TECHNOLOGY AND CHARACTERISTICS OF WEANING FOODS PREPARED FROM GERMINATED CEREALS AND LEGUMES

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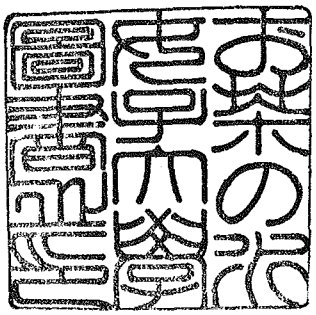

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ABSTRACT

Traditional household level food technologies in child nutrition is particularly focused on germination. This is due to reduction in viscosity of gruels prepared from germinated seeds resulting in increase in their nutrient density. Rice, corn, mungbean, and cowpea were studied for their germination characteristics, made into flour and then formulated into weaning foods. The foods were characterized for their viscosity, storage properties, nutritional quality, starch components, lipid class and vitamin E constituents, peptide composition and antinutritional factors.

The technology of weaning food includes the following processes: (1) cleaning of seeds by flotation or sorting, (2) soaking in water for 6 h, at about 32^{o}C for 72 h for rice/corn and 48 h for mungbean/cowpea, (3) washing of sprouts, (4) drying at 60$\pm$5^{o}C for about 10 to 12 h in a forced draft oven, (5) dehulling and devegetation, (6) roasting at 95^{o}C for 3 min and (7) milling into 60-mesh flour.

The formulation of germinated cereal and legume into weaning foods met 3 criteria: 1) gruel that would have a viscosity of 3,000 cPs, 2) the blend that would supply 1/3 of infants' Recommended Dietary Allowance for protein and energy and 3) the gruel from blend that would have good acceptability. Based on these criteria, the formulations were 70:30 mixtures of (1) germinated rice and mungbean (GRM), (2) rice and cowpea (GRC),(3)corn and mungbean (GCM) and (4) corn and cowpea (GCC).

Data for storage studies showed that all the four mixtures of weaning foods had a shelf-life of 6 months at room

temperature (Philippine conditions, about 32°C) packed in 0.5 mm polypropylene bags or plastic canisters. Increase in moisture and free fatty acids after 6 months did not affect the sensory evaluation results (like moderately) of the products. The foods were microbially safe with 0 count for coliforms and staphylococci. The critical moisture contents were 9.3% for GRM, 10% for GRC, 7.9% for GCM and 8.6% for GCC. The average cost of the weaning foods were about 4 times lower than the commercial ones.

Nutritional evaluation revealed that dietary bulk is decreased to 1/3 in the rice formulations and 1/2 in the corn formulations. Germination increased valine, isoleucine, leucine except in GCC, phenylalanine and tyrosine of the weaning foods. All the formulations had amino acid values that approximated the FAO reference pattern except for the sulfur-containing amino acids. The net dietary protein energy (NDpE) of GRM (9.5%), and GRC (8.5%) met PAG requirements for weaning foods while GCM with 7% met the requirement of the older children only. GCC (NDpE=6.5%) did not meet both requirements. The PER of the rice formulations were higher (2.4) than PAG requirements (2.1), but those of the corn mixes were lower (1.8). Daily 100 g servings of the 4 weaning foods as dry blends satisfied 1/3 of RDA for protein, phosphorus, iron, thiamin, riboflavin, and niacin of young children 6-11 months and 1-3 y. Germination increased the amounts of phosphorus, iron, beta-carotene, thiamin, riboflavin, niacin, and ascorbic acid but decreased calcium.

Enzymes generated during germination partially digested the starch of the materials resulting in the formation of maltooligosaccharides and soluble sugars. The additive effect of gruel preparation favored the conditions for enzyme reaction giving rise to the continued production of soluble sugars. Concentration of amylases was highest in rice, and not all the enzymes were inactivated by drying and roasting temperatures.

No changes in the lipid class composition of cereals were observed. On the other hand, changes in the lipid class of germinated legumes were noted. The major fatty acid constituents of germinated rice were oleic, linoleic and palmitic acids while those of germinated mungbeans were linoleic, linolenic and palmitic acids. Germination decreased the tocopherols of GCM. GCC was the best source of Vitamin E.

Germination increased the TCA soluble nitrogen content of rice and gruels. The TCA-soluble nitrogen constitutes about 10% of the total nitrogen of both germinated materials. Germinated rice had protease activity which was optimum at pH 8.0. It contained acidic, neutral and basic peptides.

Germination, drying, dehusking and devegetation, roasting and cooking significantly ($P<0.05$) reduced antinutritional factors in the weaning foods, namely, phytates, tannins, trypsin inhibitors, phytohemagglutinins and cyanogenic glycosides thus increasing their *in vitro* protein digestibility. All four formulations were well-tolerated by infants who showed no signs of stomach disorders, vomitting, rashes or fever

All four formulations were well-tolerated by infants who showed no signs of stomach disorders, vomitting, rashes or fever throughout the ten-day feeding test.

The potentials of germination in the formulation of supplementary foods hold great promise in helping meet the nutritional needs of young Filipino children.

INTRODUCTION

Severe malnutrition of Filipino children is undoubtedly a national and international concern. General undernutrition based on low weight-for-age data is still prevalent particularly among pre-school children despite the concerted efforts to abate it through the Philippine Food and Nutrition Program. The 1987 Food and Nutrition Research Institute (FNRI) survey findings (Villavieja et al, 1989) on the nutritional status of children showed an estimate of 2 million or 17.7% of the population as a whole, who were underweight for their age. In addition, 1.4 million or 12.4% were acutely and chronically malnourished while 0.2 million or 2.1% were severe cases considered at-risk of physical and mental retardation. The prevalence of protein-energy malnutrition (PEM), commonly indicated by a person's low weight for his age, seems to have risen over the past thirty years. About 12.7% of the pre-school children were found to be acutely and chronically malnourished with PEM as measured against their weight-for-height reference standards.

The worsening situation is indeed quite serious, considering that PEM leads to the development of diseases, to a number of physical, mental and other impairments or disabilities, and worse, sometimes to death. Some of its effects are irreversible and permanent so that even the best possible care in adulthood cannot reverse its adverse consequences.

To a very large extent, malnutrition is a problem of very low food intake. Dietary inadequacy was more pronounced in the vulnerable 0-6 year old children, as well as in pregnant

and lactating women, who hardly met 3/4 of their Recommended Dietary Allowance. Although protein intake did not fall short of desired amounts for the pre-schoolers, the calorie deficiency would have exacted a serious drain on the body's resources by burning up body proteins or slowing down metabolic processes.

Protein and energy deficiency is generally attributed to the quantity rather than to the quality of food eaten. There are various reasons for the relatively low food, especially caloric intakes of Filipinos. As in the past and continuing to the present, poverty undoubtedly is the major and root cause. Poor food production, supply and distribution, limited and inefficient facilities, services and other resources thereof, poor health and sanitation and inadequacies of mothers and caretakers also contribute to the problem. All these factors, which are both causes and effects of the general, social, economic, demographic and political milieu of the country, aggravate the malnutrition problem (Iglesias et al., 1984).

Infants and pre-schoolers stand to suffer the greatest permanent damage from malnutrition since the ages 0-6 are periods of particularly rapid and crucial physical and mental growth. Also, they are vulnerable in society as they have little or no choice as to the kinds and quantities of food they consume.

Infants have small stomachs which do not permit them to eat so much in order to meet their Recommended Dietary Allowances for several nutrients. In addition, the usual wea-

ning foods given to Filipino children are gruels of ungerminated rice and corn which are deficient in nutrients due to their high volume or dietary bulk and high viscosity characteristics. To make the situation worse, the number of feedings is much less, especially among the poor in urban and rural areas in the country, which constitute 70 % of the population. Improving calorie and nutrient in children's gruel thus, presents a challenge to food technologists.

Germination of seed results in the production of enzymes which alters the starch structures of cereals and legumes. This results in a decrease in viscosity of the gruels prepared from them thereby increasing nutrient density. Several other nutrients are synthesized during the germination process. Moreover, essential nutrients needed for growth of the plants are also made available and could be used by the growing children, if processed suitably. The use of germinated legumes at a certain level would improve the amino acid balance of the cereal-based formulations.

The production of weaning foods prepared from indigenous agricultural products is timely in improving the nutritional status of the vulnerable young Filipino children. Increasing costs of commercial weaning foods and the phasing out of foreign-donated food items deprive the young children of much-needed nutrients. Development of low-cost weaning foods with low viscosity, good nutritional value and long keeping quality is still insufficient and must, therefore, be urgently attended to. Potentials of germinating seeds from locally available cereals like rice and corn or legumes like mungbean and cowpea

should be explored. Their lowered viscosity caused by the action of amylases during germination and their subsequent increased intake will help answer the present nutritional needs of the children in the country.

This dissertation is, therefore, aimed at carrying out the following objectives: 1) to develop a technology for cereal-legume weaning foods utilizing germinated seeds and to determine their storage characteristics and cost; 2) to determine the nutritional characteristics of the formulated foods; 3) to find out the effects of germination, flour and gruel preparation on the starch composition of cereals, legumes and their food blends; 4) to determine the effect of germination on the composition of lipid classes and Vitamin E of cereal/legume materials and the formulated weaning foods; 5) to determine peptide content and analyze the peptide composition of germinated materials and 6) to assess the antinutritional factors of germinated cereals, legumes and weaning foods prepared from them.

REVIEW OF LITERATURE

Feeding children of the weaning age

FAO/WHO (1973) reported that an estimated 100 million children are suffering from protein-energy malnutrition in the developing world. The process of undernourishment begins around the age of 6 months at a time when new foods other than milk are introduced at this weaning age (Jelliffe, 1984). Fully 91% of the deficit in weight and 98% of the deficit in height at 3 years can be ascribed to the slow rate of growth between the ages of 6 to 18 months. The normal healthy infant doubles his birthweight by the age of 4 to 6 months on mother's milk alone (Ebrahim, 1983). At no time in his life afterwards does an individual grow at that speed and so, the nutritional requirements to support such rapid growth must be great. It is, therefore, important that the energy density (measure of the amount of energy per unit volume) of weaning foods are high at this crucial time of utmost need. Breast milk has the highest energy density among all infant foods, with 6 kcal/g solid matter. Most traditional weaning foods have an energy density of 1 kcal/g and the common weaning food in Western Europe had 2 kcal/g (Ibid.). On the average, an energy density of about 1.25 kcal/g of prepared food is needed in order to ingest the estimated daily requirement (FAO/WHO,1973).

Infants who have high nutrient requirements in relation to body size, and relatively small stomach volumes, may not be able to eat enough food to meet their nutrient and

energy requirements (Nicol, 1971). All traditional weaning foods tend to be in the form of gruel made from local staples, which mainly contain starch that swells in boiling water and is gelatinized on cooking (high volume). The mouth structures of infants allows only a gruel of fluid consistency which can be swallowed without choking. Thus, there is a limit to which the gruel can be thickened, and consequently, most traditional weaning gruels do not contain more than 10% flour (Mellander and Svanberg, 1984). The rest is water. On the other hand, if the amount of solids in a gruel were raised to increase the nutrient contents, the gruel will become so thick and viscous (high viscosity) that the young child can not eat it. This high volume/high viscosity factor of a diet is referred to as "dietary bulk". There is, therefore, an inverse relationship between dietary bulk and energy density.

Hence, the "bulkiness" of a diet is a major constraint in providing the children with enough food. The swelling of starch during cooking causing a decrease in the energy density and thus contributing to inadequate intake of energy has been recognized by the Protein Advisory Group of the United Nations (Cameron and Hofvander, 1980). This has focused the attention to the use of more energy-rich supplements, especially in fats, in the preparation of weaning foods (PAG, 1970).

Water binding capacity (bulk) of starch granules

The physical changes in the viscosity of starch granules depend on species of starchy food, mechanical treatment, temperature, size of starch granules, amylose/amylopectin ratio

and other factors (Hellstrom et al., 1981). In the native state, the starch granules exist as half crystalline granules composed of amylose and amylopectin. The granules have a certain degree of anisotrope order as they are birefringent in polarized light. When heated, the order breaks down at a certain temperature, the granules lose their birefringent properties and start to swell. The swelling of granules causes an increase in viscosity (Olkku and Rha, 1978). During swelling, solubilized amylose and amylopectin diffuse into the solvent and there will be an equilibrium of solubilized amylose and amylopectin in the swollen granules and the solvent. The swollen granules are sensitive to mechanical treatment. The sensitivity differs between species of starchy food.

The mechanical treatment like stirring, is the cause of a decrease in viscosity at high temperature. When the temperature is lowered, the starch paste starts to gel. This gel may consist of swollen granules amylose and amylopectin or fragments thereof. The proportion of granules in the gel depends on the starch species , the heating procedure, and the mechanical treatment. Variations in the amylose/amylopectin ratio and size of starch granules occur within each species. Measuring the viscosity of each starch species, therefore, provides for identification of cereals with favorable bulk properties.

Enzyme production during germination

During the first hours of germination, amylolytic and proteolytic activity increases rapidly in the seeds (Briggs,

1973). The alpha-amylases are synthesized within the cells of the aleurone layer from where they migrate into the starch endosperm, where hydrolysis of the starch granules begins. The activity of α-amylases in cereals increases during germination from 6 to 9 days (Lineback and Ponpipom, 1971).

The natural substrates for α-amylases are amylose and amylopectins, which are constituents of starch and glycogen. These consist of chains of glucose molecules with α-1,4 glycosidic bonds occasionally linked as branches by α-1,6 bonds. Gruels prepared from flours rich in starch have a sticky and highly viscous nature due to the formation of a three-dimensional network of swollen starch granules held together by amylose chains. These properties are rapidly demolished in the initial stage of hydrolysis by amylase, as the large amylose molecules are quickly broken down into dextrins and maltose (Whelan, 1960). This modification of the starch structure by amylases which are produced during germination results in less binding of water by the damaged starch granules giving gruels with thin consistency. More solids or dry matter (flour) can then be added while maintaining a low viscosity gruel (Ljungquist, et al., 1981). The strength of α-amylases in reducing gruel viscosity was demonstrated by Karlsson and Svanberg(1982) with 1 mg α-amylase reducing the viscosity of a 450-ml wheat flour gruel by 75% within 1 min. They also reported that 1 ml of children's saliva had the same influence on viscosity as 0.5 mg α-amylase.

Significant nutritional changes during germination

Whyte (1973) pointed out that during germination, stored materials are converted into more usable forms for both plant and man.

A considerable proportion of the insoluble protein was found to be transformed into soluble components (Piendl and Wagner, 1972). Certain essential amino acid like lysine and tryptophan were reported to have increased (Dalby and Tsai, 1976). Evancho et al.(1977) observed an increase in the relative nutritive value and PER of germinated legumes. Similar changes were noted on the vitamin contents. Banerjee et al. (1955) reported that the amounts of niacin, pyridoxine, panthothenic acid, inositol, biotin, tocopherol and Vitamin K increased in germinated legumes. The contents of ascorbic acid, riboflavin, niacin and retinol increased in germinated soybeans (Chen, 1970). There was a dramatic increase in ascorbic acid in germinated beans and peas (Fordham et al., 1975; Venugopal and Rao, 1978).

A reduction was seen in trypsin inhibitors (Bau and Debry, 1979) and other antigrowth factors (Chen et al., 1977) as well as in the phytic acid content (Ferrel, 1978). Chen et al. (1975) reported that the availability of mineral nutrients may be increased in bean and pea seeds with decreased phytic acid which chelates Ca, Fe, Mg and Zn. Germination also caused a marked reduction in the level of oligosaccharides responsible for the flatulence factors (East et al., 1972).

Improved weaning food from germinated seeds

In industrialized countries, the processing of cereal-based weaning foods usually includes some kind of procedure intended to reduce the dietary bulk. This can be accomplished by enzyme amylase treatment, pre-cooking, high-pressure extrusion, etc. Such treatment is necessary in order to obtain a level of energy density that makes it possible to cover energy and other nutrient requirements during the weaning period (Rossen and Miller, 1973; Buffa, 1969). However, they are technically sophisticated and expensive and thus, are not suitable in small-scale processing at the village level operations. A cheap method to produce enzymes naturally is by germination or sprouting (malting) of cereals and legumes. In India, malting is a traditional technology for supplementary food for the child during the weaning period (Desikachar,1982).

Brandtzaeg et al. (1981) evaluated the effect of germination on millet, ragi and sorghum in combination with green gram. They found that an increase in germination time from 30 to 48 h reduced the viscosity of a 25% gruel (25 g/100 ml water) by 100% in ragi and 150% in sorghum. Compared to the ungerminated sorghum controls, a 5-fold increase in dry weight was obtained in the 30 h-germinated seeds without any change in viscosity.

Germination, a beneficial practice, is not widely used however, because the preparation of flour from germinated seeds is a time-consuming operation that presents an additional burden to a mother's already heavy work load. Cereals with high

amylase activity are, therefore, tapped inasmuch as small amounts of these amylase-rich foods (ARF) are enough to liquefy starch gruels. Gopaldas et al. (1982) prepared an amylase-rich food from 72 h germinated bajra. This had a shelf life of 28 days when stored at room temperature (25^{o}C, 60% RH); and addition at 4 g ARF/100 g flour gave a liquefied effect on a 10% hot-paste slurry. The maximum reduction in viscosity of a hot-paste slurry made from 25 g flour/100 ml water was obtained at an addition level of 8 g fresh ARF to 42 g rice flour. Mellander and Svanberg (1984) used germinated cereals (white sorghum) which contained amylolytic enzymes to degrade the starch granules in gruels prepared from ungerminated flours of maize and cassava. The added flour from germinated white sorghum was able to liquefy the bulky starches. Thus, the practice becomes a time-saving option because the flours which contain the enzymes need to be prepared less often.

Hellstrom et al.(1981) investigated other weaning foods which had been treated with amylase namely: (1) sekmama (Turkey); (2) superamine (Egypt); (3) Lisha (Tanzania) which was an extruded product using 65% germinated white corn, and (4) two untreated foods Faffa (Ethiopia) and SEF (Sweden). The viscosity-reducing properties of the amylase-treated weaning foods were obvious when compared to the untreated foods. Malleshi and Desikachar (1982) also formulated weaning foods with low viscosity using malted ragi and green gram.

Mosha and Svanberg (1983) showed that the heat inactivation of the amylolytic enzymes in the flours of germi-

nated sorghums occurred at a range of 20-70°C. The optimum viscosity-reducing effect was observed at 40°C. About 40% activity still remained after heating at 90°C.

Desikachar (1982) summarized the relative merits of malting, as follows: 1) the process allows for the partial predigestion of starch and protein; 2) the viscosity can be reduced to any desired level depending on the extent of germination, making the process especially suitable for very young babies; 3) a desirable aroma is developed during the kilning process; 4) phytase hydrolyses the phytin to available phosphate; and 5) vitamin C is elaborated and lysine is reported increased in many cereals. Debranning of the cereal or legume can be assured after the germination process. Its limitation, however, are the long processing time and the requirement for adequate sun-drying or other mechanical drying facilities.

CHAPTER I

TECHNOLOGY OF WEANING FOOD FORMULATIONS FROM GERMINATED CEREALS AND LEGUMES

INTRODUCTION

Usually, the weaning foods given to Filipino children are gruels from rice and corn. Their starchy nature allows these foods to absorb 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, thus limiting the amount of nutrients that could have been derived from the gruel. On the other hand, if the solids in a gruel are increased to raise the nutrient contents, the gruel might be very thick (viscous), causing choking in young children.

To alter the high volume/high viscosity characteristic of starch-based gruels, various methods have been developed to modify the starch structure to lower its water-binding capacity. Some industrial methods include enzyme (amylase) treatment, precooking or extrusion and the traditional process of germination of grains and legumes. The last method has been extensively studied and found useful in increasing energy and nutrient density of infant diets. However, the dextrinogenic and amylolytic properties of rice which is the staple food of Filipinos, has yet to be studied. The present study, therefore, dealt with the feasibility of the process and the utilization of sprouted seeds. Germinated cereals and legumes were utilized to increase the protein content of prepared

blends from them. The present energy gap in the Filipino child's diet (Florentino et al.,1986) renders urgent the need to develop weaning foods with low viscosity and high caloric density from locally available cereals and legumes.

