Methodology

III

METHODOLOGY

The methodology followed in the present study entitled "**Prevalence of micronutrient deficiency in Ramanathapuram district and impact of interventions**" has been carried out under the following headings.

A. PHASE I Prevalence of Micronutrient Deficiency Disorders Among Different Age Groups of Population

1. Selection of area
2. Selection of subjects
3. Collection of background information
4. Assessment of nutritional status of the subjects

B. PHASE II Development of Micronutrient Rich Food Products

1. Selection of food sources for product formulation
2. Development of micronutrient rich products
3. Organoleptic evaluation of the developed products
4. Nutrient analysis of the developed products
5. Microbial analysis and shelf life study of the developed products

C. PHASE III Development of Education Module

D. PHASE IV Impact of Interventions Among School Going Children

1. Selection of subjects for intervention
2. Conduct of supplementation
3. Nutrition education
4. Impact evaluation of supplementation

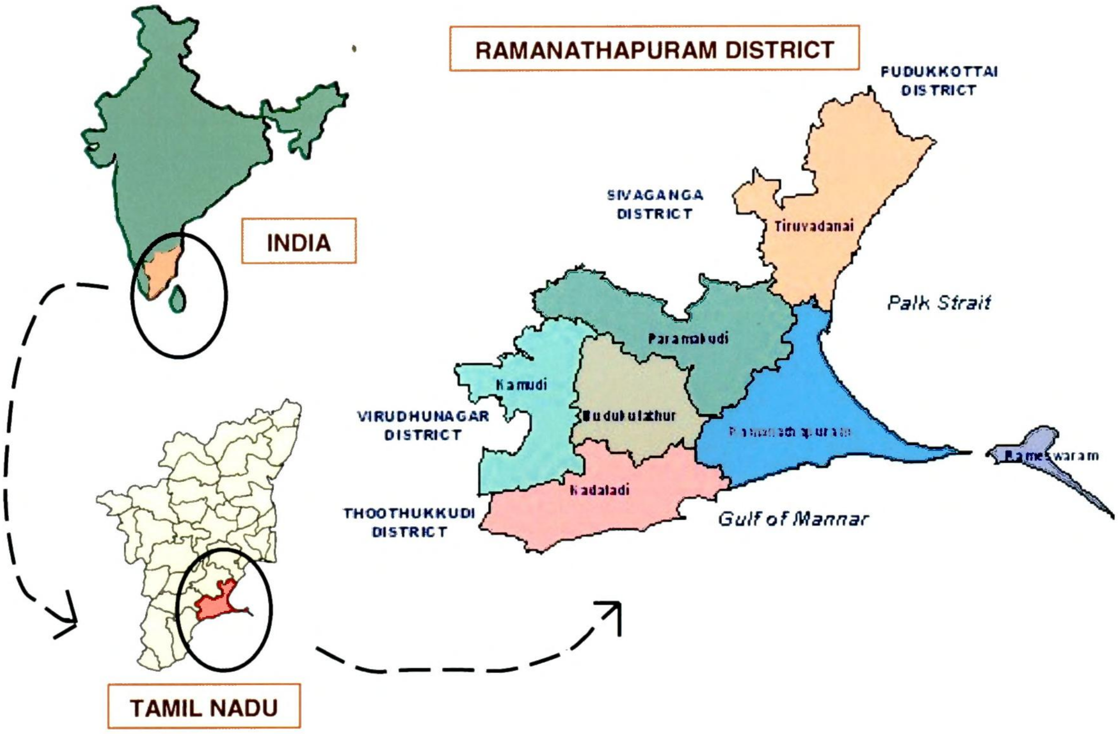
E. PHASE V Statistical Analysis and Interpretation of Data

A. PHASE I PREVALENCE OF MICRONUTRIENT DEFICIENCY DISORDERS AMONG DIFFERENT AGE GROUPS OF POPULATION

1. Selection of area

Ramanathapuram is an administrative district of Tamil Nadu with an area of 4175 sq.km. It has a total population of 11,87,604 (Ramanathapuram District Collectorate). It consists of two towns namely Ramanathapuram and Paramakudi. Ramanathapuram (Ramnad), Tiruvadanai and Rameswaram are the taluks contained in the Ramanathapuram division. Paramakudi, Mudukulathur, Kamuthi and Kadaladi are the four taluks in the Paramakudi division. In order to assess the prevalence of micronutrient deficiency, equal number of subjects were selected from all the seven taluks of Ramanathapuram district based on multistage stratified sampling.

Data on prevalence of various diseases and mortality due to these diseases in the districts of Tamil Nadu are limited. Data is available only for diseases that are targeted through disease control programmes at the national level. An important limitation of these data is that they deal with only cases that are reported in public health facilities. As a result, these data tend to underestimate incidence of diseases and incidence of deaths due to these diseases (Sen *et al*, 2008). Since Ramanathapuram is one such district where scanty information on health status are available, the present study was conducted in the seven taluks of Ramanathapuram district of Tamil Nadu. Figure 3 gives the map of Ramanathapuram district and areas selected for the study.



MAP OF RAMANATHAPURAM DISTRICT AND AREAS SELECTED FOR THE STUDY

FIGURE 3

2. Selection of subjects

Stratified random sampling method was followed to select the subjects. The sample comprised of infants, preschool children, school going children, adolescents, adults and pregnant women. From each of the age groups, a total of 200 male and 200 female subjects were selected to make up to 2200 subjects from each taluk. A total of 15,400 subjects were selected from 8,764 households the seven taluks of Ramanathapuram district by house visits and by visiting schools and colleges. The infants selected were from zero to one year of age, preschool children from three to five years, school going children from eight to 12 years, adolescents from 13 to 19 years, adults from 23 and 40 years of age. Since pregnant women were vulnerable to micronutrient deficiency and gave their consent for participating in the study, pregnant women between 19 and 27 years were also included. The pregnant women selected were in the second and third trimester of pregnancy. Table I presents the details on various subjects selected for the present study.

TABLE I

DETAILS ON SUBJECTS SELECTED FOR THE STUDY

Category	Male	Female
Infants	200	200
Preschool children	200	200
School going children	200	200
Adolescents	200	200
Adults	200	200
Pregnant women	-	200
Total in each taluk	1000	1200
Total in seven taluks	7000	8400
Grand total	**15,400**	

3. Collection of background information

The social, cultural and physical background can greatly influence the nutritional status of an individual. Social, economic, and personal factors, such as lack of knowledge or interest in healthy eating also impede the access to healthy foods (Gans *et al.*, 2010).

Background information pertaining to the present study was collected from all the 15,400 subjects using a pretested questionnaire specially designed for the purpose. Information on the socio-economic background including educational status, occupation of the head of the family, family income, family type and size along with data on the various health problems faced by the subjects and morbidity pattern were collected. The questionnaire also included questions related to basic concepts of micronutrients, their sources, deficiency symptoms and preventive measures. Both closed and open ended questions were included in the questionnaire to allow maximum flexibility and versatility in recording individual responses. After explaining the purpose of the study and obtaining consent from the participants, the questionnaires were administered for each of the subjects and filled in by the investigator. Data was collected from the parents or care takers in the case of infants, preschool and school going children. Additional information such as birth weight of the infants and preschool children were collected from the health card wherever available and were recollected by the parents wherever the health cards were not available. The subjects themselves were asked to respond to the questions in the case of the other groups (Plate 1) (Appendix I).

4. Assessment of nutritional status of the subjects

In the assessment of the nutritional status of individuals, any one or a combination of two or more of the following methods are used: dietary assessment, clinical assessment and physical signs, biochemical assessments and anthropometric measures. The selection of methods to be used often depends on the age of the individual, the reason for assessment, and the resources and expertise available (Mohammed *et al.*, 2009). The nutritional status of all the subjects was assessed using the following parameters.

a. Clinical assessment

All body systems are dependent on adequate nutrient intake, and deficiencies can be manifested at any stage of life. Every physical part of the body can reflect nutritional status. Therefore, a thorough, systematic approach to physical assessment is essential (Feeney *et al.*, 2010). A large number of clinical signs indicate nutritional deficiencies. The general expression of micronutrient deficiency is a wasted, thin individual with dry scaly skin and poor wound healing, thin hair and spooned and depigmented nails (Ahmed and Haboubi, 2010).

All the subjects selected for the present study were clinically examined with the help of a physician for exclusive clinical signs of various micronutrient deficiencies. Signs and symptoms in their physical appearance, skin, hair and eyes indicative of various deficiency disorders were noted using a clinical assessment schedule (Plate 2) (Appendix II).

b. Dietary intake

Adequate nutrition is one of the pillars of public health. Before developing and implementing effective intervention programmes to improve nutrition at the population level, it is important to know the nutritional situation of the target group (Elmad *et al.*, 2010).

Quantifying nutritional intake is best performed by a dietician. Out of the several methods used for nutritional assessment, twenty four hour recall is the most commonly used method and is based on an interview during which the person recalls all the food items consumed in the previous 24 hours (Rao *et al.*, 2009).

