3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Milk

Fresh milk (cow and buffalo) was obtained from dairy farm of NDRI, Southern Campus, Bangalore.

3.1.2 Cream

Cream was separated in Alfa Laval (500 lpd capacity) cream separator from cow and buffalo milk, as required for standardization.

3.1.3 Glass ware

Either corning/Borosil glassware was used according to requirement of various methods adopted for analysis.

3.1.4 Sugar

Refined cane sugar as per standard B1S : (1960) was used for preparation of Kunda.

3.1.5 Chemicals

3.1.5.1 Chemicals of analytical reagents (AR) grade procured from scientific supplies companies were used for various analysis and preservation studies.

3.1.5.2

(i) Protease: protease enzyme from Streptomyces griseus was procured from M/S SIGMA, USA.
(ii) Nisin: Nisalpin, 100,000 I.U per gm was procured from Applin and Barrett Ltd, Beaminstar Dorset U.K.

(iii) Potassium sorbate: Potassium sorbate A.R. grade was procured from LOBA scientific supply company. E.Merk(India) Ltd. Mumbai.

3.1.6 Packaging materials

1. Low density polyethylene (LDPE pouches) 65 µm and 200 g capacity

2. Metalised polyester (Aluminium/polyethylene) 90 µm and 200 g capacity

3. Can: Aluminium can 200 µm thickness and 200 g capacity were procured from local market for shelf-life studies.

3.1.7 Instruments and equipment


2. pH meter: mark VI: Systronics, Bangalore.

3. Remi centrifuge

4. Sartorius electronic balance (0.1 mg accuracy)


6. Steam kettle (SS): Ten liter capacity, provided with steam pressure gauge, condensate discharge tube and valve, handle, stirrer locking unit. Fixed to platform with four legs.

7. Foot operated impulse sealing machine: Sevana Co., Coimbatore, TN, INDIA.

9. Kjeldahl equipment - Kjelplus

10. Hand operated can seamer.

3.2 METHODS

3.2.1 Collection of market samples and characterisation

Belgaum is the only city where Kunda manufacturing and marketing is well known. Samples were collected from Belgaum city in sealed containers and brought to laboratory. Market samples were analysed for physico-chemical and sensory characters.

**Traditional method of Kunda preparation:** Kunda is a Khoa based delicacy. Khoa is prepared by halwais (Khoa makers) in villages under unhygienic conditions. Milk is desiccated in karahi 25 to 50 lit working capacity on firewood with continuous scrapings. Rice husk is also used as source of heating during Khoa making. After milk is half concentrated heating reduced with vigorous stirring. Concentrated milk changes to light brown colour with signs of leaving surface, which is made into a mass called ‘pat’. Then heating is stopped and the product gradually cooled to room temperature.

Kunda is prepared by using high moisture Khoa. High moisture Khoa is sold to semi-urban (sweet) Kunda manufacturers. A known quantity of Khoa, sugar and water is taken in a karahi. It is cooked till the end point with oozing of ghee like fat and changed to brown colour.
3.2.2 Standardization of various parameters of Kunda preparation

3.2.2.1 Effect of extent of desiccation on quality of Kunda

Fresh cow milk was procured from dairy farm of NDRI, Southern Campus, Bangalore and it was tested for fat, SNF and acidity (LA) according to BIS: SP: XI: 1981. Milk was preheated, filtered and standardised to 5.0 % fat and 8.5 % SNF. Milk was taken in a cleaned, sterilized Khoa kettle. Steam was let into the jacket. The milk was stirred continuously to avoid burning and heated to boiling. Then milk was desiccated to obtain Khoa.

Cane sugar crystals were added at the rate of 9.0 % on milk volume basis. The contents were heated to dissolve sugar with scraping and mixing vigorously. Further, potable water was added at about 10 to 15% (w/v) approximately to make slurry. The contents were desiccated to ‘pat’ stage. Then subsequent same lot of water was added to make slurry and desiccation was continued. This was continued till typical brown colour Kunda was obtained. The samples were drawn after 0, 2, 4, 6 and 8 such desiccations and were subjected to physico-chemical analysis (Moisture, browning index, water activity and pH). Sensory evaluation according to the procedure described in section 3.2.5.12 for different parameters was conducted.

3.2.2.2 Effect of type of milk on quality of Kunda

There are distinct differences in composition of cow milk and buffalo milk. These compositional factors influence the products texture, colour and acceptability as well as yield. To study the effect of type of milk on quality of Kunda the following experiment was conducted:
Cow milk was standardised to 5.0 % fat and 8.5 % SNF and Buffalo milk was standardised to 6.0 % fat and 9.0 % SNF. These milk lots were taken in Khoa Kettle and proceeded with preparation of Kunda as described section 3.2.2.1.

Kunda prepared from pure cow milk had a defect of gummy texture, while that prepared from pure buffalo milk had an abnormal grainy texture. Buffalo milk Kunda was dull brown in colour and cow milk Kunda was bright brown in colour. To solve the defective quality problem, buffalo milk was slowly boiled (simmering) during Khoa making and Kunda was prepared as in section 3.2.2.1.

