2.0 REVIEW OF LITERATURE

2.1 In the new millennium there is a shift in the global dairy market from bulk commodities to value added delicacies that are delightfully tasty; novel; with niche market that are unique in taste, flavour and appearance. Dairy markets in the new millennium are being increasingly shaped by the twin strands of globalization and liberalization. The major factors that are molding market profile in developing countries are increased urbanization and eating out. The new emerging trends are concerned with popular dairy products.

A recent FAO (1990) report over ethnic products in about 100 developing countries of Asia, Africa, Latin America, and Middle east have identified as ethnic food. These products are part of heritage and tradition. They are being reinvented by applying modern process technologies (Aneja et al., 2002). Little information exists on the scientific approach of several of such heritage products. The important traditional dairy products of India are Khoa, Khoa based sweets, Chhana, Chhana based sweets, Paneer, Shrikand, Kunda etc., Kunda is also one of the traditional products. Though Kunda is a very popular product in northern parts of Karnataka, published scientific literature on the subject does not exist. Since Kunda is a product obtained after further heat desiccation of Khoa, the relevant literature on Khoa is reviewed.

2.2 Factors influencing quality of Khoa

Desiccation conditions

Khoa is prepared by continuous boiling of milk until desired concentration of solids (65 - 70%). De (2004) standardized the method of preparation of Khoa. The
recommended temperature of evaporation was boiling point of milk till pan contents reach a pasty consistency and then lowering the temperature to simmering temperature (85°C to 90°C). Higher working temperatures at the later stages of Khoa making promote the development of undesirable characteristics. Temperature below the optimum produces undesirable colour and appearance, flavour, body and texture; besides being more time consuming. According to De (2004) “a low speed of stirring (30 - 40 rpm) results in an undesirable texture and flavour in Khoa. Medium speed of stirring (96 – 100 rpm) make the product highly suitable for preparation of Khoa based sweets. High speed of stirring (150 - 160 rpm) results in high oiling off in the product. The same author reported that as soon as the dehydrated mass tends together it is the right stage (Khoa pat) to remove the Khoa.

**Type of milk**

De (2004) reported 18.3% yield from pure cow milk, 21.6% yield from pure buffalo milk and 20.0% from admixture of cow and buffalo milk at 1:1 ratio. The yield of Khoa is closely proportional to the percentage increase in total solids than fat. Hence, buffalo milk is preferred for its high total solids content.

Cow milk produces pale yellow colour in the dehydrated product while buffalo milk gives it a colour, which is light greenish–white. It was observed that Khoa from cow milk has sticky body; Khoa made from buffalo milk on the other hand has a soft body and smooth texture and is highly suitable for sweets making because of high fat content.

Ranganadham and Rajorhia (1989) have reported that Khoa manufactured from buffalo milk yielded more free fat than obtained from cow milk. Cow milk gave a
product with sticky body leading to lower sensory scores than Khoa prepared from buffalo milk. Buffalo milk fat contains high amount of unsaturated milk fat of long chain which results in high free fatty acids. Stickiness in cow milk Khoa was attributed to insufficient release of free fat (Vogra and Rajorhia, 1983). Prakash and Sharma (1984) have observed that homogenization brings about reduction in size of fat globules which are less affected by heating and scraping during Khoa preparation.

**Fat/SNF Ratio**

De (2004) reported that with the increasing amount of fat in milk, the moisture content in Khoa decreases irrespective of type of milk. They have reported that if fat in milk is relatively more than certain minimum then it leads to more moisture to be removed. On the other hand if SNF in milk is relatively less than a certain minimum, the degree of dehydration must necessarily be less. Therefore, moisture content in Khoa varies directly with the ratio of SNF/Fat. The ratio is almost linear with 1.2 - 2.4. Further increase in ratio assumes a parabolic curve. The loss of total solids in handling cow milk and buffalo milk is due to milk solids sticking to surface of Karahi prior to pat formation that are difficult to scrape. As the fat % in raw milk progressively increases the loss in total solids are gradually reduced until a minimum is reached with milk testing 4 % fat for cow milk and 5% fat in buffalo milk respectively. The optimum level of fat for maximum recovery of solids in Khoa varies with the ratio of Fat/SNF. The presence of more fat in either type of milk than the optimum causes a sharp rise in the percentage loss of total solids. They have also observed that the separated milk of either type gives a unsuitable product. A progressive increase in the fat level of milk induces a gradual improvement in product. They have reported that the heat coagulation of cow milk starts
at a concentration ratio of 2.83 and ends at 3.57. The same in case of buffalo milk is at 2.48 and 3.05, respectively. Hence, heat coagulation of buffalo milk ends earlier than that of cow milk.

The quality of khoa depends on type of milk. Several heat induced changes take place during Khoa preparation. The changes in milk fat, protein, lactose and minerals components vary with the methods of preparation.

**Fat:** Fat content in milk influences the physico-chemical and sensory characteristics of Khoa. The percentage of fat recovery in Khoa decreases with the increased fat level in milk (Ranganadham and Rajorhia, 1989). According to them the combined action of scraping and agitation causes the rupture of fat globule membrane resulting in the release of higher amount free fat. Coagulation of membrane proteins also contributes to release of free fat from the fat globules.

**Proteins:** Desiccation of milk during Khoa making brings about changes in milk proteins. Aneja *et al.* (2002) have reported that β-lactoglobuliin and α-lactalbumin undergo coagulation and interaction with k-casein during heating. Rajorhia *et al.* (1990) reported that heat coagulation of milk observed during the final stages of Khoa making could be attributed to the destabilization of casein, altered salt balance and high temperature.

**Lactose:** Lactose the milk sugar having glucose and galactose, as monosaccharide is present in Khoa in the form of a super saturated solution dispersed in the form of droplets. According to Prakash and Sharma (1984a) there is increase in acidity when milk
is converted into Khoa. This could be attributed to lactose – protein interaction; cleavage of phosphate bonds, disulphide bonds thermal decomposition of lactose and the displacement of ionic calcium phosphate equilibrium which also lead to the heat coagulation of milk.

