3. MATERIALS AND METHODS

For conducting any fruitful research, the most important thing is the selection and application of right kind of methodologies to get valid results. The details of the research procedures and methodological steps used to Assess the Nutritional Status of Cardiac patients, Development of Intervention Package and Development of suitable recipes for cardiac health have been discussed here under the following heads:

- Assessment of Nutritional Status of Cardiac patients
- Development, Implementation and Validation of Intervention Package for Cardiac patients
- Formulation of suitable Recipes for the Cardiac patients

3.1.0 Domain of the study

The study entitled “Development, Implementation and Validation of Intervention Package for Cardiac Patients” was conducted in the department of Food Science and Nutrition, College of Home Science, CSK Himachal Pradesh Agricultural University, Palampur during the year 2008-2010.

3.2.0 Locale of the study

The present study was conducted in the district Kangra of Himachal Pradesh on Hundred cardiac patients were selected from Government hospital of Palampur and Baijnath. The data was collected by regular visits to their places.

3.3.0 Sampling procedure

Two government hospitals viz. Civil Hospital Palampur and Baijnath were selected, then permission was taken from SMO, Civil Hospital Baijnath and MS, Civil hospital Palampur to personally interview the respondents who visited hospital regularly for their checkup and also to obtain the records of the clinical tests of the interviewed respondents. A total of hundred respondents (male and female) formed the sample (Fig.3.1).
3.4.0 Development and Pre-testing of Questionnaire

A well structured and exhaustive questionnaire was formulated after consulting literature to collect the relevant informations specifically keeping in mind the objectives of the study. For the pretesting of the questionnaire, ten questionnaire were got filled up by cardiac patients other than the selected patients and then evaluated for responses of the patients. On the basis of collected information and difficulties faced, necessary improvement/ modification were incorporated to make it more functional.
3.5.0 Experimental layout

The study was scientifically laid out according to Complete Randomized Block Designing and replicated thrice.

3.6.0 Collection of Data

In order to meet the objectives of the study, the data was collected through pre-tested and a well structured questionnaire, given under the following subheads viz.

3.6.1. Information about Cardiac patients

i. Demographic profile
ii. Socio-economic profile
iii. Family history
iv. Personal habits
v. Physical activity
vi. Health information

3.6.2. Assessment of Nutritional Status of Cardiac patients

i. Dietary pattern
ii. Anthropometry

3.6.3. Development of Intervention Package

i. Implementation of Intervention Package
ii. Validation of Intervention Package

3.6.4. Formulation of suitable Recipes for the Cardiac patients

i. Nutritional and Chemical Composition
ii. Organoleptic Evaluation

An effort was made to develop a personal rapport with the patients to extract information as far as possible. Cross checking and indirect queries were also raised to ascertain the authenticity of the study.
3.6.1 Information about Cardiac patients

i. Demographic profile

This segment included the general information regarding age, sex, religion and community.

ii. Socio-economic

The information pertaining to education, occupation, type of family, family income, land holding pattern, animal holding and type of housing etc. were recorded by filling up questionnaire.

iii. Family history

The component was used to extract information regarding the family history of cardiac disease and related metabolic diseases such as hypertension and diabetes etc.

iv. Personal habits

This aspect include the informations regarding the smoking and alcohol consumption pattern of selected patients.

v. Physical activity

This head include the informations to get an idea to the type of activity such as sedentary, moderate and heavy activity, physical exercise and sleeping pattern of selected patients.

vi. Health information

This component of the study was used to collect informations regarding the type of measures used to control heart problems, symptoms, causation factors and history of cardiac of family members.

3.6.2 Assessment of Nutritional Status of the Cardiac patients

An effort was made to assess the nutritional status of the patients and for the purpose their dietary pattern and anthropometric measurements were evaluated.
i. **Dietary pattern**

To ascertain the dietary pattern a 24 hours recall method for three consecutive days was taken and calculated mean were used for the further analyses. The mean intake of different food stuffs was then computed for a day and compared with the balanced diet for cardiac patients given by Ghafoorunisa and Krishnaswamy1998.

