3. EXPERIMENTAL

This chapter deals with the methods and materials used during the analyses and manufacture of soy-whey weaning food (SWUF) and its ingredients. Only analytical grade reagents were used unless mentioned otherwise.

3.1 Whey

Buffalo milk Cheddar cheese whey was obtained during the manufacture of Cheddar cheese, at the Experimental Dairy of the National Dairy Research Institute, Karnal. The whey was drained from the cheese vat and passed through muslin cloth to remove any suspended curd particles. This strained whey was used for the manufacture of SWUF.
3.1.1. **Composition of whey**

Composite samples of whey were analysed for total solids, total nitrogen, non-protein nitrogen, fat, lactose and ash contents.

3.1.1.1. **Total solids (TS)**

The TS content of whey was obtained by taking approximately 10 ml of accurately weighed whey and drying over a water bath and later at 100°C for 3 hours to a constant weight (IS: 1479-1961).

3.1.1.2. **Total nitrogen (TN)**

TN in cheese whey was determined by A.O.A.C. method (1970) wherein 2-3 ml of whey was weighed in a 30 ml micro-kjeldahl flask and digested and distilled as recommended. A conversion factor of 6.38 was used to obtain crude protein content.

3.1.1.3. **Non-protein nitrogen (NPN)**

NPN was determined by the method of Rowland (1938). Whey was made to 12 percent TCA. The filtrate was used for nitrogen determination, which gave the NPN.

3.1.1.4. **Fat**

The fat in cheese whey was determined gravimetrically as per ADMI (1965), wherein approximately 10 ml of whey was weighed and used.
3.1.1.5. **Lactose**

Lactose in cheese whey was determined by Lane-Eynon method of ISI (IS:1479, Part II, 1961).

To 5 ml of Fehling solution A (34.639 g of copper sulphate, CuSO$_4$·5H$_2$O, BDH, dissolved in distilled water and made to 500 ml) was added 5 ml of Fehling solution B (173 g Rochelle salt, KNa C$_4$H$_7$O$_6$·4H$_2$O, BDH, and 50 g Sodium hydroxide, BDH, dissolved and made to 500 ml). To this mixture was added dilute whey solution (1:4) by the incremental method of titration. This mixture was boiled for 15 sec. and further quantities of the whey solution were added rapidly until only faintest perceptible blue colour remained. Then 2-5 drops of methylene blue indicator (1% _v/v_ in water) was added and the titration completed by further additions of the solution dropwise.

Before starting the titration, Fehling solutions were standardized against a standard solution of pure lactose (BDH).

From the given table, the factor corresponding to the titre was noted and correction, if any, applied before calculating the lactose content as under:

\[
\text{Lactose, percent} = \frac{\text{Factor}}{\text{Titre} \times 100}
\]
3.1.1.6. Ash

Ash content in cheese whey was estimated by ISI-method (IS:1547-1968). Ten ml of whey accurately weighed was dried in a silica crucible over a water bath then gently on a flame and finally ashed in a muffle-furnace, at 550°±20°C to a constant weight. Ash content was then calculated as under:

Total ash(percent by weight) = \( \frac{100(U_2-U)}{U_1-U} \)

where,

- \( U_2 \) = Weight in g of the dish with ash
- \( U \) = Weight in g of the empty dish
- \( U_1 \) = Weight in g of the dish with the sample.

3.2 Soybean

Mixed variety of soybean was purchased from the J.N.K.V.U., Jabalpur. The soybean was cleaned and after removal of split, undersized, wrinkled and damaged beans was packed in polythene bags and stored in dry place.

3.2.1. Composition of soybean

Soybean and its cotyledons were analysed for TN, fat, ash, carbohydrates, iron and calcium contents. In addition the percentage of cotyledons, hulls and plumules present in soybean was also determined.

3.2.1.1. Total nitrogen (TN)

The TN content of soybean or its coty-
ledons was determined by weighing 100 mg of thoroughly ground and mixed sample as per 3.1.1.2. A conversion factor of 6.25 was used to obtain crude protein content.

3.2.1.2. Fat

Approximately 5 g of accurately weighed powdered sample was used for the determination of fat by the AOAC method (1970) using Soxhlet apparatus.

3.2.1.3. Ash

Approximately 3 g of powdered sample was weighed and its ash content determined as in 3.1.1.6.

3.2.1.4. Total carbohydrates

The total carbohydrates were estimated by difference as follows:

$$\text{Total carbohydrates, } \% = 100 - (\text{moisture } \% + \text{fat } \% + \text{protein } \% + \text{ash } \%).$$

3.2.1.5. Iron

Iron content was determined from the ashed samples (3.2.1.3) according to ADMI (1965). A standard curve was prepared (Fig 1) and used for this purpose.