**Mineral composition:** Boghra and Mathur (1996) observed that a concomitant increase of about 3.5 – 6.0 and 3.5 - 7.0 times in total minerals in Khoa prepared from buffalo and cow milk respectively. Marked increase in soluble minerals from milk to coagulation stage took place. Soluble calcium, phosphorus, magnesium, and zinc attained the highest levels at this stage; and showed marked reduction thereafter up to Khoa stage. Soluble citrate, sodium, potassium, chloride, copper and iron increased gradually and reached to maximum levels at Khoa stage. Buffalo milk contained higher calcium, magnesium, phosphorus, citrate, copper, iron, zinc, lower sodium, potassium and chlorides as compared to these minerals in cow milk.

The market samples of Khoa contained 4 - 5 times more iron and about twice of zinc than laboratory made samples (Boghra and Mathur, 1996). They have concluded that iron and zinc entered as contaminants in market samples usually through the iron and galvanized karahi and kunti used by the manufacturers.

Boghra and Mathur (1996) Observed that buffalo milk contained lower soluble calcium, magnesium, phosphorus, sodium, potassium, chlorides and iron, higher soluble citrate, zinc and almost same soluble copper content as compared to cow milk. The precipitated salts then become attached to the colloidal micelles of casein - phosphate
complex (Fox, 1981a). Vigorous stirring and scraping of hot milk may discharge some of the metals attached to the fat globule membrane.

**Vitamins** : Sapre and Deodhar (1988) reported that the retention of vitamin ranged from 72.2% for vitamin B₂ and 81.6% for folic acid. The maximum loss was observed in case of vit B₂ followed by ascorbic acid, vit B₆, vita A and folic acid. Most of these losses were witnessed in the first stage (dhap) of Khoa production. It might be due to more moisture content. Subsequent to heating of Dhap during the final phase resulted in only small additional losses, which ranged between 2.50 % for Vit B₂, and 6.4% for folic acid and 18% in Vitamin A.

### 2.3 Shelf- life enhancement of Khoa

In the absence of proper packaging, the rate of chemical deterioration of Khoa such as oxidation and browning increases. Use of ply laminated pouches and tin containers increased the shelf- life of Khoa.

**Addition of preservatives**

Preservatives are the first categories of food additives. Preservatives are defined as “chemical agents intentionally added to food products to prevent or inhibit spoilage caused by mould, yeast, or bacteria or enzymes and chemical changes resulting in discoloration and off-flavors (Subbalakshmi and Shobha, 2001). The preservatives are classified into two categories. Natural preservatives are like salt, sugar, dextrose/glucose syrup, spices, vinegar and honey. Synthetic preservatives are restricted to a specific group of foods in concentrations not exceeding limits prescribed for each; like Benzoic acid /
Sulphurous acid, Sorbic acid, and their salts. Synthetic preservatives are permitted under GRAS (generally regarded as safe).

**Sorbates**

Sorbic acid or its sodium or potassium salts are very effective, even at low concentration, against yeast and fungi including those producing mycotoxins in foods such as cheese. They are safe even at the level exceeding normally found in foods as it is a metabolisable fatty acid. The antimycotic action of sorbic acid is due to the inability of microorganisms to metabolise the conjugated unsaturated structure. The methods of application for sorbates include direct addition into the product in the form of a powder and dusting the product with a sorbate powder, and incorporating (impregnating) into the wrapping or packing materials (Subbalakshmi and Shobha, 2001). The particular method of application depends on the form of sorbate to be used, the objective to be accomplished and the physical, chemical and morphological properties of the product to be preserved. The recommended level of incorporation of sorbic acid in dairy products is 2000 ppm.

Rao *et al.* (1977) studied the effect of different levels of potassium sorbate on keeping quality of Khoa at 30° and at 5° C. They found that the packaging materials impregnated with 20 per cent potassium sorbate solution and injection of nitrogen into tins containing Khoa enhanced shelf-life up to 10 and 18 days at 30° and 5°C, respectively. Concentration less than this was not effective. Under rule 55 of PFA Rules 1955, sorbic acid and its salt of sodium/calcium/potassium are permitted @ 2000 ppm in Paneer/Chhana (Mahindru, 2000).
Nisin: Nisin (Nisaplin) “Nisin – Nisaplin (commercial Nisin preparation of Aplin and Barret Ltd, England), has chemically \( \text{C}_{143}\text{H}_{230}\text{N}_{42}\text{O}_{37}\text{S}_7 \), molecular weight of 3354.25D. Yeasts and moulds tend to decompose it readily. Proteolytic enzymes like trypsin, pancreatin, digestive enzymes and salivary enzymes, but not rennet, hydrolyze and inactivate Nisin. It is an antibiotic type bacteriocin produced by *Lactobacillus lactis sub sp. lactis*. It inhibits Gram-positive bacteria and prevents out growth of bacterial spores. But it is not effective against Gram-negative bacteria, yeasts and moulds. The action of Nisin against Gram positive bacteria is said to be due to disruption of cytoplasmic membrane of the organism causing leakage of cytoplasmic compounds such as ATP (Adenosine Triphosphate), potassium ions, amino acids etc. Action against bacterial spores is due to the binding of Nisin with sulphhydryl groups of protein residues (Nanda and Sanyal, 2001). Nisin is resistant to extreme heat, effective at wide range of pH non-toxic and quickly digested. Kaushik Khamrui and Rajorhia (2000) have reported that Nisin is most widely used bacteriocin for food preservation, with around 50 countries are permitting its use in food to varying degrees. It is destroyed by digestive enzymes. It is a polypeptide. FAO/WHO has permitted the use of Nisin with ADI (Admissible Daily Intake) up to 1 mg/kg of body weight.

Kalra *et al.* (1977) have observed Khoa samples stored with Nisin up to 28, 42 and 90 days at 30 °C, 22°C and 10° C respectively showed no bacterial counts which may be attributed to the combined bacteriostatic and bactericidal properties of Nisin against micro flora of Khoa.
2.3.2 Packaging of Khoa

Packaging is a technique of using the most appropriate packaging media for the safe delivering of the contents from the centre of production to the points of consumption. Packaging is the integral part of the processing in food industry. Packaging serves as the vital link in the long line of production, storage, transport, distribution and marketing. Packaging of products contributes to the trade promotion and conserves valuable manpower and raw materials that have gone into their making. A scientifically designed package should afford protection against engross or ingress of moisture, flavour loss or odors pick up, oxygen, microbial and fungus attack apart from being compatible with food.

