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Methodology

The methodology adopted to conduct the present study entitled **"Bioavailability of Iron and Zinc from Regional Diets**" is presented under the following phases:

A. PHASE I : Consumption pattern of regional diets from households of selected districts of Tamil Nadu

B. PHASE II : Nutritional evaluation of the regional diets based on processing and cooking methods

C. PHASE III : Assessment of *in-vitro* bioaccessibility of iron and zinc from the regional diets using Atomic Absorption Spectroscopy

D. PHASE IV : Formulation and evaluation of ready to eat foods from millets incorporated with shade dried drumstick leaves

E. PHASE V : Assessment of i*n-vivo* bioavailability of ready to eat food and assessing the impact of interventions

F. PHASE VI : Statistical analysis and interpretation of the data

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A. PHASE I: CONSUMPTION PATTERN OF REGIONAL DIETS FROM HOUSEHOLDS OF SELECTED DISTRICTS OF TAMIL NADU

Ethics Approval

For conducting the study, initially the investigator presented the proposal before the Institutional Human Ethics Committee of the Avinashilingam Institute for Home Science and Higher Education For Women, Coimbatore. After obtaining ethical approval from the Institutional Human Ethics Committee of the Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, the present study was initiated. The approval number issued by the Institutional Human Ethics Committee for the present study was AUW/IHEC-13-

14/FHP-03. The study was also registered under the Clinical Trial Registry of India (CTRI), Indian Council of Medical Research and received the number 005253.

Study Design

The present study was a community based cross sectional survey, carried out in the households of four districts of Tamil Nadu, adopting multi stage random sampling procedure.

1. Locale of the study

The locale selected for conducting the present study was Tamil Nadu. The state is situated in the southernmost part of the Indian Peninsula, bordered by the Union Territory of Puducherry and the states of Kerala, Karnataka, and Andhra Pradesh. By area, Tamil Nadu ranks eleventh largest and by population, the seventh most populous state. As of 2012, in India, Tamil Nadu is the second largest state economy (http://www.tamilnadu.com). The state is one of the most urbanized states in the country with around 46 percent of the households living in the urban areas.

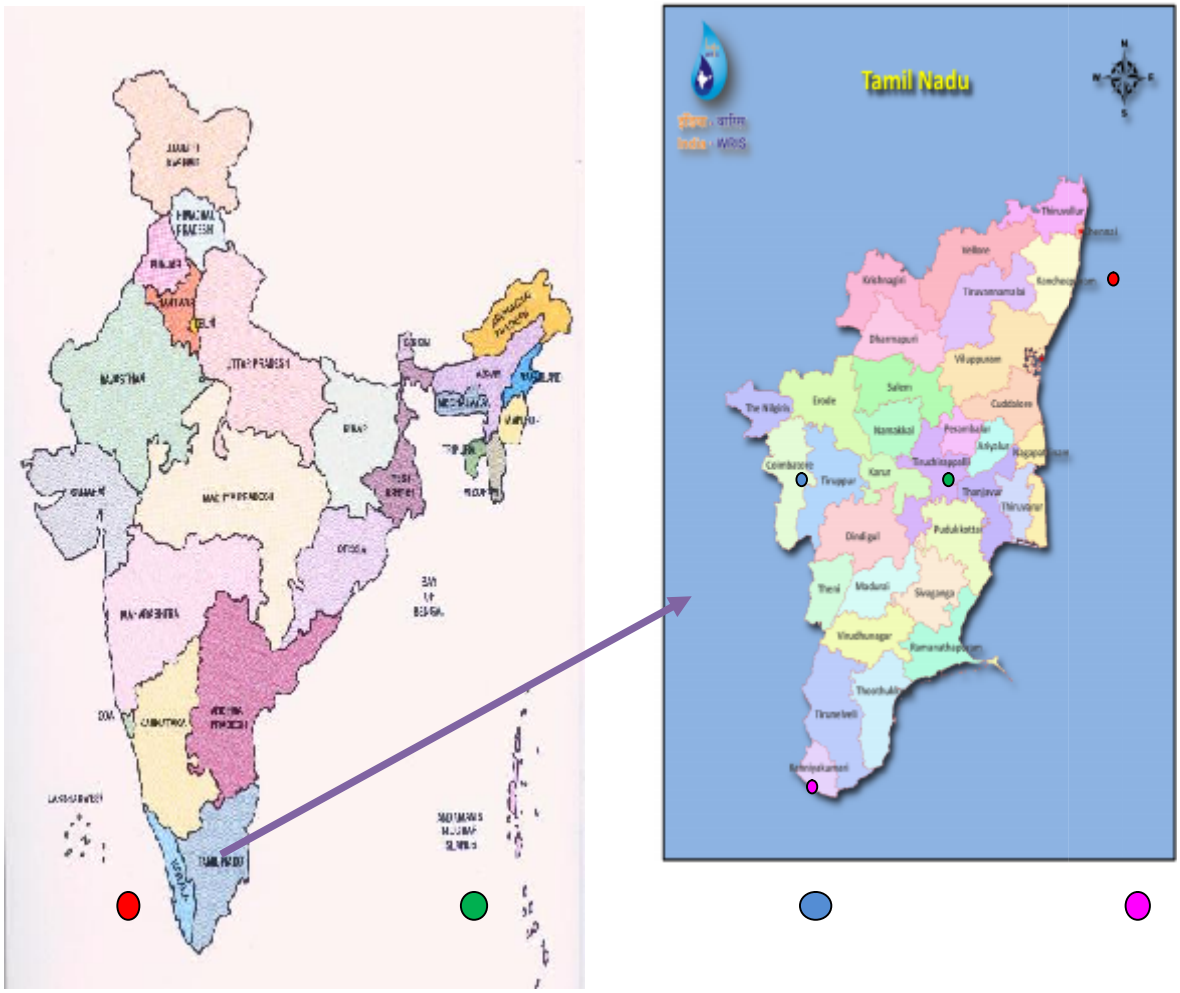
From the 32 districts of Tamil Nadu, four districts namely Chennai, Trichy, Coimbatore and Kanyakumari were selected from the four regions namely North, East, West and South. Data on regional diets and food consumption pattern of these districts were not available and hence they were considered for the present investigation. Plate I shows Locale and areas selected for the study

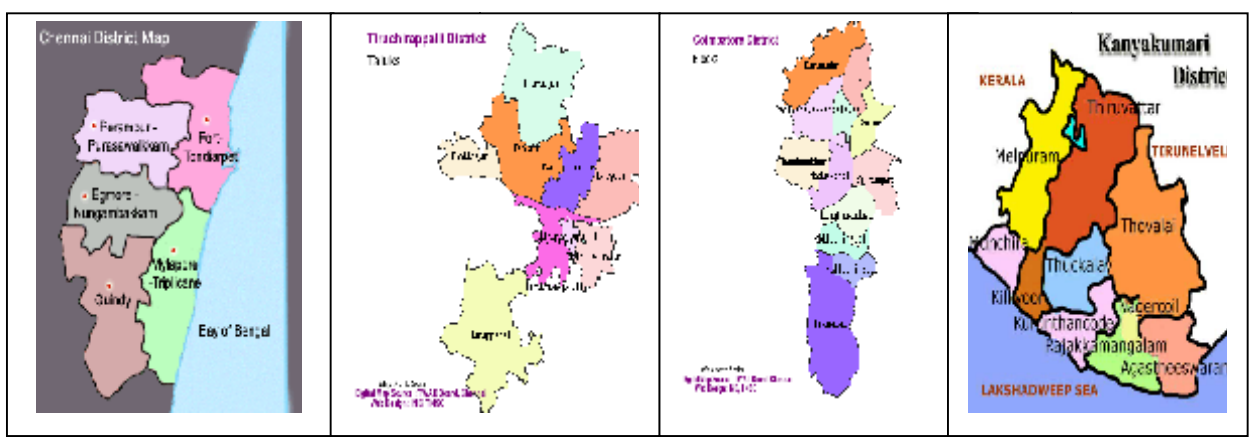
a. Selection of Taluks and villages

From the selected districts, two taluks consisting of around 100 to 110 households were selected on a random basis covering one urban area and one rural area of each district (Plate IV).

PLATE IV

LOCALE AND AREAS SELECTED FOR THE STUDY





Households were selected based on the procedure adopted by NNMB (2012). Initially 125 households were selected for the food consumption survey. Thus a total of 500 HHs from 4 districts were chosen for the study. But there were dropouts, incomplete and inappropriate responses, hence the variations in the number of households from the four different districts.

b. Selection of households

From the selected urban and rural areas, 50 to 55 households (HHs) were surveyed. From each village, the first household selected was from the North East corner. Thereafter, four adjoining households were selected and surveyed. The fifth adjoining house was left and from the next house counted as first was again surveyed. Thus, for the present study 427 households were surveyed to collect data on the socio-economic background of the households, frequency of food consumption pattern, regional diets prepared and consumed by the households during various occasions, cooking practices followed in the preparation of foods, vessels used for cooking and to collect recipes of various food preparations.

c. Selection of the respondents

For the present study, the food consumption patterns of the households were gathered from the homemaker cooking the foods for the household. The home maker was interviewed and the details regarding the food consumption pattern were collected. Initially, the investigator created a rapport with the homemakers and informed them the need for the study by showing them information sheet and written informed consent was obtained from each of them for carrying out the interview. Plate II shows the collection of data from the households.

d. Development of Questionnaire

A pretested and structured interview schedule was used to collect the details regarding personal, socio-economic characteristics such as age, type and size of family, caste and religion, sources and monthly income, details regarding family members and food expenditure pattern. Pre-testing of the questionnaire was done on 10 per cent of the households to ensure the validity and feasibility of the questionnaire before administering it on the households. Based on the pre testing, necessary modifications were made on the questionnaire. Pre-tested households were excluded from the study. Apppendix I shows the questioonaire adopted for the study.

