Methodology
CHAPTER 3

METHODOLOGY

The methods adopted and materials used for the study on “Development of millet mixes, acceptability of millet mix based recipes, their iron bioavailability and popularisation” are given below

Phase - I Identification of millets and development of millet mixes

3.1 Selection of millets.
3.2 Preparation of millet mixes.
3.3 Preliminary nutrient analysis of millet mixes.
3.4 Shelf life of the millet mixes.

Phase - II Value addition using millet mixes

3.5 Preparation of value added breakfast foods and snacks.
3.6 Organoleptic evaluation of the prepared foods.
3.7 Nutrient analysis of selected prepared foods.

Phase – III Bioavailability of Iron from selected developed products.

Phase-IV Popularisation of millet mixes.

3.8. Awareness program for public regarding millet based breakfast foods and snacks.
3.9 Statistical Analysis.
3.10 Ethical considerations.

The following Null hypotheses were framed for the above study.

- There is no significant difference between the nutrients’ content of millet mixes A, B and C.
- No significant increase subsist in the **pH, moisture** and **total bacterial count** of the three millet mixes during storage (90 days).
- The millet mix incorporated **chappathi** is not different from that of the standard.
- There is no significant difference between the standard and millet mix added **rice dosa**.
- The millet mix added **wheat dosa** is not different from that of the standard.
- There is no significant difference between the millet mix added **appam** and the control.
- There exist no significant difference between millet mix **cane jaggery porridge** and that of the standard.
- There is no significant difference between the standard and the millet mix **palm jaggery porridge**.
- Significant difference does not exist between the millet mix incorporated **puri** and the control **puri**.
- No significant difference is present between millet mix added **Idiyappam** and the control.
- There is no significant difference between the millet mix incorporated **uthappam** and the standard **uthappam**.
- Millet mix **upma** and the standard **upma** are not different from each other.
- No significant difference exist between millet mix added **onion pakoda** and the control onion **pakoda**.
- There is no significant difference between the millet mix added **sev** and the control **sev**.
- No significant difference exist between the millet mix incorporated **maemo** and that of the control **maemo**.
- There is no significant difference between the millet mix incorporated **meduvadai** and the control.
• No significance difference is present between the millet mix added *baji* and the control *baji*.

• There is no difference between the millet mix incorporated *sweet Bonda* and that of the control *Bonda*.

• Millet mix added *Bonda (salt)* and the control *Bonda* are not different from each other.

• No significant difference between the millet mix incorporated *paniyaram (sweet)* and the control.

• There is no significant difference between the millet mix incorporated *paniyaram (salt)* and the control.

• Significance difference does not exist between millet mix *kolukattai* and the control.

• There is no significant difference between the millet mix added *samosa* and the control.

• No significant difference exists between the millet mix incorporated *diamond cuts (salt)* and the control diamond cuts.

• Millet mix added *sweet diamond cuts* and the control sweet diamond cuts are not different from each other.

• No significant difference exists between the millet mix *muruku* and the control *muruku*.

• There is no significant difference between the millet mix *somas* and the control.

• There exist no significance difference between millet mix added *gulab jamun* and the control.

• The nutrients content of *bonda* (salt), *Sev, Baji, Onion Pakoda* and *Porridge* (cane and palm jaggery) do not differ significantly.

• The bioavailable iron of *bonda* (salt), *Sev, Baji, Onion Pakoda* and *Porridge* (cane and palm jaggery) is not significantly different from each other.
Development of Millet Mixes, Acceptability of Millet Mix Based Recipes, their Iron Bioavailability and Popularisation

Phase- I Identification of millets & development of millet mixes

3.1 Selection of millets

**Pearl millet** (Pennisetum glaucum) known as *cambu* in Tamil is the most important millet and is the staple food of many in lower rainfall areas of Africa and Central India. It is rich in protein and iron. It is a typical cereal grain being low in lysine, tryptophan, threonine and the sulphur containing amino acids. Pearl millet is generally not troubled with antinutritional factors, although phytic acid and trypsin inhibitors have been found. Pearl millet is the fourth most important cereal of India after rice, wheat and sorghum. It provides cheap staple food with comparatively more nutrients (protein, fat, carbohydrates, mineral etc) to millions of poor people, cattle and poultry (*Khairwal et al., 1997*).

