.1	0	0.318 (0.314-0.321)	0.127 (0.122-0.133)
Fura-Nono Without 'Kuka' Raw (3)	-	-	-	0	0.421 (0.415-0.425)	0.152 (0.149-0.156)
Cooked (3)	4259 (4248-4269)	404.6	396.39	0	0.325 (0.322-0.328)	0.143 (0.140-0.145)
Fura-Nono With 'Kuka': Raw (3)	-	-	-	0	0.415 (0.410-0.420)	0.155 (0.150-0.159)
Cooked (3)	4301 (4291-4306)	408.59	404.37	0	0.333 (0.330-0.338)	0.140 (0.139-0.142)

Table 25

Some Calculated Nutritional Indicators

Food Description		P-Cals %	NDP*Cals %
Fura:	Batch 1	9.08	5.94
	Batch 2	8.78	5.74
	Batch 3	9.85	6.44
Fura-Nono	Batch 1	13.30	8.70
	Batch 2	10.50	6.87
	Batch 3	13.70	8.96
Std. Cooked Fura		7.62	4.98
Std. Cooked Fura-Nono Without 'Kuka'		12.14	7.94
Std. Cooked Fura-Nono With 'Kuka'		9.0	5.88

* Obtained by assuming an NPU Value of 65.
Olayide et al 1972.

Table 26

Amino Acid Content of Raw Fura. (mg/100g of the Food)		Comparison of Essential Amino Acids in Raw Fura with the FAO Provisonal Pattern (mg./g of Nitrogen)	
		Raw Fura	FAO (1957)
Aspartic Acid	725		
Serine	485		
Glutamic Acid	1700		
Proline	560		
Glycine	321		
Alanine	676		
Half Cystine	68.2	47.0	126
Valine	456	314	270
Methionine	215	148	144
Isoleucine	318	220	270
Leucine	730	504	306
Tyrosine	126	85	180
Phenylalanine	220	154	180
Threonine	440	304	180
Lysine	338	232	270
Histidine	234		
Arginine	485		

Table 27

Table for Recoveries of Internal and External Standards
of Various Analytical Methods Employed

Analytical Method	Ext. Standard Recoveries	Internal Std. Recoveries
1. Kjeldahl Method for Total Nitrogen (8)	100.05% (96.6-102.9%)	99.6% (97.0-104.5)
2. Flame Photometry (a) Sodium	102% (2) (98.0-106.0)	100.8% (5) (97.5-103.5)
(b) Potassium	102% (3) (99.5-102.5)	100.9% (4) (97.5-103.5)
3. Titrimetric Oxalate Method for Ca	101.4% -	100.6% (8) (97.0-.03.5)
4. O-Phenathroline Method for Fe	98.5 (5) (98.0-99.0)	100.0% (97.5-105.0%)
5. Molybdovanadate Method for Phosphorus	107.0% (2) (106 - 108%)	97.2% (9) (95.0-100.0)
6. Gravimetric Sulphur Determination	89.7%	-
7. Modified Dye's Method for Oxalate	- -	98.2% (6) (97.0-101.5)
8. Riboflavin (Fluorometric Method) (6)	-	99.7% 95.0-106.0
9. Thiamine (Fluorometric Thiochrome Method) Column - Recovery (3)	93.3% (91.5-95.5)	- -
10. Vitamin C (Evered Method) (3)	-	104.7% (103 - 108)

Chapter Five

DISCUSSION OF RESULTS

The results of the analyses of the various foods and food items are presented in Tables 11 - 26. The actual number of determinations carried out for eadh food analyzed is given in parentheses after the name of the food item. The range of values is given in brackets immediately below the mean value. As has beem stated under "discussion of methods", duplicate or triplicate determinations for some mineral elements and vitamins consisted of two or three aliquots taken from a single digest or extract solution of the particular food item. Where more than one batch of a particular food item was analyzed, as in the case of milk, the results have been given separately and the source and breed from which sample was collected is specified.

5.1 Randomly Collected Batches of Prepared Foods:

Tables 11 - 16 and 22, give the proximate, mineral and vitamin compositions of the randomly collected batches of the prepared foods. The randomly collected batches

of fura did not show wide variations in proximate compositions (Ash, ether extract, protein, crude fibre and carbohydrate). Very wide variations were observed however, in their mineral compositions and to a lesser extent in vitamin compositions. This may be attributed to variations in handling and in preparative procedures. Since the amount of various ingredients added to millet flour in the preparation of fura is small (largely spices, see table 8), it is not likely to account for the large variations in mineral and vitamin compositions of the foods. The major factor likely to affect the fura is the amount of cooking water poured away. Part or all the cooking water may be used during pounding of the fura (see method of preparation page 30). Variations in amount of cooking water poured away must contribute to the observed variations in mineral and vitamin compositions of randomly collected batches of fura.

