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EFFECT OF PROCESSING ON CERTAIN NUTRITIONAL PARAMETERS OF COWPEAS (*VIGNA UNGUICULATA*)

By

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ABSTRACT

EFFECT OF PROCESSING ON CERTAIN NUTRITIONAL PARAMETERS OF COWPEAS (VIGNA UNGUICULATA)

By

Phillipa Olufunmilayo Ogun

The effect of processing on some antinutrients of Nigerian cultivars of cowpeas, the digestibility of carbohydrates, and the quality of proteins were investigated in three studies.

Study I assessed the levels of sucrose, flatulent oligosaccharides (raffinose and stachyose), phytic acid, tannins and trypsin inhibitory activity (TIA) in raw, dehulled, cold-soaked (twelve hours at room temperature), and hot-soaked (two minutes boiled and one hour held at room temperature) samples of fifteen cultivars. Four of the cultivars were cooked into two popular Nigerian dishes (ewa-ibeji, cooked whole cowpeas, and moin-moin, boiled dehulled cowpea paste), and assessed as above. Stachyose was significantly reduced by dehulling, hot-soaking, and cooking. Dehulling eliminated tannins and reduced sucrose, while hot-soaking and cooking significantly reduced TIA. Phytic acid was reduced by

cold- and hot-soaking. Moin-moin and ewa-ibeji contained comparable levels of all the antinutrients except tannins.

Study II determined the digestibility of carbohydrates through breath hydrogen response to ingestion of 100g and 150g cowpeas prepared into the two dishes. The dishes gave significantly higher breath H_2 response than a control meal of rice. Moin-moin appeared to be less conducive to intestinal gas production than ewa-ibeji, although the difference was not significant. Increasing the test dose resulted in a nonlinear increase in breath H_2 production. Addition of raffinose and stachyose to rice gave breath H_2 response comparable to that given by cowpeas.

Study III evaluated the quality of proteins in the two cowpea dishes prepared from American and Nigerian cultivars through *in vivo* and *in vitro* studies. Low PER values were recorded for the dishes. However, moin-moin appeared to have slightly better quality protein than ewa-ibeji. Results of the *in vitro* study correlated well with that of rat *in vivo* study. The quality of cowpea protein was not affected by cultivar difference.

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CHAPTER I

INTRODUCTION

The food legumes, of which cowpeas (Vigna unguiculata) are an example, are important sources of protein, calories, vitamins, and minerals. They are a major source of protein in the diets of people in developing countries and of vegetarians.

Cowpea is the most widely consumed legume in Nigeria and some other West African countries. It serves as the largest single contributor to the total protein intake of many rural and urban families in Nigeria. An increasing number of people are now using cowpeas as weaning food for children, and an effort is being made to increase cowpea production as a means of providing a cheaper source of protein because animal protein is not readily available.

Like other legumes, cowpeas can synthesize a wide variety of chemical substances, termed antinutritional factors, that are known to exert a deleterious effect when ingested by man or animal. These substances include such factors as phytic acid, trypsin inhibitors, oligosaccharides (mainly raffinose and stachyose) and

tannins which can cause undesirable physiological responses or diminish the availability of certain nutrients to animals or humans.

Cowpeas may be prepared in several ways. In households, they are cooked, steamed, or fried either to be eaten alone or used in various recipes or combined with other foods, especially cereals, such as rice and maize.

Many studies have been devoted to investigating the effect of such processing methods as soaking, germination, autoclaving, and cooking on the antinutritional factors of cowpeas. However, the studies on the effect of soaking have been limited to cold-soaking and those on heat treatment to autoclaving, pressure cooking, or cooking of cowpea seeds only. There is no available information on the effect of hot-soaking and dehulling; none on changes in these antinutritional factors when subjected to processes involved in the preparation of common Nigerian cowpea dishes ('ewa-ibeji' and 'moin-moin') and none on the protein and carbohydrate digestibility of these cowpea dishes.

This study was undertaken to provide information on these subjects. The experiment was divided into three parts. In Part 1, the levels of flatulent oligosaccharides, trypsin inhibitor, phytic acid, and

tannins were assessed, and the effect of dehulling, cold-soaking, hot-soaking, and traditional cooking methods on these factors were quantitatively determined in fifteen Nigerian cultivars of cowpeas. In Part II of the study, the digestibility of carbohydrates in an American cultivar was investigated by using breath hydrogen response to the ingestion of two cowpea dishes. Part III of the study involved the evaluation of the quality of the protein in the Nigerian and American cultivars of cowpeas through *in vivo* and *in vitro* protein digestibility study of the cowpea foods.

CHAPTER II

REVIEW OF LITERATURE

Legume as an Important Food Source

Plants are the most important sources of food and food proteins for human beings. Of the total world food harvest, plant products contribute approximately 81.8% of the gross tonnage, whereas animal and marine products together contribute 16.8% (FAO, 1973).

It has been estimated (Altschul, 1967) that by the end of the century, there will be approximately six billion people on the face of the earth and that a twofold increase in protein supplied by plant materials and a fourfold increase in the protein of animal origin will be needed to maintain the same level of nutrition that we have at present and this is admittedly even now suboptimal in many parts of the world.

To expect such an increase in the supply of animal protein in many of the poorer countries is unrealistic. Therefore, plant proteins are considered as the major source of dietary protein in the future, particularly in those parts of the world where poverty and high birth rate always seem to go hand in hand.

Among plant sources, dry legumes and legume products are the richest source of food proteins (Pino and Martinez, 1981). Most beans and peas contain 20-25% protein but this protein is low in sulfur amino acids (Phillips and Adams, 1983). Legumes form an important staple in many areas of the world. This importance is increasing in the diets of the poorest people. As a result, it is safe to say that anything that encourages and improves availability of legume in a form demanded by the consumer takes on particular significance. The very fact that these legumes are already a part of the diet in many parts of the world greatly simplifies efforts to increase their consumption in such countries.

However, the utilization of legumes as human food is still below their potential due to long soaking and cooking times necessary to adequately soften them, loss of valuable nutrients during their preparation, flatulence and antinutritional factors.

Origin of Cowpeas

It seems almost certain that the cultivation of cowpeas (Vigna unguiculata) originated in Africa, probably West Africa. In 1972 94% of the world's cowpea production was from Africa (FAO, 1972). The major producing countries are Nigeria, Burkina Faso, Uganda, Niger, Senegal, and Tanzania.

Cowpeas in the Nigerian Diet

Cowpea, commonly called bean, is one of the most prominent crops that is now engaging the attention of nutritionists in Nigeria. It is a cheap and important indigenous protein source. It is more popular than soybean or groundnut.

An attempt has been made to assess criteria by which consumers accept or reject a cowpea variety. Ojomo (1968) showed that solid white or brown colored seeds were preferred to speckled or black seeds. Fast-soaking varieties with easily removed testa were demanded for the preparation of local dishes such as 'akara' and 'moin-moin'. Where the beans are eaten as boiled whole seeds, varieties which cook quickly and soften readily are those that are acceptable.

Cowpea is either boiled or made into different dishes for consumption in Nigeria. The recipes include 'akara' (Yoruba) or 'kose' (Hausa), 'moin-moin' or 'olele' (Yoruba), or 'alele' (Hausa), 'Danwake' (Hausa); 'Adun' (Yoruba), 'Gbegiri' (Yoruba), and 'ekuru' (Yoruba).

However, the handling, storage, and processing of cowpea into traditional dishes is characterized by waste and drudgery (Williams, 1984). Also, the preparation of some cowpea meals, e.g., 'moin-moin' involve such labor-

intensive steps as soaking, dehulling, and grinding into paste.

Like most other foods, advances in food technology have made possible changes in the mode of consumption of cowpea seeds, so that there is now an increasing emphasis on the use of processed or semi-processed forms of cowpea seeds (Onayemi and Potter, 1976). Cowpea flours can be used to prepare such dishes as moin-moin and akara (Oyenuga, 1968).

Cowpea Composition and Contribution to Diet

On the average, American cultivars of cowpeas are composed of 9.6% moisture, 23.9% protein, 4.1% fiber, 3.3% ash, 1.6% lipid, and 57.7% carbohydrate (Table 1). These values compare favorably with those of some West African cultivars (Dovlo et al., 1976).

Onochie (1975) observed that about 60% of the total protein intake of urban families in the former Western State of Nigeria was of cowpea origin. The protein of cowpea, like other legumes, is generally considered to be of low biological value because it is deficient in sulfur amino acids. Table 2 shows the amino acid profile of raw, cooked, and overcooked cowpeas.

The contribution of cowpeas and other legumes to children's diet in Nigeria (Table 3) showed that cowpeas contributed 4% and 12% to the total daily intakes for

Table 1.--Proximate Composition of Cowpeas from 13 Cultivars (g/100g)[a]

Cultivars	Moisture	Protein (Nx6.25)	Fiber	Ash	Lipid	Carbohydrate (By difference)
California No. 5	7.9	26.3	2.2	3.0	1.3	59.3
Cabbage cowpea	9.1	26.3	2.4	3.4	1.3	57.5
Auburn 70.3	8.4	24.2	4.4	3.2	1.8	58.0
Pinkeye Purple Hull	10.1	24.5	3.4	3.5	1.9	56.6
Mississippi Silver Skin	10.1	23.6	4.6	3.3	1.7	56.7
Magnolia Blackeye	9.9	21.0	5.2	3.5	1.7	58.7
Alabama 963-8 GI-5	9.0	22.8	5.1	3.3	1.5	58.3
Alabama 562 3-1-2	10.9	22.8	3.3	3.2	1.3	58.5
Knuckle Purple Hull	10.6	24.5	3.6	3.1	1.8	56.4
Big Bog Cowpea	8.3	24.5	4.1	3.4	1.8	57.9
Sa Dandy Cowpea	10.1	23.5	5.9	3.5	1.5	55.5
Tennessee White-crowder	11.7	22.7	2.9	3.3	2.0	57.4
Giant Blackeye	8.9	24.5	5.7	3.7	1.3	59.9
Average	9.6	23.9	4.1	3.3	1.6	57.7

[a]Average of two determinations differing by less than 4% (from Akpapunam and Markakis, 1979).

Table 2.--Essential Amino Acid (EAA) Composition of Food Materials (g/16gN)

	Raw Beans	Cooked Beans	Overcooked Beans
Lysine	6.9	6.8	6.8
Tryptophan	--	--	--
Threonine	3.6	3.7	3.6
Valine	4.8	5.4	5.5
Methionine	1.7	1.8	1.8
Isolencine	4.0	4.2	4.0
Leucine	7.4	8.3	8.4
Phenylalanine	5.2	5.0	4.8
Total S-Amino Acids	2.7	2.7	2.7
Total Aromatic Amino Acids	8.6	8.4	8.0
Total EAA	38.0	39.5	39.0
	$\pm$0.2	$\pm$0.1	$\pm$0.1

Source: Oyeleke, 1983.

TABLE 3.--Mean Daily Intakes (± S.E.M.) of Nutrients from Cowpea and Other Legumes and Contribution to Mean Total Daily Intakes in Children 2-5 Years (N = 57)

Mean Intake	Energy (KCal)	Protein (g)	Calcium (mg)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)
Cowpea	48.9 ±10.6	2.9 ±0.6	12.9 ±2.8	0.0 ±0.2	0.092 ±0.019	0.024 ±0.005	0.34 ±0.07
Other legumes	150.3 ±19.2	4.9 ±0.6	24.7 ±2.9	2.0 ±0.2	0.159 ±0.020	0.043 ±0.005	1.14 ±0.15
% of Daily Intake							
Cowpea	3.9%	11.4%	4.6%	7.3%	12.8%	5.8%	4.9%
Other legumes	11.9%	19.3%	8.7%	16.3%	22.1%	10.4%	16.3%

Noge: Other Legumes: Pigeon pea (Cajanus cajan), Bambara groundnut (Voandzeia subterranea), Groundnut (Arachis hypogaea), Okpei (Parkia spp.)

Source: King et al., 1984.

energy and protein respectively. Contribution to intakes for calcium, iron, thiamine, riboflavin, and niacin were 5%, 7%, 13%, 6%, and 5%, respectively (King et al., 1984).

Cowpea Consumption Pattern in Africa

Cowpea is consumed by both children and adults in Africa. The most comprehensive review of legume consumption in Africa 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 in the pattern of legume consumption. The average consumption patterns were as follows:

25%	0 - 10g per person per day
50%	10 - 50g per person per day
23%	50 - 150g per person per day
2%	More than 150g per person per day

The study carried out by Williams (1974) in Nigeria also showed similar average consumption by household (household size of four):

45%	less than 250g per day
32%	250-500g per day
2%	500-700g per day
20%	No response

In a recent study of the dietary pattern of 250 low-income households in Nigeria, King et al. (1984) found

that 36% of the households ate cowpeas three times a week, and 23% ate it more often. Only 12% ate it once a week or less. Favorite cowpea dishes were beans and yam (35%), beans and rice (26%), 'moin-moin' (23.1%), and beans and maize (13%).

Factors Affecting the Nutritional Quality of Cowpea Proteins and Products

The nutritional quality of cowpea proteins, such as other legumes, are basically determined by the amino acid patterns, amino acid availability (digestibility), and contents of biologically active components. Of these factors, the last two are most affected by processing conditions (Rackis, 1972), whereas the amino acid profile is least affected (Table 2).

Heat treatment appears to be the factor which most affects protein nutritional quality. Generally, quality first increases with heat treatment due to inactivation of biologically active factors, passes through a maximum and then decreases due to destruction and/or inactivation of essential amino acids such as cystine. The quantity and availability of other nutrients in the cowpeas also determine its quality and that of its products.

Processing of Cowpeas

The first step involved in the processing of cowpeas is cleaning. This involves the picking out of stones, grits, and insect infected, decayed, or mildewed cowpeas. The various processing methods to which cowpeas have been subjected to include dehulling, soaking, cooking, autoclaving, germination, fermentation, etc.

Dehulling

The seed coats of legumes are sometimes removed prior to their preparation and consumption. In West Africa, some cowpea dishes are prepared only with dehulled seeds. Dehulling cowpeas is a long and tedious process. Most housewives (Williams, 1974) require about one hour to dehull cowpeas sufficient for a day's meal. Generally there are three methods of dehulling cowpeas (Dovlo et al., 1976):

--Wet method

--Combined dry and wet method

--Dry method

In the wet method, the cowpeas are soaked in water for some time. The testa are manually removed. Testa floating on the soak-water are removed by decanting.

In the combined dry and wet methods, the cowpeas are broken into smaller bits by grinding roughly. The

coats, shells, insects, etc., are then blown off. The broken, clean cowpeas are soaked in water and the remaining skins will float to the surface and can be removed.

In the dry method, the cowpeas are broken into little bits in a mill and the seed coats blown off. This method is the most suitable for cowpea flour.

The seed coat is said to be the primary barrier to the migration of water into the bean. Snyder (1936) observed and found no beneficial effect of scarification or removal of seed coat on tenderness of beans. However, Kon et al. (1973) noted a reduction of cook time when seed coat was removed. Muneta (1964) found that the presence of seed coats affects subjective evaluation of bean product. On the effect of dehulling on the antinutrients in dry beans, Deshpande et al. (1982) observed that dehulling significantly increased the phytic acid content of beans from 1.6-2.9% to 1.6-3.7%. Dehulling also increased trypsin, chymotrypsin, and α-amylase inhibitory activities of the beans. Removal of seed coasts also lowered tannin content of beans by 68-95%. Rao and Deosthale (1982) observed that decortication of the pulses studied resulted in 83-97% loss in tannins. Tannins in beans are located in the seed coat (Ma and Bliss, 1978) and thus its removal may

be expected to reduce the tannin content of beans. Similarly significant reduction in tannin content on removal of seed coats of sorghum (Jambunathan and Mertz, 1973) has been reported. Dehulling also significantly improved the *in vitro* digestibility of bean proteins (Deshpande et al., 1982).

Soaking

During soaking, beans imbibe water which leads to softening of seed coats and thus decreases cook time required to obtain desirable texture. The capacity of beans to absorb water depends greatly on their own physio-chemical composition and seed coat (Synder, 1936). Various researchers (Neely and Sistrunk, 1979; Junek et al., 1980) have used different additives, such as ethylene diamine tetraacetic acid (EDTA), sodium bicarbonate, citric acid, malic acid, acetic acid, etc. in soak water. Hoff and Nelson (1965) reported that EDTA has no significant effect on water absorption by dry beans, nor does it affect the firmness of navy, pinto, and kidney beans as shown by Junek et al. (1980). Soaking can be done with cold and hot water.

The Michigan Bean Commission developed a standard method of bean preparation in which beans are processed either by cold-soaking or hot-soaking. In cold-soaking, beans are soaked in six cups of cold water and two

teaspoons of salt for every pound of beans. Hot-soaking is done by adding a pound of beans to six to eight cups of boiling water, cooking for two minutes, removing the beans from heat, and allowing to stand for one hour at room temperature (Tittiranonda, 1984).

Ojomo and Chheda (1972) studied the rates of water absorption of different cowpea varieties with and without seed coats. They observed that varieties with rough seed coats absorbed water very fast and attained saturation in 2.3 hours. Those with wrinkled and smooth testa absorbed water more slowly and attained saturation in 3.7 and 6 hours,respectively. Without the influence of seed coats, however, the varieties absorbed water and reached saturation more or less equally.

Most of the studies done on the effect of soaking on antinutrients were carried out with cold soak water (Rao and Desthale, 1982; Ologhobo and Fetuga, 1984). However, Tittiranonda (1984) carried out her study of the physical and chemical changes during preparation and cooking of dry edible beans with both hot and cold soak water. She observed greater chemical changes with hot-soaking. Hot-soaked and cooked beans were generally softer than corresponding cold-soaked beans. She also looked at the effect of soak time and observed that prolonged soaking and cooking produced softer beans and a

greater loss of solids from the beans into the cook water.

On the effect of soaking on the antinutrients of beans, Rao and Desthale (1982) observed that as a result of overnight soaking in water, 50% of tannins were lost in pigeonpea and chickpea while in blackgram and greengram, the loss was 25%.

Ologhobo and Fetuga (1984) also observed that in the ten varieties of cowpeas studied, soaking for three days decreased trypsin inhibitory activity by a mean of 31.2%; hemagglutinin activity by 19.0%, tannic acid by 13.4%, and phytic acid by 24.4%. Tittiranonda (1984) showed that hot-soaking of navy and kidney beans resulted in 30% and 39% reduction of the sugars of the oligosaccharide family respectively, while 16-hour cold-soaking removed 35% and 50% of these sugars from the two types of beans, respectively.

Liu (1986) compared the effect of soaking on the antinutrients of immature and mature soybeans. He observed that in mature soybeans, cold-soaking slightly reduced oligosaccharides, but had no effect on phytic acid and trypsin inhibitory activity. Similar changes were observed in the oligosaccharides and phytic acid of immature soybeans.

Cooking

Most of the antinutritional or toxic effects of legumes can be partially or wholly eliminated by the proper application of heat. This effect is enhanced by a general improvement of the nutritive value of the proteins of legumes (Liener, 1962). It is this relative ease with which these toxic components can be removed, in most cases, by appropriate methods of cooking that has no doubt contributed to the popularity of legumes as a staple part of the diet in many countries. In general, the degree of improvement in nutritive value effected by heat is dependent on the temperature, duration of heating, and moisture conditions.

Some legumes, such as Phaseolus vulgaris and Dolichos labbab require preliminary soaking prior to cooking or autoclaving in order to completely eliminate the toxicity of the raw bean (Liener, 1962).

Being proteins, trypsin inhibitors are readily eliminated by heat treatments. Ologhobo and Fetuga (1983) reported that other methods of elimination, such as soaking and germination were not as effective on trypsin inhibitor as heating. Kakade and Evans (1965b) reported that autoclaving navy beans for 5 minutes at 121C destroyed about 80% of the trypsin inhibitory activity, and the growth performance of rats that were

fed beans subjected to this heat treatment was considerably improved. Recently, Liu (1986) showed that cooking or steaming completely inactivated the trypsin inhibitors in immature soybeans, but only partly in mature ones. He also showed that cooking or steaming significantly reduced the oligosaccharide content of the mature soybeans, but had little or no effect on phytic acid content.

Ologhobo and Fetuga (1984) observed that cooking and autoclaving slightly decreased the total phosphorus and phytate compounds of cowpeas by 5.0 - 8.3%.

Antinutritional Factors in Cowpeas

The antinutritional factors, also called "toxic factors" commonly refer to those substances found in foods that produce a deleterious effect when ingested by man or animals. Liener (1980) pointed out that this term can be misleading since they imply that the substance in question is lethal beyond a given level of intake. Although some plants are known to produce a violent expression of poisoning (Kingsbury, 1964), much more subtle effects, produced only by prolonged ingestion of a given plant, are commonly observed. Such effects might include an inhibition of growth, a decrease in food efficiency, a goitrogenic response, pancreatic hypertrophy, hypoglycermia, and liver damage.

