Fortification of cow milk with vegetable oil to prepare PUFA enriched mozzarella cheese

Project report submitted in the partial fulfillment for the requirement of the degree of

Bachelor of Technology

in

Biotechnology

by

Nishat Kapila-091570

under the supervision of

Dr. Gunjan Goel

May 2013

Jaypee University of Information Technology
Waknaghat, Solan-173234, Himachal Pradesh

CERTIFICATE

This is to certify that the work titled "." submitted by **Nishat Kapila** in partial fulfillment for the award of degree of **B. Tech Biotechnology** from Jaypee University of Information Technology, Solan has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of this or any other degree or diploma.

Signature of Supervisor

Name of Supervisor : Dr. Gunjan Goel

Date:

ACKNOWLEDGEMENT

We owe a great many thanks to a great many people who have been helping and supporting us during this project.

Our deepest thanks to Dr. Gunjan Goel for guiding and correcting us at every step of our work with attention and care. He has taken pain to go through the project and made necessary corrections as and when needed.

Thanks and appreciation to lab assistants and PHD scholars, for their support.

We would also thank our Institution, HOD and faculty members without whom this project would have been a distant reality. We also extend our heartfull thanks to our family members and well-wishers.

Date:-

ABSTRACT

Mozzarella cheese was prepared from fi1led milk. using sunflower oil sa the Mer fat. The milk was homogenized at 35 bar pressure at 50 - 550C and pasteurlzed at 63°C/30 m1n. Addition of 0.05% calcium chlortde to the milk before homogenization tmproved the body and texture characteristics. Direct acidification of milk (pH 5.6 - 5.2) with dtlute citric and acetic acids resulted in better qua1tity cheese compared to the conventional (culture) method. The cheese prepared by using citric acid was softer compared to the cheese prepared by using acetic acid at stmtlar pH. The melting characteristics of filled cheese compared wen wtth control cheese. The unsalted cheese packed in polyethylene pouches kept well for 8-10 day& at 8-10OC and for about 90 days in deep-freeze. The stretching characteristics deter1orated, while melting qua1t1¥ tmproved with increase in storage time at both the temperatures. The final composition of filled Mozzarella cheese was, motsture 55.74, fat 19.83, protein 21.74 and ash 1.19%.

Table of contents

CHAPTER-I

INTRODUCTION

Cheese manufacturing is one of the classical example of milk preservation, dating from 6000-7000 BC. Cheese and fermented milk products are among the great food of the world. They are also one of life's wonders because they come in great diversity of flavours and tastes, in large variety of shapes, and are highly nutritious.

Cheese is one of the most diverse groups of the dairy products and perhaps the most interesting and challenging one. More than 900 individual varieties of cheeses are being produced in the worlds (Banks, 1998) which are classified on the basis of their form, manufacturing, ripening and chemical composition (Walstra *et al.*, 2006). Many dairy products are biologically, biochemically and chemically stable provided that are properly manufactured and stored. In contrast cheese is biologically and biochemically active and consequently undergoes changes in flavour, texture and functionality as a function of storage (Fox *et al.*, 2000). In cheese manufacturing preservation of the most important constituents of milk (i.e. fat and protein) are exploited by two classical principles of preservation, i.e. lactic acid fermentation, and reduction of water activity through removal of water and addition of salt. Establishment of low redox potential and secretion of antibiotic by starter culture contribute to storage stability of cheese (McSweeney, 2004a).

The consumption of cheese in the from of pizza toppings, cheese blends, salads, sandwiches, stuffing, has increased due to dietary likeness, ease and flexibility in use and cheese quality. The increase in production of cheese is about 4% per year from the last thirty years in the world (Fox *et al.*, 2000). The basic technology for the production of all types of cheese is same with relatively small changes resulting significant differences in the final cheese. The skill of cheese manufacture consists of some key factors i.e. composition of milk, extent of acid production, moisture, curd handling and ripening conditions of cheese (Lucey *et al.*, 2003). Mozzarella cheese is the one of the most popular varieties in the world because of its primary use on the pizza topping (Kindstedt, 2004). The demand of Mozzarella cheeses is growing as the global demand for pizza and other foods has increasing manifold. It includes in the group of "Pasta filata" or stretched cheese. In the pasta filata cheese first the curd is produced as normal process of cheese making then kept in hot water or whey to consolidate into a solid mass, subsequently, stretching of this mass convert the curd into a uniform and elastic cheese consistency. Stretching is a treatment that renders the curd elastics which is a unique quality attribute of the Mozzarella cheese (Luecy *et al.*, 2003).

The physical characteristics of Mozzarella cheese like body, texture, meltability, stretchbility and colour are altered by the factors like milk composition, starter culture and ripening conditions prevalent during the cheese preparation (Luecy *et al.*, 2003). The Mozzarella cheese can be prepared from milk of various animal species such as cow, buffalo, goat, and ewe (Calandrelli, 2001). Traditionally Mozzarella was made from buffalo milk which was preferred due to its characteristic flavor. The flavor and texture of fresh Mozzarella is different from processed sliced or shredded Mozzarella, as fresh Mozzarella is moist, soft, quick in melt and delicate in taste. However, 'real' Mozzarella does not maintain ideal freshness beyond 12-24 hours (Rowney *et al.*, 2003). Guidelines set by the USDA indicate that a low moisture Mozzarella cheese shall contain moisture 45% to 52% and milk fat 30% to 45% on dry weight basis. Low moisture part skim Mozzarella is mostly utilized in the pizza industry due to exceptional properties of meltability, stretchability and elasticity (USDA, 1980). The functionality of Mozzarella cheese is important because about 75% of the total Mozzarella produced is used as an ingredient for pizza. The factors for example composition of cheese, especially fat contents and moisture, pH, starter culture, coagulating enzymes, cooking and stretching, salt concentration, and the process occur during ripening influence thefunctionality of Mozzarella cheese (Kindstedt, 1993). The factors such as degree of proteolysis, fat content, protein-protein and protein-water interactions also play a vital role to govern the functional properties of cheese (Rowney *et al.*, 2004).

Fresh manufactured Mozzarella cheese is unacceptable as a pizza ingredient because it melts to tough, very elastic possessing somewhat granular consistency with poor water holding capacity and limited stretch. However, with too much aging the cheese becomes excessively soft and fluid when melted and is no longer acceptable for pizza. So a precise ripening period is necessary to achieve desirable functionality (Rowney, 1999).

None of wide array of cheeses can be produced without the involvement of microorganism (Cogan and Accolas, 1996). The choice of the starter culture is important for proper acid production and texture and flavor profile in high-fat and reduced-fat cheeses which are vulnerable to development of off-flavour (Banks, 2004). Mozzarella is produced using a paired lactic acid bacteria starter culture comprised of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* or *Lactobacillus helveticus* (Coppola *et al.*, 2001).

The consumer is now very much conscious about diet and health. So there is a potential for production of novel low fat cheeses which gave added health benefits to the marketplace (Guinee *et al.*, 2000). In addition to nutritional significance fat also adds sensory and functional characteristics to cheese. Low moisture part skim (LMPS) Mozzarella cheese is commonly used for pizza because of its desirable functionality (Rudan and Michael, 2002).

