Studies on the Effect of Traditional Culinary Methods on the Digestibility of Local Cowpea (Vigna unguiculata) Protein.

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by

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DECLARATION

The work reported in these studies was carried out entirely by me, and nothing out of it either whole or in part, has been submitted in support of an application for another degree or qualification to any other University, or Institution of learning

.....Candidate

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D E D I C A T I O N

As I walked down the street of life, these men stood at the corners and showed me the way:-

Dr. Tai Solarin
Dr. E. Sayle
Professor J. Landon
Professor F. Aylward (late)
Professor J. Olu Mabayoje

May this volume serve as a small token of appreciation for their counsel and guidance.

Hippocrates maintained that the medical art was but a refinement of the art of good nourishment.

R.E. Hughes.

ABSTRACT

A comprehensive review of the origin of cowpea, (Vigna unquiculata) its place in the diet of the people of West Africa and in particular Nigeria, its nutritive value and the inherent anti-nutritional factors, together with the biological mechanisms affecting protein digestion is given.

The search revealed that there are about 39 varieties of cowpea in circulation within West Africa, of which 4 (with average protein content of 26.7 per cent) are major in the diet of the people. These are either eaten whole with Gari (fermented manioc), dodo (fried plantain), rice, made into stew, or paste and then either fried into Akara balls or steamed into moin-moin cakes.

A scientific approach to the culinary preparation of these dishes was undertaken, and the effect of these processes on the inherent anti-nutritional factors (polyphenols, trypsin inhibitor and lectins) and on protein digestibility was studied biochemically.

A method suitable for the extraction of cowpea polyphenols was developed. The polyphenol contents of the cowpea extracts (raw and processed) were determined using Folin-Denis redox method for the hydrolysable

tannins and the improved Vanillin-hydrochloric acid method of Price et al., (1978) for the condensed tannins. These are expressed as tannic acid equivalent and catechin equivalent respectively.

Results show that the catechin equivalents were higher than the tannic acid equivalent values in the raw cowpea; this correlates with the intensity of pigmentation. Processing however, reduced the extractable polyphenols expressed either as tannic acid equivalent or as catechin equivalent. The greater reductions in value being in the catechin equivalent, indicating that these are either structurally modified, chemically unassayable or bound in a covalent manner to the cowpea macromolecules. The use of immobilised protein in affinity chromatography technique revealed that between 72 and 97 per cent of the extractable polyphenols are protein-binding, cooking reduces this to between 9 and 42 per cent, depending on the variety.

Trypsin inhibitory activity was determined using improved Kakade et al., (1974) procedure. Processing (pre soaking of beans, removal of testa and heat treatment) caused reduction in inhibitory levels. A strong correlation (r = 0.875) between catechin equivalent level and trypsin inhibitory unit (T.I.U) was obtained indicating that

the condensed tannins are partly responsible for the lower digestibility of tannin protein complexes or proteolytic enzyme inhibition by tannins.

A weak negative correlation ($r = -0.543$) between T.I.U. and cowpea protein hydrolysis using multienzymes technique was also obtained, indicating that the polyphenol content is well correlated with the diminished digestibility of the cowpea protein.

In vivo feeding trials ('food approach') reveals differences in apparent digestibility of cowpea meal with varying nitrogen content, indicating that the apparent digestibility of food is dependent on the protein concentration in the particular meal. Average gut transit time for a bean meal is in the neighbourhood of 25.9 ± 4.2 hrs.

It is suggested that in order to get the best nutritional value out of beans, testae are to be removed preferably by dry threshing and winnowing prior to cooking into dishes requiring removal of testae. But because people have strong colour preference in beans eaten whole or made into stew, it is recommended that the beans be soaked in water for about ½ hr and the soaking water discarded prior to boiling in water.

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ABBREVIATIONS

The following abbreviations have been used in the thesis:-

BAPNA	α-N-benzoyl-DL-arginine-p-nitro-anilide HCl
BSA	Bovine serum Albumin
ca.	Cicer (about, or approximately)
cf.	Confer (refer to)
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
E.T.F.F.	Extractable Tannin Free Fraction
H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
H_2O_2	Hydrogen peroxide
SDS	Sodium dodecyl sulphate
TRIS	Tris (hydroxymethyl) amino-methane
TCA	Trichloroacetic acid
T.I.U.	Trypsin Inhibitor Unit

Agriculture is the basis upon which civilization is built, and the right production, distribution, and use of food is the basis for world health.

INTRODUCTION

Of all plants used by man, only the grasses are more important than the legumes. However, while enormous resources have been expended in recent decades on grasses like rice, wheat, corn, sorghum and barley, among the legumes, only soybeans and peanut (groundnuts) have received much attention. Yet it is the family Leguminosae that shows most promise for producing the vastly increased supplies of vegetable protein that the world will need in the near future. In developing countries especially, cultivation of legumes is the best and quickest way to augment the production of food proteins (Aykroyd & Doughty, 1964).

Leguminous plants are found throughout the world, but the greatest variety grows in the tropics and sub-tropics. With approximately 650 genera, and 18,000 species, Leguminosae is the third largest family of flowering plants (after Compositae and Orchidaceae).

Legume seeds (also called beans, grain legumes, or pulses) are second only to cereals as a source of human and animal food. Nutritionally, they are 2-3 times richer in protein than cereal grains.

Before the potato was introduced, beans constituted much of the diet of the poorer classes of Europe ('poor man's meat'), a designation of interest and importance

from historical and other standpoints. In the first place, it implies a meat substitute. A fact that is older than the discovery that legumes have a high protein content suggest that this nutritional characteristic was dimly realised before the demonstration by chemical analysis. In line with this suggestion, is the recommendation of legumes by the Church in Medieval times as suitable food during the Lenten fast, when little or no meat was eaten. Today, they remain as major foods in Latin America (especially Phaseolus vulgaris) on the Indian sub continent (Lentils - Lens esculenta; pigeon peas Cajanus cajan; and chick peas Cicer arietinum; Far East (soybean Glycine max) and in West Africa especially Nigeria, (Cowpea, Vigna unguiculata).

Legumes are natural protein supplements for cereal or cassava-based diets (although, the protein quality suffers from a deficiency of sulphur amino acids) but with proper combination of food legumes with various cereals, protein nutritive value could be raised to that of animal protein (Bressani and Elias, 1974).

Against this background, however, is the revelation that legume grains contain trypsin inhibitors (Read and Haas 1938, Bowman 1944, Ham and Sandstedt, 1944, Kunitz, 1945 and 1946; Rackis, 1965). Haemagglutinins (Liener, 1962) growth inhibitors (Weaver, 1955, Borchers, 1965)

and flatus factors (Steggerda and Dimmick, 1966, Kakade and Borchers, 1967, Rackis, 1974) which if not removed, inactivated or destroyed will have undesirable effects on the nutritive value of the food and may even result in toxic reactions.

A perhaps more important undesirable effect on nutritive value of legume proteins is the unavailability of many of their essential amino acids, even when they are cooked to the point at which all the known toxic factors have been destroyed; evidence indicates that many of the essential amino acids are not available for growth. This suggests the presence of other anti-nutritive factors. (Liener, 1977).

CHAPTER 1

A review of the current knowledge on cowpea, Nigerian foods and food habits, anti-nutritive factors in grain legumes, factors affecting protein digestion, and aims of the present study.

What little we know, what little power we possess, we owe to the accumulated endeavours of our ancestors. Mere gratefulness would already oblige us to study the history of the endeavours, our most precious heirlooms. But we are not to remain idle spectators. It is not enough to appreciate and admire what our ancestors did, we must take up their best tradition, and that implies expert knowledge and craftsmanship, science and practice.

George Sarton

LITERATURE REVIEW

1.10 Origin and Production of the Cowpea

The cowpea is one of the oldest of human food sources and has probably been used as a crop since Neolithic times (Chevalier, 1944). Due to lack of archaeological evidence the centre of origin of cowpea is uncertain and has been variously reported as possibly Asia, Africa, Persia, or even South America (Summerfield et al., 1974). However, Faris, (1965), concluded that the progenitor was wild Vigna unguiculata, probably subspecies dekindtiana, of the African savanna zone, since no other species of Vigna produces fertile progeny when crossed with cultivars. Steele (Summerfield et al., 1974) in agreement with Sauer, (1952) proposed a solely Ethiopian Centre of origin and suggested that cowpea were interplanted there with sorghum and perhaps, pearl millet, subsequently to evolve predominantly in the ancient cereal farming systems of the Savanna zone of Africa. Others consider the cowpea to be of Asiatic origin. Burkill nevertheless, concluded, that although of contraversial origin, the cowpea was introduced into Europe early enough for the Greeks and Romans to grow it under the names of Phaseolos, Phaseolus, or Phaselus, Vavilov (1950), partly agreed with Wright, (1907), in

respect of Asiatic origin and considered China and Abyssinia, now Ethiopia, as secondary source of origin. There is every possibility that the crop could have been carried along the coastal and Indian trade routes, though at present it is impossible to be certain whether migration started in Africa, Asia or both. What is certain is that the cowpea was not introduced into the New World until the late seventeenth century and probably reached the Southern States of the U.S.A. in the early eighteenth century (Wright, 1970). There it is known as the black eyed pea, although the cultivated cowpeas were known in Sanskritic times (Watt, 1908).

Cowpeas are grown extensively throughout the lowland tropics of Africa in a broad belt along the Southern fringe of the Sahara and in eastern Africa from Ethiopia to South Africa. They are mainly confined to the hot semi-arid to sub-humid areas with significant production in Nigeria (which alone produces about 61 per cent of the world crop); Niger, Upper Volta, Uganda and Senegal (Rachie and Roberts, 1974). A factor which determines the distribution of many cowpea varieties is the relationship between (a) the periodicity and degree of humidity and (b) the incidence of disease. They are extensively grown in India, South Eastern Asia, Australia, the Caribbean, lowland and coastal areas of South and

Central America, and in the Southern regions of the United States. Because of the habit of the plant, and prolonged period of pod production of many local varieties, the cowpea is more suited to subsistence, rather than commercial farming.

In Nigeria, cowpea is the most important indigenous grain legume and is found in most areas north of the confluence of the Rivers Niger and Benue. Informed opinions estimate that over 80 per cent of the total cowpeas produced in Nigeria are grown north of latitude 10°N where they are traditionally interplanted with other crops. It is an important item in the diet of West Africans (thirty-nine varieties are in use) as it is a rich source of plant protein (about 24%). It is eaten in various ways, either alone or mixed with maize, rice dodo (fried plantain) or gari. Beans flour is made into fried ('Akara' - Yoruba) or steamed cakes - ('Moin-moin' - Yoruba). It is in recognition of this importance as a good source of protein that the Food and Agriculture Organisation (FAO 1966) recommended that efforts be devoted to increasing cowpea consumption.

The classification and nomenclature of cowpeas are confused. An extensive review on this topic is produced by Sellschop, (1962) and Summerfield et al., (1974).

The correct name for the cultivated cowpea is Vigna unguiculata (L.) Walp.

Seeds vary considerably in size, shape and colour. Overall, they are 2-12 mm long and weigh 5-30 g/100 seeds. Their shape is correlated with that of the pod. Where the individual seeds are separated from adjacent ones during development, they become kidney shaped, but as crowding within the pod increases the seeds become globular and are called "crowders" in the USA. The testa may be smooth or wrinkled and white, green, buff, red, brown black and variously speckled, mottled, blotched or eyed (hilum white surrounded by a dark ring) in colour Saunders (1959 and 1960). Some varieties have probably resulted from natural hybridization, however seldom that they may have taken place, while others are the results of incidental selections and, more recently, the contributions from planned breeding projects.

1.11 Nutritional Value and Acceptability of Cowpea

The cowpea forms a major component in many African diets, not only on the basis of its high protein content, but also for calcium (90 mg/100g), iron (6-7 mg/100g; nicotinic acid (2.0 mg/100g); vitamin A (20 I.U.), and thiamine (0.9 mg/100g) (Platt, 1962). While Ogunmodede and Oyenuga (1969 and 1970) working with different

varieties (black eye; blue eye; and 'brown type') of locally grown cowpea, came out with the mean values for thiamine: 0.85 mg/100g; 0.584 mg/100g; 1.3 mg/100g, respectively. Mean values for riboflavin content are as follows: 0.144; 0.173; and 0.295 mg/100g respectively. Niacin content: 1.10; 1.17; 1.42 mg/100g respectively. Vitamin B_6 activity are 0.344; 0.292, and 0.402 mg/100g respectively. Biotin content are 18.4; 25.2; and 21.2 µg/100g respectively. Pantothenic acid content as 2.0; 2.18; and 1.82 mg/100g respectively. Folic acid content as 0.16; 0.15; and 0.16 mg/100g respectively.

The seeds of the cowpea are poor in oil content, yield is about 1 per cent, but chemically it was found to contain linolenic acid 7.1 per cent; linoleic acid 37.2 per cent; oleic acid 37.8 per cent and saturated fatty acids 17.9 per cent (Chowduri and Bagchi, 1957); Stigmasterol ($C_{29}H_{48}O$), 0.025 per cent (Chakraverti et al., 1956); Phytin 53.92 per cent Sundararajan, 1938); Carbohydrate 56-57 per cent (Johnson and Raymond, 1964).

Nigam and Giri, (1961) fractionated the sugars of cowpea and found out that it contains sucrose 1.5 g/100g; raffinose 0.4 g/100g; stachyose 2.0 g/100g; and verbascose 3.1 g/100g. Swaminathan (1937-38) found the

total nitrogen in the sample he analysed to be 4.1 per cent of which 8.8 per cent was non-protein nitrogen. Fibre and ash content are 3.9 and 3.6 per cent respectively.

In cereal based diets the lysine content of cowpea is very important and becomes more so as the proportion of the total protein in the diet derived from cereals increases (Dema, 1963). The relatively high levels of dietary lysine in South Western Nigeria are attributed to the greater use of cowpea by the Yorubas - (Annegers, 1974). It is believed that beans particularly cowpeas sometimes contribute as much as 60 per cent or more of the total protein intake for the families living in many areas of Western Nigeria (Fennell, 1963). In root and tuber diets of the humid tropics of Africa, cowpeas are important both as source of calories especially where there is much reliance on manual labour for all types of work (Lucas, 1968); and a relatively cheap source of protein, where the staple foods contain only about 2 per cent protein. Although the percentage protein in cowpea seeds is high, varying between 19 per cent and 26 per cent (Russell, 1946), like other legumes the seeds are deficient in the sulphur amino acids (methionine and cystine (Sallschop, 1962; Harvey, 1970). In a survey of the amino acid profile of six Nigerian cultivars and the wild subspecies dekindtiana, methionine was found to

vary from 0.35 to 0.90 per cent of the total protein in the cultivars, but it was 1.47 per cent in the wild cowpea. Cystine ranged from 0.38 to 0.90 per cent in cultivars, though a value of 2.0 per cent has been reported (Evans and Bandemer, 1967).

To upgrade the sulphur amino acid limiting in legumes, Boulter et al., (1973) have suggested manipulating the relative ratio of storage proteins in legumes with different sulphur amino acid content. Identical situation in maize is illustrated by the high lysine varieties such as opaque 2.

Although, seed colour and size are important determinants of consumer preference, (Ojomo, 1968) there is no evidence that variation in these characters is associated with variation in their nutritive value. Preference within varieties is due primarily to local adaptation and taste. In Maiduguri for instance, only three main varieties are commonly available; whereas in Accra and Ibadan, twenty-two and fifteen varieties respectively are available in the market, Dovlo et al., (1976). The choice of the variety to be used for a particular dish is very important. Taste, swelling capacity and quick cooking are the most important characteristics for cowpea

that are to be used in plain cooking (Ojomo, 1968). When cooking cowpeas with rice, colour is also important and a red or golden brown variety that does not "bleed" its colour is most preferred. The red or brown varieties are also the most popular for stews because they usually exhibit binding qualities rather than "grainy" characteristics Dovlo et al., (1976). Varieties that are used for processing should have different physical and chemical properties. Binding quality, short soaking time, ease of dehulling, fast grinding ability, foaming capacity, fine texture, finished appearance of the paste, and flavour are judged most important qualities. The most popular varieties amongst the Yorubas - Lagos State; Ogun State, Oyo State and Ondo State (the highest consumer of cowpea in the country as borne out by questionnaire returns:- (Appendix II) are Ibadan Brown and Ife Brown varieties for plain cooking and Frejon. Ibadan white, or black-eyed, Igbirra, and Mala varieties for processing into Akara balls and Moyin Moyin, whilst Ewa Ibeji variety is for fetish ceremonies. An early account of the place of cowpea in the dietary life of West Africans is given by Dalziel (1937).

The most comprehensive review on legume consumption in Africa was undertaken for the Bukavic Technical Meeting on legumes in Agriculture and Human Nutrition (FAO archives of meeting) was reported by Aykroyd and Doughty (1964). The results compiled from 97 surveys in 50 areas from 13 countries in Africa showed a wide range of legume consumption, 50 per cent of the respondents eat between 10-50 grammes of cowpea per head per day; 23 per cent of the respondents eat between 50-150 grammes of cowpea per head per day, whilst 2 per cent of the respondents eat over 150 grammes of cowpea per head per day and only 25 per cent of the respondents eat between nothing and 10 grammes of cowpea per head per day. Almost identical pattern of consumption was recorded in the studies carried out by Williams, (1974) in Nigerian household. As an article of diet, the people recognise the disadvantages incidental to beans in general, uncertain of digestion and degree of absorption. Hqusa folk-sayings or nicknames refer to the unsatisfactory properties of the beans in the field, in the pot and in the

stomach, Dalziel, (1937). The Yorubas too have a slogan which recognises its unsatisfactory properties and thereby crack jokes that beans is not what one takes for supper (Ewa ki nse onje ajesun). Mothers in West Africa are reluctant to feed cowpeas to their children for fear of causing indigestion Dovlo et al., (1976).

1.12 Dietary and Culinary Practices

The basic psychology of nutrition admits that what people eat depends on what they can get and what they choose and that the feeding customs which have become established through centuries of trial and error have developed as part of the people's reaction to the total environment in which they have learned to survive. The choice of food sometimes causes individuals and people to suffer from malnutrition. Ignorance of the type of food to be eaten contributes to unwise choice in many cases.

Africans are omnivorous. Some eat snakes and snails, others do not; some eat crabs, lobsters, turtle, oysters and shrimps, others do not. Lizard, crocodile and alligators are to some a horror, while to others they are delicacies. Locusts and some species of caterpillars, are in some areas highly prized. In dry seasons if locusts prevail, men, women and children give up their day's undertaking to collect them.

Nigeria is basically an agricultural nation, with more than 80 per cent of the population living in the rural areas. A typical Nigerian diet in spite of regional and state differences and variations in available foodstuffs is known to be high in carbohydrates, low in animal proteins but reputably high in plant protein (Palmer, 1972) and adequate in fat.

Most African foods do not cook long. Fufu takes about half hour to serve, and most artistocratic meal - jollof rice cooks for two hours. Unless other materials such as beans and peas are dried, they cook within an hour. Africans steam rice as the Chinese do, not liking it when it is gummy. But nowadays parboiled polished rice abound in the market and are preferred to the local grains because of its cheaper cost, cooking quality, appeal and taste. In general Nigerians cook fresh meat, fish (although scarcity in the recent past has led to the introduction of frozen, imported meat and fish, nicknamed 'Muritala' and 'Oku-Eko' respectively) and anything else until it is just tender enough to leave something chewable for the good of the teeth. Because of this and for the reason that most of the food materials were usually fresh, Africans did not need many dentists, but the introduction of convenient foods and increase

dependence on imported frozen-perishable food materials, and sugary foods, the incidence of dental caries is on the upward trend. Flavouring and spicing make food appetizing and flagrant. The onions are basic in frying tasty foods and vegetable oil (Palmoil, groundnut oil, "Egusi - Yoruba (melon seed) oil; Sheabutter, coconut oil) takes the place of lard. Whether we roast, boil, broil, stew or bake, the custom is to cook and serve many ingredients in two or three vessels. Women cook in the courtyard, or nowadays either in a kitchen separate from the house or in modern well planned 20th century kitchen with gas/electric cookers. In the villages, the kitchen is often shared by wives of the same husband and by other women of the same social group. Cooking is done in clay pots, set on three stone fire-stands (cooking supports for pots) over a fire. Fuel is a problem, usually firewood is used but this is now becoming scarce; stalks of corn are used when available. Some people use dried animal dung that could be better used for fertilizer.

In the urban centres where there are 20th century kitchens, cooking with domestic cooking gas (butane) is in vogue. Instead of clay pots the modern housewives use enamel or aluminium pots. One striking difference one notices is that meals prepared in the traditional way - i.e.

cooked in clay pots on open firewood 'oven' taste better than those prepared in aluminium or enamel pots on gas stoves. (Personal experience and group discussion feedbacks). In both cases, stews are made to last for up to 3 days and where there is no means of refrigeration, the stew is warmed daily to prevent spoilage. This may have adverse effect both on the nutrients and vitamins. In homes where there are refrigeration systems, and awareness of basic nutrition, housewives have resorted into aliquoting the stew into portions for each day's consumption. These are refrigerated and only the day's need is taken out, thawed, and warmed for the table, thereby ensuring the preservation of the nutrients.

1.13 Plant Proteins: Merit and Demerit

The nutritive importance of proteins and the dependence of animals on plants for these substances were first pointed out by Mulder around 1840. He pointed out that "in both plants and animals a substance is contained which is produced within the former, and imparted through their food to the latter. It is unquestionably the most important of all known substances in the organic kingdom. Without it, no life appears possible on this planet. Through its means the chief phenomena of life are produced".

A few years later Boussingault (1946) indicated that

the alimentary virtues of plants reside above all in the nitrogenous substance which they produce. Furthermore he asserted that their nutritive quality is proportional to the quantity of nitrogen entering into their composition.

For years, there was great controversy over the nutritional equality of plant and animal proteins. Matters however came to a head when Rubner (1897) recognised that proteins from different sources vary in their amino acid composition. This was to be further confirmed by Osborne (1907) in his monograph on 'The protein of Wheat kernel', wherein he concluded that the lysine content of wheat gluten is small when compared to that of leguminous seed. He went further to assert that histidine level in wheat gluten was almost the same as for other seed proteins. These differences have since been confirmed by later investigators. From the ongoing it became clear that all the amino acids necessary for animal nutrition are contained in plant proteins, nevertheless, certain of these amino acids are however present in such limited amounts as to restrict the extent to which the ones which are more abundant can be utilised. It is for this reason that these plant proteins are of relatively low biological value unless supplemented.

1.14 Legume Seed Protein

Protein present in the seed may be broadly described as metabolic and structural or storage protein. Metabolic protein tend to be present in relatively small quantities of a very large number of different types and are concerned with the metabolism of the seed during its development and hence in the synthesis of the storage protein and subsequent germination.

Storage protein on the other hand can be defined as that protein which is synthesised in the developing seed, and is subsequently degraded during germination, acting as a source of nitrogen for the developing seedlings.

In contrast to metabolic proteins, storage proteins often display no enzymatic activity and the very large amounts that are present (in the dry seed, storage protein frequently constitutes 80 per cent or more of the protein present) tend to be composed of relatively small number of types.

Osborne and Campbell (1897) identified the principal protein of cowpea - a globulin, naming it vignin and also reported two other globulins. Osborne and Heyl (1908) fractionated the individual amino acid of Vignin and were able to account for about 60 per cent of the nitrogen in the original protein. Other workers Brewster and

Alsberg (1919), Niyogi et al., (1932), also attempted to characterise the globulin of cowpea using Van Slyke method. Osborne and Mendel (1912) conducting feeding trials with rats found that Vignin (the major protein of cowpea) possesses a growth-promoting value slightly greater than wheat gliadin, though distinctly below that of casein.

Carasco et al., (1977) working with cowpea also found that globulin protein formed the major (80-90%) protein fraction of matured seeds of cowpea, and that it was heterogeneous when examined by using chromatography and zonal isoelectric precipitation. Both 7S and 11S globulin were present and the fraction was dissociated by sodium dodecyl sulphate (SDS) treatment into three major sub-units with apparent molecular weights: 56,000; 54,000 and 52,000 as determined in SDS-acrylamide gels. Two of the major subunits had low but different contents of sulphur-containing amino acid residues and were probably subunits of 7S glycoproteins. It was concluded that the essential amino acid content of the meal is determined by that of the globulin fraction except for cysteine/cystine which is mostly supplied by the albumin fraction, the latter probably containing some proteins rich in sulphur-containing amino acids.

Osborne and Campbell (1898) showed that much of the protein of legume seeds was salt soluble globulin and they were able to separate this fraction from Pisum sativum into 2 major fractions - legumin and vicilin. Osborne (1894) further demonstrated that similar protein fractions could be extracted from other legume seeds, but that their chemical composition would differ. Carasco (1976) on the other hand, working with cowpea cr. Prima was unable to isolate legumin and thereby concluded that legumin is either absent or it occurs in small quantity in this cultivar.

Carasco et al., (1977) admitted that the major seed proteins of legumes are globulins. These include 7S and 11S globulinsand the ratio of these is different in different species; for example it is 1:4 in Vicia faba (Wright and Boulter 1972) and 9:1 in Phaseolus vulgaris (Derbyshire and Boulter, 1976). The small number of 11S globulins which have been adequately characterised are similar to each other (Derbyshire et al., 1976a) and legumin from Vicia faba for example is typical of the group. The major protein fraction in cowpea is 7S globulin and the data obtained by ultracentrifugation together with electrophoretic data (Derbyshire et al., 1976b) indicate that it is heterogeneous; although cowpea has been placed close to Phaseolus vulgaris in traditional classifications,

the 7S globulin of cowpea is different from that of Phaseolus vulgaris (Joubert, 1957; Derbyshire et al., 1976b; Barker et al., 1976). The 11S globulin from cowpea has not been extensively characterised but the limited data available suggest that it is a typical legumin-like 11S globulin. In cowpeas, the methionine contents of vicilin and legumin are about the same, whereas that of cysteine in vicilin is much lower (Boulter et al., 1973) and much of the supplemented methionine used in rat-feeding experiments with cowpea meal is used to supply cysteine (Boulter et al., 1973). The other major protein fraction of legume seeds is the albumin fraction (Boulter and Derbyshire 1971). Although albumin proteins have not been fully separated and characterised, it is generally accepted that the various enzymes of the seed occur with this fraction. Separations of the albumins of various legume seeds on polyacrylamide gels normally give electrophoretigrams with 20-30 protein staining bands (Fox et al., 1964)

1.20 Toxic constituents

Having extolled the virtues of the plant proteins, it must be admitted that there are certain and distinct setbacks to their nutritional utility. For reasons that are yet to be explained, nature has deemed fit to endow

many plants with the capacity to synthesize a wide variety of chemical substances which are known to exert a deleterious effect when ingested by man or animal (Liener, 1973).

Toxicity of a substance is its intrinsic capacity to cause harm in a living organism; the hazard is the capacity to produce a harmful effect under conditions of use.

All substances are potentially toxic but are hazardous only if consumed in sufficiently large amounts. For example, abnormally high intake by normal individual of the common table salt and some vitamins and minerals can be toxic. Although, abnormal health or physiological make up of any individual consumer can also reveal a toxic potential toward food components. Also, dietary oxalates normally have little nutritional significance, but adverse effects may manifest in diets that are low in calcium or vitamin D or both.

Toxicity may also be species specific. For example, raw soybean meal inhibits growth of young rats and chickens, but not dogs (Rackis, 1974). Toxicity is, therefore, used when referring to those substances in food which produce some kind of deleterious effect in man or animals, whether be it an acute lethal effect, or a

chronic effect resulting in poor growth, hypertrophy of the pancreas and thyroid, or decreased availability of proteins, vitamins and minerals.

1.21. Protease Inhibitors

It was not long after soybeans were introduced into the United States that Osborne and Mendel (1917) made the significant observation that soybeans had to be heated in order to support the normal growth of rats.