Standardised milk lots also were mixed as follows for Kunda making

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Cow milk %</th>
<th>Buffalo milk %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Kunda was prepared as procedures described in section 3.2.2.1 with above treatments. Kunda samples were analysed for (Moisture, Acidity, yield and total solids) and sensory properties as in section 3.2.4.

3.2.2.3 Effect of steam pressure on quality of Kunda

Development of typical Kunda characteristics such as brown color, grainy texture and nutty flavour is mainly influenced by the extent of heating of milk, Khoa and sugar. The extent of heating has a prominent role in grain formation in Kunda.
To study the effect of temperature, two levels of steam pressure were selected which has direct relationship with temperature. During preliminary trials it was observed that steam pressure used for Khoa and Kunda making has affected the product characteristics.

Steam pressure used with gauge pressure was as follows.

1. During Khoa making- 1.0 kg/cm² (simmering)
2. During Kunda making- vigorous boiling
   i. 1.0 - 1.5 kg/cm² gauge pressure
   ii. 1.5 - 2.0 kg/cm² gauge pressure

It was observed in our preliminary trials that steam pressure 0.5 kg/cm² could not develop typical brown color and grainy texture as well flavour during desiccations. Steam pressure more than 2.0 kg/cm² caused burning of Kunda during with dark brown color and burnt flavour. Hence, above two levels of steam pressures were tried.

After preparation of Khoa, sugar and water was added and the mixture was desiccated till typical Kunda was obtained as per procedure described in section 3.2.2.1. Kunda was analysed for Browning index, water activity, yield, total solids and moisture as well as for sensory attributes on 9-point hedonic scale (Amerine et al., 1965).
3.2.2.4 Effect of levels of sugar on quality of Kunda

To study the effect of different levels of sugar on the characteristics of Kunda, the following percentage of refined sugar was used (BIS: 1960) on milk volume basis.

**Sugar levels**

1. 7.0 %
2. 9.0 %
3. 11.0 %

Kunda was prepared from fresh standardized mixed milk obtained from cattle yard. The manufacturing process was adopted as described in section 3.2.2.1. The product was analysed for quality of Kunda (yield, moisture, total solids, water activity and browning index and sensory properties) by following the procedures described in section 3.2.4 and 3.2.5.12

3.2.2.5 Optimization of kunda manufacture

A process was optimised for manufacture of Kunda as per various optimized parameters as in previous sections. Kunda prepared by the standardized method was characterized as follows:

1) Physico-chemical properties: Composition, yield, rheological, water activity, pH, acidity. Browning index

2) Sensory properties: Colour and appearance, flavour, body and texture
Traditional Method of Production

The method of production commonly adopted is summarised in the following flow diagram:

Milk
↓
High Moisture khoa* - 40% moisture
↓
Add crystal sugar (30 – 35%)
↓
Desiccation (90 – 120 min)
↓
Transfer to shallow pan
↓
*Kunda

* If low moisture khoa (< 40% moisture) is used, then about 10% buffalo milk is added.
Standardized process of Kunda manufacture

Based on the parameters standardized in earlier sections, a process has been developed for manufacture of Kunda. The flow chart of manufacture is given below:

Mixed milk:

(Cow milk (fat 5.0 & SNF 8.5%): Buffalo milk (fat 6.0 & SNF 9.0%)
(50:50)
↓
Steam kettle
(Simmering)
↓
Agitation and scraping with ladle
↓
Khoa
↓
Addition of crystal sugar @ 9% on milk basis (w/v)
(Or 30 to 35% on Khoa basis)
↓
Mixing & desiccation for dissolution of sugar
↓
Addition of water (@ 10-15%)
↓
Desiccation to pat stage
(Steam pressure 1-2 kg / sq.cm)
↓
Repeat
Such ‘desiccation’ 8 times
↓
KUNDA
3.2.2.6 Effect of desiccations on development of colour and flavour compounds in Kunda

During conversion of milk to Khoa and Khoa to Kunda, there is production of brown compounds and flavour compounds. During processing of Kunda the mixture of Khoa and sugar produces brown colour and flavour due to Maillard reaction. Maillard reaction is studied both from the point of color and flavour with equal importance in food products. It is desirable or undesirable in some food products. Many flavour compounds are produced during maillard reaction, which is an important attribute from point quality of Kunda.

Determination of flavour compounds

Standardised cow milk and bufflo milk were mixed in equal proportions. Milk was taken in a steam jacketed Khoa kettle. Kunda was prepared according to method described in section 3.2.2.5.

Samples were collected in 100 ml polystyrene cups from the following stages of manufacture and it was cooled to room temperature.

1. after ‘0’ desiccation (Khoa)
2. After ‘2’ desiccation
3. After ‘4’ desiccation
4. After ‘6’ desiccation
5. After ‘8’ desiccation

A. Flavour compounds were determined by steam distillation method and distillate was used for spectrophotometric measurement of optical density. The optical
density expressed as concentration of flavour compounds per gram of sample as in section 3.2.4.9.

