CHAPTER III

MATERIAL AND METHODS
CHAPTER - 3

MATERIALS AND METHODS

This particular chapter deals with materials which were found to be of great utilization. Again, the methodology of manufacture that faced with the odds on such a modification was affected as per detailed requirements are as follows:

3.1 SELECTION OF THE RAW MATERIALS:

3.1.1 WHEY:

The whey used in research programme was cow milk whey, which was obtained as a by-product of paneer making. The freshly drawn cow's milk used for Paneer making was obtained from the U.P. College dairy farm. The milk whey thus obtained was transferred to the laboratory for standardization and further processing.

3.1.2 STABILIZERS:

For this purpose the following stabilizer of different grades were used.

1. Pectin.
2. Carboxy-Methyl- Cellulose (C.M.C.) (Low viscosity)

As far as possible the stabilizers in their present form were obtained from the open market.
3.1.3 Sugar:

White crystal sugar of commercial value was obtained either from the departmental store for open market. It was ensured that sugar be free from all impurities like dust etc. special emphasis was laid on purity of sugar.

3.1.4 Acids:

Organic acid were used in trials. Citric acid displayed the best results. It was used in all the trials including the manufacture of the beverage on the large scale.

3.1.5 Flavour:

During the course of investigation the following edible grade flavours were used keeping in view the preference and availability of drink.

(1) Rose  (2) Vanilla  (3) Orange.

These flavours were obtained from the open market by insuring their market value. Flavours after their use were secured under refrigeration to increase their qualities.

3.1.6 COLOURS:

Edible grade orange colour was used. It is very difficult proposition to measure the liking of general masses about a specific colour as people get used to a specific intensity in a product.
3.1.7 PRESERVATIVES:

The given preservatives of different grades were used for the purpose:—

(i) Benzoic acid

(ii) Sodium benzoate

The preservatives in their purest form, as far as possible, were obtained from the open market.

3.1.8. SODA-BI-CARB:

Soda-bi-Carb was used to ascertain the keeping quality of soft drink. It had been obtained from the departmental store.

For keeping quality of soft drink the Soda bicarb was used which, was obtained from departmental store.

3.2 EQUIPMENTS:

3.2.1 For heat treatment an important and improved method was used due to absence of the HTST pasteurizer in the laboratory. The milk whey was heated up to boiling temperature and immediately cooled to room temperature.

3.2.2 Autoclave:

A laboratory autoclave to sterilize-bottle/batch under a pressure of 15 lbs/inch² with concomittant temperature of C, 121°C for 15 min, was used for sterilization of the acidified softmilk beverage-“whey soft drink”
3.2.3 Packaging Materials:

200 ml. sterilizable glass bottles and crown corks (for sealing of the bottles) were used for filling of the ready product. The bottles after filling were properly corked using a corking machine followed by desired labeling.

3.3 METHOD OF MANUFACTURE:

For the manufacture of directly acidified soft milk beverage of the type envisaged in this study, various parameters were taken into consideration which included proper standardization of the method for an effective process of manufacture. It was but natural that the procedure adopted involved modification and changes in respect of pH levels, sugar percentage, stabilizer rates, milk solid concentrations, types and rates of flavours, a highly compatible colour and amount of soda-bi-carb to go with the specific market demand.

3.3.1 Standardization of whey:

Purified water was added to the milk whey at different levels to retain predetermined total solid levels for optimisation of suitable blends.

3.3.2 Mixing of the Sugar and stabilizer:

Standardized milk whey was heated to 70-75° C. Sugar as mentioned earlier, was added to the milk gradually with constant stirring. Again, a calculated amount of the stabilizer selected for the purpose was added under constant stirring to ensure its proper mixing.
Fig. 3.1 Flow diagram for the manufacture of acidified soft drink beverage 'Whey-Soft Drink'.

Whey, obtained from Paneer making

Dilution by water (3% T.S.)

Pre-heating at 75°C

Mixing

Filtration

Cooling at 5°C ± 2°C

pH adjustment (4.5) by 10% Citric Acid Solution

Heating at 75°C

Mixing

Bottling in 200 ml capacity

Sterilization at 121°C for 15 min.

Cooling at Room Temp.