The patients were provided with different sets of standardized *katories* and glasses to record the exact amount of foods and beverages consumed by them. The amount of cooked food was converted into raw ingredients and the nutrient intake was calculated by using the value per 100 g of edible portion using the food consumption Table (Gopalan *et al.* 1995). The weight and volume of different cooked foods consumed by the patient was recorded in terms of household measures/number/kg to find out quantity of raw food intake.

In case of *chapaties*, the amount of flour used in the preparation of one *chapati* was calculated by dividing the total amount of flour used by number of *chapaties* prepared, this value was then multiplied by the number of *chapaties* consumed by the patients to find out the raw amount of flour consumed by the patients.

a. **Food preferences and avoidances**

Information regarding food preferences and avoidances of the patients in term to frequency of consumption was also collected.

b. **Food consumption pattern**

The patients were interviewed to collect information regarding the detailed dietary history with added information about his/her food likes/dislikes, general meal pattern before and after the onset of disease, number of meals and dietary intake. Information was also taken about dietary modification in relation to present condition, such as food avoided, consumption and modification of ghee/oil.

ii. **Anthropometry**
Anthropometric measurements are considered as an indispensible tool for assessing nutritional status of the patients. The height, weight, mid upper arm circumference and skinfold thickness of four different sites, i.e. triceps, biceps, subscapular and suprailliac were recorded for calculating the per cent body fat. The measurements were taken as follows:

a. Height

The height was measured according to the method described by (Jelliffe 1966) with the help of anthropometer rod. The patients were asked to stand erect without shoes with bare foot. The heels, buttocks, shoulders, and back of the head touched the anthropometer at the back, with the arms hanging on the sides. The head piece was lowered gently, crushing the hair and making contact with the top of the head. The readings were recorded to the nearest of 0.5 cm. The same procedure was repeated thrice to avoid any error and then mean was taken.

b. Weight

The weights of the patients were taken using the weighing balance calibrated in kilograms and grams. The balance was initially standardized with known weight before use and kept in a flat surface adjusted to zero. The body weight was determined to the nearest of 0.5 kg using weighing machine. The patients were not wearing any heavy garments and were asked to remove their shoes before measurements and stand erect on the centre of the platform without touching anything else. The weight was recorded in kg and the data was compared with the weight of the reference Indian man (60 kg) and women (50 kg) as given by ICMR (1999).

c. Body Mass Index

Body mass index, a measure of body weight adjusted for height is used as an indicator of nutritional status and size of the body energy stores in adult population. The index was calculated for each of the patient as weight (kg) divided by height (m²), BMI include both fat and lean tissues. It was calculated by the equation given by Garrow (1981).
**BMI**

\[
\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (m²)}} = \text{Kg/m}²
\]

**Interpretation -** BMI was compared with the classification given by WHO (1998).

<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI kg/m²</th>
<th>Risk of comorbidites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt; 18.50</td>
<td>Low (but risk of other clinical problems increased)</td>
</tr>
<tr>
<td>Normal range</td>
<td>18.50-24.99</td>
<td>Average</td>
</tr>
<tr>
<td>Overweight</td>
<td>≥ 25.00</td>
<td>Increased</td>
</tr>
<tr>
<td>Pre-obese</td>
<td>25.00-29.99</td>
<td>Increased</td>
</tr>
<tr>
<td>Obese class-I</td>
<td>30.00-34.99</td>
<td>Moderate</td>
</tr>
<tr>
<td>Obese class-II</td>
<td>35.00-39.99</td>
<td>Severe</td>
</tr>
<tr>
<td>Obese class-III</td>
<td>≥ 40.00</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

**d. Waist and Hip Circumference**

Waist and hip circumference is a measurement at the level of navel, when the patient breathes quietly, whereas, hip circumference was measured at the intertichantric level (Despres 1991) with the help of non-stretchable tape.

