CHAPTER III

MATERIAL & METHODS
MATERIALS AND METHODS

TECHNICAL PROGRAMME:

3.1 The present investigation involved preparation of WPC-70 from good quality buffalo milk and optimize the energy requirement for spray drying. This chapter encompasses details of materials and methodologies employed in the experiment.

3.2 Various Phases Of The Experiment

The experiment was divided into five phases.

3.2.1 Standardization of buffalo milk and its separation.

3.2.2 Coagulation of skim milk and collection of wash water and whey.

3.2.3 Clarification & Pasteurization of whey and feed to UF plant.

3.2.4 Spray drying of WPC-70 with single stage, double stage and three stage

3.2.5 Storage effect on shelf life at different temperatures.

3.3 Raw Materials

3.3.1 Various raw materials used during the course of this study, along with their sources, are delineated here under.
3.3.2 **Buffalo Milk:** This was obtained from various village level collection of Kosi-Kalan distt. Mathura (UP) and used for making WPC-70. (Described in section 3.4.1).

**Skim Milk:** Buffalo milk was separated at 45-47°C in Westfalia separator AG (Type MSA 160-01-076) cream separator. The skim milk (about 0.1 percent fat by Mojonnier method) so obtained was heated to 75°C/15 sec.

### 3.4 Method Of Preparation Of WPC-70:

3.4.1 Buffalo milk after preliminary treatments at village level collection, received at raw milk reception dock and after segregation, good quality milk was collected separately.

3.4.2 **Collection, Filtration And Chilling Of Milk:** Fresh, sweet, good quality of buffalo milk was collected at dock and filtered through 100 mesh clothes and chilled to 4°C temperature and stored in raw milk tank.

3.4.3 **Pasteurization Of Skim Milk:** The milk was then heated to (45-47°C), filtered and separated through Westfalia separator AG (Type: MSA 160-01-076; No. 1665 307.) and skim milk so obtained was pasteurized (75°C/15 sec.) and cooled 4°C and stored in skim milk silo having capacity 70m³.
3.4.4 Whey Preparation:

3.4.4.1 Skim milk was preheated to 35°C and acid (12 percent HCl) dosed till coagulation at pH 4.6 was obtained. Coagulated materials were heated to 45°C through tubular heat exchanger and passed through holding tube (5 minutes) and decanted through Decanter (Make Westfalia separator AG, (Type: CA365-01-00, No.8000 033) for separation of whey and curd. Whey was collected in a tank and mixed with wash water obtained from counter washing of curd.
Receiving Buffalo milk (Raw)

↓

Filtration

↓

Preheating (45-50°C)

↓

Separation

↓

Skim Milk

↓

Pasteurization (75°C/15 sec.)

↓

Cooling

↓

Evaporation (5 effect)

↓

Concentration (40-42% TS)

↓

Spray Drying

↓

Skim Milk Powder

↓

Standardization (8.5% SNF)

↓

Skim Milk (Fat % - <0.05% by Gerber or 0.11 % by Mojonnier)

(SNF % - 8.50% Gravimetrically)
Fig 3.1: Flow Diagram for preparation of skimmed milk.

SKIM MILK
↓
Preheating (35°C)
↓
Acid dosing (12% HCl)
↓
Heating 45°C
↓
Decantation
↓
Casein + Wash water
↓
Sieving (150-Mesh clothes)
↓
Wash Water
↓
Casein + Whey + Wash water
↓
Clarification
↓ NaOH dosing @6% strength
↓
Pasteurization at 72-74°C / 15 sec
↓
Cooling (16°C)
↓
Storage tank (pH 5.2)
Fig 3.2: Flow Diagram for preparation of whey.

Whey (16°C)
↓ Duplex filter (35 Micron)
↓
U.F. Plant (5 stage)
↓
WPC-70 (% TS = 21)
↓
Cooling (< 2°C)
↓
Storage tank
↓ pH adjusted 6.4 to 6.6 by KOH (8-10 percent)
Heating (59°C / 15 sec)
↓
Concentrate vat
↓
Homogenizer (100 kg/cm²), (25 kg/cm²)
↓
Spray Drying (Inlet temperature, 182°C)
↓ Stage Drying (Outlet temp., 97°C)
↓
Shifter
↓
Filling
↓
Stitching
↓
Bagging
↓
Storage

Fig 3.3: Flow Diagram for preparation of WPC-70
Clarification Of The Whey:

3.4.4.1.1 The whey was fed to clarifier (Make: Westfalia separator AG, Type: MSD-60-96-076, No.1711888) at 44-45°C and then after 6 percent NaOH dosing carried to bring the pH of whey to 5.2.

