MATERIALS AND METHODS

METHODS AND MATERIALS

Present study was designed with broad objective of exploring problems related to diet and health and developing suitable measures for the health promotion for the aged. The relative objectives included survey of problems regarding food related activities and assessment of different living arrangements, age groups and iron folic acid supplementation on nutritional status of the elderly women. The study also included formulation and evaluation of nutritious food items and to assess its effect on health and nutritional status of institutionalized elderly. The elderly women and institutionalized elderly subjects were selected for this study from the free-living population and institution of Vadodara city respectively. The details of the plan of work, tools and techniques used in the study are described in this chapter.

To achieve these objectives two approaches were used for evaluation of health of the aged. (A) Nutrient supplementation and (B) Food based approach. For collecting detailed information on various aspects concerning socio demography, diet, nutrition, activity pattern, addiction pattern, disease profile and to assess effect of nutrient and food intervention on the subjects. The study was planned as follows:

PLAN OF STUDY

Essentially the study was conducted in Vadodara city. The study was divided into different phases. The plan of each phase has been described separately. The study was phased out as follows:

I: ASSESSESSMENT OF PROBLEMS CONCERNED WITH FOOD RELATED ACTIVITIES, DIET, NUTRITION AND DISEASE PROFILE OF THE ELDERLY WOMEN WITH DIFFERENT LIVING ARRANGEMENTS AND AGE (AGED 60 YEARS AND ABOVE).

II: IMPACT OF IRON FOLIC ACID SUPPLEMENTATION ON HEALTH OF ELDERLY ANEMIC WOMEN FOR A PERIOD OF SIX WEEKS.

III: DEVELOPMENT AND EVALUATION OF SOME SELECTED NUTRITIOUS FOOD ITEMS FOR GERIATRIC POPULATION.

IV: ASSESSESSMENT OF THE EFFECT OF SOY FOODS ON HEALTH AND NUTRITIONAL STATUS OF INSTITUTIONALIZED ELDERLY SUBJECTS.

PHASE I

ASSESSMENT OF PROBLEMS CONCERNED WITH FOOD RELATED ACTIVITIES, DIET, NUTRITION AND DISEASE PROFILE OF THE ELDERLY WOMEN WITH DIFFERENT LIVING ARRANGEMENTS AND AGE GROUPS (AGED 60 YEARS AND ABOVE).

The specific objectives included:

1) To collect data on socio-demographic attributes and activity pattern.

2) To study the problems of older women regarding food related activities.

3) To assess diet, nutrition and disease profile of the older women.

The details of the study design, sample selection, parameters studied, the tools and techniques used for data collection are described below:

1. STUDY DESIGN

I) Sample Selection

2. COLLECTION OF INFORMATION AND THE PARAMETERS STUDIED:

I) Socio demographic status

II) Activity pattern

III) Problems regarding food related activities

IV) Nutritional status:

a) Dietary intake

b) Anthropometric measurements

V) Clinical parameters:

a) Hemoglobin levels

b) Blood glucose levels

c) Blood pressure measurements

VI) Disease profile: Major and minor illnesses

3. TOOLS AND TECHNIQUES:

a) Interview cum Questionnaire (pre-designed and pre-tested)

b) Bathroom scale and measuring tape

c) Set of standard cups and spoon

d) Cynmethemoglobin method

e) Sphygmomanometer

f) Glucometer

4. STATISTICAL ANALYSIS

1. STUDY DESIGN

I) Sample Selection:

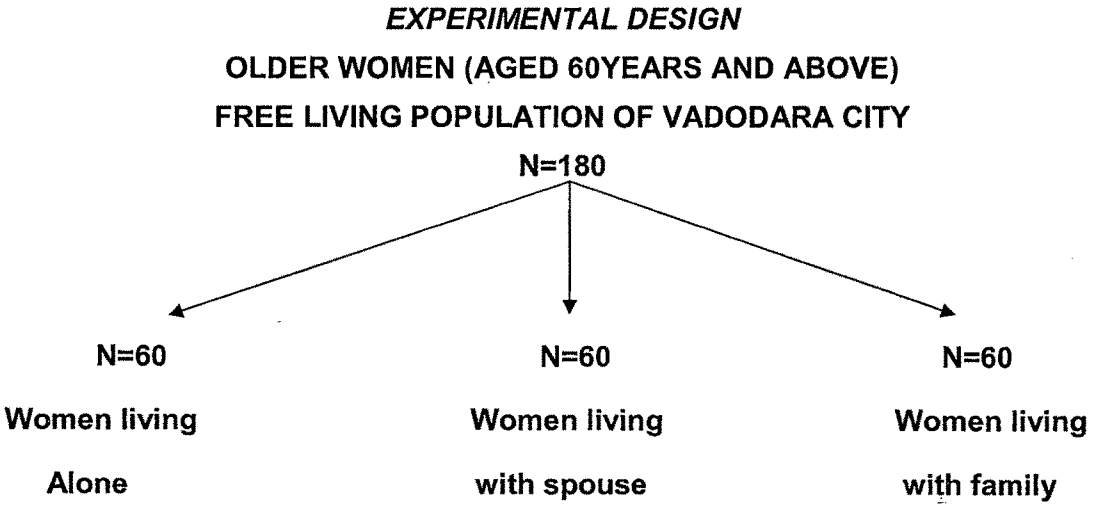
A sample was selected using purposive criteria, from the population of elderly women residing in Vadodara city. The criteria for the sample selection were age and their living arrangements.

Various organizations working with the older persons in Vadodara were contacted to identify a sample fulfilling the above-mentioned criteria. After primary identification and contacts with some respondents, snowball technique was used to identify other respondents.

One hundred and eighty elderly women aged sixty years and above were selected from the free-living population living under major five zones of Vadodara. They were distributed under three groups as per their living arrangements i.e. older women

living alone, older women living with spouse and older women living with family They were also divided into two categories according to their age (1) Young-old and (2) Old-old. The young-old group represented women in the age range from 60 – 75 years whereas the old–old group included women above the age of 75 years. The experimental design is shown in figure 3.1.

Figure 3.1



Data was collected on:

✓ **Background information**

Socio demographic status and activity pattern.

✓ **Diet and Nutrition information**

General dietary information, food frequency of specific uncooked and cooked foods, dietary intake and anthropometric measurements.

✓ **Clinical information**

Parameter like hemoglobin, blood glucose and blood pressure measurement.

✓ **Information on problems regarding food related activities**

Activities like purchasing, storing, pre-preparation, cooking, eating/serving of foods.

✓ **Information on disease profile**

Checklist of major and minor illnesses.

2. COLLECTION OF INFORMATION AND THE PARAMETERS STUDIED

Development of tool:

A semi-structured interview schedule and a questionnaire to assess the quality of life of older women were prepared. The tools were prepared in English and then translated into Gujarati for better comprehension by the respondents and ease in communication. The content validity of the tools with their sub-sections was established through feedback.