CHAPTER-II

REVIEW OF LITERATURE

Approximately one third of the total world's milk production is used for cheese manufacturing to conserve the most desirable milk components such as casein, fat, calcium and phosphorus (Adda *et al.*, 1982). Milk is believed to be a perfect food owing to its high nutrition, expediency, diversity of flavours and texture, ease of use, and good taste (Bogue *et al.*, 1999, Fox *et al.*, 2000). The global sale value of cheese represents about 30% of total dairy products sales (Fox *et al.*, 2000). Mozzarella cheese comprises 80% of all Italian style cheeses and 32% of total cheese produced in the world (Traordinary Dairy, 2002). Due to the fast growth of the pizza industry in the globe Mozzarella production has increased (IDFA, 2001). The literature regarding the factors affecting the cheese quality, cheese manufacturing and different functional properties of cheese is reviewed under the following headings.

2.1 Cheese milk quality

Cheese may be made from the milk of any species, while milk of cow's is most commonly used in the US and Western Europe, but now there is increasing trend to manufacture cheese from buffalo, goats and, to a lesser extent, sheep's milk. The compositions, microbial load, indigenous enzymes of milk are the major factors that influence different characteristics of the Mozzarella cheese.

2.1.1 Composition

The macro-constituents of milk (protein, lipids, and lactose) and some of its low molecular mass species, especially calcium, phosphate, citrate and pH are the major determinant for technological properties (Fox *et al.,* 2000). These constituents influence the casein to fat ratio, total solids, lactose, mineral content, consequently moisture levels and extent of acid development in the finished cheese (Traordinary, 2001). The milk constituents are predisposed by the breed/species of animal, season, stage of lactation, microbial quality and genetics.

Different breeds differ in their protein and fat contents. Jersey milk has higher protein and fat contents than Friesian milk and therefore possesses better cheese making properties (Auldist *et al.*, 2004). In spite of its higher fat percentage, milk and Mozzarella cholesterol content is lower for buffalo than for cow's milk (275 mg/100g vs 330 mg/100g and 1562 mg/100g vs. 2287 mg/100g respectively).

Seasonal factors had great impact on the composition of the milk which ultimately influences the cheese quality. Casein to fat ratio are higher in milk produced during May and August, while the fat in milk from February is higher and total protein is lower than in months of May and August. Cheese whey, and press whey composition are also affected by the season variation and followed the trends identical to milk composition. Fat recovery in the cheeses ranged from 83.2 to 84.2% during different seasons while the protein recovery in the cheeses was not affected by the season. February milk due to high casein content has cheese yield higher than milk of May and August (Jaeggi et al. 2005). Casein-to-fat ratio is readily manipulated seasonal variable which affects the final composition of the cheese (Barbano *et al.*, 1994). Thebuffering capacity of the cheese milk also varies seasonally as it affects the development of pH (Rowney *et al.*, 1998). The concentrations of milk constituents vary during lactation stages (Lucey and Kelly, 1994). Protein content of milk increases (Guinee *et al.*, 2007), while the lactose content decreases as lactation period progress (Fox *et al.*, 2000). At very late lactation stage, no doubt the protein content is higher but the cheese yield is low. This might be due to the low content of casein and high whey protein content at late lactation stage (Kefford *et al.*, 1995). Mozzarella cheese prepared from milk obtained at early or late lactation stages is usually unsuitable for production of pizza cheese. At early lactation, the concentration of whey and salts is higher and at late lactation, higher concentration of plasmin in milk hydrolyzes casein and gives poor cheese quality (Rowney *et al.*, 1998; Guinee *et al.*, 2000). Lucey et al. (1992) found that Mozzarella made from late lactation milk is softer, has lower apparent viscosity and higher moisture content which was attributed to more whey protein in milk, giving hydrophilic properties and high pH.

Cheese flavour and texture are overwhelmed by fatty acid composition of milk fat. The firmness of cheese reduced when milk with higher amount of long chain fatty acid is used for cheese manufacturing (Jaros *et al.*, 2001; Steinshamn *et al.*, 2006; Svanborg, 2006). Mozzarella cheese made with a low melting point fraction of milk fat has higher free oil than that made with either butter oil or higher melting point fraction (Papalois *et al.*, 1996).

2.1.2. Microbiological quality of milk

Milk used for cheese making must be of good microbiological quality as given below by (Skeie, 2007)

Total plate count (TPC) : < 104 cfu/mL

Somatic cell count (SSC) : < 105/mL

Pathogenic bacteria : absent

Antibiotics/inhibitors : absent

Psychorotropic bacteria produce heat resistant lipases and proteases which may reduce yield of cheese and produce undesirable flavours in the ripened cheese (Skeie, 2007). It is reported that proteases produce by some psychrotrophes stimulate plasminogen activators, transformed plasminogen to plasmin (Frohbieteter *et al.*, 2005).

According to Lee *et al.* (2006) increasing Somatic cell count in raw milk, the pH, free fatty acids (FFA) and chlorine increased and milk protein, lactose, fat and Ca contents decreased. The proportion of casein in total milk protein decreased from 79.58 to 75.47% with increasing Somatic cell count. Rennet coagulation time (RCT) of Cheddar cheese making increased and cheese yield decreased to approx. 11.46% with increasing mixing ratio for sub clinical mastitis milk. The texture (hardness, elasticity, brittleness, cohesiveness) of cheese samples also decreased with increasing the ratio of sub clinical mastitis milk.

In addition to microbial quality, genetic variation of casein in different milks is also considered vital with respect to cheese quality. The genetic variation in Kepa casein (K-casein) has non significant effect on cheese functionality, but there is a considerable effect on cheese yield and curd formation (Walsh *et al.*, 1998).

2.2. Milk pretreatment

The milk used for cheese making comes from the cow, goat, sheep and buffaloes as a key ingredient, its quality and preparation are of vital importance.

2.2.1. Standardization

The fat and protein content are the major constituents in milk which have an influence on yield and quality of cheese. The protein content of milk is relatively stable than fat content which is altered by various factors. Bulking of milk from multiple farms reduce variation in fat and protein composition. The variations in composition of milk are further reduced by standardization of protein and fat contents in milk according to the type of cheese.
Milk may be standardized on the basis of total protein to fat ratio or casein to fat ratio to produce a consistency in cheese quality and to meet the regulatory standards of a given variety (Farkye, 2004; Guinee *et al.,* 2006; McSweeney, 2007). Different methods are used such as addition of skim milk powder, milk protein concentrate or caseinate for increasing the casein content of cheese milk. Conversely addition of cream increases the fat content of cheese milk (Farkye, 2004). Merril *et al.* (1998) standardized cheese milk to different C/F ratios of 1.2, 1.6, 2.0, or 2.4, in the production of Mozzarella cheese. The most desirable casein to fat ratio is 0.7 in milk i.e. used for the manufacture of Cheddar and Gouda-type cheese although ratios as low as 0.64 may be desirable in some cases (Farkye, 2004). The data from Irish cheese industry between 2001 and 2003 indicate that the protein to fat ratio of milk for production of cheddar cheese varied from 0.84 to 1.02 and protein and fat contents varying from 2.99% to 3.59 % and 3.26% to 4.2 %, respectively (Guinee *et al.,* 2005).
In the last fifteen years the commercialization of low fat cheese production increased significantly. Fat reduction in the diet is important based on scientific evidence linking diet high in fat to coronary heart disease and certain type of cancer (Woteki and Thomas, 1993). This information promoted the cheese industry to investigate the fat reduction in Mozzarella cheese as an important ingredient of pizza, due to increasing popularity of pizza low Moisture Part Skim (LMPS) Mozzarella cheese is now commonly used for pizza because of its desirable functionality

(Kindstedt, 1993). However, LMPS Mozzarella cheese by definition contains 14 to 22% fat, depending on its moisture content Merrill *et al.* (1994) described a method for manufacturing of reduced fat Mozzarella cheese containing 50% less fat than Part Skim Mozzarella (LMPS).