The information regarding the food consumption pattern of the households, details of foods consumed and avoided during various minor ailments such as fever, cold, diarrhoea, vomiting; during different physiological status such weaning, childhood, school going, adolescence, pregnancy, lactation and old age; during special occasions like birthdays, wedding anniversary, weekends etc and at different festival occasions which was national as well as locally celebrated in and around their area. Foods consumed and avoided during fasting times were also gathered using a structured interview schedule. The consumption pattern of various food items were collected using a semi quantitative food frequency questionnaire Frequency of food intake was assessed from the semi quantitative questionnaire such as daily, weekly thrice, weekly twice, weekly once, fortnightly, monthly, occasionally and never. Codes were given like, 0-never, 1 -occasionally, 2 = monthly, 3- fortnightly, 4- weekly once, 5- weekly twice, 6- weekly thrice, 7- daily.

Based on the frequency of use of the various food items by the respondents, food use frequency scores were calculated as suggested by Reaburn*et al* (1979) as given below:

$$\text{Percentage of total score} = \frac{R1S1+R2S2\ldots\ldots\ldots\ldots\ldots RnSn}{n}$$

Where,

Sn = Scale of rating

Rn = Percentage of respondents selecting a rating

n = Maximum scale of rating

for example, if a respondent is rating 7 for cereal ,

$$\text{the percentage total score for cereals} = \frac{7 \times 100}{7} = 100$$

Here,

7 is the scale of rating as well as the maximum rating

100 is the number of respondents selecting that rating.

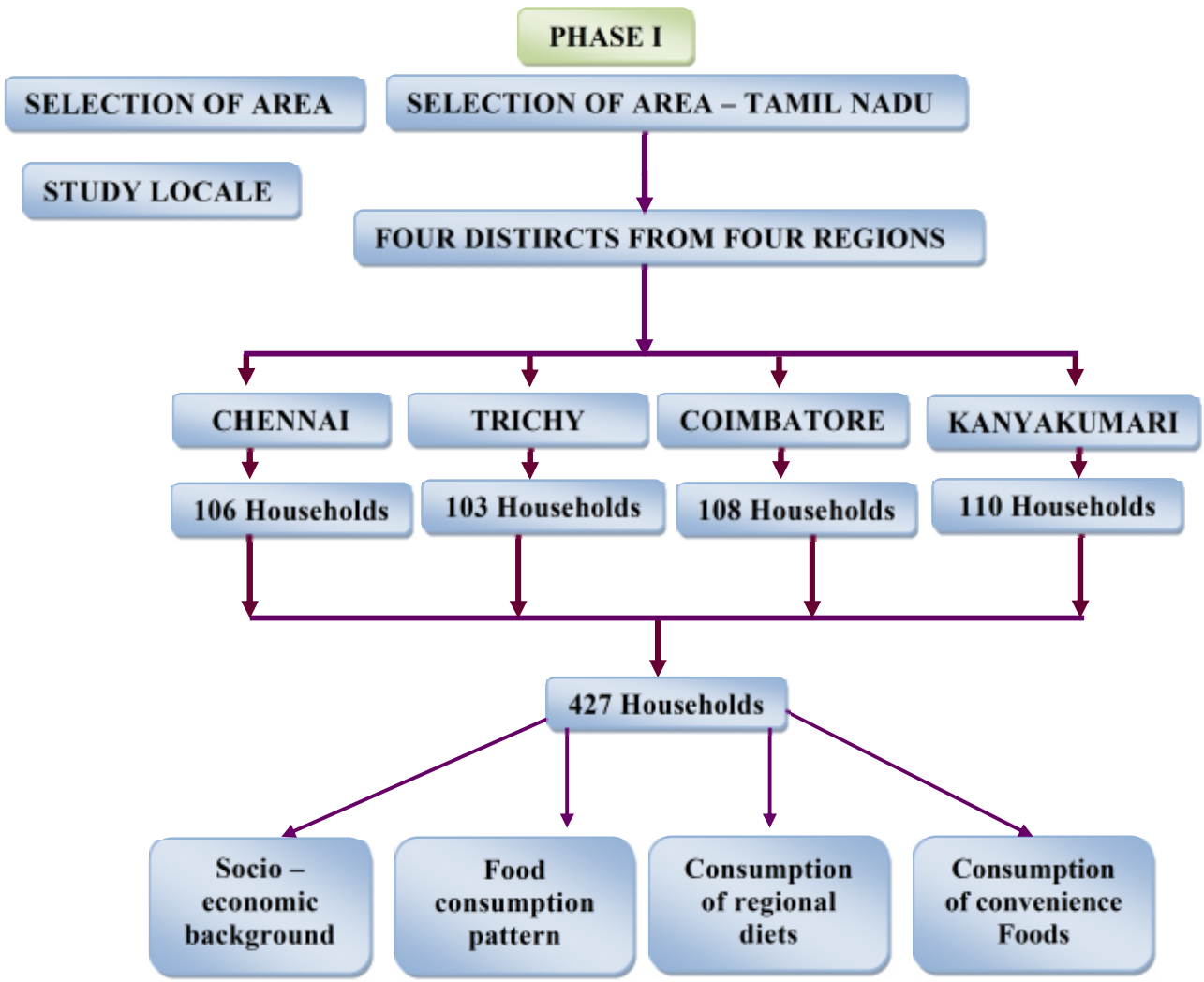
PLATE V

COLLECTION OF DATA FROM THE HOUSEHOLDS

In order to collect details regarding the regional diets of the households, 24 hour recall method was used. Using the 24 hour recall method, details regarding previous day's menu was collected from early morning tea to dinner/ bed time milk. Special attention was taken to avoid holidays as well as festival times. In order to arrive at a day's diet 24 hour recall method of three consecutive days were gathered from the house wives who were very cooperative. Figure I shows the research design of Phase I.

FIGURE I

RESEARCH DESIGN OF PHASE – I



B. PHASE II : NUTRITIONAL EVALUATION OF THE REGIONAL DIETS AND CONVENIENCE FOODS

From the 427 households surveyed among the four districts, the commonly consumed 40 regional diets were identified. It was classified as cereal preparations, pulses and legumes preparations, vegetable preparations, roots and tuber preparations, non-vegetarian preparations and milk and sweet preparations. These regional diets were subjected to nutritional evaluation for total iron and zinc content. The regional diets selected for nutritional evaluation is presented here based on food groups. The regional diets analyzed for iron and zinc content is individually classified as breakfast, lunch and dinner and vegetarian and non vegetarian diets

As per the AOAC (2005) procedure, the total iron and zinc content were analysed. Table I shows the lists of regional diets selected for the analysis.

TABLE I

REGIONAL DIETS SELECTED FOR ANALYSIS

Cereal preparations	**Pulses and legumes preparations**	**Vegetable preparations**	**Green leaves preparations**	**Root and tuber preparations**
Dosa	Masiyal	Sambhar	Chutney	Poriyal
Idli	Kottu	Poriyal	Masiyal	Kuzhambu
Appam	Poriyal	Kuzhambu	Kottu	
Wheat Dosa			Poriyal	
Rice puttu				
Upma	**Nonvegetarian preparations**	**Milk and Sweet preparations**	**Convenience food preparations**	
Daliya	Omlettee	Kesari	Porulvilanga Urundai	
Idiyappam	Egg curry	Payasam	Navadhaniya Urundai	
Chapathi	Fish Curry	Ladoo	Awalose Urundai	
Puri	Fish fry	Unniyappam		
Ven pongal	Chicken gravy	Pongal		
BajraDosa	Chicken fry	Halwa		
Ragi Dumblings				
Rice				
Lime rice				
Sesame rice				
Tomato rice				
Rice Kanji				
Fried Rice				
Curd Rice				

The commonly prepared regional diets for breakfast, lunch as well as dinner are shown in Table I. The cereals commonly consumed were raw rice, parboiled rice and wheat. Only a few households of Coimbatore had bajra and ragi in their diets. The vegetarian side dishes of Tamil Nadu are based on pulses and legumes, vegetables, green leaves and roots and tubers. From these food groups, the households used to prepare diets such as masiyal, kootu, poriyal, kuzhambu and sambhar. In this study, theses regional diets were analysed for total iron and zinc content. The non-vegetarian mostly consumed were chicken, fish and egg. Majority of households consumed non-vegetarian foods during weekends. Milk based and sweets were prepared during festivals, weekends and special occasions. The commonly prepared sweet dishes are payasam, pongal, kesari. These foods were also analysed for iron and zinc content. From, the Phase I survey, it was found that, convenience foods like ladoo or Urundai with multigrains were prepared by adding jaggery solution and kept for months. The most commonly prepared convenience foods were porulvilanga urundai, navadhaniya urundai and awalose Urundai. These ready to eat foods will be hard and will have a shelf life of 45 days or more (Anon).