Collection of information and parameters under study were as follows:

I) Socio demographic status:

An effort was made to identify the sample from the middle and upper middle class, to maintain uniformity. A carefully planned questionnaire was designed for obtaining all detailed information about the subjects. This included information on age, education, religion, income, mother tongue, marital status and type of family.

II) Activity pattern:

Data on activity pattern was collected by total self reported time spent on various activities using questionnaire. Day's activity was noted and further categorized as household, recreational, social, religious, leisure and exercise. Total time spent under each activity was then calculated.

III) Problems regarding food related activities:

Main purpose was to find out the type of activity carried out (various food related activities) and the problems faced by elderly women while doing such activities. Differences with respect to age and different living arrangements were studied.

Survey was conducted on all the subjects. A pre-tested questionnaire was used to obtain the data. Questions having multiple-choice answers pertaining to various household food related activities and their problems where asked. Activities right from purchasing, storing, pre-preparation, cooking and eating/serving were included.

Detailed information regarding need of help from other people for various activities were also collected.

IV) Nutritional status:

a) General diet related information:

Some diet related information was collected using questionnaire. It included general dietary habits. The food frequency list for specific uncooked and cooked foods was introduced to know their habits and frequency. For food frequency a list of uncooked and cooked foods were prepared (appendix-I). Frequency in terms of daily, frequently and non-frequently was noted.

b) Dietary intake:

Twenty-four hour dietary recall method was used to measure the dietary intake of all the subjects. The details of the food consumed on previous day was asked in the terms of household measures and then in the terms of standard cups and spoons. The subjects reported intake of cooked foods, which was converted into raw amounts. Information on the subject's intake of energy, iron, protein, fats vitamin C, calcium and β-carotene was calculated. Nutritive value of foods consumed was calculated using food composition tables given in the Nutritive value of Indian foods (Gopalan, 1997). The average daily nutrient intake for each subject was obtained from data collected.

c) Anthropometric measurements:

These included heights, weights and mid upper arm circumferences, the most common and important parameters used for assessing the nutritional status of the individual. BMI was calculated as per the standard formula.

i) Weight Measurements:

Weight measurements were taken for all the subjects using the bathroom scale. It is portable and can be conveniently used in the field. The subjects were asked to stand erect on the scale without touching anything with no heavy clothing or footwear and looking straight ahead. The weights were taken twice to ensure accuracy and

recorded to the nearest 0.25 kg .The scale was recalibrated to zero before each measurement. The weight measurements were taken for the all subjects enrolled for the study.

ii) Height Measurements:

Height measurements were made on all the subjects using a flexible, non-stretchable fiberglass tape. The subjects were asked to stand erect without touching anything, with no footwear, heels touching the wall and looking straight, ahead. Scale was kept on the subject's head and marks were made on the wall using a pen indicating the height of the subjects and then with the help of measuring tape the height of the subjects were taken. The height was taken twice to ensure accuracy recorded to the nearest of 0.1 cm.

iii) Body Mass Index (BMI):

BMI was calculated by the formula:

$$\text{BMI} = \frac{\text{Weight (kg)}}{[\text{Height (m)}]^2}$$

iv) Mid Upper Arm Circumference:

The mid point of the left upper arm was marked by asking the subjects to keep their left arm in right angle with the upper arm .The MUAC was then measured using glass tape.

V) Clinical parameters:

a) Hemoglobin level estimation:

Hemoglobin level estimation was carried out by Cyanmethemoglobin method (INACG, 1985).

Based on principle of treating hemoglobin (blood) with Drabkins reagent, hemoglobin presents in blood reacts with potassium ferricyanide to give hemoglobin, a rust colored compound. The intensity of which can be measured Spectrophotometrically at 540 nm. Standardization was done with cyanmethemoglobin reference standard

obtained from Glaxo Pvt Ltd. The calibration of spectrophotometer was done using this reference standard.

Suitable aliquots were taken in separate test tubes each containing 2.5ml, 2.0 ml, 1.5 ml and 1.0 ml respectively. The volume was made and aliquot remained undisturbed and was the top standard. The optical density was estimated spectrophotometrically at 540 mm, within 30 min, after adjusting the instrument to zero with the blank solution (Drabkins reagent). A factor for estimation of hemoglobin was calculated from the calibration.

Hemoglobin was estimated according to the following steps:

The finger was wiped with a cotton swab dipped in ethanol and allowed to dry. A bold prick was made using a sterile disposable lancet. The first drop of blood was wiped off. A big drop was allowed to form on the finger and then 20μl of blood was pipetted in duplicates using a micropipette. This was allowed to stand for minimum of 15minutes before taking the reading at 540 mm. Duplicate samples were collected from all the subjects for the estimation of hemoglobin levels.

Concentration of hemoglobin in the blood was calculated using the factor and optical density (O.D) of the samples as:
O.D x factor = Hemoglobin Concentration

b) Blood glucose estimation:

Random blood glucose was estimated using glucometer instrument (Johnson and Johnson).

Blood glucose was estimated according to the following steps:

The finger was wiped with a cotton swab dipped in ethanol and allowed to dry. A bold prick was made using a sterile disposable lancet. The first drop of blood was wiped off. A big drop was allowed to form on the finger, which was kept on the indicator shown on the strip. On indication strip was put gently on the meter and looked for the digital reading. The used strip was discarded and always-fresh new strip was used for separate reading.

c) Blood pressure measurements:

Blood pressure was measured using sphygmomanometer. The blood pressure was measured in millimeters of mercury (mmHg) to the nearest 10mmHg systolic and diastolic.

Certain data related to their general health was also collected using questionnaire. This included information regarding eye, ear, nose and ability to walk.

VI) Disease profile:

A checklist was prepared for both major and minor illnesses. Frequency of illnesses was noted for minor illnesses. Data were collected by the investigation through personal interviews. A pre-tested performa was used for recording the morbidity. The list included the commonly encountered ailments in elderly. (Appendex-I).

STATISTICAL ANALYSIS

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS). The baseline data collected was subjected to various statistical tests. Data were analyzed using bivariate tables with frequencies and percentages were prepared. Wherever necessary statistical tests were applied. Besides this, verbatim were used for interpreting the data. Statistical analysis was carried out using the following test:

1. Socio-demographic attributes:

Percent responses were calculated for the different socio-demographic variables.

2. Activity pattern:

Total time spent on each activity was calculated in terms of hours.

3. Problems regarding food related activities:

Percent responses were calculated for the different food related activities, difficulties, diet related information and food frequency.