Bhat et al. (1948) pioneers in study of preservation of Khoa by steaming. They steamed Khoa for 15 - 20 minutes in a bid to check the product from spoilage as the untreated product deteriorated after two days. Rudreshappa and De (1971) found that the laboratory made Khoa samples with an initial moisture content of 20 - 25% and warm packaging (80° C-90° C) at the time of packaging in tin cans ensured a maximum shelf-life of 14 days at 37° C. whereas packaging at 25°C to 30° C affected the acceptability adversely, rendering the canned product unmarketable.

Rao et al. (1977) reported that Khoa packaged in tin cans stored at 5° C were of satisfactory quality after 60 days; Khoa in aluminium foil / polyethylene remained satisfactory for 30 days. Kumar et al. (1975) found that the Khoa samples packed in parchment paper and HDPE were reported to be acceptable up to 5 days and up to 14 days at 8 ° ± 1° C, whereas laminate packaged samples were observed to be of
satisfactory grade for more than 5 days at 37°C, 30 days at 8°C ± 1°C and more than 75 days at sub-zero temperature. Four ply laminates having aluminum foil were reported to be the best for the packaging of Khoa followed by two ply packs, HDPE and parchment paper.

Goyal and Srinivasan (1989) studied the effect of three different flexible packaging materials viz. P1-posterpaper/aluminum foil/LDPE 55 - 60 gm/sq.m., 0.02 mm and 150 gauge, P2-poster paper/aluminum foil/LDPE (55 - 60 gm/sq.m., 0.009mm, 150 gauge ; P3-MST cellulose film/LDPE 300gm/sq.m. and 150 gauge and P4-tin cans on the chemical quality of Khoa stored at 37°C ± 5°C and 60% Relative Humidity for 0, 5, 10 and 15 days. Khoa samples packaged in tin cans (P4) showed the least chemical changes exhibited by the samples packed in the packaging materials P1, P2 and P3 respectively. They reported that the greatest decrease in moisture content of the Khoa samples occurred for the packaging materials (P3) due to permeability of moisture vapor. The smallest decrease in moisture content occurred for the (P4) tin cans; which were completely impermeable to moisture ingestion.

Kumar et al. (1975) observed that laminates containing aluminum foil provide good protection against moisture losses because of their superior moisture barrier properties. Goyal and Srinivasan (1989) reported that the significant differences in the values for different packages (P1, P2, P3 and P4) were perhaps mainly due to differential growth of acid producing microorganisms. They studied Khoa samples packed in P1, P2, P3 and P4 and stored at 4-5°C for 0, 20, 40, and 60 days. They observed that P1 was the best packaging material, followed by P2 and P3, respectively. Goyal and Srinivasan
(1989) observed that amongst the tried flexible packaging, P1 proved to be best package followed by P2 and P3 for storing Khoa at 4-5°C and 100% relative humidity. They also reported that the minimum peroxide values were observed in P1 and P2 flexible packaging followed by P3 and P4. All the interactions were highly significant (P<0.01) that is four types of packaging, three types of Khoa and different intervals.

2.4 Kunda

The only available literature on Kunda manufacturing and preservation is Satish Kulkarni et al. (2001) and Net work project report NDRI (SRS), Bangalore. Kunda is the milk based delicacy popular in and around Belgaum. Kunda can be defined as a “product light brown to brown color prepared by the continuous heating of milk or high moisture Khoa mixed with calculated amount of sugar. The product has rich pleasant taste with brown color, grainy texture and nutty flavour and semisolid consistency.

2.41 History

Earlier day’s buffalo milk was used for the preparation of Kunda because of its high fat content. Milk fat in the form of ghee used to be a byproduct of Kunda preparation; wherein oozing of ghee like fat from surface is the end point of Kunda preparation. In later year’s availability of cream separator made milk standardization possible. Milk standardized to 5 - 6 % fat is used for Kunda preparation and no by-product is obtained during Kunda preparation. Commercial practice of Kunda preparation has undergone a major change since then. High moisture Khoa; purchased from local market is the base material and cane sugar is the sweetening agent in the preparation of Kunda.
2.4.2 Characterization

Kunda has been characterized by sensory evaluation on a 9-point hedonic-scale (Anon, 2006) for sensory attributes as follows:

(i) Colour and appearance: The colour of the product varied from semi-brown to brown color. In certain cases solidified milk fat (white in colour) was observed all over the surface of the product.

(ii) Body and texture: Kunda has a soft body and grainy texture.

(iii) Mouth feel: The mouth feel characters indicated a smooth body, granular texture and a characteristic after taste in the mouth.

(iv) Flavour: The flavour of Kunda was observed to be sweet, nutty and pleasant. The fat flavour was observed to be prominent in the product.

2.4.3 Composition

According (Anon, 2006) the range of composition of the market samples collected from different places varied from place to place. The product from Belgaum has 73.0 - 75.0 % total solids with 15.0 - 17.25% fat, 10.1 - 10.75% protein, 25.6 - 27.9% sugar and 15.7 - 15.9% lactose. There was wide variation in the product collected from other places with the following composition. Total solids ranged from 80.4 - 82.6%, fat from 9.2 - 11.0%; protein from 5.9 -6.7%, sugar from 48.1 - 51.2%, and lactose ranged from 11.9 - 12.6%.

2.4.4 Method of preparation

According to (Anon, 2006) Kunda is prepared from milk by heat desiccation or from Khoa. Buffalo milk is preferred for preparation of Kunda.
Khoa is added with cane sugar and milk. The mixture is heat desiccated till the oozing of fat on the surface of the boiling mixture. The typical nutty flavour and brown color is obtained at the end.

2.4.5 Effect of levels of sugar on quality of Kunda

Kunda is a milk confectionary. It is made with addition of sugar. Sugar is the sweetening agent as well as a preservative in Kunda. Patel and Upadhyay (2003) have observed that the optimum sugar level in Basundi was 6.0 %. They further reported that the sugar per cent more than 7.0 % was not desirable. It did not influence the Basundi sensory quality with decline in flavour scores due to excessive sweetness. The optimum per cent of sucrose was 6.0 %.

Sachdeva and Rajorhia (1982) observed that sugar level of 25 - 30 % of the weight of Khoa was found to be highly suitable for preparation of burfi from buffalo milk. Higher percentage of sugar than the above resulted in too sweet burfi.

2.5 Compositional differences between cow and buffalo milk

Buffalo milk has higher specific gravity, viscosity, curd tension, pH, oxidation reduction potential (Eh), thermal conductance and thermal expansion. Owing to differences in chemical and physical properties, milk from two species behaves differently when processed for the manufacturing of various products (Sindhu and Singhal, 1988).

