METHODS AND MATERIALS

IV METHODS AND MATERIALS

This chapter deals with the methods and materials used in the preparation of cheese cake alongwith various analytical procedures applied in different phases of experimentation. The detailed experimental plan of the study is depicted in Fig 4.1. Various methods and materials utilized in the processing of soybean to cheese cake are discussed under the following heads:

4.1 Preparatory Methods

4.2 Analytical Methods

4.3 Statistical Methods

4.1 Preparatory Methods

Preparatory methods describe the selection of raw material, ingredients and various steps involved in the preparation of cheese cake, such as soaking and grinding of soybean to obtain soymilk, conversion of soymilk to soycurd, pre-preparation of other ingredients, and subsequent conversion of soycurd to soycheese cake.

4.1.1 Selection of raw material

The raw materials required for the preparation of soy cheese cake namely soybean (*Glycine max.*), whole egg, raw rice (*Oryza*

Phase I lopment of the Basic Product	Phase II Product Improvement by Modifying Several Process Variables	Phase III Characterisation of Standardised Products
lishing the procedure for ring soymilk, conversion ymilk to lactic curd and y to cheese cake	Effect of various additives and treatments on curd forming and cheese cake properties	Establishing gross composition, microbiological profile and product shelf-life on storage
perimental Approach	**Experimental Approach**	**Experimental Approach**
ic fermentation of soymilk reference to culture types, bation time and tempera- and different sources of entable sugars e.g. R.S.M, y, sucrose and lactose	1. Treatment of soymilk with bayleaves and lemongrass	1. Gross compositional characteristics of the final products.
s compositional changes ng different stages of con- ng soybean to cheese cake	2 Addition of egg at 20-33g% levels	2. Sensory and Microbial characteristics of the packed products fresh and stored at 4-10 C for the duration of 90 days
	3. Substituting egg with pectin, gelatin, corn starch and other natural thickening agents namely bengalgram, blackgram, greengram, rice, wheat and potato	
	4. Optimization of temperatures for steam heating of cheese cake	
	5. Incorporating artificial flavours in the product	

Fig. 4.1 Experimental Plan of the Study

sativa), wheat (*Triticum aestivum*), sugar, skimmed milk powder and bay leaves (*Cinnamomum Tamala*) were procured from the local market of Baroda. Potatoes (*Solanum tuberosum*), lemongrass (*Cymbapogon martini*) used in the preparation of cheese cake were purchased fresh whenever required. Chemicals used in this study such as Sodium bicarbonate, and lactose (for the fermentation of soymilk) were of analytical grade. Flavouring agents such as lemon, mango, pineapple, orange and vanilla in liquid essence form manufactured by Bush Boake Allen (India) and coloring agents such as lemon yellow (Jamsons Industries, Bombay) and orange colour (viola) were used in the preparation of cheese-cake.

4.1.2 Steps for cheese cake preparation

Major steps involved in the preparation of cheese cake is shown in Fig 4.2. Preparation of soycheese cake from soybean involves soaking, extraction of milk, fermentation of soymilk and conversion of soycurd to soycheese cake are discussed in detail as under:

4.1.2.1 Preparation of Soymilk

Soybeans (in 100 g amounts) were soaked in a litre of tap water containing sodium bicarbonate at 0.5% level for a period of