Prolonged storage of foods and sun-drying usually results in significant loss of thiamine and riboflavin content (FAO 1965). Thus the amount of these vitamins in fura will vary with the length of time the major ingredient - millet - has been stored.

The observed variations in proximate, mineral and vitamin compositions of the randomly collected batches

of fura-nono (Tables 13, 14 and 22) reflects variations in the amount and compositions of fura and nono in each mixed food. But this does mean that "food tables" on nutrient composition of fura-nono will not be very helful unelss the variation in proportion of fura and nono is recognized.

The proximate, mineral and vitamin compositions of the randomly collected batches of 'nono' (Tables 15, 16 and 22) did not show very wide variations compared to those observed for randomly collected batches of fura (Tables 11, 12 and 22). This is due to the fact that losses are not so likely to occur in these nutrients during preparation of nono. The variations that are likely to occur, result from inevitable variations in nutrient composition that are observed in different samples of milk. In addition, the effeciency of skimming of the soured milk and the quantity of water (which is not constant) added to nono during preparation, will all affect the percentage proximate, mineral and vitamin composition.

5.1:1 Oxalate in Randomly collected batches of Foods:

Oxalate was found to be present in each of the prepared foods (Tables 12, 14 and 16). The presence and content of this acid in fura (table 12) deserve some

special consideration. Microbial activity could lead to production of oxalate. It may be recalled here that fura is stored for a maximum period of three days after preparation (see method of preparation page 30). Part or all the oxalate in fura may therefore have arisen from microbial activity if the prepared batches of the food were stored for any length of time.

When taken into the body, oxalate combines not only with dietary calcium but also magnesium to form insoluble salts which are unavailable for body use and are excreted in faeces. If reaction between calcium and oxalate is quantitative, then 40g Ca will react with 88g oxalate (a ratio of approximately 2.2:1 oxalate/calcium). In nono, the oxalate content is well below the level of calcium, so that the latter element is fully available. In fura the ratio of oxalate/calcium well exceeds 2.2:1 while that of fura-nono is less than 2.2:1. This means that while calcium in fura will be unavailable part of that in fura-nono is present in the available form.

Crude fibre in batches of nono:

Nono batches were found to contain some crude fibre (Table 15). This crude fibre must come partly from cream of tartar added to nono and partly from the gourd used in shaking the nono during preparation. Crude fibre is known to reduce digestibility (McCance and Walsham 1948).

An increase in crude fibre of about 0.2% (over a basal figure of 0.15%) decreases digestibility by about 1.1%. In nono, the level of crude fibre found (0.14 - 0.29g per 100g) probably will not decrease greatly the digestibility of that food.

5.2 The food ingredients:

The proximate, mineral and vitamin compositions of food ingredients are presented in tables 18, 19, and 23. The four milk samples analyzed, showed marked variations in their constituents. This makes it difficult to assess the average quantitative composition of milk. The only approach to the problem is to analyze large numbers of samples taken from different herds of different breeds in various parts of the country and over different periods of the year. When results are converted to mean values, an average composition could be obtained. This variability in nutrient composition of milk will affect the values for fura-nono and 'food table' will not be very useful unless this variation is taken into account.

5.2:1 Milk Fat:

In view of the very wide variations in fat content, observed in milk samples of different cows, analyses were carried out on samples from individual cows (bred

at Shika) of known milk yield. The fat contents were found to vary with milk volume (Table 17), increase in yield being accompanied by low fat percentage and vice versa. This feature has long been observed (Ling E. R. 1963). While the fat content of milk sample analyzed was high (5.45 - 7.65%), the protein content appeared fairly constant and close to known average value of 3.6% (Table 17).

5.2:2 Mineral and Vitamin Composition of Food Ingredients:

Most of the fura minor ingredients (largely spices) show a high content of minerals (Table 19). However, supplementation of fura with minerals cannot be achieved by increasing the spices since the level of increase required may affect palatability.

The vitamin composition (table 23) for minor fura ingredients was found to be low. For example fresh pepper contains a large quantity of vitamin C (about 128mg/100g fresh weight, Watt and Merrill 1963) but the content of this vitamin in the dried pepper analyzed was just 17.9mg per 100g dry weight. No doubt, considerable lost of vitamins must have resulted from the sun-drying method employed by the people to preserve these foodstuffs. This method of storage and preservation of food ingredients must adversely affect the nutrient composition (especially

of vitamins) of some foods consumed in this part of the country and also in other parts where similar methods are employed.