Nutrient intake was assessed using the 24 hour dietary recall schedule (Appendix III). From each of the seven taluks chosen for the study, 25 per cent of the subjects from each age group excluding the infants, summing up to a total of 3,150 subjects were selected at random for the assessment of food and nutrient intake. The subjects were asked to recall all the food items consumed by them on the previous day along with the quality of each item consumed. Standard cups and measures were used in order to aid them in recollecting the exact amounts consumed. From the amounts of cooked foods consumed, the raw equivalents of each food items were computed. The daily food intake and nutrient intake were computed using the ICMR food composition tables (Gopalan *et al.*, 2009) and were compared with the recommended dietary allowances of the corresponding age groups as suggested by ICMR (2010).

c. Anthropometric assessment

Nutritional anthropometry has been defined as measurements of the variations of the physical dimensions and the gross composition of the human body at different age levels and degrees of nutrition (Jelliffe, 1966). Anthropometry remains the most practical tool for the assessment of nutritional status among members of the community in developing countries such as India (Chakraborty *et al.*, 2011). The selection of the ideal single or a combined use of anthropometric indicators depends upon the sensitivity and specificity of the indicator chosen. The following anthropometric measurements were assessed by the investigator for all the 15,400 subjects chosen for the present study.

i. Height / Crown heel length

Measures of height and weight are useful markers of nutritional status because these changes over time may reflect a change in nutritional status (Collins and Harris, 2010). Growth is the best indicator of nutritional status and its analysis remains the simplest tool for assessing changes in nutritional status (Hartman and Shamir, 2009). Height of the subjects above two years of age was measured using a stadiometer. The subjects were made to stand straight without foot wear, with minimal clothing. They were made to stand with feet apart and head straight, with hands free on either sides, knees and thighs straight with weight distributed evenly on both the feet. The feet were flat on the floor with the heels touching the base of the vertical board. The head piece was placed firmly on the crown of the head at right angles to the measuring board. Height was measured to an accuracy of 0.1 cm.

In the case of infants, crown heel length was measured from the top of the head to the sole of the foot with the body lying supine, using a neonatometer to the nearest one millimeter according to standardized methods (Wales *et al.*, 1997).

ii. Weight

Weight change is the first indication of over or under nutrition. Weight generally reflects recent changes and is an indicator of short term nutritional status (Ramzan *et al.*, 2010). Body weight of all the subjects above two years of age were measured using a weighing balance. The subjects were made to stand erect on the weighing scale with minimal clothing without foot wear and arms hanging loose without holding anything. Weight of the subjects was recorded to an accuracy of 0.1 kg. Weight of the infants was measured using an electronic weighing scale calibrated before each measurement, with an accuracy up to 5 g (Plate 3).

iii. Body mass index (BMI)

BMI has been recommended as the best anthropometric indicator of nutritional status by Burden *et al* (2005). Of the anthropometric indices, body mass index is considered to be more nutritionally than genetically related (Khongsdier, 2001). The body mass indices of the school going children, adolescents and adults were measured by the following formula

$$\text{BMI} = \frac{\text{Weight (Kg)}}{\text{Height}^2\text{ (m)}}$$

iv. Ponderal Index (PI)

In order to facilitate the diagnosis of intrauterine malnutrition of infants at birth it is useful to incorporate body length in the assessment and to calculate Ponderal Index (PI). The PI provides information on the proportionality of body growth. It allows the estimation of stunting, wasting and overgrowth that have occurred either *in utero* or postnatally and is hence more suitable for infants and children than BMI (Miller and Hassanein, 1971). Ponderal index of the infants and preschool children were calculated using the following formula (Lawrence, 2007)

$$\text{PI} = \frac{\text{Weight X 100 kg/m}^3}{\text{Weight}^3}$$

v. Head circumference

Head circumference is the maximum circumference around the head at the level of the point just above the glabella anteriorly and the occiput posteriorly (Huysman *et al.*, 2003). Head circumference was measured for infants, preschool children, school going children and adolescents by applying a non elastic measuring tape over the occipital protuberance on the back, above the ear or the sides, and on the supra orbital ridges in front (Plate 4).

Plate 1
Collection of data

Plate 2
Clinical assessment

Plate 3
Measurement of weight

Plate 4
Measurement of head circumference

vi. Chest circumference

A WHO collaborative study (1993) had identified chest circumference as one of the most appropriate indicators in the assessment of the nutritional status of infants. Chest circumference measurements were noted for all the infants, preschool children, school going children and adolescents by applying a fibre glass tape at the level of the nipple in a place at right angle with the spine at mid respiration.

vii. Mid upper arm circumference

Mid upper arm circumference measurements are indicative of the mucle mass and subcutaneous fat of an individual. This is a relatively simple measurement as it ignores age related changes, has good sensitivity and specificity and appears to be a better predictor of childhood mortality (Biswas *et al.*, 2010). The mid-arm circumference of all the 15,400 subjects was measured on the left arm. The arm was flexed at the midpoint of the line between the olecranon process and the acromium process was measured over the triceps. The arm was then allowed to hang loosely and the fibreglass tape measure was fitted to the arm without depressing the skin. The results were expressed to the nearest one cm (Plate 5).

viii. Waist hip ratio

Waist and hip girths were measured for all the adult subjects in centimetres using a fibreglass tape over light clothing. Waist circumference was measured to the nearest centimetre laterally, midway between the lowest portion of the rib cage and iliac crest, and anteriorly midway between the xiphoid process of the sternum and the umbilicus with a flexible steel tape measure while the subjects were in the standing position at the end of gentle expiration. Hip circumference was measured at the maximum protuberance of the buttocks over the greater trochanters. The waist hip ratio (WHR) was calculated for each subject using the formula

$$\text{WHR} = \frac{\text{Waist circumference (cm)}}{\text{Hip circumference (cm)}}$$

d. Biochemical assessment

Five per cent of the subjects from each age group from each of the seven taluks summing up to a total of 770 subjects from those who were previously assessed for dietary intake were chosen at random for biochemical assessment in order to identify sub-clinical levels of micronutrient deficiency. With the help of a laboratory technician, five ml of blood was drawn from the subjects and assessed for haemoglobin and vitamin A. (Plate 6).

i. Blood haemoglobin

Determining the concentration of haemoglobin, an iron containing protein in red blood cells is a sensitive and direct indicator of anaemia (CDCP, 1998). Haemoglobin measurement is the primary method for anaemia screening and to study its prevalence in populations (DeMaeyer *et al.*, 1989). Blood haemoglobin levels of the selected subjects were estimated using the cyanmethaemoglobin method (Raghuramulu *et al.*, 2003).

ii. Serum vitamin A

The serum vitamin A concentration is the most commonly used biochemical test to identify individuals and population with a high risk of vitamin A deficiency (Gershwin *et al.*, 2000). Although all biochemical indicators currently available have limitations, the biochemical indicator of choice for population assessment is the distribution of serum levels of vitamin A (Sommer and Muhilal, 1982). Serum vitamin A levels of the subjects were assessed by HPLC method using petroleum ether : dichloromethane : isopropanol in the ratio (80:19.3:0.7) as mobile phase at the flow rate of 0.5 ml/min using Micropak Si-10 column 15x0.2 cm. Retinol was used as the standard. Based on the retention time of standards and unknown and calculating the peak height ratio, vitamin A was determined (Raghuramulu *et al.*, 2003).

iii. Urinary iodine

More than 90 per cent of dietary iodine eventually appears in the urine. Therefore, urinary iodine is an excellent indicator of recent iodine intake (Marwaha and Gopalakrishnan, 2011). Urinary iodine concentration is the prime indicator of nutritional iodine status and is used to evaluate population based iodine status (Delange *et al.*, 2002). For the collection of urine, the subjects were asked to have an early dinner and arrive early in the morning. About five ml of first urine of the day was collected in clean containers for urinary iodine assessment. Urinary iodine levels of the subjects were estimated by spectrophotometric method (Raghuramulu *et al.*, 2003).

B. PHASE II DEVELOPMENT OF MICRONUTRIENT RICH FOOD PRODUCTS

Multiple approaches including nutrient supplementation, food fortification, diversification and public health measures have been suggested for prevention and control of nutritional deficiencies. Previously, there has been a tradition to prescribe high-dose iron supplements (Zhou *et al.*, 2009). These, however, have been associated with a range of side-effects including gastrointestinal perturbations, reduced zinc absorption, increased oxidative stress and diabetes risk (Bokhari *et al.*, 2012). Hence, dietary improvement becomes the most logical and sustainable strategy to prevent micronutrient deficiencies. Improving the diet is of paramount importance since it contributes to improvement in overall nutritional status. From the various methods to combat micronutrient deficiencies, dietary supplementation with micronutrient rich foods was chosen as the next phase of the present study.

1. Selection of food sources for product formulation

Different foods rich in micronutrients were selected based on the careful perusal of previous literature. Local availability, ease of procurement and cost formed the secondary criteria for the choice of ingredients.

Cereals are the major staple foods of India. Besides the high starch content as energy source, cereals provide dietary fibre, nutritious protein and lipids rich in essential fatty acids. Important micronutrients are present in cereals are vitamins, especially many B vitamins, minerals, antioxidants and phytochemicals. In general, cereals provide important amounts of most nutrients (Kadam *et al.*, 2010). Indian red rice (*Oryza sativa*) is of particular importance among these. Comparative nutrition studies on red, black and white varieties of rice suggest that pigments in red rice varieties may offer many nutrition benefits which are absent in the white rice varieties (Ling *et al.*, 2001). The bran part of red rice grain is concentrated with many phytochemicals, including proanthocyanidins, oryzanol and vitamin E, that exert beneficial effects on human health (Dixit *et al.*, 2011).

Wheat (*Triticum aestivum*) is one of the most important cultivated crops worldwide. Consumption of dietary fibre from wheat is generally recognized as having health benefits (Quraishi, 2011). The aleurone fraction of wheat has high levels of potentially health-promoting compounds (Anson *et al.*, 2009). Jowar (*Sorghum vulgare*), finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) are the three most important millets grown in the tropics (Pray and Nagarajan, 2010). Being coarse in nature, they can be used in different proportions with rice and wheat to formulate various nutritional products. They can be used to make porridges, biscuits, cakes, cookies, tortillas, bread, probiotic drinks, ladoo, ghatta, flakes and several fermented foods These cereals are laden with phytochemicals including phenolic acids, tannins, anthocyanins, phytosterols, avenanthramides and policosanols. They possess high antioxidant properties *in vitro* than staple cereals and fruits by different purported pathways (Kaur *et al.*, 2012). Pearl millet also known as Bajra, pearl millet and finger millet are known for several health benefits which may be attributed to their polyphenols, calcium and dietary fibre and other antioxidant contents (Nambiar et al., 2011). Pearl millet is rich in oil and linoleic acid accounts for four per cent of the total fatty acids in this oil, giving it a higher percentage of n-3 fatty acids (Rai *et al.*, 2008). They are also recognized for their other health beneficial effects, such as anti-diabetic, anti-tumerogenic, atherosclerogenic effects, antioxidant and antimicrobial properties (Devi *et al.*, 2011).