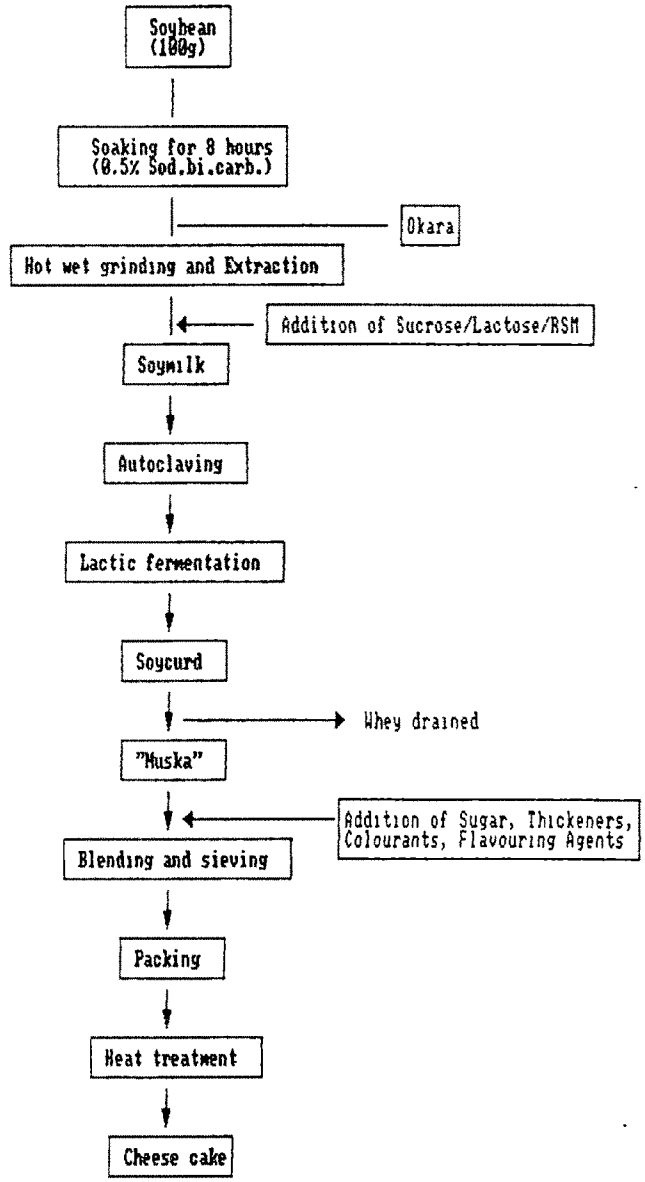

Figure 4.2 Basic steps for the preparation of soybased cheese cake

8 hours at room temperature. The water was decanted and beans were washed with fresh water, which was also drained off. Soybeans were ground to a smooth pasty consistency in a rotary mixer, for a period of 3 minutes, using 250-300 ml of boiling water. The resultant slurry was filtered through a double folded muslin cloth to extract the milk from the ground soybean slurry. Additional quantity of water was added for making up the final volume to one litre soymilk for every 100 g of soybean used. The residue (i.e., Okara) was discarded, and the soymilk thus obtained was autoclaved at 15 psi for 10 minutes in a household pressure cooker. As per the experimental requirements, sucrose, whey, lactose and reconstituted skimmed milk (as a source of fermentable sugars), bay leaves and lemon grass (flavouring enhancers) were added before autoclaving.

4.1.2.2 Whey

Whey obtained from skimmed milk was utilized after neutralising it with calcium hydroxide to precipitate calcium lactate. The resultant supernatant was used as a source of lactose, as and when required.

4.1.2.3 Preparation of lactic culture

Mixed lactic cultures commonly used in the fermentation of dairy milk were propagated in 9% skimmed milk at 40°C for 12 hours. The culture obtained in this way was stored in the deep freeze and the culture was activated by transferring at least three times before it is used in soymilk.

4.1.2.4 Conversion of soymilk to soycurd

Autoclaved soymilk was allowed to cool down to 40°C before a lactic culture was added for preparing soycurd by acid development. Soymilk inoculated with mixed lactic culture at the rate of 1% was incubated at 40°C for the duration of 14 hours.

4.1.2.5 Conversion of soycurd to cheesecake

Curd obtained with soymilk was poured on to a double folded muslin cloth which was hung freely and left undisturbed for 6 hours in order to remove the whey. The drained curd or "muska" was thoroughly blended with sugar by passing through a sieve of 60 BSS mesh to eliminate the granules and lumps. The final mix of drained curd and sugar obtained this way was thoroughly blended passed through a sieve of mesh 60 BSS with other additives as per the experimental reqirement. The product was tben heated in a waterbath at different temperatures to obtain the final cheese-cake like product.

