DEVELOPMENT AND EVALUATION OF A MILLET-BASED INFANT FOOD FORTIFIED WITH PLANT PROTEIN

By

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DEDICATION

To Steve, my dear Papa for teaching me the value of education

and

Sam, Maamie and Nana for their encouragement

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To God be the glory, great things He has done!

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ABSTRACT

Samples of low-cost protein rich millet based infant food fortified with cowpea, were formulated and analysed for their quality and acceptability. Blends were formulated with a millet base constitution of malted, roasted and raw millet with cowpea substitution at 20%, 25% and 30%. Addition of cowpea increased the protein content of the blends up to 7.5%. The crude fat and crude fibre contents were all within acceptable levels. Cooking of the blends further increased the protein content. Increase in Protein Efficiency Ratio was significant ($P<0.05$) for the 30% substituted cooked blend. SGOT and SGPT levels were normal for all the blends. Sensory scores were all above average for all the blends. However, it was observed that all the blends required fortification with minerals.

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CHAPTER ONE

INTRODUCTION

The lack of an appropriate weaning food has been identified as a major contributory factor to the widespread incidence of infant malnutrition in developing countries. Observations on traditional infant feeding practices in many developing countries revealed that the weaning period, defined as the whole period during which breast milk is gradually replaced with other foods, usually starts when the infant is four to six months old and is extended to the age of two to three years (UNICEF, 1985).

Usually, weaning foods given to Ghanaian children are gruels prepared from cereals such as rice, sorghum or millet. Their starchy nature allows these foods to bind large volumes of water to yield gruels that are inadequate in energy and nutrient content (ICMR,1977; Jelliffe, 1968). The high dilution level also increases bulk and renders the food more difficult to consume at one sitting; thus limiting the amount of nutrient that could have been derived from it. On the other hand, if the solids in the gruel are increased to improve the nutrient concentration the gruel might be too thick and cause choking in infants.

Industrial preparations of cereal-based weaning foods often include operations intended to reduce dietary bulk (i.e. high volume/viscosity of diet). Examples of such operations include enzyme (amylase) treatment, precooking and extrusion. Although these processes result in lower water binding in the gruels, as sophisticated technologies, they yield rather expensive products (Anon, 1970). In developing countries, these products are usually available only to the urban children of higher income families. A relatively inexpensive alternative to these industrial processes could be an increased use of improved traditional food preparation procedures that will modify starch structures. One such procedure that is widely known and used is, germination.

Germination of cereals is mainly associated with the production of local alcoholic beverages. In Ghana the process is associated with production of beverages like 'pito', 'nmeidan', 'brukutu' among others, from millet, sorghum or maize. To a lesser extent, however, the malting process is used in the preparation of local weaning foods with low dietary bulk (Mosha and Svanberg, 1983).

The quest for reducing the incidence of the Protein-Energy Malnutrition has led many workers in this field to produce various cereal based weaning foods which are nutritionally balanced, can be prepared domestically, as well as being affordable by the low income group. Efforts are being focused on the utilization of cowpeas, soybeans and other locally available legumes in weaning food formulations (Plahar and Swanson, 1990). Previous studies have been conducted to establish the properties, acceptability and storage stability of traditional weaning foods fortified with legume protein sources in West Africa (Ekpenyong, et al., 1977; Plahar, et al., 1983; Plahar and Leung, 1983; 1985). To improve the nutritional content and quality of these infant foods, the cereal proteins which are known to be deficient in lysine, is supplemented with Iysine in plant proteins such as legumes - pulses and oil seeds. The legume used depends on availability and acceptability.

Oil seeds and pulses have been evaluated either singly or in various combinations and found to be effective protein supplements to cereals (Bressani and Elias,1966; Eyeson and Plahar, 1988). Del Angel and Sotello (1982) reported the use of cereal and legume combinations to improve the nutritive value of food proteins for human consumption. In parts of Africa 'ladylac' and 'superamine' prepared from millet and peanut and wheat and legume respectively are being used as weaning foods (Ihekoronye and Ngoddy, 1985). In Ghana Nutrition surveys carried out over the years have indicated a high prevalence of malnutrition which has been attributed in part to poor weaning practices (Eyeson and Plahar, 1988). In 1974 it was recommended at a Food and Nutrition Conference that suitable local weaning foods are urgently needed especially mixtures that can be made and appreciated by the mothers.

Efforts of individual workers and organizations have come out with various cereal-legume blends which are nutritionally sound. At the Food Research Institute of the Council for Scientific and Industrial Research in Ghana, a great deal of work is being carried out on the formulation of vegetable protein mixtures based on locally available legumes such as wingedbean, soybean, cowpea, groundnut and pigeon pea. Some of the products developed include "Lactompea (Plain)", "Lactompea (G-Plus)", "Browinlac" and "FRI-

Weaner" (Hoyle, 1988; Eyeson and Plahar, 1988; Plahar and Hoyle 1991; Plahar, 1992).

The Nutrition division of the Ministry of Health with the help of the Joint FAO/WHO/OAU Regional Food and Nutrition Commission for Africa embarked on a pilot project to help in weaning formulations based on available legumes and cereals. The product, "Weanimix", is being promoted in towns and villages through Nutrition Extension Officers in the Regions. It is a blend of a local cereal available in a particular region with a local legume and oil seed especially cowpea and groundnut. However, the cereal-base for most of the common locally prepared weaning foods is maize (Eyeson and Plahar,1988).

The improper ways of handling infant weaning foods during preparation and feeding have been found to contribute to the high incidence of diarrhoea in these infants. This has been attributed mainly to improper cooking, lack of personal hygiene and, to a greater extent, lack of time available to nursing mothers (Hofvander and Underwood, 1987; Leverton, 1954). Women, and for that matter mothers are gradually drifting away from the home to work in crder to supplement the family income, as a result of the increasing economic constraints. Very little time is therefore available to the nursing mother for proper care of the weaning infant.

To overcome the problems of Protein-Energy Malnutrition which is being aggravated by the ever-increasing socio-economic constraints, there is the need for the development of highly nutritious, readily available infant formula based on local legumes and cereals. Such preparation must have high nutrient density and reduced bulk.

OBJECTIVES OF STUDY

1. To develop a high protein precooked weaning food based on malted millet and cowpea.

2. To determine the effects of grain malting and roasting on nutrient availability, reduced bulk and increased nutrient density.

3. To evaluate different blends of millet and cowpea developed in terms of consumer acceptability, functional characteristics, microbiological quality and nutritive value.

4. To investigate the effects of millet/cowpea precooked weaning formulations on the levels of the serum enzymes SGOT and SGPT in experimental animals.

CHAPTER TWO

LITERATURE REVIEW

2.0 Baby/Weaning Foods

The development of a baby is known to be very rapid in the first year of growth (Udo, 1980). This can only be achieved if the food ingested is nutritionally balanced to meet the growing nutritional demands of the body. Thus baby foods are critical in the life of an infant in as much as the growth, health and general well-being of the baby depends on the type of food consumed (ibid).

It has been recognized that breast milk is adequate to meet the nutritional needs of younger infants under most circumstances. Younger infants require a milk product as the major food, but formulated foods that contain a high proportion of milk are, generally too expensive for most less privileged population groups (Beaton and Ghassemi, 1982)

Baby foods are intended to be traditional foods between a diet consisting solely of milk and of staple foods that are consumed by the entire family (Young *et al.*, 1981). They may be used as part of the total diet or as the only food under unusual circumstances, but when supplementary foods are used they must be balanced, nutritional, digestible, non-irritating, high in protein, iron and vitamins (Jelliffe, 1968). They must be small in quantity but high in nutritive value, palatable and easy to prepare (Hofvander and Cameron, 1983; Leverton, 1954). They can also be formulated or manufactured specifically to meet the nutritional demands of the infant and in a form that is convenient and reassuring to the mother (Yeung *et al*, 1981).

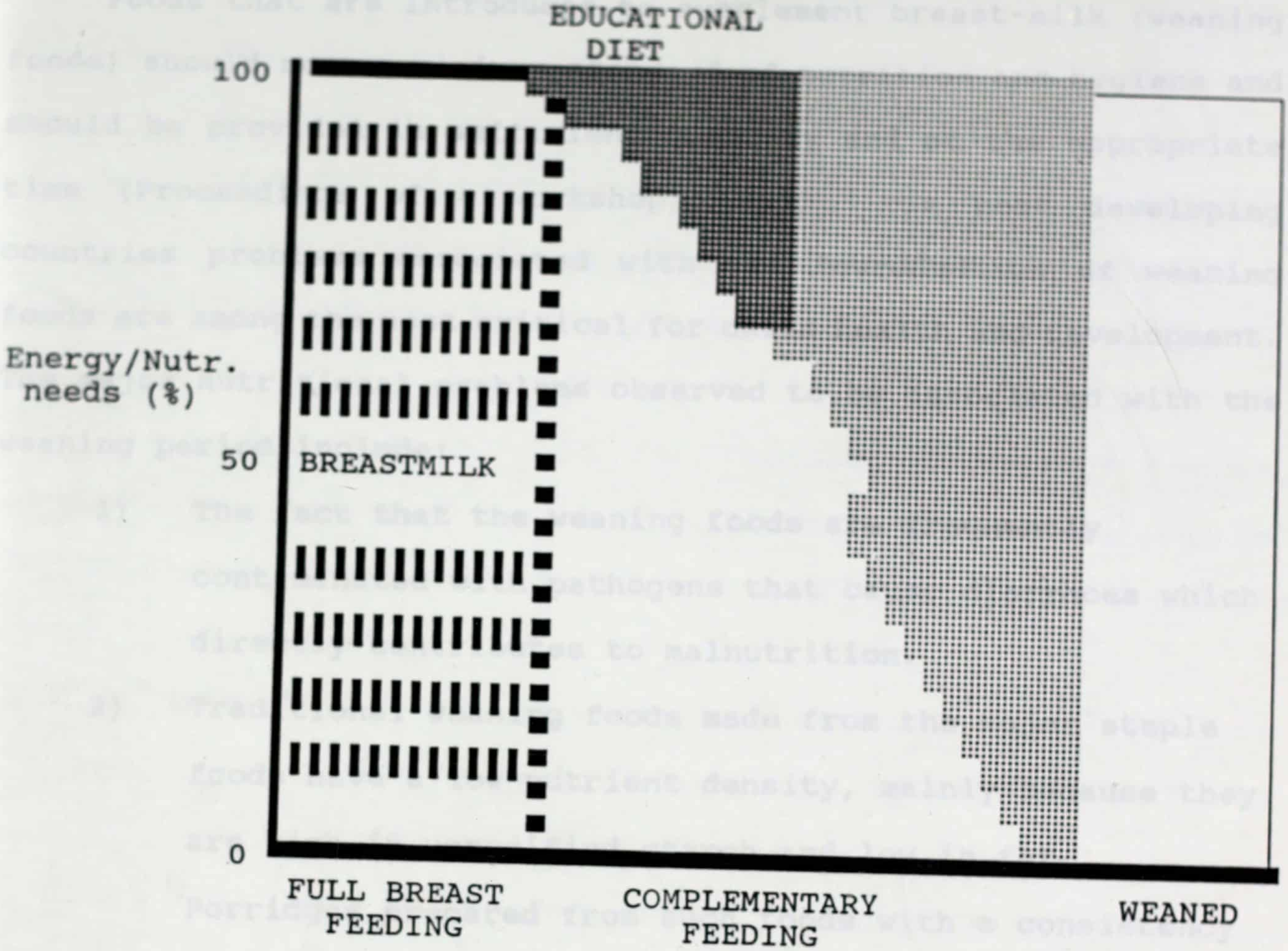
Processed supplementary foods are food mixtures meant to be used to supplement breast milk (breast-milk substitutes) for infants from the age of four to six months until completely weaned. They are also used for the progressive adaptation of infants and young children to ordinary foods (Hofvander and Cameron, 1987). Beyond the age of six months, supplementary foods should be increased gradually in amount and variety to provide not only energy but other essential nutrients including proteins, vitamins and minerals (Leverton, 1954). When such foods are given to the child before his or her needs exceed the mother's breast milk production, the child will suck less at the breast. In this way, the traditional food replaces rather than complements breast milk. The nutritional quality of the child's diet is thus lowered and she or he may be confronted with diarrhoea and other pathogens before the immune system is ready to cope with them (Mc Cann *et al*, 1981).

It has been recognized that from the fourth month of life (and under some circumstances, from the third month) breast milk alone becomes inadequate to meet the growing demands of an infant (Ashworth, 1985). Younger infants (about four to six months old) require a milk product as the major food but formulated foods that contain a high proportion of milk generally are too expensive (Beaton and Ghassemi, 1982). The emphasis, therefore, is on supplementary foods formulated from vegetable sources which when used as a complement to breast milk can provide the dietary increment in energy and nutrients that is needed to meet the increasing physiological requirements to support normal growth and development (Cameron and Hofvander, 1983). It becomes necessary therefore, to use low-cost, affordable, formulated supplementary foods.

Underwood and Hofvander (1982) have proposed a model for timing how food should be used to complement breast milk so as to meet fully the energy and nutrient needs of infants and young children. Figure 2:1 shows a graphical representation of the model.

Current trends in developing economies, like in Ghana, show a dramatic migration of low income families to urban areas. This is associated with an increased economic necessity for the mothers to participate in work outside the home. Under such circumstances, 'house helps' (often older siblings), are left to provide food to infants and young children during periods when mothers are unavailable to breast feed and/or provide supplementary foods. These circumstances necessitate that nutritionally adequate, hygienic foods be available that can be simply and safely prepared and fed, a role that low-cost formulated foods can serve (Underwood and Hofvander, 1987). Under the circumstance of a mother dying, lactation failure, natural catastrophes, decreasing available food supplies, etc.

Fig 2:1. Conceptual model for the introduction of supplementary foods into the diets of infants and young children



Source: Underwood and Hofvander (1982)

supplementary foods may provide the only suitable temporary alternative for older infants and young children (ibid).

Foods that are introduced to supplement breast-milk (weaning foods) should meet a minimum standard of nutrition and hygiene and should be provided in sufficient quantity and at the appropriate time (Proceedings of a workshop, 1987). In most developing countries problems associated with the introduction of weaning foods are among the most critical for child health and development. The major nutritional problems observed to be associated with the weaning period include:

1) The fact that the weaning foods are frequently contaminated with pathogens that cause diarrhoea which directly contributes to malnutrition.
2) Traditional weaning foods made from the major staple foods have a low nutrient density, mainly because they are high in unmodified starch and low in fat. Porridges prepared from such foods with a consistency acceptable to a one-year-old have an energy content about a third that of typical western weaning foods. A young child must consume a large volume of porridge if its energy and other requirements are to be met during the period when breast milk alone is insufficient. Many children are unable to eat such quantities, resulting in insufficient intakes of energy, protein and other nutrients. (Marero *et al.*, 1988). The problem is compounded if children are fed

infrequently (because of other demands on the mother's time) or his/her appetite is reduced because of illness (Proceedings of a workshop, 1987).

3) The major nutritional problem associated with the weaning period derives from the fact that, the duration of breast feeding have declined among certain mothers resulting in the decreased intake of an important and hygienic source of nutrients (McCann *et al*. 1981).

Generally, the criteria for choosing an infant weaning supplementary food that should meet all the nutrient requirements, be acceptable, digestible as outlined by Jelliffe (1968) and Yeung *et al* (1981) include:

1) The food should be dense in energy and other nutrients, have a high nutrient bioavailability and be low in anti-nutritional factors.
2) The food should be low in viscosity so that a large quantity may be consumed at a single sitting. This has the important effect of reducing the number of meals required to satisfy the daily energy requirements of a young child (Whitehead, 1976).
3) The food should be inexpensive to prepare, in terms of both ingredients and fuel for cooking (Proceedings of a workshop, 1987).
4) Foods should be prepared from crops that are agroclimatically suitable using dehullers, for

preparing the base materials (ibid).