Liener (1981) classified antinutritional factors into two groups according to their responses to heat: heat-labile and heat stable (Table 4).

Table 4.--Antinutritional Factors in Soybeans

Heat-Labile	Heat-Stable
Trypsin inhibitors	Saponins
Hemagglutinins	Estrogens
Goitrogens	Flatulence factors
Phytates	Allergens

Of these antinutrients, notable ones which might affect the nutritional quality of cowpeas include flatulent factors, phytates and trypsin inhibitors.

Flatulent Factors

The total carbohydrates of dry legumes range from 24.0% in winged beans to 68.0% in cowpeas (Reddy et al., 1984). Carbohydrates in legumes include starch, monosaccharides, oligosaccharides and other polysaccharides. Among the sugars, oligosaccharides of the raffinose family, namely raffinose, stachyose (Fig. 1) and verbascose predominate in most legumes and account for 31.3% to 76.0% of the total sugars (Akpapunam and Markakis, 1979; Kon, 1979; Rockland et al., 1979;

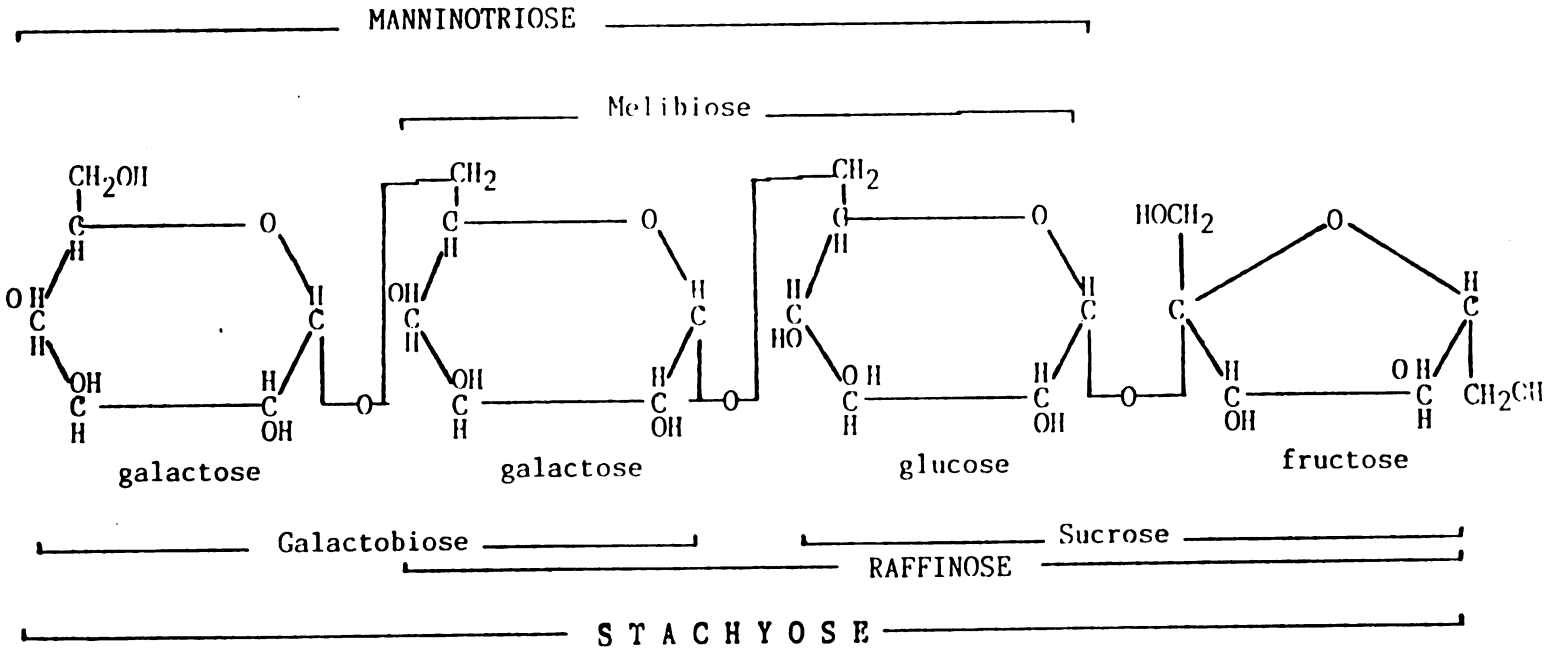


Fig 1. Structure of Oligosaccharides

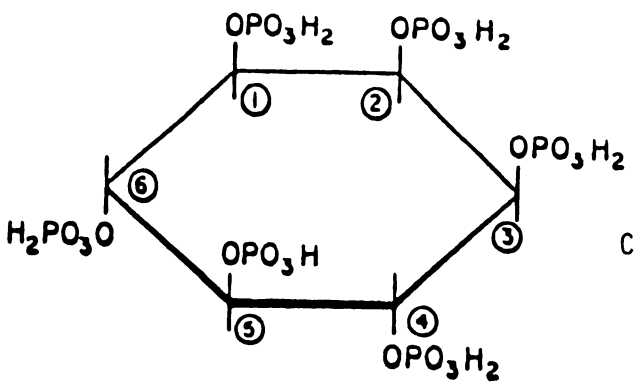
Ekpeyong and Borchers, 1980; Reddy and Salunkhe, 1980; Fleming, 1981; and Sathe and Salunkhe, 1981). Stachyose represents the major oligosaccharide in cowpeas. Raffinose is present in moderate to low amounts in most legumes.

Oligosaccharides of the raffinose family (raffinose, stachyose, and verbascose) are reported to be, at least in part, responsible for the flatulence problem in humans and animals. Several studies have been done on flatus and flatus formation (Steggarda, 1968; Levine, 1979; and Fleming, 1981). However, some studies (Olson et al., 1975 and 1982) revealed that flatus-producing capacity was not totally eliminated by removal of oligosaccharides, thus suggesting that there are other substances that contribute to flatulence. Reddy et al. (1984), in reviewing studies pertaining to this problem, mentioned that fiber, which is one major indigestible component in beans, may be involved in the fermentation by microorganisms and subsequent flatulence production.

Phytic Acid

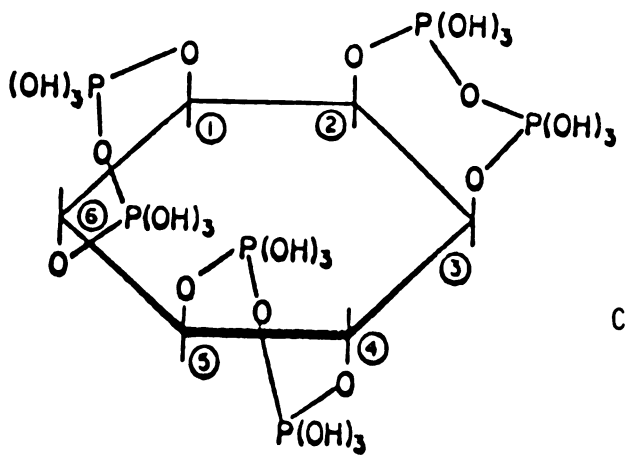
Phytic acid, a hexaphosphorylated myo-inositol (Fig. 2) is an abundant plant constituent, amounting to 1-3% of the weight of all nuts, cereals, legumes, oil

PHYTIC ACID



$C_6H_{18}O_{24}P_6$

ANDERSON STRUCTURE



$C_6H_{18}O_{24}P_6 \cdot 3H_2O$

NEUBERG STRUCTURE

Fig. 2. Proposed structures for phytic acid.

seeds, spores, and pollens (Graf, 1983). It functions as a reserve material for phosphorus and typically accounts for 60-90% of the total seed phosphorus (Lolas et al., 1976). The metabolism of phytic acid during seed development and germination has been well established (Scott and Loewus, 1986). Phytic acid serves several other important physiological functions, such as antioxidant protection during dormancy (Graf, 1986), storage of cations (Williams, 1970), and cell wall precursors (Scott and Loewus, 1986). To plants themselves, the presence of phytic acid may be desirable, but when these plants are used for food, it is undesirable because it readily chelates with di- and trivalent metal ions as Ca, Mg, Zn, Cu, and Fe to form insoluble salts. Such complexes are poorly absorbed from the intestine, resulting in reduced availability of these minerals (Jaffe, 1981; Morris, 1985). Graf (1983) cited certain potential uses of phytic acid and its derivative in medicine.

In addition to binding with minerals, phytic acid was found to bind strongly with proteins at certain pH values. This can affect the properties of proteins (Okubo et al., 1976). Phytic acid also interacts with enzymes, leading to a modification of enzymatic activity.

Tannins

As defined by Singleton and Kratzer (1973) in their review, a tannin is any polyphenolic substance that has a molecular weight greater than 500. The tannins may be classified as hydrolyzable, that is, degradable by enzymes to yield a sugar residue and a phenolcarboxylic acid, and condensed tannins, which are polymeric flavonoids, having the structure shown in Fig. 3.

Food legumes contain variable amounts of polyphenols, such as condensed tannins. Their effect on nutritional value of food legumes is also primarily detrimental. Polyphenols (as condensed tannins) are predominantly located in the pericarp and/or testa, particularly of pigmented cultivars of legumes and millets. Bressani and Elias (1980) observed a higher protein quality for white, as compared to pigmented cultivars of Phaseolus vulgaris. These authors also found tannins to be heat-stable and that they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen. Polyphenols provide agronomic benefits, such as protection of seed upon germination (Ma and Bliss, 1978). Tannins inhibit the activities of trypsin, chymotrypsin, amylase, and lipase (Griffiths, 1979). They cause growth repression in rats (Abbey et al., 1979), and poultry

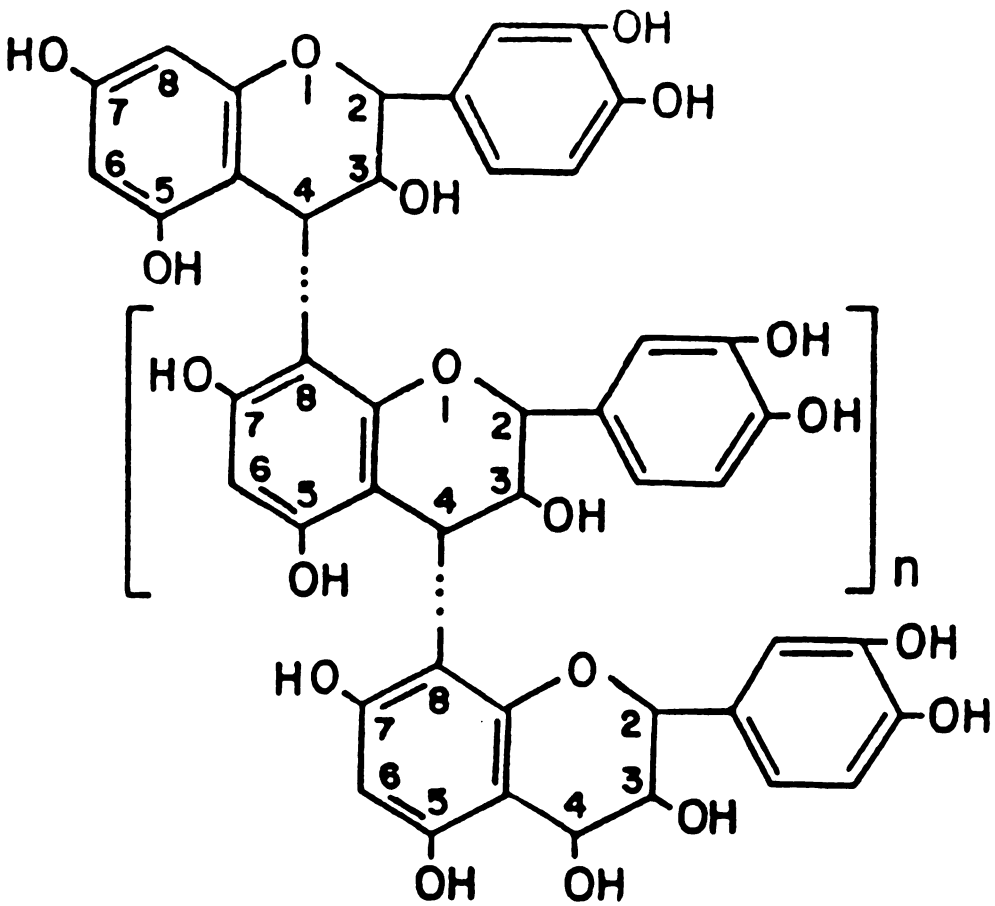


FIG. 3 . Structure of condensed tannins.

(Martin-Tanguy et al., 1977). Tannins also interfere with dietary iron absorption (Prabhavathi, 1979). They are nonspecific inhibitors of enzymes and may reduce protein quality by directly complexing with food proteins (Tan et al., 1983).

Trypsin Inhibitors (TI)

These are substances having the ability to inhibit the proteolytic activity of trypsin. They are probably the best known and certainly the most studied of all of the antinutritional factors, especially in soybeans. Osborn and Mendel (1917) made the significant observation that soybeans had to be heated in order to support growth of rats. Further evidence came from experiments which showed that addition of purified preparation of the trypsin inhibitor to heated soybeans to provide some inhibitory activity as raw soybeans, caused a significant reduction in growth (Desikachar and De, 1947; Liener et al., 1949). However, adding the trypsin inhibitor did not reduce the protein efficiency ratio (PER) to the same level of growth as was observed on raw soybeans, indicating that heat treatment was doing somewhat more than just inactivating the trypsin inhibitor. Chernick et al. (1948) found that raw soybeans and trypsin inhibitor itself could cause hypertrophy of the pancreas, an effect which is accompanied by an increase in the secretory activity of

the pancreas. This led to the suggestion that the growth depression caused by the trypsin inhibitor might be the consequence of an endogenous loss of essential amino acid being secreted by a hyperactive pancreas. Kunitz first isolated a trypsin inhibitor in 1945. All legumes that have been looked at to date--cowpeas, peanuts, navy beans, lima beans, etc.--have been found to contain trypsin inhibitors to varying degrees. The trypsin inhibitor first noted in crude extracts of cowpeas by Borchers et al. (1947b) was subsequently purified and characterized by Ventura and co-workers (Ventura and Filho, 1967; Ventura et al., 1972b). It proved to be a low molecular weight (10,000) trypsin/chymotrypsin inhibitor rich in cystine. Somewhat later Royer's group (Royer et al., 1974; Royer, 1975) using immobilized trypsin demonstrated the presence of five isoinhibitors having similar properties.

Elimination and Measurement of Antinutritional Factors in Cowpeas

Flatulent Factors

Being heat-stable, flatulent factors cannot be eliminated by heat treatments. However, techniques, such as dehulling, soaking, enzymatic hydrolysis, germination, etc., have been reported to reduce the oligosaccharide contents of legumes (Liener, 1981; Onigbinde and Akinyele, 1983).

Carbohydrates have been analyzed by classical wet chemical and enzymatic methods, as well as by several chromatographic techniques (e.g., gas, paper, thin-layer, liquid partition, ion-exchange, and gel-filtration chromatography). Most of these methods have their limitations. Ion-exchange, liquid partition, and gel-filtration methods all have excellent resolving power, but have been traditionally slow and time consuming. Rapid separation can be achieved by gas chromatography, but only after formation of volatile derivatives.

Conrad and Palmer (1979) reported a procedure for analysis of carbohydrates in legumes by high pressure liquid chromatography. This method allows direct and rapid determination of sugars in food and beverage matrices, including oligosaccharides.

In order to separate sugars from other components, several methods have been reported, including precipitation of proteins with 15% trichloroacetic acid or with lead acetate solution and purification of sugars on TLC, which is the best method according to Cegla and Bell (1977), but very tedious, or on preparative column of hydroxyapatite. The development of an accurate and simplified extracting procedure with least risk of column contamination for HPLC analysis of sugars still remains a challenge.

Phytic Acid

Man and other monogastric animals have little or no phytase to hydrolyse phytic acid. Several procedures have been used at the laboratory level to precipitate phytates as insoluble salts of barium, dialyzing them against sodium chloride solution, treating them with a strong anion exchange resin, and controlling the pH (Jaffe, 1981). The impracticality of using heat to cleave minerals from phytate was demonstrated by de Boland et al. (1975) who showed that 30 minutes autoclaving reduced the phytate content of cereal and oilseeds by less than 10%.

However, certain microorganisms containing phytase could cleave phosphates from phytates during fermentation (Erdman, 1979). Yeast has been shown (Ranhontra et al., 1974) to cleave phosphates from phytic acid during fermentation of legumes.

Phytic acid has generally been determined by modifications of the method of Heubner and Stadler (1914), which depends on the ferric chloride precipitation of the phytic acid from extracts. These methods (Wheeler and Ferrel, 1971; Ellis et al., 1977) which show that the precipitate may be digested directly or analyzed for either phosphorus or iron (Fig. 4) are not only time-consuming, but also assume certain molar

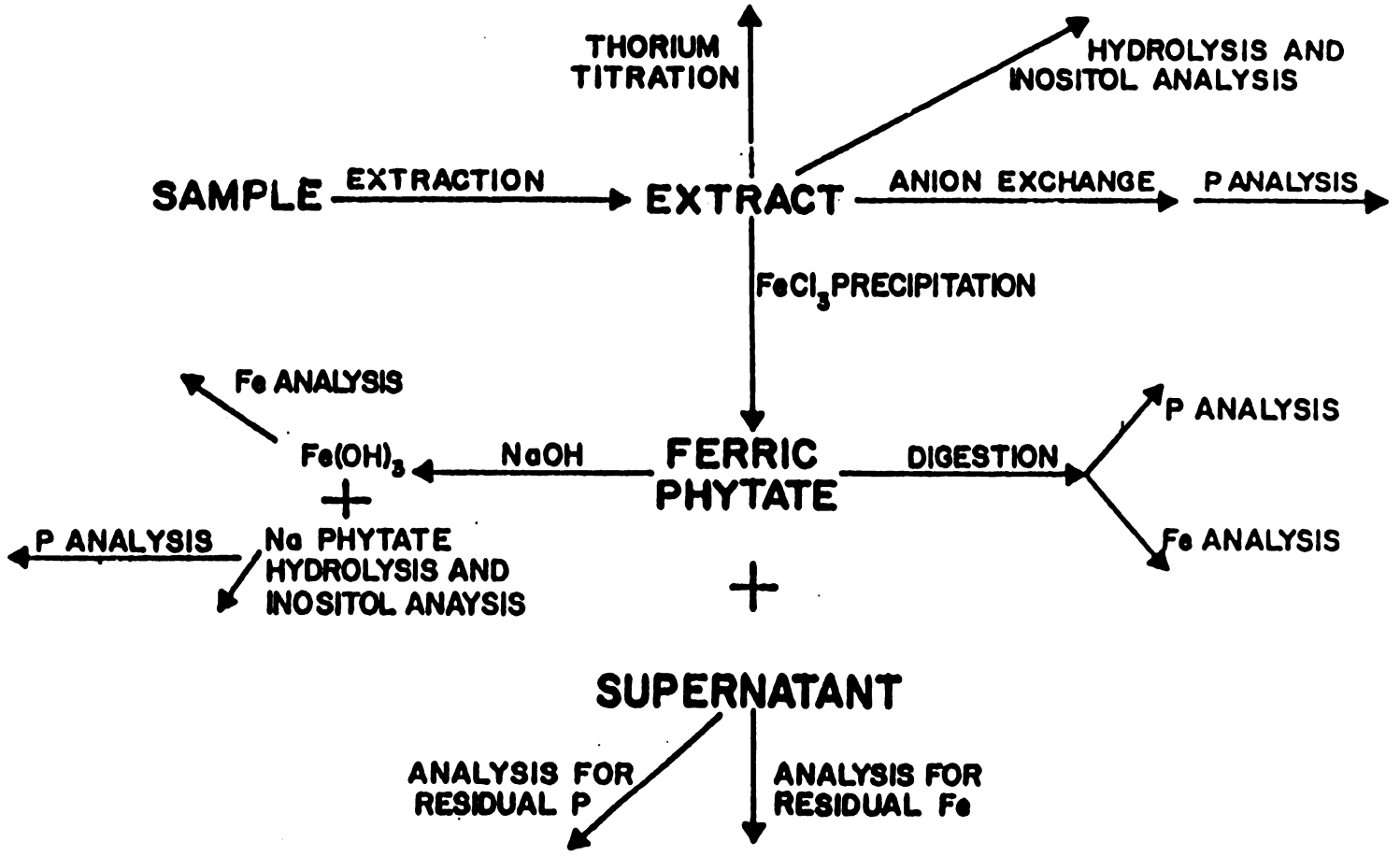


Fig. 4—Selected approaches to phytic acid analysis.

ratio of iron to phytate phosphorus (usually ranging from 3:6 to 4:6) which may result in poor reliability.