2.2.2. Pasteurization

Control of the microbiology of cheese milk is a vital issue affecting the final product (Johson and Law, 1999). The pasteurization of milk is used to kill pathogenic and some of the nonpathogenic organisms before its transformation into cheese. The main aim of pasteurization of milk for cheese making is to reduce microbial load, greater yield, quality and ripening at higher temperature (Ordonez *et al.*, 1999; Blumenthal, 2002).

Pasteurization, through the use of plate heat exchanger and holding tubes with typical time temperature relations of 72°C/15 sec, is standard practice to kill pathogens (Maubois, 2002). Pasteurization >72°C have generally not been used in milk for cheese making practices because of their adverse effects on curd formation (Guinee *et al.*, 1997; Singh and Waungana, 2001) and curd syneresis (Pearse *et al.*, 1985; Pears and MacKinlay, 1989). However, because of potential increase in cheese yield due to increased moisture content and more effective recovery of whey proteins, the effect of increasing pasteurization temperature has been studied at an experimental level and concluded that high pasteurization temperature of milk result in reducing the firmness of low fat cheese (Guinee *et al.*, 1998).

In several countries, the use of raw milk for making cheese is still in practice. In USA, cheese manufactured from raw milk must be stored at 1.7°C for at least 60 days before consumption. Cheeses made from raw milk contained higher number of non-starter bacteria compared to cheeses made from pasteurized milk (Farkye, 2004). Mozzarella from pasteurized milk is superior to raw milk cheese in functionality but with similar body and texture. Melting and stretching characteristics of cheese made from milk heated to high temperature are also inferior to the cheese made from raw and pasteurized milk. Rheological characteristics of cheese made from milk heated to high temperature can be improved with addition of 0.01% calcium chloride (Gosh and Sing 1990).

Mozzarella cheese manufactured from milk with different pasteurization temperatures (68, 75 and 78°C) have a little difference in moisture, pH and yield of cheese when compared with that prepared from raw milk. Cheese obtained at 75°C temperature was better in organoleptic properties as compared to other cheeses (Cavaliere *et al.*, 1990). In another study, increase in yield of cheese (from 2.5 to 4%) was recorded from pasteurized milk (Mc Sweeney *et al.*, 2007).

2.3. Starter culture

Dairy starter cultures are mainly the species of lactic acid bacteria (LAB) that are deliberately added to milk where their primary role is to ferment carbohydrates into energy and lactic acid for the cheese manufacturing process (Kosikowski, 1982; Caplice and Fitzgerald, 1999; Hill and Ross, 1998, Jay, 2000 and Broom, 2007). The ability of starter culture to lower pH by producing acid from sugar leads to the development of desirable organoleptic properties prevents the growth of pathogens and ensures the stability and safety of the final product (Durlu-Ozakya *et al.*, 2001). In addition, LAB produces small organic compounds that contribute towards the aroma and flavor of the fermented product (Hill and Ross, 1998; Caplice and Fitzgerald, 1999). Lactic starter culture may consists of single strain used alone or in combination or undefined mixtures of strains (mixed strain cultures) (Kosikowski, 1982; Marth and Steele, 2001). Starter cultures are now lyophilized with milk components, nutrients, and energizers and distributed commercially in the dry state or these are frozen with liquid nitrogen at - 196°C and distributed in this state (Kosikowski, 1982).

In the manufacture of most of the cheeses, carefully selected strains of different species of LAB are added to milk shortly before renneting. The major function of these strain is to produce lactic acid and in some cases flavoring compounds (Hill and Ross, 1998).

2.3.1. Classification of starter culture

Cheese starter cultures may be classified in a number of ways. They may differ from each other on the basis of cell shape, cellular display, various growth temperature, growth in different NaCl concentrations and response within litmus milk (Axelsson, 1993). The microorganisms themselves, for example, may be classified according to optimal growth temperature. Mesophilic starters comprise *Lactococcus* and *Leuconostoc* having an optimal growth temperature of 25-30°C, while thermophilic starters comprise the more widely used Lactobacillus species and *Str.*

salivarius ssp. thermophilus and *Lactobacillus delbrueckii ssp. bulgaricus* having an optimal temperature of 40-45°C (Wood and Hozapfel, 1995; Marth and Steele, 2001; Durlu-Ozakya *et al.*, 2001).

Gram positive or negative and catalase positive or negative are the key character to differentiate LAB (Gerhardt and Murry, 1981; Jay, 2000). Analytical Profile Index (API) kits are also used to characterize the LAB by their ability to ferment carbohydrates and biochemical reactions up to strain level (Muyanja *et al.*, 2003). The close synergistic relationship between *Streptococcu thermplhillus* and *Lavtobacillus bulgaricus* permit for an augmented acid production during milk fermentation. As a result of adaptation to the protein rich milk environment there is increase synthesis of amino acid. *Streptococcus thermophillus*, can produce almost all amino acids, but lacks an extra cellular protease. *Lbacillus bulgaricus* on the other hand lacks enzyme for synthesizing most amino acids, but it possesses an extra cellular caseinolytic protease.(Hols *et al.*, 2005).

2.3.2 Mozzarella cheese culture

In the production of special types of cheese, according to taste, aroma and texture , different types of microorganisms are used. For example, cheeses whose curd is scalded during the manufacture stage like Mozzarella are made by using thermophilic starters like *Streptococcus salivaris subsp. thermophilus, Lactobacillus delbrueckii subsp. helveticus, Lactobacillus bulgaricus* (Coppola *et al.*, 2001).

Starter organisms contributes to proteolysis hence, play an important role for texture, flavour and development of functional properties. Selection of the proper starter culture is very imperative for flavour and texture profile in high-fat cheeses, but it becomes additionally important in production of reduced-fat cheeses, which are vulnerable to development of off flavor (Banks, 2004).

Lactic acid bacteria used to ferment dairy products, vegetables and meat help to preserve and provide sensory and nutritional properties to food products. These bacteria synthesize short chain fatty acids, vitamins and exopolysaccharides (EPS). EPS from lactic acid bacteria have gained much attention over the past few years not only because of their role in rheology, texture and mouth feel of fermented dairy products, but also due to their immune stimulant properties

(Kitazawa, 1998). Proteolytic activity of different starter cultures vary that influence the functional properties of Mozzarella cheese (Hong *et al.*, 1998 and Oberg *et al.*, 1991).

