EFFECT OF COOKING ON THE NUTRITIVE VALUE OF SOME TRADITIONAL FOODS OF THE HAUSAS IN NORTHERN NIGERIA.

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D E D I C A T I O N

DEDICATED TO MY BELOVED DECEASED FATHER.

PA A. A. OSINUBI.

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T I T L E

EFFECT OF COOKING ON THE NUTRITIVE VALUE OF SOME TRADITIONAL FOODS OF THE HAUSAS IN NORTHERN NIGERIA.

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O. A. Osinubi (Miss).

D E C L A R A T I O N

I declare that this work is wholly the author's and has not been part of any presentation for any other qualification.

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A B S T R A C T

The three commonly consumed Hausa meals, danwake; tuwo dawa and kuka soup and koko and kosai have been extensively studied. Analyses for their proximate composition, vitamin content, mineral elements, amino acid composition, calorie value, and nutritive value have been carried out on the meals as sold in the local markets and as prepared (with known proportions of ingredients) in the Laboratory i.e. "Standard Meals".

All the meals were found to be inadequate in protein on an average level of intake, but they (i.e. Standard meals) were able to supply the daily requirements of vitamin B_1, B_2 and C if taken together per day.

The Standard meals and market meals were adequate in mineral elements although cooking was found to cause losses of minerals and vitamins.

All the meals were deficient in sulphur amino acids and marginal in tryptophan. Lysine was low in tuwo dawa and kuka soup and koko and kosai.

Cooking danwake was found to bring about major losses in sulphur amino acids, threonine, phenylalanine and tyrosine, while the amino acid pattern of the other two meals were less affected.

Attempts were made to establish the biological value of these meals by carrying out feeding experiments using albino rats.

None or the meals at the level ingested could support optimum growth of rats as compared with the balanced diet.

The digestibility of protein for danwake and tuwo dawa and kuka soup was improved by cooking while that of koko and kosai was reduced.

The calorie values of all the meals have been calculated and found to be quite inadequate at normal levels of intake, hence more food must be eaten to provide the necessary calories.

Some suggestions and recommendations were made on how to retain most of the nutrients when cooking the meals.

(ii) Clinical Studies (Mcfie, 1964; Bassir and Loebel, 1968; Edozien, 1965(a) and (b); Adadevoh, 1972; Omololu, 1972; Osuntokun, 1971; Egwin, 1975; Oluboyede and Ogunbode, 1976; Ayanru, 1974).

(iii) Biochemical analyses (Olatunbosun et al, 1972; Oomen, 1974; Osifo et al, 1974).

(iv) The direct assessment of some Nigerian diets and new food formulations using human beings and Laboratory animals (Oke and Tella, 1968; Williams, 1968; Isoun, 1974; Akinwande and Bragg, 1974; Ette and Bassir, 1974; Mba et al, 1974a;Njike et al, 1975(a) and (b); Oke and Umoh, 1974; Ette and Isoun, 1974.

From the available data, Oke (1972) showed that Nigeria could be divided into two main dietary zones according to the pattern of food consumption, (Southern zones whose staple diets are mainly roots and tubers and the Northern zone whose staple diets are mainly cereals).

Detailed reports have been written on the chemical composition of some locally produced foodstuffs. These reports include those of Platt, (1962); Oke, (1965(a) and (b)), (1965(c)), (1966), (1967(a) and (b)), (1971), (1973); Haikerwal and Mathieson, (1971); Oyenuga and Fetuga, 1975).

There have also been reports on the chemical composition of some traditionally prepared Nigerian foods (Oyenuga, 1968; Malik, 1967; Umoh, 1972; Eka and Edijala, 1972; Okoh, 1973; Abaelu, 1973;

Oyeleke, 1977; Eka and Kay, 1977; Okuwuraie, 1977; Eka, 1978; Akinrele, 1967; Ekpenyong et al, 1977; Kay et al, 1977.

1:1:2 Nutrient losses and Changes in Cooking and Processing of Food

The nutritional importance of a food in the diet depends upon the composition of the raw food (or diet) or the ingredients as purchased, the amount that is usually eaten and the extent to which nutrients are lost during preparation or cooking. Most foods have to be prepared and cooked before they can be eaten. The most popular ways of food preparation in Nigeria involve either boiling or frying. For these processes each individual has a plan of cooking and method of preparation. She determines according to her own taste, the rate at which the cooking occurs, that point at which it stops, the amount of cooking water to use, proportions of the food components and other details. These can influence the extent of nutrient losses which may occur. At each stage of preparation, some of the nutrients will be discarded or destroyed. However, these losses are usually not of major significance if a good mixed diet is eaten as this will still provide a considerable excess of nutrients over the recommended intakes. It is nevertheless, desirable that the losses are kept to a minimum.

In recent years attention has been focussed on the nutrient changes which occur during the preparation of food. Nutrients may be lost by cooking while in some cases toxic substances may be removed.

The vitamins are the most sensitive of all nutrients to processing and because they are present in such small amounts, the losses can be critical. Many of the water soluble vitamins are leached out in washing and blanching and are also lost into the cooking water which is often thrown away. (Lee, 1958; Roy and Rao, 1963; Bender, 1966).

Oke (1965a), carried out chemical studies one of the main staple crops of West Africa (Cassava), the changes in the food value, mineral content during convertion of raw cassava to 'Lafun' (flour from peeled fermented raw cassava) were duely evaluated. The report indicated that in the process, about 70% of protein is lost. He however found out that a cyanogenetic glycoside present in the raw cassava is excluded to a large extent during the preparation of the lafun. This report shows that even though processing has bad effect on the nutritive value of food, it also has a beneficial effect.

Bender (1966) reported insignificant losses of carbohydrate and minerals by leaching in wet processing of some foodstuffs. Sinclair and Hollingsworth (1969) have however reported some transformation of carbohydrates during heat processing. The most affected by heat being starch which is converted to dextrin, a soluble form of carbohydrate.

Malik (1967) reported losses of vitamin A including carotene which ranged from 25-50% from the cooking oil used in his studies. These results showed that loss of vitamin A may be expected when vegetables are prepared in stews containing oil and cooked for a long period. He also reported losses of up to 50% for riboflavin in his

diets and attributed these to leaching and open pan cooking. He however showed that mild heating used in ordinary domestic cooking has a beneficial effect apart from losses of nutrients, because cooking increased the digestibility of the dietary nitrogen of all the traditional diets of the people he studied.

Ogunmodede (1972) in his own study showed that the crude and true protein content of processed cowpea, rice and maize are reduced during processing but that the digestibility of the crude protein is increased. He also showed that losses of the vitamins depend on the method of processing.

Umoh (1972), carried out a study on the changes in the nutritive value of some Nigerian diets after cooking by certain South Eastern Nigeria traditional methods. His results on the nutrient composition of the raw mixed and the cooked diets showed that some losses of the vitamins and minerals and total fats as a result of cooking occured. He also showed that losses of total crude protein and total ash were negligible.

Abaelu (1973), showed that the handling of the foodstuffs before and after cooking leads to losses of the thiamine, vitamin B_2 and vitamin C content of the foods he studied.

Eka and Hobbs (1976) showed that the nutrient level of 'Danwake' was decreased. The indication was that the majority of the losses were into the cooking water (soup) except in the case of some vitamins that were possibly destroyed by cooking.

A lot of reports have been written on the nutritional deficiencies and impact on health in Nigeria. These reports include those of Bassir (1953), Collis et al (1962a, b), Mcfie (1964), Oomen (1974), Fleming (1970), Adadevoh (1972), Ayanru (1974).

1:2 The Project

This present study is designed to assess by chemical analyses and feeding experiments the nutritive value of three locally prepared meals of the Hausa Community and to observe the losses incurred during the preparation of the meals.

The three staple meals were chosen on the basis of the survey work carried out by Simmons (1971) and the report written by Aliu (1975). These meals are:

1. Koko and Kosai
2. Danwake
3. Tuwo dawa da miya kuka.

The main constituents of the average diet are the cereal grains and cereal products. Of the cereals which are consumed, guinea corn and millet are by far the most important. (Aliu, 1975; Fewster, 1975).

The morning meals and the evening meals are the only meals normally cooked by the women for the entire household; the morning meal in many cases include warmed up left overs of the previous evenings meal (Tuwo). The mid-day meals and most of the morning food are also always purchased with cash from the sellers of prepared food in the towns. (Simmons, 1971).

1. The chemical analysis were carried out on the meals of known ingredient composition - 'Standard Meals' such chemical analyses include proximate composition, assay of amino acids and vitamins, B_1 and B_2 and C and Carotenes that are easily affected by most food preparation methods. The standard meals consist separately of two forms.

(a) The cooked meals

(b) The raw - mixed diets - containing the prepared ingredients in the same proportion as in the cooked meals. Only then can losses in nutrients due to cooking methods can be ascertained.

2. Since it has been observed that some of the foods eaten by the Hausas are purchased from the hawkers of prepared foods, the chemical anlyses were extended to randomly collected samples of the prepared foods. And since there could be variation between different individuals preparing these foods, variations of the nutrient composition of different samples collected from different markets were investigated. Thus the magnitude of the variations in quality and quantity of the ingredients used and in handling and preparation methods by different individuals can be demonstrated.

3. Biological assay of food proteins:- It is not sufficient to deduce nutritive value of a meal from the chemical analysis of the nutrients present. This is because the presence of a particular nutrient does not mean that it is fully available and utilised by the animal.

The value of a diet as a source of protein is determined by its protein concentration, the digestibility and by its content of essential amino acid for body protein synthesis. These criteria are best determined by feeding experiment using laboratory animals (rats or chicks) or human volunteers.

The biological score of the food can then be determined and compared with the chemical score.

The results of these studies should give an indication as to whether or not the meals are adequate for regular daily consumption that is approximating in nutrient status to what dieticians and nutritionists consider to be suitable.

When the effect of traditional cooking is ascertained, some remedy may be applied where possible, when the nutritional value of the diets are greatly threatened. The people could be advised either to modify their methods of cooking or to supplement their meals with other foods that are rich in those nutrients adversely affected by the cooking methods.

Chapter 2

2:0 MATERIALS AND METHODS

Studies were carried out on the following three staple meals of the Hausas:

(i) Koko and Kosai - normally taken as breakfast
(ii) Danwake - as lunch
(iii) Tuwo da miya Kuka - as dinner.

2:1 Sources of the meals and foodstuffs

1. Each meal was bought from three different local markets around Zaria; that is Sabongari, Samaru and Tundunwada markets.

Three samples of each meal were bought from three different food vendors from each of the markets the same day and conveyed to the Laboratory for analysis.

2. The foodstuffs used for the preparation of the various meals in the Laboratory were also bought from the markets.

3. The meals were prepared in the Laboratory with known proportions of the ingredients using the method of Malik (1967) and Umoh (1972). These meals were regarded as 'Standard Meals'.

The method used involved inviting six Hausa women to the Laboratory. The foodstuffs were given out to them in greater quantities than they would have used to prepare a single meal for five people. Each was allowed to take as much as she would use in the

preparation of the meal. The quantity of each item taken by each woman was collected and weighed. Each meal was prepared by each woman so as to demonstrate the traditional cooking method of each meal. The weighings and cooking was repeated by each woman. The means of these weights for all the materials were computed. Each meal was then prepared by one of the women with these predetermined proportions of the ingredients. The raw-mixed meals were similarly compounded.

Table I - Showing the Components of the Meals

Meal	Ingredients
Breakfast	
(a) Koko	dawa (guinea corn), citta (dried ginger, and kaninfari (cloves) - spices. (Optional).
(b) Kosai	wake (cowpea), albasa (onions), tumatir (tomatoes), gishiri (salt), mangyada (groundnut oil), and atarugu (fresh pepper).
Lunch	
Danwake	dawa (guinea corn), dankeli (sweet potato), rogo (cassava), kanwa (potash), mangyada (groundnut oil), barkono (dried pepper), gishiri (salt), wake (cowpeas) and kuka (dry baobab leaves).
Dinner	
(a) Tuwo	dawa (guinea corn).
(b) Miya kuka	Miya kuka (baobab soup) - kuka (dried baobab leaves), atarugu (fresh pepper), gishiri (salt), manja (palm oil), tumatir (tomatoes) and maggi cubes.

Table II

Composition of the 'Standard Meals' (in grams)

(a) Tuwo da miya Kuka (Mean of two determination for each person) g

Person	Maggi Cube	Palm oil	Tomatoes	Onions	Kuka	Salt	Tatase	Atarugu	Meat	Dawa
1	3	120.47	104.78	46.54	34.33	16.66	47.85	33.71	248.72	849.33
2	4	121.93	108.73	47.78	25.87	14.45	37.84	22.75	252.36	738.60
3	3	111.60	112.05	50.44	26.99	16.71	38.79	30.31	256.57	800.21
4	3	98.74	104.85	46.83	27.34	9.75	0.00	29.10	287.23	771.66
5	2	92.66	109.19	0.00	28.42	6.37	38.45	18.50	224.86	808.79
6	3	88.74	120.14	58.09	22.34	6.77	33.28	35.00	281.20	794.00
Mean	3	105.69	109.95	41.61	27.55	11.79	32.70	28.23	253.49	793.77

(b) Danwake (Mean of two determinations for each person)

Person	Potash	Groundnut oil	Cowpea	Dawa	Rogo	Dankeli	Salt	Barkono	Kuka
1	27.68	256.15	305.58	357.93	280.06	264.92	13.09	13.08	23.09
2	34.79	196.47	343.28	382.86	237.35	291.24	15.45	14.30	28.12
3	36.62	165.83	320.51	371.44	227.79	234.94	16.93	15.71	22.88
4	32.80	188.72	280.88	342.00	230.36	273.41	14.40	13.90	27.66
5	28.77	166.25	288.76	358.76	206.26	211.63	12.35	13.60	27.72
6	21.56	189.80	298.28	387.93	258.00	241.26	11.42	12.33	20.05
Mean	30.37	193.87	306.22	366.82	237.97	252.90	13.94	13.82	24.92

(c) Koko/Kosai (Mean of two determinations for each person)

Person	Onions	Tomatoes	Atarugu	Cowpeas	Salt	Groundnut oil	Tatase	Dawa
1	53.30	74.59	30.70	612.41	17.16	384.65	32.71	980.94
2	37.90	82.27	28.29	593.37	22.04	438.61	32.43	1092.41
3	51.01	78.08	20.70	606.59	20.56	528.69	30.70	1468.10
4	66.83	0.00	15.64	471.88	14.98	385.13	27.30	1109.65
5	67.13	0.00	14.10	490.92	13.62	460.61	20.13	1155.20
6	68.53	89.12	29.89	502.75	16.58	446.33	21.55	1037.68
Mean	57.45	54.01	23.22	546.32	17.49	440.67	27.47	1140.66

2:2 Recipes for the preparation of the Meals

2:2:1 Koko

1. In the preparation of Koko, the dawa are ground with other ingredients after their husks have been removed, mixed thoroughly with cold water to form a thin paste. This is strained through a sieve into a container and set aside for at least 12 hours to sediment.
2. The resulting thick sediment is collected, mixed with a suitable amount of cold water to form a paste.
3. This is then added to boiling water with constant stirring and cooked for about 3 to 5 minutes to form a thick viscous paste.
4. Sugar is added (if desired) and then it is eaten.

Kosai

1. In the preparation of Kosai, the cowpeas are soaked in water for a few minutes.
2. The cowpeas are rubbed between the hands and washed vigorously until the outer skin (bawo) and the black 'eyes' are removed. At this stage the peas must be very white.
3. The cowpeas are then ground on a grinding stone (or grinding machine) to produce a fairly smooth paste. If onions and fresh peppers are to be included, these ingredients are ground along with the cowpeas after they have been cleaned and chopped. Some people prefer not to grind the onions but to chop them finely and add them later in the preparation.

4. The groundnut oil is heated in a metal pan. A little kanwa (potash) may be added to make the kosai brown nicely.

5. The ground peas with salt and enough water are mixed to make a slightly thick batter, beating vigorously with a wooden spoon to give the finished product the requisite lightness.

6. When the oil is very hot, the kosai batter is dropped into the boiling oil using a spoon.

7. When the kosai are brown on one side they are turned over and the other side fried until they are done.

8. The kosai are then removed from the pan with a slotted spoon. They are cooled and served.

2:2:2 Danwake

1. In the preparation of Danwake, the ingredients are ground separately to flour and mixed in a calabash or bowl.

2. Enough warm water to which kanwa has been soaked is added to make a fairly stiff dough and mixed thoroughly.

3. Small bits of the dough are dropped into boiling water in a large pot or kerosine tin and cooked for 15 to 20 minutes.

4. The cooked danwake are removed from the water placed in a bowl, covered slightly with mangyada.

5. Barkono and salt added and served.

2:2:3 Tuwo dawa da miya kuka

Tuwo

1. After husks have been removed by slight grinding, the dawa

grains are washed, dried and made into flour.

2. Peeled and dried cassava may also be added in the form of a powder (if desired).

3. A quantity of the mixed ingredients is mixed in a suitable amount of water to form a paste.

4. The paste is poured into a pot containing boiling water, thoroughly mixed with a long wooden stick (muciya) and allowed to set for 30 minutes.

5. A portion of the cooked tuwo is scoop out from the pot with a small piece of calabash (mara) and placed in a bigger calabash (the malmala).

6. If it is satisfactorily cooked, it is placed in an eating bowl and the required number of servings are removed and shaped.

7. The tuwo is then covered with soup (miya kuka) and served.

Miya kuka

1. Meat (if used) is cut into rough cubes.

2. Tomatoes, onions and fresh pepper are ground into a paste or chopped finely before cooking.

3. The meat is fried in oil, water added and then cooked until the meat is tender.

4. The kuka is normally added in a powdered form after the other ingredients, or approximately 10 minutes before the soup is to be served.

2:3 Formulation of the Meals (Umoh, 1972)

Two foods taken together as a meal were combined and homogenised in portions. These were then pooled and finally mixed in a food blender (Braun Multimix Mx.32). The raw forms of all the meals were similarly homogenised and mixed.

2:4 Treatment of Samples (Joslyn, 1970)

1. The cooked food samples; the raw mixed meals prepared in the Laboratory and the cooked food samples bought from the markets when conveyed to the laboratory were each dried separately almost immediately (after determining the water content) in hot air circulation oven at 70°C.

2. Where samples formed lumps on drying (especially the prepared foods), these were broken up in mortars to very fine powder before storing in hermetically sealed inert containers (wide mouth bottles with screw caps) in order to prevent changes resulting from loss or gain of moisture and other volatile constituents in the samples and stored in a refridgerator.

3. Some of the food samples (cooked and raw mixed, and the cooking water for danwake) were freeze-dried for determination of vitamins which might be destroyed during drying in a hot air circulating oven.

E X P E R I M E N T A L

2:5 Proximate Composition

2:5:1 Determination of Moisture Content

The moisture content of the meals (both Standard Meals and Market Samples) were determined by the standard method of Association of Official Agricultural Chemists. (A.O.A.C. 1975, p.222).

