Methods and Materials
MATERIALS AND METHODS

This chapter deals with the materials and methods used in manufacturing handwa as well as different analytical procedures used in various phases of experimentation. The experimental plan of the study is presented in figure 4.1 and the various methods used during the course of the study are discussed under the following heads:

4.1 Survey methods
4.2 Preparatory methods
4.3 Analytical methods
4.4 Mix formulations

4.1 SURVEY METHODS:

Under the survey methods are discussed, the selection of families, tools used for collecting information, and the statistical analysis applied on the data obtained.

4.1.1 Selection of the families:

Using purposive sampling method, 106 Gujarati families were selected from Baroda city, on the basis of their mother tongue, Native/Domicile status in Gujarat, Gujarati cultural habits and preparing routinely most Gujarati dishes including Handwa. These families were classified into low (LIG - per capita monthly income < Rs. 400), middle (MIG - per capita monthly income between Rs.
PHASE IV
Preparation of nutritionally balanced handwas •
a. To compute nutritionally balanced handwa formulations for adults, adolescents and school children
b. To prepare handwas from the nutritionally balanced formulations for three selective target groups such as adult man, adolescent boys and school goers
c. To modify the balanced formulations for these groups to improve its organoleptic qualities.

PHASE III
Technology modifications in handwa preparation in terms of:

a. Substitution of rice with other cereals such as wheat, maize, bajra, jowar at 10, 25 and 50% levels.
b. Substitution of red gram dal and bengal gram dal (1:1), with val, moth beans, peas, lentil, green gram and black gram dal at 25% and 50% level.
c. Addition of vegetables such as string beans, okra, cluster beans and bottle gourd.
d. Addition of bottle gourd in grated and ground forms.
e. Introducing steaming prior to baking.
g. Development of ready to bake handwa mixes and study its shelf life in terms of: Moisture, Free fatty acid, Peroxide value, Sensory quality of handwa prepared from them, and microbiological quality of these mixes.

PHASE II
A Standardization of handwa using the most popularly used mix in terms of:

a. Selection of rice variety (parboiled vs ordinary)
b. Baking conditions
c. Mix particle size
d. Batter moisture level
e. Propagation & maintenance of starter culture & its effect on duration of fermentation.

B. Characterization of handwa

a. Gross composition of handwa in terms of Moisture, Protein, Fat, Carbohydrates, Ash, Fibre,
b. Shelf-life studies of handwa at ref. temp. & room temp. with respect to: Moisture, Sensory qualities and Microbial Qualities.

PHASE I
Survey of Gujarati families for handwa consumption, preparation & storage practices.

Baroda based families

<table>
<thead>
<tr>
<th>HIG*</th>
<th>MIG*</th>
<th>LIG*</th>
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<tbody>
<tr>
<td>(n= 46)</td>
<td>(n= 29)</td>
<td>(n= 31)</td>
</tr>
</tbody>
</table>

* Per capita monthly income in Rs.

HIG - > 1200
MIG - 400-800
LIG - < 400

Fig. 4.1 EXPERIMENTAL PLAN OF THE STUDY
income groups on the basis of their declared income.

The families were selected from 10 localities of Baroda city as shown in the figure 4.2. The distribution of 106 families amongst these survey sites according to their income groups is also shown in the figure, totalling to 31 LIG, 29 MIG and 46 HIG.

4.1.2 Tool used for collecting data:

In total, 23 questions were asked relating to the consumption, preparation and storage practices along with quality attributes of handwa, using pretested structured questionnaire given in Ap no. 9.4.1.1.

4.1.3 Statistical analysis:

Frequency distribution for variables were plotted and chi square test was used for determining their association with various income groups.

