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COWPEA FLOUR: PREPARATION AND SOME PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES

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COWPEA FLOUR: PREPARATION AND SOME PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES

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ABSTRACT

COWPEA FLOUR: PREPARATION AND SOME PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES

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Cowpea flours were prepared by three simple methods which included soaking; blanching prior to soaking; and redrying of the presoaked raw whole beans. In each case, the beans were dehulled, autoclaved and ground to a fine powder.

The flours were free-flowing, nonhygroscopic and creamy white in color. They reconstituted very well in both hot and cold water and had relatively high bulk densities.

Chemical determinations indicated that the water soluble nitrogen, available lysine, water soluble vitamins, minerals and the phytic acid content of the flours compared to the raw cowpeas, were not affected appreciably by the process treatments.

Cowpeas are high in K, fairly high in P, Ca, and Mg and are abundant in trace mineral elements. With the exception of P and K, most of the minerals were concentrated in the seed coat which comprised only 6.0% of the whole cowpea seed. K, P, Mg, and B, decreased while Ca and Na increased following processing and blanching prior to soaking in water caused the greatest decrease.

Both the cowpeas and the oil seeds (watermelon and sesame seeds used in the protein quality evaluation) were subjected to proximate analysis before and after they were processed into flours. The flours were high in protein. On a dry basis, the protein content was as follows: dehulled autoclaved cowpea flour 28%, defatted watermelon flour 52%, and defatted sesame flour 51%. The amino acid composition of the flours showed cowpea to be limiting in total sulfur amino acids while the oil seeds were low in lysine.

The available lysine content of the flours was analysed by the 2,4,6-trinitrobenzensulfonic acid (TNBS) method. As a percent of the total lysine the available lysine in the autoclaved cowpea flour was 94.1%, 93.0% in the defatted watermelon flour, and 95.7% in the defatted sesame flour.

The nutritional quality of the proteins of the three flours and mixtures of cowpea:sesame and cowpea:watermelon flours were evaluated using Protein Efficiency Ratios (PER), Feed Efficiency Ratios (FER) and percent digestibility.

Compared to a PER of 2.50 for casein, the PER for cowpea flour (cf), sesame flour (sf), watermelon flour (wmf), cf:sf 75:25, cf:sf 50:50, cf:wmf 75:25, and cf:wmf 50:50 were 1.71, 1.22, 1.07, 2.27, 2.44, 2.20, and 2.28, respectively. On daily basis, the average weight gains for the rats were as follows: casein 4.14 g, cf 2.64 g, sf 1.62 g, wmf 1.32 g, cf:sf (75:25) 3.82 g, and cf:wmf (50:50) 4.42 g. Feed efficiency ratios showed that the

rats on diets containing cowpea, sesame, and watermelon flours used singly as the sole source of protein required more feed per gram weight gain than the casein diet or the blends of the flours. The apparent feed and protein digestibilities of the test samples were fairly high. There were no apparent adverse effects as a result of the supplementation.

To My

Mother Nwachi,

Brother I.K.,

AND TINA

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INTRODUCTION

Cowpea (Vigna unguiculata) is a tropical legume grown intensively in Nigeria and other tropical and subtropical countries. In Nigeria an area of about 2.2 million hectares was estimated to be under cowpea cultivation in 1960. Approximately 800,000 tons of cowpeas were obtained in the 1962/63 production season (Oyenuga, 1968). With improved technology, proper pest and disease control and by growing improved varieties, higher yields (approximately 2,000 kg/ha) have been realized.

Cowpeas are rich in protein. They are highly regarded in Nigeria and other West African countries and the beans are consumed in various ways; boiled and eaten alone or with maize (corn) or rice. A cowpea puree is the base for preparing two popular dishes, "moin-moin" (or Ole) and "akara" (Oyenuga, 1968; Aykroy and Doughty, 1964; Dovlo et al., 1976). Because of these and other important uses of this high protein crop, efforts have been made to:

1. Provide adequate nutritional information about the crop, and
2. To convert the cowpea into flour amenable to supplementation and better utilization.

Like most other legumes, cowpeas are deficient in sulfur amino acids, particularly methionine. Suggestions for improving the nutrient quality, include supplementation with either the pure limiting amino acid or with other plant protein sources grown locally and rich in this amino acid. Egg protein, which is very rich in sulfur amino acids would make an excellent supplement to cowpea protein, but eggs are expensive for the average Nigerian family.

Plant protein supplementation is less expensive than pure methionine and animal protein supplementation and is free from the associated flavor problems frequently found with methionine supplementation. The use of plant protein sources would improve the quality of protein, and also increase the quantity of protein in the diet.

Sesame (Sesamum indicum) seeds, also called benni-seed, and watermelon seeds are two important oil seeds grown in many tropical and subtropical countries. In Nigeria sesame is grown in many states, but its use for food locally is limited. Watermelon on the other hand is widely cultivated for its oil and meal which are used in local dishes. Both crops are high in protein with a fairly balanced amino acid composition. In particular they are rich in sulfur amino acids, but low in lysine, an amino acid, which is present in high amounts in cowpeas.

The value of watermelon seed protein as a food supplement has not been determined. Sesame seed protein has

been used as a supplement in several foods and feedstuffs with very good results. Incorporating these oil seed flours into cowpea flour would produce supplemented flours of high nutritional value in protein. This would not only provide the population with a protein rich food but also encourage the production and utilization of these high protein crops.

In this research, attempt was made first, to prepare a convenient cowpea flour suitable for preparing some Nigerian dishes and secondly, to evaluate the effect of processing on the nutritional qualities of such cowpea flour. The third objective was to compare the supplementary effects of sesame seed and watermelon seed proteins on cowpea protein.

LITERATURE REVIEW

Preparation of instant bean powder

Several methods of preparing instant bean powder have been reported (Bakker-Arkema et al., 1966; Morris, 1961; Kon et al., 1974; Miller et al., 1973; Onayemi and Potter, 1976). In general the beans are soaked overnight in water and then cooked for 1 hr. at 210 F with constant stirring. Next the beans are disintegrated and the resulting slurry dried.

Kon et al. (1974) stated that it might not be necessary to presoak the beans before cooking but unsoaked beans require at least 2 hr of cooking. In cowpea powder preparation, soaking is essential to facilitate testa removal. The cooking step may not be necessary. The dehulled beans are slurried and dried (Onayemi and Potter, 1976).

The three most used methods for drying bean slurry are single drum drying, double drum drying and spray drying. Bakker-Arkema et al. (1966) successfully dried whole precooked dry peabeans to instant precooked bean powder on a single drum and on a double drum drier. They reported equal capabilities for both types of driers, but stated that

sheet densities were considerably lower when a single drum drier was used. The same workers successfully spray dried pureed cooked beans in a vertical co-current spray drier. The resulting instant bean powder was comparable in qualities to that from a drum drier. For economical reasons and for ease of operation, the double drum drier is, however, preferred.

Effect of processing on the quality of bean powder

The effect of processing on the quality of bean powder is cumulative; beginning with the treatments (blanching, soaking, and cooking) received prior to the dehydration process, including the dehydration step itself, and continuing during the storage of the powders. The qualities most often affected include texture, color, flavor, reconstitution, solubility and the bulk density as well as the nutrient content of the bean powder.

Effect on texture

Good quality bean powder has a smooth uniform texture and flows easily. Kon et al. (1974) reported that the texture of the finished product depends on the cooking time. At atmospheric pressure, cooking for less than 45 min yields a hard product but will depend on the applied temperature and particularly the variety of the beans.

Bakker-Arkema et al. (1966) obtained a fairly good quality texture from both spray and drum dried powders.

They stated that homogenization of the slurry before drying reduced the quality of the texture, probably because homogenization caused the bean cells to rupture thus releasing free starch which led to a pasty texture.

Effect on color

Thermally dehydrated bean powder possessed a tan to brown color as opposed to the normal white color of beans (Bakker-Arkema et al., 1966). These workers reported that the white color is very difficult to produce but the colored bean powders were highly rated. Love and Dugan (1978) and Counter (1969) stated that this color change was due to non-enzymatic type browning involving the sugar and the amine groups in the bean powder. They further stated that the color change was accelerated by high temperature during processing and that the change continued during storage, if the moisture content was high.

Effect on flavor

Both Bakker-Arkema et al. (1966) and Onayemi and Potter (1976) obtained instant bean powders with highly rated acceptance in terms of flavor.

Off-flavor developments in freshly prepared and stored bean powders are known to be due to lipid oxidation and nonenzymatic browning both of which are accelerated by high temperature during processing. Kon and co-workers (1974) noted that the brinding treatment that break most or all of the cells and release cell contenta of raw

legumes prevents the subsequent development of the characteristic cooked bean flavor. They maintained that cooked powders prepared so cellular integrity is preserved retain their flavor better. Off-flavor development in finished powders is also prevented by adjusting the slurry to pH 3.5 before drying (Kon et al., 1970).

Effect on reconstitution

One of the most important physical properties of bean powder is the ability to reconstitute into a desirable soup mixture. This property is affected by the method of drying as well as the amount of heat treatment the powder has received. Bakker-Arkema et al. (1966) reported that the reconstitution of drum dried powders was not as good as that of spray-dried powders. Retorted-cooked, spray-dried powders settled out at the rate of about 5 to 7 ml in 10 min, as compared to about 10 ml for single drum-dried powders and about 30 to 40 ml for double drum-dried. These workers reported that particle size of powders did not affect reconstitution.

Kon et al. (1974) obtained good reconstitution for drum-dried regular and acidified bean powders. The regular powder reconstituted instantly in hot water while the acidified did so more slowly. Reconstitition in cold water was also better with the regular than with the acidified powder. The workers suggested that the poor reconstitution

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of the acidified powder might have been due to the free gelatinized starch in the powder, which resulted from the broken bean cells.

Effect on nitrogen solubility

The soluble nitrogen of the bean powder is related to the quality of the dehydrated powder. It is usually determined as the Nitrogen Solubility Index (NSI), which by definition, is the ratio of the water soluble nitrogen content to the total nitrogen content of the sample, following reconstitution and centrifugation of the sample under specified conditions.

NSI is affected by a number of factors including: drying system, treatment prior to drying, temperature of drying, moisture content of powder, time and temperature of storage, and the fat content of powder. Onayemi and Potter (1976), reported about 23% decrease in NSI as a result of drum drying of cowpeas.

Effect on bulk density

This property of the powder is important from an economic standpoint. Large loose volumes lead to high package cost. Packed volume is also important since excessive settling of powder during transportation could lead to more headspace in packages. Bulk density is affected by the dehydration method as well as the particle size distribution.

Effect on nutritional qualities

Nutritional studies with drum-dried instant bean powders showed that heat treatment reduced considerably the amount of trypsin inhibitor in beans thus improving the protein efficiency ratio (PER) of bean protein (Onayemi and Potter, 1976; Kon et al., 1974; Miller et al., 1973; Bressani et al., 1963). Onayemi and Potter (1976) prepared instant cowpea powder from raw cowpea puree. They reported that over 80% of the bean trypsin inhibitor was destroyed. Similar findings were reported by Carvalho and co-workers in 1977.