2.4. Transformation of milk into cheese

Milk is considered colloidal suspension of casein micelles. The formation of curd consequential from a three step process; acidification, coagulation and syneresis altered this suspension into cheese (Fox *et al.*, 2000).

2.4.1. Acidification

Acidification is the basic process in the production of the majority of cheese variety. An incessant acidification is conceded out through the processing stage, i.e., up to twenty four hours, and may be at the early stage of ripening for some varieties (Fox *et al.*, 2004). Accurate rate and time of acidification is the vital step in processing of good quality cheese therefore, affects the several aspect of cheese manufacturing which are listed below (Barbano, 1999; Fox *et al.*, 2000; McSweeney, 2007).

- Control and prevent the growth of spoilage and pathogenic organisms
- Affect the activity of coagulant during manufacturing and ripening
- Solubilises colloidal calcium phosphate which effect the cheese texture
- Promote syneresis and hence determine cheese composition
- Influence activity of enzyme which ultimately effect the flavour and quality

Acidification of milk can be carried out normally through production of lactic acid by starter cultures, although acid or amidogens are now used to acidified curd without using starter culture for some varieties, e.g., Mozzarella, Cottage and UF Feta-type cheese (Fox *et al.*, 2004).
Breene *et al.*, (1964) manufactured the Mozzarella cheese without using starter cultures by direct acidification. They acidified the milk to pH 5.6 with acetic or lactic acid and the curd produced was stretched in hot water. They reported that the time required to make directly acidified Mozzarella was reduced by 50% as compared to traditional cultured Mozzarella cheese.

Ernstrom (1965) suggested, direct acidification could be good option for a more mechanized and continuous systems for Mozzarella cheese manufacturing (Breene *et al.*, 1964). Muhammad *et al.* (2006) investigated the effect of two types of acids (lactic and citric acids) at three pH levels (5.3, 5.6, 5.8) on yield, chemical (calcium), rheological (elasticity) as well as sensory (taste and flavour) properties of cheese made by direct acidification. They found that the stretchability and sensory value were higher in cheese acidified by citric acid and the stretchability increases as the pH decrease. Moreover, variation in pH levels at different production stages appreciably affects the cheese water holding capacity and its composition in addition to this functionality of cheese product also improved (Guinee *et al.*, 2002). Change of Mozzarella cheese pH after production affect meltability and calcium distribution in the cheese (Kindstedt *et al.*, 2001).
Various starter types are used in cheese making. Starters play a role in the acidification of cheese milk to the desired pH during manufacturing. Current starter technologies include adjunct starters, genetically modified starters and fast-acid starters, which are available commercially as liquid, frozen and dried (Farkye, 2004). It is now universal practice in industrial cheese making to add a starter culture of selective lactic acid bacteria to raw or pasteurized cheese milk to achieve a uniform and predictable rate of acid production (Fox and McSweeney, 2004).

2.4.2. Coagulation

The necessary distinguishing step in production of all cheese varieties is coagulation of casein component of the milk protein system to form a gel which entraps the fat. Coagulation may be achieved by:

- Limited proteolysis by selected proteinases (mainly rennet)
- Acidification to pH 4.6
- Acidification to pH 5.2 in combination with heating to 90°C (Fox and McSweeney 2004)

Rennet coagulation involves two distinct stages, a proteolytic stage in which the casein micelle is destabilized by hydrolysis of K-casein to yield Para- K-casein micelles and a secondary,calcium mediated stage in which paracasein micelles undergo limited aggregation. The secondary stage requires quiescent conditions and a temperature above 20°C. Hydrolysis of K-casein primarily involves cleavage of the peptide bond, Phe105-Met106 which is uniquely sensitive to hydrolysis by acid proteinases. This cleavage yields a Para- K-casein common to all caseins and a glyco-macro peptide (GMP) or caseino macro peptide (CMP) (Fox *et al.*, 2000; Farkye, 2004; Fox and McSweeney, 2004; Skeie, 2007).

When roughly 85% of total K-casein is hydrolyzed, the stability of micelle is reduced to such an extent that when they colloid, they remain in contact and eventually build into three dimensional networks referred to as a coagulum or jell (Fox *et al.*, 2000). Subsequent to hydrolysis, zeta potential of casein micelles reduced from -10/-20 to -5/-7 mV (Fox *et al.*, 2000) and calcium sensitive proteins αs1 and αs2 located toward the interior of miceller structure are exposed (Lucey *et al.*, 2003). Factors which effect the rennet coagulation include protein level, fat level, pasteurization temperature, cooling and cold storage of milk, homogenization, renneting temperature, pH, rennet concentration and calcium concentration (Fox *et al.*, 2000).

Calcium plays a critical part in coagulation. The occurrence of coagulation even at a lower degree of K-casein hydrolysis increased due to increase in calcium concentration (Fox and McSweeney, 1998). Reduction in colloidal calcium phosphate made coagulation difficult. Temperature plays an important role in curd formation. According to Fox and McSweeney (1998) no curd formation will take place at a temperature lower than 20°C in spite of degree of hydrolysis and calcium concentration. It was observed channels containing closely packed fat globules which separated milk proteins that aligned into fibers during coagulation. (Oberg *et al.*, 1991; Fox *et al.*, 2000)

2.4.3. Syneresis

Rennet or acid set coagulum milk gels when cut or broken or subjected to external pressure, the paracasein matrix contracts expressing the aqueous phase of gel (whey). This process known as syneresis or dehydration enables the cheese makers to control

- Moisture content of cheese
- Activities of microorganism and enzymes in cheese
- Biochemistry of ripening
- Stability of cheese (Fox *et al.*, 2000).

The mechanism responsible for syneresis involves the shrinkage of gel (paracasein) with decrease in pH and increased in temperature but the syneresis also occurs under controlled conditions (Dejmek and Walstra, 2004). The extent of syneresis and hence the moisture content of cheese, is influenced by size of the curd particles, cook temperature, rate of acid development, stirring of the curd-whey mixture, pressing, salting, milk quality and pretreatment (Fox *et al.*, 2000). Many of these are exploited by the cheese maker to control cheese composition and thus its flavour and texture.

2.4.4 Cheese ripening

Rennet coagulated cheeses are ripened (matured) for a period ranging from 2 weeks (e.g., Mozzarella) to 2 or more years (e.g., Parmigiano Reggiano or Cheddar). The ripening is very complex process of cheese and engages biochemical and microbiological changes to the curd resulting in the development of flavour and texture characteristic of different variety (Fox *et al.*, 2000; McSweeney, 2004 a, b).

Cheese ripening is an active area of research and different aspects related to biochemistry of ripening have been reviewed comprehensively by (Grappin *et al.*, 1985; Rank *et al.*, 1985; Fox, 1989; Fox and McSweeney, 1996b; Fox and Wallace, 1997; McSweeney and Sousa, 2000; Sousa *et al.*, 2001; McSweeney, 2004; McSweeney and Fox, 2004; Upadhyay *et al.*, 2004).