5) As far as possible, the ingredients used should be ones that are available in all seasons, when ingredients are temporarily unavailable, it should be possible to substitute with others (Codex Alimentarius, 1982).

6) The food should be convenient and easy to prepare, involving few utensils, and requiring short cooking preparation and feeding times (Proceedings of a Workshop, 1987)

7) Both ingredients and finished products should be ones that can be stored safely at the household level (ibid).

8) The food should be culturally acceptable and pleasing in taste, appearance and texture to the child and to the mother (Yeung *et al.*, 1981).

9) The method of preparation should be easily understood and be based on practices that are traditionally familiar.

10) The food should be such that it would be difficult or impossible to give by bottle feeding (1DRC,1988).

2.1 Proteins in Foods

Proteins are complicated substances which contain carbon, hydrogen, oxygen, nitrogen and sometimes sulphur and phosphorus. During digestion proteins are broken down into at least 23 amino acids of which 8 are essential to adults and 10 to infants. These are essential because they cannot be synthesized by the body but can only be obtained from the food eaten. Generally, animal foods contain many of the essential amino acids, but mixtures of plant proteins can also supply all the essential amino acids thus provide a meal of high biological value (Udo, 1980).

The quality of food may be judged by its protein content, the quality and quantity of amino acids and the degree to which its protein is digested and absorbed by the body (Fox *et al.*, 1982). This makes some proteins be of a higher biological significance than others. A protein is thus said to be of good quality when it is sufficiently used for synthesis and maintenance of tissue proteins (Jansen, 1978). In Nutrition, the degree to which the proteins are utilized is a function of factors such as digestibility, amino acid composition and amino acid requirement of the organism fed the protein (Hopkins and Steine, 1978). However, protein quality is determined primarily by the amino acid composition (FAO/WHO,1973). Animal proteins have been known to be of better quality than plant proteins because animal proteins have all of the essential amino acids required for growth (Bogert, 1981). Plant proteins are of lower quality because they lack some essential amino acids. Cereal proteins have been reported to be deficient in lysine and tryptophan whilst soybean protein is limited in methionine (ibid). Some animal proteins like gelatin and keratin however, may be of low biological value (Fox et al, 1982).

The biochemical significance of proteins cannot, however, be over emphasized. If carbohydrates and lipids, generally speaking, can be considered as the fuels of the metabolic furnace, proteins may be regarded as forming not only the gears and levers of the operating machinery. Indeed, at the risk of pushing the analogy to extremes, protein hormones which act as regulator of metabolism may be regarded as the policy forming top management of the enterprise (Cantarow *et al*, 1957).

The 1985 Codex Alimentarius guidelines and the Protein Advisory Group (PAG, guidelines No. 8) proposed a model of composition for supplementary foods. It suggested that, there should be 20g protein per 100g product and that 100g product should provide about 400 kcal. It was also assumed that when no extra fat or sugar is added during supplementary food preparation, the protein energy percentage should be more than 20 kcal per cent compared to about 12 - 15 kcal per cent in ordinary mixed foods. It must however be noted that at this protein energy level the protein becomes very expensive and may be used physiologically for energy needs.

An expert committee reconsidered this suggestion about energy and protein requirements, and the safe level of protein intake. It was suggested that the safe intake level of protein for the age range of four to six months to three years should be between 14 and 15.5g (which is the protein equality equivalent to that in milk or eggs) assuming a digestibility and quality in vegetable-based supplementary foods of about two thirds that in milk or eggs.

To increase nutrient intake during the weaning period several approaches have been undertaken especially to produce low cost infant foods from plant proteins where amino acids of one plant protein complements that of the other. Legumes - oilseeds and pulses - have been evaluated in various combinations and found to be effective protein supplements to cereals (Bressani and Elias, 1966; Milner, 1969; Plahar and Leung, 1983). The high lysine content of pulses is capable of improving the nutritive value to cereals by mutual complementation of their limiting amino acids (Underwood and Hofvander, 1983).

2.1.1 Protein-Energy Deficiency Diseases

The nutritional inadequacy, whether of protein, energy and/or micronutrients, and thus a range of inadequacy states, occurs as a result of diet and nutritional requirements. Malnutrition prevents growth to their genetic potential and makes the body susceptible to infections (Kensch, 1979).

During the preparation of foods, complex interactions may occur and this may lead to the formation of products which cannot be digested and so rendered unavailable or toxic to the body.

In developing countries, like Ghana, the youngest in the society are the principal targets of malnutrition, especially Protein-Energy malnutrition (Mata, 1978).

The National Nutrition Survey of 1961 reported that less than 30% of all preschool children were underweight. A similar survey in 1986 reported that 58.4% of children aged 0 - 5 years were underweight, and 8.5% were classified clinically as suffering from marasmus or kwashiorkor.

The causes of Protein-Energy Malnutrition (PEM) in Ghanaian children are various; poverty, inadequate food production, excessive post harvest losses, ignorance, local taboos and beliefs, poor infant practices, poor sanitation leading to infections. These are but a few of the causes of PEM in Ghana (Alderman, 1990).

The tendency to consume a high carbohydrate diet with little protein especially among the low socio-economic group could be a natural response to the harsh economic conditions and a means of quenching hunger at a low cost. Information collected on socioeconomic background of families of children with kwashiorkor revealed that although public nutritional education has been going on all over the country the situation has not improved significantly because mothers are unable to afford the animal protein foods recommended to them (Kordylas, 1980).

Some nutritional experts have shown that many individuals are malnourished not because they are ignorant about what to eat or due to lack of variety of foods in the markets, but because they do not have the economic means to purchase these foods (ibid).

Inadequate dietary intake can cause weight loss or failure of growth in children and leads to low nutritional reserves. This is associated with lowering of resistance and colonisation and invasion by pathogens (Idusogie, 1977). Lower intellectual performances and inferior learning capacity are found in survivors at severe malnutrition in early life and this indicates vulnerability of the central nervous system (CNS) to nutritional insults at critical periods of development (ibid)

In human subjects protein deficiency can occur in three contexts:

1) Lack of dietary protein without concurrent insufficiency of energy intake (kwashiorkor)
2) Protein deficiency together with inadequate of energy intake (Marasmus).
3) Secondary protein deficiency, from malabsorption or from excessive body losses as in nephrosis (Osborne *et al*, 1919).

When energy intake is adequate but the calorie intake is too low general protein-energy malnutrition is observed as ingested food proteins are metabolized for energy production instead of being used for building up body protein (Silano *et al.*, 1981).

Protein-Energy Malnutrition has for a long time been associated mainly with the developing countries where the people rely on low-protein cereals and starchy roots as their main source of nourishment (Hanson, 1974; United Nations, 1973). These low-protein cereals and starchy roots are made into porridges. During processing they lose large quantities of the vitamin, mineral and even protein contents of the starting material leaving a finished food of even poorer nutritional quality (Inhekoronye and Ngoddy, 1985). Over dependence on such protein sources is the main cause for the widespread PEM in these areas (Plahar and Hoyle, 1987). This widespread problem of infant malnutrition in the developing world has stimulated a great deal of effort in the area of research, development and extension by both local and international organizations. Several methods of approach are being adopted towards the solution of the problem. In most cases however, the solution is based on formulation of blends with locally available legumes (oilseeds and pulses) and cereals to increase the protein content and also improve on the protein quality through mutual complementation of their individual amino acids (Plahar and Hoyle, 1987).

Two main approaches being used in Ghana and other developing countries to combat the PEM problem are:

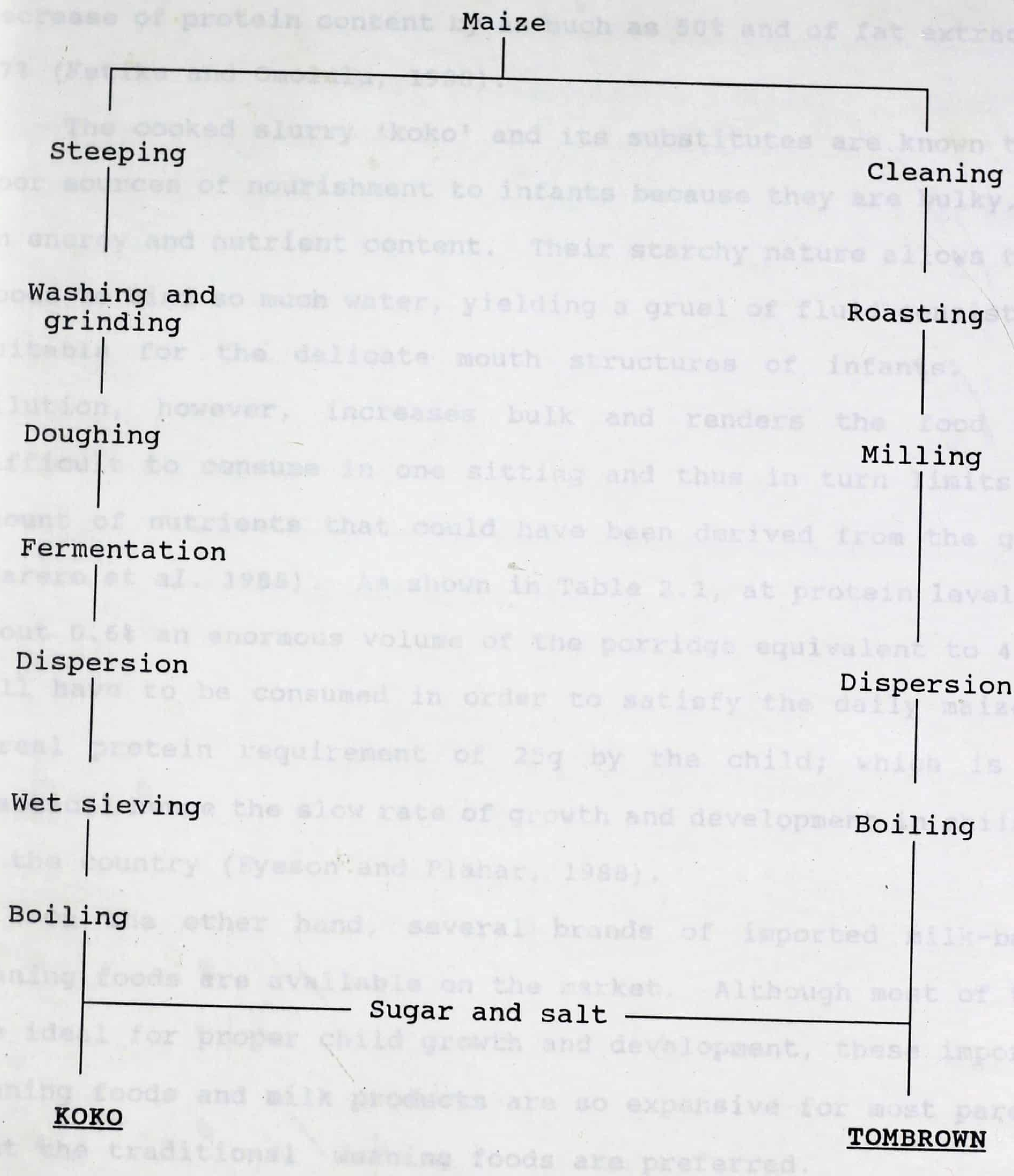
1) campaign for domestic preparation of weaning foods by mothers using predetermined proportions of available legumes and cereals; and
2) the promotion of semi-industrial units for the production of ready-made weaning foods of high nutritional quality which is also based on local cereal and legume blends (Plahar and Hoyle, 1987).

2.2 Traditional Weaning Foods in Ghana

The most common traditional weaning food for Ghanaian infants (from the age of three months) is a porridge prepared by cooking a slurry of fermented maize dough. This is the practice in many homes of average and low income earners in the Southern sector of the country. In the Northern sector the cereal used is either sorghum or millet and the process may include sprouting of the cereal before fermentation and cooking. The porridge is known as 'koko', 'Akatsa' or 'Akasa' by the different ethnic groups (Eyeson and Plahar, 1988). Another variation of 'koko' is a cooked slurry of roasted maize flour known as 'Tombrown'. This porridge like the 'koko' is given to the child with only sugar and salt added to taste. Only a few families who can afford, add milk as a protein supplement. Fig 2.2 shows a flow diagram for the preparation of the two traditional weaning foods.

The production of 'koko' involves fermentation of the dough which may improve the nutrient content (Chompreeda and Fields, 1984). Studies carried out by Van Veen and Steinkraus (1970) also showed that fermentation does not seem to improve the nutritive value of the protein. However, results of Akinrele (1970) showed slight increases of 8% and 25% in total nitrogen and amino acid nitrogen respectively during the traditional fermentation of 'ogi'. Earlier studies and more recent ones all seem to agree that fermentation increased the content of certain vitamins, particularly thiamine, riboflavin and to a lesser extent niacin (Rajalakshmi and Vanaja, 1967; Akinrele, 1970; Hamad and Fields 1979; Dhankar and Chauhan, 1987). At the same time, a decrease in some vitamins, particularly pantothenic acid, has been recorded (Akinrele, 1970). Other advantages of traditionally fermented foods include ease of digestibility better storage capability, enhanced flavour and reduction in cooking time (Van Veen, 1970; Harris and Karmas, 1975).

Fig 2.2 Flow diagram of 'koko' and Tom brown preparation



Source: Eyeson and Plahar (1988)

The method of processing the flour of 'Akatsa' or 'koko' is however wasteful of nutrients and has been shown to result in a decrease of protein content by as much as 50% and of fat extract by 27% (Ketiku and Omolulu, 1988).

The cooked slurry 'koko' and its substitutes are known to be poor sources of nourishment to infants because they are bulky, low in energy and nutrient content. Their starchy nature allows these foods to bind so much water, yielding a gruel of fluid consistency suitable for the delicate mouth structures of infants. This dilution, however, increases bulk and renders the food more difficult to consume in one sitting and thus in turn limits the amount of nutrients that could have been derived from the gruel (Marero *et al.* 1988). As shown in Table 2.1, at protein levels of about 0.6% an enormous volume of the porridge equivalent to 4.2kg will have to be consumed in order to satisfy the daily maize or cereal protein requirement of 25g by the child; which is not practical hence the slow rate of growth and development in children in the country (Eyeson and Plahar, 1988).

On the other hand, several brands of imported milk-based weaning foods are available on the market. Although most of them are ideal for proper child growth and development, these imported weaning foods and milk products are so expensive for most parents that the traditional weaning foods are preferred.

A lot is being done at the Food Research Institute to improve the nutritional status of the growing child by improving the existing weaning food through complementation with plant

Table 2.1. Composition of selected local cereals and the traditional weaning Porridge 'koko'.

	Maize	Sorghum	Millet	'Koko'
Moisture (%)	11.1	10.8	9.8	94.1
Protein (%)	8.8	9.7	8.4	0.6
Fat (%)	3.9	3.0	4.3	0.6
Ash (%)	1.3	1.6	1.7	0.2
Fibre (%)	1.2	1.2	1.2	0.2
Carbohydrate (%)	75.1	74.9	75.8	5.0

Source: Eyeson and Ankrah, 1975.

protein as well as producing ready to eat 'koko' ('koko' powder) to decrease preparation time, make it handy and hygienic to decrease the incidence of PEM.