Bressani et al. (1963) agreed that heat treatment destroyed the toxic factors in beans with consequent increase in their nutritional quality. They also maintained that the increase in PER and protein digestibility was due to the destruction of the trypsin inhibitor in the beans. This, they maintained, made the proteins available to the animals. These workers, however, pointed out that heat treatment of the beans decreased the free ε-amino groups of lysine. Further evidence on the detrimental effects of heat treatment of bean protein was reported by Onayemi and Potter (1976). They observed a slight decrease in the nitrogen solubility index (NSI) of their cowpea powder. This is an indication of protein denaturation during the drum drying process. These workers, however, did not observe any appreciable change in the amino acid content

of the powder.

The vitamins, particularly the water soluble B-vitamins, thiamine, riboflavin, niacin, pantothenic acid and folacin, are the nutrients most adversely affected by the different process methods. Consequently, they have been studied extensively.

The effect of processing varies considerably among the different vitamins. By far the largest vitamin losses occur during the dehydration step. Available data, however, show that the degree of losses depends on the treatment prior to the dehydration. Acid treated powders showed the greatest losses. Miller et al. (1973) reported a total loss of about 1% for niacin to about 63% for folacin in acid-treated powders compared to 19-20% loss in all vitamins obtained with regular drum-dried powder. Kon et al. (1974) obtained similar losses for instant pinto bean powder. The greatest loss again occured with folacin in a 2:1 ratio for acid-treated powder and regular powder.

The moisture content of the powders could be a factor in the amount of vitamin retained. Onayemi and Potter (1976) observed that drying the powder to a moisture content of 5.5% caused a greater destruction of thiamine than a moisture content of 6.5%.

Losses of mineral during soaking are very minimal. Losses during cooking on the other hand can be very high depending on the variety and the state of maturity of the

beans. Meiners et al. (1976) determined the content of nine mineral elements in raw and cooked mature dry legumes. They reported that the cooking water contained measurable amounts of all the minerals and relatively high amounts of magnesium, phosphorous, and potassium. Kumar et al. (1978) observed similar losses with respect to calcium and magnesium.

Improvement of the nutritive value of bean protein

It has been well established that bean proteins are poor in quality compared to animal proteins. In particular they are very low in sulfur-containing amino acids (methionine and cystine). Because of the scarcity of animal proteins and because the beans contain high levels of proteins, efforts have been made to improve the quality of the latter as a means of combating the ever increasing threat of protein malnutrition in many parts of the world.

Three main approaches have been adopted in studies aimed at improving the bean protein quality:

(a) through supplementation with other plant proteins

(b) pure amino acid supplementation, and

(c) supplementation with animal proteins.

The supplementation of legume protein with cereals and oilseeds has been very successful (Bressani et al., 1962; Phansalkar et al., 1957; Bressani and Elias, 1969; Boloorforooshan and Markakis, 1979). Since legume proteins have

high amounts of lysine they complement to a marked extent those of cereals and oilseeds, which are characteristically low in this amino acid. On the other hand, the low methionine content of legumes is compensated by a higher content of this amino acid in cereals and oilseeds.

Phansalkar et al. (1957) showed a good supplementary effect of chick pea, black gram, green gram and red gram proteins on those of wheat, soybeans and pear millet. These workers stated that there is a point in the combination where an optimum supplementation is achieved. In general this effect occurs when about 50% of the legume protein is replaced by cereal or oilseed proteins. Bressani et al. (1962) reported that the best combination of cooked black beans and lime-treated corn was one where each component contributed 50% of the total protein of the diet. Several combinations were reported by Bressani and Elias (1969) of cooked black beans and opague-2 corn. The best results were obtained when 50% of the protein in the diet was derived from each one of the components. Improved nutritive values were obtained by Bressani and Scrimshaw (1961) and Bressani and Valiente (1962), when polished rice and cooked black beans were combined in the range of 50-80% of rice protein and 50-20% for black beans. A combination of 19% rice, 80% legumes and 1% green vegetables, compared well to the stock rat diet which consisted of one of the vegetable rations supplemented

with milk and meat (Baptist, 1956). Boloorforooshan and Markakis (1979) fed weanling rats with different combinations of Navy beans and sesame seed proteins and considerable improvement of the protein quality of the Navy beans was noted at all levels of combination.

The improvement of biological value and protein efficiency ratio of legume proteins brought about by supplementation with methionine is well documented (Bressani et al., 1963; Sherwood et al., 1954; Onayemi and Potter, 1976; Kakade and Evans, 1965). Addition of 0.2-0.6% methionine to various legumes gave PER values similar to those obtained for casein. Onayemi and Potter, 1976, however, reported that fatty infiltration of rat liver occured at 0.4% and 0.6% levels of methionine supplementation.

Several animal protein sources including fish flour, casein, meat flour and egg, have been supplemented to cereal proteins with excellent results (Bressani and Elias, 1968). Similar results with legumes have been reported. Boloorforooshan and Markakis (1979) reported that supplementation of Navy bean protein with whole egg proteins provided a better growth for the animals than beans alone. The protein efficiency ratio of the beans was also improved. Antunes and Markakis (1977) showed that small amounts of Brazil nut protein can upgrade nutritionally the protein of beans.

Protein quality and its determination

Protein quality relates to the efficiency with which various food proteins are used for synthesis and maintenance of tissue protein (Jansen, 1978). Proteins differ a great deal with respect to their quality. This difference is largely explained by differences in their content of essential amino acids.

Several methods are available for determination of protein quality. They include:

(a) biological methods

(b) chemical methods

(c) microbiological methods and

(d) enzymatic methods

Biological methods

Biological Value (BV): Thomas (1909) introduced the concept of biological value (BV) and the method of assessing BV with humans. Since that time, the term biological value has been synonymous with protein quality. He used adult subjects, but Mitchell (1923) adopted the method to both growing and adult rats.

Biological value is calculated as the ratio of the retained nitrogen over the absorbed nitrogen and measures the efficiency of utilization of nitrogen absorbed by the test organism (McLaughlan, 1972). This method is laborious because of the extreme care which is necessary in collecting faeces and urine free of feed contamination and

difficulties in accurate measure of endogenous urinary and faecal nitrogen.

$$BV = \frac{N \text{ retained}}{N \text{ absorbed}} \times \frac{Nin-(Nf-Nmf)-(Nur-Neur)}{Nin-(Nf-Nmf)} \times 100$$

where: Nin = nitrogen intake

Nf = faecal nitrogen

Nmf = metabolic faecal nitrogen

Nur = urinary nitrogen

Neur = endogenous urinary nitrogen

The metabolic faecal N and endogenous urinary N are measured in practice for each animal during a period of non-protein feeding prior or subsequent to the test period. The assumption is made that the amount of metabolic N in urine and faeces is the same in animals consuming diets containing protein or no protein. It was shown by Mitchell (1923) that this is related linearly to the body weight and also that it is directly proportional to the roughage content of the diet.

BV does not take into account the digestibility of the foods tested, since it concerns only the utilization of absorbed nitrogen.

Net Protein Utilization (NPU): This method developed by Bender and Miller (1953) eliminates some of the laborious aspects of BV and takes into account the

digestibility of the proteins.

$$NPU = \frac{N \text{ retained}}{N \text{ intake}} = \frac{Nt-Ne}{Nc} \times 100$$

where: Nt = body nitrogen of test group

Ne = body nitrogen of the group on protein free diet

Nc = nitrogen consumed by the test group

The nitrogen retained in this method is measured as the difference in carcass nitrogen between the rats fed the test diet and rats fed the non-protein diet. This requires a very careful pairing of the animals according to body weight before the start of the experiment. The nitrogen lost by the group fed the non-protein diet represents the nitrogen required for maintenance.

Net Protein Ratio (NPR): NPR was described by Bender and Doell in 1957. This method is based on the measurement of the body weight thus eliminating the laborious body nitrogen measurement.

$$NPR = \frac{Wtgt + Wtle}{Wtct}$$

where: Wtgt = Weight gain of the group on test protein

Wtle = Weight loss of non-protein group

Wtct = Weight of protein consumed

This method takes into account the maintenance requirement of the rats. It also assumes that the body composition is constant, an assumption which may not be valid when widely different test and protein deficient diets are fed to the animals.

Slope Ratio Technique: Hegsted and co-workers (1965, 1968) proposed a slope ratio assay for the assessment of protein quality. In this procedure, the protein is fed at different levels and the slopes of the test proteins are compared with that of a standard protein, such as lactalbumin. The use of a standard protein under the same conditions as used with the test proteins aims at eliminating the causes of variability which cannot be clearly identified and controlled. The slope of the test protein is expressed as a percent of the slope of the standard protein.

A valid slope-ratio assay requires that the response curves for sample and standard be linear and that they meet at the zero dosage level. This does not always happen. Only values falling on the linear portion of the curve are used in computation of the slope assay value. However, the slope may not always be a valid index of protein quality. For example, lysine deficient proteins yield a lower slope, while threonine deficient proteins tend to yield higher slope values (Hackler, 1977).

Protein Efficiency Ratio (PER): Osborne et al. (1919) defined a method of expressing numerically the

growth-promoting value of proteins involving the determination of the gain in body weight per gram of protein consumed at level of dietary protein associated with the highest protein efficiency ratio (PER).

$$PER = \frac{\text{Gram gain in body weight}}{\text{Gram protein intake}}$$

Protein efficiency ratios appear to be related reasonably well to other methods of evaluating proteins. Block and Mitchell (1946) found that there was a good relation between PER's and biological values and also between PER's and chemical score based on amino acid composition. Bender (1956) reported a good correlation between PER and net protein utilization.

Several factors are known to influence the PER determination:

(a) Strain and sex of rats (Morrison and Campbell, 1960). Female rats grow slower than males, thus producing different PER values. There is as much difference between strains of rats as there is between sexes.

(b) Species differences: Hegsted et al. (1947) and Mitchell (1954) reported that the results of growth trials in rats could be generally applicable to the assessment of human diets. Flodin (1959) found a good correlation between PER values in rats and biological values in the adult man. Hegsted (1957) reported that the amino acid requirements of

man and of the rat are, generally speaking, the same. Allison (1957) found remarkable correlations in the nitrogen balance indices of six different proteins in man, dog and the rat.

(c) Age of the rat and assay period: Chapman et al. (1959) have shown that significant differences could be obtained in PER values between rats put on test at 22, 36 or 45 days of age. In Osborne's (1919) original method, the assay period was eight weeks. According to Chapman et al. (1959) and Morrison and Campbell (1960), the coefficient of variation of PER's had a tendency to drop after the first week and would be generally lower at the end of the third or fourth week, which indicates that the assay is becoming more stable at that point.

(d) Protein level: Obviously comparisons will be valid if the only variable between one diet and another is the nature of the protein, i.e., all diets should have the same nitrogen content. Different levels of protein in the diet have been proposed in the literature. The standard AOAC method recommends 10% protein.

Because PER determination is affected by a number of factors as enumerated above, a strict adherence to certain laid out conditions (AOAC, 1975) is necessary in order that comparison of data obtained by different researchers can be possible.

Chemical methods

The chemical methods for nutritional evaluation of proteins are based on the chemical analysis of the amino acid content of the proteins.

