METHODS AND MATARIALS

IV MATERIAL AND METHODS

This chapter deals with the materials and methods used in the development of soy cheese spread as well as standard analytical procedures used in various phases of experimentation. These are described under the following heads:

4.1 Analytical methods

4.2 Preparatory methods

4.3 Statistical methods

4.1 ANALYTICAL METHODS:

The analytical methods are discussed under three heads:

- 4.1.1 Sensory methods
- 4.1.2 Physico-chemical methods
- 4.2.3 Microbiological methods

4.1.1 Sensory methods:

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

4.1.1.1 Selection and training of judges (Griswald, 1962):

Ten panel members, experienced in quality testing of the foods were selected from the Food & Nutrition Department of the Home Science Faculty. The panel was selected on the basis of

their performance in the replicate tests. The training involved presentation of the products to be tested over a time period, until the subject's response satisfied some established performance criteria.

4.1.1.2 Development of score card:

The score card developed for organoleptic evaluation was based mainly on flavour, body and texture. spreadability and over all acceptability of cheese spreads. A sample score card along with scores are presented in Appendix -1.

4.1.1.3 Sample presentation for judge's evaluation:

Prior to the presentation of the product, each sample was coded and placed in a random order using the random tables. The cheese spreads were placed along with bread slices in an open area of the laboratory. Panelists were instructed to rate each sample as per the score card.

4.1.2 Physico-chemical methods:

The physical methods included the pH measurement of soy cheese spreads, and the measurements of curd behaviour in terms of curd tension and syneresis. The chemical methods included determining TA, analysis of proximate composition of soybean and its by products viz., soymilk, soy maska, soy residue, and whey, and soy cheese spreads in terms of moisture, protein, fat, ash, and carbohydrates, as well as the methods used for determining

the nitrogen , and lipid fractions. Particulars of these methods are detailed below:

4.1.2.1 Sampling of the cheese spread:

With the help of spatula, the cheese spread was thoroughly stirred and representative samples were taken for chemical analysis.

4.1.2.2 Measurement of pH:

The pH was measured by using a digital Electronic pH meter by making a paste of 10g cheese spread with 10ml of glass distilled water as per IS: Sp 18, part XI (1981).

4.1.2.3 Curd tension:

The curd tension of milk was determined by the method suggested by Chandrashekar et al.(1957) with the following modifications:

The temperature of soymilk was adjusted to 30oC in a water bath. From this 50 ml portions were taken in 100 ml beakers (50 x 72 mm) into which 5% of activated starter culture was added. The contents were thoroughly mixed and incubated in the water bath for 12 hrs. The curd tension was then measured and expressed in grams.

4.1.2.4 Syneresis:

Syneresis of curd was studied as follows: 100 ml of soymilk was taken in a beaker. To it was added 5% activated starter culture and incubated for 12 hrs. by keeping in water bath at 30oC. The quantity of whey expelled at intervals of 30 minutes was measured. For this purpose, the curd was poured into a measured (10"x10") piece of muslin cloth and hung on to the measuring cylinder with a funnel, and the strained whey was poured directly into the cylinder. At the end of each interval, the amount of whey drained was recorded.

4.1.2.5 Titratable acidity:

To a diluted cheese spread (10 g of soy cheese spread was diluted in 10 ml water), 3 to 4 drops of 0.5% phenophthalein indicator (1% in 50% v/w alcohol) was added and the titratable acidity was measured using 0.1N 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 0.18 so as to obtain the titratable acidity in terms of per cent lactic acid (IS: Sp 18, Part XI, 1981, pg 116).

4.1.2.6 Moisture and Total Solids:

Moisture content was determined by gravimetric method described in IS: Sp 18, part XI. (1981).

The sample (5g) was accurately weighed in a previously weighed crucible along with sand and a glass rod (W2). The sample was mixed with the sand and placed in the oven maintained at 105 ± 1oC for 4 h. It was cooled in the dessicator and weighed (W3). This process of drying and cooling was repeated at 30 min. interval until the difference between the consecutive weighings was 1 mg. The lowest weight was recorded. Moisture % in the sample was calculated using the following formula:

$$\frac{100\ (W2 - W3)}{(W2 - W1)}$$

W1 = Weight of the crucible.

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.

