CHAPTER 3

MATERIALS AND METHODS

This chapter contents methodologies regarding,

3.1 Sampling techniques

3.2 Survey work

3.3 Standardization and preparation of recepies

3.4 Assessment of Nutritive values of recepies

3.1 SAMPLING TECHNIQUES

For this study, recepies which were commonly included in Gujrati Thali particularly in Saurashtra region meals were selected for the study. The recepies selected were main meal items, snacks and sweets which were generally consumed through out the day and in special occasions like festivals and other celebrations. The recepies also were selected in such way that involved different kinds of cooking methods like boiling, steaming, shallow frying, deep frying, pressure cooking, roasting, fermentation and germination. The recepies included foods from almost all food groups like, cereals, pulses, vegetables (leafy vegetable, roots and tubers and other vegetables) milk, nuts and oils and also combination of these.

A. Cereal products
   1. Main meal items
   2. Snacks
   3. Sweets

B. Pulse products
   1. Main meal items
   2. Snacks
3. Sweets

C. Vegetable
   1. Leafy vegetable
   2. Other vegetable
   3. Sambharas

D. Cereal + Pulse products
E. Cereal + Vegetable products
F. Pulse + vegetable products
G. Cereal + Pulse + Vegetable products
H. Milk + cereals products
I. Milk + Pulse products
J. Oil seeds products

As there is a great religious impact of Hinduism, Jainism and swaminarayanism on Saurashtra and Gujarat on their meal choices. A large majority of Gujarati (Saurashtra) people do not consume eggs, non veg. and see foods. These preparations were not included for this study.

3.2 SURVEY WORK

A questionnaire was designed which was than filled up by housewives of middle economic groups. This questionnaire contented name of products, ingredients and amounts, utensils and equipments used method of preparation and cooking time up to doneness for each product.

The questionnaires were then given to the middle income groups families of urban(45 families), semi urban(45 families) and rural areas (45 families) of all the seen districts of Saurashtra region. A set of classified recepies had been filled up from middle income group families by personal visits and an interview method.

There are seven districts in Saurashtra region, namely, Rajkot, Jamnagar, Junagadh, Porbandar, Bhavnagar, Amreli and Surendranagar. 5-5- forms of each recepies were filled up from District level, Taluka level and Village level respectively from each district of Saurashtra region.
The families were selected in such a way that they were natives of that particular district/Taluka/village respectively. The forms were distributed randomly among different caste people, who were originally vegetarians. After filling up the questionnaire data gathered were tabulate for ingredients amount and cooking method at that particular time for getting a standardized recepies those forms which were having very much different ingredients/ amount / cooking procedures were excluded for getting a generalized standard recepies because extremely different recepies if included would have given a different amounts and ingredients of that particular recepies than most of the Saurashtra people were consuming.

### 3.3 STANDARDIZATION AND PREPARATION OF RECEPIES

After getting a data from middle income families of Saurashtra region for each recepies a mean value for amount was derived. And a general procedure for a making recepies was also derived. By following a general method which was obtained from survey work a standard recepies was prepared by using mean value.
of ingredients. The standard recepie was prepared in laboratory. At the time of preparing standard recepie raw weight of edible portion of ingredients was measured. Water added for soaking and cooking was also measured. And after cooking final weight of cooked recepie was also measured. Time for cooking that particular recepie excluding pre-preparation was noted. Vegetables, fruits and milk products etc. purchased on previous day from local market. Necessary cleaning and washing of raw ingredients was done before cooking. Weight of edible portion only is taken as amount of ingredients. Freshly prepared samples of 100gm recepies with in one hour cooking were used for estimation of various nutritive values. Cooking water for preparing recepies was used drinking water supplied by municipality.

3.4 ASSESSMENT OF NUTRITIVE VALUES OF RECEPIES

Nutritive value of particular recepies was assessing by different methods and results are displayed as from 100gm of recepies. Freshly prepared samples of 100gm recepies with in one hour cooking were used for estimation of various nutritive values. Analysis of all the samples was carried out for moisture, protein, fat carbohydrates, carotene, vitamin C, calcium, iron and ash. Reagents used for all analysis were either AR or LR grade. Average of three determinations was taken in all analysis.

PROCEDURE USED FOR NUTRITIONAL ANALYSIS

1. MOISTURE (AOAC, 1970)

Sample (10 gm) were taken in dry Petridis and heated in oven (for about 24 hours) at 60°C till constant weight was obtained.
2. NITROGEN ANALYSIS BY MICRO-KJELDAHL METHOD (Oser, 1976)

Principle

The nitrogen in protein or any organic material is converted to ammonium sulphate by H₂SO₄ during digestion. This salt on steam distillation liberates ammonia, which is collected in boric acid solution and titrated against H₂SO₄.