Groundnut is a healthy and commonly used ingredient in snack and chocolate recipes. The U.S. Department of Agriculture (USDA) considers it having maximum of the five important nutrients namely energy, protein, phosphorus, thiamine and niacin. Eating fresh roasted groundnuts has been considered to be very nutritious for growing children, pregnant women and nursing mothers as it builds resistance against infections, particularly tuberculosis and hepatitis (Sharma, 2005).

Green leafy vegetables provide essential carotenoids with promise in eye health (Seddon, 2007). The xanthophylls lutein, found in Ponnanganni (*Alternanthera sessilis*), has been identified as an important protective agent in several *in vitro* assays, epidemiologic studies and intervention trials examining plant food consumption and prevention of age related cataract and macular degeneration (Gupta and Prakash, 2009). Hence, food ingredients namely red rice, wheat, rice flakes, jowar, finger millet, pearl millet, roasted Bengal gram dhal, groundnut, ponnanganni and lotus stem have been chosen for the development of micronutrient rich foods.

2. Development of micronutrient rich products

Snack foods are non-meal foods that are more relished and less fussed invariably by children. Children have a broad access to a wide variety of snack foods and further, the increased engagements of the parents have increased the demand for convenience foods. However, the between-meal eating habits of children can be exploited to combat devastating deficiencies if manipulated with care.

Bakery products like biscuits are found to have high acceptability and are widely consumed by all (Salve *et al.*, 2010). All the food ingredients chosen could be easily incorporated in a biscuit recipe. Further, the process of biscuit making involves baking and supportive studies have indicated that the baking process exerted a positive effect on the dialyzability of iron, calcium, zinc and reduce the phytate content of the raw materials (Frontela *et al.*, 2011). Ready to eat multi-nutrient biscuits was hence thought to be a timely intervention to achieve the objective of the study. In order to incorporate fresh vegetables in the supplements a jam was prepared using carrot, sweet potato, tomato and beet root and kept as a filling between two biscuits (Plate 7). Hence, biscuits and jam, which are relished and readily consumed widely by children, were chosen as products for supplementation (Bokhari *et al.*, 2012).

a. Preparation of multinutrient biscuits

The food ingredients chosen for the preparation of the special flour for biscuit making comprised of all the six important food groups namely cereal, millet, pulse, nut, green leafy vegetable and vegetable in the ratio 3:3:1:1:0.7:0.7. The various foods chosen for the preparation of biscuits along with their quantities are listed in Table II.

TABLE II

COMPOSITION OF THE DEVELOPED BISCUITS

Ingredients	Quantity /100 g
Cereals	**30**
Red rice	10
Wheat	10
Rice flakes	10
Millets	**30**
Jowar	10
Finger millet	10
Pearl millet	10
Pulses	**10**
Roasted Bengal gram dhal	10
Nuts	**10**
Groundnut	10
Leafy vegetables	**7**
Ponnanganni	7
Other vegetables	**7**
Lotus stem	7
Salt	1.5
Sugar	7.3
Hydrogenated fat	14
Sodium bicarbonate	1
Ammonium bicarbonate	3
Milk (ml)	3

Plate 5
Measurement of MUAC

Plate 6
Collection of blood

Plate 7
Product developed for supplementation

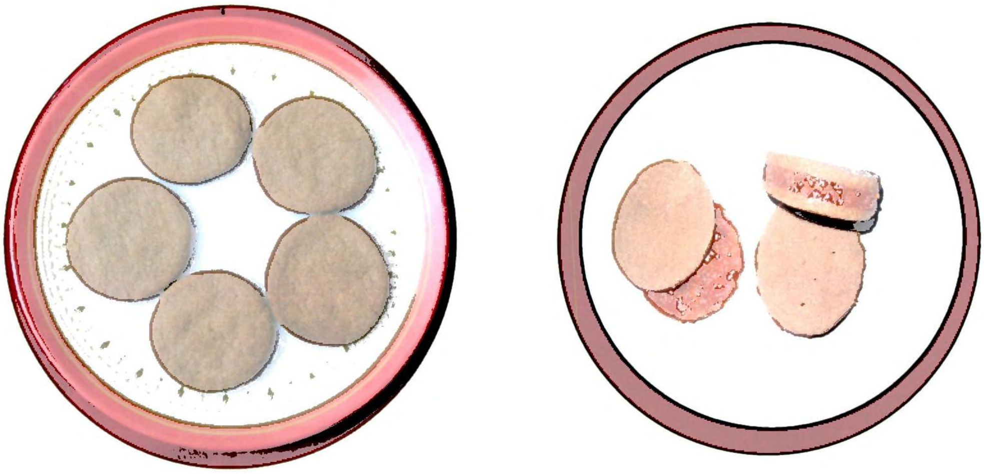


Plate 8
Spread plate analysis

Processing of ingredients

The dry ingredients were procured from the commercial market and cleaned manually by sieves and winnows. The cereals, millets and nuts in bulk were roasted in a dry pan individually at a temperature between 60 to 90° C for a duration of fifteen minutes. The process of roasting reduces the anti-nutrient content significantly and enhances the innate flavour of the ingredients. Ponnanganni greens (*Alternanthera sessilis*) were cleaned and leaves were shade dried until a residual moisture content of five to six per cent as per the procedure of Kowsalya *et al* (2001); Sharma *et al* (2000) was obtained. The same procedure was repeated for lotus stem. All the ingredients were then mixed well and ground in an electric grinder to obtain a fine flour with a particle size of 150 to 550 micron.

Preparation of biscuits

Powdered sugar and hydrogenated fat were blended and creamed well for a period of five to eight minutes. The special flour prepared as stated in the previous section 'a' was mixed along with salt, sodium bicarbonate and ammonium bicarbonate were mixed and sieved thrice. The flour was slowly added to the above mixture little by little with continuous mixing avoiding the formation of lumps. The mixture was blended and folded for another five to eight minutes to obtain a dough. Milk at a level of 15 ml per kg of flour was added so as to avoid cracks and obtain a soft dough. The dough was then sheeted to a resultant thickness of five mm using a rolling pin and cut in circular shapes with a diameter of 50 mm. It was docked with a needle and placed on a greased baking tray with a gap of 8 to 10 mm separating each biscuit. The biscuits were baked for 10 to 12 minutes at a temperature of 200° C. Biscuits were then allowed to cool completely and packed in polythene bags and stored airtight. The method of preparation of biscuits is presented in the form of flow chart in Plate 9 and Figure 4. The multinutrient biscuits rich in micronutrients developed in this study has been filed in Patent Office, Chennai, India for obtaining Indian Patent (Number : 1763/CHE/2012).

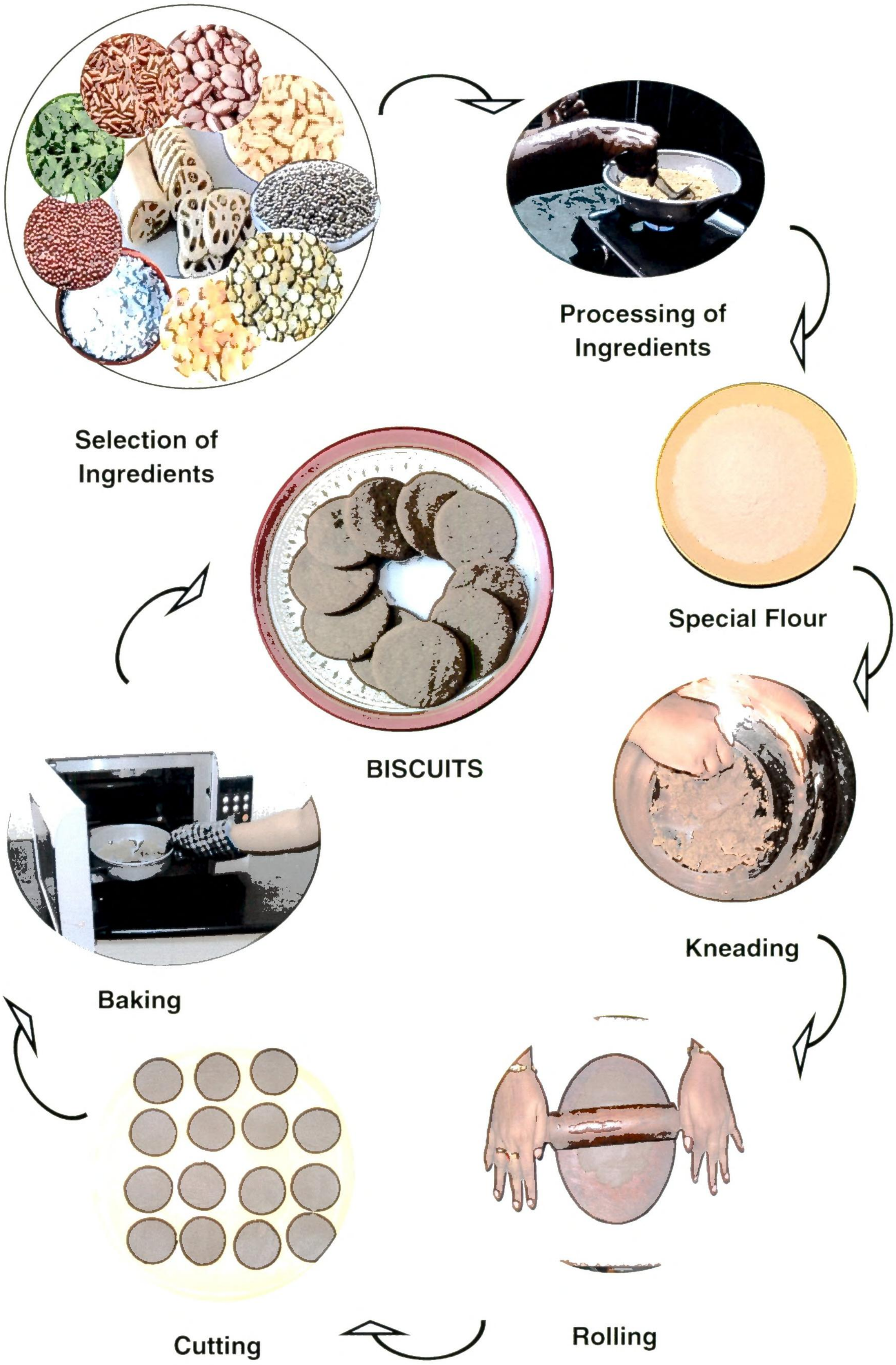


Plate 9
Steps involved in developing micronutrient rich biscuits

b. Preparation of jam

All the ingredients namely sweet potato, carrot, tomato and beet root were freshly procured from the local market. The vegetables were cleaned and washed thoroughly. Sweet potato and beet root were peeled carefully to remove the outer skin. Care was taken to avoid peeling too deeply so as to retain the nutrients present right under the skin of the vegetables. All the vegetables were diced into medium sized cubes. They were then pressure cooked with minimum water for 15 minutes and blended in a mixer after cooling to obtain a fine paste.