Buffalo milk is preferred for manufacture of Kunda because of its high fat and solids-not-fat content. Major differences between cow milk and buffalo milk are:
2.5.1 Casein differences

Buffalo milk has higher casein content (3%) than cow milk (2.65 %). It has been established that there exists distinct differences in the physico-chemical make up of casein from buffalo milk and cow milk (De, 2004). The proportion of micellar casein was 77% in buffalo milk and 67 % in cow milk. The particle size of buffalo micellar casein is larger (135 μm) than that of cow micellar casein (90 μm). The turbidity develops more quickly in buffalo casein due to its higher proportion of calcium.

Casein is the principal milk protein. Aneja et al. (2002) reported that distinct differences exist in the physico-chemical make up of casein of buffalo and cow milk. The voluminosity of buffalo milk casein is 2.68 - 3.72 ml/g at 25 - 27° C while that of cow milk casein is 4.18 ml/gm.

The particle size of buffalo casein micelle is large (110 - 160 nm), than that of cow milk casein (70 - 110nm). The sialic acid and hexose content in buffalo milk is lower than cow milk. The average range of whey proteins in buffalo milk is found to be between 0. 7 - 0.74%, as compared to 0.53% in cow milk.

2.5.2 Mineral balance

Buffalo milk contains more calcium and phosphorus than cow milk. There are more cations (calcium and magnesium) in buffalo milk, but fewer anions (phosphate and citrate). The soluble forms of calcium, magnesium and citrate are lower in buffalo milk than in cow milk (De, 2004).
2.5.3 Fatty acids profile

Free fatty acid content of buffalo milk fat is lower (0.22%) as compared to that in cow milk fat (0.33%); short chain fatty acids are low in buffalo milk fat and higher content of long chain fatty acids (Aneja et al., 2002).

2.6 Heat induced changes in milk

Heat treatment of milk is one of the most commonly used operations in the manufacture of dairy products. Various physical and chemical changes occur in casein micelles during heating and ultimately affect their functions in milk products. Heating temperature ranges from thermization (65°C /15 sec) sterilization (120°C for 10 min) and more than 120 - 140°C. Kunda manufacturing is one such process that requires the temperature more than 120 °C for 20 - 30 min. The changes that take place when milk is heated to high temperatures (>120°C) for prolonged periods are induced acid production, transfer of soluble salts to the colloidal phase, Maillard browning, dephosphorylation and hydrolysis of casein and denaturation of whey proteins and their interaction with casein micelles (Fox, 1981a).

2.6.1 Casein micelle stability

Singh and Fox (1986) reported that casein micelles are very stable to high temperature at normal pH; milk typically coagulates after heating at 140° C for about 20 minutes. However, casein micelles precipitate from solution when the pH is lowered to Iso-Electric Point (I.E.P.) of casein (pH 4.6) at 30° C. The stability of colloidal dispersion is determined by the forces acting between the particles. Three types of interactions may be involved namely (1) Attraction due to van der waals forces (2) Repulsion due to
electrostatic forces and (3) Interactions due to adsorption of macromolecules (stearic forces) which may lead to either attraction or repulsion (Overbeek, 1977).

Van der Waal’s forces are always attractive. Electrostatic forces between the electrical double layers surrounding the particles always lead to repulsion. These forces are measured as the surface charge or Zeta potential. Walstra (1979) has proposed that micelles are stearically stabilized by the macro peptide chains that protrude from the micelle surface as ‘hairy’ layer on the micelle surface; such layer explains the voluminosity of casein micelle (2.0 gm of casein) because solvent trapped in it would increase the hydrodynamic radius. The calcium / Phosphate ratio is almost constant at 1.5% suggesting that CCP is tricalcium phosphate constant \([\text{Ca}_3(\text{PO}_4)_2]\) (Schmidt, 1980).

All the constituents interact during heating and influences the changes induced in the casein micelles. Heat induced changes in the casein micelle don’t occur in isolation; simultaneously changes occur in whey proteins, lactose and milk salts. It is well established that during heating of milk, whey protein and their interaction with casein micelles are involved. In the absence of β-lactoglobulin, α-lactalbumin hardly associates with casein micelles during heating, but it does not associate with β-lactoglobulin (Baer et al., 1976). Adhikari et al. (1993).

McKenzie (1970) reported that the effect of heat on proteins should be considered as a two stage process 1) The secondary and tertiary structure is altered causing denaturation and 2) The proteins aggregate which may lead to coagulation. Webb et al. (1974) observed that β-lactoglobulin undergo such aggregation. For complete denaturation of β-lactoglobulin milk has to be heated at 90°C for 5 minutes and 80°C for
30 minutes. Prolonged heating to high temperatures during Khoa manufacture caused extensive dephosphorylation and insolubalisation of serum calcium leading to their precipitation on the casein micelles either as Ca\(^{++}\) or as Ca\(_3\) (PO\(_4\))\(_2\), which render the micelles unstable and eventually enhanced the precipitation of the micelles.

Buffalo milk containing higher proportion of calcium, phosphorus and citrate than cow’s milk might have resulted faster precipitation of calcium phosphate onto the casein micelles along with whey proteins and lactose forming a thread like loose matrix as that of Khoa, against compact and comparatively smooth body in cow milk Khoa (Adhikari \textit{et al.}, 1993). Buffalo milk k-casein contains less sialic acid content (glycoprotein) than cow milk (Sabarwal and Ganguli, 1970). The faster sedimenting micelles of buffalo milk have higher calcium content than slower sedimenting micelles of cow milk. Mathur \textit{et al.} (1999) have reported that cow milk k-casein is only the glycosylated protein. Sachdeva and Rajorhia (1982) reported that burfi prepared from Khoa with less than 5.0% fat in milk was dry and lacked smoothness. Burfi samples prepared from milk with 6.5 or higher fat content were rated as the best. Burfi prepared from cow’s milk was found to be sticky, and from buffalo milk were grainy.