Mitchell and Block (1946) used the chemical composition of food proteins and related it to their nutritive value. The basis for their method was the fact that the biological value of a protein is dictated by the limiting amino acid in that protein (the essential amino acid with the greatest percentage deficit compared to the amino acid needs of the rat or compared to its content in whole egg protein). The "chemical score" value method is defined as the lowest ratio of an essential amino acid of the test protein to the same amino acid in whole egg protein. The whole egg protein is chosen as reference protein because the amino acid requirements of the rat are not well known and also because its amino acid mixture is highly digestible and almost perfectly utilizable in rodent metabolism, being better than milk protein in this respect (Bricker and Mitchell, 1947). This was shown later to be true also for the dog (Allison et al., 1949) and the adult man (Hawley et al., 1948). Later studies (Bender, 1961) showed that whole egg proteins contain excessive amounts (relative to rats requirements) of tryptophan; also sulfur containing amino acids (methionine and cystine) are in slight excess, but lysine is present at just about the requirement level. Therefore,

the chemical score will score the lysine deficient proteins as good while it exaggerates a deficiency of sulfur-containing amino acids in proteins (McLaughlan et al., 1959).

Because of the excess of essential amino acids in the whole egg protein, the FAO/WHO 1973, proposed a provisional amino acid pattern based on the amino acid requirements of pre-school infants. The index calculated according to this pattern of amino acids is referred to as Amino Acid Score.

Oser (1951) proposed the Essential Amino Acid Index (EAAI) as a method for protein quality evaluation. This method of rating protein quality is based not only on one essential amino acid, as the chemical score, but on the contribution of all the essential amino acids. Oser felt that each essential amino acid is specific in its own right and all are equally essential. Therefore, in his method he included all the essential amino acids. The EAAI is calculated by computing the average of the logarithms of the ten "egg ratios" (the ratio of the essential amino acids in a protein relative to their respective amounts in whole egg protein) and then taking the antilogarithm of this average value.

The EAAI method modified by Mitchell (1954) by adjusting to 100 the egg ratios that happens to have values higher than that. The antilogarithm of the average of the egg ratio logarithms that includes adjusted values is called Modified Essential Amino Acid Index (MEAAI).

The major problem with the chemical methods for protein quality evaluation is the assumption that all the amino acids are biologically available. This assumption is not always valid and especially with proteins that have been heat treated or otherwise have been processed. On the other hand, the chemical methods have several advantages; (a) require small amount of sample, (b) the analysis period is short compared to bioassay and (c) they give information concerning the identity of the limiting amino acid.

Microbiological methods

These methods utilize the proteolytic activity of certain microorganisms for the estimation of the nutritional value of proteins. Different microorganisms have been used through the years.

The production of lactic acid was first used as a measure of the growth of bacteria in a media in which one amino acid was limiting.

The growth response of Streptococcus zymogenes (Ford, 1960) and the protozoa Tetrahymena pyriformis (Rosen and Fernell, 1956; Pilcher and Williams, 1954) were used for the determination of the biological value of proteins. With a rigorous proteolytic strain of Streptococcus zymogenes (Ford, 1960) obtained protein quality values for several meat meals that were closely correlated with the available lysine content as it was found by others with a combination of an enzymatic-microbiological technique. Using

Streptococcus zymogenes he also found that heating diminished the nutritive value of skim milk powder. It seems though that foods rich in carbohydrates are not suitable technically for microbiological assays for nutritional value estimation. Studies showed poor correlation between microbiologically obtained values for the biological value of cereal products and results obtained with animal experiments (Menden and Cremer, 1970). Animal protein foods assayed for methionine and lysine showed fair agreement with those for animal experiments (Rao et al., 1963; Scott et al., 1962). The microbiological tests are of shorter duration and lower cost than the animal experiments.

Enzymatic methods

Enzymatic methods are an improvement over chemical methods because they determine only amino acids that can be freed from the protein molecules and thus considered as available under physiological conditions. They can be used to measure amino acid availability or to estimate the biological value of a protein.

Melnick et al. (1946) first reported that different proteins, or proteins that had been processed differently were hydrolyzed by pancreatin at different rates. In his procedure food proteins were digested with pancreatin only.

Sheffner et al. (1956) described an amino acid index which combines the pattern of essential amino acids released by in vitro pepsin digestion with the amino acid pattern of

the remainder of the protein to produce an integrated index- the Pepsin-Digest-Residue (PDR) amino acid index. The PDR index was closely correlated with the NPU of a variety of proteins.

Akeson and Stahman (1964) used pepsin followed by pancreatin for 3 hrs and 24 hrs, respectively. They analyzed the digest with an amino acid analyzer. The calculated Pepsin Pancreatin Digest Indices (PPDI) for ten food proteins were correlated with biological values with a correlation coefficient r = 0.990 compared with the chemical score, where r was = 0.940.

One major problem with these in vitro digestion methods is the accumulation of the end products which inhibit the digestive reactions (Dimler, 1975). Mauron (1970) used pepsin and pancreatin but digested the samples in a dialysis sac, trying to simulate a physiological condition in an attempt to avoid end product inhibition. He calculated the Pepsin Pancreatin Dialysis Digestion (PPDD) index, which showed close agreement with the rats biological values. Ford and Salter (1966) digested samples on a column of sephadex G-10 to provide for chromatographic removal of small products of digestion. After digestion and deproteinization the samples were fractionated on a sephadex G-25 column. Amino acid analysis was done on the fraction with molecular weight of less than 250.

An enzyme score, calculated like the chemical score, comparing the essential amino acids released from the test protein and those released from egg protein, was determined on heat processed casein (Stahman and Woldegiorgis, 1975). Both the enzyme score and chemical score compared well with the PPDI.

Recently Hsu et al. (1977) developed a model for rapid prediction of PER of a protein by using amino acid profiles of the sample protein and a reference casein, along with their in vitro digestibilities. The in vitro digestibility is measured by the drop in pH that the enzyme system trypsin-chymotrypsin-peptidase cause in 10 minutes, and from a regression equation that correlates the drop in pH in 10 minutes by the enzyme system, to the apparent digestibility as measured by rat assays. By a combination of the digestibility and the essential amino acids profile expressed as percentage of the FAO/WHO-1973 provisional pattern a computed PER (C-PER) is derived. The average differences between the C-PER and rat based PER for 45 foods that were used in their experiments was 0.12.

This method gives a fast (72 hrs) estimate of protein quality and also gives data as to why that quality is high or low, via either high or low protein digestibility or the deficiency or abundance of one or several essential amino acids (Satterlee et al., 1977).

Biological and physiological factors in legumes

It has been recognized for many years that the nutritive value and protein digestibility of legumes are very poor unless subjected to cooking or some other form of heat treatment (Liener, 1962). The depression in protein value and digestibility has been generally attributed to the presence of certain anti-nutritional factors, including the trypsin inhibitors, hemagglutinins and flatulence factors (Liener, 1962; Liener and Kakade, 1969; Rackis, 1974).

Perhaps the best studied of all the anti-nutritional factors is the trypsin inhibitor which was first isolated from soybean by Kunitz (1945). Trypsin inhibitors have been isolated from a variety of plant materials as well as from various animal tissues. The reaction between trypsin and an inhibitor is one of the few known cases of pure protein interaction (Green and Work, 1953). Several trypsin inhibitors have been isolated and purified, examples are the inhibitors from soybeans (Kunitz, 1945; Rackis et al., 1962), lima bean (Fraenkel-Conrat et al., 1952) and navy beans (Bowman, 1948; Wagner and Riehm, 1967).

Hemagglutinins are another established anti-nutritional factor in legumes (Liener, 1974; Honavar et al., 1962). Hemagglutinins are believed to exert a non-selective adverse effect on the absorption of nutrients from the intestinal tract rather than a direct effect on the digestive process.

Both the trypsin inhibitors and hemagglutinins are heat labile. This means that proper heat treatment of the legumes can reduce the anti-nutritional factors in them. Onayemi and Potter (1976) showed that drum drying resulted in 80% destruction of the trypsin inhibitor of cowpeas. Similar results were reported by Owusu-Domfeh (1972), and Carvalho et al. (1977).

Mature bean seeds, like many other seeds, contain relatively large amounts of phytate (inositol hexaphosphate) (Makower, 1969; Kon et al., 1973; Lolas and Markakis, 1975; Chang et al., 1977). Phytate serves as a ready source of phosphate (Mayer, 1956) and inositol (Darbre and Norris, 1957) during germination. Its decomposition may also release certain metals which are essential to the developing plant. Nutritionally, phytate is probably unavailable to humans (McCance and Widdowson, 1935) due to the lack of an endogenous enzyme system (Rapoport et al., 1941) that can catalyze the hydrolysis of the molecule to its moieties. The presence of undegraded phytate in the intestines may render less available for absorption some essential di- and tri-valent cations such as Zn^{++}, Ca^{++}, Mg^{++} and Fe^{++}.

Dry bean seeds are also known to contain several low molecular weight soluble oligosaccharides which are metabolized during the germination of the seeds. A greater proportion of these oligosaccharides (60-70%) are in the form of raffinose, stachyose and verbascose (Nigam and

Giri, 1961; Lee et al., 1970; Akpapunam and Markakis, 1979), which are largely unavailable for human nutrition due to a lack of specific enzymes (α-galactosidases and β-D-fructosidases) in human digestive juices (Kuriyama and Mendel, 1917; Gitzelmann and Auricchio, 1965).

Following ingestion, phytate and those oligosaccharides which are not hydrolyzed by gastrointestinal secretions and hence not readily absorbed, pass into the large intestine where they are fermented by bacteria with formation of gas and generation of flatus (Bergey, 1957; Speck et al., 1970; Rackis et al., 1970; Steggerda, 1961; Hellendorn, 1969), commonly associated with the ingestion of cooked legume seeds.

Sesame

Sesame, Sesamum indicum L, belongs to the Pedafiaceae family, which consists of sixteen genera and sixty species generally found in tropical and subtropical areas (Caldwell, 1958).

The valuable components of sesame seed are the oil and protein and these components have been determined for several varieties of sesame which were grown in the southern and southwestern parts of the U.S.A. (Kinman et al., 1954). Oil content varied from 45-63% and averaged 54%. Protein content varied from 17-32% and averaged 26%. The average protein content of the oil-free meals was 57%.

Sesame protein is rich in sulfur containing amino acids, particularly methionine. It is deficient in lysine and somewhat in isoleucine.

Being a good source of methionine, sesame meal offers great advantage as a natural supplement to many legume proteins deficient in this amino acid. The supplementary value of sesame to soya, groundnut, chick pea, or mixtures of these legumes in different proportions has been demonstrated in several investigations using rats as experimental animals (Krishnamurthy et al., 1960; Tasker et al., 1960; Gruttikae et al., 1965).

Evans and Bandemer (1967) demonstrated the effect of fortification on the nutritive value (relative to casein) of sesame meal. Sesame alone had a protein nutritive value of 47%. The protein nutritive value of a 1:1 mixture of sesame and soybean protein was about the same as that of casein. Soybean protein has an abundance of lysine but is deficient in methionine.

In related studies (Joseph et al., 1962; Joseph et al., 1958), it was demonstrated that incorporation of 25% of sesame meal raised the PER of a 2:1 mixture of peanut and bengal gram protein from 1.79 to 2.03. Similar results were obtained with autoclaved Navy beans by Boloorforooshan and Markakis (1979).