**e. Waist to Hip Ratio**

Waist to hip ratio is a measurement of visceral obesity and is a strong indicator of risk of hypertension, cardiovascular disease and some other diseases like cancer etc (Rockville 1993). It was calculated as:

\[
\text{Waist to Hip ratio} = \frac{\text{Waist circumference (cm)}}{\text{Hip circumference (cm)}}
\]

**f. Skin fold thickness**
The Harpenden Skinfold Calliper was used to measure skinfold thickness such as triceps, biceps, subscapular and suprailliac to the nearest of 0.1 mm by the method as suggested by Jelliffe (1966).

**f.i Triceps skinfold thickness**

It measures a double layer of skin and sub-cutaneous fat. The measurement was taken half way down the left arm between the tip of acromial process of scapula (shoulder blade) and the olecraneon process of ulna (tip of the elbow). Measurements were taken while hanging freely at the side. The skin fold parallel to the long axis was picked up between the thumb and the forefinger of the left hand clear away from the underlying muscle. The calipers were applied to the fold little below the finger and reading was noted to the nearest of 0.1 mm.

**f.ii Biceps skinfold thickness**

The skinfold was picked on the front of the arm directly above the centre of the orbital fossa at the same level on which the triceps skinfold was measured.

**f.iii Subscapular skinfold thickness**

The subscapular skinfold was measured just below and laterally to the angle of left scapula. The fold should be in a line running at approximately 45° to the spine, in the natural line of skin cleavage. It has the advantage of providing a uniform layer of subcutaneous fat not requiring precise localization.

**f.iv Suprailliac skinfold thickness**

This measurement was taken above the iliac crest in the mid auxiliary line of the patients.

**g. Body fat percentage**

It was calculated from the sum of tricep, biceps, subscapular, and suprailliac from equation given by Durnin and Womersley (1977).

\[
\text{Body density} = 1.1599 - (0.0717^* \log \text{of sum of all four skin fold}).
\]

Where D is body density.

\[
4.95
\]
% Fat = \frac{\text{Body density}}{4.50} \times 100

\textbf{Interpretation}

The values thus obtained were interpreted as per the classes of body fat percentage suggested by Deurenberg \textit{et al.} (1998) as follow:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Thin</th>
<th>Normal</th>
<th>Stout</th>
<th>Obese</th>
<th>Extreme obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>\leq 10%</td>
<td>10-20%</td>
<td>20-25%</td>
<td>25-30%</td>
<td>\geq 30%</td>
</tr>
<tr>
<td>Female</td>
<td>\leq 20%</td>
<td>20-30%</td>
<td>30-35%</td>
<td>35-40%</td>
<td>\geq 40%</td>
</tr>
</tbody>
</table>

\textbf{3.6.3 Development of Intervention Package}

Based upon the information collected, need based Intervention Package was developed which includes different modules like Leaflets, Pamphlets, Games, Flash cards, Pictorial diagrams, Displays, Calendar, Desk calendar, Puppets shows and Diet charts with sample menu of different caloric diets etc.

The selected proportion of cardiac patients (N=30) were firstly evaluated to test their basic knowledge (pre-testing) regarding various aspects of disease using multiple choice questionnaire to test the awareness/ knowledge of the patients regarding general nutrition awareness, knowledge regarding heart disease and nutrition concepts (Appendix-II). They were tested for their knowledge regarding various aspects of disease before imparting nutrition education.

\textbf{i. Implementation of Intervention Package}

The developed package was then applied on selected groups of cardiac patients for its validation.

In implementation of intervention programme, the patients were educated regarding various aspects of cardiac diseases, its causative, symptoms, types, and complications associated with other diseases. Patients were also educated about the importance of various nutrients, diet charts, role of medicinal plants...
necessary dietary modification required for healthy living etc. Nutrition education was given in a group through a developed intervention package which include various printing matter like leaflet, pamphlet, games, displays, puppet show etc. Teaching materials and lesson plans pertaining to above aspects were prepared, modified and used for imparting general awareness. Teaching was carried out through lectures, discussions and demonstration were also used and medium of interaction was used in Hindi and local dialect. All queries were cleared and discussions were also made with the subjects. Personal contacts with the respondent were maintained. The developed package was also used as a medium of interaction was used in Hindi and local dialect. All queries were cleared and discussions were also made with the patients. Personal contacts with the respondent were maintained.