4. Nutritional status:

Percent responses for general diet related information was calculated for subjects belonging to different living arrangements and age groups.

a. Dietary intake:

For twenty-four hour dietary recall, mean and standard deviation was calculated for the nutrients such as energy, protein, fat, calcium, iron, β-Carotene and vitamin–C. F-test was applied to note the difference in the food intake between the groups belonging to different age group and living arrangements.

b. Anthropometric measurements:

Mean and standard deviation was calculated for the anthropometric measurements such as height, weight, MUAC and BMI.

5. Clinical parameters:

a. Hemoglobin levels:

Mean and SD were calculated for all the parameters that were expressed numerically. Percent prevalence of anemia in total, for different age groups and different living arrangement was calculated.

b. Random Blood Glucose estimations:

Mean and SE were calculated for both the groups of subjects.

c. Blood pressure measurements:

Mean and standard deviation for systolic and diastolic levels were calculated for both the groups of subjects.

6. Disease profile:

The percent of subjects suffering form various diseases with frequency were calculated for major as well as minor illnesses for both the groups.

PHASE II:

IMPACT OF IRON FOLIC ACID SUPPLEMENTATION ON HEALTH OF THE ELDERLY ANEMIC WOMEN FOR A PERIOD OF SIX WEEKS.

The specific objectives of the study were:

a. To select of anemic elderly women and to study the impact of Iron Folic Acid (IFA) tablets for a period of six weeks.

b. To collect pre and post data on dietary intake, hemoglobin levels, physical performance and cognitive performance of the elderly women.

The details of the study design, sample selection, parameters studied, the tools and techniques used for data collection are described below:

1. STUDY DESIGN

I) Sample Selection

II) Pre intervention data

III) Final Data

2. COLLECTION OF INFORMATION AND THE PARAMETERS STUDIED

I) Dietary intake

II) Hemoglobin levels

III) Physical performance tests

IV) Cognitive functions tests

3. TOOLS AND TECHNIQUES

a) Set of standard cups and spoons

b) Cynmethemoglobin method

c) Timer, paper and pencil

4. STATISTICAL ANALYSIS

1. STUDY DESIGN

I) Sample Selection:

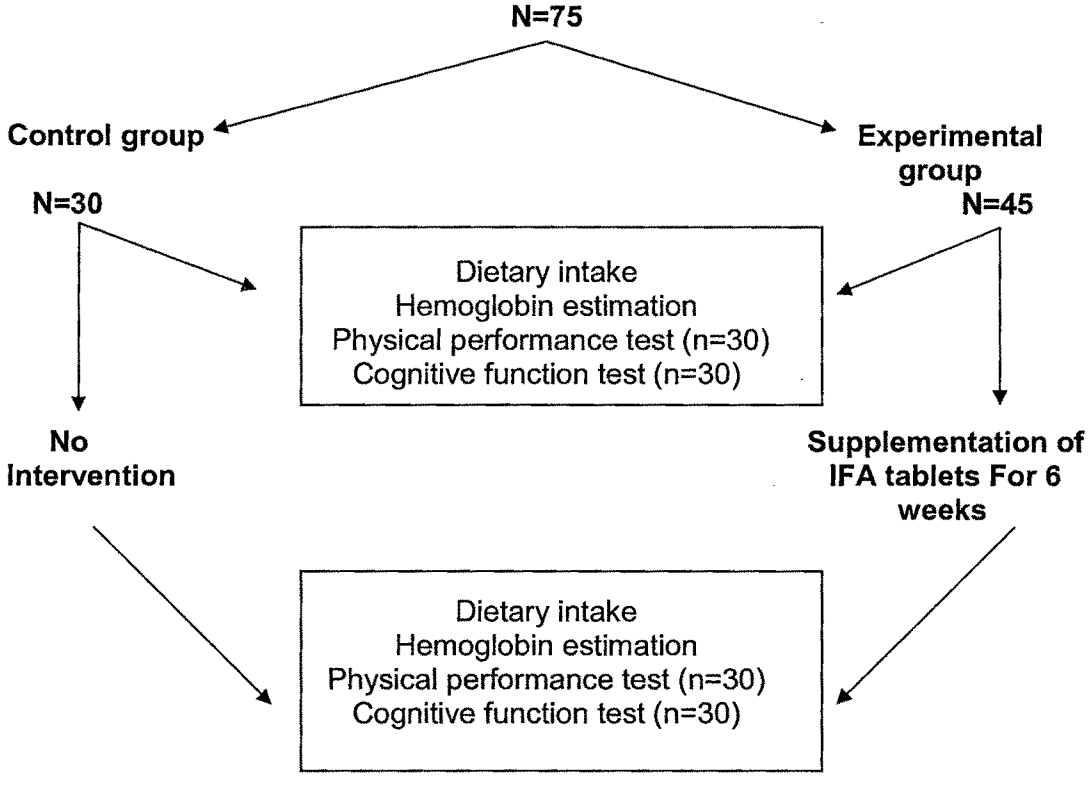
High prevalence of anemia was found in elderly women studied under phase I. Therefore it was thought to study impact of IFA supplementation on elderly women. Forty-five elderly anemic women with hemoglobin < 12gm / dl from phase–I, were selected for the supplementation of Iron folic acid tablet for a period of six weeks. IFA tablet was distributed on daily basis. It was confirmed each day, that the earlier days supplement had been consumed with the main meal and the compliance was recorded. The subjects were requested to inform in advance if they were likely to be away from the home for a day or two any time during the study period so that extra supplements could be given to them. Subjects who failed to inform before leaving were followed up till they had completed 45 days of supplementation. Iron folic acid tablets (IFA) in appropriate doses were given to the experimental group for a period of six weeks. Pre and post hemoglobin levels, physical performance tests and cognitive function tests were compared. Out of 45 experimental group subjects, only 30 subjects were ready to perform physical performance and cognitive function tests. Their age and hemoglobin matched subjects were chosen as their control group (Figure 3.2).

Figure 3.2 shows the study design of phase II.

Figure 3.2

STUDY DESIGN

SUB-SAMPLE (Elderly Anemic Subjects)



II) Pre intervention data:

Pre intervention data was collected on dietary intake, hemoglobin levels, physical performance tests and cognitive function tests.

III) Final data:

After completion of six weeks of intervention with Iron Folic Acid supplementation data was collected on same parameters.

2. COLLECTION OF INFORMATION AND THE PARAMETERS STUDIED:

I) Dietary intake: Same method was followed as described earlier in phase I.

II) Hemoglobin levels: Same method was followed as described earlier in phase I.

Elderly women who showed less than 12 gm% hemoglobin levels were selected for this phase of study.