An anion exchange separation of phytate from the extract was reported accurate (Harland and Oberleas, 1977). HPLC has been used to quantitate phytic acid (Tangendjaja et al., 1980; Camire and Clydesdale, 1981). Recently, Knucles et al. (1982) developed an improved HPLC method to quantitate phytic acid, and it has proved to be a rapid method with more reproducible results as compared with classical methods.

Extraction of phytate from legumes has been done by various acid solutions, such as 3% trichloroacetic acid (TCA) (Wheeler and Ferrel, 1971), 0.5N HCl (Makower, 1970), and 1.2% HCl + 10% Na_2SO_4 or 3% TCA + 10% Na_2SO_4 (Thompson and Erdman, 1982).

Tannins

It has been shown that decortication of pulses resulted in 83-97% loss of tannin in pulses (Rao and Deosthale, 1981). They also showed that overnight soaking in water resulted in 50% loss of tannin in pigeonpea and chickpea and 25% loss in blackgram and greengram, while cooking of the raw pulses brought about 70% decrease in their tannin content. Thus, they concluded that traditional methods employed for pulse processing are able to remove a major part of the tannin

present in the pulse. Other investigators (Elias et al., 1976; Ologhobo and Fetuga, 1984) have also observed partial removal of tannins in cowpeas and other legumes with cooking.

Methods of tannin analysis are based on precipitation of tannins, formation of colored products with tannins, oxidation of tannins, and UV spectroscopy (Puisais et al., 1968). The Association of Official Agricultural Chemists (1965) listed the Folin-Denis method for use on alcoholic beverages, and the permanganate reducing method for tea, cloves, and allspice. The hide-powder method is used in the tanning industry and some work has been reported in which UV absorbance has been used to estimate the tannin content of wines (Puisais et al., 1968), tea and beer (Owades et al., 1958). The vanillin-hydrochloric acid procedure of Burns (1963, 1971), which involves the formation of colored products with tannin, has been modified by Maxson and Rooney (1972). The modification involves extraction of samples with 1% HCl in methanol, rather than pure methanol.

Trypsin Inhibitors

TI are readily inactivated by such heat treatments as dry roasting, heating in boiling water, live steam, microwave radiation, dielectric heating,

extrusion cooking (Liener, 1981), etc. Ologhobo and Fetuga (1983) reported the elimination of TI by other methods, such as soaking and germination. However, these methods are not as effective as heat treatments.

The various methods of affinity chromatography, column chromatography, and electrophoresis which have proved valuable for the isolation and characterization of diverse TI are not suitable for quantitation. Methods that are currently employed for trypsin inhibitor activity (TIA) are mainly colorimetric, based on interaction among TI, trypsin, and a substrate. The substrates that are generally used are either synthetic, for example, benzoyl-DL-arginine-p-nitroanilide (BAPA) or natural, e.g., casein.

The method employing casein is the one originally described by Kunitz (1947), and it involves the spectrophotometric determination of the breakdown products produced by a given concentration of trypsin in the presence and absence of the inhibitor. The standard AACC method for determining the TI content of soy products (AACC 71-10) is based on use of a synthetic substrate (BAPA). This method evolved, primarily, from the work of Kakade et al. (1969, 1974) who evaluated and compared the synthetic substrate, first introduced by Erlanger et al. (1961), with a natural substrate, casein, which previously had been the generally accepted standard

method (Kunitz, 1947). Kakade concluded that the synthetic substrate was the more convenient and reliable method of assaying TI content of soy products, provided the competitive nature of the inhibition was taken into consideration. Subsequently, a collaborative study by a committee on Soybean TI analysis resulted in several modifications of the original procedure (Rackis et al., 1974; Kakade et al., 1974).

Hammerstrand et al. (1981) who found that the extrapolation procedure for data interpretation suggested by Kakade et al. (1969) could lead to an erroneously high value for TI activity, since this extrapolation used data that are not in the region in which zero order kinetics are followed, developed a modified procedure in which the TIA was determined from a sample extract that inhibited at least 40%, but no more than 60% of the trypsin.

Digestibility of Carbohydrates in Cowpeas and Its Measurement

Digestibility

The oligosaccharides of the raffinose family have been implicated in flatus formation (Fleming, 1981). They cannot be digested by humans because the intestinal mucosa does not contain the enzyme (α-1, 6-galactosidase) necessary to split these oligosaccharides into simple sugars. Gitzelmann and Aurichio (1965) found no

galactosidase activity in human intestinal mucosa. Ruttloff et al. (1967) found no enzymatic hydrolysis of raffinose in the intestinal mucosa of rats, pigs, and humans. Other studies on the absorption and degradation of oligosaccharides containing -galactosyl groups show that less than 1% of the administered dose was able to pass through the intestinal wall of man and animals (Krause et al., 1967; Taeufel et al., 1967).

The indigestible oligosaccharides pass through the small bowel and reaches the colon where bacterial fermentation produces large amounts of carbon dioxide and hydrogen and a small quantity of methane (Olson et al., 1981).

Breath Hydrogen as Index of Carbohydrate Digestibility

The hydrogen produced during bacterial fermentation of carbohydrate passes through the intestinal mucosa into the blood stream as dissolved gas and is excreted in the expired air. Intubation studies have shown a correlation between the amount of unabsorbed carbohydrates passing the terminal ileum with the expired breath hydrogen (Bond and Levitt, 1976). A similar production pattern has also been shown between flatus production and expired breath hydrogen (Fig. 5). Breath hydrogen has, therefore, been used to predict foods that

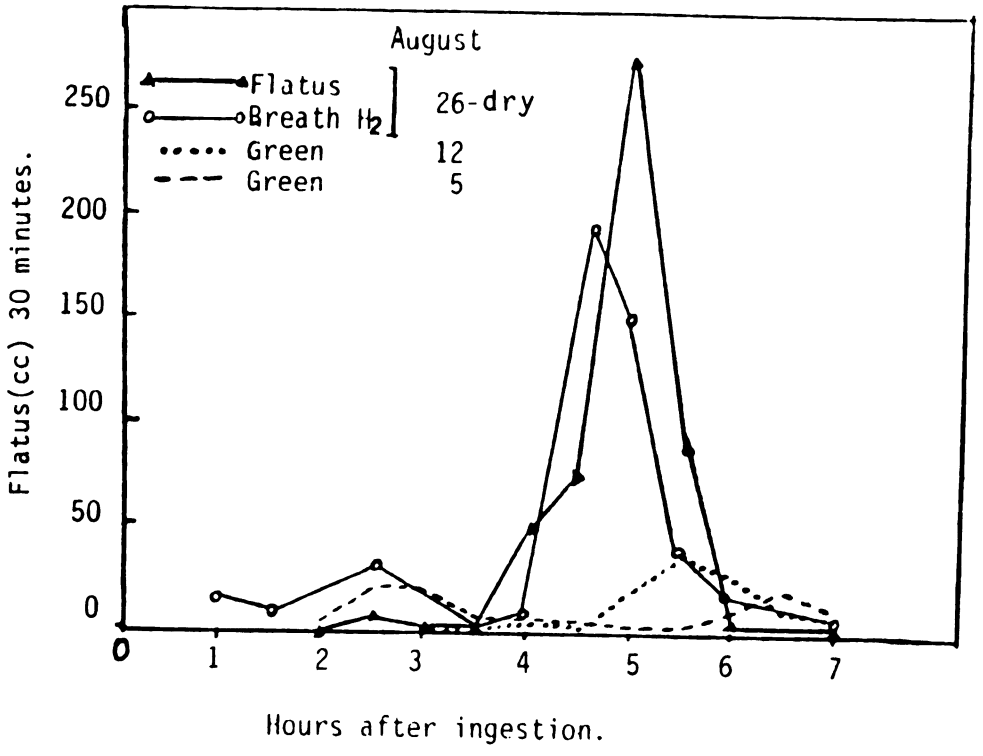


Fig. 5. Breath Hydrogen and Flatus after ingestion of beans of different maturity harvested in August.

(from Murphy,1964).

are gas-forming due to the presence of unabsorbable carbohydrates (Calloway and Murphy, 1968; Levitt, 1969). Murphy (1964) found that after a human subject eats a bean meal, the production of hydrogen gas in the breath rises in a pattern very similar to that of increase in flatus volume. He also observed that when the gas production in the intestine exceeds the specific respiratory efficiency of an individual in eliminating it from the lungs, the excess gas is then eliminated from the body as flatus. The period of maximum gas production, both as breath hydrogen and as rectal flatus occurred about 4 to 6 hours after subjects ate a sample of cooked beans (Steggerda, 1964; Murphy, 1966; Calloway et al., 1971).

Method of Measuring Breath Hydrogen

A closed rebreathing system was used to measure breath hydrogen and to quantitate carbohydrate malabsorption (Levitt and Donaldson, 1970; Bond and Levitt, 1972). This method requires special collection equipment that includes a closed hood for the subject's head during the test. However, Welsh et al. (1981) showed that interval breath samples provide an additional index of carbohydrate malabsorption and that like the closed rebreathing method, this index does not directly

quantitate carbohydrate malabsorption, but does allow meaningful intraindividual comparisons of breath hydrogen responses under different conditions.

Factors Affecting Breath Hydrogen Production

Factors, such as exercise, sleep, antibiotics, etc., have been shown to affect the production of breath hydrogen. Murphy (1966) attributed the "early morning peak" he observed in breath hydrogen to nocturnal gas accumulation in the gastro-intestinal tract during a period of reduced activity and blood flow and which is eliminated by respiration upon rising.

Solomon and Viteri (1976, 1978) observed a marked increase in breath hydrogen concentration during sleep, and they attributed this effect to be due to a combination of hypoventilation and decreased colonic motility resulting in higher intracolonic gas concentration. Metz and Jenkins (1977) agreed with Solomon (1976) that subjects undergoing breath hydrogen tests should be kept at rest and awake, and that they should be fasted prior to tests for the diagnosis of carbohydrate malabsorption so that they start the test with very low or no breath hydrogen.

Payne et al. (1983) observed a relatively rapid decrease in breath hydrogen concentration immediately after exercise. Ventilation rate changes would be one

possible explanation. Their study showed that exercise does not stabilize breath hydrogen concentration, and in fact, interferes with interpretation of a response curve after ingestion of lactose.

Antibiotic drugs have been shown to alter the activity of hydrogen-producing microorganisms (Murphy and Calloway, 1972).

Protein Quality Evaluation

Protein quality in human nutrition is mainly related to the efficiency with which food proteins are used for the synthesis and maintenance of tissue proteins. This quality is a function of the essential amino acids (EAA) and the digestibility of the proteins. The closer the EAA pattern is to a reference pattern (Table 5) required for human growth and maintenance, the better the quality of the protein. There are two types of tests used to determine the quality of protein: *in vitro* tests and *in vivo* tests.

In-Vitro Methods for Measuring Protein Digestibility

Several *in vitro* methods for the measurement of protein digestibility have been developed. Akeson and Stahmann (1964) found that a pepsin-pancreatin enzyme system gave a reasonably accurate approximation of protein digestibility. An enzyme preparation from

Table 5.--Amino Acid Patterns Required for Growth and Maintenance of Rat and Man.

	Rat (mg/kg/day)[a]		Man (mg/kg/day)[b]	
	Growth	Maintenance	Growth	Maintenance
Histidine	1.9	2.2	?	?
Isoleucine	5.0	4.7	28	12
Leucine	6.3	4.3	42	16
Lysine	8.2	3.4	44	12
Phenylalanine + Tyrosine	6.6	6.3	22	10
Methionine + Cystine	4.6	4.4	22	16
Threonine	4.6	4.6	28	8
Valine	5.1	4.7	4	3
Tryptophan	1.0	1.0	25	14

[a]Said and Hegsted, 1970

[b]Food and Nutrition Board, 1975.

Streptomyces griseus was also found to be a good predictor of protein digestibility (Ford and Salter, 1966). Buchanam and Byers (1969) described an in vitro system for measuring protein digestibility of a wheat leaf protein concentrate with an enzymatic digestion utilizing papain. The enzyme systems described by Akeson and Stahmann (1964) and by Buchanan and Byers (1969) were reinvestigated by Saunders et al. (1973). They found that the values obtained from the enzyme system used by Akeson and Stahmann (1964) showed an excellent correlation with in vivo data ($r = 0.88$). Saunders et al. (1973) developed a papain-trypsin system, the in vitro results of which correlated well with in vivo digestibility ($r = 0.91$). Rhinehart (1975) examined several enzyme systems which included trypsin, pepsin-trypsin, trypsin-chymotrypsin, and trypsin-chymotrypsin-peptidase combinations. The results were encouraging with correlation coefficients of 0.79, 0.72, 0.80, and 0.74 for the respective enzyme systems. Hsu et al. (1977) developed a multienzyme technique for the estimation of protein digestibility. The multienzyme system consists of trypsin, chymotrypsin, and peptidase. It was found that the pH of a protein suspension immediately after 10 min. of digestion with the multienzyme solution described above was highly

correlated with the *in vivo* apparent digestibility of rats.

In Vivo Methods of Measuring Protein Digestibility

Considering all the biological methods that have been used for the evaluation of protein quality, including liver regeneration, blood regeneration, liver enzyme activity, repletion of adult rats, plasma amino acids, or growth of microorganisms, there is no better validated method available than the growth of weaning rats (Jansen, 1978). The growth of weaning rats, as determined by protein efficiency ratio (PER), is widely used, and is the official assay in the U.S. and Canada. This assay (AOAC, 1980) which uses a single level of protein (10%) in the diet and measures the ratio of weight gain to protein consumed over a 28-day period has its limitations. It is a measure of protein quality as it relates to the growth requirement of the rat, but makes no allowance for the maintenance requirement of the rat; the assay yields data which are not proportional and it uses a single level of protein in the test diet (10%), a level which is biased against most plant proteins.

Bender and Doell (1957) proposed the net protein ratio (NPR), assay to overcome the major flaw of the PER assay, in that it does not consider the protein

maintenance requirement of the rat. To do this, NPR includes a second group of animals on a protein-free diet and assumes the protein needed to prevent weight loss of the protein-free group to be a measure of the maintenance requirement of the rat.

The net protein utilization (NPU) assay of Bender and Miller (1953) is similar to that of the NPR, but utilizes values of body nitrogen instead of body weight. McLaughlan (1972) stated that NPU should be equivalent to biological value (BV) x protein digestibility.

Slope ratio assays that have been used include the relative nutritive value (RNV) assay (Hegsted et al., 1968), and its modification, the relative protein value (RPV) (Hackler, 1977).

Certain assays utilize microorganisms, such as Streptococcus feccalis (Horn et al., 1952), Leuconostoc messenteroides (Terri et al., 1956), and Streptococcus zymogenes (Ford, 1960; Leleji et al., 1972). These assays were used to measure the availability of selected essential amino acids (EAA) in proteins and thereby describe protein quality based on EAA availability. The microorganism most extensively used to measure protein quality is the protozoan Tetrahymena pyriformis. This organism has definite advantages over those listed above in that it (1) has an EAA requirement similar to that of

man and the rat, and (2) can ingest particulate material, thereby not having to rely entirely on soluble nutrients for growth. By combining a proteolytic enzyme partial predigestion step along with the subsequent growth of T. pyriformis on the hydrolysate, it has been shown (Lender, 1975; Evancho et al., 1977) that Tetrahymena growth was highly correlated to rat growth on selected food proteins. Sutton (1978) modified the assay and reported protein quality as estimated by the Tetrahymena assay as a predicted PER or T-PER (Tetrahymena estimated PER).

CHAPTER III

MATERIALS AND METHODS

Cowpea Seed Collection

Fifteen Nigerian cultivars of cowpeas (Vigna unguiculata) were obtained from the National Cereal Research Institute and the International Institute for Tropical Agriculture, both in Ibadan, Oyo State, Nigeria.

The American cultivar used was obtained through the Michigan State University food store.

Cowpea Seed Processing

The cowpea seeds were processed as follows:

--Raw cowpea flour: Whole dry cowpeas were ground to a 50 mesh flour in a UDY cyclone sample Mill (UDY Corp., Fort Collins, Co.).

--Dehulled cowpea flour: Cowpea seeds were soaked in distilled water (1:4 w/v, cowpea to water ratio) for about 40 mins at room temperature. The seed coats were manually removed and the cotyledons cleaned, frozen, freeze-dried, and ground as above.

--Cold-soaked cowpea flour: Cowpea seeds were soaked in distilled water (1:4 w/v cowpea to

water ratio) for 12 hours at room temperature. The soak water was decanted and the seeds frozen, freeze-dried, and ground.

--Hot-soaked cowpea flour: A sample of cowpeas was added to boiling water (with the same cowpea to water ratio as above) and allowed to stand for two minutes, after which it was removed from heat and allowed to cool to room temperature for one hour. The soak water was then decanted and the cowpea seeds frozen, freeze-dried, and ground.

--Cooking 'Ewa-ibeji': Every 100g cowpea sample was washed, soaked for 15 mins at room temperature, and cooked for 65 mins, or until soft, after which 80g tomato sauce, 0.4g pepper, 2.5g salt, and 3 teaspoons corn oil were added. Everything was then cooked together for 10 to 15 mins. Samples for the breath hydrogen study were eaten after cooling while samples for laboratory analysis were frozen, freeze-dried, and ground.

--Cooking 'Moin-moin': Every 100g dehulled cowpea sample was blended into a paste and 80g tomato sauce, 0.4g pepper, 2.5g salt, and 3 teaspoons corn oil were added. Portions of this paste were wrapped in aluminum foil and

solidify. Samples for the breath hydrogen study were eaten after cooling while samples for other laboratory analysis were frozen, freeze-dried, and ground.

Sample Preparation

All frozen raw and processed samples were freeze-dried for 48 hours in a Unitrap II freeze-dryer (The Virtis Company) and then ground to a 50 mesh flour in a UDY Cyclone Sample Mill. The ground samples were put in screw cap glass bottles and stored at 4 C until analyzed.

Proximate Analysis of Raw Samples

Moisture, ash, crude protein (N x 6.25) and crude fat were all determined according to AOAC (1980) procedures.

Analysis for Oligosaccharides

Oligosaccharides were determined utilizing HPLC according to the method of Conrad and Palmer (1976), and the procedure developed by Agbo (1982) with very slight modifications.

Equipment

The high pressure liquid chromatography system consisted of a Model M-45 solvent delivery system, 3.9 mm i.d x 30cm long Bondapak/carbohydrate analytical column, and a model RI-401 differential refractometer

detector, all from Waters Associates, Inc., Milford, MA. The responses were recorded on a Konkes recorder 100.

Sample Extraction

A 2.5g sample of raw or processed cowpea flour was mixed with 25 ml of 80% (v/v) ethanol, put in a water bath at 80 C for 4 hr with occasional shaking. The mixture was centrifuged for fifteen minutes at 2000 rpm. The supernatant was collected in a separate tube. This extraction was performed twice more with ten and five ml of 80% ethanol, respectively. The supernatant from all three extractions was collected in the same tube. Then two ml of 10% lead acetate was added to precipitate any excess protein which was not coagulated by the heat treatment. The extract mixture was shaken in the same manner and centrifuged at 2000 rpm for ten minutes. The supernatant was removed and 1.0 ml of oxalic acid was added to it to remove excess lead acetate. The mixture was again shaken, centrifuged, and the supernatant saved. To remove the color in the supernatant, 1.0g of activated carbon was added and let stand for 30 min. The tube was centrifuged and the supernatant saved. The supernatant was then concentrated in a flash evaporator at 40 C until about 2-3 ml of the solution was obtained. The concentrated solution was then transferred into a 5 ml

volumetric flask, and brought to volume with distilled water.

Final Sample Clean-Up

Prior to HPLC analysis, the sample extract was passed through a 0.22 µ-pore diameter membrane filter (Millipore Corp.) with a prefilter, utilizing a syringe and then passed through a Waters Sep-Pak C18 Cartridge for final sample cleanup.

The Chromatograph System

A 20 µl sample of the water clear extract was injected into the HPLC. A degassed mixture of acetonitrile and water (65:35, v/v) was used as solvent with a flow rate of 1.8 ml per minute. The detector attenuator was constantly held at 8X and the chart speed of recorder at 1.0 cm per minute.

Identification and Quantification of Sugars

To identify the cowpea sugars, a standard mixture of sucrose, raffinose, and stachyose was used. Standard curves were then made from four known concentrations of each standard for quantification. Retention times and peak heights of chromatograms from cowpea samples were then compared to those from the standard sugar mixture.

