

FORMULATION OF PROTEIN RICH READY TO MIX FOOD BASED ON RICE SOYA BLEND

By

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THESIS

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COLLEGE OF AGRICULTURE
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1997

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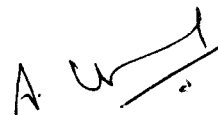

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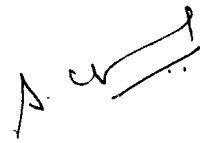
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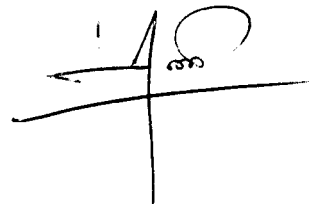
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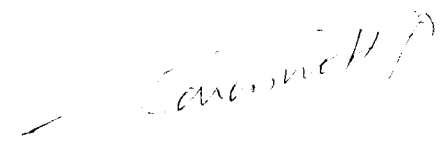


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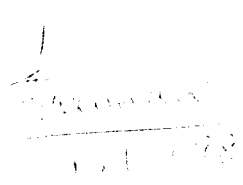
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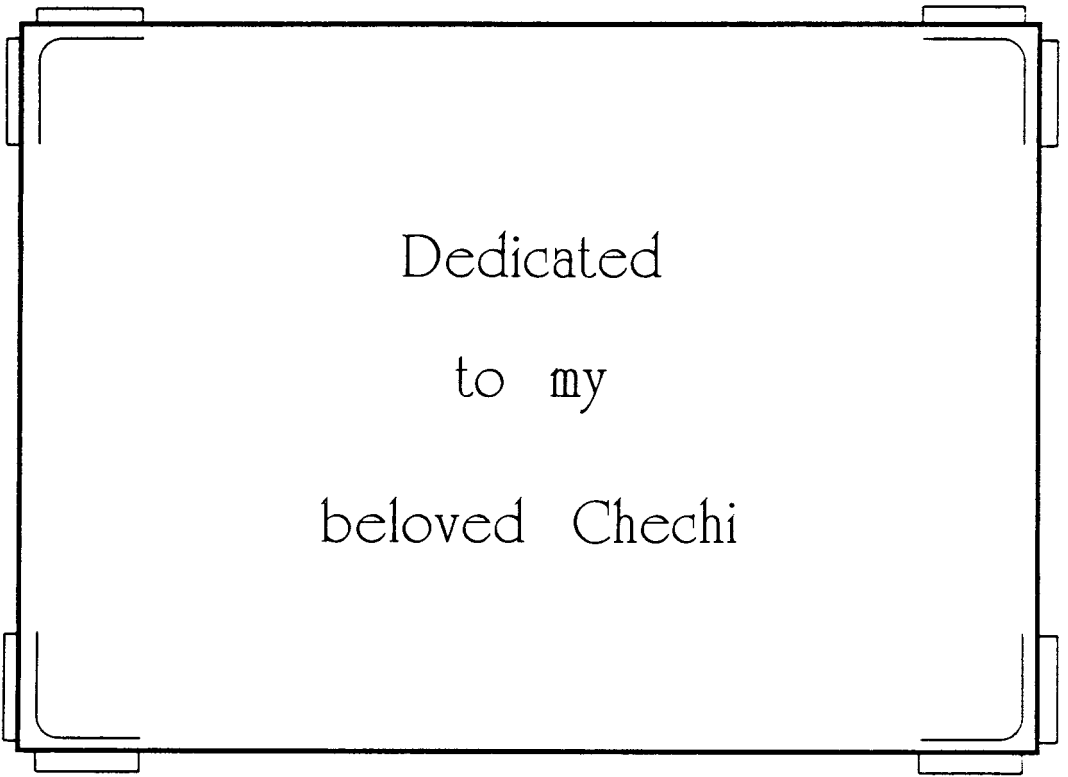
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LITTY ANDREWS



Dedicated

to my

beloved Chechi

CONTENTS

<i>Chapter</i>		<i>Page No.</i>
I	INTRODUCTION.....	1
II	REVIEW OF LITERATURE.....	4
III	MATERIALS AND METHODS.....	20
IV	RESULTS AND DISCUSSION.....	31
V	SUMMARY.....	63
VI	REFERENCES.....	i
VII	APPENDICES.....	
VIII	ABSTRACT.....	

LIST OF TABLES

Table. No.	Title	Page No.
1.	Different combinations tried	22
2.	Composition of the food ingredients (in %) in the different combinations of Mix	32
3.	Amino acid scores, consistency and cost of the combinations	35
4.	Organoleptic evaluation of the different combinations	38
5.	Nutritive value of the developed Mix	40
6.	Organoleptic qualities of the Mix	42
7.	Biological Value (BV), Digestibility co-efficient (DC) and net protein utilisation (NPU) of the mix	48
8.	Height profile of the children	52
9.	Weight profile of the children	54
10.	BMI of the experimental group	55
11.	BMI of the control group	56
12.	Serum protein level of children	58
13.	Serum iron levels of the children	60
14.	Haemoglobin levels of the children (g/dl)	61

LIST OF FIGURES

Figure No.	Between Pages
1. Flow chart for the Formulation of the Mix	
2. Organoleptic evaluation of the different combinations	
3. Experimental group after feeding trials	
4. Bio chemical estimation	

LIST OF APPENDICES

- I Amino acid scores of different combination of the Mix
- II Score card
- III Score card for preference test
- IV Procedure for measuring height
- V Procedure for estimation of haemoglobin
- VI Procedures for estimating total protein
- VII Procedure for estimation of serum iron



INTRODUCTION



INTRODUCTION

Malnutrition the man made “disaster” is an avoidable tragedy with enormous social and economic costs in wasted human potential (FAO, 1992). It affects growth and reproduction and undermines health, learning and working capacity and overall quality of life and well being. According to Devadas (1986) malnutrition has a very devastating spiral effect on the society and cripples a nations human resources.

A large part of the world’s population today is malnourished due to inadequate food of poor quality. Protein deficiency still remains the major nutritional problem of the population. The reason for this is either the short production or inadequate supply of protein rich food or the high cost of concentrated protein foods available. We can minimise this problem by incorporating low cost and easily available protein foods in our dietaries.

Rice is a vital food material for more than half of the world’s population. It is the basic ingredient of South Indian dietaries. It is the most important food crop of Kerala presently being cultivated in an area of 5.38 lakh ha. with the annual production of 10.85 lakh tonnes (Farm Guide, 1995). Munday *et al.* (1989) have stated that rice as the staple food of Keralities from ancient times has many diverse uses and is consumed in many forms.

According to Sinha and Nawab (1993) use of wheat or rice and defatted soy flour blend for preparing food items offers an unique opportunity for combating protein calorie malnutrition. So if rice could be blended with other pulses like soy bean which has protein of good biological value that would make an ideal mix which can readily be served to the needy.

The relatively low cost of soy bean as compared to animal protein and simplicity of technologies by which it can be converted to palatable high protein foods make it suitable for use as a source of protein particularly in the developing countries, where animal protein is beyond the reach of common man. It is the pulse that is accepted by nutritionists of several countries to be the 'way out' to solve the multifaceted problem of protein calorie deficiency in the world. However, inspite of its nutritive value, soy beans main application have been limited to industrial uses and animal feeds. It has not attained the prominent place in human nutrition, it rightly deserves.

The preparation of many low cost Ready to Eat food mixes has been based on complementation of the protein qualities between legumes and cereals. Cereals and legumes are the major source of protein in India because animal protein is expensive and a large part of the population is vegetarian (Naik *et al.*, 1983). Roman *et al.* (1987) have developed a multi mix with rice, cowpeas and milk powder which has been found to be highly acceptable among consumers. It has been reported by Walter (1978) that a blend of soy flour, with cereals such as corn, wheat sorghum and oats are widely used in worlds feeding programmes.

Hence processing low cost Ready to Mix food based on rice and soy which will be rich in protein with minimum preparation is most appropriate at this juncture. In the present study an attempt is made to develop a protein rich Ready to Mix food based on rice and soya and to assess its acceptability and protein quality.



REVIEW OF LITERATURE



2. REVIEW OF LITERATURE

Literature pertaining to the study entitled "Formulation of protein rich Ready To Mix food based on Rice soya blend" is reviewed under the following headings.

2.1. Prevalance of malnutrition

2.2. Role of protein rich foods in preventing malnutrition

2.3. Significance of rice and soya in supplementary foods and

2.4. Assessment of protein quality and acceptability of newly developed foods

2.1. Prevalance of malnutrition

Malnutrition is a consequence of the relation of people with food (UNICEF, 1984). According to Alexander (1985) it is the major health problem of our country and it is acute and wide spread. It is a condition when one or more **nutrients** are less or are in excess in the body.

Shukla (1982) state that the main causes for malnutrition in India include non availability of food, poverty, population growth, customes, conditioning which influence socio-economic status, education, influence of industrialisation, urbanisation and modernisation.

According to Gosh (1986) malnutrition results from the interaction of several factors. They were poor socio-economic conditions, parental ignorance and illiteracy, repeated infections, large families, closely spaced families and low birth weight.

A report by FAO (1992) state that "malnutrition a man made disaster" is an avoidable tragedy with enormous social and economic costs in wasted human potential. It affects growth and reproduction and undermines health, learning and working capacity and overall quality of life and well being.

Devadas (1986) reveals that malnutrition has very devastating spiral effects on the society and cripples a nations human resources. Malnutrition is the contributing cause in one third of the millions child deaths in the world (Grant, 1988).

The International conference on Nutrition (1992) reported that malnutrition among children is more likely to result from ignorance about hygiene and the dietary needs of children than from shortage of food.

Malnutrition continues to undermines the development of vulnerable groups in the developing countries including India (Yadav and Sihgal 1995).

UNICEF (1988) has pointed out that 33 per cent children of under five in India were suffering from mild to severe forms of malnutrition. Data compiled by NNMB (1989) has revealed that 17% of children below 5 years suffers from severe malnutrition linked to a weight deficit of over 40% and nearly 45% of children of this age group are estimated to suffer from moderate

malnutrition. India has reported to have the highest percentage of moderately and severely under weight children, when compared with Selected Asian countries (Grant 1993).

The progress of Nations report (1996) released jointly by the government of India and UNICEF indicates that as many as 53 per cent of all under five children are under weight indicating malnutrition.

Singh *et al.* (1994) feel that even today, the protein deficiency remains as the major nutritional problem of most of the developing countries.

Protein calorie malnutrition has been an important cause of infant and child mortality in many developing countries and consequently major emphasis was placed on the processing and utilization for protein rich raw material for child feeding (Subramanyam, 1980).

According to Reddy (1992) severe protein energy malnutrition is associated with infection, especially of the lower respiratory tract and with marked fluid and electrolyte disturbances as a result of diarrhoea.

Kerala is considered to be in the fore front in terms of health status, the mortality rate in the state is paradoxically high (Soman 1990 and Kurup 1992). According to NFHS (1995) though Kerala has the attention of the world for its unique achievements in health, we can notice the prevalence of malnutrition even though the percentage is less than compared with other states.

2.2. Role of protein rich foods in preventing malnutrition

Desikachar (1982) states that by introducing supplementary food of good quality and quantity at right time and in right proportions, the incidence of protein caloric malnutrition can be prevented to a larger extent.

According to Annie *et al.* (1985) protein energy malnutrition can be prevented with protein and energy rich supplements.

Ahmed *et al.* (1993) stress that to combat protein energy malnutrition, supplementation of cereals with legumes in the daily diets of the people.