High protein vegetable mixtures for human feeding containing 35% sesame flour, were developed (Scrimshaw

et al., 1961) by the Institute of Nutrition of Central America and Panama (INCAP). These low-cost mixtures were readily accepted and well-tolerated as the chief protein source of a needy population.

Availability of the amino acids in sesame is affected by the processing methods used on the seed (Villegas et al., 1968). The effect of three treatments is shown in Table 1.

Enzyme digestibility was affected very little, but methionine availability was increased by heat treatment. In the sample that was heated after oil extraction it was 2.33%, close to an average literature value. Lysine availability was not affected consistently by heat treatment and in all cases was less than 50% of an average literature value for lysine content.

Like many other common oilseed meals sesame meal contains phytic acid. A range of 1.0-1.3% P of phytic acid is reported to be present in Sesame meal (Carter et al., 1961; Lease, 1966).

Watermelon

The watermelon Citrullus vulgaris Schrad., is a creeping annual which belongs to the Cucurbitaceae family. There are two main varieties of this crop in Nigeria, the Bara and Screwe (Oyenuga, 1968).

The large, circular fruit is made up of white fleshy material inside which are embedded small flat seeds. Watermelons are principally cultivated in most countries as

Table 1. Availability of amino acids in sesame meal*

Meal preparation	Enzyme digestibility,[a] % soluble N	Available Methionine,[b] g/16 g N	Available Lysine,[b] g/16 g N
Prepressed; then extracted	90	1.85	1.08
High temperature expeller	87	2.17	0.84
Prepressed; extracted; then heated (50% H_2O) 1 hr at 121°C	92	2.33	1.04
Average literature value	-	2.34	2.71

[a]Pepsin; then pancreatin.

[b]Microbiological assay after enzyme digestion.

*Table taken from Lyon (1972).

edible fruit, but in Nigeria they are hardly used as such. Here the fruit is cultivated mainly for its seeds which form one of the most important sources of protein in the south of the country. It is also a good source of high quality oil which could be as high as 50% in shelled seeds.

Apart from its high protein and oil content, watermelon seeds contain good amounts of phosphorous, magnesium and potassium and a fair amount of carotene and vitamin D (Oyenuga, 1968).

In rat tests, watermelon seed, when used alone as the main source of protein in a well-balanced ration, was reportedly readily digestible. Its biological value and efficiency of utilization were inferior only to those of animal proteins in the form of stockfish and whole hen's egg. While rats fed on an egg ration put on a daily average live-weight gain of 1.5 g, those on stock-fish and water-melon seed put on daily average gain of 1.08 and 1.02 g, respectively, for a trial period of 9 days (Oyenuga, 1968).

MATERIALS AND METHODS

Preparation of samples

Cowpea flour

Cowpea (*Vigna unguiculata*), #76001, was obtained from the National Cereal Research Institute, Ibadan, Nigeria.

The beans were soaked in water three times their weight, at 25°C, for 18 hours. The seed coats were separated by hand rubbing and removed by flotation. Dehulled cowpeas were rinsed and drained free of water. They were then autoclaved in shallow pans at 121°C for 10 minutes. After autoclaving the samples were placed in a ventilated hood and allowed to dry at room temperature. The dried cowpeas were ground into a fine powder (50 mesh).

In another procedure the cowpeas were first blanched in water at 99°C for 3 min. They were then soaked in water at 25°C for six hours. The soaked beans were dehulled and treated exactly like in the first procedure to obtain a fine powder.

A third method involved soaking the beans in water at 25°C for 18 hrs. The water was drained and the beans left to dry in a ventilated hood for 24 hrs. at room temperature. The bean endosperm shrank moving away from the now brittle

seedcoat, which came off easily on rubbing by hand. The seedcoats were, however, removed by milling the dried beans to a coarse powder in a blender and then blowing air into it. The coarse bean powder was then autoclaved and ground to a fine powder (50 mesh) after drying in the hood at room temperature.

Defatted sesame seed flour

Dehulled sesame seed flour was obtained from the Food Stores of Michigan State University.

The oil was removed by solvent extraction with commercial hexane. The seeds were ground with twice as much hexane in a fireproof blender in a ventilated hood. After blending, the slurry was filtered under vacuum in a Buchner funnel. The extraction with hexane was repeated four to six times depending on the amount of seeds. The resulting slurry was left in a thin layer in a ventilated hood for 24 hr. at room temperature for a complete removal of the solvent. The dried slurries were broken in small pieces and ground to a fine powder (50 mesh).

Defatted watermelon seed flour

The watermelon seeds were obtained from two sources; Redwood City Seed Co., P.O. Box 361, Redwood City, California 94064, and The Vaughan-Jacklin Corporation, Ovid, Michigan 48866. The seeds came with their hulls still intact.

The unhulled seeds were ground in a blender. The hulls were then separated from the flour by passing the meal through a screen with constant agitation. The resulting coarse powder together with some hulls were solvent extracted with commercial hexane in exactly the same way as for the sesame seed flour. Repeating the extraction six to eight times further removed the remaining hulls and improved the color of the flour. The slurries were dried and ground to a fine powder (50 mesh).

All the flours were stored at $0^{o}C$ throughout the experimental period.

Proximate analysis of samples

Both the cowpeas and the oilseeds (sesame and watermelon) were subjected to proximate analysis before and after they were processed into flours. Moisture, ash, crude protein (Nx6.25 for cowpeas, and Nx5.3 for the oilseeds), crude fat, and crude fiber were determined according to AOAC (1975) procedures.

The percentage of total carbohydrate content of the samples was obtained by subtracting from 100 the percentage of contents in moisture; ash, protein, fiber and fat.

Determination of some physicochemical properties of the bean powder

Bulk density

This property was measured by transfering 50 grams of powder into a 250 ml graduated cylinder and the loose volume

determined. The cylinder was then tapped by hand until the powder had stopped settling and the packed volume was recorded. The density in gram per cubic centimeter (g/cc) was determined.

Reconstitution

Ten grams of powder were added to 100 ml of boiling water and agitated for 90 seconds. The resulting mixture was poured into a 100 ml graduated cylinder. The volume of the sediments in the cylinder was recorded after 10 minutes.

Nitrogen Solubility Index (NSI)

The Nitrogen Solubility Index was determined by the method of the AOCS (1973).

Two grams of the sample were weighed into a 400 ml beaker. Eighty ml of distilled water at 30^{o}C were added to the beaker with stirring to disperse the powder. The mixture was placed in a water bath at 30^{o}C and stirred with a mechanical stirrer for 120 minutes. At the end of this period the mixture was transferred into a 100 ml volumetric flask and made to mark with distilled water. The contents of the flask were then thoroughly mixed and allowed to stand for a few minutes. About 40 ml of the mixture was decanted into a 50 ml centrifuge tube and centrifuged for 10 minutes at 2,000 rpm. The resulting colloidal solution was further clarified by filtering through a 0.22 millipore filter. Two ml of the filtrate were used to determine

the water soluble nitrogen employing the micro-Kjeldahl method as described in the AOAC (1975) methods.

Calculation:

$$\% \text{ Nitrogen Solubility Index (NSI)} = \frac{\% \text{ Water Soluble Nitrogen} \times 100}{\% \text{ Total Nitrogen}}$$

Phytic acid determination

Phytic acid was extracted from the bean powders according to the method of Wheeler and Ferrel (1971) with some modification.

One gram finely ground sample was extracted with 25 ml 2% trichloroacetic acid (TCA) for 30 mins. with mechanical shaking. The resulting suspension was centrifuged. A 10 ml aliquot of the supernatant was transfered into a 40 ml conical tube. Four ml $FeCl_3$ solution (made to contain 2 mg ferric iron per ml in 3% TCA) was added to the aliquot which was then heated in a boiling water bath for 45 min. The mixture was centrifuged (10 to 15 min) and the clear supernatant carefully decanted. The precipitate was washed twice by dispersing well in 25 ml 3% TCA, heating in boiling-water bath 10 mins, and centrifuging. The washing was repeated once with deionized water. The precipitate was then dispersed in a few ml water, followed by addition of 3 ml 1.5 N NaOH with mixing. After bringing the volume

to the 30 ml mark with water, the tube was heated again in a boiling-water bath for 30 mins. The tubes were then cooled and centrifuged (10 to 15 mins). The supernatant was again decanted carefully. The precipitate was washed with hot water, recentrifuged and the supernatants decanted. The precipitates were dissolved with hot 40 ml 3.2 N HNO_3 and transfered to 100 ml volumetric flask. The tube was washed six times with hot water, collecting the washings in the same flask. The flask was cooled to room temperature and diluted to volume with water.

Iron was determined according to the method outlined in AOAC (1975). Ten ml aliquot of the sample preparation was pipeted into a 25 ml volumetric flask. One ml hydroxylamine hydrochloride ($H_2HOH.HCl$) solution was added followed in a few minutes by 5 ml acetate buffer solution and 1 ml O-phenanthroline solution. The mixture was made to volume with water. The absorbance was determined in a Beckman DU-Spectrophotometer at 510 nm. Iron concentration was estimated by reference to a standard curve of known iron concentration.

Phytic acid content of the samples was calculated from the iron results assuming a 4:6 iron:phosphorous molecular ratio. The phytic acid phosphorous was calculated from the equation

$$\frac{185.82}{660.8} \times \text{Phytic Acid (mg)} = \text{mg Phytate-P}$$

where 660.8 = M.wt. of phytic acid, and

185.82 = Product of atomic wt. of phosphorous and its molecular ratio (6).

Determination of vitamins

Thiamine (B_1): The thiamine content of the bean powders was determined by the thiochrome method outlined in the AOVC methods (1966) with some modifications.

Extraction: Two grams of the sample were weighed into a 100 ml volumetric flask. To this was added 70 ml 0.1N HCl. The resulting suspension was heated for 30 min in a boiling water bath with occasional shaking.

After cooling the extract for about 30 min, 5 ml of freshly prepared Taka-diastase (an α-amylase) suspension was added and the extract-enzyme mixture was incubated at 45°C for six hr. The enzyme digestion was cooled to room temperature and centrifuged (10 min). The supernatant was collected in a 100 ml volumetric flask. The precipitate was washed several times with water. The washings were collected in the flask and then diluted to mark with water.

Purification of the digest: In place of Decalso, the digest was purified in an amberlite column. The amberlite (IR-120-P, Na^+ form) a strongly acid cation exchanger was obtained from Sigma Chemical Company, St. Louis, Missouri.

Activation: A few grams of the resin (wet mesh = 16-50) were suspended in 2 liters of distilled water. When the resin had almost completely settled, some of the liquid containing the fines was decanted off. The decanting was repeated five to seven times. The resin was rinsed twice with 1 liter 95% ethanol to remove ethanol soluble impurities. It was again rinsed twice with 1 liter 2N HCl, suspended in 2N HCl and heated to 100°C. The latter operation was repeated several times until the supernatant was clear and colorless, using fresh HCl each time and allowing the resin to cool for 1 hour before repeating the heating step. The resin was then suspended in 1M NaCl and kept refrigerated.

With the adsorption tube filled with water, the resins were allowed to fall in place by gravity until a layer 4.4 cm deep was present in the column (diameter 0.9 cm). The tube was drained until no water was visible above the resin.