Mungbean and cowpea are the most common, cheap and readily available legumes almost everywhere in the Philippines. The use of mungbean in cereal-based weaning foods is known for its relative freedom from toxic factors, flatus producing substances and trypsin inhibitors (Desikachar, 1981). Cowpea was used due to its lower content of antinutritional factors compared to many other legumes (Elias et al., 1976; Phillips and Adams, 1983).

Specifically, this study aimed to (1) standardize the germination processes for rough rice, corn, mungbean and cowpea; (2) standardize the flour preparation; (3) develop acceptable, safe and nutritious cereal-legume combinations; (4) characterize the products in terms of storage stability (chemical, microbiological and sensory characteristics); and finally, (5) compute the cost of the weaning foods.

MATERIALS AND METHODS

Preparation of seeds

Seeds of unpolished rice (*Oryzae sativa* L.), corn(*Zea mays* L.), mungbean (*Vigna radiata* Wilzceck) and cowpea (*Vigna unguiculata* Walp) were obtained from dealers in a local market in Manila. Only whole viable seeds were used for germination. Prior to the process, seeds were cleaned by winnowing/hand

sorting or flotation. Three to five kg of seeds were used for each germination trial.

Germination procedure

The following parameters were considered in the germination trials: (1) soaking time (6 and 12 h); (2) type of container used (wicker basket and claypot); (3) germination period (24, 48, 72 and 96 h) and (4) root length and yield of sprouts. Whole seeds were weighed, washed and soaked separately in a volume of water which was three times the weight of seeds, for 6 and 12 h. The soaked seeds, placed in a plastic wicker basket,(see Fig. 1.1) were kept inside a closet (dark condition) at room temperature ($32\pm5^{o}C$). Germination was allowed to proceed for 24, 48, 72 and 96 h. The seeds were washed or watered three times a day. The same treatment was applied to seeds germinated in clay pots (see Fig 1.2). The length and yield of sprouts were recorded for each germination time studied.

Criteria used in germination of seeds

The criteria used to determine the optimum germination conditions were (1) desirable decrease in viscosity of gruel preparation to a maximum of about 3,000 cPs and (2) flavor acceptability of 5 and above for the gruel preparation.

The optimum conditions for germination were determined by comparing viscosity and acceptability of the germinated gruel with those of the ungerminated control.

Preparation of flour

The washed germinated seeds were drained and dried in the forced draft oven at 55°C to 65°C for a total of about 10 to 12 h after which the seeds were cleaned of sprouts and hulls by rubbing and winnowing. In the case of rough rice and corn, dehulling was done in a Parpana dehulling machine (Parpana Machinery Mfg. Inc. Manila, Philippines). The seeds were roasted in an iron skillet at varying roasting times until a uniformly light brown product was produced. The time of roasting and final temperature were recorded.

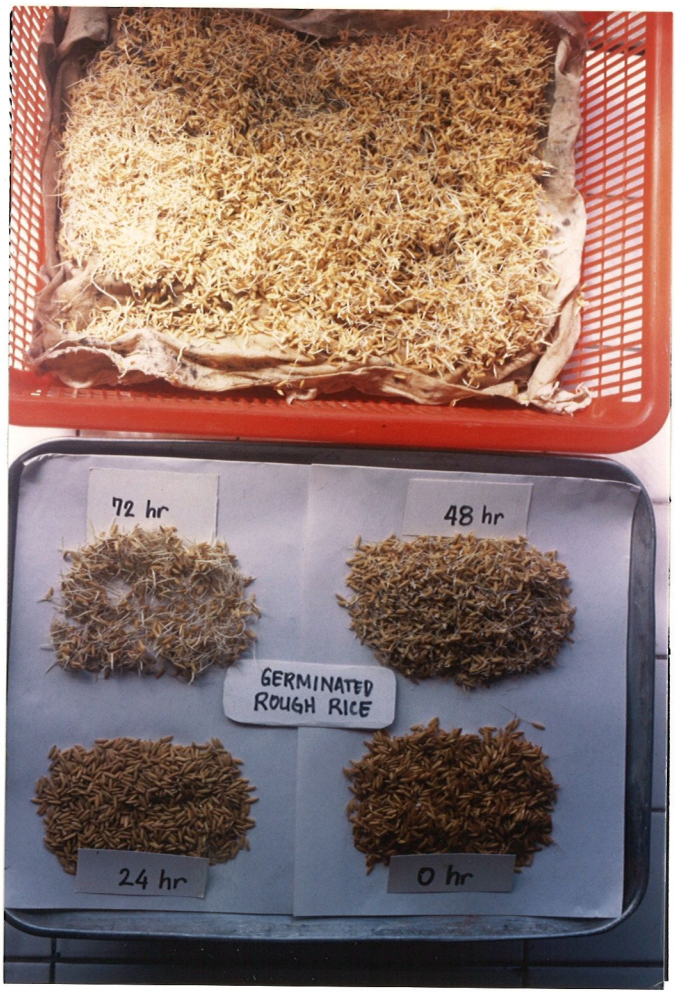


Fig.1.1 Germination of rice seeds showing plastic wicker basket.

Fig. 1.2. Germination of corn seeds showing clay flower pots.

The seeds were milled by passing three or four times through an Almeda grinder (Almeda Cottage Industry, Manila, Philippines) to yield a flour of about 60-mesh fineness. The flours were packed in thick polypropylene bags (0.5 mm), sealed and stored in the freezer (0^{o}C) until used.

Preparation of food blends

Several cereal-legume combinations were prepared using the following proportions by weight: 100:0, 80:20, 70:30, 60:40, 40:60, 20:80 and 0:100, to find out the best proportion that approximated values in the literature. Blends of germinated rice:mungbean (GRM), germinated rice:cowpea (GRC), germinated corn:mungbean (GCM) and germinated corn:cowpea (GCC) were formulated into supplementary foods (see Fig. 1.3).

Fig. 1.3 Weaning foods showing two packaging materials.

The criteria used to select the cereal:legume combinations were the following:

A. (1) Viscosity of gruels below 3,000 cPs such that the gruel ranged from a liquid to a semi-solid state or free-flowing, which was considered ideal for feeding infants (Mosha and Svanberg, 1983), and (2) Consistencies above 10,000 cPs, such that the gruel was semi-solid or had a thick consistency that might be acceptable to older children and adults.

B. The protein and calorie values according to the standards set by the Food and Nutrition Research Institute (FNRI) (FCT, 1968), Philippines, on supplementary foods which was 1/3 of the Recommended Dietary Allowance (RDA), therefore, at least 8 g protein and 383 kcal per day.

C. The flavor qualities and general acceptability after mixing with water, sugar and flavoring of above 5 in a 9-point rating scale.

The solids content of the ungerminated and germinated cereal-legume flour blend to bring about the desired viscosity (3,000 cPs) was then determined. The conditions thus established were finally adopted for the standardization of the process.

Viscosity measurement

Viscosity of gruels prepared from various proportions of rice/corn and mungbean/cowpea was measured following the method of Mosha and Svanberg (1983). One part of flour and 6 parts of water were mixed in a glass beaker and heated in a boiling water bath to reach a cooking temperature of 95^{o}C within 7 min. The gruel was kept at this cooking temperature for 15 min with occasional stirring. The gruel was then placed in a water bath maintained at 40^{o}C and its viscosity measured using a Brookfield Viscometer (B-type, Nihon Keiki, Japan) set at 30 rpm.

Packaging and storage tests

Two packaging materials, namely, high density polypropylene bags (0.5 mm) and plastic (PVC polycarbonate) canisters (see Fig 1.3) were used in storing the selected formulations at room temperature (about 32$\pm$5^{o}C) for a period of 6 months. Monthly determinations of moisture, free fatty acids, and sensory evaluation of general acceptability and initial and final assays of proximate composition, microbial, and sorption iso-

therms of the products were also made.

Analytical tests

Proximate composition of the food formulations was determined using the official methods of AOAC (1980). Food energy was determined by calculation using the Atwater factors (4 kcal x g protein, 9 kcal x g fat, and 4 kcal x g carbohydrate). Microbiological assay such as total plate count (TPC), yeast and mold count and counts for coliforms and staphylococci in the food products was done using the standardized microbiological procedure for food (APHA, 1958). The sorption isotherms were determined by the method of Wink (1946). Free fatty acid analysis was carried out using the methods of Lees (1975).

Sensory evaluation

The formulations were served to a panel of ten trained judges and were judged for general acceptability, using a 9-point rating scale, below 5 being undesirable and above 5 being desirable.

Costing of the products

Costs of production at a small-scale basis which include materials, manpower and 10% overhead cost for utilities (electricity, gas, water) for the different cereal and legume flours, as well as the food formulations, were computed. The costs of the food formulations prepared from germinated grains and legumes were compared to commercial weaning foods available in the market. The costs were also computed based on family food expenditures.

RESULTS AND DISCUSSION

Standardized germination procedure and flour preparation

The standardized germination process for rice, corn, mungbean and cowpea is shown in Fig. 1.4. The optimum soaking time for the seeds to swell was 6 h. Seeds soaked for less than 6 h had uneven germination. Beyond 6 h, the seeds started to produce a foul smell of deterioration.

The germination periods that yielded desirable changes in viscosity and imparted good flavor were 72 h for rice/corn and 48 h for mungbean/cowpea. The actual yield of sprouted materials were 150%, 200%, 250%, and 400% for rice, corn, cowpea and mungbean, respectively.

Both types of germination containers (wicker basket and clay pot) used in the study were found suitable and practical since both provided the easy flow of water during the watering process and yielded sprouts of similar length and amount. The pot, however, used less space than the wicker basket and did not require a closet or layers of cheese cloth (see Figs. 1.1 and 1.2). Furthermore, the temperature within the pot was uniform (27^{o} to 29^{o}C) which was favorable for even sprouting of the seeds. The room temperature in the Philippines (32 ± 5^{o}C) was found very conducive to fast germination of seeds as shown in this study.

Washing of seeds twice or thrice a day during germination was important to promote sprout growth and check deterioration.

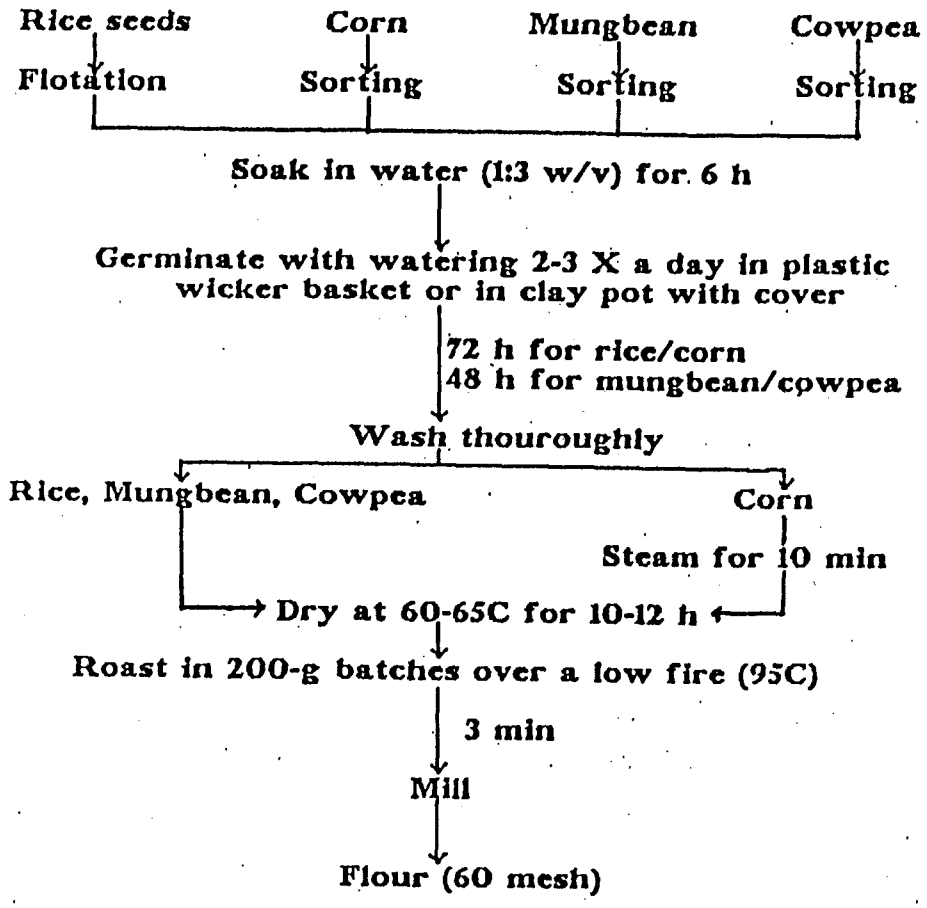


Fig. 1.4. Flow sheet for the preparation of flour from germinated cereals and legumes.

Mold growth was especially avoided, particularly those that produce the dreaded aflatoxin. Thorough washing of seeds before drying partially removed the hulls and any unpleasant smell caused by deterioration of nonviable seeds. Seed viability, therefore, is a very important consideration for germination.

The standardized technology for germinated flour is also shown in Fig.1.4. Drying of germinated seeds in a forced draft oven maintained at 55^{o}C to 65^{o}C was completed after a total drying time of 10 to 12 h, yielding a product with about 7% moisture. The final roasting required to yield a uniform brown color and pleasant aroma was 95^{o}C in all seeds studied. The flour yields from 1 kg seed were 320 g for rice; 550 g for corn; 500 g for mungbean and 640 g for cowpea.

The toasting procedure was kept at a minimum heat for a short time and in small batches to avoid Maillard reactions which can have adverse effects on the protein quality (Brandtzaeg et al., 1981). Roasting was found to contribute a pleasant aroma in the products, increasing their acceptability. Besides, toasting improved the stability of the cereals and legumes because it inactivates the lipoxygenase enzymes that cause the development of objectionable flavors during storage (Anderson et al., 1963).

Development of food blends based on selection criteria

The effect of germination (72 h for rice/corn and 48 h for mungbean/cowpea) on the reduction in viscosity can be noted in Fig. 1.5. At 15% dry matter (d.m.), the viscosity ranged from

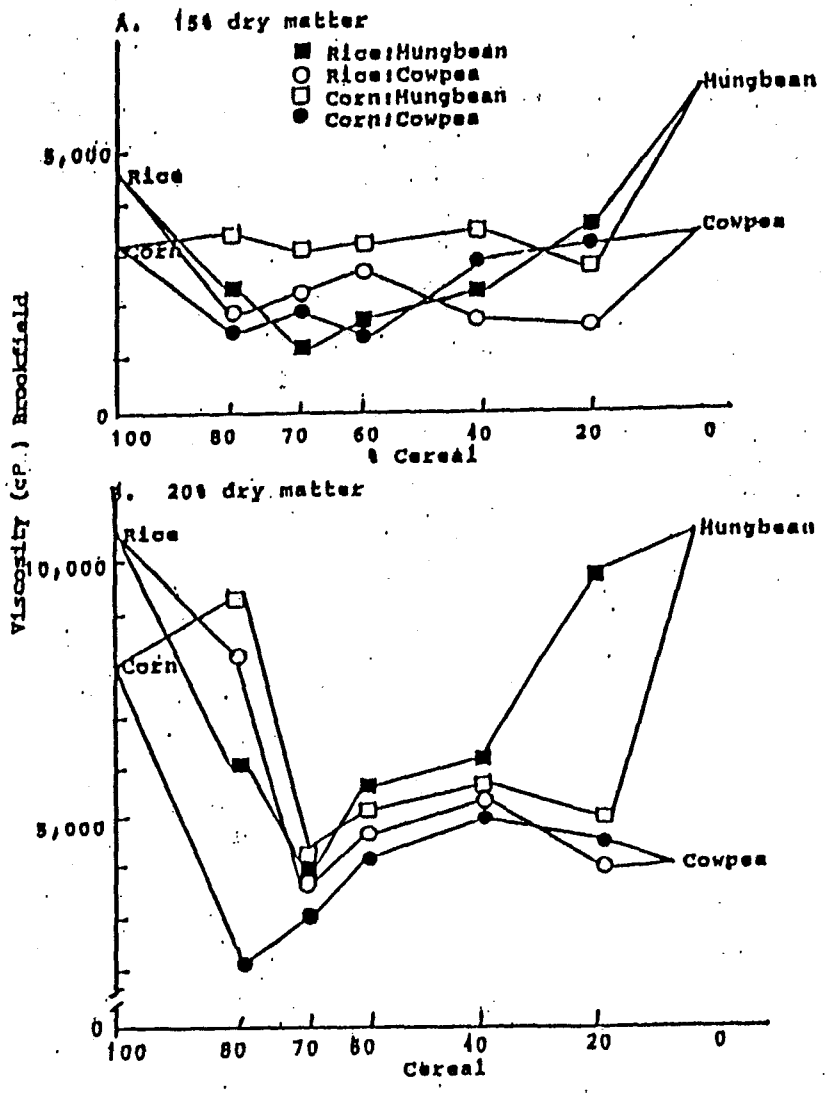


Figure 1.5 Viscosity of gruels prepared with different proportions of germinated cereal:legume

500 to 3,500 cPs (free-flowing consistency). At the denser concentration of 20% d.m., viscosity ranged from 2,000 to 9,500 cPs. These were still lower than the limit of above 10,000 cPs for semi-solid or thick consistency gruels. Noteworthy observation is that the gruels of ungerminated flours at these d.m. concentrations were too thick (paste-like) to be measured by the viscometer.

The reduction in viscosity in germinated flours is evidently a result of starch degradation caused by the action of α- and β- amylases that are produced during the germination process (Mosha and Svanberg, 1983). The amylolytic enzymes present in the seeds are responsible for the enzymatic modification of the starches of rice, corn, mungbean and cowpea during germination, resulting in the dextrinogenic (viscosity-reducing) effect on starch. Desikachar (1981) found that besides low viscosity, the germinated weaning food makes for better digestibility of the starch, inasmuch as partial breakdown to dextrins occurs during germination or malting. Lowered starch complexity and partial predigestion by enzymes during germination should help in utilization of the weaning food by a child being weaned from a lactose-based milk diet to a starch-based cereal diet.

Fig. 1.5 also shows reduction in viscosity occurring in the 70:30 proportion of cereal:legume at both 15% and 20% d.m. Based on protein and food energy composition, 100 g of the 70:30 blend contained an average of 12.5 g protein and 389 kcal. This exceeds the 1/3 RDA for both nutrients if the child were able to consume this amount in one day. The 70:30 ratio

was, therefore, used in the final formulations.

Fig. 1.6 demonstrates the difference between ungerminated and germinated flour blends compared to the acceptable eating consistency (3,000 cPs) for 1 to 3 year-old children. In rice:mungbean blends, a 7% d.m. of ungerminated flours produced the acceptable eating consistency of 3,000 cPs. However, when germinated flours were used, a high total dry matter concentration of 18% still gave the same viscosity. In fact, there was an increase of 2.5 times as much solids in the gruel from germinated flours. In the combination of rice:cowpea, a 6 % d.m. of ungerminated flour blend was equal in viscosity to that of 20% d.m. of germinated flour blend. This accounted for a 2.8 fold increase in nutrient density at the desired viscosity. In the corn:legume formulations GCM and GCC, the viscosity of 10% d.m. ungerminated flours were equal to those of 15% d.m. germinated flours at 3,000 cPs or an increase of 50% in nutrient density.

The difference in the behavior of the starches in rice and corn with regard to dietary bulk properties is explained by Mellander and Svanberg (1984). They reported that gruels from different cereals may show considerble variation in their energy density. This is due to several factors like amylose/amylopectin content of starches.