These convenience foods were also analysed for iron and zinc content using AOAC (2005) procedures.

a. Standardization of regional diets and convenience foods

According to the United States Department of Agriculture (USDA), a standardized recipe is defined as a recipe which has been tried and retried several times by a person and it produces the same food each time when the procedures used were exact with the same type of equipment's and the quantity and quality of ingredients used were also the same". For preparing each recipe, a written set of description was followed. Each ingredient used for preparing a recipe was weighed using a weighing scale before and after preparation.

Standardization of a recipe is essential as it leads to the development of cost effective products, nutrients per serving can be known and gives customer satisfaction. The components of standardized recipes includes a recipe title, ingredients used in the recipe, amount of each ingredient used, method of

preparing the recipe, cooking temperature and time, serving size, recipe yield or volume and number of servings and serving equipment to be used in preparing and serving the recipe, nutrients per serving, purchase quantities for ingredients that have a preparation loss or gain before they are ready to use in a recipe.

In this study, for ascertaining the regional diets of a district, the 24-hr recall method for three consecutive days were used. Using the 24-hr recall method, details regarding the menu of each household were collected for three days. The data gave a clear picture of the regional diets of the four districts. Based on these information gathered, the regional diets were prepared and standardized and the convenience foods prepared in the household were developed.

In the laboratory, the investigator prepared each diet and standardized the recipes thrice following the procedures and ingredients collected from the house wives. Each time the prepared recipes were subjected to sensory evaluation by semi trained panel members for accurateness (http://fns. dpi.wi.gov/files/fns /pdf/ra_stand .pdf). Thus, the regional diets of each district was prepared and standardized by the investigator in the laboratory. Figure II shows the steps involved in the standardization of regional diets.

Regional diets prepared and standardized in the present study include breakfast, lunch and dinner menus. Apart from these, convenience food prepared during special occasions such as festivals, foods prepared and served during different physiological conditions, diseased conditions and special days. Plates IVa to IVd show the regional diets and convenience foods standardized.

After the standardization of the regional diets and convenience foods, selected foods were subjected to nutrient analysis for total iron and zinc content.

FIGURE II

STEPS INVOLVED IN THE STANDARDIZATION OF REGIONAL DIETS AND CONVENIENCE FOODS

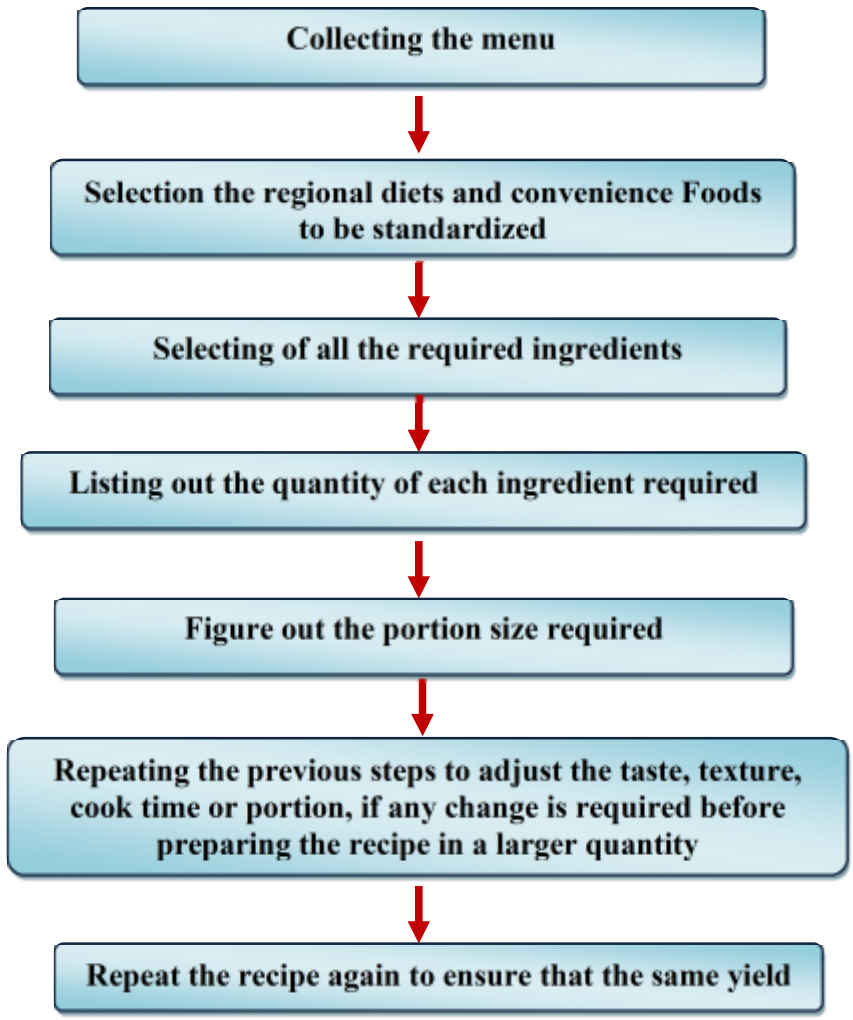


PLATE VI

ONE DAY'S DIET OF HOUSEHOLDS OF CHENNAI DISTRICT

Semiya Upma
with Chutney

Lemon Rice, Amaranth Dal
Poriyal and Pickle

Idly Tomato Chutney

PLATE VII

ONE DAY'S DIET OF HOUSEHOLDS OF TRICHY DISTRICT

Upma chutney

Tomato rice, brinjal kootu

Rice, cabbage poriyal, sambar

PLATE VIII

ONE DAY'S DIET OF HOUSEHOLDS OF COIMBATORE DISTRICT

Dosa chutney

Rice, bittergourd sambar, ridge gourd kootu

Ragi dosa, bittergourd sambar

PLATE IX

ONE DAY'S DIET OF HOUSEHOLDS OF KANYAKUMARI DISTRICT

Rice puttu, green gram, pappad

Rice, sambar, aviyal

Rice, pulissery, fish fry

C. PHASE III ASSESSMENT OF *IN-VITRO* BIOACCESSIBILITY OF IRON AND ZINC FROM THE REGIONAL DIETS USING ATOMIC ABSORPTION SPECTROPHOTOMETRY

In the present investigation, from the standardized regional diets and convenience foods, bioaccessibility of iron and zinc were assessed by *in-vitro* method of Luten *et al* (1996) and by atomic absorption spectrophotometer.

According to Jackson (1997), the fraction of nutrient present in the food that can be absorbed, stored and used by the body forms the bioaccessibility or bioavailability of the nutrient. The chemicals used in the experiments were of analytical grade. All the solutions were prepared in triple distilled water. The analysis was carried in triplicates and the results are presented as mean $\pm$ SD.

1. Assessment of bioaccessibility of iron and zinc from the regional diets and convenience foods by *in-vitro* method

The *in- vitro* bioaccessibility of iron and zinc from the regional diets of each district were assessed by the method developed by Luten *et al* (1996). This method involves simulated gastrointestinal digestion with appropriate modifications and has two major steps – the initial step is the gastric phase and the subsequent step is the intestinal phase.

In the initial step, that is the gastric phase, the food samples of regional diets as well as convenience foods were finely ground in a mixer grinder with known quantity of water to form a gruel consistency. In a conical flask, 10 g of the food in gruel consistency was taken and to this, triple distilled water was added to makeup to 80 g. To this, 3 ml pepsin solution was added and the pH of the digest was adjust to 2 by adding 6N HCl. The digest was made upto 100 g with triple distilled water. It was then subjected to simulated gastric digestion by incubating in a shaking water bath at 37 °C for 2 hrs. After 2 hrs, from an aliquot of gastric digest (20 g of the gastric digest), titratable acidity was measured and the pH was adjusted to 7.5 with 0.2 M sodium hydroxide in the presence of pancreatin-bile extract mixture. Titratable acidity can be defined as the quantity of 0.2 M sodium hydroxide required to attain a pH of 7.5.

In the intestinal digestion phase, in order to imitate the intestinal digestion, segments of dialysis tubing (Molecular mass cut off 10 kDa) was used. In each dialysis tubing, 25 ml aliquots of sodium bicarbonate solution which is equivalent to the sodium hydroxide (in moles) needed to neutralize the gastric digest (derived in measuring titratable acidity) was taken, tied with rubber band at both ends and the dialysis tubing was placed in the conical flask containing the gastric digest. It was then subjected to incubation at 37°C in shaking water bath for 30 min or longer until the pH of the digest reaches 5.0. Five ml of the pancreatin–bile extract mixture were added to this and incubation at 37°C in shaking water bath was continued for 2 hrs or longer until the pH of the digest reaches 7.0.

At the end of simulated gastro intestinal digestion, iron and zinc present in the dialyzate was analyzed by atomic absorption spectrometry. The dialyzable portion of the total minerals present in the sample (expressed as percent) represented the bio accessible minerals.