It was well known that the nutritive value of legume proteins can be significantly enhanced by complementation with cereal proteins due to mutual supplementing and these supplements could very well be processed Yadav and Leiner (1978).

According to Udani (1990) to save time and energy protein rich ready to cook or ready to eat mixes need to be developed for supplementary feeding.

Sinha and Nawab (1993) reveal that use of cereals and medium fat soy flour blend for making traditional food items can offer an unique opportunity for combating protein calorie malnutrition prevailing among our masses.

Solanki (1986) feels that there is an urgent need to develop low cost ready to eat mixes to improve the nutritional status of people.

Inamder (1981) has developed malted and roasted powdered multi mixes of staple, viz. wheat, bengal and ground nut in ratios of 4:1:2, 8:1:1 and 8:1:0 respectively. A malted RTE Mix with ragi, greengram and groundnut was developed by Tajuddin (1981) who found that the mixture was superior to roasted RTE mixes in meeting the calorie deficit.

Pandya (1982) processed five malted mixes based on the locally available cereals and oil seeds and a high consumption of calorie was observed.

A soya ragi multimix formulated by Vijayalakshmi *et al.* (1985) was recommended, as a low cost, nutritious multimix, used as a preschool child snack and also a remedial and preventive measure against protein energy malnutrition.

Chellammal and Prema (1995) processed a multipurpose food with cassava flour, soy flour and skim milk powder. Wheat flour, green gram dhal and soyabean were the ingredients used by Malleshi (1995) for the preparation of protein rich multipurpose food.

Schapiro (1980) developed a mix using protein isolates, sodium calcium caseinate and whey powders which could be mixed with water milk, fruit juice etc. to yield a nutritious beverage. Patil and Gupta (1981) prepared flavoured protein rich beverage from soyabean and wheat.

A protein rich beverage using whey and soyabean was formulated by Patil *et al.* (1981). The method compared of heat processed soyabean and

lactose reduced whey. The beverage had soy protein whey protein ratio 3:1 and contained 4% protein.

A beverage was developed in USA named Gempro-70. It is a powder containing soy protein concentrates and lecithin. The product is easily dispersible in H₂O and remains suspended in hot or cold beverage like fruit juices. It contains all amino acid required for human nutrition (Morris 1982).

'Soy even' a nutritious low cost infant formula based on oats and soyabean has developed in Mexico. It was used to alleviate malnutrition. (Merinelstein, 1983).

Patil *et al.* (1984) have reported that soyabean and milk whey in combination provide a low cost nutritious beverage which can be utilised in institutional feeding programmes.

Leslie *et al.* (1992) had developed a concentrated soy milk beverage of 32% total solids content using soyabean extract, palm kernel oil, sucrose and water.

2.3. Significance of Rice and Soy in supplementary foods

Rice is the most important cereal raised in tropical and subtropical regions of the world. Besides being the main source of energy for more than two-thirds of Indian population, it is important staple because of its high digestability, biological value and protein efficiency ratio among all the cereals (Sood 1989).

According to Juliano (1985) about 90 per cent of the world's rice crop is grown and consumed in Asia. Rice is the major staple food in Asia and in some countries like Africa and Latin America (Lii and Chang 1986). Rice has been recognised as a food material of more than half of the world's population as pointed out by Pilliayar (1988) and Stephane (1990).

Rice as the staple food of Keralities from ancient times has many diverse uses and is consumed in many forms (Mundy *et al.* 1989). It is the most important food crop of Kerala presently being cultivated in area of 5.38 lakh ha. with the annual production of 10.85 lakh. tonnes (Farm Guide, 1995).

Rice is the chief source of carbohydrates. According to Grist (1986) being a staple food, rice is reported to provide 80 per cent of the caloric requirement of the diet. The calorific value is meant to determine the CHO content of rice which is composed of amylose and amylopectin.

Rice is reported to be a moderate source of protein. Okazaki and Oki (1961) found that rice varieties that cook well contained considerable amount of protein constituents, such as amino acids like glutamic acid, aspartic acid and arginine.

Prema and Menon (1969) have reported that protein content was higher in exotic rice varieties like Tainan-3 and IR-8, compared to local varieties like Kunchuvithu.

According to Srinivasan *et al.* (1969) Indian rice varieties contained protein in the range of 11 to 13 per cent.

Rice contains negligible amount of fat. According to Itoh and Kawanura (1987) free fatty acid values of parboiled rice stored for 25 to 30 days were lower than those of untreated.

Aberg (1994) had found that starch is the major constituent in cereal grains and is the nutritional reservoir in plants and exists in two different forms. Amylose, the unbranched type of starch, consists of glucose residues in 1-4 linkage, amylopectin, the branched form has about 1-6 linkage per every thirty 1-4 linkage.

Rice is reported to have a moderate source of minerals. Roberts (1978) reported that the iron content in different rice varieties decreased with different degree of milling.

Pederson and Eggum (1983) had reported that the mineral content in different rice varieties decreased considerably during milling and the extent of decrease differed between minerals. Damir (1985) stated that the minerals, in rice grain increased as a result of parboiling.

Rice is reported to be a moderate source of fibre. Dutta and Barua (1979) found that rice with only the husk removed had more crude fibre than well milled rice and showed a lower digestability and retention of nitrogen. Eggum (1979) had stated that the low content of tannin and crude fibre in rice had positively influenced the digestability of rice protein and energy.

According to Juliano (1970) among water soluble B-Vitamins, thiamine was present in a larger proportion in brown layers and embryo than riboflavin and niacin. Narunnabi *et al.* (1975) had found that thiamine, riboflavin and niacin contents of husked rice varied significantly and were influenced by different parboiling methods.

Venkat Rao (1976) formulated a weaning food with rice flour, barely flour, roasted ground nut cake flour, roasted bengal gram flour, hydrogenated ground oil, common salt, calcium carbonate (CaCO_3), tricalcium phosphate, Vitamine premix and protein.

Bushra *et al.* (1983) developed a protein rich weaning mix with rice, wheat, chickpea, milk and drumstick leaves. Dhananitta *et al.* (1983) have reported that in Thailand the distribution of supplementary food mixtures containing rice, soy beans and ground nuts or rice, mung beans and sesame provides 13.2-16.5g/100g protein.

Roman *et al.* (1987) developed a weaning food based on rice, cowpea and milk powder. Their experiments revealed that the protein quality of the developed weaning food was good.

Capanzana and Maleshi (1989) reported that rice protein is of good quality and its carbohydrates are also easily digested and hence forms an ideal base for weaning foods.

A feeding trial was conducted to assess the nutritional quality of rice-soya supplementary food by Sailexmi (1995). The trial was conducted for a

period of six months and a comparison between the initial and final anthropometric measurements of the pre school children revealed a significant improvement in height, weight and upperarm circumference.

Soybeans were introduced in India during mid sixties (Sandeep *et al.* 1993). Soya bean has long been recognised mostly as a valuable source of an edible oil and as an excellent source of protein for feeding both animals and man (Irvin, 1994).

As a protein source, soybean contributes two-third's of world's consumption of protein grains. It is also the major source of oil providing for one-third of the world's consumption by man (Gornov, 1989).

Soybeans has great potential in overcoming protein calorie malnutrition on account of its higher protein contents (Chauhan and Bains, 1985). Soya beans has great promises to combat malnutrition in the third world countries owing to its very high content of quality protein (Aswathi *et al.*, 1991). Increased use of soya bean in human diet is of importance in view of the prevalent protein calorie malnutrition (Krishna, 1993). Incorporation of defatted, soy flour in diets will not only enhance the protein content of the diet but also raise its nutritive value, thereby helping in combating malnutrition (Anita *et al.*, 1994). Popularising soya beans in the diet will help in combating protein calorie malnutrition in children to a certain extent (Kanchana and Neelakantan, 1994).

A report in world health (1983) had revealed that soybean is totally acceptable to Hindus and Buddhist as a substitute for fish and meat.

According to Gandhi (1991) soyabean is one of most suitable food ingredient for diabetes; since it contains about 40 per cent good quality protein, 22 per cent carbohydrates and 20 per cent cholesterol free oil, minerals and vitamins. Patil and Shukla (1990) have reported that soya bean contains 40 per cent protein and 20 per cent oil.

According to Natarajan (1989) soya bean is currently the largest commercially available vegetable protein source in most parts of the world.

Studies conducted by Ruales *et al.* (1988) has revealed that soyabean contains 34 per cent protein and depending on the processing it may contain as much as 50 per cent protein of the total solids.

Sinha *et al.* (1993) reported that soyflour is a very good source of quality protein which can supplement lysine deficiency in cereal flour.

Brand and Label (1988) also feel that defatted soyflour contains two times as much protein as in dhal, three times as much as in eggs and 15 times as much as in milk.

Soybean is reported to be a good source of essential amino acids except for methonine and tryptophan and the high lysine content of soybean proteins can complement lysine deficiency in flour proteins (Wolf, 1969). Adbel *et al.* (1980) reported that defatted soybean are found to be a higher in protein and some other essential amino acids.

According to Jimbu (1990) the quality of Vitamin-A is low in soybean, but the vitamin is present to a level of 1.4mg/100g and prevents the oxidation of fatty acids.

The soybean and soybean foods are consumed in significant amounts in Asian countries because of its inexpensive high quality protein and the anticarcinogenic effects (Wang and Murhpy, 1994).

According to Jayalakshmi and Neelakantan (1988) soybean and its products have become increasingly popular but a large section of the population in India is still not aware of the food value of soybean. The demand for soybean products is consistently increasing in India as they have a great potential in solving the food shortage created by over expanding population (Gandhi *et al.*, 1985).

Soy based food products can be formulated to contain reduced concentration of lactose, saturated fats and cholesterol (Morr, 1979).

According to Karleskind *et al.* (1991) soy based food products can be manufactured with good physical and functional properties and at comparable or lower cost than some traditional food products.

Defatted soya flour is a common form in which soybean can be incorporated in various food preparations reports (Chauhan, 1985).

Studies have shown that addition of 15 per cent soy flour to degermed corn meal and sorghum meal separately increased the protein efficiency ratio.

The study also showed that incorporation of the soyflour not only improved the nutritional quality of the sorghum flour but also improved its keeping quality (Jayalakshmi *et al.*, 1987).

Soyflour is most attractive in price, quality and quantity and has been extensively studied and generally recommended for fortifying purpose (Gupta *et al.*, 1991). The fortified soy flour is used for making a number of soy fortified food items such as biscuits, bread and cookies (Patil and Ali, 1990).

In a study it was found that soy flour could be incorporated with sorghum flour upto 50 per cent level for making deep fat fried products (Jayalakshmi and Neelakantan, 1987). Srealathan *et al.* (1987) prepared south Indian recipes with soya bean and were found to be acceptable.

Tandon and Singh (1987) feel that soyflour can be incorporated in various food preparation its use in bread, biscuits, chappathis, snacks and textured products has been successfully demonstrated by them.

2.4. Assessment of protein quality and acceptability of newly developed foods

Ritchy and Taper (1981) report that the most reliable way to assess the nutritional quality of proteins is through feeding trials.

According to Swaminathan (1989) the quantitative data regarding the relative digestability co-efficient and nutritive value of proteins could be obtained only through experiments on animals or human beings.

Shande and Chakrabarti (1980) studied the effect of methionine supplementation on the BV, DC and NPU of lentil protein in male albino rats. The result obtained that methionine supplemented lentil protein showed increased N retention by the body and positive N balance was observed.