4.2 PREPARATORY METHODS:

The preparatory methods include selecting raw materials for handwa preparation, grinding and seiving of handwa ingredients, germination of pulses, preparation of batter, development of natural culture along with its storage, propagation and activation, use of baking devices, and steps involved in preparation of standard and instant handwas.
**LOCALITIES**

I ... Alkapuri, Race Course  
II... Pratap Gunj  
III... Fateh Gunj  
IV ... M.S.U. Campus(Tara Baug Colony, Adhyapak Kutir)  
V ... Sama  
VI ... Kareli Baug  
VII... Mandvi  
VIII... Makarpura  
IX ... Nizampura  
X ... Panigate

<table>
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<tr>
<th>Income Groups</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>H I G.</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>-</td>
<td>9</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>M I G.</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>L I G.</td>
<td>-</td>
<td>4</td>
<td>7</td>
<td>13</td>
<td>4</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>9</td>
<td>8</td>
<td>13</td>
<td>26</td>
<td>7</td>
<td>32</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>106</td>
</tr>
</tbody>
</table>

**Figure 4.2** Localities of Baroda city selected for Handwa survey.
4.2.1 Selection of raw materials used for handwa preparation:

The raw materials used for handwa preparation were procured from the commercial market of Baroda. These included a variety of cereals such as parboiled rice (*Oryza sativa*), wheat (*Triticum aestivum*), jowar (*Sorghum vulgare*), baira (*Pennisetum typhoidium*), and pulses such as red gram dal (*Cajanus cajan*), bengal gram dal (*Cicer arietinum*), green gram dal (*Phaseolus aureus roxb*), black gram dal (*Phaseolus mungo roxb*), peas (*Pisum sativum*), field beans (*Dolichos lablab*), and moth beans (*Phaseolus aconitifolius, Jacq.*).

In this study, sesame (*Sesamum indicum*), mustard (*Brassica nigra*) and garden cress (*Lepidium sativum*) seeds were also used. Oil used was a doubled filtered groundnut oil (Dhara, NDDB, Anand) purchased in one litre pack.

Fresh vegetables were purchased on day to day basis which included bottle gourd (*Lagenaria vulgaris*), ladies fingers (*Abelmoschus esculentus*), cluster beans (*Cyamopsis tetragonoloba*), string beans (*Vigna catjung*), and fresh spices including green chillies (*Capsicum annuum*), ginger (*Zinziber officinale*), and garlic (*Allium sativum*).

Other ingredients included sugar, commercially available 'amchur' powder (dried green mango powder), freshly squeezed and filtered lemon juice, salt and turmeric powder. Citric acid, and sodium bicarbonate of ANALAR grade were used in the preparation of Ready-To-Bake handwa mixes.
4.2.2 Grinding and sieving of cereal, pulse and other ingredients for handwa mix:

The ingredients in a predetermined ratio (typically rice, red gram dal, and bengal gram dal, in the ratio of 3:1:1), used for handwa making were ground coarsely in a horizontal stone grinder (Baby prince, model no. 158-10-85, Tushar Enterprises, Baroda) having the motor capacity of 1 horse power. The machine has provision for making adjustment for varying degree of coarseness of the flour. For preparing coarse mix flour, the knob was fixed at the seventh slot from the left, and the grain flow during grinding was controlled by using a ring of size '3'. The flour obtained by above grinding treatment was passed through sieve of 150 μ (100 BSS), 75 μ (200 BSS) and 25 μ (600 BSS) to obtain fraction F1 (>150 μ), F2 (150-75 μ), F3 (75-25 μ) and F4 (<25 μ).

4.2.3 Germination of pulses:

Germination of pulses was carried out according to Mushini (1990). The clean seeds (red gram whole and bengal gram whole) were steeped at room temperature with triple volume of water for 12 h. The steeped grains were drained off excess moisture, and germinated using the glass jar method (Chen, 1982). The glass jars were kept in a dark place. The grains were washed with tap water once a day to prevent drying and mold growth, and were allowed to germinate for 36 h.

The germination process was terminated by drying in an oven at 65°C, followed by devegetating the dried grains by hand.
4.2.4 Preparation of batter:

A batter was prepared by adding predetermined quantity of potable water (at room temperature) to the mix and kept ready for subsequent steps of fermentation and baking.