B. Browning compounds were determined by method described in section 3.2.4.8. Optical density was expressed as concentration of browning compounds per gram of sample.

C. Kunda sample was analysed for pH, total carbonyls, Browning index and sensory properties as in section 3.2.4

3.2.3 PROCESS UPGRADATION STUDIES

3.2.3.1. Effect of addition of caramelized sugar on desiccation time reduction during Kunda manufacturing

Kunda processing requires prolonged desiccation of Khoa and sugar mixture in slurry form to get desirable brown color. Prolonged desiccation requires more heat energy as well as labour.

In view of the above facts, a study was undertaken with addition of cane sugar partly in caramelized form.

(i) Preparation of caramelized sugar solution

Required quantity (@ 30%, 40% and 50%) of refined cane sugar was taken in a shallow pan (S.S) and heated on a L.P.G stove till it melted and developed brown color. Then minimum quantity of water was added to stop further browning. Then heating was continued to dissolve the gummy sugar. This caramelized sugar solution was used in Kunda manufacturing. Optimum level of caramelized sugar addition was determined based on the sensory quality of Kunda.
(ii) Use of caramelized sugar solution in Kunda making

Standardized mixed milk was taken in Khoa kettle (S.S) and desiccated to make Khoa. The required quantity of cane and water sugar was added and further desiccated. Then caramelized sugar solution (40%) was added and the desiccation was continued. The required dilutions and desiccations were continued till desirable brown color was obtained (section 3.2.2.5).

(iii) Energy computation for Kunda manufacturing

During the process of Khoa making as well as Kunda making, the time and energy requirements were computed.

Time and energy consumed for Kunda manufacture was divided into two stages:

I. Milk to Khoa stage

II. Khoa to Kunda stage

i. Time taken

The initial temperature of standardized mixed milk was noted down. As it was fresh milk, without chilling, the temperature was about 30°C.

Time taken for first boiling of milk (latent heat) was noted. Then the time taken from boiling of milk to Khoa making and then Khoa to Kunda making was recorded. The time required for each dilution and desiccation was also recorded.
ii. **Collection of condensate**

As the quantity of steam used is equal to the quantity of condensate discharged, the quantity of condensate discharged from Khoa Kettle was collected in a vessel (SS), condensate volume and temperature were recorded immediately. Kunda was analysed for moisture, acidity, yield and sensory properties according to Section 3.2.4.

**Computation of energy consumption**

The total heat energy required for preparation of Kunda was computed as follows.

1. Quantity of condensate = ‘X’ kg
2. Temperature of condensate = t °C
3. Enthalpy of steam at absolute pressure (A) = y + 1.013 kg/cm². (Obtained from steam Table)
4. Operational steam pressure = ‘y’ kg/cm²
5. Total enthalpy of steam = AxX (kJ/Kg)
6. Enthalpy of condensate (B) = Xx 4.18X t °C
7. Total enthalpy of condensate = BxX (kJ/kg)
8. Total energy consumption = (AxX) - (BxX)

**3.2.3.2 Effect of food additives on quality of Kunda**

Browning is expected to increase with additives like trisodium citrates and disodium phosphate due to increase in pH so that the drudgery of prolonged heat desiccation during Kunda manufacturing can be reduced. In view of the above facts,
an experiment on effect of addition of disodium phosphate and trisodium citrate to milk during Kunda manufacturing was conducted.

Milk was standardised and mixed in equal proportions and added with 0.05% and 0.10% trisodium citrate or disodium phosphate.

Kunda was prepared from above milk according to section 3.2.1.5. Kunda was analyzed for pH, moisture, water activity ($a_w$), yield and sensory characteristics by following standardized procedures as in section 3.2.4.

Sensory evaluation was carried out by a panel of experienced judges for the following attributes.

1. Color and appearance
2. Flavour
3. Body & texture
4. Overall acceptance

on a nine point hedonic scale.

3.2.3.3 Effect of homogenization of milk on quality of Kunda

I. Homogenization of milk results in increased availability of proteins due to breakdown of globular proteins and adsorption on newly formed fat globules. These broken down proteins increase the surface area of milk fat globule about 10,000 times than the unhomogenized milk.

In view of the above increase in surface area, there might be increase in maillard reaction during Kunda manufacturing. Hence, an experiment was conducted to study the effect of homogenization milk on the physico-chemical properties of Kunda.
**Homogenisation of milk** Cow Milk was standardized to 5.0% fat and 8.5% SNF and buffalo milk was standardized to 6.0% fat and 9.0% SNF respectively. These standardized whole milks were separated into two lots. First lot (1) was homogenized at (1) 70 kg/sq.m and (2) 140 kg/sq.m without addition of sugar. Homogenized milk was taken on Khoa kettle and Kunda was prepared according to standardized procedure as in section 3.2.1.5. Refined cane sugar was added to second lot (2) @ 9.0% on milk volume basis and homogenized at (1) 70 kg/sq.m and (2) 140 kg/sq.m.