The protein quality of soya, fermented soyabean product, fermented soy milk drink, germinated soybean bread was determined by chemical analysis and by animal feeding studies. Results indicate a slight increase in essential amino acids content. (Khader 1983).

Fashakin *et al.* (1986) assessed the protein quality of vegetable protein diet including soyabean, melon and cowpea and found that it could be compared favorably with the milk powder based control diet terms of growth rate, protein efficiency ratio PER and net protein ratio (NPR).

The studies of Fomon *et al.* (1959) have shown that N-balance in infants fed soyabean milk providing 1.7g protein/kg was of the same order as that observed in infants fed on pasteurized human milk providing 1.5g protein/kg thus showing that soyabean milk could meet the protein needs of infants for satisfactory growth. The nitrogen balance technique has been successfully used for assessing the protein requirements of pregnant and lactating women (Jayalekshmi *et al.* 1959).

Studies on the effect of supplementation of diets based on rice and sorghum with limiting amino acids on N-retention and NPU of dietary protein have been carried out by CFTRI (Swaminathan 1967).

The impact of the consumption of soy food on a range of diet and health issues analysed by Erdman (1989) revealed its protein quality, growth promoting effects, hypocholesterolaemic effects, glucose tolerance, bioavailability of zinc and iron and also on the prevalence of allergies in children.

Scientific methods of sensory analysis of food are becoming increasingly important in evaluating the acceptability of the food product. When the quality of food is assessed by means of human sensory organs, the evaluation is said to be sensory analysis. Sensory evaluation through the use of our senses only by applying exact scientific testing methods (Skeleton, 1984).

According to McBernott (1922) sensory method is the method in which palatability is evaluated by a panel of judges, because they answer all important questions of the food taste, smell, looks and feels.

Rao *et al.* (1975) conducted acceptability trials with ready to eat foods on pre school children and found that the food was acceptable.

Gandhi *et al.* (1984) had reported that full fat soy flour blended with cereal, millet or pulse flour could be made use of to make a variety of Indian traditional products and it was found that all products were acceptable.

According to Vaidehi *et al.* (1985). High protein biscuits made with ragi and soya flour were found to be both acceptable and nutritious.

Soyflour incorporated chapathi was prepared by Chellammal and Prema (1986) and its acceptability was successfully demonstrated.

Sensory evaluation conducted by Verma *et al.* (1987) showed that chapathi containing 10 per cent full fat soy flour was acceptable while those containing 25 per cent full fat soy flour neither like or disliked.

Veidehi and Shivaleela (1989) reported that soy flour can be incorporated in the place of whole milk and skim milk powder at an acceptable level to develop instant jamoon mix. It has acceptable sensory qualities, increases volume and yield and equally good keeping quality as that of any commercially available jamoon.

The feasibility of incorporating soyflour in extruded food macaroni as well as its acceptability was studied by Chellammal and Prema (1993).

Groven and Singh (1994) studies were undertaken to evaluate the effect of supplementation of fine commercial defatted soy flour samples on physical and sensory characteristics of cookies. Overall acceptability values of cookies with these flours did not differ appreciably.

Ahluwalia *et al.* (1995) reported that three ready to eat snack foods viz., savoury sev, sweet sev and Murukku were made substituting defatted soy flour for chick pea flour consumer acceptable studies showed high acceptability of three snacks.



MATERIALS AND METHODS



3. MATERIALS AND METHODS

The present study entitled “Formulation of protein rich Ready To Mix” was undertaken to develop a Ready to Mix food based on rice soy blend and to assess its acceptability and protein quality.

Plan of action

3.1. Formulation of the Mix

3.2. Assessing the acceptability of the Mix and

3.3. Determination of the protein quality of the Mix through feeding trials

3.1. Formulation of the Mix

3.1.1. Selection of raw ingredients

The ingredients selected for the Ready To Mix food formulated in the study were based on the local availability, nutritional value, economic significance, shelf life qualities, acceptability and digestability. As pre-determined rice flour and soy flour were selected as basic ingredients. Other ingredients tried along with these were milk powder and wheat flour.

Rice is the staple food of various state in India including Kerala. Rice contributes to about 70 per cent of the dietary energy and is major protein

source of Indian population. It is the only major cereal in the world consumed commonly as a cooked kernal and it is the chief source of carbohydrates (Govindaswamy, 1985). According to Juliano (1990) rice provides 68 percent of total energy and 69 percent of total dietary protein in South Asia. To Indians it is the most important food crop supplying, on an average, one third of the calories required (Saikia, 1990).

Soyflour is used as a protein supplement since animal proteins are in short supply and costly, plant proteins have received considerable attention by virtue of their availability, low cost as well as nutritional and functional attributes. Among different vegetable proteins, soybean is the cheapest source of protein, supplying 40-50 per cent. Sinha *et al.* (1993) reported that soyflour is a very good source of quality protein which can supplement the lysine deficiency in cereal flours. Incorporation of defatted soyflour in diets will not only enhance the protein content of the diet but also raise its nutritive value (Anita *et al.* 1994).

Supplementation with milk solids in weaning foods could increase the nutritive value and make them nutritionally complete (Desikachar 1983).

For the majority of the world's human population, wheat-based foods constitute the important source of energy and other nutrients. (Kent and Evers, 1994). Accordingly these four ingredients were selected for the development of the Mix.

3.1.2. Selection of the best combination for the development of the Mix

The principles governing the selection of the proper combination of these foods were protein quality, consistency, cost and over all acceptability. The different combinations tried for the development of Ready To Mix food are presented in (Table 1).

Table 1. Different combinations tried

Combinations	Ingredients			
	Soy flour	Rice flour	Milk powder	Wheat flour
1	60	20	10	10
2	60	15	15	10
3	50	15	25	10
4	50	20	20	10
5	50	20	15	15
6	60	15	20	10
7	60	25	10	5
8	55	25	10	10
9	56	24	10	10
10	40	40	10	10
11	70	10	10	10
12	65	20	10	5
13	50	25	15	10
14	60	10	20	10
15	50	10	25	15

According to Jansen and Harper (1985) amino acid score provides an useful estimate of protein quality of blended foods and is an acceptable substitute for the biological assays. The amino acid of the different combinations tried were computed (ICMR 1991) to assess the protein quality. (Appendix 1).

Cost of the different combinations were computed as per the market price of the ingredients.

Overall acceptability of the combination were assessed by organoleptic evaluation. Organoleptic qualities were assessed in the laboratory with the selected panel of judges. The panel members were selected from a group of 30 healthy women in the age group of 20-23 years. Triangle tests (Jellink 1964) was employed to select the panel members. From 30 women who participated in the triangle test, ten women were selected as judges for the conduct of organoleptic quality studies.

The organoleptic quality evaluation was conducted using score cards. The major quality attributes included in score card were appearance, colour, flavour, texture, taste and overall acceptability (Appendix-2). The judges were requested to taste the first sample and score it. They were requested to taste the second sample after washing their mouths. Each quality was assessed by the pannel members after tasting the same sample several times if needed. The testing was conducted in the afternoons between 3 pm and 4 pm. since this time is considered as the ideal time for conducting the acceptability studies (Swaminathan, 1975). The combination which secured the highest overall score was selected for the formulation of the Mix.

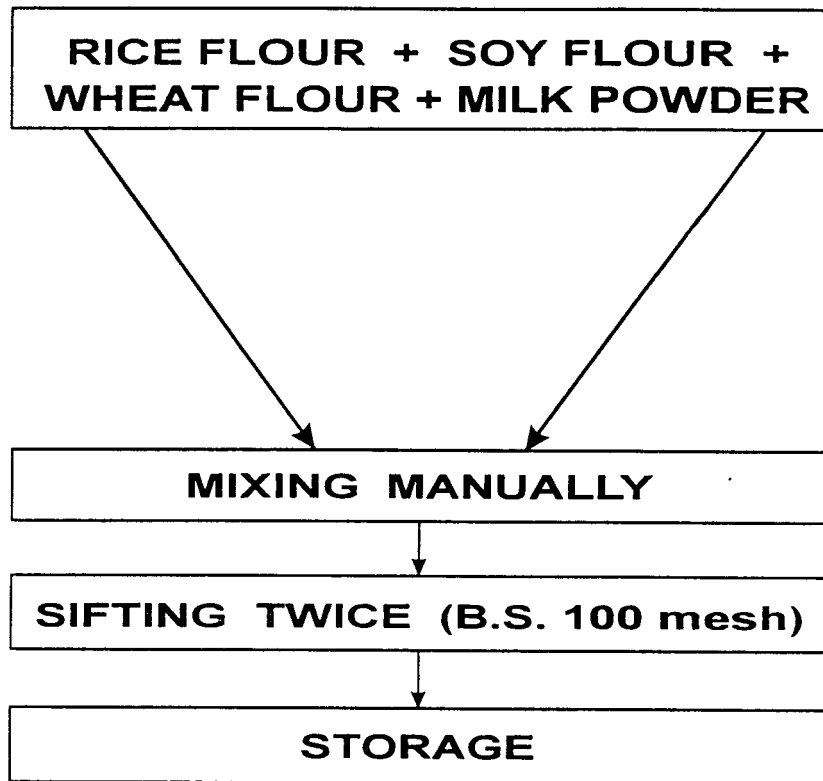


Fig. 1. Flow chart for the formulation of Mix

3.1.3. Processing of the ingredients

Rice flour : For the preparation of Mix, 1 kg good quality rice was purchased from the market and was cleaned to remove all the impurities. It was fully dried in shade. Dried rice was roasted, powdered and sieved.

Soy flour : Defatted soyflour is the common form in which soy bean can be incorporated in various food preparations (Chauhan *et al.*, 1989). 2½ kg of defatted soy flour was purchased in bulk from shakthi soyas, Pollachi, Tamil Nadu.

Whole milk powder was used for the preparation of Mix. 1 kg of milk powder was purchased from the local market.

For the preparation of the Mix, 500g of good quality wheat was purchased from the local market and was cleaned. The cleaned wheat was soaked in water for 12 hours and germinated for 24 hours. The germinated wheat was dried in shade, roasted, milled and sieved.

3.2. Assessing the acceptability of the Mix

Acceptability of the Mix was assessed with special reference to nutritional significance, organoleptic qualities, preference, and physiological tolerance.

3.2.1. Nutritional significance of the Mix

Nutritional significance of the Mix was assessed by determining energy, protein, fibre, iron, calcium, magnesium and zinc. The methods ascertained for the estimation of nutrients were :-

Energy	Swaminathan (1984)
Protein	Microkjeldhal (ICMR, 1983)
Fibre	Raguramule's <i>et al.</i> (1983)
Calcium	
Iron	Jackson (1973)
Magnesium	
Zinc	

3.2.2. Organoleptic quality trials among technical experts

According to Araullo (1976) organoleptic qualities play an important role in evaluating the quality of the food product. Consumer acceptability evaluation of any food product is essential before advocating or marketing the product. The acceptability of the Mix was assessed through organoleptic evaluation in the laboratory with the selected pannel of judges.

3.2.3. Preference for the developed Mix among rural women

The main purpose of our research is to take the technology from lab to home. To test the consumer preference of the product, the Mix prepared in the laboratory was distributed amongst a group of farm women.

Hundred farm women were selected at random from Kalliyoor Panchayath in Nemom block. A good rapport had already been established through several extension education programmes and the university personnel were very familiar, to the women in this area. The prepared Mix was served to the women and they were requested according to give preference. A scale from nine to one was used, nine representing optimum (Appendix-3).