4.2.5 Cultivation of Natural Culture:

In order to obtain reproducible acidity in the fermented batter, a natural culture was developed by using the so called 'back slopping method'. Standard mix (10 g) was taken in a set of 3 sterilised screw capped test tubes, 15 ml of water was added to it and mixed thoroughly. The tubes were tightly closed and the batter was fermented at room temperature (28-30°C) for 12 h.

At the end of 12 h period, a small portion of fermented mix was taken from the tube that had the most acceptable aroma and developed acidity and was inoculated into another set of 3 sterilised tubes. This process of inoculation, incubation and fermentation was continued until all the 3 tubes gave an acceptable pleasant aroma and uniform rate of acid development.

The natural culture developed in this way was stored in deep freeze and removed for use, after activating the culture by transferring at least three times. Such an activated culture was used at the rate of 1.2 g for 50 g of typical handwa mix, to develop desired acidity level after 12 h of incubation at room temperature (28 to 30°C).
4.2.6 Use of baking Ovens:

For this study, the traditional handwa oven was used for standardizing a sand bath for the bulk of experimentation. In addition, solar cooker and microwave oven were also used for handwa preparation.

4.2.6.1 Handwa oven:

As shown in the figure 4.3-I, handwa oven consisted mainly of three parts (a) a circular aluminium body with a hole in center which holds the batter, (b) small circular ring filled with sand with a hole in the center to be placed below the larger vessel and (c) a 2" high domed cover, to be placed on the larger vessel. In order to study the time temperature profile of handwa being baked in handwa oven, about 1500 g of typical handwa batter was poured in the previously greased and dusted larger vessel, and was kept on the sand filled ring and was then kept on gas stove. The flame of the gas was kept high (Knob at position I) for initial 10 min. after which it was slowed down to minimum (Knob at position II) and baked for 70 min. The set-up used for determining the changes in temperature at 10, 20, 30, 40, 50, 60, 70 and 80 min interval, using thermometers inserted at three different positions in the batter (Top, center and bottom) is shown in figure 4.4a.

4.2.6.2 Sand bath:

A sand bath was used as a means of baking for the convenience of experimental work so that it was possible to prepare handwa in small quantities (130 g approx.). This simple device...
Different components of Handwa oven

- Lid with holes
- Main pot
- Sand filled bottom container

- Empty containers embedded in a sand bath

Main pot filled with handwa batter ready for baking on gas stove

Batters being poured into the pre-heated handwa containers in the sand bath on gas stove

Freshly baked handwa in a sand-bath

Set-up indicating handwa baking in traditional handwa oven

Sand-bath Set-up for handwa baking
Figure 4.4 a Placement of thermometers indicating the time-temperature profile of handwa during baking in handwa oven.

Figure 4.4 b Placement of thermometers indicating the time-temperature profile of handwa during baking in sand-bath.
consisted of a large aluminium container of about 4.5 l capacity, as shown in figure 4.3-II and 4 small containers, of about 180 ml capacity. The larger container was first filled with 600 ml of sand (of 25-150 u mesh size). Four smaller containers previously labeled, greased, and dusted with mix flour were placed on it at a uniform distance. The smaller containers were covered individually and about 900 ml of sand was poured all around the smaller containers so that they were fully immersed in the sand bed.

The smaller covers were removed and the sand bath thus prepared was placed on fire at a high flame for initial 5 min followed by covering the larger vessel in such a way that it allowed for some vent (a gap of half a centimeter) during baking. The flame was then reduced to a minimum, by adjusting the knob of the burner to position II, followed by pouring of about 162 ml of handwa batter, as shown in figure 4.3 IIb, in each of the smaller containers.

The handwa in sand bath with cover on (and a space for venting out vapour) was then allowed to bake for the remaining period (75 to 90 min) at a low flame. The changes in temperature of handwa batter while baking was recorded at an interval of 10, 20, 30, 40, 50, 60, 70 and 80 min from the thermometers, which were inserted previously into the batter through an aluminium cover at top, center and bottom positions as shown in figure 4.4b.