Heating milk up to 90\(^{\circ}\) C causes only minor changes in the size of the casein micelles but severe heat treatment (90\(^{\circ}\)C -140 \(^{\circ}\)C) at pH 6.7, denatures whey proteins and complexes on to the micellar surfaces. Heating milk at 140 \(^{\circ}\)C for 10 min causes an increase in the diameter of the casein micelles (Mohammad and Fox, 1987), Carroll \textit{et al.} (1970) have observed that the increased level of calcium in concentrated milk may lead to calcium bridging between micelles with a concomitant increase in micelle size.
Singh and Fox (1985, 1986) have found that in milk the dissociation of k-casein rich protein from the micelles was pH dependent. pH values lower than 6.7 showed no dissociation of micellar k-casein on heating at 140°C for 1 minute; because of complex formation of whey proteins with micellar k-casein; but at higher pH values >6.9, dissociation of micelle K-casein increases with increasing pH.

Electrostatic interactions are involved in the dissociation of micellar k-casein. The dissociation of k-casein from the micelles is markedly affected by pH, ionic strength and ionic detergents. It appears that when the negative charge on the micellar surface increases to a certain value, hydrophobic bonds are insufficient to hold k-casein on the micelles and dissociation of k-casein then occurs due to electrostatic repulsions (Singh and Fox, 1986).

2.6.2 Maillard reaction

The interaction between amino and carbonyl compounds resulting in complex changes in biological food system are called ‘the amino-carbonyl’ reaction. Milk and milk products are sensitive to the effects of heat treatment encountered under conventional process and storage conditions (Fox, 1960).

French chemist “Louis-Camile-Maillard” in 1912, first noted the effects of heating at 120 °C -140°C on browning of malt during the course of his studies on biosynthesis of proteins. It is called Maillard reaction. Interaction of free functional groups of protein and aldehydes is controlled by browning (Maillard reaction). It is very desirable in making food as appetizing. Browning color produced during Maillard
reaction involves proteins, reducing sugars and is influenced by pH, temperature, moisture content and other constituents (IDF, 1995)

**Mechanism**

There are four mechanism of production of brown color in food system viz (i) Maillard reaction (ii) caramelization (iii) Ascorbic acid oxidation and (iv) Enzymatic reaction. Out of these, first two are of primary concern in dairy products.

The primary step of Maillard reaction involves a condensation reaction between ε-amino groups of amino acid or protein and the carbonyl groups of reducing sugars. Hodge (1953) divided scheme of browning into three stages 1) initial stage 2) intermediate stage and 3) final stage.

**(1) Initial stage:**

The first step in the reaction is the simple condensation between the carbonyl group and the free amino group of protein to give N-substituted glycosyl amino compound followed by the reversible formation of the Schiff base derivative and Amadori rearrangement.

The scheme proposed by Hodge (1953) for the initial stage of the Maiillard reaction is the condensation. This condensation reaction is initiated by an attack of nucleophilic amino nitrogen with an unshared electron pair on the carbonyl carbon. The maximum rate occurs when the product concentration of \[>\text{C=O}]\[\text{RNH}_2\] was maximum. The rate of condensation reaches maximum at a weak acidic pH in the reaction involving aldose and amine. The remaining step for formation of the Schiff base usually occurs
faster than first combination step. This increases the rate of carbonyl-amino reaction (Feeney et al., 1975) up to this step the reaction is reversible because the glycosylamine can be hydrolyzed into parent compound in aqueous solution.

The glycosyl amines derived from amino acids are immediately converted into the Amadori products (N–substituted 1-amino -1 deoxy -2-ketoses). This Amadori rearrangement of N-substituted glycosyl amines the second step of 1st stage reaction is the most important in the Maillard reaction.

(2) The second stage reaction:

This reaction involves dehydration and fractionation of sugars and amino acid degradation. Kirigaya et al. (1968) has reported that the Amadori compounds in Maillard reaction mixture could exist as enaminol structures. The reducing power of the reaction mixture probably attributable to such reactions increases with browning and browning products have been shown to posses’ antioxidative activity.

Namiki and Hyashi (1975) found a novel free radical in the very early stage of the sugar-amino acid reaction mixtures. Radical formation was observed only in the case of the primary amino compounds. Carbonyl compounds that are highly reactive in the formation of a free radical are also effective in browning, while carbonyl compounds such as furfural show high activity only for browning (Namiki, 1988). Based on the Electron Spin Resonance ESR (spectrometry experiments) on various amino compound-sugar-system; Hayashi et al. (1977) found that as shown by α- and β–alanine the difference in hyper fine structure depends upon the difference in the number of protons on the α-carbon; of amino compound and not upon the carbon number of common sugar.
N-substituted glucosyl amines derived from amino acids are internally unstable and they are either hydrolyzed to the parent amino acid and reducing sugar or react via a spontaneous rearrangement to form the corresponding keto (1-amin-1-deoxy-2-ketose) or aldo (2-amino-2-deoxyaldose) derivative, depending on whether the parent sugar was an aldose or a ketose, respectively.

The aldo→keto transformation is referred as the Amadori rearrangement and the corresponding keto→aldo rearrangement as the Heyns rearrangement. Thus the chemistry of the reacting carbonyl compound has a major influence on the mechanism of the Maillard reaction. Because the Amadori and Heyns compounds differ considerably in their reactivity in general, the rate of browning of Amadori products appears to be faster than that of Heyns products. (O’Bien, 1995).

(3) Final stage reaction:

Hodge (1967) reported that Amadori products may decompose by dehydration reaction or by thermally induced non-hydrolytic scissions (C-C and C-N bond cleavages). The latter become more important at elevated temperatures: (I) under acidic conditions, the nitrogen atom of the Amadori compound is protonated and 1, 2 enolation (1,2 E pathway) is promoted by the withdrawal of electrons from C₁ of the sugar residue by the positively charged electrons (II) 2, 3 enolisation (2, 3 E pathway) becomes more favorable. The 2, 3, E pathway is particularly important source of flavour volatiles in food system yielding furonones, pyronones and strecker degradation precursors.

The flavour compounds produced during degradation reactions are glycerides, Dihydroxy acetones glycoldehyde, 3-Deoxyglucosene, 5-HMF, furfural, glyoxal,
crotonaldehyde, proponaldehyde, etc. 5-Hydroxy Methyl-2-Furfuraldehyde (5 HMF) was formed by the decomposition of Amedori compounds under acidic conditions via the 3-deoxyosulose intermediate. It has a high molar extinction coefficient at 280 nm and is probably a major contributor to the increase in $A_{280^\circ}$ (Feather, 1989). Furfuraldehydes are key intermediates in the formation of the higher molecular weight melanoidin pigments. 2-Furfuraldehyde has been shown to react readily with glycine via its aldehydes group and 5-HMF reacts with reducing sugars at high temperatures via both the aldehydes and hydroxyl methyl groups (Urashima et al., 1988).