3.2.4. Physiological tolerance

The physiological tolerance of the developed Mix was assessed by conducting nitrogen balance studies among human volunteers. Children of 12-14 years of age were selected for the experiment. They were free from infection and infestation.

The subjects were fed weighed quantities of the experimental diets for a preliminary period to make them acclimatized to the diet and experimental conditions. The preliminary period was 4 days. Water intake of the subjects were kept constant throughout the experiment. The subjects were fed at fixed hours viz. morning (breakfast), noon (lunch), evening (tea) and night (dinner) with weighed foods.

Quantitative data regarding food intake were maintained for a subsequent period of 3 days. 1/10th of the aliquot portion of all the cooked foods was collected, preserved in HCl and stored in refrigerator. The preserved food was homogenised and weighed amounts were analysed for nitrogen balance studies using standard techniques.

Collection of excreta was made for a subsequent period of 3 days. Carmine was used as a marker for the collection of faeces. Urine was collected in bottles in which 50ml of conc. hydrochloric acid and 5g of phenol were kept to prevent decomposition of urine and loss of ammonia. The urine samples were made upto volume and 1/5th of the daily collection is transferred into a clean bottle and stored in refrigerator. The faeces collected daily were mixed with 100 ml of 3 per cent oxalic acid and 100 ml alcoholic solution containing 10g phenol. They were dried in an air oven at 70°C. The dried faeces collected for 3 days period were weighed, powdered and portion used for analysis of Nitrogen.

Faecal and urinal nitrogen is estimated by microkjeldhal method. From the values obtained biological value, digestability co-efficient and Net protein utilization were computed by,

$$BV = \frac{IN - (Fn - Fe) - (Un - Ue)}{IN - (Fn - Fe)} \times 100$$

$$DC = \frac{IN - (Fn - Fe)}{IN} \times 100$$

$$NPU = \frac{BV \times DC}{100}$$

BV = Biological value

DC = Digestability co-efficient

NPU = Net protein utilisation

Fn = Nitrogen in faeces

Fe	=	Endogenous nitrogen
Un	=	Nitrogen in urine on protein diet
Ue	=	Nitrogen in urine on protein free diet
IN	=	Nitrogen intake

3.2.5. Microbiological profile of the Mix

500 g of the developed Mix was stored in poly propylene covers for a period of six months. After the storage period the Mix was analysed for the presence of microbes using standard technique (Mehrotora, 1980).

3.3. Determination of the protein quality of the Mix through feeding trials

The protein quality of the developed Mix was assessed by conducting feeding trials.

3.3.1. Selection of the centre

Sri. Chitra Poor Home, Thiruvananthapuram was selected for the conduct of the experiment. The children were the inmates of the Home from the same socio-economic background and they consume the same diet. Their willingness in participating in the experiment was the other criteria for selection of this Institution.

3.3.2. Selection of children

As a first step fifty children in the age group of 12-13 years were selected randomly with approximately same height and weight. From this



EXPERIMENTAL GROUP

group 20 children were further selected consisting of experiment group (10 children) and control group (10 children).

3.3.3. Conduct of the experiment

The experiment was conducted for a period of six months. Every month the Mix was processed at the laboratory by the investigator. 15 kg of the Mix was prepared in the beginning of the month and stored in the feeding centre. The Mix was prepared in the form of beverage and served to the children along with break fast for a period of six months.

3.3.3.1. Anthropometric measurements

Height and weight of the children from both the experimental and control group were recorded every month for 6 months. The height is a measure of long standing nutritional status. Height was measured using the stadiometer. The procedure is presented in (Appendix-4). Weight of the children were measured using a beam balance with children wearing very light clothes.

3.3.3.2. Biochemical evaluation

Biochemical measurements represent the most objective assessment of the nutritional status of an individual, frequently providing pre or subclinical information (Sausberluh *et al.* 1977). Biochemical examination was conducted at the beginning and completion of the feeding programme. In the present study haemoglobin level, serum protein and serum iron of the selected children were assessed initially and after the completion of the study.



BIOCHEMICAL ESTIMATION

The haemoglobin content of blood was estimated by the cyanmethaemoglobin method as described in Manual of laboratory Techniques (NIN. 1983) (Appendix-5).

Total protein content of the serum was estimated by Biuret method described in manual of laboratory techniques (NIN. 1983) (Appendix-6) and the serum iron was estimated using wong's method (Appendix-7).

Statistical analysis

The data collected during the study were compiled analysed statistically and presented under results and discussion.



RESULTS AND DISCUSSION



4. RESULTS AND DISCUSSION

The present study entitled "Formulation of protein rich Ready To Mix" was undertaken to develop ready to Mix food based on rice soy blend". The results of the study are presented and discussed under the following heads.

4.1. Formulation of the Mix

4.2. Assessing the acceptability of the Mix and

4.3. Determination of the protein quality of the Mix through feeding trials

4.1. Formulation of the Mix

The newly emerging era of fast and convenient foods, instant foods are becoming increasingly popular among Indian house holds (Singh and Shurpalakar, 1989). In the present experiment a protein rich Ready To Mix food was formulated based on rice soy blend. According to Solanki (1986) there is an urgent need to develop low cost ready to eat mixes to improve the nutritional status of the vulnerable population.

The major steps observed in the product development were selection of raw ingredients and best combination for the development of the Mix, ensuring quality of the Mix, assessing nutritional significance, physiological tolerance and conducting feeding trials.

4.1.1. Selection of raw ingredients

The preparation of many low cost Ready to Eat Mixes has been based on the complementation of the protein qualities between legumes and cereals (Naik and Gleason, 1988). The basic ingredients selected for the present study were rice and soy. Other ingredients tried were milk powder and wheatflour. Fifteen combinations were tried with these food ingredients in different proportions for the processing of the Mix. The different combinations of food materials attempted for processing of the Mix are presented in Table 2.

Table 2. Composition of the food ingredients (in percentage) in the different combinations of Mix

Sl. No.	Combinations	Rice flour	Soy flour	Milk powder	Wheat flour
1	C ₁	20	60	10	10
2	C ₂	15	60	15	10
3	C ₃	15	50	25	10
4	C ₄	20	50	20	10
5	C ₅	20	50	15	15
6	C ₆	15	60	20	10
7	C ₇	25	60	10	5
8	C ₈	25	55	10	10
9	C ₉	24	56	10	10
10	C ₁₀	40	40	10	10
11	C ₁₁	10	70	10	10
12	C ₁₂	20	65	10	5
13	C ₁₃	25	50	15	10
14	C ₁₄	10	60	20	10
15	C ₁₅	10	50	25	15

As indicated in Table 2 rice was added in the proportion ranging from ten to forty per cent. Rice is the most important cereal raised in the tropical and subtropical regions of the world. Besides being the main source of energy for more than two thirds of Indian population, it is an important staple because of its high digestability, biological value and protein efficiency ratio among all the cereals (Sood, 1989). Capanzana and Malleshi (1989) reported that rice protein is of good quality and its carbohydrates are also easily digested and hence forms a ideal base for the Mix. Apart from above facts which merits inclusion of rice as an item of the basic Mix, it has been well recognised that rice is capable of imparting desirable characters like smoothness, flavour, colour and taste in the reconstituted product.

Defatted Soyflour, which is the protein supplement, was added to rice in different proportions of the Mix. Rice is found to be deficient in essential amino acid of cereals and millet proteins. By the addition of soyflour to rice the deficiency is compensated and a better quality protein is obtained.

The soybean and soybean foods are consumed in significant amount in Asian countries because of their inexpensive high quality protein and anti-carcinogenic effects (Wang and Murphy, 1994).

The above mentioned facts justifies the inclusion of soyflour in the Mix. Soyflour has been added in the proportion ranging from forty to seventy percent in the Mix.

Singha and Nawab (1993) have reported that wheat is usually used as the cereal base for supplementary foods. For the majority of world's human

population, wheat based foods constitute the important, source of energy and other nutrients (Kent and Evers, 1994). Due to above reason wheat flour has been added in proportion ranging from five to fifteen per cent.

According to Desikachar (1983) supplementation with milk solids could increase the nutritive value of weaning foods and make them nutritionally complete. Therefore in this study milk powder was used as the protein vitamin and mineral supplement. Hence milk powder was also tried in ratios ranging from ten to twenty five per cent.

4.1.2. Selection of best combination for the development of the Mix

The principles governing the selection of the proper combination were protein quality, consistency, cost and overall acceptability.

The protein quality of each combinations were assessed on the basis of amino acid score.

Amino acid scores provide a careful estimate of the protein quality of blended foods and are an acceptable substitute for the biological assays. Therefore amino acid scores of the different combinations of the Mix were worked out using the food composition tables of ICMR (1991) and scores were presented in Table 3.

Waninik *et al.* (1993) reports that high quality dietary protein can be created with an ideal amino acid pattern required for human growth and metabolism, by combining two or more protein sources to compensate for the limiting amino acids in any of them.

Table 3. Amino acid scores, consistency and cost of the combinations

Combinations	Amino acid scores	Consistency (Rank means) (n=10)	Cost/kg
C ₁	82.89	73.30	34.00
C ₂	83.37	58.60	24.00
C ₃	84.35	53.00	38.00
C ₄	84.42	141.70	25.00
C ₅	82.45	130.30	24.00
C ₆	88.77	82.40	29.00
C ₇	87.30	77.85	26.00
C ₈	85.90	72.25	31.00
C ₉	86.02	88.00	31.00
C ₁₀	85.82	84.00	26.00
C ₁₁	84.38	58.60	36.00
C ₁₂	83.81	53.00	33.00
C ₁₃	86.40	53.00	35.00
C ₁₄	85.37	53.00	37.00
C ₁₅	84.93	53.00	35.00
X ² (Kruskal - Walli's)		64.67	
Critical value	38.082		

As indicated in Table 3 amino acid scores were ranged from 82.45 to 88.77. The lowest amino acid score of 82.4 was obtained for combination five containing rice twenty, soy fifty, Milk powder fifteen and wheat fifteen per cent. The highest amino acid score of 88.7 was observed for combination six which had soy 60, rice 15, Milk powder 20 and wheat 10. The amino acid scores of combinations one, two three and four were 82.89, 83.37, 84.35 and 84.42 respectively. Combination four is found to be better than combination one, two and three with rice 20, soy 50, Milk powder 20, and

wheat 10. An amino acid score of 87.30 was obtained for combination seven, while the amino acid score of combination eight was 85.90. Combination nine (soy 56, rice 24, milk powder 10, wheat 10) was found to be superior to combination ten (soy 40, rice, 40, milk powder 10, wheat 10). The amino acid score obtained for combination eleven was 84.38 which contains seventy per cent soy flour, ten per cent rice flour, ten percent milk powder and ten percent wheat flour and for combination twelve was 83.81 which had sixty per cent soy, twenty per cent rice, ten per cent milk powder and five per cent wheat flour. Combinations thirteen, fourteen and fifteen have got an amino acid score of 86.40, 85.37 and 84.93 respectively. Combination thirteen was found to be superior to combinations fourteen and fifteen.

Difference in scores are not pronounced. So amino acid score alone could not be considered as basic for the choice of the best proportion.

The consistency of combinations were assessed by ten technical experts using a standardised pre tested score card. The variation in consistency of the combinations were statistically analysed using Kruskal Wall's test and the results obtained are presented in Table 3.

The results of statistical analysis revealed that the consistency scores of C_4 and C_5 was significantly high in comparison with other combinations which were on par.