4.2.5.3 **Solar baking**

The solar cooker used for handwa was a commercially
available metal, box type cooker, with a black painted interior, covered with a double glass heat trap, and fitted with a glass mirror on the inside of the lid. Handwa was baked in this device by pouring 132 ml of typical handwa batter in the previously greased and dusted aluminium container, which was kept uncovered in the solar cooker, kept facing the sun on a bright sunny day for 2 h (11.30 a.m. to 1.30 p.m.).

4.2.8.4 Microwave Baking:

The microwave oven used for baking handwa was a Microwin MX 1100 operating at a frequency of 2450 MHz and of 1250 watts (electrical with 650 w microwave) capacity. The oven allowed for variations in microwave power settings, ranging from 100% (high or full power) to 10% (warm). It allowed the choice of time of exposure from 59 min, 59 sec to 0 sec. A rotating base was provided to give the materials a uniform exposure to microwave radiations.

Handwa batter was poured in a previously greased and dusted 250 ml glass beaker and baked for 4.5 min in 3 stages. The power level was set at 9 initially for 10 sec in the first stage, at power level 5 for 4 min in the second stage and at power level 8 for 20 sec in the third stage.
4.3 ANALYTICAL METHODS:

The analytical methods are discussed under three heads:

4.3.1. Sensory methods
4.3.2. Physical and chemical methods,
4.3.3. Microbiological methods.

4.3.1 Sensory methods:

Under the sensory methods are discussed, the selection of judges, preparation of a score card for product evaluation, method for sample presentation and evaluation, and statistical analysis of the data.

4.3.1.1 Selection of judges. (Griswald, 1882):

Twelve panel members, experienced in organoleptic testing of the foods were selected from the Foods and Nutrition Department of the Home Science Faculty. The selection was made on the basis of their performance in the replicate tests. They were presented with the handwa samples to be tested over a time period until the subject's response satisfied some established performance criteria. (e.g. standard handwa given along with other handwa samples could be identified easily by the panel members for all its quality characteristics.)

4.3.1.2 Development of score card:

The score card developed for organoleptic evaluation was based on the guidelines given by Hunter et al. (1950) for evaluation of cake quality. A sample score card along with scores (used
for statistical analysis) is presented in Ap no. 9.4.1.2.

4.3.1.3 **Sample presentation for judges' evaluation**:

Before presentation, each sample was coded and placed in a random order using the random tables. Cut handwa samples were placed on a white porcelain dish at room temperature in a clean, odour free, open area of the laboratory (figure 4.5). Panelist were instructed to rate each sample as per the score card.

4.3.1.4 **Data Analysis**:

The statistical analysis of data was performed according to Gupta (1991). In all cases, the experiments were carried out atleast in triplicates at different times. Means and standard deviations were calculated for each quality characteristics. ANOVA was used for estimating the differences between the means of various treatments for each quality characteristics. Correlation coefficient between the sensory qualities and physical qualities of handwa were calculated wherever necessary, particularly when differences were statistically significant.

4.3.2 **Physical and chemical methods**:

The physical methods included the volume measurement of the product as well as the pH measurement of batters, where as the chemical methods included determining titratable acidity, analysing the proximate composition of the handwa in terms of moisture, protein, fat, ash, fibre and carbohydrates as well as the methods used for determining the shelf-life of the product e.g. peroxide
Figure 4.5 Freshly prepared handwa indicating the porous crumb texture and golden brown chewy crust. (Sesame seeds can be seen on the top).
4.3 2.1 Volume measurement of the Handwa:

Volume of baked handwa was measured using the seed displacement method (Griswold, 1962). The volume of handwa was determined from the differences in the volumes of seeds held by the empty container, and that with handwa.

4.3.2.2 Measurement of the specific volume of the handwa:

This was calculated by using the ratio of the volume of the product to the weight of the product. The Specific volume was expressed as ml/g.

4.3.2.3 Measurement of pH of the batter mix:

The pH of batter mix was measured with a digital electronic pH meter by directly immersing the combination electrode into the batter at room temperature.