Analysis of Phytic Acid

The method of Wheeler and Ferrel (1971) was used. Method involved the extraction of phytic acid, iron chelation, and determination of the chelated iron. Color measurement was done according to the method of Makower (1970).

Extraction and Precipitation

A 2.0 g sample of finely ground cowpea (50 mesh) was extracted with 40 ml of 3% Trichloroacetic acid (TCA) + 10% sodium sulfate solution in 125 ml Erlenmeyer flask for 45 mins using a mechanical shaker. The suspension was poured into a 40-50 ml round bottom polyethylene centrifuge tube and centrifuged at 20,000 x g for 15 mins. Then a 10 ml aliquot of the supernatant was transferred to clean centrifuge tubes containing 5 ml ferric chloride solution (6mg $FeCl_3$ per ml of 3% TCA solution). The content was heated in boiling water for 60 min after which it was cooled. The tube was centrifuged for 15 min. The precipitate was washed twice by dispersing it in 20 ml 3% TCA + 10% sodium sulfate solution and then heated in boiling water for 5 min and centrifuged. A third washing of the precipitate was done, but with water. The precipitate was dispersed in 5 ml water and 5.0 ml 0.6N NaOH was added to it to coagulate ferric hydroxide. The content was then heated

in boiling water for 45 min, after which it was centrifuged for 15 min. The precipitate was washed in 30 ml hot water and centrifuged. The precipitate was then dissolved in 5 ml of 0.5N HCl with heating in boiling water for 15 min. The content was transferred to 100 ml volumetric flask and made to volume with 0.1N HCl solution.

Iron Standard Solution

Iron stock solution was made by dissolving 0.0484g $Fe_2Cl_36H_2O$ (Analytic Reagent grade) in 200 ml volumetric flask and made to volume with 0.1N HCl solution. This stock solution containing 100 g Fe/ml was diluted with 0.1N HCl solution to obtain standard solutions ranging from 2.0 to 10.0 g Fe/ml.

Color Measurement

1 ml aliquot of standard or sample solution was transferred to a 25 ml Erlemeyer flask and the following were added:

--9 ml 0.1 N HCl solution

--1 ml 10% hydroxylamine HCl solution

--10 ml 2M sodium acetate solution

--1 ml 0.1% orthophenanthroline solution

The content was mixed and allowed to stand for 5 mins, after which it was poured into transparent glass test

tubes (Pyrex) for color reading at 510 nm in a spectrophotometer (Spectronic 70, Bausch and Lomb Co.).

Calculation of Phytate

A Standard curve of iron concentration against absorbance was prepared (Fig. 6). The phytate phosphorus content was calculated based on the assumption that the molecular ratio of iron:phosphorus is 4:6.

Analysis of Tannins

The method of Burns (1971) as modified by Maxson and Rooney (1972) was used.

Extraction

A 200 mg sample was extracted with 10 ml of 1% HCl in methanol for 20 min at room temperature, with mechanical shaking. The mixture was then centrifuged at 1000 x g for 5 min and the supernatant saved for color measurement.

Standard Solution

A stock D-catechin solution was prepared by dissolving 10 mg catechin in 10 ml methanol. This stock solution was diluted with methanol to obtain standard solutions with concentration ranging from 0.1 mg/ml to 1.0 mg/ml.

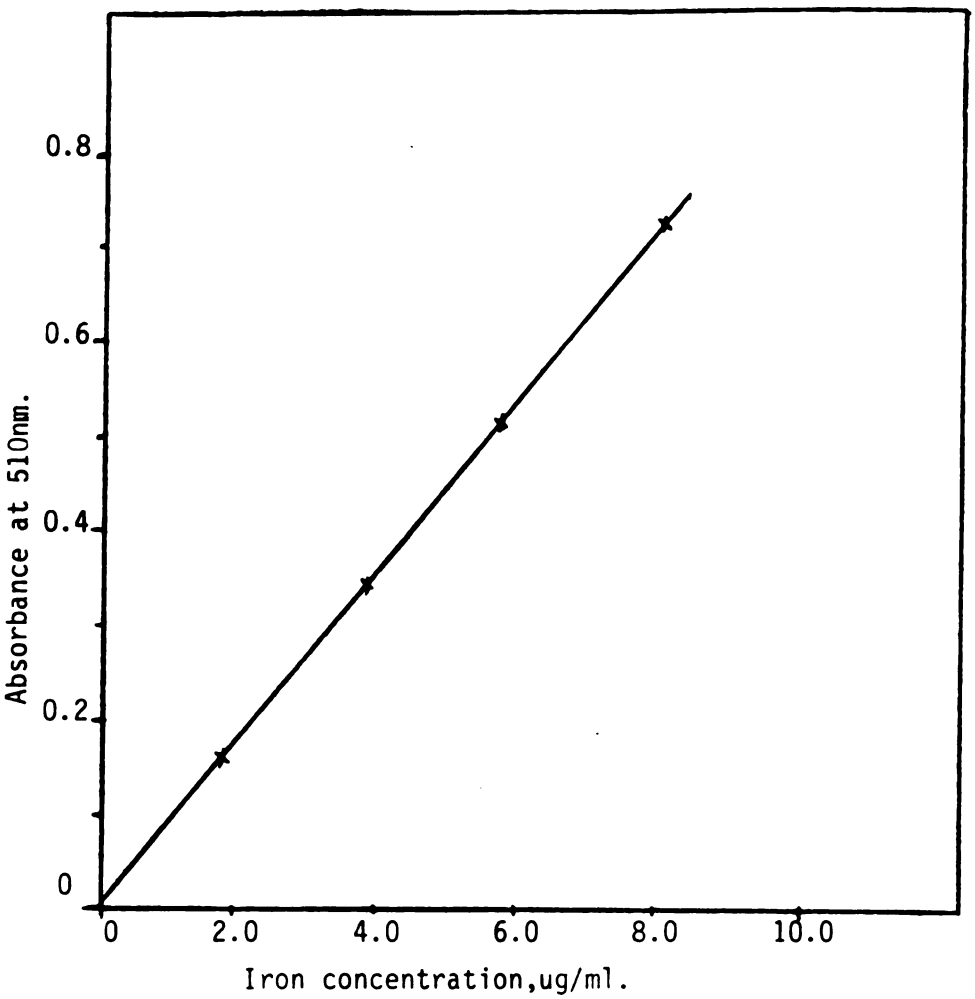


Fig. 6. Standard curve for Fe determination.

Color Measurement

Vanillin-HCl reagent was prepared by combining equal volume of 8% concentrated HCl in methanol and 4% vanillin in methanol just before use. Then 5.0ml of this reagent was quickly added to 1.0 ml aliquot of sample extract or standard solution. A blank containing methanol instead of sample or standard was also prepared. Absorbance was then read at 500 nm after 20 min using a Spectronic 70 spectrophotometer (Bausch and Lomb Co.). Values of tannins were read as D-catechin equivalent from a standard curve of catechin concentration against absorbance (Fig. 7).

Analysis for Trypsin Inhibitor

The method of Hamerstrand et al. (1981) was used.

Preparation of Reagents

Tris-buffer. Tris (hydroxymethyl amino-methane) (6.05g) and 2.95g of $CaCl_2 2H_2 0$ were dissolved in 900ml of distilled water. The pH was adjusted to 8.2 and the volume brought to 1 liter with water.

Substrate solution: 80mg of benzoyl-DL-arginine-p-nitroanilide (BAPA) was dissolved in 2ml of dimethyl sulfoxide and diluted to 200ml with tris-buffer prewarmed to 37 C. The solution was prepared before each run and was kept at 37 C after preparation.

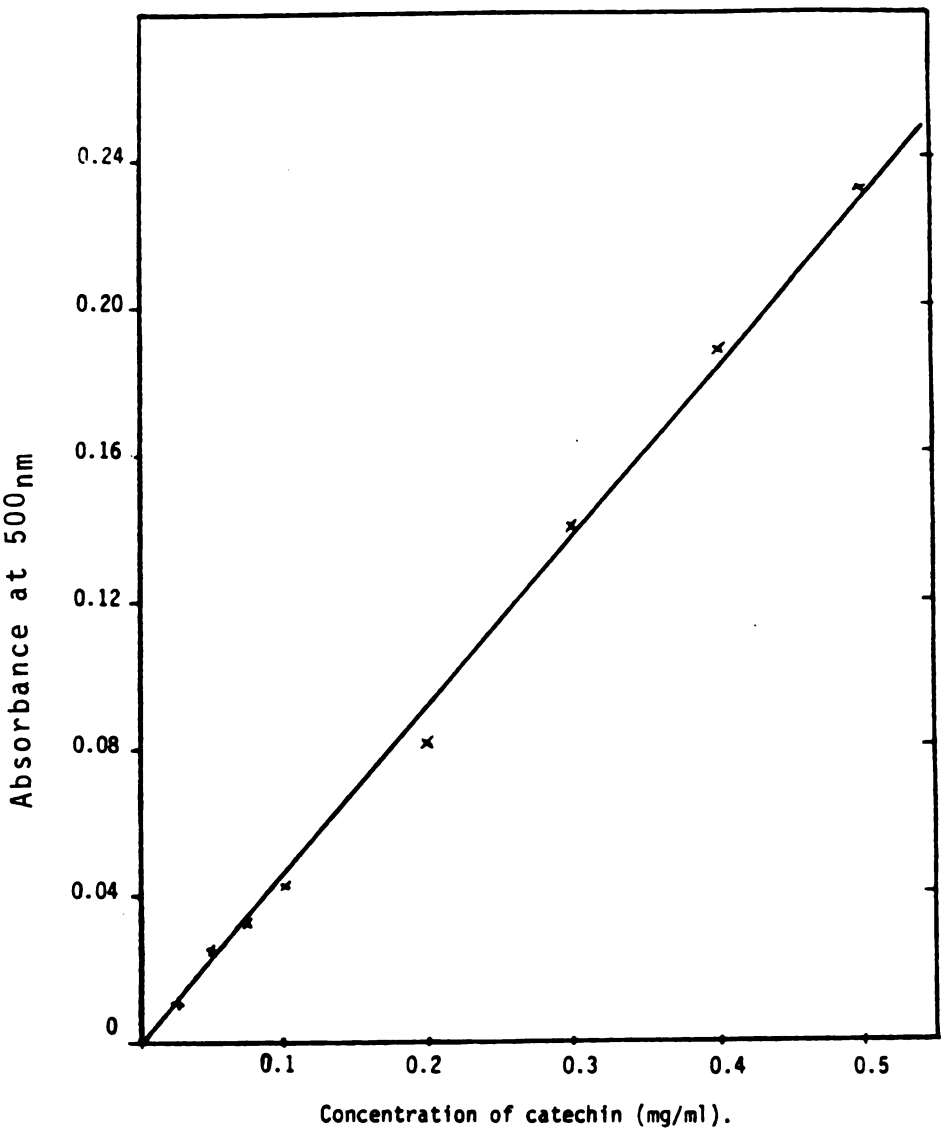


Fig. 7. Standard curve for measurement of tannins as catechin equivalent.

Trypsin solution: Trypsin (40mg) was weighed into a 200-ml volumetric flask and diluted to 200ml with 0.001 N HCl. A fresh solution was prepared after two weeks.

Extraction of Sample

Cowpea sample (1.0g) was extracted with 50ml of 0.1N NaOH for 3 hr. on a mechanical shaker. The pH of the suspension was adjusted, when required, to 8.4 - 10.0. A 1.0 ml aliquot of this suspension was diluted to 12.0 ml with water. For raw cowpea samples, 2ml of this diluted suspension inhibited 40-60% of the trypsin used as a standard in the analysis.

Procedure

To each of three test tubes, 2-ml aliquots of the diluted sample extract were added. A fourth tube was prepared for the trypsin standard by adding 2 ml of distilled water. To two of the three tubes containing the sample extract, 2 ml of the trypsin solution was added, and the tubes were placed in a constant temperature bath (37 C) for 10 min. Then, 5 ml of BAPA solution (prewarmed to 37 C) was rapidly blown into each tube. The contents were stirred immediately on a vortex mixer, and the tubes were replaced in the constant temperature bath. The reaction was terminated exactly 10

min later by blowing in 1 ml of 30% acetic acid with immediate mixing with a vortex mixer. A sample blank (the third tube containing sample extract) was prepared by the same procedure, except that the trypsin solution was added after the reaction was terminated by the addition of acetic acid.

The absorbance of each solution was determined at 410nm against the sample blank. The value obtained from each of the two sample extract was subtracted from the trypsin standard to get the differential absorbance reading, Astrd-Asample (Hamerstrand et al., 1981).

Expression of Activity

To obtain an expression of TI activity, the differential absorbance (Astrd-Asample) is first converted to trypsin inhibitor unit (TIU). One such unit is arbitrarily defined as that giving an increase of 0.01 in absorbance at 410nm per 10ml of reaction mixture under the conditions used. The value is then divided by aliquot size (in ml) to yield TIU per millimeter for each sample extract used in the analysis. The TI activity is then expressed as trypsin unit inhibited (TUI) per milligram sample.

Digestibility of Carbohydrates

The digestibility of the carbohydrates in cowpeas was determined through breath hydrogen response to ingestion of cowpea foods.

Test Meals

The test meals, Ewa-ibeji (cooked whole beans) and moin-moin (steamed cowpea paste),were prepared as outlined in (Fig. 8).

Amount of Cowpeas Eaten

The quantity of cowpeas fed to the subjects was expressed on a dry weight basis. Whole or dehulled cowpeas (100g and 150g) were prepared as 'ewa-ibeji' and 'moin-moin' and fed to the subjects randomly during the study. Two of the subjects also ate 200g of cowpeas as tests meals.

Other Foods Included in Study

In addition to cowpeas, the following foods were also eaten during the study:

1. Control meal of rice (85g), plus all other ingredients added to the cowpea meals; this meal was used to establish the baseline gas production throughout the day--this was determined only once for each subject during the study. The rice (85g) supplied an amount of carbohydrates equal to 100g cowpeas.

2. A light lunch meal consisting of

--milk-free French bread

--ham

--Swiss cheese

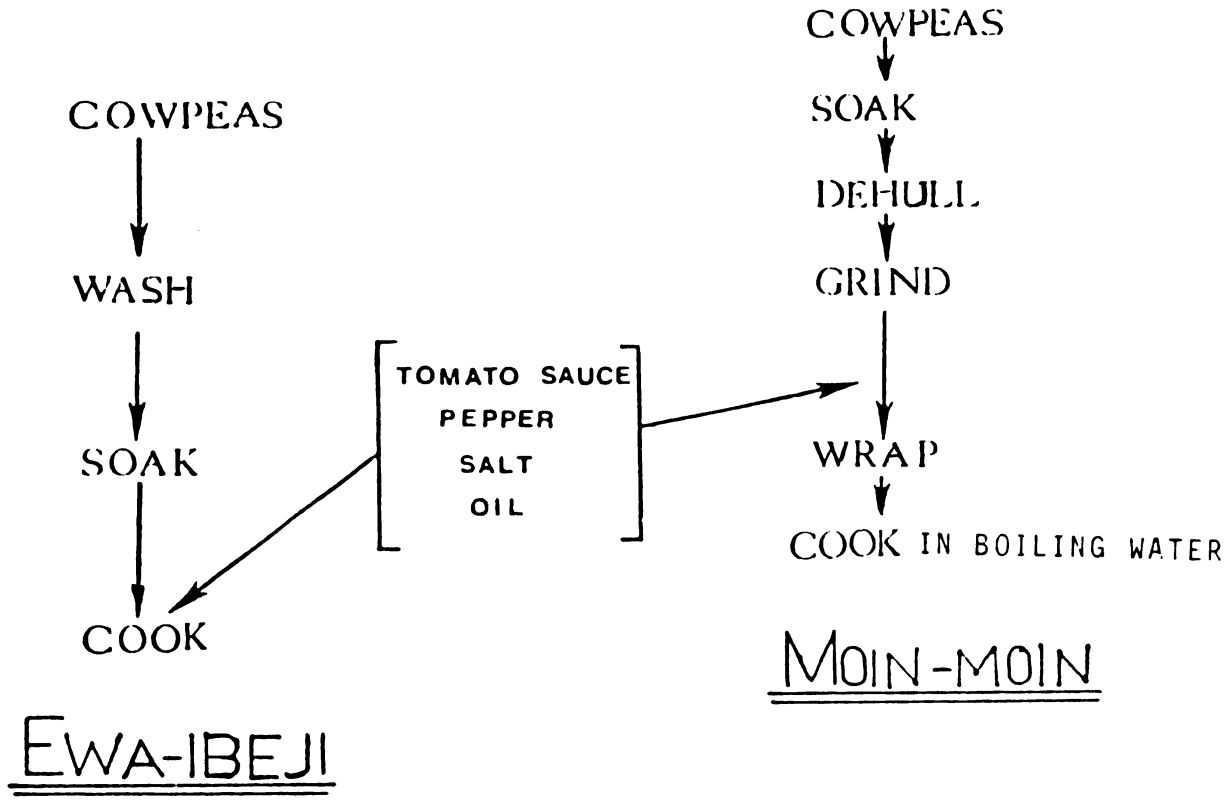


Fig. 8. Preparation of two popular Nigerian Cowpea dishes: Ewa-ibeji and Moin-moin.

--canned pineapple

--water

3. Control meal (1) to which was added 1.2g raffinose or 3.4g stachyose, amounts comparable to those found in 100g cowpeas. The cowpea and control meals were served with Tang, an orange-flavored beverage.

Testing Procedure

Time of test meals. Test meals were served at 8 a.m. on every test day. Noon meals were served at 1 p.m. The test meal was administered randomly for each subject. Subjects were allowed 30 min to finish each meal.

State of subjects. The five subjects used in the study had an overnight fast of at least 10 hr., i.e., they took their last meal by 10 p.m. on the days preceding the test days. Subjects kept a record of foods eaten on the evenings before the test days.

Sampling schedule. Two basal breath samples were obtained before the test meal was eaten. Other breath samples were obtained every 30 mins after the test meal over a 7-hr period (i.e., 3rd to 10th hour after meal). Subject breathed into plastic breath hydrogen bags and the breath samples were analyzed for hydrogen by a Quin Tron Microlyzer which uses the principle of chromatographic separation of gases. The column material

has an attraction for all the ordinary components in the breath, except hydrogen. These components are retarded in their passage through the column. This delay permits hydrogen to be detected in the presence of all other components present in the expired air in concentration which can be detected with the gas sensor of the Quin Tron Microlyzer.

Estimation of Breath Hydrogen Concentration

The cumulative breath hydrogen concentration in the expired air was estimated by calculating the area under the curve of hydrogen concentration against time with the following equation for the sum of the areas of consecutive trapezoids (Kotler et al., 1982):

$$A = (1/2H_1 + H_2 + H_3 \ldots + H_{n-1} + 1/2\ H_n)\ \Delta t$$

where A = ppm x min

H = Breath H_2 concentration (ppm)

Δt = Time interval between sample collection = 30 min

Protein Quality Evaluation

Protein Efficiency Ratio (PER)

Diet. The PER test was performed according to the AOAC method (1980). Five diets were prepared using the following protein sources:

1. Casein (as control)
2. Ewa-ibeji prepared from Nigerian cultivar of cowpea (Ife brown)
3. Moin-moin prepared from Nigerian cultivar of cowpea
4. Ewa-ibeji prepared from American cultivar of cowpea (California No. 5)
5. Moin-moin prepared from American cultivar of cowpea

The nitrogen content of the samples was determined by the micro-Kjeldahl method, and the fat content by the Goldfisch diethyl ether extraction method. Protein was determined by multiplying nitrogen content by a factor 6.25. All feeds were standardized to meet the following composition:

Protein = 10%

Fat oil = 8%

Salt mixture USP = 5%

Vitamin mixture = 1%

Cellulose = 1%

Water = 5%

Corn starch + Sucrose (1 + 1) to make 100%. The vitamin mix AOAC was from Tekland Test Diets, and the Salt mix and vitamin-free casein from United States Biochemical Corporation, Cleveland, Ohio.

Experimental animals. Fifty laboratory male rats, $\geq$ 21 days of age, but $\leq$ 28 days of age, from Harlan Sprague Dawley, Inc., were used.

The animals, with an average weight of 45g, were allowed to acclimatize for 4 days on rat chow diet. At the beginning of the test, ten rats were assigned to each of the five diet types. Average weight of rats in any one group on day beginning assay period did not exceed average weight of rats in any other group by more than 5g.

Assay Period. The rats were kept in individual cages and provided with appropriate assay diet and water ad libitum. Body weight and diet consumed were recorded every 3 days for rats on test diets from the Nigerian cultivar and every 4 days for rats on casein diet and test diets from the American cultivar. The experiment was terminated after 28 days from the beginning of the assay.