The cost of the Food Mix in the different combinations were worked out by the actual cost of ingredients and processing charges. The cost of the different combinations are presented in Table 3.

Sinha *et al.* (1993) has recommended soy flour as an ideal substitute for expensive ingredients like non fat dry milk thereby offering nutritionally better food at lower cost. As revealed in Table 3 the cost was lowest for combination four, five and two (25, 24 and 24). The highest cost was for the combination three and fourteen (38 and 37). The cost of the selected combination (C_4) was very low as it is only twenty five rupees per kg of the food Mix compared to Food Mixes currently marketed of similar nutritional significance. Organoleptic qualities can be defined as qualities affecting a bodily organ or sense particularly of the combination of the taste (perceived in the mouth) and aroma (perceived in the nose) Sindhu (1995). The organoleptic qualities can be assessed by sensory evaluation. Sensory evaluation of food is assumed to be of increasing significance as this provides information which may be utilised for development of a product and its improvement. Organoleptic evaluation was carried out by a group of ten judges selected by a triangle test.

Using Kruskal Walli's test, organoleptic qualities of 15 combinations were tested and the results are presented in Table 4 and Figure 2.

The combination C_4 and C_5 were scored to be superior in appearance in comparison with other combinations which were on par.

Colour of C_4 and C_5 were better than others but C_5 was on par with $C_1, C_2, C_3, C_6, C_7, C_8, C_{11}, C_{13}$ whose colour difference were not significant. Also the colour of C_9, C_{15}, C_{10} and C_{14} were on par with $C_1, C_2, C_3, C_6, C_7, C_8, C_{11}, C_{13}$. However colour of C_{12} found to be inferior to all the other combinations except C_{14} .

Table 4. Organoleptic evaluation of the different combinations (n = 10)

Combinations	Mean scores			
	Appearance	Colour	Flavour	Taste
C ₁	81.20 (4)	81.00 (3)	58.75 (9)	69.50 (5)
C ₂	65.65 (8)	81.00 (3)	68.65 (6)	59.20 (7)
C ₃	81.10 (5)	81.00 (3)	73.55 (5)	79.80 (3)
C ₄	145.00 (1)	143.50 (1)	144.00 (1)	139.80 (1)
C ₅	124.75 (2)	106.00 (2)	120.60 (2)	120.10 (2)
C ₆	50.00 (13)	81.00 (3)	58.75 (9)	69.50 (3)
C ₇	75.95 (6)	81.00 (3)	68.65 (6)	79.80 (3)
C ₈	75.95 (6)	81.00 (3)	68.65 (6)	69.50 (5)
C ₉	70.70 (7)	60.60 (4)	88.70 (3)	74.65 (4)
C ₁₀	86.45 (3)	53.80 (5)	88.35 (4)	74.65 (4)
C ₁₁	39.50(14)	81.00 (3)	48.90(10)	48.90 (8)
C ₁₂	60.50(10)	13.00 (7)	48.90(10)	48.90 (8)
C ₁₃	55.05(12)	81.00 (3)	73.55 (5)	64.35 (6)
C ₁₄	65.55 (9)	47.00 (6)	58.80 (8)	69.50 (5)
C ₁₅	55.15(11)	60.60 (4)	63.70 (7)	64.35 (6)
X ² (Kruskal Walli's)	64.63	102.75	56.46	49.53

Critical value = 38.082

Values in parenthesis indicate ranks

Ranganna (1984) stated that flavour is an important factor which enriches the consumer preference to a particular product. The flavour of C₄, C₅ were significantly superior to others. The flavour of C₉ and C₁₀ were significantly superior to C₁₁ and C₁₂. All others did not registered any significant difference in flavour.

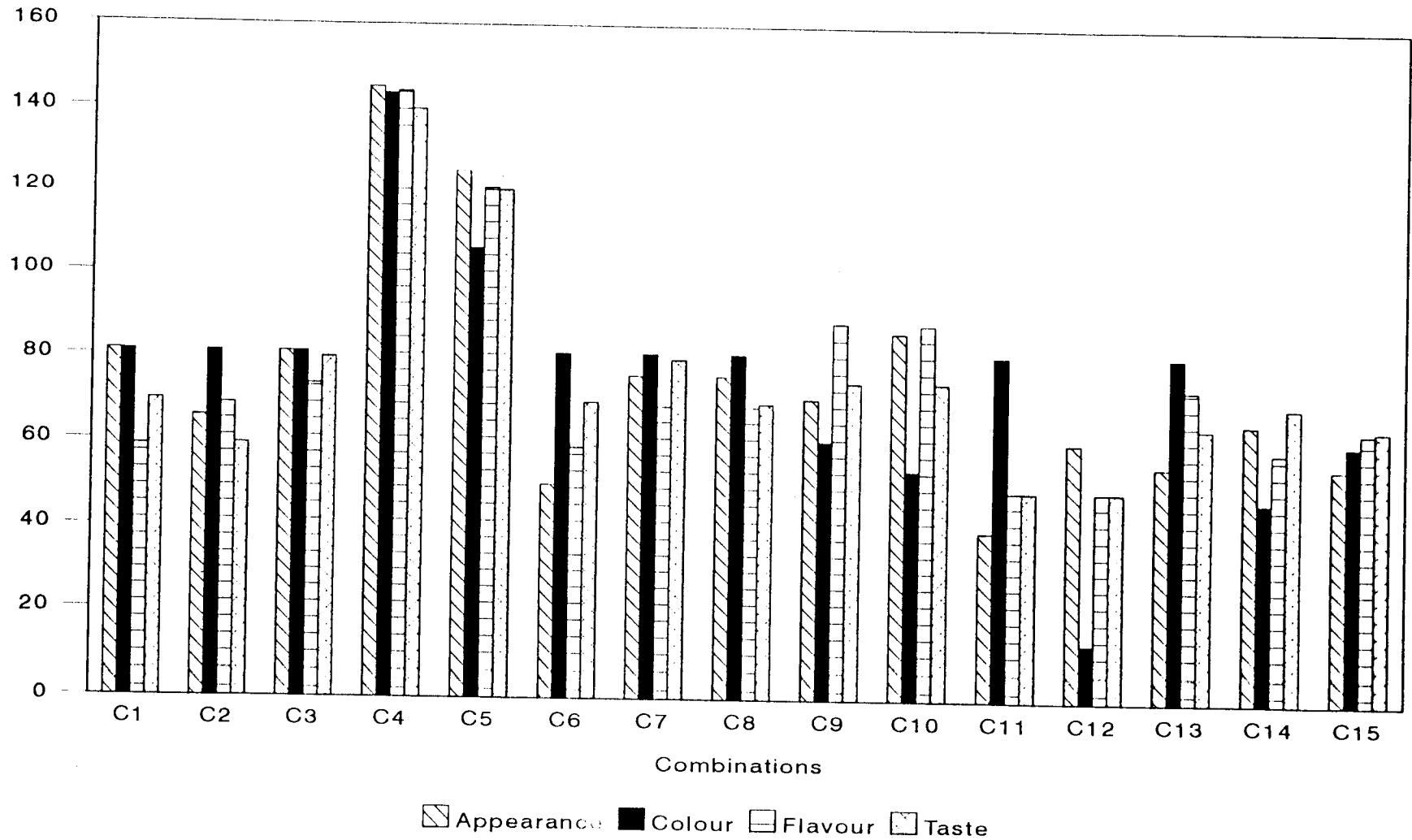


Fig. 2. Organoleptic evaluation of the different combinations

Fifteen combinations were scored equally by the judges for the quality of texture. Mean score of four was obtained for all the fifteen combinations. The data on texture of the product were not subjected to any statistical analysis owing to the fact that the responses remained the same across all combinations and respondents.

The taste of C₄ and C₅ were higher than the other combinations which were on par.

The result of the organoleptic evaluation revealed that combination four was superior in all parameters viz. consistency, cost and over all acceptability. So combination four was selected for the formulation of the Mix. It contains twenty per cent rice, fifty per cent soy twenty per cent milk powder and ten per cent wheat flour.

4.2. Assessing the acceptability of the Mix

Acceptability of any product can be influenced by their constituents as well as by the procedure selected for processing (Sindhu 1995). In general, while examining the acceptability of any product, the nutritive value, organoleptic and the shelf life qualities of the product should be considered.

The acceptability of the developed Mix was assessed with special reference to nutritional significance, organoleptic qualities, preference and physiological tolerance.

4.2.1. Nutritional significance of the Mix

Nutritional significance of the Mix was ascertained by suitable laboratory techniques and the data is presented in Table 5.

Table 5. Nutritive value of the developed Mix

Nutrients	Quantity/100 g
Protein (g)	23.68
Energy (Kcal)	349.72
Calcium (mg)	19.40
Magnesium (mg)	1.46
Iron (mg)	29.84
Zinc (mg)	2.70
Fibre (g)	2.72

Protein is one of the most important nutrient required by the body to carry out a wide range of functions essential for the maintenance of life. When the protein content of the developed food was analysed, it was found to be 23.68g. The high protein content of the developed Mix may be due to the addition of soy flour. Nikoli and Salihodzic (1988) also report that additional of soy flour generally increases the protein content.

The human body utilises the potential energy contained in food for maintaining life and doing work. As depicted in Table 5, the calorific value

of the developed Mix was found to be 349.70 Kcals. Both rice and soy are rich in calorie and this may be the reason for this high value.

Minerals play a vital role in nutrition and slight change in the concentration of the important minerals may rapidly endanger life. Hence, the mineral content of any food product is highly essential. Minerals like Ca, Fe, Zn and Mg were essential for normal up keep and maintenance of the body. The Ca, Fe, Zn and Mg contents of the Mix were found to be 19.40 mg, 29.84 mg, 2.70 mg and 1.46 mg respectively. Sayokoikeda and Tomomi (1995) have also, proved the presence of zinc in several traditional foods prepared from soybean.

Rounet *et al.* (1992) reported soybean has a fibre content of 9.90 g. It was also found that soy fibre contains twenty to twenty three per cent soluble fibre which could help in controlling cholesterol. As revealed in Table 5 the fibre content of the developed Mix was 2.72 g.

From the Table it is clear that the developed Mix contains adequate amounts of protein, energy and minerals.

4.2.2. Organoleptic qualities of the developed Mix

Assessment of the organoleptic qualities is carried out mainly to draw conclusion about a particular food from a large population through selection of a limited number of panel members. According to Mahony (1985) the organoleptically assessed samples form a true representative of the products developed and organoleptic assessment stands essential for the further

development of the products. Through sensory evaluation tests, the judges will provide clues about physical characteristics of the developed product.

Organoleptic quality parameters such as appearance, colour, flavour, texture and taste were assessed by ten technical experts selected by triangle test and the results are presented in Table 6.

Table 6. Organoleptic qualities of the Mix

Sl. No.	Quality parameters	Meanscores (maximum - 5)
1.	Appearance	5.0
2.	Colour	4.0
3.	Flavour	5.0
4.	Texture	4.0
5.	Taste	4.8
6.	Overall acceptability	4.6

Colour, one of the important visual attributes, has been, used to judge the overall quality of food for a very long time. If the colour is unattractive, a potential consumer may not be impressed by the major attributes. colour is associated with every aspect of our lives and influences many of our day-to-day decisions, involving food. According to the reports from CFTRI (1990) the aesthetics, safety, sensory characteristics and acceptability of food are all affected by colour. The colour influences other sensory characteristics and in turn, food acceptability choice and preference. (Fergus 1993). Here in this study the developed Mix has got an average score of four for colour. By the

inclusion of soyflour the colour of the product become cream and that might have contributed to the low score.