Biochemical reactions which occur during ripening are grouped into 4 major categories: 1. Glycolysis of residual lactose and catabolism of lactate, 2. Catabolism of citrate, 3. Lipolysis and the catabolism of free fatty acids 4. Proteolysis and catabolism of amino acids (McSweeney, 2004).

Most of the lactose is lost in the whey as lactose or lactate during cheese manufacture. However, low levels of lactose (e.g. 0.8–1%) remain in the curd at the end of manufacture (Huffman and Kristoffersen, 1984).It is necessary that the lactose is fermented completely in cheese otherwise there may be unwanted development of secondary microflora. In early stages of ripening residual lactose is metabolized rapidly to L-lactate (Turner and Thomas, 1980; Parente and Cogan, 2004). Non starter lactic acid bacteria (NSLAB) metabolized lactose that remains unfermented (McSweeney and Fox, 2004).During the carbohydrate catabolism there is formation of organic acids as major products of lactic acid bacteria (Gonz!alez-de Llano, Rodr!ıguez, and Cuesta, 1996). Organic acids play an integral role in quality, and flavor development of cheese (Califano and Bevilacqua, 2000).

There is approximately 1750 mg/ L of citrate present in milk. Mainly citrate is present in the soluble form and so most of it is lost on whey drainage (Fox *et al.* 1993). Approximately citrate levels in cheese are three times more as compare to the whey (Fryer *et al.*, 1970), most probably owing to the concentration of colloidal citrate (McSweeney and Fox, 2004). Lipids contribute to cheese flavour in three ways; 1. As a source of short chain fatty acids, which have strong and characteristic flavour, 2. Polyunsaturated fatty acids, which undergo oxidation and lead to the formation of aldehydes that are strongly flavoured, 3. Serve as a solvent for sapid and aromatic compounds (Fox *et al.*, 2000). Extensive ripening of cheese results in an exceptionally soft texture and increased free oil release making it inappropriate for use on pizza toppings (Kindstedt, 1995).

One of the primary biochemical events, which occur in cheese manufacturing during ripening, proteolysis, is the most complex and important. Proteolysis plays a major role in the development of flavor and texture in most of the rennet- curd cheese varieties. Ripening of Mozzarella cheese is done to get most of the desired functional characteristics.

2.4.4.1. Proteolysis

During cheese ripening proteolysis is a recognized group of biochemical reactions for several cheese varieties, especially for Cheddar, but it still remains less studied in pasta filata cheeses, such as Mozzarella (Johnson and Law, 1999; Feeney *et al.*, 2002). The extent and depth of proteolysis in low-moisture Mozzarella cheese plays an important role in the development of functional properties of this type of cheese (Fox, 1989; Lucey *et al.*2003). Sources of proteolytic enzyme in cheese are from milk, rennet, LAB, NSLAB and secondary cultures. Residual coagulant and endogenous proteases (plasmin) are mainlyresponsible for the proteolysis of Mozzarella within the first two weeks of storage (Barbano *et al.*, 1993; Barbano *et al.*, 1995). Rennet enzymes are extensively denatured in high cooked cheese such as Mozzarella and Parmesan (Creamer, 1976; Matheson, 1981; Garnot and Molle, 1987) Therefore the contribution of plasmin to primary proteolysis is considerably higher in these varieties of cheese than in cheddar and Dutch-type cheeses (Fox *et al.*, 2000). Grufferty and Fox (1988) provide the evidence of plasmin activity in Mozzarella cheese during ripening because of degradation of β-casein with the formation of γ-casein. But the recent studies show the considerable residual coagulant activity.

The starter culture contributes primary to secondary proteolysis i.e., degradation of products of primary proteolysis to small peptides and amino acid. On the other hand the starter culture may contribute to the initial hydrolysis of intact casein (Barbano *et al.*, 1995). Two of the most important factors influencing chemical properties are the state of the casein particles in cheese that involve molecular interactions within and between, in addition to this the content of Ca linked with these particles and the degree of proteolysis (Lucey *et al.*, 2003). These in turn are predisposed by various environmental conditions such as pH development, temperature, and ionic strength. Hence, how individual casein molecules, or aggregates of many casein molecules interact, is very important in understanding the physical and chemical properties of the cheese (Lucey *et al.*, 2003). The stretchability of Mozzarella cheese is related to high concentration of intact casein and to critical concentration of Ca and PO4 (Lawrence *et al.*, 1982).

Decrease in firmness and fracture stress is due to the initial hydrolysis of alpha s1-casein at the Phe23- Phe24 peptide bond because of residual chymosin results in a marked weakening of para-casein matrix (Creamer and Olson, 1982; Fenelon *et al.*, 2000). β-casein generally undergoes markedly less breakdown than αs1-casein during storage of most of the cheeses, including Cheddar, Gouda, and Mozzarella (Visser and de Groot-Mostert, 1977; Yun *et al.*,1993; Fox *et al.*, 1996). According to Fox (1970) that the individual casein in milk especially αs1-casein, become progressively more susceptible to rennet induced proteolysis at pH 6.6 as the level of miceller calcium phosphate is reduced. This effect was attributed to the increased accessibility of individual caseins to rennet, owing to the disruption of micelles on removal of colloidal calcium phosphate.

The extent of hydrolysis of the whole and αs1-casein or β-casein by chymosin generally decreased as the pH increased from 3.5-6.0 (Tam and Whitaker, 1972). Mulvihill and Fox (1977) reported that in pH range 4-7, the hydrolysis of αs1-casein to αs1-casein (f 24-199) by chymosin was optimum at pH 5.8 and minimum at pH 4.6, where the αs1-casein (f 24-199) is aggregated. Further, the rate of degradation of αs1-casein (f 24–199) to αs1-casein (f 102–199) is influenced by reaction pH, being optimal at pH 5.8 (Mulvihill and Fox, 1977). However, the influence of pH on the proteolytic activity and specificity of chymosin on αs1-casein (f 24–199) is influenced by NaCl (Mulvihill and Fox, 1980). However, hydrolysis of αs1- casein to αs1- casein (f 24–199) and further hydrolysis to αs1- casein (f 24–199) is markedly inhibited at 5% NaCl in the pH range 5.8 to 7.0. Fife *et al* .,(1996) found the amount of intact αs1- casein decreased by at least 48% in cheeses containing different fat to casein ratios as a result of proteolysis during 28 days of storage. Urea PAGE (poly acrylamide gel electrophoresis) is powerful tool for monitoring proteolysis during early stages of cheese maturation and comparing casein hydrolysis pattern in cheese manufactured from the milk of different species (Marcos *et al.*, 1979: Sousa and Malcata, 1997). Urea PAGE is widely used to monitor proteolysis as it resolves protein on a combination of mass to charge while sodium dodecylsulphate (SDS) - PAGE which is used more widely in biochemistry is less suitable for studying proteolysis in cheese because this technique resolves protein based on the size and caseins have similar molecular mass.