3.4.4.2 Pasteurization Of Whey:

3.4.4.2.1 Whey at pH 5.2 was pasteurized to 72-74°C for 15 seconds (Through preheat exchanger) and cooled to 16°C through regeneration process and then stored in 20m³ tank capacity. as shown in Fig.3.2

3.4.5 Whey Protein Concentrate-70 Percent (WPC-70%):

3.4.5.1 Whey in section (3.4.4.2.1) at pH 5.2 and 18-20°C temperatures was fed to ultrafiltration plant (UF) (Make: Filtration Engg. Co. Inc. Champlin, Minnesota 55316 USA).

3.4.5.2 Ultrafiltration (UF) Plant:

3.4.6.2.1 UF Plant consisted of five stages and each stage contains eight vessels. Whereas each vessel contained four membranes (Make: Sinder, USA). However the mil spacer was 65; 45; 45; 31 and 31 of fifth; fourth; third; second (A&B) and first (A&B), respectively. The membrane areas were 141.44; 236.8; 236.8; 275.2 m² of fifth, fourth, third, second and first stages, respectively. The
UF unit consisted of five stages and each stage had its own mass balance during running of plant. During feeding of whey, it entered in fifth stage and made mass balance as per concentrate/ feed ratio valve. As the baseline pressure reached to > 40 Psi, next (fourth) stage opened and pressure reduced. However the mass balance started in fourth stage. Similarly as and when pressure reached > 40 Psi immediately next (third) stage opened and pressure reduced on third stage, mass balances circulation started. In the same way pressure touched > 40 Psi, second A stage opened and on increased pressure stage first A opened; on 40 Psi, second B stage and at last stage first B opened and mass balance each stage continue till the proteins level reached to 70 percent. Temperature of each stage increased by 1-2°C because of friction losses and maintained by tubular heat exchanger provided in each stage.

3.4.6.3 Fractionation:

3.4.6.3 Whey protein concentrate increased to protein level after circulation through each stage and permeate get separated out. Whey protein concentrate purified by addition of reverse osmosis water (hardness < 20 ppm and TDS - 70 ppm) through stage third, fourth, and fifth. Addition of
R.O. water was approximately 27 percent of feed. However the retentate obtained 4-5 LPM on 215 - 220 LPM feed.

3.4.6.4  Retentate:

3.4.6.4.1 Retentate obtained was cooled to 2°C through chiller having capacity of 600 liter/hour and stored in S.S. 316 tank.

3.4.6.5  pH Adjustment

3.4.6.5.1 pH of retentate was adjusted with 8-10 percent KOH solution up to 6.4-6.6 pH in the storage tank manually. Homogeneous mixture was made by agitator.

3.4.6.6  Heating Of Retentate

3.4.6.6.1 Retentate was heated to the various temperatures 55-70 deg C for 15 seconds through tubular heat exchanger and collected in concentrate vat.

3.4.6.7  Homogenizer

3.4.6.7.1 Retentate at various temperatures was homogenized in cleaned and sanitized homogenizer (Double stage, Make: Gaulin, Denmark) at 100 kg/cm² first stage and 25 kg/cm² at second stage and fed directly to the spray dryer.
3.4.6.8 SPRAY DRYING

1. High Pressure Pump
2. Feed Flow
3. Spraying Nozzles
4. Inlet Air Fan
5. Air Heater (Radiators)
6. Drying Air
7. Drying Chamber
8. Primary Cyclone I
9. Primary Cyclone II
10. Exhaust Fan
11. Integrated Fluidized Bed
12. Pressure Conveying System
13. Vibrofluidiser Air Fan
14. Vibro Fluidized Bed
15. Fine Recirculation System
16. Rotary Valve
17. Powder
18. Vibro Cooling Fan
19. Vibro Exhaust Fan