3.2.1.6. Calcium

Calcium content was determined from the ashed sample solution (3.2.1.5) by ISI method (IS:1479, part II,1961) wherein 20 ml of
FIG. 1. STANDARD CURVE FOR IRON ESTIMATION
the ashed solution was transferred to a beaker and
diluted to about 50 ml. The mixture was made
slightly alkaline with 1:1 ammonium hydroxide (BDH).
While still hot, saturated ammonium oxalate solution
(BDH) was added dropwise as long as any precipitate
formed and then an excess sufficient to convert
magnesium salts also to oxalate was added. The
mixture was heated to boiling and left to stand for
3 hours or longer. The clear solution was decanted
through a Whatman No. 42 filter paper. Fifteen to 20
ml of hot water was poured on the precipitate and clear
solution again decanted through the filter paper.
Any precipitate remaining on the filter paper was
dissolved by washing with hot 1:9 hydrochloric acid
(BDH) into the original beaker and washed six times
with hot water. It was then reprecipitated by the
addition of ammonium hydroxide and a little ammonium
oxalate solution. It was allowed to stand as before,
filtered through the same filter paper and washed
with hot water until chloride free.

The apex of the filter paper cone
was punctured and calcium oxalate precipitate was
washed into the beaker used for precipitation. The
filter paper was then washed with hot 1:4 sulphuric
acid (BDH) and titrated at 85°C-90°C with 0.1 N
potassium permanganate solution. Calcium content in the sample was calculated as under:

$$\text{Calcium \% by weight} = \frac{1.002 \times V}{W}$$

where,

- $V = \text{volume in ml of standard potassium permanganate}$
- $W = \text{weight in g of prepared sample taken for the determination of ash}$.

3.2.1.7. Components of soybean

The percentage of cotyledons, hulls and plumules were determined after drying 10 g of seeds in oven at $100 \pm 1^\circ \text{C}$ for 3 hours. The dried seeds were cracked and content of individual components determined by weighing.

3.3. Soy-whey weaning food (SWUF)

SWUF means soy-whey weaning food fortified with vitamins, iron and methionine unless otherwise indicated. SWUF was analysed for its physico-chemical properties as under:

3.3.1. Moisture

Moisture content of all samples was determined gravimetrically by ISI method (IS: 1547-1968) wherein the samples were dried at $100 \pm 1^\circ \text{C}$ for 3 hours to a constant weight.
3.3.2. **Total nitrogen (TN)**

The TN content of SUUF was determined as for soybean (3.2.1.1). A conversion factor of 6.25 was used to obtain the crude protein content.

3.3.3. **Non-protein nitrogen (NPN)**

NPN in SUUF was determined by the method of Becker et al. (1940), wherein 1 g of sample was extracted with 40 ml of 0.8 N (13.6%) TCA (Riedel, Germany) for 30 min in a mechanical shaker. The suspension was centrifuged for 5-6 min at 1975xg and 10 ml of clear supernatant liquid used for nitrogen determination as for soybean 3.2.1.1.

3.3.4. **Fat**

Approximately 1 g of accurately weighed SUUF was used for fat determination as per ADMI (1965) 3.1.1.4.

3.3.5. **Soluble nitrogen (SN)**

The SN of SUUF was determined by the method of Albrecht et al. (1966) wherein 2.5 g of sample was added to 100 ml distilled water and pH adjusted to 7.2 with sodium hydroxide solution (1:10). The sample was agitated at 125 rpm with a flat blade agitator for 2 hrs at 30°C. After centrifugation for 15 min at 1000 rpm, nitrogen was determined in 10 ml of supernatant as per
A conversion factor of 6.25 was used to obtain crude protein content in the supernatant. Percent soluble nitrogen was calculated on the basis of the weight of the sample.

3.3.6. Free fat

The method suggested by Bums (1971) was used for the determination of free fat in SWUF with slight modifications, as under:

In a 250 ml glass stoppered Erlenmeyer flask, 2.5 g SWUF was transferred and 100 ml petroleum ether (40°-60°C) was added to it. During the first 10 min the suspension was twirled frequently and thereafter every 5 min. Total time given for extraction was 30 min at room temperature (approx. 30°C). The dispersion was filtered through a sintered glass (Corning) disc (porosity grade 3). The clear filtrate was collected in a flat bottom dish and the solvent removed by evaporation on a steam bath. The residue was then dried at 100°±1°C for 2 hrs to a constant weight. The amount of fat so obtained was designated as free fat.

3.3.7. Free fatty acids (FFA)

The FFA content was estimated by the method of Thomas et al. (1954), with minor modifications as under:
Five g of sample was dispersed in 20 ml of hot water in a separatory funnel. When completely dispersed, 25 ml of neutralised absolute ethyl alcohol was added to it. It was shaken for 2 min and then 40 ml of diethyl ether (Sarabhai M. Chemicals, Baroda) and 60 ml of petroleum ether (40°-60°C) were added and after each addition shaken for at least 2 min. The separatory funnel was allowed to remain undisturbed for a few min. The separated solvent was removed and evaporated at reduced pressure on a water bath (45°C). To the oil so recovered, 30 ml of neutralised alcohol was added along with 1 ml of phenolphthalein indicator and was titrated against 0.1 N sodium hydroxide. The FFA content was then calculated using the following formula:

$$\text{percent FFA (as oleic acid)} = \frac{\text{Vol of alkali} \times 28.2 \times 0.1}{\text{Wt. of oil}}$$

3.3.8. Ash

Three g of SWUF was used for the determination of ash as in 3.2.1.3.