III) Physical performance tests (Seshadri, 1996):

Impairment of physical work capacity and work output is one of the adverse effects. Thus in order to measure the impact of IFA supplementation on physical performance, an assessment of standing balance, a timed 2.4m walk and timed test of five chair rises were used to assess physical performance. Each physical performance test was categorized into a 5 level score, with 0 representing the inability to do the test and 4 representing the highest level of performance. Score on the three tests were added together as a summary performance test. Physical Performance Test was assessed by measures of standing balance, walking speed, and ability to rise from a chair.

a) Standing balance:

For tests of standing balance, the subjects were asked to attempt to maintain their feet in the side-by-side, semi-tandem (heel of one foot beside the big toe of the other foot), and tandem (heel of one foot directly in front of the other foot) positions for 10 seconds each. The subjects were given a score of 1 if they could hold a side-by-side standing position for 10 seconds but were unable to hold a semi-tandem position for 10 seconds, a score of 2 if they could hold a semi-tandem position for 10 seconds but were unable to hold a full tandem position for more than 2 seconds, a score of 3 if they could stand in the full tandem position for 3 to 9 seconds, and a score of 4 if they could stand in the full tandem position for 10 seconds.

b) Walking speed:

An 8-ft (2.4-m) walk at the subjects' normal pace was timed, and the participants were scored according to quartiles for the length of time required. The time of the faster of two walks was used for scoring, as follows:

Time in seconds	Score
≥ 5.7	1
4.1 to 5.6	2
3.2 to 4.0	3
≤ 3.1	4

c) Rise from a chair:

Subjects were asked to fold their arms across their chests and to stand up from a sitting position once; if they successfully rose from the chair, they were asked to stand up and sit down five times as quickly as possible. Quartiles for the length of time required for this measure were used for scoring, as follows:

Time in seconds	Score
≥ 16.7	1
13.7 to 16.6	2
11.2 to 13.6	3
≤ 11.1	4

Performance measure included tests of standing balance, walking speed and ability to rise from a chair.

IV) Cognitive function test (Seshadri, 1996):

The association between iron deficiency and poor cognitive performance has been well documented. A deficiency of iron impairs cognitive performance and hence supplementation with iron improves selected aspects of mental functions. Two aspects of mental functioning were assumed namely attention and concentration & memory.

a) Attention and concentration test:

Each elderly woman was given a sheet of paper on which numbers from 1 to 20 were written in random order. Fifteen numbers were selected randomly, out of the total 20, prior to administration to the test by the investigator. Each number was called at intervals of 2 seconds. The women were asked to strike off numbers, which are being called out. Scoring was done by giving mark for each correctly mark off numbers and 1 mark deducted for every numbers that was incorrectly struck off the marks upon which scoring was done was 15.

b) Memory Test:

Prior to actual administration of the test 30 elderly women, not included in the study, were asked to list down 20 objects present in the environment, which were familiar to them. Out of the objects listed by them, most commonly mentioned items were selected and check listed. The selected items were placed on the table and shown at a time for 30 seconds. The object were covered by a thick table cloth and the elderly women were asked to list down the objects from memory, within 1min. One mark was given for correctly listed objects and 1mark wad deducted for every objects listed in correctly. Marks ware given upon a total of 15 marks.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences was used for analysis. Data were analyzed using bivariate tables with frequencies and percentages were prepared. Statistical tests were applied wherever necessary. Besides this, verbatim were used for interpreting the data. Statistical analysis was also carried out using paired 't' test.

1. Dietary intake:

For twenty-four hr. dietary recall, mean and standard deviation was calculated for the nutrients such as energy, protein, fat, calcium, iron, β-Carotene and vitamin–C. Paired t-test was applied to note the difference in the food intake at the 0 month and 1 ½ month. Mean nutrient intake was also calculated.

2. Hemoglobin levels:

Mean and SD were calculated for all the parameters that were expressed numerically. Independent "t" test was used to compare differences between the mean hemoglobin in two groups.

Paired "t" test was used to assess the difference between the mean hemoglobin of the same group before and after the study period.

3. Physical Performance and Cognitive Functions:

Mean and median values were compared within each intervention group separately. Appropriate statistical tests i.e. 't' test was applied.

PHASE III:

DEVELOPMENT AND EVALUATION OF NUTRITIOUS FOOD ITEMS FOR GERIATRIC POPULATION.

The study was carried out in two stages.

Stage I: Development and sensory evaluation of food items.

Stage II: Processing of cooked food items for various analysis.

Stage I: Development and sensory evaluation of food items:

Since majority of the elderly women projected problems with food related activities, especially young elderly and elderly living alone and with spouse it was also decided to formulate items, which required minimum cooking and is suitable for geriatric population.

Selected food items were planned under two categories. A) Soy based food items and B) Quick cooking food items. Following considerations were made for developing food items:

(a) Easy digestibility (b) Culturally acceptable (c) Nutrient rich and (d) Possible to prepare at home

Category (A), food items were planned with special consideration of meeting needs of elderly population along with its benefits. All soy based food items (except soy roti) were containing 12 – 15 gm of soy protein in accordance of the study subjects and their overall health. (B) This category included quick cooking food items, which were nutrient rich and required less time and efforts for preparation.

SOYBEAN was selected, as it is a functional food and could fulfill the first part of the requirement. Nutritional composition and amino acid profile is given in appendix III-A and III-B. Few items were formulated using soybean as main ingredient namely:

1. **Soybean usal.**
2. **Soybean sambhar.**
3. **Soybean dhokli.**
4. **Soybean stuffed paratha.**
5. **Soybean roti.**

Considering the ease of preparation, digestability and nutritive value two food items were formulated. Therefore one item was prepared using carrots and another with spinach.

1. **Carrot kheer.**

2. **Spinach with white sauce.**

All the items were developed using weighed quantities of ingredients and cooked for a fixed period of time. The quantity of various ingredients used and the method of preparation are given in the appendix III-C. All the items were cooked using deionized water for analysis.

Sensory evaluation of the food items:

This was carried out in two different manners. Soy based food items were selected considering the requirement of the elderly population and its health benefits. Since the items were to be freshly prepared and intervened, the judges selected for the sensory evaluation were also taken from the field only. Therefore institutionalized elderly were purposively selected for evaluation as items were to be intervened on them. Initially to help in the standardization of the amount of the food items and to observe the side effects if any, firstly the soy items were prepared and given to 10 untrained elderly people which were selected randomly from the free living population. All the inmates of the vridh ashram were selected as untrained judges that were enrolled for the study later on.

Hedonic rating test:

The Hedonic scale (Appendix III-D) of 1 - 9 points ranging from dislike extremely to like extremely was used in the institution for evaluating overall acceptability of the food items.

Selection of the trained panel members for the sensory evaluation of quick cooking food items

Selection of judges to evaluate the food items was done on the following criteria

(a) accuracy and consistency in identifying the sample in the triangle test and threshold test.
(b) availability throughout the experimental period.
(c) Willingness for evaluation.

Using these criteria 21 judges were selected to evaluate the food items. The selection was based on their performance for triangle test and threshold test.