4.3.2.4 Protein estimation (AOAC, 1965):

Nitrogen content was estimated by the micro Kjeldahl method which is based on the determination of the amount of reduced nitrogen (NH₂ and NH) present in sample. The various compounds are converted into ammonium sulphate, which is decomposed with an alkali and the ammonia liberated is absorbed in excess of neutral boric acid solution and then titrated with a standard acid.

Procedure: Finely powdered handwa samples (800 mg) was digested...
in 100 ml digestion tubes using 1 g catalyst mixture (finely ground 99 g K₂SO₄, 4.1 g HgO and 0.8 g CuSO₄) and 5 ml nitrogen free concentrated H₂SO₄ (Sp. Gr. 1.84). The digested sample was steam distilled along with 40% NaOH solution until 20 ml of distillate was collected in 2% boric acid solution in water containing 2 to 3 drops of mixed indicator (one part of 2% methyl red in ethanol with five parts of 0.2% bromocresol green in ethanol. The distillate was then titrated against 0.02 N HCl solution until the pink colour turned green. Blank was determined (without the sample) using same quantity of reagents, similar digestion and distillation methods as for the sample determination. Nitrogen percent was calculated as per the following:

\[ \frac{\text{Titre reading of sample} - \text{Titre reading of blank} \times 100 \times \text{Normality of HCl} \times 14.007}{\text{Wt. of the sample (mg)}} \]

4.3.2.5 Fat (AOAC, 1984):

Fat was estimated as crude ether extract of the dry material using soxhlet apparatus. The dry sample (5 g) was accurately weighed into a thimble and plugged with cotton. The thimble was then placed in a soxhlet apparatus and extracted with anhydrous ether for about 18 h. The ether extract was filtered into a weighed conical flask. The flask containing the ether extract was washed 4 to 5 times with small quantities of ether and the washings were also transferred. The ether was then removed by evaporation and the flask with the residue dried in an oven at 80-100 °C, was cooled in a desiccator and weighed.
Fat content (g/100 g sample) = \( \frac{\text{Wt. of ether extract} \times 100}{\text{wt. of sample}} \)

### 4.3.2.6 Total Ash (IS. 1984):

In the porcelain dish, previously dried in an oven and weighed, 5 g of handwa sample was accurately weighed. The dish was gently heated on a flame at first and then strongly heated in a muffle furnace at 550 ±20°C till gray ash resulted. The dish was then allowed to cool in desiccator and weighed. This process was repeated until the difference between two successive weighing was less than 1 mg.

Total ash percent by weight = \( 100 \frac{(W2-W)}{W1-W} \).

Where:
- \( W2 \) = weight in g of dish with ash.
- \( W \) = weight in g of the dish only.
- \( W1 \) = weight in g of the dish and handwa sample.

### 4.3.2.7 Fibre (AOAC. 1984):

Five g of moisture and fat free sample (determined as in 4.3.2.6 and 4.3.2.9) was weighed into 500 ml beaker and 200 ml of boiling 0.255 N (1.25% W/V) sulfuric acid was added to it. The mixture was boiled for 30 min, keeping the volume constant by the addition of water at frequent intervals (a glass rod inserted in the beaker helped smooth boiling).

At the end of this period, the mixture was filtered through a muslin cloth and the residue washed with hot water till free from acid. To the residue in a beaker, 200 ml of boiling 0.313 N (1.25%) NaOH added. After boiling for 30 min (keeping the volume constant as before) the mixture was filtered through muslin cloth.
The residue was washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80-100°C and weighed (We). The crucible was heated in a muffle furnace at 600°C for 2 to 3 h, cooled and weighed again (Wa). The difference in the weights (We-Wa) represented the weight of crude fibre.

\[
\text{Crude fibre} = \frac{\text{Wt of fibre} \times 100}{\text{Wt of sample}}
\]

4.3.2.8 Moisture (IS, 1984):

The sample (3-5 g) was accurately weighed in a previously weighed crucible along with sand and a glass rod (W1). The sample was mixed with the sand and placed in the oven maintained at 105±1°C for 4 h. It was cooled in the desiccator and weighed (W3). This process of drying and cooling was repeated at 30 min interval until the difference between two consecutive weighings was < 1 mg. The lowest weight was recorded. Moisture % in the sample was calculated using the following formula:

\[
\text{Moisture} \% = \frac{100(W2 - W3)}{(W2 - W1)}
\]

W1 = weight of the crucible + sand + glass rod

W2 = weight of the crucible + glass rod + sand with material in gram before drying.