2.3 Improving Plant Proteins by Complementation

The realisation of the nutritional inadequacy of the Ghanaian traditional weaning foods in properly supporting growth and development of infants has given rise to extensive research and development efforts aimed at providing relatively inexpensive but suitable local substitutes for children and babies.

Plant protein sources have been shown to be cheaper than animal protein sources in the formulation at infant weaning foods. Moreover, animal protein sources are scanty and expensive in most developing countries (Fashakin *et al*, 1991).

Plant proteins are classified as second class proteins because their amino acids are not a balance of the essential animo acids (Onabarino, 1953). However, they have been known to complement each other to produce proteins that are of high quality. The usefulness of a protein depends on which amino acid is present in the least amount, so that it is possible that two protein sources each limited by a different amino acid can have a combined nutritional value superior to either (Ihekoronyo and Ngoddy,1985).

The major sources of plant proteins in Ghana are the grain legumes and cereals. The most important legumes in terms of adult nutrition and in terms of production and consumption are cowpeas and groundnuts (Eyeson and Plahar, 1988). Bambara beans, soybeans, pigeon peas, winged beans and french beans are also grown but at subsistence level with relatively low yields. Table 2.2 gives the protein content and the essential amino acid composition of the most important grain legumes in Ghana. Table 2.3 gives the main cereals grown in Ghana.

The contribution of the protein content of these cereals to any diet may be low and the quality relatively poor (Eyeson and Plahar, 1988). However, the combined use of legumes and cereals has the advantage of the fact that cereals (except rice) are relatively deficient in lysine while pulses have a high lysine content. A mutual amino acid complementation is achieved by combining them. This way the protein quality is improved.

Various workers have shown the possibility of protein complementation to produce infant foods of acceptable nutritional values. Akinrele, (1970) developed a corn-based 'soy-ogi' of acceptable quality complementing lysine deficient corn with soybeans. Bressani (1985) reported that a food product for weaning small children made of 75% cereal grain and 25% cowpea would give about 13% good quality protein. A lot more combination of cereals and legumes have been tried but Cameron and Hofvander (1983) suggested that the ratio of cereals to pulses should be in the range of 3:1 or 4:1 in order to obtain the best mutual lysine complementation. Table 2.4 gives examples of cereal/legumes infant food combinations. In Ghana, 'weanimix' a product made from cereal, cowpea and groundnut is doing quite well and is an improvement on the traditional all cereal 'koko'.

Table 2.2. Protein Content and Essential Amino Acid Composition of the major legumes Grown in Ghana.

	Cowpeas	Groundnut	Pigeon Pea	Soybean	Winged bean
Protein content (%)	25	25.60	22.30	39.2	33.8
Essential aa (g/16g N)					
Isoleucine	3.82	3.38	3.10	4.54	5.00
Leucine	7.04	6.40	6.30	7.78	8.90
Lysine	6.83	3.54	7.70	6.38	7.70
Phenylalanine	5.17	4.98	8.27	4.94	5.3
Tyrosine	2.61	3.90	2.02	3.14	3.20
Phe.+Tyro.	7.78	8.88	10.29	8.08	8.50
Methionine	1.17	1.15	0.572	1.26	1.20
Cysteine	1.09	1.25	0.98	1.33	2.10
Met + Cys	2.26	2.40	1.49	2.59	3.30
Threonine	3.60	2.61	2.91	3.86	4.40
Trytophan	1.09	1.04	0.56	1.28	1.00
Valine	4.53	4.18	3.60	4.80	5.30
Protein score	64.6	63.6	42.6	74.0	94.3

Based on 50% moisture content.

Source: FAO (1970)

Table 2.3. Protein Content and Essential Amino Acid Composition of the major cereals grown in Ghana.

	Maize	Millet	Sorghum	Rice
Protein Content (%)	10.2	10.4	10.8	8.2
Essential aa (g/16gN)				
Isoleucine	3.68	4.10	3.92	4.20
Leucine	12.55	9.57	13.31	8.22
Lysine	2.67	3.42	2.02	3.62
Phenylalanine + tyr	6.70	0.07	7.57	8.05
Methionine	1.92	2.46	1.39	2.13
Cysteine	1.55	2.37	1.50	1.54
Met + Cys	3.47	4.89	2.89	3.67
Threonine	3.60	3.86	3.02	3.31
Tryptophan	0.70	1.95	1.22	-
valine	4.85	5.52	5.01	5.78
Protein Score (%)	48.5	62.2	36.7	65.8

Source: FAO (1970)

Fashakin *et al*. (1991) have also reported a further improvement when more than two plant proteins are mixed together. Data available from East Africa also show different ways by which complementation is practised to achieve a high protein quality especially from plant sources (Proceedings at a Workshop, 1987).

When appropriate levels of all the essential amino acids are provided through complementation or fortification of foods lower in protein quantity and quality, the nutritive value of the plant protein will apparently be equal to that of egg protein which is used as the standard (Cameron and Hofvander, 1985). Therefore, the wider range of foods included in a diet during a meal the greater the variety and the lower the risk of nutritional deficiency (Jelliffe, 1968).

Although certain traditional diets contain both cereals and legumes, the maximum benefits from this complementation is not achieved because of the unbalanced proportions of the components. Table 2.5 shows the effect of different levels of complementation on the amino acid composition in millet/legume blends.

The level of complementation/supplementation is very important since fortifying cereals especially with protein may affect the functional characteristics. Several reports are available on the effects of some fortification attempts on the physico-chemical, nutritional and sensory characteristics of resulting blends (Glover, 1976; Ekpenyong *et al*., 1977; Plahar *et al*., 1983; Plahar and leung, 1983). The main purpose of protein mixtures may be defeated especially when the extent of the fortification is not known,

Table 2.4. Protein-rich Foods on novel Proteins (Partial list)

Country	Product Name	Source of principal ingredient
Algeria	superamine	wheat, legumes
Brazil	Fortifier	corn, soy
	Enriched maize	corn, soy
	Cerelina	corn, soy
Ethiopia	Faff	Teff, cereal, milk, soy
Guatemala	Incaparina	corn, cotton seed, soy
Mexico	Protea	corn, soy
Nigeria	Amana	Groundnut
South Africa	Pronutro	corn, soy, yeast
Senegal	Ladylac	millet, peanut

source: Ihekoronye and Ngoddy (1985)

since above a certain percentage of fortification a different amino acid becomes limiting which may create a different problem in the weaning process rather than a solution. Plahar and Hoyle (1987) reported that lysine remained the limiting amino acid in different blends of cowpea/maize, cowpea/millet, and cowpea/sorghum up to 25% cowpea substitution except the cowpea/millet blend which still had lysine to be the limiting amino acid up to 30% substitution. They also reported that imposing a change in the limiting amino acid through high levels of cowpea fortification results in a decrease in the blend protein score and hence a decrease in the rate of increase in the net protein value (Plahar and Hoyle,1991).

2.3.1 <u>Cereals</u>

Cereal grains are the staple food of the people of the tropics providing them with about 75% of their total caloric intake and 67% of their protein intake. The grains are eaten in so many ways, sometimes as pastes, roasts, porridges and pottages or other preparations of the seeds. More often they are milled and further processed into flour, starch bran, oil, or breakfast or dinner cakes (Ihekoronye and Ngoddy, 1985).

Cereals are essentially carbohydrate concentrates with the protein content normally ranging between 8 and 12% (McDonald *et al.*, 1976). Except for two amino acids, lysine and tryptophan, most cereals contain the essential amino acids required by man as well as vitamins and minerals. All the cereal grains are plant

Table 2.5. Estimated Protein Quality of weaning Blends containing 20%, 25% and 30% legume and 80%, 75% and 70% cereal (summary)

Legume	Protein content (%)	Protein score (%)	NPV (%)	Limiting amino acid
20%				
Cowpea	13.3	85.6	11.4	Lysine
Groundnut	13.4	63.1	8.5	Lysine
Pigeon Pea	12.8	88.3	11.3	Threonine
Winged Bean	15.1	97.3	14.7	Lysine
Soy Bean	16.2	89.6	14.5	Lysine
25%				
Cowpea	14.1	89.8	12.6	Lysine
Groundnut	14.2	63.3	9.0	Lysine
Pigeon Pea	13.4	86.5	11.6	Threonine
Winged Bean	16.3	102.0	16.3	-
Soy Bean	17.6	93.3	16.5	Lysine
30%				
Cowpea	14.8	93.3	13.8	Lysine
Groundnut	15.0	63.5	9.5	Lysine
Pigeon Pea	14.0	85.0	11.8	Threonine
Winged Bean	17.4	104.0	17.4	-
Soy Bean	19.0	96.5	18.4	Lysine

seeds and so contain a large centrally located starchy endosperm which is also rich in protein. The crude fat content of the grains range between 1.0 and 6.0%, Cereals are deficient in calcium, containing less than 0.15% (Ihekoronye and Ngoddy, 1985).

The main cereals grown in Ghana as part of the traditional staples are maize (*Zea mays*), millet (*Pennisetum typhoideum*), rice (*Oryzea* spp) and sorghum (*Sorghum vulgare*). Although the protein content of these cereals is low and the quality relatively poor, their contribution to human protein nutrition cannot be under estimated in view of the large amounts consumed (Eyeson and Plahar, 1988). Table 2.4 (refer) gives the protein content and the essential amino acid composition of the cereals grown in Ghana. The 1986 estimates of cereal production in Ghana are as follows;

Maize	495,000 tonnes
Sorghum	190,000 tonnes
Millet	140,000 tonnes
Rice	80,000 tonnes

(Eyeson and Plahar, 1988)

2.3.1.1 <u>Production and food uses of millet in Ghana</u>

Millet (*Pennisetum typhoideum*) is one of the widely cultivated crops in Ghana and in the savannah regions immediately south of the Sahara. In Ghana it is widely grown in the North Eastern part of the country. It produces a larger quantity of grain than does other cereals under conditions of infertile soil, intense heat and scanty rainfall (Rakhimbaes, 1968).

Millets are easy to grow; the seeds which are usually cheap can mature in 3 - 4 months (Robinson, 1966). They have a greater advantage over other cereals in that they can be stored for a longer period and are attacked by fewer insect pests as compared with the other cereals.

There are two varieties of millet grown in Ghana, the late maturing millet (*Pennisetum spicatum*) and early maturing millet (*Pennisetum americanum*). The late millet is planted in May to July and matures after five to six months, and the plant grows to a height of 5.2m. The early millet as the name implies matures between 3 and 4 months. It is planted around April and May. The plant grows to a height of 2.2m. In the Northern Regions the local names for the late and early maturing millets are 'Nyasa' and 'Manganara'.

The millets consist of carbohydrates, nitrogenous compounds (mainly proteins), lipids, mineral matter and water together with small quantities of vitamins, enzymes and other substances some of which are important nutrients in the human diets. Millets are rich in lipids as compared with other cereals like sorghum and wheat. It has a higher mineral content than most cereals (Rachie, 1975). A look at the chemical composition shows that it has a high carbohydrate content of 54.0% to 67.3%, 10.6 - 12.1% protein, 3.6 - 5.0% ash and fat content of 3.5 - 4.5% (FAO/WHO,1976). It has a food energy of 414 calories and fibre content of 2%. Millet has a high percentage of indigestible fibre because the seeds are enclosed in hulls which are not easily removed by ordinary processing methods. It is a good source of thiamine and probably contains appreciable amounts of other B vitamins. Millet ranks high in ash content and contains good levels of calcium, phosphorus, magnesium and iron (Ihekoronye and Ngoddy, 1985).

In most developing countries millet provides a large portion of the dietary carbohydrates, proteins and other nutrients to the large number of human populations that consume it (Wang and Fields, 1978) As a cereal for human consumption, millet is considered highly palatable and is among the most nutritious of grain crops. However, the nutritional quality of millet protein is only moderate because its level is low in the grain and it is deficient in some of the essential amino acids (Rehman *et al*., 1974). One of the possible ways of improving this quality is through controlled germination of the grain, a process that has been employed for other seeds (Opoku *et al*. 1981; Hamad and Fields, 1981; Wang and Fields, 1978)

Millet is widely used in the Northern part of Ghana basically for food. 'Fula' is a very common formulation obtained from millet and spices which is eaten as snacks. 'Massa', a fried spiced millet flour meal is usually served with 'koko' for breakfast. The cracked grain may also be used and cooked like rice and consumed with stew. Malted or germinated millet is also used to brew traditional beers, 'pito' and 'brukutu'; a preparation which is very common in Northern Ghana and gaining popularity in the south.

Millet is now being promoted for use in weaning food formulations among the people of the Northern regions of Ghana where millet is a staple crop. The weaning food 'weanimix' is formulated from roasted millet flour and legumes, basically cowpeas and groundnuts. Hariharan *et al* (1965) reported a significant weight gain in experimental rats when 'fingermillet' used in poor vegetarian diets were supplemented with groundnut flour to provide 2.5 - 5.0% extra protein. Supplementation with calcium salts and vitamins did not significantly increase or improve growth.

Of economic importance is the pigment in millet which is known to contain polyphenols such as tannins and phytates which some populations find objectionable. However, soaking the seeds in 0.IN HCl, fermentation, and germination have been found to drastically reduce these antinutrients to a minimum (Reicher *et al.*, 1979).

2.3.2. Legumes

Legumes refer to the edible seeds of leguminous plants belonging to the Leguminosae family. Those used for food can be divided into the pulses and oilseeds. Pulses are the dried edible seeds of cultivated legumes such as lentils, beans and peas. The oilseeds consist of those legumes used primarily for their oil content. These include the groundnuts and soybeans. Another category of legume crops, pasture or forage legumes, are those used as fodder, green manure and cover crops. In many of the developing countries and tropical areas beans and peas are important sources of proteins (Ihekoronye and Ngoddy, 1985).

The world production of grain legumes is about a tenth that of cereals and contribute about 12% of the total protein available to mankind (Silano *et al.*, 1981). In terms of adult nutrition, grain legumes contribute a significant proportion of the overall protein intake of the Ghanaian population (Eyeson and Plahar, 1988). The most important legumes, in terms of production and consumption, are cowpeas and groundnuts. Other legumes such as bambara nuts, soybeans, pigeon peas, winged beans and french beans are also grown but their production has remained at subsistent level with relatively low yields.

Based on a nitrogen conversion factor of 6.25, the crude protein content of most raw, dry legume seeds is relatively constant, varying between 16.0% in bambara beans to 35.1% in soybeans (Ihekoronye and Ngoddy, 1985). A lipid content of 1.3% and 2 - 3% mineral content have been reported (King *et al.*, 1976). The legume proteins have a well recognized deficiency of the essential sulphur bearing amino acids, methionine and cysteine but are comparatively rich in lysine. Thus, a combination of cereal and legume proteins (eg. millet and cowpeas), comes very close to providing an ideal source of dietary proteins for human consumption (Sood *et al.*, 1982). The seeds contain significant amounts of thiamine, niacin, folic acid, phosphorus, calcium and particularly, iron. With the exception of the germinated seeds legumes, as consumed, are almost devoid of ascorbic acid (Levy *et al.*, 1936).

Legumes contain toxic components which interfere with digestive processes and prevent efficient utilization of the legume proteins. Examples of such toxic substances include trypsin inhibitors, polyphenols, tannins among others. Sugars like raffinose and stachyose present in legumes, which are indigestible to mammalian enzymes, are the causes of flatulence among susceptible individuals and may cause extreme discomfort. Restricted use and consumer antipathy to dry beans may be attributed in part to the discomforting and socially unacceptable rectal elimination of odorous flatus gases (Ihekoronye and Ngoddy, 1985). However, heat processing conditions have been shown to minimize some of these effects. Sessa *et al.* (1990) reported a 90% reduction in trypsin inhibitor activity in cowpea after giving a moist heat treatment. Heat treatment reduced phytate in peas by 13% (Beal and Mehta, 1985). Phytate is also hydrolysed during fermentation to inositol and orthophosphate (Reinhold, 1985). Clydesdale and Camire (1983) reported that the removal of fractions of seeds especially the bran leads to reduction in phytate content of food.