4.1.3 Preparation of other ingredients

In the replacement of egg in the cheese cake following thickening agents such as rice , wheat and potato were used.

Rice : One hundred grams of rice was soaked for 8 hours in tap water at room temperature. The soaked rice was ground in a rotary mixer for 3 minutes with 300 ml of water and passed through a sieve (mesh 60 BSS). The volume was made upto 500 ml with water and the slurry was heated to 75^{o}C for 5 minutes to promote gelatinization before it was added to the "muska".

Wheat : One hundred grams of wheat was soaked for 8 hours in cold water at room temperature. The soaked wheat was ground in a rotary mixer for 3 minutes with 300 ml of water and passed through a sieve (mesh 60 BSS) to eliminate the bran. The final volume was made upto 500 ml with water and was heated to 75^{o}C for 5 minutes before it was added to the "muska".

Potatoes : Cut potatoes were pressure-cooked for five minutes (at 15 psi),deskinned and mashed thoroughly before mixing with "muska".

4.2 Analytical Methods

Analytical methods utilised in the study are grouped under the following heads:

4.2.1 Sensory methods

4.2.2 Physical and chemical methods

4.2.3 Microbiological methods.

4.2.1 Sensory methods

Sensory method included the selection of judges, preparation of score card, and presentation of sample for evaluation.

4.2.1.1 Selection of judges (Griswald 1962)

The taste (sensory evaluation) panel was composed of 10 panelist experienced in organoleptic testing, selected from the Department Foods & Nutrition , Faculty of Home Science, M S University of Baroda. These panelists were selected according to their performance in the repeated tests. The panelists were presented with cheese cake samples to be evaluated repeatedly until their recognition for the product characteristics became consistent.

4.2.1.2 Development of score-card

Important character notes such as flavour, body & texture, colour and appearance and its overall acceptability were evaluated on a 5 point hedonic scale in which 5 = "like very much ", 4 = " only liked ", 3 = "like slightly", 2 = "disliked slightly" and 1 = "dislike extremely". Judges were required to tick the relevant comments about the product included in the score-card (sample score card in Fig 4.2).

4.2.1.3 Sample presentation for panelists evaluation

Samples were presented in aluminium dishes in a random order to judges. Sensory evaluation was carried out atleast in triplicates with the trained panel members.

4.2.2 Physical and chemical methods

Various physical methods employed in different stages of preparation of soycheese cake includes yield, pH, synerisis, curd tension, and gel strength of the final product. The chemical methods included tests such as titratable acidity, analysis of gross compositional changes during different stages of processing of soybean to cheese cake in terms of moisture, protein, fat, ash and carbohydrates. These are described in the following sections.

Sensory Attribute		Sample no.						Date : Name :
Colour and appearance								
Highly desirable	: a							Criticism :
Desirable	: b							1. Ununiform colour
Moderately desirable	: c							2. Too dark/Too pale
Slightly undesirable	: d							3. Gas holes
undesirable	: e							
Body & Texture								
Highly desirable	: a							Criticism :
Desirable	: b							1. Sticky
Moderately desirable	: c							2. Too coarse
Slightly undesirable	: d							3. Creamy/Fine
undesirable	: e							
Flavour								
Highly acceptable	: a							Criticism :
Moderately acceptable	: b							1.Off Flavour
Acceptable	: c							2.After taste
Slightly unacceptable	: d							3.Acidic
unacceptable	: e							4.Very sweet
Overall acceptability								
Like very much	: a							Numerical Conversion:
Only liked	: b							a = 5
Like slightly	: c							b = 4
Dislike slightly	: d							c = 3
Dislike extremely	: e							d = 2
								e = 1

Other comments :

Figure 4.3 Score card for sensory evaluation of Cheese cake

4.2.2.1 Measurement of yield

The yield was expressed as weight of *muska* /fresh cheese cake obtained from 100 gm of soybean.