Bhagvat (1937) however was the first to demonstrate the slower rate in which certain plant proteins were digested by pancreatic enzymes than those of animal proteins. The subsequent discovery (Ham and Sandstedt, 1944, Bowman, 1944) and purification (Kunitz, 1945; Kunitz, 1946) of a heat labile protein in soybeans which had the unique property of combining with the digestive enzyme trypsin to form an inactive complex, strengthened the concept that this trypsin inhibitor was responsible, at least in part, for the observed growth depression. Furthermore, active antitryptic fractions extracted from unheated soybeans, when incorporated into diets were shown to retard the growth of rats (Klose et al., 1946, Borchers et al., 1948, Liener et al., 1949), chicks (Ham et al., 1945, Borchers et al., 1948) and mice (Westfall et al., 1948). Since the protein efficiency of partially heated

soyflours was found to increase in proportion to the destruction of the trypsin inhibitory activity, Westfall and Hauge (1948) concluded that the trypsin inhibitor was the major cause for the poor utilization of the protein in raw soybeans. Hayard and Hafner (1941) also showed improved utilization of unheated (raw) soybean meal (to the same extent as proper heating) when supplemented with methionine or cystine. It was however interesting to find that addition of methionine did not raise the nutritive value of raw soybean meal to the level of heated one similarly supplemented with methionine.

Carrol et al., (1952) demonstrated that the net absorption of nitrogen sulphur and methionine itself from the digestive tract of the rat was essentially the same for both raw and heated soybeans and that difference in nitrogen absorption in the rat seems to be confined to the terminal end of the small intestine, whereas over twice as much nitrogen is absorbed from heated soybean than from the raw. It therefore shows that a considerable portion of the nitrogen from raw soybean meal which escapes hydrolysis in the small intestine must be absorbed from the large intestine, and has little utility for growth. Therefore, according to Carrol et al., (1952), the site of absorption rather than the net absorption is of significance

in the rats. In the chick, however, the net absorption of protein is significantly less with the unheated soybean meal (Evans and McGinnis, 1948; Bouthilet et al., 1950; Nesheim and Garlich, 1966). Thus the basic difference between the rat and chick (an important species difference) as far as the absorption of nitrogen and sulphur from raw and heated soybeans is concerned is that less net nitrogen and sulphur is absorbed from raw soybean by the chick than by the rat.

From these studies there appeared to be little doubt that the poor growth-promoting quality of raw soybeans could be attributed to a large extent to a trypsin inhibitor. How this inhibitor functioned was not readily understood, since a difference in the digestibility between raw and cooked soybean did not appear to account for this observed growth depression. Melnick et al., (1946) on the basis of experiments involving the in vitro release of amino acids from soybean protein by pancreatin suggested that methionine was liberated more slowly by the proteolytic enzymes of the intestine than the other essential amino acids and was thus ineffectively utilized for protein synthesis. However, later in vitro studies did not appear to support this hypothesis since it was shown that the trypsin inhibitor does not

specifically retard the enzymatic release of methionine, but seemed to affect all amino acids to the same degree (Riesen et al., 1947; Ingram et al., 1949; Liener and Fevold, 1949). Further, active antitryptic preparations have been shown to retard growth of rats and chicks even when added to diets containing predigested protein (Desikachar and De 1947; Liener et al., 1949). Under these conditions intestinal proteolysis would not play a part with respect to the availability of essential amino acids.

When fed raw soybean meal (Chernick et al., 1948; Booth et al., 1960; Alumot and Nitsan, 1961), or crystalline trypsin inhibitor (Lyman and Lepkovsky, 1957) chicks and rats were found to develop marked hypertrophy of the pancreas. Contrary, to what had been generally assumed, the amount of trypsin found in the intestine of those experimental animals was actually greater than that found in the control animals fed heated soybean meal. These observations indicated that the growth depression was not a result of inhibition of proteolysis but rather the result of an endogenous loss of essential amino acids from a hyperactive pancreas which is responding in a compensatory manner to the effects of trypsin inhibitor (Booth et al., 1960). This is further supported by the

findings of Green (1972) and Niess (1972), who have presented evidence to indicate that trypsin or chymotrypsin in the intestine suppresses pancreatic enzyme secretion by feed-back inhibition and that trypsin inhibitors evoke increased enzyme secretion by counteracting the supression produced by trypsin thereby releasing pancreas-stimulating hormone cholecystokinin from the intestinal mucosa (Wilson et al., 1978). Since soybeans and legumes in general are low in the sulphur amino acids this would explain why, on addition of cystine or methionine to unheated soybean meal to counteract the loss of endogenous amino acids, protein utilization was found to be improved, essentially to the same extent as adequate heat treatment (Hayward and Hafner, 1941; Almquist et al., 1942; Russell et al., 1946; Evans and McGinnis, 1946; Clandinin et al., 1946; McGinnis and Evans 1947; Evans and McGinnis, 1948; Evans et al., 1951). There is increasing evidence to suggest that human trypsin exists in two forms, a cationic species which is the major component of human pancreatic juice and an anionic species which comprises about 10 to 20 per cent of the total trypsin activity (Figarella et al., 1975). While the latter is fully inactivated by the soybean inhibitor, the predominant cationic species is only weakly inhibited (Figarella et al., 1974).

Further support of the probability that the soybean inhibitor is relatively ineffective against human trypsin is the rather interesting relationship that appears to exist between the size of the pancreas of various species of animals and their sensitivity to pancreatic hypertrophy induced by raw soybeans or the inhibitor (Liener, 1977). It was demonstrated that the pancreas of these species of animals whose weight exceeds 0.3% of their body-weight become hypertrophic when fed raw soybeans, whereas those whose weight are below this value are insensitive to this effect. Since man has a pancreatic weight of 0.09 to 0.12% of his body weight it was assumed that human pancreas would be insensitive to this effect of soybean trypsin inhibitor. Gatehouse et al., (1980) characterized cowpea trypsin inhibitor and showed it to have molecular weight of ca. 17,000. The isolated inhibitor was a mixture of several iso-inhibitors. Some were able to inhibit chymotrypsin as well as trypsin. Complex formation with trypsin was demonstrated.

On the other hand, diets high in tannins have been shown to cause interference with pancreatic digestion (Drieger and Hatfield, 1972) irritation of the intestinal tract (Mitjavila et al., 1971), and to give rise to methionine deficiency (Singleton and Kratzer,

1969) by requiring active methyl groups in the detoxication process. Plant polyphenols and tannins have been demonstrated as heat stable factors which may reduce protein digestibility. Thus in high and low tannin sorghum (Armstrong et al., 1973), and broad beans (Martin-Tanguy, et al., 1977), the presence of plant phenolics in seeds adversely affect the utilization of protein. It has been found using Phaseolus vulgaris that colour of the seed coat and polyphenol content are correlated in their effect on the digestibility of the protein (Jaffe and Floves, 1975; Elias et al., 1979).

1.22 Polyphenols and Vegetable Tannins

Polyphenols form a heterogenous group of secondary plant products the majority of which in combination with compounds of a carbohydrate nature, either as esters or glycosides are located in the cell vacuole in the vegetative tissues of plant. Knowledge of their distribution, particularly in quantitative terms, is still fragmentary, although in some plants they make a substantial contribution to the weight of the tissue. Thus for example, up to 40 per cent of the dry matter of leaves of the tea plant, Camellis sinensis, is reported to be polyphenolic in character. The range of compounds collectively designated as vegetable tannins are those plant polyphenols characterised by high molecular weight

(500-3,000) and containing a sufficiently large number of phenolic groups to enable them to form effective cross-linkages with proteins and other macromolecules. This distinctive property has permitted their use for at least 2,000 years in the conversion of raw animal hides to durable permeable leathers. Tannins are categorized into two groups (as first suggested by Freudenberg, 1920) hydrolysable and condensed and according to Loomis and Battaile (1966) they react differently with polypeptides.

The study of tannins has been hindered by their complexity and discouraged by the absence of any apparent physiological role. It is believed, but not proven, that they contribute to plant protection by providing resistance against consumption by birds (Halloran and Maunder, 1971) and attack by insects and microorganisms (Axtell and Oswalt, 1972; Karrer, 1958). Most food tannins are polymeric condensed tannins in the molecular weight range of 500-3,000 (Haslam 1966; Swain, 1966), wherein they are thought to impart certain tastes and flavours (Bate-Smith 1973; Goldstein and Swain, 1965). Tannins occur universally in higher plants (more especially dicotyledonous plant families) and are present in many food crops in significant amount. The precise molecular structure of only a few of these compounds is known (Robinson, 1967;

Weingers and Piretti, 1971). Their interaction with proteins is believed to take place through hydrogen bonding (Gustavson, 1954, Haslam, 1966; Robinson, 1967) but only a few systematic studies (Calderon et al., 1968; Van Buren and Robinson, 1969) have been made of the structural, spacial and functional group requirements of the interaction.

Owing, presumably, to long evolutionary adaptation, the common plant phenolics as usually consumed are readily detoxified. It appears (Singleton and Kratzer, 1969) that carnivores tend to be more susceptible than herbivores to the acute toxicity of phenols, such as those from plants. Omnivores, for example man and rats, appear to be intermediate. This relative sensitivity seems to be true parenterally as well as orally, so that it does not appear to be exclusively an effect of digestive-tract microflora. Considering that the diet of a herbivore may contain nearly 20 per cent of its dry weight as lignin and other plant phenols, and that the diet of a carnivore has nearly none, an evolved difference in phenol tolerance therefore seems reasonable (Singleton and Kratzer, 1973). All phenols have some properties in common that render them potentially toxic to animals; the fact that most plant phenols are harmless as usually encountered in the diet

is largely the result of the animal's effective detoxification or non-absorption of them. But if the animal's detoxification mechanism be overloaded by massive amounts of phenolic derivatives in the diet or circumvented by unusual structures of the phenols or unusual circumstances of ingestion, toxicity is likely to become manifest.

Tannic acid is typical of the hydrolysable tannins. It is readily hydrolyzed enzymatically or hydrolyses spontaneously to glucose and gallic acid with about seven or less gallic acid units per glucose. Other tannins of this group may yield as hydrolysis products ellagic acid, which replaces gallic acid, or quinic acid which replaces glucose (Haslam, 1966). Tannic acid has been the tannin most used in medical treatment. It has been used in barium enemas to improve definition of the colon wall in diagnostic x-rays. Severe acute liver damage, sometimes fatal, was produced in a small proportion of the patients (Singleton and Kratzer, 1969; Janower et al., 1967). The toxicity of tannic acid when administered rectally is about twice that of the substance when given orally (Boyd et al., 1965). The incidence of serious reactions after tannin enemas was greater when administered to juvenile patients or when the enemas were given repeatedly or when the patient had pre-existing ulcers or inflammation or when the enemas were retained in the bowel, for increased period of time or

when the tannic acid concentration was increased (Zboralske et al., 1966). One of the symptoms of continued tannin feeding is gastritis as well as irritation and oedema of the intestines. Under such a condition, it appears that tannin oligomers may be absorbed. The attendant result of possible carcinogenesis as evident by the production of liver cancer when tannins are applied to burns, or administered repeatedly by subcutaneous injection (Bichel and Bach, 1968; Kirby 1960; Korpassy, 1961) is worthy of consideration.

Tannic acid inhibits the absorption of glucose and methionine in mouse intestine; this is believed to be the result of denaturation of the proteins of the protective outer cellular layer of the mucous membrane (Mitjavila et al., 1970). Localised destruction of epithelium of the gastrointestinal tract has been found to occur following oral administration of tannic acid and to be more severe in newborn than in older rats (Weinberg et al., 1965). Since they tend to break down more, hydrolysable tannins (e.g. tannic acid) would be expected to be more toxic than condensed tannins in the systemic sense. The no-effect level for food-grade tannic acid in rats has been established to be 800 mg per kilogram body weight per day and the total acceptable daily intake

for a man is 560 mg (Anonymous, 1969). One reason for the low oral toxicity of tannic acid is that it is hydrolysed in passing through the normal gut, and only gallic acid (the methyl donors: choline and methionine provide the methyl group required to methylate gallic acid to 4-O-methyl gallic acid, a major excretory product) or metabolic products thereof appear in blood or urine. On the other hand, the condensed tannins (Flavolans) are polymeric flavonoids composed predominantly of leucoanthocyanidin units linked carbon to carbon from the 4-position of one unit to the 6- or 8- position of the next (Haslam, 1966). Unlike the hydrolysable tannins, they do not breakdown readily under physiological conditions, but when treated drastically, they usually produce either less soluble polymeric "phlobaphenes" or flavonoid monomers, particularly catechins and antho cyanidins (Haslam, 1966).

Experimental feeding of diets with known or added tannins to chicks and to rats have shown growth depression with levels of the order of 1 per cent of the diet, but effects are somewhat variable. Chicks are apparently more sensitive than rats.

Vohra et al., (1966) reported a decrease in nitrogen retention upon feeding high (0.5%) levels of tannic acid

to chicks and at 5% high mortality occurs. Condensed tannins were however found less detrimental than tannic acid.Glick and Joslyn (1970) also noted an increased excretion of protein in the faeces of rats fed 2 per cent or more of tannic acid in the diet. They demonstrated that proteolytic activity of intestinal contents of rats fed 5 per cent tannic acid was over three times that of control rats and concluded that protein of endogenous origin accounted for most of the increased protein excretion.

Martin-Tanguy et al., (1977), however found that condensed tannin of the horse bean seeds appear to depress the growth of muscovy ducklings in addition to causing reduction in the weight of eggs produced by laying hens and to decrease the digestibility of nitrogen compounds in growing chicks. Eggum and Christensen (1975) working on barley found out that tannin exerted a severe negative effect on protein digestibility. These workers also found out that with tannin in the diet of rats, availability of all amino acids decreased significantly but to different degrees, glutamic acid, proline and glycine being severely affected. The sulphur amino acids (methionine and cystine) were however least affected by the addition of tannins. These workers therefore, have their reservations

as to the validity of the theory of detoxification effect of methionine (as far as rats are concerned), since a higher demand for these amino acids when tannin is fed did not apply in their study; concluding that tannin gelatin complex is very strong (Van Buren and Robinson, 1969) and that the high concentration of these three amino acids (proline, glycine and glutamic acid) suggest a specific affinity for them. However, Fuller et al., (1967), reported that supplementation with methionine, choline, and arginine reduced the toxicity of 1 per cent tannic acid and completely removed the adverse effect of 0.5 per cent tannic acid. This is believed to result from the need for methyl groups for the O-methylation of gallic acid derived from tannic acid, (Williams 1959).

It appears however, that there are several causes of the growth-depressing and toxic effects of tannins, and the interplay between them and the experimental conditions account for variable observations. High tannin in the diet makes it astringent and the animals must be starved to force them to eat it. Weight loss during this period is more serious for smaller, younger animals and seems to be at least one reason that larger animals are more tolerant of a high tannin diet (Glick and Joslyn, 1970). Paired feeding shows that feed intake and presumably palatability are major but not the only factors

in growth depression by high tannin diets (Vohra et al., 1966, and Glick and Joslyn, 1970).

The feeding of tannin also leads to lowered energy conversion from food and to excretion of high levels of nitrogen in the faeces as earlier mentioned. The high nitrogen excretion results largely from the binding of dietary protein by tannin into an indigestible form. Any protein added in excess of the amount required to bind the tannin is utilized by the animal, resulting in greatly improved growth; the residual nitrogen in the faeces remains about as it was before the addition (Glick and Joslyn, 1970b). Supplementation with 40 per cent casein and 5 per cent tannic acid gave growth equal to that of pair-fed rats without these supplements (Glick and Joslyn, 1970a). The protein-tannin complex appears to be formed by multiple hydrogen bonding between phenolic hydroxyl groups of the tannins and the carbonyl groups of the protein peptide bonds of enzyme proteins; and since the body responds to high-tannin diets by synthesizing severalfold as much proteolytic enzyme (Glick and Joslyn, 1970b) part of the high nitrogen excretion is endogenous enzyme protein. A net loss of nitrogen would be possible on protein-poor and high-tannin diets, and this would be expected to be most deleterious to young animals during

the period of rapid muscular growth. The tannins also react in a similar non specific, multiple hydrogen bonding fashion with dietary protein forming undigestible precipitates as in leather tanning, because treatment of faeces with urea liberates some of the tannin (Tamir, 1970). Free amino acids do not however bind strongly to tannin.

However, the absorption of an intact protein-binding non-dialysable tannin macromolecule whether hydrolysable or condensed seems quite unlikely, and the best evidence seems to be that it does not occur in the normally functioning alimentary tract of an animal (Singleton and Kratzer, 1969). Moreover, since microorganisms may not only modify phenols but possibly also destroy them entirely, the microflora of the alimentary tract can greatly reduce the apparent toxicity of orally administered phenols, especially in animals which are adapted to plant diets (Singleton and Kratzer, 1969). Presently, it appears that the hypothesis of a lower digestibility of the tannin-protein complexes, or proteolytic enzyme inhibition by tannins as mentioned above should be eliminated, at least as far as hydrolysable tannins are concerned (Mitjavila *et al.*, 1977).

1.23. Lectins (Phytohaemagglutinins)

Lectins are defined as carbohydrate-binding proteins and because of their unique property of being able to agglutinate erythrocytes, they are also known as (phyto)haemagglutinins. Like the protease inhibitors, the lectins appear to be widespread throughout the leguminosae.

The first description of phytohaemagglutinin was given by Stillmark (1889), who studied the toxicity of castor beans and press cakes from the production of castor oil. He concluded that the toxic action was due to the presence of a protein which was capable of agglutinating the red cells from human and animal blood; this he called "ricin". Landsteiner and Raubitschek (1908) later observed that the relative haemagglutinating activities of various seed extracts were quite different when tested with red blood cells from different animals, and compared this specificity with that of the antibodies of animal serum. No toxicity was detected at that time. However, Liener (1953) subsequently isolated a haemagglutinin from soybeans and demonstrated its ability to inhibit the growth of rats. The isolation of pure lectins and subsequent nutritional testing have now thrown much light on the toxicity of lectins. Thus, a

lectin component isolated from navy (haricot) bean (Andrews, 1974) was found to be toxic for Japanese quail (Jayne-Williams and Burgess, 1974). Similarly, a correlation was found between lectin content in white kidney bean (cv. "Processor") and the extent of depression of protein utilization for the rat (Pusztai et al., 1975).

The seeds of Phaseolus vulgaris (kidney bean) contain about the highest lectin content when compared to those of other grain legumes, and hence most work concerning lectin toxicity has been carried out using the lectin extracted from the seeds. Jaffé et al., (1968) undertook a systematic study of the haemagglutinating activities of a large number of different varieties and cultivars of Phaseolus vulgaris and observed that only those extracts which agglutinated trypsinised bovine erythrocytes were toxic when injected into rats; feeding trials further confirmed this. Why this should be the case is uncertain, indeed the basis of lectin toxicity, when ingested is not understood and the fact that these proteins are readily digested and inactivated is even more puzzling. However, it has been suggested that the ingested lectin might exert its toxic effects on the recipient animals by interfering with their digestion and/or absorption (Jaffé and Vega Lette, 1968), by the formation of intestinal and other lesions (Tedeschi et al., 1965) or

by an impairment of body defences and the consequent tissue invasion by normally innocuous gut bacteria (Jayne-Williams and Burgess, 1974). Although absorption was depressed to a certain extent Pusztai et al (1979) concluded that it still occurred, probably through the non-disrupted cells of the small intestine. It was suggested that toxicity was the result of ensuing systemic effects, such as for example the observed high N excretion possibly through increased tissue catabolism.

Improperly processed bean meals have been reported to cause an outbreak of poisoning (Griebel, 1950). Korte (1972) has, in fact, observed that when mixtures of ground beans (Phaseolus vulgaris) and ground cereals were prepared under conditions prevailing in Africa (Tanzania) the agglutinins were not always destroyed. It is evident therefore that caution should be exercised in recommending the use of beans under conditions where proper heat treatment may not be ensured.

Baker (1978) studying the basis of insect resistance in cowpea (Vigna unguiculata) looked into the probable role of lectins in cowpea. She tested both the albumin fraction at a concentration of 10 mg per ml, as well as total protein extracts from 4 varieties of cowpea, using concanavalin A and Vicia faba lectins as positive

agglutination controls. She in addition added bovine serum albium (BSA) to some of the assays at a concentration of 1 mg per ml; since some lectins appear to require added protein for activity (Toms and Western, 1971). The results were however negative with respect to the cowpea extracts against human groups A, O, and rabbit (with and without BSA), pig, sheep and cow bloods. Lis and Sharon, (1973) indicated that many lectins, especially concanavalin A require calcium and magnesium ions for activity whilst trypsin and neuraminidase increase the sensitivity of the assays. These effects were also tested on cowpea extracts, but the results were equally negative. It was further found out that no material from Vigna unguiculata seed extracts will bind to either Sephadex G100 or Sepharose 4B (Gatehouse, private communication as quoted by Baker 1978) whereas most lectins will bind to one or other of these resins.

The significance of these findings with respect to the consumption of these beans in human diet is that almost all the food legumes have little nutritive value unless subjected to some form of heat treatment. The presence in the seeds of legumes of antiphysiological activity such as lectins and trypsin inhibitors, do not pose a problem in practice as man rarely consumes raw

legumes and that the activity is usually virtually destroyed by the usual cooking procedures, as long as sufficient heat has been applied to ensure complete destruction of these factors.

1.30 Effect of Processing and other Nutrients on Protein Digestibility

Most people recognise that beans are difficult to digest and may give rise to stomach upsets. Nutritionists are aware that beans have a low protein digestibility or impaired amino acid bio-availability. At present, it is not known whether these effects are caused by a more rapid discharge from the intestine, or by a resistance to protein hydrolysis by the gastro-intestinal enzymes. In any case, significant losses of nitrogen occur in faeces when beans are consumed (Bressani, 1972).

The low digestibility of legume grains has been observed not only among species, but also among varieties of the same species. For example, Jaffé (1950) observed that Cajanus indicus had a protein digestibility of 59 per cent, in contrast to other varieties that showed values as high as 90 per cent. On the other hand, Vigna sinensis showed digestibility coefficients that varied between 86 per cent and 90 per cent. The value for Lens esculenta was 93 per cent; for Pisum sativum about 92

per cent. The grain size has been incriminated as a possible cause for decreased digestibility, because it is thought that small seeds have heavier cotyledons (Bressani, 1972). Seidl et al., (1969), Jaffé and Vega (1968) obtained a globulin fraction from black beans that was resistant to in vitro digestion by ten proteolytic enzymes even after protein denaturation.

Venkataraman et al., (1976) on the other hand reported a decrease in digestibility of germinated cowpea, while Onayemi et al., (1976) reported a decrease in trypsin inhibitory activity in cowpea soaked for 36 hours. Whilst heating has been shown to increase the digestibility of cowpea nutrients and its protein utilization (Owusu-Domfeh, 1972), Bressani and associates (1977), however, in an attempt to throw more light on the problem of low digestibility of protein of legume foods, fed different types of beans with varying colours to young growing dogs. The data produced suggest that total protein digestibility of legume grains was significantly lower than that of casein, but increased with intake with the exception of red beans. Their regression analyses also showed that as bean intake increased, so too was faecal nitrogen. These were higher for the Phaseolus species, than cowpea, pigeon peas and soybean; and

among the bean samples, it was higher for the red beans.

In 1966, Ford and Salter pointed out the possible effect of heat damage on the digestibility of heat processed proteins. Storage conditions have been known to affect protein digestibility by indirectly affecting cooking time, a phenomenon known as "hard cook" Molina et al., (1975). Hard cook is caused by storage of beans at high temperature (>70°F) and high relative humidity (>75%) the type that prevails in the tropics. As a result the beans have been known to take an unusually long time (up to six hours) to cook as compared to 30 minutes if stored at low temperatures and low relative humidity.

Protein foods are cooked for reasons which include making them more digestible, increasing their keeping qualities and for safety. Eggs, meat and fish may be cooked for aesthetic reasons. But above all, for the majority, foods are cooked to make them more appetizing, that is, to increase their organoleptic appeal.

There are two main methods of cooking - dry and moist. The most important differences between the two methods arise as a result of higher external temperatures and the greater possibility of surface evaporation of moisture in dry methods. Dry methods include grilling

(broiling), baking, frying and roasting. Moist methods include steaming, boiling, braising, stewing, poaching and pressure cooking.

In cooking foods containing protein, the Maillard reaction is of considerable importance. Melanoidin pigments produce characteristic changes of colour and flavour (Ellis, 1959) combined with possible reduction in biological value (Clegg, 1960). Increased temperatures and high pH favour this reaction.

1.31 Protein Digestion

The amino acid composition of a food protein as revealed by chemical analysis, may be said to represent its potential nutritive quality, but other characteristics may be of importance in determining its value for the animal. Of these, digestibility and the biological availability of its amino acids are of first importance, perhaps more especially among protein foods that have been heated in manufacture.

Review of the literature however, shows that ingested food is stored in the stomach where it is further moistened, softened and mixed with hydrochloric acid and pepsin, the combination of which results in denaturation of native proteins and their partial or complete solubilization. The stomach thus plays an important part in digestion.

It acts as an osmotic shield (Hunt and Pathak, 1960) to prevent the passage into the intestine of large volumes of hyperosmotic material.

The evaluation of the pH of optimal activity of pepsin toward protein substrates is complicated by the fact that a prior acid denaturation of the substrate markedly facilitates digestion, or may be an absolute prerequisite for proteolysis in many cases (Linderstrøm-Lang et al., 1938). Thus Christensen (1955) has presented evidence which suggests that the rate-limiting step in the peptic digestion of ovalbumin and lactoglobulin is acid denaturation, and has found that although the pH optimum for the digestion of the native proteins is about 1 that for digestion of the previously denatured proteins was near 1.7. Other studies by Northrop (1922); Desnuelle et al., (1950); Sri Ram and Maurer (1957) and many others have substantiated the observation that the practical pH of optimum activity of pepsin toward protein substrate is between pH 1 and 3.

The chyme is passed from the stomach into the duodenum as protein or large peptides where it is mixed with bile, its pH is raised, and is finally, subjected to the hydrolytic action of pancreatic and intestinal proteases and peptidases secreted in response to discharge

of chyme into the duodenum (Twombly-Snook and Meyer, 1964).

Many native proteins are quite resistant to hydrolysis by trypsin and chymotrypsin, and are digested only after undergoing chemical or physical treatments which cause denaturation and thus expose all or some of the susceptible bonds to the action of the protease (Desnuelle, 1960). It has been suggested that the proteolytic enzymes themselves can produce denaturation of a protein substrate by virtue of its ability to form a complex (Linderstrøm-Lang et al., 1938 and Green and Neurath, 1954), but it has proved difficult to distinguish this possibility from an alternate one in which the hydrolysis of a single exposed susceptible bond results in exposure of others (Desnuelle, 1960).

Chymotrypsin and trypsin both attack susceptible proteins optimally in the pH range from 7.5 to 9. Apart from the distinctly different amino acid residues preferred in substrates by these enzymes, the major distinguishing characteristic is the rapid autolysis undergone by trypsin at the pH of optimum catalytic activity, and the inhibition of this autolysis by calcium ions (Northrop et al., 1948). It has been suggested that autolysis results from the fact that native trypsin exists in an equilibrium with a reversibly denatured form which is susceptible to proteolysis by the native form

(Northrop et al., 1948, and Gorini, 1951) and that the binding of calcium ion shifts the equilibrium toward the native form, but the situation is not completely understood.