The total solids content of the samples were estimated by substracting the moisture content in 100.

4.1.2.7 Total nitrogen:

Total nitrogen was estimated by Micro-kjeldhal method (A.O.A.C.,1965) which is based on the determination of the amount of reduced nitrogen (NH_2 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. In order to obtain the protein (%), the total nitrogen per cent obtained was multiplied by 6.25 factor.

4.1.2.8 Fat:

Fat content of soybean, soy residue, and soy maska, were estimated as crude ether extract of the dry material using Soxhlet apparatus (A.O.A.C. 1984). The dry sample (5 g) was accurately weighed into a thimble and plugged with cotton. The timble was then placed in a Soxlet apparatus and extracted with anhydrous ether for about 16 h. The ether extracted 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 to 100oC, was cooled in a desiccator and weighed.

$$\text{Fat content (g/100 g sample)} = \frac{\text{Wt. of ether extract} \times 100}{\text{Wt. of sample}}$$

The fat content of soymilk, and soy whey was estimated by as per Rose-Gottlieb method (IS: Sp 18, Part XI, 1981).

4.1.2.9 Total ash:

In the porcelain dish, previously dried in an oven and weighed, 5 g of cheese spread sample was accurately weighed. The dish was gently heated on a flame at first then strongly heated in a muffle furnace at 550 $\pm$ 20oC till gray ash resulted. The

dish was then allowed to cool in a desicator and weighed. This process was repeated until the difference between the two successive weighings was less than 1 mg (IS:Sp.18, part XI,1981).

Total ash per cent by weight = 100 (W2-W)/W1-W.

W2 = weight in g of dish with ash.

W = weight in g of the dish only.

W1 = weight in g of the dish and cheese sample.

4.1.2.10 Carbohydrates (by subtraction):

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

4.1.2.11 Salt:

Salt content of cheese spread was determined as per IS: Sp:18 (part XI), 1981.

4.1.2.12 Nitrogen fractions:

The nitrogen fractions viz., total nitrogen (TN), water soluble nitrogen (SN), non-protein nitrogen (NPN) were determined as per the method of El-Sokkary et al.,(1952) following semi-micro-kjeldhal method of Manifee and Overman (1940).

4.1.2.12.1 Water Soluble nitrogen:

Five grams of cheese spread sample was accurately weighed and mixed thoroughly with 25-30 ml of distilled water (50oC). The mixture was allowed to stand for 30 min. and decanted into a 100

ml volumetric flask through Whatman No.1 filter paper. Similarly, two other extractions were carried out and volume was made up to the mark with distilled water. A 20 ml portion was digested and the nitrogen content was determined.

4.1.2.12.2 Non-protein nitrogen:

Two grams cheese was thoroughly mixed and transfered into a 100 ml beaker with 40 ml distilled water and 10 ml of 20 percent tri-choloro acetic acid (TCA). The contents were heated to 70oC for 10 min. with continuous agitation, cooled and filtered into 100 ml flask through Whatman No.42 filter paper. The precipitates on the filter paper were washed with one percent TCA till the volume was made up to the mark. A 20 ml portion of the filtrate was digested and nitrogen content was determined.

4.1.2.12.3 Amino acid nitrogen:

The amino acid nitrogen was determined by the method of O'Keeffe et al. (1976). A 6% cheese homogenate in water was prepared and heated to 75oC for 5 min. with continuous agitation. The mixture was cooled and filtered through Whatman No.1. filter paper. A 50 ml portion of the filtrate was made with 12% TCA and filtered through Whatman No.42 filter paper. The filtrate was washed 3-4 times with solvent ether to make it free from TCA. The nitrogen content of 20 ml of the filtrate was determined.

4.1.2.13 Fatty acid fractions:

The fatty acid fractions included the estimation of total free fatty acids and the total volatile fatty acid content in the soy cheese spreads.

4.1.2.13.1 Total free fatty acids (TFFA):

The cheese fat was isolated as per method of Ramamurthy and Narayanan (1974). The cheese spread was packed tightly into 100 ml centrifuge tubes. These were placed in water-bath maintained at 50-55oC for an hour. The tubes were centrifuged (1500 x g) for 5 min. and the fat decanted. The TFFA of cheese fat was determined by the method of Lowry and Tinsley (1976).