Flavour is the unique character of colour and taste. According to Rolls *et al.* (1981) flavour is the quality attribute which stands next to taste. Brich (1988) has also stated that flavour is the mingled but unitary experience of sensation produced by a material taken in the mouth perceived principally by the senses of basic smell and by the other cutaneous sensations in the mouth. The percentage score of flavour was found to be maximum (5).

The first impression of food is usually visual and a major part of our willingness to accept a food depends on its appearance.

As the consumer preference to appearance is one of the major factor leading to the increasing demand of the product, it is very essential to keep the appearance of the product quite attractive (Christensen, 1988). The result revealed that the developed Mix had obtained a score of five for appearance.

Texture constitute a physical property of food stuffs apprehended by the eyes, the skin and muscle senses located in the mouth. According to Ranganna (1991) texture is the property of food which is associated with the sense of feel or touch experienced by the fingers or the mouth. The score secured by the Mix for texture was four.

Pallavi *et al.* (1991) showed that in the preparation of some traditional foods, defatted soyflour can be incorporated to the extent of 100 per cent without significantly affecting their textural properties.

According to Rolls *et al.* (1981) in the various quality attributes tests, the first preference goes to taste followed by flavour, appearance texture and colour. Taste is the major attributes which determines the acceptability of a food material. It is not only a sensory response to soluble materials but also aesthetic appreciation of the mouth. In this study the developed Mix has got an average score of 4.8 for taste. This is because of the reason that, since the quality of the ingredients used has got direct impact on taste the score percentage is found to be high. The use of milk powder in the Mix also helps to improve the taste of the product.

According to Kordylus (1990) the overall acceptability depends on the concentration or amount of particular components, the nutritional and other hidden attributes of a food and its palatability or sensory quality. After analysing each quality attribute, the score of over all acceptability was determined for the Mix. A score of 4.6 was attained by the Mix for over all acceptability.

The result of organoleptic evaluation revealed that the quality parameters such as colour and texture had a low score compared to other qualities. The reason for this may be due to the inclusion of soyflour in the Mix. Rastogi and Gurmuk Singh (1988) reported that at higher levels of full fat soyflour the acceptability was reduced due to undesirable flavour.

4.2.3. Preference test for the developed Mix among rural women

During the development of new food product or the reformation of existing products the identification of changes caused by processing method,

by storage or by the use of new ingredients, their acceptability could be assessed by conducting preference test on a large number of consumers (Watts *et al.* 1989). Preference studies are designed to determine consumers subjective reactions to external phenomena and their reasons for having them. Swaminathan (1984) reports that for preference test a large number of persons are required and their evaluation should come spontaneously based on their judgement. While conducting preference test the consumer expects to be favourably impressed with the food he tastes and expressed displeasure if the product does not measure upto his anticipation.

In the present study the developed Mix was tested by a group of hundred farm women and their opinion on the degree of liking were obtained. Data was collected using a point rating scale varied from like extremely (9) to dislike extremely (1). Since, none of the farm women gave scorings for 'like reasonably', 'like some what', 'do not like' or 'dislike' and 'dislike extremely', these four rating were deleted from discussion of the data.

The Mix prepared in the laboratory was distributed in the form of porridge amongst the farm women. The result revealed that 97 per cent of the panel members scored the porridge as like extremely. Studies conducted by Neelakantan *et al.* (1987) also revealed the acceptability of soy-sorghum blends in few South Indian traditional recipes were well preferred by the consumers. It could also be observed that addition of 50 per cent defatted soyflour in the Mix did not negatively affected the organoleptic qualities of the Mix. The opinion of the farm women were in accordance with the experts. Only 3 per cent of the farm women neither liked nor disliked the product.

This may be due to the strong flavour of soy flour in the Mix. Tandon and Singh (1987) have also observed that the effect of addition of defatted soy flour on the quality characteristics and acceptability of moong bari and revealed that consumer acceptability of bari decreased with the increase in the level of defatted soy flour.

4.2.4. Physiological tolerance of the Mix

Physiological tolerance is one of the important criteria to be analysed before the production of any new food. Ritchey and Harper (1981) reported that the most reliable way to assess the nutritional quality of proteins is through feeding trials. According to Swaminathan (1989) quantitative data regarding the relative digestibility coefficient and nutritive value of proteins could be obtained only through experiments on animals or human beings.

Several biological measurements have been proposed as indicators of the physiological utilisation of foods. Among these, nitrogen balance study is the most important one which is considered as a measure to find out the extent of utilization of proteins from any food in the body. In the present investigation, human balance studies were conducted to assess BV, DC and NPU.

Biological Value

Biological Value (BV) measures the quantity of dietary proteins utilised by the animal for meeting its protein needs for maintenance and

growth. (Swaminathan, 1995). The biological value of proteins can be determined by Nitrogen balance experiments.

In this experiment, the biological value of experiment diet was significantly higher when compared to the control diet (Table 7). The biological value of the Mix was 66.88 per cent, where as the biological value of control diet was 65.09 per cent. In a study conducted by Ifon (1980), the biological value of soybean millet porridge was found to be 78.93 + 1.01 per cent. The percentage of protein in soybean is very much higher than the protein contents of almost all vegetables (Margareta *et al*, 1989).

Murlin *et al* (1944) found an average biological value of 81 for soybean proteins.

Net protein utilisation (NPU)

Net protein utilisation (NPU) is the ratio of nitrogen retained to the total nitrogen intake and determined from BV and DC and the data are presented in Table 7.

When the net protein utilisation of the Mix was analysed, significant difference among the groups could be observed. The experiment group registered the highest value (58.00) when compared to control group (55.40). The difference between the groups were significantly different. High content of soy in the Mix may be the reason for this. Studies on the effect of supplementation of diets based on ragi, sorghum and rice with limiting amino

acids on N - retention and NPU of dietary proteins have been carried out at CFTRI, and the result indicate that supplementation with lysine brings about a significant increase in N - retention and NPU in children on diets based on ragi or sorghum.

Digestability Co-efficient (DC)

The term digestability co-efficient of protein refers to the percentage of the ingested protein absorbed in to the blood stream after the process of digestion is complete (Swaminathan, 1995).

In the present study the DC was found higher in the experimental diet than the control diet. As indicated in Table 7 the digestability Co-efficient of the experimental group was observed to be 86.72 per cent and that of control group was 85.24 per cent. The statistical analysis of the data revealed a significant difference between the two groups.

Table 7. Biological Value (BV), Digestability co-efficient (DC) and net protein utilisation (NPU) of the Mix (percentage)

Group	B.V	D.C	NPU
Experiment	66.88	86.72	58.00
Control	65.09	85.24	55.40
't' Value	11.45**	5.34**	9.43**

** Significant at 1 per cent level

The result of several investigation showed that the digestability coefficient of soybean proteins varied from 80 per cent to 97 per cent in adults. Cahill *et al* (1944), from their studies on adult human subjects reported biological values of 94 and 92 and true digestability coefficients of 90 and 94 for cooked whole soybean and for cooked soy flour proteins respectively.

4.2.5. Microbiological profile of the Mix

500 g of the developed Mix was stored in poly propylene cover for a period of six month. After the storage period the Mix was analysed for preference of microbes using standard techniques.

No microbes were found in the Mix. The result is in line with the study conducted by Kapoor *et al.* (1978) in which the storage life of low cost soybean weaning food packed in cans was found to be 8 months at 5°C and 6 months at 30°C. Gupta *et al.* (1991) have proved that dried soybean could be stored in polyethylene bags for more than 20 days at 37° C without deterioration.

4.3. Determination of the protein quality of the Mix through feeding trials

The diets consumed by a large majority of school children in India and other developing countries are lacking in protein rich foods. The growth rates and physical development of children on such diets are poor (Swaminathan, 1995). Studies carried out by several workers have shown that supplementation of the diets with protein rich foods brings about marked

improvement in their growth and nutritional status. Vaidehi (1990) found that a soybean product produced satisfactory rates of growth in young children and recommended its widespread use as a supplementary food.

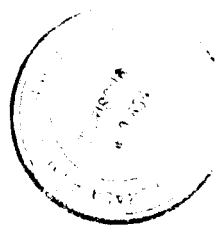
Protein quality of the developed Mix was assessed by conducting a feeding trial at the Sri Chitra Poor Home, Thiruvananthapuram. Children, the inmates of the Home, were from the same socio-economic background. The selected children were between the age group of 12-13 years. Care was taken to select children of approximately same height and weight.

The C₄ Mix which was found superior to others with respect to all the factors studied was fed on 10 children and their height, weight, serum protein, serum iron and haemoglobin were examined after a period of six months. A control group of 10 children was also maintained. The initial and final values with respect to the above characters were recorded and subjected to statistical analysis viz. Analysis of co-variance (Panse and Sukhatme, 1961).

The experiment was conducted for a period of six months. Every month the Mix was processed at the laboratory by the investigator. The Mix was prepared in the form of beverage and served to the children along with breakfast for a period of six months.

4.3.1 Anthropometric measurements of the children

School children are considered as the best tool to ascertain the impact of various nutrition intervention programmes. As indicated by



Scrimshaw *et al.* (1967), ICMR (1972), NIN (1973), Swaminathan (1973) and Gopaldas (1975) : a well accepted procedure for evaluating the impact of supplementary feeding is to measure absolute weight gains and the significant growth differences in the beneficiaries as compared with age matched non-beneficiaries. Hence the impact of the feeding trial was assessed by recording the anthropometric measurements.

Anthropometric measurements such as height and weight were measured at monthly intervals for a period of six months.

4.3.1.1. Height profile of the children

Height is a measure of long standing nutritional status. The extent of height deficit in relation to age, can be regarded as a measure of the past nutritional history of a subject. Height profile for the experimental and control group are presented in table 8.

The initial height of the experimental group ranged from 127 to 142 cms and that of the control group ranged from 127 to 135 cms. After the experiment, height of the experimental group ranged from 127.80 to 143. It was observed that only negligible changes were noted in both the groups as far as height is concerned. When the increase in height of the experimental group was compared with that of control group it was more in experimental group.

Table 8. Height profile of the children

	Experimental group		Control group	
	Initial (cm)	Final (cm)	Initial (cm)	Final (cm)
	134.00	135.00	133.00	134.00
	137.50	138.20	135.00	135.50
	136.00	137.00	131.00	132.00
	136.00	137.10		
	142.00	142.80	129.00	130.00
	127.00	127.80		
	134.00	134.50	131.00	131.00
	142.00	142.80	130.00	131.00
	140.00	141.50	129.00	129.80
			128.00	128.50
	142.00	143.00		
	141.00	142.00	132.00	132.50
Mean	138.45	139.39	130.00	131.21
Mean adjusted for initial		135.41		135.19
$F_{1,17}$		0.60 ^{ns}		

The average height of experiment children did not show any significant difference. The experiment group recorded 135.41cm height while it was 135.19cm in control children, the mean height being not significantly

different. Height is a parameter which does not reveal a significant difference in six months period. However the experimental group had recorded a mean increase in height though not statistically significant. Similar results were observed in the feeding trials conducted by NIN (1969), Hafñander and Eksmyr (1971) and CFTRI (1974).

4.3.1.2. Weight profile of the children

The most recognised indicator of protein energy malnutrition is weight (Gelliffee, 1969) and this index is effectively used to determine the current state of nutritional status (Gopaldas and Seshadri, 1987).