5.3 Calorie Value of foods:

Metabolizable energy values obtained by two independent procedures are compared. The results are presented on tables 22, 23, and 24.

The Miller and Payne factors which are used to allow for faecal and urinary losses are based on those of Atwater, (Miller and Payne 1959). However, use of Atwaters factors involves determination of ether extract (assumed to be fat), Nitrogen (protein assumed to be 6.25 x N), ash assumed to have no calorie value and fibre (1); the remainder is assumed to be carbohydrate (best called Nitrogen-free extract or "soluble" carbohydrate). The numerical value of 'Nitrogen-free extract' will therefore be affected by any errors in chemical analysis of the other four groups. Unlike the method based on chemical analysis, the Miller and Payne procedure involves fewer assumption since the gross energy of each foodstuff is obtained by direct calorimetry. It is then converted to metabolizable energy using factors based on Atwater's to allow for faecal and urinary losses. On this basis,

calorie value obtained by chemical analysis would be expected to show marked difference when compared to metabolizable energy for same food obtained by the Miller and Payne direct calorimetry procedure. However, apart from food ingredients with high ash and/or crude fibre content (e.g. Black pepper, Pepper, Kaninferi, Ginger and Kuka in Table 18), no two values showed a difference as high as 3% (Table 23). It is therefore likely that the errors arising from the analysis method at least on the average compensate one another. Computation of calorie values of these foods from chemical analysis (using Atwater's factors) appear therefore to be justified. Although some crude fibre may be broken down in the gut by bacterial action, this is likely to be quantitatively insignificant. When foods show considerable content of crude fibre, it might therefore be necessary to substract the contribution of the crude fibre to gross energy value in the Miller and Payne procedure. In the chemical analysis method, crude fibre content is excluded in computing metabolizable energy values. Very high metabolizable energy values (as observed for some foods, table 23) will therefore be obtained for foods with high crude fibre content when no allowance is made for the contribution of this indigestible portion to the gross energy.

Metabolizable energy values obtained either by the Miller and Payne procedure or by the use of AtWater's factors for fat protein and carbohydrate content will show close agreement provided the mentioned constituents are obtained by chemical analysis and not from table of food composition. Difficulties arising from estimating energy value of human diets from 'tables of food composition' include, (1) the problem of fitting the condition of the food to that to which available chemical analysis apply, (2) the problem of fitting the description of the food to that in the table of food composition, (3) difficulties in relating to the method of expressing carbohydrate. Hollingswort (1955). The Miller and Payne procedure is direct, it is more rapid and enables numerous determinations to be carried out within a reasonably short time and with few assumptions.

5.4 Protein Value and Calorie Supply:

It is obvious that the quantity of protein provided by a diet depends both on the amount of food consumed and on the concentration of protein in the food. However, the amount of food eaten is determined largely by the requirement for ~~protein~~ Calories. For example, a minimum supply target of 65 grams of crude protein and 2,420 K-cals per capita per day is suggested to ensure the nutritional

needs of a Nigerian. (Olayide et al 1972). On this basis, his diet must provide 10.7% of the total recommended calories as protein. If the diet provides less than 10.7% of the required total calories as protein, irrespective of the quality of the protein, the subject will not consume enough of it to meet requirements, since his appetite will limit his food intake before he has obtained the necessary amount of protein.

The number of calories obtained from protein when expressed as a percentage of total metabolizable calories (P-cals %) offers a good index for assessing the protein and calorie supply of a food or diet. The total protein value is the actual amount of protein utilized by the body, and this is expressed in terms of calories, as a percentage of the total calories called Net-dietary protein calories percent (NDP-cals %). Results of NDP-cals % calculated by assuming an NPU value of 65 (Olayide et al 1972) are shown on table 25. The results indicate that a good mixture of fura and nono will not only show NDP cals % value well above the minimum of 6 recommended for adults but will also reach the minimum value of 8 recommended for children. All except one batch of fura showed an NDP-cals % value below 6. Thus while fura-nono will form good food for adults and children, a diet based

on fura alone will not meet the protein requirement of both groups of people.

5.5 Minerals and Vitamins in prepared Foods:

A good supply of mineral elements is indeed met in an adequate fura-nono 'mixed food' (Table 14). This is because, the limiting mineral elements (mainly calcium and phosphorus) in fura (Table 13) are supplied by the nono in the fura-nono mixed food.