3.3.9. Total carbohydrates

The total carbohydrates content of SWUF was determined as in 3.2.1.4.
3.3.10. Vitamins

Prior to fortification of the SUUF, it was essential to determine the initial content of vitamins. Vitamin A, B₁, B₂, niacinamide and C were determined. Since the facilities for the analysis of B₁, B₂ niacinamide were not available in this laboratory, the samples were sent to Shriram Test House, New Delhi. The other vitamin contents were analysed as under:

3.3.10.1. Vitamin A

Vitamin A content of SUUF was determined by the Carr-Price method (IS: 1547-1968), wherein 10 g of material was taken instead of 5 g. The reading was taken at 620 μμ (Red Filter) in a EEL colorimeter (Evans Electroselenium Limited, U.K). The quantity of vitamin A was calculated with the help of a standard curve (Fig 2).

3.3.10.2. Vitamin C

The vitamin C content of SUUF was determined as per A.O.A.C. (1970). After preparation of the standard reagents the procedure consisted of weighing 5 g of the sample into 100 ml graduated flask, addition of 40 ml water at 50°C, mixture was then shaken thoroughly and the metaphosphoric acid - acetic acid reagent was added to make up to 100 ml. This was mixed thoroughly and filtered.
FIG. 2. STANDARD CURVE FOR VITAMIN A
Ten ml of the filtrate was titrated rapidly with the indophenol solution. Blank determination was carried out with 11 ml of the reagent along with sufficient water to make the volume of the mixture equivalent to 15 ml plus the volume of the indophenol solution required in the direct titration. The amount of vitamin C in the sample was calculated as follows:

\[
\text{Vitamin C content in mg percent by weight} = \frac{A \times B \times 1000}{W}
\]

where,

- \( A \) = volume in ml of indophenol solution used for titration
- \( B \) = weight in mg of the ascorbic acid equivalent to 1 ml of the indophenol solution, and
- \( W \) = weight in g of the sample.

3.3.11. Methionine

As is evident from literature, soybean is deficient in this sulphur containing amino acid. It was therefore necessary to determine the initial content of methionine in SWWF prior to fortification. Methionine content was estimated by the method of Horn et al. (1946). The values were estimated
using a standard curve (Fig 3) prepared by taking known quantities of methionine and reading in Klett-Summerson colorimeter using filter 54.

3.3.12. Available lysine

Available lysine content was estimated by suitably modifying the methods of Baliga et al. (1959) and Booth (1971) as follows:

One g of the sample was weighed and transferred to a round bottom flask, containing glass beads. Ten ml of sodium bicarbonate (80 g sodium bicarbonate U/V per 1000 ml) was added to it and the flask gently shaken and left to stand until the sample was completely wet. Four tenths of a ml of 1-fluoro 2,4 dinitro benzene (FDNB) obtained from E. Merck, U. Germany, was dissolved in 15 ml absolute alcohol and added to the flask. The flask was stoppered and shaken for 4 hrs. Half way through the shaking period, the flask was twirled to disperse the sample and to ensure that all particles were wet with FDNB solution. The ethanol, but not all the water, was evaporated on a boiling water-bath. When the mixture cooled, 30ml of 8.1 N hydrochloric acid (BDH) was added which neutralised the sodium bicarbonate and made a total of 40 ml (to 6N). The mixture was refluxed for 16 hours. After refluxing
FIG. 3. STANDARD CURVE FOR METHIONINE ESTIMATION
the condenser was washed with a small aliquot of water and the flask disconnected. The acid was removed by distillation, at reduced pressure. The hydrolysate was extracted with peroxide free diethyl ether to remove excess of reagent and small amount of ether soluble DNP-amino acids which may be present. Two more extractions were carried out this way. Excess ether was evaporated over water bath (80°C). Boiling distilled water was added to the flask in small amounts and the material filtered through Whatman No.41 filter paper. Water soluble amino acids washed down with hot water to the filtrate. The filtrate was received in 100 ml volumetric flask.

The DNP-lysine so obtained was separated by paper chromatography using Whatman No.3 mm paper measuring 18.5 x 22.25 cm. A 0.2 ml sample was applied to the paper and was irrigated with n-butanol: acetic acid:water (4:1:1) for 6 hrs and air dried in a dark cabinet. \(\varepsilon\)-DNP-lysine was spotted with the help of a similarly run standard on the same paper. Then this spot was cut and eluted in 1N hydrochloric acid in 25 ml volumetric flask and the volume made to 25 ml. Optical density was measured at 363 μμ on a Beckman-Model DU-spectrophotometer. The amount of available
lysine was calculated with the help of a standard curve using 0 to 140 μg of ε-DNP-lysine (Fig 4).