Sugar was taken in the ratio of one part of sugar for four parts of the vegetable paste. The sugar was dissolved in twice the amount of water and brought to a boil. The vegetable paste was slowly added to this with continuous stirring and cooked in a low flame till it attained jam consistency. The jam was then cooled down and stored airtight. The method of preparation of jam is presented in the form of flow chart in Figure 4 and Plate 10.

3. Organoleptic evaluation of the developed products

Sensory qualities of the developed products were assessed by a 35 member expert panel and categorised using the nine point rating scale (Appendix IV). The biscuits were tested for organoleptic evaluation by studying sensory attributes, namely crust colour, surface characteristics, crumb texture, crumb colour, taste, flavour, mouth feel and overall acceptability. The jam was also assessed similarly for colour, texture, taste, flavour and acceptability.

4. Nutrient analysis of the developed products

The developed biscuit and jam were analyzed for their nutrient content. Carbohydrates, protein, thiamine, riboflavin and niacin were determined by AOAC method (AOAC, 2000). Iron was determined spectrophotometrically (Wong, 1928). Other nutrients namely β carotene, vitamin C, pyridoxine, vitamin B12, folic acid, calcium, phosphorous, iodine and zinc were assessed using the procedures recommended by Raghuramulu *et al* (2003).

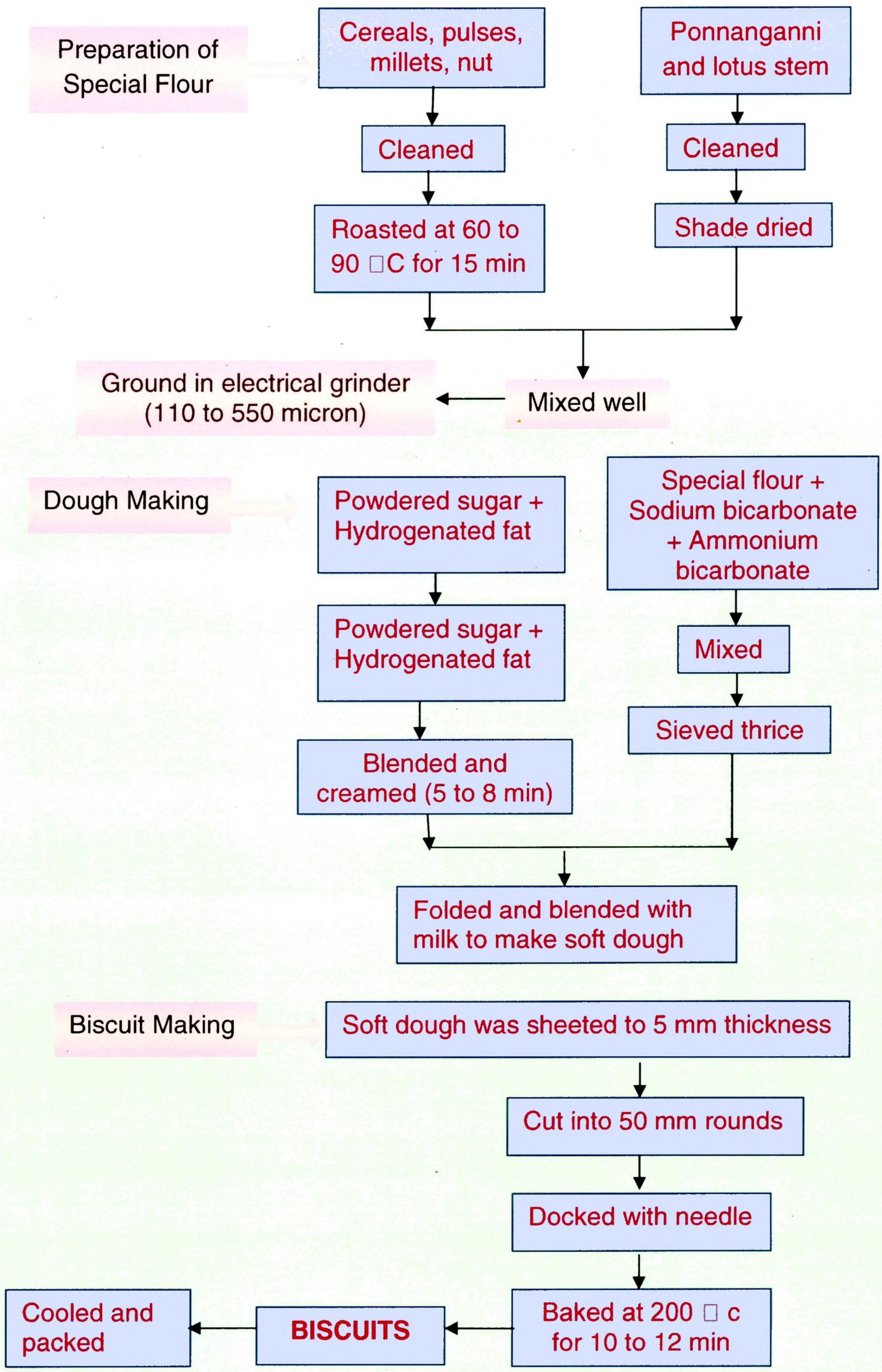
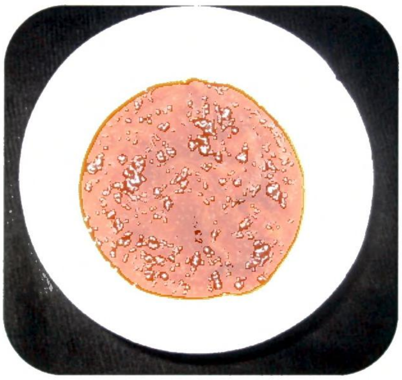


FIGURE 4

FLOW CHART FOR BISCUIT PREPARATION

1. Cutting of Ingredients

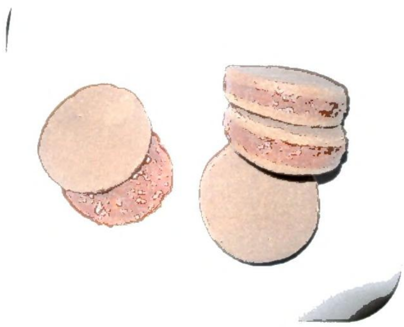
2. Pressure cooked



3. Ground vegetables

4. Cooked with sugar syrup

5. Prepared jam



6. Packed between biscuits

Plate 10
Steps involved in developing micronutrient rich jam

5. Microbial analysis and shelf life study of the developed products

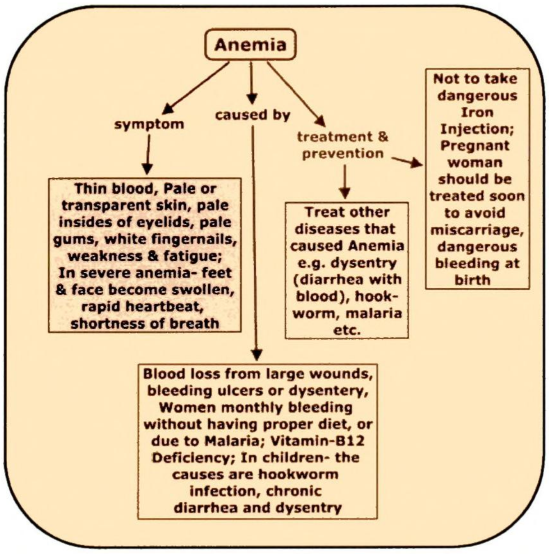
Storage changes in foods include not only development of off-flavors, but also loss of color, nutrient value, and more importantly, the accumulation of deleterious compounds and microbes which might be connected with higher incidence and mortality rates of numerous human illnesses. Therefore, the keeping high quality of baked food such as biscuits is of great nutrition and economic importance (Szkudlarz *et al.*, 2009). Biscuits were stored in sealed polyethylene packs under room temperature in a clean dry atmosphere for a period of six months and analyzed on the first and sixth months for microbial growth. Microbial analysis of the biscuits was done using the standard plate count technique (Speck, 1984) (Plate 8).

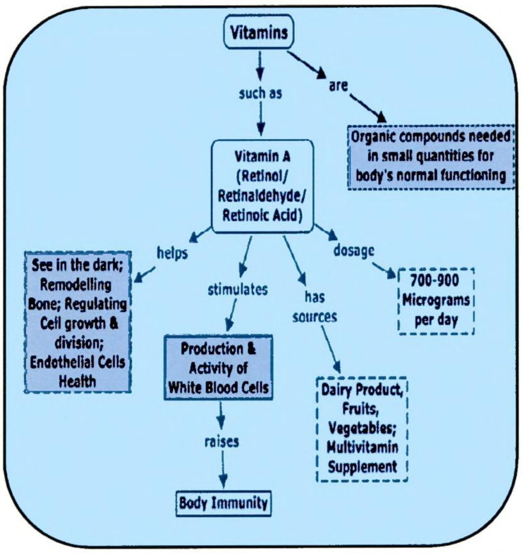
Peroxide value is an indicator of rancidity development during storage and serves as a good tool for the measurement of degree of oxidation. Peroxide value of the biscuits was determined according to method described in AOCS (1993).