Final Product Whey soft drink

Addition of Sugar 10% CMC stabilizer 0.4%

Addition of Benzoic Acid 0.5%

Addition of Orange Flavour 0.5 ml/l Orange Colour 0.5 ml/l Soda-Bi-Carb 0.1%
3.3.3 Addition of preservatives:

As per plan, preservatives viz, Benzoic acid and Sodium benzoate were added to the mixtures in appropriate amount to enhance the shelf-life of the product.

3.3.4 Acidification and Cooling:

The mixture thus found after addition of the Preservatives was cooled to 6-8°C and its pH was adjusted to the predetermined levels through additions of 10% cetic acid solution added gradually with constant but slow stirring. The acidified mixture was then kept aside at low temperature over a period of 30 min.

3.3.5 Mixing of flavour and colour:

The required concentration of liquid flavours and colour were added to the mixture to develop appropriate flavour and colour intensities prior to filling of the contents in bottles.

3.3.6 Mixing of the Soda-bi-carb:

Soda-bi-carb was added before filling the soft drink in bottles to enhance the foaming and to maintain the keeping quality of the product. After that the soft drink bottles were stored at refrigeration temperature.

3.3.7 Packaging and sterilization:

The product was filled in 200 ml capacity clean and sterilised glass bottles which were eventually sealed with crown corks using a corking machine. Propely sealed bottles were transferred into an autoclave exercising due precautions and sterilized using a pressure of 15 lbs/inch$^2$ at 121°C for 15 min.
3.3.8. Cooling:

The sterilized bottles were later on further cooled gradually to room temperature (30°C). Some of the bottles as per plan were secured at refrigeration temperature (5°C).

3.4 OPTIMISATION OF THE PROCESS:

The final index of the quality attributes of a product rest with the opinion of the clientele. In the present study, a penal of five judges having expertise in the field was drawn to stamp their approval and/or suggest measures to modify/improve the product. The parameters involved were to be optimised through trial. Detail of the process involved is described in the following paragraphs:

3.4.1 Optimization of total solid, pH, Sugar levels.

A series of laboratory trials were conducted to optimise suitable pH, proper total solids and the appropriate sugar concentration so as to evolve a product of appreciable acceptability. For the manufacture of soft beverage envisaged during the current studies, it was necessary to reduce the Total solids (T.S.) level of whey through addition of pure water. Three levels of T.S. viz. 3.0, 4.0 and 5.0 were computed.

Acidification of the beverage was carried out through acid solution with the objective to maintain three pH level viz, 4.0, 4.50 and 5.0. Further three sugar concentrations i.e. 8,10 and 12% were tried for the purpose. The combinations involved in respect of these parameters are shown in table 3.1.
Table 3.1: Showing various pH, T.S. and Sugar levels during the investigation:

<table>
<thead>
<tr>
<th>pH level</th>
<th>Total solids (T.S.) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>Sugar %</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>8</td>
</tr>
<tr>
<td>4.5</td>
<td>8</td>
</tr>
<tr>
<td>5.0</td>
<td>8</td>
</tr>
</tbody>
</table>

On the basis of three extensive trials conducted in the laboratory, the most suitable combination was obtained based on the judgments in respect of sensory scores as revealed by the experts.

3.4.2 Selection of Stabilizers

We had conducted laboratory trials to bring the best of suitable types and the levels of stabilizers on the basis of their impact on protein-stability body and texture and consistency of the product for this purpose the types of stabilizers used and their levels are details as under:

<table>
<thead>
<tr>
<th>Name of the stabilizers</th>
<th>Concentration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pectin</td>
<td>0.2, 0.4 and 0.6</td>
</tr>
<tr>
<td>Carboxy methyl cellulose (CMC) (Low viscosity)</td>
<td>0.2, 0.4 and 0.6</td>
</tr>
</tbody>
</table>
The best type of stabilizer and its appropriate level was selected on the basis of protein stability of the final during sterilization along with its status of viscosity.

### 3.4.3 Selection of Preservatives:

For the preparation of soft drink milk beverage the preservatives and the levels that we tried are as:

(i) Benzoic acid - 0.05 and 0.10 ml/100 ml.

(ii) Sodium Benzoat 0.05 and 0.10 ml/100 ml.