A 0.2-0.5 g of fat sample was weighed into a screw-capped culture tube in duplicate and 5 ml of benzene solution was added to it and mixed thoroughly. After adding 1 ml of cupric-acetate pyridine solution, the biphasic system was shaken for 2 min. The contents were centrifuged in a clinical centrifuge at 2000 rpm for 5 min. The upper layer was read at 700 um in spectrocol and absorbance was recorded. The blank was prepared by adding 1 ml of reagents to 5 ml of benzene.

The standard curve was prepared by dissolving 0.34 ml of 99.7% pure oleic acid in benzene and the volume made to 100 ml. Out of this, 1 ml was made to 100 ml with benzene which is equivalent to 2000 um. Hence, 1 ml contains 20 um. A series of dilutions were prepared from the stock solution and the

development of colour was read in the same manner as described for the test sample. Standard curve was drawn by plotting the absorbance values against the oleic acid concentration, and was used for determining the TFFA of samples.

4.1.2.13.2 Total volatile fatty acids (TVFA):

The TVFA contents were determined as per method of Kosikowski (1966) from 10 g of well mixed cheese and the value expressed as ml of 0.01N NaOH per 10 g of cheese.

10 g of the well mixed cheese spread sample was taken in a beaker. A measured quantity (50 ml) of 10% sulphuric acid was added to it and the sample was mixed till complete emulsion was formed. The contents were transferred to 800 ml Kjeldhal flask. The beaker was washed thoroughly with remaining quantity of 10% sulphuric acid and washings were transferred to the Kjeldhal flask. Thirty five grams magnesium sulfate, 250 ml of distilled water, and a few glass beads were added into flask and shaken thoroughly. The flask was placed on Kjeldhal distillation apparatus and exactly 250 ml of distillate was collected and titrated against 0.01 N NaOH solution using phenolphthalein indicator. The insoluble fatty acids were collected by washing the condensor with 25 ml neutral alcohol and filtering it through Whatman No.1 into a separate flask. This was also titrated against 0.01 N NaOH.

The sum of these two titrations gave the TVFA in terms of ml of 0.01 N NaOH/10 g of cheese spread.

4.1.3. Microbiological analysis:

The analysis was carried out as per the methods described in the Recommended Methods for Microbiological Examination of Foods' by American Public Health Association (APHA, 1965). The ready-made media were procured from "Hi Media" (Loba Chem, Bombay).

4.1.3.1. Preparation of dilution blanks:

The dilution blanks consisted of phosphate buffer, (0.0041% KH_2PO_4), adjusted to pH 7.2, and filled in 99 ml screw capped dilution bottles. They were autoclaved at 121oC for 15 mins. The bottles were brought to room temperature before using for sample preparation.

4.1.3.2 Sampling of Cheese spread:

With a sterile spatula, the spread (150 g portion) was mixed thoroughly and 11 g of sample was weighed asceptically and transferred into a 99 ml dilution blank (representing dilution 1 in 10). After thorough mixing, the solid particles were allowed to settle and 1 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.

4.1.3.3 Total plate count:

The total plate count was taken using autoclaved (121oC for 15 min) tryptone glucose yeast extract media (TGEA), (2.4% in water), cooled to 45oC before use. The plates were incubated at 37oC for 48 hours ± 3 h before the counts were taken, and was reported as count per g of sample.

4.1.3.4 Yeast and mold count:

For yeast and mold count, 3.9% potato dextrose agar media autoclaved at 121oC for 15 min. and cooled to 45oC was used. One ml of sterilised tartaric acid was added to the media (200 ml) to adjust the pH to 3.5. The plates were then incubated at 2 ± oC and the colonies developed were counted after 5 days of incubation period.

4.1.3.5 Coliform count:

4.1% of violet red bile agar (VRBA) media was boiled and cooled to 45oC before plating. The plates were incubated at 37oC for 24 h. and the colonies developed were counted and recorded.

4.1.3.6 Aerobic total spore count:

The total aerobic thermophilic spore count was determined by using the dextrose tryptone agar by pipetting out 20 ml of 1:10 cheese spread suspension into a flask containing 100 ml of dextrose Tryptone agar held at a temperature of 55oC (±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 soy cheese spread-agar mixture was distributed equally between 5 petri plates and then plates were incubated for 48 h at 55oC. The combined count from the 5 plates represented the number of aerobic thermophilic spores in 2 g of product.