2:5:2 Determination of Ash Content and Organic matter

The ash and organic matter were determined by incineration of a known weight of the sample until ash was obtained (A.O.A.C., 1975 p.222). The organic matter was obtained by substracting the weight of ash from weight of dry material (Lighstein and Oginsky, 1965).

2:5:3 Determination of Lipid Content or Pet - Ether Extract

The lipid content (Pet - Ether extract) was estimated by extraction exhausively of a known weight of the material with fat solvent (petroleum ether - 60^{o}-80^{o}C) - (A.O.A.C. 1975, p.185).

Discussion of the Method

The method used is a Standard Method. The loss in weight of the thimble was compared with the gain in weight of the round bottomed flask for each sample to establish if all extractable lipid was obtained in the round bottomed flask.

2:5:4 Determination of Crude Protein

The crude protein was determined using Macro Kjeldahl method outlined by Association of Official Agricultural Chemists. (A.O.A.C. 1975, p.186).

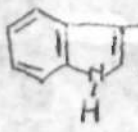
Discussion of the Kjeldahl Method

In this method it is assumed that the nitrogen of the food is converted by digesting with concentrated sulphuric acid into ammonium sulphate. This is liberated as ammonia on addition of sodium hydroxide and distilling. The quantity of protein is then calculated from the nitrogen content by multiplying by a factor of 6.25.

To establish whether or not there was nitrogen contaminant either in the chemicals used or in the atmosphere, a blank was prepared containing all the chemicals except the food sample and subjected to same conditions of the experiment as the food sample.

An internal standard employing 10mg of ammonium sulphate was included to establish the percentage nitrogen recovery. The recoveries was found to range between 108.5 and 112% for the three meals.

Tryptophan which contains a ring nitrogen was digested to further establish if the digestion was complete within the time used in the digestion procedure.



CH_2. CH. (NH_2) COOH

tryptophan.

The mean value for the recovery was found to be 98.88% for four digests and the value was found to range between 97.5 - 100% and the standard error is ± 3.217.

2:5:5 Determination of Fibre Content

Fibre content of the standard meals was determined using the methods of Official Analysis of A.O.A.C. (A.O.A.C. 1965, p.332).

Dried fat free samples were used for the estimation of the crude fibre content and the method is based on the separate extraction of substances soluble in both boiling sulphuric acid and boiling sodium hydroxide.

2:5:6 Determination of Carbohydrate Content including Nucleic acids

Carbohydrate content was determined by the difference method, that is, by substracting the total protein and Lipid from the organic matter.

2:6 Mineral Elements Determination

2:6:1 Determination of total Phosphorus

Phosphorus content of the meals was determined using the Molybdovanadate Method of Official Analysis of A.O.A.C. (A.O.A.C. 1975, p.143).

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

2:6:2 Determination of Mineral Elements

The following mineral elements were determined by atomic absorption method of the Association of Official Agriculatural Chemists. (A.O.A.C. 1975, p.22), using Perkin-Elmer Atomic Absorption Spectrophotometer Model 306 with digital read out.

(i) Calcium (ii) Potassium (iii) Zinc
(iv) Sodium (v) Iron (vi) Copper
(vii) Magnessium (viii) Manganese.

The method operates on the principle of absorption. When ray of light of a particular wavelength passes through the flame containing a cloud of free atoms of the tested element, intensity of the ray is diminished or reduced due to absorption. The magnitude of the decrease in intensity is proportional to the concentration of the atoms in the flame and consequently the atoms in solution.

Discussion of the Method

Differences observed between separate digested solutions of a particular food sample were commonly less than 2%.

Strontium and Lanthanium Salt were added to potassium and sodium, calcium and magnessium salts respectively to eliminate any ionisation of the standard salts which might occur in the acetylene flame.

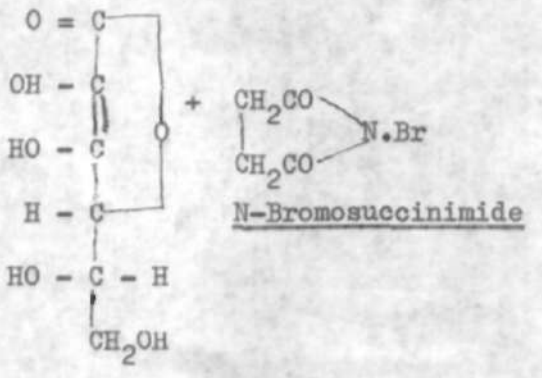
2:7 Vitamins Determination

2:7:1 Determination of Vitamin C. (Ascorbic acid)

Ascorbic acid content of the 'Standard meals' was determined by Evered's Method. (Evered, 1960).

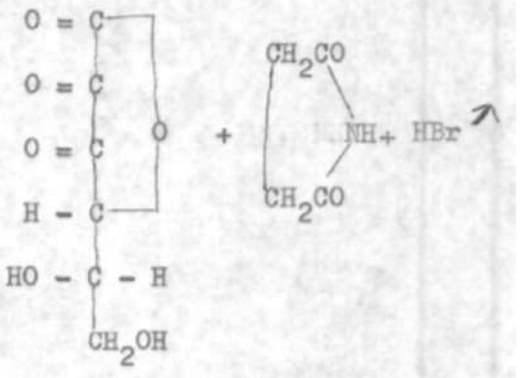
Ascorbic acid oxidation by N-Bromosuccinimide is a satisfactory method for assaying ascorbic acid and the reagent unlike 2:6 dichlorophenolindophenol is unaffected by reductones, reductic acid and Iron salts (Evered, 1960).

Excess of N-bromosuccinimide that is, the end point of titrating the ascorbic acid, is detected by liberation of Iodine from potassium Iodide in acid solution and formation of the blue colour with starch. However, pigments also interfere with this end point, so with pigmented material, the liberated Iodine can conveniently be detected by extraction into an organic solvent, when the ascorbic acid present has been completely oxidised.



Ascorbic acid

N-Bromosuccinimide



Oxidised Ascorbic acid

Discussion of the Method

There are two common methods of estimating Ascorbic acid in biological materials. These methods are the 2,6-dichlorophenol-indophenol titration method and the N-Bromosuccinimide titration method.

Estimation of Ascorbic acid with 2,6 dichlorophenol-indophenol is limited by the presence of interfering substances such as reductones and reductic acid. Slow reducing substances that may be present in biological fluids render the end point less distinct (Mohammed et al, 1955). This defect of this method, induced the author to investigate the estimation of ascorbic acid by N-Bromosuccinimide, the oxidising action of which is selective. The fact that ascorbic acid is selectively oxidised before other reducing substances that may be present, provides a reliable titrimetric method of extensive applicability.

Three tubes were included with known amount of ascorbic acid added to each meal sample so as to check the titration results, that is percentage recovery.

The percentage recovery was found to vary between 98.95 and 103% for the three meals.

2:7:2 Determination of Thiamine (Vitamin B_1)

Thiamine content of the Standard meals (raw mixed and cooked forms) were determined by the Thiochrome method of the Association of Vitamin Chemists (A.O.V.C. 1966, p.127).

Thiochrome procedures depend upon the oxidation of thiamine to thiochrome which fluoresces in Ultra Violet light. Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present, and hence to the thiamine originally in solution. If necessary the thiamine may be freed from interfering substances by treatment with adsorbents which retain thiamine but not the impurities. Subsequent elution usually provides an extract sufficiently pure for analysis.

The apparatus used was photofluorimeter:- Single sided M.K.4.W129 R.T.

Discussion of Thiochrome Method

None of the present known methods for determination of thiamine and its phosphoric esters allow thiamine to be determined separately.

Chemical methods are based either on the difference of the solubility of the thiochrome resulting from the oxidation of thiamine and its phosphoric esters or on the determination of thiochrome fluorescence before and after treatment with phosphatase which converts phosphate esters into free thiamine.

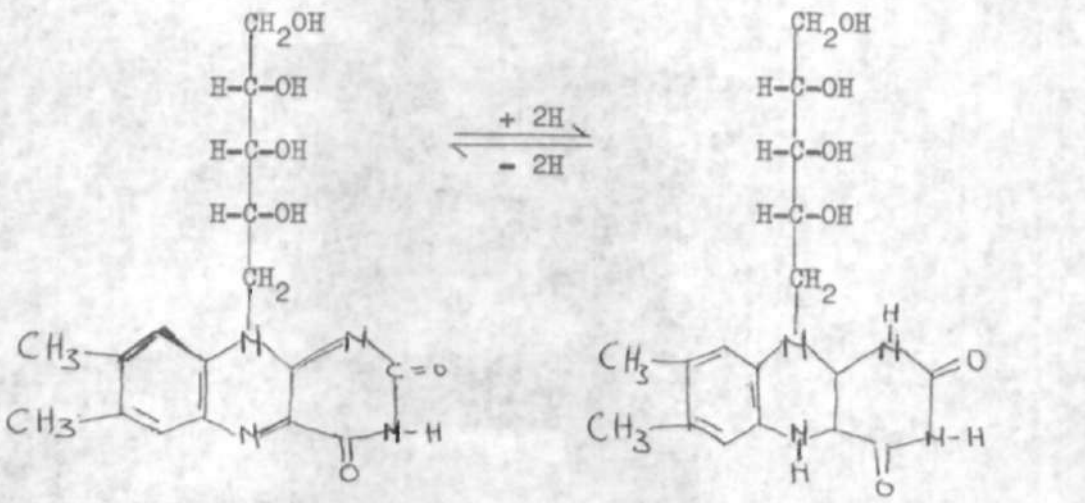
For this study, the fluorometric method was used, the percentage recovery using Standard thiamine solution was tested after activation of the resin used for the purification of vitamin extracts. This was found to be necessary in order to ensure that there was complete activation of the resin and that the quantity employed for

absorption of the thiamine was neither too small or too large. An average column recovery of 97.3% was obtained for the resin employed and the recovery value was found to range between 96.8-97.6% for four determinations. This recovery value is allowed for in the method for calculation of the thiamine content in the samples. The standard error was found to be ± 0.36 for the method.

2:7:3 Determination of Riboflavin (Vitamin B_2)

Riboflavin was determined by fluorometric method of Association of Vitamin Chemists. (A.O.V.C. 1966, p.158).

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



Riboflavin
(fluorescent form).

Leucoriboflavin
reduced, nonfluorescent form

Discussion of Method used for Riboflavin estimation

Riboflavin is naturally bound to proteins, hence to liberate riboflavin from its natural protein bound forms, it is necessary to treat the samples with suitable solvents at room temperatures or at the boiling point of the solvent. For this determination, aqueous acid solution was used.

Impurities in the extract were precipitated within pH 4.5 and 6.0. Reducing agent such as sodium dithionite, zinc in acid solution, catalytically activated hydrogen and titanous chloride transform riboflavin in alkaline, neutral or acetic acid solutions directly into an almost colourless pale yellow dihydroflavin which is re-oxidised on shaking with air, hence it was necessary to take the blank reading within 10-30 seconds of addition of the dithionite.

In the riboflavin estimation using the fluorometric method, the problem encountered was that of avoiding formation of bubbles which can interfere with the reading of the fluorescence. The level of hydrogen peroxide just necessary to decolourise the permanganate without much bubble formation was therefore determined by trial. This concentration of hydrogen peroxide varied with sample type. Pouring the oxidised sample rapidly down the side of the cuvette was also found to reduce formation of bubbles in the cell.

2:7:4 Determination of Carotene

Carotene is determined by Chromatographic method of Association of Vitamin Chemists. (A.O.V.C. 1966, p.104).

The method is based upon the separation of the biologically active carotenoid pigments from the total carotenoid pigments in an extract by an adsorbent with varying affinities for the different pigments.

Under the proper conditions, the various materials may be separated into relatively distinct zones or bands. The individual pigments exhibit characteristic absorption maxima at which their concentration may be determined by photometric measurement.

After the extraction of the carotenes, the carotene from each meal was run along with the Standard Carotene on an S.P.800 Spectrophotometer to determine the actual wavelength at which maximum absorption of the extract occurs.

The wavelength was found to be 450nm, hence the carotene was estimated at this wavelength using Spectronic 20.

The percentage recovery of the purification column was tested using a known concentration of carotene. The mean of three determinations was found to be 98.63% and the Standard error ± 0.097.

2:8:1 Amino acid Analysis

The amino acid was analysed using the original method of Spackman et al (1958) as adopted by Haikerwal (1969), with a Beckman 120C automatic amino acid analyser.

Preparation of the Sample for amino acid analysis (hydrolysis)

Direct acid hydrolysis

1. 50mg of the dry and well ground food sample was weighed into a clean dry pyrex ampoule.

2. 5 ml of 6N HCl was added and the neck constricted slightly using a flame. (Nitrogen gas was bubbled into the 6N HCl before use to exclude oxygen).

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

4. The ampoule containing the frozen sample was evacuated for about 20 minutes through a stop cock. During evacuation, the ampoule was tapped gently to exclude dissolved gases.

5. The evacuated ampoule was sealed off at the constriction.

6. The ampoule was then placed in an oven at 110°C for 24 hours, cooled and opened carefully.

7. Excess HCl in each sample was evaporated off by rotary evaporator and washed thrice with 5 ml of distilled water each time.

8. After final evaporation, the thin film of the hydrolysate was taken up in 10 ml pH 2.2 citrate buffer and then stored in a deep freeze until needed.

Running of Sample

1. The Beckman 120C automatic amino acid analyser was used to estimate the amino acid composition of the hydrolysate.

2. Just before analysis, the hydrolysate was diluted if needed with pH 2.2 citrate buffer. 0.5 ml and 1.0 ml were applied to short and long columns respectively.

3. 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.

Calculations

The concentration 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).

The Standard

The analyser was standardised by running a calibration mixture containing 0.25 μmoles of each amino acid.

HW constants (HW^c) are calculated for each amino acid by dividing the area occupied by a peak by the concentration in the calibration mixture.

Three calibration runs were made for the initial standardisation of the analyser and the average of the HW constants (Standard HW constants) were obtained for the individual amino acid.

The concentration of unknown components in other samples were obtained by dividing their HW values by the appropriate Standard HW constants.

Discussion of Method used for the Amino acid analysis

The working of the Beckman 120C automatic amino acid analyser assembled by Spackman, Moore and Stein (1958) is based on Ion exchange resin column chromatography.

The developing buffers are mechanically pumped through the column at a constant rate under carefully regulated condition after volume of buffer is quantitatively applied to the top of the resin. The effluent emerging from the column, brings out in definite sequence the resolved amino acid components and is joined into a stream of ninhydrin where it is mixed up and pumped at constant rate.

The ninhydrin mixture is then passed through a long coil heated in a boiling water to produce colour. The products emerging from the coil are led through a three cell photoelectric photometer where absorbance output at 570nm is traced on a three channel recorder.

Quantitative calculation for amount of each amino acid components in the original sample mixture can then be made from integration of areas in the resulting automatic plots of absorbance versus time as described under materials and methods. The major problem in this ion exchange chromatographic estimation of the amino acid composition of the protein, 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, various

other reactions take place during the hydrolysis and the amino acid analysis is influenced by this side reactions. For example racemization and oxidation of some of the amino acids.

The hydrolytic procedure is therefore of crucial importance in obtaining an accurate knowledge of composition of protein.

Hydrolytic agents generally employed are of three kinds; acids, bases and enzymes.

Acid hydrolysis

This method was used to determine the amino acid composition of 'Standard meals' - raw and cooked form. It involves hydrolysis of the sample with 6N HCl in an evacuated ampoule at 110°C for 24 hours.

Limitations

Tryptophan is largely destroyed by this method. Asparagine and glutamine are completely converted to aspartic acid and glutamic acid respectively. Cysteine and Cystine are partially destroyed during the acid hydrolysis, but better result can be obtained by previous oxidising them with performic acid to cysteic acid which is stable (Tyrosine, histidine, tryptophan are destroyed by performic acid oxidation. (Byers, 1971).

Serine and threonine are slowly destroyed during acid hydrolysis and they are usually obtained by carrying out analysis on the samples which have been hydrolysed for different periods of time. (Byers, 1971; Haikerwal and Mathieson, 1971).

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 Barium hydroxide in an evacuated tube at 120°C. (Haikerwal and Mathieson, 1971, Blackburn, 1968).

Enzyme hydrolysis

This usually involves the use of more than one enzyme to cleave the peptide bonds of the protein. This enzyme hydrolysis makes it possible, the determination of glutamine and asparagine residues of proteins in addition to the increased recovery of amino acids. The method is however limited in use, since the extent of digestion can only be judged after comparing values obtained with that from any acidic hydrolysis. (Oke and Umoh, 1974).

pH of hydrolysate

The pH of the hydrolysate applied to the column was found to be very critical in the elution time of the amino acids. Repeated washing and evaporation of the sample hydrolysate was therefore carried out four times, before the 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 strongly to the resin and their

elution time therefore increases. This will create some difficulty in identification of the amino acids on the chromatogram.

Oyeleke (1977) demonstrated that period of hydrolysis may have effect on the amount of amino acids produced. He found that the amino acids usually found in sorghum could be divided into four major groups in terms of duration of the hydrolysis:-

(1) The acid labile amino acids with yields decreasing with increased time of hydrolysis, i.e. Cystine, Methionine, Tyrosine, glutamic acid, tryptophan and Arginine.

(2) The amino acids showing no significant trend in yield, i.e. threonine, lysine, histidine and aspartic acid.

(3) The acid stable amino acids with maximum yield at 48 hours, alanine, proline and glycine.

(4) The bulk acid stable (sterically hindered) amino acids whose release required longer than 72 hours of hydrolysis i.e. phenylalanine and valine.

2:8:2 Chemical Determination of Tryptophan by the method of Horacio and Lynn, 1969 as adopted by Osaniyi (1977).

The method involves the hydrolysis of the protein by papain releasing tryptophan which then complexes with ferric chloride to give a brown colour which absorbs maximally at 420nm.

Reagents:-

1. Papain enzyme solution:- 0.4% W/V enzyme in phosphate buffer pH 7.0.
2. 5% W/V sodium cyanide solutions in distilled water.
3. 0.1M formic acid.
4. Ferric chloride reagent:- 270mg of ferric chloride hexahydrate were dissolved in 0.5 ml distilled water and then transferred to a 100 ml volumetric flask and made up to volume with glacial acetic acid. Equal volumes of this solution and concentrated sulphuric acid were mixed 1-2 hours before use.

Procedure:-

1. A stock solution of tryptophan 1mg/ml was prepared using distilled water.
2. A calibration curve was prepared using dilutions of stock solution containing 1.0mg to 50mg tryptophan per 100 ml.
3. 1 ml. of each solution was pipetted into a test tube containing 4 ml of the ferric chloride reagent.
4. Mixture was vigorously shaken and incubated for 15 minutes in a water bath at 65°C for colour development.
5. After cooling to room temperature, the absorbance of the solutions were measured using Spectronic 20 at 420nm against a reagent blank prepared using 1 ml. of distilled water instead of 1 ml. of tryptophan solution above.