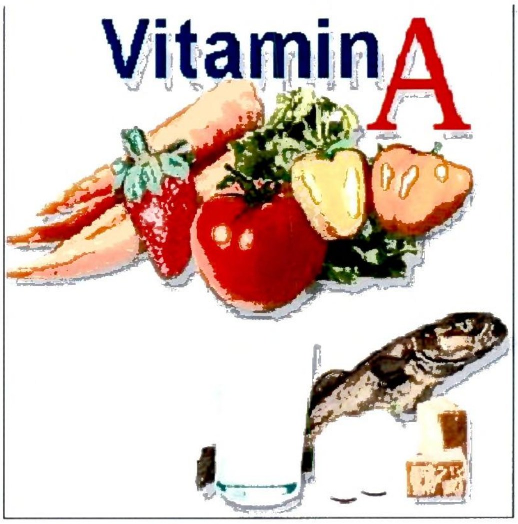
PHASE III DEVELOPMENT OF EDUCATION MODULE

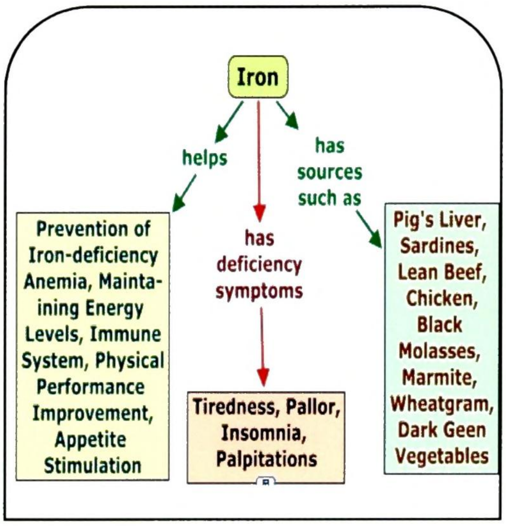
Nutrition education and knowledge enhancement are key components in improving health and nutrition in a sustainable way. By providing the tools and knowledge to make choices, a resilient food and nutrition system can be developed that ensures environmental integrity, economic self-reliance and social wellbeing (Burchi *et al*, 2011). A nutrition education module containing all the essential aspects of nutrition in general and micronutrients in particular was developed. Aspects covered included the basic nutrients present in food, their roles, sources of micronutrients, deficiency signs and symptoms, balanced meal and efficient cooking methods. Several nutrition education materials such as charts, posters, pamphlets, leaflets and calendar were prepared in order to facilitate easy teaching and better understanding of the various concepts of the nutrition education module (Plate 11 and 12).

Plate 11
Charts developed for nutrition education









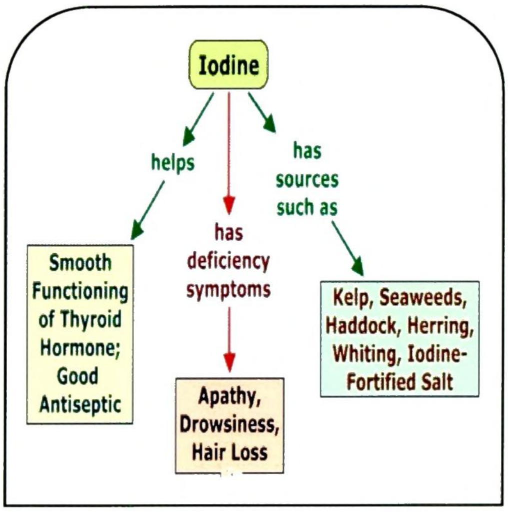
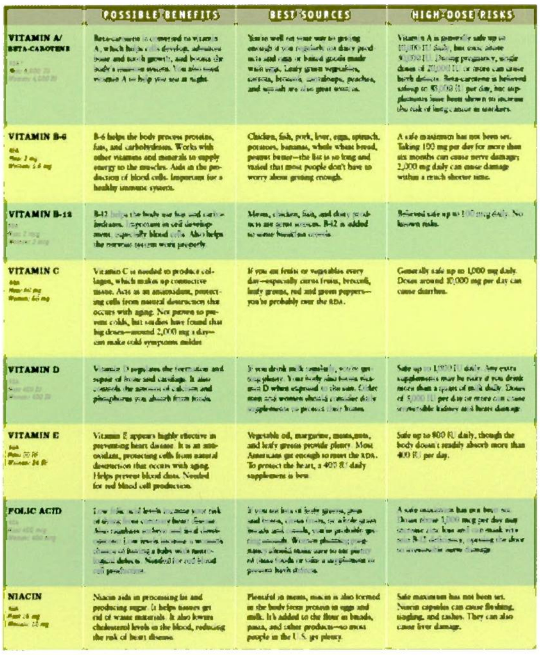
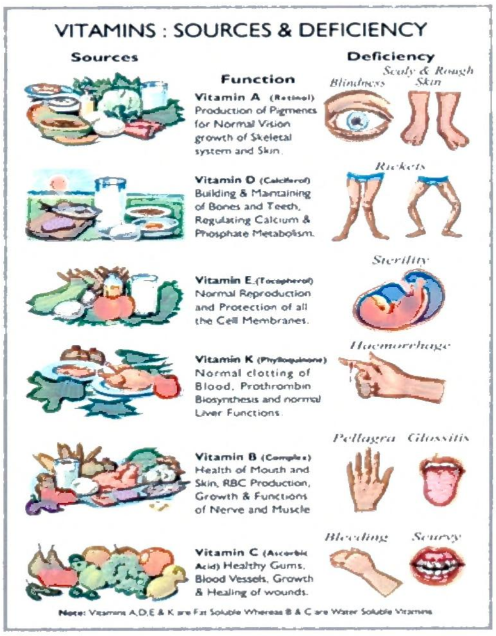
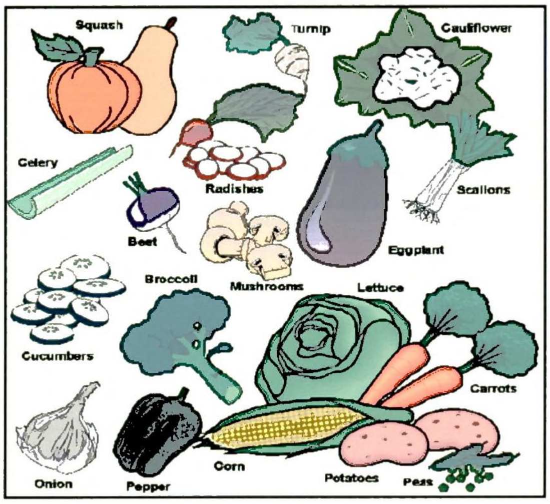
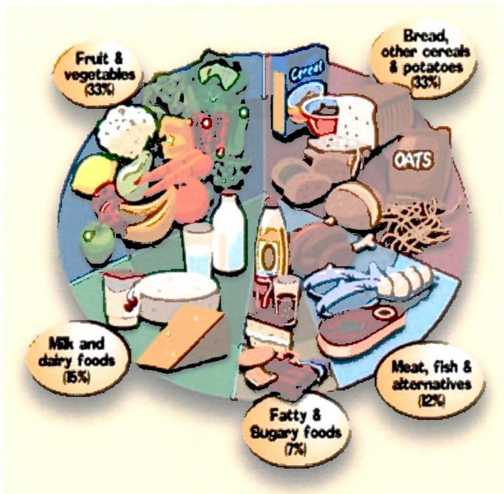


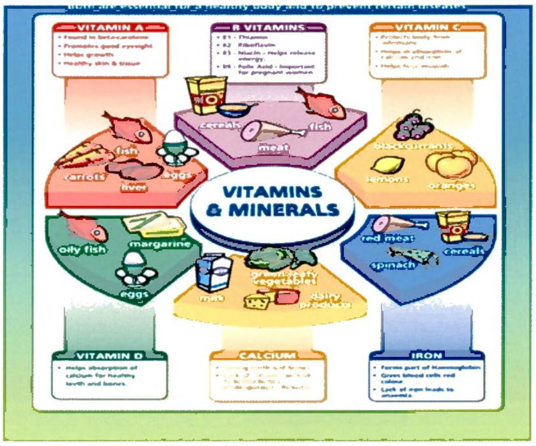
Plate 12
Posters developed for nutrition education











PHASE IV IMPACT OF INTERVENTIONS AMONG SCHOOL GOING CHILDREN

Data collected in the preliminary phases of the study revealed that there existed a considerable gap in the diets of the school going children in terms of micronutrient intake and the major reason behind this could be the lack of diversity in the diets of the subjects and inadequate knowledge of nutrition among the parents resulting in poor choices.

Despite its economic success, India has made little progress towards meeting its Millennium Development Goal targets of reducing undernourishment, particularly among children (Maitra *et al*, 2010). The right mix of interventions tailored to the local circumstances should be applied in achieving the objectives of deficiency control (Edem, 2009). Therefore, supplementation and nutrition education were chosen to be the most appropriate steps in order to achieve the objective of the present study.

It could be inferred from the dietary assessment data that the diet of school going children was deficient in all the essential micronutrients. The mean deficit in the diet were 12.5 mg iron, 4184 µg vitamin A and 79.5 µg folic acid. Hence, in order to correct the deficiencies, it was planned to provide four biscuits each weighing 20 g, with 10 g of jam spread between two biscuits. On the whole, 80 g biscuit and 20 g of jam was given to each child which provided 11.5 mg iron, 3201 µg β carotene, 0.5 mg folic acid and 1.29 mg iodine.