Bioaccessibility (%) was calculated as follows:

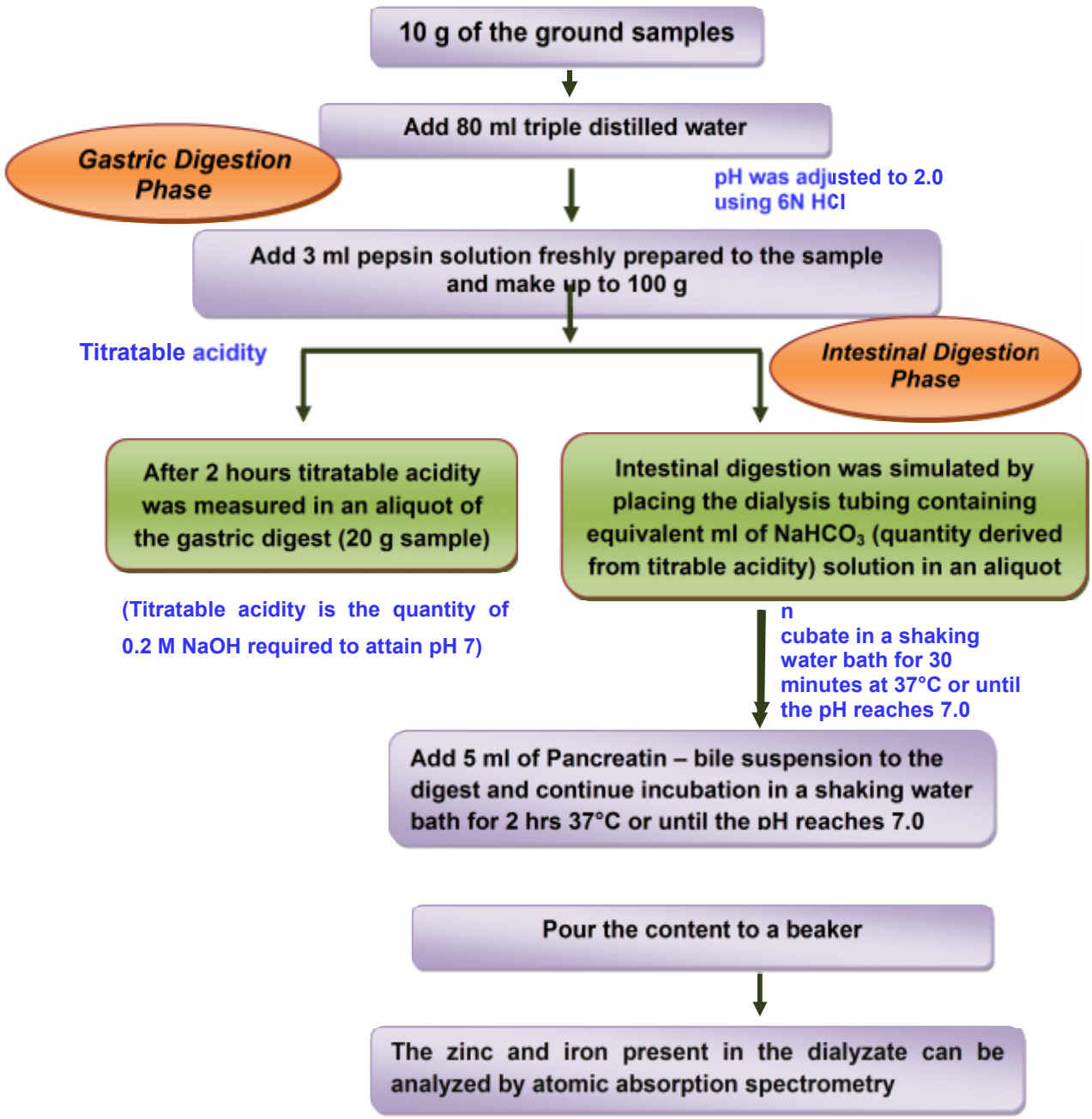
$$\text{Bioaccessibility (\%)} = 100 \times \frac{Y}{Z},$$

Where, Y is the element content of the bio accessible fraction (mg mineral element/100 g food sample), and Z is the total zinc or iron content (mg mineral element/100 g food sample).

Figure III shows the steps involved in the *in-vitro* bioaccessibility of iron and zinc.

FIGURE III

STEPS INVOLVED IN THE *IN-VITRO* BIOACCESSIBILITY OF IRON AND ZINC (Luten *et al*., 1996)



2. Assessment of bioaccessibility of iron and zinc from convenience foods by Atomic Absorption Spectrophotometer

The total iron and zinc content of the convenience foods and regional diets were assessed using Atomic Absorption Spectrophotometer.

Atomic Absorption Spectrophotometer (AAS) is a method for measuring concentration of chemical elements present in the samples by measuring the absorbed radiation of the chemical element. The principle of Atomic Absorption Spectrophotometer is that the element of a neutral or ground state atom can absorb electromagnetic radiation of defined wavelengths.

For analysing the iron and zinc content of the regional diets as well as convenience foods, triple acid digest was made up for each samples using triple distilled water. The acid solution was aspirated into a flame where it is converted into atomic vapor. Since, most of the atoms continue in the ground state, they are capable of absorbing radiation of a particular wavelength.

Atomic absorption method measures the amount of energy in the form of photons of light which is absorbed by the sample. This was done by analysing the spectra formed when the sample was excited by radiation. The atom absorbs ultraviolet or visible light and makes transitions into higher energy levels. The detector measures the wavelength of the ultraviolet or visible light transmitted by the sample and compares them with the wavelengths which actually passed through the sample. A signal processor which integrated the differences in wavelength absorbed, that appears in the readout as peaks of energy absorption at discrete wavelengths. An atom of an element emits a characteristic spectral line and every atom has its own pattern of wave lengths at which it will absorb energy, due to the distinct configuration of electrons in its outer shell. This enables the qualitative analysis of a sample. The concentration was calculated based on the Beer-Lambert law. Absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions. The concentration was determined from a calibration curve, obtained using standards of known concentration.

For the present investigation, 5 g of dried food sample was placed in a previously weighed porcelain crucible and heated. The resulting white ash was weighed, dissolved in 3ml of concentrated nitric acid and diluted with triple distilled water in a 25ml calibrated flask. The solution was then used to determine iron and zinc. Standard stock solution of iron and zinc was prepared from AAS grade chemicals (Sigma, USA). Plate-6 shows the bioaccessibility of iron and zinc using AAS.

D. PHASE IV FORMULATION AND EVALUATION OF READY TO EAT FOODS FROM MILLETS INCORPORATED WITH SHADE DRIED DRUMSTICK LEAVES

To combat the problem of anaemia, the simplest and the most effective method is food to food fortification. Incorporation of iron rich foods with suitable processing methods would enhance the iron content of a product when all these ingredients are used together in a recipe. According to Kowsalya and Shimpray (2008), iron supplementation resulted in positive impact on the haemoglobin levels of adolescent girls.

Even though cereals are good sources of iron, other iron rich foods were required to enhance the iron bioavailability. Development of a nutritious and organoleptically acceptable product from locally available food is a strategy to prevent micronutrient malnutrition. Products developed from locally available ingredients cost-effective, sustainable, culturally acceptable and feasible to implement. Indian diets provide mostly non-anaemia iron, which is very poorly absorbed (only 2 to 20 per cent bioavailability). Thus it has been suggested that vegetarians may be at a greater risk of iron deficiency than non-vegetarians.

Millet is a generic term used for the grains from the heterogeneous group of forage grasses. They are small sized grains and are grouped along with maize and sorghum as 'coarse cereals' perhaps because of their typical grain texture, which makes them difficult to process as well as cook in convenience form similar to rice and wheat (Malleshi and Desikachar,1985).

1. Selection and processing of ingredients

The formulation adopted for the development of iron rich food was based on the ICMR recommendations of the basic five foods groups. From the cereals and millets group, Bajra or *Pearl millet* (*Pennisetumglaucum*), Ragi or finger millet (*Eleusinecoracana*), Jowar or Sorghum (*Sorghum vulgare*), Maize (*Zea mays L*), from the pulses group, green gram (*Phaseolusaureus*) and roasted Bengal gram (*Cicerarietinum*), from leafy vegetable group drumstick leaves (*Moringa oleifera*), and from nuts group ground nuts (*Arachishypogaea*) were selected.

All these ingredients were purchased from the market, cleaned thoroughly and were further subjected to suitable processing methods for the development of bioavailable ready to eat iron food supplement.

a. Cereals and millets group

The Bajra or *Pearl millet* (*Pennisetumglaucum*), Ragi or finger millet (*Eleusinecoracana*), Jowar or Sorghum (*Sorghum vulgare*) and Maize (*Zea mays L*) were selected as the major ingredient. These millets were procured from local market and were subjected to visual inspection and then removed stones, dirt, present in it. It was washed in water for four times and later each of these millets was malted as per the standard procedures of Malleshi and Deschikachar, 1985. The malted millets were dry roasted separately for a period of 10 to 15 minutes in a medium flame. When the millets get dried completely, it was removed from the fire. On cooling the four millets were powdered separately in a blender and kept it in air tight containers with labels.

b. Pulse group

From the pulses group, green gram (*Phaseolusaureus*) and roasted Bengal gram (*Cicerarietinum*) was chosen for the development of bioavailable iron food. These pulses were brought from the local market and were subjected to visual inspection and then removed stones, dirt present in it. Green gram washed with water and was dry roasted to a period of 10 to15 minutes in a medium flame. When the green colour of the gram changed to olive brown

colour, it was removed from the fire. On cooling green gram and roasted Bengal gram were powdered separately in a blender and kept it in air tight containers with labels.

c. Nuts group

From nuts group, ground nut (*Arachishypogaea*) was selected for the formulation of bioavailable ready to eat iron food supplement. The nuts were purchased from the local market and were subjected to visual inspection and then removed stones, dirt present in it. It was dry roasted for a period of 10 to15 minutes in a medium flame. When a nutty aroma came, it was removed from the fire. On cooling ground nut was powdered separately in a blender and kept it in air tight containers with labels.

d. Leafy vegetable group

Drumstick leaves (*Moringa oleifera*) was chosen from leafy vegetable group. The reason behind the selection of drumstick leaves for incorporating in the food supplement was that throughout the survey carried out in the Phase I, drumstick trees were seen in most of the houses of the four districts. House wives reported that they are not consuming drumstick leaves frequently, in spite of its availability at all times. Hence, the investigator has chosen the leaves of drumstick for the bioavailable ready to eat food supplement. Dehydration is one of the traditional and most popular household processing method, which doesn't requires any sophisticated instruments or preparations. Moreover, the mineral content are not lost during dehydration, thus dehydrated drumstick leaves were utilized to enrich a ready to eat convenience food, which is adapted from the households.