6. The hydrolysate of the sample were run concurrently with the standards.

Hydrolysis of Sample Powder

1. Three 300mg portions of dried food powder were weighed into 50 ml. Erlenmeyer flasks and 8 mls. of the papain solution added.
2. Three drops of 5% $^{w}/_{v}$ sodium cyanide solution were added and the flask lightly capped.
3. The samples were incubated in a water bath for 36 hours at 60°C.
4. At the end of the hydrolysis period 1 ml. of 0.1M formic acid was added to each flask to stop the reaction.
5. The reaction mixtures were centrifuged.
6. A blank containing 8 ml. of enzyme solution and three drops of 5% w/v sodium cyanide solution in the flask (without the sample) was run along with the sample.

Determination of hydrolysate

1. 1 ml. of the papain hydrolysate was diluted 10 times.
2. 1 ml. of the diluted hydrolysate was mixed with 4 ml. of ferric chloride reagent.
3. Mixture was vigorously shaken and the colour allowed to develop in a water bath maintained at 65°C for 15 minutes.
4. After cooling to room temperature the absorbance of the solutions were measured in a Spectronic 20 at 420nm against the reagent blank.

2:9 Animal Feeding Experiment Using Albino Rats

The diets used were prepared using the proportions of the ingredients in the 'Standard Meals' shown in Table II.

In preparing the various meals the traditional procedures was used by one of the Hausa women employed for computing the proportion of the ingredients in the 'Standard Meal' so that the meals would be identical with the food as actually consumed by the Hausas.

The cooked homogenised food were dried (Bassir and Umoh, 1973) and the lumps of the dried meals were broken down to small pellet sizes which could be managed easily by the rats.

Non-protein diets

The non-protein diet was made up of the following composition on a dry weight basis - Sucrose 7 percent, mineral elements mixture (Rippon, 1959), 4 percent, Butter, 10 percent; Vitamin mixture (Cuthbertson 1957) - 5 percent; Cod-liver oil, 1 percent; and corn starch (Rippon, 1959), 73 percent. The corn starch was added after all other ingredients have been thoroughly mixed together.

Table III

Composition of Basal Protein free diet

Ingredients	Amount (g)
Sucrose	140
Butter	200
[1]Vitamin mixture	100
[2]Salt mixture	80
Cod liver oil	20
Corn starch	1,460

[1]Vitamin Mixture Oke and Umoh (1974)

Ingredients	Amount
Vitamin A	4000 I.U = 1.2mg.
	1. I:U = 0.0003mg Vit A
Vitamin D (calciferol)	2,000 I.U = 0.05mg.
	1.I.U = 0.000025mg Vit D_3
Vitamin E (tocopherol acetate)	280 mg.
Vitamin K	2 mg
Thiamine	30 mg
*Riboflavin	30 mg
Pyridoxine	8 mg
Ca D-panthothenate	100 mg
Nicotinic acid	100 mg
Choline Chloride	1000 mg
Inositol	220 mg
Vitamin B_{12}	50 mg
p-amino benzoic acid	75 mg
*pteroyl glutamic acid	1.0 mg
Biotin	0.2 mg

(* Riboflavin and pteroyl glutamic acid were added last)

Table III Contd.

[2]Salt Mixture (Oke and Umoh, 1974)

Ingredients	Amount
Sodium Chloride (NaCl)	50g
$Ca_3(PO_4)_2$	400g
$FeC_6H_5O_7 \cdot 3H_2O$	35g
$MgSO_4 \cdot 7H_2O$	80g
NaH_2PO_4 (anhydrous)	105g
KCl	250g
KI	1g
$MnSO_4 \cdot 4H_2O$	0.2g
NaF	0.04g

Animals and grouping of animals (Bender and Doell, 1957)

Sixteen Albino rats of a single strain from the Department of Biological Sciences were used in the experiment. The animals were weaned at 21 days and fed for one week on stock diet (mice cubes, produced by Pfizer Nig. Ltd.).

The rats were divided into 8 groups of 2 rats in such a way that each group has 1 male and 1 female and each group totalled the same weight within 1-2g and combined body-weights were used (Bender and Doell, 1957).

Procedure:

One group was fed on the non protein diet and one group on mice cubes at 21% protein level, produced by Pfizer and each of the

remaining groups were fed on separate test protein diets for 10 days simultaneous. Water and food were given ad libitum. The first three days were regarded as an acclimitization period during which no record was kept of the food consumption and no collection of faeces was made. Daily records of food consumption were kept for the last 7 days, by weighing the food given and what remained together with that spilled after 24 hours. The spilt food and faeces were separated by hand which also allowed the investigator to separate and discard all particles of hair.

The weight of each group of rats was recorded every day.

Collection of faeces were made daily for the last 7 days of the feeding experiment. The faeces of each group of rats were pooled dried at 105°C for 24 hours and ground into powder for faecal Nitrogen estimation. The parameters considered are:-

1. The Net Protein Ratio (N.P.R.) was determined as:-

$$\frac{\text{gain weight of test group + loss in weight of non-protein diet}}{\text{protein intake.}}$$ (Bender and Doell, 1957).

2. Protein efficiency ratio was also calculated thus:-

$$\text{P.E.R.} = \frac{\text{Gain in body weight}}{\text{N intake.} \times 6{\cdot}25}$$

3. Protein retention efficiency, P.R.E.

$$= \text{N.P.R.} \times 16$$ (Bender and Doell, 1957)

4. Coefficient of digestibility; C.D. or % absorbed

$$= \frac{100(\text{N intake} - \text{faecal N})}{\text{N intake.}}$$

The experiment was repeated using another set of 21 days old rats.

Modification of Bender and Doell Method (1957)

In this experiment, the investigator used two rats per group instead of four used per group by Bender and Doell. This was because the investigator could not get up to 32 rats of the same age for each trial run.

Discussion of Method:-

N.P.R. is defined as gain in weight of test group plus loss in weight of non protein group, divided by protein intake. It thus measures the protein used for maintenance. (Bender and Doell, 1957).

In practice the test groups and the non protein group were run simultaneously. Protein retention effeciency:- (P.R.E.) - this term was given to the value obtained by converting N.P.R. into a percentage scale by use of an experimentally determined factor, 16. This modification of the P.E.R. method, described overcomes its major disadvantages but is still apparently subject to the criticisms of any growth method, namely, that the gain in body weight does not necessarily bear a constant relation to the new protein tissue. However, P.R.E. has been shown to correlate extremely highly with the N.P.U. determined by Carcass analysis (Bender and Doell, 1957). It has been shown to be independent of food intake. It includes the maintenance requirements of the test animal as well as the growth.

CHAPTER 3.

Table 1

Proximate Composition of Danwake Market Samples

*(Mean ± Standard Error)

Sample		% Wet Weight	% Dry Weight				
		Moisture	Ash	Organic Matter	Crude Protein	Pet-Ether Extract	Carbohydrate + Fibre
Samaru	1	30.57 ± 0.06	3.06 ± 0.01	96.94 ± 0.01	8.23 ± 0.05	6.63 ± 0.11	82.08 ± 0.07
	2	30.80 ± 0.05	2.89 ± 0.07	97.11 ± 0.07	5.38 ± 0.08	7.25 ± 0.10	84.48 ± 0.22
	3	30.29 ± 0.05	2.39 ± 0.15	97.61 ± 0.15	9.02 ± 0.12	6.38 ± 0.11	82.21 ± 0.02
Sabo	1	26.82 ± 0.02	2.56 ± 0.13	97.44 ± 0.13	4.64 ± 0.03	3.75 ± 0.09	89.05 ± 0.24
	2	30.14 ± 0.09	2.63 ± 0.09	97.37 ± 0.09	11.85 ± 0.09	6.75 ± 0.08	78.77 ± 0.25
	3	29.30 ± 0.06	1.94 ± 0.08	98.06 ± 0.08	4.94 ± 0.12	8.38 ± 0.08	84.74 ± 0.28
Tundunwada	1	28.20 ± 0.01	3.02 ± 0.06	96.98 ± 0.06	11.64 ± 0.07	3.50 ± 0.10	81.84 ± 0.15
	2	37.36 ± 0.06	2.69 ± 0.05	97.31 ± 0.05	7.36 ± 0.07	22.50 ± 0.10	67.45 ± 0.02
	3	42.21 ± 0.09	2.36 ± 0.02	97.64 ± 0.02	11.64 ± 0.13	12.63 ± 0.06	73.37 ± 0.16

*Mean of triplicate determinations for each nutrient plus the Standard error from the mean.

Table 2

Proximate Composition of Danwake Market Samples
Comparison Within Markets
Danwake

α = 0.05 df = 4 (degree of freedom)

$t_{\alpha/2}$ = -2.7764 $t_{1-\alpha/2}$ = 2.7764

Proximate	Market	Vendor	t - statistic	df	Result	P-value
Lipid	1	1 vs. 2	-4.3266	4	S.	$p < 0.01$
		1 vs. 3	1.6287	4	N.S.	-
		2 vs. 3	5.9143	4	S.	$p < 0.005$
	2	1 vs. 2	-25.9740	4	S.	$p < 0.005$
		1 vs. 3	-39.40	4	S.	$p < 0.005$
		2 vs. 3	-14.7913	4	S.	$p < 0.005$
	3	1 vs. 2	-147.1727	4	S.	$p < 0.005$
		1 vs. 3	-74.40	4	S.	$p < 0.005$
		2 vs. 3	98.4048	4	S.	$p < 0.005$
Protein	1	1 vs. 2	30.8442	4	S.	$p < 0.005$
		1 vs. 3	-6.1622	4	S.	$p < 0.005$
		2 vs. 3	-26.0186	4	S.	$p < 0.005$
	2	1 vs. 2	-78.1148	4	S.	$p < 0.005$
		1 vs. 3	-2.4038	4	N.S.	-
		2 vs. 3	46.5633	4	S.	$p < 0.005$
	3	1 vs. 2	43.3198	4	S.	$p < 0.005$
		1 vs. 3	0.00	4	N.S.	-
		2 vs. 3	29.0169	4	S.	$p < 0.005$
Ash	1	1 vs. 2	1.3912	4	N.S.	-
		1 vs. 3	3.7960	4	S.	$p < 0.01$
		2 vs. 3	3.0637	4	S.	$p < 0.025$
	2	1 vs. 2	-0.4442	4	N.S.	-
		1 vs. 3	3.9871	4	S.	$p < 0.01$
		2 vs. 3	5.6098	4	S.	$p < 0.005$
	3	1 vs. 2	4.2746	4	S.	$p < 0.01$
		1 vs. 3	10.4928	4	S.	$p < 0.005$
		2 vs. 3	6.6398	4	S.	$p < 0.005$
Moisture	1	1 vs. 2	-3.0144	4	S.	$p < 0.025$
		1 vs. 3	3.9052	4	S.	$p < 0.01$
		2 vs. 3	7.2961	4	S.	$p < 0.005$
	2	1 vs. 2	-35.7374	4	S.	$p < 0.005$
		1 vs. 3	-42.3208	4	S.	$p < 0.005$
		2 vs. 3	7.9171	4	S.	$p < 0.005$
	3	1 vs. 2	-162.46	4	S.	$p < 0.005$
		1. vs 3	-155.011	4	S.	$p < 0.005$
		2 vs. 3	-45.8412	4	S.	$p < 0.005$

Table 3

Proximate Composition of Market Samples (Danwake)

Comparison Between Markets

Danwake

Proximate	Market	t - statistic	df	Result	P-value
Lipid	1 vs. 2	0.6637	16	N.S.	-
	1.vs. 3	-2.2292	16	S.	$p < 0.025$
	2 vs. 3	-2.3294	16	S.	$p < 0.025$
Protein	1 vs. 2	0.3072	16	N.S.	-
	1 vs. 3	-2.9513	16	S.	$p < 0.01$
	2 vs. 3	-2.2275	16	S.	$p < 0.025$
Ash	1 vs. 2	2.4179	16	S.	$p < 0.025$
	1 vs. 3	0.5968	16	N.S.	-
	2 vs. 3	-2.0109	16	N.S.	-
Moisture	1 vs. 2	3.5688	16	S.	$p < 0.005$
	1 vs. 3	-2.6089	16	S.	$p < 0.01$
	2 vs. 3	-3.3894	16	S.	$p < 0.005$

S = Significant; N.S. = Not Significant

df = 16; $t_{\alpha/2} = -2.1199$

$t_{1-\alpha/2} = 2.1199$

$\alpha = 0.05$

Market: 1 = Samaru

2 = Sabo

3 = Tundunwada

Table 4

Proximate Composition of Tuwo dawa and Kuka Soup (Market Sample)

*(Mean ± Standard Error)

		% Wet Weight	% Dry Weight				
Sample		Moisture	Ash	Organic Matter	Crude Protein	Pet-Ether	Carbohydrate + Fibre
Samaru	1	87.64 ± 0.002	3.42 ± 0.001	96.58 ± 0.001	14.31 ± 0.13	11.63 ± 0.16	70.64 ± 0.15
	2	87.39 ± 0.004	2.46 ± 0.002	97.54 ± 0.002	13.91 ± 0.09	12.21 ± 0.06	71.42 ± 0.15
	3	87.72 ± 0.004	2.30 ± 0.003	97.70 ± 0.003	11.82 ± 0.006	8.38 ± 0.09	77.50 ± 0.14
Sabo	1	87.85 ± 0.003	3.91 ± 0.003	96.09 ± 0.003	13.48 ± 0.09	16.78 ± 0.06	65.83 ± 0.16
	2	85.61 ± 0.004	2.33 ± 0.002	97.67 ± 0.002	11.88 ± 0.02	4.00 ± 0.12	81.79 ± 0.40
	3	86.23 ± 0.003	2.82 ± 0.003	97.18 ± 0.003	17.62 ± 0.33	15.20 ± 0.05	64.36 ± 0.41
Tundunwada	1	89.97 ± 0.004	5.04 ± 0.003	94.96 ± 0,003	17.77 ± 0.11	14.40 ± 0.08	62.79 + 0.24
	2	87.18 ± 0.003	4.25 ± 0.02	95.75 ± 0.02	15.36 ± 0.16	22.42 ± 0.11	57.97 ± 0.34
	3	85.32 ± 0.005	3.83 ± 0.02	96.17 ± 0.02	14.31 ± 0.31	19.40 ± 0.05	62.46 ± 0.28

*Mean of three determinations for all the nutrients with the exception of Moisture Content where two determinations were carried out plus the Standard error from the mean.

Table 5

Proximate Composition of Market Samples
Comparison Within Markets

Tuwo dawa and Kuka Soup

df = 4, $t_{\alpha/2}$ = -2.7764, $t_{1-\alpha/2}$ = 2.7764 α = 0.05

df = 2, $t_{\alpha/2}$ = -4.3027, $t_{1-\alpha/2}$ = 4.3027

Proximate	Market	Vendor	t - statistic	df	Result	P-value
Lipid	1	1 vs. 2	-3.3066	4	S.	$p < 0.025$
		1 vs. 3	17.223	4	S.	$p < 0.005$
		2 vs. 3	34.8861	4	S.	$p < 0.005$
	2	1 vs. 2	16.0635	4	S.	$p < 0.005$
		1 vs. 3	20.7349	4	S.	$p < 0.005$
		2 vs. 3	-158.4634	4	S.	$p < 0.005$
	3	1 vs. 2	-60.1425	4	S.	$p < 0.005$
		1 vs. 3	-54.8847	4	S.	$p < 0.005$
		2 vs. 3	25.1417	4	S.	$p < 0.005$
Protein	1	1 vs. 2	2.508	4	N.S.	-
		1 vs. 3	19.2492	4	S.	$p < 0.005$
		2 vs. 3	13.8997	4	S.	$p < 0.005$
	2	1 vs. 2	17.7358	4	S.	$p < 0.005$
		1 vs. 3	-12.1683	4	S.	$p < 0.005$
		2 vs. 3	-17.4180	4	S.	$p < 0.005$
	3	1 vs. 2	12.2908	4	S.	$p < 0.005$
		1 vs. 3	10.6237	4	S.	$p < 0.005$
		2 vs. 3	3.0318	4	S.	$p < 0.025$
Ash	1	1 vs. 2	429.911	4	S.	$p \ll 0.005$
		1 vs. 3	353.42	4	S.	$p \ll 0.005$
		2 vs. 3	42.6593	4	S.	$p < 0.005$
	2	1 vs. 2	436.842	4	S.	$p \ll 0.005$
		1 vs. 3	257.3651	4	S.	$p \ll 0.005$
		2 vs. 3	-134.3490	4	S.	$p < 0.005$
	3	1 vs. 2	38.8861	4	S.	$p < 0.005$
		1 vs. 3	61.2354	4	S.	$p < 0.005$
		2 vs. 3	14.9964	4	S.	$p < 0.005$
Moisture	1	1 vs. 2	59.1335	2	S.	$p < 0.005$
		1 vs. 3	-21.3486	2	S.	$p < 0.005$
		2 vs. 3	-62.6522	2	S.	$p < 0.005$
	2	1 vs. 2	515.2558	2	S.	$p < 0.005$
		1 vs. 3	450.1385	2	S.	$p < 0.005$
		2 vs. 3	-142.4639	2	S.	$p < 0.005$
	3	1 vs. 2	386.241	2	S.	$p \ll 0.005$
		1 vs. 3	635.161	2	S.	$p \ll 0.005$
		2 vs. 3	342.1101	2	S.	$p \ll 0.005$

Table 6

Proximate Composition of Market Samples
Comparison Between Markets

Tuwo Dawa/Kuka Soup

df = 16, $t_{\partial/2} = -2.1199$, $t_{1-\partial/2} = 2.1199$, $\partial = 0.05$
df = 10, $t_{\partial/2} = -2.2281$, $t_{1-\partial/2} = 2.2281$.