2.6.2.2 Factors affecting Maillard reaction:

Maillard reactions are strongly dependent on the reaction conditions. The most important parameters are duration and temperature of heating, moisture content, pH and type of sugar present.

(a) Duration and temperature of heating: Temperature is perhaps the most important criterion. Hurrell and Carpenter (1974) reported that browning and flavour compounds are formed mainly at higher temperatures as a result of advanced Maillard reactions.

(b) Moisture: The Maillard reaction proceeds in aqueous solution more readily at low moisture levels. Very low moisture levels however retard the reaction and complete dehydration results in a rapid halt to the process. Moisture level of 15 - 18% resulted in the maximum loss of $\varepsilon$-amino lysine groups from their casein-glucose system stored at $37^\circ$ C, although much more browning occurred at 30% moisture. Wolfrom and Rooney (1953) reported that maximum browning occurred at 30% moisture. When the moisture content was either 0 or above 90% no browning was observed.
(c) **pH:** The Maillard reaction rate is increased by a rise in pH. Foods of high acidity are therefore less susceptible to the reaction. Phosphates and citrates increase the rate of browning due to their catalytic activity and to their buffering capacity (Saunders and Jervis, 1966). The acidic products formed during the course of the reaction appear buffered by these salts, thus maintaining an alkaline environment favourable for the reaction to proceed.

(d) **Sugars:** Only reducing sugars can take part in Maillard reactions as they provide the necessary carbonyl groups. The reaction is not confined to monosaccharides and can also proceed in the presence of the reducing disaccharides such as maltose and lactose. Lewis and Lea (1950) found that at 37°C and 15% moisture, the order of reactivity was xylose > arabinose > glucose > lactose > maltose > fructose. Fructose was about one tenth as reactive as glucose. Spark (1969) confirmed that the aldopentoses are more reactive than the aldohexoses and that the reducing disaccharides exhibit still less activity.

The participation of sucrose, a non-reducing sugar, in Maillard reactions is less clear-cut. At temperatures of 37°C and below, food systems containing sucrose appear to be stable for long periods of storage. At higher temperatures the glycosidic bonds of sucrose may be hydrolyzed, so releasing its constituent monosaccharides and Maillard reactions can then precede in the normal way. More severe heat is needed with sucrose to obtain the same degree of reaction as can be under mild conditions with glucose. Low pH values and relatively high moisture levels appear to increase the reaction rate in protein–sucrose systems, due to their influence on sucrose hydrolysis rather than to their effect on
the subsequent reactions (Hurrell and Carpenter, 1977). It is well known that low pH favours sucrose inversion but retards early Maillard reactions.

(e) Water activity: The non-enzymic browning rate generally increases with increasing $a_w$ at low moisture range, reaches a maximum at $a_w$ 0.4 to 0. 8 and decreases with a further increase in $a_w$ (Jayaraj Rao, 2000). Jayaprakasha et al. (1997) have reported that complex relationship exists between $a_w$ and non-enzymatic browning. Minimum browning is observed at low and high $a_w$. The decrease in reaction at high $a_w$ is due to the dilution of reacting species. The decreased reaction rate at low $a_w$, when the amount of mobile water is greatly reduced, has been ascribed to an increasing diffusion resistance, which lowers the mobility of the reactants.

Diffusion of solutes can take place at water contents above the monomolecular layer value on the sorption isotherms, corresponding to the maximum amount of very strongly bound water. Generally, maximum browning occurs in the range, of $a_w$ 0.3 to 0.7 depending on the type of food. Maximum browning not only varies with $a_w$, but also with type of humectants, pH and temperature (Jayaprakasha et al 1997).
2.6.2.3 Methods for determination of Maillard reaction products:

(A) Flavour: The flavour and aromas from Maillard reactions include the numerous desirable and undesirable flavours that develop during heat processes such as baking, roasting, concentration and dehydration. These food aromas can be described as toasted, baked, nutty and roasted; corn like (Hodge, 1967).

Strecker degradation aldehydes arise from the reaction of free amino acids with dicarbonyls formed on breakdown of the Amadori compounds. These flavour compounds include N-heterocyclics, S-heterocyclics, furans, carbonyls and melanoidins. Ferretti et al. (1970) have extensively studied the Maillard reaction products from lactose-casein browning system. They have isolated and identified 40 compounds, which were grouped as 13 furans, 9 lactones, 9 N-heteroclyics, and 9 other volatile compounds.

Hodge (1967) reported that different aromas are produced by the same reaction mixture heated at different temperatures i.e. 100° C- 150° C and 150° - 180° C indicating that reactions other than Maillard reaction have taken place between (150° - 180° C). Hodge et al. (1972) have classified Maillard reaction flavour into four groups viz. 1 (i) Nitrogen heterocyclic ii) Caramel aromas (iii) Burnt and pungent aromas and (iv) Volatile aldehydes and ketonic aromas

(i) Nitrogen heterocyclics: Hodge et al. (1972) reported that roasted nutty, corny bread aromas include pyrazines, pyroles pyridines and thiazoles. (a) Pyrazines contribute directly to the roasted or cooked flavours of various foods. They have been reported to give roasted nutty, chocolate, coffee and baked flavours (Maga and Sizer, 1973). The pyrazine ring was thought to result from the condensation of two- carbon fragments with
nitrogen. (b) Pyroles, pyridines: they can be formed by the reaction of furfural and its homologous with α-amino acids; 2- acetyl furan was one of the reaction products in greatest concentration in lactose-casein browning system (Ferretti et al., 1970). (c) Thiazoles: these compounds contain both nitrogen and sulphur in the ring structure and are potential flavour compounds, possessing strong nut like odour

(ii) Caramel aromas: Sugars are normally colorless and are generally sweet. However when heated above their melting point, they darken through yellow; orange/brown, red/brown and finally at almost black. The pleasant caramel flavour is produced by carefully controlling the process of heating (150° C -180° C) (Hodge et al., 1967).

Marmion (1984) reported that according to FDA, Caremal is “the dark brown liquid or solid material resulting from the carefully controlled heat treatment of food grade carbohydrates such as invert sugar, lactose, malt syrup, molasses, starch hydrolysates and fractions there of or sucrose”.