1. Selection of subjects for intervention

Schools are the centres of communities and school feeding programmes for the improvement of nutritional status of the children. Schools can therefore be a good place for promoting nutrition. School feeding programs based on healthy food choices could be a suitable method for improving the nutritional status among children (Rahmani *et al.*, 2011). In order to assess the impact of the intervention programmes, Sourashtra Matriculation Higher Secondary School was chosen from Paramakudi taluk of

Ramanathapuram district (Plate 13). The school was easy to access and provided a congenial atmosphere for carrying out the research. The study protocol was approved by the Institutional Ethical Committee Higher Education for Women (HEC.2010.17). Permission and cooperation of the school administrative authorities were obtained. The study design was explained to the parents of the children and their consent was obtained and rapport was established (Appendix IV). A total of 150 children studying in the sixth class in the age group of 10 to 12 years, both boys and girls were chosen for the study (Plate 14). In order to facilitate efficient conduct of the study and to avoid differences among the children, all the 50 children belonging to each section were divided into supplementation, nutrition education and control group respectively.

2. Conduct of supplementation

It was computed that about 200 biscuits were required for one day's supplementation. Around 35 biscuits could be prepared from one kg of biscuit flour. The monthly requirement was calculated to be 17 kg of flour which required 18 kg of cereals, pulses and nuts and 7 kg of Ponnanganni greens and lotus stem. All the dry ingredients were procured in bulk from the local market and stored. Fresh ponnanganni greens and lotus stem were obtained from local vendors, shade dried and powdered once in 15 days. The requirement for jam was similarly computed to be seven kg per week which required about two kg of each vegetable and two kg of sugar. The jam was prepared on weekly basis by procuring fresh ingredients from the local market. Since the biscuits had a good shelf life, they were prepared once in every month and stored airtight. Jam was prepared once every week and refrigerated.

The children belonging to the supplementation group were enlightened on the nutritional benefits of the biscuits. It was found that all the children were eager and enthusiastic to cooperate with the investigator throughout the study period. Prior to the commencement of feeding trials, all the children were treated for deworming by administering Albendazole tablets. Each child was supplemented with four biscuits weighing 80 g for five days a week, in the

Plate 13
Area selected for supplementation study

Plate 14
Subjects selected for the supplementation study

school premises during the fifteen minute interval period in the mid morning. Just before distribution, 20 g of the jam was spread between two biscuits and distributed to the children. The children were made to consume the biscuits immediately under the supervision of the investigator (Plates 15 and 16). Supplementation was carried out for a period of six months. The children were instructed that they must feel free to report any inconvenience or discomfort felt due to the consumption of the biscuits and they were allowed to quit from the study if they decided to do so at any point of time.

3. Nutrition Education

Well designed educational interventions and behaviour change communication focused on key messages of micronutrient malnutrition can go a long way in improving the nutritional status of the community. Only those programmes which included additional components such as nutrition education produced improved nutrition outcomes (Ruel, 2001). Since parents are seen as nutritional gatekeepers for their children's diets, their views and beliefs are of crucial importance.

Parental perceptions play an important role in targeting dietary advice and prioritising of public health issues for children (Brands *et al.*, 2012). Nutrition education was imparted in the school premises to the parents of the children along with their children belonging to the nutrition education group. Education was imparted for a period of six months in twelve sessions each with a duration of two hours (Plate 17). During the course of education, the participants were also taught to prepare and serve nutritionally adequate meals and snacks to school going children. Didactic sessions providing basic knowledge of the contribution of individual foods and food groups to nutrient intake were included. Emphasis was placed on improving consumption of underutilised indigenous foods and low-cost animal source foods and incorporation of these into traditional recipes. The last half hour of each session was allotted for interaction and all the doubts and queries of the parents with regard to the day's lessons were cleared and the lessons of the previous sessions were reviewed. The parents were intimated about the sessions through telephone calls and their attendance was noted. Additional

Plate 15
Conduct of supplementation among the subjects

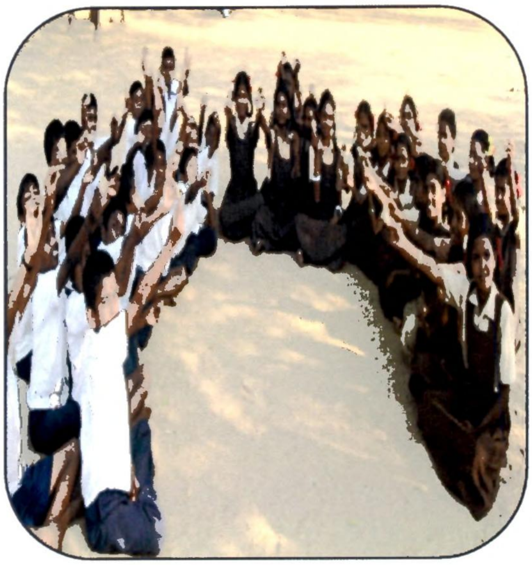



Plate 16
Children consuming the multi nutrient biscuits


sessions were conducted whenever necessary at the time of their convenience for those parents who could not attend the regular sessions. All the teaching aids developed for the purpose were used at appropriate times and take-home handouts were distributed which contained important tips and guidelines that have to be followed by them.

4. Impact evaluation of supplementation

The impact of the nutrition interventions was assessed in terms of anthropometry, clinical profile, biochemical assessment, cognitive test and performance parameters and nutritional KAP. The following parameters were assessed both at the beginning and at the end of the six months intervention period.

a. Anthropometry

Various anthropometric measurements such as height, weight, BMI, mid upper arm circumference and head circumference were measured for all the 150 children belonging to the supplementation, nutrition education and control groups both at the beginning and at the end of the intervention period.

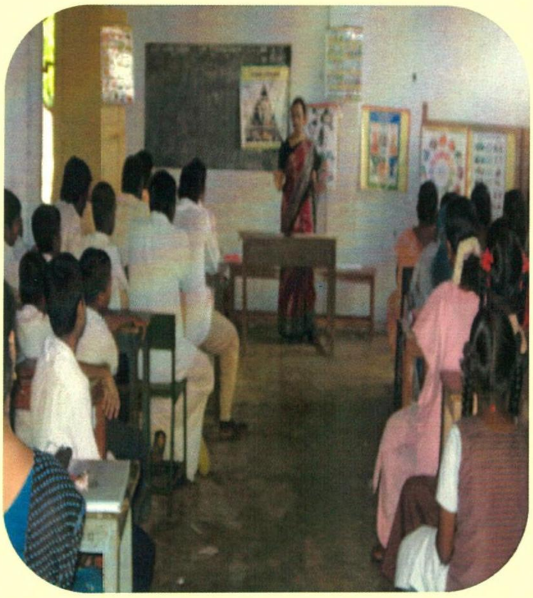
b. Clinical profile

All the children included in the intervention programme were examined for the presence of various clinical signs and symptoms of micronutrient deficiencies with the help of a medical physician. Clinical examination was carried out at the beginning and at the end of the intervention programmes in the school premises (Plate 18).

c. Biochemical assessment

Biochemical assessment was done for all the children before and after the intervention programmes. Five ml of blood was drawn from each child with the help of a laboratory technician. Various biochemical parameters namely haemoglobin, serum iron, total protein, vitamin A and zinc were estimated using standardised procedures (Raghuramulu *et al.*, 2003). Urinary iodine was also estimated by the procedures explained before.

Plate 17
Conduction of nutrition education for the subjects



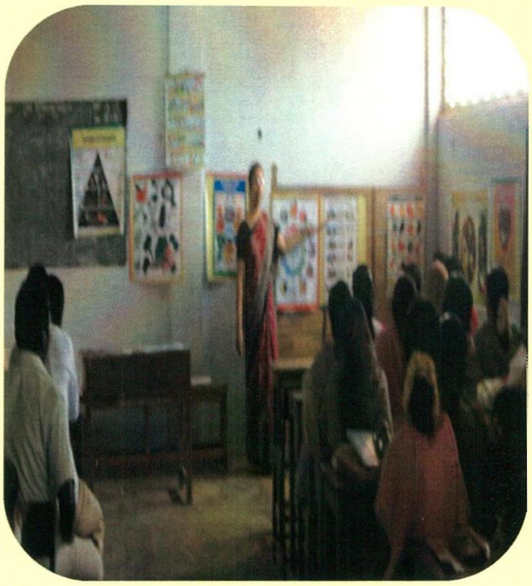
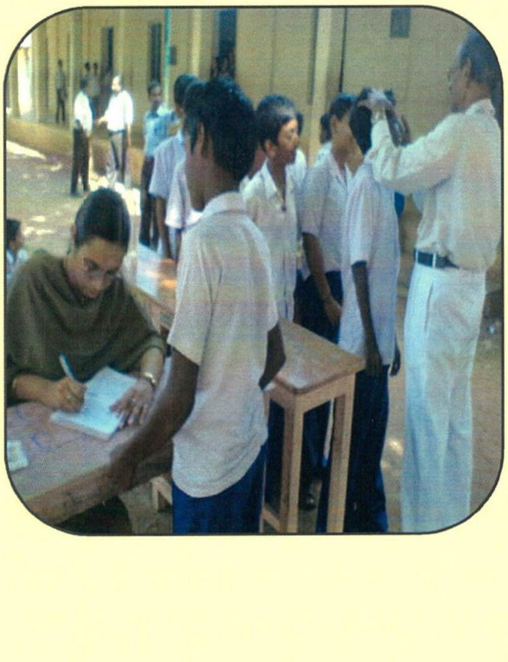


Plate 18
Clinical assessment of the children



d. Cognitive test

Micronutrient deficiency has direct effects on the cognitive abilities of children. The cognitive functioning of the children was assessed using the Malin's Intelligence Scale for Indian Children (MISC), an Indian adaptation of Wechsler's Intelligence Scale for Children. The MISC is an individual intelligence test for children from the age of 6 to 15 years. The whole test comprises of eleven subtests of which the following tests were selected for assessment in the present study. The test was administered to all the children participating in the intervention at baseline and at the end of the intervention and the performance were recorded in the record form (Plate 19) (Appendix VI).

i. Information test

In this test, a set of questions were read one by one to each child in the order given. At each correct response the next question was asked and each item was scored one or zero based on the correct or wrong response. The test was discontinued if the child gave five consecutive wrong answers.

ii. General comprehension test

This test was performed similar to information test with a specific set of questions asked one by one to the child and the test was discontinued after three consecutive failures.

iii. Arithmetic test

This test evaluates the numerical reasoning and concentration of the children. Various problems in the increasing order of difficulty were administered for each child. The children were asked to solve the problems in a limited time without involving paper work. Test was discontinued after three consecutive failures.

iv. Vocabulary test

Each child was given a set of test words for which they had to state what the word means. The test was discontinued after five consecutive failures and scores were given accordingly.