2.5. Functional properties of cheese

Cheese that is used as an ingredient in prepared food must satisfy certain performance requirement that are determined by the function of cheese in the particular food application in which it is used (Kindstedt *et al.,* 2004). Functionality of cheese is defined by its rheological, physicochemical and micro-structural, which affect the behavior of cheese in food systems during preparation, processing, storage, cooking, and or consumption. Cheese contributes to the quality and improves organoleptic attributes of the food in which used (Fox *et al.,* 2000). Low moisture Mozzarella cheese is used mostly as an ingredient in pizza. Therefore functional properties are essential determinant of the quality and acceptability of low moisture Mozzarella cheese (Kindstedt *et al.,* 2004). LMMC is usually produced in block form, ranging from 2.3 to 9.5 kg and must be shredded or diced before it is used as an ingredient in pizza. Problem from shredability may occur when the body of the cheese is soft and pasty or wet, causing shredability machine to become clogged with cheese and resulting in shreds with ragged edges and deformed geometry.

Such cheese is likely to undergo excessive matting after shredding, which makes it difficult to handle, store and apply uniformly on the product. At the apposite extreme, cheese that is firm and dry may take longer to shred and fracture excessively to produce shattered shreds and fines, which are more difficult to handle (Kindstedt, Caric and Milanovic, 2004). Other functional properties namely stretchability, meltability, browning and free oil formation of Mozzarella cheese are highly dependent cheese composition and structure. The milk pretreatment, pH, moisture content, fat and minerals including salt and extent of proteolysis govern the cheese functionality (Yun *et al.*, 1993; Farkye *et al.*, 1991; Rowney *et al.*, 1999; McMahon and Oberg, 1998; Kindstedt and Guo, 1998; Metzger *et al.*, 2001; Guinee *et al.*, 2002).

2.5.1. Meltability

The ability of cheese particles to flow in a continuous uniform melted mass is meltability (Kindstedt, 1993). The fat content and the protein-protein interactions with water are the two main factor determinant factors of Mozzarella meltability (McMahon *et al.*, 1999). The meltability of LMMC has been evaluated extensively by empirical tests such as Schreiber (Kosikowski and Mistry, 1997) and Arnott (Arnott *et al.*, 1957) tests, which measure the increase in diameter or decrease in height of a cylinder of cheese upon melting under standard conditions. Muthukumarappan *et al.* (1999) proposed several modifications to improve the efficiency of the Schreiber test. More recently, Wang and Sun (2002) applied computer vision image analysis to quantify the increase in the area of cheese sample upon melting as an index to meltability.

It was concluded by many researchers that the type of the protein matrix play a key role in determining Mozzarella cheese melting properties (Guo *et al.*, 1997; McMahon and Oberg, 1998; McMahon *et al.*, 1999). It was further suggested that during ripening, proteins absorb serum from the surrounding, thinning accumulation and enhancing melt by water transfer from the fat-serum channels to the protein matrix. End result is decreased hydrophobic interactions within the protein matrix (McMahon *et al.*, 1999).

Milk Homogenization prior to cheese-making decrease the size of fat globules offering higher buffering in the casein matrix and as results meltability is decreased. Mozzarella cheese meltability as a Homogenization of the cream did not affect the meltability of cheeses, but use of ultra filtered buttermilk lowered meltability (Tunick, 1994). Yun *et al.* (1998) prepared low moisture part skim Mozzarella cheese by the incorporation of incorporated nonfat dry milk which has no significant impact on meltability. Rudan *et al.* (1999) suggested hydrophobic surface coating to provide low fat Mozzarella cheese appropriate melt characteristics during pizza baking.

Fat content of cheese strongly relates to free oil formation and melt. Low fat Mozzarella cheeses made from milks containing casein to fat ratios of 3.0, 5.0, 7.0, and 8.0 did not melt as well as did the part-skim Mozzarella cheese (19% fat in cheese). Even the storage for 28 days only marginally increased the meltability of low fat cheese (Fife *et al.*, 1996).

The functional properties of unmelted and melted Mozzarella cheese also depend on the coagulant type. Three different coagulants (*Endothia parasitica* protease, chymosin derived by fermentation and *Mucor miehei* protease) were used in Mozzarella manufacturing in a study. During 50 days of storage at 4°C it was found that cheese made with E. parasitica protease was more meltable and had lower apparent viscosity and more free oil release on melting than other cheese. In general, cheeses made with chymosin and Mucor miehei protease were similar in functional characteristics (Yun *et al.*, 1993). Imm *et al.* (2003) compared the functional properties of Mozzarella cheese prepared from caprine and bovine milk. They found that meltability of ripened cow and goat cheeses were not different when fat content of both milks were standardized. The melting characteristics showed high positive correlation (r =0.51 to 0.80) with proteolysis, whereas it was negatively correlated with textural properties.

2.5.2. Stretchability

Stretchability is a tendency of a thing to form extended fibrous string (Kindstedt, 1993). This unique property of Mozzarella cheese, a type of pasta filata makes it satisfactory use as toppings on pizza (Guinee and O'Callaghan, 1997). The curd is transferred into molten mass by heat during cheese manufacturing. Plasticization necessitates para casein matrix arrangement and combination of fat and moisture into larger pools parallel to the protein fibers (Fox *et al.*, 2000). The thermal treatment applied to Mozzarella cheese during stretching also affects the microbiological and proteolytic properties during refrigerated storage. The temperature remains below 60ºC, thermophilic starter bacteria and residual coagulant remain alive in cheese during ripening while when curd temperature exceeds 66ºC during stretching there may be inactivation of starter bacteria and rennet that may affect the functional properties of cheese (Kindsted *et al.*, 2000). Arrangement of curd strand develops by kneading and stretching stages of cheese production. The scientists suggested that pH of curd, whey pH and colloidal phosphate content of Mozzarella cheese significantly affect the stretchability (Lawrence *et al.*, 1987; Kiely *et al.*, 1992).

Apparent viscosity (AV), stretchability and elasticity ("strength of stretch"), is often used to describe cheese. Denatured whey protein addition to milk @ 0.4% produced cheese with considerably greater apparent viscosity (AV) than Mozzarella cheese produced by standard milk and exhibit unacceptable stretch properties (Mead and Rupas, 2001). During the refrigerated storage the AV first reduce and then stabilized (Bertola *et al.*, 1996). This is due to the proteolysis within the system (Kindstedt *et al.*, 1992). During ripening, proteolysis result in increased porosity of casein matrix and therefore resistance to stretching has reduced (Bertola *et al.*, 1996; Tunick *et al.*, 1997). During the ripening period, protein to protein and calcium to protein interactions undergo partial reversal as calcium dissociates from and water interact with the Para casein fibers. This in turn triggers microstructure changes and the development of a more flowable, stretchable and less chew melted consistency. During the measuring of rheological properties of melted cheese there are some difficulties. Industry tends use empirical tests like "fork test" due to its simplicity (USDA, 1980). While this type of information has an advantage that fork test can be performed at location but on the other hand fork test is highly sensitive and tester with experience is required to carry out the test. Guinee and O'Callaghan (1997) developed a method to determine stretchability using uniaxial extension in combination with an environment liked by the consumer. All of these tests used to measure stretchability produce acceptable results but no one can decide that which method provides more precise information on stretchability.

