CHAPTER II

MATERIALS AND METHODS
The experimental procedures adopted and the survey methods followed for the proposed study are outlined as under:

I. SELECTION OF THE SAMPLES

As mentioned the area which forms the kamar habitat is situated in Bindranawagarh and Dhamtari tahsils of Raipur district. Kamar habitat comes under the ITDP Gariaband which is comprised of four blocks Chhura, Gariaband, Mainpur and Nagri; the former three blocks are in Bindranawagarh tahsil and the last one is in Dhamtari tahsil. These blocks are continuous and constitute a compact area.

The samples were selected from different Kamar settlements of all the four blocks, using simple random sampling method. According to 1991 census the number of identified Kamar families was 2866 residing in 249 villages 10 per cent families that is 286 families from 32 villages were selected for the present study. (see in appendix) Out of 13,165 total Kamar population 1,355 persons were covered under this survey which consisted of infants, children, adolescents, adults and elderly people. As suggested by Jelliffe the adult subjects were divided into 3 age groups – Young adults (19-35 years). Adults (36-55 years), and Old adults (> 55 years) as depicted in Table No. 2. The subjects surveyed were homogenous in their character and way of living.

II. SOCIO-ECONOMIC SURVEY

Socio-economic survey was conducted on a pretested schedule with certain modifications (proforma-1) as described by NIN, Hyderabad. The Socio-economic details were assessed by oral questionnaire.

III. DIET SURVEY

Diet survey was conducted on a pretested schedule with certain modifications (proforma 2) as described by ICMR. The food intake for three consecutive days was assessed by oral questionnaire (24 hour recall), using standard measures.
a. FOOD CONSUMPTION

To determine their food consumption, raw food ingredients were measured by NIN standard cups. The weights were taken for three consecutive days in each household. Food consumed by guests was also included. The average consumption per consumption unit was finally analysed from food intake and nutritive value was calculated using food tables. Comparison with recommended allowances of ICMR (1990)\textsuperscript{111} was then made.

b. CALCULATION OF CONSUMPTION UNIT

The intake in terms of consumption unit or per person per day may be expressed as follows:

\[
\text{Intake per consumption unit/day} = \frac{\text{Total raw amounts of each food stuffs}}{\text{Total consumption units of the family who partake the meal} \times \text{Total number of days of the survey.}}
\]

\[
\text{Intake per person per day} = \frac{\text{Total raw quantity of each food stuff}}{\text{Total number of members in the family who partake the meal} \times \text{Total number of days of the survey.}}
\]

c. CALCULATION OF NUTRITIVE VALUE

The data collected through the diet survey were converted in terms of weight and tabulated. The food composition of the dietaries was computed from the standard tables (ICMR 1990)\textsuperscript{111} and comparison with recommended allowances was made.

d. SEASONAL VARIATION

Kamars are forced to change their foods, from season to season all throughout the year. This type of behaviour is the availability of governed
by food stuffs. So diet survey was performed in all the three seasons of the year, that is summer, monsoon and winter, to know the pattern of food consumption and the seasonal variations in food intake.

e. **COOKING PRACTICES**

The major food plants that require cooking include all of the grains, tubers, beans, plantains etc. All of these foods are important staples in various areas of the world. Cooking practices of the tribals are said to be unique. Some groups have mastered the technique of cooking many plant foods, such as tubers, by covering them with hot ashes. Cooking practices observed by the Kamars were also noted in the present study.

**IV. FOOD ANALYSIS**

Kamars due to their low economical status and non availability of edible food stuffs, sometime consume some types of food items which are not generally used by other human races. These rare food items taken by the Kamars are not very well known to the people in general.

Some rare food items, characteristic of Kamar diet, were analysed for their nutritive value

a. **ENERGY**

Energy was calculated by sum of physiological energy values of carbohydrate, fat and protein (at water bryant values).

b. **PROTEIN**

Protein content was estimated by Micro-Kjeldahl method as described by AOAC (1980).112

Principle

The estimation of nitrogen was done by Macro-Kjeldahl method. The
against standard acid. Since 1 ml of 0.1 N acid was equivalent to 1.401 mg N, calculation was made to arrive at the nitrogen content of the sample.

Reagent

1. Catalyst mixture: \( K_2\text{SO}_4 (100 \text{ g}), Cu\text{SO}_4 (20 \text{ g}) \)
2. Sodium Hydroxide (40%)
3. Sodium Hydroxide (0.1N)
4. Sulphuric acid (0.1N)
5. Mixed indicator: Bromocresol green (0.099 g), Methyl red (0.066 g), dissolved in 100 ml of ethanol.
6. Boric acid (2%)

Procedure

Plant sample, (0.5 g) has taken in digestion tube. 10 ml of concentrated \( H_2\text{SO}_4 \) and 2 g of digestion mixture (100 g of \( K_2\text{SO}_4 \) and 20 g of \( Cu\text{SO}_4 \), \( 5H_2O \)) were added and the material was digested in the digestion chamber. The digested material was transferred to 10 ml volumetric flask and volume was made up using distilled water. A 10 ml aliquot was pipetted into distillation apparatus and then stem distilled in presence of 10 ml of 40% NaOH solution (kjeldahl method).