**Finger millet** (Eleusine Coracona) also called as *ragi* in Tamil. In India, finger millet (*ragi*) is consumed generally by a small segment of the population in the form of dumpling, porridge and *roti*. In recent years, *ragi* has regained importance, because of its nutritional strength in terms of dietary fibre, starch pattern as well as high calcium and iron contents. (*Wadikar et al., 2006*). Finger millet is highly nutritious as it is a rich source of minerals and dietary fibre in addition to primary nutrients (*Usha and Chandra, 1998*) and is consumed by sizable populations in South India.

**Barnyard millet** (Echinochloa spp) known as *kuthiraivolly* in Tamil; also called as *sawan* is grown in the kharif season in shallow soils. Little has been published on the uses of barnyard millets. It is used as "rice" (boiled whole). It is also used as stiff porridge (*saragati*) and also as *chappathi*. (*David et al., 2005*). Pearl millet, Finger millet and Barnyard millet were chosen for millet mix formulation.
Plate 1

SELECTION OF MILLETS
3.2 Preparation of millet flours

Millets (Pearl millet, Finger millet and Barnyard millet) produced in Kholli hills were purchased through M.S Swaminathan Research Foundation, Namakkal, Tamil Nadu, India, soon after harvest and processed (Figure I).

Figure I
General processing of the selected millets
After purchase the millets were cleaned (manually) thoroughly to remove dust, dirt, mud, clay and unwanted particles. Roasting was done at 60°C for 15 minutes, in order to reduce the moisture content, improve flavour, enhance shelf life and to destroy the anti-nutritional factors present in the millets (Makokha et al., 2002, Elyas et al., 2002 and Sankara Rao and Deosthale 1983). Further the millets were ground and prepared into coarse grain (rava) and also flour using the milling machine. The rava was sieved (25 mesh size) to remove any fine flour present, but the flour was sieved well (60 mesh size) repeatedly to get a fine powder. The rava and flour thus obtained were packed in HDPE (High Density Polyethylene) covers which have excellent chemical resistance, good water vapour barrier and great impact resistance. The covers were stored at room temperature in air tight containers.

3.2:1 Formulation of millet mixes

The millet mixes were developed from Barnyard millet, Finger millet and Pearl millet flour by mixing the three flours in different proportions as indicated in Table 1.

<table>
<thead>
<tr>
<th>Variations</th>
<th>Pearl millet</th>
<th>Finger millet</th>
<th>Barnyard millet</th>
<th>Total gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM - A</td>
<td>40</td>
<td>20</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>MM - B</td>
<td>40</td>
<td>15</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>MM - C</td>
<td>45</td>
<td>10</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>
3.2:2 Analysis of antinutritional factor – Phytate:

Oxford dictionary of biochemistry and molecular biology (2006) defines the antinutrients as natural or synthetic compounds that interfere with the absorption of nutrients. Nutrition studies focus on the antinutrients commonly found in food sources. Phytic acids are common in the hulls of nuts, seeds and grains. Phytic acid has a strong binding affinity to minerals such as calcium, magnesium, iron, copper, and zinc. This makes the minerals unavailable for absorption in the intestines (Tait et al., 2005, Ekholm et al., 2003, Cheryan et al., 1980). The simple traditional household methods such as roasting, germination and fermentation, cooking and soaking improve the nutritional quality (Nadeem et al., 2010). Gahlawat and Sehgal, 1994 had found an increase iron bioavailability in weaning foods prepared after roasting of barnyard and finger millet. Hence the developed millet mixes A, B and C were analysed for their phytate content before and after roasting at a moderate temperature of 50-60ºC for 15 minutes. The procedure for the above analysis is given in Appendix I

3.3 Preliminary nutrient analysis of millet mixes

Proximate analysis of energy, moisture, carbohydrate, protein, fat, crude fibre, ash, calcium, phosphorus and iron were carried out using standard procedures for the prepared millet mixes A, B and C.