From the pioneering studies of Bergmann (1942) and his associates (1941), as well as later investigations of the hydrolysis of simple amides and peptides by these enzymes (Green and Neurath, 1954, Desnuelle and Rovery, 1961), it was learned that trypsin acts rapidly only on those peptide bonds in which arginine or lysine provides the acyl portion, while chymotrypsin acts on those bonds in which tyrosine, phenylalanine, tryptophan and, to a lesser extent, methionine and leucine, provide the acyl portions. The products of protein digestion are absorbed in the intestinal mucosa into the portal circulation. It is now established that, though in general, only free amino acids enter portal blood during protein absorption, protein digestion products leave the intestinal lumen in two forms: as free amino acids and as small peptides (Matthews, 1972).

Melnick et al., (1946) argued that time and concentration relationships determine the efficiency with which the amino acids are utilised after enzymic release and postulated that, for optimum utilization of a food protein, all the

essential amino acids must not only be made available for absorption, but must also be liberated during digestion *in vivo* at rates permitting mutual supplementation. Denton and Elvehjem (1953 and 1954) in a series of experiments to measure the rate of hydrolysis of proteins, both *in vitro* and *in vivo*, and the concentration of amino acids absorbed from the intestinal tract into the portal vein, demonstrated that the amino acids from digestion of casein or beef enter portal vein within a brief enough period of time to allow for maximum utilization. The time of absorption of each amino acid in relation to others is therefore important.

Elman (1939), for example, found that tryptophan and methionine had to be injected simultaneously to produce positive nitrogen balance in the dog. Geiger and associates (1947, 1948, and 1950), showed that delayed supplementation of diets with either tryptophan, lysine, or methionine resulted in poor growth of rats. Cannon *et al.*, (1947) on the other hand, reported that rats did not grow if 5 of the 10 essential amino acids were fed one hour and 5 the next hour, while Henderson and Harris (1949), demonstrated that protein anabolism decreased if there was a delay of 3 hours or longer in the feeding of lysine as a supplement to a lysine-low diet. The importance of simultaneous availability of

essential amino acids is illustrated also by feeding one protein to supplement another, simultaneous feeding of the proteins resulting in much better growth of animals than when the proteins are supplied at separate feedings (Henry and Kon, 1946; Mertz et al., 1952; Eggert et al., 1953).

The finding, that free amino acids supplement is absorbed relatively quickly may explain reports that supplementation does not necessarily make the nutritive value of a protein equal to that of the model (Hogan et al, 1955, Banks et al, 1964). However, it does not necessarily follow that it would be advantageous for a supplemented protein to be digested more rapidly. If the amino acids were made available faster than the rate at which they could be utilized in the tissue, they might be subjected to wasteful deamination (Gitler, 1964).

1.32 The rate of stomach emptying on Protein Digestibility

The rate of emptying of the stomach after a meal is known to be influenced by a variety of factors; such as the nature and level of the protein (Porter and Rolls, 1971), the presence of other dietary constituents particularly carbohydrate and fat (Rosenthal and Nasser, 1958; Peraino et al., 1959; Hunt and Knox, 1968; Pirk and Skala, 1970); the pH of intestinal contents (Rune, 1968),

osmotic pressure (Hunt and Pathuk, 1960), the physical state of the meal i.e. liquid or finely divided meals have been noticed to leave the stomach more quickly than do coarser meals (Marcus and Lengemann, 1962). Porter and Rolls (1971), have observed wide variations in the rate of stomach emptying in healthy animals under emotional states, such as fear or excitement. Whilst confinements have been noticed to delay stomach emptying, on the other hand, frequent small meals result in an increase in the rate of gastric emptying.

Buraczewski et al., (1971), found that when different proteins were fed with the same carbohydrate, the nature (raw, heat treated, or heat damaged) of the protein component largely determined the rate of stomach emptying, whereas the effect of feeding different carbohydrates with a particular protein could be related to the properties of the carbohydrates - very soluble carbohydrates especially those which were poorly absorbed such as lactose, tended to delay stomach emptying.

Krehl et al., (1946), observed a better growth of animals fed low protein diets containing dextrin rather than sucrose and concluded that this may be the result of the effect of rates of digestion and absorption on the utilization of amino acids. Lyman and Elvehjem (1951)

have also suggested that the lower tryptophan requirements of rats receiving dextrin rations deficient in this amino acid and in niacin may be a result of slower passage of this ration through the digestive tract, permitting more complete digestion and utilization of the diet. Buraczewski (1966), however, compared the levels of free amino acids in the portal and systemic blood plasma of rats at two hours after being given test proteins (raw unheated; heated at 135°C and heated at 145°C) with and without sucrose.

In rats given the unheated protein without sucrose, the levels of free amino acids were markedly higher in the portal than in the systemic blood plasma. This difference was much smaller in rats given the meal heated at 135°C and was negligible for the meal heated at 145°C. Feeding sucrose with the test proteins gave a different picture. For the unheated protein the differences between portal and the systemic levels were smaller, and for the heated meals the differences were increased, especially for the meal heated at 135°C. Buraczewski then concluded that besides the effect on amino acid uptake, sucrose had a pronounced effect in retarding the passage of the heated protein from the stomach.

Still another interesting problem involving digestion

is the effect of processing of food on the rate of hydrolysis in the gastrointestinal tract.

Buraczewski et al., (1967) in their study of the effects of heating on the course of digestion of cod muscle protein by rats concluded that with increasing severity of heating, the solubility of the test protein decreased as did the rate at which it was hydrolysed by pepsin and subsequently by pancreatin and erepsin; and that digestion in vivo of heat-damaged meals was far from complete and was comparatively inefficient and therefore concluded that much of the fall in nutritive quality can be attributed to this difference between the rates of enzymic release of different amino acids.

Heating, particularly dry heat, has been known to reduce the nutritive value of proteins which contain little or no carbohydrate by reducing the liberation of lysine and possibly arginine and histidine all of which become tied up in new chemical linkages (Beuk et al., 1949; Clandinin et al., 1951 and Clegg 1960). Overheating proteins in the presence of carbohydrate causes another type of destruction of amino acids, particularly lysine, arginine and tryptophan, through reactions with carbohydrates (Schroeder et al., 1951), a reaction that seems to be inhibited by water (Schroeder et al., 1953).

It must therefore be noted that the rate of gastric emptying is clearly a determinant in the rate of absorption of a substance and that the latter in return may also affect emptying. Thus digestibility is a variable of first importance to the determination of nutritive value, particularly so since the methods for determination of nutritive value of proteins are designed to measure the degree of retention of that portion of the dietary nitrogen which is absorbed into the body of the animal. The nitrogen retained is determined as a function of growth, nitrogen balance or repletion measurements of the whole or parts of the animal.

1.40 Aims of the Present Study

Many developing countries, Nigeria inclusive, are probably protein poor. One potentially large source of supplementary protein is legume grains, which are already part of the people's diet, but they are known to contain proteolytic enzyme inhibitors that decrease digestibility and inhibit growth.

In Nigeria, consumption of legumes (Cowpea - *Vigna unquiculata*) is relatively high, although this could be increased several fold if some of these offending factors such as digestive inhibitors and flatus factors are greatly reduced or removed, especially now that National and State banquets and first class hotels have on their menus, bean ('Gbegiri - Yoruba) soup as a substitute or alternative choice to foreign soup courses (in a bid to be self reliant) and moreover, bean meal as a possible weaning food. This is even made more pertinent by the findings of Gatehouse and Gatehouse (1979), that insect resistance in the cowpea is due to an elevated level of trypsin inhibitor. These findings would undoubtedly encourage the plant geneticists to produce insect resistant varieties in order to reduce post harvest loss due to insect infestations. Information on the possible nutritional effect of such a venture is now more urgent than hitherto.

Most studies up to date on the anti-nutritive factors of legumes have been on soybeans and Phaseolus vulgaris. The few (Onayemi et al., 1976; Venkataraman et al., (1976) Bressani and Molina, 1977; Owusu-Domfeh, 1972, Carasco, 1976, and Gatehouse and Gatehouse, 1979), there are on cowpea, have not looked into the ways our traditional culinary methods affect the nutritive quality of our local beans.

Much attention has been given in the past to the destruction of the well known anti-nutritional factors in legume foods by some sort of heat treatment. Likewise some reports have focused on the establishment of nutritional standards (Hulse et al., 1977) which besides protein and specific essential amino acids, include other characteristics related to acceptability and ease of preparation for consumption. Even though the amino acid profile is important in evaluating the nutritive quality of a protein, the digestibility of that protein is the primary determinant of the availability of its amino acids. Legume grain proteins has been known to have low digestibility, which has not been adequately explained.

The aims of this study therefore are to:

1) Find out the protein content of some of the most locally consumed varieties of cowpea.

2) Determine the level(s) of anti-nutritive factors (Trypsin inhibitors, Polyphenols, and Tannins; and phytohaemagglutinins - lectins) present therein.

3) Determine the effect(s) of contemporary Nigerian culinary processes on these factors.

4) Determine the effect of these culinary processes on the digestibility of the bean meal.

5) Finally, to attempt to give explanation(s) for the likely decreased digestibility of the cowpea protein even when they are cooked to the point at which all the known toxic factors have been destroyed, and give possible suggestion(s) for improving the digestibility.

CHAPTER 2

EXPERIMENTAL

Materials and Methods

We all stand on the shoulders of our predecessors, is it surprising therefore, that we have a wider view than they?

F.A. Kekule

MATERIALS

2.10 Biological Materials

Seeds of *Vigna unguiculata*, (Local Brown; Ife Brown, or Ibadan Brown[12]; Local White; Ibadan White; Igbirra; Mala[7,13]; and Ewa Ibeji[6,8] varieties Ojomo private communication) were purchased from three major markets (Iddo, Badagry, and Agege) in the Lagos State of Nigeria. Each variety from each of the respective markets were pooled together, mixed to obtain a near representative sample of what goes into the homes. These samples were used throughout for the respective assays.

The patients that took part in the metabolic studies were volunteers in-patients of the Orthopaedic Ward of The Lagos University Teaching (LUTH) Hospital by kind permission of Professor Jaja. These were in all respect in good health except for simple fractures, sustained in motor accidents. My family and myself volunteered as controls.

2.12 Chemicals and Reagents

Except for those listed below, chemicals were obtained from British Drug Houses (BDH) Ltd., Poole, Dorset, England or Hopkin and Williams Ltd., Chadwell

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Figures in parenthesis represent the varietal types in Appendix I.

Heath, Essex, England. They were of analytical grade, or nitrogen free where necessary.

The water used were all glass distilled, using the Super Four Autostills supplied by Jencons (Scientific) Ltd., Hemel Hampstead, England.

Sepharose CL 4B, was obtained from Pharmacia Ltd., Uppsala, Sweden.

Dimethyl sulphoxide (DMSO) puris was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks, England. Dairy hyprochloride (11.5% w/w) from Boots farm Sales Ltd., Nottingham, England.

Trypsin (T8003) from Bovine pancreas, activity approximately 10,000 BAEE unit per mg protein; Trypsin inhibitor (T9003 type 1-S-lyophilized from soybean activity 1 mg inhibits 1.3 mg of trypsin with activity approximately 10,000 BAEE unit per mg protein; Peptidase (P7625) grade 1 from Porcine intestinal mucosa, activity 15-25 unit per gram solid; Chymotrypsin (C7762) type 1-S from Bovine pancreas, activity 40-50 units per mg protein. Catechin; α-N-Benzoyl-DL-arginine-p-nitro-anilide HCl (BAPNA), and Tris (hydroxy methyl) amino-methane (TRIZMA BASE T-1503) were obtained from SIGMA Chemical Company, St. Louis, U.S.A. or Fancy Road, Poole, Dorset, BH17 7NH, England, whenever available.

Kjeltabs Auto containing 1.5g K_2SO_4 and 7.5 mg selenium used in the total nitrogen analysis were obtained from Thompson and Capper Ltd., Runcorn, Cheshire, England.

2.13 Instruments

The beans were either cooked on a gas or electric cooker, till soft. Cooked bean samples were freeze dried on an Edwards Modulyo freeze drier Model EP03, supplied by Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex, RH16 2LW, England.

Cooking temperatures were taken using Baileys electronic thermometer, model BAT-4, supplied by Bailey Instrument Co. Inc., Saddle Brooke, N.J. 07662.

The extracted polyphenols were either concentrated or solvent distilled off under vacuum, using rotary evaporator (Rotavapour-R-Buchi) with high vacuum pump supplied by Edwards High Vacuum Ltd.

Fractionation of the polyphenols on 7 x 1 cm column was done using LKB Minivac fraction collector 17000, supplied by LKB-Produkter AB-5-16125 Bromma 1 Sweden.

Weighings were carried out on either Mettler H51 microbalance, or Oertling (micro or top loading) balance as the occasion demanded.

All pH measurements were carried out using pH meter 7020 supplied by Electronic Instruments Ltd., England.

All glasswares (rimmed, rimless, boiling and centrifuge) test tubes, pipettes, beakers, and volumetric flasks were either acid or decon 90 washed, tap water, and distilled water rinsed, and hot air dried.

Test tube mixing of solutions was done using Whirlimixer supplied by Fisons Co. Both macro and micro digestion of samples used in nitrogen estimations were carried out on macro and micro digesters. These and the water bath used were supplied by Gallenkamp.

The bean samples were milled using Janke and Kunkel mill with cooling water jacket. The test sieves (BS410, 425 micron ASTM36 mesh) was supplied by Endcott Ltd., London, SW 19. Milled samples were stored in Kilner air-tight jars.

All optical density measurements were carried out either on Perkin-Elmer 402 u.v.-visible spectrophotometer or a Pye Unicam SP800 spectrophotometer with attachment Speedomax recorder model XL681 and a Gilford 2000 spectrophotometer attachment.

Food and faecal samples were dried over reflector lamps in a fume cupboard overnight until constant weight was obtained.

Nitrogen analyses were carried out on either Gallenkamp macro digester and distiller followed by titrimetric method, or on a MacFarlane Robson Carlo Erba Model 1510/3 with a speedomax recorder model XL681 attachment after micro digestion, by courtesy of the Botany Department, Durham University.

The extracted seed protein was clarified by spinning at 16,000 rpm on an MSE18 centrifuge at 4°C. *In vitro* digestibility, measured as a function of pH drop from the protein chain when proteolytic enzyme was added, was recorded automatically over a 10 minute period using PHM61 Laboratory pH meter, with TTT60 titrator, ABU13 autoburette and REC61 servo graph attachments (REA160 tetrigraph module):- pH stat, supplied by V.A. Howe & Co. Ltd., 88, Peterborough Road, London, SW6, England, by courtesy of the Botany Department, Durham University.

METHODS

2.20 Preparation of Bean Meal

Samples of the dry beans were subjected to different methods of preparation and cooking that are commonly used throughout Nigeria. These methods include:

a) Boiling: Boiling was done according to the local conventional method which involves the stewing of a known weight of dry bean (Local Brown, Local White, or Ewa Ibeji varieties), to which a measured amount of water was added. This was brought to boil in either enamel or aluminium cooking pot over a gas or electric stove until very tender to touch and the cooking water dried up in the cooked beans. The time and volume of water required were noted. For the human feeding experiment the cooked beans were garnished with other ingredients as in Appendix IV (page 129) to make it appetising. This is the familiar preparation of dishes like Bobo, cowpea porridge, rice and cowpea, cowpea and plantain pottage, Frejon, Egwa Hikaje, Adalu and Awuje - Dovlo et al., (1976).

b) Steaming/Frying in oil: Dry beans were soaked in ordinary tap water for about 30 minutes to remove the testa. The cotyledons are then homogenised in a blender to a fine paste. The paste is then mixed with ingredients (see Appendix III), air whipped, and either deep fried in

vegetable oil to make 'Akara' balls, or wrapped in special leaves and then steamed to make 'Moin-Moin'. This process of removing testa and homogenising the cotyledons on a grinding stone or in a blender is a common step to other (Gbegiri, Jogi, Ekuru, Ikoko, Apapa, Adayi, Kengbe) culinary preparations of cowpea meal Dovlo et al., (1976). Only the Local Brown and Local White varieties are used in this way. The Ewa Ibeji variety is only used for stewing.

2.21. Preparation of Bean sample

The bean samples used in this study were prepared as in above except that no ingredient was added, and instead of wrapping in leaves as in Moin-Moin preparation or deep frying as in Akara balls preparation, the decorticated beans were heat (stewed) treated. In each case, the cooked bean and the cooking water was frozen and later freeze dried in the Modulyo freeze-drier.

The dried (raw and processed) beans were milled in a Janke and Kunkel water cooled mill to pass through sieve of 425 microns mesh size.

The milled beans were then stored in Kilner jars from where samples were taken from time to time for analysis.

2.22 Determination of Moisture in sample

Weighed duplicate samples were dried for 24 hours at 105°C (until a constant weight was obtained) allowed to cool in a dessicator and then reweighed to determine moisture content by difference.

2.23 Determination of ratio of testa to cotyledon

A weighed amount of dried bean was soaked for 30 minutes in 60% (v/v) ethanol, the testa gently removed by rubbing between the palm. Both the cotyledon and the testa are then dried separately in an oven 105°C for 24 hours and the weights noted.

2.24 Determination of Total Nitrogen Content

The method used is a modification of Varley's (1966) by Evans and Boulter, (1974), and consists in reacting a solution containing ammonium ions with alkaline phenol and hypochlorite. On heating the solution, an intense blue colour develops which is closely related to that of indophenol, and read at 625 nm automatically.

Duplicate samples (40 to 60 mg) were weighed out into paper cups and then dried at 105°C for 2 hours to determine their moisture content. For digestion, the sample together with a couple of glass beads, and one Kjeldahl tablet were placed in a Kjeldahl flask. Then 5 ml of 95% (v/v) H_2SO_4 : 5% (v/v) H_3PO_4 were added

gradually followed by 3 ml of hydrogen peroxide, and the mixture digested on Gallenkamp micro-Kjeldahl digestion stand until the solution was clear (this takes about 40 minutes). Flasks were removed, cooled, and the contents transferred to a 100 ml volumetric flask and made up to volume with ammonia free distilled water. Duplicate analyses were run on the Carlo Erba autoanalyser. Nitrogen values were obtained from a calibration curve constructed using standard (9.6 to 15.89 ppm N) tyrosine solution.

The efficiency of the operation was monitored by putting through the entire procedure, analytical grade urea, and correction factors made accordingly.

The macro-Kjeldahl method involves the conversion of the protein into ammonium sulphate by the action of concentrated sulphuric acid and a catalyst (potassium, sulphate, mercuric oxide, cupric sulphate mixture). The ammonium sulphate formed is reacted with strong alkali and the ammonia evolved, is distilled into a weak 5% boric acid containing mixed (1% bromocresol green and 1% methyl red 2:1 v/v) indicators. This (ammonium borate) is later back titrated with 0.01M hydrochloric acid. The nitrogen equivalence (1 ml of 0.01M HCl = 1.4×10^{-4}gN) and efficiency factor, using urea as standard are worked out, and applied to the test runs.

Blanks were put up using analytical grade sucrose. This is the method used for Food and faecal nitrogen estimation.

The crude protein contents were calculated by multiplying the total nitrogen figures by a factor of 6.25; in both methods employed (Table 3.02).

2.25 Extraction of seed Polyphenols

Experiments were carried out to ascertain the best system of extraction of cowpea polyphenols. Solvents like water, methanol, methanol and water (60:40 v/v) mixture and dimethylformamide were used.

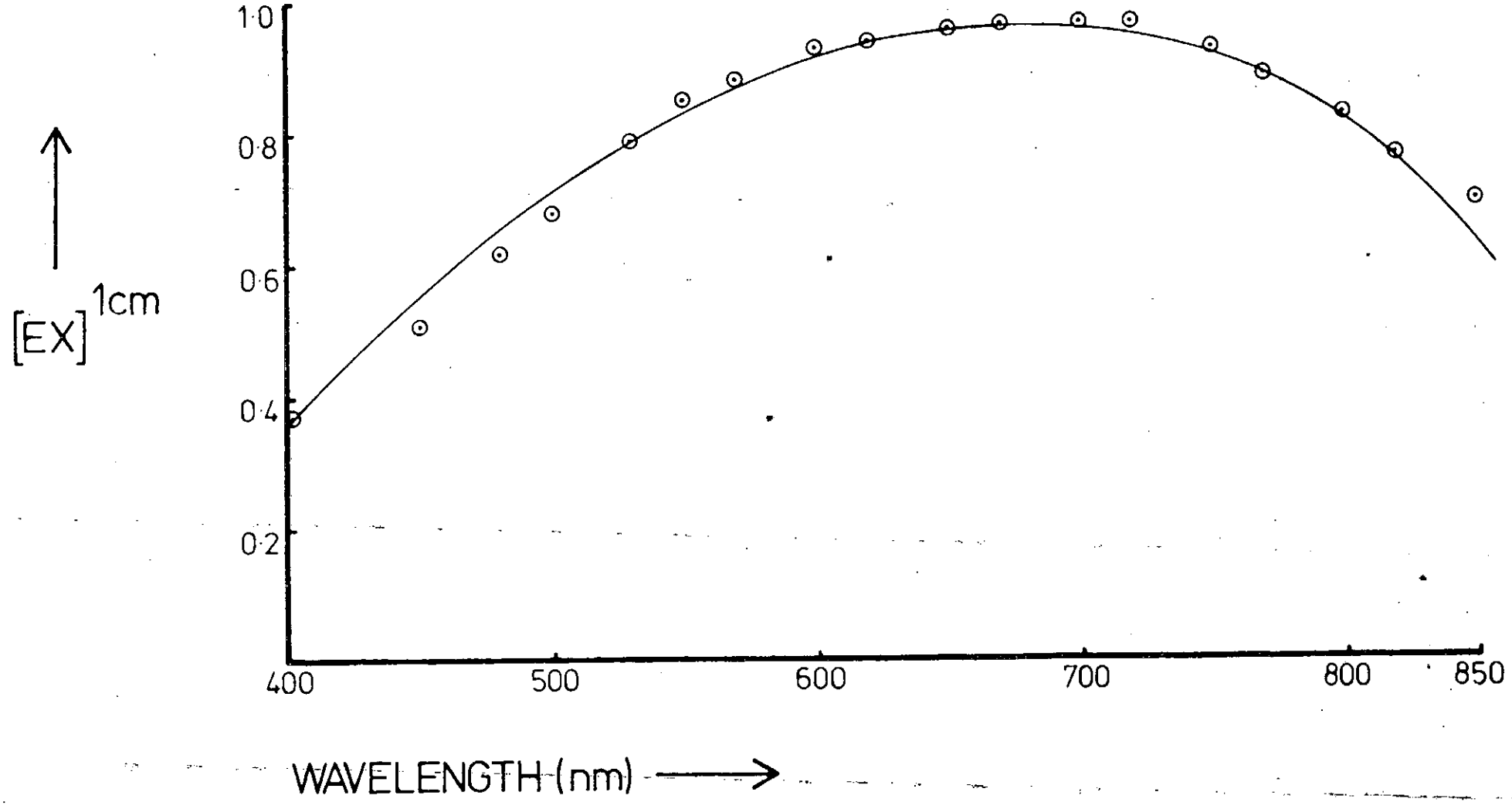
The effect of using whole grain or ground grains was also looked into: (Table 3.03).

The most appropriate solvent (Dimethylformamide) as judged suitable for cowpea tannin extraction, by this experiment, was used for extracting the chemically extractable polyphenols (in raw and processed meal) prior to analysis. The residual bean meals were vacuum dried and later freeze dried. These were respectively referred to in these studies as "Extractable Tannin Free" fractions.

The procedure involved taking a known weight (5g for whole seed, or 1g of ground seed) of cowpea in

Figure 1

Typical absorption spectrum of Folin and Denis Colour Reaction using Perkin Elmer 402 spectrophotometer.


1·0
0·8
0·6
0·4
0·2
[EX] 1cm
400
500
600
700
800
850
WAVELENGTH (nm)

a conical flask or test tube as the case may be, and to add ten times its weight of the respective solvents. The mixture is left at room temperature with occasional agitation for up to 3 hrs after which the coloured liquid is pipetted off, centrifuged for clarity, and analysed for both 'tannin-like' and 'catechin-like' polyphenols.

2.26 Assay of Cowpea Polyphenols

Both hydrolysable and condensed tannins were assayable from cowpea extracts, using the redox method of Folin and Denis (Burne, 1963) with slight modification, and the improved vanillin assay of Price et al., (1978) and Dalby and Shuman, (1978) which is specific for a narrow range of flavanols and dihydrochal-cones which have a single bond at the 2,3 position and free meta or lented hydroxy groups on the B ring (Sarkar and Howarth, 1976). The values were expressed as either tannic acid or catechin equivalent in mg per 10g dry sample (Table 3.05).

(a) Cyanogen Bromide Activation of Sepharose CL 4B and the coupling with BSA for Affinity Chromatography

This was carried out using essentially the simplified method of March et al., (1974). The procedure involves taking a 60 ml of sepharose slurry in water (when fully packed this gives a 50 ml packed sepharose which is

equivalent to 1 volume) for packing under pressure using sintered glass funnel. The packed sepharose is washed with distilled water, carefully scrapped off the sintered glass funnel, into a measuring cylinder and made up to 100 ml with distilled water. This was added to 100 ml (1 vol) of 2M Sodium Carbonate in a litre plastic beaker and mixed by stirring slowly using magnetic stirrer. The rate of stirring was speeded up on the addition of 5 ml (0.05 vol) of an acetonitrile solution of cyanogen bromide (100 g of CNBr per 50 ml of acetonitrile). The slurry is kept stirring vigorously for 2 minutes after which it was poured onto a sintered glass funnel, filtered under pressure, washed with 5-10 volume each of 0.1M Sodium Carbonate pH 9.5; distilled water; and 0.2M Sodium Carbonate pH 9.5. After the last wash, the slurry was filtered still under vacuum to a moist, compact cake and transferred to another plastic bottle containing 200mg (0.2g) albumin dissolved in 100 ml of 0.2M Sodium Carbonate pH 9.5. The bottle was rotated end-to-end for 20 hrs. at 4^{o}C for coupling. After coupling, the beads were washed with 20 vol each of 0.1M Sodium acetate (pH 4), 2M urea, and 0.1M Sodium bicarbonate (pH 10) and 0.5M Sodium chloride. The activated, coupled, and washed sepharose beads were

Figure 2

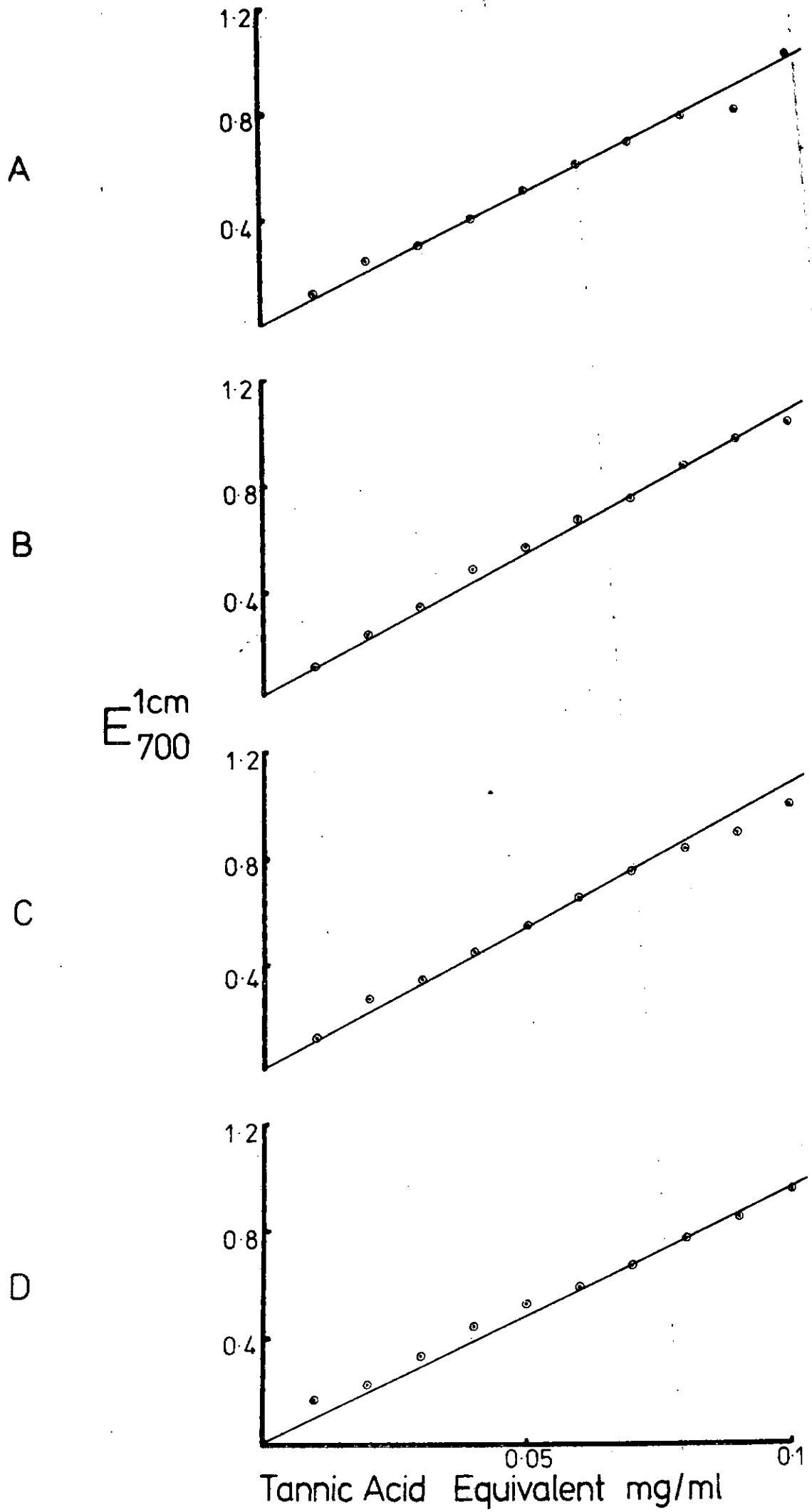
Effect of different solvents on tannic acid calibration curve

A. Dimethylformamide (DMF)
slope (K = 9.254)

B. Methanol
slope (K = 9.89)

C. Methanol/water (60:40 v/v)
slope (K = 9.37)

D. Water
slope (K = 8.77)


A
B
C
D
1·2
0·8
0·4
E^{1cm}_{700}
0·05
0·1
Tannic Acid Equivalent mg/ml

stored at 4°C in a plastic bottle containing the buffer (Acetate buffer pH 4) to be used, until required.