4.1.3.7 Proteolytic count:

For proteolytic count, TGEA was used. To this, 5% sterilised skim milk was added asceptically just before plating, thoroughly mixed and poured in the plates. The plates were incubated at 37oC for 4 days. The colonies having a digested zone surrounding each colony represented proteolytic one. The same experiment was repeated using soymilk as a source of protein in the media.

4.1.3.8 Lipolytic count:

For lipolytic count, nutrient agar with 5% butter fat added was used. The media was thoroughly mixed, pH adjusted to 7.8, and autoclaved at 121oC for 15 min. At the time of plating, the temperature of media was kept at 45oC. The plates incubated at 30oC for 3 days were counted for colonies. The same experiment was repeated using soyoil as a source of fat in the media.

4.1.3.9 Lactic count:

Lactic count was taken by using tomato juice agar media, autoclaved at 121oC for 20 min. and cooled (45oC) before plating.

The plates were incubated at 37oC for 3 days and then counted for the colonies.

4.2 PREPARATORY METHODS:

Under the preparatory methods, are discussed, selection of raw materials used for soy cheese spread, preparation of starter culture , methods followed for preparing soy cheese spreads based on maska and slurry approaches, and shelf-life studies.

4.2.1 Selection of raw materials:

The raw materials procured for the soy cheese spread preparation are detailed below:

4.2.1.1 Soybean:

Soybean (Glycine Max.) of **Monnato** variety was procured from the Main Research Station, University of Agricultural Sciences, Dharwad.

4.2.1.2 Salt:

Commercial grade fine grain salt was procured from M/S Tata Chemicals, Bombay.

4.2.1.3 Skimmed milk powder:

Sagar skimmed milk powder was procured from the local market.

4.2.1.4 Cream:

Fresh cream of 60% fat was procured from a local dairy for the purpose of the study.

4.2.1.5 Calcium chloride (dihydrate):

Calcium chloride of GR grade from S.D. Fines Chem. Pvt. Ltd., Bombay was used.

4.2.1.6 Tri-Sodium citrate:

I.P. Grade Tri-Sodium citrate was procured from M/S SD Fines Chem. Pvt. Ltd. Bombay. for processed cheese spread trials.

4.2.1.7 Tri-Sodium phosphates:

I.P. Grade Tri-sodium-phosphates was procured from M/S SD Fines Chem. Pvt. Ltd. Bombay for processed cheese spread trials.

4.2.1.8 Spices:

The required spices were purchased from the local market.

4.2.1.9 Papain:

Purified papain was procured from Loba-Chemie Indo-Austranal Co. Bombay.

4.2.1.10 Low methoxy pectin:

Amidated pectin type 2000 low methoxyl was procured from HP Bulmer limited, Hereford, England.

4.2.1.11 Agar Agar:

5 star quality, china grass (Agar Agar) packed by Agar Distributors, Bombay was used in the processed cheese spread experiments.

4.2.1.12 Reduced- Gluthathione:

AR grade reduced gluthathione was procured from SD Fine-Chem Pvt. Ltd. Bombay.

4.2.1.13. Natural Cheddar cheese:

Natural cheddar cheese (cured for 6 months) was procured from Amul Dairy, Anand for the soy spread making trials. The average composition of cheese was moisture, 30%, protein, 28%, and fat, 30%.

4.2.2 Starter Culture:

Mother Culture of multiple-strain mixed culture was obtained from a local dairy, Baroda. Bulk starter culture was prepared from cow's skimmed milk (10% TS), autoclaved at 15 psi for 10 min. and cooled to room temperature. The prepared milk was inoculated with 1% mother culture under asceptic conditions and incubated at 30oC for 12 hours to attain a desirable titratable acidity of 0.7 to 0.8 % lactic acid. The culture was activated atleast three times before use.