Twenty-one individuals including the post graduate students and students from second and third year class from the Department of Foods and Nutrition participated in the initial trial. Based on their performance finally 15 judges were selected for evaluation of quick cooking food items.

Selection of sensory evaluation tools for trained judges

Hedonic rating test was selected for sensory evaluation of quick cooking food items. The Hedonic scale (Appendix III-D) of 1 - 9 points ranging from dislike extremely as score 1 to like extremely as score 9 was used for evaluating overall acceptability of the food items.

Stage II: Processing of cooked food items for various analyses:

In determining the overall composition of a food sample or content of a specific component, generally following steps are carried out:

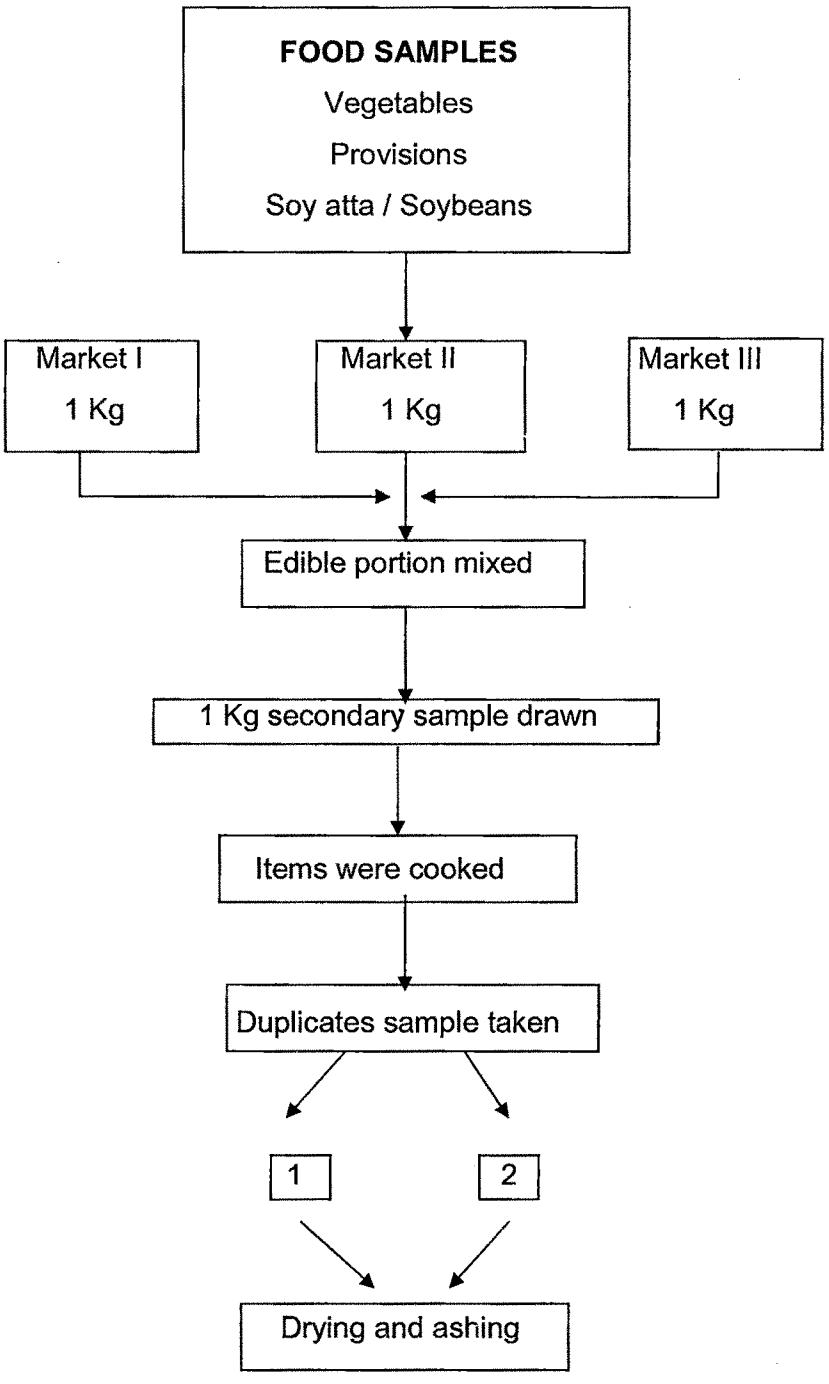
(1) Obtaining a representative sub-sample for analysis.
(2) Converting the components into a form that permits an assay.
(3) Performing the assay.
(4) Calculating and interpreting the data.

(1) Obtaining a representative sub-sample for analysis

The food samples namely vegetables; provisions and soy atta were purchased from 3 different markets of Baroda. One kilogram of each was pooled together to obtain a primary sample of three kilogram. Out of it a secondary sample of one kilogram was drawn. This was then used for the preparation of the items. The flow chart is depicted in Figure 3.3.

Figure 3.3

FOOD SAMPLING FOR ANALYSIS



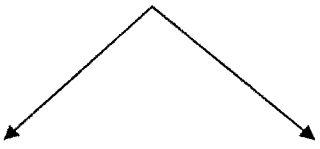
(2) Assay

The processing of items for various laboratory estimations was done as shown in the table 3.1.

Table 3.1

PARAMETERS FOR PROCESSED FOOD ITEMS

SEVEN ITEMS



Drying	Ashing
Protein Fat	Calcium Iron

Note: β–carotene was estimated in quick cooking food items only.

Processing of Items:

Different types of processing were used for the estimation of different analyses. The two processing methods that were used for the study were: **Drying** and **Ashing.**

Drying: Some of the nutrients were analyzed using the dry powder of the items. The items were dried in an air oven at 60-80 ^{0}C by uniformly spreading the items on an aluminium foil. For sambhar and usal, bowls made of stainless steel were used. The dry items were powdered in a stainless steel jar. The powders were packed in double sealed auto seal polythene bags and stored in a box with lid in the refrigerator. Suitable portions were taken for analysis of proximate principles (protein, crude fibre and fat).

Ashing: For the estimation of the mineral content of the items, 5 g of the dry powder in duplicates was accurately weighed into preweighed and preignited crucibles. The crucibles were put on a burner and heated over low flame till all the material was completely charred. They were heated in a muffle furnace at 600 C for 6 hours, cooled in a dessicator and weighed. This process of heating and cooling was repeated till two consecutive weights were identical and the ash was almost white or grayish in color. The ash content was calculated as follows for all the items:

$$\text{Ash (gm)} = \frac{\text{Ash content for 5 gm X dry weight of recipe (gm)}}{5}$$

Preparation of ash solution

The ash was moistened with 1 ml of deionized water and 5 ml of conc. HCL. Mixture was evaporated to dryness on a sand-bath. After evaporation, the process was repeated 2 times and the contents were evaporated again. Then 4 ml of conc. HCL was added with a few drops of deionized water and the solution was warmed over a boiling water bath. After cooling, the volume was made up to 100 ml with deionized water in a volumetric flask.