Optical measurement at 280 nm of the coupling buffer after reaction, indicated that almost 70% of the albumin had been bound. The effectiveness of the bound beads was tested using commercial tannic acid loaded into a column of the beads. The tannic substances were eluted with 3 ml dimethylformamide - 0.05M acetate buffer pH 4.0 (7:3 v/v) as is to be used in the experiment. This gave a recovery of between 98% and 100% (Figure 4).

(b) Separation of Tannin from non-tannin polyphenolics using Affinity Chromatography

The method with slight modification was that used by Hoff and Singleton (1977). The separation is achieved chromatographically by taking advantage of the ability of tannins to complex with proteins. The extracts are passed through a small column (7 x 1.0 cm) of immobilized protein. The tannic substances adhere to the column while the remaining substances including simple polyphenolics that have no affinity for proteins pass unhindered through the column. The tannic substances are eluted with a solution containing dimethylformamide, and can then be quantified using the modified Folin and Denis (redox) method. Results as shown in Table 3.05.

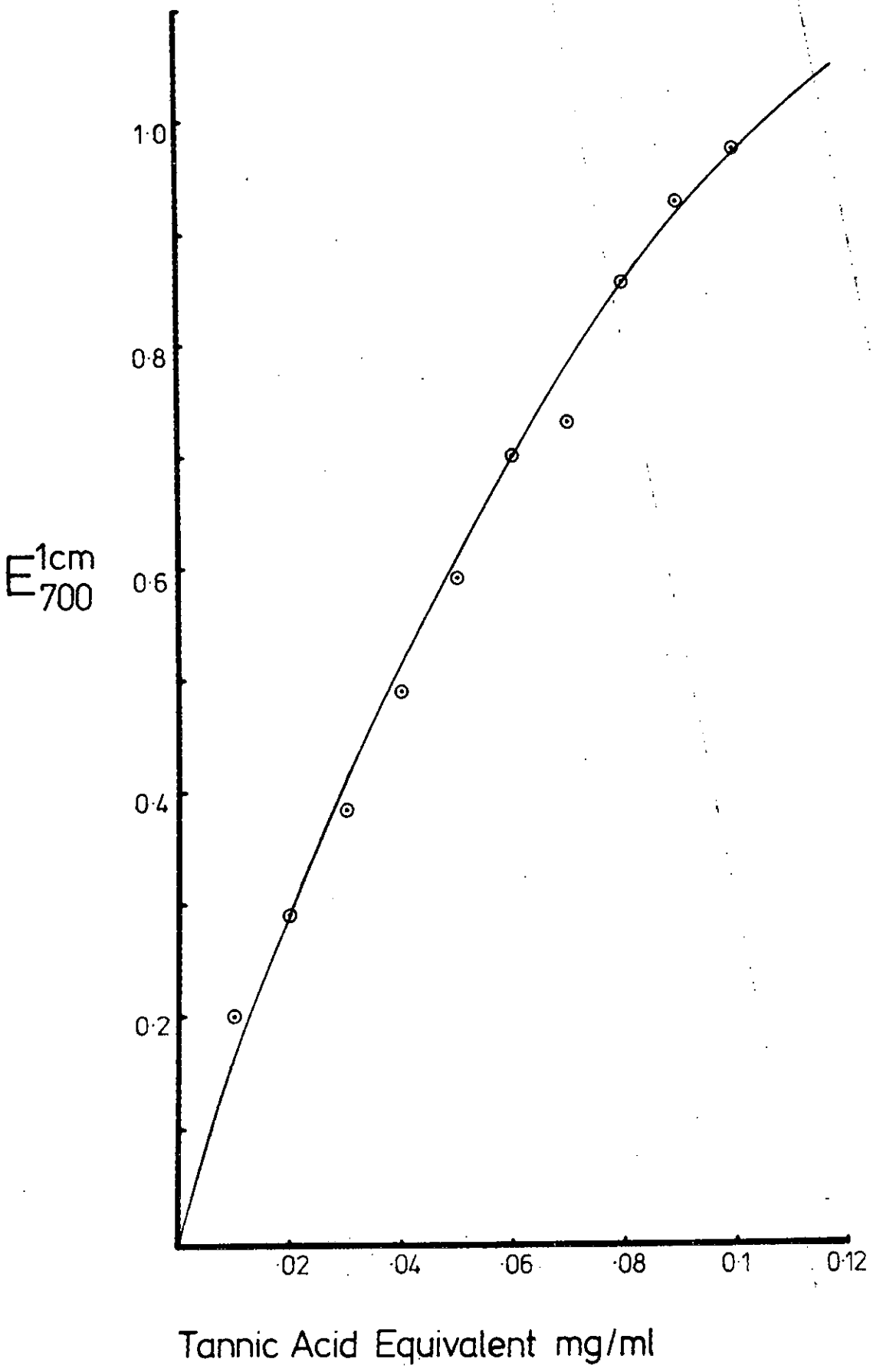
2.27 Extraction and Determination of Trypsin Inhibitory Activity

Both the extraction and determination of trypsin inhibitory activity in raw and processed bean samples were in accordance with the improved procedure of Kakade et al., (1974) with slight modifications.

The method involves extracting 200 mg finely ground (425 micron) sample with 10 ml of 0.01N sodium hydroxide (suspension pH 9 - 10.2), in screw capped sample bottles, at room temperature with occasional agitation for 3 hrs. At the expiration of 3 hr, portions (0; 0.6; 1.0; 1.4 and 1.8 ml of the bean suspensions were pipetted into duplicate sets of test tubes and adjusted to 2.0 mls with distilled water. A control/standard, commercial soybean trypsin inhibitor was similarly treated. Afterwards, 2 ml of trypsin solution were added to all the tubes. The extracts and trypsin were mixed, and placed in water bath at 37°C for 5 minutes. At the end of 5 minutes, 5 ml of 0.04% (w/v) BAPNA solution previously warmed to 37°C was added to each tube, and exactly 10 minutes later, the reaction was terminated by adding 1 ml of 30% (v/v) acetic acid. After thorough mixing, the contents of each tube was centrifuged and the absorbance of the supernatant read at 410 nm (the optimum

Figure 3

A typical Folin and Denis (tannic acid) calibration curve for polyphenols. Standard made up in dimethyl-formamide (DMF)


E^{1cm}_{700}
1·0
0·8
0·6
0·4
0·2
·02
·04
·06
·08
0·1
0·12
Tannic Acid Equivalent mg/ml

absorption spectrum of p-nitroanilide - Fig. 9).

Reagent blank was prepared by adding 1 ml of 30% (v/v) acetic acid to a test tube containing trypsin and water (2 ml each) before the 5 ml of 0.04% (w/v) BAPNA solution was added.

Endogenous BAPNA-ase activity was monitored by incubating 5 ml of 0.04% (w/v) BAPNA with 1.8 ml of the raw bean extract adjusted to 4 ml with distilled water, and incubating the mixture at 37°C for 10 minutes, and then adding 1 ml of 30% (v/v) acetic acid solution. There was no appreciable absorption at 410 nm.

Expression of Activity

One trypsin unit (TU) is arbitarily defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of the reaction mixture under the conditions used herein. Trypsin inhibitor activity is expressed in terms of trypsin unit inhibited (TIU). The reference soybean trypsin inhibitor activity was similarly used as equivalent unit of expression. Results as indicated in Table 3.06.

2.28 Effect of Tannic acid/Catechin on tryptic activity

From the results obtained from 2.27 procedure, it became necessary to look into the possible effect of both hydrolysable (as typified by tannic acid) and condensed (as typified by catechin) tannins on tryptic activity of BAPNA.

The experimental materials were selected to stimulate probable biological parameters. Tannic acid/catechin solutions ranging between 0.8 and 4 mg (4.7 to 23.5 and 22.1 to 110.3 x 10^{-4} mM respectively) were incubated with trypsin (40 µg) as in 2.27 and the hydrolysis of BAPNA allowed to continue for 10 minutes after which the reaction was stopped by the addition of 30% (v/v) acetic acid, centrifuged and absorbance read at 410 nm. Trypsin inhibitory activity was measured and this is expressed as percentage as indicated in Figure 11.

The second experiment stemmed also from the foregoing findings. It was designed to (a) determine the effect of protracted (7 1hr) cooking time on the level of trypsin inhibitor; (b) the contributory effect of endogenous polyphenols before and with increasing cooking period on the inhibition of trypsin if any.

For these experiments, cowpea (Vigna unguiculata) Local brown variety was used. The reason being that this

Figure 4

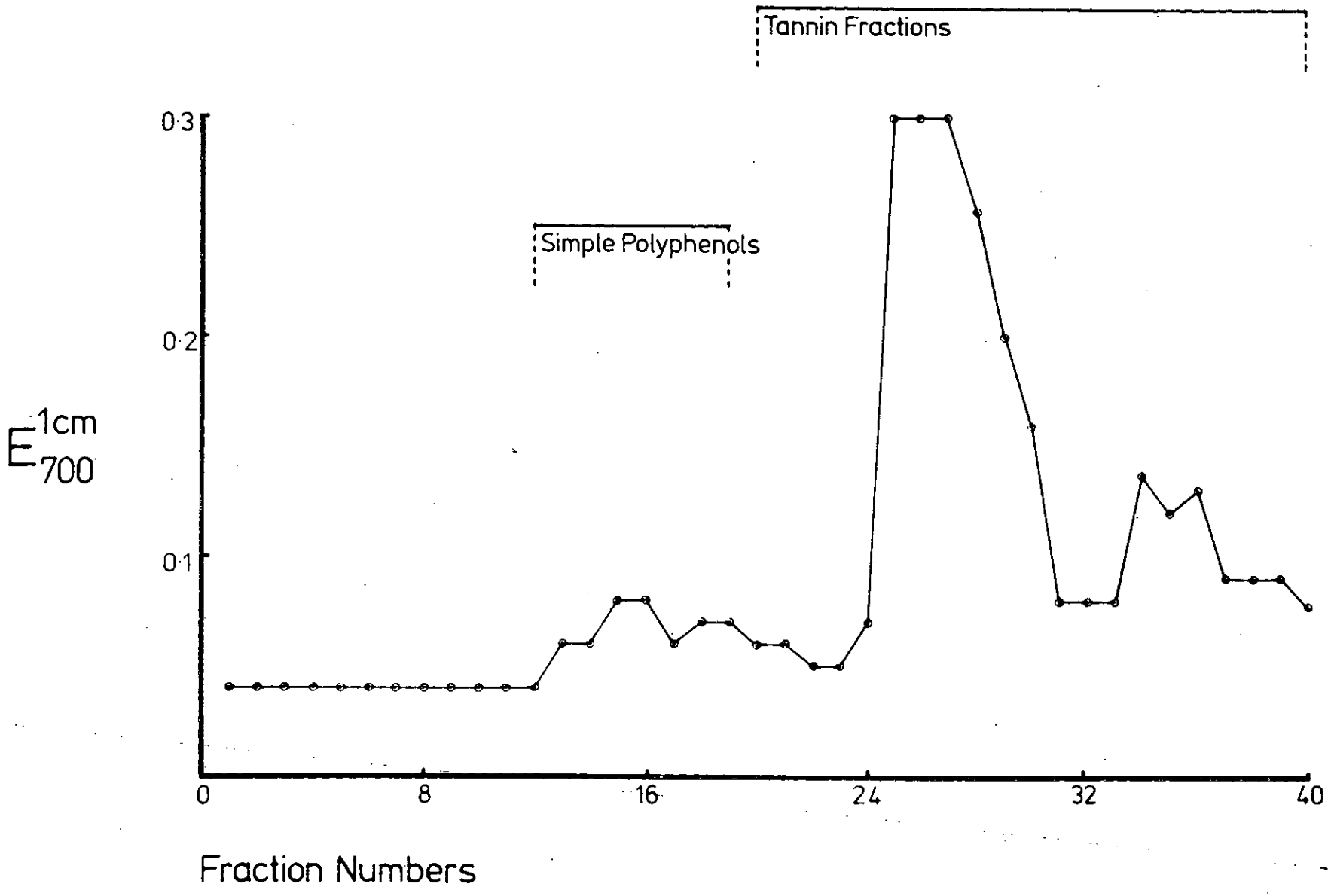
Elution profile for Affinity Chromatography of Local Brown tannin extract

Column dimension = 7 x 1.1 cm

Flow rate = 0.75 mls/min

Elution flow rate with DMF = 0.22 mls/min

Tannic acid recovery 95 - 100%


Tannin Fractions
Simple Polyphenols
0·3
0·2
0·1
E^{1cm}_{700}
0
8
16
24
32
40
Fraction Numbers

variety has moderate level of polyphenol, it is the most versatile - Ojomo (1968), and the pigmentation appeared easy to remove by gentle scrapping without touching the cotyledon when compared to others - Ewa Ibeji variety.

A portion of Local brown variety was "detannined" by gently scrapping off (with a sharp scalpel) the light brown colouration without touching the cotyledon. In all about 80-90% efficiency was achieved.

For the experiment proper, 5g each of the whole grains and the "detannined" grains were each weighed out separately in boiling tubes, in seven places to correspond to 0; 15; 30; 45; 60; 90 and 180 minutes (cooking times) respectively.

Into 15 and 30 minutes tubes were added 10 mls of boiling distilled water and into 45 to 180 minutes tubes were added 20 mls (proportion of water needed for proper cooking as judged from previous experiment) of boiling distilled water. All the tubes were then stood in boiling water in a cooking pot, only to be taken out at their respective cooking periods. Nothing was done to both zero time tubes.

At the expiration of the respective cooking times, the boiling tubes were quickly cooled under running tap

water, after which, all the tubes (together with the cooking water) including the zero timed, were frozen in liquid air, prior to freeze drying in the Modulyo freeze drier, for a period of four days.

The freeze dried bean meals were afterwards milled to pass through 425 micron sieve. Samples from these were taken for trypsin inhibitor assay, as detailed in 2.27. A summary of the results is illustrated in Figure 12.(page 112)

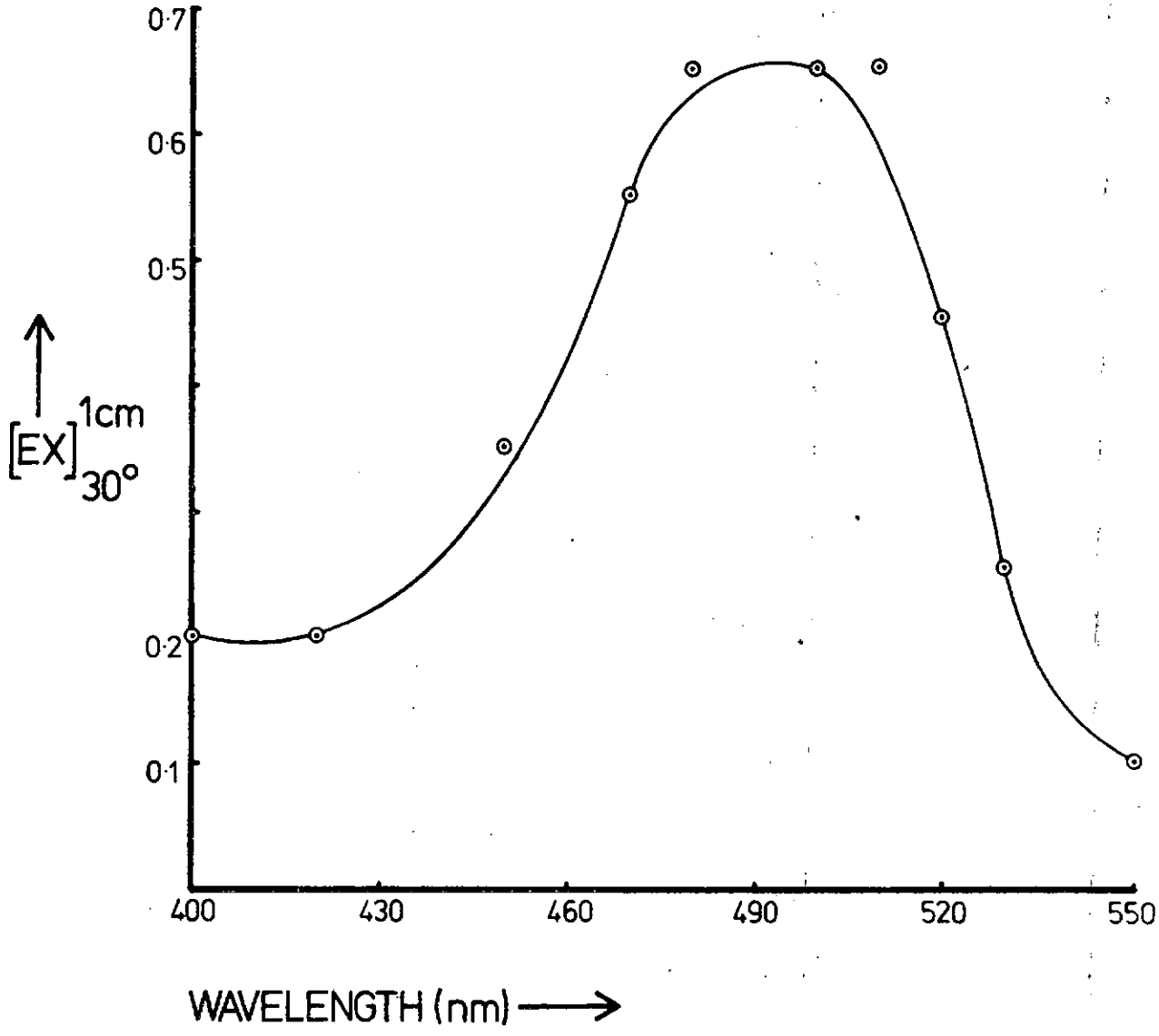
2.29 Extraction and measurement of phytohaemagglutinin (Lectin) activity

Phytohaemagglutinins were extracted from finely powdered samples (425 microns) by suspending powdered bean in 1.0ml of phosphate buffer saline for 3 hrs with occasional agitation. The extraction residue was removed by centrifugation for 5 minutes at 3000 xg. The supernatant was used for the determination of the phytohaemagglutinin activity.

Phytohaemagglutinin activity was determined by the agglutination of a 2% (v/v) suspension of rabbit erythrocytes in phosphate buffer saline. The assay was conducted by micro-titration plates by serial dilution of an extract of the phytohaemagglutinins, with a visual estimation of the end point after 2 hr. A positive

Figure 5

Absorption spectrum of vanillin-HCl Colour Reaction using Perkin Elmer 402 spectrophotometer.


0·7
0·6
0·5
0·2
0·1
[EX]1cm 30°
400
430
460
490
520
550
WAVELENGTH (nm) ⟶

control using concanavalin A was similarly put up.

2.30 Determination of apparent *in vitro* Digestibility of Crude Protein

The *in vitro* methods used are slight modifications of that of Hsu *et al.*, (1977), using a multienzyme system. The multienzyme system consists of trypsin, chymotrypsin and peptidase. In the original method, it was found that the pH of a protein suspension immediately after 10 minutes digestion with the multienzyme solution was highly correlated with the *in vivo* apparent digestibility of rats. Regression analysis of 23 samples tested showed that the correlation coefficient between pH at 10 minutes and *in vivo* apparent digestibility was 0.90 with a standard error of estimate of 2.23. The method is reputably sensitive and claimed to detect the effects of trypsin inhibitor, chlorogenic acid, and heat treatment on protein digestibility. Strong buffer salts are known to affect the measurement of protein digestibility, but it was claimed that the buffering effects found in general food proteins and products tested did not create any problem with the procedure.

In a typical experiment, 10 ml of aqueous bean meal suspensions (6.23 mg protein/ml) in glass distilled water were adjusted to pH 8.0 with 0.1N HCl and/or NaOH, while

stirring in a 37°C water bath. The multienzyme solution (1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase/ml) was maintained in an ice bath and adjusted to pH 8.0 with 0.1N HCl and/or NaOH; 1.0 ml of the multi-enzyme solution was then added to the protein suspension which was being stirred at 37°C. A rapid decline in pH occurred immediately. This was caused by the freeing of amino acid carboxyl groups from the protein chain by the proteolytic enzymes. The pH drop was recorded automatically (using the pH stat) over a 10 minute period.

The graphical relationship of pH drop against time was determined for the various bean meal preparations. This was compared with those of casein, and potato protein of known true digestibility values (courtesy of Mr. M.D. Eyres, Department of Agric. Biochemistry, Newcastle University), similarly treated. The regression equation $210.46 - 18.10X_1$; where X, equals pH at 10 min was used for the calculations.

The buffering capacities of the various bean meal preparations were determined by slowly titrating a 10 ml suspension (pH 8.0) of each meal with 0.0096 N HCl to pH 6.45 over a 10 minute period. The amount of acid consumed was used as an index for the buffer capacity of the protein source. All analyses were in duplicate, summary of result as in 3.08.

Figure 6

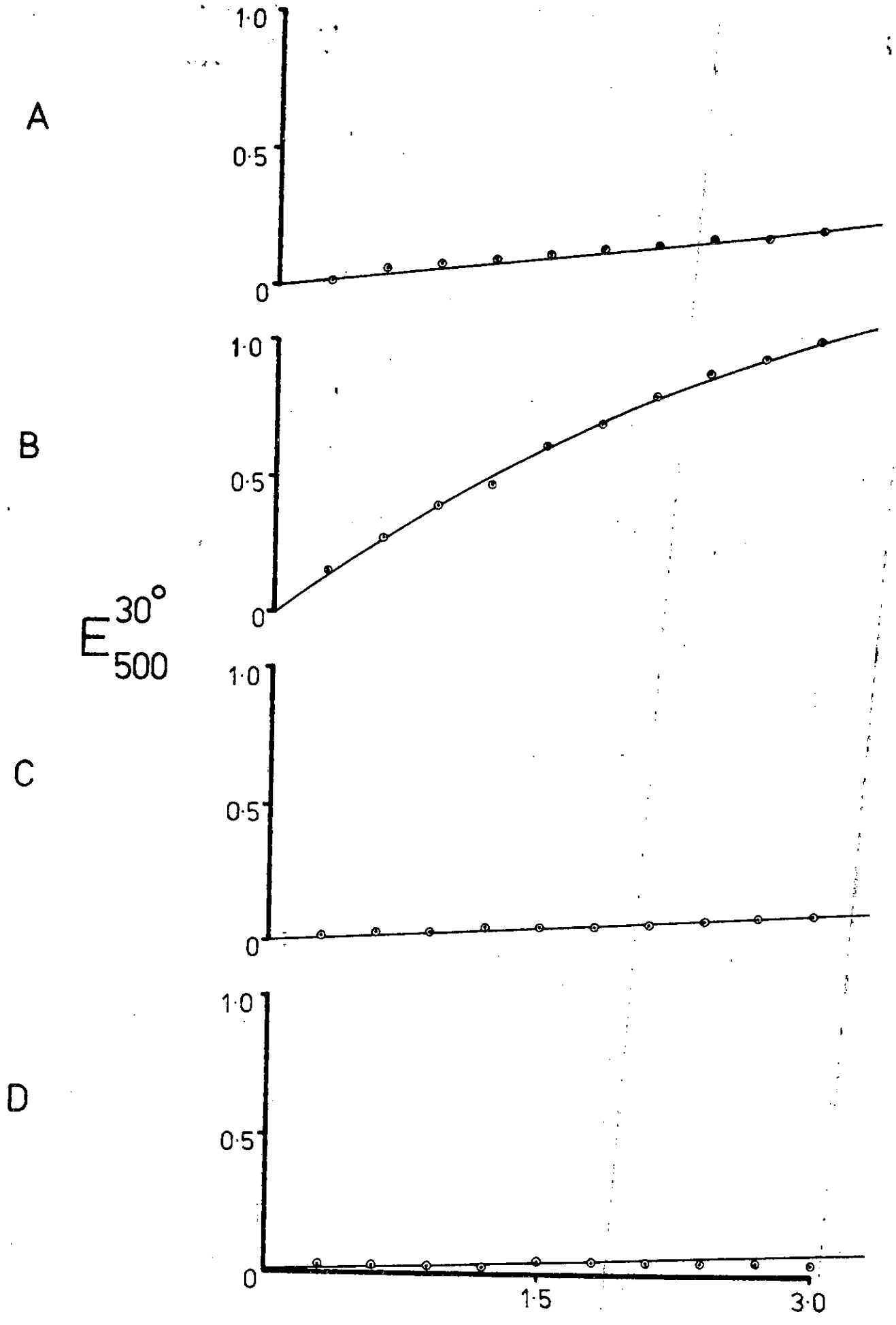
Effects of different Solvents on Catechin-Vanillin/HCl Colour Reaction.

A. Calibration curve in DMF

B. Calibration curve in Methanol

C. Calibration curve in Meth/Water (60:40 v/v)

D. Calibration curve in Water


A
B
C
D
1·0
0·5
0
$E^{30°}_{500}$
1·5
3·0
(+) Catechin Equivalent in mg/ml

In the other modification of this multienzyme system employed, 5 ml of buffered bean meal suspension (6.25 mg/ml) in phosphate buffer (0.1M KH_2PO_4/NaOH) pH 8.0, and 0.5 ml of equally buffered multienzyme (1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase/ml) pH 8.0 were incubated at 37°C for 3 hrs, and hydrolysis allowed to proceed at this temperature. After 3 hrs the reaction was stopped by the addition of 2 ml aqueous 10% w/v trichloroacetic acid. The solutions were whirlimixed, allowed to stand for 30 minutes and then centrifuged. The supernatants were analysed by Kjeldahl method to determine the TCA-soluble nitrogen released by hydrolysis. Reagents controls were put up containing inactivated trypsin (addition of trypsin after TCA). All samples were done in duplicate and allowance made for reagent controls.