Twenty-five ml aliquot of sample enzyme-digest was added to the adsorption tube. The solution was allowed to pass slowly through the resin. The filtrate was collected and discarded. The resin was then washed three times with hot distilled water. The washings were also discarded.

After the washing, the column was eluted twice with 10 ml 1M NaCl. The eluate was collected in a 25 ml volumetric flask and then made to mark.

The entire procedure from the purification step to the elution of the thiamine was repeated, this time with 25 ml of working thiamine solution containing 0.2 μg of thiamine per ml.

Conversion to Thiochrome: Five ml of the NaCl-eluate was pipetted into a separation funnel. To this was added 3 ml alkaline ferricyanide solution followed by addition of 15 ml isobutyl alcohol. The contents of the funnel were mixed vigorously for about 90 seconds. The operation was repeated using 5 ml of the working thiamine eluate.

The mixture was allowed to settle. The lower aqueous layer was siphoned out, leaving the alcohol layer to which 2-3 grams of anhydrous Na_2SO_4 were added. The solution was then mixed by swirling the funnel for 30 seconds. After allowing to settle for awhile, a 10 ml portion of the clear solution was taken and the thiochrome content measured with a Turner fluorometer.

A blank was prepared using 5 ml of the NaCl eluate. It was treated exactly in the same way as the sample except that 15% NaOH was added rather than alkaline ferricyanide. Blank determinations were also carried out for the working thiamine standard.

Thiamine content of the sample was calculated from the equation below:

μg thiamine per g sample =

$$\frac{U-UB}{S-SB} \times \frac{1}{5} \times \frac{25}{V} \times \frac{100}{\text{wt. of sample}}$$

where U and UB = deflections of unknown and its blank

S and SB = deflections of standard and its blank

V = volume applied to column if different from 25 ml

Niacin: Niacin was determined according to the chemical method I of the AOVC method (1966).

Hydrolysis-Extraction: One gram bean powder was weighed into a 50 ml centrifuge tube and diluted to 15 ml with water. Five ml concentrated HCl was added from a buret. The centrifuge tube was placed in a rack in a boiling water bath and stirred occasionally. After 1 hr the tubes were removed from the hot water bath and cooled to room temperature by placing in a cold water bath. The solution was then diluted to 25 ml with distilled water. After stirring thoroughly, the mixture was centrifuged for 20 min at 2,000 rpm.

Adsorption and Elution-Partial Decolorization: Ten ml aliquot of the extract was transfered into a 50 ml beaker. To this was added 2 ml 10N NaOH. The mixture was cooled, and the pH adjusted by drop-wise addition of concentrated HCl (pH 0.5-1.0). The mixture was transfered quantitatively to a centrifuge tube containing 2 g Lloyd's reagent (Hydrated aluminum silicate) using a small amount

of 0.2N H_2SO_4 as the wash solution. The mixture was stirred for one min and the stirring rod and the sides of the tube washed down with 0.2N H_2SO_4. It was then centrifuged (5 min at 2,000 rpm) and the supernatant liquid discarded. Next the precipitate was washed with 10 ml 0.2N H_2SO_4. It was then stirred to break up clumps of Lloyd's reagent. After washing the rod and the sides of the tube, it was centrifuged again and the supernatant discarded. Fifteen ml 0.5N NaOH was added to the precipitate which was again stirred for one minute after breaking up the clumps. The rod was washed and the solution diluted to 21.2 ml with distilled water. The solution was again centrifuged and the supernatant drained completely into another centrifuge tube containing 1 g of pulverized lead nitrate and one drop of phenolphthalein solution. The solution was stirred until the pink color disappeared and then centrifuged. It was decanted into another centrifuge tube containing one drop of phenolphthalein solution. The pink color was restored by adding a few crystals of K_3PO_4. Enough 20% H_3PO_4 solution to bring the pH to 4.5 was then added.

Color Development: Three corrections were made for the sample: (1) Aromatic amine correction

(2) Cyanogen bromide (CNBr) correction and

(3) Inert colored material correction

The tubes were set up as shown below; Four ml aliquots of sample extracts were added to the proper tubes, and 1 ml

Total Color Series	Amine Correction Series	CNBr. Correction Series	Color Correction Series
Instrument Blanks	(1)	(2)	(3)
7 ml H_2O	9 ml H_2O	7 ml H_2O	9 ml H_2O
1 ml KH_2PO_4	1 ml KH_2PO_4	1 ml KH_2PO_4	1 ml KH_2PO_4
2 ml CNBr.	-	2 ml CNBr.	-
10 ml Metol	10 ml Metol	-	-
-	-	10 ml 0.5N HCl	10 ml 0.5N HCl
Standard			
1 ml niacin working standard			
6 ml H_2O			
1 ml KH_2PO_4			
2 ml CNBr			
10 ml Metol			
Sample Tubes			
4 ml extract	4 ml extract	4 ml extract	4 ml extract
3 ml H_2O	5 ml H_2O	3 ml H_2O	5 ml H_2O
1 ml KH_2PO_4	1 ml KH_2PO_4	1 ml KH_2PO_4	1 ml KH_2PO_4
2 ml CNBr	-	2 ml CNBr	-
10 Metol	10 ml Metol	-	-
-	-	10 ml 0.5N HCl	10 ml 0.5N HCl

of working standard containing 25 µg niacin was added to the standard tube. To all the tubes 1 ml 10% KH_2PO_4 was added followed by water in amounts shown. All tubes were placed in a water bath at 70°C for 5 mins. Two ml CNBr was added to tubes indicated from a buret in a ventilated hood. Five min after the addition of CNBr to the first tube, the tube was transfered to a water bath at 25°C. Subsequent tubes were transfered at 15 sec intervals. Next 10 ml freshly prepared acid metol solution was added to those tubes indicated. After mixing, 10 ml 0.5N HCl was added to the designated tubes. The mixture was again mixed and placed in the dark for one hour. The absorbance readings were measured with a Beckman DU Spectrophotometer (400 nm).

Niacin in sample was calculated from the following formula:

µg Niacin/g Sample =

$$\frac{25}{\text{Standard absorbance}} \times \frac{\text{Corrected sample absorbance}}{\text{Sample wt}} \times$$

dilution factor

Corrected Absorbance = Total absorbance -((1)+(2)-(3))

Riboflavin (B_2): Riboflavin was determined according to the AOAC (1975) official method.

Seven grams of the bean sample were weighed into a 125 ml Erlenmeyer flask. Fifty ml of 0.1N HCl was added and the flask autoclaved for 30 mins. at 15 lb pressure.

After autoclaving and cooling, the sample was adjusted to pH 6.0 with NaOH. The NaOH was added in small increments with constant agitation to avoid local concentration, which would destroy the vitamin. Immediately following this 0.1N HCl was added to bring the pH to 4.5. The solution was diluted to 100 ml with water and filtered. To a 50 ml aliquot of the filtrate was added 0.1N HCl dropwide with constant agitation until no more precipitate occured. Next an approximately equal amount of drops of 0.1N NaOH was added to the solution, which was then diluted to mark with water.

Ten ml of sample solution and 1 ml H_2O were added to each of two test tubes. Another 10 ml of sample solution and 1 ml solution containing 0.5 µg riboflavin were added to another set of two test tubes. To each of the four test tubes, 1 ml glacial acetic acid was added followed by 0.5 ml 3% $KMnO_4$. After allowing to stand for 2 min 0.5 ml 3% H_2O_2 was added to all the tubes.

The fluorescence was measured with a Turner fluorometer (Model III), which was preset at lowest sensitivity and zeroed with a dummy cuvette. Blank measurements were made on the samples after adding with shaking, approximately 20 mg of sodium hydrosulfide ($Na_2S_2O_4$) to the each of the cuvettes.

$$\frac{\text{Riboflavin in sample}}{(\mu g/g)} = \frac{A-C_1}{B-C_2} \times \frac{\text{Riboflavin added}}{10 \text{ ml aliquot}} \times$$

$$\frac{\text{Dilution factor}}{\text{Sample wt.}}$$

A = Sample fluorometer Reading

B = Sample + Added Riboflavin Reading

C_1 and C_2 = Blanks of Sample, and Sample plus Riboflavin Standard

Mineral analysis

Dry finely ground samples were analyzed directly by Inductive Couple Plasma Emission Spectrograph at the Research Extension Analytical Lab., Ohio Agricultural Research and Developmental Center, Wooster, Ohio.

Sodium was also determined by the method outlined in the Perkin Elmer Corp. Laboratory Manual (1971).

One gram powdered sample was decomposed with 17 ml HNO_3 (conc) plus 3 ml perchloric acid ($HCLO_4$). The mixture was heated over an electric hot plate and the volume reduced to about 2 ml. It was then filtered and diluted to 25 ml with deionized water. The solution was analyzed in a Beckman DU spectrophotometer with the flame photometry attachment at 589 nm. The sodium concentration was read off from a standard curve prepared from standard solutions

of sodium chloride with potassium chloride solution as buffer.

Amino acid analysis

The samples were analyzed for their amino acid content using the Beckman amino acid analyzer, model 121. The analysis was performed according to the procedure of Moore et al. (1958).

About 18 mg of cowpea flour and 10 mg watermelon seed flour were weighed into 20 ml glass vials. Five ml 6N HCl was added to the vials. The mixtures were frozen by placing the vials in a dry-ice acetone bath. The vials were evacuated with a high vacuum pump. The mixtures were then refrozen and the caps tightly fitted. The vials and contents were then autoclaved for 16 hrs. at 121^{o}C and 15 lb pressure.

After hydrolysis the vials were cooled, and 1 ml of 2.5 μM norleucine solution was added to each vial as an internal standard to measure mechanical losses during transfer. The hydrolysates were evaporated to dryness on a rotary evaporator. The dried samples were washed three times with small amounts of deionized water and again brought to dryness. The dried hydrolysates were dissolved in lithium citrate buffer (pH 1.9) and diluted to a volume of 5 ml. The solutions were then filtered with a 0.22 μ Millipore filter.

A 0.5 ml aliquot of the sample solution was applied to the columns for amino acid analysis. The chromatograms were quantitated by the peak area method. Standard amino acid mixtures were analyzed by the same ninhydrin solution and used to identify the amino acids of the samples.

Sulfur containing amino acids

Methionine and cystine were analyzed separately because of their instability during acid hydrolysis. They were analyzed by the methods of Schram et al. (1954) and Lewis (1966). These methods involve oxidizing methionine and cystine to methionine sulfone and cysteic acid respectively with performic acid.

The performic acid solution was prepared by mixing one ml 30% (w/w) hydrogen peroxide with 9 ml 88% (w/w) formic acid. The mixture was cooled to $0^{o}C$.

Ten mg watermelon seed flour and about 18 mg cowpea flour were weighed into 25 ml pear-shaped flasks. Ten ml performic acid solution was added and oxidation carried out at $4^{o}C$ for 24 hrs.

After oxidation, the performic acid was removed in a rotary evaporator (30-$40^{o}C$). The dried sample was quantitatively transfered to 20 ml glass vials with 5 ml 6N redistilled HCl. Hydrolysis and amino acid analysis were performed as previously described.