This ash solution was used for the estimation of minerals like calcium and iron.

(3) Performing the assay and calculations

This deals with the chemical analysis of the food items. All the estimations whether on dry food powders or on ash solutions, were carried out on two replicate samples. The details of the parameters and the methods used for each are given below:

A. Protein

Protein content of the food item was estimated using the standard micro kjeldhal method (AOAC, 1980).

Principle:

The procedures comprises of 3 steps, namely digestion, distillation and titration. Whereas digestion with strong acid converts organic nitrogen into ammonium sulphate, a strong alkali instantly liberates ammonia from ammonium sulphate. The

liberated ammonia is made to complex loosely with boric acid after steam distillation and condensation. The final step involves the titremetric estimation of basic ammonia by a standard using red bromocresol green mixed indicator.

Digestion:

In the digestion flask, 1 gm of the dry fat free powder (food sample) was taken to which 2.5 to 3.0 gm of catalyst mixture (copper sulfate and potassium per sulfate in the ration of 1:5) was added. To this about 15 ml of concentrated sulphuric acid was added and the digestion flask was placed in the digestion chamber. The digestion got completed in around 7-8 hours after which the digested contents were allowed to cool and then it was subjected to the distillation procedures.

Distillation:

To the digested contents about 15 ml of distilled water was added. From these mixture 10 ml of sample is taken in a distillation flask, to which 10 ml of 40% sodium hydroxide and 10 ml of distilled water is added and flask is made ready for 6-7 minutes of distillation (or till the color of the boric acid indicator changes). On other end of the distillation unit 10 ml of boric acid indicator is kept in a conical flask to trap the liberated ammonia. After steam distillation and condensation, the ammonia vapors liberated from ammonium sulphate, loosely complexed with boric acid indicator. At this point the color changed from maroonish red to bluish green.

Titration:

The flask containing the ammonia vapors was titrated manually against 0.1 N HCL until the color changed from bluish green to maroonish red. This titre value was used to estimate the nitrogen content in the food sample, after which the nitrogen content was multiplied by 6.25 factor to get the protein content per gm of food sample.

The protein content of the food items was calculated using the following formula:

$$\textbf{Protein content of the item (gm)} = \text{titre value (ml)} \times 1400\ \mu g \times \frac{1}{1000} \times \frac{1}{1000} \times \text{dry powder weight (gm)} \times 6.25$$

B. Fat

The fat content of the recipes was estimate by the SOXHLET method (AOAC, 1980).

Principle:

Fat is estimated as crude ether extract of dry material.

Estimation:

About 5 gm of the dry food sample was put into a pre-weighed fat free thimble. Diethyl ether was added to the weighed flask. This was attached to the butt tube containing thimble and the condenser. The unit was kept in a heating chamber and the temperature was maintained at 30 ^{0}C. The extraction procedure was run for around 8 hours. The soxhlet unit was dismentallled and the flask containing ether with crude fat was kept for evaporation on a hot plate at 35 ^{0}C and flask was weighed with fat only. The percent was determined by calculation.

$$\textbf{Fat (gm)} = \frac{\text{Crude fat in gms}}{5}$$

C. Crude fibre

This deals with the undigested fiber remains in the food sample that was estimate by the standard method given in AOAC, 1980.

Principle:

Dry food sample was subjected to digestion with dilute acid followed by dilute alkali. The difference in weight of the residue between the moisture and the ash is said to crude fibre.

Estimation:

Transfer a weighed amount of moisture and fat free food sample to a graduated beaker of 500 ml capacity. Very dilute acid (0.255 N sulphuric acid) was added till the mark of 200 ml and boiled for 30 minutes. The volume was made to remain constant by adding water gradually. After 30 minutes the content was cooled and drained through a muslin cloth. The residue was washed with warm distilled water to

make it acid free. Again transfer the residue to the same beaker and 200 ml of dilute alkali (0.33 N sodium hydroxide) was added. Content was boiled for 30 minutes in a similar manner, keeping the volume constant. Drain the residue and washed with warm water to make it alkali free. Finally the residue was washed with solvent (1:1, alcohol: ether) to remove fat if any. The remaining residue was transferred to a preignited and preweighed porcelein crucible. Crucible was kept for drying at 80 ^{0}C in a hot air oven for 3-4 hours. Weight of dry residue was taken and then the crucible was kept for ashing in the muffle furnace for 4-5 hours at 600 ^{0}C. On cooling weight of the ash was taken.

The difference in the weight of dry residue and the ash was considered as the crude fibre in the sample.

D. Iron

Iron content of the recipes was estimated by the method of Wong (Oser, 1980).

Principle:

Iron present in the sample gives a brick red color on reaction produces thiocyanin. The intensity of this color can be noted calorimetrically at 540 nm.

Estimation:

One ml of ash solution containing iron was taken in the test tube. To the sample, 0.5 m of potassium per sulphate (7%) and 2 ml of potassium thiocyanate (3N). The total volume in test tube was made up to 10 ml with deionized water. The tubes were mixed by inversion and the color developed was read within the next 30 min in the spectrophotometer, setting the instrument to zero with the blank at 540 nm.

Blank was prepared by taking 0.5 ml potassium per sulphate and 2 ml of potassium thiocyanate.

Aliquots of standard iron solutions prepared from crystalline ferrous ammonium sulphate, containing 10-50 μg ferric iron were also run simultaneously through the entire procedure. The readings of the unknown were compared with those of the standard solutions in order to obtain the concentration of iron in the unknown solutions.

The amount of iron present in the food sample was calculated as follows:

$$\textbf{Iron content of the food item (mg)} = \frac{\text{Reading of the sample}}{\text{Reading of standard}} \times \text{Concentration of standard } (\mu g)$$

$$\times 50 \times \frac{1}{1000} \times \frac{\text{dry powder wt (gm)}}{\text{weight of sample (gm)}}$$

E. Calcium

Calcium from the ash solutions was estimated by the method of Clarke and Collip (Oser,1980).

Principle:

Calcium was precipitated as calcium oxalate, then dissolved in an acid and titrated against potassium permanganate.

Estimation:

Estimation involves 2 steps: Precipitation and Titration.

Precipitation:

To 2 ml of ash solution, 2 ml of double distilled water was added followed by the addition of 1 ml of 4% ammonium oxalate. The mixture was vortexed for 1 minute and left overnight. Next day it was centrifuged for 5 min at 2000 rpm, the supernatant was drained off and the mouth of the centrifuge tube was wiped off with filter paper. Three ml of dilute ammonia (2%) was added to the precipitate and it was vortexed and recentrifuged. Washing with ammonia was repeated thrice to ensure complete removal of ammonium oxalate.