The high calorie density per unit volume of food attained by germinating rice, corn, mungbean and cowpea was shown by the reduced viscosity of gruels prepared from them. Because of this observation, the formulations of four weaning foods, namely GRM, GRC, GCM and GCC were developed. Seventy parts rice/

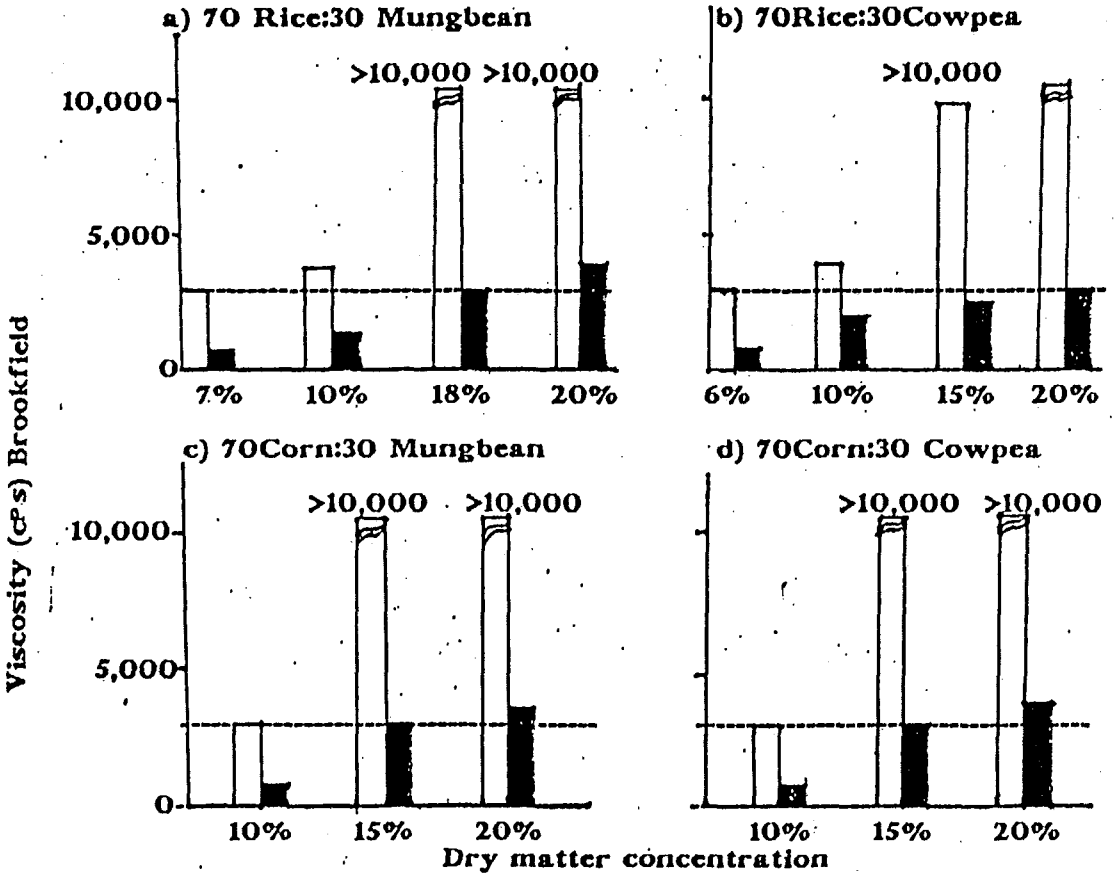


Figure 1.6 Viscosity of gruels prepared with 70:30 cereal-legume blend of ungerminated (□) and germinated (■) flours compared to the ideal eating consistency (-----).

corn were combined with 30 parts mungbean/cowpea (by weight) to balance the protein and amino acid profile of the blend. The profile is reported elsewhere (Marero et al., 1987b).

Table 1.1 shows the acceptable weaning foods that yield 386-560 ml gruel depending on the formulation, with a 3,000 cPs viscosity. These gruels had protein content of 8.6% to 10.8% and food energy that ranged from 426 to 468 kcal, which can adequately supply 1/3 RDA for the 6-11 months and 1-3 y groups of infants. These volumes seem to be just enough to be consumed as weaning food in one sitting.

Table 1.1. Standardized formulation of gruels[a] prepared from a 70:30 blend of germinated cereal (72 h) and legume (48 h) flours measuring 3,000 cPs viscosity.

Ingredients of gruel	GRM[b]	GRC[b]	GCM[b]	GCC[b]
Weight of flour (70:30 cereal:legume)blend,g	75	80	70	80
Volume of water. mL	400	400	460	525
Sugar, g	35	35	40	50
Flavoring (optional)				
Pandan leaf (*Pandanus copelandii* Merr.),piece	1	1	-	-
banana flavor, g	-	-	5	-
vanilla flavor, mL	-	-	-	0.5
Yield (gruel),mL	437	386	495	560

[a]The gruels contained 8.6 to 10.8% protein and food energy that ranged from 426 to 468 kcal.

[b]GRM-germinated rice-mungbean; GRC- germinated rice-cowpea, GCM-germinated corn-mungbean; GCC- germinated corn-cowpea.

Packaging and storage properties of formulations

Table 1.2 shows the storage data of the formulations kept for a period of 6 months at room temperature packed in polypropylene bags and plastic canisters. No noticeable change was found in the proximate composition throughout the storage period. A significant increase in moisture and free fatty acids were, however, observed at the end of the storage period. Nevertheless, these changes did not affect the sensory qualities of the formulations (scores were all above 6, implying maintenance of acceptability).

Results of the sorption isotherm studies shown in Fig.1.7 revealed that the critical moisture content was a_w=0.60, a value needed for safety from deterioration particularly molding. The study showed GRM was 9.3%; GRC 10%; GCM 7.9% and for GCC 8.6%. Results indicated the suitability of both packaging materials in maintaining the desirable moisture content of stored food.

Table 1.3 shows that all four formulations were microbiologically safe for consumption by the infants, being negative for coliforms and staphylococci. Furthermore, they showed a much lower value for Total Plate Count (TPC),(10^1 to 10^2), and yeast and molds (10^1) compared to the FAO/WHO (Christian, 1983) limit for infant foods and to the values of Hobbs and Greene (1976).

Costing of germinated flours and food formulations

The average cost of the weaning food formulations was about 4 times lower than commercially available ones. Based

Table 2—Effect of storage of germinated cereal:legume formulation for 6 months at room temperature in two packaging materials

	GRM[a]			GRC[a]			GCM[a]			GCC[a]		
		Final			Final			Final			Final	
	Initial	PP	PC[b]	Initial	PP	PC	Initial	PP	PC	Initial	PP	PC
1. Moisture, g/100g (n = 4)	4.0	4.4	4.5	4.3	5.2	6.6**	2.0	3.4*	4.6**	2.0	1.8	5.2**
2. Free fatty acid, % (n = 2)	0.41	4.09**	3.34**	0.94	3.90**	3.52**	0.67	3.81**	3.03**	0.76	4.44**	2.53**
3. Sensory test[c] (n = 10)	7.2	7.5	7.4	7.1	7.0	7.2	7.2	7.0	7.1	7.2	6.7	6.7

[a] GRM - Germinated rice:mungbean; GCM - Germinated corn:mungbean; GRC - Germinated rice:cowpea; GCC - Germinated corn:cowpea.

[b] PP - Polypropylene bags; PC - Plastic canister.

[c] Score: 9 = like extremely, 1 = dislike extremely

Note: Unmarked number - not significant

*Final values differ significantly at $P < 0.05$ from initial value

**Final values differ significantly at $P < 0.01$ from initial value

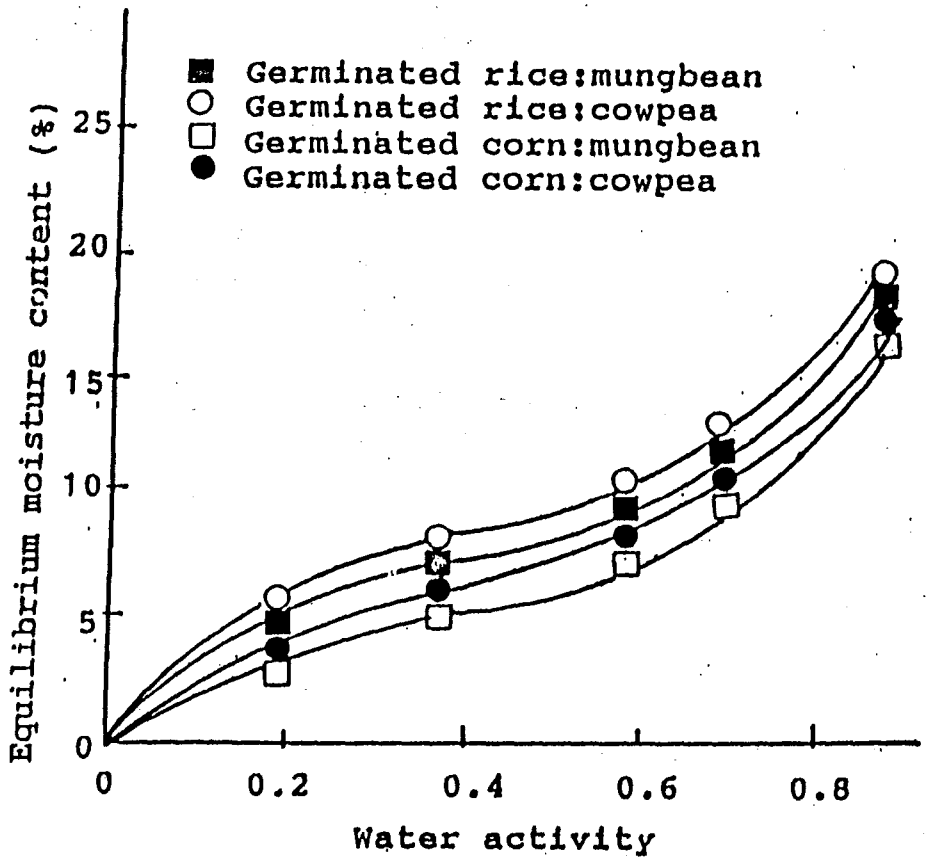


Fig.1.7. Sorption isotherm for germinated cereal and germinated legume blends (70:30).

Table 1.3 Microbiological assay of germinated cereal:legume blends.

Sample	Total plate count(col/g) Initial	Final	Yeast and mold count (col/g) Initial	Final	Coliforms (col/g) Initial	Final	Staphylococci (col/g) Initial	Final
GRM*	11×10^2	17×10^1	3×10^2	5×10^1	neg	neg	neg	neg
GRC	42×10^1	22×10^1	5×10^1	6×10^1	neg	neg	neg	neg
GCM	17×10^2	26×10^1	9×10^1	7×10^1	neg	neg	neg	neg
GCC	46×10^2	41×10^1	1×10^1	1×10^1	neg	neg	neg	neg
Std[b]	$10^4 - 10^6$		-		10		0	
Std[c]	$10^2 - 10^6$		-		-		-	

*GRM-germinated rice-mungbean; GRC-germinated rice-cowpea; GCM-germinated corn-mungbean; GCC-germinated corn-cowpea

[b] Christian (1983)

[c] Hobbs and Greene (1976)

on a 6-member family's monthly food expenditure of P1,590.10 or U.S. $77.57 (1985 values, FNRI Survey, 1985), a month's supply of the weaning food costs only about 3.34% of the food budget. Indeed, these inexpensive weaning foods hold great promise in solving the malnutrition problem faced by the vulnerable groups.

CHAPTER II

NUTRITIONAL CHARACTERISTICS OF WEANING FOODS FROM GERMINATED CEREALS AND LEGUMES

INTRODUCTION

During the transition period from a lactose diet to a solid food, thin gruels with viscosities ranging from 1,000 to 3,000 cPs were found ideal in feeding young children (Mosha and Svanberg, 1983). In the Philippines, weaning foods prepared at home usually consist of bulky monocereal food which is inadequate in terms of energy and nutrient content. To reduce the bulk and increase the nutrient density of weaning food formulations, a technology was standardized for processing flours from germinated rice/corn and germinated mungbean/cowpea (Marero et al., 1987). Four weaning food formulations were prepared from these materials, namely, germinated rice:mungbean (GRM), germinated rice:cowpea (GRC), germinated corn:mungbean (GCM) and germinated corn:cowpea (GCC). Supplementation of the cereals with 30 parts legumes was found efficient in increasing the quantity of protein and energy. But the changes in the quality of the protein of the formulations were not known. Also, their micronutrients such as minerals and vitamins were not given emphasis. Only the high protein and calorie contents were emphasized. This is usually the case in most supplementary foods produced at the village level among member countries in the Association of South-East Asian Nations or ASEAN (Graham et al., 1983).

During the process of sprouting, enzymes released from the scutellum digest some of the starch into dextri-maltose which, of course, does not swell when cooked into a gruel (Ebrahim, 1983). Flour prepared from sprouted grain, therefore, can be used in greater amounts to give the same viscosity as flour from unsprouted grain. As such, nutrient and energy density are increased (Mosha and Svanberg, 1983; Desikachar, 1981; Brandtzaeg et al.,). It was, therefore, interesting to compare the amounts of gruels prepared from ungerminated and germinated flour blends based on the Recommended Daily Allowance (RDA) (FCT, 1968) of Filipino infants for protein and energy.

The present investigation dealt with the nutritional evaluation of GRM, GRC, GCM and GCC formulations. Specifically, the study aimed to characterize the weaning food formulations in terms of: (1) contribution of the food formulations to the RDA of infants for energy and protein; (2) protein quality evaluation using amino acid composition (chemical score and net dietary protein energy or NDpE measures) and biological assay (Protein Efficiency Ratio or PER); and (3) contribution of the food formulations to the RDA for vitamin A (beta-carotene), thiamin, riboflavin, niacin, and vitamin C (ascorbic acid) as well as minerals, specifically, iron, calcium, and phosphorus.

MATERIALS AND METHODS

Samples

Weaning food formulations prepared from blends of 70 parts of germinated rice/corn and 30 parts (by weight) of germinated mungbean/cowpea were used. The four formulations, namely, GRM, GRC, GCM, and GCC were prepared according to standardized methods (Marero et al., 1987a), packed in plastic canisters and stored in the freezer until analysis.

Contribution of formulations to RDA of infants for protein and energy

Gruels were prepared from 70:30 blends of ungerminated and germinated rice/corn and mungbean/cowpea flours which measured 3,000 cPs in viscosity. The gruel yield was recorded. The protein and energy contents of the gruels were computed based on the nutrient composition per 100 g dry blend. Likewise, the volume of gruels required to meet 1/3 of the RDA for protein and energy, i.e.,8.3 g protein and 383 kcal for the 6-11 months old and 8.6 g protein and 437 kcal for the 1-3 years old was computed.

Analytical tests

Proximate composition of the weaning food formulations was analyzed using AOAC methods (Horwitz, 1980).

Amino acids analysis was performed as follows: About 100 mg sample was hydrolyzed with 3 ml of 6N HCl at 110^{o}C for 24 h. The hydrolyzed solutions were filtered (Whatman No. 2) into a 50 ml evaporating flask and dried in a rotary evaporator at

$40^{o}C$. The dried samples were dissolved in 2 ml 0.01 N HCl. Amino acids were determined with a Hitachi Amino Acid Analyzer (Model 835). Tryptophan was analyzed by the method of Sato et al.(1984). About 100 mg sample was hydrolyzed with 10 ml of 4.2 N NaOH at $110^{o}C$ for 20 h, and tryptophan was analyzed in a Hitachi 835 automatic amino acid analyzer. Net Dietary Protein Energy (NDpE) was computed using the nomograph method of Cameron and Hofvander (1980).

Vitamin and mineral analyses were performed using the methods modified and standardized by the Food Composition and Quality Research Program (FCQRP, 1987), Food and Nutrition Research Institute (FNRI), Philippines. Analysis of beta-carotene (AOAC, 1980) involved extraction of powdered samples with 40 ml acetone and 60 ml hexane with 0.1 g $MgCO_3$, adsorption in a column chromatograph using 1:2 MgO:hyflosupercel and elution with 150 ml at 4% acetone in n-hexane. The beta-carotene concentration was determined spectrophotometrically at 453 nm. Thiamin was analyzed by the Henessey and Cerecede thiochrome method reported by Munsell et al. (1949), niacin by the USP microbiological method (Anonymous, 1950). Ascorbic acid was analyzed by the method of Roe and Hall (1939) by extraction with 0.5% oxalic solution.

Calcium was analyzed by the AOAC (1980) methods with dry ashing until C-free; phosphorus by the Fiske and Subbarow method (Lowry and Lopez, 1946); and iron by the Hahn (1945) method.

Protein Efficiency Ratio (PER) experiments on the formulations were performed on 10% protein level in the diet using the method of Campbell (1963) modified by the Food Composition and Quality Research Program, FNRI. Each formulation was fed to a group of ten Sprague-Dawley rats for 28 days. A control group was fed with casein. The rats were fed ad libitum daily and the diet replenished every other day. The rats were weighed once a week.

RESULTS AND DISCUSSION

Dietary bulk of the germinated formulations

In terms of dietary bulk, gruels prepared from the four weaning food formulations, compared to their ungerminated controls, are shown in Table 2.1. The formulations that yielded 3,000 cPs viscosity in ungerminated and germinated flour blends contained only 0.81 to 1.3 g protein per 100 ml gruel in the former, and 1.7 to 2.5 g in the latter. The caloric content of the ungerminated blends was almost trebled when germinated flour blends were used. In the corn formulations, however, the increase in nutrient density was not as high as in the rice formulations. These trends are also evident when comparing the volume of the gruels needed to meet one third of the RDA for the 6-11 months and 1-3 years old infants. The bulk needed was reduced to about 33% for the rice formulations and to about 50% for corn.

Germination period of 72 h for the rice/corn and 48 h for the mungbean/cowpea was very effective in reducing the bulk of

Table 2.1 Dietary bulk of ungerminated and germinated weaning food formulations[a] compared to 1/3 of the RDA for protein and energy for children.

	Rice:Mungbean		Rice:Cowpea		Corn:Mungbean		Corn:Cowpe	
	U[b]	G[b]	U	G	U	G	U	G
Protein, g/100g dry blend	12.5	11.4	11.3	12.3	13.3	12.9	12.2	13.
Energy, kcal/100g	374	384	376	381	385	387	395	390
Formula to yield 3,000 cp-viscosity, g blend	28	75	28	80	46	70	53	80
Water, mL	400	400	400	400	460	460	525	525
Gruel yield, mL	430	437	383	386	496	495	560	560
Protein content, g/100 mL gruel	0.81	1.95	0.84	2.5	1.3	1.7	1.2	1
Energy content, kcal/100 mL gruel	24.4	65.9	27.4	79	35.7	54.7	37.3	55.
Volume (mL) needed to meet 1/3 RDA for 6-11 months:								
for protein (8.3 g)	1020	427	993	327	675	489	726	430
for energy (383 kcal)	1568	581	1397	485	1074	700	1026	687
Volume (mL) needed to meet 1/3 RDA for 1-3 years:								
for protein (8.6 g)	1057	442	1029	339	699	507	753	446
for energy (437 kcal)	1790	663	1594	553	1224	798	1170	784

[a] Formulations 70:30 cereal:legumes, to yield gruel with viscosity of 3,000 cps

[b] U - ungerminated; G - germinated.

the resulting gruels. The formulations, however, showed higher nutrient density than the corn formulations.

Amino acid analysis

Table 2.2 shows the amino acid pattern of the various ungerminated and germinated formulations compared to the FAO reference pattern. The results showed that the combination of 70:30 cereal and legume flours gave efficient complementation of the amino acids, which met the FAO reference pattern, except for the S-containing amino acids. An increase in valine, isoleucine, leucine except in GCC, phenylalanine and tyrosine was noted in the germinated samples compared to the ungerminated blends. However, germination generally did not increase threonine, lysine and tryptophan in the formulations.

Hamad and Fields (1979) reported a significant increase in lysine of germinated rice compared to the ungerminated rice. Tsai et al. (1975) also noted an increase in lysine, tryptophan, and methionine in germinated corn compared to the ungerminated grain. Wang and Fields (1978) reported an increase in available lysine by 2.5 times, tryptophan by 6.5 times and methionine by 5 times for corn germinated for 3 days at $35^{o}C$. In this study, an increase in methionine was noted only in the rice formulations. The decrease in lysine in the corn formulations may have been caused by the 30-min steaming process.