(Fig.: Three Stage Spray Dryer with recirculation System)

3.4.6.8.1 Spray drying was done in three-stage dryer (Make: S.S. P. Pvt. Ltd. Faridabad). Retentate at 170-kg/cm² pressure, sprayed through two
3.4.6.8.2 Inlet temperature of air were 182°C and outlet temp 68.5 °C first stage drying and powder get second stage drying at static fluid bed dryer at inlet air temperature 80°C and this product passed to vibro-fluidized bed zone at inlet air temperature were kept at 75°C for hot zone and at 25°C for cold zone. Afterwards, the product was shifted through shifter of 30 mesh (Make: Dalal Engg. Thane) and filled in 25kg liner and bagging carried out.

(a) Vacuum was measured by standard manometer

(b) Temperature was measured by NPL Certified thermometer

(c) The volicity of the air was measured by Pitot tube and standard anemometer.

3.4.6.9 Storage Of The Product: The product was stored at 6°C/ 50% R H; 24°C/ 60% R H and at ambient temperatures 38° C/65% R H and was analyzed for sensory, certain physio-chemical and bacteriological attributes on the month 0, 2, 4, 6, 8, 10 and 12 months.
3.5 Compositional Analysis Of Milk:

3.5.6 The representative samples of buffalo milk were analyzed for the physico-chemical parameters as described in this section. The chemicals employed in the analysis were of analytical grade, or otherwise specified in the text.

3.5.7 Estimation Of Total Solids

3.5.7.2 Total solids in milk were estimated by gravimetric method using Saritorius balance (Make: Saritorius AG, Germany) according to procedure described in BIS Handbook of Food analysis (1981).

3.5.8 Estimation Of Fat Content

3.5.8.2 Fat contents in milk were estimated by gravimetric method using Roese - Gottlieb method (by using Mojonnier fat extraction) as per procedure described in BIS Handbook of food analysis (1981).

3.5.9 ESTIMATION OF ASH

3.5.9.2 Ash was estimated according to procedure described in BIS Handbook of food analysis (1981).

3.5.10 Determination Of pH:

3.5.10.2 pH of milk was determined by digital pH meter (Make: Cyber Scan 500pH; Eutch cybernetics, New Delhi).
3.5.11 Determination Of Titratable Acidity

3.5.11.2 Titratable acidity of milk was measured as per the procedure mentioned in BIS Handbook of food analysis (1981).

3.5.12 Estimation Of Total Protein

3.5.12.2 Protein was estimated by the method of Menefee and Overman (1940). For estimation of total nitrogen (Protein) 0.2 gm milk was weighed and transferred into a 300 ml Kjeldahl flask. To this 25 ml concentrated sulphuric acid (E. Merk India Ltd. Bombay) was added along with 4g digestion mixture consisting of copper sulphate and sodium sulphate (1:20, W/W). The material was then digested over a heater (Make: Buchi, Germany). After cooling, the mixture was transferred to a distillation assembly (Make: Buchi Switzerland). 90 ml of 50 percent sodium hydroxide (E. Merk India Ltd. Bombay) solution was added to the distillation (automatically with the pump) flask. The mixture was heated and distilled and collected in a 250ml beaker containing 50 ml of 4 percent boric acid. (E. Merk India Ltd. Bombay) and 3-4 drops of mixed indicator mixture of equal volume of 0.1 percent solution of methyl red (GR Grade, BDH, India) and 0.1 percent solution of methylene blue (GR Grade, BDH, India) in absolute alcohol.
About 100ml of distillate was collected and titrated against 0.1 N hydrochloric solution (E. Merk India Ltd. Bombay).

3.5.12.3 The total nitrogen (g / 100g) of the sample was determined by multiplying the net titration volume (Sample reading - Blank reading) by 1.4 N/W, where N is the normality of hydrochloric acid and W is the milk sample in grams.

3.5.12.4 Total protein was obtained by multiplying the value obtained from above equation (3.5.7.2) with a factor of 6.38.