Tryptophan

Tryptophan is very labile during acid hydrolysis, and after prolonged hydrolysis, little or none of the amino

acid is left. Therefore, it was determined colorimetrically after hydrolysis with the enzyme pronase as described by Spies (1967).

Thirty mg sample was weighed directly into a 10 ml glass vial with a screw cap.

To each vial 0.3 ml pronase hydrolytic solution and a drop of toluene, as preservative, was added. Pronase hydrolytic solution was prepared fresh by adding 50 mg pronase to 5 ml 0.1M phosphate buffer, pH 7.5. The suspension was shaken gently for 15 min., then clarified by centrifugation for 15 min. at 10,000 rpm.

The vials were closed and incubated for 24 hrs. at 40°C. After incubation, 0.9 ml 0.1M phosphate buffer, pH 7.5 was added to each vial. The uncapped vials were placed into 50 ml Erlenmeyer flasks containing 9.0 ml 21.2N H_2SO_4 and 30 mg dimethylaminobenzaldehyde (DAB). The vials were tipped over and the contents quickly mixed by rotating the flasks. Samples were cooled to room temperature and kept in the dark at 25°C for 6 hrs.

At the end of this period 0.1 ml 0.045% sodium nitrite solution was added to each flask. After gentle shaking, the flasks were left standing for 30 mins. for the development of the color. The absorbance was measured at 590 nm, with a Beckman DU spectrophotometer.

Duplicate blanks of the pronase hydrolytic solution were treated similarly and the tryptophan content of

pronase was subtracted from the total tryptophan content of sample.

A standard curve from zero to 120 μg tryptophan was prepared according to the procedure E described by Spies and Chambers (1948).

D,L tryptophan (2.4 mg) was dissolved in 20 ml 21.2N H_2SO_4 containing 60 mg of dimethylaminobenzaldehyde (DAB). 0, 1, 2, 4, 6, 8 and 10 ml of this solution were made up to 10 ml with solutions of 21.2N H_2SO_4 containing 600 mg DAB/200 ml, and placed in 50 ml Erlenmeyer flasks. The mixtures were kept in the dark at 25°C for six hrs., then 0.1 ml of 0.045% sodium nitrite was added to each flask. The flasks were allowed to stand 30 mins. for color development and the absorbance was measured at 590 nm, with a Beckman DU spectrophotometer. A straight line relationship was obtained between absorbance and tryptophan content.

Available lysine

The available lysine in the samples was analyzed according to the 2,4,6-trinitrobenzenesulfonic acid (TNBS) method of Kakade and Liener (1969).

Ten mg finely ground sample were weighed into a test tube containing 1 ml of $NaHCO_3$ solution. The suspension was placed in a constant-temperature shaking bath at 40°C for 10 min. One ml of 1.0% TNBS solution was then added to the suspension. The reaction was allowed to proceed at 40°C for 2 hr. at which time 3 ml concentrated HCl was

added. Small round marbles were placed over the mouth of each tube, and the reaction mixture was autoclaved at 121°C (15-17 psi) for 1 hr. After the hydrolysate had been allowed to cool to room temperature, 5 ml of distilled water was added, and the mixture was filtered. The filtrate was transfered to a glass-stoppered test tube and extracted twice with 10 ml ethyl ether in order to remove TNP-N-terminal amino acids or peptides as well as picric acid, which is also produced during the course of the reaction. Residual ether was eliminated from the aqueous phase by placing each tube in hot water bath for 10 mins.

The aqueous solution was read at 346 nm with a Beckman DU spectrophotometer against a blank carried through the same procedure except that the HCl (conc.) was added to the protein solution prior to the addition of the TNBS reagent. (The TNBS in acid medium has no appreciable absorption at a wavelength longer than 300 nm).

The amount of ε-TNP-Lysine was calculated from equation;

$$C(\text{mole/L}) = \frac{A}{\varepsilon b}$$

where A = absorbance

ε = molar absorptivity (1.46×10^4 M^{-1} cm^{-1})

b = path length (1 cm)

Biological evaluation of protein quality

Protein Efficiency Ratio (PER)

Weanling male rats of the Sprague Dawley strain, 21 days of age were used for the experiment. The rats were purchased from Spartan Research Animals, Inc., Haslett, Michigan. The experiment was carried out according to the AOAC (1975) official method.

Ten rats were assigned to each diet, and there were eight diets with one of them containing the ANRC reference casein. Rats were housed individually in stainless steel cages in a room at 23°C. Assay diets and water were offered ad libitum. The animals were weighed once a week and their food intake and waste also measured once a week. The total experimental period for PER determination was 21 days.

Prior to the 21 days test period, the animals were weighed and distributed randomly among the groups. They were also weighed on the first day of the experimental period. The initial average weight of the groups varied from 58.4 g to 63.6 g. The acclimatization period was 3 days during which the rats were fed standard rat diet.

The materials under test were fed as the sole source of protein at the 10% protein level. The composition of the basal diet is shown in Table 2.

The average 21 days weight gain and protein (Nx6.25) intake per rat for each group were calculated. The Protein

Table 2. Composition of basal diet for the PER

Ingredients	Amount %
Protein[1]	10
Corn Oil	8
Salt mixture[2]	5
Vitamin mixture[3]	1
Non-nutritive fiber	1
Water	5
Corn Starch To Complete	100

[1]Vitamin-free Casein was purchased from Teklad Test Diets, P.O. Box 4220, Madison, Wisconsin. Protein Content: 87.0%. All the rations contained the equivalent of 10% protein, coming primarily from; (1) Casein 100%, (2) Cowpeas 100%, (3) Sesame flour (Sf) 100%, (4) Watermelon flour (Wmf) 100%, (5) Cowpeas/Sf 50/50, (6) Cowpeas/Sf 75/25, (7) Cowpeas/Wmf 50/50, (8) Cowpeas/Wmf 75/25 (ratios refer to protein).

[2]USP XVIII, (ICN Nutritional Biochemicals, 26201 Miles Road, Cleveland, OH, 44128). Composition (%): Sodium chloride (NaCl), 13.93; Potassium iodide (KI), 0.079; Potassium phosphate monobasic (KH_2PO_4), 38.90; Magnesium sulfate ($MgSO_4$), 5.73; Calcium carbonate ($CaCO_3$), 38.14; Ferrous sulfate ($FeSO_4 \cdot 7H_2O$), 2.7; Manganese sulfate ($MnSO_4:H_2O$), 0.548; Cupric sulfate ($CuSO_4 \cdot 5H_2O$), 0.0477; Cobalt chloride ($CoCl_2 \cdot 6H_2O$), 0.0023.

[3]Vitamin mixture contained (mg/100 g ration): Vitamin A, 2000 (IU); Vitamin D, 200 (IU); Vitamin E, 10 (IU); Menadione, 0.5; Choline, 200; P-Aminobenzoic acid, 10; Inositol, 10; Niacin, 4; Ca-D-pantothenate, 4; Riboflavin, 0.8; thiamine·HCl, 0.5; pyridoxine.HCl, 0.5, folic acid, 0.2; Biotin, 0.04; Vitamin B_{12}, 0.003.

The nitrogen sources used in the diets (except Casein) contained different amounts of oil, ash, fiber and water and these were taken into account in computing the above percentages.

Efficiency Ratio (Weight gain/Protein intake) was then determined for each group.

Digestibility Studies (Apparent)

1. Protein: The apparent protein digestibility was determined during the PER studies. It started after the animals have been on the test diets for 14 days and lasted for seven days. Carmine Alum Lake (Carmine-red) was added to the diet as a marker. Appearance of marked faeces (red) indicated the start of faecal collection. At the end of the seventh day, the rats were placed back on the dye-free diet and the marked faeces collected for two more days (that is at the first appearance of unmarked faeces). The feed intake for the period, 14th to 21st day, was calculated and from this the protein intake was calculated. The total faecal output was measured, a representative portion was dried, ground and used for the faecal protein (Nx6.25) determination.

Apparent protein digestibility =

$$\frac{\text{Protein intake (g) - Faecal Protein (g)}}{\text{Protein intake (g)}}$$

2. Feed digestibility: This was calculated from the data on food consumed and the faecal output both on a dry basis during the seven days protein digestibility studies.

Apparent Feed digestibility =

$$\frac{\text{Feed Intake (g) - Faecal Output (g)}}{\text{Feed Intake (g)}}$$

Feed Efficiency Ratio

This ratio was calculated from the average total food consumed and the average total weight gain of the animals during the 21 days experimental period.

$$\text{Feed Efficiency Ratio} = \frac{\text{Average Total Feed Intake (g)}}{\text{Average Total Weight gain (g)}}$$

RESULTS AND DISCUSSION

Cowpea flour preparation

Three simple methods for preparing a convenient cowpea flour were described. In each of these methods soaking was emphasized. Soaking was necessary to facilitate the removal of the seed coat. Soaking for up to 18 hr. softens the seed coat making it easier to rub off with the hand. (The presence of dark specks from the hilum or "eye" of the beans in most cowpea dishes is considered unacceptable. It is therefore necessary to remove the seed coats which contain these "eyes". Also, removal of the seed coats results in bean flours with improved binding quality (Dovlo et al., 1976)). With a large quantity of beans to be dehulled, like in this experiment, however, the operation became a painful chore. Another method was thus sought which could reduce the labor involved. Blanching the beans in water before soaking was then tried. While this method reduced the soaking time from 18 hr. to 6 hr., it did not improve the ease with which the seed coats were removed. Moreover, like in the first method, the rubbed off seed coats were removed by floatation in water, a process which could prove very expensive in most developing countries

where water is often in short supply.

To overcome these problems the third method was tried. This method reduced the labor involved in the hand rubbing operation by grinding the soaked dried whole raw beans in a blender. The seed coats now brittle came off easily and were then removed by air blast which again reduced the water wastage.

One important advantage which was common to all the three methods was their simple drying process. The room temperature air drying operation can be replaced by sun drying thus making these methods possible at the village level.

All the flours from the three methods were free-flowing, non-hygroscopic and creamy white in color as opposed to the white color of the raw beans. The color change was probably due to non-enzymatic type browning involving the sugar and the amine groups in the bean flour (Love and Dugan, 1978; Counter, 1969).

Composition of raw and processed samples

Table 3 shows the results of the proximate analysis of the cowpea, the watermelon and sesame seeds before and after they were processed into flours. The moisture, protein, fiber, ash, lipids, and carbohydrate contents of the samples agree well with reported literature values (Oyenuga, 1968; Lyon, 1972; Boloorforooshan and Markakis, 1979; Akpapunam and Markakis, 1979). There was

Table 3. Proximate composition of two varieties of watermelon seed (defatted and full fat), sesame seed (defatted and full fat), and cowpea flours (raw whole and processed). % dry sample.