Calculation of PER. Weight gain and protein intake (10% of food intake) per rat for each group were calculated and the PER for each group was calculated as the ratio of weight gain per protein intake. Corrected PER was calculated by multiplying the PER of each treatment with 2.50/PER of casein.

In Vitro Protein Digestibility Assay

The pepsin-pancreatin method of Saunders et al. (1973) was used.

Procedure. In a centrifuge tube, 250mg of sample material was suspended in 15ml of 0.1N HCl containing 1.5mg pepsin, and gently shaken at 37 C for 3 hours. The solution was neutralized with 0.5N NaOH and treated with 4mg of pancreatin in 7.5ml of 0.2M phosphate buffer (pH 8.0) containing 0.005 M sodium azide. The mixture was gently shaken at 37 C for 24 hours. The solids were separated and cleaned by centrifuging at 20,000 x g for 5 min and washing with 30 ml of distilled water five times. The solids were finally filtered through a 1.2 μ- filter (Millipore) air-dried, weighed, and analyzed for nitrogen.

Calculations. The following relationship was used to calculate protein digestibility:

$$\text{Protein Digestibility (\%)} = \frac{[(\text{N in sample}) - (\text{N in undigested fragment})]}{(\text{N in sample})} \times 100$$

Statistical Analysis of Data

Data were analyzed using analysis of variance at 5% level. Paired comparison on the treatments was done

by using Tukey's multiple comparison test. Regression analyses were done to see if there were any correlation among some of the outcomes.

CHAPTER IV

RESULTS AND DISCUSSION

Proximate Composition of Cowpeas

The proximate composition of the Nigerian cultivars of cowpeas used in this study are given in Table 6.

Ash

The mean ash content was 3.04% with values ranging from 2.8 to 3.3%. These results agree with the findings of Akpapunam and Markakis (1979) who recorded a mean ash content of 3.3% for American cultivars of cowpeas.

Protein

The mean protein content was 22.4%. There is a wide range in the protein content among cultivars: 20.1 - 24.8%. A similar observation was made by Akpapunam and Markakis (1979).

Lipid

The lipid contents of the cowpeas ranged from 1.1 to 1.6% with a mean value of 1.4%. This result agrees with the data of Ojomo and Chheda (1970) who reported a

Table 6. Proximate Composition of Nigerian Cultivars of Cowpeas.*

Cultivar	Composition (% Dry Weight)			
	Moisture	Protein (Nx6.25)	Ash	Lipids
IT-81D-1137	10.1	22.4	3.3	1.1
IT-81D-994	9.9	22.8	3.0	1.4
IT-81D-1096	9.8	21.6	2.8	1.1
IT-82D-716	10.6	20.1	2.8	1.5
IT-82D-789	9.9	20.8	3.1	1.4
IT-82E-9	10.6	23.1	3.2	1.3
IT-82E-16	10.2	20.5	3.0	1.3
IT-83D-442	10.1	21.6	3.0	1.4
TVX-3236-01G	9.6	22.9	2.9	1.4
TVX-4659-03E	10.2	21.8	3.0	1.5
NIGERIA A104	9.6	24.1	3.3	1.6
IT-3	10.2	21.7	2.9	1.2
NIGERIA B7	10.4	24.2	2.9	1.6
Kano 1696	9.6	23.3	3.2	1.4
Ife Brown	10.3	24.8	3.2	1.4
Mean + S.D	10.1±0.3	22.4±1.3	3.0±0.2	1.4±0.2

*Each value is an average of two determinations differing by less than 4%.

range of 1.0 to 1.7% with a mean value of 1.3% for Nigerian cultivars of cowpeas. However, Akpapunam and Markakis (1979) reported a higher mean value of 1.6% for the American cultivars of cowpeas.

Oligosaccharides

HPLC Chromatography

The HPLC separation of three standard sugars: sucrose, raffinose, and stachyose is shown in Fig. 9a. Fig. 9.b shows the HPLC chromatogram of the sugars present in an 80% (v/v) ethanol extract of cowpeas. A comparison of the two chromatograms indicates that cowpeas contained sucrose, raffinose, and stachyose in appreciable amounts. This fact was also recorded by Akpapunan and Markakis (1979) and Onigbinde and Akinyele (1983) in their studies of the oligosaccharides of American and Nigerian cultivars of cowpeas, respectively. These authors used paper chromatography for their analysis.

Oligosaccharides in Cowpeas

The data obtained from the raw samples, shown in Table 7, revealed the concentration of stachyose, compared with other oligosaccharides, to be the highest in all cowpea varieties. This is followed by raffinose. The cultivar had a significant ($p < 0.05$) influence on

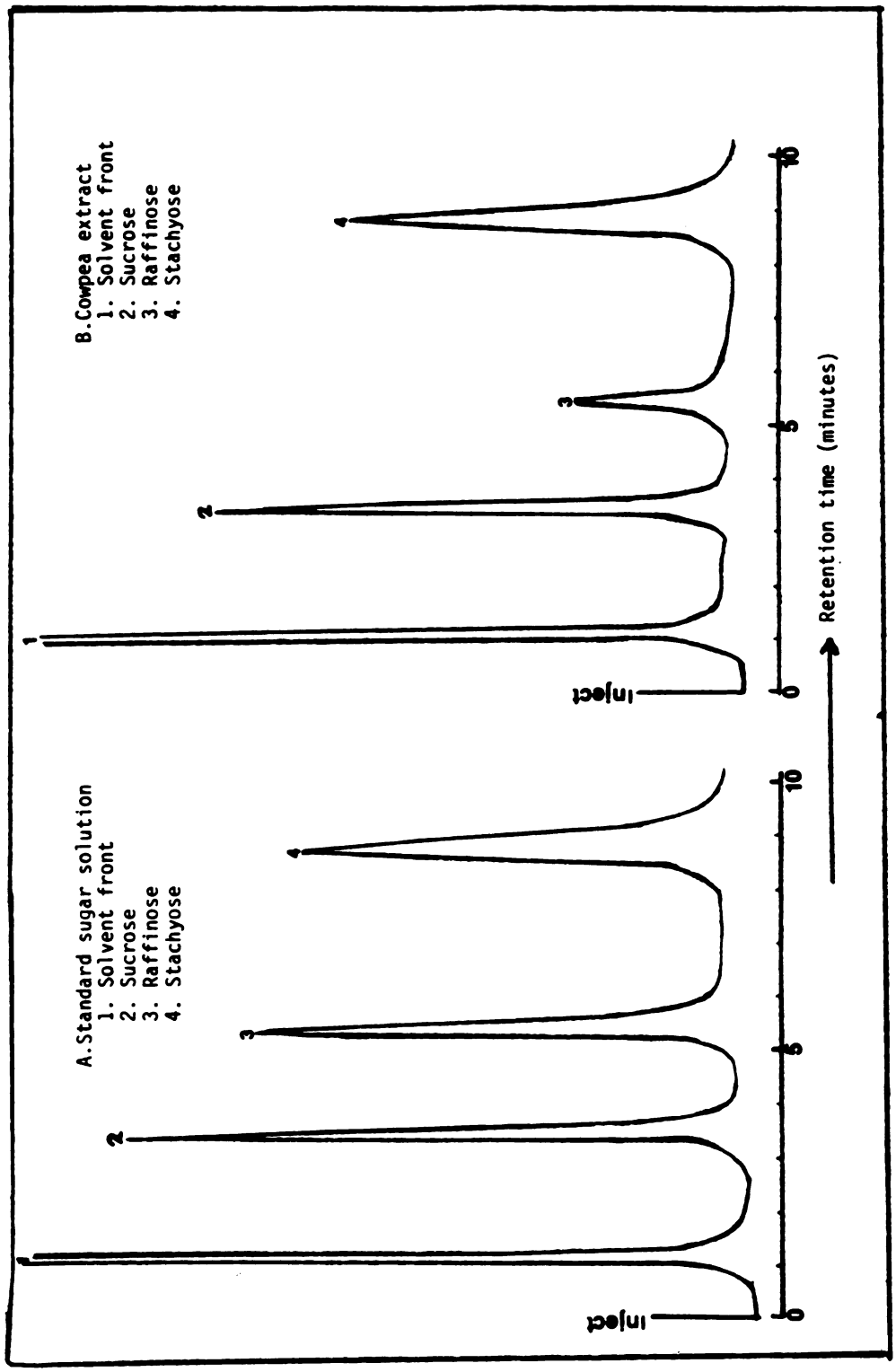


Fig. 9. HPLC chromatograms for sugar analysis:
Solvent: acetonitrile/water(65/35); Flow rate: 1.8ml/min.; Attenuation: 8X;
Chart speed: 1.0cm/min.

Table 7.--Oligosaccharides (% Dry Weight) of Raw Cowpeas.*

Cultivar	Sucrose	Raffinose	Stachyose
IT-81D-1137	0.8	1.9	2.3
IT-81D-994	1.0	1.3	3.7
IT-81D-1096	0.8	1.1	2.9
IT-82D-716	0.7	0.8	3.0
IT-82D-789	1.0	2.3	2.7
IT-82E-9	1.4	2.0	3.1
IT-82E-16	0.9	1.6	2.7
IT-83D-442	0.9	0.7	2.7
TVX-3236-01G	1.2	2.4	2.8
TVX-4659-03E	0.7	2.0	3.4
NIGERIA A104	1.0	1.1	3.3
IT-3	0.8	0.9	3.5
NIGERIA B7	0.8	1.4	2.6
Kano 1696	1.0	1.1	3.1
Ife Brown	1.0	2.6	3.2
Mean	0.9	1.5	2.9
S.D	$\pm$0.19	$\pm$0.60	$\pm$0.41

*Each value is an average of two determinations differing by less than 4%.

the level of oligosaccharides. The data showed that 1T-81D-1137 had the least concentration (2.3%) of stachyose and TVX-4659-03E had the highest (3.46%) on dry weight basis. 1T-83D-442 had the least concentration of raffinose (0.7%) and Ife Brown had the highest (2.6%) on dry weight basis. The sucrose concentration of 1T-82D-716 was the least, while 1T-82E-9 had the highest concentration on dry weight basis. Akpapunam and Markakis (1979) and Onigbinde and Akinyele (1983) also observed that stachyose, when compared with other oligosaccharides, occurred in highest concentration in cowpeas. A similar observation was made by Tittiranonda (1984) for dry edible beans (Phaseolus sp). However, in his study of the oligosaccharides of soybeans, Liu (1986) reported the highest concentration for sucrose. This is followed by stachyose, with raffinose having the least concentration. Since flatulence in legumes is mainly ascribable to raffinose and stachyose (Steggarda, 1964), it is not surprising that these oligosaccharides occurred in appreciable amounts in the cowpea cultivars studied and also in other legumes.

Effect of Processing on Sucrose

The sucrose content of raw and processed cowpeas is given in Table 8. The ranges are: for raw cowpeas, 0.7-1.4%; for dehulled, 0.5-1.1%; for cold-soaked, 0.5-

Table 8.--Sucrose Content of Raw and Processed Cowpeas (% dry weight).**

	Processing Methods			
Cultivar	Raw	Dehulling	Cold-soaking	Hot-soaking
IT-81D-1137	0.8	0.6	0.6	1.0
IT-81D-994	1.0	0.9	0.8	0.9
IT-81D-1096	0.8	0.6	0.7	0.8
IT-82D-716	0.7	0.6	0.7	0.7
IT-82D-789	1.0	0.8	0.9	0.9
IT-82E-9	1.4	1.1	1.3	1.5
IT-82E-16	0.9	0.8	0.9	1.0
IT-83D-442	0.9	0.8	0.8	1.0
TVX-3236-01G	1.2	0.7	0.8	1.2
TVX-4659-03E	0.7	0.5	0.7	0.8
NIGERIA A104	1.0	0.9	0.9	0.9
IT-3	0.8	0.6	0.7	0.8
NIGERIA B7	0.8	0.5	0.5	0.6
Kano 1696	1.0	0.9	1.0	1.0
Ife Brown	1.0	0.7	0.9	1.0
Mean*	9.0^{a}	0.7^{b}	$0.8^{a,b}$	0.9^{a}
S.D	$\pm$0.19	$\pm$0.17	$\pm$0.19	$\pm$0.21

*Mean bearing different superscript differ significantly at 5% level.

**Each value is an average of two determinations differing by less than 4%.

1.3%; and for hot-soaked, 0.7-1.5%. The effect of processing, including cooking, on the oligosaccharides of four of the cultivars studied is shown in Fig. 10. Of all the processing methods, dehulling and cold-soaking reduced the sucrose content. However, this reduction was only significant ($p \leq 0.05$) with dehulling. This observation is probably an indication that the testa of cowpeas contain an appreciable amount of sucrose.

The result of this study agrees with the findings of Onigbinde and Akinyele (1983) who also observed a significant reduction in sucrose with dehulling. These authors also reported about 100% increase in sucrose with cooking.

Tittiranonda (1984) observed a higher decrease of sucrose in kidney beans with cold-soaking, than with hot-soaking. This is in agreement with the result of this study.

Liu (1986) observed that steaming increased the sucrose content of soybeans only in immature seeds. He also reported a combination of soaking and cooking to cause the highest decrease in sucrose content. Such an observation was not made in this study with moin-moin, the cowpea dish in which the preparation involves soaking, dehulling, and cooking. The combined effect of soaking, dehulling, and cooking may not be additive.

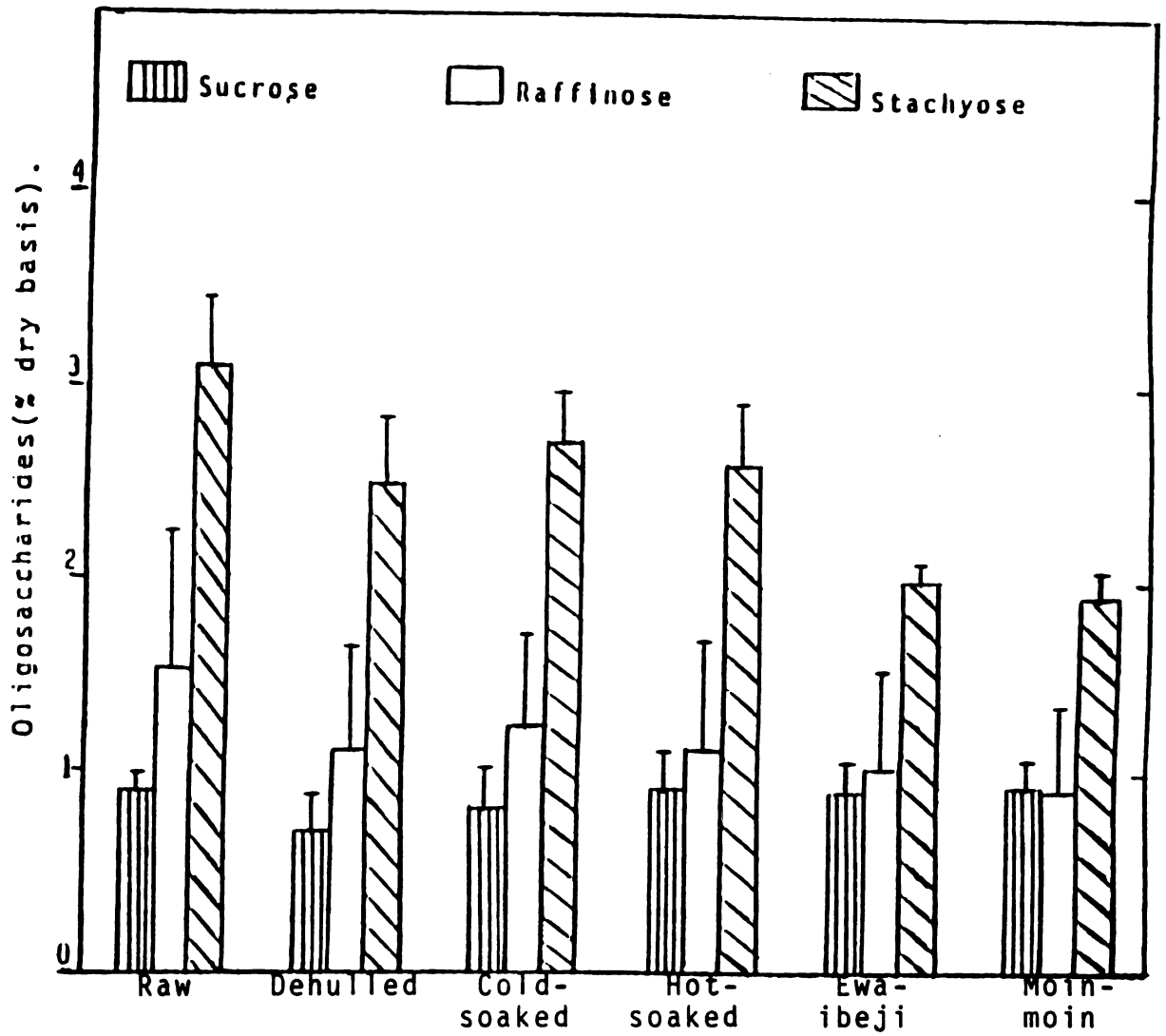


Fig. 10. Effect of Processing on the Oligosaccharides of cowpeas(average for four cultivars).

Effect of Processing on Raffinose

The raffinose content of raw and processed cowpeas is given in Table 9. The ranges are: for raw cowpeas, 0.7-2.4%; for dehulled, 0.6-1.8%; for cold-soaked, 0.7-2.0%; and for hot-soaked, 0.6-1.8%. Fig. 10 shows the effect of processing, including cooking, on the oligosaccharides of four of the cultivars.

All of the processing methods slightly reduced the raffinose in all cultivars studied, but this reduction was not significant ($p \geq 0.05$). A 20% decrease in the mean raffinose content observed with dehulling is slightly lower than the 30% decrease reported by Onigbinde and Akinyele (1983). These authors observed a 50% decrease with cooking while a 40% decrease was observed in this study. However, since they did not report any statistical testing, it is difficult to conclude whether the decrease they observed was significant or not. Liu (1986) observed that a combination of soaking and cooking caused the largest reduction in the raffinose content of soybeans. Nonsignificant decrease in the raffinose content of navy beans and kidney beans subjected to cold- and hot-soaking was also reported by Tittiranonda (1984).

Table 9.--Raffinose Content of Raw and Processed Cowpeas (% Dry Weight).**

	Processing Methods			
Cultivar	Raw	Dehulling	Cold-soaking	Hot-soaking
IT-81D-1137	1.9	1.5	1.5	1.4
IT-81D-994	1.3	1.0	1.1	1.1
IT-81D-1096	1.1	0.9	1.1	0.9
IT-82D-716	0.8	0.7	0.7	0.7
IT-82D-789	2.3	1.7	1.9	1.5
IT-82E-9	2.0	1.2	1.7	1.2
IT-82E-16	1.6	1.4	1.5	1.3
IT-83D-442	0.7	0.6	0.7	0.6
TVX-3236-01G	2.4	1.7	2.0	1.8
TVX-4659-03E	2.0	1.6	1.8	1.5
NIGERIA A104	1.1	0.8	1.0	0.9
IT-3	0.9	0.6	0.8	0.6
NIGERIA B7	1.4	0.8	0.8	0.7
Kano 1696	1.1	0.8	0.8	0.7
Ife Brown	2.1	1.8	1.8	1.8
Mean*	1.5^{a}	1.2^{a}	1.3^{a}	1.2^{a}
S.D	+0.60	$\pm$0.43	$\pm$0.46	$\pm$0.39

*Means bearing the same superscript do not differ significantly at p = 5%.

**Each value is an average of two determinations differing by less than 4%.

Effect of Processing on Stachyose

The stachyose content of raw and processed cowpeas is given in Table 10. The ranges are: for raw cowpeas, 2.3-3.7%; for dehulled, 2.0-3.1%; for cold-soaked, 2.2-3.3% and for hot-soaked, 1.5-2.8%. The effect of the processing methods, including cooking, on four cultivars is shown in Fig. 10. Dehulling, hot-soaking, and cooking significantly decreased stachyose content. The level of stachyose in cooked cowpeas was also significantly lower than the level in dehulled cowpeas. These findings are in agreement with the results reported by Onigbinde and Akinyele (1983). The decrease in the concentration of stachyose with cooking may be due to heat hydrolysis of this oligosaccharde probably to simple monosaccharides and other compounds. The testa probably contain an appreciable amount of stachyose, hence the removal of the testa during dehulling resulted in significant decrease.

Liu (1986) reported the greatest decrease for stachyose in soybeans with a combination of soaking and cooking. Tittiranonda (1984) observed that soaking did not produce any significant effect on the stachyose of navy and kidney beans.