3.5.13 Estimation Of Soluble Nitrogen (NPN)

3.5.13.2 Soluble nitrogen content of milk was estimated as follows.

3.5.13.3 Five gram of milk was taken in 100ml volumetric flask, sharp's extraction solution (Appendix-I) at 50°C was added to make up the volume to 100 ml. The content was tempered at 50±1°C for 1 hour with intermittent shaking followed by filtration through Whatman No. 41 filter paper. From this 20 ml filtrate was used for estimation of soluble nitrogen by Kjeldahl method. Digestion, distillation and titration were performed as per the method describe in section 3.5.7.1 for total nitrogen protein of milk. The soluble nitrogen in percent was obtained by multiplying corrected titration volume.
(Burette reading-Blank reading) in ml by 1.4 N/W, where N is the normality of hydrochloric acid and W is weight of sample in gram.

3.5.14 Estimation Of Lactose By Lane - Eynon Method.

3.5.14.2 In Lane - Eynon method, 10gm of the well-mixed milk sample was weighed and transferred in 100ml volumetric flask.

Add 10 ml of distilled water and well mixed with milk. Add 10 percent acetic acid (E. Merk India Ltd. Bombay) solution to adjust the pH 4.5 and kept till precipitation occur followed by making of the volume up to 100 ml mark with distilled water. After mixing the content thoroughly it was filtered through Whatman No. 40 filter paper. The filtrate was titrated against 10ml boiling solution of Fehling A and B (5 ml each) containing 20 ml distilled water till the colour of methylene blue indicator (5 drops of 0.2 percent W/V methylene blue in water) disappeared and appearance of brick red colour. Rest was followed from BIS Handbook (1981).

3.5.15 Determination Of Calcium

3.5.15.2 Calcium was determined as per the method given in BIS Handbook of food analysis (1981). Ash (3.5.4.1) dissolved in 15 ml diluted hydrochloric acid (1:10) (E. Merk India Ltd. Bombay) in the same
crucible dish. Transfer the solution in 100ml volumetric flask and
total volume was made to 100 ml with distilled water. Transfer 20 ml of this solution to a beaker and dilute to about 50 ml with
distilled water and make slightly alkaline with ammonium hydroxide (E. Merk India Ltd. Bombay). Heat it and add saturate ammonium oxalate (E. Merk India Ltd. Bombay) solution drop wise as long as any precipitate formed and then as excess sufficient to convert magnesium salts to oxalate. Heat to boiling. Allow standing for three hours. Decant the clear solution through filter paper Whatman No 42. Pour 15 ml to 20ml of hot water on precipitate and again decant the clear solution through the filter paper. Dissolve any precipitate remaining on the filter paper by washing with hot hydrochloric acid (1:9) into the original beaker and washed six times with hot water. Reprecipitate by adding ammonium hydroxide and little ammonium oxalate solution. Allow standing as before. Filter through the same filter paper and washed with hot water until chloride free. Perforate the apex of filter cone, wash the calcium oxalate precipitate into the beaker and then wash the filter paper with hot dilute sulphuric acid (E. Merk India Ltd. Bombay) and titrate at 85-90°C with standard
potassium permanganate (E. Merk India Ltd. Bombay) 0.1 N solution.

3.5.16 Determination Of Chloride:

3.5.11.1 Chloride was estimated by the method outlined by Jeffry et al., (1989) in Vogel's Text book of Quantitative chemical analysis (1989), with certain modification. 10 ml milk was taken a 250 ml conical flask and add few drops of 5 percent potassium dichromate (E. Merk India Ltd. Bombay). Titrate with 0.1 N silver nitrate solution (E. Merek India Ltd. Bombay) till red colour formed by the addition of silver nitrate. This faint reddish brown colour should persist after brisk shaking. Volume of silver nitrate x 35.3 will give the chloride in mg/g of sample.

3.6 Compositional Analysis Of Whey.

3.6.11 The pasteurized whey samples were subjected to physico-chemical analysis for pH, Total Solids, Fat, Ash, Protein, Soluble Nitrogen (NPN), Calcium, Chloride, and Acidity.

3.6.12 Determination of pH.

3.6.12.1 pH of whey was determined by pH meter (Make: Cyber Scan 500pH Eutch cybernetics New Delhi) as described in section 3.5.5.1 at 25°C.
3.6.13 **Determination Of Titratable Acidity**

3.6.13.1 Titratable acidity of whey was determined as per the procedure mentioned in BIS Handbook (1981) except whey was taken in place of milk.