3.3:1 Energy

The components of foods such as (Protein, fat, carbohydrate, alcohol, polyols, Organic acids and novel compounds) provide energy. This can be determined either by burning food in a bomb calorimeter and measuring the heat produced or by adding the energies of all components together to represent the nutritional gross energy value of the food. The calculation adopted for the above is given in Appendix-II.
3.3:2 Moisture

Moisture assay is one of the most important analysis performed on a food. The moisture content of foods varies greatly. The propensity of microorganisms to grow in foods depends on their water content and for this reason many foods are dried below the critical moisture content. The procedure adopted for the above analysis is given in Appendix III.

3.3:3 Carbohydrate

Carbohydrates are important component of foods as a major source of energy. Although FAO (1998) had pointed that it can be calculated by difference method, in the present study it was estimated according to the procedure as specified by Hedge and Hofreiter (1962) and is appended (Appendix IV).

3.3:4 Protein

Food proteins are complex and abundant component in all cells and are important for biological functions and cell structure. The proteins and other organic food components of the sample was digested with sulphuric acid in the presence of catalysts, then neutralised, distilled into boric acid solution and titrated with standardised acid. The result of the analysis represents the crude protein content of the food. The protein estimation was carried out using the micro kjeldhal method and is enclosed (Appendix V).

3.3:5 Fat

Fat is mainly a fuel source and it contains more than twice the calories found in an equal dry weight of protein or carbohydrate. The procedure of AOAC method for the above is given in Appendix VI.

3.3:6 Fibre

The crude fibre is the undigestable carbohydrate in foods. Crude fibre was determined by sequential extraction of the sample with sulphuric acid and sodium hydroxide as per the procedure specified in Appendix VII.
3.3:7 Ash

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in food stuff. Three major types of ashing are available: dry ashing for the majority of samples, wet ashing (oxidation) for samples with high fat content (meat and meat products) and low-temperature plasma dry ashing (also called simply plasma ashing or low temperature ashing) for samples with volatile components. The procedure of Raghuramulu, Nair and Kalyanasundharam, (2003) was adopted for the ash determination and is detailed in Appendix VIII.

3.3:8 Calcium

Calcium is an important component of a healthy diet and a mineral necessary for life. “Calcium plays an important role in bodily activities, structure development and regulation of various cell functions. Insufficient calcium levels lead to deterioration of the cell membrane resulting in the loss of cell compounds and eventually death of the cell and plant tissue. The procedure for the above analysis is detailed in Appendix IX.

3.3:9 Phosphorus

Phosphorus; the mineral is a major structural component that is vital for energy transfer and stored as phytin in plants. Phosphorus is found in many foods. The procedure for its estimation is given in Appendix X.

3.3:10 Iron

Iron is also an important component of various enzyme systems, such as the cytochromes, which are involved in oxidative metabolism. Iron lends itself for colorimetric analysis as it forms coloured complexes with reagents. This was analysed by AOAC (1965) and it is enclosed in Appendix XI.
3.4 Shelf Life

The shelf life of a food is defined as “the time it takes for a product to decline to an unacceptable level of consumption”. In many cases, shelf life is taken as “the time a product remains stable”. The actual length of the shelf life of any product will depend on a number of factors such as moisture content of the product, processing method, packaging and storage conditions. The shelf stability of the millet mixes developed for the present study was checked for a period of three months. The mixes were weighed (10gms each) and packed in ten different air tight HDPE (High Density Polyethylene) covers which have low water vapour transmission properties (Barnwal, Purushottam and Sharma, 2013). The covers were numbered and dated. All the ten covers were placed in a large HDPE covers and then sealed. The covers were stored in air conditioned room, because relative humidity and temperature during storage are the two major factors that affect overall quality of the product (Mridula et al., 2009). Once in ten days one small cover containing ten grams of the millet mix was taken for analysis of pH (at 27°C), moisture content and the total bacterial count (at 37°C). The procedures adopted for the above three estimations are appended (Appendix III, XII and XIII).