Reagent

(1) Sulphuric acid,

(2) Potassium sulphate and copper sulphate (catalysts)

(3) Sodium hydroxide 50% solution

(4) Indicator solution: Methyl red 0.2g/100ml ethanol, methylene blue 0.2g/100 ml ethanol. For mixed indicator, two parts or methyl red solution were added to one part of methylene blue solution.

(5) Boric acid 2% solution

(6) Standard HCl or H₂SO₄, 0.02N

Procedure

The sample was weighed (0.5 g) and transferred to digestion flasks. Each of the catalyst (1 mg) was added and the samples were digested with 10 ml of concentrated sulphuric acid till the solution became colorless. After digestion the volume was made to 100 ml with distilled water. From this 10 ml of digested and diluted sample(s) was taken in Kjeldahl flask along with 10 ml of 50% NaOH and heated to liberate ammonia. The liberated ammonia was collected in a 100 ml conical flask containing 5 ml of boric acid solution with a few drops of mixed indicator. The flask was placed with the tip of the condenser dipping below the surface of the solution. The solution was titrated against the standard acid until the
first appearance of violet color, the end point. A reagent blank was run with an 
equal volume of distilled water and the titration volume was subtracted from that 
of sample titre volume.

**Calculation**

\[
\text{Protein} = \frac{\text{Titre reading} \times \text{Normality of H}_2\text{SO}_4 \times 0.28 \times \text{Volume made up} \times 100 \times 6.25}{1 \times 0.02 \times \text{aliquot} \times \text{sample taken} \times 1000}
\]

3. **ESTIMATION OF OIL** (Sadasivam and Manickam, 1991)

**Principle**

Oil from a known quantity of the seed is extracted with petroleum ether. It is then 
distilled off completely, dried, the oil weighed and the % oil is calculated.

**Reagent**

(1) Petroleum ether [40 – 60 C°]

(2) Whatman filter paper No. 2

**Procedure**

Standard procedure of Association of Official Analytical Chemistry (AOAC) 
(1970) was adopted for the determination of fats and oils. The sample 5.0 gms 
was weighed on the fat free paper and hanged firm on the upper portion on the 
extraction chamber gripped on its sides. The bottom flask was filled with ether. It 
was heated to nearly 4 to 6 hours depending upon the material. The thimble 
containing fat free material was carefully removed from the chamber. The residue 
was air-dried at room temperature and the dried material was weighed on after 
evaporation of ether from the extracted material and the percentage composition 
of fat were calculated.

\[
\text{Fat} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100
\]
4. **TOTAL CARBOHYDRATES BY ANTHRONE METHOD** (Sadasivam and Manickam, 1991)

**Principle**

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is converted into hydroxymethyl furfural. This compound reacts with anthrone to a green colored product with absorption maximum at 630 nm.

**Reagent**

1. 2.5 N – HCl

2. Anthrone reagent: Anthrone (200mg) was dissolved in 100 ml of ice cold 95% H$_2$SO$_4$. The reagent was prepared fresh before use.

3. Standard glucose: Stock – Glucose (100 mg) was dissolved in100 ml water. Working standard – 10 ml of stock was diluted to 100 ml with distilled water.

4. Sodium carbonate

**Procedure**

The samples (raw and cooked) were weighed (100 mg) into boiling tubes. HCl (2.5 N, % ml) was added and the tubes were kept in boiling water bath for 3 hours for hydrolysis. The tubes were cooled to room temperature. Sodium carbonate was added to the samples until the effervescence ceased. This neutralized the samples. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.1 ml aliquots were taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.5, 0.8, and 1 ml of the working standards. 0 served as blank. The volume was made up 1 ml in all the tubes including the sample tubes by adding distilled water. 4 ml anthrone reagent was added to all tubes. The tubes were heated for eight minutes in a boiling water bath. The contents of the tube were cooled rapidly and the dark green color was read at 630 nm.
nm. A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.

**Calculation**

\[
\text{Amount of carbohydrate present in 100 mg of sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100
\]

5. **ENERGY** (swaminathan, 1986)

The energy value of foods were calculated from their contents of carbohydrates, fats and proteins using the physiological energy values of 4 Kcal per gram of carbohydrate or protein and 9 Kcal per gram of fat.

6. **CAROTENE** (colorimetric method) (AOAC, 1970)

**Principle**

Carotene dissolves in n-butanol and solution absorbs energy at specific wavelength (435 nm).