3.3.13. Trypsin inhibitor (TI) activity

The TI activity of SUUF was determined by the ISI method (IS: 6387-1971), wherein 0.2 to 1.0 ml trypsin solution (200 mg trypsin, 20,000 F-G units per g obtained from E. Merck, W. Germany, in 100 ml 0.0025 N hydrochloric acid, prepared fresh every time) was transferred to test tubes. Phosphate buffer (0.1 M, pH 7.6 prepared by mixing 43.5 ml of 0.2 M sodium phosphate dibasic, with 6.5 ml of 0.2 M sodium phosphate monobasic, made to 100 ml mark) was added to make the volume to 2 ml. The test tubes were incubated at 35°C for 5 min in a water bath. Two ml of casein solution (1 g casein suspended in water, dissolved by adding a few drops of 0.1 N sodium hydroxide, warming and then diluting to 100 ml mark with 0.1 M phosphate buffer, pH 7.6 was added to the first test tube and time noted. To the remaining test tubes, 2 ml casein solution was added at 30 sec interval. To the first tube, 6 ml of 5 percent TCA was added after 20 min incubation. To the remaining test tubes 6 ml of 5 percent TCA was added at 30 sec intervals. Contents of the tubes were mixed thoroughly by vigorously shaking the test tubes. Test tubes were then removed from
FIG. 4. STANDARD CURVE FOR AVAILABLE-LYSINE ESTIMATION

O.D. AT 363 mÅ

CONCENTRATION OF DNP-LYSINE (µg)
the water bath and kept at room temperature (25°C) for 30 min before filtering. Corresponding blanks were prepared by mixing 6 ml of 5 percent TCA with 2 ml casein solution and adding trypsin solution (0.2 - 1.0 ml) and then buffer to make up to 2 ml dilution as before and filtered through Whatman No. 42 filter paper. Optical density (OD) was read at 280 μm in a Beckman DU-spectrophotometer against the corresponding blanks.

For the determination of TI activity in the SWUF, 0.1 to 0.5 ml of sample solution (1 percent extract prepared with 100 ml 0.1 M phosphate buffer, pH 7.6 in a homogenizer, this was filtered and 0.2 percent solution prepared by diluting 20 ml of 1 percent solution to 100 ml mark) was taken in a test tube. Tubes were incubated at 35°C for 5 min by adding appropriate amounts of trypsin solution and phosphate buffer (0.1 M, pH 7.6) so as to make up the final volume to 2 ml. Two ml of 2 percent casein solution was added to all the test tubes. Six ml of 5 percent TCA solution was added after 20 min incubation at 35°C as described above. Corresponding blanks were prepared with sample solution as described above. OD was read at 280 μm against the corresponding blank, calculations were done as follows:
Antitryptic activity of sample/g = \frac{\text{Difference in OD (between control and sample)}}{0.1 \times 20 \times \text{amount of sample in g}}

The unit of antitryptic activity, here, was defined as the difference of 0.1 OD between the control (without sample) and the sample at 280 µg per min per g of sample at 35°C.

3.3.14. Peroxide value

Peroxide value was determined by the method of Smith (1939) with minor modifications as under:

Ten g sample was taken in a 100 ml graduated flask, to which 50 ml of glacial acetic acid (BDH) was added. In order to facilitate dissolution of fat, the mixture was warmed to 35°C for 5 min, and shaken vigorously at intervals. Chloroform was then added to the mark and after vigorous shaking the solution was filtered through a Buchner-funnel over a Whatman No. 42 filter paper. The flask and the funnel were washed with 25 ml acetic acid: chloroform mixture (3:2). The liquid so obtained was included in the filtrate and transferred to a iodomteric flask. To this 1 ml saturated potassium iodide (BDH) solution was added. The mixture was shaken for exactly one min and was then diluted with 100 ml distilled water, and titrated against

\[ 2I_2 + 2I^- \rightarrow I_2 + 2I^- \]
0.01 N sodium thiosulphate (BDH) until the yellow colour just disappeared. Half ml of starch indicator (1% starch, BDH, U/V in water) solution was then added and titration continued until the blue colour just disappeared.

A blank determination using distilled water was also carried out and the peroxide value calculated using the following formula:

\[
\text{Peroxide value as meq per 1000 g of fat} = \frac{(S-B)(N)(1000)}{\text{Weight of fat (in 10 g sample)}}
\]

where,

\[
B = \text{ml of 0.01 N sodium thiosulphate required for blank}
\]

\[
S = \text{ml of 0.01 N sodium thiosulphate required for sample}
\]

\[
N = \text{normality of sodium thiosulphate.}
\]

3.3.15. Colour

The colour of SUUF was determined by the use of the Lovibond Tintometer (The Tintometer Ltd., England) using carbonate of magnesia as reference white standard.

3.3.16. Solubility index

The solubility index of SUUF was estimated as per ADNI (1965).
3.3.17. Wettability

Wettability of SWWF was determined by the method of Muers and House (1962), with slight modifications in the apparatus and temperature used. The fabric used, weighed approximately 270 g per M², having about 40 threads per cm in the warp and 25 threads per cm in the weft. A circular piece of this cloth measuring about 10 cm in dia was stretched over one end of the body (Fig 5) of a pyrex glass tubing (B) of 6.5 cm dia and 4.5 cm high, open from both the ends and held on with a rubber band (C). Another open ended pyrex glass tubing (D) of 5 cm dia and 7 cm high was fitted inside the larger tube using a rubber "O" ring (E) as a spacer. A dish (F), 10 cm dia and 5 cm high, was marked to a height of 2.5 cm from the bottom and filled with distilled water to this point. A triangle (G) of 4 mm thick glass rod with sides 8 cm long rested in the dish and served to prevent close contact of the cloth with the bottom of the dish.