Homogenized milk was taken on Khoa kettle. Kunda was prepared according to standardized procedure under section 3.2.1.5.

Kunda was analysed for physico-chemical properties (yield, moisture, water activity, browning index, pH and sensory properties) according to procedure followed in section 3.2.5 and 3.2.5.12.

All these trials were compared with control (1:3:1) (mixed standardized unhomogenized milk) as in section 3.2.1.5

**II. Homogenization of skim milk**

Cow milk and buffalo milk were separated fat in a Alfa Laval cream separator, to get skim milk (<0.10% fat). The skim milk was divided into two lots. First lot was homogenized at (1) 70 kg/sq.m and (2) 140 kg/sq.m and with out addition of sugar (1). Kunda was prepared from homogenized skim milk after adding required milk fat in the form of cream, according to standardized procedure as in section 3.2.1.5. Second lot of milk was added with sugar (2) @ 9.0% on milk basis and
Homogenized after heating to 65 -70°C @ pressure of (1) 70 kg/sq.m and (2) 140 kg/sq.m. This homogenized skim milk was mixed and required fat percent was maintained by mixing cream to prepare Kunda according standardized procedure as in section 3.2.1.5. Kunda was analyzed for physico-chemical and sensory characteristics as in section 3.2.4.

**Homogenisation experiment Plan**

Kunda was manufactured from homogenized milk so that the some of difficulties faced during using cow milk and buffalo milk for Kunda making could be solved. Effect of homogenization of milk on the quality of Kunda was studied. Milk (1) was homogenized either in absence (1) or presence (2) of sugar. The effect of homogenization of milk in the absence of fat i.e. skim milk (2) was also studied. The following treatment combinations were tried for Kunda manufacture. Comparison was made with unhomogenised milk (3) as control.

(1) Standardized mixed milk was homogenized at 70 kg/sq.m without addition of sugar (1:1:1), (2) Standardized mixed milk homogenization at 140 kg/sq.m without sugar (1:2:1), (3) Control (1:3:1), (4) Standardised and mixed milk was added with sugar and homogenized at 70 kg/sq.m (1:1:2), (5) Standardised mixed milk added with sugar, homogenized at 140 kg/sq.m (1:2:2) and sugar added after Khoa, (6) Standardised mixed milk added with sugar and un homogenized ((1:3:2), (7) Skim milk, homogenized at 70 kg/sq.m and added with cream, and sugar at Khoa stage (2:1:1), (8) Skim milk, homogenized 140 kg/sq.m, and added with cream, and sugar at Khoa stage (2:2:1), (9) Skim milk added with cream and sugar and unhomogenized (2:3:1), (10) Skim milk, added with sugar homogenized at 70 kg/sq.m and added with
cream (2:1:2), (11) Skim milk added with sugar homogenized at 140 kg/sq.m and added with cream (2:2:2) and (12) Skim milk added with sugar unhomogenised and added with cream (2:3:2).

3.2.4 SHELF-LIFE STUDIES

3.2.4.1 Effect of preservatives on shelf-life of Kunda

The various substances, which are added to food for production processing, packaging and storage are called food additives. They may be added to prevent the deterioration or enhance the quality of foods during production, processing and storage.

Food additives under PFA rules part XIII A rule 64C; include color, flavoring agents, antioxidants anti caking agents emulsifying agents, stabilizing agents and preservatives.

1. Addition of preservatives

After preparation of Kunda as per standardised method (section 3.2.1.5), preservatives i.e., potassium sorbate and nisin @ 2000ppm were dissolved in some sterilized distilled water. The solution was added to Kunda when the product was still hot (about 85 - 90°C). Then Kunda was mixed thoroughly and packaged in 200 g capacity LDPE pouches. The pouches were sealed by impulse sealing machine. The warm sealed samples were cooled to room temperature and stored at 30°C for 0, 7, 14, 21, 28, 35 and 42 days. Another set of sealed samples were stored at 5°C for 0, 15, 30, 45, 60, 75 and 90 days.
Stored Kunda samples were analyzed at regular intervals for the following parameters according to procedure as in section 3.2.4. The samples were analyzed for physico-chemical, microbiological and sensory parameters at regular intervals (moisture, water activity, acidity, bacterial counts, yeasts and moulds counts, coliform counts and sensory properties).

### 3.2.4.2 Effect of packaging materials and storage temperature on quality of Kunda

Indigenous dairy products for large scale production face hindrance from lack of standard processing technology as well as proper packing technology. Khoa and Khoa based sweet meats are spoiled due to improper packaging. Khoa is produced in countryside and packaged in bamboo baskets lined with leaves and they are transported to urban areas for sales.

Kunda is packaged in loose LDPE pouches or collapsible bags. So the shelf life is very limited. The deterioration due to high moisture and unhygienic production and preservation methods, Kunda get spoiled within few days.