W3 = weight of the crucible + glass rod + sand with material in gram after drying.
4.3.2.9 Carbohydrates (by subtraction):

The total carbohydrate was calculated by subtracting the values of protein, fat, ash and moisture from 100.

4.3.2.10 Peroxide value (B S 684:section 2.14:1976):

The peroxide value is determined by fractionating lipid portion of the sample and determining its peroxide content. This depends on reaction of KI in acid solution with the bound O₂ followed by titration of the liberated iodine with sodium thiosulphate.

Procedure: Fat was extracted from 10 g sample using 100 ml of petroleum ether (with soxhlet apparatus as mentioned in 4.3.2.6) and was weighed in a glass stoppered conical flask. To the fat was added a mixture of 10 ml chloroform and 15 ml of glacial acetic acid solution. The flask was stoppered and shaken for 1 min and placed in dark for 5 min, followed by the addition of 75 ml water and was titrated against 0.001 M sodium thiosulphate using 1 % freshly prepared soluble starch solution as an indicator.

Calculations:

Peroxide value/Kg of fat = [(V-V₀) T/M X 1000] meq/Kg

M = weight of the fat obtained.
V = titre reading for sample.
V₀= titre reading for blank.
T = Normality of sodium thiosulphate.

4.3.2.11 Measurement of free fatty acid (IS. 1984):

The sample (5 g) was weighed in a conical flask and 50 ml
of 90% alcohol was added. This alcohol was previously neutralised
to a slight pink colour using phenophthalein indicator (1% in 50% v/w alcohol). The mix was allowed to stand for 24 h with occasional shaking. The alcohol extract was filtered through a dry filter paper (Whatman no.1) and 10 ml of the filtrate was titrated against 0.05 N NaOH using phenophthalein (4 drops) as an indicator. The free fatty acid (expressed as % H₂SO₄) was calculated as:

\[
\text{Free fatty acid (as percent H}_2\text{SO}_4 \) = \frac{24.52 \times A \times N}{W}
\]

\[A = \text{Volume of NaOH required}\]
\[N = \text{Normality of NaOH}\]
\[W = \text{Weight of the sample}\]

4.3.2 Measurement of titratable acidity for handwa batter:

To a diluted batter, (25 ml of water was added to 5 g of handwa batter and mixed thoroughly) 3-4 drops of 0.5% phenolphthalein indicator (1% in 50% v/w alcohol) was added and the titratable acidity was measured using 0.1 N standard NaOH solution. The mixture was then titrated against the alkali until a faint pink colour was observed. The titre value obtained was multiplied by a factor of 0.18 so as to obtain the titratable acidity in terms of percent lactic (IS.sp : 18 (part XI) 1981 pg 118).

4.3.3 Microbiological analysis:

The ready made media were procured from "Hi Media" (Loba Chem, Bombay). The microbiological analysis was carried out as per the methods described in the Recommended Methods for Microbiological Examination of Foods by APHA (1965).
4.3.3.1 **Preparation of dilution blanks:**

The dilution blanks consisted of phosphate buffer (0.0041% KH$_2$PO$_4$), adjusted to pH 7.2 and filled up to 99 ml in screw capped dilution bottles. These were autoclaved at 121°C for 15 min. The bottles were brought to room temperature before using for sample dilution.