ii. Validation of Intervention Package

After imparting nutrition education, any changes in the knowledge of the patients were reassessed by asking them to fill up the same questionnaire (post-test).

a. Gain in knowledge

In the present study, the effect of implementation package was assessed in terms of gain in knowledge calculated by finding out the difference between post and pre exposure knowledge of the patients. For evaluating the questionnaires, one score was awarded for each correct and zero for wrong answer, respectively. Effect of nutrition education was assessed by applying impaired’t’ test to see its impact on gain in knowledge which was calculated using the following equation.

\[
\text{Gain in knowledge} = \text{Score of post test} - \text{Score of pre test}.
\]

b. Physical fitness

To assess the physical fitness, Harvard Step test was conducted as per the method suggested by Weiner and Laurie (1969).
This test was performed by the subject stepping on and off a bench 14" height 30 times a minute or until the subject was able to do it. Stepping should be in time to metronome beating at half second interval. Stepping up with one leg on the first beat, up with the other leg on the second beat, down with the first leg on third beat and down with the other leg on final beat of the cycle. It was permissible to change the step from time to time. The subject must stand erect at each step on the bench. If the subject failed to keep-up with the metronome for 15 seconds exercise was stopped and duration of exercise was recorded at the time of stoppage. To note the duration of exercise stop watch was used. The pulse count was made from one to one and half minutes. The Rapid Fitness Index (RFI) was calculated as follows:

\[
\text{RFI} = \frac{\text{Duration of exercise in second}}{5.5 \times (\text{Pulse count 1-1.5 min after the exercise})} \times 100
\]

The norms followed for the test were:

- Below 50 - Poor fitness
- 50 - 80 - Average fitness
- Above 80 - Good fitness

### 3.6.4 Formulation of suitable Recipes for the Cardiac patients

Different products \textit{viz} beverages, juices, RTS, squash and syrup were prepared by using \textit{bottle gourd} and \textit{aloe-vera}. An effort was made to prepare low caloric products by substituting in place of sugar suiting to the palate of cardiac patients as well as other health conscious strata of the society. \textit{Bottle gourd} (\textit{Lagenaria siceraria}) and \textit{Aloe-vera} (\textit{Aloe barbadensis}) were mainly taken for the study as these have a therapeutic uses and act as functional food for cardiac patients. The various products \textit{viz.} \textit{aloe-vera} and \textit{bottle gourd} juice, dietetic based \textit{aloe-vera} beverage, RTS, squash and syrup were also prepared by using these two functional foods. Since Arjuna bark (\textit{Terminalia arjuna}), Dalchini (\textit{Cinnamomum zeylanicum}), linseed(\textit{Linum usitatisimum}), Oat(\textit{Avena sativa}) and Giloy(\textit{Tinapora cordifolia}) are also known to their medicinal value and functional
constituents of cardiac health, an effort was made to develop and formulate certain recipes *viz.* Beverages, Soups, Porridges, Breakfast items, Dry mixtures and Main meals by using these ingredients. The detailed recipes are given in appendix (III).

**a. Procurement of selected raw ingredients**

*Bottle gourd* was procured from the local market and *Aloe-vera* from local area of the Palampur at optimum maturity period.

**a.1 Processing of raw materials**

After selecting fruits of *bottle gourd*, were washed to remove dirt and extraneous matter, removed heads, peeled and cut into approximately 12 mm thick pieces. *Aloe-vera* leaves were also washed, cleaned and then lower part of leaf base or the white part attached to the stem of plant, and the tapering point of the top, and sharp spines located along the leaf margins were removed by a sharp knife. Then with the help of knife mucilage layer was removed lie below the green top rind then similarly bottom rind was also removed (Plate 3.1).