Very little information exists in the literature on the oligosaccharides of dehulled, soaked and cooked cowpeas. A comparison of the mean concentration of

Table 10.--Stachyose Content of Raw and Processed Cowpeas (% Dry Weight).**

		Processing Methods		
Cultivar	Raw	Dehulling	Cold-soaking	Hot-soaking
IT-81D-1137	2.3	2.1	2.2	1.5
IT-81D-994	3.7	3.1	3.3	2.5
IT-81D-1096	2.9	2.2	2.7	2.3
IT-82D-716	3.0	2.2	2.8	2.3
IT-82D-789	2.7	2.0	2.5	2.1
IT-82E-9	3.1	2.8	2.9	2.4
IT-82E-16	2.7	2.2	2.6	2.2
IT-83D-442	2.7	2.4	2.5	2.2
TVX-3236-01G	2.8	2.2	2.7	2.3
TVX-4659-03E	3.4	2.4	2.9	2.5
NIGERIA A104	3.3	2.7	2.9	2.8
IT-3	3.5	2.5	3.0	2.8
NIGERIA B7	2.6	2.1	2.4	2.4
Kano 1696	3.1	2.9	2.9	2.8
Ife Brown	3.2	2.4	2.6	2.5
Mean*	2.9^{a}	2.4^{b}	2.7^{a}	2.4^{b}
S.D	$\pm$0.41	$\pm$0.33	$\pm$0.27	$\pm$0.33

*Means bearing different superscript differ significantly at p = 5%.

**Each value is an average of two determinations differing by less than 4%.

sucrose, raffinose, and stachyose content of raw cowpeas with earlier studies showed that the values compared favorably with those of Onigbinde and Akinyele (1983) with a difference of less than 14% for all the three sugars. However, the values differ from those reported by Longe (1980) and the sucrose content differs from those reported by Akpapunam and Markakis (1979).

On the effect of processing, Bianchi and Silva (1983) reported that soaking promoted no reduction in oligosaccharide contents regardless of the soaking time (3, 6, 12, 18, and 24 hrs). In contrast, Lo et al. (1968) found that as soaking time increased, large quantities of water-soluble solids leached into the soak water, including oligosaccharides.

A suitable comparison cannot be made based on these divergent results because of the different varieties of legumes and different processing conditions used in each study.

Since appreciable amounts of stachyose and raffinose were still detected in the cowpea dishes, the result of this study showed that cooking alone may not be enough to totally eliminate flatulent factors in cowpeas.

Phytic Acid

Phytic Acid in Cowpeas

Data on the phytic acid content of raw and processed cowpeas are presented in Table 11.

The mean phytic acid content for raw cowpea is 1.3% on dry weight basis with values ranging from 1.0% in 1T-82E-9 and Kano 1696 to 1.5% in 1T-81D-1096. These values agree with those reported for some cowpea cultivars by Ologhobo and Fetuga (1984).

Results obtained indicate that dry cowpea seeds are fairly rich in phytic acid, the principal source of phosphorus in many seeds. These results, in agreement with other findings, show that phytic acid is a characteristic and abundant constituent of legume seeds.

Liu (1986) reported a mean phytic acid value of 1.4% for soybeans while Ologbobo and Fetuga (1984) reported 1.5% and 0.9% for soybeans and lima beans, respectively.

The values obtained for cowpeas in this study appear to be higher than those reported for black beans (Lolas and Markakis, 1975); chickpeas, cowpea, greengram (Kumar et al., 1978); peas and broad beans (Gad et al., 1982). All these results suggest that if unprocessed, the nutritive value of the raw seeds may be impaired to a great extent since phytic acid is capable of forming

Table 11.--Phytic Acid Content of Raw and Processed Cowpeas (% Dry Weight).**

	Processing Methods			
Cultivar	Raw	Dehulling	Cold-soaking	Hot-soaking
IT-81D-1137	1.2	1.3	1.2	1.2
IT-81D-994	1.3	1.4	1.1	1.2
IT-81D-1096	1.5	1.5	1.4	1.2
IT-82D-716	1.3	1.3	1.3	1.3
IT-82D-789	1.4	1.4	1.3	1.2
IT-82E-9	1.0	1.0	1.0	1.0
IT-82E-16	1.2	1.2	1.2	1.2
IT-83D-442	1.2	1.3	1.2	1.1
TVX-3236-01G	1.4	1.4	1.3	1.2
TVX-4659-03E	1.2	1.2	1.2	1.2
NIGERIA A104	1.3	1.3	1.3	1.3
IT-3	1.1	1.2	1.1	1.0
NIGERIA B7	1.3	1.3	1.2	1.2
Kano 1696	1.0	1.0	1.0	1.0
Ife Brown	1.4	1.4	1.3	1.3
Mean*	1.3^{a}	1.3^{a}	1.2^{b}	1.2^{b}
S.D	$\pm$0.15	$\pm$0.14	$\pm$0.12	$\pm$0.11

*Means bearing different superscript differ significantly at p = 5%.

**Each value is an average of two determinations differing by less than 4%.

complexes with di- and trivalent cations thereby making them unavailable.

Effect of Processing on Phytic Acid

Fig. 11 shows the effect of dehulling, cold-soaking, hot-soaking, and cooking on phytic acid in four cowpea cultivars.

Dehulling did not have any effect on the phytic acid content (Table 11). This may be due to the fact that this substance is not contained in any appreciable amount in the testa of cowpeas. No information is available in the literature on the effect of dehulling on the phytic acid content of cowpeas. However, Deshpande et al. (1982) reported that dehulling significantly increased the phytic acid content of beans (Phaseolus vulgaris L.). These authors explained this increase by assuming that dehulling improves the extraction efficiency in determining the phytic acid content of beans. The results of this study did not agree with their findings. This difference could be attributed to the differences in legume species studied or in the method of analysis.

Soaking either in cold water or hot water significantly reduced the phytic acid content of cowpeas (Table 11). The mean value for soaked samples is 1.2%. Ologhobo and Fetuga (1984) also reported a significant

reduction with soaking. However, they reported a higher decrease (20-28%) than the 8% reported in this study for 15 cultivars. This difference may be due to the fact that the authors soaked their cowpea seeds for 3 days, as opposed to the 12-hour soaking time used in this study. The effect of soaking may, therefore, be time-dependent. Both cold-soaking and hot-soaking had similar effect on the phytic acid content. Ologhobo and Fetuga (1984) also observed similar reduction with soaking for the phytic acid content of lima beans and soybeans. However, Liu (1986) reported a very slight, but nonsignificant, increase in the phytic acid content of soaked Pella and Beeson 80 soybeans. Sudarmadji and Markakis (1977) have reported that overnight soaking did not reduce the phytic acid content of soybeans.

Upon cooking, phytic acid was reduced by about 17% in four cultivars (Fig. 11). However, this decrease was not significant at the 5% probability level. A decrease of 10-13%, 7.5-8.5%, and 9.2-13.2% was reported by Ologhobo and Fetuga (1984) for cowpeas, lima beans, and soybeans, respectively. Sudarmadji and Markakis (1972) reported a 14% reduction with boiling for soybeans. Cooking or steaming in boiling water for 20 mins had a slight decreasing effect on the phytic acid content of soybeans (Liu, 1986). All these results are in agreement with the findings in this study.

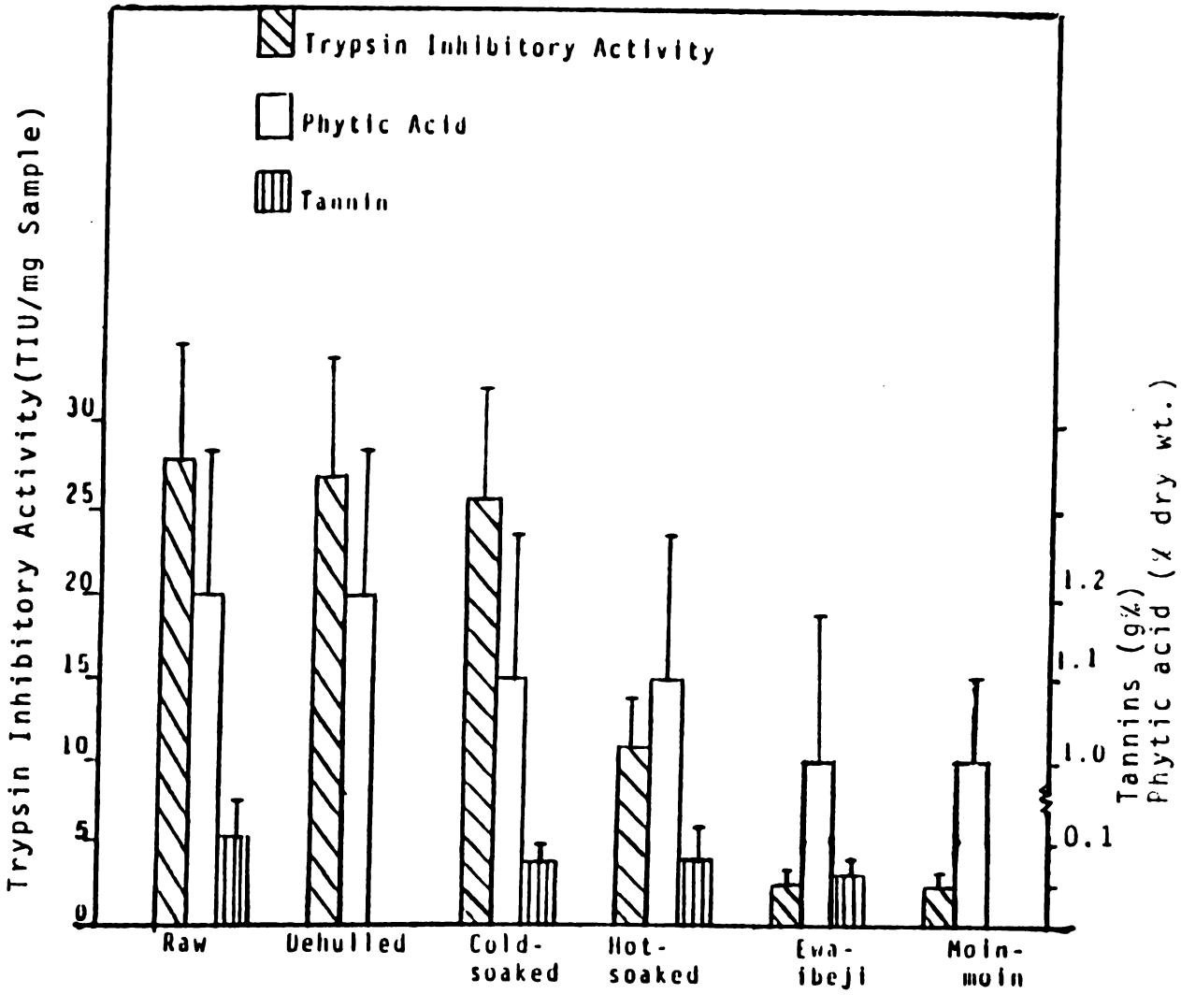


Fig. 11. Effect of Processing on certain Antinutrients of cowpeas(average for four cultivars).

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None of the processing methods considered in this study is able to completely eliminate phytic acid from cowpeas or even to decrease it by up to 50%. Several reports regarding the effect of various processing treatments on phytate content have appeared in the literature. Ologhobo and Fetuga (1984) reported that germination was very effective in lowering phytic acid. The advantages of decreased phytate content through germination are not likely to be counteracted by increased fiber content in sprouts (Aman, 1976). So, cooking of sprouted cowpeas may be encouraged.

Fardiaz and Markakis (1981) reported that the phytic acid content of peanut press cake fermented with Rhizopus oligosporus was reduced from 1.3% to 0.05% after 72 hr of fermentation. This decrease was attributed to the activity of phytase released by the mold. Sudarmadji and Markakis (1977) also demonstrated that R. oligosprus used in tempeh fermentation has strong phytase activity.

Tannins

Tannins in Cowpeas

The tannin content of cowpeas are presented as catechin equivalents in Table 12. The mean tannin content for raw cowpeas is 0.53% on dry weight basis and it agrees with a mean value of 0.56% reported by Ologhobo and Fetuga (1984). The cultivar had a tremendous

Table 12.--Tannin Content (Catechin Equivalent, g%) of Raw and Processed Cowpeas.**

	Processing Methods			
Cultivar	Raw	Dehulling	Cold-soaking	Hot-soaking
IT-81D-1137	0.06	0	0	0.02
IT-81D-994	0.04	0	0	0.03
IT-81D-1096	0.18	0	0.13	0.13
IT-82D-716	0.48	0.01	0.01	0.06
IT-82D-789	0.60	0	0.51	0.48
IT-82E-9	2.62	0	1.50	1.67
IT-82E-16	1.31	0.11	0.12	0.18
IT-83D-442	0.74	0	0.53	0.66
TVX-3236-01G	0.05	0	0.02	0.03
TVX-4659-03E	0.35	0.01	0.31	0.32
NIGERIA A104	0.04	0	0	0.02
IT-3	0.08	0	0.06	0.07
NIGERIA B7	1.13	0	0.08	0.09
Kano 1696	0.05	0	0.03	0.04
Ife Brown	0.15	0	0.09	0.11
Mean*	0.53^{a}	0.01^{b}	0.29^{a}	0.33^{a}
S.D	$\pm$0.71	$\pm$0.001	$\pm$0.45	$\pm$0.49

*Means bearing different superscript differ significantly at p = 5%.

**Each value is an average of two determinations differing by less than 4%.

Table 13.--Comparison of Seed Color and Catechin Equivalent Values of Cowpeas.

Color of Seed	Catechin Equivalent Value (g%)
White	0.06
White	0.04
Brown	0.18
Brown with White	0.48
Brown	0.60
Black	2.62
Brown	1.31
Brown	0.74
Brown with White	0.05
Brown with White	0.35
White	0.04
White	0.08
Brown	1.13
White	0.05
Brown	0.15

influence on the tannin content of the cowpeas. This could be seen in the very wide range from 0.04% in 1T-81D-994 to 2.62% in 1T-82E-9. A comparison of seed color to tannin levels showed the color of the skin to be related to tannin content (Table 13). Cowpeas with white skin color had tannin levels of 0.06% or less while cultivars with brown skin color had tannins ranging from 0.15% to 1.31%. This wide range may be due to varying degree of darkness among the brown cultivars. The cultivar with the black skin color (1T-82E-9) had the highest tannin level of 2.62%.

Rao and Deosthale (1982) reported a mean tannin level of 0.96% for pigeonpea, 0.61% for greengram, 0.18% for chickpea, and 0.86% for blackgram, while Ologhobo and Fetuga (1982) reported mean tannin levels of 0.77% for lima bean.

An agreement with the observation in this study is the report by Tan et al. (1983) that revealed a 25-fold variation in the tannin content (0.03 - 0.75%) of winged beans. All of these authors observed a correlation between seed color and tannin content.

The minimum amount of dietary tannin needed to elicit a negative growth response has not been established. It is still unclear what level of tannin would be noticeably harmful (National Academy of Sciences, 1973). However, from the contentions of Chang

and Fuller (1964) that tannins in plants do not affect their nutritional potentials unless at very high levels, often 10% or more of the dry weight, the highest level obtained in this study in cultivar 1T-8E2-9 (2.62%) may not be very harmful.

Effect of Processing on Tannins

The effect of processing on the tannin content of cowpeas are presented in Table 12 and Fig. 11.

Dehulling significantly reduced the tannin content by an average of 98%. The reduction was 100% in some of the cultivars. This observation confirms that tannins are predominantly located in the pericarp and/or testa of legumes.

Cold-soaking, hot-soaking, and cooking as ewa-ibeji decreased tannin content by 45%, 38%, and 40%, respectively. However, these decreases were not significant at the 5% probability level. When cowpeas were cooked as moin-moin, tannin was completely eliminated. This total elimination, as opposed to the 40% decrease observed in ewa-ibeji was probably due to the dehulling of cowpeas involved in the preparation of moin-moin.

Several reports which agree with the findings in this study appear in the literature on the effect of processing on the tannin content of legumes. Deshpande

et al. (1982) reported a 68-95% reduction with dehulling for dry beans (Phaseolus vulgaris, L.) while Tan et al. (1983) reported 83-97% loss of tannin with the decortication of four different pulses. As a result of overnight soaking, they observed that 50% of tannin was lost in pigeonpea and chickpea while in blackgram and greengram, the loss was 25%. Cooking of the raw pulses brought a 70% decrease in their tannin content, a reduction higher than that observed in this study. Ologhobo and Fetuga (1984) however, reported that cooking reduced the tannin content of ten cowpea varieties by 31.0 - 47.3%.

The loss of tannins during soaking may be attributed to the leaching of a small fraction of hydrolyzable phenolic compounds located in the seed coats of cowpea varieties (Elias and Bressani, 1979) into the soaking medium. Some amounts of polyphenols have been found in the soaking and cooking waters of Phaseolus vulgaris, indicating that large amounts of polyphenols could be eliminated by discarding washing, soaking, and cooking waters (Fukuda Suzuki, 1978).

Trypsin Inhibitory Activity

Trypsin Inhibitory Activity in Cowpeas

The trypsin inhibitory activity in raw and processed cowpeas is given in Table 14. The mean trypsin

Table 14.--Trypsin Inhibitory Activity (TUI/mg sample) in Raw and Processed Cowpeas**

	Processing Methods			
Cultivar	Raw	Dehulling	Cold-soaking	Hot-soaking
IT-81D-1137	27.0	26.1	25.9	10.2
IT-81D-994	25.6	23.3	23.3	7.0
IT-81D-1096	15.1	13.4	12.5	4.5
IT-82D-716	20.6	19.2	18.2	5.8
IT-82D-789	23.1	19.8	21.4	4.8
IT-82E-9	15.4	13.6	14.5	3.8
IT-82E-16	30.5	26.1	26.0	6.3
IT-83D-442	20.7	19.5	19.1	4.8
TVX-3236-01G	25.0	23.1	22.4	6.5
TVX-4659-03E	17.5	14.8	14.5	9.9
NIGERIA A104	24.8	24.8	24.0	7.7
IT-3	31.8	31.2	30.2	15.5
NIGERIA B7	26.8	26.7	26.7	9.9
Kano 1696	35.3	33.0	30.6	9.5
Ife Brown	16.5	15.6	15.3	7.9
Mean*	23.7^{a}	22.0^{a}	21.7^{a}	7.6^{b}
S.D	$\pm$6.13	$\pm$6.12	$\pm$5.67	$\pm$3.04

*Means bearing different superscript differ significantly at p = 5%.

**Each value is an average of two determinations differing by less than 4%.

inhibitory activity in raw cowpeas is 23.7 TUI/mg sample with values ranging from 15.1 to 35.3 in the fifteen cultivars studied. The mean value observed was in agreement with that reported by Ologhobo and Fetuga (1984) for ten cultivars of cowpeas.

The TI activity in the cowpea cultivar studied appears to be higher than the values of 15.5 and 18.5 TUI/mg sample reported for navy beans and chickpeas, respectively (Kakade et al., 1969), but lower than the mean values of 32.6 for lima beans (Ologhobo and Fetuga, 1983), 64.3 TUI/mg for Beeson 80 soybeans (Liu, 1986), and of 37.5 and 36.4 TUI/mg reported for protein isolates from soybean and lima bean, respectively (Jaffe, 1950).

The values reported for cowpeas in this study would tend to suggest that the nutritive value of the raw seeds would be impaired to a great extent.

Effect of Processing on Trypsin Inhibitory Activity (TIA)

The effect of dehulling, soaking, and cooking on the trypsin inhibitory activity in cowpea is presented in Table 14 for fifteen cultivars and Fig. 11 for four cultivars.

Dehulling and cold-soaking slightly decreased activity by 7.2% and 8.4%, respectively. These decreases are not significant at 5% level. However, hot-soaking

significantly reduced the activity of trypsin inhibitors by about 68%. Cooking also significantly reduced activity by 91%. A comparison of the effect on trypsin inhibitory activity of hot-soaking and cooking showed a significant difference. The results obtained with dehulling and soaking appear to indicate that trypsin inhibitors are not present in the testa of cowpeas to any appreciable level, and they are not leached into soak water.

Being proteins, the trypsin inhibitors were probably inactivated by the heat treatment involved in hot-soaking. However, heat treatment greater than the two-minute boiling in hot-soaking was necessary to further reduce the activity of the inhibitors. This is confirmed by the 91% reduction in activity reported for cooking. The level of the trypsin inhibitory activity in the two cowpea dishes are similar, indicating that dehulling as was done in preparing moin-moin played no significant role.

Ologhobo and Fetuga (1983) reported a mean total TIA decrease of 34.6% for lima bean after six days of soaking. This long-soaking period is not practiced in common household preparation of legumes. Liu (1986) reported a decrease in the trypsin inhibitory activity from 64.3 TUI/mg to 64.0 TUI/mg for soaking, to 31.1

TUI/mg for steaming, and to 9.2 TUI/mg for cooking in soybeans. Total elimination of the trypsin inhibitors was observed only with combined steaming and cooking.