3.6.14 **Determination Of Total Solids**

3.6.4.1 Total solids in whey were estimated by gravimetric method using saritorius balance (Make : Saritorius AG, Germany MA 30-000v3) according to the procedure described in BIS Handbook (1981) for milk.

3.6.5 **Estimation Of Fat**

3.6.5.1 Fat content in whey was determined as per procedure given in section 3.5.3.1 used whey in place of milk.

3.6.6 **Determination Of Ash**

3.6.6.1 Ash in whey was determined as in section 3.5.5.1 except whey sample was taken in place of milk.

3.6.7 **Determination of Proteins**

3.6.7.1 The procedure of determination of protein was same as in section 3.5.7.1 except sample was taken whey.
3.6.8 **Determination of soluble nitrogen**

3.6.8.1 Soluble nitrogen of whey was same as in section 3.5.8.1 except whey was taken sample instead of milk.

3.6.9 **Estimation of lactose by lane-eynon method**

3.6.9.1 Lactose was determined in whey by Lane-Eynon method as described in section 3.5.9.1. Whey was titrated against 10-ml boiling solution of fehling A and B (15 ml each) containing 20 ml distilled water till the colour of methylene blue indicator (5 drops of 0.2 percent W/V methylene blue in water) disappeared and appearance of brick red colour.

3.6.9.2 **Determination Of Calcium**

Calcium was estimated through same procedure as given in section 3.5.10.1 except whey was taken as sample.

3.6.10 **Determination Of Chloride**

3.6.10.1 Chloride content of whey was determined as per procedure given in section 3.5.11.1 except whey was taken sample.

3.7 **Compositional Analysis Of Retentate.**

3.7.4 The retentate samples were subjected to physico-chemical analysis for pH, Total Solids, Fat, Ash, Protein, Soluble Nitrogen, Calcium, Chloride, and Acidity.
3.7.5 Determination of pH.

3.7.5.1 PH of retentate was determined by pH meter (Make: Cyber Scan 500pH, Eutch cybernetics, New Delhi) as described in section 3.5.5.1 at 20°C.

3.7.6 Determination of Titratable Acidity.

3.7.6.1 Titratable acidity of retentate was determined as per the section 3.5.6.1 except retentate was taken as sample.

3.7.7 Determination of Total Solids.

3.7.7.1 Total solids in retentate were estimated by gravimetric method using Saritorius balance (Make: Saritorius AG, Germany MA 30-000v3) according to the procedure described in BIS Handbook (1981).

3.7.8 Estimation of Fat.

3.7.8.1 Fat content in retentate was determined as per procedure given in section 3.5.3.1. used retentate in place of milk.

3.7.9 Determination of Ash.

3.7.9.1 Ash in retentate was determined as in section 3.5.5.1 except retentate sample was taken in place of milk.
3.7.10 Determination of Proteins.

3.7.10.1 The protein of determination of retentate was same as in section 3.5.7.1 except sample was taken retentate.

3.7.11 Determination of Soluble Nitrogen.

3.7.11.1 Soluble nitrogen of retentate was same as in section 3.5.8.1 except retentate was taken sample instead of milk.

3.7.12 Estimation of Lactose By Lane-Eynon Method.

3.7.12.1 Lactose was determined in retentate by Lane-Eynon method as described in section 3.6.9.1.

3.7.13 Determination of Calcium.

3.7.13.1 Calcium in retentate was estimated through same method as given in section 3.5.10.1 except retentate was taken as sample.

3.7.14 Determination of Chloride.

3.7.14.1 Chloride content of retentate was determined as per procedure given in section 3.5.11.1 except retentate was taken as sample.

3.8 Compositional Analysis of Whey Proteins Concentrate – 70

3.9.1 The samples of whey protein concentrate (WPC-70) were subjected to sensory, physico-chemical and bacteriological analysis during manufacturing and storage.
3.9.2 Physico - Chemical Analysis of WPC-70

3.9.2.1 WPC-70 was analyzed for pH, Acidity, Total Solids, Fat, Ash, Protein, Soluble Nitrogen, Calcium, Chloride, Solubility- Index and bulk density.