2.3.2.1 <u>Cowpeas</u>

Cowpea (*Vigna unguiculata*) are probably the pulse crop grown in the largest quantity in tropical West Africa, South America and other tropical countries, and it constitutes an important source of protein in developing countries (Reichert *et al.*, 1979).

Cowpeas are now gradually finding their way into more dishes especially in Africa because of the increasing awareness that grain legumes may provide a cheaper source of protein. In some areas of the semi-humid tropics, cowpea provides more than half the plant protein in human diets (Singh and Rachie, 1985)., Fennels (1963) observed that beans, particularly cowpea, contribute as much as 60% of the protein intake in some families in Nigeria. It is highly palatable, very nutritious and relatively free of metabolites and other toxic substances if well cooked (Kay, 1979). In Ghana cowpeas are eaten with cereals in dishes like rice and beans, yam and beans, jollof rice, akara, moinmoin, and gari and boiled beans among others. The fresh, mature seed pods are sometimes eaten boiled as a vegetable as in the case of winged beans.

The chemical composition of cowpeas show that it is very nutritious. Kay (1979) reported a crude protein content of between 18 - 29% and up to about 35% in some cultivars. He also reported 11% moisture, 1.3% fat, 56.80% carbohydrate, 3.9% fibre, and 3.0% ash. Cowpeas have been known to significantly contribute to dietary mineral intake (Levy *et al*., 1936). Some workers have reported improved health and body development when one quarter dry weight of cowpea supplement was added to the diet of some West Africans (Fuller *et al*., 1972; Sundrez *et al*., 1972).

Table 2.6 gives the proximate analysis of cowpeas as reported by Inglett and Charalambous(1979).

The disgestibility co-efficient of cowpea is low hence the proper utilization of the minerals present in cowpea is also affected due to the presence of antinutritional factors which form complexes with some of the minerals making them unavailable (Bressani *et al*., 1976) Experiments with Haryana calves in 1976 by Meiners *et al*., showed that only 45% of the total calcium present in the grains was available.

Various workers have worked on the presence of antinutrients in cowpea and reported that it is markedly reduced by heating, dehulling, soaking in 0.1N HCl, moist heat treatment, fermentation, germination or pressure cooking (Kay, 1979).

2.4 Grain malting for Improved Nutrient Density

The dietary bulk problem in weaning diets has recently attracted much attention because of its clear association with malnutrition in young children (Svanberg, 1987). The two characteristics that determine dietary bulk are volume (or energy density) and consistency. Dietary bulk properties appropriate to young children would be high energy density (low volume) and semi-liquid consistency (ibid).

2.4.1 Dietary Bulk Properties

To evaluate the influence of dietary bulk properties on the nutrition of young children the factors that determine daily energy and nutrient intake must first be considered; vis a viz number of meals per day, amount of food consumed at each meal, energy and nutrient density of the food consumed and bio-availability of energy and nutrients in the food (Svanberg, 1987). Of these the second and the third are the ones most closely related to the dietary bulk properties of the food.

Nicol (1971) was one of the first workers to make quantitative estimates of the food intake in children. He concluded that the volume of starch-based foods required to cover the energy needs of

Table 2.6. Proximate analysis of cowpea

Parameter	**Amount**
Protein (%)	27.3
Lipid (%)	1.4
Fibre (%)	5.4
Ash (%)	4.0
Potassium (mg)	1.99
Calcium (mg)	117
Iron (mg)	9.1
Zinc (mg)	5.2
Manganese (mg)	3.3
Copper (mg)	5.2
Nickel (mg)	0.28

Source: Inglett and Charalambus, (1979)

preschool children is between 900 and 1650 ml. The amount of food eaten ranged between 660 to 1250 ml, divided into two meals per day but this was not enough to meet the energy requirements stipulated by FAO/WHO (1973). Even if the food were to be divided into many meals per day, Nicol concluded, it would be impossible to expect a child of 1 - 3 years of age to consume 1450 ml of thick sticky-porridge. For cereal based diets however, it was considered possible for a child to consume a sufficient volume of porridge (900 - 980 ml) to meet his energy requirements if the food were divided into four servings per day.

Rutishauser (1975) compared energy density, feeding frequency, and appetite (depending on the presence or absence of illness), with regard to the energy needs of preschool children and concluded that energy density is the most decisive factor. She also pointed out that, in cases of discontinued breast feeding milk was the only food to provide full compensation. It was suggested then that milk may have desirable bulk properties that distinguish it from other starch based foods.

Formon *et al*. in 1969, 1971 and 1975 carried out a series of studies on food intake in infant given, ad libitum, milk formula with different energy concentrations. In spite of the greater quantity of food consumed by infants fed the low calorie formula, the mean calorie intake (107 Kcal/kg per day) was considerably less than that of infants fed the high-calorie formula (126Kcal/ kg per day).

After the age of 4 months, the mean quantity of food consumed was 939 ml/day for the high low-calorie group, and 582 ml/day for the high calorie group. This resulted in an equal intake of calories per day and in weight gain for all infants. All these however refer to milk based formulae which do not have relevance to starch-rich weaning foods.

Thicker foods necessitate more effort in chewing and in passing the chewed food on to the stomach. It is possible that this increased effort can limit food intake in young children who have not fully developed their abilities in these respects. Liquid foods, of course, require very little effort to be passed on to the stomach. At the same time however there will be (probably) less amylase from the saliva, this reduction in amylase may delay further digestion (Svanberg, 1988). This bulk affects the rate of gastric emptying (Hunt and Knox, 1968). The rate is also regulated by the energy density to all energy units, whether the origin of these units is fats or carbohydrates. These regulating mechanisms will not however, fully equalize the differences between meals of a higher-energy density which will still release more energy into the intestines per unit time than will a meal of lower energy density (Svanberg, 1985). The viscosity of gel fibres like pectin, have been shown to delay gastric emptying in adults (Holt *et al.*, 1979). Quantitative dietary surveys have revealed that although a diet may contain all the essential nutrients, it may still be difficult for the individual to satisfy his or her nutrient requirement due to dietary bulk. (Ljungvist *et al.*, 1978).

The final digestion and absorption of food components are usually concentrated to the proximal parts of the intestines, but these processes may be extended to more distal parts of the intestines as a result of high rates of unavailable carbohydrates (Johansson, 1975; McCance *et al.*, 1953). These unavailable carbohydrate or 'dietary fibre' constitute a source of unavailable energy and they may also affect the availability of proteins and minerals (Southgate, 1973). Further more, the indigestible carbohydrates bind large amounts of water and therefore contribute to dietary bulk such as pectin, carrying five times its own weight of water (Latham, 1975).

2.4.2. Reducing Dietary Bulk through Germination

Traditional food handling technologies have been shown to be able to reduce the water-holding capacity of cereals and legumes, and thus make it possible to prepare gruels with acceptable energy and nutrient densities but with semi solid consistencies. Germination or malting of cereals and legumes and fermentation are examples of two traditional processes used to reduce dietary bulk of food (Brandtzaeg *et al.*, 1981; Mosha and Svanberg, 1983).

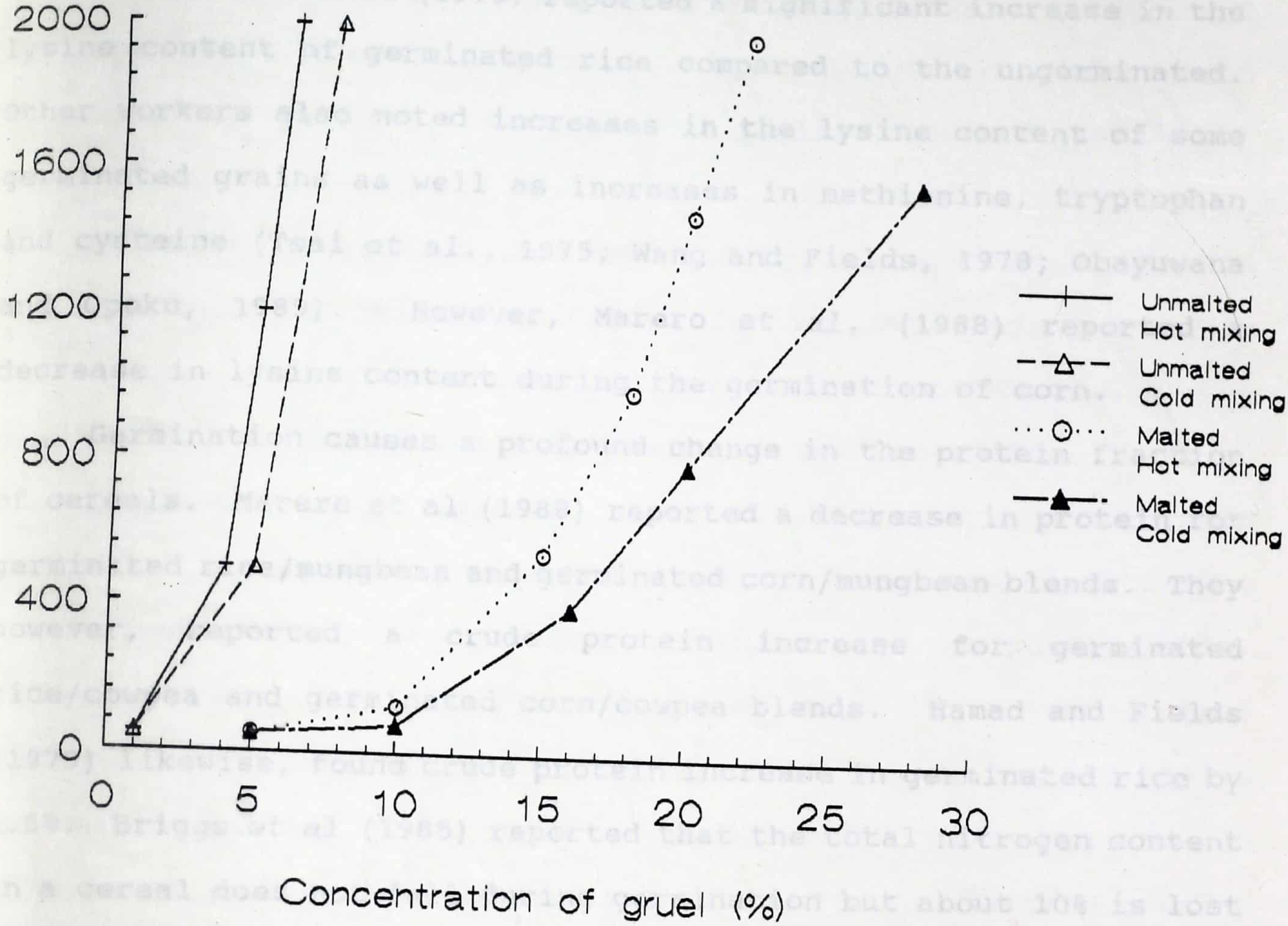
The bulk reducing effect of germination depends on the formation of amylases. Enzymatic activity increases rapidly in germinating seeds, in some cereal varieties such as barley, sorghum and millets the amylolytic activity is especially high. The alpha-amylases are synthesized in the cell within the aleurone layer, and migrate into the starchy endosperm where hydrolysis of the starch granules begin. These starch granules will therefore not swell to the same-extent during cooking and the amylose chains supposed to form the gel network will be broken down (Brandtzaeg *et al*., 1981). The alpha-amylase activity developed during germination will thus reduce the water-holding capacity of gruels prepared from germinated flours. Sorghum (white variety) and millets have been shown to develop significant amylolytic activity after 48 hrs of germination. Figure 2.3. shows the dramatic effect on the bulk properties of a millet variety after germination. At least three times as much germinated flour can be mixed into the same volume of water while maintaining the same consistency of gruel.

Malleshi and Desikachar (1982) reported that because of the high amylase content of germinated cereals 50% addition of malted barley flour can be used to substantially reduce the viscosity of a 15% hot paste slurry of branded Indian weaning foods such as Nestum, Farex, Cerelac and Balamue. This means that with the addition of a small amount of germinated flour, a sticky porridge of about 20% flour concentration will become a semi liquid gruel (Mosha and Svanberg, 1983).

2.4.3 Effect of Germination on Nutrient quality

Germination is characterized by the reactivation of enzymes present in the grain during the steeping process prior to germination. During germination part of the carbohydrates present in the grain is broken down into maltose, dextrin, glucose and fructose units by alpha-and Beta-amylases which contribute to the

Fig 2.3. Effect of cooking method on the viscosity of gruels prepared from unmalted and malted dehusked ragi flour.



Hot mixing: **Flour mixed directly with boiling water and cooked for three minutes**

Cold Mixing: **Flour mixed with cold water and heated slowly. Viscosity was measured at 40°C in a Brookfield Viscometer.**

Source: Svanberg (1988).

decrease in bulk density observed (Chen *et al.*, 1975; Mosha and Svanberg, 1983). The sugar content of a cereal is therefore increased after germination.

Hamad and Fields (1979) reported a significant increase in the lysine content of germinated rice compared to the ungerminated. Other workers also noted increases in the lysine content of some germinated grains as well as increases in methionine, tryptophan and cysteine (Tsai *et al.*, 1975; Wang and Fields, 1978; Obayuwana and Opoku, 1989). However, Marero *et al.* (1988) reported a decrease in lysine content during the germination of corn.

Germination causes a profound change in the protein fraction of cereals. Marero et al (1988) reported a decrease in protein for germinated rice/mungbean and germinated corn/mungbean blends. They however, reported a crude protein increase for germinated rice/cowpea and germinated corn/cowpea blends. Hamad and Fields (1979) likewise, found crude protein increase in germinated rice by 1.5%. Briggs *et al* (1985) reported that the total nitrogen content in a cereal does not fall during germination but about 10% is lost through translocation into the rootlets and shoots which are removed after kilning.

During germination lipids are partially hydrolysed to free fatty acids and glycerol by lipolytic enzymes (De Clercke, 1958). There is almost no change in the soluble ash content of raw grain and malt, but the major change which occurs is the formation of inorganic phosphate by the hydrolysis of phytin. There is an increase from 20% of the total phosphate content in raw grain to

40% in the malt (ibid). Marero *et al.* (1988) reported an increase in the amounts of phosphorus, iron and zinc which were otherwise bound to phytates. The breakdown of phytates during germination releases the minerals (Mosha and Svanberg, 1988). Luhila and Chipulu (1987) have also reported an increase in the *in vitro* iron availability due to a decrease in phytate content and other inhibitors.

Various researchers have reported the improvement of vitamin quality during germination. They further reported a dramatic increase in vitamin C content. This was also reported by Hamilton and Vandestoep (1979) and also Venugopal and Rao (1978).

Antinutritional factors such as haemaglutinin, trypsin inhibitors among others have been reported to be the cause of the unavailability of some minerals (Luhila and Chipulu, 1987). However, some workers have reported a decrease in antinutritional factors during germination making available minerals and increasing protein digestibility and quality. Germination is also reported to enhance vitamin content of food materials, reduce flatus producing factors (stachyose and raffinose) and also impart desirable flavour and taste to the product.