A total elimination of TI by cooking alone has been reported by Ologhobo and Fetuga (1983, 1984) for lima bean and cowpeas, respectively.

Heat treatments are commonly used to inactivate the protease inhibitors of legumes (Liener, 1981). Striking differences were noticed on the effect of soaking on the reduction of TIA among various legumes (Al-Bakir et al., 1982). These differences may be due to differences in chemical composition and seed coat texture of the legumes.

Digestibility of Carbohydrates

Measurement of the hydrogen (H_2) content in breath samples provides an index of carbohydrate malabsorption. The breath H_2 response of one subject to all the test meals administered in this study is presented in Fig. 12.

Basal Breath Hydrogen

The basal breath H_2 concentration in all subjects after eating a self-selected evening meal and without food after 10 p.m. on days preceding test days varied between 1.0 and 16.0 ppm. Kotler et al. (1982) reported a wider range of 1.6 to 40.0 ppm in a similar study of

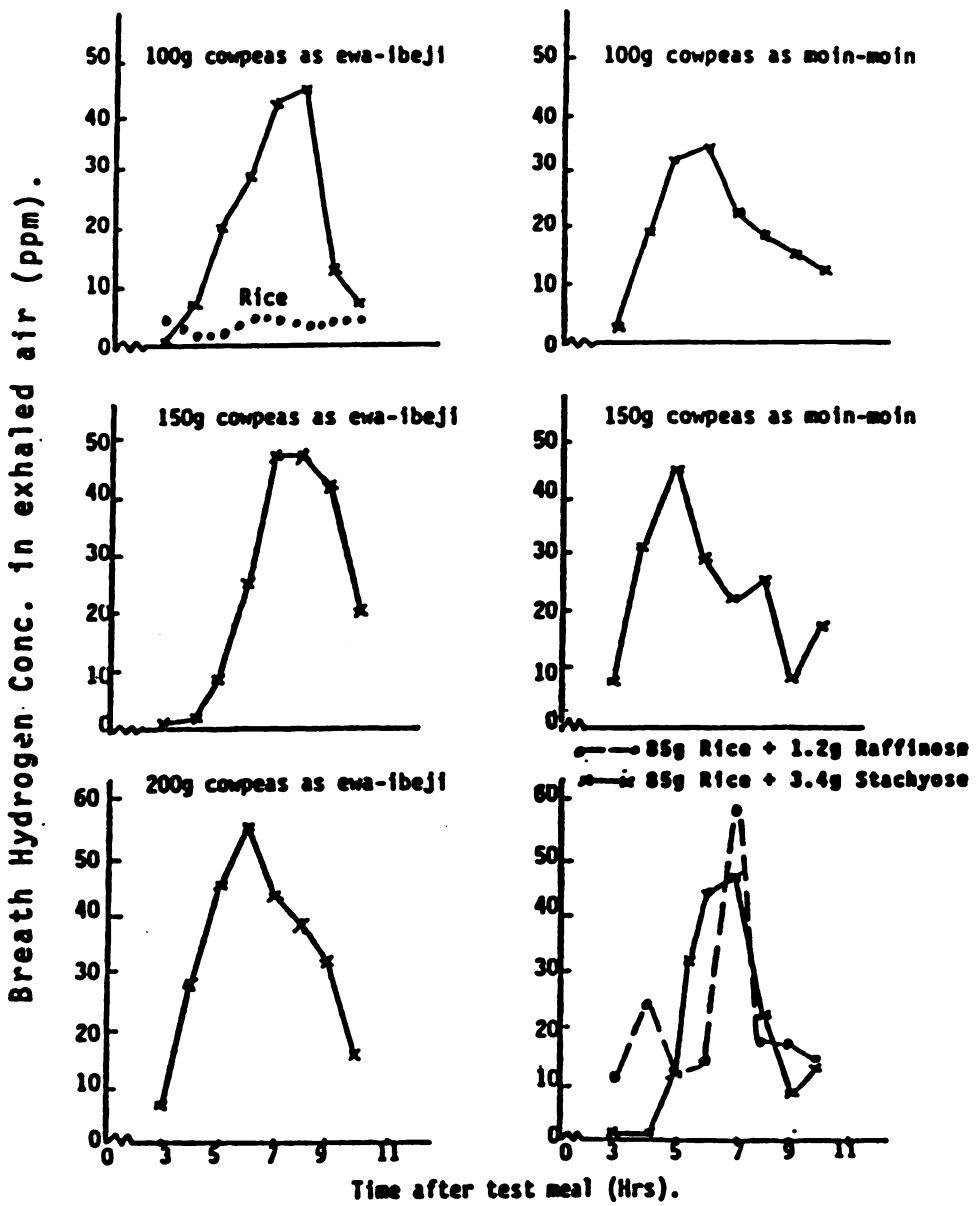

Fig. 12. Breath Hydrogen Response of one subject to all test meals.

twelve subjects eating self-selected evening meals. These variable elevations in basal breath H_2 concentration after an unrestricted dinner supports the suggestion that some degree of carbohydrate malabsorption may be common in people (Anderson et al., 1981).

A preliminary study revealed that less than 20% of the total cumulative breath H_2 was produced during the first three hours after ingestion of test meals, therefore collection of breath samples in this study was limited to the third through the tenth hour after meal ingestion. This observation is in agreement with that of Kotler et al. (1982) who reported that 10.6% or less was produced during the first two hours following ingestion of lactulose. Elevated basal breath H_2 levels have been identified as a pitfall to breath H_2 tests because they make it difficult to attribute subsequent changes in H_2 concentration after a carbohydrate test dose as due totally to the dose (Payne et al., 1983). In this study, a cowpea-free meal of rice was incorporated to establish baseline production levels of breath H_2 for the subjects. This will also enable the increase in breath H_2 concentration after cowpea foods to be obvious and attributable only to the cowpeas ingested.

Breath H_2 Response to
Cowpea-Free Food

Breath H_2 recovery following ingestion of all test meals is presented in Table 15. The mean cumulative breath H_2 production after the rice meal was 1464 ppm x min. Throughout the study, concentration of breath H_2 after the test meal of rice did not exceed 7.0 ppm, except in one subject in which 14.0 ppm was recorded during the seventh hour after the ingestion of rice. This sudden elevation was not considered a problem because it dropped down again in about 30 minutes. For all subsequent tests, significant H_2 excretion was defined as cumulative H_2 excretion greater than two standard deviations above the mean cumulative H_2 excretion after cowpea-free meal. Calloway et al. (1971) also observed an unusually high breath H_2 response in one of their subjects to a bland baseline formula. No explanation was given for this phenomenon. However, a study of the microflora of the subjects and of their tolerance of the various components of the formulas might prove enlightening.

Breath H_2 Response to
Cowpea Foods

The breath H_2 excreted after all the cowpea test meals administered in this study was significantly higher

TABLE 15.--Breath H_2 Recovery Over Different Time Intervals

Meal	N[a]	Cumulative H_2 Conc. (PPM x min) $\bar{M} \pm SD$	% Increase Over the Baseline	3rd-6th hr[b] %	6th-9th hr[b] %
85g rice (baseline)	5	1464 ± 578	--	33	50
100g cowpeas as ewa-ibeji	5	8871 ± 2079	505	27	55
150g cowpeas as ewa-ibeji	5	10707 ± 1807	631	31	52
200g cowpeas as ewa-ibeji	2	14722 ± 1007	905	35	51
100g cowpeas as moin-moin	5	6813 ± 1770	365	37	49
150g cowpeas as moin-moin	5	7383 ± 2775	404	32	48
85g rice + 1.2g raffinose	5	5966 ± 2077	307	20	55
85g rice + 3.4g stachyose	2	7079 ± 1806	383	29	54

[a]Number of subjects.

[b]Data listed as percent of 7-hour cumulative H_2 excretion.

(i.e., $\geq$ 2 S.D.) than that excreted after a control meal of rice. This increase ranged from 365% in 100g cowpeas prepared as moin-moin to 631% in 150g cowpeas prepared as ewa-ibeji. This observation probably indicates that cowpeas contain certain components, absent in rice, which are not digestible and are responsible for the elevated breath H_2. Bond and Levitt (1976) have observed that when unabsorbed carbohydrates reach the colon, bacterial fermentation produces H_2 gas as one of the resulting products. This H_2 is absorbed through the intestinal mucosa, passes into the blood stream as dissolved gas, and is excreted in the expired air. The excess H_2 (i.e., breath H_2 concentration greater than the baseline) was excreted for about nine hours after the test meals were ingested. Maximum excretion of breath hydrogen, however, occurred between the fifth and ninth hour after meal ingestion. Even at the tenth hour after a test meal, the H_2 concentration in the exhaled air was still higher than that of the control rice meal. The exhaled H_2 concentration between the sixth and ninth hour after ingesting test meals accounted for about half of the total H_2 exhaled. The concentration during this period ranged 48 to 55% of the total (Table 15). In nearly all cases, a

gradual increase in breath H_2 excretion was observed during the first few hours, followed by a slight decrease before a later, higher rise in H_2 concentration.

There is no available data on flatus or breath H_2 production following ingestion of cowpea foods. Legumes, the family of plant foods to which cowpeas belong, have achieved notoriety as sources of flatus. They are known to be important sources of oligosaccharides whose alpha-galactosidic bond is not hydrolyzed in the upper intestinal tract due to the absence of alpha-galactosidase activity in the mammalian intestinal mucosa (Rackis, 1975).

Calloway et al. (1971) reported an increase in flatus and breath hydrogen production after test meals of soybeans, mung beans, navy beans, and their products.

The maximum H_2 excretion observed between the fifth and ninth hour after cowpea meal ingestion in this study was longer than the two-hour maximum production period reported by Murphy (1966) after a meal of cooked beans. Calloway et a al. (1971) also observed maximum gas production during the fourth and sixth hour after ingestion of cooked beans. Such difference is not unreasonable, considering the opportunities for variation in composition due to bean variety, maturity at harvest, storage conditions, and method of preparation. The result

of this study showed that cooking as a processing method is not enough to completely eliminate flatus factors in cowpeas.

Effect of Preparation Method on Breath H_2 Response

The cumulative breath H_2 production after ingestion of varying quantities of two different cowpea dishes is given in Table 16 while the response to these meals over a seven-hour period is graphically presented in Figs. 13 and 14.

Table 16.--Cumulative Breath H_2 Concentration After Ingestion of Varying Quantities of Two Different Cowpea Dishes (ppm x min)

Quantity	Cowpea Dishes	
	Ewa-ibeji $\bar{M} \pm$ S.D.	Moin-moin $\bar{M} \pm$ S.D.
100 g	8871 ± 2079	6813 ± 1770
150g	10707 ± 1807	7383 ± 2775

Both types of cowpea dishes resulted in breath H_2 production significantly higher than that produced after ingesting the control rice meal. The method of preparation slightly affected breath H_2 excretion. The concentration of H_2 in the air exhaled after ingesting moin-moin, the dehulled cowpea dish, was less than that produced after ingesting similar quantities of cowpeas

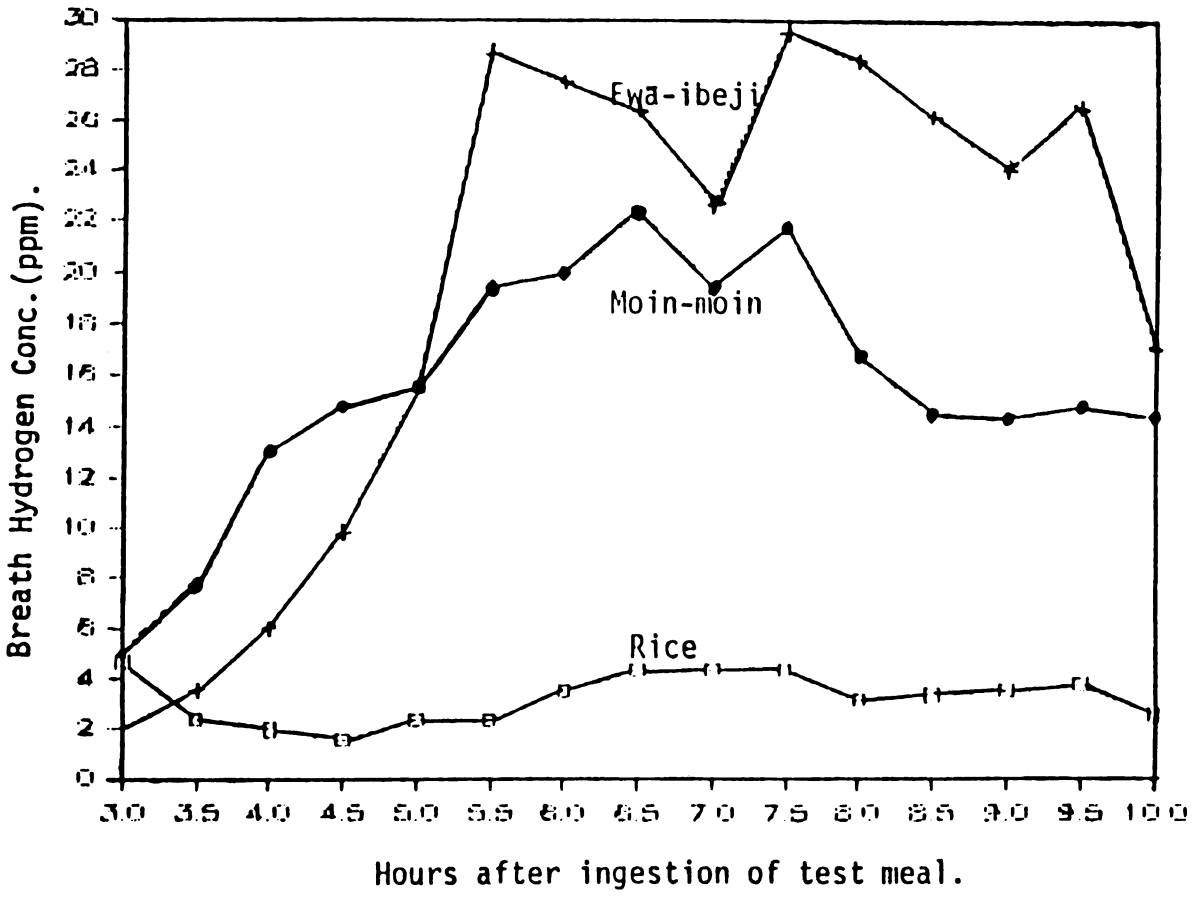

Fig. 13. Breath Hydrogen Response to ingestion of 100g cowpeas prepared as ewa-ibeji and moin-moin.

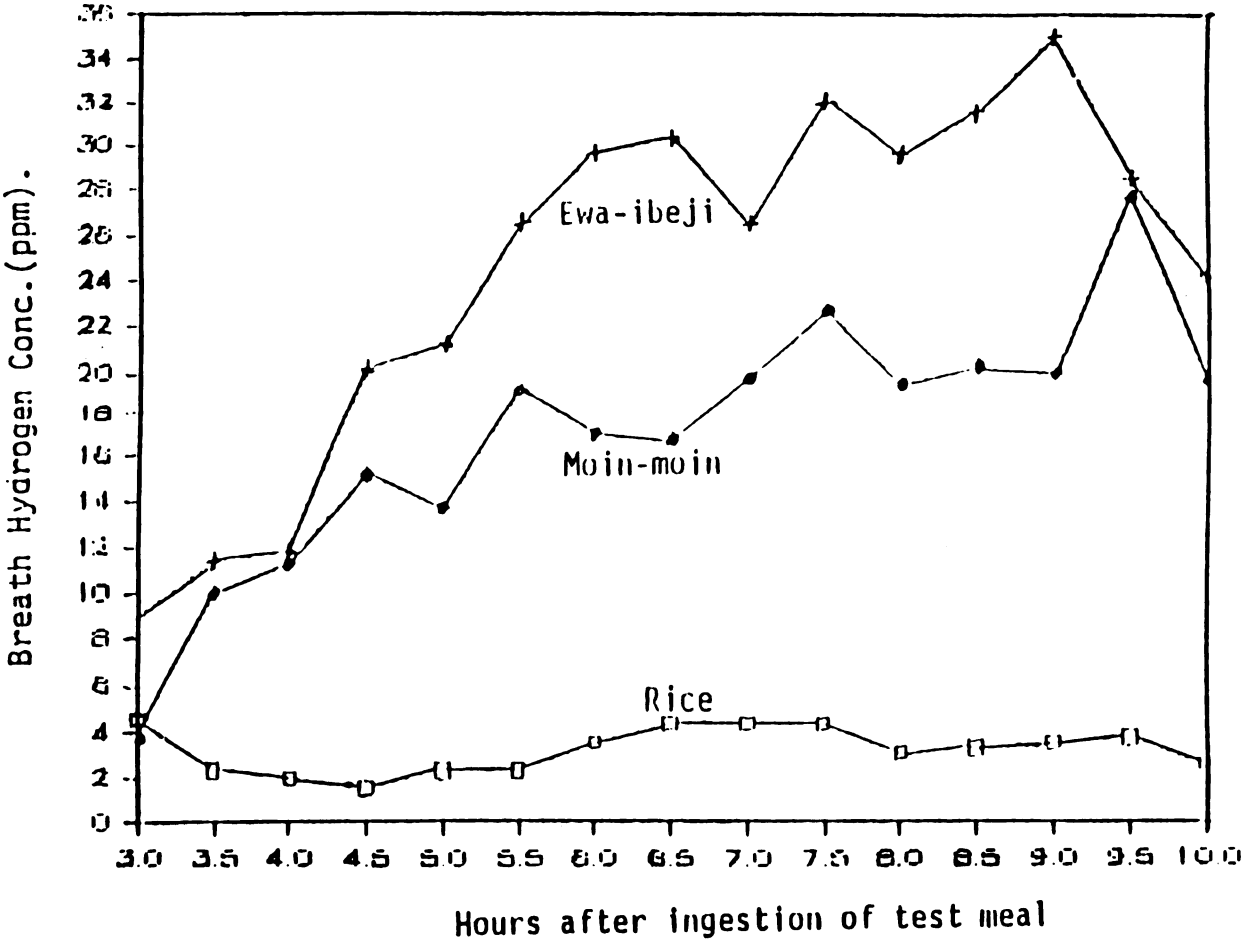

Fig. 14 Breath Hydrogen Response to ingestion of 150g cowpeas prepared as ewa-ibeji and moin-moin.

prepared whole as ewa-ibeji. This probably suggests that moin-moin is less conducive to intestinal gas production. However, the difference observed was not statistically significant at the 5% probability level. The dehulling process to which moin-moin was subjected, probably removed some flatus-causing component present in the testa of cowpeas. This removal of the testa which has earlier been shown to significantly reduce stachyose (Table 10) did not cause any significant reduction in the raffinose content of cowpeas (Table 9). There may also be the possibility of leaching during the soaking process that preceded dehulling in the preparation of moin-moin. All of these may be responsible for lesser breath H_2 production after eating moin-moin. The slightly greater amount of breath H_2 produced after eating whole cowpeas prepared as ewa-ibeji may also be attributed to the presence of polysaccharide fractions of the seed coat which are also fermentable products. However, all of these are not enough to cause significant differences between ewa-ibeji and moin-moin.

There is no available information in the literature on flatus or breath H_2 response to these cowpea dishes. The results obtained in this study showed that neither cooking as in ewa-ibeji, nor dehulling with boiling as in moin-moin was enough to eliminate flatus factors in cowpeas. This agrees with the findings of

Calloway et al. (1971) who observed significant flatus and breath H_2 values in their subjects after eating cooked legumes. They, however, observed that tempeh, made from soybean grits by mold fermentation, did not increase gas production over baseline values and caused a significant delay in the time of gas formation, suggestive of temporary suppression of intestinal bacteria. Soybean and mung bean sprouts appeared to retain most of the flatulent factors present in the whole bean. This does not agree with the findings of Alani (1987) who observed that germination reduced the oligosaccharides of cowpeas.

Effect of Dose (Quantity) on Breath H_2 response

The breath H_2 excretion after ingestion of different quantities (100g and 150g) of cowpeas is given in Table 16. The cumulative breath H_2 concentration after eating 100g cowpeas prepared as ewa-ibeji was 8871 ppm x min. This increased by about 20% to 10707 ppm x min when 150g cowpeas was eaten as ewa-ibeji. A similar nonlinear and lesser increase, was observed with moin-moin when the quantity eaten was increased from 100g to 150g.

The difference due to quantity eaten was not statistically significant at the 5% probability level. Apparently, intakes of flatulent compounds above a certain level does not cause proportional production of gas.