Casein, potato protein and egg albumin of known true digestibilities were similarly treated. The percentage hydrolysis was calculated as a fraction of the equivalent amount of unhydrolyzed protein or bean meal put through Kjeldahl for total nitrogen. Results, are as tabulated in Table 3.09 (page 116)

2.31 Human Feeding Experiment

It is generally admitted that the availability of the different amino acids may vary within the same feed-

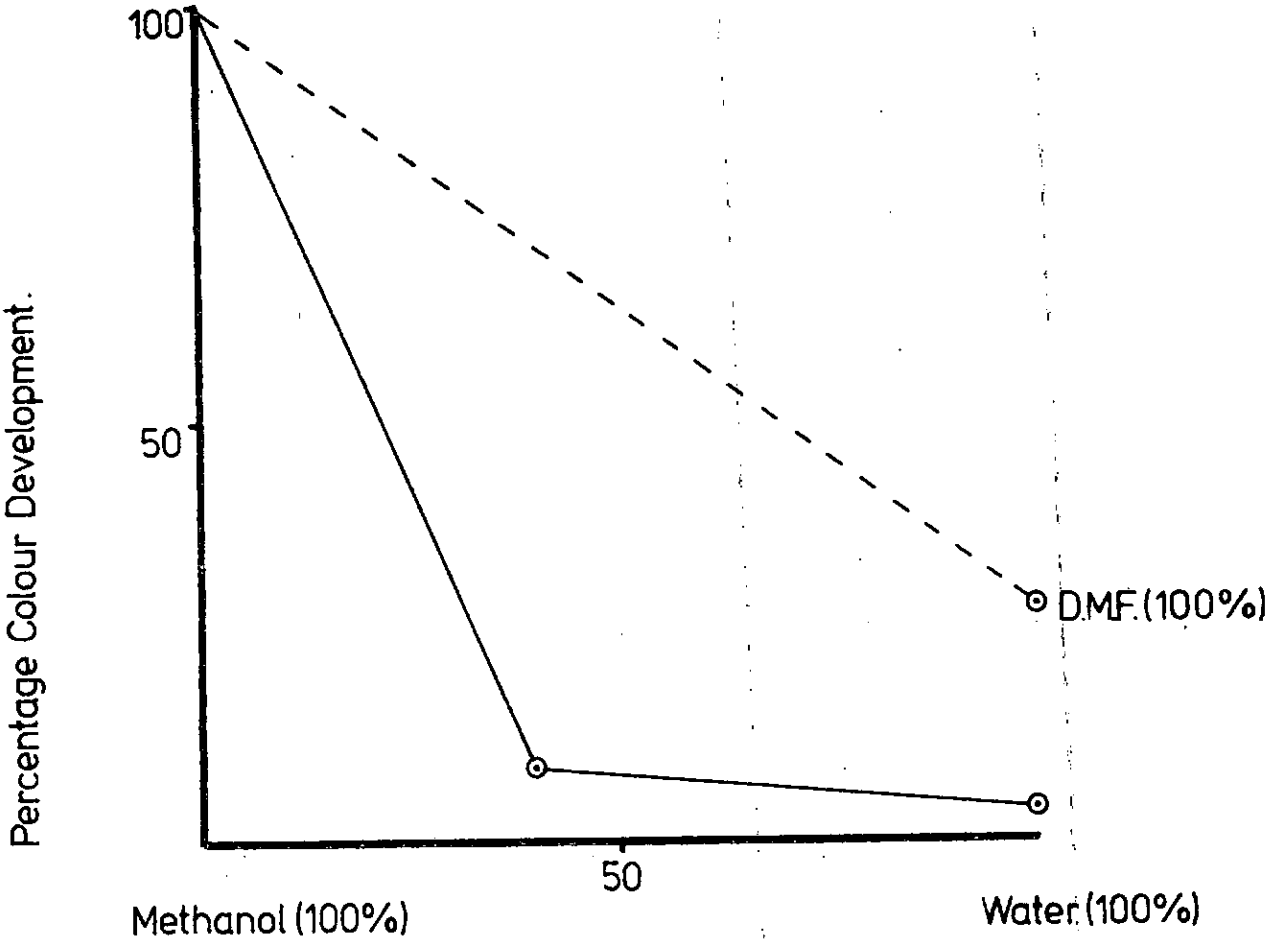
stuff, and that several of the indirect biological tests employed as indices of nutritional value of dietary protein suffer from some degree of inaccuracy, whilst the ultimate procedure is practical feeding tests with the respective animals on which production is to be done as a necessary step to verify data collected in the laboratory.

To this end, it was thought necessary to look into the nutritional fate of bean meal as consumed by Nigerians. The technical difficulties and costs associated with such an exercise were too familiar, but the exercise was remodified from the standard nitrogen balance experiment involving trials at different levels of protein intake to only what happens (as regards "crude" gut transit times as well as apparent digestibilities) to bean meals subjected to different culinary processes since human beings eat food and not diet. As there is no metabolic ward designed for such a study, the obvious choice then became patients that could be relatively controlled dietarily, these being orthopaedic patients.

In practice, the procedure involved preparing bean meal as whole grain (washing and boiling or soaking for 1 hr and throwing away the soaking water prior to boiling) and as Moin-Moin - Appendix III.(page 128) The ingredients added

Figure 7

Inhibition of Catechin/Vanillin-Hydrochloric acid colour reaction by some solvents (DMF and Water)


100
50
Percentage Colour Development.
D.M.F. (100%)
50
Methanol (100%)
Water (100%)

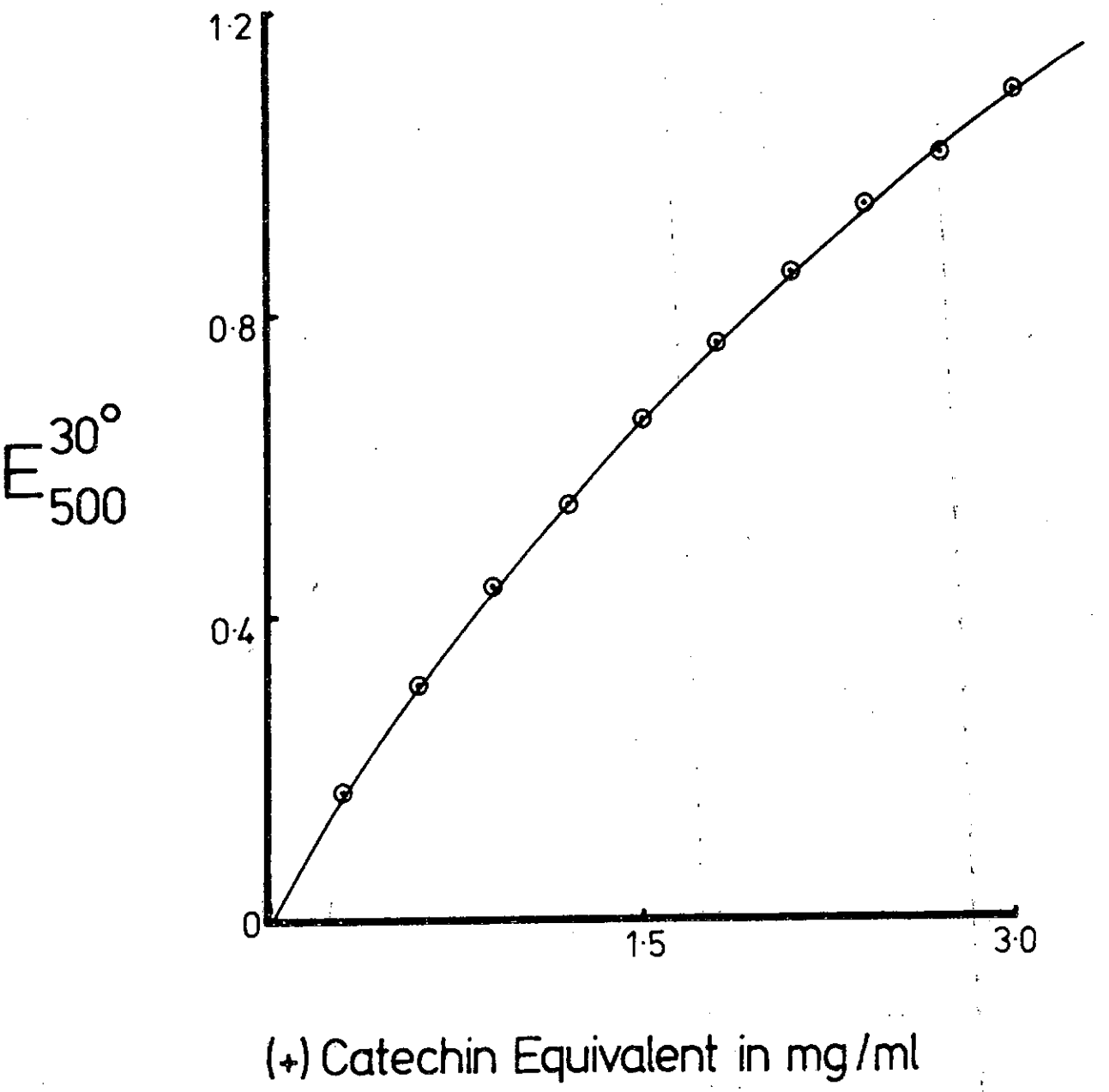
in the three procedures were the same as in Appendix IV. (page 129). The Local Brown variety was used. The rationale behind this, is to see if tannin and/or bean seed coat has any appreciable effect on gut transit time and thereby indirectly influencing digestibility.

Volunteer patients after an overnight (14 hrs) fast were dished out weighed quantities of bean meal as much as each wanted. Before and after the meal one activated charcoal tablet (supplied by Merck Pharmaceuticals) each was given to subjects to swallow with water. No other meals were allowed except water until approximately 6 hrs later. Any leftover (plate waste) was weighed and the weight taken out of the original portion. A portion of the meal was taken to the laboratory for analysis (moisture and nitrogen content). The subjects and nursing staffs were asked to look out for all dark stools. These were collected free from urine contamination, and the times passed equally noted.

In this study all the marked meal was deemed to have fully passed through immediately brighter stools start appearing. The 'crude' gut transit time for the marked meal is taken as the mean of the time the first marked stool was passed, and the time the last marked stool appeared. Each procedure was done in duplicate. The

Figure 8

A typical calibration curve for Catechin-Vanillin/ Hydrochloric acid. Standard made up in methanol using Perkin Elmer 402 Spectrophotometer and 1 cm light path.


1·2
0·8
0·4
0
1·5
3·0
$E^{30°}_{500}$
(+) Catechin Equivalent in mg/ml

stools were all reflector lamp dried and samples taken for nitrogen estimation by macro Kjeldahl methods. Apparent digestibility was calculated as:-

$$\frac{\text{Total Nitrogen Intake - Total Faecal Nitrogen X100}}{\text{Total Nitrogen intake}}$$

collated results as in Table 3.10.

2.40. Statistical Analysis

Data were subjected to a non-parametric statistics (Mood et al., 1974) in assessing correlation between the catechin level, Trypsin Inhibitor levels and Multiple enzyme hydrolysis of the bean meal under studies.

CHAPTER 3

RESULTS AND DISCUSSION

It is after all as fallacious to believe that lack of convincing evidence is proof of no effect as it is to urge the indiscriminate human consumption of any substance purely on the basis of a theoretical argument or an animal experiment.

T.W. Aderson (1977)

3.10 General characteristics of cowpea

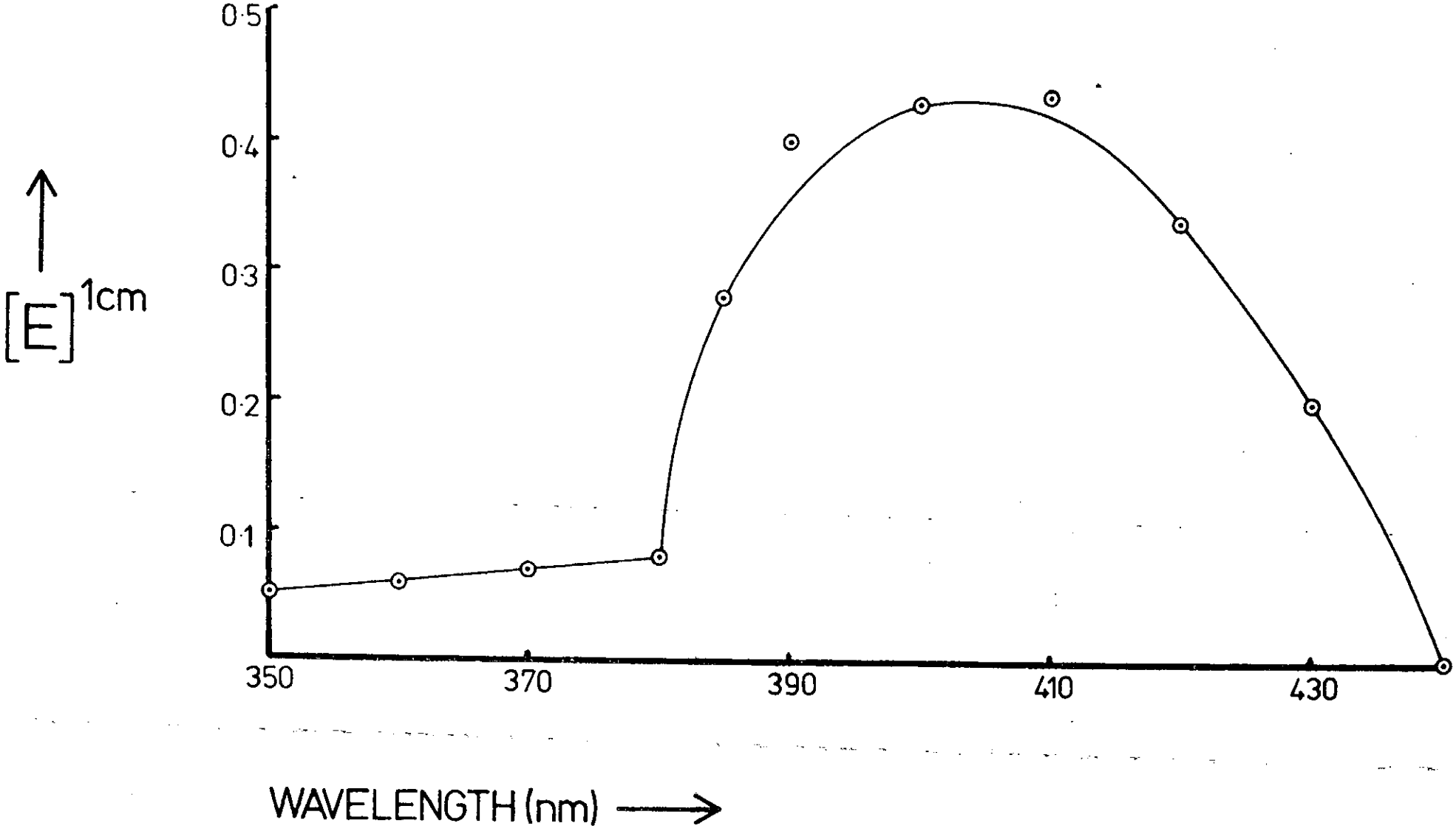
Several general characteristics of cowpea (Vigna unguiculata) found during the traditional culinary preparation of cowpea meal in this study are presented in Table 3.01 (page 95)

The various varieties are unique in size and weight, generally ranging between 12 to 30 gram per 100 seeds. It appears that seeds that are not within the average weight and size of its variety abort instead of developing to maturity.

The moisture content of the beans as bought from the market ranges between 9 to 10 per cent. The nutritional importance of this low water content could only be speculative in the light of Carpenter et al., (1962) finding that the loss of available lysine was greatest at 5-14 per cent moisture. Thus suggesting that drying to a moisture content below 5 per cent should only take place when absolutely necessary. In the case of cowpea, this is absolutely necessary especially in the humid tropics, in order to prevent post harvest loss due to mould growth. On the other hand high available lysine has been reported in cowpea dishes of Western Nigerians, Annegers (1974). Therefore the low or reduced moisture has not resulted in an appreciable loss of dietary lysine.

Figure 9

Typical absorption spectrum of the p-nitroanilide liberated from BAPNA (maximum absorption at 410 nm).


0·5
0·4
0·3
0·2
0·1
[E]1cm
350
370
390
410
430
WAVELENGTH (nm) ⟶

However, Miller (1956) reported that dry materials are resistant to heat. In this study, most cowpea dishes cooked within 2 hrs, although it was found out that pre-soaking the beans in cold water reduces the cooking time by about 10 to 15 minutes. Using household pressure cookers was also found to reduce cooking time and preserves flavour. The ratio of bean to cooking water being in the neighbourhood of 1 part of dry seeds to 2.5 - 3.0 parts of cooking water, giving a final cooked product 2.5 to 2.7 times (wet weight) its original dry weight, depending on the variety. It must be borne in mind however, that prolonged storage and the several procedure employed in processing of cowpea can have deleterious as well as beneficial effect on protein quality. The principal factors involved are the duration of heat treatment (as it operates in Nigerian traditional way of life - repeated day to day warming of food), temperature level and the presence of moisture and reducing substances. The content and availability of several amino acids may be affected during processing of this type.

Moderate heat treatment on the other hand facilitates the enzymatic decomposition of the proteins and thus improves digestibility. Heat treatment can also result in the decomposition of hydrogen bonds,

salt bonds, and peptide linkages, thereby increasing the number of reactive groups in the protein molecule, in addition to inactivating enzyme inhibitors and neutralising toxic substances (Lang, 1960 and Bender 1970). The improvement of taste by the formation of various aromatic substances is another beneficial effect brought about by heat treatment.

Most of the cooked (whole bean & moin-moin) cowpea dishes contains between 60 to 70 per cent moisture. This illustrates the importance of water to the body, both from the point of view of structure and function. Water is the solvent of most of the constituents of the living cell, it is the vehicle that transports nutrients to the cells, the medium wherein all chemical changes take place and solvent wherein the end-products of these chemical changes are discharged from the body. The functional efficiency of the digestive tract, the normal production of the digestive juices, the normal absorption of food and the normal action of the bowels may be cited as conspicuous examples of the need of the body for adequate supply of water. Insufficient ingestion of water gives rise to headache, loss of appetite, disturbance of digestive functions and of the action of the bowels, nervourness and impaired capacity for work, mental or physical. Its composition and function in the diet

Table 3.01

General Characteristics of Cowpea (Vigna unquiculata)

Table 3.02

Crude protein content of the most relished cowpea varieties from Nigerian Markets. Raw[1] and cooked[2] respectively.

	%age moisture	PH. in distilled water	Ratio of seed to water for thorough cooking	Cooking temperature	Cooking time (mins)	Weight/100 dry seeds	% Seed coat
Local Brown (raw)	9-10	6·2-6·5	1:2·5	100-104	70-90	18-20g	3·5-4·0
Local White (raw)	9-10	6·2-6·5	1:2·5	100-104	70-90	26-27g	4·7-5·0
Ewa Ibeji (raw)	9-10	6·2-6·5	1:3	100-104	120-150	12·5-13g	
Cooked Bean	62-65						
Moin-Moin	69-70				60-80		

①

VARIETIES	% Nitrogen	G.Protein/100g (N×6·25) dry wt
Local Brown	4·03	25·19
Local White	3·96	24·75
Ewa Ibeji (A variety)	4·50	28·13
Ewa Ibeji (B variety)	4·60	28·75
RANGE	4·6-3·96	28·75-24·75

②

% Nitrogen	G.Protein/100g (N×6·25) dry wt
4·14	25·88
4·20	26·25
4·40	27·50
4·75	29·69
4·75-4·14	29·69-25·88

therefore, presupposes that water could be regarded in a strict sense as food.

3.20 Protein content

The crude protein contents of the varieties of cowpea (raw and processed) under study are presented in Table 3.02. The crude protein content ranges between 25 and 29 grams per cent in the raw, and 26 to 30 gram per cent in the processed cowpea respectively. The slight increase in crude protein content, as a result of processing is due to damage (gelatination) to starch grains, resulting in relative increase in nitrogen to carbohydrate.

However these figures should be interpreted with caution, in view of the method used.

The Kjeldahl method employed estimates the total nitrogen which is converted to total crude protein by means of an empirical factor (x6.25), which depends on the proportions of the various nitrogenous compounds present in the particular food. The figures therefore include non-protein nitrogen but nitrates, nitrites, nitroso and nitro-compounds for instance are not estimated by the procedure. When these figures are compared with those of animal proteins, it becomes clear that cowpea has higher total nitrogen content. This is explainable on the basis of the amino acids which differ from those of animal

proteins. There are more basic and dicarboxylic amino acids in plant proteins with a considerable part of the carboxylic groups present in amide form, hence the total nitrogen content of plant proteins are higher than those of animal proteins (Agren 1970).

3.30 Extraction and Determination of Polyphenols

The experiments using different solvents (water, dilute methanol, 100% methanol and dimethylformamide), together with various studies in this thesis show that most seed polyphenols are localized in the tissues other than the cotyledons (e.g. the testa). Seeds having buff, or white testae had small amounts of both hydrolysable and condensed tannins as compared to the highly pigmented (darker coloured) seeds.

Table 3.03 shows the results obtained on extraction procedure of the cowpea under study.

In both whole grain and ground (milled) grain, water gave better extractive power than other solvents. This is followed by dimethylformamide. The seeds used in this experiment was the Ewa Ibeji (twin beans) variety. Both Folin and Denis (redox) and Vanillin-HCl colorimetric reactions with water extracts gave either cloudiness or low values even after centrifugation, Figures 2, 6 and 7 (pages 72, 84 & 87) indicating that although water has the greatest

Table 3.03

Extractable polyphenol levels of whole or ground grain in different Solvents. Concentration expressed in mg. per 10g dry weight "Tannic acid equivalent".

Table 3.04

The extractable polyphenols content of some common varieties of cowpea (Vigna unguiculata) from Nigerian market expressed as "Tannic acid and catechin equivalent" respectively in mg per 10 g dry weight.

	Water.	Methanol / Water 60/40v.v	Methanol 100%	Dimethyl-Formamide D.M.F.
Whole Grain	12·2	5·5	0·9	5·8
Ground Grain	39·8	21·0	7·2	33·7

	RAW		PROCESSED		% Raw Bound as a result of processing	
	A	B	A	B	A	B
Local Brown	11·2±1·8	25·4	3·6	1·6	67·9	93·7
Local Brown (decorticated)	—	—	2·7	N.C.D.		
Local White	5·0	16·6	2·5	N.C.D.	50	100
Local White (decorticated)	—	—	2·0	N.C.D.		
Ewa Ibeji (A variety)	35·2±4·0	109·5	3·9	5·6	88·9	94·9
Ewa Ibeji (B variety)	38·2	75·4	3·9	2·4	89·8	96·8

A Tannic Acid equivalent.

B Catechin equivalent.

N.C.D. Not Chemically Detectable.

extractive power it is unsuitable for the subsequent chemical reactions. On the strength of this the next polar solvent dimethylformamide (DMF) was chosen. This produced less cloudiness in the Folin and Denis reaction. Folin and Denis calibration curve in DMF is experimentally acceptable (slope 9.25) amongst the four solvents under investigation. A summary of the results is given in Figure 2. The solvent of choice for cowpea hydrolysable tannin assay was therefore dimethylformamide. The maximum absorption spectrum of the Folin and Denis colourimetric reactions using this procedure was found to be 700 nm on the Perkin Elmer 402 spectrophotometer in use. A typical spectrum and calibration curve of the Folin and Denis colourimetric reaction (tannic acid equivalent) are shown in Figures 1 and 3 (pages 69 & 75 respectively).

Although an ideal solvent for polyphenol extraction and tannin acid equivalent assay, dimethylformamide was found unsuitable for the catechin equivalent (condensed tannin) assay, causing quenching of the Vanillin-HCl colourimetric reactions. This was circumvented by rotary evaporating the DMF extracts and taking it up in methanol for the Vanillin-HCl colour reaction. A summary of the effect of the respective solvent on Vanillin-HCl colour reaction is illustrated by Figures 6 and 7 (pages 84 & 87). The maximum absorption spectrum (Fig. 5) (page 81) of the Vanillin-HCl was

found to be 500 nm using the Perkin Elmer 402 spectrophotometer, and a typical calibration curve using this procedure and catechin as standard is presented in Figure 8.(page 89)

3.40 Polyphenol Contents

The polyphenol contents of cowpea (raw and processed) are expressed as either 'Tannic acid equivalent' or 'Catechin equivalent' as shown in Tables 3.04 and 3.05 respectively. The extractable polyphenol was further characterised into 'tannin-like' (protein binding) and simple polyphenols by means of affinity chromatography on immobilised protein Figure 4.

When polyphenols are determined by the Vanillin-HCl method and values expressed as catechin equivalents, the values (16.6 to 109.5 mg per 10 gram dry sample) are higher in the raw samples than when determined by the Folin and Denis method and the result expressed as tannic acid equivalent (5 to 40 mg/10 gram dry sample). In either of the methods however, the highly pigmented cowpea has the highest polyphenol content, suggesting a probable correlation between colour of the seed and its polyphenol content.

Knowledge of the chemistry and function of plant polyphenols is still fragmentary but the seemingly

Table 3.05

Extractable polyphenols and the relative 'Tannin-like' portion in some varieties of raw and processed cowpea (Vigna unguiculata) using affinity chromatography technique. Contents expressed in mg per 10 g dry weight.

	RAW			PROCESSED			
	Total Extractable Polyphenols	Tannin-like Equivalent	%age Tannin-like	Extractable Polyphenols	%Extractable Polyphenol relative to raw	Tannin-like	%age Tannin-like
Local Brown	11·2	8·8	78·6	3·6	32·1	3·2	88·9
Local Brown (decorticated)	—	—	—	2·7	—	2·6	96·3
Local White	5·0	3·6	72	2·5	50	1·5	60
Local White (decorticated)	—	—	—	2·0	—	2·0	100
Ewa Ibeji (A variety)	39·8	38·7	97·2	3·9	9·8	3·8	97·4
Ewa Ibeji (B variety)	39·9	38·8	97·2	3·9	9·8	3·7	94·9

explanation advanceable for the relative discrepancy in values between 'tannic acid and catechin equivalents', is that the latter is not specific for polyphenols that are tannins, but will react with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus activated toward electrophilic substitution in the molecule. It is however specific for a very narrow range of flavan-3-ols, dihydrochalcones and proanthocyanidins, but will not discriminate between these on the basis of molecular weight. The higher value is therefore due to the (vanillin hydrochloric acid) method measuring low as well as high molecular weight phenols and not tannin alone (Gupta and Haslam, 1979).

The use of immobilised protein in the affinity chromatography (Figure 4) therefore affords the means of assessing the 'true' protein binding polyphenols in the bean extracts. The values for these expressed as tannic acid equivalent are shown in Table 3.05; Columns 2 and 3; 6 and 7 for the raw and processed beans respectively. These are termed 'Tannin-like' equivalents.