Investigator purchased drumstick leaves from the market, visually examined and removed the infected and damaged leaves and washed it thoroughly under running tap. It was then allowed to drain the excess water present in it. The drumstick leaves were shade and then incorporated in the formulated food mixes. Shade drying was adapted to dry drumstick leaves as sun drying may cause loss of β carotene. β carotene plays an essential role in the

bioavailability of iron. Joshi and Mehta (2010) also reported that 100 g of shade dried drumstick leaves powder contains 24 mg iron. So, for the present study, shade drying was adopted for drying drumstick leaves.

In order to shade dry the drumstick leaves, a well-ventilated room having a temperature of 25 ± 2°C was selected. The cleaned, washed and water drained drumstick leaves were spread on a tray covered with cotton sheets and the tray was kept on a stand in the room for drying. Net was used to cover the leaves so that dust particles do not get adhered to the leaves. It took about three to four days for the leaves to dry completely and become crisp and brittle to touch. When the leaves became brittle to touch, it was ground in a blender then stored in air tight containers with label.

2. Formulation of the food mixes

For the formulation of the food, four standard mixes were developed from millets namely Bajra or p*earl millet*, Ragi or finger millet, Jowar or Sorghum, and Maize as the base. The other ingredients were green gram, roasted Bengal gram and ground nuts. The malted millet flour was 65 g, green gram flour was 20 g, roasted Bengal gram flour was 10 g and ground nut powder was 5 g. These formulated food mixes were then subjected to *in – vitro* bioaccessibility of iron as per the procedure discussed in Phase - III. It was found that, the *in – vitro* bioaccessibility of iron from p*earl millet* based mix was high when compared to the other three millets. Since one of the objectives of the study was to enhance *in-vivo* bioavailability of iron, p*earl millet* based mix was chosen for supplementation Plate X shows the processing of the ingredients.

PLATE X

PROCESSING OF THE INGREDIENTS

3. Nutrient analysis of the food mixes

The formulated food mixes were analyzed for nutrients and the bioavailable iron food was analyzed by *in –vitro* method for bioaccessibility of iron and zinc. The nutrients analyzed were, for proximate composition

a. Moisture :

Estimation of moisture is one of the most often performed determinations in food analysis. Moisture is lost when food is heated not much higher that the temperature of boiling water or by allowing to stand overnight over dehydrating agent or by heating over vaccum.

b. Ash :

By continuous heating, the substance gets charred which can be used for the determination of minerals presents.

c. Fat :

Ether extraction of the crude fat in vegetable products is carried out in a continuous extractor that is an apparatus in which the ether, after dissolving a portion of the fat of the materials and discharging into the extraction flask, is volatized, condensed and again allowed to act on the material. The steps in the process are repeated continuously and automatically until the extraction is complete.

The soxhelt extraction used depends on the intermittent action of a glass syphon. The ether gradually condenses into the extraction tube containing the material until it rises to top when it is discharged into the extraction flask.

d. Fibre

The term “crude fibre” ordinarily meant in agriculture and food analysis is the organic residue consisting largely of cellulose, that is left after other carbohydrates and proteins have been removed by successive treatment with boiling acids and alkalis. The crude fibre obtained in this way is not cellulose but contains distinct properties of hemicelluloses, and nitrogenous substances. These however are not sufficient to prevent the results from being reasonably accurate and comparable.

e. Protein

The given sample is digested with concentrated sulphuric acid in a macrokjeldahl flask when nitrogen gets converted to ammonium sulphate. Ammonia is liberated by the action of strong alkali in a macrokjeldahl steam distillation apparatus. This nitrogenous substance is converted to ammonium borate by absorbing 2% boric acid is titrated against N 70 sulphuric acid. The volume of acid required to bring the test sample to the colour of the blank of the blank gives the equivalent to the ammonia.

f. Carbohydrate

Carbohydrates are hydrolyzed into simple sugar using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This compound forms with anthrone, a green colour with an absorption maximum at 630nm

g. β carotene

Carotene present is extracted with petroleum ether and the intensity of the color of the colour of the extract is compared with that of the standard solution using colorimeter.

h. Vitamin C

Vitamin c is a good reducing agent and it reduces the dye 2,6dichlorophenol indophenol. In this reaction the ascorbic acid itself is oxidized to dehydro ascorbic acid. In the absence of interfering substances, the capacity of the extract of the sample to reduce a standard solution of a dye as determined by titration is directly proportional to the vitamin C content .oxalic acid is not only used to reduce the pH of the extracting medium, there by establishing the vitamin C but also form complexes with metals eg. Copper thereby preventing the catalytic oxidation of vitamin.

i. Iron

The food sample is oxidized with ignition or oxidation .Iron as ferric iron reacts with ammonium thiocyanate or with potassium thiocyanate to give ferric thiocyanate which is red in color .The color which is a measure of the concentration is measured colorimetrically.

j. Calcium

Calcium is determined by the precipitating it as calcium oxalate and titrating the oxalate solution in dilute sulphuric acid against standard potassium permanganate.

k. Phosphorus

When the ash solution is treated with ammonium molybdate, phosphomolybdic acid is formed. Phosphomolybdic acid is reduced by the addition of 1, 2,4 amino naphtholsulphonic acid regent to produce a blue colour which is apparently a mixture of oxides of molybdenum. The intensity of the colour developed is the measure of phosphorous present.

l. Energy

The energy content of the food were calculated from carbohydrate, protein and fats.

The procedures used for the estimation of ntrients are givne in Appendix II.

4. Formulation of bioavailable ready to eat iron rich food

For the formulation of bioavailable ready to eat iron rich food supplement, the formulated pearl millet based mix was kept as standard. Three variations of the formulated pearl millet based mix were formulated by incorporating three variations of shade dried drumstick leaves from five per cent to 15 per cent in the standard mix. The standard mix was without drumstick leaves, variation 1 was with five per cent dehydrated drumstick leaves, variation 2 with 10 per cent dehydrated drumstick leaves and variation 3 with 15 per cent dehydrated drumstick leaves. Convenience foods were developed from the four mixes and were then subjected to sensory evaluation. Plate XI shows the four variations of Bajra based ready to eat food and Table II shows the formulation of the food mixes.

TABLE II

FORMULATION OF THE FOOD MIXES

Variations (g)	Bajra (g)	Green Gram (g)	Roasted Bengal Gram (g)	Ground Nut (g)	Drumstick Leaves (g)	Total (g)
Standard	65	20	10	5	0	100
Variation 1	60	20	10	5	5	100
Variation 2	55	20	10	5	10	100
Variation 3	50	20	10	5	15	100

5. Analysis of anti-nutritional factors

Studies have showed that anti-nutritional factors such as phytates, oxalates and dietary fibres may hinder the bioavailability of iron and zinc. For the study, from the fours mixes developed these anti-nutritional factors such as phytates, oxalates and dietary fibres were analyzed.

a. Oxalates

Analyzed by extracting with HCl and then precipitated as calcium oxalates from the deproteinized extracts and was then estimated by titration with potassium permanganate (Baker, 1952).

b. Phytates

Determined by extracting as phytic acid and analyzed by procedure described by Odunayo and Singh (2007).

6. Sensory evaluation of the food

Sensory evaluation of a food depends on the characters such as appearance, flavour, taste, texture, doneness and overall acceptability. Sensory evaluation gives an index of the overall acceptability of the food (Nambiar and Parnami, 2008).

For supplementing the anaemic adolescent girls, ladoos were prepared from the four mixes by adding jaggery solution. The jaggery solution was made

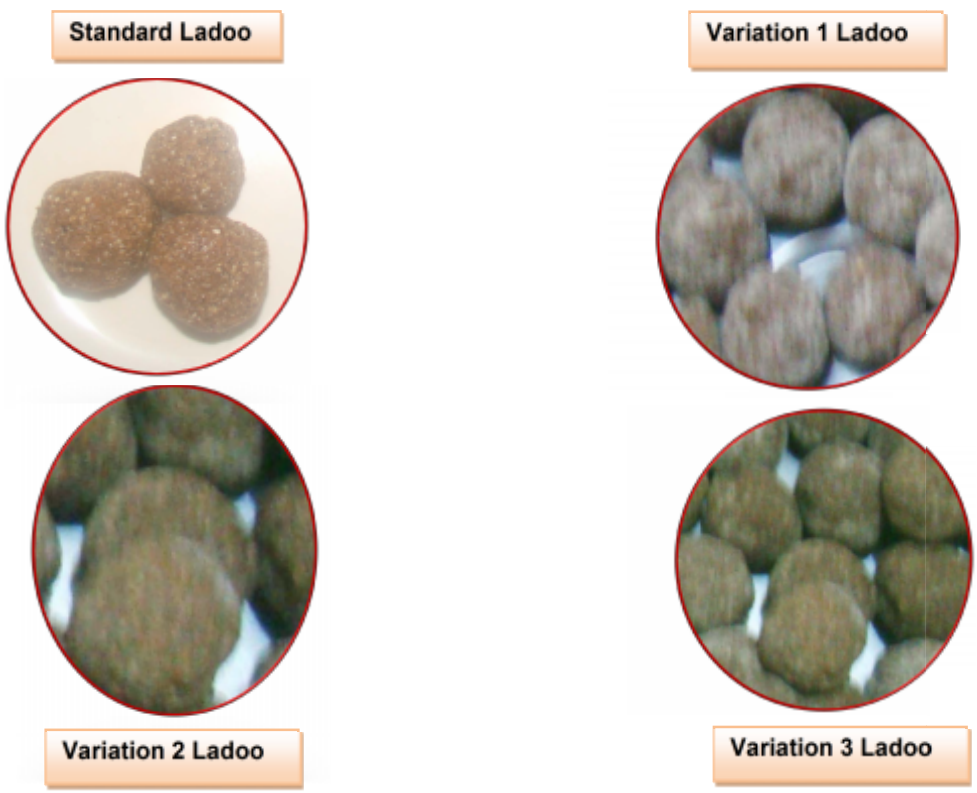
by boiling jaggery in water to form a thick consistency. For preparing 100 g of jaggery solution, 100 g of jaggery was dissolved in 25 ml of water.