At higher temperature fat globules are in liquid state and appear smooth in the observed channels. As the cheese cools (to 4°C) fat globules become rigid and protein matrix adopted the shape as govern by solidified fat globules. As a results of this function fat appears as a break up agent for fusion of the protein matrix during resulting in retention of excess serum during cooking and stretching stages (McMahon *et al.*, 1999). Hekken *et al.* (2007) found that microfluidization of cheese milk at different temperatures and pressures altered the meltability and rheological properties of Mozzarella cheese. They observed that microfluidization of the cheese milk did not improve the melt or rheology of low fat cheeses. Microfluidization of milk with fat in the liquid state at higher pressures resulted in smaller lipid droplets that altered the component interactions during the formation of the cheese matrix and resulted in low fat and high fat Mozzarella cheeses with poor melt and altered rheology.

2.5.3. Free oil

Free oil formation is generally known as fat leakage or oiling off affinity of liquid fats to detach from melted cheese and ultimately build up in pools or pockets specifically at the surface of cheese. Limited or excessive free oil formation is two severe defects while considering the quality of Mozzarella cheese.

Liberation of oil is the outcome from the lapse of the casein matrix, consequently allowing the fat globules to combine and then move to the surface where pools of free oil are produced (Rowney *et al.*, 1999). Previous researchers revealed that free oil formation can easily be estimated by utilizing a modified Babcock test (Kindsted and Rippe, 1990) where as Kindsted and Fox (1991) also concluded that free oil can be assessed by Gerber apparatus. Explication for the changeability of free oil production examined from various kinds of Mozzarella cheese has been the decisive concern of some researchers. The processes of free oil formation in Mozzarella cheese depends upon fat globule size, degree of agglomeration and position within the casein matrix (Rowney *et al.*, 1998).

A correlation amongst magnitude of agglomeration and fat globule size exists for emulsion consistency of the globule and interaction of the water/fat interface (Oberg *et al.*, 1993, Cano-Ruiz and Richter, 1997). Furthermore, in cheese production, the melting profile of milk fat has also been correlated with free oil content of Mozzarella (Rowney *et al.*, 1998). It has also been noticed that when emulsifying salts are added to Mozzarella cheese, a change in the polymorphic framework of cheese has occurred (Tunick *et al.*, 1989). In contrary, Kindstedt *et al.* (1992) studied that a little salt concentration in Mozzarella cheese leads to an improvement in free oil formation originated by decreased emulsification of fat by casein owing to less Na/Ca exchange. In contrast, intensification in salt content would decline the fat overflow at some point in melting. In other investigation Kindstedt and Rippe (1990) reported a reduction in free oil formation as a result of proteolysis after refrigerated storage. Conversely otherresearchers stated a significant rise in free oil when samples of cheese were frozen (Apostolopoulos *et al.*, 1994). Kindstedt and Rippe (1990) and McMahon *et al.* (1993) concluded that higher cheese fat result in excessive oiling off while a lack of available fat have less oiling off which produce tough and rubbery melted cheeses.

Firstly, Mozzarella is hard, not uniform, and has a very elastic consistency caused by lack of free oil release and separation of free water. After 21 days the cheese becomes more workable, consistent in form, and more fluid due to the break down of casein components of the cheese (Kiely *et al.*, 1993).Imm *et al.* (2003) observed a significantly larger amount of free oil in bovine Mozzarella cheese than caprine throughout the storage experiment.

2.6. Cheese texture

Rheological and textural properties of cheese are affected by numerous factors. Many such effects are fairly well documented and yet others are still the subject of continued research. Several factors that affect rheological and textural properties of cheese also have an effect on flavour, appearance and functional properties important to consumers. Texture is very important property used to discriminate numerous cheese varieties. Wendin *et al.* (2000) revealed that texture characteristics are more important in unfolding disparities between cheese samples than the taste and flavor attributes. Antoniou *et al.* (2000) grouped and distinguished French cheeses based upon instrumental and sensory measurements of texture. Generally, suitable cheese texture is preferably considered by the consumer to be assessed the overall quality. Textural attributes are a determinant of consumer fondness in cheese (Lee *et al.*, 1978; Adda *et al.*, 1982; McEwan *et al.*, 1989). Texture can be described in two fundamental terms: texture assesses through the genuine, physical structure of material and that which is observed by visual and perceptible elements of the material (Szczesniak , 1963). Different characteristics of cheese both perceptual and physical are originated from significant rudiments of foodstuff makeup and sensory scheme evaluation. Therefore this signify that in standing imperative information regarding both the human being's sensory processes and physical prototype of the material employed in mastication is vital in an attempt to depict conclusions in relation to a material's texture (Christensen, 1984).

Methods to improve evaluation techniques have also been explored. Drake *et al.* (1999) showed that both hand and mouth evaluation similarly differentiated the texture of a variety of cheeses. Many researchers have used the texture profile analysis (TPA) and similar uniaxial compression tests at a temperature range from 10 to 20°C to characterize hardness and firmness of cheese. During these analyses Mozzarella cheese showed a significant softening trend with increasing age and level of proteolysis (Tunik *et al.*, 1993, 1995; Yun *et al.*, 1993, 1995; Kindstedt *et al.*, 1995a, b) with increasing fat and/or moisture content (Tunik *et al.*, 1991, 1993,1995 Rudan *et al.*, 1999) and with decreasing Ca content pH (Guinee *et al.*, 2002).

Foods are material and therefore have the mechanical properties. Large parts of perception of food involve communication, which can be related to fracture mechanism. Texture is governed by a combination of mechanical and fracture properties and their modification and expression within the mouth during chewing. Difficulties arise due to number of factors.

1. Mechanically food are very complex
2. Mechanical processing and chewing combine many processes that material science needs to separate to quantify and understand them.
3. Food in mouth is continually changing its properties as temperature, water content, pH and so on changes.
4. Most food scientists are not trained in material science
5. Most material scientists do not admit that food is a material

It is therefore probably impossible to measure in a machine, although it is probably possible to identify the main factors that govern the texture of a food material and to measure them. Thus accounting for a large part of texture there are two main approaches. The first more common and intrinsically easier, is to apply any type of mechanical deformation to measure the response and try to correlate the result with the result from a sensory panel (Kilcast, 1999).

2.7. Evaluation of cheese on pizza

Mozzarella cheese is predominately used as an ingredient in pizza. Mozzarella cheese undergoes significant change in temperature during baking on a pizza (Kindstedt, 1993). Low moisture Mozzarella cheese and low moisture part skim (LMPS) Mozzarella cheese, approximately 20% fat on a wet basis, are types commonly used for pizza baking because of their desirable functional characteristics (Kindstedt, 1993). Pizza is baked at 232°C and at the end of baking the cheese temperature is approximately 60 to 70°C (Kindstedt et al, 1989). It normally consumed at 40 to 50°C. Wide temperature range in baking and successive cooling influence the appearance of Mozzarella cheese. Generally, proper functionality during pizza baking includes complete melting and shred fusion without the molten cheese becoming too soupy, combined with some free oil (FO) release, giving the surface a shiny appearance without forming pools of oil, some blistering and browning should occur but without producing a burnt appearance (Kindstedt, 1991).