Phase-II Value addition using millet mixes

3.5 Preparation of value added breakfast foods and snacks

Common breakfast foods (Nine) such as chappathi, rice dosa, wheat dosa, appam, porridge, puri, idiyappam, uttapam, upma and snacks (Thirteen) like onion pakoda, sev, maemo, meduvadai, baji, bonda(sweet & salt), paniyaram (sweet & salt), kolukattai, samosa, diamond cuts (salt & sweet), muruku, somas and gulab jamun were selected and standardised. The millet mixes MM-A, MM-B and MM-C; were incorporated in the above listed foods at 10, 15 and 20 percent. In the preparation of baji, bonda (sweet & salt), samosa, somas the millet mix flour was incorporated into the batter used for their preparation. “Millet mixes rava” was used for the preparation of upma. The standard recipe adopted for the
above preparations are given in Appendices XIVa – XIVy. The Breakfast foods and snacks prepared are given below.

3.5:1 Chappathi

*Chappathi* made from wheat flour is an unleavened flatbread (also known as *roti*) in India. It is a common staple cuisine of South Asia as well as amongst South Asian expatriates throughout the world.

3.5:2 Rice Dosa

It is a crispy savoury pancake / crepe of South Indian cuisine. It is prepared by grinding rice and black gram dhal in a fixed proportion (4:1 or 3:1) fermented and then made on a flat *dosa* pan.

3.5:3 Wheat Dosa

It is a very easy and fast to prepare instant pancake. It is prepared with whole wheat flour on a *dosa* pan and does not require fermentation.

3.5:4 Appam

*Appam* is a pancake made with fermented rice batter and coconut milk. It is a staple diet and a cultural synonym of the South Indian mainly the Tamils and Keralites. It is eaten most frequently for breakfast or dinner.

3.5:5 Porridge

Porridge is also called as “*kanji*” or “*koozh*” in Tamil. It is made by boiling ground, crushed or chopped cereal in water, milk or both, with optional flavourings. It is usually served hot in a bowl or dish. It may be sweetened with sugar/jaggery/palm sugar or also served as a savoury dish with salt and buttermilk.

3.5:6 Puri

*Puri* is commonly served as a breakfast or dinner item. It is prepared with whole wheat flour or in combination with refined wheat flour. The flour is kneaded into dough, flattened, deep fried and served with a side dish.
3.5:7 Idiyappam

*Idiyappam* or string hoppers is a traditional Kerala and Tamil food. It is made of rice flour or wheat flour, salt and water; kneaded into a dough, extruded as strings and then steamed. It is generally served as the main course at breakfast or dinner together with coconut milk and sugar or gravy.

3.5:8 Uttapam

*Uttapam* is sometimes called as an Indian pizza. *Uttapam* or *Ooththappam* or *Uthappa* is a dosa-like dish made by cooking the fermented batter of rice and black gram dhal on a *tawa*. Unlike a *dosa*, which is crisp and crepe-like, *uttapam* is a thick pancake, with toppings cooked right into the batter.

3.5:9 Upma

*Upma* or *Uppuma* or *Uppittu* is a common South Indian breakfast dish, cooked as a thick porridge from dry roasted semolina i.e. white *rava* / wheat *rava* and vermicelli. Various seasonings and/or vegetables are often added during cooking, depending on individual preferences.

Snack Foods

3.5:10 Onion Pakoda

Onion *Pakoras* or Onion Fritters is a popular Indian street food. It is one snack that is easy to prepare and tastes good too. In India *pakoras* are usually served with “coriander greens and green chillies chutney” or sweet chutney made with dates and jaggery.

3.5:11 Sev

*Sev* is essentially small pieces of crunchy noodles. It is eaten as standalone snack as well as topping on dishes like *Bhelpuri* and *sevpuri*. *Sev* is a popular variety of Indian snack food. *Sev* can be made with whole bengal gram flour and rice flour mix at home and stored for weeks in airtight containers.
Maemo

*Maemo* is a dish prepared using *Dosa* and *Idli* batter with coconut toppings in south East Asian countries in which the batter is mixed with sugar or jaggery for sweetness.