Final solution of the source and the level of the preservative was made on the basis of sensory evaluation and the resultant keeping quality as judged by a panel of experts after manufacture and during the storage.

### 3.4.4 Selection of Flavours

These flavours were tried at two levels for the preparation of “Whey Soft Drink”

(i) Rose 0.5 and 1.0 ml/litre

(ii) Vanilla 0.5 and 1.0 ml/litre

(iii) Orange 0.5 and 1.0 ml/litre

The panel of experts selected one of the best flavour with concentration on the basis of acceptability of the flavour intensity.

### 3.4.5 Selection of Colour

Orange colour with two concentrations viz 0.5 and 1.0 ml/litre was used for the purpose suitable concentration was finally arrived on the basis of sensory scores.
3.4.6 Selection of soda-bi-Carb

Soda-bi-carb was used with three concentrations viz. 0.1, 0.3 and 0.5 percent. It is also based on sensory evaluation and keeping quality of the product.

3.4.7 Use of packaging materials

Glass bottles of 200 ml capacity were used for the purpose of dispensation. The bottles were crown corked using a corking machine followed by necessary labelling.

3.4.8 Sterilization of the product - optimisation of Temperature / Time relationship:

Suitable temperature-time combinations in relation to the basic objectives of getting 'No bacteria' drink were arrived through trials and errors. The final temperature-time combination was computed to ensure the complete destruction of micro-organisms commensurate with maximum stability of the product with least viscosity or sluggishness in consistency.

3.4.9 Keeping quality of the beverage

Studies were undertaken to observe the shelf-life of the product in relation to storage temperatures and time. The prepared samples stored to two temperatures viz.,

(i) 30°C ± 2°C (Room temperature)
(ii) 5°C ± 2°C (Refrigeration temperature)

For 15 days, 30 days, and 45 days.
The stored product was analysed at regular interval of fifteen days for various physico-chemical properties as also for their sensory evaluation.

It may be worth while to maintain that quite a large number of factors affect the keeping quality of products particularly those in liquid form. As such systematic enquiries were undertaken to evaluate various changes i.e. sensory, physico-chemical and microbial in the product at different intervals of time.

3.5 Quality Evaluation:

Acidified beverage 'Whey-soft drink samples were analysed for:

3.5.1 Fat content:

In the light of Garber's method fat content of whey soft drink was determined.

10 ml. of sulphuric acid (sp. gr. 1.825) was taken in the clean and dry skim milk butyrometer and to this was added 10.75 ml. whey (which was measured by 10.75 ml pipette). Then put 1 ml of amyl alcohol (sp. gr. 0.825). To makeup the volume of skim milk butyrometer, few ml of distilled water was added as required. Rubber stopper was inserted after drying the neck with the help of chalk. The tube was shaken by holding it by the stem, until content mixed well. Buryrometers were then placed in an electric centrifuge for 4-5 minutes at a speed of 1000-1400 rpm. The centrifuge stopped and the tube was transferred to a water
bath maintained at 70°C. After 5 minutes the reading was noted on graduated stem of the butyrometer.

### 3.5.2 Total solids

The total solids content of the beverage samples was determined on following the methods prescribed under Indian Standard IS : 1479 (Part II, 1961). About 15-20 gm of washed dry sand was taken in a clean dry procelain dish and transferred to an oven maintained at 102°C ± 1°C to a constant weight. 5.0 ml aliquot of the beverage was evenly and uniformly spread on the sand to ensure maximum surface exposure. After weighing the contents precisely the dish was placed on a vigorously boiling water bath for 40-45 minutes. After partial evaporation the dish was transferred into an oven maintained at 90-100°C over a period of 3h. Later, the sample was placed in a dessicator, cooled and weighed. The process was repeated, if necessary till the difference in weights was reduced to less than 5 mg. the least weight obtained was used to calculate the percentage of total solids following the formula:

\[
\text{Total solid Wt. of residue after drying} = \left( \frac{\text{percentage by wt.}}{\text{Wt. of the sample taken}} \right) \times 100
\]

### 3.5.3 pH

pH values of the product both during its process development and storage intervals were determined using pH meter.
3.5.4 Total protein:

The total protein content of the sample was determined by the method of Menaffe and Overmann (1940) with slight modification. An accurately weighed (0.5 gm) sample was transferred into a 300 ml. Kjeldahl Flask and digested after addition of 2.0 gm sodium sulphate, 0.14 gm mercuric oxide and 5ml. conc. sulphuric acid for 45 minutes or till the mixture was free of carbon. After cooling the content of the flask, 50 ml Nitrogen free distilled water was added. The ammonia from the sample was distilled off after adding 15 ml. (50 percent w/v) sodium hydroxide solution. The distillate was collected into a 25 ml saturated boric acid solution containing 4 drops of mixed indicator (made by dissolving 100 gm methyl red and 30 gm Methylene blue in 60 ml of 95 percent ethyl alcohol and then making up the volume to 100 ml with distilled water). Nearly 60 to 70 ml distillate was collected in 100 ml conical flask. The contents of the flask were titrated against - 0.02 N sulphuric acid. A blank was run simultaneously using distilled water in place of the sample, the total Nitrogen and the percent protein were calculated as:

\[
\text{Percent Nitrogen} = \frac{(A-B)N \times 14.0 \times 100}{W}
\]

Where,

\[A = \text{ml sulphuric acid required for sample}\]
\[B = \text{ml sulphuric acid required for blank}\]
\[N = \text{Normality of sulphuric acid used}\]
\[W = \text{Weight of the sample in mg}\]

Percent protein = Percent total nitrogen x 6.38
3.5.5 Total spore count

Total spore count of the acidified soft-drink beverage 'Whey-soft-drink' was carried out in the department under ideal conditions. The method followed is given here under:

Composit beverage sample was thoroughly mixed by inverting it 25 times in a specific fashion. Sterilized water added to the sample to get the following dilutions:

(i) 1 in 10 - 90 ml water + 10 ml Sample

(ii) 1 in 100 - 90 ml Water + 10 ml of (i)

(iii) 1 in 1000 - 90 ml Water + 10 ml of (ii)

These liquids were thoroughly but gently mixed avoiding any vigorous shaking. One ml aliquot from each dilution was put in a sterile petridish (4" diameter) with the help of a sterile pipette. Melted agar media of specific composition and cooled at 45°C was added to each of the petriplates and mixed with the sample thoroughly through a rotating motion.

After getting the media solidified the plate were incubated in an inverted position over a period of 48 h at 30°C. The number of colonies was counted in each plate and their mean number recorded. Calculation for the number of spores/ml of beverage was computed by multiplying the dilution factor by the number of colonies observed.

3.6 SENSORY EVALUATION:

The sensory assessment of beverage samples was carried out by a panel of five Judges. Scoring of the samples was done on a 9-
point Hedonic scale as recommended by Peryam and Pilgrim (1957). The samples were evaluated for various selected sensory parameters like flavour, body and texture, colour and appearance and overall acceptability.

3.7 ACCEPTABILITY OF THE SOFT DRINKS BY THE CONSUMERS:

Consumer's acceptability trials were conducted at public places within the University Campus. The beverage was served to the consumers selected at random. A questionnaire with premonitored parameters was provided to each consumer selected to list his/her order of preference by placing a tick mark (✓) on the questionnaire.

3.8 STATISTICAL ANALYSIS:

As mentioned earlier, the samples approved by the panel of experts were finally prepared on a large scale and exposed for public exceptions. The data regarding physico chemical properties of the acidified soft milk beverage sample collected both during the developmental process and during public acceptability trials were statistically analysed and analysis of variance (ANOVA) was estimated for each parameters.

3.9 COST OF PRODUCTION OF THE SOFT DRINKS:

Although, it is difficult to arrive at tangible conclusions in regard to the cost estimations, yet it was though desirable to arrive at approximations. The cost, it may be emphasised, shall
depend to a large extent, on the scale of production, raw material cost processing expenses and the cost of packaging etc. Since, whey was obtained as a by-product of paneer, therefore, the production cost of Whey was considered as nil. An attempt has however, been made to computed the cost of production on a semi-commercial scale based on certain assumptions.

It is very difficult to come to a conclusion regarding the cost estimations. It was easy to arrived approximations. The general impression is that cost depends upon the scale on production, the cost of raw material processing expenses and the cost of packaging.