4.2.2.2 Measurement of pH of the milk curd and "muska" mix

A digital pH meter (Digichem 9201) was used for measuring pH, using a combination electrode, which was directly immersed into the sample of milk, curd or "muska" to measure the pH at room temperature.

4.2.2.3 Synerisis

One hundred ml of soycurd was poured onto a double folded muslin cloth (25 x 25 cms) and it was tied and hung freely on a funnel from which the whey expelled was collected directly in a graduated measuring cylinder. Quantity of whey expelled from the curd was monitored for 6 hours duration with 30 minutes interval.

4.2.2.4 Curd tension

The method of Chandrasekara et al (1957) was modified for measuring firmness of the curd using curd tension meter.

Curd tension knife was placed in a 100 ml beaker containing 50 ml soymilk innoculated with 0.1% lactic culture

before incubation. After incubation for the required duration the curd tension was measured by loading the pan with lead shots till the curd tension knife cut its way through the curd. The weight of the lead shots expressed in grams was taken as a measure of curd tension.

4.2.2.5 Gel strength/Hardness of cheese cake

To measure the textural quality of cheese cake, curd tension meter detailed in the Section 4.2.2.4 was used. Curd tension knife was placed in 100 ml beaker containing 50 g of final mix to be heat treated consisting of soy "muska", sugar and other thickening agents. The beaker was then placed in a thermostatically controlled waterbath at required temperature and duration. After the heating process was completed, the hardness of the cheese cake was measured by loading the pan with lead shots till the curd tension knife cut its way through the product. The weight of the lead shots expressed in grams was taken as a measure of gel strength.

4.2.2.6 Measurement of titratable acidity of milk, curd, "muska" and "muska" mix

Titratable acidity of milk and curd was measured using 0.1 N standard NaOH solution. Ten ml of milk or curd was taken in a 50 ml conical flask, to which 3-4 drops of phenolpthalein indicator

(1% in 50% v/w alcohol) was added. The mixture was then titrated against the alkali until a stable faint pink colour was observed for atleast 30 seconds.

Developed titratable acidity was calculated by subtracting the initial titratable acidity of milk from final titratable acidity of curd.

In the case of "muska", 1 g of the sample was diluted with 10 ml of distilled water and the mixture was titrated against 0.1N standard NaOH using phenophalein as an indicator for the end point. Titratable acidity was expressed in terms of percent lactic acid.

4.2.2.7 Moisture (IS : 1981)

Moisture content of the sample was determined gravimetrically by drying at $105\pm1^{o}C$ for a period of 4-6 hours until the difference between two consecutive weighings was less than 1 mg. Moisture percentage in the sample was calculated using the following formula:

$$\text{Moisture } \% = \frac{100\ (W_2 - W_3)}{(W_2 - W_1)}$$

W_1 = weight of the empty crucible with material

W_2 = weight of the crucible with material in gram before drying

W_3 = weight of the crucible with material in gram after drying.

4.2.2.8 Protein estimation (I S 1981)

Nitrogen content was estimated by the microKjeldahl method which determines the amount of reduced nitrogen (NH_2 and NH) present in the sample. The nitrogenous compounds present in the sample are converted into ammonium sulphate with sulphuric acid, which decomposes with the addition of excess alkali and the ammonia liberated is absorbed and forms ammonium borate, which is titrated directly against standard sulphuric acid.

Total protein present in the sample was calculated by multiplying total nitrogen percent by 5.71 factor.

4.2.2.9 Fat

Fat content of soy bean, soy muska , soy residue and final product were estimated as ether extract of the dry material using soxhlet apparatus (I S 1984). The dry sample was weighed accurately in to a thimble and plugged with cotton . The thimble was then placed in a soxhelet apparatus and extracted with ethyl ether for about 16 hours. The ether extract after evaporation was transferred in to a crucible and the residue was dried in an oven at 100^{o} C, cooled in a dessicator and weighed .