One g of powder was transferred to the inner tubing and spread over evenly with the help of soft camel hair brush. The inner tube was then removed and the outer one lowered in the dish and made to rest on the glass triangle, and held in
FIG. 5. WETTABILITY APPARATUS - SECTION
place until the water level inside the tube ceased to rise. The moment the cloth touched the water, the time was recorded till the powder was completely wetted. The average of five replications was taken as the wetting time.

3.3.18. Dispersibility

Dispersibility of SUUF was determined by the methods of Baker and Bertok (1959) and ADMI (1965) with modifications as under:

A 400 ml beaker was fitted with a siphon while keeping the mouth of the siphon as close to the bottom as possible. The other end of the siphon was fitted with a rubber tubing and pinch cock.

For determination of dispersibility, 50 ml water at 25°C was taken in the beaker and carefully sucked through the siphon to maintain continuity of flow, (the siphon contained 5 ml of water). A mechanical stirrer was adjusted to such a height so that the blades were close to the bottom of the beaker but did not touch. The speed of the stirrer was adjusted to 250 rpm. Five g of sample was gently placed on the surface of the water and the stirrer was switched on for exactly 15 sec. The dispersed material was quickly siphoned
off and filtered through a 70 mesh screen into a dish. Five ml of the filtrate was transferred to a weighed aluminium dish and the total solids determined (3.1.1.1). The amount of solids obtained in the filtrate represented the dispersed powder and therefore the percent "dispersibility" calculated by multiplying the resultant with 20.

3.3.19. Viscosity

The viscosity of the reconstituted SUUF was determined by Hoppler viscometer (type BH, manufactured by Veb Prufgeratwerk Medingen/Dresden). The sample was adjusted to 12 percent solids at 30°C prior to determining dynamic viscosity. The temperature was kept constant to 30°C±1°C with the help of Colora Ultrathermostat (Germany). After levelling the Hoppler viscometer, the measuring tube was filled with the sample and ball No.2 (15.627 mm dia) inserted through the open end of the tube (15.937-15.938 mm dia). Time required for the ball to travel 100 mm distance was recorded for 4 such events and averaged.

The specific gravity of sample was also determined at 30°C using a specific gravity bottle.

The dynamic viscosity of the sample was calculated by the following formula (based on
Stokes law:  
\[ \eta = F (SK - Sf) K \]

where,  
\[ \eta \] = dynamic viscosity in centipoise  
\[ F \] = fall time of the ball in seconds  
\[ (\text{angle of inclination } 80^\circ) \]  
\[ SK \] = specific gravity of the ball (2.409)  
\[ Sf \] = specific gravity of the sample, and  
\[ K \] = ball constant (0.07338).

3.3.20. **Bulk density, average particle density**  
and **percent volume occupied by powder particles**

Bulk and average particle densities were estimated by the method of Beckett et al. (1962), wherein a 100 ml graduated cylinder was filled with 50 ml hexane (BDH) and covered with aluminium foil. The volume of hexane \( (V_1) \) and the total weight \( (W_1) \) were recorded. Enough powder was added slowly through a funnel to increase the volume by about 40 ml. The cylinder was left to stand for 1 hr after which the volume of powder \( (V_3) \), the volume of powder + hexane \( (V_2) \) and the total weight \( (W_2) \) were recorded. The calculations were made as under:
Bulk density  
\[ \text{g per ml} = \frac{W_2 - W_1}{V_3} \]

Average particle density  
\[ \text{g per ml} = \frac{W_2 - W_1}{V_2 - V_1} \]

Percent volume occupied by powder particles  
\[ = \frac{V_2 - V_1}{V_3} \times 100 \]

3.4. Packaging and shelf life

The world prices of tin have been steadily rising and for this reason packaging of low cost weaning food in tin containers is obviously not recommended. However, in order to study the comparative value of polythene bags (300 gauge), lacquered tin containers, with or without nitrogen packing were used.

In order for any product to be successful it is essential that it should have a good shelf life not only on basis of acceptability, for flavour and reconstitutability, but its ability to retain the nutritive value for as long period as possible.

It is generally an accepted practice to assume that \(30^\circ \pm 1^\circ C\) and 80 percent RH represent the average year round room temperature and humidity, respectively. But in order to see the effect of storage under refrigerated condition, a study at \(5^\circ \pm 2^\circ C\) representing refrigeration
temperature was also undertaken on the two packa-
ging systems.

The stored (air packed) SUWF was
analysed for moisture, NPN, SN, free-fat, FFA,
available lysine and peroxide value and assessed
for colour, solubility index, wettability, dispersa-
bility, viscosity, bulk density, average particle
density, percent volume occupied by powder particles,
and organoleptic characteristics at regular
intervals.

3.5. Sensory evaluation

Sensory evaluation of the product was
carried out at monthly intervals, during storage
trial of the food, with a selected panel of judges,
for acceptability of reconstituted sweetened
flavoured SUWF (12% SUWF solids + 7% sugar). A
nine point hedonic scale (Table 13) was used.

3.6. Protein efficiency ratio (PER)

PER of the soybean and its prepared
products was determined as per the method of
A.O.A.C. (1970) with slight modifications. The
various materials and animals used in this study
are described as under:

3.6.1. Reference casein

The casein fed as control diet to
rats was prepared by the method of Newport (1967)
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<tr>
<td>Moderately</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Dislike</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Very much</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dislike</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extremely</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comments

Initials
from buffalo milk, in the Experimental Dairy, National Dairy Research Institute, Karnal.