Proximate	Markets	t - statistics	df	Result	P-value
Lipid	1 vs. 2	-0.2421	16	N.S.	-
	1 vs. 3	-5.4512	16	S.	$p < 0.005$
	2 vs. 3	-0.2723	16	N.S.	-
Protein	1 vs. 2	-0.8204	16	N.S.	-
	1 vs. 3	-2.1316	16	S.	$p < 0.05$
	2 vs. 3	-0.8309	16	N.S.	-
Ash	1 vs. 2	-0.7859	16	N.S.	-
	1 vs. 3	-5.2316	16	S.	$p < 0.005$
	2 vs. 3	-3.6611	16	S.	$p < 0.005$
Moisture	1 vs. 2	2.3809	10	S.	$p < 0.05$
	1 vs. 3	0.6372	10	N.S.	-
	2 vs. 3	-0.7513	10	N.S.	-

S = Significant N.S. = Not Significant

Markets
1 = Samaru
2 = Sabo
3 = Tundunwada

Table 7

Proximate Composition of Koko and Kosai (Market Samples)

*(Mean ± Standard Error)

		% Wet Weight	% Dry Weight				
Sample		Moisture	Ash	Organic Matter	Crude Protein	Pet-Ether	Carbohydrate + Fibre
Samaru	1	90.15 ± 0.16	2.79 ± 0.06	97.21 ± 0.06	14.01 ± 0.01	21.50 ± 0.07	61.70 ± 0.13
	2	89.64 ± 0.10	2.80 ± 0.08	97.20 ± 0.08	14.88 ± 0.07	14.42 ± 0.05	67.90 ± 0.16
	3	90.10 ± 0.54	3.50 ± 0.07	96.50 ± 0.07	12.52 ± 0.05	18.42 ± 0.06	65.56 ± 0.10
Sabo	1	74.34 ± 0.20	2.13 ± 0.001	97.87 ± 0.001	12.26 ± 0.02	33.10 ± 0.12	52.51 ± 0.14
	2	84.71 ± 0.10	2.43 ± 0.002	97.57 ± 0.002	14.27 ± 0.05	29.80 ± 0.08	53.50 ± 0.20
	3	89.20 ± 0.41	3.03 ± 0.001	96.97 ± 0.001	11.12 ± 0.06	19.60 ± 0.13	66.25 ± 0.10
Tundunwada	1	82.69 ± 0.203	3.20 ± 0.003	96.80 ± 0.003	14.88 ± 0.05	18.99 ± 0.11	62.93 ± 0.09
	2	89.42 ± 0.11	2.99 ± 0.001	97.01 ± 0.001	13.83 ± 0.09	20.91 ± 0.01	62.27 ± 0.13
	3	83.53 ± 0.11	3.13 ± 0.003	96.87 ± 0.003	14.53 ± 0.14	21.99 ± 0.004	60.35 ± 0.31

*Mean of triplicate determinations for each nutrient with the exception of Moisture where two determinations were carried out, plus the Standard error from the mean.

Table 8

Proximate Composition of Market Samples
Comparison Within Markets

Koko/Kosai

Proximate	Market	Vendor	t - statistic	df	Result	P-value
Lipid	1	1 vs. 2	80.2605	4	S.	$p < 0.005$
		1 vs. 3	33.7524	4	S.	$p < 0.005$
		2 vs. 3	-51.216	4	S.	$p < 0.005$
	2	1 vs. 2	23.9695	4	S.	$p < 0.005$
		1 vs. 3	79.2895	4	S.	$p < 0.005$
		2 vs. 3	69.8152	4	S.	$p < 0.005$
	3	1 vs. 2	-17.2172	4	S.	$p < 0.005$
		1 vs. 3	-27.0297	4	S.	$p < 0.005$
		2 vs. 3	-118.3206	4	S.	$p < 0.005$
Protein	1	1 vs. 2	-13.2375	4	S.	$p < 0.005$
		1 vs. 3	28.8007	4	S.	$p < 0.005$
		2 vs. 3	28.3114	4	S.	$p < 0.005$
	2	1 vs. 2	-36.467	4	S.	$p < 0.005$
		1 vs. 3	19.0619	4	S.	$p < 0.005$
		2 vs. 3	42.0694	4	S.	$p < 0.005$
	3	1 vs. 2	9.9338	4	S.	$p < 0.005$
		1 vs. 3	2.2786	4	N.S.	-
		2 vs. 3	-4.0721	4	S.	$p < 0.01$
Ash	1	1 vs. 2	-0.1436	4	N.S.	-
		1 vs. 3	-7.3595	4	S.	$p < 0.005$
		2 vs. 3	-6.5019	4	S.	$p < 0.005$
	2	1 vs. 2	-141.6667	4	S.	$p < 0.005$
		1 vs. 3	-570.2532	4	S.	$p \ll 0.005$
		2 vs. 3	-241.40	4	S.	$p < 0.005$
	3	1 vs. 2	64.8734	4	S.	$p < 0.005$
		1 vs. 3	16.4978	4	S.	$p < 0.005$
		2 vs. 3	-42.7215	4	S.	$p < 0.005$
Moisture	1	1 vs. 2	5.1131	2	S.	$p < 0.025$
		1 vs. 3	0.0852	2	N.S.	-
		2 vs. 3	-0.8314	2	N.S.	-
	2	1 vs. 2	-46.8513	2	S.	$p < 0.005$
		1 vs. 3	-32.5828	2	S.	$p < 0.005$
		2 vs. 3	-10.6147	2	S.	$p < 0.005$
	3	1 vs. 2	-63.4166	2	S.	$p < 0.005$
		1 vs. 3	-7.8705	2	S.	$p < 0.01$
		2 vs. 3	39.1849	2	S.	$p < 0.005$

S = Significant N.S. = Not Significant

Market	df	$t_{\alpha/2}$	$t_{1-\alpha/2}$	$\alpha = 0.05$
1 = Samaru	4	-2.7764	2.7764	
2 = Sabo	2	-4.3027	4.3027	
3 = Tundunwada				

Table 9

Proximate Composition of Market Samples
Comparison Between Markets

Koko and Kosai

Proximate	Markets	t - statistics	df	Result	P-value
Lipid	1 vs. 2	-3.3899	16	S.	p 0.005
	1 vs. 3	-2.2607	16	S.	p 0.01
	2 vs. 3	3.3012	16	S.	p. 0.005
Protein	1 vs. 2	2.1762	16	S.	p 0.025
	1 vs. 3	-1.603	16	N.S.	-
	2 vs. 3	-3.8284	16	S.	p 0.005
Ash	1 vs. 2	2.4098	16	S.	p 0.025
	1 vs. 3	-0.5935	16	N.S.	-
	2 vs. 3	-3.3566	16	S.	p 0.005
Moisture	1 vs. 2	2.5847	10	S.	p 0.025
	1 vs. 3	3.5129	10	S.	p 0.005
	2 vs. 3	-0.7991	10	N.S.	-

S = Significant N.S. = Not Significant

Market	df	$t\alpha/2$	$t_{q-\alpha/2}$	$\alpha = 0.05$
1 = Samaru	16	-2.1199	2.1199	
2 = Sabo	10	-2.2281	2.2281	
3 = Tundunwada				

Table 10(a)

Mineral Status of Danwake (Market Sample) - Minor Elements

(Mean ± Standard Error)

Sample		mg/g Dry Weight of Sample				
Market	Vendor	Sodium	Manganese	Iron	Copper	Zinc
Samaru	1	1.950 ± 0.084	0.013 ± 0.0012	0.187 ± 0.014	0.008 ± 0.002	0.058 ± 0.006
	2	2.870 ± 0.00*	0.014 ± 0.0005	0.197 ± 0.001	0.021 ± 0.001	0.067 ± 0.001
	3	2.990 ± 0.167	0.015 ± 0.0019	0.212 ± 0.008	0.010 ±0.00014	0.094 ± 0.005
Sabo	1	2.150 ± 0.117	0.011 ± 0.0004	0.128 ± 0.062	0.007 ± 0.00*	0.055 ± 0.00*
	2	2.600 ± 0.200	0.021 ± 0.0014	0.191 ± 0.005	0.023 ± 0.007	0.072 ± 0.007
	3	2.070 ± 0.002	0.010 ± 0.0005	0.207 ± 0.00*	0.007 ± 0.0001	0.134 ± 0.00*
Tundunwada	1	2.900 ± 0.100	0.021 ± 0.0004	0.187 ± 0.002	0.019 ± 0.00*	0.075 ± 0.001
	2	2.550 ± 0.050	0.014 ± 0.0007	0.050 ± 0.017	0.0005± 0.0002	0.047 ± 0.0007
	3	2.365 ± 0.065	0.016 ± 0.0008	0.125 ± 0.060	0.017 ± 0.00*	0.074 ± 0.0043

* No variation in the determinations.

Mean of two separately digested Samples for each meal.

Table 10(b)

Mineral Status of Danwake (Market Sample)- Major Elements

(Mean ± Standard Error)

Sample		mg/g Dry Weight of Sample		
Market	Vendor	Potassium	Calcium	Magnessium
Samaru	1	1.350 ± 0.050	0.783 ± 0.083	1.015 ± 0.015
	2	0.630 ± 0.100	1.132 ± 0.029	1.025 ± 0.015
	3	0.830 ± 0.100	1.433 ± 0.167	1.233 ± 0.067
Sabo	1	1.250 ± 0.184	1.400 ± 0.200	0.865 ± 0.035
	2	3.850 ± 0.170	1.815 ± 0.215	1.350 ± 0.050
	3	1.770 ± 0.100	1.732 ± 0.185	0.900 ± 0.100
Tundunwada	1	3.200 ± 0.100	1.900 ± 0.010	1.467 ± 0.101
	2	0.520 ± 0.170	1.515 ± 0.085	1.117 ± 0.017
	3	2.130 ± 0.033	0.730 ± 0.00*	1.250 ± 0.050

* No variation in the determinations.

Mean of two separately digested Samples for each meal.

Table 11(a)

Mineral Status of Tuwo dawa and Kuka Soup (Market Samples) - Minor Elements

(Mean ± Standard Error)

Sample		mg/g Dry Weight of Sample				
		Sodium	Manganese	Iron	Copper	Zinc
Samaru	1	1.859 ± 0.0002	0.008 ± 0.0002	0.007 ± 0.0002	0.008 ± 0.0002	0.061 ± 0.001
	2	3.033 ± 0.0	0.017 ± 0.0	0.043 ± 0.0	0.017 ± 0.0	0.086 ± 0.0
	3	3.093 ± 0.0002	0.018 ± 0.0002	0.047 ± 0.0003	0.005 ± 0.0	0.068 ± 0.006
Sabo	1	6.984 ± 0.0002	0.017 ± 0.0	0.030 ± 0.0	0.006 ± 0.0001	0.069 ± 0.0
	2	2.960 ± 0.0	0.014 ± 0.0002	0.100 ± 0.0002	0.007 ± 0.0	0.065 ± 0.003
	3	3.129 ± 0.0002	0.013 ± 0.0	0.060 ± 0.0002	0.009 ± 0.0001	0.096 ± 0.0
Tundunwada	1	9.323 ± 0.002	0.018 ± 0.0002	0.033 ± 0.0	0.001 ± 0.0	0.134 ± 0.002
	2	7.643 ± 0.003	0.018 ± 0.0001	0.050 ± 0.0002	0.016 ± 0.0001	0.085 ± 0.003
	3	7.316 ± 0.002	0.017 ± 0.0	0.033 ± 0.0	0.007 ± 0.0001	0.081 ± 0.001

Mean of two separately digested Samples for each meal, plus standard error from the mean.

Table 11(b)

Mineral Status of Tuwo dawa and Kuka Soup (Market Samples)- Major Elements

(Mean ± Standard Error)

Sample		mg/g Dry Weight of Sample		
		Potassium	Calcium	Magnessium
Samaru	1	2.490 ± 0.010	0.698 ± 0.0005	0.963 ± 0.010
	2	3.470 ± 0.010	1.330 ± 0.0	1.466 ± 0.033
	3	3.460 ± 0.0	0.766 ± 0.100	1.331 ± 0.001
Sabo	1	3.660 ± 0.110	1.360 ± 0.0	1.463 ± 0.002
	2	2.060 ± 0.0	0.897 ± 0.002	1.363 ± 0.0
	3	3.940 ± 0.020	1.330 ± 0.0	1.331 ± 0.100
Tundunwada	1	4.225 ± 0.015	1.532 ± 0.002	1.232 ± 0.0
	2	3.760 ± 0.0	1.760 ± 0.0	1.264 ± 0.001
	3	3.860 ± 0.02	1.395 ± 0.015	1.264 ± 0.002

Mean of two separately digested Samples for each meal, plus Standard Error from the mean.

Table 12(a)

Mineral Status of Koko and Kosai (Market Samples) - Minor Elements

(Mean ± Standard Error)

		mg/g Dry Weight of Sample				
Sample		Sodium	Manganese	Iron	Copper	Zinc
Samaru	1	2.666 ± 0.111	0.014 + 0.0	0.026 ± 0.0003	0.009 ± 0.0	0.061 ± 0.001
	2	3.021 ± 0.02	0.017 ± 0.003	0.023 ± 0.0001	0.007 ± 0.0001	0.086 ± 0.0
	3	4.723 ± 0.0	0.011 ± 0.0001	0.018 ± 0.0	0.010 ± 0.00004	0.068 ± 0.006
Sabo	1	2.914 ± 0.013	0.010 ± 0.0001	0.020 ± 0.0	0.009 ± 0.0	0.069 ± 0.0
	2	2.196 ± 0.002	0.013 ± 0.0	0.013 ± 0.002	0.006 ± 0.0	0.065 ± 0.003
	3	3.181 ± 0.101	0.012 ± 0.001	0.020 ± 0.0	0.006 ± 0.0	0.096 ± 0.0
Tundunwada	1	3.545 ± 0.010	0.015 ± 0.0	0.019 ± 0.001	0.013 ± 0.00001	0.134 ± 0.002
	2	3.355 ± 0.032	0.012 ± 0.0002	0.030 ± 0.002	0.077 ± 0.010	0.085 ± 0.003
	3	3.498 ± 0.001	0.014 ± 0.0	0.020 ± 0.0	0.014 ± 0.0	0.081 ± 0.001

Mean of two separately digested Samples for each meal.

Table 12(b)

Mineral Status of Koko and Kosai (Market Samples) - Major Elements

(Mean ± Standard Error)

Sample		mg/g Dry Weight of Sample		
		Potassium	Calcium	Magnessium
Samaru	1	4.440 ± 0.220	0.658 ± 0.003	0.922 ± 0.002
	2	4.555 ± 0.045	0.797 ± 0.002	0.997 ± 0.002
	3	3.420 ± 0.0	0.619 ± 0.0	0.708 ± 0.008
Sabo	1	2.375 ± 0.295	0.827 ± 0.005	0.795 ± 0.110
	2	3.260 ± 0.0	0.732 ± 0.100	0.998 ± 0.001
	3	3.027 ± 0.007	1.027 ± 0.007	0.927 ± 0.012
Tundunwada	1	3.020 ± 0.020	1.060 ± 0.010	0.995 ± 0.001
	2	3.390 ± 0.030	0.798 ± 0.001	0.865 ± 0.021
	3	5.06 ± 0.0	0.766 ± 0.0	1.033 ± 0.0

Mean of two separately digested Samples for each meal.

Table 13

Comparison of the Mineral Composition of the 3 different meals
Result of the analysis of variance test

A. Within the same market*

S = Significant N.S. = Not Significant

	Danwake			Tuwo dawa/Kuka Soup			Koko/Kosai		
Mineral	F-Ratio	Result	P-value	F-Ratio	Result	P-value	F-Ratio	Result	P-value
Ca	1.774	N.S.	-	40.00	S.	$p \ll 0.01$	2.867	N.S.	-
K.	12.176	S.	$p < 0.01$	219.29	S.	$p \ll 0.01$	17.143	S.	$p \ll 0.01$
Mg	0.420	N.S.	-	12.85	S.	$p \ll 0.01$	2.411	N.S.	-
Na	7.543	S.	$p < 0.05$	**			321.91	S.	$p \ll 0.01$
Fe	0.106	N.S.	-	686.49	S.	$p \ll 0.01$	11.67	S.	$p \ll 0.01$
Mn	5.000	N.S.	$p < 0.05$	0.020	N.S.	-	1.25	N.S.	-
Cu	4.960	S.	$p < 0.05$	206.67	S.	$p \ll 0.01$	59.154	S.	$p \ll 0.01$
Zn	183.750	S.	$p \ll 0.01$	11.22	S.	$p \ll 0.01$	**		

Notes:

1. The level of significance for all tests was set at $\alpha = 0.05$
2. For all tests the tabled value of the F-statistic is $F = 4.26$
3. The degrees of freedom of the F-statistic were 2 and 9 for the numerator and denominator mean squares respectively.
4. *Due to the small number of observations (i.e. two) per vendor within each market, the analysis of variance test was applied to see if there is any significant difference between vendors across all three markets.
5. **Na from Tuwo dawa/Kuka soup and Zn from Koko/Kosai have equal total and subtotal sums of squares. Consequently, the within groups sum of squares equals zero thus making the analysis of variance test unapplicable. A pairwise comparison of these elements was carried out using the t-test.

Table 13. Continued

Mineral	Food Item	Vendors	t-Statistic	df	Result	P-value
Na	Tuwo dawa/ Kuka Soup	1 vs. 2	0.8866	10	N.S	-
		1 vs. 3	0.9339	10	N.S.	-
		2 vs. 3	0.0240	10	N.S.	-
Zn	Koko/Kosai	1 vs. 2	1.645	10	N.S.	-
		1 vs. 3	0.6262	10	N.S.	-
		2 vs. 3	-2.4240	10	S.	$p < 0.025$

$\alpha = 0.05$

$t_{\alpha/2} = -2.2281$

$t_{1-\alpha/2} = 2.2281$

Table 14

Comparison of Mineral Elements of the 3 meals

B. Between the three markets

	Danwake			Tuwo dawa/Kuka Soup			Koko/Kosai		
Mineral	F-Ratio	Result	P-value	F-Ratio	Result	P-value	F-Ratio	Result	P-value
Ca.	13.774	S.	$p < 0.01$	44.600	S.	$p \ll 0.01$	21.990	S.	$p < 0.01$
K.	174.588	S.	$p \ll 0.01$	64.291	S.	$p \ll 0.01$	82.575	S.	$p \ll 0.01$
Mg.	1.357	N.S.	-	14.004	S.	$p < 0.01$	4.445	S.	$p < 0.05$
Na.	9.650	S.	$p < 0.01$	**			212.850	S.	$p \ll 0.01$
Fe	0.5389	N.S.	-	432.43	S.	$p \ll 0.01$	43.300	S.	$p \ll 0.01$
Mn.	15.000	S.	$p < 0.01$	0.0473	N.S.	-	5.800	S.	$p < 0.05$
Cu.	0.3923	N.S.	-	99.000	S.	$p \ll 0.01$	264.620	S.	$p \ll 0.01$
Zn.	453.125	S.	$p \ll 0.01$	140.306	S.	$p \ll 0.01$	**		

S = Significant N.S. = Not Significant

Notes:
1. The level of significance for all tests was set at $\alpha = 0.05$
2. For all tests the tabled value of the F-Statistic is $F = 4.26$
3. The degrees of freedom of the F-Statistic were 2 and 9 for the numerator and denominator mean squares respectively.

** Na from Tuwo dawa/Kuka Soup and Zn from Koko/Kosai have equal total and subtotal sums of squares. Consequently, the within groups sum of squares equals zero thus making the analysis of variance test unapplicable. A pairwise comparison of these elements was carried our using the t-test.