Plate 19
Cognitive performance test among the children

v. Digit span

This test measures the memory of the children. Each child was told a set of numbers in a series and the child had to repeat it in the same sequence. The difficulty increased as the test advanced. The test was discontinued if the child failed to repeat a sequence in two consecutive trials.

vi. Digit symbol (coding)

This is used to test the visual motor coordination speed and concentration of the children. The child had to mark specific symbols to specific numbers. The total time taken by the child to finish the test was noted.

e. Performance parameters

Physical fitness is a state or a condition that permits the individual to carry out daily activities without undue fatigue, and with sufficient reserve to enjoy active leisure pursuits (Malina and Katzmarzyk, 2006). Several micronutrients have been implicated in physical performance. At a biochemical level, this is explained on the basis of their participation in energy yielding pathways and indirectly in oxygen carriage through the synthesis of haemoglobin and as regulatory factors in erythropoeisis. Additionally, vitamins A, C,. and E have antioxidant properties and may reduce muscle damage associated with high muscle usage (Vaz *et al.*, 2011). The following tests were performed for the children in order to assess their physical performance parameters in terms of endurance (Squat thrust test), strength (Push up test), speed (Fifty yard dash test) and flexibility (standing toe-touch test).

i. Squat thrust test

The squat thrust test is used to assess the endurance level, power of the hip and thigh, stability of knees and ankles. The child was asked start from a standing position with eyes straight forward and arms folded or straight outward, and then to squat with knees bent, back flat, and eyes forward. From the squat position, the child was made to drop to a push-up position. The child was then asked to bring the legs back toward the chest and prepare to thrust upward from the squat position and finally return to the start position. The procedure was repeated till the child was exhausted. The child scored one point for a completed squat thrust. Each quarter movement was given ¼ point (Plate 20).

ii. Push up test

This test measures the upper body strength and endurance by counting the number of push-ups the child can do at a rhythmic pace. The child being tested was asked to assumed a prone position on the floor with hands placed under the shoulders, fingers stretched out, legs straight and slightly apart, and toes tucked under. The child was asked to push up off the floor with the arms until arms were straight, keeping legs and back straight. The child was instructed to keep the back in a straight line from head to toes throughout the test. The child was then asked to lower the body, using the arms, until the elbows bend at a 90-degree angle and the upper arms were parallel to the floor. This movement was repeated as many times as possible. Push-ups were performed until the child could no longer continue according to the prescribed procedure. The number of push-ups performed with proper form and rhythm by each child was recorded (Plate 21).

iii. Fifty yard dash test

The aim of this test is to determine acceleration and speed. The test involved running a single maximum sprint over 50 meters, with the time recorded. A thorough warm up was given and the child was asked to run a distance of 50 m and the time was accurately recorded (Plate 22).

iv. Standing toe-touch test

This is a commonly used indirect test of flexibility. The child was instructed to stand with the feet shoulder-width apart with the knees straight and to bend forward slowly until a firm resistance and slight discomfort in the back of the thighs were felt, thus defining the end point of motion. The child was then told to flex the head and neck in an effort to encourage full forward flexion of the trunk. After reaching the endpoint in the toe-touch position, the child returned to upright standing, thus completing one trial. The test was performed until the child was exhausted. The number of trials performed by each child was recorded.

Plate 20
Squat thrust test

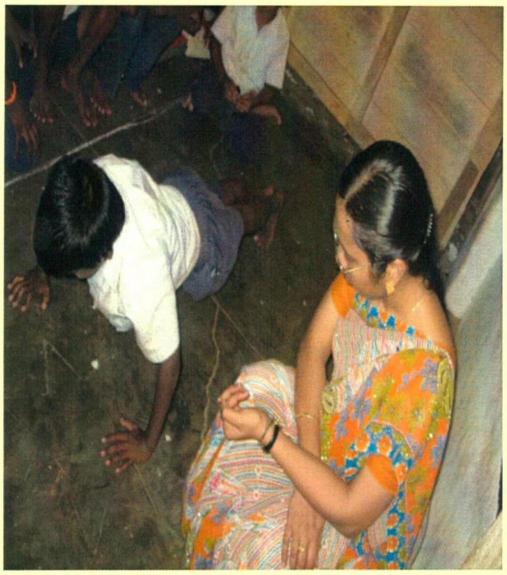


Plate 21
Push up test

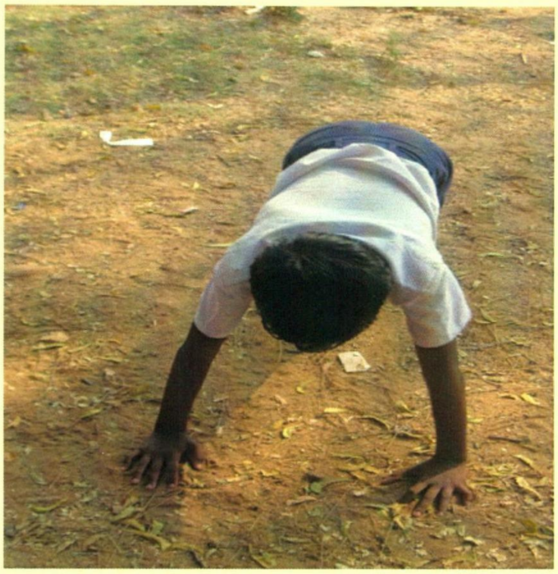


Plate 22
50 yard dash test

f. Nutritional KAP

The impact of nutrition education was assessed using a nutritional KAP questionnaire. The questionnaire comprised of a total of 30 questions with ten questions pertaining to each of the three categories of nutritional Knowledge, Attitude and Practice. The questions were framed in such a way that they were easy to understand and covered all the aspects of micronutrient deficiency that were included in the nutrition education schedule. Each question was given three choices and the subject had to choose the correct answer. One correct response was given one score. The questionnaire was administered to the parents of the children belonging to all the three groups both at the beginning and at the end of the intervention programme (Plate 23).

F. PHASE V STATISTICAL ANALYSIS AND INTERPRETATION OF DATA

Descriptive statistics (mean and standard deviation) was used to represent the basic distribution of various parameters. Chi-square correlation was used to determine the degree of linear relationship between two or more variables. Paired t- test was used to determine the significance of the impact of nutrition interventions using pre and post intervention scores. Correlation analysis was performed between biochemical and diet as well as anthropometric parameters.

Validation of data

Anthropometric measurements of height, crown heel length, head, chest and mid upper arm circumferences were measured by the investigator with the help of an assistant to record the data. The initial prevalence study was done over a one year period. Blood for biochemical assessment was drawn by a lab technician because the subjects were not willing to give blood if it was drawn by the investigator. The questionnaire developed was pretested for a subgroup and validated. Both cognitive and performance tests were done by the investigator herself there was hence no probability of bias in data collection. The supplementation was done for a period of six months and evaluation was carried out both at the beginning and at the end of six months.

The research design of the study is depicted in Figures 5, 6 and 7.

Plate 23
KAP test analysis among the subjects

PHASE I Prevalence of Micronutrient Deficiency Disorders among Different Age Groups of Population