4.2.3 Preparation of soy cheese spreads:

The soy cheese spreads preparation by using two approaches viz. slurry and maska are outlined below:

4.2.3.1 Preparation of soy slurry based spreads:

Milk solids blended in soy slurry based cheese spreads were prepared according to the modified procedure of Singh and Mittal (1984). The modified procedure followed for slurry preparation are detailed below:

4.2.3.1.1 Preparation of soy slurries:

The soy slurry was prepared from dehulled soybeans. The dehulling was accomplished by rubbing the soaked beans (overnight in slight alkaline (0.04%) cold water) against each other with the hands. The seed coats freed from the beans were floated away with water. The dehulled soy bean was cleaned with water twice and boiled in an open pan with 10 times its volume of 1% sodium-bi-carbonate (weight basis of soybean) solution for 60 min. The water was decanted and the boiled beans were washed 2 to 3 times to wash away the excess alkali and ground in an electrical grinder to the consistency of slurry with sterile saline water.

4.2.3.1.2 Preparation of slurry spreads:

The detailed procedure of spread making is schematically depicted in fig. 4.1. Soybeans were cleaned, boiled in an open pan with 10 times its mass of 1% sodium bi carobonate solution

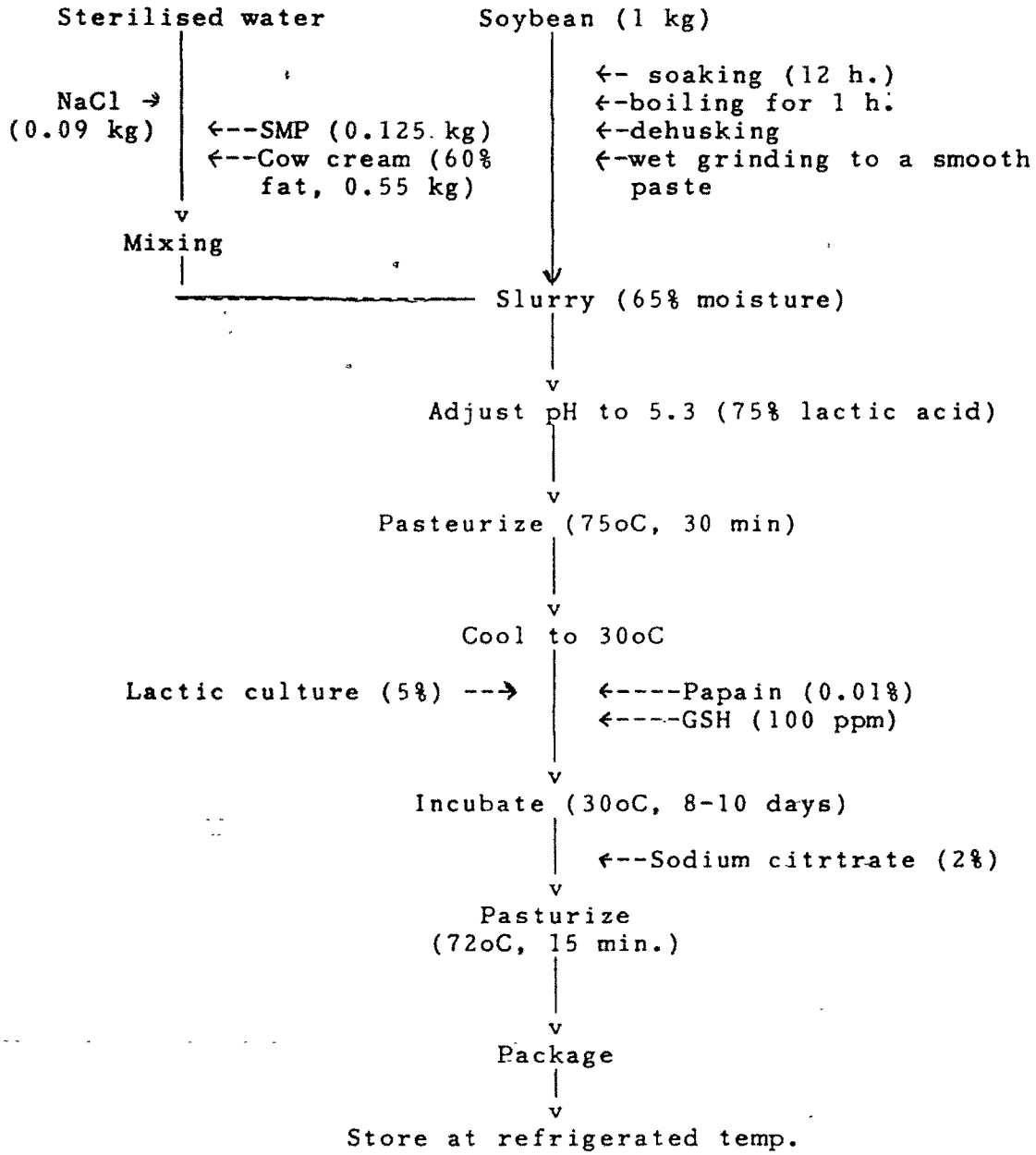

Fig.4.1 Preparation of soy slurry based cheese spread.

(weight basis of soybean) for 60 mts. and ground in an electric grinder to a smooth paste. The cream, skim milk powder and salt were added and mixed thoroughly. The pH of soy slurry was adjusted to 5.3 with 75% lactic acid. Soy slurry was pasteurized at 72oC for 30 mts. and cooled to 30oC. The dairy culture (5%), 0.01% papain and 100 ppm GSH were added. The inoculated slurry was maintained at 25 to 30oC for 8 days. The pH of the slurry was maintained to 5.3 with 50% NaOH when necessary.