Inspection of Table 3.05 columns 1, 2 and 3 show that between 72 to 97 per cent of the extractable total polyphenols expressed as tannic acid equivalent is protein binding. These values increase with increase in

pigmentation. Processing however, reduces this to between 10 and 50 per cent (Table 3.05, column 5) indicating that between 90 and 50 per cent (Table 3.04 column 5) were bound in one form or the other to the protein or carbohydrate or both, or chemically modified during heat processing to the extent that it became chemically unextractable or unassayable. What is particularly interesting is that highly pigmented beans firmly held on to their polyphenols on processing. In particular is the case of Ewa Ibeji varieties. These are comparatively small in grain size but have high polyphenol contents; of these only 10 per cent became extractable after processing. This could be another case of small bean grains having heavier cotyledons (Bressani, 1972). However, Ma Yu and Bliss (1978) in their study on tannin content and inheritance in common bean, concluded that correlation between seed size and tannin content for all parental lines and F_2 populations studied were not significant either among all samples within a population or among only black-testa beans within a population, suggesting that tannin content is independent of seed size. They however affirmed that smaller seed may have higher tannin concentration since most tannin is located in the seed coat and smaller seed usually have more seed coat area than larger seed by weight.

A noteworthy observation is the demonstration that the removal of the testa either by scrapping or soaking of the seeds in water greatly reduces the water soluble tannins or tannin-like substances so that in effect, bean dishes like moin-moin, Akara balls, Gbegiri soup and the like that involves removal of testa (Appendix III) page 128 in preparation, a low tannic acid equivalent as well as catechin equivalent levels are to be expected. Although there is a strong consumer preference for coloured beans when eaten as whole grain because of the flavour and appeal the pigmentation offers; there are data however linking inhibition of digestive hydrolysis of dietary protein and carbohydrate with tannins especially the condensed tannins (Gadal and Boudet, 1965; Feeny, 1969). Hydrolysable tannins on the other hand have been implicated in the endogenous loss of protein and methionine by its astringency/detergency on the gut lining, (which is noticeable in the faeces of subjects on high tannin bean meal being smeared in streaks of heavy mucous) and the requirement of methyl group in detoxication of the gallic or ellergic acid produced by the hydrolysis of hydrolysable tannin.

Turning the searchlight on the catechin equivalent values and the effect of processing thereon it is worth noting that between 95 and 100 per cent of the initial

(raw sample) value became bound to the seed macromolecules or became chemically modified and as such became unextractable Table 3.04, (page 98) columns 2, 4 and 6. Feeny (1969), studying the inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin found out that as little as 10 per cent condensed tannin caused a reduction of 20 per cent in hydrolysis, and that casein complexed with its own weight of condensed tannin was almost 80 per cent less hydrolysed than the uncomplexed casein control. The nutritional effect of the findings in this study with respect to the bound catechin equivalent tannin becomes apparent by reference to Tables 3.06 and 3.09. (Pages 107 and 116)

Pierpoint (1970) in his review of group of reactions that are thought to involve o-quinones, pointed out that oxidation of phenols (generally o-dihydroxyphenols e.g. flavanols produce o-quinones, and once these are formed they react non-enzymically with many compounds. They may either polymerise, be reduced, or suffer nucleophilic attack by substances possessing amino, thiol and 'activated' methylene groups. Proteins are of course known to have a range of chemical groups; amine; α-amino and thiol, potentially able to react with o-quinones. Although some of these may be 'buried' in the interior of the protein and consequently unreactive, exposed

groups however will probably be reactive because of their peptide bonding. Another possible reaction between quinones and proteins is that the polymer formed from some phenols, especially the flavanols (and possibly enhanced by the cooking procedure) might have 'tannin-like' properties and, as do the classical vegetable tannins, complex with and possibly precipitate proteins. The reactions between phenolic groups and peptide bonds are initially through hydrogen-bonding and although this is reversible by detergents or alkali, they would become less reversible with time as o-quinone groups formed in the phenols react covalently with suitable groups on the protein. Processing and storage of plant materials are known to cause oxidation of any phenols present and the polymerisation of o-quinones.

3.50 The Phytohaemagglutinin and Trypsin Inhibitor Levels

There was no detectable agglutination of rabbit erythrocyte by both raw and processed cowpeas as compared to the control concanavalin A. This may be due to a significant low level in Vigna unguiculata species as a whole, which is further degraded during storage (Toms and Western, 1971). Whatever be the case, the heat treatment usually given to cowpea meal is adequate to inactivate any lectin that may be present.

Table 3.06

Trypsin inhibitory levels of some common varieties of cowpea and the effect of processing on their activities

* 1 µg of soybean TI (activity 1 mg = 1.2 mg trypsin) is equivalent to 39.375 TIU in this assay

Table 3.07

Trypsin inhibitory (TI) levels of solvent treated bean (raw and processed) meals - "Extractable tannin-free fractions" (E.T.F.F.)

* 1 µg soybean TI is equivalent to 39.375 TIU

VARIETIES	RAW TIU*/mg. sample	PROCESSED TIU*/mg. sample	PROCESSED %destruction due to processing
Local Brown	97·9	22·5	77
Local Brown (decorticated)	—	14·6	85
Local White	55·0	21·2	61·5
Local White (decorticated)	—	5·4	90
Ewa Ibeji (A variety)	105	22·9	78·2
Ewa Ibeji (B variety)	105	22·9	78·2

VARIETIES	RAW• TIU*/mg.	PROCESSED•• TIU*/mg.	PROCESSED•• TIU/mg. extractable polyphenol. ($\times 10^{-3}$)
Local Brown E.T.F.F.	42·7	18·8	• 55·2/1·12 •• 3·70/0·36
Local Brown (decorticated) E.T.F.F.	—	14·2	0·40/0·27
Local White E.T.F.F.	50	18·8	• 5/0·5 •• 2·40/0·25
Local White (decorticated) E.T.F.F.	—	5·4	0·0/0·2
Ewa Ibeji (A variety) E.T.F.F.	85	22·9	• 20/3·98 •• 0/0·39
Ewa Ibeji (B variety) E.T.F.F.	88·3	20·4	• 16·70/3·99 •• 2·50/0·39

Trypsin inhibitory activity of raw and processed cowpea using the improved Kakade et al., (1974) procedure which allows the estimation of less than 10 trypsin inhibitor unit/mg sample is as shown in Tables 3.06 and 3 07. The calibration curve of trypsin using BAPNA as substrate is shown in Figure 10. This is linear within the concentration of trypsin used in this study, maximum extinction (410 nm) of the p-nitroaniline liberated from BAPNA is as shown in Figure 9.

Inspection of Table 3.06 shows that the raw sample ranges between 55 and 105 T.I. Units depending on the variety and pigmentation. The highly pigmented (Ewa Ibeji varieties) have the highest trypsin inhibitory unit whilst the Local White has the least. Processing significantly, reduced the levels in all the whole grains to just well over 20 (21.2 - 22.9) T.I. Units which is a 62 - 78 per cent destruction as a result of heat treatment. When beans are soaked, testae removed and cooked, the residual trypsin inhibitor is slightly further decreased. Trypsin inhibitors are known to readily leaching out on soaking (Onayemi et al., 1976). This difference is probably due to combination of leaching effect of trypsin and tannin (Ma Yu and Bliss 1978, and this study).

Table 3.07 illustrates the valuesof extractable

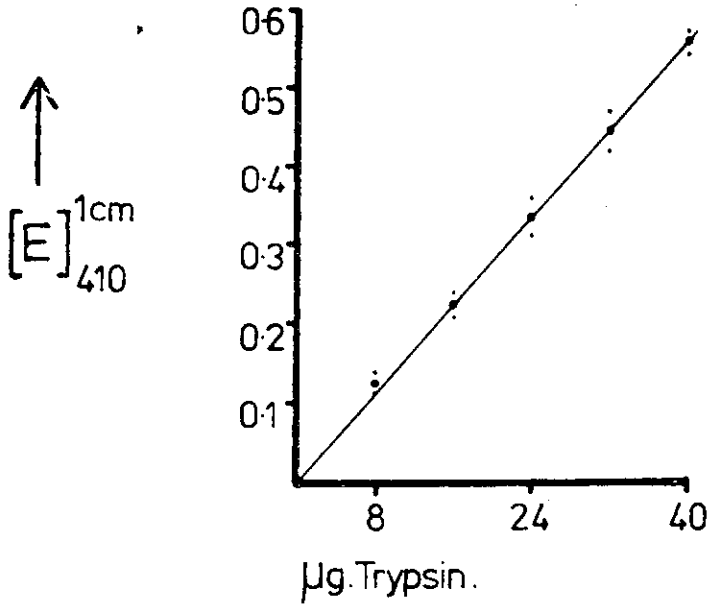
tannin free fractions (E.T.T.F) of raw whole grain as ranging between 42 and 88 T.I.U. a reduction of between 56 and 16 per cent in trypsin inhibitory unit as a result of removal of soluble tannins. The Local brown variety has the lowest T.I. value after extraction which is an indication that the inhibitory effect is partly due to extractable polyphenol. The processed values were those obtained on fractions that were extracted (E.T.F.F.) after processing. Thus the reduction (0 - 16 per cent) in trypsin inhibitory value is as a probable result of contributory extractable tannin. This is nutritionally insignificant if we admit that hydrolysable tannin (which generally forms the major fraction of extractable tannins, cf. Table 3.05) is hydrolysed in the gut into gallic or ellagic acid and a sugar moiety and that its hydrogen bonding with protein is equally reversible at pH above 8 and in a medium containing detergent of which intestinal deoxycholate is a probable one. However, it is important to note as well, that this reduction in trypsin inhibitor level after extraction may be due to protein solvent effect of dimethylformamide and therefore trypsin inhibitor will in the like manner leach out as proteins do. It is equally possible that the use of D.M.F. may likely denature trypsin inhibitor resulting in loss of activity. However, when trypsin inhibitory unit of the

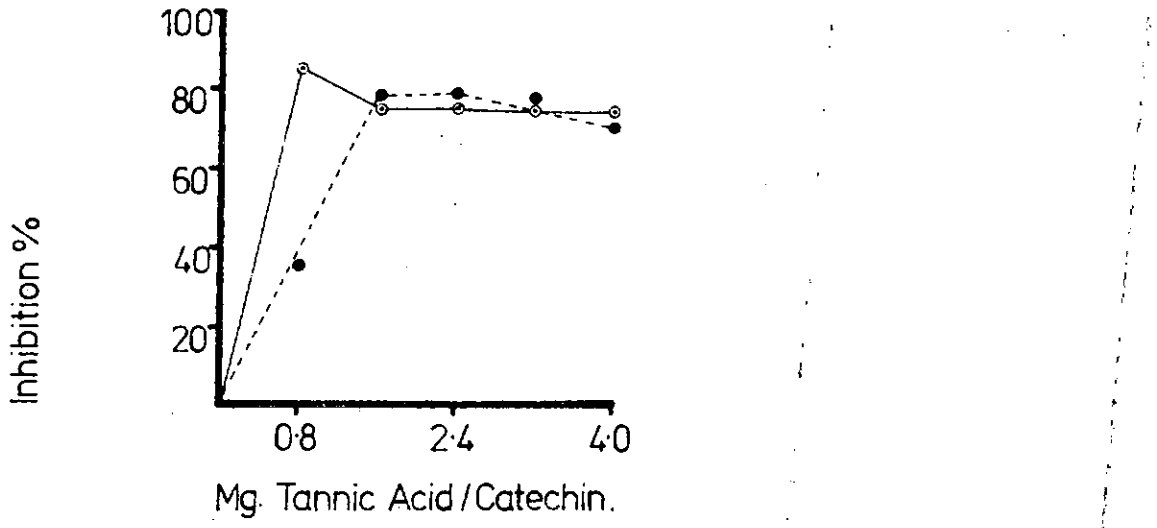
Figure 10

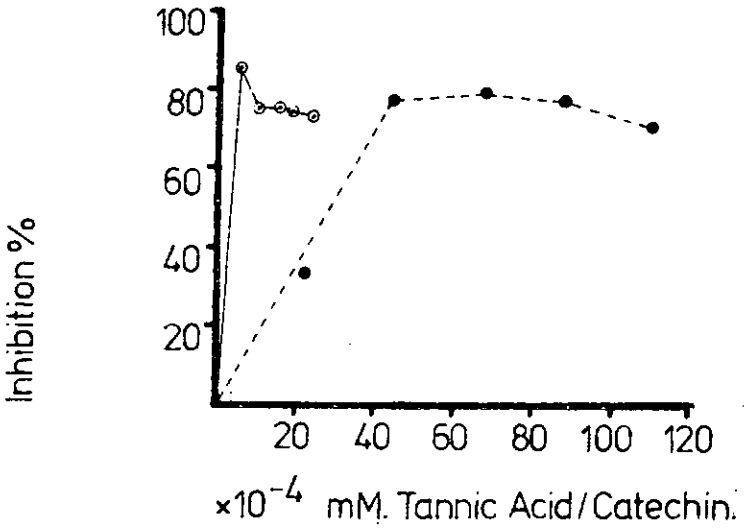
Calibration curve of tryptic activity on BAPNA

Figure 11

Effect of commercial tannic acid and catechin on tryptic activity (40 µg trypsin/tube as in the normal assay)


$[E]^{1cm}_{410}$
0·6
0·5
0·4
0·3
0·2
0·1
8
24
40
µg. Trypsin.

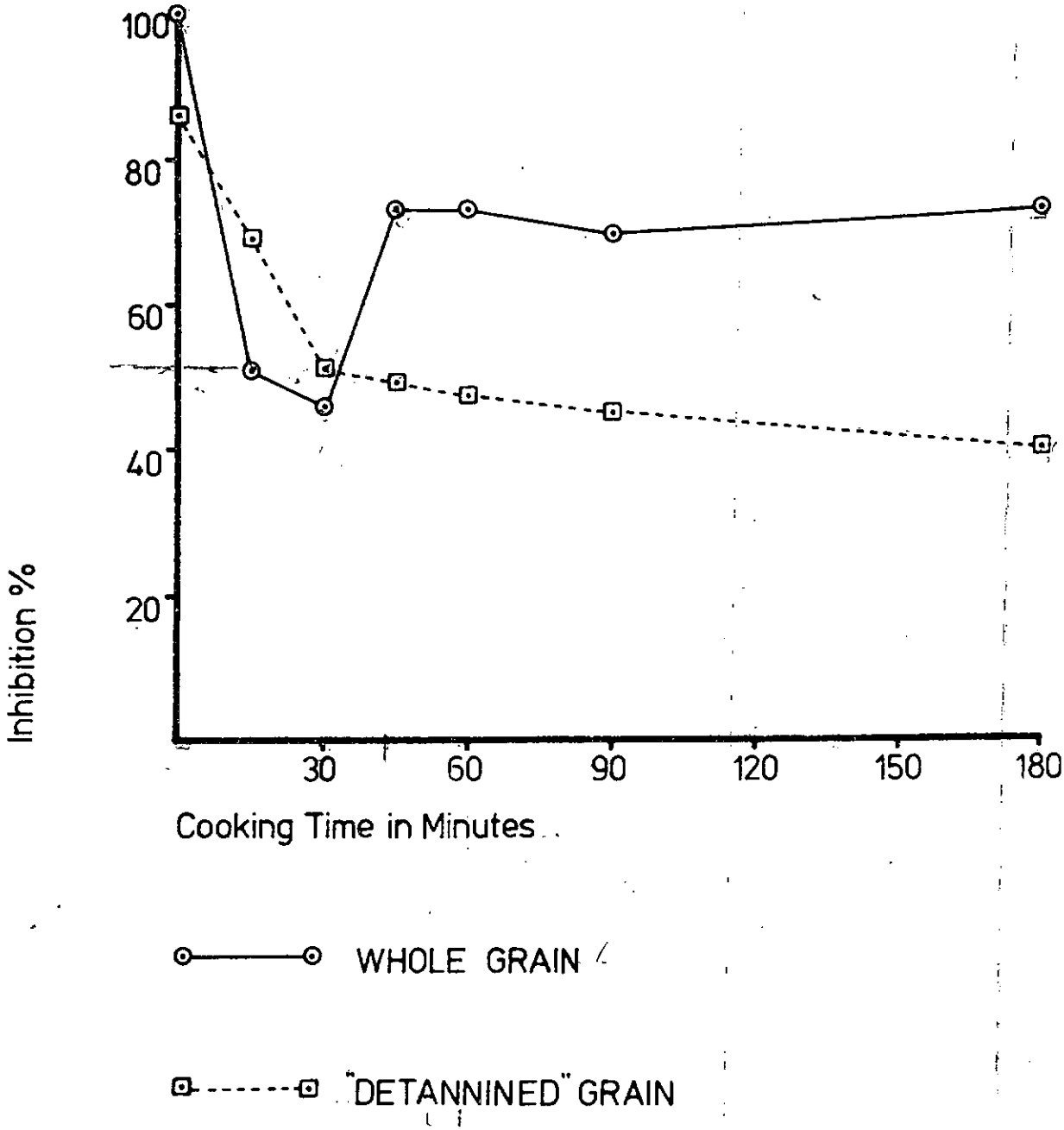

Inhibition %
100
80
60
40
20
0·8
2·4
4·0
Mg. Tannic Acid / Catechin.
Tannic Acid.
Catechin.


Inhibition %
100
80
60
40
20
20
40
60
80
100
120
$\times 10^{-4}$ mM. Tannic Acid / Catechin.

processed is ranked with catechin equivalent level of the raw bean meal using a non-parametric statistics, a strong positive rank correlation (0.875) between catechin level and T.I.U. was obtained. This in effect is a probable indication that the simple polyphenols must have undergone modification during processing and become bound covalently with the protein of the seed to the extent that they became chemically undetectable but enzymically causing inhibition. This finding is in conformity with those of Feeny (1969). Figures 11 and 12 respectively illustrate the molar effect of both tannic acid and catechin on tryptic activity. Figure 12 shows vividly the comparative effect of duration of cooking time on trypsin inhibitor level of both whole grain and 'detannined' grain. From this it could be seen that in both, inhibition dropped sharply after half an hour of heat processing, probably due to heat denaturation of the endogenous trypsin inhibitor. The 'detannined' grain continues with this drop in inhibition up to 3 hr cooking but never below 40 per cent inhibition, whilst after the ½ hr sharp drop in the whole grain, there followed a sharp rise in inhibition. This sharp rise could be assumed to be the effect of modification of the simple polyphenol with possible polymerisation resulting

Figure 12

Comparative effect of cooking time on trypsin inhibitor level of whole grain and 'Detannined' grain respectively.


100
80
60
40
20
Inhibition %
30
60
90
120
150
180
Cooking Time in Minutes
WHOLE GRAIN
"DETANNINED" GRAIN

in complexing with the trypsin or precipitating it.

3.60 In vitro Digestibility (Hydrolysis) of the bean meals

It is generally assumed that if a protein is readily digested by several enzymes in vitro, it is reasonable to expect that it will be digested (in vivo)[ ] in the gut, if it is not digested in vitro, it may still be digested in vivo, because of the simultaneous action of several proteases and of possible cooperation from the gut flora.

Although the bioavailability of protein must, in the final analysis, be established by exact feeding trials, nevertheless, in vitro methods of evaluating protein digestibility are of great importance because of their rapidity and sensitivity. Several methods ranging from one enzyme system (Sumner & Howell, 1935, Kunitz, 1947, Shaffner, 1956, MacDonald, 1962, Byers, 1967, Feeny, 1969, Tamir & Alumot, 1967, and Bond, 1976) to double and even multi-enzyme methods (Saunders et al., 1972 and Hsu et al., 1977) have been employed in the past. In all of these methods the extent of hydrolysis (digestibility) is expressed as the percentage of the substrate nitrogen or amino acid appearing as either TCA soluble nitrogen or tungstate/sulphuric mixture soluble amino acids. However of late, Hsu et al., (1977)

Table 3.08

Digestibility of Bean Meal using Multienzyme technique

a) Newcastle University, Dept. of Agric. Biochem

b) Average of triplicate runs

c) Average of duplicate runs

Regression equation $Y = 210.46 - 18.10x$, where

x, = pH at 10 min (cf. Hsu *et al.*, 1977)

Sample	In Vivo (Rat) Digestibility	In Vitro	
		HSU's (pH drop method) (c)	HSU adapted to Feeny's (c)
Local Brown (raw)		74·84	56·50
Local Brown(cooked)		78·36	71·74
Local Brown (decorticated)		81·69	70·65
Local White (raw)		77·72	65·93
Local White (cooked)		78·00	67·03
Local White (decorticated)		79·53	68·13
Ewa Ibeji (A variety) (raw)		76·56	46·74
Ewa Ibeji (A variety) (cooked)		77·19	62·64
Ewa Ibeji (B variety) (raw)		74·48	53·85
Ewa Ibeji (B variety) (cooked)		76·83	66·30
Casein (BDH)	90·00 (a)	86·08 (b)	85·71
Potato Protein	96·55 (a)	79·17	86·25
Egg Albumin	98·13 (a)	74·48	41·60

employed pH drop after 10 minutes of incubation to determine degree of digestibility.

In this study, Hsu et al., (1977) method is used because it is purported to detect the effect of trypsin inhibitor, chlorogenic acid, and heat treatment on protein digestibility. Results are as in Table 3.08. Because the multienzyme should give a better approximation of protein digestibility than the single enzyme system which is liable to specific inhibitor thereby under predicting the digestibility of proteins containing the specific inhibitor; the multienzyme system was also adapted to the single system of Feeny (1977) and of Byers (1967). Results as in Table 3.09 (page 116)

(page 114)

Reference to Table 3.08, column 2 indicates that there is a slight difference between the "apparent digestibility" figures of the raw and processed samples, using the pH drop after 10 minutes hydrolysis method. When this is compared with Table 3.09, (page 116) in which the hydrolysis after 3 hr incubation is expressed as the percentage of substrate nitrogen appearing as TCA-soluble nitrogen, we find a sharper distinction in hydrolysis as a result of processing, and of the presence of anti-enzymatic factors. Although, the higher hydrolysis index of the processed bean meal never exceeded 74 per cent; that

Table 3.09

In vitro Hydrolysis (Digestibility) of bean meal using the multienzyme of Hsu *et al.*, adapted to Feeny's method

*Unhydrolysed substrate precipitated by 10% TCA, and soluble nitrogen (X6.25 = Crude Protein) in supernatant determined by Kjeldahl analysis: Means of duplicate values, after allowance for reagent controls containing inactivated (trypsin added after TCA) trypsin. The extent of hydrolysis is stated as the percentage of the substrate N appearing as TCA - soluble N, converted to crude protein

Substrate	Total Crude * Protein (g)	TCA (g) Soluble Protein after hydrolysis	% Hydrolysis
Local Brown (raw)	0·092	0·052	56·50
Local Brown (cooked)	0·092	0·066	71·74
Local Brown (decorticated, cooked)	0·092	0·065	70·65
Local Brown (detannined, cooked 60 mins.)	0·081	0·060	74·07
Local Brown (detannined, cooked 90 mins.)	0·081	0·060	74·07
Local White (raw)	0·091	0·060	65·93
Local White (cooked)	0·091	0·061	67·03
Local White (decorticated, cooked)	0·091	0·062	68·13
Ewa Ibeji (A variety) raw.	0·092	0·043	46·74
Ewa Ibeji (A variety) cooked.	0·091	0·057	62·64
Ewa Ibeji (B variety) raw.	0·091	0·049	53·85
Ewa Ibeji (B variety) cooked.	0·092	0·061	66·30
Casein (BDH)	0·098	0·084	85·71
Potato Protein	0·080	0·069	86·25
Local Brown Protein Extract	0·070	0·042	60·00
Egg Albumin	0·089	0·037	41·60

the hydrolysis index is a better indicator of the presence of anti-enzymatic factor is vividly exhibited by the figures obtained on the controls (casein, potato protein and egg albumin). For example the protein structure of casein-based products is highly randomised and thus enzymatic treatment results in a rapid rate of hydrolysis. Hence there is a close relationship between pH drop after 10 minutes and degree of hydrolysis after 3 hr incubation as in the hydrolysis index method. By contrast however, vegetable proteins (e.g. soyprotein) are structurally highly organised proteins and are thus more resistant to enzymatic attack. This could be the possible explanation for low figures obtainable in the short time (10 minutes) incubation as compared to the 3 hr hydrolysis index.

Of particular interest is the great discrepancy in indices obtained in respect of egg albumin. One of the most interesting constituent of egg white is ovomucoid which has been identified as an inhibitor of trypsin (Lineweaver & Murray, 1947). Ovomucoid inhibits trypsin by the formation of an equimolar complex, and this complex is relatively stable for the periods which are necessary to conduct many biochemical experiments (Lineweaver & Murray, 1947). In addition to the ovomucoid in chicken

egg white, Matsushima (1958) reported another inhibitor of proteolytic enzyme which he named ovoinhibitor. Rhodes et al., (1960) reported that ovoinhibitor was also active against chymotrypsin and that it was capable of inhibiting both trypsin and chymotrypsin simultaneously. However, Feeney et al., (1963) reported that chicken ovomucoid has essentially no inhibitory activity against chymotrypsin, and that the several previous observations that ovomucoid had weak activity against chymotrypsin was due to contamination with ovoinhibitor; that the ovoinhibitor of avian egg white should be considered as a 'double headed' (distinct inhibitory sites, therefore inhibition of both trypsin and chymotrypsin occurs simultaneously, and excess of one enzyme does not affect the inhibition of the other enzyme) type of inhibition. This could probably be the explanation for the low hydrolysis index value, whereas the slight pH drop (8.0 to 7.55) compared to that of casein (8.0 to 6.87) as a probable effect of peptidase results in such a high 'apparent digestibility'.

Having said so much so far, it must be admitted that both techniques gave a pointer to the possible effect of gastro intestinal proteolysis of the different processed bean meal. It is therefore reasonable to

assume that heat processing have beneficial effect by rendering the protein more susceptible to hydrolysis due to structural changes or by destroying all or a portion of the antinutritive factors which may be present. Removal of testa by scrapping (or threshing and winnowing) resulted in slight improvement in hydrolysis (74 per cent) as compared to testa removal by soaking (70 per cent). However, a weak but significant negative rank correlation ($r = -0.543$) between trypsin inhibitory unit (TIU) and hydrolysis was obtained, indicating that the polyphenols and more particularly condensed tannins are correlated in their effect on digestibility of the cowpea protein. The low levels (ca. 40 per cent) trypsin inhibitory activity found after scrapping off the testa and heat treatment could be related to heat stable trypsin or residual polyphenol content which are known to be heat stable.

3.70 The Level of Protein intake on Apparent Digestibility: In vivo Study

As was enumerated in 2.31, this part of the study was initially initiated as a possible means of identifying the fate of bean meal through 'food approach' rather than 'diet approach' as is generally adopted in many animal experiments. It became evident later that human

Table 3.10

Effect of level of protein intake and gut transit time on the apparent digestibility of cowpea protein - meals containing more than 3 gram nitrogen

Table 3.11

Effect of level of protein intake and gut transit time on the apparent digestibility of cowpea protein - meals containing less than 3 gram nitrogen

Initials	Age	Sex	Nitrogen Intake (g)	Gut Transit Time (hrs)	Faecal Nitrogen (g)	Apparent Digestibility
R.O.O.	38	F	4·94	26	1·16	76·5
J.A.O.	41	M	4·94	26	2·01	59·3
E.A.O.	13	M	3·71	28	1·67	55·0
C.De.	35	M	3·14	22	1·39	55·7
J.A.O.	41	M	4·18	18	1·39	66·7
A.D.	21	M	4·18	27	0·93	77·8
A.D.	21	M	5·23	27	1·84	64·8
F.O.	28	M	6·28	22	0·69	89·0
A.A.	30	M	3·80	31	1·50	60·5
					Average Digestibility	67·3

Initials	Age	Sex	Nitrogen Intake (g)	Gut Transit Time (hrs)	Faecal Nitrogen (g)	Apparent Digestibility
A.O.	8	M	2·47	25	1·90	23·1
R.O.O.	38	F	2·03	28	1·28	36·8
J.A.O.	41	M	2·15	27	1·66	22·7
C.De.	35	M	2·86	32	2·02	29·4
J.A.O.	41	M	2·86	29	2·27	20·6
A.A.	30	M	2·80	17	1·59	43·2
C.De.	35	M	2·80	29	1·87	33·2

beings have some degree of freedom in their food choices, this coupled with the medicolegal aspects of human experimentation B.M.J. Editorial (1964) restricted the investigation to free choice (uncontrolled) of bean meal as in everyday life with the hope of salvaging any useful information as regards 'crude gut transit time' and possibly a one-point-meal apparent digestibility index to complement in vitro studies. The results are as shown in Table 3.10 and 3.11. (page 120) These figures are from the different culinary processing (whole grain, moin-moin) of beans which could not be analysed under the different treatment because of the small number of test patient yielding complete faecal output. On thorough scrutiny, however, a trend was observed between nitrogen intake, gut transit time and apparent digestibility. Hence the results have been collated to reflect the trends (> 3g nitrogen and < 3g nitrogen intakes) observed. Table 3.10 (page 120) shows the gut transit time, and apparent digestibility on meal containing more than 3g nitrogen whilst Table 3.11 (page 120) shows values for meal containing less than 3g nitrogen.