Increase in breath H_2 production was observed barely three hours after the ingestion of 150g cowpeas prepared as ewa-ibeji (Fig. 15). The increase, which was more gradual, came about 1.5 to 2.0 hours later for 100g. This trend was not observed for moin-moin where elevated breath H_2 production started at about the same time in both 100g and 150g (Fig. 16).

Results of this study are in agreement with those of Calloway et al. (1971) who also observed that intestinal gas response is not precisely dose-dependent. They observed an increase, but not a linear response, to doubling the soybean test dose in their study. The 200g dose of soybeans used caused an insignificant increase in breath H_2 and flatus volume as compared with the 100g meal. The lack of proportionality between the quantity of cowpeas consumed and the concentration of exhaled H_2 also agrees with the finding of Kotler et al. (1982).

The observed response in this study emphasizes the importance of accounting for intra- and interindividual variation when assaying for flatulent factors. It also suggests that the response to malabsorbed sugar in the colon may vary because of alteration in bacterial sugar metabolism, a finding with possible clinical implication. It is also possible that the flatulent factors in legumes has a threshold level above which increased quantity may not significantly elevate breath H_2 production.

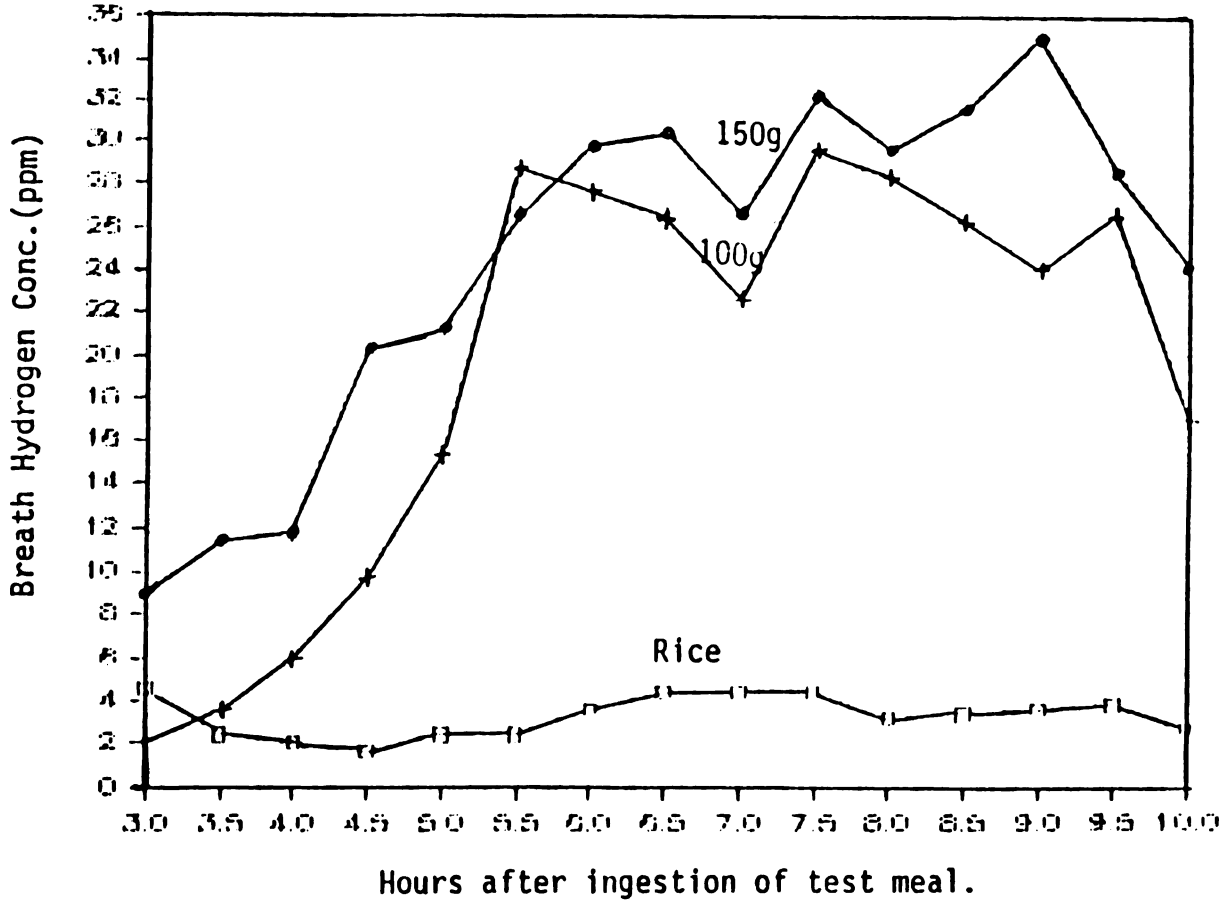

Fig. 15. Breath Hydrogen Response to ingestion of 100g and 150g cowpeas prepared as ewa-ibeji.

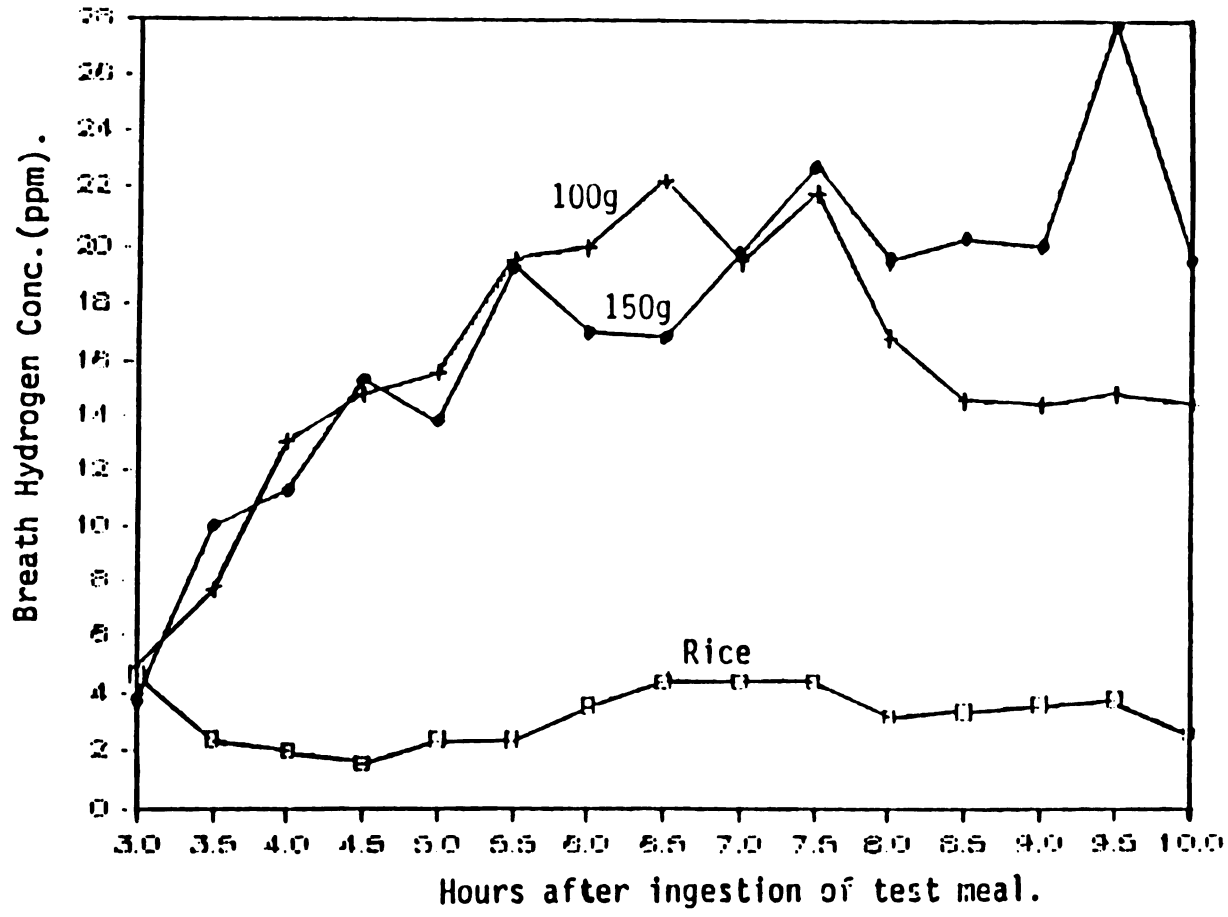

Fig. 16. Breath Hydrogen Response to ingestion of 100g and 150g cowpeas prepared as moin-moin.

Raffinose and Stachyose in Breath H_2 Production

Breath H_2 response to ingestion of rice with raffinose or stachyose added in amounts comparable to those found in 100g cowpeas is presented in Table 17. Elevated response in breath H_2 production came earlier with stachyose than with raffinose (Fig. 17).

Table 17. Cumulative Breath H_2 Response to Raffinose or Stachyose in Amounts similar to Those Found in 100g Cowpeas.*

Test Meal	Cumulative Breath H2 Conc. (PPM x min)
Rice	$1464^{a} \pm 578$
Rice + Raffinose	$5966^{b} \pm 2077$
Rice + Stachyose	$7079^{b,c} \pm 1806$
100g cowpeas as ewa-ibeji	$8871^{c} \pm 2079$
100g cowpea as moin-moin	$6813^{b,c} \pm 1770$

*Mean values + S.D. Values with different superscript differ significantly at 5% level.

The cumulative breath H_2 concentration of 1464 ppm x min following ingestion of rice increased significantly ($p < 0.05$) when 1.2g raffinose or 3.4g stachyose was added to the rice. Ingestion of rice + stachyose gave a breath H_2 response comparable to those found with 100g cowpeas prepared either as ewa-ibeji or as moin-moin. However,

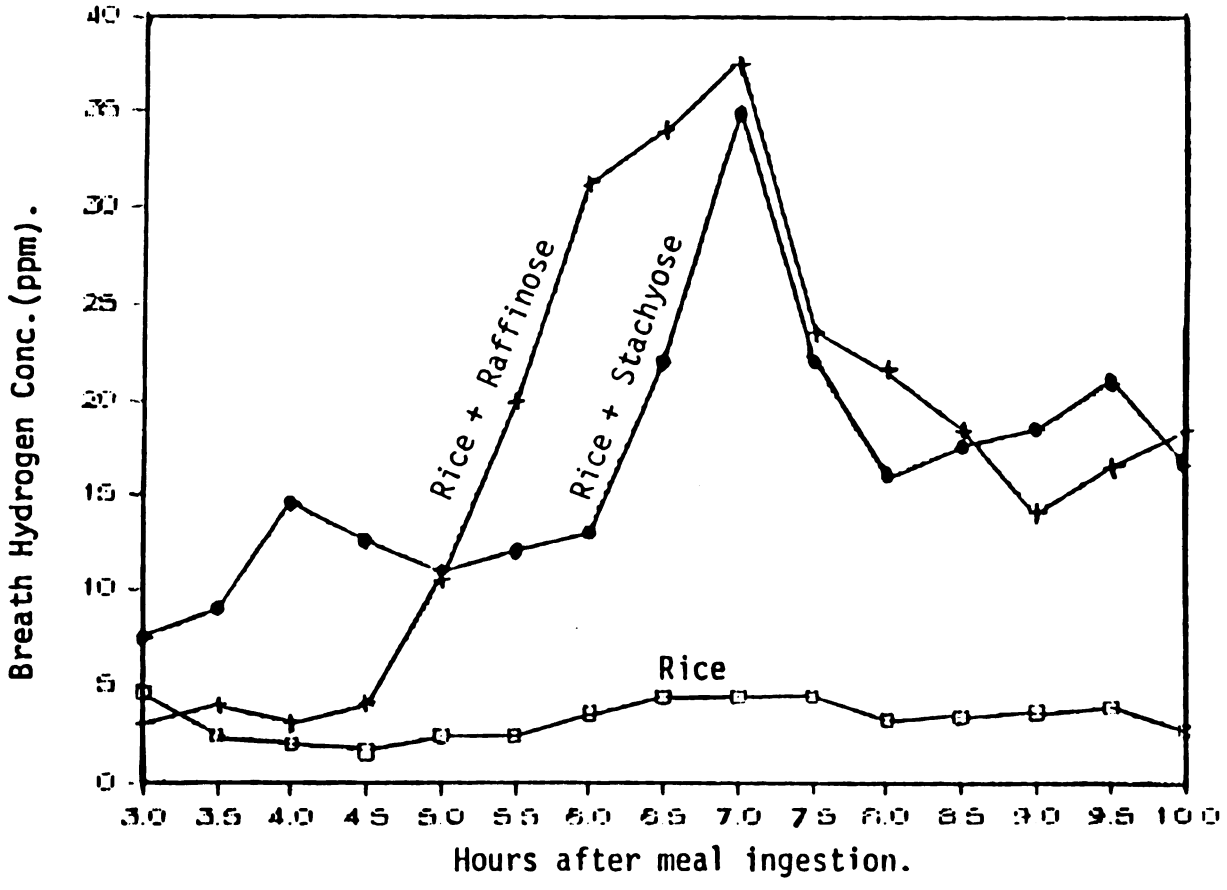

Fig. 17. Breath Hydrogen Response to ingestion of rice containing 1.2g raffinose or 3.4g stachyose.

rice + raffinose gave a response that was significantly lower ($p < 0.05$) than that found with ewa-ibeji.

This observation probably suggests that the tetrasaccharide, stachyose plays a greater role in intestinal gas production than the trisaccharide, raffinose. This is understandable since stachyose is also found in larger quantity than raffinose in cowpeas (Table 7). Therefore, the probable removal of stachyose with dehulling led to the lesser gas production observed with moin-moin. The significantly elevated production of breath H_2 following addition of raffinose or stachyose to rice suggests that these substances, probably among others, are responsible for the gas produced after eating cowpeas since they resulted in breath H_2 responses comparable to those found with cowpeas.

The indigestible and undigested carbohydrates of cowpeas, especially the low molecular weight oligosaccharides, have been associated with gas production and gastrointestinal discomfort experienced by cowpea consumers (Steggarda et al., 1966). Oligosaccharides of the raffinose family (raffinose, stachyose, and verbascose) predominate in most legumes and account for 31.3% to 76% of the total sugars (Akpapunam and Markakis, 1979; Onigbinde and Akinyele, 1983) in cowpeas.

Protein Quality Evaluation

A summary of the utilization of proteins from ewa-ibeji and moin-moin prepared from both Nigerian and American cultivars of cowpeas is showwn in Table 18.

Protein Efficiency Ratio (PER)

Casein diet had the highest PER which was significantly different from those of the other cowpea test diets. The low PER values of the two very popular cowpea dishes in Nigeria indicate the need for complimentary sources of protein in the diet of the people involved. Proteins from legumes are known to be deficient in sulfur-containing amino acids.

In both cowpea cultivars, the PER of moin-moin was higher than that of ewa-ibeji. This difference was statistically significant ($p < 0.05$) only in the American cultivar. Apparently, moin-moin has a slightly better quality protein than ewa-ibeji, a difference which may be due to the milder heat treatment of the former food, rather than to the removal of seed coats involved in its preparation. Ketiku and Ladoye (1984) observed that the absolute protein digestibility coefficient from rice and peeled beans mixture did not differ significantly from that obtained for rice and unpeeled beans mixture. This indicates that dehulling does not affect protein quality. Heat denaturation of cowpea proteins had been cited by

TABLE 18.--PER and % Digestibility (in vitro) of Ewa-ibeji and Moin-moin Perpared from American and Nigerian Cultivars of Cowpeas.

	Casein	Cowpea Foods			
		Ewa-ibeji		Moin-moin	
		Nigerian Cultivar	American Cultivar	Nigerian Cultivar	American Cultivar
Average daily[x] weight gain, g	1.97 ±0.51	0.79 ± 0.17	0.77 ±0.19	0.89 ±0.16	1.21 ±0.28
Average feed intake, g	9.48 ±1.54	6.55 ±0.86	6.56 ±1.33	6.82 ±0.60	8.27 ±1.11
PER[y]	2.08^{a} ±0.23	1.22^{b} ±0.29	1.17^{b} ±0.15	$1.30^{b,c}$ ±0.20	1.46^{c} ±0.19
Corrected PER	2.50	1.45	1.41	1.56	1.77
Protein quality (Sample PER/Casein PER x 100)	100	59.2	57.3	63.6	70.9
% Digestibility (in vitro)	--	68.5	66.9	69.0	73.6

[x]Average for 10 rats ± SD

[y]Values with different superscript differ significantly at p = 5%.

Williams (1984) as the most likely reason for the very low PER value of 0.53 that she recorded for akara, a fried paste prepared from presoaked, dehulled, and ground cowpeas.

In-Vitro Protein Digestibility

The in vitro protein digestibility of cowpea proteins following digestion by pepsin and pancreatin are presented in Table 18.

The % digestibility of proteins in all the cowpea dishes ranged from 66.9% to 73.6% with moin-moin giving slightly higher values than ewa-ibeji. This result confirms that of the PER study which showed moin-moin to have a slightly higher quality protein. The in vitro % digestibility shows a very good correlation ($r = 0.97$) with PER data.

Sheffner (1967) has pointed out that in vitro methods are useful for nutritive evaluation of protein quality or digestibility, but one can only interpret the results with confidence when they are a complement to in vivo animal tests. The pepsin-pancreatin method of Saunders et al. (1972) used in this study shows good correlation with in vivo data. Akeson and Stahman (1964), in devising a pepsin-pancreatin digest index of protein quality evaluation, obtained excellent correlation between biological values for the growing rat and quality of the

protein calculated from the amino acids released by the pepsin-pancreatin digest.

Both the *in vivo* and *in vitro* results indicate that cowpea proteins need to be complimented in the diets of the people involved.

CONCLUSION

The levels of certain antinutrients in cowpeas were assessed and the effects of processing on them investigated. Raw cowpeas of 15 Nigerian cultivars were found to contain significant quantities of oligosaccharides (raffinose and stachyose), phytic acid, tannins, and trypsin inhibitory activity (TIA).

In Study I, effects of dehulling, cold-soaking (twelve hours at room temperature), hot-soaking (two minutes boiled and one hour held at room temperature), and cooking into two popular Nigerian dishes (ewa-ibeji, cooked whole cowpeas and moin-moin, boiled, dehulled cowpea paste) were as follows: dehulling completely eliminated tannins and significantly reduced stachyose and sucrose. Hot-soaking significantly reduced stachyose, phytic acid, and TIA, but had no effect on sucrose, raffinose, and tannins. The reducing effect of cold-soaking was only significant in phytic acid. Cooking significantly decreased stachyose and TIA, but had no effect on sucrose, phytic acid, and tannins. Moin-moin and ewa-ibeji contained comparable levels of all the antinutrients, except tannins. Dehulling

eliminated tannins which have been shown to render ionizable iron in foods unavailable to the body. Consequently, dehulling may be encouraged more in cowpea food preparation, especially in a country such as Nigeria where iron deficiency anemia is common. Also, hot-soaking, which is not commonly used in household cooking, may be a good practice.

In Study II, the indigestibility of the carbohydrates in cowpeas was evidenced by the significant increase in breath H_2 production when moin-moin and ewa-ibeji were eaten, compared to a control meal of rice. The implicated oligosaccharides in cowpeas appear to be raffinose and stachyose because they are known to cause flatulence and because addition of these substances to the control meal of rice gave breath H_2 response similar to that given by comparable amounts of cowpeas. Cooking cowpeas as is normally done in many Nigerian homes did not completely eliminate flatulent factors. However, moin-moin appeared to be less conducive to gas production. Increasing the test dose result in a nonlinear increase in breath H_2 production. Cowpea consumers may be advised to take lesser quantities of cowpeas at any particular time. Of course, other foods should be used to supplement cowpea diets.

In the Study III, which evaluated the quality of cowpea proteins in American and Nigerian cultivars, ewa-ibeji and moin-moin gave very low PER values of 1.17 and 1.46, respectively, for the American cultivar, and 1.22 and 1.30 for the Nigerian cultivar. These results indicate the need for complimentary sources of protein in the diet of the people involved. It is interesting that moin-moin appeared to have slightly better quality protein than ewa-ibeji. The quality of cowpea protein in the tested dishes was not affected by cultivar difference. In vitro protein digestibility of the cowpea dishes ranged from 66.9 - 73.6% and the values correlated very well with those obtained for the animal study.

Future Studies

--Determining breath hydrogen response to cowpeas ingested in quantities less than 100g. This is an attempt to establish a valid dose-response pattern.

--Preparing cowpea dishes from germinating seeds, determining the acceptance of such dishes and the breath hydrogen response to their ingestion.

--Measuring the color in the brown cultivars of cowpeas and correlating the degree of darkness to the content of tannins.

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