3.6.2. **Salt mixture**

The salt mixture included in the diet of all animals was prepared by grinding, to fine powder, and thorough mixing of salt mixtures 1 and 2 as given below:

<table>
<thead>
<tr>
<th>Salt mixture-1</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride, NaCl</td>
<td>9.30 g</td>
<td></td>
</tr>
<tr>
<td>Potassium Iodide, KI</td>
<td>0.79 g</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salt mixture-2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride, NaCl</td>
<td>130.000 g</td>
<td></td>
</tr>
<tr>
<td>Potassium phosphate, KH₂PO₄</td>
<td>389.000 g</td>
<td></td>
</tr>
<tr>
<td>Magnesium sulphate, MgSO₄</td>
<td>57.300 g</td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate, CaCO₃</td>
<td>381.400 g</td>
<td></td>
</tr>
<tr>
<td>Ferrous sulphate, FeSO₄·7H₂O</td>
<td>27.000 g</td>
<td></td>
</tr>
<tr>
<td>Manganese sulphate, MnSO₄·H₂O</td>
<td>4.010 g</td>
<td></td>
</tr>
<tr>
<td>Zinc sulphate, ZnSO₄·7H₂O</td>
<td>0.548 g</td>
<td></td>
</tr>
<tr>
<td>Copper sulphate, CuSO₄·5H₂O</td>
<td>0.477 g</td>
<td></td>
</tr>
<tr>
<td>Cobalt chloride, CoCl₂·6H₂O</td>
<td>0.023 g</td>
<td></td>
</tr>
</tbody>
</table>

The prepared salt mixture was kept in screw cap polythene bottles.
3.6.3. **Vitamin mixture**

Since B complex vitamins were not available individually, a vitamin Premix was obtained from Roche Laboratories, Bombay. The final mixture used contained the following vitamins in proportions mentioned against them. Although vitamin C is not needed for the rat growth, it was present in the Premix.

<table>
<thead>
<tr>
<th>Vitamin A (Dry, stabilized)</th>
<th>2000 I.U.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td>200 I.U.</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>10 I.U.</td>
</tr>
<tr>
<td>Menadione</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline</td>
<td>200</td>
</tr>
<tr>
<td>p-Aminobenzoic acid</td>
<td>10</td>
</tr>
<tr>
<td>Inositol</td>
<td>10</td>
</tr>
<tr>
<td>Niacin</td>
<td>5.28</td>
</tr>
<tr>
<td>Calcium D-pantothenate</td>
<td>4</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.88</td>
</tr>
<tr>
<td>Thiamine hydrochloride</td>
<td>0.53</td>
</tr>
<tr>
<td>Pyridoxine hydrochloride</td>
<td>0.5</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.2</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>42</td>
</tr>
<tr>
<td>Glucose, to make</td>
<td>1000</td>
</tr>
</tbody>
</table>

The final mixture used contained the following vitamins in proportions mentioned against them. Although vitamin C is not needed for the rat growth, it was present in the Premix.
3.6.4. **Vegetable oil**

Commercially available Dalda brand hydrogenated vegetable oil, manufactured by Hindustan Lever Ltd. was used in this study.

3.6.5. **Cellulose**

Powdered cellulose was obtained from Vallabhai Patel Chest Institute, Delhi University, Delhi.

3.6.6. **Composition of basal diet**

The basal diet consisted of following proportions of the ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>10</td>
</tr>
<tr>
<td>Fat</td>
<td>11</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1</td>
</tr>
<tr>
<td>Yeast powder</td>
<td>1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>1</td>
</tr>
<tr>
<td>Water</td>
<td>5</td>
</tr>
<tr>
<td>Sucrose and Lactose</td>
<td>66</td>
</tr>
</tbody>
</table>

As far as possible, all the diets contained approximately the same proportions and contents of nitrogen, fat, ash, moisture and crude fibre.
3.6.7. **Experimental animals**

Weaned albino rats, bred and brought up by the Human Nutrition and Dietetics Division, National Dairy Research Institute, Karnal, were used.

The experimental diets, after preparation, were packaged in double layer polythene bags and stored in deep freeze, till PER determination.

The experimental animals were fed the basal diets (Table 14) and water *ad libitum*, for a period of 20 days. Weight gained by the rats as well as the amount of protein taken was recorded weekly. On the basis of these data, protein efficiency ratio was calculated as under:

\[
\text{PER} = \frac{\text{g weight gain}}{\text{g protein intake}}
\]