Research has revealed that during germination mycotoxins such as aflatoxin are developed (Frazier and Westhoff, 1978). These mycotoxins however are not destroyed by washing, cooking or heating. Akazawa *et al.* (1960) reported the presence of dhurrin - a cyanogenic glycoside that yields hydrocyanic acid upon hydrolysis - which exists in the form of heat stable non volatile cyanogenic glycoside in sprouted sorghum. The amount intended for use in porridge to reduce dietary bulk is however, very small 5 - 10g/100 - 125 ml which constitutes 18.6 - 37.23 ppm/100 - 125 ml of hydrocyanic acid which is less than the average permissible consumption level of 200 ppm/day of hydrocyanic acid (Conn, 1973). Panasiuk and Bills (1984) therefore opposed the idea of home sprouted sorghum because of its potential cyanide poisoning. Dada and Dendy (1987) however reported that when dhurrin is hydrolysed, HCN is released which can be eliminated either partially or fully by toasting at 180°C. This reduced HCN by 90%. Toasting wet germinated sorghum for 15 min at 100°C or at 180°C resulted in a loss of 83 to 96.5%. Fermentation also reduced HCN levels by 90% (ibid).

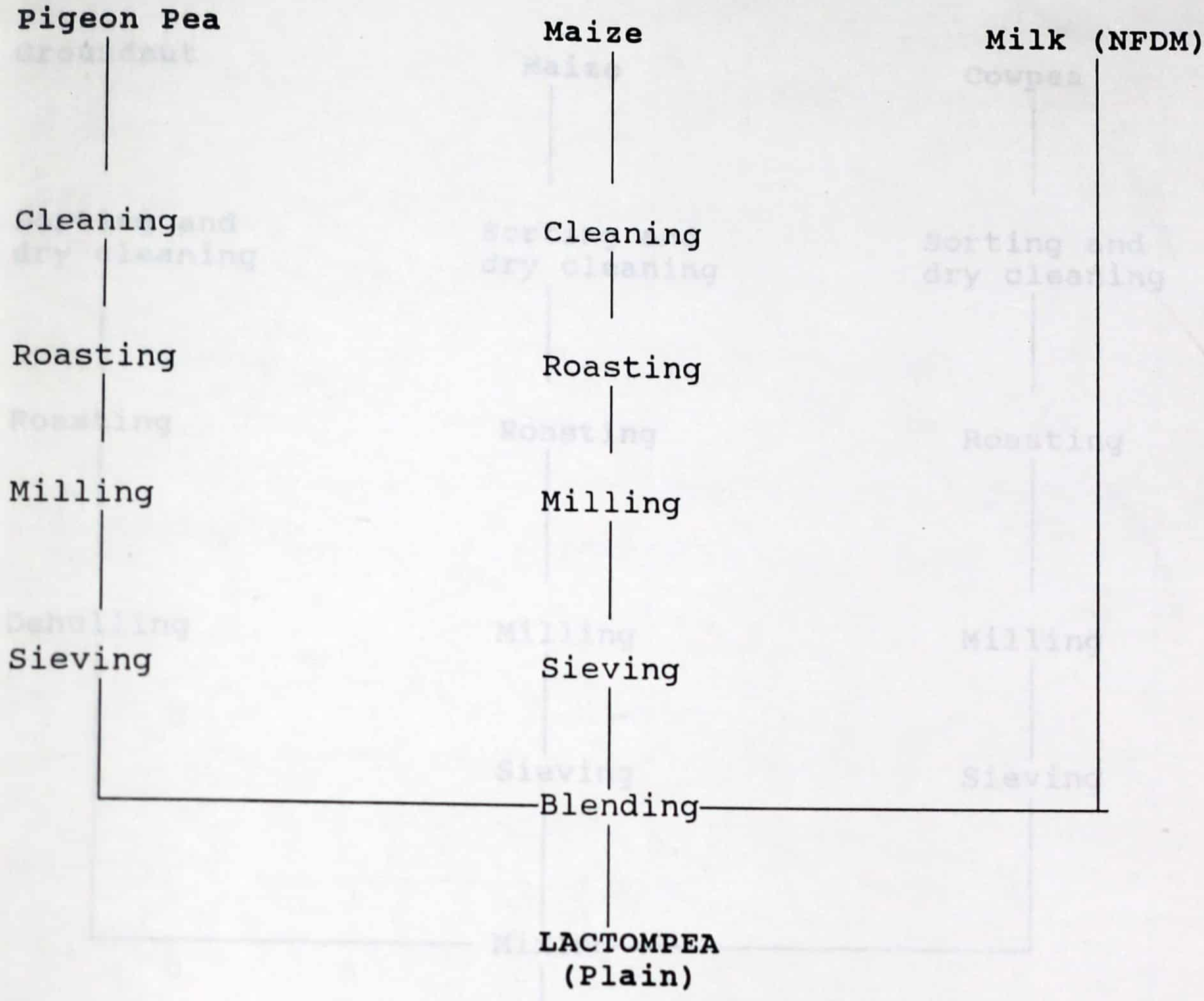
2.5 <u>Advances in Weaning Formulations in Ghana</u>

The realization of the nutritional inadequacy of the Ghanaian traditional weaning foods in supporting proper growth and development, has given rise to extensive research and development efforts aimed at providing relatively inexpensive local substitutes for children and babies (Eyeson and Plahar, 1988). At the Food Research Institute of the Council for Scientific and Industrial Research (CSIR), work is far advanced on the formulation of vegetable protein mixtures based on locally available legumes such as winged bean, soybean, cowpea, groundnut and pigeon peas. Techniques developed could yield legume flours with high protein content for use as protein base for weaning formulations with local cereals like maize, millet and sorghum (Eyeson and Plahar, 1988).

The efforts of the grain legume research team at the Institute have already yielded three high-quality protein weaning foods for babies aged four months and above. In terms of content and consistency, therefore, different formulations are considered for specific age groups. The products developed include 'Lactompea' (plain), 'Lactompea (G-plus)' and 'Browinlac'. Figures 2.4, 2.5, and 2.6 give the flow charts for the preparation of the three products.

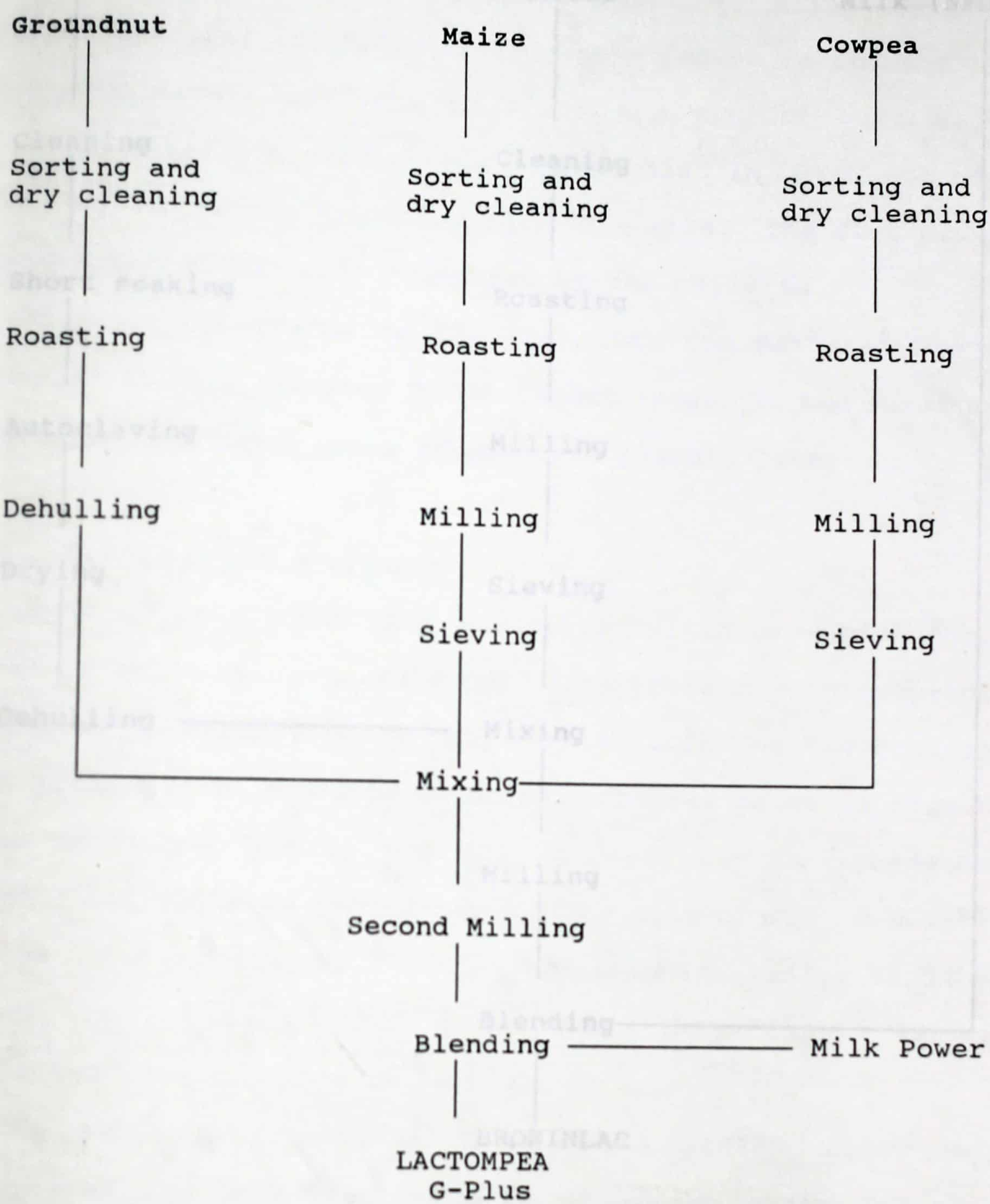
The development of weaning foods based on local legumes and cereals has not been confined to the Food Research Institute alone. According to Eyeson and Plahar (1988), The Nutrition Division of the Ministry of Health with the help of the joint FAO/WHO/OAU Regional Food and Nutrition Commission for Africa, embarked on a pilot project to help people in selected villages with simple weaning formulations based on available legumes and cereals. The project has lately been revitalised by the National Food and Nutrition Coordinating Committee with equipment provided by organizations such as UNICEF and World Vision International. The product 'Weanimix' is being promoted in towns and villages through Nutrition Extension Officers in the Regions. It is a blend of a major local cereal in a particular region with two local legumes, especially cowpeas and groundnuts. Table 2.7 gives the protein content and quality of the 'Weanimix' and other legume/cereal blends being promoted in Ghana. Although the nutritional quality of the 'Weanimix' is not as ideal as the Food Research Institute

Figure 2.4. Process flow sheet for the production of 'Lactompea (Plain)' weaning food.



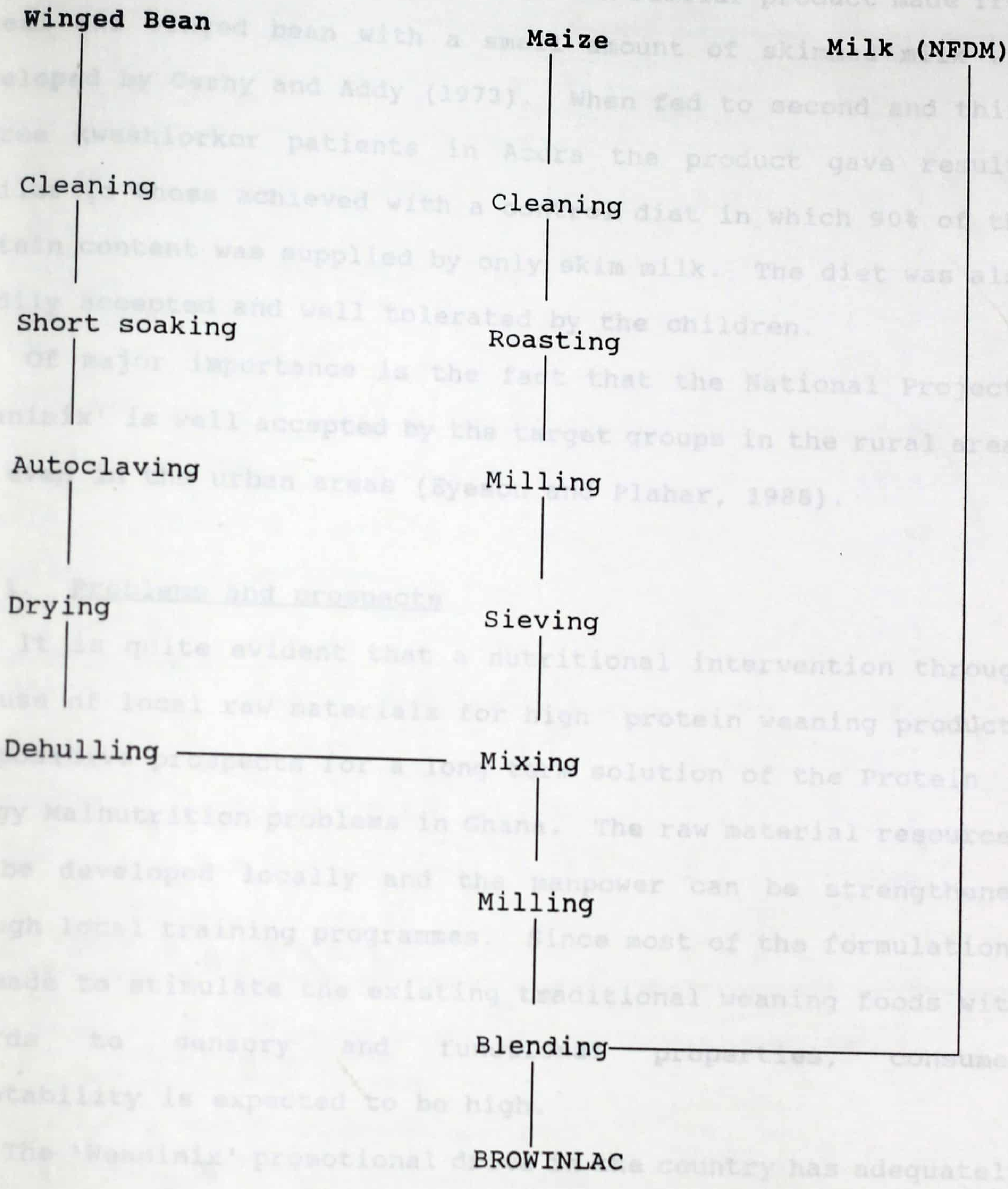
Source: Eyeson and Plahar, (1988)

Figure 2.5. Process flow sheet for the preparation of 'Lactompea G-Plus' weaning food.



Source: Eyeson and Plahar, 1988

Figure 2.6. Process flow sheet for the Production of 'Browinlac' Weaning Food.



Source: Eyeson and Plahar (1988)

products, it is a great improvement on the traditional weaning foods currently being used by mothers. A similar product made from cereal and winged bean with a small amount of skimmed milk was developed by Cerny and Addy (1973). When fed to second and third degree kwashiorkor patients in Accra the product gave results similar to those achieved with a control diet in which 90% of the protein content was supplied by only skim milk. The diet was also readily accepted and well tolerated by the children.

Of major importance is the fact that the National Project, 'Weanimix' is well accepted by the target groups in the rural areas and even in the urban areas (Eyeson and Plahar, 1988).

2.5.1. Problems and prospects

It is quite evident that a nutritional intervention through the use of local raw materials for high protein weaning products has positive prospects for a long term solution of the Protein Energy Malnutrition problems in Ghana. The raw material resources can be developed locally and the manpower can be strengthened through local training programmes. Since most of the formulations are made to stimulate the existing traditional weaning foods with regards to sensory and functional properties, consumer acceptability is expected to be high.