	Moisture	Protein	Fiber	Ash	Lipid	Carbohydrate (by difference)
Watermelon (American) Full fat	3.1	28.8	5.8	4.2	48.4	9.7
Watermelon (American) Defatted	6.6	51.5	5.6	7.9	0.8	25.2
Watermelon (Nigerian) Full fat	2.7	24.2	3.0	3.7	54.8	11.6
Watermelon (Nigerian) Defatted	6.5	51.4	2.6	7.8	4.0	27.7
Sesame, full fat	3.3	23.1	3.4	2.9	56.9	10.6
Sesame, defatted	8.7	50.7	3.1	5.8	7.5	24.2
Raw whole cowpea	7.0	24.0	1.5	3.2	2.3	62.0
Cowpea powder, (Soaked 18 hr. dehulled and autoclaved)	4.5	27.9	1.8	3.9	2.1	59.8

not much difference between the contents of these components in the Nigerian watermelon and the American variety. The reason for the lower oil content in the American variety could be due to the higher fiber content of the meal rather than varietal difference. The high fiber content was due to the fact that not all the pieces of the shells of the watermelon seed were removed during the processing. The carbohydrate content of the samples was obtained by difference. The result shows that the oil seeds were very low in carbohydrate. The cowpea on the other hand was very high in carbohydrate.

Effect of processing on some physical and chemical properties of cowpea flour

Reconstitution

The flours produced from the soaked, dehulled, and autoclaved beans reconstituted fairly better than the flours from beans blanched prior to being soaked, dehulled, and autoclaved. Both flours reconstituted much better than the raw bean flour. They both settled out at the rate of 6 and 8 ml in 10 mins. respectively, as compared to 45 ml for the raw bean flour (Table 4). The fairly good reconstitution of the processed flours showed the need for some form of heat treatment of the beans before or after they are processed into flour. The heat treatment probably gelatinized the starches in the beans, resulting

Table 4. Effect of processing on the nitrogen solubility index, reconstitution, and bulk density of cowpea flour

Treatment	Nitrogen solubility index, %	Reconstitution (ml sedimentation after 10 min.)	Bulk density (g/cc.) Loose	Packed
Raw whole cowpea (50 mesh)	30.5	45	0.44	0.68
Cowpea dehulled, and autoclaved after;				
Soaking (18 hr.) 50 mesh	26.6	6	0.57	0.69
Blanching, and soaking (6 hr.) 50 mesh	27.1	8	0.61	0.72

in flours that disperse readily in water to give a moderately stable suspension.

Bulk density

The results of the bulk densities of the raw and processed cowpea flours are given in Table 4. The relatively higher bulk density of the processed flours as compared to the raw flour shows that they can be packaged more economically. The processed flours appear to settle equally after mechanical agitation. The flours settled at a rate one-half that of the raw flours. This is advantageous, because less flour will settle out during transportation causing less headspace or empty volume in the package.

Nitrogen Solubility Index (NSI)

The NSI of the raw cowpea flour was about 31% as compared to about 27% for the two processed cowpea flours respectively (Table 4). This is a 13% decrease in NSI compared to 23% decrease reported by Onayemi and Potter (1976) for their drum-dried cowpea flour. This is an indication that the blanching and autoclaving processes were less destructive to the proteins than the drum-drying operation.

Phytic acid and phytate-phosphorous of cowpeas

The raw whole cowpea was found to contain about 1% phytic acid and about 0.3% phytate-phosphorous (Table 5). These values are much higher than that reported for an

Indian cowpea by Kumar et al. (1978). Apart from varietal differences the lower values reported by these workers could have been due to the method used to extract the phytate compounds. They extracted the phytate with 0.5N HCl, a method reported to extract less phytate phosphorous (Wheeler and Ferrel, 1971) than the TCA extraction used in this experiment. The cowpea seed coat contained about 0.2% phytic acid, this indicates that a large amount of the phytate is concentrated in the endosperm. Neither the 18 hr soaking nor the blanching prior to soaking and autoclaving of the dehulled beans had any effect on the phytate content. Based on the total phosphorous of the raw beans and its processed flours (Table 7) it is seen that a large part of the phosphorous in the beans is present as phytin. The phytate-phosphorous as percentage of total phosphorous is as follows; 80% in the raw whole cowpea, 67% in the seed coat, and 83% and 100% in the unblanched and blanched flours, respectively. The last two values are unique in that they indicate that only the free phosphorous was lost during the processing.

Vitamin Content of Cowpeas

Table 6 shows the effect of processing on the thiamine, riboflavin and niacin contents of cowpea in mg/100 g dry sample. The thiamine, and the riboflavin values agreed very well with values reported by Ogunmodede and Oyenuga (1969). The niacin value was much higher than the value

Table 5. Phytic acid and phytate-phosphorous contents of raw whole cowpeas, cowpea seed coat, and processed cowpea flour. g/100 g dry sample.

Treatment	Phytic acid	Phytate-phosphorous
Raw whole cowpea	0.98	0.28
Cowpea seed coat	0.22	0.06
Cowpea, dehulled, and autoclaved after;		
Soaking (18 hrs.)	1.02	0.29
Blanching, and soaking (6 hrs.)	1.05	0.29

reported by these workers, but falls into the range reported for cowpea by Elias et al. (1963) and Dovlo et al. (1976). The greatest effect of processing was on niacin with 31% destruction in the unblanched beans and 34% in the blanched beans, compared to 11% and 14% destruction of thiamine and 12% and 13% destruction of riboflavin for the two process treatments respectively.

Mineral composition of cowpeas

Table 7 shows the distribution of minerals in the raw cowpeas and the effect of processing on these minerals. The values for the dehulled raw cowpeas were obtained by calculation. The results show that cowpeas are high in potassium, fairly high in phosphorous, calcium and magnesium and contain a host of trace mineral elements. The values reported here agree fairly well with literature values (Oyenuga, 1968; Singh et al., 1968; Meiners et al., 1978).

Although the seed coat contains large quantities of minerals, its removal does not amount to considerable impoverishment of the processed cowpea flour, because the seed coat representa a very small fraction (6.0%) of the whole seed.

When the mineral content of the processed cowpea flours is compared to that of the dehulled raw cowpeas, a loss of phosphorous, potassium, magnesium and boron is observed on the flours. This loss may be attributed to leaching

Table 6. Effect of processing on the vitamin content of cowpeas. mg/100 g dry sample.

Treatment	Vitamins		
	Thiamine	Riboflavin	Niacin
Raw whole cowpea	0.91	0.105	2.30
Cowpea dehulled, and autoclaved after;			
Soaking (18 hrs.)	0.81	0.092	1.59
Blanching, and soaking (6 hrs.)	0.78	0.091	1.51

during the soaking in water. A reversed trend to the above is observed with calcium and sodium. These two minerals increased in the processed flours. The reason for the increases is probably absorption of these two minerals from the soaking water by the cowpeas.

A third comparison can be made between flours 1 and 2. Flour 1, which was only soaked, shows higher retention of certain minerals namely phosphorous, potassium, and magnesium, than flour 2, which was blanched prior to soaking. This is probably due to wilting of the seed coat by blanching and thereby easier escape of these minerals into the soak water. Although both flours 1 and 2 show considerable increases in their calcium and sodium contents, higher increases are observed in flour 2, which was blanched before soaking. As stated earlier, the increases are probably due to absorption of these two minerals from the soaking water by the cowpeas. The wilting of the seed coats by blanching again might have facilitated the increased uptake of these minerals in the water.

Amino acid composition of cowpeas, watermelon and sesame flours

Table 8 shows the total amino acid composition of autoclaved cowpea flour, two varieties of watermelon flour and sesame flour. The sesame seeds used in this experiment came from the same batch as that used by Boloorforooshan and

Table 7. Mineral composition of raw whole cowpeas, dehulled raw cowpeas, cowpea seed coat, and processed cowpea flours. mg/100 g dry sample.

Minerals	Cowpea[a] seedcoat	Whole raw cowpea	Dehulled[b] raw cowpea	Processed cowpea flours 1	2
Phosphorous	87.3	354.4	371.5	328.8	277.9
Potassium	172.9	1515.1	1600.8	611.9	459.5
Calcium	901.3	115.5	65.3	110.1	115.8
Magnesium	461.0	216.1	200.4	167.1	120.9
Sodium	104.6	10.6	4.60	21.5	38.1
Manganese	2.42	1.70	1.65	1.65	1.75
Iron	11.7	7.42	7.15	6.60	7.14
Boron	4.17	2.30	2.18	1.55	1.30
Copper	5.57	0.97	0.68	0.73	0.65
Zinc	3.89	4.53	4.50	4.57	4.06
Aluminum	0.47	Trace	--	Trace	Trace
Strontium	2.93	0.23	0.01	0.27	0.39
Barium	1.50	0.07	--	0.12	0.20

[a]Seed coat forms 6.04% of whole cowpea seed. [b]Value obtained by calculation.
[1]Cowpea dehulled and autoclaved after soaking 18 hrs.
[2]Cowpea dehulled and autoclaved after blanching and soaking 6 hrs.

Markakis (1979); consequently the amino acid composition was that reported by them. The result shows that cowpeas, like most legumes, are a good source of the essential amino acids isoleucine, leucine, phenylalanine, threonine, lysine, and valine, but a poor source of sulfur amino acids (methionine and cystine). The amino acid composition of the watermelon and sesame proteins are very similar. Both are fairly balanced in their amino acid composition. Unlike cowpeas they are poor sources of lysine, but very good sources of sulfur amino acids, particularly sesame protein. In the light of this there is a chance of supplementation between the cowpea protein and the oil seed proteins.

Because the American variety of watermelon seed was used for this experiment, an attempt was made to analyse and compare the amino acid composition of the Nigerian variety with the American variety. The result show that the two varieties compared very well in almost all the amino acids (Table 8).

Available lysine of the flours

The nutritional quality of vegetable proteins is said to correlate directly with the number of epsilon amino groups of lysine in the proteins that are free to react with 1-fluoro-2,4-dinitrobenzene (FDNB). The number of such groups is decreased when the sources of proteins are subjected to processing (El-Nockrashy and Frampton, 1967;

Table 8. Amino acid composition of cowpeas (Vigna unguiculata), defatted watermelon seed flour, and sesame seed flour. (g/16 g N)

	Cowpea[A]	Watermelon seed		Sesame seed[B]
		Nigerian Variety	American Variety	
Aspartic acid	11.2	7.4	7.8	6.7
Threonine	4.2	3.9	3.8	3.0
Serine	5.4	4.4	4.2	3.6
Glutamic acid	15.2	13.8	14.2	17.1
Proline	3.9	2.7	2.6	3.0
Glycine	3.8	4.8	4.4	3.6
Alanine	5.5	5.5	5.4	3.5
Valine	5.8	4.6	4.2	3.9
Isoleucine	4.7	3.5	3.3	3.0
Leucine	8.8	6.6	6.4	5.4
Tyrosine	4.9	2.6	3.0	2.9
Phenylalanine	6.6	5.0	4.9	3.9
Histidine	3.0	1.8	1.8	2.2
Lysine	6.4	2.9	2.7	2.3
Arginine	8.2	12.0	12.2	11.6
Methionine	1.4	2.4	2.5	3.1
Half-Cystine	0.5	1.0	1.1	1.6
Total sulfur amino acid	1.9	3.4	3.6	4.7
Tryptophan	1.3	1.8	1.9	1.5

[A](processed cowpea powder - soaked 18 hrs., dehulled and autoclaved).

[B]Data taken from Boloorforooshan and Markakis (1979).

Villegas et al., 1968; Carpenter, 1973). The possibility of such changes was investigated in this experiment. The determination was done by the 2,4,6-trinitrobenzene sulfonic acid (TNBS) method of Kakade and Liener (1969). This method is simple and rapid and correlates very well with the commonly used FDNB method.