Table 2.3 shows that in some cases, (such as in GRM and in GCM) germination resulted in the decrease in protein, while in other cases (GRC and GCC), an increase was noted. Hamad and

Table 2.2 Amino acid composition of 70:30 blends of ungerminated and germinated flours compared to the FAO reference pattern

Amino acid, mg/gN	Rice:Mungbean		Rice:Cowpea		Corn:Mungbean		Corn:Cowpea		FAC referer patter
	U[a]	G[a]	U	G	U	G	U	G	
Threonine	219	429	307	331	205	229	206	204	250
Valine	376	457	459	495	312	341	554	571	310
Methionine	99	138	119	129	80	61	66	66	
Cystine	88	76	60	68	76	78	82	81	
Total sulfuramino acids	187	214	179	197	156	139	148	147	220
Isoleucine	261	287	357	388	263	287	462	475	250
Leucine	516	521	673	719	661	708	616	602	440
Phenylalanine	334	388	472	511	365	408	319	333	
Tyrosine	202	319	230	252	155	176	153	157	
Total aromatic amino acids	536	707	702	763	520	584	472	490	380
Lysine	360	351	446	448	311	293	355	361	340
Tryptophan	109	104	103	101	47	72	52	67	65

[a] U - Ungerminated; G - Germinated.
[b] PAG (1971).

Table 2.3 Effect of germination on the supplementation of cereals with legumes at levels of 30 parts per 100.

Blend and treatment	% Protein	Limiting amino acids	Chemical score*	Protein value**
Rice-Mungbean				
Ungerminated	12.5	CysMet	85	10.6
Germinated	11.4	CysMet	97	11.1
Rice-Cowpea				
Ungerminated	11.3	CysMet	81	9.2
Germinated	12.3	CysMet	90	11.1
Corn-Mungbean				
Ungerminated	13.3	CysMet	71	9.4
Germinated	12.9	CysMet	63	8.1
Corn-Cowpea				
Ungerminated	12.1	CysMet	67	8.1
Germinated	13.5	CysMet	67	9.1

*Based on mg amino acid/g essential amino acid nitrogen, compared to whole egg.

**Chemical score x %Protein/100

Fields (1979), likewise, found crude protein increased in germinated rice by 1.5%. The first stage in the germination of seeds involves the breakdown of seed reserves and their utilization by the growing root and shoot. In pea seeds, the carbohydrate reserves may be exhausted and protein is used as a respiratory substrate (Chen et al., 1975). During the germination process, some amino acids are produced in excess of the requirements for protein synthesis and tend to accumulate in the free amino acid pool. This may explain the increase in the protein content of the other weaning foods. The limiting amino acids were methionine and cystine in all the formulations, particularly in GCM with chemical score of 63 and in GCC with a chemical score of 67 (Table 2.3). The chemical scores of the rice based formulations were higher than their ungerminated counterparts.

The NDpE of GRM formulation was 9.5% and GRC was 8.5%, which met PAG requirements for weaning foods (8% for the 6-11 months and 7% for the 1-3 years infants, PAG, 1971). The NDpE of GCM was 7%, which satisfied the requirement of the older group only. Only the GCC formulation (NDpE= 6.5%) did not meet the requirement.

As in the chemical score results, the NDpE values on the GCM and GCC formulations were found to be inferior to the rice formulations due to the poorer quality protein of corn.

Protein efficiency ratio

The Protein Efficiency Ratio (PER) of GRM and GRC (2.4) was higher than the Protein Advisory Group requirements for

weaning foods (PER=2.1) (PAG, 1970), while those of GCM and GCC were lower(PER=1.8) as shown in Table 2.4. PER measures were in agreement with the findings of lower protein quality as measured by chemical score and NDpE in the corn-based formulations compared to the rice formulations. Hamad and Fields (1979) found that the relative nutritive value (RNV) for flour of germinated rice is significanly higher than that of the ungerminated rice. Bau and Debry (1979) reported that germination tended to improve the nutritional quality of the protein products as measured by PER. Geervani and Theophilus (1980) found that roasting decreased the PER of green gram, and theorized that germination beyond the optimum period (16 h) altered the protein quality adversely. Germination certainly improved the protein quality of the rice-based formulations in this study.

Table 2.4 PER of germinated cereal-legume formulations prepared from 70 parts rice/corn and 30 parts (by weight) mungbean/cowpea.

Formulation	PER*
Germinated rice-mungbean	2.4
Germinated rice-cowpea	2.4
Germinated corn-mungbean	1.8
Germinated corn-cowpea	1.8
Protein Advisory Group requirement**	2.1

*Corrected to PER of casein= 2.5; variability: n=10.

**PAG (1971).

Vitamin and mineral composition of the formulations

A daily 100 g dry blend serving of each formulation of GRM, GRC, GCM and GCC met one third of the RDA (RDA/3) for protein, phosphorus, iron, thiamin, riboflavin, and niacin (Table 2.5) in both the young and older infants. Vitamin A was met only in the corn formulations, GCM and GCC, which were higher than the rice formulations due to the use of yellow corn. Nevertheless, GRM contributed 40% of the RDA/3 for Vitamin A, while GRC supplied only 20% for both ages. Vitamin C, likewise, was low in the rice formulations, contributing about 37% and 32%, respectively, for the young and older groups. The corn formulations, on the other hand, contributed 84% of the RDA/3 to the first group and 72% to the second group. Calcium content of the formulations was lowest among the nutrients studied, providing only 17% of the RDA/3 to both groups.

Germination caused a decrease in calcium but it did increase the amounts of phosphorus, iron, β-carotene, thiamin, riboflavin and niacin in different legumes. Banerjee et al (1955) reported that the concentration of niacin and other vitamins in certain legumes increased after germination. The increase in thiamin in the weaning food formulations ranged from 3% to 55%, while that of riboflavin ranged from 22% to 211%. This behavior of the B-vitamins during germination in the dark was explained by Simpson et al (1953) who cited that mungbean seeds rely largely on the stored reserve of thiamin, while there was apparently a synthesis of riboflavin. They further reported that thiamin is concentrated largely in the

Table 2.5 Chemical composition of weaning food formulations prepared from germinated cereals and legumes compared to the Recommended Da Allowance (RDA)[a]

Nutrient per 100 g	Rice:Mungbean U[b]	Rice:Mungbean G[b]	Rice:Cowpea U	Rice:Cowpea G	Corn:Mungbean U	Corn:Mungbean G	Corn:Cowpea U	Corn:Cowpea G	1/3 RDA 6-11 months	1/3 RDA 1– yea
Moisture (g)	5.7	4.0	5.5	4.3	3.2	2.0	3.0	2.0		
Protein (g)	12.5	11.4	11.3	12.3	13.3	12.9	12.1	13.5	8.3	8.
Fat (g)	0.7	1.2	0.8	1.0	2.5	1.5	2.7	2.3		
Ash (g)	1.6	1.6	1.4	1.8	1.8	1.8	1.6	1.5		
Carbohydrates (g)	79.5	81.8	81.0	80.6	77.2	80.4	80.6	80.7		
Energy (kcal)	374	384	376	381	385	387	395	390	383	437
Calcium (mg)	62	27	38	29	57	27	33	25	200	167
Phosphorus (mg)	210	284	218	379	205	313	213	277	150	133
Iron (mg)	2.4	3.2	2.7	4.3	2.9	3.1	3.1	3.3	3	2
Vitamin A (IU)	24	33	2	17	136	325	114	292	83	83
Thiamin (mg)	0.27	0.30	0.27	0.42	0.30	0.31	0.32	0.33	0.16	0.
Riboflavin (mg)	0.10	0.31	0.13	0.33	0.15	0.31	0.18	0.23	0.16	0.
Niacin (mg)	2.7	4.0	2.6	6.6	1.9	3.5	1.8	3.3	2	3
Ascorbic acid (mg)	3.0	3.7	0	3.6	3.0	8.4	0	8.3	10	11.

[a] FCT (1968).

[b] U - ungerminated; G - germinated.

cells of the germ and in the cells of the cotyledons adjacent to the germ, while riboflavin appeared to occur in the cotyledons of the dormant seed. During germination, a more general diffusion of thiamin and dispersion of riboflavin occurred. This is caused by absorption and diffusion of water into the tissues.

A dramatic increase in vitamin C of legume sprouts compared to the unsprouted seeds was reported. Hamilton and Vanderstoep (1979) reported a three- to four-fold increase in alfalfa seeds. Hsu et al.(1980) noted an increase of 29- to 86-fold after 4 days germination of yellow peas, lentils and faba beans. Venogupal and Rao (1978) indicated that 42 h was the optimum germination time for the increase in ascorbic acid. Fordham et al (1975) found a range of 2.2 to 9 mg Vitamin C per 100 g for different legumes.

The gruels prepared from the four formulations were intended to supplement a child's main diet, and as supplementary foods, they must supply one third of the RDA for nutrients of the children.

CHAPTER III

EFFECT OF GERMINATION AND GRUEL PREPARATION ON THE STARCH OF CEREALS, LEGUMES AND WEANING FOODS

INTRODUCTION

The Protein Advisory Group of the United Nations Guideline on Protein-rich Mixtures for Use as Weaning Foods (PAG, 1971) recommended the processing of starchy components with amylases which will reduce the viscosity and water retention capacity or bulkiness of the mixture. This allows for the feeding of a more concentrated preparation. Responding to this challenge, four weaning food formulations, namely, germinated rice:mungbean (GRM), germinated rice:cowpea (GRC), germinated corn:mungbean (GCM) and germinated corn:cowpea (GCC) were prepared from a combination of 70% 72-h germinated rice/corn and 30% 48-h germinated mungbean/cowpea (Marero et al., 1988a). The formulations containing from 15 to 20% dry matter in the gruels were found to have 3,000 cPs viscosity, which was suitable for infant feeding. Due to the reduced viscosity of the gruel, more solids could be added, thus increasing their nutrient composition (Marero,et al.,1988b). During germination, starch is partially broken down to dextrins by enzymes (Desikachar, 1980). The partially digested starch may be taken advantage of by a child being weaned from a lactose diet to a cereal diet. It was considered to be of interest to investigate the breakdown of starches into maltooligosaccharides through the germination process.

The purposes of this study were to: (1) show the TLC pattern of sugars formed from starch of germinated rice, corn, mungbean, and cowpea and of their selected combinations into weaning foods; (2) compare the amount of soluble, reducing and dispersible sugars to the total sugars in the flour or gruel samples; and (3) show the amylase activities in the flour and in weaning food samples.

MATERIALS AND METHODS

Germination, flour preparation and food formulation

Rice, corn, mungbean, and cowpea flours with germination periods of 0, 24, 48, 72 and 96 h and weaning food formulation samples for analysis were drawn from 1 kg of thoroughly mixed flour of each of the germination treatments. The samples were sealed in thick (0.5 mm) polypropylene bags and were kept in the freezer (-18^{o}C) until use.

Preparation of samples and detection of sugars on TLC

A 1-g representative flour sample was added to 10 ml of boiling water, mixed in a vortex mixer for 1 min, and filtered through Whatman No. 2 filter paper. The filtrate was immediately used in the TLC experiments. Six µl of the hot-water extracts was spotted on 20 x 20 cm silica gel TLC plates (Whatman K5F, 250 µm thick) along with 3 µl of standard sugars (40 mg/ml): glucose (G1), maltose (G2), maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7). These oligosaccharides were obtained from Nihon Shokuhin Kako, Tokyo.

The plates were developed with a solvent system of ethyl acetate-methanol-water, 37:40:23 by volume (Maeda et al.,1978). The developed plates were dipped in glucoamylase (Sumichimu, obtained from Shin-Nihon Kagaku Kogyo, Aichi prefecture) solution, prepared from 125 mg of glucoamylase in 200 ml of cold ($5^{o}C$) acetone, for 1 min and allowed to air-dry. Formation of glucose was accompanied by incubation in a closed chamber maintained at $40^{o}C$ for 30 min. The enzymes were inactivated by allowing the plates to stand at $100^{o}C$ until the plates were thoroughly dried (5 to 10 min). The plates were soaked for 1 min successively in 3 solutions as follows: solution 1 consisted of 1 ml of saturated silver nitrate in 200 ml of acetone; solution 2 was a mixture of 4 g of sodium hydroxide, 10 ml water, and 190 ml of ethanol; and solution 3 was 240 g of sodium thiosulfate, 25 g of sodium sulfite, and 10 g of sodium bisulfite dissolved in 1 L of distilled water. The plates were dried with a hair blower after each dipping in the solutions. After the third solution, the plates were immersed in water first to eliminate the reagents before drying completely.

Preparation of samples and determination of sugars

a) Total sugars in the flour:

One hundred milligram flour samples were placed in screw-capped test tubes and added with 10 ml of 0.1 N HCl. The tubes were placed in a block heater for 100 min at $80^{o}C$. The mixture was neutralized with 0.1 N NaOH, centrifuged, and the supernatant was analyzed for total sugar by the anthrone method (McCready et al., 1950). Results were expressed as mg glucose equivalent per g sample.

b) Soluble sugar in the flour sample:

One gram of each representative flour sample was suspended in 8 ml of 0.1 N HCl, mixed for 1 min (votex mixer), and allowed to stand for 30 min at ambient temperature to inactivate the enzymes. It was neutralized with 0.1 N NaOH and centrifuged at 3,000 rpm for 10 min. The supernatant was analyzed for soluble and reducing sugars by the anthrone method (Ibid) and Somogyi-Nelson method (Nelson, 1944), respectively. Results were expressed as mg glucose equivalent per g sample.

c) Soluble sugars in the gruel samples:

A 1-g representative flour sample was added to 6 ml of distilled water ($37^{o}C$) and mixed in glass centrifuge tubes for 1 ml in a vortex mixer. Gruels were prepared by the method of Mosha and Svanberg (1983) to simulate actual gruel preparation, i.e., the mixtures were heated in a boiling water bath for 10 min to reach a cooking temperature of $95^{o}C$, then kept at the cooking temperature for 15 min, and cooled to $40^{o}C$. The gruels were added with 2 ml of 0.4 N HCl in order to inactivate enzyme and allowed to stand for 30 min. The supernatant solution was analyzed for soluble and reducing sugar as above. Results were expressed as mg glucose equivalent per g sample.

d) Total sugar dispersible in the gruel:

One gram sample was prepared into gruel as described in c). The gruel was allowed to stand still for 5, 10, 15, and 20 min at ambient temperature; then they were determined for total sugar. Samples were carefully taken from the surface of the tube by means of a 1 ml pipette without disturbing the gruel.

The tubes were left undisturbed throughout the 20-min sampling period.

Enzyme activity

A 1-g representative flour sample from each of the 48-, 72-, and 96-h germination periods for rice, corn, mungbean, cowpea and the four weaning foods (GRM, GRC, GCM and GCC) was suspended in 10 ml of 0.2% calcium chloride solution (Maeda et al., 1979), mixed in vortex for 1 min, and centrifuged at 3,000 rpm for 10 min. The supernatant solution was immediately tested for enzyme activity using 1% soluble starch in 0.05 M acetate buffer, pH 4.8, as substrate. To tubes containing 0.5 ml of the substrate solution, 0.5 ml of the supernatant solution was added. The samples were incubated at 30^{o}C for 0, 5, 10, 15, and 30 min. As control (0 min)of reaction, 1 ml of 1 N HCl was added before the addition of the sample to stop enzyme reaction. At the end of each incubation period, 1 ml of 1 N HCl was added to the samples to inactivate the enzymes. Reducing sugar was measured as described above. The amount of sugar produced from the different samples was shown as glucose equivalent (mg/g) and plotted against incubation time.

RESULTS AND DISCUSSION

Qualitative determination of sugars by TLC

Preliminary detection of the digestion products of germinated rice, corn, weaning food formulations (Fig. 3.1, top photo), mungbean and cowpea (Fig. 3.1, bottom photo) revealed formation of maltooligosaccharides in the germinated materials.

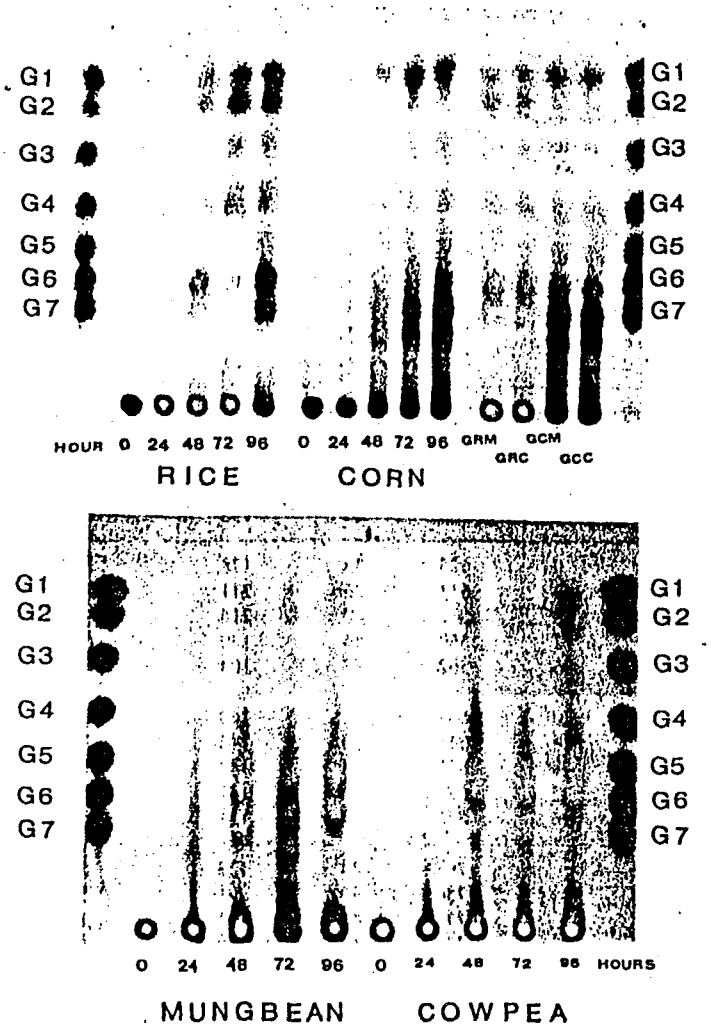


Fig. 3.1 Thin-layer chromatograms of hot-water extracts of germinated rice, corn, weaning foods (top photo), mungbean and cowpea (bottom photo). Formulation: 70 cereal: 30 legume. Germination period: 72 h, cereal; 48 h, legume. Abbreviations: GRM, germinated rice-mungbean; GRC, germinated rice-cowpea; GCM, germinated corn-mungbean; GCC, germinated corn-cowpea.

The sugars were more visibly clear at 48, 72 and 96 h of germination. Presence of glucose and maltooligosaccharide series was shown in all the materials studied. Cereals and the weaning food formulations showed a better separation of the sugars on TLC,. compared to the legume samples. In the latter, the presence of other constituents like peptides and/or minerals may have overlapped with, or disturbed the chromatography of the sugars.

The ratio of amylose to amylopectin is different in every material. The amount of glucose and the speed of its production during hydrolysis or starch breakdown was also observed to differ. In this study, germination of the seeds caused by the breakdown of the starch into maltooligosaccharides by the action of amylolytic enzymes during the germination process was shown.

Determination of sugars

Results in Table 3.1 showed that the total sugars in the flour samples generally decreased after a 96 h germination period in all the materials studied. The soluble sugar increased in both the flour and gruel samples. In rice flour, the average chain length of the sugars was 2, as shown in the ratio of soluble to the reducing sugar in Table 3.1. TLC also showed that G1 and G2 were the major components of rice germinated at 48, 72, and 96 h (see Fig. 3.1). A notable increase in the soluble sugar occurred during the gruel preparation, amounting to 23, 48, and 51% of the total sugars, for the 48-, 72-, and 96-h samples, respectively. Sugars corresponding to

TABLE 3.1 Total and soluble sugar content of flour and gruel prepared from germinated cereals and legumes and their combinations.[a]

Sample	Germination period (h)	Total sugar[b]	Soluble sugars			
			Flour		Gruel	
			Reducing sugar[b]	Soluble sugar[b]	Reducing sugar	Soluble sugar
Rice	0	805	24	50	24	66
	24	791	25	48	26	56
	48	791	36	48	37	187
	72[c]	791	32	64	63	382
	96	791	33	75	80	400
Corn	0	763	32	48	37	70
	24	763	32	56	36	64
	48	763	40	67	42	94
	72[c]	763	56	123	85	216
	96	750	60	155	108	248
Mungbean	0	625	35	128	35	130
	24	611	36	211	38	287
	48[c]	562	40	216	49	316
	72	569	56	120	60	320
	96	562	57	104	65	315
Cowpea	0	659	35	160	35	163
	24	645	36	144	37	188
	48[c]	631	48	144	54	308
	72	631	56	141	58	380
	96	631	57	131	65	315
GRM		763	34	123	57	392
GRC		756	40	91	54	407
GCM		777	40	171	54	464
GCC		756	32	139	54	306

[a] Average of two determinations, no significant difference between values at $p < 0.05$. [b] Reducing sugar and total/soluble sugars were determined by Somogyi-Nelson and anthrone method, respectively, and the values were expressed as glucose equivalent (mg/g sample). [c] These germination products were made into gruels of GRM, GRC, GCM, and GCC. Abbreviations: GRM, germinated rice-mungbean; GRC, germinated rice-cowpea; GCM, germinated corn-mungbean; GCC, germinated corn-cowpea.

about G6 was formed in the gruel samples of the 96-h sample. TLC (Fig 3.1) also showed , in addition to G1 and G2, the prominence of G6 and G7. In corn, polymers of 2 to 3 chain length (Table 3.1) were shown to be formed in both the flour and gruel samples. The soluble sugars in 72 h germinated corn were 16 and 28%, respectively for the flour and gruel samples, of their total sugars. At 96 h, the soluble sugars increased to 20 to 33% in the flour and gruel, respectively.