Table 14: Continued

Mineral	Food Item	Markets	t-Statistic	df	Result	P-value
Na	Tuwo dawa/ Kuka Soup	1 vs. 2	-1.951	10	N.S.	-
		1 vs. 3	-11.600	10	S.	$p < 0.005$
		2 vs. 3	-4.06	10	S.	$p < 0.005$
Zn	Koko/Kosai	1 vs. 2	2.33	10	S.	$p < 0.05$
		1 vs. 3	1.833	10	N.S.	-
		2 vs. 3	-1.225	10	N.S.	-

$\alpha = 0.05$

$t_{\alpha/2} = -2.2281$

$t_{1-\alpha/2} = 2.2281$

Table 15

Proximate Composition of Standard Meals (Raw-Mixed and Cooked Forms)

(Mean ± Standard Error)

Sample	% Wet Weight	% Dry Weight					
	Moisture	Ash	Organic Matter	Crude Protein	Pet-Ether Extract	Carbohydrate + Fibre	Fibre
Danwake:							
Raw	50.73 ±0.02	3.77 ± 0.04	96.23 ± 0.04	8.43 ± 0.13	21.17 ± 0.08	66.63 ± 0.23	3.11 ± 0.01
Cooked	74.95 ± 0.32	3.57 ± 0.01	96.43 ± 0.01	8.02 ± 0.08	20.00 ± 0.12	68.41 ± 0.18	2.67 ± 0.03
Cooking water	96.20 ± 0.12	4.86 ± 0.03	95.14 ± 0.03	9.67 ± 0.58	9.5 ± 0.06	75.97 ± 0.56	1.50 ± 0.05
Tuwo dawa and Kuka Soup:							
Raw	64.48 ± 0.08	3.96 ± 0.02	96.04 ± 0.02	11.35 ± 0.03	22.00 ± 0.06	62.69 ± 0.06	0.29 ± 0.01
Cooked	87.16 ± 0.28	3.98 ± 0.04	96.02 ± 0.04	11.14 ± 0.45	20.98 ± 0.02	63.90 ± 0.37	0.21 ± 0.01
Koko and Kosai:							
Raw	66.68 ± 0.04	1.89 ± 0.001	98.11 ± 0.001	11.17 ± 0.19	45.37 ± 0.17	41.57 ± 0.13	0.74 ± 0.01
Cooked	70.08 ± 0.02	1.88 ± 0.002	98.12 ± 0.002	10.46 ± 0.15	43.00 ± 0.10	44.66 ± 0.23	0.68 ± 0.01

Mean of three determinations for each nutrient (for each Sample)

Table 16

Proximate Composition of Standard Meals
Paired Comparisons in the Raw and Cooked Forms

Food Item	Proximate	t-Statistic	df	Result	P-value
Danwake	Lipid	-11.5842	2	S.	$p<0.005$
	Protein	-2.5247	2	N.S.	-
	Ash	-4.1685	2	S.	$p< 0.05$
	Moisture	80.7667	2	N.S.	-
	Fibre	-11.2564	2	S.	$p< 0.005$
Tuwo dawa/ Kuka Soup	Lipid	13.9315	2	N.S.	-
	Protein	-0.4602	2	N.S.	-
	Ash	0.5052	2	N.S.	-
	Moisture	63.00	2	N.S.	-
	Fibre	-4.00	2	S.	$p<0.05$
Koko/Kosai	Lipid	-8.778	2	S.	$p< 0.01$
	Protein	-3.1605	2	S.	$p<0.05$
	Ash	-9.000	2	S.	$p< 0.01$
	Moisture	61.9091	2	N.S.	-
	Fibre	*	2	-	-

* Sd = 0; No variation. It is safe to assume that there is no change in fibre content.

S = Significant; N.S. = Not Significant.

df = 1, $t\alpha/2 = -6.3138$; df = 2, $t\alpha/2 = -2.92$

$\alpha = 0.05$.

Table 17

Vitamin Content of 'Standard Meals'

Raw-mixed and Cooked Forms

**(Mean ± Standard Errors)

Meals-Sample	Per 100g Dry Weight			
	Vitamin B_1 (mg)	Vitamin B_2 (mg)	Vitamin C (mg)	Carotene (mg)
Danwake:				
Raw	6.24 ± 0.01	0.53 ± 0.01	Trace	108.00 ± 1.00
Cooked	5.49 ± 0.06	0.35 ± 0.03	Trace	79.00 ± 2.00
Cooking water	5.28 ± 0.05	Trace	N.D.	71.00 ± 1.00
Tuwo dawa and Kuka Soup:				
Raw	4.37 ± 0.12	0.60 ± 0.01	*33.00 ± 0.00	1836.00 ± 10.00
Cooked	4.08 ± 0.02	0.53 ± 0.002	*33.00 ± 0.001	1639.50 ± 2.50
Koko and Kosai:				
Raw	6.11 ± 0.11	1.99 ± 0.01	10.50 ± 0.50	130.00 ± 5.00
Cooked	4.89 ± 0.12	1.08 ± 0.075	6.60 ± 0.001	117.50 ± 2.50

* No variations in the determinations.

** Mean of three determinations for each Vitamin (for each meal).

N.D = Not detected

Table 18

Vitamin Content of 'Standard Meals'

Paired Comparison in the Raw and Cooked Forms

Food Item	Vitamin	t-Statistics	df	Result	P-value
Danwake	B_1	-10.7857	2	S.	$p < 0.005$
	B_2	-4.50	2	S.	$p < 0.025$
	C*	-	-	-	-
	Carotene	-9.6667	2	S.	$p < 0.01$
Tuwo dawa and Kuka Soup	B_1	-2.9	2	N.S.	-
	B_2	-6.7	2	S.	$p < 0.025$
	C**	-	2	-	-
	Carotene	-15.72	2	S.	$p < 0.005$
Koko and Kosai	B_1	-122.0	2	S.	$p \ll 0.005$
	B_2	-10.7647	2	S.	$p < 0.005$
	C	-7.8	2	S.	$p < 0.01$
	Carotene	-5.0	2	S.	$p < 0.025$

* The amount observed is in trace form or sometimes not detectable at all.

** Sd = 0; No variation. It is safe to assume that there is no change in Vitamin C content of Tuwo dawa and Kuka Soup before and after cooking.

S = Significant; N.S. = Not Significant

$\alpha = 0.05$; df = degree of freedom
df = 2, $t_{\alpha/2} = -2.92$.

Table 19(a)

Mineral Status of the Standard Meals (Raw-Mixed and Cooked forms) - Minor Elements

*(Mean ± Standard Error)

Sample	mg/g Dry Weight of Sample			
	Sodium	Iron	Copper	Zinc
1. Danwake:				
Raw	5.46 ± 0.04	0.49 ± 0.01	0.01 ± 0.001	0.67 ± 0.002
Cooked	5.04 ± 0.03	0.16 ± 0.013	0.005 ± 0.0004	0.62 ± 0.001
Cooking water	4.90 ± 0.05	1.64 ± 0.12	0.03 ± 0.004	0.30 ± 0.02
2. Tuwo dawa and Kuka Soup:				
Raw	6.50 ± 0.05	1.18 ± 0.0	0.01 ± 0.001	0.04 ± 0.002
Cooked	6.44 ± 0.06	1.17 ± 0.005	0.006 ± 0.002	0.05 ± 0.002
3. Koko and Kosai:				
Raw	3.92 ± 0.03	0.15 ± 0.003	0.01 ± 0.002	0.12 ± 0.001
Cooked	3.92 ± 0.04	0.14 ± 0.0	0.01 ± 0.004	0.09 ± 0.003

Table 19(b)

Mineral Status of Standard Meals (Raw and Cooked Forms) - Major Elements

*(Mean ± Standard Error)

mg/g Dry Weight of Sample

Sample	Potassium	Calcium	Magnessium	Phosphorus
1. Danwake:				
Raw	5.50 ± 0.10	1.06 ± 0.005	1.20 ± 0.09	6.08 ± 0.01
Cooked	4.76 ± 0.07	1.05 ± 0.01	1.12 ± 0.001	5.25 ± 0.005
Cooking water	13.40 ± 0.07	1.42 ± 0.01	1.70 ± 0.004	7.25 ± 0.10
2. Tuwo dawa and Kuka Soup:				
Raw	3.36 ± 0.02	1.04 ± 0.01	1.05 ± 0.01	5.25 ± 0.05
Cooked	3.36 ± 0.10	0.98 ± 0.006	1.01 ± 0.007	4.75 ± 0.03
3. Koko and Kosai:				
Raw	2.04 ± 0.01	0.46 ± 0.04	0.80 ± 0.006	4.42 + 0.01
Cooked	1.57 ± 0.02	0.39 ± 0.007	0.71 ± 0.04	3.83 ± 0.01

*Mean of two separately digested Samples for each meal.

Table 20

Mineral Status of the Standard Meals

Paired Comparison in the Raw and Cooked Forms

Mineral	Danwake			Tuwo dawa and Kuka Soup			Koko and Kosai		
	t-Statistics	Result	P-value	t-Statistics	Result	P-value	t-Statistics	Result	P-value
Na	-42.00	S.	$p < 0.01$	-0.7692	N.S.	-	0.00	N.S.	-
K	-47.742	S.	$p < 0.01$	0.250	N.S.	-	-55.255	S.	$p < 0.01$
Mg	-1.00	N.S.	-	-88.367	S.	$p < 0.005$	-4.8857	N.S.	-
Cu	-0.00	N.S.	-	-1.0033	N.S.	-	-0.7072	N.S.	-
Fe	-132.6	S.	$p \ll 0.005$	-3.000	N.S.	-	-18.0796	S.	$p < 0.025$
Zn	-80.0	S.	$p \ll 0.005$	1.095	N.S.	-	-23.261	S.	$p < 0.025$
Ca	-0.243	N.S.	-	-10.646	S.	$p < 0.05$	-0.7835	N.S.	-
P	-20.29	S	$p < 0.025$	-6.25	N.S.	-	-194.33	S.	$p \ll 0.005$

S = Significant N.S. = Not Significant

Degree of freedom in each case = 1

$t_{\alpha/2} = -6.3138$

Table 21(a)

Amino Acid Composition of the Standard Meals in mg/g of Food Sample (Mean of 2 determinations)

Amino Acids	Raw Danwake	Cooked Danwake	Raw Tuwo dawa and Kuka Soup	Cooked Tuwo dawa and Kuka Soup	Raw Koko and Kosai	Cooked Koko and Kosai
Aspartic acid	9.33	6.92	11.90	9.17	10.25	9.17
Serine	3.56	2.69	3.50	4.38	5.25	4.56
Glutamic acid	13.00	11.00	19.18	17.09	18.91	17.91
Proline	2.43	3.14	5.86	6.14	4.07	4.14
Glycine	1.32	1.44	5.20	3.56	2.88	2.76
Alanine	3.15	2.85	6.00	5.50	3.75	3.80
Histidine	1.35	1.29	1.87	1.77	2.87	2.71
Arginine	2.47	1.75	4.31	4.19	6.81	6.58
Threonine	3.21	2.21	4.79	4.00	7.21	7.07
*Cysteine (half)	0.00	0.00	1.07	Trace	0.00	0.00
*Methionine	0.45	0.18	1.57	1.57	1.09	1.00
Valine	8.07	7.86	15.00	14.29	6.25	5.46
Isoleucine	4.33	3.58	4.42	4.30	4.17	4.25
Leucine	6.00	5.42	10.10	9.80	12.17	10.92
Tyrosine	3.40	2.50	2.50	2.40	8.10	7.10
Phenylalanine	3.60	3.00	4.00	4.50	6.20	5.10
**Tryptophan	0.75	0.65	0.87	0.75	0.93	0.84
Lysine	6.82	6.35	2.77	2.73	4.50	3.77
Organic Nitrogen (mg) content in the food Sample.	13.50	12.84	18.16	17.82	17.57	16.74
Calculated N (mg) from recovered amino acid.	10.82	9.33	15.64	14.33	15.92	14.77
% recovery	80.15	72.66	86.12	80.42	90.61	88.23

* partial destruction by acid hydrolysis. ** determined chemically using ferric chloride reagent.

Table 21(b)

Essential Amino Acid Composition (mg/g N) of Standard Meals
Comparison with FAO/WHO Provisional Pattern (1973)

	Isoleucine	Leucine	Lysine	Methionine + Cysteine	Phenylalanine + Tyrosine	Threonine	Typtophan	Valine	Limiting amino acid	% Protein Score
FAO/WHO Provisional Pattern (1973)	250	440	340	220	380	250	60	310		100
Raw Danwake	321	444	595	33	518	238	56	598	Met + Cys.	15
Cooked Danwake	279	422	495	14	428	176	51	612	Met + Cys.	6.4
Raw Tuwo dawa and Kuka Soup	243	556	153	86	358	264	48	826	Met + Cys. Lysine	39.09 45.00
Cooked Tuwo dawa and Kuka Soup.	241	550	153	88	359	224	42	802	Met + Cys. Lysine	40.05 45.00
Raw Koko and Kosai	237	693	256	62	814	410	53	356	Met + Cys. Lysine	28.2 75.3
Cooked Koko and Kosai	254	652	225	60	729	422	50	326	Met + Cys. Lysine	27.3 66.2

Table 22

*Food Intake, gain/loss in Weight, Protein intake of albino rats fed on the different Meals (both raw and cooked and control diets)

Meals		Nitrogen % in the diet	Food Intake (g)	Protein Intake (g)	Gain/loss in Weight (g)	Energy Cals.
Danwake:-	Raw	1.35	30.89	2.60	-6.22	147.74
	Cooked	1.28	42.83	3.43	0.97	203.85
Tuwo dawa and Kuka Soup:-	Raw	1.82	43.02	3.86	0.20	207.94
	Cooked	1.78	55.79	6.22	9.62	272.30
Koko and Kosai:-	Raw	1.76	27.96	3.12	-7.25	172.28
	Cooked	1.67	39.48	4.13	4.00	238.78
Protein free diet		0.00	43.14	-	-17.90	N.C.
Balanced diet		3.36	92.01	19.32	56.99	373.56

- Sign denotes loss in weight.

* Average of two rats over a period of 10 days

N.C. = Not calculated.

Table 23

P.E.R., NPR and P.R.E. and digestibility of the different Meals (both raw and cooked)

Meals		P.E.R.	N.P.R.	P.R.E.	Protein % digestibility	Corrected P.E.R
Danwake:-	Raw	-14.95	4.49	71.84	87.00	-2.39
	Cooked	1.77	5.50	88.00	92.36	0.28
Tuwo dawa and Kuka Soup:-	Raw	0.32	4.69	75.04	89.16	0.05
	Cooked	9.67	4.42	70.72	93.38	1.55
Koko and Kosai:-	Raw	-14.53	3.41	54.56	95.43	-2.32
	Cooked	6.05	5.30	84.80	91.40	0.97
Balanced diet		18.44	3.88	62.08	98.18	2.95

Table 24(a)

Calorie values of the Standard Meals and the Net Protein Calorie %

Sample		Cals./100g dry weight	
		Calorie Value	Net Protein Calorie %
Danwake:-	Raw	478.33	7.00
	Cooked	475.04	6.80
Tuwo dawa and Kuka Soup:-	Raw	493.00	9.20
	Cooked	487.50	9.10
Koko and Kosai:-	Raw	616.33	7.20
	Cooked	604.76	6.90

Table 24(b)

Calorie Values of the Actual Intake of the Cooked Standard Meals (per day)

Sample	Dry Weight (g)	Calorie Supply (Cals.)
Cooked Danwake	105.2	499.76
Cooked Tuwo dawa and Kuka Soup.	64.2	313.39
Cooked Koko and Kosai	164.56	995.16

Total Calorie intake = 1,808.

4.2 Mineral Status of Food and Diet Brought from the Family.

Tables 10 to 12 show the mineral element [illegible],
It is observed that the sodium is rich in all [illegible]
content of sodium could be easily due to the [illegible] salt
added during [illegible] mastication.

Potassium, was the dietary [illegible] existing [illegible] was
also over 1mg/day on average in all the meals. [illegible]
among of calcium and magnesium in each of the meals [illegible] on
by their comparable functions in biological systems.

[illegible] elements or trace elements [illegible], manganese
and zinc are present in trace amounts (Tables 10 - 12).

Recommended daily intake of calcium and iron [illegible]
and women respectively. (F.A.O. [illegible] for man and women.

Judging from the results shown in Tables 10 to 12, it is
observed that the daily intake of 430g of [illegible] boiled
rice and boka noun, and 450g boka and kosai (wet vegetable containing
92%, 84.2% and 83.1% (dry matter) respectively, the daily average
38 mg of iron and 320 mg calcium while two days and [illegible] provides
2.56mg of iron and 43 mg of calcium, and koko and kosai provides
3.16 mg of iron and 51 mg of calcium, the total [illegible] of
43.8mg Ca. From these results it can confidently [illegible] that
eater per day by an [illegible] can easily supply the necessary [illegible]
mineral element requirements.

small due to the high water content. The presence of ascorbic acid in the cooking water may be due to the leaching out of water soluble proteins into the water.

The crude protein in tuwo dawa and kuka soup and koko and kosai are fairly high; being 11.14 and 11.35% for cooked and raw forms of tuwo dawa and kuka soup and 10.46 and 11.17% for cooked and raw forms of koko and kosai. These results show that tuwo dawa and kuka soup and koko and kosai is richer in crude protein than sorghum. The lower result in the crude protein of cooked [illegible] may be due to [illegible]

of carotene present in tuwo dawa and kuka soup may be due to the amount of palm oil used in the preparation of the soup. Palm oil has been shown to be a good source of carotene.

All animal and plant tissues contain tocopherol and it is therefore present in all whole natural foods, but cereals, nuts, peas, beans and other pulses in addition yeast are particularly rich sources (Davidson et al, 1975). All the foods studied contain one or more of these.

these mineral elements into the cooking oil. Therefore, it would be necessary to take the cooking water used in preparing the danwake as soup and to use the oil (used in the frying of kosai) to make stew for other meals; by so doing most of the nutrients lost in them can still be consumed.

It is observed that the Ca: P ratios for the meals are as follows:-

(a) For danwake it is 1:6, 1:5 for cooked and raw forms respectively.

(b) For tuwo dawa and kuka soup is 1:5 for both. While it is 1:9 for both in the case of koko and kosai.

The satisfactory ratio is 1:2 (Oke 1965b).

Since the danwake is not taken along with other food items at a meal, the phosphorus intake will not be augmented at that meal. However, danwake is not the only food consumed per day and with adequate supply of Vitamin D, produced by sunlight, this ratio may not lead to rickets or osteomalacia and accentuate the deformities of the bones.

Considering the intake of each meal (as discussed earlier on, under proximate composition of standard meals); the intake of danwake is 105.2g (dry weight) and this provides 110.47mg of Ca, and 16.8mg of Fe. Similarly the daily intake of tuwo dawa and kuka soup provides 62.9mg Ca and 75mg Fe. For koko and kosai the intake is 64.78mg Ca, and 23mg Fe. Therefore the total daily intake supplied

by the three meals is 238.15mg Ca and 114.8mg Fe.

From the foregoing results it can be concluded that all the meals cannot supply the daily requirement of 0.4 - 0.5 g of calcium but can supply up to 28mg of iron for both adult man and woman.

4:6 Losses of Nutrient during preparation and cooking of the meals.

Tables 16, 18, 20 show the statistical analysis of the effect of cooking on the nutrient status of the standard meals.

4:6:1 Effect of Cooking on the Proximate Composition.