Of the vitamins estimated, fura-nono is mostly deficient in Riboflavin and ascorbic acid. Fura is indeed devoid of vitamin C, and although this vitamin is present in milk sample (0.67 - 1.2mg per 100gm) the level present in nono was not detectable. This is probably due partly to the high dilution of milk with water in nono preparation, and partly to oxidation of ascorbic acid that may have occured during milk souring. A diet based on fura-nono will not therefore meet the daily recommended allowance, (of about 40 mg for children and 60mg for adults) unless supplemented with fruits or vegetables. The amount of riboflavin in nono (Table 22) is not high enough to augment the low content of this vitamin in fura in order to meet the recommended daily requirement of 0.44mg per 1000K-cals.

The calcium phosphorus ratio in cooked standard fura-nono mixed foods (Table 21) are 1:1.5 and 1:1.7 for

one with 'Kuka' and one without 'Kuka' respectively. The satisfactory ratio is 1:2, since fura-nono is not taken along with other food items, the phosphorus intake will not be augmented at that meal. However, fura-nono is not the only food consumed per day and with adequate supply of vitamin D, this ratio may not lead to rickets or osteomalacia and accentuate the deformities of the bones. All or part of the iron content in most of the samples must have arisen from contamination from metal containers (usually with partly scrapped coatings) used either for storage of foods or at certain stages during food preparations. For example, milk is known to be deficient in iron, but while most milk samples showed no detectable iron, some nono samples showed iron content as high as 11.5mg/100g (Table 21).

5.6 Losses incurred during the Preparation of the foods:

Changes in the total content of various nutrients during the preparation of food are shown on tables 20, 21 and 24, and the raw and cooked foods are compared.

Minerals are usually lost from most Nigerian cooked foods by leaching (Malik 1965). Losses in mineral elements were not observed however in the standard prepared foods. This is because nearly all the cooking water was poured back to the fura during pounding. In nono also, skimming

of the soured milk during preparation did not lead to losses in mineral elements except for phosphorus. Lecithin content of milk varies from 0.035 - 0.042% and this may account for the drop in phsophorus content of soured skimmed milk. The standard cooked fura-nono samples showed remarkable increase in calcium content as compared to the raw; while there was a drop in iron content of cooked fura-nono samples as compared to the raw (Table 21). The only explanation that could be offered for these observations is that variable amount of contamination may have occured for these elements in samples prepared in the laboratory and those prepared at Hanwa.

The vitamins, (Thiamine and riboflavin) appear to have survived the souring of milk in nono preparation, as there is no significant change in their content (Table 24). With fura, losses in thiamine and riboflavin content were observed. Leaching losses of these vitamins will not occur since cooking water was almost used up in fura preparation. While the loss in riboflavin was fairly small (less than 10%), that of thiamine was quite high (about 22%). This is because ordinary cooking do not destroy appreciable quantities of riboflavin except in an alkaline medium. Thiamine on the other hand is not only destroyed in both alkaline and neutral solutions, but also exposure

to heat as in cooking can cause loss of thiamine content (F.A.O. 1965).

5.7 Amino Acid content and essential amino acid pattern:

The amino acid composition of raw fura is presented on table 26. Mention have to be made however, that owing to circumstances beyond my control (break-down of Amino Acid Analyzer), only a single determination (involving d irect acid hydrolysis) was carried out. The validity of the amino acid content of the raw fura as presented cannot therefore be fully ascertained especially for those amino acids affected by the hydrolytic conditions employed (see discussion of methods). The complete amino acid composition of raw and cooked fura and 'fura-nono' will be obtained before the publication of this work.

The essential amino acids are compared to F.A.O. provisional pattern and of those that survive the hydrolytic condition employed, Lysine is the limiting amino acid. All millet proteins are characterized by high methionine and low lysine content (Baptist and Perera 1956). It is interesting to recall here the high lysine content of cow's milk, because the combination of these two groups of foodstuffs in fura-nono, which forms a major food in various parts of the North is likely to give a well balanced mixture of essential amino acids.

Chapter Six

C O N C L U S I O N

From results obtained, both fura and nono did not show any major loss of assayed nutrients as a result of local preparation methods, but fura preparation in which all the cooking water is poured back during pounding should be encouraged. Even the vitamins assayed are not so drastically affected by the usual method of preparing fura.