The principal classes of compounds responsible for the desirable or undesirable flavour in milk and milk products are carbonyl compounds. Carbonyl compounds have been extensively studied among the various flavour compounds. At optimum levels carbonyl compounds are responsible for good flavour but varied off-flavours can arise at higher concentrations.
Hemavathi and Prabhakar (1973) have isolated and identified carbonyls compounds in Burfi. The major classes of carbonyl identified in Khoa and Burfi were methyl ketones and saturated aldehydes. Bhat and Rama Murthy (1982) isolated steam volatile monocarbonyls from milk skim milk as well as cultured milk and skim milk from cow and buffalo milk by steam distillation method. They have classified the monocarbonyls into methyl ketones, 2,4 dienals, and alk-2-enals. Methyl ketones are the major monocarbonyls class in steam distillates of both cow and buffalo milk fat.

2.7 Energy conservation

Kunda manufacturing is more heat energy consuming as it needs prolonged heat desiccation. Energy saving is need of the hour. It affects cost of production. Various options are available for reduction in manufacturing time as well as energy requirement.

Dairy industries use considerable amount of energy in processing of milk and milk products. The cost of these energy sources are increasing continuously. These energy sources are non-renewable. The limitation of availability of non-renewable energy
sources has necessitated the critical evaluation of the energy requirement for various operations in dairy processing. The energy conservation is in short, without large investment improving house keeping, minimizing losses and thus increasing efficiency (Verma, 1988).

Steam is used in organized dairies for most of the operations. Removal of water from dairy products is the main process used for food preservation. Energy costs in dairy industry can reach 7.5% of the total costs. If the energy costs are reduced by 20% without increasing capital cost or other costs, then profit margins would increase by 50% (Darlington, 1982).

Evaporators are used in dairy industry mainly to remove around 80 to 90% of the total water contained in liquid milk. Evaporation implies a change of phase and the energy input required to evaporate 1 kg of steam at 100 °C under atmospheric conditions is 2257 kJ. Dairy processors should seek new ways of reducing costs by modifying the processing. Verma (1988) reported that the amount of steam consumed for processing milk to Khoa varies from 1.2 to 1.35 kg/kg of milk when steam pressure varied from 0.5 to 1.5 kg/sq.cm. There was about 35.5% loss of heat in Khoa making including 3.24% by radiation and convection. The heat losses with condensate were 14.3%.

Verma and Giridhari Lal (1989) have studied the heat transfer coefficient of indigenous milk products during manufacturing. The average values of heat transfer coefficient of Khoa were estimated to be 125.32 Kcal/sq.m. Efficient heat transfer depends on the heat transfer and thermal properties of the dairy products. More and Verma (1989) studied the heat transfer properties of Khoa, ghee and Paneer. Verma and
Dodeja (2000) found out the heat transfer coefficient of various indigenous dairy products during processing.

More and Verma, (1989) observed that heat transfer properties of the dairy products are estimated by over all heat transfer coefficient. Thermal properties of indigenous milk products are necessary for determining their heat transfer properties, which in turn are depending upon composition, temperature, and physico-chemical characteristics of milk. Verma and Giridhari Lal (1989) estimated the heat loss during Khoa making by method of the thermal energy balance.

“Thermal energy in---Thermal energy out = 0”

The thermal energy enters the system with steam and the raw material (milk, cream or butter). Thermal energy leaves the system with product, with condensate, as heat loss from product surface as well as by convection and radiation.

Thermal energy can be computed using the formula.

“Thermal energy input with steam+thermal energy input with raw material = Thermal energy output with condensate + thermal energy losses due to convection and radiation”. Agrawala et al. (1987) reported that the amount of steam consumed for converting milk to Khoa in an open kettle was found to be1.17 kg/kg of milk and 6.802 kg/kg of Khoa. Rajashekar et al. (2003) reported that with the change in shape of the vessel for the same quantity of milk, its wetted area, surface area exposed to atmosphere, height of milk, area of flame contact with the vessel vary. The decrease in liquid height decreases the thermal resistance. It is easier to control time, temperature, and pressure in a scraped surface heat exchanger than in a kettle. It is well established that steam is the
best heating medium which can cover the entire heating area of the kettle with uniform steam pressure. Steam consumption decreases with the increase in steam pressure (Verma, 1988).

Khoa preparation is tedious and more heat energy consuming. Verma and Giridhani Lal (1989) reported that on an average thermal energy loss in Khoa manufacturing by traditional methods is 32.03%. Cheryan et al. (1987) Observed that the energy consumption will be markedly reduced using either vacuum evaporation or reverse osmosis compared to partial preconcentration of milk. On an average the energy consumption in Reverse osmosis is half of that for Pasteurization. An additional energy (about 200 cal/kg of milk) will be required for the open pan boiling of milk to Khoa (65%TS).

Kunda manufacture requires large amount of steam for prolonged repeated heat desiccation. The energy consumption can be reduced by advancing the browning time. Caramel solution is used for manufacture of many food products especially in manufacturing of cakes, candies and other bakery products. Caramel is produced by heating of sugar. According to Anon, 1997 (PFA 1954:A26.08:) Caramel shall be prepared from the food grade carbohydrate or their combination by the action of heat, in the presence or absence of acids or alkalis or salts. The materials shall be amorphous or liquid and dark brown in color. It shall be water soluble free from extraneous coloring matter.

Hodge (1967) reported that sugar caramelization can occur in certain foods subjected to intense heating in the region of 150 °C to 200 °C. Sugars are normally colour
less and are generally sweet. When they are heated above their melting point they darken becoming yellow; orange/brown, red/brown and finally to almost black. This process proceeds under acid or alkaline conditions and is associated with a change in flavour as burnt, bitter and acrid flavour which gradually over come initial sweetness. The pleasant caramel flavour is produced by careful controlling the heat process. According to BIS: (1960) refined cane sugar can be used for preparation of caramel. The cane sugar should be crystalline white, odorless and free from dirt and other extraneous matter. It should contain 99.8 per cent sucrose by weight.

2.8 Changes induced by homogenization of milk

Homogenization primarily ruptures the fat globules into much smaller ones, hence, causes an increase in globule surface to 1000 times the original area. The original membrane material may tend to spread on the enlarged fat-plasma interface.