The 'Weanimix' promotional drive in the country has adequately demonstrated the beneficial effects of adding grain legumes to cereal foods. With the awareness and understanding created among mothers, legume/cereal weaning blends may replace the traditional

Table 2.7. Protein content, Essential Amino Acid composition and Sensory Scores of selected cereal/legume based weaning foods developed in Ghana.

	Weanimix [a]		Lactompea [b]		Browinlac [b]
	1	2	Plain	G-Plus	
Protein Content (%)	10.8	12.3	19.6	17.0	20.7
Protein Score (%)	58.0	61.0	98.3	94.4	98.7
Essential aa (g/16gN)					
Isoleucine	3.9	4.1	4.6	4.4	4.8
Leucine	11.9	11.1	11.2	11.0	10.5
Lysine	3.3	3.4	5.8	5.2	6.0
Methionine + Cysteine	4.4	3.7	4.0	3.8	3.4
Phenylalanine + Tyrosine	8.7	8.0	10.1	9.0	9.1
Threonine	3.4	3.2	4.0	3.9	9.1
Tryptophan	0.6	0.8	1.3	1.2	1.1
Valine	5.0	5.1	5.7	5.6	5.5
Sensory score[c]	7.0	7.0	7.8	8.2	7.0

[a] Developed by the Ghana National Nutritional Coordinating Committee. :
1 = maize/groundnut/cowpea,
2 = sorghum/groundnut/cowpea

[b] Developed by the Food Research Institute (CSIR):
Plain lactompea = maize/pigeonpea/milk
G-Plus Lactompea = maize/cowpea/groundnut/milk
Browinlac = maize/winged bean/milk

[c]. Based on a 9-point hedonic scale (1=dislike extremely, 9=like extremely)

Source: Eyeson and Plahar, (1988).

weaning foods in most homes. (Eyeson and Plahar, 1988).

To achieve a large scale production and widespread utilization of legume/cereal weaning foods for effective replacement of both the poor quality traditional weaning foods and the expensive imported milk-based baby foods, Eyeson and Plahar (1988) have suggested the need:-

1) for extensive agricultural programmes aimed at cultivating the traditional legumes and cereals above the present subsistence level as well as promoting the large scale cultivation of the non-traditional legumes such as soybeans and winged beans.

2) for extensive training programmes on the basis of grain legumes and cereal formulations with special emphasis on the complementary nature of the individual limiting amino acids

and 3) to stress the beneficial effects of legume/cereal mixtures in solving the Protein Energy Malnutrition problem based on closely monitoring blend formulations and controlled techniques, since any improper combinations and processing procedures can result in a completing waste of effort and money.

Plahar and Hoyle (1987) also suggested that semi industrial units should be set up in towns and villages to produce weaning blends for distribution among mothers; which is more likely to be effective than the home preparation of weaning foods by mothers themselves. This they said was very important since problems associated with the use of soybeans and winged beans, which require special treatment when used as protein base in weaning formulations, is anticipated when their production is stepped up.

CHAPTER THREE

MATERIALS AND METHODS

3.1 RAW MATERIALS

Millet (*Pennisetum americanum*) and cowpea (*Vigna unguiculata*) were obtained from the Nyankpala Agricultural Research Station and the Tamale Central Market. Albino rats (*Mus Nervegicus albinus*) were obtained from the Biological Sciences Animal House.

3.2 CHEMICALS AND MEDIA

Chemicals and media used in the study were of analytical grade. The list is given in Appendix A.

3.3 METHODS

3.3.1 Processing of Raw Material

3.3.1.1 Preparation of Millet Samples

The millet was screened for any foreign materials like pebbles, twigs and broken grains. The lot was divided into three parts. One part was washed and surface sterilized with 0.1% potassium metabisulphite solution and steeped in stagnant water overnight, that is, to give about 6-12 hours soaking time. The grains were germinated using the method of Marero *et al* (1988) in a cane basket lined with jute sack. The grains were watered twice a day and stirred to get rid of excess heat due to respiration. The process was carried on for 48 hours and the grains dried at 40°C until crisp dry. The vegetative parts (rootlets and shoots) were removed mechanically, and then the grains milled and stored in an air-tight container. The germination process is summarized in the flow sheet in Figure 3.1.

The rest of the grains were washed and steeped in 0.1N HCl for 12 hours to get rid of the colour typical of millet. They were drained and washed several times with tap water to get rid of the 0.1N HCl used in the steeping process. They were dried at 105°C and divided into 2 portions. One portion was roasted at 150°C until golden brown. The two portions were milled separately and stored in air-tight containers (Figure 3.1).

3.3.1.1 Preparation of Cowpea

The cowpea was screened and roasted at 150°C until brown to get rid of the beany flavour. The grains were dehulled by mechanical rubbing whilst hot, winnowed, milled and stored in an air tight container.

3.3.2 Formulation of Millet base

The millet base involved a combination of malted, roasted and raw millets in three different proportions (Figure 3.1). Based on the results of chemical and sensory analysis of the different combinations, the 40:30:30 combination was chosen as the

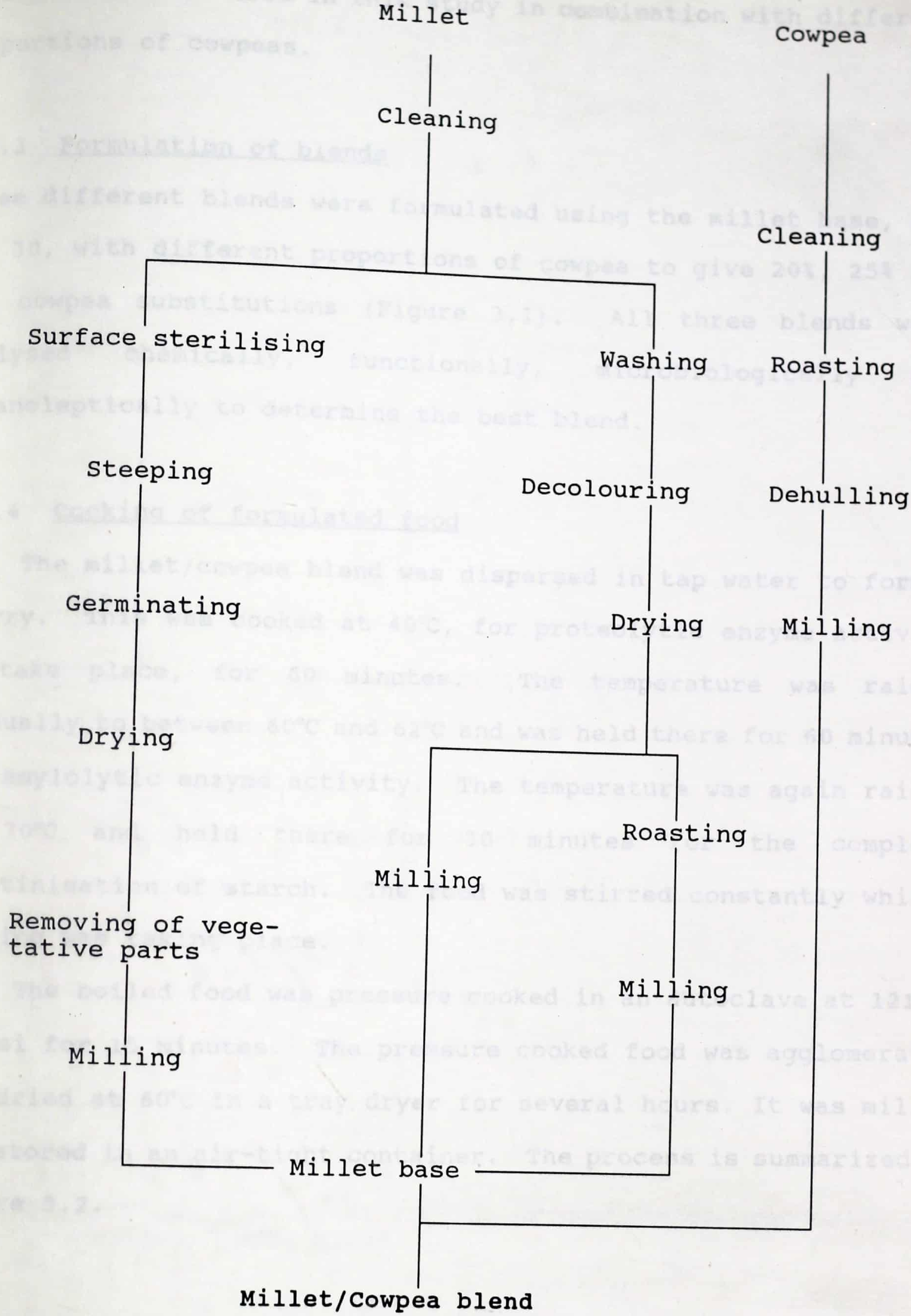


Fig 3.1.Process flow sheet for millet/cowpea blend production

millet base to be used in this study in combination with different proportions of cowpeas.

3.3.3 Formulation of blends

Three different blends were formulated using the millet base, 40: 30: 30, with different proportions of cowpea to give 20%, 25% and 30% cowpea substitutions (Figure 3.1). All three blends were analysed chemically, functionally, microbiologically and organoleptically to determine the best blend.

3.3.4 Cooking of formulated food

The millet/cowpea blend was dispersed in tap water to form a slurry. This was cooked at 40°C, for proteolytic enzyme activity to take place, for 60 minutes. The temperature was raised gradually to between 60°C and 62°C and was held there for 60 minutes for amylolytic enzyme activity. The temperature was again raised to 70°C and held there for 30 minutes for the complete gelatinisation of starch. The food was stirred constantly whilst cooking was taking place.

The boiled food was pressure cooked in an autoclave at 121°C, 15 psi for 15 minutes. The pressure cooked food was agglomerated and dried at 60°C in a tray dryer for several hours. It was milled and stored in an air-tight container. The process is summarized in Figure 3.2.

Millet/Cowpea blend

Dispersion

Cooking at 40°C (Protease activity)

Cooking at 60-62°C (amylase activity)

Cooking at 70°C to boiling

Pressure cooking

Tray drying

Milling

Fig 3.2 Process flow sheet for the production of precooked millet/cowpea blend.

3.4 Chemical Analysis

The raw materials - millet in the various forms of raw, malted, or roasted; and cowpea - were analysed for their chemical composition using the methods described in the sections that follow.

3.4.1 Moisture Determination

The moisture determination was according to AOAC (1970) method 7.008. A 2g portion of the sample was transferred into a previously dried and weighed sintered glass crucible and dried in an oven thermostatically controlled at 105°C to a constant weight. It was cooled in a desiccator. The difference in weight was recorded as moisture loss and the percentage moisture loss calculated (Appendix B.2).

3.4.2 Crude Fat Determination: (AOAC, 1970 method 7.048)

The moisture free sample (3.4.1.) was transferred into a paper thimble and plugged with glass wool. Extraction was done using a soxhlet extractor with petroleum ether (BP 60-80°C) in an already weighed flask. The extraction was carried out at 60°C for 16 hours. The flask was placed on a steam bath to evaporate excess petroleum ether and later in an oven at 105°C. It was cooled in a desiccator and reweighed. The increase in weight of flask was recorded as crude fat and the percentage crude fat calculated (Appendix B.2)

3.4.3 Crude Protein Determination (AOAC, 1975)

The Kjeldahl method using the Kjeltec system was used. 1g of each sample was weighed into Kjeltec digestion tubes and 2 Kjeltec tablets were added. 12 ml of concentrated H_2SO_4 was added to each tube and carefully mixed. Digestion was carried on until a clear solution was obtained (about 40 mins). The tube was cooled to room temperature and 75 ml of distilled water added. The diluted samples were then distilled in a Kjeltec distilling unit with 50 ml of alkali for 6 minutes, into boric acid and mixed indicator. The distillates were titrated with 0.1N HCl and the percentage nitrogen and crude protein calculated (Appendix B.3).

3.4.4 Crude Fibre Determination (AOAC, 1970; 7.054)

The fat free sample obtained from the crude fat determination was transferred into a 750 ml Erlenmeyer flask and approximately 0.5g asbestos added. 200 ml of boiling 1.25% H_2SO4 was added and immediately the flask was transferred into hot plate and connected to a cold finger-type condenser. The contents were stirred frequently by shaking the flask whilst boiling. After 30 minutes, the flask was removed and immediately filtered through linen cloth in a funnel. The cloth was washed into funnel with hot water until washings were no longer acid to litmus paper. The residue (sample and asbestos) were washed back into flask with 200 ml boiling 1.25% NaOH solution. The flask was connected to a condenser and allowed to boil, with shaking for 30 minutes. It was filtered again using linen cloth and washed thoroughly with boiling water until washing as not basic to litmus paper.

The residue was transferred to an already weighed gouche crucible and washed with 15 ml alcohol. The crucible was dried in an oven at 100°C for 1 hour. It was then cooled in a desiccator and weighed. It was ignited in an electric furnace for 30 minutes, cooled and reweighed. The loss in weight was recorded as crude fibre and the percentage crude fibre calculated (Appendix B.4)

3.4.5 Ash Determination (AOAC, 1970; 14006)

2g of each sample was weighed into porcelain crucible which had been ignited and weighed. The crucible and content were ignited at 600°C in a muffle furnace for 3 hours, cooled in a desiccator and weighed. The increase in weight of crucible was recorded as ash and the percentage ash calculated (Appendix B.5).

3.5 Functional Properties

3.5.1 Water absorption Capacity

The water absorption capacity was estimated by the method of Cegla *et al* (1977) with some modifications. One gram of sample was weighed into an already weighed centrifuge tube. Ten millilitres distilled water was added and stirred vigorously for 5 minutes at room temperature and centrifuged at 3,000 rpm for 15 minutes. The supernatant was decanted and the tube reweighed. The increase in weight was recorded as water absorbed and the percentage absorbed calculated.

3.5.2 Fat Absorption Capacity

The fat absorption capacity was determined according to the method of Lin et al (1974) with some modifications. One gram of sample was stirred vigorously for 5 minutes in 50 ml Olive oil in a centrifuge tube. The tube was left standing for 30 minutes and centrifuged at 3,000 rpm for 15 minutes. The volume of supernatant was measured and the decrease noted. The oil absorption capacity was calculated as the percentage of oil absorbed. Specific gravity of Olive oil is 0.91g cm^{-3}.

3.5.3 Bulk Density

The method of Wang and Kinsella (1976) was used. A previously tarred graduated cylinder was filled with 10g sample. The bottom of the cylinder was gently tapped on the laboratory bench several times until there was no further decrease in the sample level. The bulk density was calculated as weight per unit volume of sample (g/ml).

3.6 Microbiological Assay

Petri dishes and canisters were sterilized at 300°C for 6 hours. The agar and distilled water blanks were sterilized by autoclaving at 121°C, 15 psi for 15 minutes. Serial dilutions were prepared from a stock solution of 2g sample dissolved in 10ml distilled water.

3.6.1 Total Plate Count

The total plate count of the diets was determined on plate count agar. The 10^{-3} to 10^{-5} dilutions were used to prepare pour plates and they were incubated at 37°C for 48 hours. Growth of colonies on the 10^{-3} plates were counted using a Quebec colony counter and the colonies per gram sample calculated.

3.6.2 Coliform

The presumptive test for coliforms was determined on MacConkey agar. Pour plates were prepared using the 10^{-3} to 10^{-5} dilutions and they were incubated at 37°C for 72 hours. All plates were investigated for growth of colonies.