Protein efficiency ratio of a 12 month stored sample of SWWF was also carried out, against fresh casein as control. The basal diets were prepared as per group A and E. The SWWF was packaged in polythene bags and tin containers and stored at 5°C±2°C and 30°C±1°C for 12 months.
Table 14. Basal diet given to different groups, in kg.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>0.591</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raw soyflour</td>
<td>-</td>
<td>1.230</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blanched soyflour</td>
<td>-</td>
<td>-</td>
<td>1.140</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SUUF</td>
<td>-</td>
<td>-</td>
<td>2.130</td>
<td>2.130</td>
<td></td>
</tr>
<tr>
<td>SUUF + methionine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0052</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>0.980</td>
<td>0.980</td>
<td>0.980</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat</td>
<td>0.543</td>
<td>0.304</td>
<td>0.322</td>
<td>0.130</td>
<td>0.130</td>
</tr>
<tr>
<td>Yeast powder</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Cellulose</td>
<td>0.050</td>
<td>0.005</td>
<td>0.008</td>
<td>0.035</td>
<td>0.035</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Salt mix</td>
<td>0.233</td>
<td>0.185</td>
<td>0.209</td>
<td>0.150</td>
<td>0.150</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.200</td>
<td>0.200</td>
<td>0.200</td>
<td>0.200</td>
<td>0.200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>2.303</td>
<td>1.996</td>
<td>2.041</td>
<td>2.255</td>
<td>2.255</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5.000</strong></td>
<td><strong>5.000</strong></td>
<td><strong>5.000</strong></td>
<td><strong>5.000</strong></td>
<td><strong>5.000</strong></td>
</tr>
</tbody>
</table>

Group A control - casein
Group B Raw soyflour - obtained by grinding raw whole soybean to 50 mesh
Group C Blanched soybean- hot air dried after blanching and ground to 50 mesh
Group D SUUF - Prior to fortification with methionine
Group E SUUF - after fortification with methionine
3.7. **Statistical analysis**

The data obtained in this investigation were subjected to analysis of variance and critical difference by 't' value, wherever necessary.

3.8. **Cost estimation**

The cost of manufacturing soy-whey weaning food was estimated as per the guidelines of Peter and Timmerhaus (1968), and of the case study by Patel and Arora (1976) with some assumptions, and modifications.

3.8.1. **Assumptions**

In order to arrive at a realistic cost of the raw materials and the end product, certain assumptions were made. The prevailing practices in the dairy industry on disposal of products or their byproducts were taken into account. Such as whey. It was assumed, as is the practice in the dairy industry, that cheese whey would be available at no cost, because it is generally thrown away that acts as a pollutant. It was further assumed, in the absence of a big capacity spray drier, that the Anhydro spray drier installed in the Experimental Dairy, National Dairy Research Institute, Karnal (capacity 35 kg water evaporation per hr) would be used and run continu-
ously for 24 hrs, except of course stopped for maintenance whenever needed. With the provision of 4,500 litres of fresh Cheddar cheese whey, the spray drier would run for 24 hrs, and on an average of 300 working days per year (after subtracting the time for maintenance etc.) it would produce 155.7 tons of weaning food.

Since the SUFF manufacture is dependent on the availability of cheese whey, its proximity to a cheese factory, if separately owned or as an annexe of the cheese factory is obviously required. The latter is most desirable, since the facilities such as steam, electricity, water etc. can be shared and for this calculation the same has been taken as the case.

3.9. Standardisation of method of manufacture

The method for the manufacture of SUFF was standardized, after several trials and is presented in (Fig 6).

3.9.1. Processing of soybean

Mixed-variety soybean was cleaned and after removal of split, undersized, wrinkled and damaged beans, was used for further processing.

3.9.1.1. Presoaking

The cleaned soybeans were soaked in
FIG. 6. FLOW SHEET FOR MANUFACTURE OF WEANING FOOD

WHOLE SOYBEAN

SOAKING in 0.5 percent sod. bicarbonate-12 hrs.

BLANCHING in boiling water for 30 min.

COOLING AND DEHULLING in tap water

DISINTEGRATION with whey

PRE-HEATING to 98°C

CONDENSING to 35% solids

HOMOGENISATION 3000±500 psi

CHILLING AND STORAGE at 5°C

SPRAY-DRYING

FORTIFICATION ferrous sulphate

DRY MIXING vitamin and methionine

PACKAGING nitrogen/air

FLAVOURING

PACKAGING nitrogen

STORAGE
10 volumes of 0.5 percent sodium bicarbonate solution for 12 hrs at 25°C to 20°C. The soak water was drained off.

3.9.1.2. Blanching

Presoaked soybeans were blanched in boiling water, in a double jacketed stainless steel kettle for 30 min as recommended by Nelson et al. (1971). After blanching the soybeans were left to drain on a screen.

3.9.1.3. Cooling and dehulling

The drained soybeans were cooled to room temperature by showering with tap water. The blanched soybeans were dehulled manually and the hulls removed by floatation. The cotyledons were separated from plumules and other sediment with the help of a sieve. Cotyledons were kept at 5°C to 20°C, if necessary, prior to grinding.

3.9.2. Disintegration

The soybean cotyledons were disintegrated in a 250 lit per hr capacity Micropulveriser (Frigimaires Engineers, Bombay) (Fig 7) fitted with a 325 mesh screen, to a slurry with about 75 percent of the total whey and redisintegrated.

3.9.3. Filtration

The soy-whey mix thus prepared was passed through a muslin cloth to remove any large particles which might have fallen into it.
Filtration was made easier by pouring the remaining 25 percent of the whey along with the mix.

3.9.4. **Pre-heating**

The soy-whey mix was heated to 98°C in a Silkeborg (Denmark) plate-heat-exchanger.