There is no significant difference between the raw-mixed and cooked forms of tuwo dawa and kuka soup when their proximate composition are compared (see Table 16). For danwake, variation is observed between the raw and the cooked forms in terms of Lipid, Fibre and the ash and this variation is seen to be of significant value, $p < 0.005$ for lipid $p < 0.05$ for ash and $p \ll 0.005$ for fibre.

The variation between the raw and the cooked forms of koko and kosai in terms of the Lipid; protein and ash are also of significant value, $p \ll 0.01$, $p < 0.05$, $p \ll 0.01$ respectively. In other words, it can be concluded that cooking brings about significant losses in Lipid, ash and Fibre in danwake and no significant loss in the proximate composition of tuwo dawa and kuka soup with the exception of fibre ($p < 0.05$). There is a significant loss in protein, Lipid and ash in the case of koko and kosai. The apparent increases observed

in the percentage of total carbohydrate in the cooked meals is probably due to the losses reported for the other nutrients.

Slight increases observed in total ash in the case of tuwo dawa and kuka soup may be due to absorption from the cooking medium. This result agreed in principle with the reports of Stewart, (1946) and Bender (1966). The losses of Lipid and crude protein are too small to be of nutritional significance.

4:6:2 Effect of Cooking on the Mineral Status of the Standard Meals.

Judging from the data in Table 19, it can be seen that cooking affects the mineral status of danwake, for example, the sodium content of danwake is reduced from 5.46mg/g (dry weight) in the raw form to 5.04mg/g (dry weight) in the cooked form i.e. 7.7% loss and this loss is found to be statistically significant (see Table 20) with a p value <0.01. Also losses of Iron, phosphorus, potassium and zinc are found to be significant (Table 20), with the losses being 45.4%, 13.7%, 13,5%, 7.5% respectively. In the case of tuwo dawa and kuka soup only losses of magnessium and Calcium are found to be significant $p<0.005$, $p<0.05$ respectively and the losses are 3.8%, 15.2% respectively. Koko and kosai show significant losses of potassium, iron, zinc and phosphorus and were found to be 23%, 6.7%, 25%, 13.3% respectively.

Minerals are usually lost from most Nigerian cooked foods by leaching (Malik, 1967). However, losses in tuwo dawa and kuka soup were not marked, this is because no cooking water is discarded during

the preparation. This does not apply to danwake from the mineral content of the dry matter in the cooking water. There was quite a considerable difference in the case of potassium, a result agreeing with the findings of Eka and Hobbs (1976). The actual amount of potassium for example, in the cooking water is calculated to be 0.51mg.

Similar losses of some mineral elements in domestic cooking have been reported by various workers e.g. Noble and Halliday (1937) reported losses between 13% and 14% of phosphorus during cooking of some vegetables in an open pan. Pfund and Nutting (1942) observed an average of 10% loss for Iron in potatoes soaked for 6 hours prior to boiling. Phosphorus retention in some vegetables ranged from 60-87% when cooked in open cookers and from 68 to 83% when cooked in water less cookers and from 81 - 82% when cooked in pressure sauce pan (Brinkman et al, 1942).

Bender (1966) reported an increase in sodium content of peas from 1.7 mg/100g in the raw vegetable to 12mg per 100g in cooked form. This increase was probably due to the absorption of the mineral from the cooking water. Losses of minerals during domestic cooking by South Eastern traditional methods was also reported by Umoh (1972).

4:6:3 Effect of Cooking on the Vitamin Content of the Standard Meals.

The result of the effect of cooking on the vitamin content of the meals after performing paired comparisons in the raw and the cooked

forms is presented in Table 18.

It is observed, that vitamin B_1 and B_2, carotenes, of danwake are reduced during cooking and that this reduction is of significant value, $p \ll 0.005$, $p < 0.025$ and $p < 0.005$ respectively.

The reduction of the vitamins due to the heat treatment may be explained by the fact that the vitamins are heat labile under alkaline condition and water soluble. The potash used during the preparation of the danwake (see the recipe for this meal) makes the cooking water alkaline, and under such a condition, there are probably losses of vitamin B_1 and B_2 and some other vitamins. (Davidson et al, 1975; A.O.V.C., 1966; Bender, 1966 and Lee, 1958).

The losses of Vitamins B_2 and carotene are found to be of statistically significant value for tuwo dawa and kuka soup $p < 0.025$ and $p \ll 0.005$ and the losses are 11.7% for Vitamin B_2 and 10.7% for carotene, while the losses for danwake are 12% Vitamin B_1, 33.96% Vitamin B_2 and 26.85% carotene. The loss of all these vitamins is found to be significant for koko and kosai, $p \ll 0.005$ for B_1 and B_2, $p < 0.025$ for carotenes and $p < 0.01$ for vitamin C. The losses are 20% vitamin B_1, 46% vitamin B_2; 37% vitamin C and 9.6% carotene.

Losses of carotene in the three meals vary between 12.5mg per 100g and 196mg per 100g during cooking. The losses may be due to the high temperature reached during the cooking and presence of Oxygen (Harris and Von Leescke, 1960) and to the presence of traces of copper and iron in the meals (Bender, 1966).

Similar losses of these vitamins have been reported in the Literature by some workers. Roy and Rao (1963) observed a loss of about 35-85% of thiamine in gruel cooked with water of moderate to high alkalinity, cooking in distilled water caused a loss of about 5%. With tap water there was 8-10% loss while well water caused a loss of up to 36%. Fish lost up to 50% of its thiamine content on boiling and 75% on canning. Losses of vitamin B_1 content of meat during cooking varied with size of the cut, fat content and the average was about 15-40% on boiling, 40-50% on frying, 30-60% on roasting and 50-70% on canning. (Harris and Von Leesecke, 1960).

Losses of riboflavin occurs mainly through leaching and due to open pan cooking. The vitamin is stable to oxygen and to acid conditions but unstable to light and alkaline. Malik (1967) reported losses of up to 50% for riboflavin in his diets and attributed this to leaching and open pan cooking.

Umoh and Bassir (1977), found out that losses of vitamin C in all the six diets they studied were between 40 and 100%, there were mild losses of vitamin A, and β carotene. Of the B vitamins, they found that the most affected were thiamine and pyridoxal. Their losses were between 29 and 48% and 3.4% and 49% respectively.

Vitamin C is the most labile of all the vitamins in foods of plant origin and its retention is often used as an index of severity of processing and storage conditions. (Bender, 1966). Loss of vitamin C occur through oxidation aided by the presence of ascorbic

acid oxidase, heat, sunlight, oxygen, alkali and by leaching into the processing water which depend on the volume of water used (Bender, 1966; Stafford et al, 1976). Prolonged heating completely destroys vitamin C. Platt et al (1963) observed that peeled potatoes when soaked overnight in water lost 45-60% of their ascorbic acid content. Losses of vitamin C vary also with the method of cooking. Kohman (1942) showed that losses became progressively greater with steaming, boiling and baking. The handling of the material before and after cooking also provides opportunities of Vitamin C loss.

4:7 Amino Acid Composition of the Standard Meals (Raw and Cooked)

Unfortunately owing to the break down of the amino acid analyser only two determinations involving direct acid hydrolysis were carried out. The mean value of the amino acid analysis are set out in Table 21(a).

Valine, glutamic acid, aspartic acid, arginine and leucine, were the most abundant amino acids. Similar high values of glutamic acid, aspartic acid and arginine have been reported by Mba et al (1974b) in the different oil seed meals they studied. Hence, even though these meals could be generally regarded as good sources of these three amino acids, they are of little nutritional significance, in the human body, since they can be easily synthesised from other amino acids. They are in other words regarded as non-essential amino acids.

The meals however contain very low amounts of sulphur amino acids; methionine and cysteine. Even though, some destruction of these amino acids might have occurred during the hydrolysis, they are likely to be the most limiting amino acids in these meals.

The essential amino acids are compared with the F.A.O. provisional amino acid scoring pattern (FAO/WHO, 1973) (Table 21b).

Of those that survive the hydrolytic condition employed, the sulphur amino acids, lysine, tryptophan, isoleucine and threonine are deficient in some of the meals vis:-

(i) Sulphur amino acids and tryptophan in all.
(ii) Isoleucine and lysine in tuwo dawa and kuka soup and koko and kosai.
(iii) Threonine in danwake and in cooked tuwo dawa and kuka soup.

The limiting amino acids are sulphur amino acids in all and lysine next, in tuwo dawa and kuka soup and koko and kosai. The protein scores are also listed.

Cereals are mainly deficient in Lysine but with high methionine (Baptist and Perera, 1956; Kakade, 1974). The high content of lysine therefore in some of the meals studied e.g. danwake was probably contributed by the other ingredients present in the food e.g. cowpeas. The methionine is however low, this may be due to some destruction of this amino acid by the method of hydrolysis employed.

It is interesting to note that this high lysine content is achieved because of the combination of the plant proteins used in

preparing the meals. For this reason the major meals of the Hausas of Northern Nigeria is likely to give a well balanced mixture of essential amino acids when the required quantity of each ingredient is used.

Mathieson (1964) reported super abundance of leucine in sorghum as compared with other amino acids. All the meals studied contain a fairly large proportion of sorghum contributing greatly to the high leucine content of the meals.

The low level of histidine, lysine (in most cases), tryptophan, cysteine and methionine agrees with the findings of Oyenuga (1968a). He found that the biological value of cereal proteins is generally not very high and as a group, these proteins are low in some or all of the following amino acids, histidine, lysine, tryptophan, cysteine and methionine. For example, Nigerian maize with a true protein content of 10.6% (dry weight) is low in tryptophan, histidine, lysine and cysteine, while Nigerian rice with a true protein content of 12.5% (dry weight) is low in tryptophan, histidine and cysteine. (Oyenuga, 1968a).

Protein deficiency in man is usually a relative deficiency for one or another of the essential amino acids although total Nitrogen intake may also sometimes be limiting. Since all amino acids must be present at the site of protein synthesis in adequate amounts for protein synthesis to proceed, an equal percentage deficit of any essential amino acid would lead to wastage of other amino acids and also limit protein synthesis to a comparable degree.

Hepatic and renal damages have been reported to result from methionine deficiency and it has been found that this amino acid is also necessary for normal hair growth. (Davidson et al, 1975). The low values of lysine (in most of the meals) and methionine may lead to enlarged and fatty liver and hepatic and renal damages respectively.

Since all the meals are deficient in one or more essential amino acids, they can be regarded as low quality protein suppliers.

4:7:1 Effect of Cooking on the amino acid Composition of the meals.

When the raw and cooked forms of the meals are compared it is observed that, cooking danwake brought about major losses in sulphur amino acids, threonine and phenylalanine and tyrosine. The amino acid pattern of the other two meals were less affected.

The loss of these amino acids may be due to the heat treatment. Possibly loss of the water soluble amino acid into the cooking water or oil may occur. Loss could also be due to combination of one or more of the amino acid in linkages that are not hydrolysed during digestion. One such linking is the browning reaction, in which the amino group ($-NH_2$) from a protein reacts with the aldehyde group of a sugar ($-C\overset{O}{\underset{H}{\diagdown\!\!\diagup}}$) to produce a brown coloured material in the food product which is not easily digested.

In the case of danwake, the cooking water is found to contain some protein hence some amino acids. But this does not occur in the case of tuwo dawa and kuka soup, where there is no cooking water to be discarded. The losses in amino acid observed on cooking

koko and kosai may be attributed to loss to the cooking oil and in addition to the break-down of the amino acid due to heat treatment.

Similar losses have been reported due to cooking and processing of food. Studies by Patton and Hill (1948) on purified casein and soy-globulin in 5% glucose solution maintained at 96.5°C for 24 hours showed significant losses of lysine, arginine, tryptophan and histidine. The losses were 27.4, 22.7, 15.3 and 14.6% respectively in the case of soy-globulin as measured by microbiological assay after acid and alkaline digestion.

Losses in amino acid from diets due to South Eastern Nigeria traditional methods of cooking were also reported by Umoh (1972).

4:8 Feeding Experiment

Feeding experiment with animals were designed on previous experiences and observations of the early nutritionists.

For this experiment, the investigator fed the rats on the whole meal without supplementation. This was done in order to find out whether this whole meal could support the growth of rats without any supplementation.

Table 22 shows the changes in body weight of the experimental animals and the food intake and Table 23 shows the different Biological indices calculated.

The nutritive value of a diet prepared for human consumption is influenced by a number of inherent factors, including the way the food is prepared, the quality of the protein, the dietary level of

vitamins and minerals, the amount of food consumed (Miller, 1970) and because of their protein sparing action (Platt and Miller, 1958), the levels of fats and carbohydrates. When human diets are assayed by rat feeding experiments for the determination of the nutritive value, it is essential that the rats are normal and not suffering from any infection. (Dema et al, 1959) or hookworm (Orraca-Tetteh and Platt, 1967).

None of the raw meals, in this study supported growth. This was achieved to a small extent with the cooked meals although it was still poor compared to the balanced diet. The food intake was always poor about 50% relative to the balanced diet. This poorer intake of the meals may be attributed to both poorer digestibility and palatability of the meals compared to the control balanced diet.

There is a definite improvement in the digestibility of danwake, and tuwo dawa and kuka soup on account of cooking, danwake containing beans and tuwo dawa and kuka soup containing dawa and soup with beef. In the case of meal 3, koko and kosai, the digestibility of the raw-mixed is higher than that of the cooked form.

The most obvious conclusion is that the meals are deficient at the level eaten in one or more essential nutrients and as such fail to maintain optimum growth. However, comparing the meals with the protein free diet, it is apparent that at least the meals contain some useful protein, vitamins and elements which brought about an increase in weight of the rats but at a suppressed rate. The result

supported the previous findings of Fraps and Carlyle, (1939).

The gain in weight produced in the rats by feeding with these meals was seen to roughly increase with increasing amount of Nitrogen intake. This therefore suggests that enough protein is not being consumed in these meals.

The fact that none of the rats died as a result of the meal indicated that all the meals were able to sustain the animals but at varying levels. Thus while the rats on raw danwake and raw koko and kosai were inactive and tiny, the rats on cooked tuwo dawa and kuka soup and cooked koko and kosai were active like the rats fed on the balanced diet. Rats on raw tuwo dawa and kuka soup and cooked danwake were midway between the above stated categories.

On the whole, the Standard diet which was used as the control gave the best productivity.

The values of true digestibility of dietary nitrogen in all the diets including the balanced diet are high being within the range 87-98%. These results agree with the view expressed by Henry and Kon, (1957) that for good quality proteins, the changes in levels of intake do not always appear to affect digestibility. Such differences when they do occur are often small and insignificant.

Table 23 presents the different biological indices calculated.

N.P.R. (Net protein ratio) is defined as gain in weight of test group plus loss in weight of non protein group, divided by protein intake (Bender and Doell, 1957). It thus measures the protein used for maintenance. For danwake it is observed that this value is

higher in the cooked forms than the raw, whereas in the case of koko and kosai, tuwo dawa and kuka Soup, the cooked forms have lower N.P.R. values than the raw form. The N.P.R. values for all the meals are however higher than that for the balanced diet except in the case of raw koko and kosai. Hence it can be said that the protein used for maintenance of the rats in the case of the balanced diet is less than that for the three meals, which probably gives an indication to the better quality of the protein of the balanced diet.

The same trend is observed for the P.R.E. value, this term is given to the value obtained by converting N.P.R. into a percentage scale by use of an experimentally determined factor, 16. It has been shown to be independent of food intake. It includes the maintenance requirements of the test animal as well as the growth requirements.

P.E.R. (Protein Efficiency Ratio) is the gain in body weight divided by the nitrogen intake. From Table 23, this is seen to be higher in the case of the balanced diet than in the case of the three meals. This thus show that the food intake in the case of the balanced diet was fully utilised for body growth, while it is only slightly utilised in the cooked forms of the meals.

The comparison of the meals with a balanced diet at a lower protein intake (i.e. 10%) could have been carried out to further establish the nutritive value of these meals, but there was no time to do this. Hence further work is suggested on this aspect of the study.

4:9 Caloric Values of the Standard Meals (raw and cooked)

Tables 24(a) and (b) show the caloric values per 100g of dry food, and that of the actual intake of the three cooked standard meals along with the Net Protein Calorie percent.

The values were obtained by multiplying the values of the total or crude protein, oils (pet-ether extract) and carbohydrate by 4, 9, 4 respectively and taking the sum of the products for each meal. Crude fibres were excluded from the calculations.

The Nigerian reference man's (i.e. an adult man weighing 63kg and adult woman weighing 53kg) daily energy requirement is 2893 calories while the reference woman requires 2154 calories. (Oyenuga 1968b). These figures are quite close to the 2960 calories (reference man) and 2,130 calories (reference woman) later calculated by Idusogie (1972). The first set of figures will be used here to compute the minimum daily requirements.

From Table 24(b), it is observed that the cooked danwake as actually taken per day can only supply 500 calories, while tuwo dawa and kuka soup and koko and kosai can supply 313 calories and 995 calories respectively. Therefore the total calories per day that could be supplied by these meals is 1,808 cals. which is too low to meet the daily requirement for both man and woman. To meet these requirements therefore, more food has to be eaten than the three meals recorded.

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If no other energy containing food is taken, then part of the protein, low enough, will go directly to provide calories. Fortunately enough, these people consume a lot of sugar cane as snacks in between their meals, which could provide them with more calories. Hence the problem of calorie deficiency will probably not occur.

Chapter 5

5:1 CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDIES

From the results obtained the following conclusions could be drawn.

1. (a) Danwake samples bought from the market are found to be good energy sources. The normal intake provides 20g lipid and 186.62g carbohydrate.

   (b) Tuwo dawa and kuka soup and koko and kosai are found to be low in energy sources, 7.08g of lipid and 34.97 carbohydrate; 14g of lipid and 39g carbohydrate are supplied respectively by these meals.

   The three meals combined supplying 41.06g of Lipid 260g of carbohydrate.

2. All the meals were found to be low in crude protein. The three meals combined are inadequate to supply the daily requirement for an adult. The total protein intake being 35g.

3. (a) The Standard Danwake sample is richer in Lipid and ash but less in carbohydrate and comparable in crude protein to the market sample.

   (b) The market sample of tuwo dawa and kuka soup is richer in protein but poorer in ash content than the standard meal.

   (c) Market sample of koko and kosai is richer in ash, protein and carbohydrate but poorer in petroleum ether extract than the Standard.

Hence it can be concluded that there was a marked variation between the Laboratory prepared meals i.e. Standard meals and local markets' meals and this was attributed to economic considerations.

4. Vitamin B_1 was found to be high in all the meals but Vitamin B_2 and Vitamin C were very low. Carotenes were found to be low in both danwake and koko and kosai but high in tuwo dawa and kuka soup, All the meals were found to be adequate to supply the daily requirements of Vitamin B_1 for an adult man but inadequate to supply the required Vitamin B_2 or Vitamin C when the meals are taken singly. But the three meals taken together in a day could supply the daily recommended intake of these vitamins.

5. All the Standard meals and market meals were adequate in mineral elements.

6. Cooking has been found to cause a loss of 45.4% Fe, and 13.7% P in danwake, 15.2% Ca in tuwo dawa and kuka soup and 6.7% Fe, 13.3% P, 25% Zn and 23% K in koko and kosai.