3.9.2.2 Determination of pH.

3.9.2.2.1 The WPC-70 analyzed for its pH by making 10% solution and determined by digital pH meter (Make: Cyber Scan 500pH, Eutch cybernetics, New Delhi) at 25°C.

3.9.2.3 Determination of Titratable Acidity

3.9.2.3.1 Titratable acidity was determined after dissolving and dispersing 6.5 g of dry powder in 100 ml distilled water by using mixer. Allow the sample to stand for one hour, stir gently and then pipette 17.6 ml into porcelain dish. Rinse out the same pipette with 17.6 ml distilled water and add this to the sample in the porcelain dish. Add 0.5 ml phenolphthalein indicator and titrate with standardized 0.1 N NaOH until a faint pink colour obtained.

3.9.2.4 Estimation of Fat

3.9.2.4.1 Fat content of WPC-70 was determined as per AOAC (1980) by using 1g sample.
3.9.2.5 **Estimation of Total Solids**

3.9.2.5.1 Total solids of WPC-70 were determined by the procedure given on ADPI manual. Weighed 1.5g dried WPC into a previously oven dried, (Make: Tempo, Bombay) desiccated (Cooled to room temperature), and weighed (Make: Schimdzu Libror, Model AEF - 200 G) in a covered weighing pan. Placed the weighing pan into vacuum oven, (Make: Tempo Bombay) after removing the cover inside the vacuum oven and placed the cover in oven. Dried for 16 hour (over night) at 64-65°C under 20mm mercury. Released vacuum, passed air through drying column. Place the pan with cover into a desiccator. Cooled for 1 hour (to room temperature) and weigh.

3.9.2.5.2 **Calculation**

\[
\text{Percent Moisture} = \frac{\text{Weight initial samples} - \text{weight dried sample}}{\text{Weight initial sample}} \times 100
\]

Total Solids = 100 - Percent Moisture.

3.9.2.6 **Determination of Ash**

3.9.2.6.1 Determination of Ash was done as per ADPI method (1988)
3.9.2.7 Determination of Protein

3.9.2.7.1 Estimation of protein in WPC-70 was done as in section 3.5.7.1 only 0.1 gm weight of sample was taken.

3.9.2.8 Determination of Soluble Nitrogen

3.9.2.8.1 Soluble nitrogen of WPC-70 was same as in section 3.5.8.1 except 1 gm weight of sample was taken.

3.9.2.9 Determination Of Denatured Whey Proteins.

3.9.2.9.1 Extent of denaturation was measured by estimating the whey protein nitrogen in sodium chloride filtrate by Kjeldahl method. Whey protein nitrogen was determined before and after sodium chloride precipitation to assess the extent of denaturation. The samples for the estimation were prepared as per the method suggested by Mahmoud et al. (1990). Accurately 1g of powder was transferred to a test tube (25 x 150 mm size), 25 ml of 0.1M phosphate buffer (pH 6.7) and 10g of sodium chloride were added to the mixture and the tubes shaken vigorously and transferred to a water bath maintained at 37°C for a period of 30 min with intermittent shaking for about 15 min. Thereafter, samples were cooled to 20°C temperature and filtered through Whatman No.1 filter paper. The nitrogen content of the filtrate was determined by
Kjelfoss Automatic 16210. The extent of denaturation was estimated by using the formula:

\[
\%\text{Denaturation} = \frac{(\text{Total whey protein nitrogen - Whey protein nitrogen after salt precipitation})}{\text{Total whey protein nitrogen before precipitation}} \times 100
\]

3.2.9.10 Determination of Lactose

3.2.10.1 Lactose was determined as in section 3.6.9.1.

3.2.11 Determination Of Calcium

3.2.11.1 Calcium in WPC-70 was analyzed as in section 3.5.10.1.

3.9.2.12 Determination Of Chloride

3.9.2.12.1 Chloride content of WPC-70 was determined as per procedure given in section 3.5.11.1.

3.9.2.13. Determination Of Solubility Index

3.9.2.13.1 Solubility of index of WPC-70 powder was determined as per BIS Handbook of food analysis (1981).

3.9.2.14 Determination Of Bulk Density

3.9.2.14.1 Bulk density of WPC-70 powder was determined as per BIS Handbook of food analysis (1981).
3.9.2.15  **Microbiological Analysis:**

3.9.2.15.1 In this section, various microbiological methodologies employed for WPC-70 during the present investigation are described.