To each 100 g of the mix, 25 g of jaggery solution was added to prepare the ladoos. In this way, ladoos of four variations were made and was subjected to sensory evaluation by semi trained panel members,

The semi trained panel members carried out the sensory evaluations of the prepared convenience foods on a 9 point hedonic scale from liked extremely (9) to disliked extremely (1) (Larmond, 1977). For carrying out the sensory evaluations of the developed convenience foods, characters such as taste, texture, flavour, doneness and over all acceptability of the developed food was evaluated. The food receiving highest overall acceptability score was chosen for supplementation. (Appendix - III shows the schedule for sensory evaluation). Plate XI shows the variations of the ready to eat foods (Ladoos)

PLATE XI

VARIATIONS OF THE READY TO EAT FOODS



7. Shelf life evaluation of the food

The shelf life of the developed food mixes was analyzed by storing the mixes in airtight containers and at ambient temperature for a period of 0 days, 30 days, and 90 days respectively. The pour plate method of knowing and counting the number of viable bacteria present in the sample was adopted as described by Jideani and Jideani (2006). For the shelf life evaluation of the formulated food mixes, the parameters studied were moisture content, peroxide value, total bacterial count and total fungal count.

The peroxide value of the foods were analysed as per the procedure given by AOAC (2005). Two grams of the samples were weighed for analysis and evaluated. Quarter strength of peptone water solution was prepared by dissolving 3 g in 200 ml of distilled water. Nutrient Agar (NA) was prepared by dissolving 4.6 g in 200ml of distilled water. Serial dilution was carried out on the food samples and plating of the samples was performed. The ingredients were dissolved in distilled water and autoclaved. The content were cooled to 45 to 50° C and 20 ml of hot molten medium was poured into the sterilized petri plates and was then allowed to solidify. Plate count method for microbial number: 1 g of aseptically handled sample was added to 10 ml sterile distilled water and mixed well. 0.1 ml of this diluted sample was transferred to the center of a solidified agar plate and then spread uniformly over the surface of the medium with a sterile bent rod (spreader). The plates were incubated at 37°C for 24 to 48 hours following which the colonies of bacteria were counted.

$$\text{cfu/ml} = \frac{\text{No. of colonies formed x dilution factor}}{\text{Volume sampled}}$$

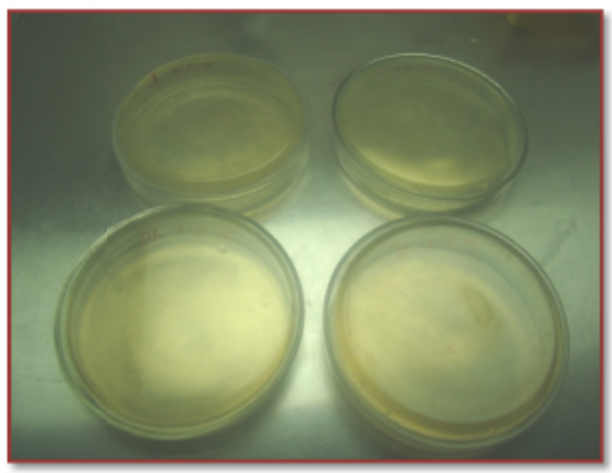
Potato Dextrose Agar (PDA) was used as medium for fungal population. Samples were diluted in ringer solution and plated in duplicate using the surface plating method. The plates were incubated at optimum temperature of 37°C for bacteira and at 30°C for fungi for 48 hour. Later the counting of the colonies were done and mean readings were recorded. Plate XII shows the evaluations of the ready to eat foods.

PLATE XII

EVALUATIONS OF THE READY TO EAT FOODS

Sensory evaluation ready to eat foods

Nutritional evaluation of ready



Shelf life evaluation of ready to eat foods

PLATE XIII

FORMULATED READY TO EAT FOODS

Prepared ready to eat food

Packed ready to eat food

8. Calculation of the cost of the developed food

The total cost of the developed food was calculated by calculating the market cost of all the ingredients used in the formulation of the food mixes. Later, the cost of the developed food was compared with the other similar products available in the market.

E. PHASE - V: ASSESSMENT OF *IN-VIVO* BIOAVAILABILITY OF READY TO EAT IRON FOOD SUPPLEMENT AND IMPARTING NUTRITION EDUCATION TO ADOLESCENT GIRLS AND ASSESSING THE IMPACT OF INTERVENTIONS

Anaemia continues to be a major public health problem worldwide (Raghuram *et al*., 2012) due to its widespread association of micronutrient deficiencies such as iron, vitamin C, folic acid, vitamin A, niacin, panthothenic acid and vitamin B_{12}. These micronutrients are essential for maintaining the Haemoglobin level of the individuals (Kaur, 2014). Among adolescents, girls form a vulnerable group and the prevalence of anaemia is excessively due to poverty, inadequate diet, frequent worm infestations, lack of proper knowledge of nutrition and poor access of health services and excessive bleeding during menstruation (Joshi and Gumashta, 2013).

The World Health Organization estimates that worldwide, 2 billion people are anemic and twice as many are iron deficient (Kolb and Beard, 2007) With regard to zinc deficiency, an estimated 1.3 billion worldwide are at risk due to inadequate zinc intake (Hotz and Brown, 2004) . Deficiencies of iron and zinc often coexist because iron and zinc are most bio available from many of the same foods and their absorption is inhibited by many of the same dietary substances (Yokoi *et al*., 2007).

A study was undertaken by Chellappa and Karunanidhi (2012) to examine the relative effects of iron, zinc and combined iron and zinc supplementation on certain cognitive functions and behavioral outcomes of female adolescents of Chennai reported that the benefits of combined iron and zinc supplementation as a logical strategy was felt since deficiencies of iron and zinc may coexist in vulnerable populations. Hence for the present study also apart from haematological parameters, serum zinc levels of the adolescent girls were also assessed.

For the present investigation, in order to assess the *in- vivo* bioavailability of iron and zinc, adolescent girls in the age group of 16-18 years were selected. Prior permission was obtained from the parents to conduct the study. The adolescent girls and the parents were informed about the need for the study by showing them information sheet and consent was obtained from each of them for carrying out the interview as well as the intervention. Only those adolescents willing to participate in the supplementation and give blood samples were included in the study.

A. Criteria for selection of samples

Inclusion Criteria:

i. Adolescent girls in the age group of 16-18 years
ii. Adolescent girls free from illnesses
iii. Haemoglobin levels should be between < 7.0 to 9.9 g/dl (Moderately anaemic)
iv. Haemoglobin levels > 11.5 g/dl (Non anaemic)
v. Willing to sign consent forms
vi. Willing to participate in the intervention programmes
vii. Willing to give blood samples

Exclusion Criteria:

i. Adolescent girls less than 16 and more than 18 years
ii. Adolescent girls suffering from illnesses and taking medications
iii. Haemoglobin levels below < 6.9 g/dl (Severely anaemic)
iv. Haemoglobin levels between 10.0 to 11.4 g/dl (Mildly anaemic)
v. Not willing to sign consent forms
vi. Not willing to participate in the intervention programmes
vii. Not willing to give blood samples

After obtaining ethical approval from the Institutional Human Ethics Committee of the Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (AUW/IHEC-13-14/FHP-03) and registering under the Clinical Trial Registry of India (CTRI), Indian Council of Medical Research (005253); the study was carried out.

1. Selection of samples

From different areas of Coimbatore district, 140 adolescent girls in the age group of 16-18 years were selected based on the inclusion criteria. Samples were selected based on the prevalence of anaemic adolescent girls in Tamil Nadu.

a. Assessment of socio-economic and personal characteristics of the adolescent girls

Socio-economic characteristics gives the total measure of an individual's or family's economic and social position. The socio - economic characteristics include caste, religion, type and size of family, education and occupation status

of all family members, and sources of income and total monthly income (National Center for Educational Statistics, 2008). The socio - economic and personal characters of an adolescent includes age, birth order, educational level, type and size of the family, caste, religion, educational level of father and mother, monthly income etc. Socio-economic and personal characteristics of the adolescent girls were collected using a structured interview schedule.

b. Assessment of the dietary characteristics of the adolescent girls

The various dietary characteristics such as vegetarian or non-vegetarian, meal skipping pattern, frequency of consumption of fast foods, frequency of consumption of micronutrient rich foods were assessed using the semi quantative food frequency questionnaire.

c. Assessment of the Haemoglobin level of the adolescent girls

In order to carry out the interventional studies, the adolescent girls were screened for haemoglobin level using finger prick method. Later, they were classified based on the levels of anaemia as per the WHO (2008) recommendations. From these classified respondents, moderately anaemic adolescent girls with Hb level 7 -9.9 g/dl and Non - anaemic adolescent girls Hb level > 12 g/dl were selected for interventional study.