Medu vadai

*Medu Vadai* is a traditional South Indian dish which can be served as a main course, side dish, or a snack. This crispy delicious dish is made with spiced urad dal batter and fried in oil as donut shaped dumplings. They are crispy outside and soft inside.

Baji

*Baji* is a spicy Indian snack similar to potato fritters, with a variety of vegetables dipped in batter made with bengal gram flour and rice flour and then deep fried. It has become popular as a “eat alone snack”.

Bonda (sweet and salt)

*Bonda* is a typical South Indian snack that has various sweet and spicy versions of it at different regions. The process of making *bonda* involves deep frying, a filling of potato with other vegetables dipped in gram flour batter.

Paniyaram (sweet and salt)

The *Kuzhi paniyaram*, is a South Indian dish made by shallow frying the batter in a mould. The batter used is the same as that for *idli* and *dos*.* Kuzhi paniyarams* can be made sweet or spicy.

Kolukattai

*Kolukattai* is a popular South Indian “sweet dumpling” made from rice flour, grated coconut and jaggery, and is similar to *modak* made in other parts of India. For the present study the rice flour was replaced by millet mix flour entirely.
3.5:18 *Samosa*

*Samosa* or *samoosa* is a fried or baked pastry with a savoury filling, such as spiced potatoes, onions, peas, lentils, ground lamb, ground beef or ground chicken. Its size and consistency may vary, but typically, it is distinctly triangular.

3.5:19 **Diamond Cuts (sweet and salt)**

Diamond cuts can be made sweet or spicy. Savoury *Diamond Cuts* is an easy and good Indian tea time snack. It is also known as maida biscuits, maida chips and *khara* biscuits.

3.5:20 **Murukku**

*Murukku* means “twisted” in the Tamil language. It is a South Indian snack of savory crunchy twists made from rice and dhal flour.

3.5:21 **Somas**

Sweet *somas* are perfectly sweetened coconut gratings with sugar inside a crispy maida outer cover.

3.5:22 **Gulab Jamun**

Gulab Jamun is a traditional Indian enjoyable desert dish. Deep fried sweet dumplings are stewed in sugar syrup. These syrupy doughnut-like dumplings can be made for any occasion. The traditional method of preparing Gulab Jamun is a complex process, but this new recipe is simple and easy to make.

3.6 **Organoleptic evaluation of the prepared foods**

Quality is the ultimate criterion of the desirability of any food product. Sensory evaluation consists of judging the quality of food by a panel of judges. The evaluation deals with measuring, analyzing and interpreting the qualities of food as they are perceived by the senses of sight, taste, touch and hearing. Sensory evaluation may be designed to reflect common preference, to maintain the quality of food at a given standard, for the assessment of process variation, cost reduction,
product improvement, new market development and market analysis. Human judgement is individual and is not always consistent. Physical conditions of the individual, psychological factors and environment factors may affect one’s judgement. Further, one individual may not be able to discriminate different aspects of the food quality (Manay and Shadaksharaswamy, 2007). For these reasons, for sensory evaluation, a panel of (twenty) judges was used. The panelists were provided with the prepared foods an hour after any normal meal (mid-morning or mid-afternoon), when the judges were neither too well fed nor too hungry. The panelists were also provided with room temperature water for rinsing between samples. Organoleptic evaluation was done for different sensory attributes such as colour and appearance, flavour, texture and taste with a maximum score of five each for each of the food items prepared. The desirable and undesirable qualities of each attribute were provided in descriptive terms to help in proper evaluation of the products. The data were tabulated, averaged and analyzed because for a correct interpretation of results, their statistical analysis is necessary (Ranganna, 1986). The organoleptically evaluated products were arranged in descending order using the “overall acceptability score percentage”. From this; five highly acceptable foods which also had a higher iron content as per nutritive value calculation (bonda (salt), baji, onion pakoda, sev and porridge) were chosen for further analyses.