3.9.2.16  **Standard Plate Count**

3.9.2.16.1 WPC-70 sample were analyzed by the standard plate count (SPC) procedure described of whey powder in Bulletin W-16 of American Dairy Products Institute (ADPI), except for medium which was used (given in Annexure-1).

3.9.2.16.2.1 Weigh 1gm of sample into wide mouth dilution bottle containing 99 ml of sterile, phosphate buffered distilled water to which 1.25 % sodium citrate has been added to dissolve the dry WPC-70. While preparing dilutions, special precautions were taken to completely dissolve dry WPC-70 to obtain a homogenous mixture. Mildly agitate the dilution blank to completely wet the sample. Allow soaking 2 min; shaking dilution bottle making 25 completely up and down movements of about 1 feet in 7 seconds.
3.9.2.17 Coliform Count

3.9.2.1.1 Coliform counts of fresh and stored WPC-70 were obtained. The analysis was done in accordance with the procedure laid down in American Dairy Products Institute (ADPI), Bulletin, 1986.

3.9.2.17.2.1 Preparation of sample was done in the same way as in 3.9.3.2.2 as first dilution 1:1 dilution was prepared by pipetting 10ml of the first dilution (section 3.9.3.2.2) and distribute into three Petridishes. Add 15-20ml of tempered Violet Red Bile Agar at temperature 44-46°C to each dish and thoroughly mix contents. After agar has solidified, add an extra 3-4 ml of the agar into each dish so as to evenly cover the surface and allow solidifying.

Inverted the Petridishes and incubated for 24 hour + 2 hour at 32 ±0.5°C. Coliform count (dark red colonies at least 0.5 mm in diameter) of three dishes reported as Coliforms per gram of dry WPC-70. No colories reported as less than 1 per gram.
3.9.2.18 **Escherichia Coli:**

3.9.2.18.1 E.Coli counts of fresh and stored WPC-70 were carried during the investigation. The analysis was done in accordance with the procedure laid down in American Dairy Product Instituent (ADPI), Bulletin, 1986.

3.9.2.19 **YEAST AND MOULD COUNT**

3.9.2.19.1 The analysis for Yeast and Mould count was done according to the method described in BIS Handbook (1981). The media composition and method of preparation is given in Annexure-1.

3.9.2.19.1 Suitable dilution of the sample was prepared as in section 3.9.3.2.2 and transferred to duplicate Petridishes. The Petridishes containing the dilution sample were poured with the PDA medium adjusted to pH 3.5 ± 0.1 with 10 percent solution of sterilized tartaric acid (The medium so adjusted for pH was utilized within 30 minutes) and allowed to solidify after mixing. The plates were incubated at 22 to 25°C for 3-5 days and colonies of yeast and mould were counted.

3.9.2.20 **Bacillus Cereus:**

3.9.2.20.1 The analysis for Bacillus cereus count was done according to Bureau of Indian standards (BIS) in "ISI" Handbook of Food analysis (1981).
3.9.2.20.1.1 MEDIA: Mannitol Egg Yolk Polymixin Agar Base (Hi Media M636) MYP).

<table>
<thead>
<tr>
<th></th>
<th>Gram / Liter</th>
</tr>
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<tbody>
<tr>
<td>Meat extract</td>
<td>01.0</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Mannitol</td>
<td>10.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>10.0</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>00.025</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>07.1+0.2</td>
</tr>
</tbody>
</table>

3.9.2.20.1.2 Additives:

Egg Yolk Emulsion - 50 ml/litre (Hi Media FD045).

Bacillus cereus selective, supplement (Polymixin B, (Hi Media FD003) 50,000 units.

3.9.2.20.1.3 PREPARATION: Suspend 46g media powder in 900 ml distilled water. Heat to boiling Sterilize by autoclaving at 151bs (121°C) for 15 min. Cool to 55°C. Aseptically add sterile polymixin. B-sulphate solution to final concentration of 1000 U/ml and 100 ml sterile egg yolk emulsion to 1 l of medium. Mix well and pour into plates.