3.7 Organoleptic Tests

The method described by Mosha and Loni (1987) was used. A panel of 20 untrained personnel from the University of Science and Technology, Kumasi, aged between 18 and 30 years (including students, mothers and workers) was used. Rating was done using a 5 point score scale where 5 represented extreme likeness, 1 - extreme dislike and 3 - borderline of acceptability. Sampling was done in a room with the various samples in a labelled bowl. The panellists were encouraged to rinse their mouth between samples. Figure 3.3 shows a sample of the judgement sheet used. The score for each parameter and an overall acceptance were computed.

Sample	Taste	Flavour	Colour
A B C			

Score: 5 - Extreme likeness

4 -

3 - Borderline of acceptability

2 -

1 - Extreme dislike

Fig 3.3 Rating Sheet for Organoleptic tests

3.8.2 Serum Glutamate Oxalate Transaminase (SGOT) Determination

The Reitman and Frankel (1957) method was used. 0.5ml SGOT buffered substrate was pipetted into 14 test tubes and labelled S, B, T_c; a_1, a_2; b_1, b_2; c_1, c_2; and d_1, d_2. The various letters indicating serum from rats fed on different diets. S, B, and T_c, indicating Standard, Blank and Test controls (with subscripts a,b,c,d indicating which test serum). The 14 test tubes were incubated at 37°C in a water bath for 5 minutes. The tubes were removed and 0.1 ml distilled water pipetted into one test-tube for the blank reading. 0.1 ml pyruvate standard solution was pipetted into the test tube, S.

0.1 ml test serum was pipetted into the test tubes labelled a to d. All 14 test tubes were incubated again in a water bath at 37°C for 60 minutes. 0.5 ml 2,4-dinitrophenol hydrazine was added and thoroughly mixed. 0.1 ml test serum was added to the T_c labelled tubes and all test tubes were allowed to stand for 20 minutes at room temperature.

Finally, 5 ml 0.4M NaOH solution was added to all tubes and allowed to stand for 5 minutes and the absorbance against the blank was read at 505 nm. Pyruvate was calculated and converted using Table values (Appendix B.7).

3.8.3 Serum Glutamate Pyruvate Transaminase (SGPT) Determination.

The Reitman and Frankel (1957) method was used. 14 test tubes were labelled S, B, T_c, a_1, a_2; b_1, b_2; c_1, c_2; d_1, d_2.

0.5ml SGPT buffered substrate was pipetted into all test tubes and incubated in a water bath at 37°C for 5 minutes. The test tubes were removed and 0.1 ml pyruvate standard pipetted into test tube S. 0.1 ml test serum from specific rats was pipetted into test tubes labelled a to d and all tubes incubated at 37°C in a water bath for 30 minutes.

0.5 ml 2,4-dinitrophenol hydrazine was added and mixed thoroughly. 0.1 ml test serum was added to the test tubes labelled T_c (with subscript depending on rat) and all tubes allowed to stand for 20 minutes at room temperature.

Finally, 5 ml 0.4M NaOH solution was added to all tubes and allowed to stand for 5 minutes. Absorbance against blank was read at 505 nm. The calculation was performed as in Appendix B.7.

3.9 Statistical Analysis

The results were analysed statistically using Analysis of Variance (ANOVA). The computation is as shown in Appendix B.9.

CHAPTER FOUR

RESULTS AND DISCUSSION

The proximate, microbiological, functional and biochemical compositions of formulated diets were investigated, and an enzyme assay conducted on the blood of experimental rats fed on the diet for 28 days. The results and discussion are given in the sections that follow. The results of chemical and sensory analysis for millet base blends are shown in Table 4.0. A base ratio of 40:30:30 (malted:roasted:raw millet) was chosen for test diet formulations based on these results.

4.1 Proximate chemical composition

The proximate chemical compositions of raw materials used are shown in Table 4.1. Malting and roasting did not affect the chemical composition of millet adversely apart from the crude fat and crude fibre contents which were low in the malted samples. During malting enzymes present in the grains use the fat as energy during their respiration processes and also during grain modification process. During grain germination process carbohydrates (especially) and proteins are broken down into smaller units: amylose, maltose, glucose and peptides respectively. The decreases caused by malting in the fat and carbohydrate contents observed in this study were also reported by Marero *et al.* (1988).

The protein contents were apparently similar for the raw,

Table 4.1. Effect of malting and/or roasting on the proximate composition of millet and cowpea used in weaning formulations.[1]

Component	Millet			Cowpea
	Raw	Malted	Roasted	(Roasted)
Moisture (%)	11.8 ± 1.9	9.5 ± 1.2	5.0 ± 1.7	5.7 ± 1.6
Crude Protein (%)	10.2 ± 1.9	10.4 ± 1.8	10.6 ± 1.8	23.5 ± 1.7
Crude Fat (%)	9.0 ± 1.5	7.8 ± 1.1	8.5 ± 1.3	1.6 ± 0.3
Ash (%)	1.3 ± 0.7	1.4 ± 0.5	1.3 ± 0.6	3.6 ± 1.3
Crude Fibre (%)	1.23±0.01	1.09±0.15	1.23±0.00	1.38±0.15
% Carbohydrates By difference	66.5	69.8	73.4	64.4

[1] Values are means ± standard deviation of triplicate determinations.

roasted and malted millets which confirms reports by other workers (Eyeson and Ankrah, 1975). Generally during malting majority of the grain protein is not affected but the quality is affected since the amino acid profile may be improved by the malting process. For instance Opoku *et al.* (1989) reported increases in lysine content after the germination of millet. Other workers have also reported both increase and decrease in protein values during malting depending on various circumstances (Hamad and Fields, 1979; Marero *et al.* 1988). Crude fibre content has been reported to decrease during the germination process due to the activity of enzymes (Briggs *et al*, 1985). In this investigation crude fibre decreased by 11.38% after malting.

The proximate analysis values obtained for the cowpeas fell within literature values except that of crude fibre which was rather low due to the fact that the cowpeas were dehulled. These results confirm the observations of Eyeson and Ankrah (1975). The dehulling also has another advantage of decreasing antinutrients especially raffinose and stachyose, mainly associated with the testa of pulses, which are responsible for indigestion and subsequently passing of odorous gases after their consumption (Ihekoronye and Ngoddy, 1985).

The proximate compositions and sugar content of the uncooked and cooked formulated foods are shown in Tables 4.2 and 4.3 respectively. Generally, there were increases in all the parameters after cooking apart from crude fat which decreased from 4.51% to 4.28% and from 4.48% to 4.15% for 80:20 and 70:30

Table 4.2. Composition of different blends of millet base and cowpea in weaning formulation[1] (Uncooked).

Component	Millet base : Cowpea blend ratio		
	80 : 20	75 : 25	70 : 30
Moisture (%)	7.96 ± 0.08	7.85 ± 0.03	7.83 ± 0.05
Ash (%)	1.36 ± 0.23	1.68 ± 0.09	1.72 ± 0.14
Crude Protein (%)	10.75 ± 0.69	11.36 ± 0.08	12.22 ± 0.78
Crude Fibre (%)	1.30 ± 0.04	1.30 ± 0.02	1.60 ± 0.04
Crude Fat (%)	4.51 ± 0.04	4.06 ± 0.41	4.47 ± 0.01
Total Sugars (mg/g)	27.50 ± 0.03	27.50 ± 0.03	27.40 ± 0.07
Reducing Sugars (mg/g)	6.10 ± 0.07	6.20 ± 0.03	6.20 ± 0.01
Residual Carbohydrate (%)	40.52 ± 0.60	40.05 ± 0.33	38.79 ± 0.93
Food Energy (Kcals)	380.07	376.98	378.67

[1]Values are means ± standard deviation of triplicate determinations.

Table 4.3. Composition of different blends of millet base and cowpea in weaning formulation[1] (Cooked).

Component	Millet base : Cowpea blend ratio		
	80 : 20	75 : 25	70 : 30
Moisture (%)	7.30 ± 0.34	8.01 ± 0.37	7.60 ± 0.04
Ash (%)	1.62 ± 0.09	1.68 ± 0.03	1.83 ± 0.12
Crude Protein (%)	11.63 ± 0.87	12.59 ± 0.09	13.28 ± 0.76
Crude Fibre (%)	1.30 ± 0.02	1.30 ± 0.02	1.36 ± 0.06
Crude Fat (%)	4.23 ± 0.05	4.15 ± 0.03	4.15 ± 0.03
Total Sugars	29.30 ± 1.93	34.70 ± 1.30	35.10 ± 1.44
Reducing Sugars	18.40 ± 2.23	18.40 ± 1.43	18.40 ± 1.71
Residual Carbohydrate (%)	26.22 ± 2.23	19.17 ± 1.43	18.29 ± 1.71
Phosphorus (%)	0.33	0.50	0.54
Calcium (%)	0.01	0.01	0.01
Iron (%)	0.01	0.01	0.01
Zinc (%)	0.01	0.01	0.01
Food Energy (Kcal)	380.27	376.79	377.59

[1]Values are means ± standard deviation of triplicate determinations.

combinations respectively. The decrease in the crude fat is expected since at the initial stages of the cooking the temperature may favour increased lipase activity. Despite the decrease, the resulting value for the crude fat was within acceptable levels of 2.3 - 5.0% for baby foods (PAG 1971).

The addition of 10% cowpea did not significantly alter the crude protein content of the millets but the addition of 25% was able to raise the crude protein content by an average of 5.67%. A significant increase of 7.57% was recorded when 30% cowpea was added to the millet base. The increase though short of the PAG requirement for infant foods, is an improvement over the traditional 'koko' and 'weanimix' currently being used by mothers in the country.

Addition of cowpea at all levels reduced the crude fat content of the blend by an average of 48.39%. This could be due to the fat that cowpea has a low crude fat content of 1.6% and therefore could lower the overall crude fat when blended with the millet. The ash content increased as the cowpea flour concentration was increased giving an indication that the cowpea flour contributed to the increase observed. The cowpea flour had an ash content of 3.6%.

Dietary fibre is very important in a diet since a high dietary fibre reduces intestinal transit time of food, improves glucose tolerance and lowers plasma lipids among others. A low fibre content is associated with chronic disorders such as constipation, obesity, and divertulitis among others (Brigitt and Ivowel, 1975). The Expert Committee on Guidelines for Supplementary foods for older infants and children (1985) therefore have suggested a fibre content of up to 2.1% in the diet of an infant. The average crude fibre content of 1.4% for the blends is therefore acceptable .

During the cooking process the foods were held at specific temperatures of 40 - 42°C and 60 - 63°C for proteolytic and amylolytic enzymes activities. The proteolytic enzyme activity improves the protein by breaking the protein down to smaller peptide units and free amino acids. It must be emphasized that during the malting process some amino acids might have been produced in excess of the requirements for protein synthesis and tend to accumulate in the free amino acid pool thus increasing the protein nitrogen in the samples (Bhise *et al.*, 1988; Marero *et al.*, 1988). The increase in protein could also be attributed to dry matter losses through respiration (Luhila and Chipulu, 1988) Obayume and Opoku (1939) also observed significant increases in water soluble proteins and free amino acids in weaning foods prepared from germinated millet. Breakdown of the total reserve proteins have been reported to occur during the first 18 hours of millet germination and thereafter, there is a period of protein accumulation (ibid). Apart from the 70:30 combination which had a protein content of 13.28% the rest had protein contents below 13.00% (Tables 4.2 and 4.3). Bressani (1985) reported that a food product for weaning small children made of cereal/legume blend of up to 30% substitution would give about 13% good quality protein. Therefore the 70:30 combination should be within an acceptable range of protein quality .

Malting did not apparently affect the ash content of the grains nor did the cooking of the formulated food. Malting has been reported to improve the quality of the minerals which were otherwise unavailable through chelation with phytates (Mosha and Svanberg, 1983). Soaking millet in 0.1N HCl also removes phytates and other antinutrients from the grains. Luhila and Chipulu (1988) have reported *in vitro* iron availability in germinated millet. Ogun *et al.* (1989) observed that dehulling of cowpea completely eliminated tannins from the seed and suggested the use of dehulled cowpea for improved mineral availability. Plahar and Swanson (1990) have also reported 98% decrease in the tannin content of Ghanaian cultivars of cowpea as a result of dehulling. The small increase observed in the mineral content could be due to the state of the raw materials used in the food preparation. Part of the millet was soaked in O.IN HCL overnight, part germinated and the rest roasted. The cowpea was roasted and dehulled so that the processes which could improve the quality of minerals had already been taken care of before blending and the cooking process. The vegetative parts (rootlets and shoots) of the germinated seeds which contain antinutrients especially polyphenols which affect the availability of minerals were also removed before the seeds were milled as suggested by Gopaldas *et al.* (1988). The mineral levels were however below the expected hence the formulations had to be fortified with minerals to meet the required values.

During germination the starch content of the seed decreases rapidly with a concomitant increase in soluble carbohydrates which is due to the increase in alpha-amylase activity (Opoku *et al.*, 1983). The enzymes reduced the carbohydrate in the uncooked food by 35.29%, 52.13 and 52.85% in the 80:20, 75:25 and 70:30 combinations respectively. There was a general increase in the reduction rate as the cowpea content was increased. The vast difference between the 80:20 combination may be due to either an activator substance from the cowpea which activated the amylase activity, or the cowpea itself has active amylases which greatly enhanced the hydrolysis of the carbohydrates. The cowpea amylase could have been activated to optimum activity after the 5% addition of malt, hence the small increase observed for the 70:30 combination.

The reducing sugars increased by an average of 198.39%, that is, 201.64% for 80:20 and 196.77% for 75:25 and 70:30 combinations. The concentration of malt in the various combinations are 32%, 30% and 28%. The differences in the malt concentrations are not high enough to give a significant difference in their activities. This is shown in the results for the 75:25 and 70:30 combinations. The difference of about 5 units in the increase in total sugars observed between the 80:20 combinations and the rest could also be attributed to the effect of increase in the concentration of cowpea flour.

More than 15% of the food was needed to show the acceptable viscosity of 3000 cps of infant foods. This could be attributed to the high enzyme activity and the concomitant decrease in carbohydrate content. This also means an increase in nutrient density since more food will be needed in reconstitution to give the same effect as other traditional infant foods (Mosha and Svanberg, 1988).

The comparatively low level of residual carbohydrate give an indication of a high digestibility rate which will prevent delay of food in the gut during digestion.

4.2 Microbial Quality of Formulated Foods

Table 4.4 shows the results of the microbial assay for the cooked and uncooked formulated foods. The results indicate that the formulations were safe for consumption being negative for coliforms as well as having a low count for total plate count (TPC) test. The values obtained were far below the limits of contamination of 10^2 - 10^6 colonies (Christian, 1983). These could be attributed to the methods of preparation which involved dry heat and autoclaving. These treatments are known to be microbicidal. The moisture contents are within the limits of 0 -10% for infant foods (ibid). A longer shelf life will be favoured since a low moisture content retards microbial activity of microorganisms. Personal hygiene and water quality for reconstitution remain the two factors to check the incidence of diarrhoeal diseases which is a major cause of malnutrition in infants.

Table 4.4. Microbial quality of uncooked and cooked formulated foods.