The average amount of nono alone consumed per head per day in three villages around Zaria (Doka, Dan-Mahawayi, and Hanwa) has been found to be about 15.6gm, 80.9gm and 83.0gm respectively (Simmons 1972). These quantities of nono will provide 3.3 K-cals, 16.5 K-cals and 17.2 K-cals of energy and 0.37gm, 1.04gm and 1.08gm of crude protein per head per day in the respective villages (calculated from average metabolizable energy value, and average protein content in Tables 22 and 15 respectively). Although the energy supply from nono consumed is low, the quantity

of protein form it may help to balance the proportion of essential amino acids in fura when both are consumed. At present, no data is available on the amount of fura or fura-nono consumed per head per day in the three villages around Zaria, but since fura-nono forms a favourite lunch food (personal observation) it is likely that the amount of fura consumed with nono is high.

Souring of milk inhibits the growth and later destroys many pathogenic bacteria, particularly typhoid and parathyphoid organisms and noxious coliforms, so that outbreaks of intestinal disease so common with untreated milk in hot countries, are much less likely in fermented milk. However, souring cannot be relied on to control all pathogenic organisms; for example the tuberculosis and brucellosis micro-organisms survive for days if not weeks in fermented milks of quite high acidity (Kon 1972).

It is now known that souring is due to the action of acid-forming micro-organisms which convert milk sugar - lactose to lactic acid, and that if certain yeasts and probably specific bacteria are present lactose is also fermented to alcohol. The modification of lactose will make nono more acceptable to a group of Nigerians (especially from non-dairy areas) whose lactose absorption have been found to be impaired (Olatunbosun and Adadevoh 1972).

Finally, the nutritive value of fura-nono has been assessed to a reasonable measure, on grounds of chemical composition and likely effects of preparation methods, and a summary of conclusions is given below:

1. No wide variation occurs in proximate composition of fura batches, but variations occur in fura-nono batches.
2. Both fura and fura-nono batches show wide variations in mineral and vitamin compositions.
3. Part of calcium in fura-nono mixed food is present in the available form since level of oxalate present will not make all the calcium unavailable.
4. Metabolizable energy values obtained by the Miller and Payne procedure and that obtained by the chemical analysis method show close agreement.
5. While fura-nono mixed food will meet the protein requirement of adults and children, fura alone will not.
6. Marked losses in nutrients do not occur during the preparation of fura and nono by traditional method.
7. Fura-nono mixed food is likely to give a balanced mixture of essential amino acids, but more analytical work is essential.

To establish fully however, the nutritive value of fura-nono, microbiological studies and biological evaluation of the food need be carried out.

A P P E N D I X

Table 2 Contd.

Food	Ingredients	
	Local Name	Common Name
Alele	Wake*	Cowpeas
	Manja or	Palm oil
	Man gyada	Groundnut oil
	Barkono	Red Pepper
	Gishiri	Salt
	Citta	Ginger
	Kaninferi	Cloves
	Kubewa	Okro
	Albasa	Onion
Daddawa (fermented locust bean cakes)	Kalwa*	Locust beans
	Dargaza	
	Kanwa	Potash
	Daddawabaso	
Dakuwu	Aya*	Tiger nut
	Gyada*	Groundnut
	Barkono	Red pepper
	Gishiri	Salt
	Suga	Sugar (brown or white)
Dan Wake (Cooked bean cakes)	Rogo*	Cassava
	Dawa*	Guinea corn
	Wake*	Cowpeas
	Dankali*	Sweat potatoes
	Dargaza	
	Kuka	Baobab leaves
	Man gyada	Groundnut oil
	Barkono	Red pepper
	Kanwa	Potash

Appendix Contd.

Food	Ingredients	
	Local Name	Common Name
Guada da Miya	Dawa*	Guinea corn
	Wake*	Beans, Cowpeas
	Kargo leaves	
	Rogo*	Cassava
	Dankali*	Sweet potatoes
	Tumatir	Tomatoes
	Gyada	Goundnuts
	Manja	Palm oil
	Daddawa	Locust bean cakes
	Barkono	Pepper
	Kubewa	Squash, Pumpkin
	Gishiri	Salt
Kubele	Genye	Leaves
	Wake*	Cowpeas
	Dawa*	Guinea corn
	Man gyada	Groundnut oil
	Barkono	Red pepper
	Gishiri	Salt
Kuli-Kuli	Gyada*	Groundnuts
	Gishiri	Salt
	Barkono	Red pepper
	Man gyada	Groundnut oil
Shinkafa da Wake	Shinkafa*	Rice
	Wake*	Beans
	Gishiri	Salt
	Man gyada	Groundnut oil
	Barkono	Red pepper
Soyeyen Gyada	Gyada*	Groundnuts
	Gishiri	Salt
	Toka	Ashes

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