**a.2 Preparation of juice**

The prepared pieces of *bottle gourd* and *aloe-vera* gel were divided into four different lots and then subjected to following treatments.

i) Untreated

ii) Water blanched for 3 minutes

iii) KMS (1% w/v) treated

iv) Citric acid (1% w/v) treated

Then the treated pieces and extracted gel were crushed in a blender to extract the juice which stored still further use. The detailed process is shown in Figure 3.2 and 3.3.
Fig. 3.2: Flow sheet for preparation of Bottle gourd juice
Fig 3.3: Flow sheet for preparation of Aloe-vera juice
Plate 3.1: Steps in processing of Aloe-vera
a.3 Development of Low Calorie *Aloe-vera* based RTS, Squash and Syrup

The extracted juice of *aloe-vera* was further used for the preparation of sorbitol and sugar based RTS, squash and syrup. Zero caloric beverage was prepared by substituting stevioside in place of sugar (Figure 3.4). The detailed procedure is given in appendix III.

i. **Nutritional and Chemical Evaluation**

a. **Moisture (AOAC 1990)**

The moisture was determined on the basis of AOAC method. Weighed samples (5 g) in triplicate were dried for eight hours in a hot air oven at 105°C in pre-weighed moisture dishes. The dishes were transferred immediately to desiccators, cooled and weighed. The losses in weight represented the moisture content of the samples.

**Calculations**

\[
\text{Weight of empty dish} = W \text{ (g)}
\]

\[
\text{Weight of sample} = W_1 \text{ (g)}
\]

\[
\text{Weight of dish + sample before drying} = X \text{ (g)}
\]

\[
\text{Weight of crucible + sample after drying} = Y \text{ (g)}
\]

\[
\text{Per cent moisture} = \frac{\text{Loss of weight}}{\text{Weight of sample (g)}} \times 100
\]

\[
\text{Per cent Moisture} = \frac{(X-W) - (Y-W)}{W_1} \times 100
\]
Fig 3.4: Flow sheet for preparation of Sugar and Dietetic *Aloe-vera* based Beverage, RTS, Squash and Syrup

b. Per cent Ash

The ash content was obtained as laid down in literature (AOAC 1990).
After determining the moisture content, the samples in the crucibles were burnt on a hot plate and then placed in a muffle-furnace at 600°C for 4 hours. From the weight of residue left in crucible, the total ash content was calculated as follows:

Calculations

\[
\text{Weight of empty crucible} = W \text{ (g)}
\]

\[
\text{Weight of empty crucible + sample before drying} = W_1 \text{ (g)}
\]

\[
\text{Weight of empty crucible + sample after drying} = W_2 \text{ (g)}
\]

\[
\text{Per cent Ash} = \frac{\text{Weight after ashing (g)}}{\text{Weight of sample (g)}} \times 100
\]

\[
\text{Per cent Ash} = \frac{W_2 - W}{W_1 - W} \times 100
\]

c. Fat (AOAC 1990)

Reagent

Petroleum ether (B.P. 60-80°C)

Procedure

Weighed samples of 5.0 g each in triplicate were extracted with petroleum ether (60-80°C BP) in Soxhlet extraction apparatus for 18 hours. The ether extract was filtered through a sintered funnel in a pre-weighed beaker and was washed with small volume of petroleum ether 2-3 times. The petroleum ether was completely evaporated and the beakers were weighed. The increase in the weight of beakers represented the fat content present in the samples.

Calculations

\[
\text{Weight of sample} = W \text{ (g)}
\]
Weight of empty beaker = $W_1$ (g)

Weight of beaker + fat extract = $W_2$ (g)

Per cent Fat = \[
\frac{\text{Amount of ether extract (g)}}{\text{Weight of sample (g)}} \times 100
\]

Per cent Fat = \[
\frac{W_2 - W_1}{W} \times 100
\]

d. Crude Protein (AOAC 1990)

Reagents

Digestion mixture: One part of copper sulphate + 10 parts of potassium sulphate

Boric acid solution: 4.0 per cent

Sodium hydroxide: 40.0 per cent

Standard $H_2SO_4$: 0.1 N

Mixed indicator: 0.1 (g) Methyl red and 0.5 (g) Bromocreasol green in 100ml of 95.0 percent ethanol

Procedure

Weighed sample (2 g) was digested with concentrated sulphuric acid (25 ml) and digestion mixture (5 g) in Kjeldahl digestion flask. The content were cooled and transferred to 250 ml volumetric flask. The volume was made upto the mark with distilled water and mixed. Measured aliquot (5 ml) was taken in a distillation flask followed by 40.0 per cent Sodium hydroxide and ammonium borate was collected through a condenser in a flask containing (10 ml) of 4.0 per cent boric acid solution. The distillate was titrated with 0.1 N sulphuric acid. A blank sample was also run along with the sample.