Sample[1]	Total Plate Count (col/g)	Coliforms (col/g)
Uncooked formula		
80 : 20	$2.4 \pm 0.97 \times 10^3$	- ve
75 : 25	$8.1 \pm 0.23 \times 10^3$	- ve
70 : 30	$4.6 \pm 1.23 \times 10^3$	- ve
Cooked formula		
80 : 20	$1.2 \pm 1.77 \times 10^2$	- ve
75 : 25	$8.4 \pm 1.77 \times 10^2$	- ve
70 : 30	$6.2 \pm 0.93 \times 10^2$	- ve
Standard	$10^2 - 10^6$	10

[1] Millet base : cowpea

4.3 Functional Characteristics of Formulated Foods

The functional characteristics determines the overall acceptability of a product since these characteristics appeal to the sensory organs. Table 4.5 shows the functional characteristics of the precooked formulated foods. The water absorption has a direct link on the amount of water that a food product can absorb and the ultimate body and texture of the food products (Chou and Morr, 1979). There was an increasing water absorption capacity as the cowpea was increased which suggests that the protein component was responsible for this (probably the water soluble proteins). Catsimpoolas and Meyer (1970) have suggested that proteins consist of sub unit structures which when denatured may have more water binding sites. The results compare favourably with work done by Abbey and Ibeh (1988).

Oil absorption enhances flavour retention and improves mouthfeel. As can be seen from Table 4.5 the 70:30 combinations had the highest oil adsorption of 3.2g/g. Kinsella (1976) reported that the chemical modifications of protein which increases the bulk density also enhances fat absorption so that the 70:30 combination with the highest protein substitute should have the highest fat absorption. The results compare favourably with work done by Abbey and Ibeh (1988) who recorded an oil absorption capacity at 3.2g/g.

Bulk density measures the weight per unit volume of a sample. There were no significant differences in the bulk densities of the different combinations tested. The average value was 0.833g/ml.

The high bulk density can be improved by using a 0.5 mm mesh size or a hammer mill to reduce the particle size of the grits. Okesie and Bello (1988) recorded 0.33g/ml bulk density of soy protein isolates by the hammer mill technique.

4.4 Sensory Characteristics

The results of the sensory characteristics are also recorded in Table 4.5. According to Kinsella (1976), the taste and flavour of a product is a consequence of the oil absorption which he has attributed to the degree of chemical modification of the protein present in the sample. There was an increase in the scores obtained with 80:20 scoring lowest and 70:30 highest. The overall acceptance for all the combinations was above average, 2.5. This could be attributed to the effect of the malting on the various blends. Malting is said to greatly enhance flavour, since certain flavour enhancers could be released during the malting process. The flavour could also be enhanced through Maillard reaction of the sugars and free amino acids present during the roasting and cooking processes. The taste was improved by the presence of reducing sugars, low fibre content and a decrease of starch due to thermal dextrinisation, and the high oil absorption (Faubion *et al.*, 1982). The colour was above average probably due to thermal browning during the roasting period.

Even though the results for the various combinations are different they are not significant at 5% ($P > 0.05$).

Table 4.5. Functional characteristics and consumer preference scores for precooked millet base/cowpea blends.

Property	Millet base:Cowpea blend ratio 80 : 20	75 : 25	70 : 30
Functional characteristic			
Water absorption (g/g)	1.53 ± 0.33	1.54 ± 0.32	2.57 ± 0.65
Oil absorption (g/g)	2.30 ± 0.40	2.60 ± 0.10	3.20 ± 0.50
Bulk density (g/ml)	0.82 ± 0.02	0.85 ± 0.01	0.84 ± 0.00
Sensory attribute			
Colour	2.60 ± 0.38	2.95 ± 0.03	3.40 ± 0.42
Taste	2.75 ± 0.55	3.50 ± 0.17	3.75 ± 0.47
Flavour	2.90 ± 0.42	3.30 ± 0.02	3.75 ± 0.43
Overall acceptability	2.75 ± 0.46	3.25 ± 0.07	3.63 ± 0.42

Interpretation of sensory scores:
1 = Dislike extremely
5 = Like extremely

4.5 Animal Studies

Table 4.6 shows the results of the animal assay with respect to Protein Efficiency Ratio, Serum Glutamate Oxalate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT).

4.5.1 Protein Efficiency Ratio (PER)

The PER values were highest for 70:30 combination with a value of 2.31 which was higher than the Protein Advisory Group requirement for weaning foods (PER = 2.1; PAG, 1970). The values for the 75:25 and 80:20 were lower than the PAG requirement. The PER increase for the 70:30 is significant at 5% ($P<0.05$). This could be due to the protein quality as the cowpea concentration was increased even though the increase observed in protein quantity (content) was not significant ($P>0.05$). Plahar and Hoyle (1987) reported that up to 30% substitution with cowpea in millet/cowpea blend, lysine (the limiting amino acid) remained the limiting amino acid and that imposing a change in the limiting amino acid through high levels of cowpea fortification result in a decrease in the blend protein score and hence a decrease in the rate of increase in the net protein value (NPV) even though the crude protein value may be high. The quality of the cowpea protein therefore had a significant effect on the blend with 30% cowpea substitution. Bressani (1985) also reported that a cereal/legume blend of at least 13% crude protein content gives good quality protein. The results for the 70:30 combination was thus expected.

Table 4.6. Protein quality (PER) of formulated diets and serum enzyme assay of test animals.

Sample	SGOT	SGPT	PER
80 : 20	64	43	1.80
75 : 25	33	36	1.95
70 : 30	30	31	2.31
Casein	44	34	2.54
Normal value (infants)	1 - 64	27 - 54	2.10[a]

[a] PAG (1971).

Johasen *et al.* (1961) also observed that animals receiving diets of low protein quality will show loss of appetite which eventually affects the amount of protein eaten. This probably explains the PER values obtained for the 80:20 and 75:25 combinations, which compares with results reported by Gonzalez-Agramon and Ferna-Selvidar (1988). They reported PER value of 1.77 for test diets while Marero *et al.* (1988) reported a PER value of 1.80 for germinated maize:cowpea diet.

The difference in PER values observed could also be due to the differences in rates of digestion and absorption due to residual carbohydrate which invariably affects the availability of protein in the body.

4.5.2 Enzyme Assay in Test Animals on Formulated Diets

Serum Glutamate Oxalate Transaminase, SGOT and Serum Glutamate Pyruvate Transaminase, SGPT levels were determined in weaning rats after 28 days of feeding on the formulated diets. The results indicate that the diets did not show any contraindications after being consumed (Table 4.6). The values for both SGPT and SGOT show a decrease as the cowpea content was increased which probably was due to the increase in protein content and quality. The SGOT value for the 80:20 was however marginal which may be attributable to the low protein of 11.63. The PER was also below the acceptable levels for the 80:20 combination. Values for organ weights indicate that the food had a significant effect on the heart of rats which were fed on the 80:20 blend. The weight of the heart was about three times that for the control. Generally, the enzyme levels in the blood depends on the physiology of any individual and may be random even when the same diet is consumed. Therefore if an individual is within the range it does not matter which value except when it is marginal. Then the individual may be healthy but is more susceptible to infections. Increased levels of SGOT are observed after myocardial infarction and in liver disease whilst SGPT is not elevated after myocardial infarction but in liver disease. The two enzymes however, catalyse different transamination reactions.

4.5.3 Organ Weights

The organ weights of test animals were weighted and the percentage of organ weight to body computed. The results are summarised in Table 4.7. Observation of the various organs after sacrificing did not reveal any fatty organs. However, analysis of variance revealed significant differences at 5%, as shown in the Table, for the heart and the kidney. The 70:30 combination formula gave significantly similar results as the standard casein diet for the heart and the kidney which is a reflection of the values obtained for the PER and the enzyme assays. This probably confirms the observation made by Bressani (1985). The quality of the 70:30 combination may be similar to that of the casein diet. The values obtained for the 80:20 combinations can be said to be high as compared with the standard casein diet. The diet had a significantly different effect on the heart and kidney at 5% level as compared with the standard.

4.6 Food Energy

The results are shown in Tables 4.2 and 4.3. The foods were formulated intending to supplement the child's main diet, and as supplementary foods, they must supply one-third of the Recommended Dietary Allowance for nutrients and energy for children.

All the foods were within acceptable values of 383 Kcals with the highest recording 385.47. This is due to the 80:20 having more carbohydrate than the rest. Marero *et al.* (1988) also recorded similar energies whilst working with baby foods used in formulations.

Table 4.7. Organ weights of rats fed on formulated weaning foods for 28 days (expressed as percentage of final body weight)[1].

Organ	Test diet (millet base:cowpea blend)			
	80:20	75:25	70:30	Casein
Heart (%)	1.55±0.07a	0.84±0.30b	0.53±0.08b	0.53±0.08b
Liver (%)	5.05±0.06a	5.13±0.64a	4.37±0.71b	4.40±0.32b
Kidney (%)	1.50±0.35a	1.67±0.22a	1.30±0.00b	0.93±0.08c
Pancreas (%)	1.25±0.07a	0.93±0.29b	0.73±0.22b	0.60±0.09c

[1] Values are means of triplicate determinations.

[abc] Means in a row followed by same letter are not significantly different ($P>0.05$).

CONCLUSIONS

The 40:30:30 millet base had good functional characteristics and above average sensory scores. This was critical in formulating the food since any changes in such characteristics caused by the addition of cowpea flour, could affect the overall acceptability of the products.

From the discussions it is apparent that the addition of cowpea improved the nutritional characteristics of the blends. The nutritional characteristics increased with increasing levels of cowpea flour. Cooking of blends also improved the nutritional aspects further.

Cooking the food decreased the bulk of the food caused by the high carbohydrate content. This was reduced by the amylases. The cooking also increased the nutrient density which is an advantage in weaning food formulation.

It is apparent from the results that the diets can be consumed without any negative effect on the heart and liver since the enzymes SGOT and SGPT were all within acceptable limits except for the 80:20 combination which may not be consumed alone since the SGOT level was marginal.

The sensory attributes were all above the borderline for acceptability and therefore there is a high probability of the food being accepted based on these characteristics, as well as the functional properties.

The microbial load was found to be within the acceptable limits which may help to improve the shelf life of the products, as well as making the food safe for consumption.

From the discussion, it is apparent that the 70:30 combination produced the best characteristics in all the analysis and especially since the PER was within the acceptable limits with a significant ($P<0.05$) effect on growth. The 70:30 combination was the best formulation.

The 70:30 combination can thus be recommended for use as a weaning food after fortification with minerals and vitamins because of decreased bulk, increase in nutrient density and above average sensory scores.

The proximate analysis suggests that the products are a better alternative to the Weanimix which has a crude protein content of about 10.8%, to be used as a local weaning food.

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APPENDIX A

LIST OF CHEMICALS AND MEDIA

Petroleum Ether,

Concentrated Sulphuric acid,

a- Ketoglutaric acid,

D.L - Aspartate,

Ethylene diamine tetra acetate (EDTA) disodium salt,

Glucose,

MacConkey Agar,

Concentrated hydrochloric acid,

Bromocresol green,

Ethanol,

Potassium metabisulphite,

Glacial Acetic acid,

Potassium dihydrogen Phosphate,

Sulphosalicylic acid,

2,4 - Dinitrophenol hydrazine,

D.L -Alanine,

Sodium pyruvate,

Anthrone,

Plate Count Agar,

Sodium Hydroxide,

Boric acid,

Methyl red,

Chloroform,

Kjeldahl tablets (Selenium),

di-Sodium Hydrogen Phosphate

Olive oil.

APPENDIX B

CALCULATIONS

B1. Moisture

$$\% \text{ moisture} = \frac{a - b}{a} \times 100$$

where a = weight of wet sample
b = weight of dried sample

B2 Crude Fat

$$\% \text{ crude fat} = \frac{\text{Increase in weight of extraction flask}}{\text{weight of sample}} \times 100$$

B3 Crude Protein

$$\% \text{ Nitrogen} = \frac{V_1 - V_o \times m \times V_2 \times 100 \times 14}{V_3 \times w \times 1000}$$

where V_o = Volume of HCl for blank

V_1 = volume of HCl for test sample

V_2 = Volume of digested sample in 100ml

w = weight of sample

m = Molarity of acid.

% Protein = of Nitrogen x 6.25

B4 Crude Fibre

$$\% \text{ Crude fibre} = \frac{w^1 - w^2}{w_o} \times 100$$

where w_o = weight of sample

w_1 = weight of dried sample

w_2 = weight of ashed sample

B5 Ash

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

B6 PER

$$\text{PER} = \frac{\text{weight gain}}{\text{protein consumed}}$$

B7 Serum Glutamate oxalate Transaminase

calculated pyruvate μmol/min/L

$$= \frac{\text{Ab.T} - \text{Ab.T}_c}{\text{Abs}} \times 33.33$$

where Ab.T_c = Absorbance of test control

Ab.T = Absorbance of test

Ab.s = Absorbance of standard

33.33 = constant

B8 Serum Glutamate Pyruvate Transaminase

calculated pyruvate μmol//min/L

$$= \frac{\text{Ab.T} - \text{Ab.T}_c}{\text{Ab.s}} \times 66.66$$

where Ab.T_c = Absorbance at test control

Ab.T = Absorbance at Test

Ab.s = Absorbance of standard

66.66 = constant

B9 The relation of the μmol of pyruvate per min per litre in the colorimetric reaction to I.U. determined spectrophotometrically at 37°C.

Calculated Pyruvate μmol/min/L	SGOT results in I.U. at 37°C
2	4
4	6
6	10
8	12
10	15
12	19
14	23
16	27
18	31
20	35
22	40
23	42
24	44
26	48
38	52
30	56
32	60
34	64
36	69
38	73
40	77
42	81
44	85
46	92
48	98
50	106
52	114
54	128

B.10 The relation of the umole of pyruvate per min per litre in the colorimetric reaction is I.U. determined spectrophotometrically at 37°C.

Calculate Pyruvate (μmol/min/L	SGPT results in IU
2	1
4	2
6	2
8	3
10	4
12	4
14	5
16	6
18	7
20	7
22	8
24	9
28	10
30	11
32	12
34	13
36	14
38	15
40	16
42	17
44	18
46	19
48	20
50	21
52	22

APPENDIX C

REAGENT DETAILS

C.1 Boric acid/mixed Indicator solution

40g of boric acid was dissolved in 600 mls boiled deionised water and made up to 900 mls with more hot deionised water. The solution was cooled to room temperature and 10 ml bromocresol green solution (containing 10 mg in 10 ml alcohol) and 7 ml methyl red solution (containing 7 mg in 7 ml of alcohol) were added. The mixture was diluted to 1 liter with deionised water and mixed well.

C.2 2.4 - dinitrophenylhydrazine 1 mol/l

23.8 mg of 2,4-dinitrophenylhydrazine was dissolved in 1 M HCl in a 100 ml volumetric flask. This solution in stable for 2 months at 2-8°C when stored in a brown bottle.

C.3 Phosphate Buffer 0.1 M, pH 7.4

11.9g of disodium hydrogen phosphate (anhydrous) and 2.2g of potassium dihydrogen phosphate (anhydrous) were dissolved in distilled water and made up to 1 liter with more distilled water. The pH checked and adjusted to pH 7.4 by adding small amounts of potassium dihydrogen phosphate.

C.4 SGOT Buffered Substrate

2.66g of DL - Aspartic acid and 29.2mg of alpha- ketoglutarate were dissolved in about 22 ml of 1 M NaOH. The pH was checked and adjusted to 7.4 with 1 M NaoH.

The solution was transferred into a 100 ml volumetric flask with phosphate buffer, pH 7.4 1 ml of chloroform was added as preservative and stored at 2-8°C.

C.5 SGPT Buffered Substrate

1.8g of DL alanine was dissolved in 18 ml distilled water and 0.5 ml of I M NaOH to adjust pH to 7.4. 29.3 mg of alpha-ketoglutarate was added and dissolved by adding a little more NaOH solution. The pH was adjusted to 7.4 and the whole transferred into a 100 ml volumetric flask with phosphate buffer, pH 7.4. 1 ml chloroform was added as preservative and stored at 2-8°C.