Initial weight of the experimental group ranged from 25 to 32 kg and that of the control group ranged from 23 to 28 kg. Final weight of the experimental group ranged between 27.5 to 34.50 kg and for the control group it was 23.50 to 28.50 kg (Table 9).

It was observed from the table that a wide range of increase in weight was noted in the experimental group, after the experiment. The mean increase was 2.80 kg. In the control group, the mean weight gain was only 0.65 kg which was lower than the experimental group.

The average weight of children fed with C₄ diet for six months feeding was significantly higher than that of control group. 8.5 per cent increment in weight was observed in experiment group. A significant increase in body weight of the experimental group in feeding trials was also reported by

Subrahmanyam *et al.* (1961). Sindhu (1995) and Sailaxmi (1995). High content of good quality protein in the Mix may be the reason for the increase in the body weight of the experimental group.

Table 9. Weight profile of the children

	Experimental group		Control group	
	Initial (kg)	Final (kg)	Initial (kg)	Final (kg)
	25.00	28.50	25.00	26.00
	29.00	32.50	26.00	27.00
	25.00	28.00	26.00	26.50
			25.00	27.50
	29.00	32.00	24.00	25.00
			23.00	23.50
	27.00	29.50	27.00	27.50
	30.50	33.00	25.00	25.00
	29.00	31.50	24.00	24.50
			23.00	24.00
	32.00	34.50		
	29.00	31.50	28.00	28.50
Mean	28.05	30.85	25.10	25.75
Mean adjusted for initial		29.46		27.14
F _{1,17}		122.41**		

** Significant at 1% level

4.3.1.3. Body mass index of the children (BMI)

Body mass index is regarded as a good indicator of nutritional status (NIN, 1991). According to Visweswara Rao and Singh (1970) weight in kilogram divided by height in square meter ratio is known as body mass index. Body mass index of the children were computed initially and after the completion of the experiment and details are presented in table 10 and 11.

Table 10. BMI of the experimental group

Age	Initial	Final
12 years	Boys	
	13.92	15.64
	15.34	17.02
	13.52	14.92
	13.52	14.63
	14.39	15.69
	Girls	
	15.04	16.31
	15.13	16.18
	14.80	15.73
13 years	Boys	
	Nil	
	Girls	
	15.87	16.87
	14.59	15.62
BMI class	Presumptive diagnosis	
< 16.0	CED grade	111 (severe)
16.1 - 17.0	CED grade	11 (moderate)
17.1 - 18.5	CED grade	1 (Mild)
18.6 - 25.0	Normal	Source NNMB (1991)

Table 11. BMI of the control group

Age	Initial	Final
12 years	Boys	
	14.13	14.48
	14.27	14.71
	15.15	15.21
	Girls	
	15.73	16.02
	14.79	14.57
13 years	14.42	14.54
	14.04	14.53
	Boys	
	14.42	14.79
	14.26	14.39
	Girls	
	16.07	16.23

Initial body mass index of the experimental group ranged between 13.52 and 15.87 and for the control group this was 14.04 to 16.07. But after the completion of the experiment it was found that the BMI of the experimental group showed increase (14.63 to 17.02) and for control group the final BMI ranged from 14.48 to 16.23.

Initial body mass of index of the experimental group showed that all the children were severely malnourished (grade III) (NNMB 1991). On completion of the feeding trial, it could be observed that 30 per cent of children could be shifted to moderate (grade II).

As revealed in table 11, ten per cent of the control group were showing signs of moderate malnutrition and 90 per cent of the children were found to be severely malnourished before the experiment. After the experiment severely malnourished children were 80 per cent.

4.3.2. Bio chemical profile of the children

Aebi (1983) has stated that bio chemical parameters proved to be very informative in order to detect marginal cases of deficiencies. Bio chemical assessment can reveal sub-clinical states of deficiency due to lowered intake or absorption or impaired transport or abnormal utilisation of a nutrient as reported by Swaminathan (1990). Bio chemical parameters such as serum protein, serum iron and haemoglobin levels were assessed in the beginning and completion of the feeding programme for both the experimental and control group.

Serum total protein levels is considered as a good index for estimating protein energy malnutrition and also to determine the severity of the disease (Ukkuru 1993).

Serum protein levels of the experimental group at initial stage ranged from 6.0 to 7.40 g and for control group it ranged from 5.6 to 7.20 g. After the experiment, the serum protein levels of the experimental group ranged from 6.2 to 7.55 and for the control group it ranged from 5.8 to 7.20 g (Table 12). In the experimental group 30 per cent children had serum protein value below normal level and in the control 40 per cent children had serum protein levels below normal before starting the experiment.

Table 12. Serum protein level of children (g)

	Experimental group (n = 10)		Control group (n = 10)	
	Initial (g)	Final (g)	Initial (g)	Final (g)
	7.10	7.25	6.60	6.60
	6.40	6.50	6.80	6.90
	7.40	7.55	7.00	7.10
	6.50	6.70	7.20	7.20
	5.90	6.00	5.60	5.80
	5.70	5.90	5.90	5.90
	6.00	6.20	6.50	6.60
	7.00	7.20	6.00	6.00
	6.60	6.80	6.20	6.30
	7.00	7.20	5.80	5.80
Mean	6.56	6.73	6.36	6.42
Mean adjusted for initial		6.63		6.51
$F_{1,17}$		16.92**		

** Significant at 1% level

Normal value 6.5 to 8.5g

Table 12 reveals that after feeding trials there is an increase in the serum protein value of the experimental group, the mean increase being 0.17 g for the experimental group and for the control group mean increase being 0.06 g.

The average serum protein level was significantly higher in experimental group when compared with control group. There was 1.8 per cent increase in serum protein levels. Protein rich supplement may be the reason for this observation.

The direct measurement of iron is very important in detecting iron deficiency status (Indira, 1993). Hence the serum iron level of both the groups were assessed in the initial and final stage of the experiment (Table 13). Serum iron levels of the experimental group ranged from 40.00 to 41.50 in the initial stages and for the control group ranged from 40.00 to 41.00. After the experiment the serum iron levels of the experimental group ranged between 40 to 41.6 and for the control group ranged between 40 to 41.1. Most of the children have normal value for serum iron in the experimental and control group. Only few children have below normal value in both groups.

The average serum iron levels of the children fed with C₄ diet for six months feeding was significantly higher than that of control group. 0.59 per cent increment in serum iron levels was observed in experiment group.

Swaminathan (1990) has opined that haemoglobin level of blood is a reliable index of the overall state of nutrition. Initial and final haemoglobin levels of the 20 children were estimated and data are presented in table 14.

It was also observed that children from both experimental and control group have normal haemoglobin levels. Only three children have lower values than the standard (12-14 g/dl).

Table 13. Serum iron levels of the children ($\mu\text{g}/\text{dl}$)

	Experimental group (n = 10)		Control group (n = 10)	
	Initial ($\mu\text{g}/\text{dl}$)	Final ($\mu\text{g}/\text{dl}$)	Initial ($\mu\text{g}/\text{dl}$)	Final ($\mu\text{g}/\text{dl}$)
	40.00	41.00	40.00	40.00
	40.00	40.00	40.20	40.21
	41.00	41.20	40.00	41.00
	41.00	41.50	41.50	40.51
	40.00	40.00	40.00	40.00
	41.00	41.30	41.00	41.10
	40.80	40.90	40.20	40.20
	41.00	41.00	40.00	40.00
	41.50	41.60	40.00	40.10
	40.50	40.70	40.00	40.10
Mean	40.68	40.92	40.29	40.32
Mean adjusted for initial		40.74		40.50
$F_{1,17}$		4.59*		

* Significant at 5% level

Normal value 41 to 132 $\mu\text{g}/\text{dl}$

Table 14. Haemoglobin levels of the children (g/dl)

	Experimental group (n = 10)		Control group (n = 10)	
	Initial (g/dl)	Final (g/dl)	Initial (g/dl)	Final (g/dl)
	40.00	41.00	40.00	40.00
	11.80	12.01	11.90	11.90
	11.80	11.80	11.80	11.80
	13.50	13.60	12.10	12.20
	12.10	12.30	13.00	13.10
	11.90	12.00	11.90	11.90
	12.70	12.80	12.20	12.30
	12.80	13.00	12.50	12.60
	13.00	13.20	11.90	11.90
	12.70	12.90	12.60	12.60
	12.10	12.25	12.30	12.40
Mean	12.44	12.59	12.22	12.27
Mean adjusted for initial		12.47		12.38
$F_{1,17}$		9.64**		

** Significant at 1% level

Normal = 12-14 g/dl

The results revealed that among the experimental group a wide range of variation in the increase of haemoglobin content could be observed. The

initial reading among experimental group ranged between 11.80 to 13.50 and final reading ranged from 11.80 to 13.60. The mean increase being 0.15.

Among the control group only negligible changes in the haemoglobin level was seen during the experimental period. The initial reading among the control group ranged between 11.80 to 13.00 and the final reading ranged from 11.80 to 13.10.

The average haemoglobin levels of the experimental group was significantly higher than that of control group there was 0.72 per cent increase in haemoglobin levels. The formulated Mix contained 29.84 mg iron that may be the reason for the result. A significant improvement in haemoglobin levels of the experiment group in feeding trials were also reported by Nagammal (1989).

The protein rich Mix developed with rice-20 per cent, soy-50 per cent, milk powder-20 per cent and wheat-10 per cent had satisfied all quality parameters. Nutritional significance of the Mix was assessed and the results revealed that the Mix contained all the nutrients in the optimum quantity. Organoleptic evaluation and preference test of the developed Mix revealed that it was acceptable to all categories of consumers. The physiological tolerance and shelf life of the Mix were also good. Feeding trials for about six months were conducted among the children. Results revealed that there was significant improvement in the nutritional status of the children.



SUMMARY



SUMMARY

The present study on “Formulation of protein rich Ready To Mix Food” based on rice and soya blend the basic ingredients selected were rice and soy flour. Other ingredients tried along with these were milk powder and wheat flour. Fifteen combinations were tried with these ingredients.

The principle governing the selection of the proper combination were protein quality, consistency, cost and overall acceptability. The combination which got the highest score for all these parameters was selected for the formulation of the Mix.

Acceptability of the Mix was assessed with special reference to nutritional significance, organoleptic qualities, preference test, and physiological tolerance.

Nutritional significance of the Mix was assessed by analysing energy, protein, fibre, iron, calcium, magnesium and zinc. It was found that the Mix contained 23.68 g protein, 349.72 K cal, 2.72 g fibre, 29.84 mg iron, 19.4 mg calcium, 1.46 mg magnesium and 2.7 mg zinc.

The organoleptic qualities of the developed Mix was assessed in the laboratory with ten panel members. Selected through triangle test using a standardised pre tested score card. The major quality attributes included in

the score card were appearance, colour, flavour, texture, taste and overall acceptability. The result revealed that the Mix was found to be acceptable.

Preference test for the developed Mix was conducted among rural women. Hundred rural women were selected from Kalliyoor panchayath in Nemom block. The prepared Mix was served to the rural women and they were requested to mark their preference.

The physiological tolerance of the developed Mix was assessed by conducting nitrogen balance studies among human volunteers. Children of 12-14 years of age were selected for the experiment from Sri. Chitra Poor Home. They were free from infection and infestation. Biological value, Digestibility coefficient, and net protein utilisation of the Mix was analysed. The results revealed that there was significant difference between experimental and control group. The experimental group exhibited higher values for digestibility coefficient, biological value and net protein utilization. The BV, DC and NPU of the Mix were 86.72, 66.88 and 58.0 respectively and for control group 85.24, 65.09 and 55.40 respectively.