To study the suitability of modern packaging materials for storage of Kunda the following experiment was conducted.

### 3.2.4.2.1 Packaging of Kunda

Kunda was prepared from standardised cow milk and buffalo milk according to standardised method.
Hot Kunda was divided into two lots for storage at 30° and 50°C. Hot Kunda (85°-90°C) was packaged in the following packaging materials for storage at 30°C. Packaging materials.

1. P₁ (LDPE pouches) 65μm, 200g capacity
2. P₂ (Metalised polyester) 90μm, 200 g
3. P₃ (Aluminum cans) lacquer coated 200 μm and 200 g capacity.

The samples were drawn at the following intervals for analysis, at 0, 7, 14, 21, 28, 35, and 42 days of intervals. Each packaging containing 200 gm Kunda sample was sealed immediately. P₁ and P₂ were sealed by foot-operated impulse sealing machine. P₃ was sealed by can seamer.

Another set of above packaging materials (P₁, P₂ and P₃) were used for packaging and storage of Kunda at 5°C. They were stored for 0, 15, 30, 45, 60, 75 and 90 days.

Above packaged samples containing 200 gm were labeled accordingly for storage at different temperatures and intervals. The samples were drawn at regular intervals and analyzed for physico-chemical, rheological, microbiological and sensory properties (moisture, water activity, acidity, bacterial counts, yeast and mould counts, coliform counts and sensory properties) as per procedure given in section 3.2.5.
3.2.5. Chemical analysis of Kunda

Analysis of any product reveals its quality. Based on the analytical results the uses, shelf-life of the product can be decided. Analytical methods are chosen according to the category of products, its constituents and its effect on in future.

Analytical methods for the Indigenous dairy products are rarely standardized. However the methods available for other products are modified and adopted to analyze Kunda.

Moisture

The moisture percent in Kunda influences the physical properties like texture and body, water activity as well as shelf-life. The optimized moisture percent results in good quality with desirable properties.

Moisture percentage was determined according to BIS: 1981(SP: 18(part XI: 205).

Fat

Fat content in the Kunda influences the texture and flavour. Fat percentage in dairy products is determined by either Gerber method or Mojonnier extraction method. Kunda contains high total solids, with about 30 % sugar. With Kunda ‘s grainy texture determination of fat by Gerber method was difficult due to blocking of butyrometers as well as charring of contents. To overcome these difficulties Mojonnier extraction method was adopted according to BIS: 1981; (SP: 18: part XI:
22.3.2) with slight modification in weight of Kunda which was decided based on the fat percent equivalent to that in standardized milk.

**Protein**

Determination of protein by Kjeldahl method is the standard method. Microkjeltech method was BIS: 1981 (SP: 18: part XI: 17.1) was slightly modified. Accordingly quantity of Kunda sample taken for analysis is also modified based on protein equivalent to standardized milk protein.

**Carbohydrates**

Determination of carbohydrates in Kunda is a complex procedure. Determination of sucrose in Kunda was followed according to BIS 1981: (SP: 18(Part XI): 114.2) Polarimetric Method

**Ash:**

Kunda contains mineral matter that is same as in milk. Determination of ash was done according to the procedure BIS: 1980 (SP: 18 (Part I):33)

**Acidity**

Acidity was determined according to procedure laid down in BIS: 1980 (IS: 4883):Appendix.A-2: Direct Method.

**pH**

pH of Kunda was determined in all experiments as it was required according to O’Keefe et al (1976)
**Procedure:** Ten gm of Kunda was taken in a clean dry 100 ml beaker. 10 ml warm distilled water was added. The contents were mixed to uniform suspension using glass rod. Then the pH was determined using a previously standardized pH meter (Mark-IV: Systronics Make).

**Browning index:**

Kunda is brown in colour. It is one of the desirable characteristics. Brown colour in Kunda is due to Maillard reaction. During progressive heating Kunda develops typical brown colour with nutty flavour as a result of protein-carbohydrate interaction.

Gothwal and Bhavadasan (1992b) have modified the Palomobo *et al.* (1984) procedure to determine the browning index in whole milk powder. The modifications are as follows:

1. Calcium was avoided in the assay system.
2. Tris-HCl buffer (0.05M) pH 7.2 was used instead of Tris-Maleate buffer (pH 7.0).
3. Three ml of milk were taken instead of 1.5 ml sample.

Determination of browning index in kunda was carried out with proportionate changes in sample and the chemical used for the experiment.

**Preparation of reagents:**

a. **Tris-HCl stock solution (pH 7.2)**

i. Tris stock solution: Dissolve 2.42 gm Analytical Reagent(AR) grade. Tris in distilled water and make up the volume to 100 ml.
ii. 0.02M HCl: Dissolve one ml concentrated hydrochloric acid in 600 ml distilled water.

I. Tris-HCl buffer solution (pH 7.2): For preparation of Tris-HCl buffer solution, mix 6.25 ml Tris-stock solution with 5.75 ml of 0.02M HCl. Distilled water (88 ml) was added to make up 100 ml.