Ripened slurry was pasteurized at 72oC for 15 mts. after addition of 2% sodium citrate in a double jacketed kettle. The coarsely ground spices were added after heating for 10 min. The slurry spread was packed in clean, hot water dipped polystyrene cups, covered tightly with alcohol flammed aluminium foil and stored in a refrigerator (8 to 10oC) for subsequent use.

4.2.3.2 Preparation of Maska based soy spread:

The maska based soy spread was prepared according to the method developed by Chakraborty (1990). The detailed procedure involved the preparation of soymilk from soybean and soy maska from the soymilk.

4.2.3.2.1 Preparation of Soymilk:

Soybeans were cleaned of foreign matters, broken beans etc. Beans were soaked for 8-10 hours using three times their weight of fresh cold tap water. A pinch of sodium-bi-carbonate was added to the soaking water (0.25% in water) to inactive the lipo-

oxygenase enzyme system. At the end of the soaking period, the unswollen dead seeds were sorted out and removed. The beans were then ground to a smooth paste for about three min. at high speed in Sumeet electrical grinder, and made up to a final mass 10 times the original bean weight by adding water. The homogenised mass was then strained through a double layered muslin cloth to extract the 'milk' portions. The milk was autoclaved for 10 minutes in pressure cooker (15 psi) and cooled to room temperature.

The process of obtaining milk from soybean is schematically presented in figure. 4.2.