Inspection of both tables indicates that the mean gut transit times (25.2 ± 3.9; 26.7 ± 4.8 hrs) are not statistically different. $P > 0.05$. Striking differences are however noticeable in the apparent digestibilities. Table 3.10 having an average apparent

digestibility of 67.3 per cent whilst Table 3.11 has an average apparent digestibility of 29.9 per cent. This clearly demonstrates that the apparent protein digestibility and possibly biological value are dependent on the protein concentration in the diet and must therefore be estimated at a fixed level of nitrogen concentration. The implication of this in the true 'food approach' as compared to 'diet approach' is however hard to postulate, except to leave it as an academic exercise, because no one, not even the best nutritionist approaches his/her meals with the preset intention of acquiring x, y, z amount of protein or vitamins at a sitting per se.

CHAPTER 4

CONCLUSION

Eat all kind nature doth bestow,
It will amalgamate below
If the mind says "it will be so"
But, if once you begin to doubt
Your gastric juice will find it out.

Cathcart (1937)

4.00 CONCLUSION

Vegetable protein virtually supplies over 80 per cent of the dietary protein in the developing countries of the world, and it is estimated that legumes especially cowpea accounts for the bulk of the dietary protein intake for adults and are virtually an important source of proteins for many children in West Africa (Dema, 1963). Although possessing high protein content, poor protein digestibility as a result of the presence in the seeds of antinutritive factors such as protease inhibitors have been suggested as one factor contributing to the poor utilisation of the cowpea meal. The poor digestibility of the legume proteins has also been attributed to the presence of resistant "core" with a high cystine content and to trypsin inhibitors which have been demonstrated not to release their cystine when incubated with proteolytic enzymes (Almquist et al., 1966). The digestibility, absorption and tolerance depends on the type of legume consumed, the culinary methods, the amount consumed and the condition of the alimentary tract.

It would appear from the present studies that most of the bean polyphenols are located in the testa, and that the pigmentation of the testae correlates with the polyphenols contents whether this is expressed as

tannic acid equivalent or catechin equivalent. The legume polyphenols and more particularly the condensed tannins expressed as catechin equivalent are partly responsible for the lower digestibility of tannin-protein complexes or proteolytic enzyme inhibition by tannins as a result of heat modification of the simple phenolics, possibly resulting in quinones formation with the subsequent polymerisation of these, culminating in a probable covalent (irreversible) bonding with the cowpea macro-molecules. Any hydrolysable tannin, hydrogen bonded to the macromolecule, however, may become reversible through the gut surfactant (bile salts) apart from its ease of hydrolysis to gallic or ellagic acid and a sugar moiety.

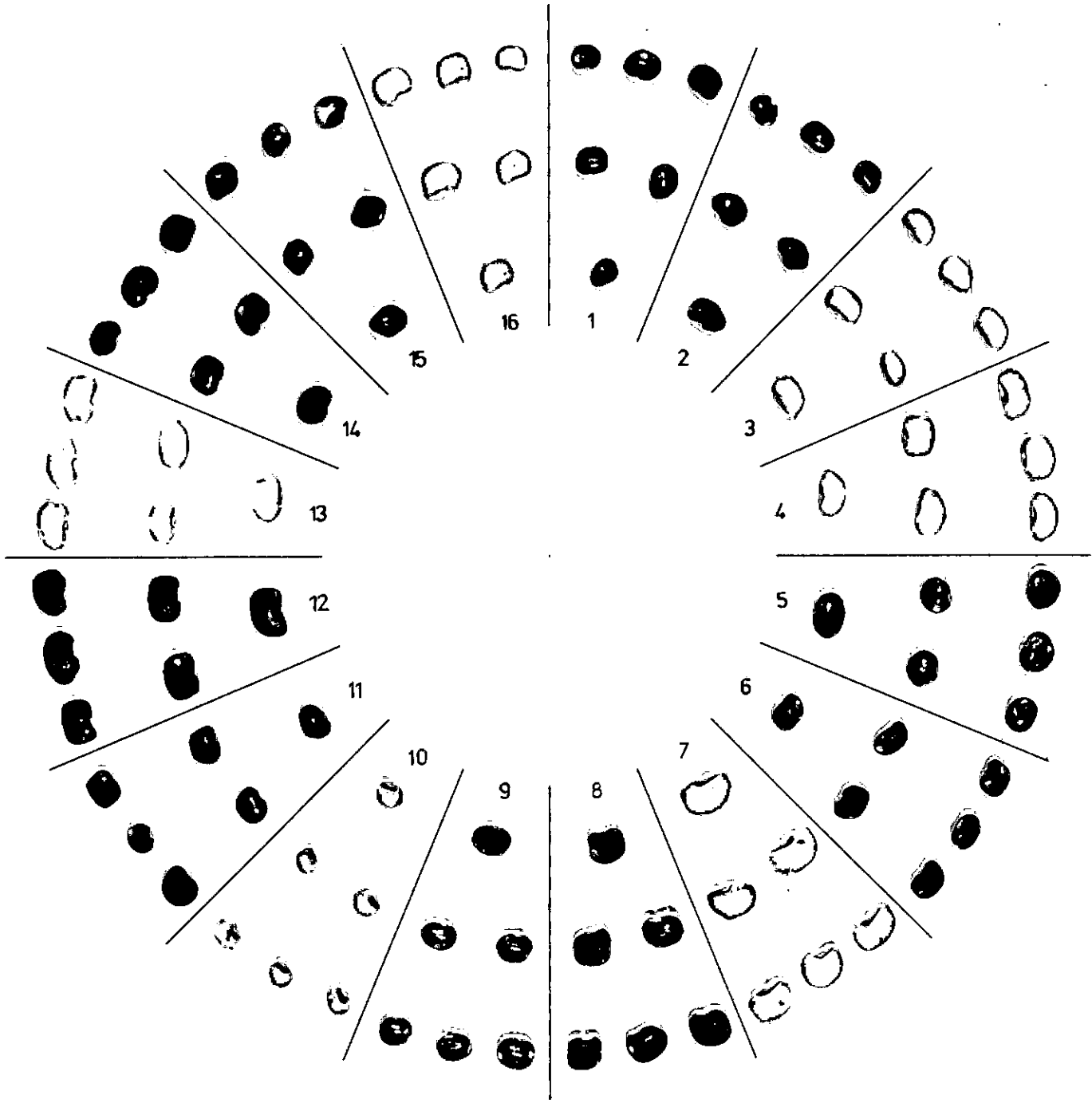
Culinary methods such as pre-soaking or better still removal of testae by threshing and winnowing coupled with adequate heat processing improved the digestibility of cowpea meal. The present studies show that boiling grain in water does not seem to have any serious effect, although acid labile amino acid may be disturbed. The normal cooking temperature (100-104^{o}C) and time (70-90 min) appears sufficient for the inactivation of trypsin inhibitor, but this seems not to be reduced to zero level even after prolong (3 hr) cooking period. This heat stable trypsin inhibitor

activity may be related to the polyphenol already coupled with bean macromolecule or both. However, the apparent digestibility of the cowpea meal as judged by "food approach" method is dependent on the nitrogen (protein) concentration in the particular meal.

Further investigations are however, required on the oxidation and polymerisation of phenolics and how phepholics covalently bind to proteins. The use of anti-oxidants such as sodium metabisulphite or ascorbic acid in the preparation of high phenolics bean meal to prevent possible oxidation culminating in reduced protein digestibility and the organoleptic acceptability of this approach in the traditional dishes needs looking into. Furthermore, in view of the acclaimed nutritional superiority of animal protein over vegetable protein (as determined by rapid growth rate and bodily conformation) and the high prevalence of degenerative diseases in animal protein consuming nations, a better understanding of dietary source of protein (its quality and quantity in the diets of the people) in relation to growth, senescence, longetivity and disease pattern is required.

APPENDIX

Some of the varieties of cowpea (Vigna unguiculata L. Walp) in Nigerian market.



EATING, USAGE AND HABIT ENQUIRY IN RESPECT OF BEANS

1. EATING

	West/Lagos	North	East
No. interviewed eating beans	100	30	40

2. HOW DISHES WERE PREPARED

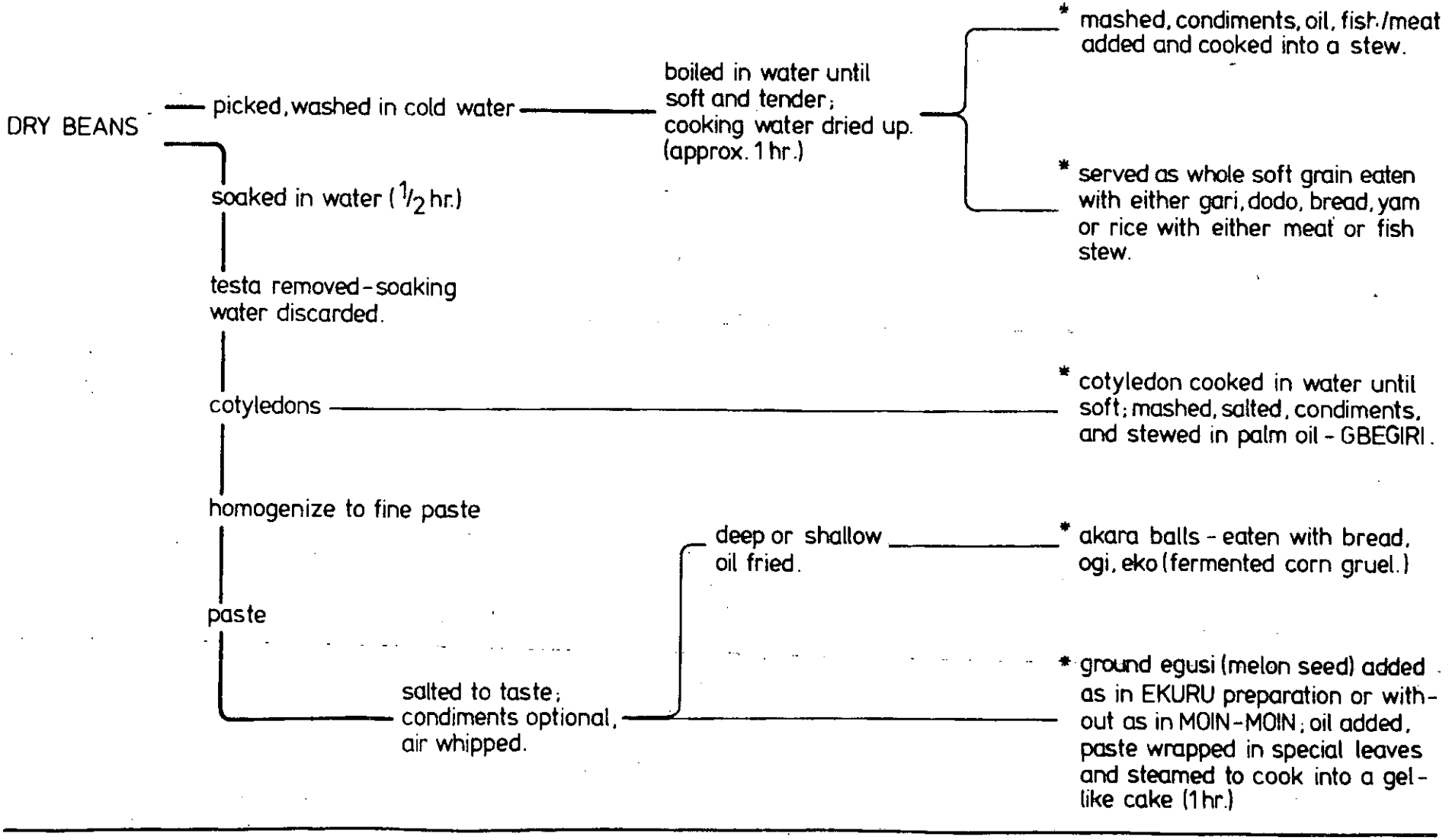
Boiled whole	90%	90%	87%
Made into Moin-Moin	5%	2%	-
Made into Akara balls	5%	8%	13%

3. USAGE

Had rice and beans yesterday	50%	30%	30%
Had yam and beans yesterday	40%	5%	10%
Had bread and beans yesterday	10%	65%	60%

4. INGREDIENTS ADDED

Meat (any type)	68%	50%	50%
Fish (Fresh/dried)	30%	20%	36%
Chicken/Fowl	-	2%	-
Nothing	2%	28%	14%



Culinary processing of beans for consumption in Nigeria and some West African countries.

asterisks indicate fractions and/or products consumed.

TYPICAL RECIPE

Beans (Ife Brown variety)	2 kg
Tomatoes (Fresh)	½ kg
Tomato Puree	180 g
Onions	¼ kg
Dry pepper	30 g
Palm oil	½ kg
Salt	to taste
Water	6 litres

BIBLIOGRAPHY

BIBLIOGRAPHY

Agren, G. (1970) Discussion on nutritional evaluation of proteins by laboratory animals, by B.O. Eggum. In Evaluation of Novel protein products pp 117-128 Edited by Bender et al., Pergamon Press, Oxford 390 pp.

Almquist, H.J., Mecchi, E., Kratzer, F.H. and Grau, C.K. (1942) Soybean Protein as a source of amino acids for the chick. J. Nutr. 24, 285-392

Almquist, H.J., Christensen, H.L., Maurer, S. (1966) Sulfide liberation from raw soybean protein. Proc. Soc. Exptl. Med. Biol. 122, 913-914

Alumot, E. and Nitsan, Z. (1961) The influence of soybean anti-trypsin on the intestinal proteolysis of the chicks. J. Nutr. 73, 71-77

Andrews, A.T. (1974) Navy (Haricot) Bean (Phaseolus vulgaris) Lectin: Isolation and Characteristics of two components from a toxic agglutinating extract. Biochem. J. 139, 421.

Annegers, J.F. (1974) Protein quality in West African foods. Ecology of Food and Nutrition 3(2), 125-130

Anonymous (1969) Tannic acid again. Food Cosmetol Toxicol 7, 364

Armstrong, W.D., Featherstone, W.R., Rogler, J.C. (1973) Influence of methionine and other dietary additions on the performance of chicks fed bird resistant sorghum grain diets. Poultry Sci (US) 52, 1593

Axtell, J.D. and Oswald, D.L. (1972) Inheritance and improvement of protein quality and content in sorghum bicolor (L). Moench Research Progress Report No. 9. A.I.D. Department of State, Washington D.C. January 1 - December 31.

Aykroyd, W.R. and Doughty, J. (1964) Legumes in Human Nutrition. F.A.O. Nutritional Studies No. 19 Rome p. 138.

Baker, A.M.R. (1978) Protease inhibitors and the biochemical basis of insect resistance in Vigna unquiculata. Ph.D. Thesis, Durham University.

Banks, W.L., Allinson, J.B. and Wannemacher, R.W. Jr. (1964) Supplementation of wheat gluten protein J. Nutr. 82, 61-66

Barker, R.D.J., Derbyshire, E., Yarwood, A. and Boulter, D. (1976) Purification and characterization of the major storage proteins of Phaseolus vulgaris seeds and their intracellular and cotyledonary distribution. Phytochemistry 15, 751-757

Batesmith, E.C. (1973) Haemanalysis of tannins The concept of relative astringency. Phytochemistry 12, 907-912

Bender, A.E. (1970) Factors affecting the nutritive value of protein foods p. 319 In Evaluation of novel protein products (A.E. Bender, R. Kihlberg, B. Löfquist, and L. Munck eds) Pergamon Press, Oxford 390 pp

Bergmann, M. and Fruton, J.S. (1941) The specificity of proteinases. Advan. Enzymol. 1, 63-98

Bergmann, M. (1942) A classification of proteolytic enzymes. Advan. Enzymol. 2, 49-92

Beuk, J.F., Chornock, F.W. and Rice, E.E. (1949) The effect of heat on the availability of pork protein in vivo and in vitro J. Biol. Chem. 180, 1243-1251

Bhagvat, K. (1935) Studies in the proteins of Indian foodstuffs. Part IV: The Globulins of cowpea J. Indian Inst. Sci. 18A, 39-47

Bichel, J., Bach, A. (1968) Investigation on the toxicity of small chronic doses of tannic acid with special reference to possible carcinogenicity. Acta. Pharmacol. Toxicol. 26, 41-45

Bond, D.A. (1976) In vitro digestibility of testa in tannin-free field bean (Vicia faba L.) J. Agric. Sci. Camb. 86, 561-66

Booth, A.N., Robbins, D.J., Ribelin, W.E. and DeEds, F., Smith, A.K. and Rackis, J.J. (1964) Prolonged pancreatic hypertrophy in rats. Proc. Soc. Exptl. Biol. Med. 104, 681-683

Borchers, R., Ackerson, C.W. and Kimmett, L. (1947) Trypsin inhibitor, iv. Occurrence in seeds of the Leguminosae and other seeds. Arch. Biochem. 13, 291.

Borchers, R., Ackerson, C.W. and Mussehl, F.E. (1948) Trypsin inhibitor. vi. Effect of various heating periods on the growth promoting value of soybean oil meal for chicks. Poultry Sci. 27, 601-604.

Borchers, R. (1965) Raw soybean growth inhibitors. Federation Proc. 24, 1494-1497

Boulter, D. and Derbyshire, E. (1971) Taxonomic aspects of the structure of legume proteins, In Chemotaxonomy of the Leguminosae. Ed. Harborne, Boulter and Turner pp 285-308. Academic Press, London, N. York.

Boulter, D., Evans, I.M. and Derbyshire, E. (1973) Proteins of some legumes with reference to environmental factors and nutritional value. Qualitas Plantarum (plants foods for Human Nutrition) XXIII 1/3, 239-50.

Boulter, D., Evans, I.M., Thompson, A. and Yarwood A. (1973) The amino acid composition of Vigna unquiculata (cowpea) meal in relationship to nutrition. In nutritional improvement of food legumes by Breeding Protein Advisory Group U.N. New York. 205-215

Boussingault, J.B (1851) Economic Rurale (Paris)

Bouthilet, R.J., Hunter, W.L., Luhman, C.A., Ambrose, D. and Lepkovsky, S. (1950) The metabolism of raw versus heated soybeans in bird with colostomies Poultry Sci. 29, 837-840.

Bowman, D.E. (1944) Fractions derived from soybeans and navy beans which retard the tryptic digestion of casein. Proc. Soc. Exptl. Biol. Med. 57, 139-140

Boyd, E.M., Bereczky, K. and Godi, I. (1965) The acute toxicity of tannic acid administration intragastrically Can. Med. Assoc. J. 92, 1292

Bressani, R. (1972) Legumes in Human Diets and how they might be improved. In Nutritional Improvement of Food Legumes by Breeding pp 15-42 P.A.G. United Nations System

Bressani, R. and Elias, L. (1974) "Legume Foods" In New Protein Foods Altschul (Ed.) Academic Press, London, and New York.

Bressani, R., Elias, L. and Molina, M.R. (1977) Digestibility of Protein of different species of legumes. Archives Latino Americanos de Nutricion 27(2), 215-231

British Medical Journal (1964) Human Experimentation Code of Ethics of the World Medical Association. Editorial Comment B.M.J. 2, 177-180

Brewster, J.F. and Alsberg, C.L. (1919) Determination of the distribution of nitrogen in certain seeds J. Biol. Chem. 37, 367-371

Buraczewski, S. (1966) Factors affecting amino acids in the blood. Reading University Ph.D. Thesis

Buraczewski, S., Buraczewska, L. and Ford, J.E. (1967) The influence of heating of fish proteins on the course of their digestion. Acta Biochimica Polonica 14(1), 121-131

Buraczewski, S., Porter, J.W.G., Rolls, B.A. and Zebrowska, T. (1971) The course of digestion of different food proteins in the rat. Effect of feeding carbohydrate with proteins. Br. J. Nutr. 25, 299-306

Burns, R.E. (1963) Georgia Experimental Station "Methods of Tannin Analysis for Forage Crop Evaluation"

Byers, M. (1967) The *in vitro* hydrolysis of leaf proteins II - The action of papain on protein concentrates extracted from leaves of different species. J. Sci. Food Agric. 18(1), 33-38

Calderon, P., Van Buren, J.P., Robinson, W.B. (1968) Factors influencing the formation of precipitates and hazes by gelatin on condensed and hydrolysable tannins. J. Agric. Food Chem. 16: 479

Cannon, P.R., Steffee, C.H., Frazier, L.J., Rowley, D.A. and Stepto, R.C. (1947) The influence of time of ingestion of essential amino acid upon utilization in tissue-synthesis. Fed. Proc. 6, 390

Carasco, J.F. (1976) Seed proteins of cowpea (Vigna unquiculata) Durham University Ph.D. thesis

Carasco, J.F., Croy, R., Derbyshire, E. and Boulter, D. (1978) The Isolation and characterisation of the major polypeptides of the seed globulin of cowpea (Vigna unquiculata L. Walp) and their sequential synthesis in developing seeds. J. Exptl. Botany 29(109), 309-323.

Carpenter, K.J., Morgan, C.B., Lea, C.H. and Parr, I.J. (1962) Chemical and nutritional changes in stored herring meal. 3. Effect of heating at controlled moisture contents on the binding of amino acids in freeze-dried herring presscake and in related model system. Brit. J. Nutr. 16, 451

Carroll, R.W., Hensley, G.W. and Graham, W.R. Jr. (1952) The site of nitrogen absorption in rats fed raw and heat-treated soybean meals. Science 115, 36-39

Chakravarti, R.N., Chakravarti, D., Mitra, M.N. Dasgupta, B. and Maiti, P.C. (1956) Stigmasterol from Indian beans. J. Sci. Industr. Res. (Indian) 15C, 89-90.

Chernick, S.S., Lepkovsky, S. and Chaikoff, I.L. (1948) A dietary factor regulating the enzyme content of the pancreas. Changes induced in size and proteolytic activity of the chick pancreas by the ingestion of raw soybean meal. Am. J. Physiol. 155, 33-41

Chevalier, A. (1944) Cowpeas in Africa. Revue de Botanique Appliquee et d'Agriculture Tropicale 24, 128-152

Chowduri, D.K. and Bagchi, R. (1957) Naturwissenchaften 44, 263.

Christensen, L.K. (1955) Concerning the pH optimum of peptic hydrolysis. Arch. Biochem. Biophys. 57, 163-173

Clandinin, D.R., Cravens, W.W., Haplin, J.G. and Hart, E.B. (1946) Supplementary value of methionine, cystine and choline in a practical soybean oil meal starter ration. Poultry Sci. 25, 509-516

Clandinin, D.R., Stevens, J.M., Morrison, A.B. and Robblee, A.R. (1951) Liberation of lysine by acid and enzyme from heated lysine - Glucose mixture J. Biol. Chem. 190, 219-222

Clegg, K.M. (1960) The availability of lysine in ground-nut biscuits used in the treatment of kwashiorkor Br. J. Nutr. 14, 325-329

Dalby, A. and Shuman, A.C. (1978) Temperature - Induced errors in the colorimetric determination of tannins. Anal Biochem. 85, 325-327

Dalziel, J.M. (1937) The useful plants of West Tropical Africa. Crown Agents for Overseas Governments and Administrations. London, England.

Dema, I.S. (1963) In Proceeding 1st. Nigerian Grain Legumes Conference, Samaru Zaria, Nigeria.

Denton, A.E. and Elvehjem, C.A. (1953) Enzymatic Liberation of amino acids from different proteins J. Nut. 49, 221-229

Denton, A.E. and Elvehjem, C.A. (1954) Availability of amino acids in vivo J. Biol. Chem. 206, 449-454.

Derbyshire, E. and Boulter, D. (1976) Isolation of Legumin -like protein from Phaseolus aureus and Phaseolus vulgaris. Phytochemistry 15, 411-414.

Derbyshire, E., Wright, D.J. and Boulter, D. (1976a) Review: Legumin and vicilin, storage proteins of legume seeds. Phytochemistry 15, 3-24

Derbyshire, E., Yarwood, J.N., Neat, E. and Boulter, D. (1976b) Seed proteins of Phaseolus and Vigna New Phytol. 76, 283-288

Desikachar, H.S.R. and De, S.S. (1947) Role of inhibitors in soybeans. Science 106, 421-422

Desnuelle, P., Rovery, M., and Bonjour, G. (1950) Quelques Nouvelles Observations sur le mode D'attaque de la globine de cheval et de l'albumine doeuf par la pepsine. Biochem. Biophys. Acta. 5, 116-137

Desnuelle, P. (1960) Trypsin. In: "The Enzymes" vol iv part A pp 119 Edited by Boyer, P.D., Lardy, H. and Myrback, K. Academic Press, New York.

Desnuelle, P. (1960) Chymotrypsin. In: The Enzymes vol. iv. part A pp 93-118 edited by Boyer Lardy and Myrback Academic Press, New York.

Desnuelle, P. and Rovary, M. (1961) The proteins of the exocrine pancreas. Advan. Protein Chem. 16, 139-195

Dovlo, F.E., Williams, C.E. and Zoaka, L. (1976) Cowpea - Home preparation and use in West Africa. IDRC 055e

Driedger, A. and Hatfield, E.E. (1972) Influence of tannins on the nutritive value of soybean meal for ruminants. J. Anim. Sci. 34, 465

Eggert, R.G., Brinegar, M.J. and Anderson, C.R. (1953) Delayed protein supplementation of corn diets for growing swine. J. Nut. 50, 469-477

Eggum, B.O. and Christensen, K.D. (1975) Influence of tannin on protein utilisation in feedstuffs with special reference to barley. In Breeding for seed protein improvement p. 135 International Atomic Energy Agency (IAEA) Vienna.

Elias, L.G., De Fernández, D.G., Bressani, R. (1979) Possible effect of seed coat polyphenolics on the nutritional quality of bean protein. J. Food Sci. 44(2), 524-527

Ellis, G.P. (1959) The Maillard Reaction. Adv. Carbohyd. Chem. 14, 63-134

Elman, R. (1939) Time factor in retention of nitrogen after intravenous injection of a mixture of amino acids. Proc. Soc. Exper. Biol. & Med. 40, 484

Evans, R.J. and Bandemer, S.L. (1967) Nutritive value of legume seed protein. J. Agric. Food Chem. 15, 439-443.

Evans, I.M. and Boulter, D. (1974) Chemical methods suitable for screening for protein content and quality in cowpea (Vigna unguiculata) meals. J. Sci. Fd. Agric. 25, 311-322

Evans, R.J., Croschke, A.C. and Butts, H.A. (1951) Studies on the heat inactivation of cystine in soybean oil meal. Archives Biochem. 30, 414-422

Evans, R.J. and McGinnis, J. (1946) The influence of autoclaving soybean oil meal on the availability of cystine and methionine for the chick. J. Nut. 31, 445-461

Evans, R.J. and McGinnis, J. (1948) Cystine and methionine metabolism by chicks receiving raw or autoclaved soybean oil meal. J. Nutr. 35, 477-488

F.A.O. (1966) Agricultural Development in Nigeria 1965-1980. pp 392-400 Food and Agricultural Organisation, Rome.