The distilled ammonia was absorbed in 10 ml of boric acid (2%) containing mixing indicator. The Nitrogen was determined by titrating the collected distillate against \( H_2\text{SO}_4 \). Total protein was estimated by total nitrogen value multiplying with 6.25 will give crude protein content which includes non protein nitrogen.

c. FAT

Fat content was estimated in dry material by using a Soxhlet apparatus.\(^{114}\)

Principle

Oil from a known quantity of the food stuff was extracted with petroleum ether. (40-60\(^{\circ}\)C) It was then distilled off completely, dried, the oil weighed and the percentage oil was calculated.
Procedure

A piece of filter paper was folded in such a way that it could hold the food stuffs. The second filter paper which was left open at the top like a thimble was wrapped; a piece of cotton wool was placed at the top for evenly distributing the solvent as it dropped on the sample during extraction.

Then the sample packet was placed in the butt tube of the Soxhlet apparatus. After this the petroleum ether (150 drops/minute) was extracted for 6 hours without interruption by gentle heating. The extraction flask was allowed to cool and dismount. The ether was evaporated on water bath until no odour of ether remained and it was cooled at room temperature.

The dirt or moisture outside the flask was carefully removed and the flask was weighed. Heating, cooling and weighing processes were repeated until two constant weights were obtained.

a. MOISTURE

Moisture content was estimated by drying the material in an oven at 100 to 105°C and weighing it before and after drying (NIN, 1983).113

Procedure

About 10g of the material was weighed into a weighed moisture box and dried in an oven at 100 to 105°C and cooled in a desiccator. The process of heating and cooling was repeated till a constant weight was achieved.

\[
\text{Moisture } \% = \frac{\text{Initial weight} - \text{final weight}}{\text{Weight of the sample}} \times 100
\]

e. CRUDE FIBER

Crude fiber was estimated using the method described by NIN (1983).113

Procedure

About 2-5 g of moisture and fat free sample were weighed into a 500 ml beaker and 200 ml of boiling 0.255 N(1.25%w/v) Sulphuric acid was
added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals (a glass rod was inserted in the beaker for smooth boiling). At the end, the mixture was filtered through a whatman No 41 filter paper and the residue was washed with hot water till free from acid. The material was then transferred to the same beaker and 200ml of boiling 0.313N (1.25 percent) NaOH (keeping the volume constant as before), the mixture was filtered through whatman No. 41 filter paper. The residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We). The crucible was heated in a muffle furnace at 600°C for 2 to 3 hours, cooled and weighed again (Wa). The difference in weights (We-Wa) represented the weight of crude fibre.

f. ASH

Ash was also estimated using the method described by NIN (1983) for the procedure.

About 5-10 g of the sample was weighed accurately into a tarred porcelain crucible (which was previously heated to about 600°C and cooled). The crucible was placed on a clay pipe triangle and heated, first over a low flame till all the material was completely charred, followed by the heating in a muffle furnace for about 3-5 hours at about 600°C. It was then cooled in a desiccator and weighed. To ensure completion of ashing, the crucible was again heated in the muffle furnace for ½ hour, cooled and weighed. This procedure was repeated till the two consecutive weights arrived were the same and the ash was almost white or grayish white in colour.

g. ASCORBIC ACID

Vitamin C in the dietaries was estimated using 2, 6 dichlorophenol, endophenol dye. (NIN 1983) for the principle.

The blue colour produced by the reduction of 2, 6 - dichlorophenol indophenol by ascorbic acid is estimated colorimetrically.
Reagent

1. Acetate buffer, PH 4.0: 300g of anhydrous sodium acetate dissolved in 700 ml of water and 1 litre of glacial acetic acid were mixed.

2. Dye solution: 25 mg of sodium salt of 2, 6 dichlorophenol indophenol is dissolved in distilled water and it was made up to 200 ml.

3. 6% Metaphosphoric acid (HPO$_4^-$)

4. Ascorbic acid standard (1mg/ml)

Procedure

5 g of the sample was blended with 6% metaphosphoric acid to make 50 ml and 5 ml of the slurry was further diluted to 50 ml.