The amount of protein adsorbed is at least 10 mg/sq. m. on new surface area. This depends upon conditions during homogenization, such as pressure, temperature, ratio of fat to plasma, plasma composition and denaturation of protein (Mulder and Walstra, 1974)

2.8.1 Protein migration

Mulder and Walstra (1974) reported that proteins are the main constituents of new membrane. Fox et al. (1960) found that large amount of protein adsorbs onto the new interface. Casein micelles have now been clearly demonstrated at the surface of homogenized fat globules by electron micrograph. Fox et al. (1960) assumed that a complex between fat and protein is formed by casein micelles opening up in the
homogenizer and then enclosing small fat globules. Walstra (1979) suggested that casein may be homogenized in fat-serum interface by interfacial tension (\(\gamma\)). The interfacial tensions are in the correct range. \(\Gamma\) Gama (Casein-fat) will not be higher than 10; (casein-serum) will be very small 1; (fat-serum) is initially about 40 and may decrease to about 14 dynes/cm.

### 2.8.2 Appearance

Homogenization makes milk look little whiter probably because yellow colour of fat is masked by light scattering. Taste and flavour, mouth feel of homogenized product will be different, if milk is homogenized at very low temperature. It may acquire a chalky taste, presumably from clusters formed and excessive adsorption of protein on to fat globules. Fat globules in milk are surrounded by a thin layer of surface active membrane material. Milk fat globule membrane (MFGM) consists of a complex mixture of protein, glycoproteins, triglycerides, phospholipids, cholesterol, enzymes and other minor compounds.

Composition of milk fat globule membrane can be altered by various factors such as animal, environmental and processing factors. Animal factors include diet, season and stage of lactation and breed. Environmental factors include the presence of bacteria in milk from pre or post pasteurization contamination or presence of mastitis pathogens in mastitis milk.

Processing factors include cooling, drying, separation, agitation, heating and homogenization. Among these factors, heat treatment of milk causes whey proteins to denature and interact with membrane components as well as with k-casein in the casein
micelles (Sung Jee Lee and John W. Sherbon, 2002). Dalgleish and Sharma (1993) have reported that homogenization also causes significant compositional changes in the milk fat globule membrane by disrupting fat globules decreasing size while increasing surface area. Newly created surfaces are covered by the adsorption of skim milk proteins, preferentially casein micelles over whey proteins.

Lee and Sherbon (2002) have observed that when raw milk was homogenized, significant amounts of casein in the membrane were detected; but whey proteins were only detected when milk was heated either before or after homogenization. As a result of homogenization, casein interaction increased. Protein load on the casein based membrane expressed milligram protein per square meter of fat surface area was almost twice.

Sharma and Dalgleish (1994) reported the presence of more whey proteins in the casein based membrane when milk was heated after homogenization. They suggested that more binding sites for interaction of whey proteins become available as adsorbed casein micelles are spread over the fat globule surface rather than being in their native configuration.

2.8.3 Heat stability

The homogenization effect produced a gradual reduction in heat stability with increased pressure. This could have been a consequence of the increased adsorption of protein, especially casein forming centre of high protein concentration favorable to coagulation. Homogenization of milk is known to produce sulphhydryl (SH) compounds and denature proteins (Maurice Sweetsur and Donald Muir, 1983a). Two stage (low
pressure 3.5 M Pa and high pressure 20.7 M Pa) resulted in a more heat stable product than the single stage process.

Maurice Sweetsur and Donald Muir (1983 a) found that homogenization at pressure up to 31.0 M Pa did not destabilize skim milk whereas the heat stability of the whole milk was markedly reduced. They have also observed that the higher the homogenization temperature the greater was the disruption of milk fat globule.

Maurice Sweetsur and Donald Muir (1983 b) also reported that heat stability of homogenized concentrated milk was found more susceptible than that of unhomogenised concentrated milk to changes in the ratio of β-lactoglobulin to k-casein which were deliberately designed to alter the extent of disulphide linked complex formation during heating.

2.9 Changes induced by additives in milk

Food additives are substances added to food in small quantities to improve its functional properties, freshness, sensory and nutritional qualities or as processing aids in the manufacture of food. Food additives help to achieve some degree of uniformity needed for large scale production to enhance the flavour or improve the appearance or texture of product.

The additive must be (i) Technologically effective. (ii) Safe in use. (iii) Absolutely necessary. (iv) Never mislead the consumer. (v) Confined to minimum use of non nutrient dynes.
Food additives under PFA rules mentioned under part XIII A Rule 64 C, include colour, flavoring agents, antioxidants, anticaking agents, emulsifying and stabilizing agents and preservatives.

The salts of weak acids namely citric and phosphoric acid present in milk are distributed in accordance with dissociation constants of the acids depending on the pH and the total ionic concentrations. Phosphates and citrates ordinarily exert an action opposite to that of calcium and magnesium thus decreasing the effectiveness of the calcium and magnesium ions concentration. Sodium and potassium effects oppose those of calcium and magnesium because these tend to cause a higher degree of dispersion and hydration of caseinate particles (Ismail et al., 1971b).

Ismail et al. (1971c) found that calcium and magnesium ions carry electrical charges different to those carried by phosphate and citrate. The binding capacity of casein for calcium varies according to the citrate, phosphate and protein content and is influenced by both the duration and temperature of heating. It is the soluble part of the salts which could balance and control the influence of calcium and citrate but not the total phosphate and citrate.

Ismail et al. (1971d) reported that titrable acidity, pH, butterfat, protein and SNF content had no significant effect on stability. The phosphate and citrate in milk are always in competition with the proteins in forming salts with calcium and magnesium. Ismail et al. (1971a) observed that the casein is primarily stabilized in solution by the charge carried by the particles and also for binding of divalent ions such as calcium and magnesium very tenaciously.
On heating the milk the precipitation and deionization of both calcium and magnesium increased with increasing the heating temperature. The change was always higher in buffalo milk than in cow milk at any particular heat treatment.

Singh et al. (2000) reported that hydrolytic changes in protein, lactose and salt balance as a result of heat induced chemical changes increase the acidity of milk. Harwalkar and Vreeman (1978) observed gel formation in orthophosphate added (@ 1.5 g/kg) UHT milk during storage at 28°C. Gel formation apparently results from a network formation through cross links between protein particles (casein micelles). At the time of gelation the casein micelles were aggregated with longer chains which were connected to each other to give a continuous three dimensional network.