In the legumes, decrease in the soluble sugars for the flour samples was noted in mungbean germinated for 72 h and in cowpea germinated for 24 h. However, the amount of soluble sugars increased during the preparation of gruel, compared to the flour. The soluble sugars in mungbean had an average of 4 to 7 chain length (Table 3.1) in the flour and gruel samples. Fig. 3.1(bottom photo) also showed the prominence of the higher maltooligosaccharides. In the flour samples, the soluble sugars ranged from 21 to 38% of the total sugars at 48, 72, and 96 h of germination. During gruel preparation, the amount of soluble sugars increased to 56% of the total sugars. In cowpea, the major sugars formed at 48, 72 and 96 h of germination were sugars with 4 chain length for both flour and gruel samples. Cowpeas germinated for 96 h, though, showed the formation of all the maltooligosaccharides, as shown by TLC (Fig.3.1). The conditions during gruel preparation contributed to the production of the higher sugars with about 5 to 6 chain length at 96 h of germination. In the cowpea flour, the soluble sugars were 20 to 27% of the total sugars, but in the gruel, a range of 48 to 60% was found at 48, 72, and 96 h of

germination.

The decrease in the total sugars of all the materials studied could be due to increased α-amylase activities during germination. In the flour samples, the increase in the soluble sugars could be attributed to the increased rate of mobilization of soluble carbohydrates in the endosperm of cereals during germination. Hsu et al (1973) cited that soluble carbohydrates are an important energy source during the early stages of germination. In the case of the legumes, the decrease may have been caused by the more rapid utilization of the sugars in respiration than the rate at which they are formed by the degradation of reserve carbohydrates. Aman (1979) also found that the total and soluble sugars decreased during the germination of mungbean. During gruel preparation, the soluble sugars were increased in all the materials. Aside from the effect of germination, it was found in this study that there was also an additive effect of the process of gruel preparation, in increasing the solubility of sugars. The conditions during gruel preparation, such as addition of water, heating, and stirring favored amylolytic activity, thus making possible the continuous production of maltooligosaccharides. Mosha and Svanverg (1983) found that the amylases in germinated cereals are active at over $85^{o}C$.

Increase in the reducing sugars of all the germinated materials implies that higher polysaccharides, like starch, have undergone sequence of enzymatic degradation.

The soluble sugars in Table 3.1 were those that were

obtained in the supernatant, i.e., after centrifugation. In actual conditions, however, the gruel administered to a child is not centrifuged, so it contains all the dispersible sugars. The values in Table 3.2 represent the amount of sugars taken at the surface of the container when the gruel was allowed to stand without disturbing the tubes, at different times of sampling (5, 10, 15 and 20 min). The dispersible sugars in the gruel of the 72-h rice sample were 89, 92, 96 and 97% of the total sugar at 5, 10, 15, and 20 min sampling, respectively. In corn germinated for 72 h, the dispersible sugars in the gruel ranged from 95 to 98% over the 20-min standing period. Mungbean gruels prepared from 48-h germination showed a firm, gelatinized structure, thus making it impossible to take samples.

Table 3.2. Total sugar* dispersible in the gruels prepared from germinated cereals and legumes.**

Sample	Germination period (h)	Time of standing after gruel preparation, min			
		5	10	15	20
Rice	72	710	735	764	769
Corn	72	725	738	748	751
Cowpea	48	591	605	612	623
Mungbean	48	solid-not determined			

*Sugars were analyzed by the anthrone method and expressed as mg glucose equivalent/g sample.

**Average of three determinations, no significant differences among samples at $p < 0.05$.

The amount of soluble sugars obtained with centrifugation may indicate the partial digestibility of the weaning foods prepared from the germinated materials. However, the amount of dispersible sugars (Table 3.2) available to the child, i.e., without centrifugation, therefore include the soluble sugars, dextrins, and other partial degradation products of starch, which were stabilized in the starch gruel during cooking.

Enzyme reaction on soluble starch

Fig. 3.2 shows the activity of the enzymes generated at the 48, 72, and 96 h of germination in the four materials and in the weaning food formulations prepared from their selected combinations. Fig. 2A and 2B show that the enzymes produced in rice had the highest amylolytic reaction to soluble starch substrate, compared to the other materials. When the 72-h germinated cereal was used in the weaning foods, at a level of 70%, there was a higher concentration of the enzymes in the rice formulations, GRM and GRC, as compared to the corn formulations GCM and GCC (Fig. 3.2D).

The results in Fig. 3.2 showed that the total enzyme activity was highest in rice, followed by cowpea, corn, and mungbean. Although rice had the least amount of soluble sugars in the flour sample (Table3.1) compared to the other materials, its total enzyme activity (Fig. 3.2) was high. This may be due to the localization of the enzymes at the germ area, or due to the characteristics of the substrate, i.e., raw starch in Table 3.1 as compared to soluble starch in Fig 3.2, or may be due to the rigid structure of the rice starch and so enzymes produced

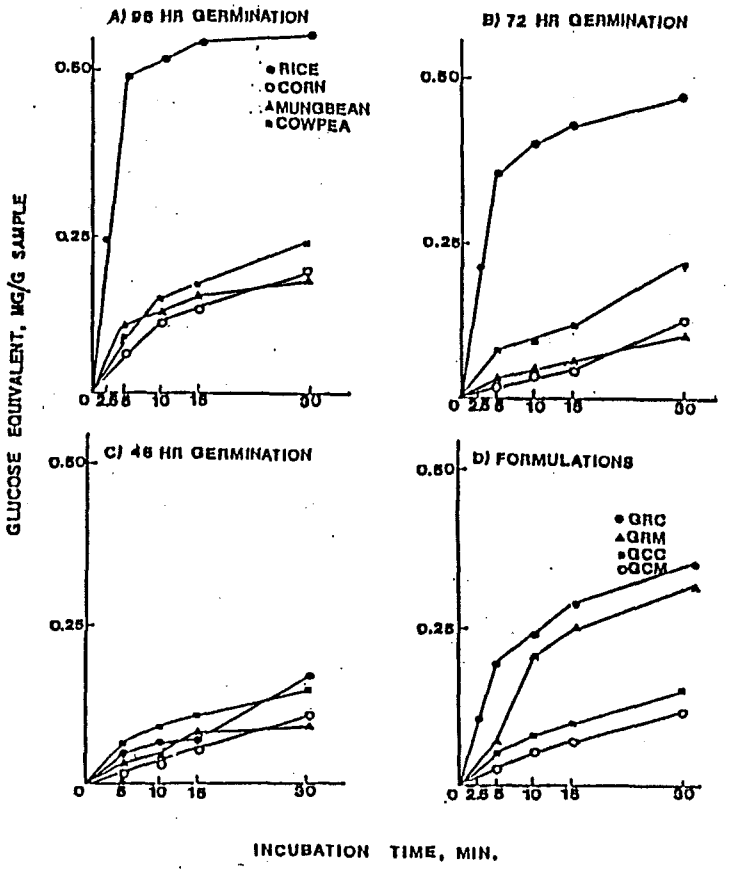


Figure 3.2. Enzymatic reaction expressed in mg glucose equivalent released by 1 g of germinated samples at 30 C acting on 0.5 ml 1% soluble starch from 0 to 30 min.

were not involved in the reaction with starch. Murata et al (1968) found that maltose series oligosaccharides showed a gradual increase from 6 to 12 days of germination at 30^{0}C, and that the activity of α-amylase is very low at the initial stages, up to 4 days, and then increases abruptly in this period. Nevertheless, the amylolytic enzymes produced in rice also increased the production of reducing sugars in GRM and GRC (Fig. 3.2D) at 70% addition of germinated rice in the weaning foods. Palmiano and Juliano (1972) found that the major enzyme for starch degradation in germinating rice was α-amylase. Literature data (Acharaya, 1934; Kneen, 1944) also support that germinated rice had more than one amylase. In fact, three independent amylases were present - a starch liquefying, a dextrinizing, and a saccharifying enzyme.

The enzymes generated during germination were shown to have been retained in the flour regardless of heating treatments during drying and roasting. This remaining enzyme activity continued to produce oligosaccharides during the gruel preparation. Changes in the starch composition, shown by the pattern of maltooligosaccharides in TLC and amylase activities of flours and gruels, have a favorable impact on the digestibility, as well as on the viscosity reduction, of the weaning foods prepared from germinated cereals and legumes.

CHAPTER IV

CHANGES IN LIPID CLASS AND VITAMIN E CONSTITUENTS OF GERMINATED CEREALS, LEGUMES AND WEANING FOODS

INTRODUCTION

Safe weaning foods free from antinutritional factors prepared from germinated cereals and legumes (Marero et al., 1990b) is becoming interesting as a subject of study. This is due to several changes occurring in the seeds during germination. There was a need to investigate the changes in the composition of lipids and tocopherols of the germinated materials since these constituents were concentrated in the germs of embryos which are physiologically involved during germination.

An adequate lipid supply and absorption are especially important for infants and adults with a high energy requirement. Recently, interest in the nutritional effects of phospholipids was renewed (Beil, 1980). Oral or intraduodenal administration of soya phosphatidylcholine will decrease markedly absorption of cholesterol and as in rats (O'Mullane and Hawthorne, 1982) and in men (Beilj, 1980) it was a more potent hypocholesterolemic agent than triglyceride with a similar fatty acid composition. Furthermore, phosphatidylcholine given orally is more efficient in raising blood choline levels than equimolar amounts of free choline (Zeisel, 1980) which might have significant effects on biosynthesis of acetyl

choline in the brain (Cohen and Wurtman, 1976). This is considered to have interesting therapeutic implications in diseases such as Alzeimer's (pre) senile dementia, tardine dyskinesia, and other conditions probably caused by a failing cholinergic activity (Linscheer and Vengroesen, 1988).

Phospholipids play an important role in the digestion and absorption of triglycerides. Practically, all phospholipids in vegetable fat have been removed during preparation for consumption except for the addition of lecithin as emulsifier (FAO/WHO, 1978). An adult human being has a great capacity for fat absorption. Newborns, however, have no capacity, so the source of fat is important. Infants who consume mother's milk, which contains a lipase that is resistant to gastric acid and pepsin may have no problems of fat absorption. Infants reared on cow's milk, though, may have up to 1 year to adjust to have a certain degree of fat absorption (Linscheer and Vergroesen, 1988). Babies have a relative deficiency of bile salts and the intestinal bile salt concentration is frequently below the critical micellar concentration for efficient fat absorption.

Our study aimed to look into the changes during germination of cereals and legumes on the following aspects: (1) lipid content (2) lipid class and composition (3) TLC pattern of lipid classes (4) fatty acid composition (5) tocopherol constituents (6) effects of supplementation of cereals and legumes based on tocopherol contents.

MATERIALS AND METHODS

Sample Preparation

Weaning foods were prepared from 70% germinated (72 h) rice/corn and 30% germinated (48 h) mungbean/cowpea according to standardized procedures (Marero et al., 1988a). Control weaning foods were prepared from combinations of ungerminated materials using the same 70:30 formulation.

Lipid composition

Extraction of lipid in germinated cereals and legumes was carried out by the Folch method with some modifications (Marero et al., 1986). A 30 g sample was homogenized with 150 ml of chloroform:methanol (2:1) mixture and allowed to stand overnight. The mixture was homogenized and centrifuged. The extraction of the residue with 150 ml of the same solvent mixture was repeated two times, and the extracted solutions were combined and condensed in vacuo. The condensed residue was dissolved in the same solvent mixture, which was swirled with one fifth volume of 0.75% of potassium chloride solution. The washing of the separated chloroform layer with the potassium chloride solution was repeated two times and the chloroform was dried with sodium sulfate.

Separation of lipid class by silicic acid column chromatography

Silicic acid (100 mesh, Mallinckrodt Inc.)was suspended in methanol, swirled and allowed to stand. The supernatant was decanted and the silicic acid was washed with water. Washing

with water was repeated until the supernatant became clear. The washed silicic acid was heated at 110 to 120°C for 5-6 h for activation. The activated silicic acid was stored in a dessicator. Prior to use, the silicic acid was heated again for 1 h. Fifteen grams of silicic acid was suspended in 40 ml chloroform and poured into a column (2 cm i.d. x 40 cm). The lipid (200 mg) was dissolved in 5 ml chloroform and charged on the column. The column was successively eluted with 10 column volumes of chloroform, 40 column volumes of acetone and 10 column volumes of methanol. The eluates were collected into 3 fractions according to each solvent, and the above-mentioned volumes were enough to eluate all the organic compounds in the sample until organic compound were no longer detected by sulfuric acid spray on a silica gel TLC plate. Each eluate was condensed in vacuo with a rotary evaporator and then by a vacuum pump. Recovery of lipid in fractions by weight was determined.

TLC of Lipid

Lipid in each fraction was subjected to TLC of silica gel (Merck). The developing solvent was hexane:ethyl ether:acetic acid 80:20:1 or chloroform:methanol:water 65:25:4. TLC was visualized by spraying 80% of sulfuric acid for general detection. Alpha naphtol sulfuric acid was used for glycolipid which gave a blue or red purple color. Dittmer reagent was used for phospholipid which gave a blue spot. In this experiment, chloroform fraction was regarded as neutral lipid, acetone fraction as glycolipid and methanol fraction as phospho-

lipid.

Fatty acid composition

The fatty acid composition of extracted lipid was determined by gas chromatography. Sample lipid (10 g) was saponified with 2 ml of N-KOH at 100^{0}C for 30 min. To this reaction mixture was added 10 ml water and 10 ml petroleum ether and mixed. The ether layer was discarded. The aqueous layer was acidified and added with 10 ml of petroleum ether and well-swirled. The extracted petroleum ether was washed with water and dried with sodium sulfate, then condensed in vacuo. The liberated fatty acid was transformed into methyl ester according to the BF_3-methanol (Morrison and Smith, 1964) method. The fatty acid was analyzed in a Shimadzu GC 7A with flame ionization detector fitted with a column (3 mm i.d. x 3 m) of Unisol 3000 on Uniport C 80-100 mesh. The operating conditions were as follows: Column temperature at 240^{0}C, nitrogen (carrier gas) flow, 50 ml/min. The peaks were recorded and determined with a Shimadzu Chromatopak (Model CR-1A) computerized recorder.

Tocopherol constituents

The weaning foods (5 g) were separately extracted by magnetically stirring 1 h with 50 ml chloroform-methanol (2:1 v/v) following the method of Folch et al (1957). Saline solution was added to the extract and the lower chloroform layer was separated. The chloroform was washed with water and condensed in vacuo. The extract was mixed with 10 ml 3% ethanolic

pyrogallol and 2 ml 60% KOH, and saponified at 60-70^{o}C for 30 min. The tocopherols were added with a 22.5 ml 1% NaCl solution and 15 ml ethyl acetate:n-hexane (1:9 v/v), and mechanically stirred for 15 min and the organic or upper phase was separated. Extraction was done twice. An internal standard (2,2,5,7,8-pentamethyl-6-hydroxy-chroman) was added to the hexane extracts and the mixture was subjected to HPLC.

Apparatus and HPLC specifications

The equipment consisted of a Shimadzu LC-3A pump, a Shimadzu RF 535 fluorescence spectrometer equipped with a microflow cell and a Shimadzu CR3A Chromatopac integrator. The conditions for fluorometry were excitation wavelength 295 nm, emission wavelength 325 nm and both slits 10 nm. The column was a Nucleosil 5 NH_2, 15.0 x 4.0 mm i.d. The column was eluted with n-hexane-isopropyl alcohol (98.5:1.5) at a flow rate of 1.0 ml/min.

RESULTS AND DISCUSSION

Lipid composition of germinated seeds

Table 4.1 shows the lipid composition of germinated rice, corn, mungbean and cowpea at different germinations times (h). Rice and cowpea samples showed an increasing amount of lipids at 24, 48 and 72 h of germination but decreased after 96 h. On the other hand, mungbean and corn showed a decreasing pattern up to 72 h but increased at the 96th h of germination. The increase in the lipid of rice and cowpea during the early

Table 4.1. Lipid content of each germinated seed (g/100 g sample)

Sample	Germination period, h				
	0	24	48	72	96
Rice	1.17	2.48	2.00	2.69	1.93
Mungbean	1.72	1.72	1.45	1.40	1.66
Corn	3.12	2.39	2.10	2.00	2.95
Cowpea	1.86	2.10	2.11	2.57	1.77

stages might be due to the relative weights caused by the decrease in carbohydrates which was used up during germination. Corn had the most lipids (3.12 g), followed by cowpea (1.86 g), mungbean (1.72 g) and rice had the lowest (1.17 g per 100 g samples).

Composition of lipid class of germinated rice and mungbean

The lipid class composition of germinated rice is shown in Table 4.2. It is clearly shown in the table that neutral lipids are the major components of rice while glycolipids and phospholipids are present in minor quantities. In the germinated samples, the neutral lipids ranged from 70 to 88.5; glycolipids from 4.6 to 5; and the phospholipids from 3 to 5.1 mg/100 li pid sample. At 72 hr of germination the neutral lipids decreased from 84.5 to 70 and the glycolipids from 7.1 to 5, while the phospholipids increased from 2.3 to 4.2 mg/100 mg lipid sample.

Table 4.2. Changes in the lipid composition of germinated rice (mg/100 mg lipid).