2.5 ml filtrate was placed in a 50 ml separating funnel (A). The same amount of the 6% metaphosphoric acid was taken in two more separating funnels (B) and (C). Funnel 'B' serves as the dye blank and to funnel 'C' which serves as a standard was added 0.1 ml (equivalent to 0.1 ascorbic acid) of the ascorbic acid standard solution. An amount of acetate buffer equal to the volume of the extract taken was then added to all three funnels, followed by 2ml of the dye solution. Xylene 10ml was then added quickly and the contents shaken for 6-10 sec. After the layers separate, the lower water layer was removed and the colour in the xylene extract was measured in a photoelectric colorimeter at 500 mm.

The ascorbic and contained in the amount of the extract taken for reaction with the dye in equal to $0.1 \left(\frac{b-a}{b-c}\right)$ mg

The ascorbic and content of the material can then be calculated by applying the necessary solution factors.

h. CARBOHYDRATE

The total per cent of the carbohydrates was determined spectrophotometer method by using anthron.
Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound with anthron forms a green coloured product with an absorption maximum at 630 nm.

**Principle**

**Reagent**

1. Hydrochloric acid (2.5 N)
2. Anthron reagent: 200 mg anthrone dissolved in 100 ml of ice cold 95% H₂SO₄. This reagent should always be prepared fresh before use.
3. Standard glucose: (a) Stock - 100 mg glucose was dissolved in 100 ml distilled water (b) Working Standard - 10 ml of stock was diluted to 100 ml with distilled water. It was stored in refrigerator.

**Procedure**

100 mg of the sample was weighed into a boiling tube. It was hydrolysed by keeping it in boiling water for three hours with 5 ml of 2.5 N HCl and cooled to room temperature.

It was neutralised with solid carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.1 ml aliquotes were taken for analysis and makeup volume 2 ml as standard. Glucose solution (Conc. $1 \times 10^{-2}$ g/ml) standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2 ml of the working standard. '0' serves as blank. The volume was made up to 2 ml in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added in samples and standard solution and heated for eight minutes in a boiling water bath. It was cooled rapidly, and the green colour was read at 630 nm.

A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph the percent of carbohydrate present in the sample was calculated.
I. MINERALS

The mineral content of the food stuffs was determined by spectroscopic atomic absorption.115

Principle

Representative sample in a suitable liquid form is sprayed into the flame of an atomic absorption spectrophotometer and the absorption or emission of the mineral to be analysed is measured at the specific wavelength.

Ash solution

The ash was moistened with a small amount of glass distilled water (0.5 - 1ml) and 5 ml of distilled HCl was added to it, the mixture was evaporated to dryness as before. 4 ml of HCl and a few ml of water were then added and the solution warmed over a boiling water bath and filtered into a 100ml volumetric flask using whatman No. 40 filter paper. After cooling, the volume was made up to 100ml and suitable aliquots were used for the estimation of Co, Cu, Mg and Zn.

Digestion for Ca and Fe

1g sample was moistened with distilled water and 10ml of concentrated HNO₃ was added. Then it was digested till dryness and again 5-10 ml of HNO₃ was added. It was then digested till syrupy. Again 5ml of HNO₃+5ml perchloric acid (HClO₄) was added and again digested till fuming. It was removed from hot plate and volume was made up to 100ml by distilled water.

RECOMMENDED INSTRUMENT PARAMETERS FOR MINERAL ANALYSIS

<table>
<thead>
<tr>
<th>Element</th>
<th>Lamp current</th>
<th>Fuel</th>
<th>Support</th>
<th>Wavelength nm</th>
<th>Spectral band pass nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
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<td>Acetylene</td>
<td>Air</td>
<td>240.7</td>
<td>0.2</td>
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<td>-!-</td>
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<tr>
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<td>-!-</td>
<td>285.2</td>
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<tr>
<td>Zn</td>
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<td>-!-</td>
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<tr>
<td>Fe</td>
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<td>-!-</td>
<td>-!-</td>
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</tr>
<tr>
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<td>-!-</td>
<td>nitrous oxide</td>
<td>422.7</td>
<td>0.1</td>
</tr>
</tbody>
</table>
V. STATISTICAL CONSIDERATION

a. Arithmetic mean

Arithmetic mean was calculated using the formula -

\[ x = \frac{\sum x}{n} \]

Where 'x' was the Arithmetic mean, \( \sum \) means the sum of, 'x' represented the measurements and 'n' represented the total number of individuals.

b. Standard deviation

\[ S.D = \sqrt{\frac{\sum (x-\bar{x})^2}{n-1}} \]

Where \( (x-\bar{x}) \) was the deviation from the mean, in which 'x' was the mean value and 'x' was the individual value. \( \sum (x-\bar{x})^2 \) represented summation of the squares of mean deviation and 'n' was the total number of individuals.