3.7 Nutrient Analysis of selected prepared Foods

Moisture, protein, fat, energy, carbohydrate, crude fibre, ash, calcium, phosphorus and iron were analysed for the five most acceptable products. The procedures adopted for the analyses are given in the Appendices II to XI.

Phase – III Bioavailability of Iron from selected developed products

Bioavailability is a measure which scores the absorbability of a nutrient by comparing it with that of a reference nutrient that is considered as having the most efficient absorbability (Allen et al., 2006). While Bilal (2002) defines bioavailability
as the portion of the total ingested nutrient utilized by the body; Anderson et al. (2004) explains it further that bioavailability is equated with absorption of a mineral element after its digestion from food and before its use in tissue and cells. Organic acids such as ascorbic, citric and tartaric acids enhance bioavailability of iron by preventing the precipitation of ferric iron and this is achieved by reducing it to the ferrous state and by forming suitable soluble ligands that are available for absorption. Ascorbic acid is the most clearly documented enhancer of non-heme iron absorption (Monsen, 1988).

In the present study for “invitro bioavailability analysis”; five foods (bonda (salt), baji, onion pakoda, sev and porridge) were chosen based on the overall acceptability score percentage and their iron content. Each one of the chosen products’ “invitro - iron bioavailability” was done with the addition of iron absorption promoter (lemon juice) because ascorbic acid is one of the major enhancers of iron absorption (Tait et al., 2005) and inhibitor (tea) and without the addition of neither the promoter nor the inhibitor. Pepsin-HCL digestion procedure of Rao and Prabhavathi (1978) and Govindaraj, KrishnaRau and Prakash (2007) was adopted for assessing the invitro iron bioavailability. According to Rao and Prabhavathi (1978) the ionizable and soluble iron at pH 7.5 can be directly correlated with “percent in-vivo iron absorption” and the highest correlation with physiological availability in humans could be observed with ionizable iron at pH 7.5. Based on this correlation the prediction equation Y= 0.4827 + 0.4707 X, where Y is the percent iron absorption in adult men and X is the percent ionizable iron at pH 7.5 for iron absorption using percent ionizable iron at pH 7.5; that is derived by the above authors was used in this study. The results were tabulated and statistically analysed for further interpretation.
Phase-IV Popularisation of millet mixes

3.8 Awareness program for public regarding millet based breakfast foods and snacks

India is the largest producer of many kinds of millets, which are often referred as coarse cereals. However, realizing the nutrient richness of these grains they are now considered as” nutria-cereals (Michaelraj and Shanmugam, 2013). In the present study to create awareness about millets, their uses and methods of incorporating the same in regular food preparation among urban public the following tools were developed and used.

Plate 2

Awareness Program – Lecture
A special questionnaire (Appendix XV) was developed to collect information on the existing awareness about millets from women. The questionnaire consisted of fifteen Yes / No or multiple choice questions. The questionnaire was distributed to the three hundred volunteers and their level of awareness was also assessed prior to the education programme (Plate – 2) and cooking demonstration.

1. **Charts** (Appendix XVI) were made to highlight the nutritional benefits of millets. The charts prepared for the popularisation of millets covered the nutritional profile of the three millets selected, their nutritional value compared to rice and wheat. These charts were used during the lecture given to the public to be more informative.

2. **Cooking Demonstration** of the millet mix added products was done in an apartment of houses, for a group of 50-60 people at a time. Demonstrations were given frequently to cover the three hundred people who respondent by filling up the awareness questionnaire. The demonstrations included on the selection of millets, cleaning of millets, duration of roasting, milling and sieving techniques, combination of millets that can be used, proportion that could be added in the recipes selected were given in detail.

### 3.9 Statistical Analysis

Results of the analyses were statistically analysed by SPSS 16.0 for mean, standard deviation and analysis of variance. Duncan’s multiple range test was also used to determine the significance of differences between the samples.

### 3.10 Ethical Considerations

The sensory panel members were informed about the study, knew the composition of the products and participated voluntarily. The study was approved (Appendix XVII) by the Institutional Research ethics committee of the PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.