3.9.5. **Condensing**

The preheated soy-whey mix was drawn into a vacuum pan (John Chamber Sons, Auckland, NZ) (Fig 8) and condensed to a solids content of 35±0.5 percent under 61.0-63.5 cm vacuum. When the desired concentration had reached, the vacuum was released and the product (at 60°C) filled into cans for further processing.

3.9.6. **Addition of vegetable oil, fat soluble vitamins and sodium citrate**

In the absence of an acceptable quality refined polyunsaturated oil, calculated quantity of locally available fresh hydrogenated vegetable oil (Dalda of Hindustan Lever Ltd., Bombay) was added to the concentrate (at 60°C) to achieve the desired level of fat in SUUF. Fat soluble vitamins (Roche Laboratories, Bombay) (Table 15) and 0.25 percent (on solids basis) sodium citrate were added along with the vegetable oil.
3.9.7. **Homogenisation**

The concentrate was then homogenized in a 2 stage Gaulin (U.S.A) homogeniser, by maintaining a pressure of 3000 psi in the first stage and 500 psi in the second.

3.9.8. **Cooling**

The soy-whey concentrate was cooled, after homogenisation, in a DESC0 (Dairy Supply Co. Ltd., London) chilled water tank and kept at 5°C till spray drying.

3.9.9. **Fortification with iron**

Soy-whey concentrate was fortified with IP grade Ferrous sulphate (BDH) to obtain the desired level of iron in SWUF (Table 15). The ferrous sulphate was dissolved in distilled water and mixed thoroughly in the concentrate, just before spray drying.

3.9.10. **Spray drying**

The soy-whey concentrate was spray dried in an Anhydro (Denmärk) spray drier (Fig 9) of capacity 35 kg water evaporation per hr. The outlet temperature was kept constant at 95°±1°C while the inlet temperature was 190°±1°C. The speed of the atomiser was controlled manually to 25,000 ± 1,000 rpm. The powder was collected and packed in polythene bags and then stored in kraft-paper bags.
3.9.11. **Dry mixing of ingredients**

The SUUF was fortified with water soluble vitamins and methionine (Table 15) with the help of a APV horizontal mixer (APV Engineering Co., Calcutta) (Fig 10) of 10 kg capacity. The mixer had all stainless steel construction and was sterilised before use. Fortification was done in batches of 7.5 kg SUUF at a time. Calculated and weighed amounts of vitamin mixture (Roche Laboratories, Bombay) and DL-methionine (Sigma Chemical Co. USA) were mixed with the powder for 20 min.

3.9.12. **Addition of flavours**

In order to study the effect of flavouring agents such as vanilla, pineapple and strawberry (obtained in encapsulated form from Naarden India, Bombay), these were dry mixed in the SUUF at the rate of 0.8 g/kg and packaged in 200 g capacity lacquered tin cans, under nitrogen.

3.9.13. **Packaging**

The fortified SUUF was packaged in either polythene bags (300 gauge) or 1 kg capacity lacquered tin cans under air. In addition 200 g capacity lacquered tins were used for comparative study under nitrogen packing. For the purpose of acceptability trials during storage, the SUUF flavoured with different flavouring agents was
Fig. 7. Micropulveriser
Fig. 8. Vacuum pan and DESCO chilling tank
packaged in 200 g capacity lacquered tins under nitrogen. The tins and bags were stored for shelf life study (3.4)

Table 15. Rates of fortification of vitamins, iron and methionine

<table>
<thead>
<tr>
<th>Item</th>
<th>Initial content per 100 g</th>
<th>Amount added per 100 g</th>
<th>Final content per 100 g</th>
<th>ISI specification min per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>200 I.U.</td>
<td>1500 I.U.</td>
<td>1700 I.U.</td>
<td>1500 I.U.</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>Not estimated</td>
<td>300 I.U.</td>
<td>300 I.U.</td>
<td>300 I.U.</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>-do-</td>
<td>1.5 I.U.</td>
<td>1.5 I.U.</td>
<td>1.5 I.U.</td>
</tr>
<tr>
<td>Vitamin B₁</td>
<td>5.50 mg</td>
<td>0.53 mg</td>
<td>6.03 mg</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>4.04 mg</td>
<td>0.88 mg</td>
<td>0.92 mg</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>Not estimated</td>
<td>0.265 mg</td>
<td>0.265 mg</td>
<td>0.3 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>-do-</td>
<td>0.88 mg</td>
<td>0.88 mg</td>
<td>1 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Nil</td>
<td>42 mg</td>
<td>42 mg</td>
<td>30 mg</td>
</tr>
<tr>
<td>Nicinamide A</td>
<td>Nil</td>
<td>5.28 mg</td>
<td>5.28 mg</td>
<td>5 mg</td>
</tr>
<tr>
<td>Folid acid</td>
<td>Not estimated</td>
<td>95 mg</td>
<td>95 mg</td>
<td>50 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>2.5 mg</td>
<td>1.5 mg</td>
<td>4.0 mg</td>
<td>-</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.68 per 16 g N</td>
<td>1.12 per 16 g N</td>
<td>2.80 per 16 g N</td>
<td>-</td>
</tr>
</tbody>
</table>

* IS: 6387-1971