3.9.2.20.1.4 PROCEDURE: After depositing 0.1 ml of test portion (dilution 10-2) on the well dried Agar plates, store the plates for drying.
3.9.2.20.1.5 INCLINATION: 48 hrs at 30°C+1°C.

3.9.2.20.1.6 RESULT: Count all colonies surrounded by a halo offense precipitate with distinct violet-red background.

3.9.2.20.2 Conformation Of Bacillus Cereus.


1. Transfer a loopful of "Positive Colony" to "Phenol Red dextrose broth" (Hi Media M056) and incubate aerobically at 37°C for 24 hrs. A yellow colour indicates the positive test.

2. Transfer a loopful of "Positive Colony" to "Nitrate broth" (Hi Media M439) and incubate at 37°C for 24 hrs. Add 0.25 ml each of sulfanillic acid 0.8 percent (Hi Media R015) and alpha Naphthylamine solution (Hi Media R009). An orange colour within 10 minutes constitutes the positive test.

3. Modified VP Broth (Hi Media M637) is inoculated and incubated at 30°C for 48 hrs. Add 0.2 ml 40 percent potassium hydroxide and 0.6 ml of 5 percent alcoholic
alpha-naphthol solution to 1 ml of culture in a test tube. A purple colour within 15 minutes constitutes the positive test.

4. Inoculate Nutrient broth W/1 percent peptone (Hi Media M244) containing a final concentration of 0.001 percent lysozyme for 48 hrs at 37°C. Growth after incubation constitutes a positive test.

3.9.3 SENSORY EVALUATION:

A technical panel of 3 judges was selected on the basis of triangle test for sensory evaluation. The judges were suitably trained about the sensoric attributes associated with WPC-70. Score Card for organolaptic evaluation of WPC-70 was used as per IS: 6273 (Part-II) 1979 with slight modification as in Table-3.1.

Table 3.1. Scorecard for organoleptic evaluation of WPC-70 was used as per IS: 6273 (Part-II) 1979 with slight modification as tabulated here under.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Score</th>
<th>Sample No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max.</td>
<td>Min.</td>
</tr>
<tr>
<td>Colour of product</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Appearance of reconstituted WPC</td>
<td>15</td>
<td>09</td>
</tr>
<tr>
<td>Body and Texture of reconstituted WPC</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Flavour of reconstituted WPC</td>
<td>45</td>
<td>27</td>
</tr>
</tbody>
</table>

NOTE: If the sample scores less than the minimum for any characteristic, it is to be rejected.
### B: Degree of defect

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Defect</th>
<th>Degree of defect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Suspicion</td>
</tr>
<tr>
<td>Colour of Dry Product</td>
<td>Sl. Yellowish/Yellowish cream/Light Yellow/Light or Dark brown</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Brown particles/Extraneous matter</td>
<td>2</td>
</tr>
<tr>
<td>Appearance of WPC</td>
<td>Lumpy, Sl. Yellowish, Brown particles/Dark Brown/Churned/Grainy</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Undispersed lumps</td>
<td></td>
</tr>
<tr>
<td>Flavour</td>
<td>Oxidized/stale/rancid</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Chalky/neutralizer/cheesy/salty/acid</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Metallic/cooked/scorched</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Weedy/bitter/foreign</td>
<td>5</td>
</tr>
</tbody>
</table>

Score obtained by the powder sample

<table>
<thead>
<tr>
<th>Score obtained by the powder sample</th>
<th>Grade of the powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 and above</td>
<td>Excellent</td>
</tr>
<tr>
<td>80-89</td>
<td>Good</td>
</tr>
<tr>
<td>60-79</td>
<td>Fair</td>
</tr>
<tr>
<td>59 and below</td>
<td>Poor</td>
</tr>
</tbody>
</table>

**NOTE:** In order that the product is acceptable it must score a minimum of 60% for each sensory attribute.

**Date:**

**Name:**

**Signature:**

3.9.4.1 The flavour and odour was determinated on a sample by reconstituting 8.5 g to 100 ml distilled water and thoroughly mixing in a mixer. Sample was allowed to stand for 1 hour in an airtight glass container, gently stirred and determined flavour and odour at 75°C. Reported sample flavour as "Good", "Fair", "Poor" where off flavour characteristics was identified (Bulletin W-16 ADPI).