The result in Table 9 shows that over 90% of the lysine in all the flours are available. This is a fairly good stability for lysine in these flours. It indicates that the flours did not undergo substantial Maillard-type changes or the 1-2 glycosidic interactions between the nonreducing sugars and the lysine of the flours (El-Nockrashy and Frampton, 1967).

Biological evaluation of protein quality

The protein sources used for this part of the studies came from casein, autoclaved cowpea flour (soaked 18 hrs. and dehulled), defatted watermelon (American variety) and defatted sesame flours.

A. Nutritional qualities of cowpea, watermelon and sesame proteins

The average values of the daily weight gain, daily feed intake, PER, feed efficiency ratio and digestibility are shown in Table 10. Also shown in Figure 1 are the growth curves for the rat feeding experiment. As indicated in these results, the cowpea, sesame and watermelon proteins were inferior to the reference casein in all the parameters

Table 9. Available lysine content of dehulled, autoclaved cowpeas, watermelon, and sesame flours

	Cowpea flour	Watermelon flour		Sesame flour
		American	Nigerian	
Total lysine (g/16 g N)	6.4	2.7	2.9	2.3
Available lysine (g/16 g N)	6.0	2.5	2.7	2.2
% Availability	94.1	92.6	93.1	95.7

measured. The cowpea protein with its high lysine content supported greater growth and had higher PER than the sesame and watermelon proteins respectively. This seems to highlight the greater requirement for lysine over the sulfur amino acids by the growing rat. The watermelon protein was slightly lower in total sulfur amino acid than the sesame protein. This slight difference was evidenced in the poorer nutritional quality of the watermelon protein. Since all the diets were well digested one may conclude that the poor nutritional qualities of the cowpea, sesame, and watermelon proteins were due to a poor over-all amino acid balance or perhaps to the lower digestibility of the proteins, resulting in a decreased availability of the amino acids for the growing rat. The results of this experiment agreed fairly well with reported literature values. Elias et al. (1964) reported a PER range of 1.42 to 2.30 for eight varieties of cooked cowpeas. Onayemi and Potter (1976) obtained a PER of 1.64 (casein = 2.50) and an average daily gain of 1.4 g for a drum dried cowpea powder. Boloorforooshan and Markakis (1979) obtained a PER of 1.57 and an average daily gain of 1.31 g for sesame protein. On a 9 day trial period, Oyenuga (1968) reported a daily gain of 1.02 for rats fed watermelon flour as the sole source of protein in the diet.

Table 10. Weight gain, feed intake, PER, feed efficiency ratio, and digestibility of the feed samples fed to the rats for 21 days

Diets	Average daily weight gain, g	Average daily feed intake, g	PER	Adjusted PER[2]	Feed[a] efficiency ratio	% digestibility[b] Feed	% digestibility[b] Protein
Casein	4.14	12.9	3.20±0.23[C]	2.50	3.12	95.2	90.0
Cowpea flour (cf)	2.64	12.1	2.19±0.18[B]	1.71	4.60	93.9	80.1
Sesame flour (sf)	1.62	10.3	1.56±0.11[A]	1.22	6.38	91.8	81.2
Watermelon flour (wmf)	1.32	9.6	1.37±0.11[A]	1.07	7.30	91.5	79.2
Cf + sf (75:25)	4.46	15.3	2.91±0.14[C]	2.27	3.43	93.8	81.1
Cf + sf (50:50)	4.93	15.8	3.12±0.22[C]	2.44	3.20	92.6	80.5
Cf + wmf (75:25)	3.82	13.6	2.82±0.21[C]	2.20	3.57	93.5	80.7
Cf + wmf (50:50)	4.42	15.1	2.92±0.17[C]	2.28	3.42	92.8	79.4

1 (PER=Protein Efficiency Ratio) Means with the same letter are not significantly different. (Duncan's Multiple Range Test. $P<0.05$).
± Standard Error of the Mean (SEM).
2 Corrected for Casein = 2.50.
a Feed intake (g)/Weight gain (g).
b Protein = (Protein intake - Fecal protein)/Protein intake x 100.
Feed = (Feed intake - Fecal output)/Food intake x 100.

B. Comparison of the supplementary effects of watermelon and sesame proteins on cowpea protein

Supplementing watermelon and sesame flours into cowpea flours at 75:25 and 50:50 protein ratios improved the weight gain of the rats considerably (Table 10 and Figure 1). On a daily basis, the blends, with the exception of the cowpea flour:watermelon flour 75:25 ratio, supported greater gains than the reference casein. The protein efficiency ratios (PER) of the blends were significantly greater ($P<0.05$) than those of either the cowpea or the oil seeds alone but did not differ significantly ($P<0.05$) from that of the casein control. The efficiency with which the feeds were utilized was greatly improved by supplementing watermelon and sesame proteins into cowpea protein. There were no appreciable changes in the digestibility of either the feed or the protein as a result of the supplementation. Based on these results, one may conclude that the poor nutritional qualities of the diets in which all the proteins were contributed by either the cowpea or each of the oil seeds was due to a poor over-all amino acid balance, rather than low protein digestibility. Similar views were expressed by Onayemi and Potter (1976), who upgraded the PER of their drum dried cowpea powder by supplementing methionine at levels of 0.4% and 0.6% respectively and by Sherwood et al. (1954). Lyon (1972) attributed the

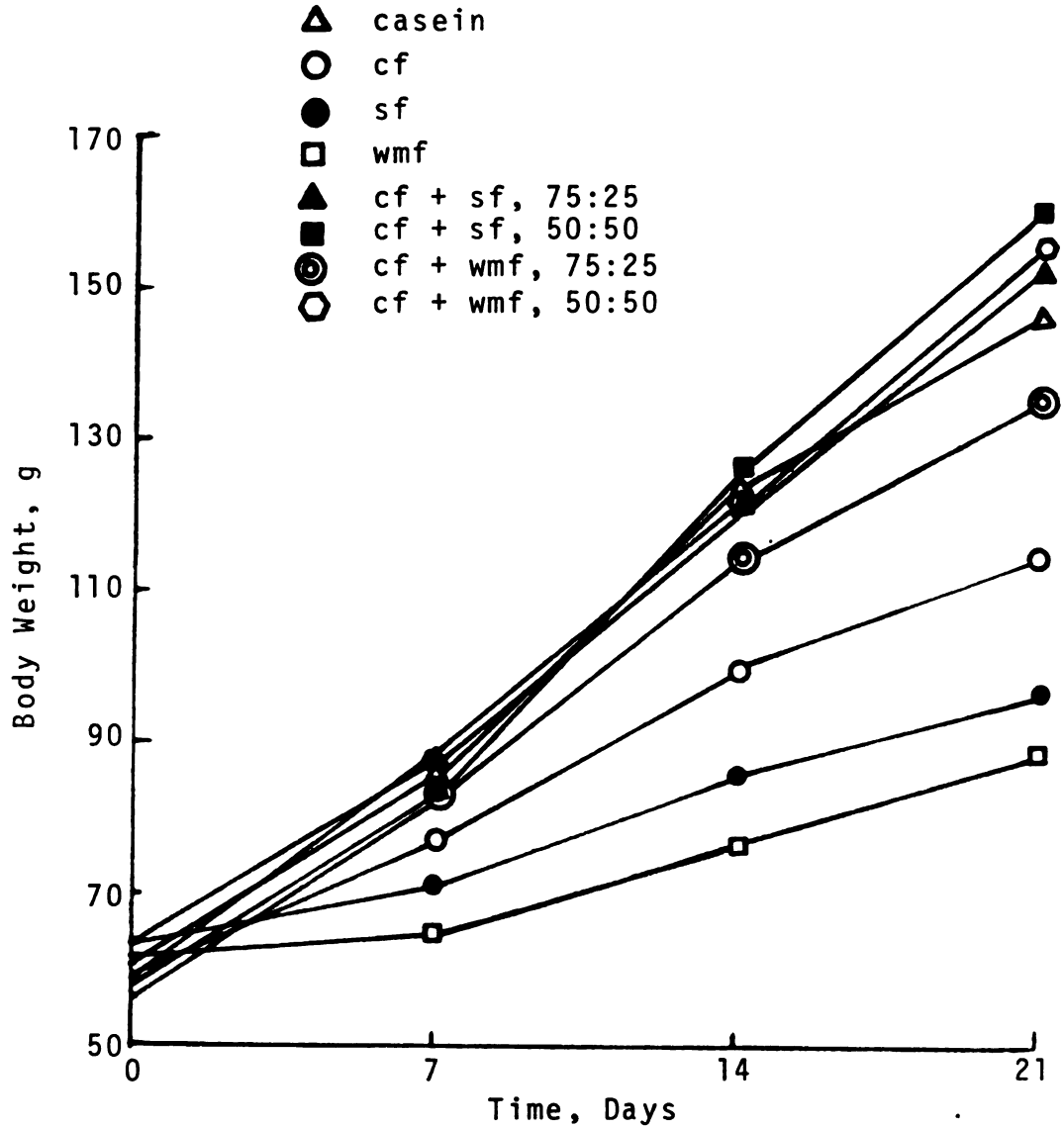

Figure 1. Average body weight of weanling rats fed standard diets containing: casein, autoclaved cowpea flour (cf), sesame flour (sf), watermelon flour (wmf), cf + sf, 75:25, and 50:50 protein ratios, and cf + wmf, 75:25 and 50:50 protein ratios.

relatively low protein quality of sesame meal to its deficiency in lysine and somewhat in isoleucine.

In general supplementation of sesame protein with cowpea protein produced greater weight gain and higher PER (differences not significant at P 0.05) than the watermelon:cowpea combinations. This seems to reflect the differences in the amino acid profiles of these oil seeds. Both protein sources were low in lysine and added to this, watermelon protein was border line in total sulfur amino acid content. This result also confirmed the earlier result which showed that sesame protein was slightly superior to watermelon protein, when both were added individually to the diet as the sole source of protein. It is important to note that with each of the combinations the protein quality improved when the protein from either the sesame or watermelon was increased from 25% to 50%. The most efficient diet combination contained 50% of protein from cowpea and 50% from sesame, which is equivalent to 65% cowpea and 35% sesame by weight.

SUMMARY AND CONCLUSIONS

Three simple, practical procedures have been developed for the preparation of convenient cowpea flour. For economic reasons and for greater ease of testa removal, the method which involved the redrying of the presoaked, raw whole bean is recommended. The autoclaved convenient cowpea flours were free-flowing, non-hygroscopic and creamy white in color. The flours reconstituted very well in both cold and hot water.

The process treatments did not affect seriously the minerals, vitamins and the protein content of the flours.

The results of the rat growth experiment showed that the quality of cowpea protein can be greatly improved by supplementation with watermelon or sesame proteins. Because of the greater demand for lysine and methionine by the growing rat than man, it is believed that the combination of either cowpea and sesame or watermelon is capable of supporting growth in man very effectively.

RECOMMENDATION

Because the samples were in short supply it was not possible to prepare products for organoleptic analysis. It is therefore recommended that future experiments should include human sensory testing, particularly of local dishes containing the new flours. Storage stability of the autoclaved cowpea flour and the blends should also be studied at temperatures simulating tropical environment.

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