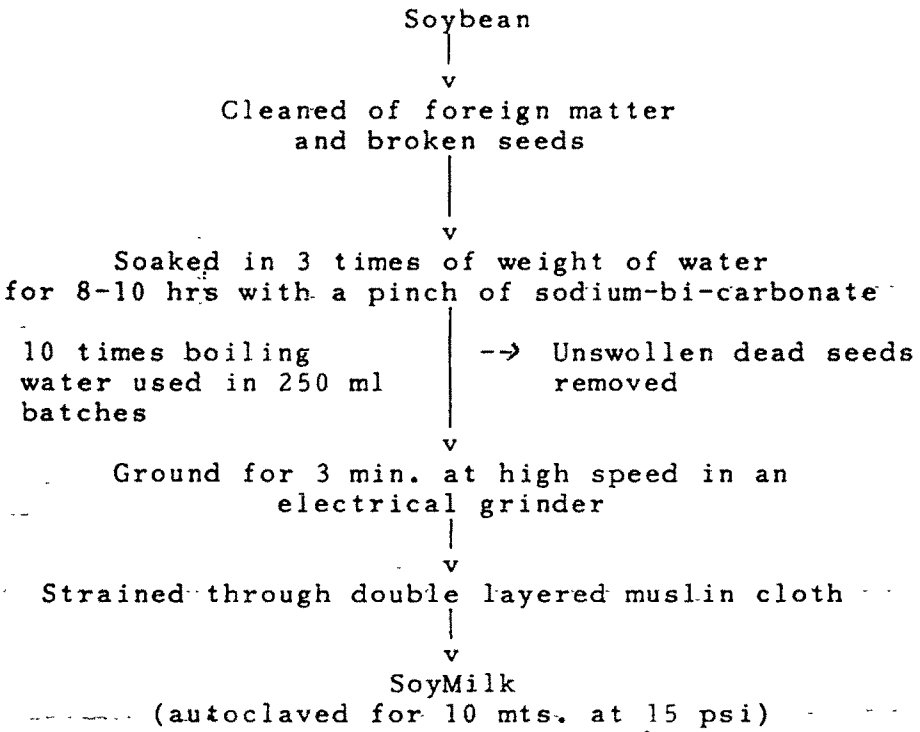

Fig 4.2 - Preparation of soymilk from soybean.

4.2.3.2.2 Preparation of Soy maska:

The autoclaved and cooled (30oC) soymilk was fortified with 5% reconstituted skimmed milk (10% TS)and 1% lactose. Further, the soymilk was inoculated with 5% active dairy culture in sterile conical flasks under asceptic conditions and incubated for 16 hrs. at 30oC. The fermented curd was subsequently drained to separate the whey and the concentrate, the maska' was stored in the refrigerator for future use. The process of preparing the soy maska is schematically presented in fig. 4.3.

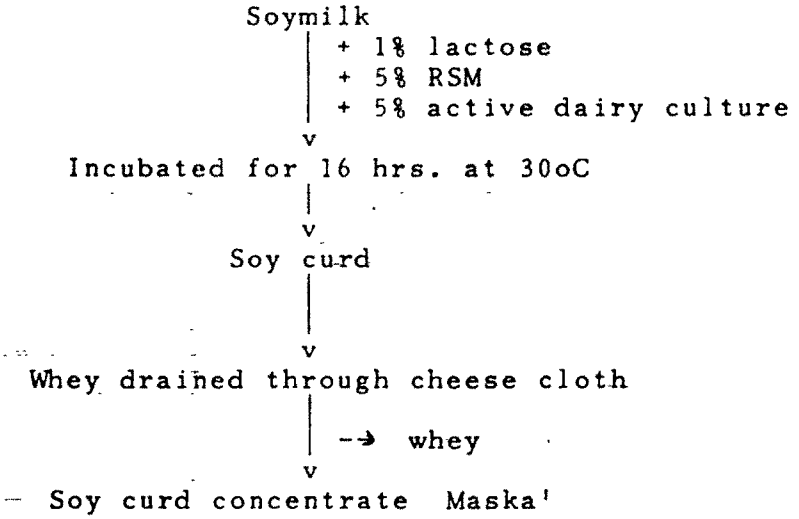

Fig.4.3 Preparation of soy maska from soymilk

4.2.3.2.3 Preparation of maska based cheese spread:

The detailed method followed is schematically represented in fig. 4.4. The 60 parts of soy maska was mixed thoroughly with 40 parts grated natural cheddar cheese (6 months old), along with 1.8 parts of salt. The blend was stored in the sterilised glass