Fraction	Germination period, h				
	0	24	48	72	96
Chloroform (Neutral lipid), mg	84.5	88.5	86.5	70	81.5
Acetone (Glycolipid), mg	7.1	4.6	5	5	5
Methanol (Phospholipid), mg	2.3	3	5.1	4.2	5.1
Total	93.9	96.1	96.9	79.2	91.6

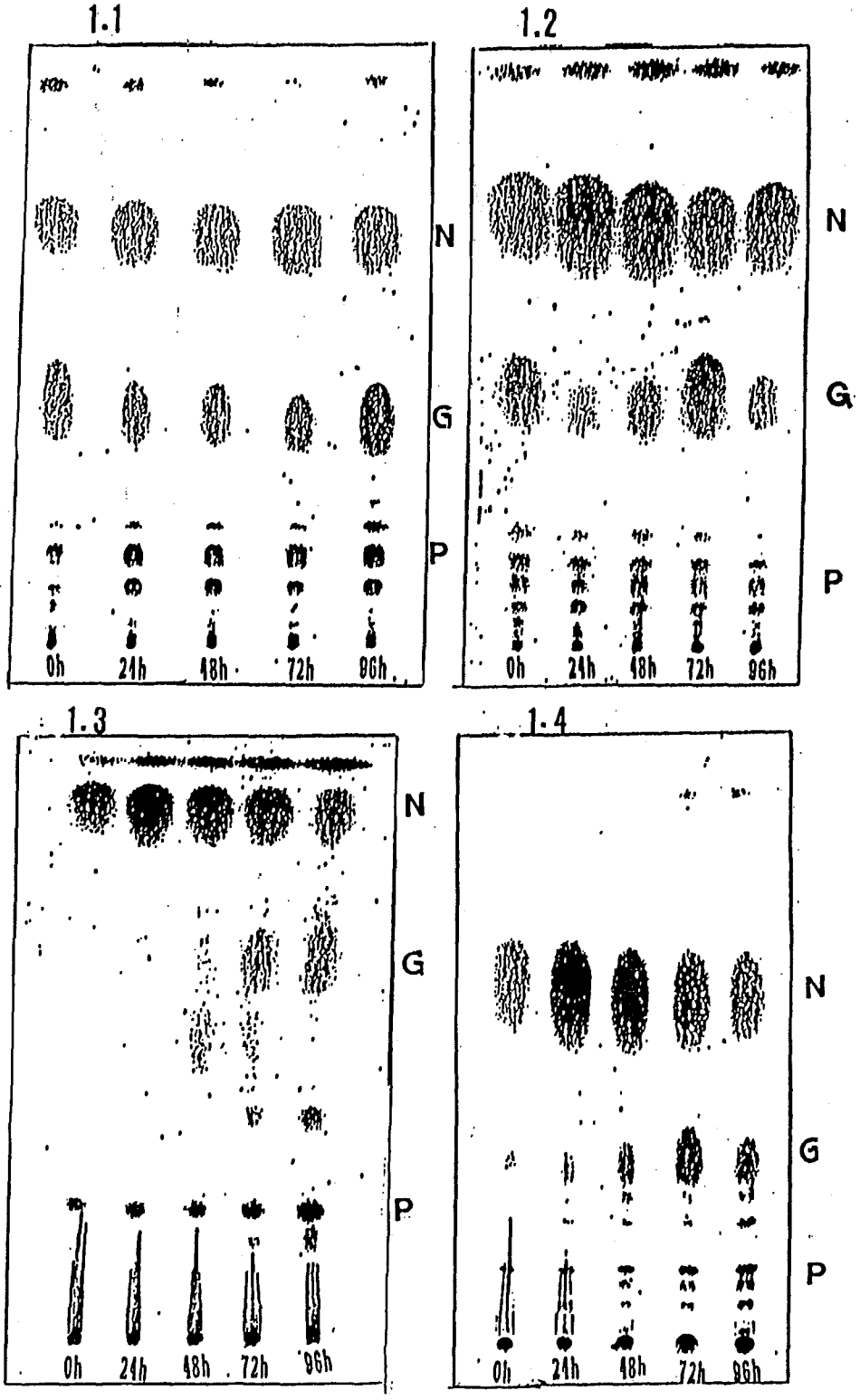
It is shown in Table 4.3 that the lipid class composition of germinated mungbean changed with germination time. The neutral lipids comprise the major constituents followed by the phospholipids and the glycolipids were the least. In the germinated samples the neutral lipids ranged from 50 to 64.3; the glycolipid from 5.8 to 10.7; and the phopholipids from 12.5 to 25.6 mg/100 mg lipid. At the 48 h of germination, the neutral lipids increased from 35.6 to 64.3; but the glycolipids decreased from 9.2 to 5.8 and the phospholipids from 42.1 to 20.9% mg/100 mg lipid.

Table 4.3 Changes in the lipid composition of germinated mungbean (mg/100 mg lipid).

Fraction	Germination period, h				
	0	24	48	72	96
Chloroform (Neutral lipid), mg	35.6	57.8	64.3	60.5	50.0
Acetone (Glycolipid), mg	9.2	7.3	5.8	10.7	20.2
Methanol (Phospholipid), mg	42.1	25.6	20.9	12.5	18.8
Total	86.9	90.7	91.0	83.7	89.0

TLC chromatograms of lipid class in germinated seeds

Fig. 4.1 shows the TLC pattern of the lipids of germinated cereal and legume seeds as detected by 80% sulfuric acid reagent. There were obvious differences between the cereals (Figs. 4.1.1 and 4.1.2) and the legumes (Figs. 4.1.3 and 4.1.4) in the three lipid classes, namely the phospholipids at the lower part, the glycolipids at the middle and the neutral lipids at the top portion of the frame. There were apparent



Developing solvent- Hexane:Ether:Acetic acid = 80:20:1

Detector: 80% H_2SO_4 reagent

Fig. 4.1. TLC chromatograms of lipid class in germinated seeds. .1) rice, .2) corn, .3)mungbean, .4)cowpea.

changes in these lipid classes particularly in the glycolipids of both legumes where increasing intensity is shown. The results in Fig. 4.1 confirms the results in Tables 4.2 and 4.3 in which there were no noticeable changes in rice as compared to the obvious changes in the amounts in each class of lipids in germinated mungbean.

Fatty acid composition of germinated rice and mungbean

Table 4.4. shows the fatty acid composition of germinated rice. The major fatty acid constituents of germinated rice were C 18:1 or oleic acid ranging from 33.4 to 38.1%; followed closely by C 18:2 or linoleic acid ranging from 33.4 to 37.8; and C 16:0 or palmitic acid ranging from 16.4 to 17.8%. The minor components in decreasing order were C 18:3 or linolenic, C 14:0 or myristic, C 18:0 or stearic, C 20.1 or eicosenoic, C 22:0 or behenic and C 24:0 or lignoceric acids. Germination for 72 h did not notably change the amount of the monounsaturated oleic acid. It has, however, increased the polyunsaturated C 18:2 or linoleic acid from 35.8 to 37.8% and decreased the C 16:0 or palmitic acid from 19.1 to 17.8%. Rice is comprised of about 2/3 unsaturated fatty acids.

Table 4.5 shows that germinated mungbean had the predominance of C 18:2 or linoleic acid, followed by C 18:3 or linolenic and C:16:0 or palmitic acids. It contained minor amounts of the unsaturated C 18:1 or oleic acid and the rest of the constituents were the saturated fatty acids C 12:0 or lauric, C 14:0 or myristic, C 18:0 or stearic, C 20:0 or arachidic, C 22:0 or behenic and C 24:0 or lignoceric acids.

Table 4.4. Changes in the fatty acid composition of germinated rice.

Fatty acid, %	Germination period, h				
	0	24	48	72	96
C 12:0				2.0	
C 14:0	2.8	2.5	1.3	1.6	1.7
C 16:0	19.1	17.2	16.4	17.8	16.8
C 16:1		0.5	0.5	0.5	0.7
C 18:0	1.9	1.8	1.8	2.1	2.2
C 18.1	34.7	37.7	36.6	33.4	38.1
C 18:2	35.8	35.0	33.4	37.8	34.7
C 18:3	3.1	3.0	2.7	3.3	3.3
C 20:0	0.9	0.8	0.8	0.6	0.8
C 20:1	0.9	0.8	0.8	0.6	0.9
C 22:0	0.4	0.3	0.4	0.3	0.3
C 24:0	0.4	0.4	0.1	0.4	0.4

Germination for 48 h increased C 18:2 or linoleic acid from 36.8 to 40%, but decreased the C 18:3 content from 26.6 to 21.3%. Palmitic acid remained fairly stable. The presence of linolenic acid in high amounts complements the small amounts in rice in formulating weaning foods, thus improving the quality of the resulting products. Since the 48-h germinated mungbean is used in the formulation of weaning foods, linoleic acid which peaked at this period is, therefore, made available.

Table 4.5 shows that germinated mungbean had the predominance of C 18.2 or linoleic acid (34.7 %) followed by C 18:3 or linolenic (21.6% and C 16:0 or palmitic acid (20.4%). It contained minor constituents of the unsaturated C 18:1 or oleic acid (3.9%). The rest of the constituents were the saturated fatty acids in minor amounts, namely C 12:0 or lauric acid (5.1%), C 14:0 or myristic acid (2.1%), C 18:0 or stearic acid (4.8%), C 20:0 or arachidic acid (2.1%), C 22:0 or behenic acid (1.9%) and C 24:0 or lignoceric acid (1.7%). The presence of linolenic acid in high amounts complements the small amounts in rice in formulating weaning foods, thus improving the quality of the resulting products. Germination increased stearic acid from 17.3 to 22.4% after 96 h of germination of mungbeans. Linoleic acid increased from 36.8 to 40% after 48 h of germination and it remained high (38.9%) at 72 h but decreased to 24% after 96 h. Since in the formulation of weaning foods, 48 h germinated mungbean is used the linoleic acid which peaks at this period, is therefore, made available. Changes in the linolenic acid were unstable, decreasing from 26.6% at 0 h to 15.9% at 24 h and increasing to 21.3% at 48 h and 25% at 96 h.

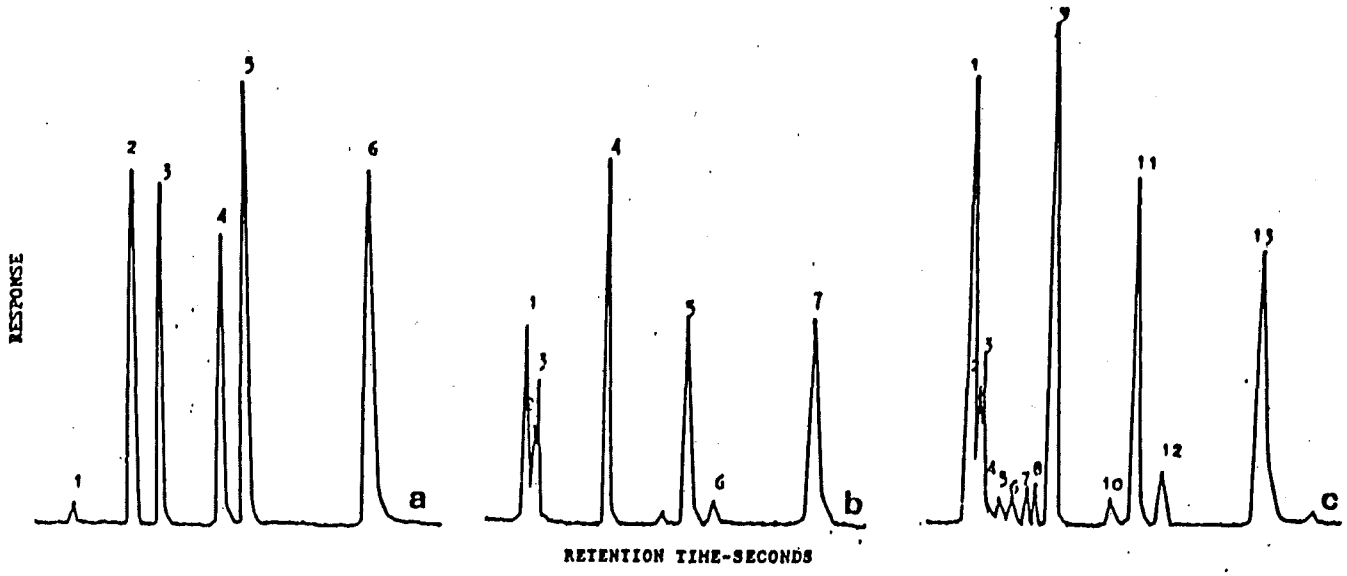
Table 4.5. Changes in the fatty acid composition of germinated mungbean.

Fatty acid	Germination period, h				
%	0	24	48	72	96
C 12:0		6.5	4.3	4.5	
C 14:0	2.0	3.3	2.3	2.3	5.2
C 14:1	0.6				
C 16:0	17.3	25.5	17.0	19.6	22.4
C 18:0	5.1	5.3	4.6	4.6	4.4
C 18:1	4.2	4.9	3.5	3.6	3.2
C 18:2	36.8	33.8	40.0	38.9	24.0
C 18:3	26.6	15.9	21.3	20.3	24.0
C 20:0	2.3	1.7	2.2	1.9	2.2
C 22:0	2.9	1.1	1.9	1.9	1.5
C 24:0	1.0	1.7	2.4	1.8	1.6

Tocopherol constituents

Due to its selectivity and sensitivity, the fluorescence detector was essential for reliable quantification of tocopherols (Fig. 4.2).

The amount of the tocopherols found in the weaning foods is shown in Table 4.6. In the rice-mungbean weaning food, the major tocopherols were γ-tocopherols; β- and δ-tocopherols were present in small amounts while α-tocopherol was minimal. In the rice-cowpea combinations, δ- and γ-tocopherols



Peak No.	Time (Sec)	Identification
1	2.287	
2	5.313	α-Tocopherol
3	6.860	Internal Std
4	9.997	β-Tocopherol
5	11.248	γ-Tocopherol
6	17.9	δ-Tocopherol

Peak No.	Time (Sec)	Identification
1	2.202	
2	2.692	
3	2.842	
4	6.53	Internal Std
5	10.9	γ-Tocopherol
6	12.29	
7	17.488	δ-Tocopherol

Peak No.	Time (Sec)	Identification
1	2.212	
2	2.69	
3	2.845	
4	3.177	
5	3.733	
6	4.365	
7	5.093	
8	5.582	α - Tocopherol
9	6.58	Internal Std
10	9.658	β - Tocopherol
11	10.965	γ - Tocopherol
12	12.378	
13	17.632	δ - Tocopherol

Fig. 4.2. Chromatograms of tocopherols in germinated rice and corn measured by fluorescence spectrometer.

predominated. β- and α-Tocopherols were present in small amounts. Corn-mungbean formulation consisted mainly of γ-tocopherol; α- and δ-tocopherols were present in almost the same amounts and β-tocopherols were the lowest. In terms of Vitamin E composition the corn-cowpea formulations appeared best among the four weaning foods. Predominance of γ-tocopherols could be noted in weaning foods where mungbeans were added while those in which cowpeas were used, δ- and γ-tocopherols were high. α-tocopherols appeared to originate from corn.

Generally, the tocopherol constituents slightly decreased after germination of cereals 72 h and legumes 48 h, except for the γ-tocpherols of germinated corn-mungbean.

Among the materials studied, corn may be a good source of tocopherols as it is used as oil resources, but after germination and processing (drying, winnowing, milling, roasting) removal of the germ and husk and partial digestion of starch, little of the lipids may have remained, thus decreasing the amounts of tocopherols. It could be inferred, therefore, that the tocopherols came from the legumes since they did not have germ and the lipids or tocopherols were present in the cotyledons.

Chen et al (1975) reported 117 to 662 mcg/100 g tocopherol constituents in germinated beans and peas compared to 24 to 2300 mcg/100 g in dry seeds. Banerjee et al (1955) reported tocopherols increased in certain legumes after germination.

The tocopherols as quantitated in our study may have contributed to stability of the products during storage. They have been shown to keep well for more than 6 months when packed

Table 4.6. Tocopherol constituents of control and germinated 70 cereal:30 legume weaning foods (72 h germination period for cereals and 48 h for legumes).

Weaning Food	Tocopherol, µg/g sample			
	α	β	γ	δ
Rice-mungbean				
ungerminated	++	23.8	178.5	11.7
germinated	2.3	6.3	166.0	16.0
Rice-cowpea				
ungerminated	++	4.1	128.0	184.0
germinated	3.5	6.2	120.0	164.7
Corn-mungbean				
ungerminated	18.0	8.9	323.6	19.2
germinated	11.7	7.7	190.0	17.1
Corn-cowpea				
ungerminated	13.9	10.0	145.9	154.9
germinated	13.5	9.1	142.0	156.1

++ Present in minimal amounts

in 0.5 mm polypropylene bags, stored at room temperature ($32\pm5^{o}C$) (Marero et al., 1988a).

CHAPTER V

PEPTIDE COMPOSITION OF GERMINATED RICE AND MUNGBEAN

INTRODUCTION

During germination, the reserve proteins which are stored in the endosperm and cotyledons are slowly used up by the growing seed, and thus proteolytic activity occurs. Koller (1966) showed that the profile of the proteins changes significantly in the early stages of germination with the breakdown of low molecular weight fractions, followed by high molecular weight components. This results in an increase in the levels of free amino acids and peptides which are translocated to the embryonic axis (Altschul et al., 1966).It is interesting to find out changes in the peptide composition of weaning foods prepared from germinated cereals and legumes which have recently been shown to have changes in their tocopherol (Marero et al.,1990c) and fatty acid compositions (Marero and Homma, 1990d).

The products of peptic and pancreatic digestion in the gastrointestinal lumen are peptides and a proportion of free amino acids. The mechanics of peptide absorption by the intestine are now the focus of intense interest, since it has recently been proven that mucosal uptake of small peptides are instrumental in protein absorption by mammals (Matthews and Payne, 1975). The absorptive capacity of the intestine is greater for mixtures of peptides and amino acids than for amino acids alone (Adibi and Phillips, 1968). It has been

demonstrated that in patients with congenital inability to absorb certain groups of amino acids, protein nutrition is maintained by the ability of the intestinal mucosa to take up peptides (Adibi, 1980). The purposes of this study were to determine: (1) the amount of peptide in germinated materials by the amount of TCA-soluble nitrogen; (2) protease activity; (3) amino acid composition of the peptidic constituents and (4) HPLC of peptide fractions.

MATERIALS AND METHODS

Sample Preparation

The germinated seed samples were prepared as described in the preceeding chapters. Samples were obtained from a batch of 1 kg processed flour. The samples were stored in the freezer (-18^{0}C) until analysis.

Estimation of nitrogenous constituents

Total nitrogen and peptide and amino acid nitrogen (TCA-soluble) of the flour from germinated materials were estimated by Kjeldahl method.

TCA-soluble peptides content during gruel preparation

Seventy two hour germinated rice , and 48-h germinated mungbean and weaning food from 70:30 germinated rice-mungbean (72 h germination period for rice and 48 h for mungbean) were separately prepared into gruel following the method of Mosha and Svanberg (1983). Sampling was done at 0 min

(control), 10 min come-up time to reach 95°C, 15 min cooking at 95°C and 20 min cooling to 40°C. The gruel samples were added with equivolume of 10% TCA to a final concentration of 5%, and the filtrates were assayed for their nitrogen content by Kjeldahl method.

Protease activity

Rice and mungbean (0, 24, 48, 72 and 96 h germinated) samples (0.1 g) were added separately with 10 ml distilled water incubated for 1, 2, and 3 h at 37°C. After incubation, 10 ml 10% TCA was added, mixed in vortex for 5 min and filtered. Five ml of the filtrate was determined for their nitrogen content by Kjeldahl method.

Enzyme activity at different pH conditions of casein solution

Casein solutions (10%) was prepared at pH 5, 6, 7, 8 and 9 using buffer systems (acetate buffer for pH 5 and 6 and phosphate buffer for pH 7, 8, and 9). Two grams 72-h germinated rice and 48-h germinated mungbean were separately added to 10 ml casein solution at each pH condition, incubated at 37°C for 3 h and added with 10% TCA solution to a final conentration of 5%. Control samples (no incubation) were prepared by adding 2 g germinated (72 h)rice sample to 10 ml 10% TCA solution and added with 10 ml casein solution for each pH condition. Nitrogen in the filtrate was determined by the Kjeldahl method.

Separation of Peptide

Ten grams 72-h germinated rice and 48-h germinated mungbean were added with 60 ml distilled water and prepared into gruel. Gruel was cooled and added with TCA (5% final concentration) and allowed to stand in an ice bath for about 30 min. The solution was centrifuged at 9,000 rpm at 4°C for 10 min, then the supernatant was washed with ethyl ether to remove TCA. The washing was repeated until the final pH value of the aqueous solution was more than 3. The aqueous solution was evaporated (rotary evaporator) at 40°C, then freeze-dried.

Analysis of peptides in the free and acid hydrolyzed samples

About 100 mg of the freeze-dried TCA-soluble peptides was analyzed for their amino acid constituents by hydrolyzing the sample with 3 ml 6 N HCl at 110°C for 24 h. The hydrolyzed solutions were filtered into a 50-ml evaporating flask and dried in a rotary evaporator at 40°C. The dried samples were dissolved in 10 ml 0.01N HCl. Amino acids were separated with a Hitachi Amino Acid Analyzer Model 835). Free amino acids were determined on the freeze dried TCA-soluble peptides by dissolving 200 mg in 3 ml distilled water, after which it is filtered through Whatman No. 2. The filtrate was subjected to amino acid analysis.

Fractionation of peptide into groups by ion exchange column chromatography

Two hundred mg freeze-dried sample was dissolved in a small amount of water and passed through an ion-exchange column (1.5 x 25 cm) of IRA-4B equilibrated to pH 3.3. The column was previously washed with 1 N HCl, distilled water, 1 N NaOH, distilled water and pH 3.3 (0.2 M formic acid with pyridine) buffer. The column was eluted with pH 3.3 buffer, and 300 ml was collected for neutral and basic peptide analyses. One hundred fifty ml 1 N HCl was added to the column and the adsorbed material in the eluate was collected for assay of acidic peptide components. The acidic eluate was evaporated in vacuo and the concentrate was dissolved in 5 ml distilled water then subjected to HPLC.