Faris, D.G. (1965) The origin and evolution of the cultivated forms of Vigna sinesis Can. J. Genetics and Cytology, 7, 433-452

Feeny, P.P. (1969) Inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin Phytochemistry, 8, 2119-2126

Fennell, M.A. (1963) In Proceeding of the 1st Nigerian Grain Legumes Conference Samaru, Zania, Nigeria.

Figarella, C., Negri, G.A. and Guy, O. (1974) Studies on Inhibition of the two human trypsins. In Bayer Symposium on proteinase inhibitors p. 213 (Fritz, Tochesche, Greene and Truscheit, editors) Berlin Springer-Verlag.

Figarella, C., Negri, G.A. and Guy, O. (1975) The two human trypsinogen. Eur. J. Biochem. 53, 457-463

Fox, D.J., Thurman, D.A. and Boulter, D. (1964) Studies on the proteins of seeds of the Leguminosae 1. Albumins. Phytochemistry, 3, 417-419

Ford, J.E. and Salter, D.N. (1966) Analysis of Enzymatically digested food proteins by Sephadex-gel filtration. Br. J. Nutr. 20, 843-860

Freudenberg, K. (1920) Die Chemie der Naturlichen Gerbstoffe Springer-Verlag, Berlin

Fuller, H.L., Chang, S.I., Potter, D.K. (1967) Detoxication of dietary tannic acid by chicks J. Nutrition 91, 477

Gadal, P. and Boudet, A. (1965) Sur -'inhibition des enzymes par les tannins des feuilles de Quercus Sessilss Ehrh. Inhibition de la β-amylase C.R. Acad. Sci. Paris 260, 4252-4258

Gatehouse, A.M.R. and Gatehouse, J.A. (1979) Biochemical basis of insect resistance in Vigna unquiculata J. Sci. Food Agric. 30, 948-958

Gatehouse, A.M.R., Gatehouse, J.A. and Boulter, D. (1980) Isolation and characterisation of the trypsin inhibitors from cowpea (Vigna unquiculata) Phytochemistry 19, 751-756

Geiger, E.J. (1947) Experiments with delayed supplementation of incomplete amino acid mixtures. J. Nutrition 34, 97-111

Geiger, E.J. and Geiger, L.E. (1948) The role of the time factor in feeding supplementary proteins. J. Nutrition 36, 813-819

Geiger, E. and Hagerty, E.B. (1950) Importance of time factor upon utilization of amino acids in 'maintenance' of adult rats. Federation Proc. 9, 359

Gitler, C. (1964) Protein digestion and absorption in non-ruminants. In Mammalian protein metabolism vol. 1 p 35 (H.N. Munro and Allinson J.B. editors) Academic Press London and New York.

Glick, Z. and Joslyn, M.A. (1970) Food intake depression and other metabolic effects of tannic acid in the rat. J. Nut. 100, 509

Glick, Z. and Joslyn, M.A. (1970b) Effect of tannic acid and related compounds on the absorption and utilization of proteins in the rat. J. Nut. 100, 516

Goldstein, J.L. and Swain, T. (1965) changes in tannins in ripening fruits. Phytochemistry, 4, 185

Gorini, L. (1951) Role du calcium dans le systeme Trypsine - Serumalbumine Biochim. Biophys. Acta 7, 318-334

Green, N.M. and Neurath, H. (1954) In "The Proteins", vol. 2 part B p. 1057 edited by Neurath, H. and Bailey, K. Academic Press, New York.

Green, G.M. and Lyman, R.L. (1972) Feedback regulation of pancreatic enzyme secretion as a mechanism for trypsin inhibitor-induced hypersecretion in rats Proc. Soc. Exptl. Biol. Med. 140, 6-12.

Griebel, C. (1950) Erkrankungen durch bohuen flocken Phaseolus vulgaris L. und plattererbsen Lathyrus tingitanus; Z. Untersuch. Forsch 90, 191

Gupta, R.K., Haslam, E. (1979) Vegetable tannins - Structure and Biosynthesis. In Polyphenols in cereals and legumes, IDRC - 145e edited by J.H. Hulse pp 15-24.

Gustavson, K.H. (1954) Interaction of vegetable tannin with polyamides as proof of the dominant function of peptide bond of collagen for its binding of tannins. J. Polymer Sci. 12, 317

Hallovan, H.R. and Maunder, A.B. (1971) Nutritional evaluations with bird resistant and yellow endosperm sorghums Poultry Sci. 50, 1582

Ham, W.E. and Sandstedt, R.M. (1944) A proteolytic inhibitory substance in the extract from unheated soybean meal. J. Biol. Chem. 154, 505-506

Ham, W.E., Sandstedt, R.M. and Mussehl, F.E. (1945) The proteolytic inhibitory substance in the extract from unheated soybean meal and its effect upon growth in chicks. J. Biol. Chem. 161, 635-642

Harvey, D. (1970) Commonwealth Bureau Animal Nutrition Technical Communication No. 19 2nd Edition

Haslam, E. (1966) Chemistry of vegetable tannins. Academic Press, New York.

Hayward, J.W. and Hafner, F.H. (1941) The supplementary effect of cystine and methionine upon the protein of raw and cooked soybeans as determined with chicks and rats. Poultry Sci. 20, 139-150

Henderson, R. and Harris, R.S. (1949) Concurrent feeding of amino acids. Fed. Proc. 8, 385

Henry, K.M. and Kon. S.K. (1946) The supplementary relationships between the proteins of dairy products and those of bread and potato as affected by the method of feeding. With a note on the value of soyabean protein. J. Dairy Research 14, 330-340

Hoff, J.E. and Singleton, K.I. (1977) A method for determination of tannin in foods by means of immobilised protein. J. Food Science 42(6), 1566-1574

Hogan, A.G., Gillespie, G.T., Kocturk, O., O'Dell, B.L. and Flynn, L.M. (1955) The percentage of protein in corn and its nutritional properties. J. Nut. 57, 225--239

Hsu, H.W., Vavak, D.L., Satterlee, L.D. and Miller, G.A. (1977) A multienzyme technique for estimating protein digestibility. J. Food Sci. 42(5), 1269-1273

Hulse, J.H., Rachie, K.O. and Billingsley, L.W. (1977) Nutritional standards and methods of evaluation for food legume breeders. International Development Research Centre (IDRC) Canada Ottawa IDRC-TS7e

Hunt, J.N., Pathak, J.D. (1960) The osmotic effects of some simple molecules and ions on gastric emptying J. Physiol. 154, 254-269

Hunt, J.N. and Knox, M.T. (1968) A relation between the chain length of fatty acids and the slowing of gastric emptying. J. Physiol. 194, 327-336

Ingram, G.R., Riesen, W.H., Cravens, W.W. and Elvehjem, C.A. (1949) Evaluating soybean oil meal protein by enzymatic release of amino acids. Poultry Sci. 28, 898-902.

Jaffe, W.G. (1950) Protein digestibility and trypsin inhibitor activity of legume seeds. Proc. Soc. Exptl. Biol. Med. 75, 219

Jaffe, W.G. and Vega Lette, C.L. (1968) Heat labile growth inhibiting factors in bean (Phaseolus vulgaris) J. Nut. 94: 203.

Jaffe, W.G., Flores, M.E. (1975) Coacion de frijoles (Phaseolus vulgaris) Arch. Latinoam Nutr. (Venezuela) 25, 79

Jayne-Williams, D.J. and Burgess, C.D. (1974) Further observations on the toxicity of navy beans (Phaseolus vulgaris for Japanese Quail (Coturnix coturnix japonica) J. Appl. Bact. 37, 149-169

Janower, M.L., Robbins, L.L. and Wenlund, D.E. (1967) A review of the use of tannic acid in patients with ulcerative colitis. Radiology, 89, 42

Johnson, R.M. and Raymond, W.D. (1964) The chemical composition of some tropical food plants. 11. Pigeon Peas and Cowpeas. Tropical Science 6, 68-73

Joubert, F.J. (1957) Ultracentrifuge studies on seed proteins of the family Leguminosae Part III. Proteins from different bean species J.S. Afr. Chem. Inst 10, 16-20

Kakade, M.L. and Borchers, R. (1967) Gastrointestinal gas production in rats fed raw and heated navy beans with or without added antibiotics. Proc. Soc. Exptl. Biol. Med. 124, 1272

Kakade, M.L., Rackis, J.J., McGhee, J.E. and Puski, G. (1974) Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chemistry 51(3), 376-382

Karrer, W. (1958) Konstitution und Vorkommen der organischen Pflanzenstoffe Birkhauser, Basel

Kirby, K.S. (1960) Induction of Tumours by tannin extracts. Brit. J. Cancer 14, 147-150

Klose, A.A., Hill, B. and Fevold, H.L. (1946) Presence of a growth inhibiting substance in raw soybean. Proc. Soc. Expt. Biol. Med. 62, 10-12.

Korpassy, B. (1961) Tannins as hepatic carcinogens Progr. Exptl. Tumor. Res, 2, 245-290

Korte, R. (1972) Heat resistance of phytohaeagglutinins in weaning food mixtures containing beans (Phaseolus vulgaris). Ecol. Food Nut. 1, 303

Krehl, W.A., Sarma, P.S., Tepley, L.J. and Elvehjem, C.A. (1946) Factors affecting the dietary niacin and tryptophane requirement of the growing rat J. Nutr. 31, 85-106

Kunitz, M. (1945) Crystallisation of a trypsin inhibitor from soybeans. Science 101, 668-669

Kunitz, M. (1946) Crystalline soybean trypsin inhibitor. J. Gen. Physiol. 29, 149-154

Kunitz, M. (1947) Crystalline soybean trypsin inhibitor 11. General properties. J. Gen. Physiol. 30, 291

Landstenier, K. and Raubitschek, H. (1908) Beobachtungeniuber Hamolyse und Hamag glutination. Zentr. Bakteriol. Parasistenk Abt. 11. Orig 45, 660

Lang, K. (1960) Denaturierung der Nahrungsproteine und Zerstorung von Aminosauren durch die thermische Behandlung von Lehensmitteln Veranderungen der Nahrung durch industrielle und haushaltmassige Verarbeitung. 3. Symposium 1959, Wiss. veroffenti d. Deutschen Gesellschaft fur Ernahrung. Steinkapff-Verlag Darmstadt.

Liener, I.E., Deuel, H.J. Jr. and Fevold, H.L. (1949) The effect of supplemental methionine on the nutritive value of diets containing concentrates of the soybean trypsin inhibitor. J. Nutr. 39, 325-339.

Liener, I.E. and Fevold, H.L. (1949) The effect of the soybean trypsin inhibitor on the enzymatic release of amino acids from autoclaved soybean meal. Arch. Biochem. Biophys. 21, 395-407.

Liener, I.E. (1953) Soyin, a toxic protein from the soybean I. Inhibitor of rat growth. J. Nutr. 49, 527

Liener, I.E. (1962) Toxic factors in Edible legumes and their elimination. Am. J. Cli. Nutr. 11, 281

Liener, I.E. (1973) Toxic factors associated with legume proteins. India. J. Nutr. Diet. 10, 303.

Liener, I.E. (1977) Toxicological considerations in the utilisation of New Protein Foods. In. Biochemical Aspects of New Protein Foods p. 129 J. Alder-Nissen edt. Pergon Press.

Linderstrøm-Lang, K., Hotchkiss, R.D. and Johansen, G. (1938) Peptide bonds in globular proteins. Nature, 142, 996

Lineweaver, H. and Murray, C.W. (1947) Indentification of the trypsin inhibitor of egg white ovomucoid J. Biol. Chem. 171, 565-581

Lis, H. and Sharon, N. (1973) The Biochemistry of Plant Lectins. Ann. Rev. Biochem. 42, 541

Loomis, W.D. and Battaile, J. (1966) Plant phenolic compounds and the isolation of plant enzymes J. Phytochemistry 5, 423-438

Lucas, J.W. (1968) The role of plant food in solving the world food problem - energy requirements In Plants Food for Human Nutrition 1, 13-21

Lyman, R.L. and Elvehjem, C.A. (1951) Further studies on amino acid imbalance produced by gelatin in rats on Niacin-tryptophan low ration J. Nutrition 45, 101-117

Lyman, R.L. and Lepkovsky, S. (1957) The effect of raw soybean meal and trypsin inhibitor diets on pancreatic enzyme secretion in the rat. J. Nutr. 62, 269-284

Ma Yu and Bliss, F.A. (1978) Tannin content and Inheritance in common bean Crop Science 18(2), 201-204

MacDonald, R.P. and Le Fave, R.O. (1962) Serum lipase determination with olive oil substrate using a three-hour incubation period. Clin. Chem. 8, 509-518

March, S.C., Parikh, I. and Cuatrecasas, P. (1974) A simplified method of cyanogen bromide activation of agarose for affinity chromatography. Analytical Biochemistry 60, 149-152

Marcus, C.S. and Lengemann, F.W. (1962) Use of radioyttrium to study food movement in the small intestine of the rat. J. Nutr. 76, 179-182

Martin-Tanguy, J., Guillaume, J. and Kossa, A. (1977) Condensed tannins in horse bean seeds: Chemical structural and apparent effects on Poultry. J. Sci. Fd. Agric. 28, 757-765

Matsushima, K. (1958) An undescribed trypsin inhibitor in egg white. Science 127, 1178-1179

Matthews, D.M. (1970) Intestinal Absorption of amino acids and peptides. Proc. Nutr. Soc. 31, 171-177

McGinnis, J. and Evans, R.J. (1947) Amino acid deficiencies of raw and overheated soybean oil meal for chicks. J. Nut. 34, 725

Melnick, D., Oser, B.L. and Weiss, S. (1946) Rate of enzymic digestion of proteins as a factor in nutrition. Science 103, 326-329

Mertz, E.T., Baxter, E.J., Jackson, L.E., Roderick, C.E. and Weis, A.E. (1952) Essential amino acids in self-selected diets of older women. J. Nutr. 46, 313-322

Miller, D.S. (1956) The nutritive value of fish proteins. J. Sci. Food Agric. 7, 339

Mitjavila, S., De Saint-Blanquat, G. and Derache, R. (1970) Effect de l'acide tannique sur l'absorption intestinale chez le souris. Food Cosmatol. Toxicol. 8, 27

Mitjavila, S., Carrera, G., Derache, R. and Bouss, M.T. (1971) Effect of tannic acid on growth body composition and biological utilisation of foods in rats. Ann Nutr. Aliment. 24(4), 297

Mitjavila, S., Lacombe, C., Carrera, G. and Darache, R. (1977) Tannic acid and oxidised tannic acid on the functional state of rat intestinal epithelium. J. Nut. 107(12), 2113-2121

Molina, M.R., de la Fuenta, G. and Bressani, R. (1975) Interrelationships between storage, soaking time, cooking time, nutritive value and other characteristics of black beans (Phaseolus vulgaris) J. Food Sci. (US) 40, 587

Mood, A.M., Graybill, F.A. and Boes, D.C. (1974) Introduction to the Theory of Statistics, 3rd Edition chapter X1. McGraw-Hill ISBN 0-07-042864-6

Mulder, G.J. (1840) In The chemistry of animal and vegetable physiology as quoted by Block, R.J. and Mitchell, H.H. (1946) Nutrition Abstracts and Review 16, No. 2

Nesheim, M.C. and Garlich, J.D. (1966) Digestibility of unheated soybean meal for laying hens. J. Nutr. 88, 187-192.

Niess, E., Ivy, C.A. and Nesheim, M.C. (1972) Stimulation of gallbladder emptying and pancreatic secretion in chicks by soybean whey protein. Proc. Soc. Exptl. Biol. Med. 140, 291.

Nigram, V.N. and Giri, K.V. (1961) Sugars in pulses. Candanian J. Biochem. Physiol. 39, 1847-1853

Niyogi, S.P., Narayana, N. and Desai, B.G. (1932) Studies in the Nutritive value of Indian vegetable foodstuffs. Indian J. Med. Res. 19, 859-866

Northrop, J.H. (1922) The mechanism of the influence of acids and alkalines on the digestion of proteins by pepsin or trypsin. J. Gen. Physiol. 5, 263-274

Northrop, J.H., Kunitz, M. and Herriot, R.M. (1948) In Crystalline enzymes, 2nd edition, Columbia University Press, New York.

Osborne, T.B. (1894) The proteins of the kidney bean (Phaseolus vulgaris) J. Am. Chem. Soc. 16, 633-643

Osborne, T.B. and Campbell, G.F. (1898) Proteins of the pea J. Amer. Chem. Soc. 20, 348-410

Osborne, T.B. (1907) Carnegie Inst. Washington Publ. No. 84.

Osborne, T.B. and Heyl, F.W. (1908) Hydrolysis of Vignin of the cowpea (Vigna sinensis) Amer. J. Physiol. 22, 362

Osborne, T.B. and Mendel, L.B. (1912) Brobachtungen uber Wachstum die Futteringsuchen mit isolierten Nahrungsubstanzen Z. Physiol. Chem. 80, 307-309

Ogummodede, B.K. and Oyenuga, V.A. (1969) Vit B content of cowpea (Vigna unguiculata) 1-Thiamine riboflavin and Niacin. J. Sci. Food Agric. vol 20. 101-103.

Ibid (1970) 11 - Pyridoxine Pantothenic acid, biotin and folic acid. J. Sci. Fd. Agric. 21, 87-91

Ojomo, O.A. (1968) Criteria for consumer acceptance of cowpea (Vigna unguiculata) in Western Nigeria West State Agric. Res. Memo 50 pp 1-4.

Onayemi, O., Pond, W.G. and Krook, L. (1976) Effects of Processing on the Nutritive value of cowpea (Vigna sinesis) for the growing rat. Nutrition Report International 13(3), 299-305.

Owusu-Domfeh, K. (1972) Trypsin inhibitor activity of cowpea (Vigna unguiculata) and bambara beans (Voandzeia subteranea) Ghana J. Agric. Sci. 5, 99-102.

Palmer, I. (1972) Food and New Agricultural Technology UNRISD. Genera

Peraino, C., Rogers, Q.R., Yoshida, M., Chen, M.L. and Harper, A.E. (1959) Observations on Protein Digestion in vivo: Dietary factors affecting the rate of disappearance of casein from the gastrointestinal tract. Can. J. Biochem. Physiol. 37, 1475-1491.

Pirk, F. and Skala, I. (1970) Mobility of the digestive tract after administration of medium chain triglycerides (M.C.T) as compared with long chain triglycerides (L.C.T) Digestion 3, 73-80.

Platt, B.S. (1962) Tables of Representative values of foods commonly used in Tropical Countries M.R.C. Special Report 302. Her Majestys Stationery Office London.

Porter, J.W.G. and Rolls, B.A. (1971) Some aspects of the digestion of Proteins Proc. Nutr. Soc. 30, 17-25

Price, M.L., Van Scoyoc, S. and Butler, L.G. (1978) A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26(5), 1214-1216

Pusztai, A., Grant, G. and Palmer, R. (1975) Nutritional Evaluation of kidney bean (Phaseolus vulgaris) isolation and partial characterisation of toxic constituents. J. Sci. Fd. Agric. 26, 149-156

Pusztai, A., Clarke, E.M.W. and King, T.P. (1979) The nutritional toxicity of Phaseolus vulgaris lectins Proc. Nutr. Soc. 38, 115

Rachie, K.O. and Roberts, L.M. (1974) In Advances in Agronomy 26, 44

Rackis, J.J. (1965) Physiological properties of soybean trypsin inhibitors and their relationship to pancreatic hypertrophy and growth inhibition of rats. Federation Proc. 24, 1488-1493

Rackis, J.J. (1974) Biological and Physiological Factors in soybeans. J. Am. Oil. Chem. Soc. 51(1), 161A-174A

Read, J.W. and Haas, L.W. (1938) Studies on the baking quality of flour as affected by certain enzyme actions V. Further studies concerning potassium bromate and enzyme activity. Cereal Chem. 15, 59

Rhodes, M.B., Bennett, N. and Feeney, R.E. (1960) The trypsin and chymotrypsin inhibitors from avian egg white. J. Biol. Chem. 235, 1686

Riesen, W.H., Clandinin, D.R., Elvehjem, C.A. and Cravens, W.W. (1947) Liberation of essential amino acids from raw, properly heated and overheated soybean oil meal. J. Biol. Chem. 167, 143-150

Robinson, T. (1967) The organic constituents of higher plants. Their Chemistry and Interrelationships 2nd ed. Burgess Minneapolis MN

Rosenthal, S. and Nasset, E.S. (1958) Gastric emptying and intestinal absorption of carbohydrate and protein as influenced by the nature of the test meal. J. Nutr. 66, 91-103

Rubner (1897) as quoted by Block & Mitchell (1946) The correlation of the amino acid composition of proteins with their nutritive value. Nutr. Abstr. & Review 16, No. 2.

Rune, S.J. (1968) An electrode for pH measurement in the Gastrointestinal Tract. Scand. J. Gastroent. 3, 91-94.

Russel, W.C., Taylor, M.W., Mehrhof, T.G. and Hirsh, R.R. (1946) The nutritive value of the protein of varieties of legumes and the effect of methionine supplementation J. Nut. 32(3), 313-325

Sarkar, S.K., Howarth, R.E. (1976) Specificity of the vanillin test for flavanols. J. Agric. Food Chem. 24, 317-320.

Sauer, C.O. (1952) Agricultural origins and dispersals American Geographical Society New York p. 110

Saunders, A.R. (1959) Inheritance in the cowpea 1. Colour of the seed coat. South African Journal of Agricultural Science 2, 285-307

Saunders, A.R. (1960) Inheritance in the cowpea 2. Seed coat colour pattern, flower, plant and pod colour. South African Journal of Agricultural Science 3, 141-161

Schroeder, L.J., Stewart, R.A. and Smith, A.H. (1951) The nutritive effect of heating lactalbumin in the presence of various reducing sugars J. Nut. 45, 61-74

Schroeder, L.J., Iacobellis, M., Lees, H. and Smith, A.H. (1953) The effect of heat on the nutritive value of milk protein as influenced by water and fat. J. Nut. 50, 351-360.

Seidl, D., Jaffe, M. and Jaffe, W.G. (1969) Digestibility and inhibitory action of a kidney bean globulin. Agr. Food Chem. 17, 1318

Sellschop, J.P.F. (1962) Cowpea (Vigna unquiculata L.) Walp) Field Crops Abstract 15(4), 259-266

Sheffner, A.L., Eckfeldt, G.A. and Spector, H. (1956) The pepsin-digest-residue (PDR) amino acid index of net protein utilization. J. Nutr. 60, 105-120

Singleton, V.L., Kratzer, F.H. (1969) Toxicity and related physiological activity of phenolic substances of plant origin. J. Agric. Food Chem. 17(3) 497-512

Singleton, V.L. and Kratzer, F.H. (1973) Plant phenolics. In Toxicants occuring naturally in Foods. National Academy of Sciences, Washington D.C. 2nd ed. pp 309-345

Sri Ram, J. and Maurer, P.H. (1957) Modified bovine serum albumin. III. Hydrolysis studies with trypsin chymotrypsin and pepsin. Arch. Biochem. Biophys. 70, 185-204.

Steggerda, F.R. and Dimmick, J.F. (1966) Effect of bean diets on concentration of carbon dioxide in flatus Amer. J. Clin. Nutr. 19, 120.

Stillmark, H. (1889) Über Ricin Arch Pharmacol Inst. Dorpat 3, 59

Summerfield, R.J., Huxley, P.A. and Steele, W. (1974) cowpea (Vigna unqiculata (L) Walp) Field Crops Abstract 27(7), 301-312

Summer, J.B. and Howell, S.F. (1935) A method for determination of Saccharase activity. J. Biol. Chem. 108, 51-54.

Sundararajan, A.R. (1938) Phytin-phosphorus content of Indian Foodstuffs. Ind. J. Med. Res. 25, 685-691

Swain, T. (1966) Comparative phytochemistry. Academic Press New York.

Swaminathan, M. (1937-38) The relative amounts of the protein and non-protein nitrogeneous constituents occurring in Foodstuffs and their significance in the determination of the digestibility co-efficient of proteins. Indian J. Med. Res. 25, 847-853

Tamir, M. and Alumot, E. (1959) Inhibition of digestive enzymes by condensed tannins from green and ripe carobs. J. Sci. Fd. Agric. 20; 199-202

Tamir, M. and Alumot, A. (1970) Growth depression and levels of insoluble nitrogen in the digestive tract of rats. J. Nut. 100, 573

Tedeschi, G.G., Petrelli, F. and Amici, D. (1965) Toxicity of a protein fraction extracted from Phaseolus vulgaris (Giorn) Italian J. Biochem. 14(4) 238-252

Toms, G.C. and Western, A. (1971) Phytohaemagglutinins In Chemotaxonomy of leguminosae (Edt. by Harborne, Boulter and Turner) pp 367-462 Academic Press, London.

Twombly-Snook, J. and Mayer, J.H. (1964) In the role of the gastro-intestinal tract in protein metabolism (H.N. Munro, ed.) p. 97 Blackwells, Oxford, England

Van Buren, J.P. and Robinson, W.B. (1969) Formation of complexes between protein and tannic acid. J. Ag. Food. Chem. 17, 772

Varley, J.A. (1966) Automatic methods for the determination of nitrogen, phosphorus and potassium in plant material. Analyst. 91, 119-126

Vavilov, N.L. (1950) Selected writings on the origin, variation immunity and breeding of cultivated plants Chronica botanica 13, 21-38

Venkataraman, L.V., Jaya, T.V. and Krishnamurthy, K.S. (1976) Effect of germination on the biological value, digestibility coefficient, and net protein utilisation of some legume proteins. Nutrition Reports International 13(2), 197-205

Vohra, P., Kratzer, F.H. and Joslyn, M.A. (1966) The growth depressing and toxic effects of tannins to chicks. Poultry Sci. 45, 135

Watt, G. (1908) Commercial products of India pages 1107-1118. John Murray, London

Weaver, J.M. (1955) Inhibitory compounds in raw soybeans. Federation Proc. 14, 301.

Weinberg, M.S., Goldhamer, R.E. and Sarson, S. (1965) Acute oral toxicity of various drugs in newborn rats after treatment of the dam during gestation. Toxicol Appl. Pharmacol, 9, 234

Weingers, K. and Piretti, M.V. (1971) Isolierung des $C_{30}H_{26}O_{12}$ Pro cyanidin B1 aus Weintrauben Liebigs Ann. Chem. 748, 218

Westfall, R.J., Bosshardt, D.K. and Barnes, R.H. (1948) Influence of crude trypsin inhibitor on utilization of hydrolysed protein. Proc. Soc. Exptl. Biol. Med. 68, 498-500

Westfall, R.J. and Hauge, S.M. (1948) The nutritive quality and the trypsin inhibitor content of soybean flour heated at various temperatures. J. Nutr. 35, 374-389

Wight, W.F. (1907) The history of the cowpea and its introduction with America. Bulletin US Department of Agriculture and Bureau of Plant Industry 102, 43-59

Williams, R.T. (1959) Detoxication mechanisms. Wiley New York 2nd Edt.

Williams, C.E. (1974) A preliminary study of consumer preferences in the choice of cowpeas. Western and Kwara States Headquarters and Areas of Nigeria. University of Ibadan Press

Wilson, P.A., Melmed, R.N., Hampe, M.V. and Holt, S.I. (1978) Immunocytochemical study of the interaction of soybean trypsin inhibitor with rat intestinal mucosa. Gut 19, 260-266.

Wright, D.J. and Boulter, D. (1972) The characterisation of vicilin during seed development in Vicia faba (L) Planta 105, 60-65

Zboralske, F.F., Harris, P.A. Riegelman, S.I., Rambo, D.N. and Margulis, A.R. (1966) Toxicity studies on tannic acid administered by enema. 3, studies on the retention of enemas in humans 4. review and conclusions. Am. J. Roentgen 96, 505.