PLATE XIV

CONDUCT OF IN VIVO STUDY

Explaining about the study

Getting consent letter signed from the respondentsstudy

Collection of data from the respondents

2. Conduct of the Interventions

a. Grouping of the adolescent girls

For the conduct of the intervention trials, 67 the adolescent girls were divided in to four groups based on Haemoglobin level and the interventions received. Experimental Group I were moderately anaemic (Hb level 7 to 9.9 g/dl) received ready to eat food supplement and nutrition education. Experimental Group II were also moderately anaemic (Hb level 7 to 9.9g/dl) received nutrition education alone. Experimental Group III were Non - anaemic (Hb level > 11.5g/dl) received ready to eat food supplement and nutrition education. Non anaemic adolescent girls with Hb level >11.5g/dl formed the control group of the study were received nutrition education alone. Figure – 10 shows the grouping the adolescent girls, the interventions to be given and the assessments to be carried out.

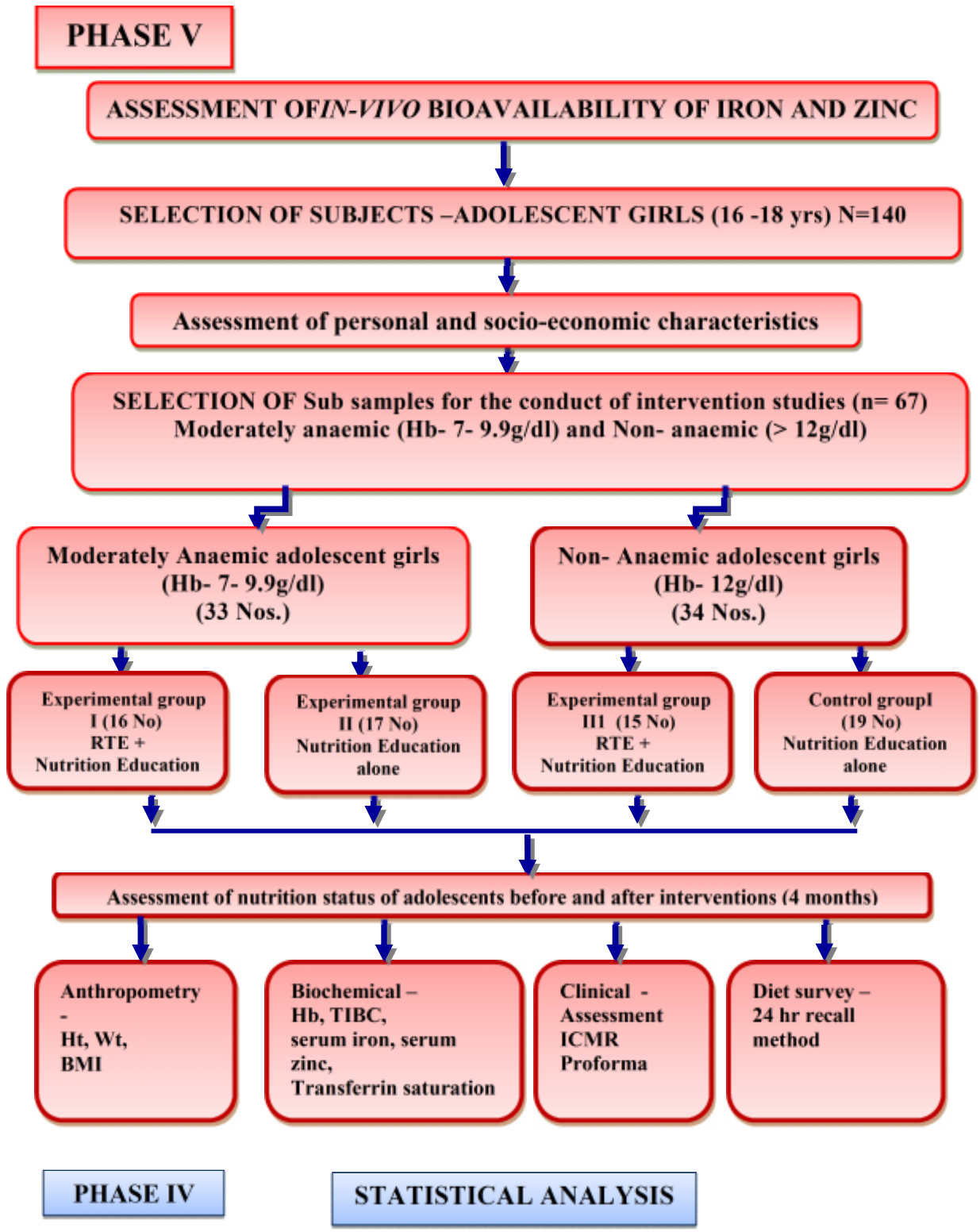
b. Assessment of Knowledge Attitude and Practices (KAP) of the adolescent girls

Knowledge Attitude and Practices (KAP) of the adolescent girls were assessed using questionnaire. The developed questionnaire consists of 20 questions each for Knowledge Attitude and Practices thus a total of 60 questions. These questions were related to micronutrient rich foods, sources, processing methods which will enhance bioavailability, good food combinations, and ways to overcome anaemia. After getting responses from the adolescent girls, one mark was given for the right answer and zero for the wrong answer and the totaling was done for each adolescent girl. Later, the adolescent girls were classified based on their mean $\pm$ SD KAP scores as low, medium and high.

Assessment of the Knowledge Attitude and Practices of the adolescent girls were done before and after interventions (Supplementation and Nutrition Education).The mean $\pm$ SD of the KAP scores were compared with‘t’ test.

FIGURE IV

RESEARCH DESIGN OF PHASE V AND VI



c. Supplementation with ready to eat food

As indicated in the Phase – IV, a ready to eat food in the form of ladoo (50g) made of the variation 1 (consisting of 5g shade dried drumstick leaves powder) was supplemented to Experimental Group I (moderately anaemic) and Experimental Group II (Non – anaemic) adolescent girls for a period of 120 days. The ready to eat foods were supplemented weekly in sealed pouches and the adolescent girls were asked to keep them in closed containers. They were instructed to touch the containers as well as the foods with dry hands and keep the containers closely after taking the food and store the container in room temperature and not in freezer. Every week, investigator collected the sealed pouched without the food and replaced with a new sealed pouch with foods.

Investigator instructed the adolescent girls to take the supplement as an evening snack without tea or coffee.

Before starting the supplementation, the selected groups of adolescent girls were given deworming tablets - Albendazole 400mg, fortnightly twice.

d. Nutrition Education

Nutrition Education plays an important role to enhance the nutritional status of the population and is very essential for the wellbeing of the community

In the present study, sixty adolescent girls - group I and II of experimental as well as the control group, their mothers and neighbourhood people were imparted nutrition education. Nutrition education was provided fortnightly for four months during the supplementation period.Nutrition education was imparted on topics such as micronutrients or hidden hunger– causes, deficiencies, symptoms, ways to prevent micronutrient malnutrition, household processing methods to enhance bioavailability of iron and zinc, anti-nutritional factors present in the diet, importance of micronutrients among adolescent girls etc. Nutrition education materials like folders, software etc were developed for imparting nutrition education.

4. Assessment of nutritional status of adolescent girls

The nutritional status of individual is the result of many interrelated factors such as social, economical, hereditary. However, it is influenced by the sufficiency of food intake both in terms of quality as well as quantity and also by the physical health of the individual (WHO, 2008)

Nutritional status can be defined as the health condition of an individual influenced by the intake and utilisation of nutrients. Nutritional status of the selected adolescent girls was assessed using anthropometric measurements such as height, weight and BMI, biochemical parameters such as Haemoglobin, TIBC, serum iron, serum zinc, serum ferritin and clinical examination with the help of a qualified physician using the ICMR proforma and diet survey by 24-hr recall method.

Techniques used for the assessment of the nutritional status of the respondents in this study include: -

a. *Anthropometric Measurements*

Anthropometric measurements help in the assessment of nutritional status and monitor changes in growth of adolescents. Investigating on the anthropometric measurements of adolescents is an important determinant of a nation's health. Measurements of height, weight and nutrient intake are the reliable means to evaluate the nutritional status of individuals and it is very much in need.

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). In this study the anthropometric measurements recorded were height, weight, and Body Mass Index.

i. Height

The height of the individual is influenced both by genetic and environmental factors. Height is affected only by long-term nutritional deprivation and is considered an index of chronic or long duration malnutrition.