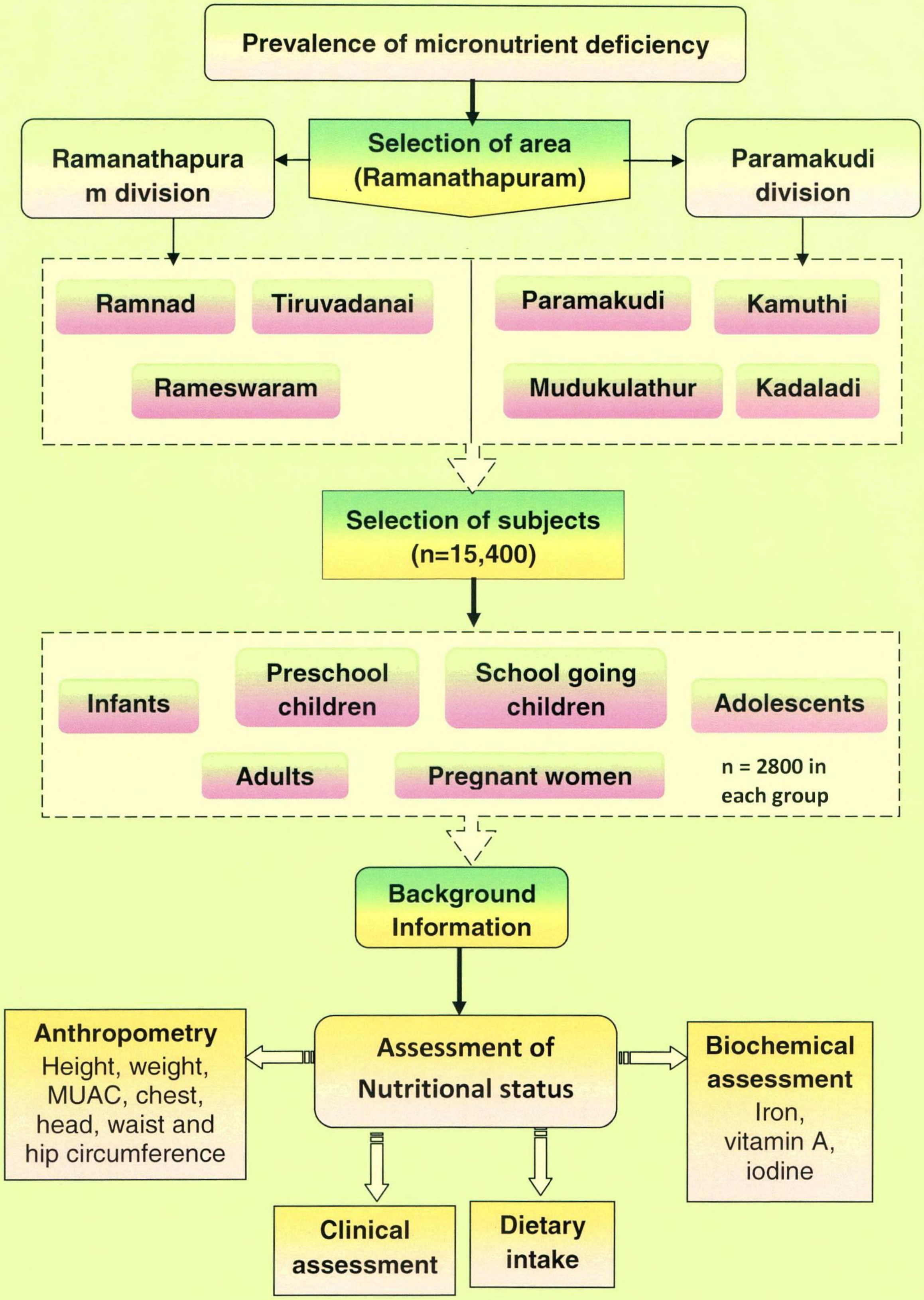
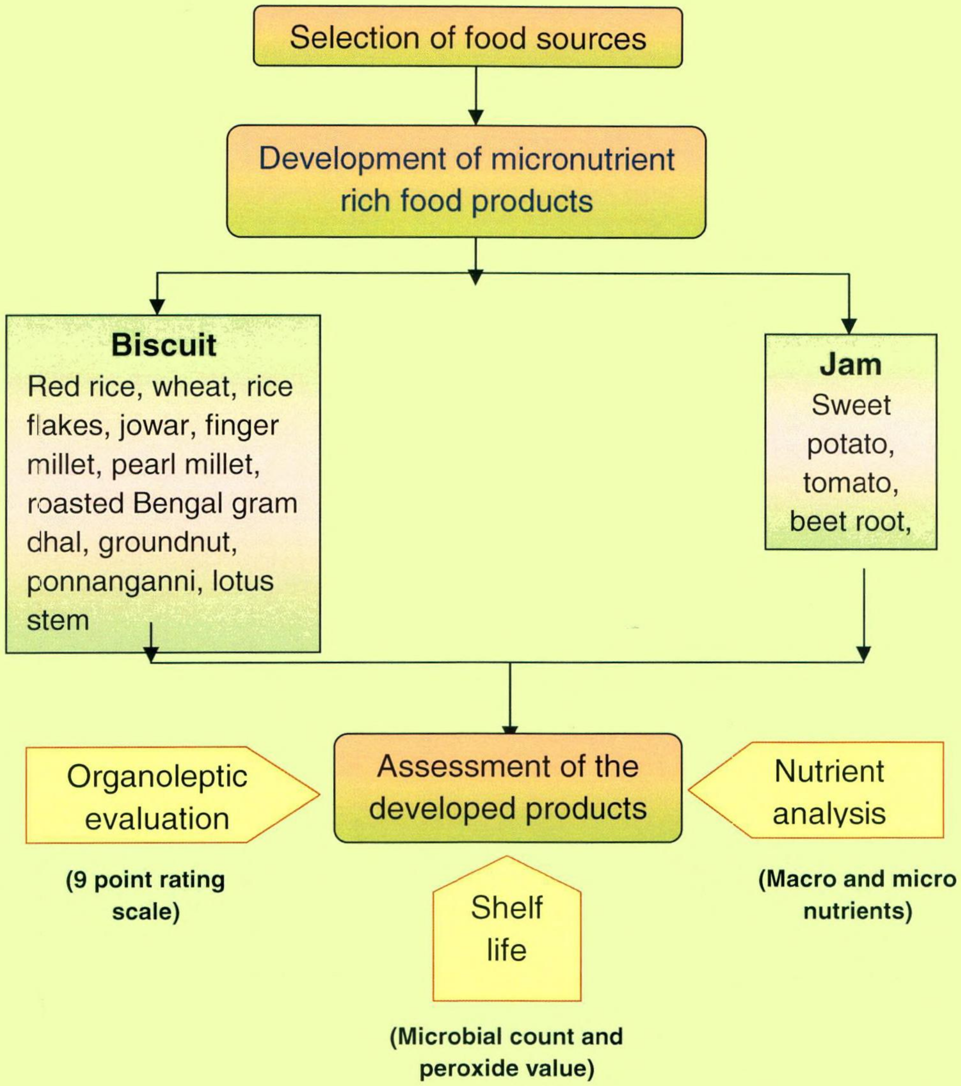


FIGURE 5
RESEARCH DESIGN (Phase I)

PHASE II Development of Micronutrient Rich Food Products



PHASE III Assessment of Knowledge, Attitude and Practice (KAP) and Development of Education Module

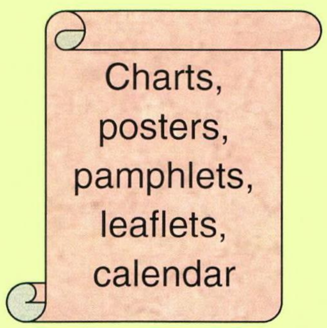


FIGURE 6
RESEARCH DESIGN (Phase II and III)

PHASE IV Impact of Interventions among School Going Children

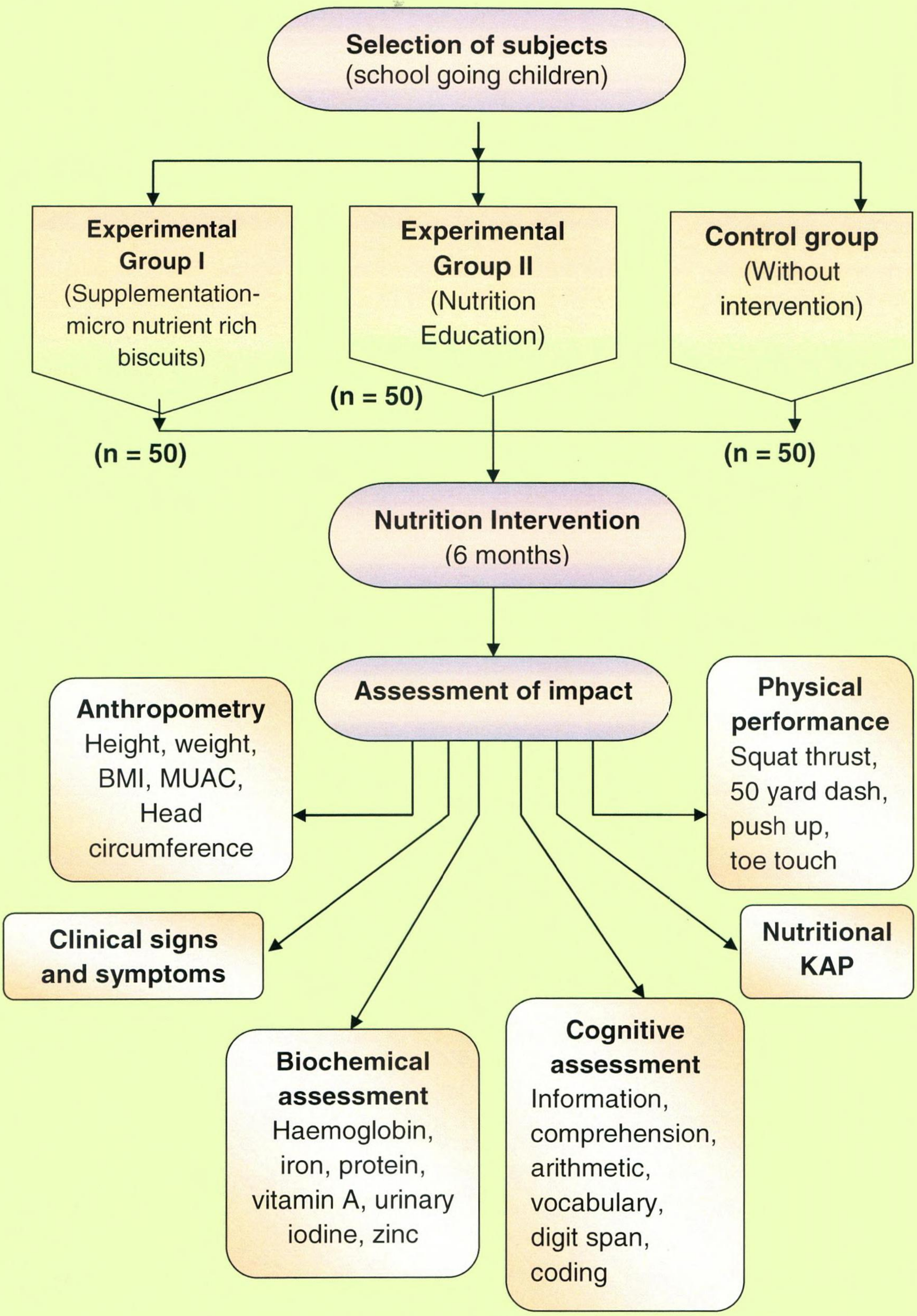


FIGURE 7
RESEARCH DESIGN (Phase IV)