Titration:

To the ammonia washed precipitate, 2 ml of 1 N sulphuric acid was added and the tube was placed in a water bath (60-70 C) for 1 minute and titrated immediately against 0.01N Potassium permanganate till a definite pink color persisted for at least 1 minute. The reading of a blank consisting of 2 ml of 1 N sulphuric acid was subtracted from that of the sample.

Aliquots of standard prepared from fused calcium chloride, containing 0.5-2.0 mg were also run simultaneously through the entire procedure. The calcium content of the food item was calculated using the value obtained based on standardization.

*1 ml of 0.01 N $KMnO_4$ = 0.227 mg of Calcium.

Calculation:

$$\text{Ca content (mg/item)} = \frac{(\text{Titer value (ml)} - \text{Blank value (ml)}) \times 0.027^{*}\text{ (mg)} \times 50 \times \text{dry powder wt(g)}}{\text{weight of sample(g)}}$$

F. Beta-carotene

This method was estimated by the method given in AOAC, 1980. Method was used as described by Raghuramulu et. al., (1983).

Principle:

The individual carotenoids are separated on a column of calcium hydroxide or alumina and determined spectrophotometrically. The values for their respective vitamin A potency are used to arrive at the total vitamin A value of the foodstuff.

Estimation:

Ten gms of dry sample was taken in mortar pestle. To it 10 ml of acetone was added and crushed properly. A pinch of hydroquinone was added. After this 20 ml of petroleum ether (60-80 ^{0}C) was added. Again the content was crushed and the supernatant was collected in a lumber colored separating funnel. Washing of the residue was collected in the same manner till it becomes colorless. To this equal

volume of distilled water was added in the funnel and the two layers were separated by shaking. Water layer was separately taken out and green layer was again washed with around 1-1.5 liter distilled water. Water layer was totally removed carefully and a pinch of sodium sulphate anhydrous was added to the green layer. Now, a known volume was made with petroleum ether. On other side column was filled with activated alumina, which was washed with petroleum ether. To this 10 ml of sample was applied. Over it 10 ml of acetone (10% in petroleum ether) was applied (it helps to elute β carotene). After some time yellow color band was collected in brown test tube which was directly measured spectrophotometrically at 460 nm against blank as petroleum ether.

The obtained reading was calculated further as follows:

1 O.D. = 4μg / ml of β-carotene.

STATISTICAL ANALYSIS

For sensory evaluation the ranks of hedonic rating were converted to scores. The mean scores were calculated.

Nutritive value of cooked food items: Means and standard deviations of four replicates for the parameters were calculated. The percent retention of nutrients based on the raw ingredients was calculated.

PHASE IV

ASSESSMENT OF EFFECT OF SOY FOODS ON HEALTH AND NUTRITIONAL STATUS OF INSTITUTIONALIZED ELDERLY SUBJECTS.

The related objective of the study included

a) Assessment of dietary intake, nutritional status and health profile of the institutionalized elderly.
b) Intervention with soy foods for a period of three months after which various parameters for diet, nutrition and health profile were studied.

The details of the study design, selection of sample, parameters and the tools and the technique used for the data collection are presented under following sub-headings.

1. STUDY DESIGN

I) Sample Selection

II) Baseline Data

III) Soy Food Intervention

IV) Final Data

2. COLLECTION OF INFORMATION AND THE PARAMETERS STUDIED

I) Socio demographic profile

II) Activity pattern

III) Addiction pattern

V) Nutritional status

a) Anthropometric measurements

b) Dietary intake

V) Clinical parameters

a) Hemoglobin

b) Serum total protein

c) Lipid profile

d) Random blood glucose

e) Blood pressure measurement

V) Morbidity profile: Major and minor illnesses and psychological problems

3. TOOLS AND TECHNIQUES

a) Interview cum Questionnaire

b) Bathroom scale and measuring tape.

c) Set of standard cups and spoon

d) Sphygmomanometer

e) Cynmethemoglobin method

g) Glucometer

h) Enzymatic kits

STATISTICAL ANALYSIS

1. STUDY DESIGN

I) Sample selection:

All the occupants (20) were selected from Jalaram Vridh Ashram, Warasia, Vadodara. (This ashram admits people aged 60 years and above and most of them come from middle-income group).

The criteria for the selection of the subjects were:

✓ They should be residing regularly in the ashram.

✓ They should be willing to co-operate in providing information and taking the soy foods regularly for three months.

✓ They should be willing to provide blood for clinical findings.

II) Base line Data:

Basic information on socio demographic data, activity pattern, addiction pattern, psychosocial and health profile (free listing method), disease profile (checklist method) and morbidity profile for minor complaints was collected using a questionnaire.

Nutritional status was determined by anthropometric measurements and dietary intake, which was evaluated using 24hr dietary recall with respect to energy, proteins, fats, calcium, iron, β-carotene and vitamin-C. Clinical parameters included

hemoglobin, serum total proteins, random blood glucose and serum lipid profile. Biophysical parameter like blood pressure measurement.

III) Soy food intervention

1. Selection of food supplement:

Keeping in mind the health benefits of soybeans, food items prepared from it were selected as the food source that provides high quality protein with minimal saturated fat for supplementation. Four different food items were prepared namely soy usal, soy sambhar, soy dhokli and soy stuffed parantha along with soy roti.

Four food items were standardized and than supplemented to a group of ten free-living elderly for assessing its acceptability and find out if any digestive problems such as diarrhoea, constipation, flatulence and acidity occurred after consumption. The food items were acceptable and tolerable with no other complaints and therefore the four food items alternately along with soy roti were administered in old age home to elderly subjects and their acceptability was studied using Hedonic scale. Finally any one-food item was served each day to break the monotony in the diet.

2. Preparation of the soy food items: (see appendix-III-C)

3. Administration of food supplement:

Daily any one of the food items was prepared which consisted of ingredients stated before. Each subject was personally given a bowl of soy recipe, which contained 25gms of soybeans every morning at around 8:30 am in their breakfast. The investigator remained present till every subject consumed the full amount given. Soy roti was provided in two major meals of the day and served fresh.

4. Final data:

After six weeks of intervention the data was collected to see the impact of soybean and again on completion of three months post data was collected with respect to baseline data except socio demographic profile, activity pattern and addiction pattern.

The experimental design of the study is presented in figure 3.4.