In the present study, height of each adolescent girl was measured using a stature meter. The subject was asked to stand erect looking straight on a levelled surface with heels, buttocks, shoulders and back of the head touching upright without slippers. The head should be held erect with the arms hanging at the sides in a natural manner. The moving headpiece of the stature meter was lowered to rest flat on the top of the head and the measurement was taken. Height was read to the nearest 0.5 cm. An average of three measurements was taken as final measurement of the height of the respondent.

ii. Weight

Body weight is the most widely used sensitive and simplest reproducible anthropometric measurement .It indicates the body mass and is a composite of all body constituents like water, mineral, fat, protein and bone. It reflects more recent nutrition.

For taking weight of the respondent, digital - weighing balance was used, as it is portable and convenient to use in the field. The weighing scale was adjusted to zero before taking each measurement. The subjects were asked to remove slippers and were asked to stand on the plat form of the scale without touching anything and looking straight ahead. The weight was recorded to the nearest 0.25 kg. Each reading was taken thrice and the average was taken as the final measurement.

iii. Body Mass Index (BMI)

BMI can be used to grade Chronic Energy Deficiency (CED) and is regarded as a good indicator of nutritional status. BMI, which is expressed, as the ratio of Weight to Height Square is an indicator of general obesity and also gives the magnitude of protein calorie malnutrition (WHO, 1995).

Body Mass Index of the respondents was computed using the formula,

BMI = Weight (kg)/ Height (m^2).

b. Assessment of Diet

Dietary assessment enables to assess the eating habits of the individuals both food intake and nutrient intake of the individuals although the assessment of eating habits.

i. Food intake

For this, a set of cups and spoons were first standardized by the investigator following the procedure given by Thimmayamma *et al* (2003). The respondents were asked about the types of food preparations made for breakfast, lunch, teatime and dinner and the raw ingredients used for each of the preparations. Information on the total cooked amount of each preparation and the quantity consumed by the respondent was then assessed using the standardized cups. The cups were used to aid the respondent recall the quantities prepared and eaten. Later the raw food equivalents of the food consumed by the respondent were found out.

Dietary intake of the overweight adolescent subjects was adjudged by 24-hr recall method for three consecutive days using standardized containers. Those days were avoided which include fasting, festivals or wedding or any other occasion.

ii. Measurement of mean nutrient intake

The different food items consumed were converted into their raw equivalents; categorized into their respective food groups and average daily intake of energy, protein, fat, calcium, iron, beta- carotene and vitamin C were calculated from the values per 100 g of edible portion using the Gopalan *et al* (2010). The nutritive value of some of the foods like noodles, potato chips, biscuits etc. were taken from the information provided on the packaging of product. The calculated nutrient intake was compared with the recommended dietary allowances (RDA) for the respective age group (16-18 years).

c. Clinical examination

Examining for presence or absence of clinical signs of nutritional deficiencies is an important method of assessment of the nutritional status. It involves identification changes occurring in the surface epithelium of various parts of the body such as skin, hair, eyes, buccal mucosa or the organs such as thyroid gland which may be due to insufficient (or sometimes excess) nutrition. Clinical examination assess level of health the individuals in relation to their food

consumption. It is a nutritional assessment tool which provides direct information of the signs and symptoms of dietary deficiencies.

A proforma was prepared, standradised and used to assess the presence or absence of clinical deficiency symptoms, was carried out by a qualified physician. For the present investigation, the investigator with the help of a physician assessed the adolescent girls for the occurrence of diseases or illness in the past six months.

d. Biochemical Estimation

Biochemical test determines the various nutrients present in the body as well as forms an estimate of the nutrients stored in the body. Biochemical tests enable us to detect the deficiencies before the symptoms gets clinically evident and thereby giving an early chance of getting the deficiencies corrected and preventing further complications.

For the present investigation, the biochemical parameters evaluated for assessing the iron status of the adolescent girls were Haemoglobin, serum ferritin, serum iron, serum zinc, Total iron binding capacity (TIBC) and transferrin saturation (Yoon *et al*., 2015). From each respondent venous blood samples were collected for assessing the biochemical parameters in a fasting state. The biochemical estimations of the adolescent girls were carried out before and after interventions.

i. Haemoglobin estimation

Haemoglobin estimation is the most practical method of diagnosis of anaemia as it is cost effective and can be easily performed by trained technician. The Haemoglobin level of adolescent girls was estimated by cyanmethaemoglobin method since this method appears to be the most accurate. It is measured in terms of g/dl. Cuvette tube was pre-filled with cyanmethaemoglobin reagent and then incubated for 5 minutes. Later, the final readings were recorded. For each sample a blank containing tube was placed in the haemoglobin analyzer to check accuracy.

ii. Total Iron Binding Capacity (TIBC)

Serum was prepared from venous bloods by centrifugation after clotting and was stored at minus 20°C for determination of iron status indices**.** Iron status was determined by measuring serum iron and total iron binding capacity with a commercial reagent kit. Total Iron Binding Capacity gives the blood's capacity to bind iron with the transferrin. It is measured by drawing the blood and measuring the maximum amount of iron that it can carry which indirectly measures the transferrin, since it is the most dynamic carrier.

For the present study, TIBC was measured using a commercial reagent kit. Dipyridyl method was followed to estimate TIBC of the blood (Raghuramulu *et al*., 2003). The cutoff level for defining iron deficiency in adolescent girls is 200-450 µg / dl

iii Serum Iron

Serum iron measures the quantity of iron (Fe++) in the blood. Iron status can defined in relation to the amount of iron contained in the storage room temperature. Serum iron shows a consistent and progressive fall when a negative iron balance occurs. During iron efficiency anaemia, serum iron gets reduced.

For the present study, serum iron status was determined by measured using a commercial reagent kit. Dipyridyl method was followed to estimate serum iron content of the blood (Raghuramulu *et al*., 2003). The cutoff level for defining iron deficiency in adolescent girls is 50 – 175 µg / dl.

iv. Estimation of transferrin saturation

Transferrin saturation reflects the quantity of iron in transit from the reticuloendothelial system to the bone marrow and gets decreased during iron deficiency. Percentage of transferrin saturation was calculated using the formula

$$\text{Transferrin saturation \%} = \frac{\text{Serum Iron}}{\text{Total Iron Binding Capacity}} \times 100$$

v. Estimation of Serum Ferritin

Serum ferritin is a protein which helps store iron in our body. Reduction in the serum ferritin level is used to diagnose iron deficiency. The serum ferritin (SF) levels of the adolescent girls were assessed using an electro-chemiluminescence immunoassay. A decrease in serum ferritin level <12 µg/L represents the point of total depletion of iron store. Storage iron depletion was defined in these subjects as a persistent decrease in hemoglobin concentration rather than on the basis of a subnormal serum ferritin.

vi. Estimation of Serum Zinc

Serum zinc levels of the adolescent girls were estimated by atomic absorption spectrophotometry (AA 7000 Series; Shimadzu). Serum zinc level <74 µg/dL for male children and < 70 µg/dL for female children above 10 years of age was considered as zinc deficiency as recommended by International Zinc Consultative Group (2004).

All serum samples were analysed simultaneously to avoid deviation in assay conditions during the study. All the samples were assessed in triplicates and the results are presented in mean $\pm$ SD.

e. Impact of Interventions

The impact of supplementation of Bioavailable iron food and nutrition education to adolescent girls was assessed after four months of supplementation. After four months of the interventions, the body mass index and the biochemical parameters such as Haemoglobin, Total iron Binding Capacity, serum iron, serum ferritin and serum zinc was assessed.

F.PHASE- VI: STATISTICAL ANALYSIS AND INTERPRETATION OF THE DATA

The data collected was scored, coded, consolidated and subjected to suitable statistical analysis and interpretations. The data was analyzed using the Statistical analysis Package for Social Sciences (SPSS). Descriptive statistics were computed using standard methods like frequencies and percentages for men and women individually. Means and Standard deviations were calculated for

all the parameters and were presented in the tables. ANOVA was used to compare between standard and variations of formaulated foods. Dunans Multiple range test was used for mean comparison. t'-test was used to compare heamatological parameters and KAP of experimental and control groups. P>0.05 was set for significance.

The results of the statistical analysis and findings were presented in the next chapter.

Validation of Data

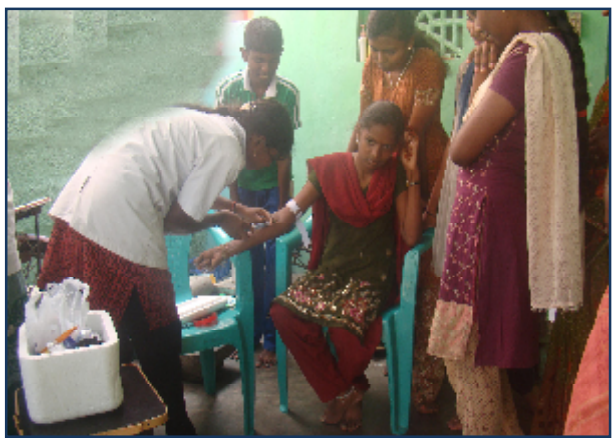
The investigator got trained in assessing the nutritional status of the population by attending workshop conducted by eminent scientists of National Institute of Nutrition. Each protocol was standardized and the investigator analysed the samples with meticulous care and precision. This resulted in avoiding bias in data collection and analysis.

Limitations of the study

Complete analysis of for all regional diets could not be performed and hence only iron and zinc contents were analysed due to paucity of time. Sample size for invivo study is small since consent was given only by this group.

PLATE XV

ASSESSMENT OF NUTRITIONAL STATUS



Biochemical assessment

Assessment of height

Assessment of weight

FIGURE V

RESEARCH DESIGN OF THE STUDY