II. Enzyme solution: Three mg of protease (type XXV) (Protease E from Sigma Co., USA) was dissolved in 0.8 ml of Tris-HCl buffer and used for each assay.

Procedure: Three gram of Kunda sample was taken in a mortar, three ml of warm distilled water was added and made into a slurry with the help of pastle. Then remaining 15 ml of warm distilled water was added and made into a suspension. Six ml of Kunda suspension was pipetted out into a 15 ml sedimentation index tube. An aliquot of 1.6 ml of pronase enzyme solution was added and mixed. The sample enzyme mixture was incubated at 45°C in a water bath for 2½ hours. Then the mixture was chilled by keeping in the chilled water. Then mixture was removed from chilled water and 0.6 ml of TCA (100% solution) was added and mixed. This was centrifuged at 7000 rpm for 15 min in a high speed Remi centrifuge.

Then it was filtered thorough Whatman No. 1 filter paper. Then about five ml of filtrate was taken in a transparent cuvette (1 mm thickness) for reading optical density (OD) at 420 and 550 nm in Anthelite spectrophotometer according to the instruction manual.

Optical density (OD) was calculated as follows:

\[
OD = \frac{420 \text{ nm} - 550 \text{ nm}}{\text{Quantity of sample}}
\]
Browning index is expressed directly as optical density per gram of sample.

Note: Certain substances might interfere with absorbance at 420 nm, whose maximum absorbance is found at 550 nm. Therefore, to nullify the interference effect, the absorbance value is deducted from that of 420 nm.

3.2.5.9 Total carbonyls

Kunda has typical nutty flavour. It is because of protein–carbohydrate interaction during heat processing in presence of water. Many flavour compounds are produced during manufacturing of Kunda. These contribute to the acceptance of the products.

Carbonyls are the main desirable flavour components contributing to flavour of Kunda. Total steam volatile carbonyls in Kunda were determined according to procedure laid out in Ravindra Kumar et al. (1993).

Reagents prepared (i) 3N HCl: mix 3.6 ml of concentrated HCl in 96.4 ml of distilled water. (ii) Silicon oil.

**Apparatus**: Steam volatiles distillation apparatus.

**Procedure**: Eighteen gram of Kunda was taken in a mortar and it was ground with pestle after adding about five to eight ml warm distilled water out of 72 ml. Then remaining warm distilled water was added mixed and transferred completely to the round distillation flask (500 ml capacity). Glass beads were added to prevent bumping during distillation. Seven ml of 3N HCl was added and the contents were mixed. The round flask was fixed to distillation assembly and heating was started. The steam
generated dissolved kunda slurry releasing the steam volatile compounds. Distillation was carried out at control rate to collect 50 ml of distillate in 10 to 12 minutes. The UV absorption of the steam distillate was read in a spectrophotometer at 280 nm against distilled water as blank and the results were expressed directly in terms of optical density.

### 3.2.5.10 Water Activity

Water activity is one of the most significant parameters for the shelf stability of food system. It is the free water rather than total water content that is important as far as food microbial proliferation is concerned. Water activity controls the microbial, chemical and physical changes in food during storage.

Water activity of Kunda was measured by using Rotronic Hygrokop (BT-RS1, Switzerland).

**Procedure:** Water activity instrument was switched on for about 15 minutes for equilibration at room temperature. After the instrument shows equilibration for temperature and water activity, the sample was taken.

Kunda sample was taken in a equilibration cups. It was spread in 5 mm depth, 10 mm deep and 40 mm diameter circular plastic cups.

The sample cup was kept in the enclosed equilibration chamber without covering the lid. Water activity of the sample was observed after about 30 minutes. The readings were recorded after equilibration in temperature and water activity ($a_w$), which was displayed digitally.
3.2.5.11 Microbiological analysis

Microbial quality is the most important factor, which spoils the food products. Assessment of microbial load in dairy products is necessary function of the dairy industry.

**Bacterial counts (SPC):** Bacterial load in dairy products is estimated by Standard Plate Counts Method according to BIS:1980 (SP: 18 (Part-I):11.0.) Experiment was carried out in duplicate. Measured volumes of different dilutions of sample are plated on suitable nutrient media, incubated and number of colonies (cfu) is counted. The total viable counts per gram are calculated.

i. **Apparatus:** All apparatus were standardized according to BIS1980 (SP: 18 (Part I) 11.3).

ii. **Sterilisation:** All glassware, i.e., pipettes (1.0, 1.1, 2.0 and 10.0 ml), conical flasks (250, 500 and 1000 ml), petri plates, dilution test tubes were sterilized at 160° to 180°C for three hours in a hot air oven. Pestle and mortars sterilized at 80°C for three hours in an oven.

iii. **Diluent:** Two per cent trisodium citrate solution was prepared by dissolving two gram trisodium citrate A/R in 100 ml distilled water in diluent bottles with stopper. It was autoclaved at 121°C for 15 min and naturally / gradually cooled to room temperature.