Microbiological profile of the developed Mix was ascertained using standard technique. 500 g of the developed Mix was stored in a poly propylene cover for a period of six months. After the storage period the Mix was analysed for the presence of microbes and found that the Mix was free from microbes.

A feeding trial was conducted to assess the protein quality of the Mix. The experiment was conducted for a period of six months. Twenty

children were selected for the experiment consisting of the experimental group of 10 children and the control group of 10. The study was conducted in Sri. Chitra Poor Home, Thiruvananthapuram.

Improvement in their nutritional status were assessed by determining gain in weight, height, haemoglobin level, serum protein and serum iron. Height, weight, haemoglobin levels, serum protein and serum iron of the selected children were assessed initially and after the completion of the experiment. It was observed that the height and serum iron levels of the experimental group were on par with control group. Significant difference between control and experimental group with special reference to weight, serum protein and haemoglobin levels were observed.

From the above observation and findings it can be concluded that the developed Mix is low cost and nutritious. More over it is easy to prepare and it has good shelf life qualities. The developed Mix can be popularised as a food supplement to eradicate malnutrition in the community. As this Mix has got a high protein content in it, helps to improve the growth of the children by building up the tissues and controls the problem of protein calorie malnutrition to a great extent.



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* - Originals not seen



APPENDICES



APPENDIX - I

AMINO ACID SCORES OF DIFFERENT COMBINATION OF THE MIX

Ingredients	Arginine	Histidine	Lysine	Tryptophan	Phexylalanine	Tryosine	Methonine	Cystine	Theronine	Leucine	Isoleucine	Valine
1. Soya - 60	270.00	90.00	240.00	48.00	180.00	126.00	48.00	60.00	144.00	288.00	192.00	192.00
Rice - 20	96.00	26.00	46.00	16.00	56.00	58.00	30.00	18.00	46.00	100.00	60.00	76.00
Milk powder - 10	22.00	18.00	49.00	9.00	31.00	30.00	17.00	5.00	28.00	63.00	36.00	42.00
Wheat - 10	29.00	13.00	17.00	7.00	28.00	18.00	9.00	14.00	18.00	41.00	22.00	28.00
	417.00	147.00	352.00	80.00	295.00	232.00	104.00	97.00	236.00	492.00	310.00	338.00
Amino Acid Score - 82.887												
2. Soya - 60	270.00	90.00	240.00	48.00	180.00	126.00	48.00	60.00	144.00	288.00	192.00	192.00
Rice - 15	72.00	19.50	34.50	12.00	42.00	43.50	22.50	13.50	34.50	75.00	45.00	57.00
Wheat - 10	29.00	13.00	17.00	7.00	28.00	18.00	9.00	14.00	18.00	41.00	22.00	28.00
Milk powder - 15	33.00	27.00	73.50	13.50	46.50	45.00	25.50	7.50	42.00	94.50	54.00	63.00
	404.00	149.50	365.00	80.50	296.50	232.50	105.00	95.00	238.50	498.50	313.00	340.00
Amino Acid Score - 83.368												
3. Soya - 50	225.00	75.00	200.00	40.00	150.00	105.00	40.00	50.00	120.00	240.00	160.00	160.00
Rice - 15	72.00	19.50	34.50	12.00	42.00	43.50	22.50	13.50	34.50	75.00	45.00	57.00
Milk powder - 25	55.00	45.00	122.50	22.50	77.56	75.00	42.50	12.50	70.00	157.50	90.00	105.00
Wheat - 10	29.00	13.00	17.00	7.00	28.00	18.00	9.00	14.00	18.00	41.00	22.00	28.00
	381.00	152.50	374.00	81.50	297.50	241.50	114.00	90.00	242.50	513.50	317.00	350.00
Amino Acid Score - 84.358												

Contd...

APPENDIX - II

Place :

Product :

Date :

Tested by :

SCORE CARD

Combinations

1. Appearance

Excellent	5
Good	4
Satisfactory	3
Mediocre	2
Poor	1

2. Colour

White	5
Cream	4
Pale yellow	3
Yellow	2
Dark yellow	1

3. Flavour

Excellent	5
Good	4
Satisfactory	3
Mediocre	2
Poor	1

4. Texture

Smooth	5
Soft	4
Fibrous	3
Hard	2
Tough	1

5. Taste

Excellent	5
Good	4
Satisfactory	3
Medicore	2
Poor	1

APPENDIX - III

SCORE CARD FOR PREFERENCE TEST

Sl. No.	Products	Like extremely	Like very much	Like reasonably	Like some what	Do not like or not	Dislike some what	Relatively dislike	Dislike very much	Dislike extremely

APPENDIX - IV

PROCEDURE FOR MEASURING HEIGHT

Height is measured with stadiometer

1. The subject will stand erect with heels together after removing his shoes.
2. He will look straight, the head comfortably erect, the arms hanging at the side.
3. The buttocks, shoulders and back of the head will be in the same line and touching the rod.
4. The wooden block used as head piece was gently lowered crushing the hair, making contact with the top of the head.
5. The measurements were done to 0.5cm accuracy.

APPENDIX - V

PROCEDURE FOR ESTIMATION OF HAEMOGLOBIN (Cyanmethaemoglobin method)

Principle

Haemoglobin is converted into cyanmethaemoglobin by the addition of KCN and Ferricyanide. The colour of cyanmethaemoglobin is read in a photoelectric colorimeter at 540nm against a standard solution since cyanide has the maximum affinity for haemoglobin, this method estimates the total haemoglobin.

Reagent

Drabkin's solution - Dissolve 0.05g of KCN, 0.20g of potassium ferricyanide and 1.00g of sodium bicarbonate of 1 litre of distilled water.

Procedure

20ml of blood is transferred with the help of a haemoglobin pipette and delivered on to a whatman No. 1 filter paper disc. The filter paper is air dried, labelled and can be stored upto one week. The portion of filter paper containing the blood is cut and dipped in 5ml of Drabkin's solution taken in a test tube. Wait for 30 minutes and Mix the contents in the tubes, take the readings in a photoelectric colorimeter. The reagent blank (Drabkin's diluent) is adjusted to zero.

Construction of standard curve

If the blood drawn from the subject contains haemoglobin 15g/dl after estimation then prepare three reference standards as follows.

1. Reference standard A

4ml of blood in 100ml Drabkin's reagent contains haemoglobin 15g/dl.

2. Reference standard B

300ml of reference standard A + 200ml Drabkin's reagent contains haemoglobin concentration of 10g/dl.

3. Reference standard C

200ml of reference standard A + 300ml Drabkin's reagent contains haemoglobin concentration of 7.5g/dl.

Thus we have three reference standards at three levels of haemoglobin concentration use 5ml from each standard when ever haemoglobin estimations are done.

APPENDIX - VI

PROCEDURES FOR ESTIMATING TOTAL PROTEIN (Biuret method)

Principle

The CONH gps in the protein molecule react with copper sulphate in alkaline medium to give purple colour which is then read at 540nm.

Reagents

1. Biuret reagent : Dissolve 4.25g of potassium sodium tartarate, 1.5g of cupric sulphate and 2.5g potassium iodide in about 500ml of distilled water. Dissolve 4g of NaOH in the solution and make up the volume to 1 litre.
2. Standard : The standard protein solutions may be either a pooled normal human serum or a solution of pure albumin saline.

Procedure

To 0.1ml aliquot of standard test plasma and blank (saline and distilled H₂O) add 5ml of biuret reagent. Mix well and keep for 30 minutes. Read absorbances of test and stand and against blank at 540nm.

$$\text{Calculations} = \frac{\text{Reading the test}}{\text{Reading of the standard}} \times \text{Conc. of standard}$$

APPENDIX - VII

PROCEDURE FOR ESTIMATION OF SERUM IRON (Wong's method)

Principle

Iron is determined colorimetrically making use of the fact that ferric iron gives a blood red colour with potassium thiocyanate.

Reagents

1. 30% H_2SO_4
2. 7% of potassium persulphate solution : 7g potassium persulphate is dissolved in glass distilled water and the solution made upto 100ml.
3. 40% potassium thiocyanate solution : 40g KCNS is dissolved in 90ml glass distilled water. 4ml acetone added and the volume made upto 100ml.
4. Standard iron solution : 702.2mg ferrous ammonium sulphate is dissolved in 100ml glass distilled water and after addition of 5ml of 1:1 HCl, the solution is made upto 1 litre and mixed thoroughly (0.1mg Fe/ml). The standard solution is prepared fresh once in 6 months.

Working standard solution (10 μ g/Fe/ml) is prepared by diluting the above solution 10 fold.

Procedure

Two ml of cone H_2SO_4 is taken in a 50ml volumetric flask. Add exactly 0.5ml of well mixed blood, mix and to this add 2ml of potassium persulphate, agitate the flask, cool and dilute with about 25ml distilled water. Then 2ml of sodium tungstate is added and the volume made upto the mark. Filter using whatman No.42 filter paper. Transfer 15ml of the filtrate to a fresh tube, add 1ml of potassium persulphate and 4ml of potassium thiocyanate Mix and read the colour at 540 nm in a colorimeter. A standard (10-100 μ g) is run similarly and a standard graph is prepared.

**FORMULATION OF PROTEIN RICH
READY TO MIX FOOD BASED ON RICE
SOYA BLEND**

By

LITTY ANDREWS

ABSTRACT OF A THESIS
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ABSTRACT

The present study on "Formulation of protein rich Ready To Mix based on rice and soya blend", rice and soy flour were selected as basic ingredients. Other ingredients tried along with these were milk powder and wheat flour. Fifteen combinations were tried using these ingredients. The principles governing the selection of the proper combination of ingredients were protein quality, consistency, cost and overall acceptability. The combination of rice, soya, milk powder and wheat flour in a 20, 50, 20 and 10 per cent respectively got the highest score for all parameters selected.

Acceptability of the mix was assessed with special reference to nutritional significance, organoleptic qualities, preference among consumer and physiological tolerance.

The developed mix contained nutrient such as (Energy, protein, fibre, calcium, magnesium and zinc) in adequate amounts. Organoleptic qualities of the mix was assessed in the laboratory with the selected panel of technical experts as judges and the mix was found to be acceptable. Preference test conducted among farm women revealed that most of the farm women (97%) preferred the mix.

The physiological tolerance of the developed mix was assessed by conducting nitrogen balance studies among human volunteers. Children of

12-14 years of age were selected for the experiment. Biological Value (B.V.), Digestability Coefficient (D.C.) and Net Protein Utilisation. The results revealed that experimental group exhibited higher values for DC, BV, and NPU. The BV, DC and NPU of the developed mix were 86.72, 66.88 and 58.00 respectively.

Microbiological profile of the mix was ascertained using standard techniques and found that the mix was free from microbes after a storage period of six months.

Protein quality of the mix was assessed by conducting feeding trials in children for a period of six months. Sri. Chitra poor home, Thiruvananthapuram was selected for the conduct of the experiment. Improvement in the nutritional status of the children was assessed by determining gain in height and weight, Hb level, Serum protein and serum iron. The result revealed that there was significant improvement in their nutritional status with special reference to weight gain and serum protein.

On taking in to consideration the above observation and findings got from the study it was found that the mix was highly nutritious and low cost. The processing of the mix is less time consuming and have a good shelf life quality. This mix would very well be used as a food supplement to eradicate malnutrition that persists with in the community.