Calculations

Initial burette reading = a

Final burette reading = b
Volume used (Titre value) = (a – b) ml
Volume made up = 250 ml
Aliquot taken = 5 ml
Weight of sample = 1.0 g

Per cent nitrogen = \( \frac{\text{Titre value} \times 0.00014 \times \text{Volume made} \times 100}{\text{Aliquot taken (g)} \times \text{Weight of sample (g)}} \)

Per cent nitrogen = \( \frac{(a-b) \times 0.00014 \times 250 \times 100}{5 \times 2} \)

Per cent crude protein = Per cent nitrogen \times 6.25

e. **Crude Fibre (AOAC 1990)**

**Reagents**

- Sulphuric acid : 1.25 per cent
- Sodium hydroxide : 1.25 per cent

Weighed defatted samples (5 g) each in triplicate were digested with 200 ml of 1.25 per cent sulphuric acid by gentle boiling for half an hour. The contents were filtered and the residue was washed free of acid using hot distilled water. Acid free residue was then transferred to the same flask to which 200 ml of 1.25 per cent sodium hydroxide was added. The contents were digested again for half an hour, filtered and again washed free of alkali using hot distilled water. The residue was dried in an oven overnight at 105°C, weighed and then placed in the muffle furnace at 600°C for 4 hours. The loss in weight after ignition represented the crude fibre in the sample.

**Calculations**

Weight of sample = \( W \) (g)
Weight of crucible = \( W_1 \) (g)
Weight of empty crucible + Sample before ignition = \( W_2 \) (g)

Weight of empty crucible + Sample after ignition = \( W_3 \) (g)

\[
\text{Per cent Crude fibre} = \frac{\text{Weight of sample (before ignition)} - \text{Weight of sample (after ignition)}}{\text{Weight of sample}} \times 100
\]

\[
\text{Per cent Crude fibre} = \frac{(W_2 - W_1) - (W_3 - W_1)}{W} \times 100
\]

**e.1 Neutral Detergent Fibre (NDF)**

NDF was estimated by the method suggested by Van Soest and Wine (1967).

**Reagents**

**Neutral detergent solution**

- Sodium borate decahydrate: 6.18 g
- Sodium lauryl Sulphate: 30.00 g
- 2-ethoxy ethanol: 10.00 ml
- Disodium ethylene diamino tetra acetate (EDTA): 18.16 g
- Water: 1.00 L
- Disodium hydrogen phosphate: 5.00 g

**Disodium hydrogen phosphate:**

Weighed together EDTA and sodium borate decahydrate in a large beaker. Added some amount of water and heated the solution till the content dissolved. Then sodium lauryl sulphate was added to the solution. In a separate
beaker disodium hydrogen phosphate was weighed and dissolved by heating in remaining water and added to the beaker containing other ingredients.

**Procedure**

Weighed out 500 mg air-dried sample (triplicate) and transferred into a beaker of the refluxing apparatus. Added to this 100 ml of neutral detergent solution and heated to boiling. As it started boiling, heat was reduced to avoid foaming and allowed to reflux for 60 minutes. Then filtered through weighed Gooch crucible with minimum of hot water. Liquid was filtered and repeated the washing procedure. Then washed with acetone in the same manner. Dried the crucible in hot air oven at 100°C for 8 hours and weighed after cooling.

**Calculations**

\[
\text{Per cent NDF} = \frac{(\text{Weight of crucible + Fibre content}) - \text{Weight of crucible}}{\text{Weight of sample (g)}} \times 100
\]

**e.2 Acid Detergent Fibre (ADF)**

ADF was estimated by method suggested by Van Soest and Wine (1967).