7. Losses of Vitamins B_1, B_2, C and carotenes were incurred by cooking in all the meals. The loss of Vitamin B_1 varies between 6.6 and 20% in all the meals; loss of Vitamin B_2 varies between 11.7 and 46%; 37% and 100% for Vitamin C and 9.6 and 26.85% for carotene.

8. All the meals were found to be limiting in sulphur amino acids i.e. methionine and cysteine and marginal in tryptophan. Lysine was low in tuwo dawa and kuka soup and koko and kosai.

9. Cooking danwake was found to bring about major losses in sulphur amino acids, threonine, phenylalanine and tyrosine while the amino acid pattern of the other two meals were less affected.

10. The animal experiment shows that none of the meals could support optimum growth of rats at the level eaten. The digestibility of protein for danwake and tuwo dawa and kuka soup were improved by cooking while that of koko and kosai was reduced.

11. The meals are all inadequate in calorie when compared with daily requirement for an adult man and woman. Hence more of the foods must be eaten to provide the necessary calories.

In general, all the meals assayed are found to be fairly high in nutritive value. However, the knowledge of their methods of preparation and the effect of cooking on the nutrient composition allows the following recommendations and suggestions to be made:-

(a) Using the water from preparation of danwake as soup and also using less cooking water in the preparation so as to lessen loss of nutrients.

(b) The volume of cooking oil used in frying the koko and kosai should be reduced and the oil left over after frying should be made use of in preparing stew at other meals.

(c) More meat should be eaten along with the tuwo, and more kosai should be eaten along with the koko.

(d) The proportion of cowpeas should be greater than those of other ingredients in preparing danwake. Even though the food will be more costly, quality of food is better than quantity.

(e) Vegetables and fruits should be taken along with the meals to supply necessary vitamins.

The nutritional values of the meals would then be improved.

Poisonous substances like hydrocyanic acid, phytic acid and oxalate, may be present in these meals and these might have been one of the causes of the suppressed growth observed when the meals were fed to rats. Attempt should be made to identify possible toxic substances in the meals and to establish their toxic levels in each, since there seems to be no available reports on them. Work in this direction should be encouraged.

In order to establish the nutritive value of the meals, more work need to be done on the feeding trials using experimental animals.

It might also be necessary to carry out some clinical and biochemical observations on human volunteers (Hausas) who are fond of consuming these meals daily.

Household survey work should be encouraged so as to establish the quantity of each meal taken so as to find out whether each meal is adequate in amount or not.

Services of Health workers should be sought, to try to advise the Hausa people how to prepare their meals so as to retain most of the nutrients particularly vitamins.

5:2 Contribution to Knowledge.

The present study has made the following important contributions to knowledge of nutrition especially in Nigeria.

1. Levels of nutrients in the three commonly consumed Hausa meals as sold in the markets have been determined.

2. At the average level of intake, danwake (market sample) provides 19.25g of crude protein, tuwo dawa and kuka soup and koko and kosai provide 7.42g and 8.58g of protein per day respectively. The total protein intake per day as supplied by the three meals is 35.26g and this is not able to meet the requirement for a reference woman needing 41g of lower quality protein (Net protein utilisation 70) and reference man needing 53g of protein per day.

3. The calorie values of all the three meals have been ascertained and found to be inadequate at the normal level of intake.

4. All the meals have been established as adequate in vitamins and mineral elements i.e. magnessium, sodium, potassium, zinc, copper, iron, phosphorus and manganese.

5. The effect of cooking on the chemical composition of the three meals have been established. The most affected are the minerals and vitamins.

6. The essential amino acid contents have been ascertained and compared to FAO provisional amino acid scoring pattern of an ideal protein. (egg protein or milk protein). The three meals are low in sulphur containing amino acids.

7. The effect of cooking on the digestibility of the crude protein of the three meals has been worked out by feeding these meals to rats.
8. At the same time by using the rat growth as an index of nutritional adequacy, the nutritive values of these meals have been established.
9. Hence the Nutritive status of these three commonly consumed Hausa meals in tne quantities normally eaten has been elucidated.

APPENDIX 1

PROXIMATE COMPOSITION DETERMINATION

(A) Determination of Moisture Content A.O.A.C. 1975, p.222

Apparatus:- Vacuum Oven

Dessicator - using reignited Calcium oxide as dessicant.

Crucibles with lids.

Procedure:-

1. 3-5g of sample was weighed in a weighed crucible and kept in a vacuum oven at 60°C and at a pressure of about 25mm mercury for 24 hours.
2. The crucible was removed, cooled in a dessicator and weighed.
3. The drying was continued until two consecutive readings differed by not more than 1-3mg.
4. The loss in weight represents the moisture content.

(B) Determination of Ash Content (A.O.A.C., 1975, p.222)

Apparatus:- Crucibles with lid.

Furnace

Dessicator using reignited calcium oxide as drying agent.

Procedure:-

1. 3-5g of dried powdered food sample was weighed in a weighed crucible with its lid and ignited for about 24 hours at 550°C until grey ash was formed.

2. Crucible containing the ashed sample was removed cooled and weighed.

3. Weight of the ash was obtained by subtracting the weight of the crucible.

(C) Determination of Lipid Content or Pet-ether extract
A.O.A.C., 1975, p.185

Apparatus:- Soxhlet extractor set.
Thimbles.
Heating mantle.
500ml round bottomed flasks.
Antibumping granules.
Rotary evaporator.

Reagent:- Petroleum ether 60^{o}-80^{o}C.

Procedure:-

1. 5-10g of sample powder was weighed into a thimble of known weight.
2. 300ml of petroleum ether was poured into a previously weighed 500ml round bottomed flask containing antibumping granules.
3. Soxhlet extractor into which a thimble with its contents had been introduced was fitted into the flask and the solvent boiled under reflux.
4. The extraction process was continued for about 12 hours.
5. Thimble with its content was removed dried in oven maintained at 60^{o}C for 4 hours and then weighed.

6. Solvent containing the extracted lipid was distilled off and finally evaporated to dryness using a rotary evaporator.

7. Round bottom flask with lipid extract residue was then weighed.

8. Amount of lipid extracted was then obtained from the difference between weight of flask before and after extraction and loss in weight of the thimble and its content after drying.

(D) Determination of Crude Protein:- Macro-kjeldahl Method. A.O.A.C. 1975, p.186.

Apparatus:- Digestion rack with electric heater.
Kjeldahl distillation apparatus
Digestion flasks - 500ml.

Reagents:- Concentrated Sulphuric acid (H_2SO_4)
0.1N Sulphuric acid (H_2SO_4)
0.1N Sodium hydroxide solution.
50% Sodium hydroxide solution (NaOH)
Sodium Sulphate anhydrous (Na_2SO_4)
Anhydrous cupric sulphate ($CuSO_4$)
Methyl red indicator.
Antibumping granules.

Procedure:-

(a) Digestion of Material

1. 1g of dried powdered sample was weighed into the kjeldahl digestion flask.

2. 5g of Sodium Sulphate and few crystals of anhydrous cupric sulphate were added.

3. 25ml of concentrated sulphuric acid was also added.

4. The flasks were then set up in a slanting position on the Kjeldahl's digestion heating mantle and then heated in the fume cupboard for 12 hours (when colourless solutions were obtained).

5. The tubes were allowed to cool.

(b) Distillation and Titration

1. The digestion flask and its content was immersed in an ice bath, the digested sample was diluted with 100ml distilled water.

2. Four drops of methyl red indicator were added.

3. 100ml of 50% NaOH was added slowly into the solution.

4. 50ml of 0.1N H_2SO_4 was pipetted into the receiving flask and four drops of methyl red indicator added.

5. Distillation apparatus was assembled and the distillation was carried out until about 150ml of distillate has been collected.

6. Excess acid was titrated with 0.1N NaOH.

7. A blank was set up using water instead of the sample.

8. A number of digestions using tryptophan, ammonium sulphate were carried out to determine recoveries in the Kjeldahl method.

9. Three separate digests, distillation and titrations were made for each sample.

10. Protein content was obtained by multiplying percentage nitrogen by 6.25.

(E) Determination of Fibre Content

Apparatus:- A rubber tipped glass rod.

400ml beakers

Buchner funnels

Furnace

Dessicator - using reignited calcium oxide as dessicant.

Reagents:- 5% Sodium hydroxide solution (NaOH)

5% Sulphuric acid (H_2SO_4)

1% Hydrochloric acid (HCl)

95% ethanol.

Whatman filter paper No.40.ashless.

Procedure:-

1. 3g of the fat free residue from pet-ether extract already described, was transfered to a 400ml beaker which had been previously marked at 200ml level.

2. 50ml 5% H_2SO_4 was added and the mixture made up to 200ml mark with distilled water.

3. The contents of the beaker were heated to boiling and kept boiling for exactly 30 minutes.

4. During the boiling, the mixture was stirred with a rubber-tipped glass rod to remove all particles from the sides and the volume kept constant by addition of hot water from time to time.

5. The mixture was then filtered immediately through the ashless filter paper on a buchner funnel attached to a filter pump.

6. 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.

7. The residue was scraped off carefully from the filter paper with a spatula and replaced in the same beaker removing the last traces with a jet of hot water.

8. 50ml 5% sodium hydroxide was added and then distilled water to make up to 200ml mark.

9. 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.

10. The content was filtered immediately through the same filter paper of the same buchner and residue transferred to the filter paper by means of a jet of hot water.

11. Residue was washed few times with hot water until acid free.

12. Finally, the residue was washed twice with a little 95% alcohol and transferred to a 100ml porcelain crucible, removing traces with a jet of alcohol.

13. After drying in an oven at 100°C to constant weight, the crucible with the contents was cooled in a calcium oxide dessicator, weighed and ignited in a furnace at 550°C.

14. Then it was cooled again in the dessicator and weighed.

15. The crude fibre content was determined from the loss in weight of crucible after ignition.

APPENDIX 2

MINERAL ELEMENTS DETERMINATION

(A) Determination of total phosphorus by a Chemical Method. A.O.A.C. 1975, p.143 - Molybdovanadate method

Apparatus:- Porcelain crucibles with lid.

Spectronic 20; Furnace; Dessicator

2000ml, 1000ml, 250ml beakers.

Volumetric flasks:- 100ml, 1000ml and 2000ml, 250ml.

Test tubes; Pipettes;-5ml, 10ml, 20ml.

Reagent:- Ammonium molybdate tetrahydrate.

Ammonium metavanadate.

Perchloric acid.

Potassium dihydrogen phosphate.

Concentrated hydrochloric acid.

Procedure:-

(a) Molybdovanadate reagent:-

1. 40g of ammonium molybdate was weighed, dissolved in 400ml hot distilled water in a 1000ml beaker and cooled.

2. 2g of ammonium metavanadate was dissolved in 250ml hot distilled water in a 2,000ml beaker, cooled and exactly 313ml of 60% perchloric acid added.

3. Molybdate solution was then added gradually to metavanadate solution with stirring and the solution diluted to 2 litres.

(b) Phosphorus Standard Solution

1. Stock solution was prepared by dissolving 8.788g of potassium dihydrogen phosphate accurately weighed, in distilled water and diluting to 1000ml (this solution contains 2mg phosphorus per ml i.e. 2mgP/ml.

2. Working Standard Phosphorus Solution: (0.1mg P/ml), this was prepared by diluting 50ml of stock solution to 1000mls with distilled water.

(c) Preparation of Calibration Curve:

1. Aliquots of Working Standard phosphate solutions containing 0.5, 0.8, 1.0 and 1.5mg phosphorus were pipetted separately into 100ml volumetfic flasks.

2. 20ml of molybdovanadate reagent was added to each flask and the mixture diluted to volume with distilled water.

3. After thorough shaking, the solutions were allowed to stand for 10 minutes and absorbance determined at 400nm against a blank. Blank was prepared by pipetting 20ml molybdovanadate ragent into 100ml volumetric flask and diluted with distilled water to mark.

(d) Determination of Sample

1. 1.5g of powdered sample was accurately weighed into a porcelain crucible and ashed in a furnace at 500^{o}C until carbon free.

2. Ash was allowed to cool to room temperature in a dessicator and washed into a 250ml beaker with 40ml hydrochloric acid (1 volume concentrated hydrochloric acid plus 3 volumes distilled water)in small portions.

3. Several drops (10-15) of concentrated nitric acid were added.

4. The solution was heated to boiling, cooled and transferred into 250ml volumetric flask and diluted to volume with distilled water.

5. The solution was filtered.

6. 10ml of this solution was pipetted into 100ml volumetric flask.

7. 20ml of Molybdovanadate reagent was added and mixture diluted to mark with distilled water. The mixture was shaken and left for 10 minutes for colour development.

8. Absorbance was determined at 400nm against the reagent blank.

9. The concentration of phosphorus in the sample was determined from the calibration curve.

(B) Determination of Mineral Elements by Atomic Absorption. A.O.A.C. 1975, p.22.

The mineral elements determined were:-

Calcium, Potassium, Zinc, Sodium, Iron, Copper, Magnessium and manganese.

Apparatus:- Furnace

Perkin-Elmer Atomic Absorption Spectrophotometer model 306 with digital read out.

100ml volumetric flasks.

Porcelain crucibles.

Dessicator.

Water bath; Ashless filter paper.

Reagents:- Concentrated hydrochloric acid (HCL)

Concentrated Nitric acid (HNO_3)

Lanthanium chloride solution.

Strontium chloride solution.

Magnessium ribbon.

Calcium carbonate.

Potassium chloride

Zinc ribbon.

Cupric chloride.

Iron wire

Manganese chloride.

(a) Preparation of Standard Solutions

1. Calcium

2.497g oven dried calcium carbonate was dissolved and diluted to 100ml with water. This solution contains 1000ppm calcium ions per ml. From this stock solution, calcium Standard Solutions of concentration, 1.0, 3.0, 6.0 and 9.0ppm were prepared.

2. Magnessium

1g. of Magnessium ribbon was accurately weighed and dissolved in 10ml concentrated hydrochloric acid, the solution was boiled and evaporated almost to dryness on a water bath. Then water was added and solution transferred into 1000ml volumetric flask and made up to volume with distilled water.

From this stock solution which contains 1000mg/ml magnessium ions, four standard solutions of concentration, 0.1, 0.5, 1.0 and

1.5ppm were prepared. A stock solution of Strontium containing 10,000µg/ml strontium was prepared using oven dried strontium chloride. To each of the calcium and magnessium standard solution was added enough strontium solution such that there was 1,500µg/ml of Strontium in the final solution.

3. Potassium

A stock solution containing 1000µg/ml potassium ions was prepared by dissolving 1.907g potassium chloride (KCl) in water and diluting to 1000ml with distilled water. From this, standard solutions of concentration, 1.0, 2.0, 4.0, 6.0ppm were prepared.

4. Sodium

A stock solution containing 1000µg/ml sodium ions was prepared by dissolving 2.542g oven dried sodium chloride (NaCl) in distilled water and diluted to 1000mls. From this standard, sodium solutions of concentration 0.1, 0.2, 0.3, 0.4ppm were prepared.

A 1% stock solution of Lanthanium chloride was prepared and added to the potassium and sodium standard solutions such that the concentration of Lanthanium in the final solution was 0.1%.

5. Zinc

A stock solution containing 1000µg/ml zinc was prepared using exactly 1g of zinc ribbon. This was treated like the magnessium ribbon. Standard solutions of concentration 0.5, 1.0, 1.5, 2.0ppm were prepared from stock solution.

6. Copper

A stock solution containing 1000µg/ml Cu^{++} ions was prepared by dissolving 2.682g cupric chloride in distilled water and diluting the final volume to 1000ml. Standard solutions of concentrations 0.1, 0.5, 1.0, 2.0ppm were prepared from the stock solution.

7. Iron

A stock solution containing 1000µg/ml Iron was prepared from pure Iron wire. 1g of the wire was dissolved in 10ml concentrated Nitric acid boiled on a water bath, cooled and diluted to 100ml with water. From this stock solution, standard solution of concentration 0.0, 0.5, 1.0, 2.0 and 4.0ppm were prepared.

8. Manganese

A stock solution containing 1000µg/ml Manganese ions was prepared by dissolving 1.582g of manganese oxide in 5ml of hydrochloric acid. This solution was evaporated almost to dryness and then diluted to 1 litre with 0.1N hydrochloric acid.

(b) Preparation and analysis of Sample for the mineral elements.

1. 3g of dried powdered sample was accurately weighed and ashed in the furnace at 550°C.
2. Ash was cooled in the dessicator and weighed.
3. 2ml concentrated hydrochloric acid was added to dissolve the ash. Few drops of concentrated nitric acid (10-15 drops) were added.
4. Solution was boiled on the water bath for a few seconds and evaporated almost to dryness.

5. Distilled water was added and the solution transferred into 100ml volumetric flask through ashless filter paper and made up to volume with distilled water.

6. Appropriate dilutions were made for each element determination.

7. Standards were first determined in the instrument before the sample solutions using appropriate lamps and correct wavelength as specified in the instrument manual.

Element	Wavelength (nm).
Calcium (Ca)	422.6
Magnessium (Mg)	285.2
Potassium (K)	766.5
Sodium (Na)	589.5
Copper (Cu)	324.7
Zinc (Zn)	213.9
Iron (Fe)	248.8
Manganese (Mn)	279.5

8. Amounts of various elements were then determined from the calibration curve prepared for each element.

APPENDIX 3

VITAMINS DETERMINATION

(A) Determination of Vitamin C. (Ascorbic acid) by Evered's Method. (1960).

Apparatus:- 100ml volumetric flasks.

Test tubes:- 25 x 150mm.

Test tube racks.

Pipettes:- 1ml, 5ml, 10ml.

10ml microburette.

Mechanical shaker.

Reagents:

1. N-Bromosuccinimide Solution:- A stock solution was prepared by dissolving 0.2g of the reagent in warm water cooling and diluting to 100ml. This solution is stable for only two days when stored in a refrigerator.

Just before use, the stock solution was diluted 10ml to 100ml with distilled water. 1ml of this solution is approximately equivalent to 0.2mg of ascorbic acid.

2. Acetic acid - glacial analytical reagent grade.

3. Potassium Iodide solution:- 4% w/v aqueous solution prepared from Iodate free potassium Iodide, by dissolving 4g of potassium Iodide in distilled water and making up to mark with distilled water in an 100ml volumetric flask.

4. Di-ethyl ether:- analytical grade.

5. Standard Ascorbic acid solution:- This solution (0.2mg/ml ascorbic acid) is freshly prepared in 1% v/v acetic acid by dissolving 0.02g in 100ml.

Procedure:

1. 5g of the freeze dried well powdered sample was accurately weighed and transferred to a 100ml volumetric flask with 1% v/v aqueous acetic acid and diluted to mark with the same reagent.

2. The mixture was poured into a conical flask and shaken vigorously with a mechanical shaker for about 30 minutes to extract the ascorbic acid into solution.

3. The solution was centrifuged.

4. 5ml of the extract was transferred into a 25 x 150mm test tube.