iv. **Preparation of media:** Ready mix plate count agar medium (Hi-Media) was prepared by dissolving 30 g in 1000 ml distilled water. pH was adjusted to 7.0±1 at 25°C. Medium was sterilized by autoclaving at 121°C for 15 minutes, then slowly cooled to 43 to 45°C.
Standard formula of PCA medium was:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gm/lit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein enzymatic hydrolysate</td>
<td>5.00</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>2.50</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>6.50</td>
</tr>
<tr>
<td>Agar</td>
<td>15.00</td>
</tr>
</tbody>
</table>

v. *Labeling of dilutions petri plates:* Petri plates were labeled with markers according to dilution factors, and sample numbers. The dilution tubes were also labelled according to dilutions and sample numbers. $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilutions were selected.

Dilution test tubes were filled with 99 ml, 9 ml sterilised diluent, as it was required.

vi. *Preparation of dilutions:* Eleven gram Kunda sample was taken from the package opened under sterilised condition. It was ground in pestle and mortar. It was transferred to dilution bottles containing 99 ml of diluent. From this, one ml of dilution was transferred to $10^{-1}$ dilution tube using a sterilised one ml pipette under sterilised condition. Again one ml of $10^{-1}$ dilution sample was pipetted out into $10^{-1}$ petri dishes. Again using a one ml sterile pipette, One ml of sample was transferred to $10^{-2}$ dilution test tubes and mixed. Similarly $10^{-3}$ and $10^{-4}$ dilutions were made under sterile environment (in UV laminar flow chamber).
vii. *Pouring of medium:* About 15 to 20 ml sterilized PCA medium at 43° to 45°C was poured to petri dishes which were transferred with dilutions under sterile conditions.

viii. *Incubation:* Poured petri dishes were incubated at 30°C for 48 h after solidification of medium and they were inverted and piled in rows (4 no.), taking care.

ix. *Selection and counting of plates:* Spreader free, colonies ranging from 30 to 300 and contamination free plates were selected. Colony forming units were counted in a colony counter.

x. *Reporting:* Counts were computed by multiplying colonies per plate by dilution factor and taking arithmetic average as cfu/gm. The results were reported as log \(10\) cfu/ml

**Yeast and Mold counts**

Growth of yeasts and moulds in sugar containing slightly acid food product is common. Kunda contains about 30% sugar. Most spoilage of Kunda is due to growth of yeasts and molds. The most prolific source is aerial contamination.

Yeast and molds counts were carried out according to BIS: 1980: (SP: 18 (Part I): 12:0).

Experiment was carried out in duplicate.

i. *Apparatus:* Standard apparatus as in 12.2 to 12.2.7 in BIS: 1980;( SP: 18 (Part I): 12:0)
ii. **Sterilisation**: Glassware (Borosil) i.e., pipettes (1.0, 1.1, 2.0 and 0.1 ml capacity), conical flasks (250, 500 and 1000 ml), dilution tubes (test tubes 20 ml) and Petri dishes with covers (100 x 15 mm) were sterilised at 180°C for three hours and cooled to room temperature. Pestle and mortars were also sterilised.

iii. **Preparation of diluent**: Two per cent trisodium citrate diluent solution was prepared using distilled water (500 ml).

iv. **Preparation of medium**: A known quantity (30 gm) Malt yeast extract agar base (MEA) was suspended in 1000 ml distilled water and soaked for 15 minutes. It was sterilised by autoclaving at 121°C for 15 min. pH was adjusted to 3.5±1 by using 10 % citric acid solution and mixed well before pouring.

v. **Preparation of dilution blanks**: All dilution tubes and petri plates were labeled with markers according to number of samples and dilutions. The experiment was conducted in duplicates and compared with blank.

Eleven gm sample was drawn aseptically into 99 ml diluent blank. It was ground into slurry in pestle and mortar and transferred to dilution bottle. One ml of 10⁻¹ dilution was transferred to nine ml of 10⁻² dilution; as well as one ml of 10⁻¹ dilution was also transferred to 10⁻¹ petri plates. Again one ml dilution was transferred to 10⁻³ dilution tube and one ml to 10⁻³ petri plates.

vi. **Pouring plates**: Melted MEA medium at 43° to 45°C about 15 to 20 ml was added to each petri dishes aseptically. Plates were cooled slowly for uniform spreading and cooled to room temperature.
vii. *Incubation:* After solidification, petri plates were inverted and incubated at 25°C for three to five days in an incubator.

viii. *Counting of colonies:* After three days, yeasts and mould colonies were counted and again same plates were incubated for growth of molds for five days and counts were taken in a colony counter.

ix. *Reporting:* Average of two consecutive dilutions was reported as \( \log_{10} \text{ cfu/gm} \) of Kunda sample which represents the yeasts and mould population that would have multiplied in the product.

**Coliform counts**

Coliform counts indicate the unhygienic handling during manufacturing and storage. Coliforms can be transmitted by water and personnel handling. Indigenous sweet meets are manufactured in unhygienic conditions by not following aseptic procedures. Hence, coliforms count is necessary for Kunda, during preparation and storage.