The neutral and basic peptide fraction was passed through a column of IRC-50 (1.5 x 25 cm) ion exchange. The column was previously washed with 1 N NaOH, distilled water, 1 N HCl, distilled water and 0.2 N sodium acetate. The column was washed with 100 ml distilled water and the break-through fraction was collected for neutral peptide analysis. The eluate was evaporated in vacuo at 40^{o}C, and the concentrate was dissolved in 5 ml distilled water for HPLC analysis.

The adsorbed material was eluted with 150 ml 1 N ammonia water and the eluate collected was analyzed for basic peptides. The eluate was evaporated, dissolved in 5 ml distilled water and subjected to HPLC.

HPLC Conditions

The HPLC was performed on a Hitachi 635-A HPLC apparatus with a reverse phase column(4.6 mm i.d.x 250 mm) of Capcell Pak (C18 SG, 120 A) using a solvent system of 0.1% TFA in water vs. 80% acetonitrile (acetonitrile:0.1% TFA, 80:20). The peptides were eluted by a gradient increase of acetonitrile concentration at a flow rate of 1 ml/min at room temperature. The peptides were detected by their absorbance at 213 nm with a UV-visible spectrophotometer, Hitachi Model L-4200.

RESULTS AND DISCUSSION

Total and TCA-soluble nitrogen in germinated materials

During the increasing stages of germination (Table 5.1), total nitrogen increased from 1.23 (0 h) to 1.37% after 24 h but decreased to 1.18% after 96 h in the case of rice. In mungbean samples there was a steady increase from 3.86% to 4.43% after 96 h of germination. Table 5.1 also shows that there seemed to be a slight protease activity as germination period progressed in rice. The activity in germinated mungbean was more noticeable especially in the 72- and 96-h samples. The increase in the TCA-soluble nitrogen was especially seen after 3 h incubation period at 37^{o}C in both germinated samples. This suggests that germination favored protease activity in flour of rice and mungbean, despite drying (60^{o}C, 10-12 h) and roasting (95^{o}C, 3 min) processes.

Table 5.1. Total and TCA-soluble nitrogen content of germinated rice with incubation period of 3 h at 37°C.

Sample	Germination period (h)	Total nitrogen (%)	TCA-soluble nitrogen, (%) Incubation time (h) at 37°C			
			0	1	2	3
Rice	0	1.23	0.06			
	24	1.37	0.07	0.08	0.09	0.10
	48	1.23	0.10	0.11	0.12	0.13
	72	1.23	0.12	0.14	0.17	0.18
	96	1.18	0.14	0.15	0.18	0.18
Mungbean	0	3.86	0.40			
	24	4.10	0.45	0.45	0.51	0.52
	48	4.11	0.67	0.71	0.71	0.73
	72	4.32	0.74	0.83	0.85	0.86
	96	4.43	0.85	0.94	0.95	0.97

TCA-soluble peptides during gruel preparation

Table 5.2 shows the results of sampling during the different stages of gruel preparation (initial sampling at 37°C, after 10 min come-up time to 95°C, cooking at 95°C for 15 min and cooling to 40°C). Changes in the TCA-soluble nitrogen was noticed particularly in the rice-mungbean weaning food combination where there was an increase from 0.25% at 0 min to 0.29% after cooking at 95°C. Also, in the case of mungbean, an increase from 0.62 to 0.67 after 10 min heating was noted but the activity has stabilized to 0.67% after cooking and cooling.

pH dependence of protease activity

Table 5.3 shows the dependence of pprotease activity of 72-h germinated rice and 48-h germinated mungbean on 10% casein prepared at different pH conditions, after incubation at 37°C for 3 h. The maximum activity was found at pH 8.0 with an

Table 5.2. TCA-soluble nitrogen content of 72 h germinated rice and germinated rice-mungbean weaning food gruels.

Sample	Sampling time (min)	Sampling temperature (^{o}C)	Nitrogen (%)
Rice	0	37	0.18
	10	95	0.20
	25	95	0.20
	40	37	0.19
Rice:Mungbean (70:30)	0	37	0.25
	10	95	0.26
	25	95	0.29
	40	37	0.29
Mungbean	0	37	0.62
	10	95	0.67
	25	95	0.67
	40	37	0.67

Table 5.3. pH dependence of protease activity in germinated rice.

pH	TCA-soluble nitrogen (%)			
	Incubation period,(h) at 37^{o}C			
	0		3	
	Rice 72-h	Mungbean 48-h	Rice 72-h	Mungbean 48-h
pH 5.0	0.18	-	0.21	-
pH 6.0	0.20	0.73	0.26	0.81
pH 7.0	0.20	0.73	0.28	0.85
pH 8.0	0.18	0.66	0.30	0.79
pH 9.0	0.18	0.71	0.19	0.83

increase from 0.18% to 0.30% TCA-soluble nitrogen in germinated rice and from 0.66% to 0.79% in germinated mungbean. The increase in the TCA-soluble nitrogen content suggests that a protease activity exists in both the flours of the germinated materials.

Amino acid composition of peptides

Table 5.4 shows the amino acid composition of TCA-soluble peptides from a sample of 72-h germinated rice gruel. The difference between the total and free amino acids, that is, before and after acid hydrolysis , may show the amount of peptidic constituents. The major peptidic constituents consisted of glutamic acid (0.2580 m mol/g N), aspartic acid (0.1840), glycine (0.1564) and alanine (0.1319). There appeared to be moderate amounts of peptidic constituents with valine)0.0923), leucine (0.0738), arginine (0.0633) and serine (0.0501). PPeptidic constituents with isoleucine (0.0440), lysine (0.0396), phenylalanine (0.0290), methionine (0.0284), histidine (0.0269) and tyrosine (0.0160) were in minor amounts.

HPLC Chromatograms of peptides

Attempts to isolate the peptides were done to identify the various constituents by HPLC. Fig 5.1 shows the HPLC chromatograms of the neutral peptide fractions of 72 h germinated rice gruel. The acidic fractions contained a total of 14 peaks; between 0 to 11 min, there were 10 peaks, 5 of which are major and 5 are minor peaks. Minor peaks, two of which are shown at 17 min and 1 each at 32 and 36 min were also shown.

Table 5.4. Amino acid composition of TCA-soluble peptides from 72-h germinated rice gruel (m mol/g N).

Amino acid	Total amino acid (ave, n=4)	Free amino acid (ave, n=3)	Peptidic constituents
Asp	0.2552	0.0712	0.1840
Thr	.1078	.0814	.0264
Ser	.1373	.0872	.0501
Glu	.3292	.0712	.2580
Gly	.2081	.0517	.1564
Ala	.2231	.0912	.1319
Val	.1799	.0876	.0923
Met	.0489	.0205	.0284
Ile	.0814	.0374	.0440
Leu	.1519	.0781	.0738
Tyr	.0933	.0773	.0160
Phe	.0941	.0651	.0290
Lys	.0885	.0489	.0396
His	.0388	.0119	.0269
Arg	.1391	.0758	.0633

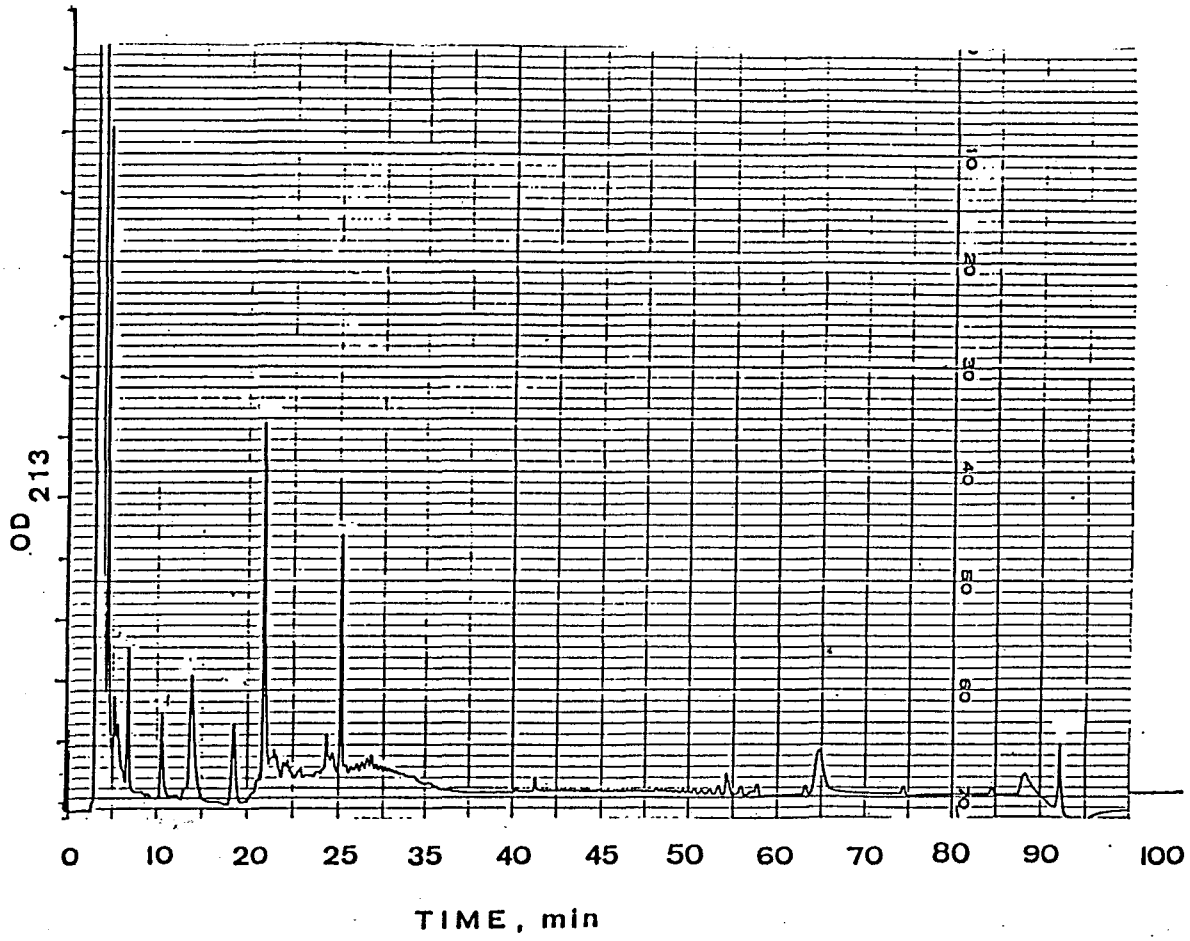


Figure 5.1. HPLC chromatograms of neutral peptides in gruel of germinated rice.

For the first 12 min in the HPLC chromatography of the neutral fraction (Fig 5.1) , 5 major and 4 minor peaks were shown. Minor peaks were shown at 14 min (2 peaks) and 1 each at 17, 20 and 21 min, giving a total of 14 peaks.

The basic fraction showed peptide peaks for the first 6 min and one minor peak each at 22 and 30 min giving a total of 6 peaks.

Fig. 5.2 shows the peptide chromatograms of 48-h germinated mungbean. A total of 22 peaks were shown for the neutral peptides (Fig. 5.2.1) and 16 peaks for the basic fraction (Fig. 5.2.2).

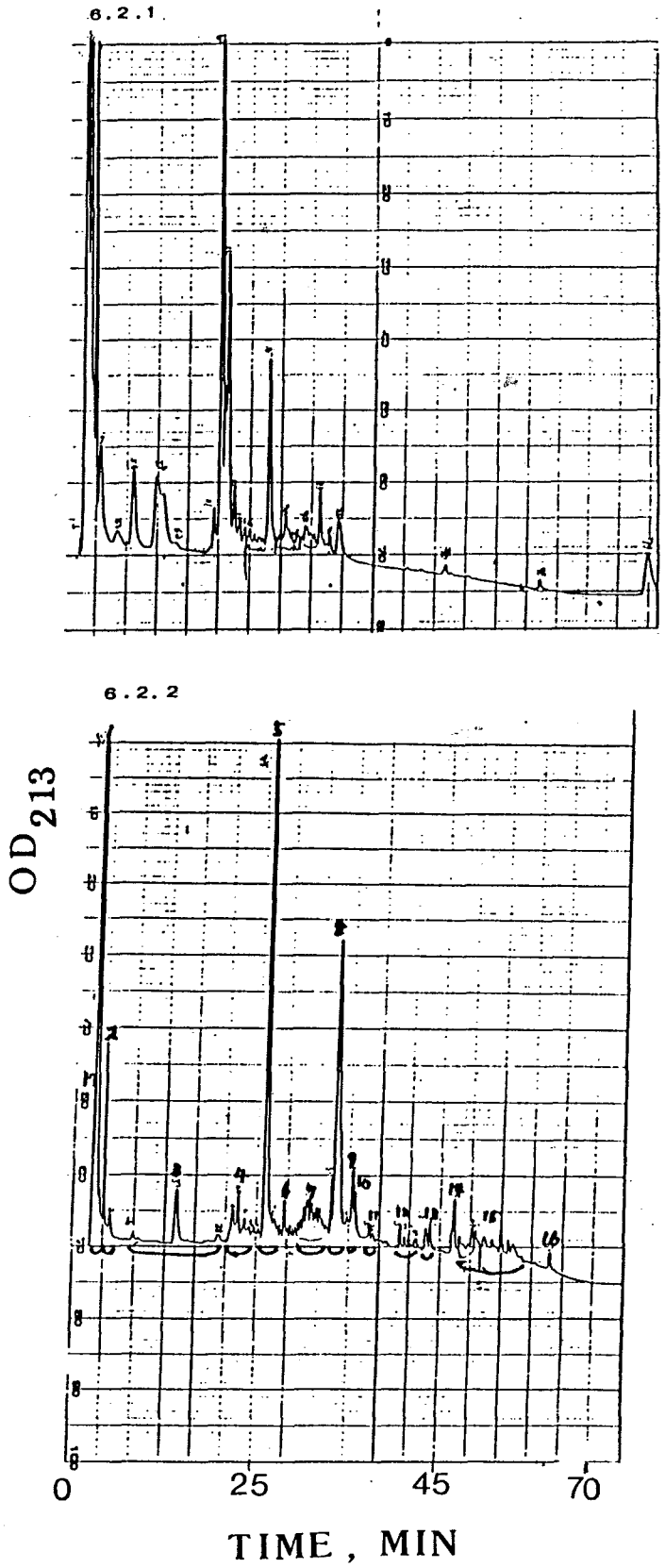


Fig. 5.2. HPLC chromatograms of peptides of 48-h germinated mungbean. .1) neutral and .2) basic peptides.

Peptide composition of both rice and mungbean showed that germination was able to pre-digest the proteins into peptides. The impact of this finding has relevance to the amounts of amino acids that can be available and absorbed as peptides by the infants.

The characteristics of absorption of the normal products of protein digestion, oligopeptides and amino acids, are different from those of simple amino acid mixtures. Further breakdown of peptides to small peptides of 2 to 3 amino acid residues probably takes place largely in the brush border peptidases (Kim et al., 1974). Amino acids, dipeptides, (Heading and Schedl. 1973), tripeptides (Silk et al.,1973) and tetrapeptides (Adibi, 1989) then enter the absorptive cells by a number of carrier mechanisms (Caspary, 1972). Peptide uptake involves one or more active transport systems and at certain concentrations, peptide uptake is more rapid than uptake of the equivalent free amino acids (Walker et al., 1972). Since peptides are absorbed through their specific transport systems, there is thus no competition for transport occurring with the free amino acids. Those amino acids which are particularly absorbed slowly from free solution may then be more rapidly absorbed from peptides, resulting in a more rapid protein absorption. In general, peptides do not enter the portal blood on an appreciable scale, though any peptide which is unusually slowly hydrolyed, is likely to be absorbed into the blood intact to some extent (Wilson, 1962).

CHAPTER VI

ANTINUTRITIONAL FACTORS IN WEANING FOODS PREPARED FROM GERMINATED CEREALS AND LEGUMES

INTRODUCTION

Beneficial effects of germination in improving nutritional quality of protein in cereal-legume weaning foods provide efficient utilization of locally available crops as solution to worsening protein deficit of young Filipino children. Germinated mixtures of rice-mungbean, rice-cowpea,corn-mungbean and corn-cowpea prepared from 70% 72-h germinated grains and 30 % 48-h germinated legumes proved to be potential protein-calorie sources due to reduced dietary bulk (Marero et al., 1988a) and improved nutritional value for protein quality and micronutrients (Marero et al., 1988b). Also, the weaning foods contained maltooligosaccharides which were produced during germination and gruel preparation, resulting in better carbohydrate digestibility (Marero et al., 1990). Since the foods were prepared from plant products, it was necessary to determine their antinutritional factors after heat treatment, if they were to be depended upon as supplementary foods for the vulnerable target groups.

Literature data (Eskin and Wiebe, 1983; Chang, 1967) support that germination reduced phytic acid concentration due to increased phytase activity. Germination reduced tannin content by 23-36% in mungbean after 48 h (Barroga et al.,1985). Trypsin inhibitor activity of cereal or legume seeds sometimes

increased but, generally, decreased during seed germination (Puztai, 1972). Lectins have disappeared after seed germination (Smets et al., 1985), and toxicity outbreak of hemagglutinins caused by beans cooked in oven at 150-160^{o}C for 3 h or in crocker cooker cooked for 5.5 h was reported (Noah et al., 1980). The cyanogenic glycoside, linamarin, increased during germination of mungbeans (Noel and del Rosario, 1987). Germination increased in vitro protein digestibility of beans (El-Hag et al., 1978).

Specifically, the purposes of this study were to: (1) assess effects of germination, flour and gruel preparation on the amounts of antinutritional factors, namely, a) phytic acid, b) tannins, c) antitrypsin activity, d) hemagglutinating activity and d) cyanogenic glycosides; 2) to determine in vitro protein digestibility of the flour, gruel and weaning foods; and 3) to g determine, in a feeding trial, tolerance of infants to four weaning foods previously shown to have superior nutritional quality.

MATERIALS AND METHODS

Preparation of samples

Seeds of brown rice (Indica var.), yellow corn, green mungbean and white cowpea were separately soaked in water for 6 h, drained and germinated for 0, 24, 48, 72 and 96 h at room temperature conditions in the Philippines (about 32^{o}C) according to standardized procedures (Marero et al., 1988a). Samples for analysis of antinutritional factors were drawn from

each germination period. Flour samples for all grains were prepared from each germination batch by drying seeds at 60+5^{o}C, dehusking and devegetation with the aid of corn mill and by winnowing, roasting at 95^{o}C for 3 min, and lastly, milling into 60-mesh flour. Four weaning foods, namely, germinated mixtures of rice-mungbean (GRM), rice-cowpea (GRC), corn-mungbean (GCM), and corn-cowpea (GCC), were prepared from flours of 70% 72-h germinated rice/corn and 30% 48-h germinated mungbean/cowpea. Gruel with solids: water ratio of 1:6 (w/v) was prepared (Mosha and Svanberg, 1983) to simulate actual kitchen conditions by cooking at 95^{o}C for 15 min with come-up time of 10 min.

Analytical Measurements

Phytic acid was determined by a combination of two methods: Wheeler and Ferrel's (1971) for the extraction and precipitation of phytate, and Makower's (1970) for the measurement of iron of the precipitate. A 4:6 Fe/P molecular ratio was used to calculate phytate content.

Total polyphenols (tannins) were determined by protein precipitation method (Hagerman and Butler, 1978) and expressed as tannic acid (Koso Chemical Co., Ltd., Tokyo, Japan) equivalent.

Trypsin inhibitor activity was determined colorimetrically (Liu and Markakis, 1989).