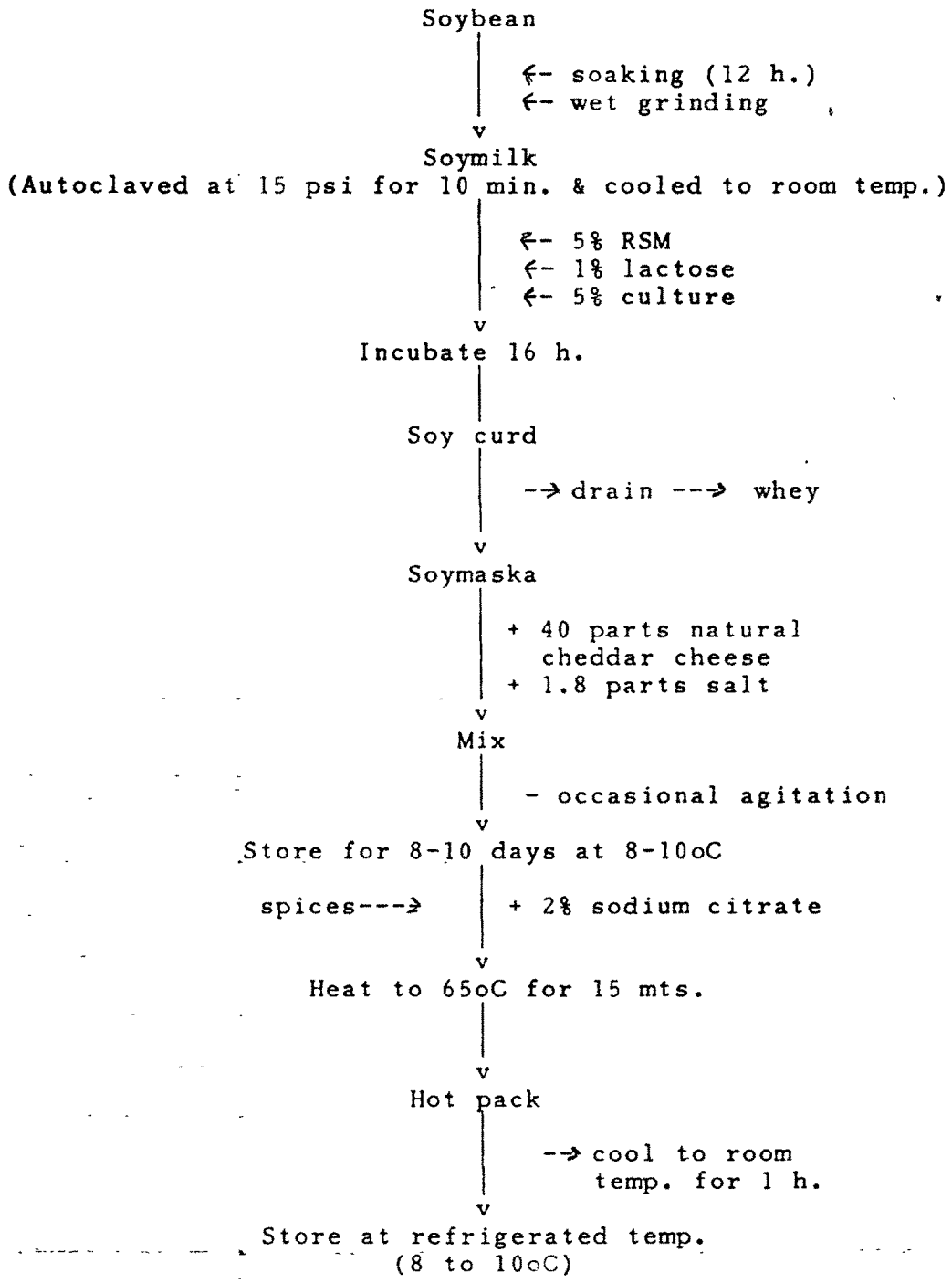

Fig. 4.4 Preparation of soymaska based cheese spread

jars with steel screw caps at 8 to 10oC for 10 days with occasional agitation (once in 2 to 3 days) with the help of sterilised steel spatula.

The cured maska cheese blend was heat processed at 65oC along with 2% sodium citrate on a water bath with continuous agitation. The blend pasteruized at 72oC for 15 mts. (as suggested by Singh and Mittal (1984) for soy slurry spread) resulted in destabilization of proteins and formation of precipitation. The spices (0.5 parts of green chillies and 1.0 part of garlic) were coarsely ground into a paste, and added to the blend after 10 mts. heating. The processed soy cheese spread was hot filled into hot water sterilised poly- styrene cups and tightly covered with an alcohol flammed aluminium foil. The spread was allowed to cool for one hour at the room termperature, and stored in the refrigerator (8 to 10oC) either for sensory evaluation or subsequent storage studies.

4.2.3.3 Shelf life studies:

For the purpose of shelf-life studies, the polystyrene cups, hot filled with heat processed soy cheese spreads were asceptically packed tightly with alcohol flammed aluminimum foil followed by wrapping in polyethylene bags. The samples were allowed to cool for an hour at the room temperature, and subsequently stored in the refrigerated temperature (8 to 10oC). The spreads were evaluated for moisture, pH, TA, sensory attributes, and microbiological quality.

4.3 STATISTICAL 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 and student t' test was used for estimating the differences between the means of various treatments for each quality parameters. Correlation coefficient between sensory qualities, and various microbial counts and the chemical paratmeters.