Figure 3.4

EXPERIMENTAL DESIGN

INSTITUTIONALISED ELDERLY

(N=20)

Pre data collection on SES, activity pattern, health and nutritional status and morbidity profile

Supplementation of soy foods in the morning breakfast and soy roti in two major meals for 3 months

Post data collection after 45 days and at the end of 3 months of intervention

2. COLLECTION OF INFORMATION AND PARAMETERS UNDER STUDY

Development of tool:

A semi-structured interview schedule and a questionnaire to assess the quality of life and study effect of soy supplementation on health and nutritional status of institutionalized elderly were prepared. The tools were prepared in English and then translated into Gujarati for better comprehension by the respondents and ease in communication. The content validity of the tools with their sub-sections was established through feedback.

Collection of information and parameters under study were as follows:

I) Socio demographic profile:

A carefully planned Questionnaire was designed for obtaining all detailed information about the subjects. This included information on age, sex, education, income and martial status. Information was also collected regarding recreational activities.

II) Activity pattern:

The activities performed throughout the day by an elderly were noted and then they were classified into sedentary, moderate or heavy worker. The time spent in doing each activity was also noted.

III) Addiction pattern:

Addictions such as alcoholism, tobacco or pan/gutkha chewing and smoking were noted.

IV) Nutritional status:

a) Anthropometric Measurements:

These included heights, weights and mid upper arm circumferences. The methods used for each of the parameters are described under phase – I earlier.

b) Dietary intake:

Information on dietary intake was collected and analyzed as described earlier in phase–I.

V) Clinical parameters:

a) Hemoglobin levels:

Hemoglobin was estimated as per method described in phase - I.

b) Serum total proteins:

Total proteins were analyzed employing the Biuret method (Amino 1976) using the Glaxo Diagnostic kit.

Based on principle, Proteins bind with copper ions in an alkaline medium of the Biuret Reagent and produce a purple colored complex whose absorbance is proportional to the protein concentration.

c) Lipid profile:

Lipid profile included estimation of serum triglyceride, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol. The following procedures were used for estimating serum lipid profile.

i) Estimation of Triglyceride:

The TG were estimated by GPO/PAP method using enzymatic kit (Mc Gowan,1983).

Based of the principle the TG is hydrolyzed by lipase to glycerol and free fatty acids. Glycerol is phosphorylated by ATP in the presence of glycerol kinase (GK) to glycerol-3-phosphate (G-3-P) which is oxidized by the enzyme glycerol-3-phosphateoxidase (G-3-P-O) producing hydrogen peroxide which reacts with 4-aminoantipyrine and 3-5 dichloro 2-hydroxy benzene sulphonic acid (DHBS) in the presence of enzyme peroxidase (POD) to produce red quinoeimine dye. The intensity of the colour develop is proportional to the TG concentration in the sample.

Reactions

$$TG + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol + fatty acid}$$

$$\text{Glycerol + ATP} \xrightarrow{GK} \text{Glycerol – 3-P + ADP}$$

$$\text{Glycerol–3–Phosphate} + O_2 \xrightarrow{GPO} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{aminoantipyrine + DHBS} \xrightarrow{POD} \text{Quinoneimine} + H_2O$$

ii) Estimation of Total Cholesterol:

TC was estimated by CHOD-PAP method using enzymatic kit provided by Human (Flegg, 1973)

Principle of cholesterol is that cholesterol esters are hydrolyzed by cholesterol esterase (CE) to free cholesterol and fatty acids. Free cholesterol is oxidized by cholesterol oxidase (CO) to cholest-4-en—3-one and hydrogen peroxide. Hydrogen peroxide produced, couples with 4-aminoantipyrene and phenol in the presence of peroxidase to form a pink colored quinoneimine dye. The intensity of colour is proportional to the cholesterol concentration.

Reactions:

$$\text{Cholesterol ester} + H_2O \xrightarrow{CE} \text{cholesterol + fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CO} \text{Cholest} - 4 - \text{en - 3one} + H_2O_2$$

$$2\,H_2O_2 + \text{4amino antipyrine + phenol} \xrightarrow{POD} \text{Quinoneimie dye} + 4H_2O$$

iii) Estimation of High Density Lipoprotein –Cholesterol:

The VLDL and LDL fractions of serum sample were precipitated using phosphotungstic acid (Warnick et. al. 1982).

The supernatent was then used for HDL-C estimation by enzymatic kit as described in TC-estimation.

iv) Estimation of Low Density Lipoprotein – Cholesterol:

This was done by calculation method i.e. LDL-C = TC – (HDL+VLDL).

v) Estimation of Very Low Density Lipoprotein – Cholesterol

This was done by calculation method i.e. VLDL = TG/5.

d) Blood glucose estimation: Same as mentioned earlier in phase I.

e) Blood pressure: Same as mentioned earlier in phase I.

IV) Morbidity profile:

The proforma to study morbidity profile included questions regarding common illnesses and frequency of such illnesses experienced by them. Besides this a detailed checklist was also used which consisted of:

1. Problems of oral cavity.
2. Problems of digestive system.
3. Problems of hepato-biliary system.
4. Problems of pancreatic system.
5. Problems of respiratory system.
6. Problems of cardio vascular system.
7. Problems of genito-urinary system.
8. Problems of locomotor system.
9. Problems of neurological system.
10. Problems of endocrine system.
11. Miscellaneous problems.

Information on use of different prosthetic aids was also collected, which included hearing aid, walking stick, glasses, dentures and inhalers.

For 'Clinical interview for depression' (Amin, 1995) a questionnaire was used to learn various minor complaints and psychological problems in the institutionalized elderly.

STATISTICAL ANALYSIS

The data collected were subjected to various statistical tests using Statistical Package for Social Sciences. They were as follows:

1.Socio-demographic attributes, activity pattern and addiction pattern:

Percent responses were calculated for the different socio demographic variables, activity and addiction pattern.

2. Nutritional status:

a) Anthropometric measurements:

Mean and standard deviation was calculated for the anthropometric measurements such as height, weight, MUAC and BMI. Paired t-test was applied to assess the differences in the status before and after the intervention.

b) Dietary intake:

Mean and standard deviation was calculated for the nutrients such as energy, protein, fat, fibre, calcium, iron, β carotene and vitamin C. Paired t-test was applied to note the difference in the food intake at 0 month, one and half month and three months. Percent RDA for all nutrients stated above was also calculated to assess the differences in the status before and after the intervention.

3. Clinical parameters:

For various clinical parameters like hemoglobin levels, lipid profile, serum total protein, random blood glucose and blood pressure mean and standard deviations were calculated. Paired t-test was applied to note the difference in the values at 0 month, one and half month and three months. Percent prevalence of anemia, abnormal and normal blood glucose and blood pressure were also calculated.

4. Morbidity Profile:

The percent of subjects suffering from various diseases were calculated. Minor complaints and psychological problems before and after intervention were noted for each individual in a questionnaire and percentage of total number of individuals suffered from each complaint was reported.

Using above plan and methodology for the parameters described in this chapter, data was collected for each of the phases. Results are described and discussed in the following chapter.