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

Fat content of the soy milk and whey was analysed using Mojonnier fat extraction method (I S 1981).

4.2.2.10 Total ash (IS : 1984)

Five grams of the sample was weighed in a dry porcelain dish and the dish was gently heated on a flame first and heated in a muffle furnace at 550 ± 20°C till gray ash resulted. The dish was allowed to cool in dessicator and weighed. This process was repeated until the difference between two successive weighing was less than 1 mg. Total ash percent by weight was calculated using the following formula :

$$\text{Total ash percent by weight} = \frac{100\ (W_2 - W)}{W_1 - W}$$

W_2 = weight in g of dish with ash

W = weight in g of the dish only

W_1 = weight in g of the dish with sample.

4.2.2.12 Carbohydrates (by subtraction)

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

4.2.3 Microbiological analysis

The microbiological analysis of the final product was carried out by the Recommended Methods for Microbiological Examination of Foods by APHA (1965). The dehydrated readymade media from "Hi media" (Loba Chem, Bombay) was used for culturing microorganisms.

4.2.3.1 Preparation of dilution blanks

Dilution blanks required for the sample dilution were prepared with phosphate buffer (0.0041% KH_2PO_4), adjusted to pH 7.2 and filled up to 99 ml in screw capped dilution bottles. These were autoclaved at 121^{o}C for 15 minutes. The dilution blank was brought to room temperature before it is used for sample dilution.

4.2.3.2 Sampling of cheese cake

The cheese cake undergone incontainer heat treatment (portion size of 75 g) was transferred with a sterile spoon into a sterile pestle and mortar. It was ground to 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

pipetted out into another 99 ml dilution blank - bottle (dilution 1 in 100). Subsequent serial dilutions were prepared whenever necessary.

4.2.3.3 Total plate count

The total plate count was done using tryptone glucose yeast extract agar media (2.4% in water), which was autoclaved (121^{o}C for 15 min) and cooled to 45^{o}C before use. After innoculating with the sample plates were incubated at 37^{o}C ($\pm$ 1) for 48 hours before the counts were taken, and was reported as count per gram of sample.

4.2.3.4 Yeast and mould count

For yeast and mould count, 3.9% dextrose agar media autoclaved at 121^{o}C for 15 minutes and cooled to 45^{o}C was used. The pH of the media (200 ml) was adjusted to 3.5 by adding 1 ml of tartaric acid. The plates were then incubated at 21$\pm$1^{o}C and the colonies developed were counted after 5 days of incubation period.

4.2.3.5 Coliform count

For coliform count, 4.1% of violet red bile agar media was boiled and cooled to 45^{o}C before plating. The plates were

incubated at 37°C and the colonies developed were counted after 24 hours of incubation period.

4.2.3.6 Psychrophillic count

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

4.2.3.7 Total aerobic spore count

The total aerobic thermophillic spore count was determined by using the dextrose tryptone agar. 20 ml of 1:10 sample suspension was pipetted out in to a flask containing 100 ml of dextrose tryptone agar held at a temperature of 55°C (±2). The flask containing the media with the sample was placed in a boiling waterbath for approximately 3 minutes and heated at 80°C for 30 minutes with occasional shaking. The entire heated sample agar mixture was distributed equally between 5 petriplates and the plates were incubated for 48 hours at 55°C. The combined count from the plates represented the number of aerobic thermophillic spores in 2 g of product.

4.3 Statistical Methods

The data obtained in different phases of experimentation was subjected for statistical analysis according to the methods suggested by Gupta 1991. The chemical,physical and sensory evaluations involved in the development of the product was carried out atleast in triplicates . ANOVA and students 't ' test was applied for finding the effect of various treatments and in the selection of most suitable treatment. Correlation coefficient was calculated wherever applicable.