**Reagents**

1) n-Butanol, 
2) Distilled water

**Procedure**

Use clean and dry glassware. Weigh 10.0 gm of sample in 150 ml. conical flask. Add 50 ml n-butanol and shake well for 5 minutes. Stand it for 15 minutes protected from sunlight. Again shake well and filter it through 12.5 cm folded Whatman no. 1 filter paper. Collect the filtrate in 100 ml flask. Measure absorbance (A) at 435 nm wavelength against blank as n-butanol on spectrophotometer.
Calculation

Calculate carotene content in ppm from standard curve or in absence of standard carotene from following formula.

\[ C = 5.0 \times \frac{A}{b \times k} = 30.1 \times A \]

Where, \( C \) = Pigment as carotene in ppm.
\( b \) = Cell thickness
\( k = 0.16632 \)

1 ppm = 0.1 mg

Ppm x 0.1 x 10 = mg in 100 gm sample

ppm = mg in 100 gm sample

7. ASCORBIC ACID (Sadasivam and Manickam, 1991)

Reagents

1) Oxalic acid 4%

2) Dye solution: Sodium bicarbonate (42 mg) was added to a small volume of distilled water. 2,6-dichlorophenol indophenols (52 mg) was dissolved in it and the volume was made up to 200ml with distilled water,

3) Stock standard solution: Ascorbic acid (100 mg) was dissolved in 100 ml of 4% oxalic acid (1 mg/ml).

4) Working standard: The stock solution (10 ml) was diluted to 100 ml with 4% oxalic acid. The concentration of working standard was 100 mcg/ml.

Procedure

To 5 ml of working standard, 10 ml of 4% oxalic acid was added and titrated against the dye (\( V_1 \) ml) till the appearance of pink color which persisted for few minutes. 5 gm sample was extracted in 4% oxalic acid. Volume was made 100 ml and centrifuged. 5 ml of the supernatant was pipette to which 10 ml of 4% oxalic acid wa added and titrated against the dye (\( V_2 \) ml).
Calculation

Ascorbic acid (mg) in 100g sample = \( \frac{0.5 \text{ mg}}{V_1 \text{ ml}} \times \frac{V_2}{5 \text{ ml}} \times \frac{100 \text{ ml}}{\text{ Wt. of sample}} \times 100 \)

8. ASH (AOAC, 1970)

Dried samples after removal of moisture were kept/ ignited in muffle furnace at 600°C until white ash was obtained. From the weight of the residue amount of total ash per 100g of the sample was calculated.

9. ASH SOLUTION

The ash (refer food analysis) is moistened with a small amount of glass distilled water (0.5-1.0 ml) and 5 ml of distilled hydrochloric acid is added to it. The mixture is evaporated to dryness on a boiling water bath. Another 5 ml of hydrochloric acid is added again and the solution evaporated to dryness as before. Four ml of hydrochloric acid and a few ml of water are then added and the solution warmed over a boiling water bath and filtered into a 100 ml volumetric flask using Whatman No. 40 filter paper. After cooling, the volume is made up to 100 ml and suitable aliquots are used for the estimation of phosphorus, iron and calcium.

10. CALCIUM

Principle

Calcium forms complex with EDTA at definite pH

Reagents

1) 0.02N EDTA
2) Buffer
3) Eriochrome black – T
Procedure

Fill the burette with 0.02N EDTA solution up to zero mark. Take 10 ml of ash solution of sample in conical flask. Add 2 ml of 0.01N NaOH as buffer solution and 4 to 5 drops of Eriochrome black – T indicator. Titrate it against 0.02N EDTA solution, the color change will be wine red to blue. Note the burette reading and repeat it twice or thrice.

Calculation

1N 1000 ml EDTA = 40,000 mg calcium

1N 1 ml EDTA = 40 mg calcium

0.02N 1 ml EDTA = 40 X 0.02 mg calcium = 0.8 mg calcium

0.02N A ml EDTA = 0.8 X A mg calcium in 10ml of ash solution sample.

11. IRON (Wong’s method) J. Biol. Chem. &:&409(1928).

Principle

Iron (Fe) is determined colorimetrically with ferric iron which gives a blood red color with potassium thiocynate.

Reagents

1. 30% H₂SO₄
2. 7% Potassium persulphate solution
3. 30% Potassium thiocynate solution: 40 g KCNS is dissolved in 90 ml glass distilled water, 4 ml acetone is added and the volume made up to 100 ml.
4. Standard iron solution: 702.2 mg ferrous ammonium sulphate is dissolved in 100 ml glass distilled water and after addition of 5 ml of 1:1 HCl, the solution is made up to 1 L and mixed thoroughly (0.1 mg Fe/ml). The standard solution is prepared fresh once in 6 months.
5. Working standard solution (10 µg Fe/ml) is prepared by diluting the above solution 10 fold.
Procedure

Food iron

To an aliquot (6.5 ml or less) of the mineral solution (Reer food analysis, enough water is added (if necessary) to make up to a volume of 6.5 ml followed by 1.0 ml of 30% H$_2$SO$_4$, 1.0 ml potassium persulphate solution and 1.5 ml 40% KCNS solution. The red colour that develops is measured within 20 min at 540 nm.

Calculation

$\text{g Hemoglobin} = 0.348 \times \text{mg Fe}$

NOTE

If use of reagent containing traces of iron cannot be avoided, it should be seen that the final solutions of standard and test contain identical quantities of these reagents. Potassium thiocynate should be added just before taking the readings.