Phytohemagglutinin (PHA) activity was determined by hemagglutination assay as follows: rabbit blood was washed 3 times with 0.9% NaCl solution (saline) to remove soluble blood constituents (Kitagaki and Seno, 1985). Sensitivity of

erythrocytes to agglutination was increased by treatment with trypsin (lyophilized powder from porcine pancreas, Type IX, Sigma Chemical Co., St. Louis, MO). Following incubation for 1 h at 37^{o}C, treated cells were washed three times with saline and 5% cell suspension was then prepared for the assay. Samples were prepared (Chen et al., 1980) by homogenizing and then stirring 1 g composite sample in 10 ml extracting solution for 3 h. PHA activity was assayed by diluting extracts in two-fold increments and mixing with 25 µl of 5% trypsinized rabbit erythrocytes in U-type microtiter plates. The final sample volume was 50 µl. The plate was carefully shaken to mix contents. Agglutination was observed visually after 1 h.

Detection of cyanogenic glycosides was by AOAC procedures (AOAC, 1980).

In vitro protein digestibility

Measurement of in vitro protein digestibility was by multienzyme technique (Hsu et al., 1977). Fifty ml of aqueous protein suspension (6.25 mg protein/ml) from flour or gruel was adjusted to pH 8.0 at 37^{o}C and added with multienzyme (porcine pancreatic trypsin, bovine pancreatic trypsin, and porcine intestinal peptidase) solution and pH drop after 10 min was recorded. Results were computed using regression equation $Y= 210.46.10\ X_1$, where X_1 was pH at 10 min. Results were expressed as % in vitro protein digestibility.

Feeding Test

GRM, GRC, GCM, and GCC were subjected to ten-day feedi study at an orphanage in Paco, Manila, Philippines. Feedi trial was conducted according to standard operating procedur of the Nutritional Products Development Section of the Food a Nutrition Research Institute (FNRI). In the biologic evaluation, five children aged 6 to 11 months and ten childr aged 1 to 3 years were used as subjects. Gruels were prepar from the formulations by mixing 800 g dry matter to 400 water and cooked at 95^{o}C for 15 min. It was served as mi morning snack daily to the subjects, with variations in way preparation (e.g. as soup with ginger, served with chocolat artificial flavoring like vanilla, banana or pandan leaf). T hundred ml gruel were given to each child and an additional 2 ml portion was given to each child as desired. The fo consumed by each child was recorded. Any signs of unfavorab reaction or gastrointestinal upsets were carefully observed.

RESULTS AND DISCUSSION

Phytates

Results in Table 6.1 show that phytic acid content of ri was 0.86% in ungerminated sample (control) and decreas significantly to 0.29% after 96 h of germination. Since phyt acid is concentrated in germ and aleurone layers or pericarp rice kernel cells (Reddy et al., 1982), this antinutrient w therefore destroyed during germination because the germ w involved physiologically by the growing plant. Moreove

Table 6.1. Effect of germination, flour and gruel preparation on the phytic acid and tannin contents of cereals, legumes and weaning foods.[1]

Sample	Germination period,h	Phytic acid,%			Tannins,%		
		Germinated, fresh	Flour	Gruel	Germinated, fresh	Flour	Gruel
Rice	0	0.86^{a}	0.44^{a}	0.30^{a}	0.05^{a}	0.03^{a}	0.01^{a}
	24	$.84^{ab}$	$.28^{b}$	$.21^{ab}$	$.04^{a}$	$.02^{a}$	$.00^{a}$
	48	$.75^{bc}$	$.12^{b}$	$.05^{b}$	$.03^{a}$	$.01^{a}$	$.00^{a}$
	72	$.48^{c}$	$.02^{b}$	$.01^{b}$	$.03^{a}$	$.00^{a}$	$.00^{a}$
	96	$.29^{c}$	$.01^{b}$	$.00^{b}$	$.02^{a}$	$.00^{a}$	$.00^{a}$
Corn	0	0.97^{a}	0.68^{a}	0.50^{a}	0.04^{a}	0.02^{a}	0.01^{a}
	24	$.79^{bc}$	$.42^{ab}$	$.35^{bc}$	$.03^{a}$	$.01^{a}$	$.00^{a}$
	48	$.55^{c}$	$.21^{b}$	$.10^{c}$	$.03^{a}$	$.01^{a}$	$.00^{a}$
	72	$.43^{c}$	$.05^{b}$	$.03^{c}$	$.02^{a}$	$.00^{a}$	$.00^{a}$
	96	$.39^{c}$	$.01^{b}$	$.00^{c}$	$.01^{a}$	$.00^{a}$	$.00^{a}$
Mungbean	0	0.59^{a}	0.30^{a}	0.21^{a}	0.32^{a}	0.09^{a}	0.07^{a}
	24	$.48^{a}$	$.27^{a}$	$.18^{a}$	$.26^{a}$	$.07^{a}$	$.05^{a}$
	48	$.35^{b}$	$.15^{ab}$	$.10^{b}$	$.20^{ab}$	$.06^{a}$	$.05^{a}$
	72	$.26^{b}$	$.13^{b}$	$.04^{b}$	$.18^{b}$	$.06^{a}$	$.04^{a}$
	96	$.18^{b}$	$.12^{b}$	$.02^{b}$	$.16^{b}$	$.06^{a}$	$.02^{a}$
Cowpea	0	0.37^{a}	0.20^{a}	0.17^{a}	0.15^{a}	0.06^{a}	0.05^{a}
	24	$.23^{ab}$	$.16^{a}$	$.14^{a}$	$.13^{a}$	$.06^{a}$	$.03^{a}$
	48	$.18^{b}$	$.12^{a}$	$.09^{ab}$	$.09^{a}$	$.05^{a}$	$.01^{a}$
	72	$.15^{b}$	$.11^{a}$	$.08^{b}$	$.08^{a}$	$.05^{a}$	$.00^{a}$
	96	$.12^{b}$	$.10^{a}$	$.05^{b}$	$.06^{a}$	$.04^{a}$	$.00^{a}$
Weaning Food	GRM		0.06^{a}	0.03^{a}		0.02^{a}	0.00^{a}
	GRC		$.05^{a}$	$.03^{a}$		$.01^{a}$	$.00^{a}$
	GCM		$.08^{a}$	$.05^{a}$		$.02^{a}$	$.00^{a}$
	GCC		$.07^{a}$	$.04^{a}$		$.01^{a}$	$.00^{a}$

[1] Each value represents the mean of two observations and shown by dry basis percentage.

[a,b,c] Means in the same column with the same superscript for each commodity are not significantly different at $P=<0.05$.

during flour preparation, phytic acid contents decreased from 0.44% at 0 h to 0.01% after 96 h of germination. The significant ($P<0.05$) decrease was due to dehusking and devegetation or removal of sprouts, drying and roasting processes. Cooking of the flour into gruel totally eliminated phytic acid contents of rice.

Phytic acid content of corn (Table 6.1) registered the highest among the four grains studied, with 0.97% at 0 h in ungerminated sample which decreased significantly ($P=<0.05$) to 0.39% after germination for 96 h. Corn germ contains about 88% phytic acid (O'Dell et al., 1972) and only 3.2% is found in the endosperm, thus decreasing the amounts after germination. Phytic acid was no longer found after gruel preparation.

In legumes, (Table 6.1), phytic acid also decreased during germination and some phytase activity may have remained regardless of the treatments in which seeds have been subjected to during flour preparation. Heat treatment during gruel preparation, however, remarkably reduced phytic acid of mungbean and cowpea to very low amounts of 0.02 and 0.05%, respectively, in cooked 96-h samples.

Levels of phytic acid (Table 6.1)in gruels of GRM,GRC, GCM and GCC, were too low (0.03 to 0.05%) to be of nutritional significance.

Tannins

Cereals contained small amounts of tannins (Table 6.1)even in the ungerminated state with rice having only from 0.05% to 0.02% and corn with 0.04 to 0.01% after 96 h of germination.

In legumes, however, tannins in fresh samples were highest in mungbean (0.32% which decreased to 0.16% in 96 h), then in cowpea (0.15% to 0.06%). In addition to the effect of germination, further reduction of tannins was observed in the flour samples due to removal of seed coats which contained 81 to 85% of the tannins in mungbean (Barroga et al,1985) and in other legumes. A 90 to 98% polyphenol removal has been reported upon dehulling cowpea (Laurena et al., 1984).

Trypsin Inhibitor (TI) Activity

Antitryptic acitivity of rice and corn (Table 6.2) significantly ($P<0.05$) decreased during germination for 96 h. No activity was found in rice flour samples and only a trace (0.05 TUI/mg sample) was found in corn flour. The highest antitrypsin activity was exhibited by ungerminated mungbean with 6.5 TUI/mg sample which significantly ($P<0.05$) decreased to 4.94 TUI/mg after 96 h germination. Heat treatment of 96 h germinated mungbean during drying (65^{0}), however, reduced the activity effectively to 0.15 TUI/mg sample. Cowpeas showed also a slight decrease but activity was lower than in mungbean. Cooking destroyed the trypsin inhibitor activity of the flours. Negligible amounts (0.01 to 0.06 TUI/mg) were found in weaning foods, which was contributed by legumes.

Phytohemagglutinin (PHA) Activity

As shown in Table 6.2, positive PHA responses were found with ungerminated samples of rice, corn, mungbean and cowpea at

Table 6.2. Effect of germination, flour and gruel preparation on the antitryptic activity and hemagglutinating activities of cereals, legumes and weaning foods.[1]

Sample	Germination time, h	Antitryptic Activity,[2] TUI per mg sample			PHA Activity,[3] titer/mg sample		
		Fresh	Flour	Gruel	Fresh	Flour	Gruel
Rice	0	1.46^{a}	0.08^{a}	0.00^{a}	2^{0}	2^{0}	-
	24	0.86^{ab}	.00^{a}	.00^{a}	-	-	-
	48	.20^{b}	.00^{a}	.00^{a}	-	-	-
	72	.10^{b}	.00^{a}	.00^{a}	-	-	-
	96	.08^{b}	.00^{a}	.00^{a}	-	-	-
Corn	0	2.96^{a}	0.80^{a}	0.03^{a}	2^{3}	2^{2}	-
	24	1.86^{ab}	.70^{a}	.02^{a}	2^{0}	-	-
	48	1.80^{b}	.40^{a}	.00^{a}	-	-	-
	72	0.98^{b}	.21^{a}	.00^{a}	-	-	-
	96	.10^{c}	.05^{a}	.00^{a}	-	-	-
Mungbean	0	6.50^{a}	0.20^{a}	0.13^{a}	2^{3}	2^{0}	-
	24	5.61a	.19a	.11a	-	-	-
	48	5.16ab	.17a	.10a	-	-	-
	72	5.00b	.15a	.09a	-	-	-
	96	4.94b	.15a	.09a	-	-	-
Cowpea	0	1.18a	0.19a	0.11a	22	20	-
	24	0.98a	.18a	.10a	-	-	-
	48	.82a	.17a	.08a	-	-	-
	72	.75ab	.15a	.06a	-	-	-
	96	.55b	.13a	.03a	-	-	-
Weaning Food	GRM		0.05a	0.02a	-	-	-
	GRC		.04a	.01a	-	-	-
	GCM		.14a	.06a	-	-	-
	GCC		.19a	.04a	-	-	-

[1] Each value represents the mean of duplicate observations.

[2] TUI=Trypsin unitsa inhibited, where 1 TU is defined as 0.01 of A_{410} under the assay conditions of pH 8.1 at 37^{0}C with 4 ml assay volume and porcine trypsin.

[3] PHA=phytohemagglutinin activity; Titers are expressed as the highest dilution at which the extracts still agglutinated a suspension of trypsin-treated rabbit erythrocytes.
2^{0}=100,000 µg sample/ml; 2^{2}=25,000µg/ml; 2^{3}=12,500µg/ml;
- = negative.

[a,b] Means in the same column for each commodity which have the same superscript are not significantly different at p=<0.05.

a titer of 2^0. Germinated samples as early as 24 h showed negative phytohemagglutinin (PHA) activity except in corn, which showed a titer of 2^0 at 24 h. In all the later stages of germination (48, 72, 96 h), no hemagglutination activity was observed. In flour sample, only ungerminated control exhibited positive reaction. Weaning food formulations showed no PHA activity reactions.

Germination decreased agglutinating activities in the first three days in rice (Peumans and Stinissen, 1983) which occured only in the embryo (Nachbar and Oppenheim, 1980), and lectins found in corn (Ibid), mungbeans (Hankins and Shannon, 1978), and cowpea (Agrawal and Mahadevan, 1984) had no effect on human erythrocytes.

Cyanogenic Glycosides

Qualitative determination of cyanogenic glycosides in flour and gruel samples showed negative reaction of the materials to the picrate paper test. No data has been listed in tables. Although data (Noel and del Rosario, 1980) showed that linamarin, a cyanogenic compound, had increased during germination of mungbeans, the heating applied during drying, roasting and cooking may have reduced this antinutrient.

In vitro protein digestibility

Table 6.3 shows that the longer cereals and legumes are germinated, the greater the digestibility of the protein in their flour becomes. Gruel preparation from each batch of flour further improved protein digestibility. Thus, increase

Table 6.3. Effect of germination and gruel preparation on the in vitro protein digestibility of cereals, legumes and weaning foods.[1]

Sample	Germination period, h	In-vitro protein digestibility,%	
		Flour	Gruel
Rice	0	73.8^{a}	77.3^{a}
	24	74.7^{b}	78.6^{b}
	48	75.0^{b}	79.0^{bc}
	72	77.4^{c}	79.9^{cd}
	96	77.6^{c}	80.2^{d}
Corn	0	76.8^{a}	79.5^{a}
	24	78.5^{b}	80.0^{ab}
	48	78.6^{b}	80.1^{b}
	72	78.8^{b}	80.7^{b}
	96	79.2^{b}	80.9^{b}
Mungbean	0	76.2^{a}	79.3^{a}
	24	76.8^{a}	80.2^{a}
	48	77.0^{ab}	80.3^{ab}
	72	77.3^{b}	80.5^{ab}
	96	78.5^{c}	80.9^{b}
Cowpea	0	75.0^{a}	78.8^{a}
	24	75.2^{a}	79.2^{ab}
	48	76.0^{b}	79.2^{ab}
	72	77.0^{c}	80.1^{b}
	96	77.6^{c}	80.9^{b}
Weaning Foods	GRM	77.3^{a}	80.6^{a}
	GRC	77.0^{a}	80.4^{a}
	GCM	78.3^{b}	81.6^{b}
	GCC	78.0^{b}	81.4^{b}

[1]Each value represents the mean of two observations.

^{a,b,c,d}Means in the same column for each commodity with the superscript are not significantly different at $P=<0.05$.

in in vitro protein digestibility was caused by the effective reduction of antinutritional factors through germination, in addition to the heat treatments during flour and gruel preparation.

Tolerance Test

The preliminary acceptability and tolerance test for the products in a ten-day feeding test provided promising results as the children were able to take more than the 1/3 RDA-serving (Fig. 6.1). Table 6.4 shows average intake of two age groups of young children in a ten-day feeding study. There were no signs of stomach disorder, vomitting, fever or rashes among children fed the formulations in both 6 to 11 months and 1 to 3 years groups served. The tolerance of infants to weaning foods throughout the ten-day feeding test indicates that these cereal-legume formulations were safe for infant feeding.

Fig. 6.1. Photograph showing the subjects fed with the weaning foods for ten days.

Table 4. Average daily intake (g gruel) of two age groups of children in a ten-day feeding test.

Subject		Age	GRM	GRC	GCM	GCC
6-11 months group						
1	Angeline	7 mo	296^{bc}	240^{a}	295^{b}	279^{b}
2	Joanne	9 mo	331^{c}	249^{a}	233^{a}	278^{b}
3	Mark	11 mo	293^{bc}	256^{ab}	246^{a}	250^{ab}
4	Jamil	11 mo	479^{f}	360^{d}	271^{b}	300^{c}
5	Jonah	11 mo	301^{c}	263^{b}	321^{c}	363^{d}
1-3 y group						
1	Katrine	1 y 4 mo	313^{c}	266^{b}	263^{b}	329^{c}
2	Leizyl	1 y 11 mo	230^{a}	248^{ab}	320^{c}	286^{b}
3	Benedict	2 y	368^{d}	380^{d}	255^{ab}	334^{c}
4	Jeffrey	2 y 1 mo	440^{d}	320^{c}	432^{e}	216^{a}
5	Normel	2 y 5 mo	339^{c}	460^{f}	356^{d}	343^{c}
6	Robert	2 y 11 mo	247^{a}	226^{a}	297^{b}	329^{c}
7	Babylou	3 y	231^{a}	250^{ab}	328^{c}	336^{c}
8	Michael	3 y	289^{b}	250^{ab}	295^{b}	300^{b}
9	Freddielex	3 y	440^{e}	395^{d}	271^{b}	336^{c}
10	Enrico	3 y	500^{f}	420^{e}	380^{d}	368^{d}

^{a,b,c,d,e,f}Means in the same column with the same superscript are not significantly different at the 5% level of significance.

CONCLUSIONS AND RECOMMENDATIONS

Blends of the germinated flours at a 70 cereal: 30 legume ratio exhibited reduction in viscosity to about 3,000 cPs, which was ideal for young child feeding. With this decrease in viscosity, the dietary bulk was reduced, and the nutrient density of the gruels was increased, such that the infants were able to consume enough of the food to supply 1/3 of their daily dietary requirements in one sitting.

Germinated rice, corn, mungbean and cowpea were effective in reducing the bulk of resulting cereal-legume gruels compared to the ungerminated samples. Germination increased the essential amino acids of 70:30 cereal:legume blends except for total sulfur-containing amino acids. NDpE and PER values were higher in rice formulations than in corn. Germination increased the content of micronutrients such as phosphorus, iron, and especially in the corn formulations, beta-carotene, thiamin, riboflavin, niacin, and ascorbic acid but decreased calcium. The formulated products were good sources of protein, energy, B-vitamins, iron and phosphorus.

Reformulation of the corn-based formulations by supplementation or fortification with foods rich in cystine and methionine, such as sesame flour, for example, is recommended to improve their protein quality. Calcium and Vitamin A fortification in all formulations by addition of sources like fish and squash flours need further study.

The enzymes generated during the germination process were shown to have been retained in the flour regardless of heating

treatments during drying and roasting. This remaining enzyme activity continued to produce oligosaccharides during the gruel preparation. Changes in the starch composition, shown by the pattern of maltooligosaccharides in TLC and amylase activities of flours and gruels, have a favorable impact on the digestibility, as well as on the viscosity reduction, of the weaning foods prepared from germinated cereals and legumes.

Further study to quantify the kinds of sugars present in the germinated cereals and legumes and in weaning foods by the HPLC method is recommended.

Lipids increased with germination time in rice and cowpea up to 72 h but decreased after 96 h. The reverse pattern was found true for mungbean and corn. Corn contained 3.12 g lipids; cowpea 1.86 g; mungbean, 1.72 g and rice, 1.17 g per 100 g samples. The lipid class composition of cereals were more stable with germination time compared to the marked changes observed in legumes. The major composition of both rice and mungbean were the neutral lipids. Phospholipids and glycolipids were higher in mungbean compared to rice. TLC pattern also confirmed the stability of the cereals during germination while the legumes changed in their lipid class compositions. The major fatty acid constituents of germinated rice were oleic, linoleic and palmitic acids while those of germinated mungbeans were linoleic, linolenic and palmitic acids.

The major tocopherol constituents of weaning foods from 72 h germinated rice/corn and 48-h germinated mungbean/cowpea were as follows: GRM, GCM (γ-), GRC, GCC (δ- and γ-). Germination

decreased γ-tocopherol contents, especially in GCM. The tocopherols of the weaning foods might have been derived mainly from germinated legumes due to the presence of vitamins in the cotyledons. In cereals, they might have been concentrated in the germ area which was used up during germination.

Peptide composition showed that germination was able to pre-digest the proteins into peptides . The impact of this finding is that infants and children of the weaning ages can avail of the predigested proteins in the peptide form which was recently shown to be absorbable in the small intestines of humans.

Germination, drying, dehusking and devegetation, roasting, and cooking significantly ($P<0.05$) reduced antinutritional factors in the materials. Phytates and tannins in cooked gruel, ultimately, were too small to be of nutritional significance. Antitrypsin activity was almost nil in cooked gruel, while phytohemagglutinin activity was virtually eliminated. Increase in in vitro protein digestibility was noted from the flour form to the cooked gruel. All four formulations were well-tolerated by infants who showed no signs of stomach disorders, vomitting, rashes or fever throughout the ten-day feeding test.

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