5. 1ml of glacial acetic acid and 5ml of potassium Iodide solution were added with mixing after each addition.

6. 3ml of diethyl ether was added and the mixture titrated with N-bromosuccinimide solution from a 10ml semi-microburette. The test tube was shaken vigorously after each addition of titrant and the organic layer allowed to separate.

7. The end point was indicated by the 1st appearance of the brown colour of liberated Iodine in the upper ether layer.

8. Comparison against an untitrated 'dummy' mixture was found to be necessary in the establishment of the end point.

9. A blank titration was carried out using 5ml of water plus the volume of distilled water equivalent to the titre to replace the diluted sample.

10. The blank titration gives the volume of N-bromosuccinimide solution necessary to impact a definite brown colour to the ether layer.

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

(B) Determination of Thiamine. (fluorometric method)
A.O.V.C. 1966, p.127 - Thiochrome Method.

Apparatus:- Volumetric flasks, 25ml, 100ml, 1000ml.
Funnels; 100ml erlenmeyer flasks
Beakers:- 250ml, 400ml.
Base exchange tubes with 25-30ml reservoir constricted gradually into a column 6 to 8mm inside diameter and 15cm long.
Pippettes:- 5ml, 10ml, 25ml.
Photofluorimeter:- single sided, M.K.4 W.129 RT.

Reagents:-

15% Sodium hydroxide (NaOH).

Anhydrous sodium sulphate granular.

1% Potassium Ferricyanide solution.

Alkaline potassium Ferricyanide - 3ml of 1% potassium ferricyanide solution was diluted to 100ml with 15% sodium hydroxide solution prepared daily and kept out of sunlight.

0.1N hydrochloric acid (HCl).

0.1N Sulphuric acid (H_2SO_4).

2.5M Sodium acetate solution:- 205g of anhydrous sodium acetate or 345g of sodium acetate trihydrate was dissolved in water and diluted to 1 litre.

Isobutyl alcohol.

Enzyme solution:- Diastase, prepared fresh daily - 6g of enzyme was suspended with thorough shaking in 2.5M sodium acetate solution and diluted to 100ml with additional sodium acetate solution.

25% potassium chloride (KCl).

Acid 25% potassium chloride:- 8.5ml of concentrated hydrochloric acid was diluted to 1 litre with 25% potassium chloride solution.

Activated Decalso:- 200ml of hot 3% acetic acid was added to 100g of Decalso and the acid was allowed to remain in contact with it for 10-15 minutes. Mild vacuum was applied to drain off the solution. The acid wash was repeated. The washing was repeated once more with 250ml hot 25% KCl solution, then washed once more with acetic acid solution.

After draining off the wash solution, the remaining KCl was flushed out by washing the decalso with 500ml-600ml portions of hot water and applying mild vacuum continually. The final wash solution was free of chlorides as indicated by failure to give precipitate with 1% silver nitrate solution. The decalso was dried at room temperature and stored in a stoppered wide mouth bottle.

Stock Thiamine solution:- 100mg of thiamine was dissolved in 25% ethanol and diluted to 1 litre with same reagent and kept in a refridgerator.

Intermmediate thiamine solution:- 5ml of stock solution was diluted to 100ml with water after warming to room temperature.

Working thiamine solution:- 4ml of intermmediate thiamine solution:- was transferred into a flask containing 75ml of 0.1N H_2SO_4 and 5ml of sodium acetate solution and adjusted to 100ml with water (final concentration - 2µg. of thiamine per ml.).

Stock Quinine Sulphate Solution:- 100mg of quinine sulphate was dissolved in 0.1N H_2SO_4 and diluted to 1 litre with same solvent.

Working quinine sulphate solution:- 3ml of stock solution was made up to 1 litre with 0.1N H_2SO_4 to give a final concentration of 0.3µg/litre.

85% ethanol.

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

Procedure

(a) Extraction

1. 5g of freeze dried powdered sample was accurately weighed into a conical flask, 75ml of 0.1N HCl, was added and then autoclaved for 30 minutes.

2. The extract was cooled to 50°C and 5ml of freshly prepared enzyme suspension was added and incubated at 45°C for about 3 hours.

3. The extract was cooled to room temperature and diluted to 100ml with distilled water. It was mixed thoroughly and filtered.

(b) Purification

1. Each adsorption tube was filled with distilled water and the decalso was allowed to fall in place by gravity, until a layer 5mm deep was present in the reservoir above the column. The tube was allowed to drain until no water was visible above the decalso.

2. 25ml of the filtrate from a(3) above was pipetted into the reservoir of the prepared adsorption tube and allowed to pass slowly through the Decalso column. The filtrate was discarded.

3. The reservoir and column was washed with three successive portions (about 10ml) of hot water discarding the washings.

4. After washing, 10ml of acid KCl was placed in the reservoir and the effluent solution was collected in a 25ml volumetric flask. A second 10ml portion was added when all the 1st had entered the Decalso, the eluate was collected into the same 25ml flask. When this second portion has drained into the receiver, the content was diluted to mark with acid KCl solution and mixed well.

5. Steps 1, 2, 3 and 4 were repeated with a new column and using 25ml of working thiamine solution in place of the sample aliquot.

(c) Conversion to thiochrome

1. 5ml of acid KCl eluate from b(4) above was pipetted into a reaction vessel.

2. To the vessel, 3ml of alkaline ferricyanide solution was added and mixed gently. 15ml of isobutyl alcohol added and then shaken vigorously for 90 seconds.

3. Steps (1) and (2) were repeated using 3ml of the working thiamine eluate b(5).

(d) Separation of thiochrome solution

1. The reaction vessels were centrifuged for 1 to 3 minutes.

2. The aqueous layer was siphoned out and 2-3g of anhydrous sodium sulphate was added to the alcohol solution.

3. The solution from (2) was allowed to stand until sparkling clear.

(e) Measurement of Thiochrome

1. 10ml of the clear colourless isobutyl alcohol solution was decanted from d(3) above into separate matched cuvette.

2. Using the working quinine sulphate solution, the photofluorimeter was adjusted to give a deflection of 1.00 on the digital scale.

3. The fluorescence of the extract in isobutyl alcohol was determined on the digital scale.

4. To guard against fluctuation due to unstable current fresh working standard quinine sulphate solution was used as a check in between readings.

(f) Preparation of Blank

1. 1 drop of concentrated hydrochloric acid was added alternatively to the cuvet containing the isobutyl alcohol solution of thiochrome whose fluorescence has been measured, mixed and the fluorescence determined again as in e(2) above.

2. Step f(1) was repeated with working thiamine hydrochloric acid standard solution.

Calculation:- Thiamine content of sample in $\mu g/g$

$$= \frac{U - UB}{S - SB} \times 1/5 \times \frac{25}{V} \times \frac{100}{\text{Weight of Sample used}}$$

Where U = readings of unknown

UB = readings of unknown blank

S & SB = readings for the Standard and Standard blank respectively.

V = volume of solution used for adsorption on Decalso.

(C) Determination of Riboflavin (fluorometric method).
A.O.V.C. 1966, p.158

Apparatus:- Autoclave

150ml erlenmeyer flasks

funnels

Centrifuge

100ml conical flasks.

Pipettes:- 1ml, 5ml, 10ml.

Graduated cylinders:- 100ml, 1000ml.

Volumetric flasks:- 50ml, 100ml, 1000ml, 2000ml.

Test tubes; 25 x 150mm.

Test tube racks.

Fluorophotometer:- Single sided fluorimeter M.K.4. Locarte Scientific Instrument London.

pH meter.

Reagents:-

10N Hydrochloric acid, (HCl)

1N hydrochloric acid

0.1N HCl.

10N Sodium hydroxide solution

1N Sodium hydroxide (NaOH).

Solution:- 100ml of 10N solution of NaOH diluted to 1 litre with distilled water.

0.1N NaOH - 100ml of 1N NaOH diluted to 1 litre with distilled water.

Glacial acetic acid.

3% potassium permanganate solution, $KMnO_4$ - 3g $KMnO_4$ was dissolved in sufficient water to make 100ml.

3% hydrogen peroxide (H_2O_2) - prepared at time of use by diluting 30% H_2O_2 1:10 with distilled water.

Stock Riboflavin Solution:- (25 µg/ml)

50mg of riboflavin which has been dried over concentrated sulphuric acid in an oven for 24 hours was accurately weighed and transferred quantitatively to 2,000ml volumetric flask. About 1,500ml of water and 2.4ml glacial acetic acid were added and mixture warmed to aid solution. After cooling to room temperature, the solution was diluted to mark with distilled water. This stock solution was preserved under toluene in a dark bottle.

Working Riboflavin Standard Solution - 20ml stock solution was diluted to 100ml with distilled water. From this working standard (i.e. 0.5 µg riboflavin per ml.) the following concentrations of riboflavin were made, - 0.10, 0.20, 0.30, 0.40 µg/ml.

Solid Sodium hydrosulphite - (Dithionite).

Procedure:

(a) Extraction:-

1. 5g of freeze dried sample was weighed into 150ml erlenmeyer flask.
2. 50ml of 0.1N HCl was added to the flask and autoclaved for 30 minutes.

(b) Precipitation of Interfering Impurities

1. After autoclaving, the sample was cooled and adjusted to pH 6.0 with 1N NaOH using a pH meter. Since riboflavin is unstable in alkaline solution the extract was swirled constantly during the addition of alkaline to prevent local areas of high pH.

2. Immediately, 1N HCl was added in drops to bring the pH to 4.5 The solution was then diluted to 100ml with distilled water and filtered.

3. 1N HCl was added dropwise to about 5ml of the filtrate to see if further precipitation occurs.

(c) Acidification of Extract

1. 10ml of sample solution (extract) and 1ml of water were added to each of 3 test tubes and mixed.

(d) Internal Standard

1. 10ml of sample solution and 1ml of riboflavin solution containing 0.5 µg/ml were added to each of two test tubes and mixed.

2. 1ml of glacial acetic acid was then added to all the five tubes and mixed.

(e) Oxidation

1. 0.5ml of 3% $KMnO_4$ was added to each tube, mixed and allowed to stand for exactly two minutes.

2. After two minutes, 0.5ml of 3% hydrogen peroxide were added and mixed thoroughly. The purplish colour disappeared within 10-15 seconds.

(f) Fluorometry

1. The instrument was operated according to manufacturers instruction.

2. Before attempting to measure the fluorescence of sample solutions containing riboflavin, it was necessary to determine that the fluorometer gave a linear response to different concentrations of standard riboflavin solutions.

3. It was adjusted before each reading to give a reading of 1.00 on the digital scale using the riboflavin working standard of highest concentration.

Blank Reading

1. About 20mg of dithionite was added with mixing to the samples of standard solution in the cuvet after sample reading had been taken ('A') and fluorescence taken within 10 to 30 seconds ('C').

2. This reading corresponding to the blank for the riboflavin is converted into non fluorescent form by the dithionite.

3. Fluorescence of the extracts containing added riboflavin were measured. - reading 'B'.

Calculation:- Riboflavin: Content of Sample in $\mu g/g$

$$= \frac{A - C}{B - A} \times \frac{\text{riboflavin increment}}{\text{10ml aliquot.}} \times \text{dilution factor} \times \frac{1}{\text{Sample weight}}$$

$$= \frac{A - C}{B - A} \times \frac{0.5}{10} \times \frac{100}{50} \times \frac{20}{100} \times \frac{1}{\text{Weight}}$$

$$= \frac{A - C}{B - A} \times \frac{10}{\text{weight.}}$$

(D) Determination of Carotene by Chromatographic Method.
A.O.V.C. p.104

Apparatus:- 50ml beakers.

fitted glass funnels.

filter flasks, 500ml.

Separatory funnels

Funnels

500ml, 250ml erlenmeyer flasks.

Steam bath.

Adsorption tube.

Plunger.

Volumetric flasks:- 25ml, 250ml, 100ml.

Spectronic 20, Antibumping granules.

Reagents:-

Acetone; petroleum ether 60-80°C; Glycerine; anhydrous sodium sulphate granular; glass wool.

The adsorbent employed was Aluminium oxide, which allows separation of isomers which cannot be distinguished on other adsorbent. (Strain, 1942).

Eluent:- 3% in petroleum ether.

Carotene standard solution: 50mg of a 90% β , 10% α carotene mixture was dissolved in 500ml petroleum ether. From this calibration curve was prepared using 0.1 to 0.5mg carotenes/ml.

1. Procedure:

(a) 5g of the freeze dried powdered sample was weighed into a 50ml beaker.

(b) The weighed sample was transferred into a fritted glass funnel rinsing with 5 to 10ml portions of water.

(c) The water was drawn off under reduced pressure until only a thick paste remained. Suction was discontinued.

(d) 15 to 20ml of acetone was added and stirred for at least 30 seconds.

(e) Most of the acetone was then drawn off with suction but not to dryness to avoid oxidation of the pigments.

(f) The extraction was continued by stirring with successive 20ml portions of acetone and removing the solvent each time until the extract and residue are colourless.

(g) The contents of the filter flask was quantitatively transferred into a separatory funnel.

(h) The flask was rinsed with two 10ml portions of water and three 15ml portions of petroleum ether.

(i) The acetone water solution was extracted 3 to 4 times with 50ml petroleum ether.

(j) The combined extracts were washed three times with 100ml distilled water.

(k) The petroleum ether extract was filtered through approximately 10g of sodium sulphate contained in a funnel into an erlenmeyer flask.

(L) The separatory funnel and Na_2SO_4 was rinsed with small portions of petroleum ether until both were free of pigment. Several antibumping granules were added and the extract was evaporated to about 20ml in a rotatory evaporator.

2. Purification

(a) Preparation of Column:- Packed dry under vacuum to depth of about 10cm.

(b) 1 cm of Na_2SO_4 was placed with 25-50ml of the petroleum ether, while the last 5ml of the petroleum ether was still above the sodium sulphate, the adsorption column was transferred to a clear filter flask.

3. Adsorption and Elution

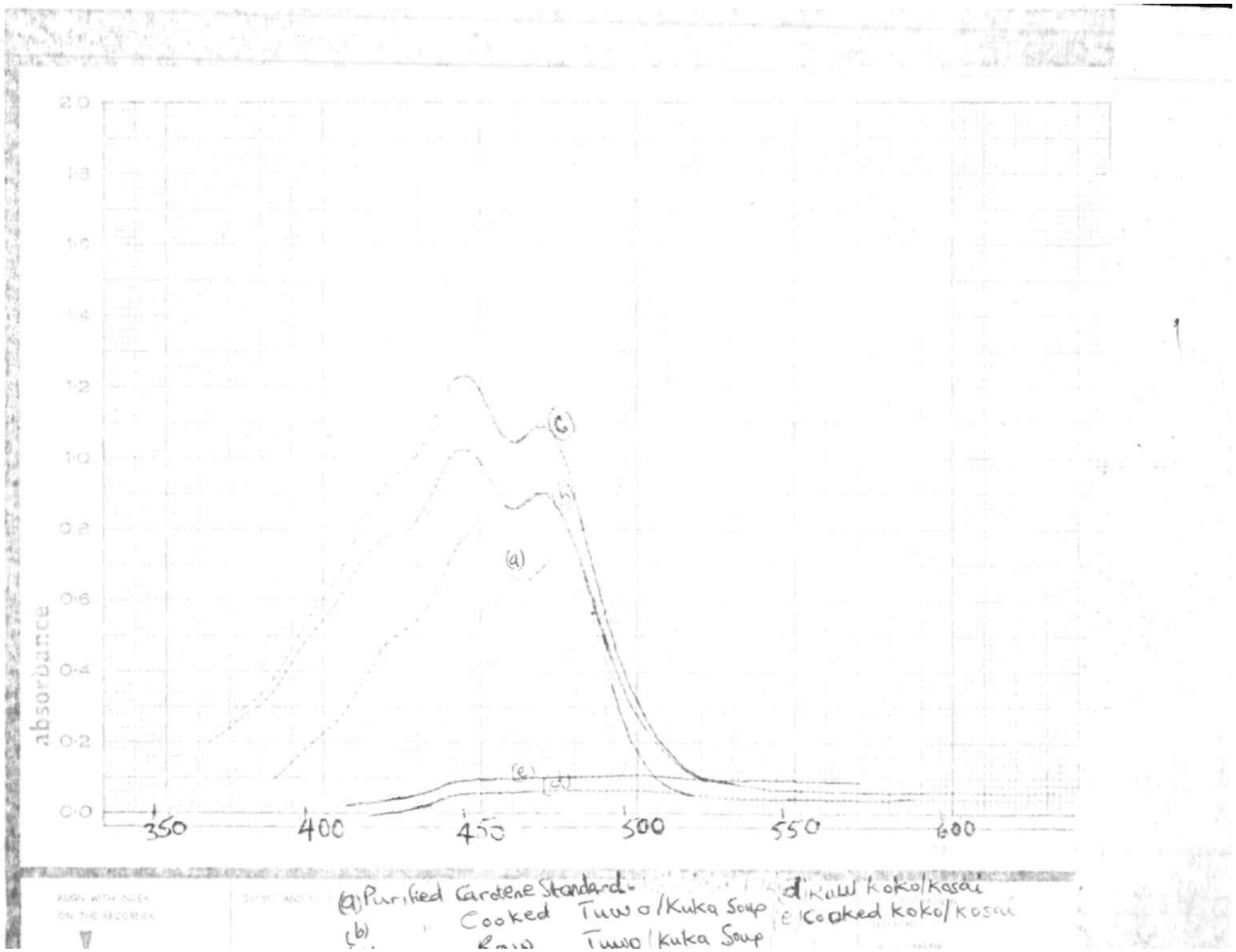
(a) The extract was poured onto the column.

(b) The flask was rinsed with 10ml portions of eluent, pouring each rinsing onto the column before the previous one has been entirely absorbed into the sodium sulphate.

(c) The washings were continued until the desired pigments had moved off the column and the filtrate colourless.

(d) The contents of the filter flask was transferred into a 100ml volumetric flask and diluted to volume with eluent.

(e) The total eluate was then evaporated to a convenient volume using rotary evaporator.



(a) Purified Carotene Standard.
(b) Cooked Tuwo/Kuka Soup
(c) Raw Tuwo/Kuka Soup
(d) Raw koko/kosai
(e) Cooked koko/kosai

4. Determination of Concentration of Carotene

(a) The extracts and the standard carotene of a particular concentration (0.4mg/ml) were first scanned through in an S.P.800 to determine the wavelength of maximum absorbance.

(b) The absorbance of the extracts and the standard carotene solutions were determined at this wavelength, i.e. 450nm using Spectronic 20.

(c) The concentration of carotene in the extract was determined by reference to a calibration curve prepared by plotting the absorbance against concentration for a series of carotene standard solutions.

Calculation:- mg of carotene per g.

$$= \frac{\text{Concentration} \times \text{final volume}}{\text{Wt. Sample} \times \text{dilution}}$$

$$= \text{Concentration} \times \frac{\text{final Volume}}{\text{Wt. of Sample}} \times \text{dilution.}$$

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