4.3.3.2 **Sampling of handwa:**

With a sterile knife the entire 130 g portion of handwa was removed from the baking container under aseptic conditions into a sterile pestle and mortar. It was ground into a uniform mixture. Aseptically 11 g was weighed and transferred into a 99 ml dilution blank (dilution 1 in 10). After thorough mixing, the solid particles were allowed to settle and 10 ml supernatant was subsequently pipetted out into another 99 ml dilution blank bottle (dilution 1 in 100). Subsequent serial dilutions were prepared similarly as needed.

4.3.3.3 **Total plate count:**

The total plate count was taken using autoclaved (121°C for 15 min) tryptone glucose yeast extract agar media (2.4% in water), cooled to 45°C before use. The plates were incubated at 37°C (±1) for 48 h before the counts were taken, and was reported as count per gm of sample.

4.3.3.4 **Yeast and Mold count:**

For yeast and mold count, 3.9% potato dextrose agar media
autoclaved at 121°C for 15 min and cooled to 45°C was used. 1 ml of sterilized tartaric acid was added to the media (200 ml) to adjust the pH to 3.5. The plates were then incubated at 21 ±1°C and the colonies developed were counted after 5 days of incubation period.

4.3.3.5 **Coliform count** :

4.1% of violet red bile agar media was boiled and cooled to 45°C before plating. The plates were incubated at 37°C and the colonies developed were counted after 24 h of incubation period.

4.3.3.6 **Psychrophillic count** :

This was obtained by using 2.4% tryptone glucose yeast extract media which was autoclaved at 121°C for 15 min and cooled to 45°C. The plates were incubated at 10 (±2)°C in the refrigerator for 7 days and the count obtained was taken as Psychrophillic count.

4.3.3.7 **Aerobic spore count** :

The total aerobic thermophilic spore count was determined by using the dextrose tryptone agar by pipetting out 20 ml of 1:10 handwa suspension into a flask containing 100 ml of dextrose Tryptone agar held at a temperature of 55°C (±2). The media along with sample was placed in a boiling water bath for approximately 3 min and then in flowing steam for 30 min with occasional shaking. The entire heated handwa-agar mixture was distributed equally between 5 petri plates and then plates were incubated for 48 h at 55°C.

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The combined count from the 5 plates represented the number of aerobic thermophilic spores in 2 g of product.

4.4 Preparation of nutritionally balanced handwa formulations:

The preparation of handwa involved almost all the major food groups such as cereals, pulses, vegetables, fats and curd. Using these ingredients, handwa recipes were developed to meet the requirements of a balanced meal for various target populations. The balanced handwa recipes were formulated using a computer programme in BASIC language developed by Prof. B.K. Chakraborty (Ap no. 9.4.2.1).

The programme required a standard RDA table having the values for five important nutrients viz., calories, protein, fat, calcium and iron, for various population groups such as moderately working adult man and woman, preschool children (3-6 y), school goers (7-9 y), adolescent boys and girls (13-15 y) per 100 g food.

The nutrient requirement per 100 g of the food was calculated by multiplying the recommended nutrient by a factor 0.1052 for adult man, 0.1204 for woman (moderately working), 0.1852 for preschoolers, 0.1129 for adolescent boys, and 0.1142 for adolescent girls (13-15 y). The factors were calculated according to the total food requirement per day of the population groups as per the tables on balanced diet (Gopalan et al., 1981). The food table on the other hand included 50 items commonly used in handwa along with their nutritional composition, such as calories, proteins, 

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fat, Fe & calcium.

In the computer, the process of obtaining balanced handwa formulations for a particular population group along with the ingredients and their nutrient content as well as its comparision with the RDA was carried out in 4 steps. The first step involved choosing a standard RDA table for a particular population group. In step two, necessary ingredients for the recipe formulation were selected. The third step included calculation of the mix formulated and its comparision with the RDA. Finally, in the fourth step, changes were made in the quantity of ingredients to match the RDA. The effect of such quantity changes was immediately visible in the monitor. The best solution for a given set of ingredients was then printed out. If not satisfactory, new formulations with different ingredient combination could be tried in the same manner.

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