**Reagents**

**Acid detergent solution**

- Cetyl trimethyl ammonium bromide (CTAB) - 20.0 g
- \(1\text{N } \text{H}_2\text{SO}_4\): Added 20.0 g of CTAB to \(1\text{N } \text{H}_2\text{SO}_4\) to make the volume 1 litre and stirred.

**Procedure**

Five hundred milligram of air-dried sample was weighed and transferred to the beaker of the refluxing apparatus. Hundred ml of acid detergent solution was added to it and the mixture was heated to boil and refluxed for 60 minutes. The
mixture was then filtered through a weighed Gooche crucible on filter manifold. The sample was rinsed into the crucible with minimum amount of hot water. Filtered the liquid and washing was repeated. Then the sample was dried at 100°C for 8 hours in hot air oven and weighed.

**Calculations**

\[
\text{Per cent ADF} = \frac{(\text{Weight of crucible} + \text{Fibre content}) - \text{Weight of crucible}}{\text{Weight of sample}} \times 100
\]

**f. Total Soluble Solids (Ranganna 2006)**

The TSS was determined in triplicate using Hand Refractometer and the values were expressed in degree Brix. A temperature correction was also applied when the temperature was above or below 20°C (Appendix iv).

**g. pH (Ranganna 2006)**

The pH of the samples was determined with the help of pH meter. The equipment was standardized with buffer solution of pH of 4.0 and 9.0.

**h. Acidity (Ranganna 2006)**

**Reagent**

- NaOH solution : 0.1 N NAOH
- Phenolphthalein indicator : 1 per cent

**Procedure**

Five gram sample was taken and transferred into a flask. The volume was made upto 50 ml with distilled water. The mixture was boiled for half an hour by replacing water losses. Five milliliter aliquot was taken and titrated with 0.1 N NaOH and phenolphthalein was used as an indicator. The end point was the development of faint pink colour which persisted for 15 seconds.

\[
\text{Per cent Acidity} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Volume made up} \times 64}{\text{x100}}
\]
(as citric acid) \( \text{Volume of aliquot taken} \times \text{Wt of sample(g)} \times 1000 \)

i. **Ascorbic Acid (Ranganna 2006)**

The ascorbic acid estimation was done by the method of Ranganna (2006). Ascorbic acid was estimated by using 2, 6-dichlorophenol indophenol dye.

**Procedure**

5 g sample was taken and blended with 3 per cent HPO\(_3\) and made the volume to 50 ml with HPO\(_3\) and filtered. Then 5 ml aliquot was taken and titrated with standard dye to a pink colour persisted for 15 seconds.

**Calculations**

\[
\text{Ascorbic acid} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot taken for estimation} \times \text{Wt of sample(g)}}
\]

j. **Sugars**

Sugars were estimated by Lane and Eynon’s method (1923) as reported by Ranganna (2006).

1. Total sugars
2. Reducing sugars
3. Non-reducing sugars

**Calculations**

1. Total sugars percent \( = \frac{\text{mg of invert sugar} \times \text{Dilution}}{\text{Titre value} \times \text{wt of sample (g)}} \times 100 \)

2. Reducing sugars percent \( = \frac{\text{mg of invert sugar} \times \text{Dilution}}{\text{Titre value} \times \text{wt of sample (g)}} \times 100 \)

3. Non-reducing sugars percent \( = \frac{[\text{Total sugar} – \text{Reducing sugars}] \times 0.95}{\text{Total sugars percent}} \)
ii. **Organoleptic Evaluation** (Gould 1978)

The sensory attributes evaluated were colour, flavour, texture and overall acceptability of the prepared products. A minimum of 10 judges were selected at random. The judges were required to record their preferences and acceptability of products on the evaluation card (Appendix I).

**Statistical Analysis**

The data obtained by questionnaire was tabulated using frequency tables and percentage. The effect of nutrition education was assessed by applying paired-t test (Snedecor and Cochran 1988) and chi-square test was also used to test the association of different independent variables with the dependent variables that were being studied by using the following formula:

\[ X^2 = \frac{(O_i - E_i)^2}{E_i} \]

- \( O_i \) = observed frequency
- \( E_i \) = expected frequency

Significance was tested at 0.05 level