Coliforms counts were conducted as per BIS: 1980; (SP: 18 (Part I):10:0 in duplicate.

i. *Apparatus:* Apparatus as in BIS: 1980 (10.3 of SP: 18 (Part-I) were used.

Glassware ware of Borosil makes: Pipettes (1.0, 1.1, 2.0 and 10.0 ml), conical flasks (250, 500 and 1000 ml), dilution tubes (test tubes 25 ml can) and petriplates (100 mm x 15 m) with lids.

ii. *Sterilisation:* All glassware, pestle and mortars were sterilized at 180°C for three hours in a hot air oven.
iii. *Preparation of diluents*: Two per cent trisodium citrate diluent solution was prepared using distilled water (500 ml). It was sterilized at 121°C for 15 min.

iv. *Preparation of media*: Violet Red Blue Agar (VRBA) 41.53 g ready media was dissolved in 1000 ml distilled water. The contents were boiled for few minutes. The pH was adjusted to $7.4^\circ \pm 1^\circ$C at $25^\circ$C (Hi-Media).

Standard formula of VRBA:

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>gm/lit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Peptic digest of animal tissue</td>
<td>7.00</td>
</tr>
<tr>
<td>2. Yeast extract</td>
<td>3.00</td>
</tr>
<tr>
<td>3. Lactose</td>
<td>10.00</td>
</tr>
<tr>
<td>4. Bile salt mixture</td>
<td>1.50</td>
</tr>
<tr>
<td>5. Sodium chloride</td>
<td>5.00</td>
</tr>
<tr>
<td>6. Neutral Red</td>
<td>0.03</td>
</tr>
<tr>
<td>7. Crystal violet</td>
<td>0.002</td>
</tr>
<tr>
<td>8. Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

v. *Labeling of dilutions petri plates*: Petri plates were labeled with markers according to dilution factors, sample numbers. Dilution tubes were also labeled.

vi. *Preparation of dilution*: Eleven gram of Kunda sample was drawn aseptically into a pestle and mortar. It was ground with some quantity of diluent and made into slurry. It was completely transferred to dilution bottle for uniform mixing.

It forms $10^{-1}$ dilution, and then one ml from $10^{-1}$ dilution was pipetted out into nine ml dilution tube and mixed uniformly. It forms $10^{-2}$ dilution. One ml of $10^{-1}$ dilution was pipetted out into $10^{-2}$ dilution tube. One ml of $10^{-2}$ dilution was
pipetted out into $10^{-3}$ dilution and also was transferred aseptically into $10^{-3}$ petri plates.

vii. *Pouring of plates:* About 5 to 10 ml VRBA medium at 43 to 44°C was poured into petri plates. Petri plates were stirred and cooled. After cooling a over laying medium was poured. Then the plates were cooled to room temperature and inverted.

viii. *Incubation:* Inverted petri plates were incubated at 30°C for 24 to 48 h in an incubator.

ix. *Selection and counting of plates:* Petri plates were counted where total number of cfu/gm was less than 10. The deep red colonies with shining indicates coli form colonies in the medium.

*Reporting of results:* The arithmetic average from two consecutive dilutions was multiplied by dilution factor and reported as $\log_{10}$ cfu/gm.

### 3.2.5.12 Sensory evaluation of Kunda

The expert panelists consisting the scientists at NDRI, Southern Campus, Bangalore and KVAFSU, Bangalore were selected for evaluation of the product. The parameters for sensory evaluation were (i) colour and appearance, (ii) body and texture, (iii) flavour and (iv) overall acceptance.

Panelists were asked to evaluate Kunda quality on a 9-point hedonic scale with their preferences according to the scale (Amerine *et al* 1965).
9-Point hedonic Scale (Amerine et al 1965)

<table>
<thead>
<tr>
<th>Preference</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Like extremely</td>
<td>9</td>
</tr>
<tr>
<td>2. Like very much</td>
<td>8</td>
</tr>
<tr>
<td>3. Like moderately</td>
<td>7</td>
</tr>
<tr>
<td>4. Like slightly</td>
<td>6</td>
</tr>
<tr>
<td>5. Neither like nor dislike</td>
<td>5</td>
</tr>
<tr>
<td>6. Dislike slightly</td>
<td>4</td>
</tr>
<tr>
<td>7. Dislike moderately</td>
<td>3</td>
</tr>
<tr>
<td>8. Dislike very much</td>
<td>2</td>
</tr>
<tr>
<td>9. Dislike extremely</td>
<td>1</td>
</tr>
</tbody>
</table>

******

The judges were also requested to record the specific observations regarding quality of the product other than above parameters.

3.2.6 Statistical analysis

The data were analysed by one way or two way or three way factorial ANOVA wherever appropriate by SPSS package (Version 11.0). Significance of treatments was determined by Critical Difference at 5% levels as per Snedecor and Cochran (1994).