Given the healthier eating goals of consumers and the continued demand for pizza, there has been interest in developing a lower fat Mozzarella cheese (Rudan *et al.*,1998; Tunick *et al.*, 1998,) It has been demonstrated that a reduction in fat content leads to poor functionality during pizza baking; for example, during pizza baking for 5 min at 232°C, lower fat cheeses exhibited incomplete shred melt and fusion, followed by burning of intact shreds, resulting in a pizza with a burnt appearance (Rudan *et al.*, 1994). As the cheese temperature increases during pizza baking, the interactions of protein and protein, protein and water, as well as protein and calcium change, and the AV decreases, causing the cheese shred to collapse and flow because of gravity (Ruegg *et al.*, 1991). The relative expansion of the melted fat is larger than that of the protein matrix, which forces some of the fat to move from the protein matrix and to the cheese shred surface (Visser, 1991). Shred flow and fusion probably begin within the temperature range of 55 to 80°C. However, more work is needed to understand better the effect of temperature on softening point, flow, and apparent viscosity (AV) of Mozzarella cheese. Furthermore, additional work is needed to identify the important interactions between cheese components (i.e., among proteins, proteins and water, and proteins and calcium) that are responsible for the observed effect of temperature on softening point, flow, and AV of Mozzarella cheese (Kindstedt, 1991).

Above 100°C (at the cheese surface and below), water is converted to steam; this steam and the trapped air between cheese shreds collect in bubbles under the molten cheese surface. Next, the cheese surface over these bubbles begins to rise from the expansion of the trapped air and steam, initiating the formation of a blister. The size and characteristics of the blister are dependent on the size of the bubble and, with respect to the cheese, the amount of expressible serum, proteolysis, and ratio of moisture to protein, pH, and any other factors that influence the AV of the cheese (Rudan and Barbano, 1997).

From the review of literature it is concluded that quality of Mozzarella (pizza) cheese is influenced by several factors. Type of milk and standardization of fat and protein (casein) in milk, pasteurization, homogenization and microfluidization effect the yield, proteolysis, texture, functionality and sensory attributes of cheese. Starter type and their rate of acid production are very important in cheese. pH at whey drainage determines the residual level of rennet and the amount of insoluble Ca in cheese. Higher the amount of insoluble Ca in cheese, more firm will be the texture. Ripening and milk composition has more effect on the functionality of pizza cheese.

CHAPTER-III

MATERIALS AND METHODS

3.1 SELECTION OF MILK SOURCE AND RAW MATERIALS

1. Raw milk (cow milk)
2. Citric acid
3. Calcium chloride
4. Starter cultures (S.thermophilus&L.bulgaricus)
5. Rennet

There are basically two methods used for the production one is direct acidification and the other one is using starter cultures

Direct acidification:- It has gained commercial interest as it doesn't rely on the starter performance (unpredictable, risk of phage infection and milk contaminated with antibiotics) and helps towards mechanization of production. Cheese produced using this method has higher calcium chloride content and good melt ability and stretch ability.

Using starter cultures:- In this method two starter cultures S.thermophilus and L.bulgaricus are used.Pasteurized milk is incubated at 33 C with rennet or starter culture containing lactobacilli and streptococci is used. The separated curd(ph 5.2)is heated in water at 80C kneaded and formed into balls 150-200 g balls which are cooled under cool water at 10-12C followed by immersion in brine for 30 min.

3.2. Procurement of raw materials

3.2.1. Milk

Raw milk of buffalo and cow were procured from local milk vendor in Waknaghat for the preparation of Mozzarella cheese.

3.2.2. Starter cultures

3.2.2.1. Indigenous starter culture

Streptococcus thermophilus and *Lactobacillus delbrueckii subsp. bulgaricus* cultures isolated, identified and purified in Jaypee University of information technology were used for cheese manufacturing as indigenous cultures.

3.2.2.2. Commercial starter culture

Commercially available culture for Mozzarella cheese (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus*) from Chr. Hansen's Laboratory (Ireland) Ltd, Cork, Ireland was also used for cheese manufacturing for comparison with locally isolated culture.

3.2.3. Rennet

The enzyme chymosin (Double strength Chy-max, 500000 MCU/mL, Pifzer Inc, Milwaukee, WI, USA) was used to coagulate the milk in the present study.

3.3. Preparation of cheese milk

3.3.1. Fat standardization

Buffalo milk and blend of cow and buffalo (50:50) milk were standardized for the fat content prior to manufacturing of Mozzarella cheese. The milk samples (buffalo and blend of cow and buffalo milk) were decreamed and content of both cream and milk was determined by Gerber method. The fat level was standardized in milk by using the Pearson's square method at a level of 1.5 and 2.5%.

3.3.2. Physicochemical analysis of milk

The standardized milk samples were analyzed for physico-chemical composition such as pH, acidity, fat, protein, casein and calcium content.

3.3.2.1. pH

The pH of Milk was measured through electronic digital pH meter (Inolab WTW Series 720). Buffer solution of pH 4 and 7 were used to calibrate the pH meter. Milk sample was taken in a beaker; electrode of pH meter was immersed in the sample to determine pH.

3.3.2.2. Acidity

Acidity in milk samples was determined by the method (No. 947.05) given in AOAC (2000). Nine mL of milk sample was taken in a titration flask and 2-3 drops of phenolphthalein were added to it. The sample containing indicator was titrated against 0.1N NaOH until light pink end point appeared and for few seconds. Volume of 0.1 N NaOH used was recorded to determine acidity of milk in terms of lactic acid by using following expression % Acidity (as lactic acid) = Volume of NaOH used X 0.1

3.3.2.3. Fat

The Gerber method was used to determine fat content in milk (Marshall, 1993). Sulphuric acid (10mL) was poured into butyrometer and then 10.94mL of milk sample was slowly added into butyrometer followed by 1mL of amyl alcohol. The contents of butyrometer were thoroughly mixed and centrifuged at 1100 rpm for 5 minutes. Butyrometer was transferred to a water bath at 65°C for at least 3 minutes and the percent fat was recorded directly from the butyrometer scale.

3.3.2.5. Casein

The casein content in milk was determined by method (998.06) given in AOAC (2000). Casein was precipitated from milk at pH 4.6 using acetic acid and sodium acetate solutions. The acidified solution, which contains the noncasein N components of the test portion, was separated from casein precipitate by filtration. The filter paper along with residue was placed in hot air oven for drying. After drying filter paper wrapped casein was subjected to Kjeldhal's method to determine casein nitrogen and than multiplied with 6.38 to get casein % in the sample.

The pH of Milk was measured through electronic digital pH meter (Inolab WTW Series 720). Buffer solution of pH 4 and 7 were used to calibrate the pH meter. Milk sample was taken in a beaker; electrode of pH meter was immersed in the sample to determine pH.

3.3.2.2. Acidity

Acidity in milk samples was determined by the method (No. 947.05) given in AOAC (2000). Nine mL of milk sample was taken in a titration flask and 2-3 drops of phenolphthalein were added to it. The sample containing indicator was titrated against 0.1N NaOH until light pink end point appeared and for few seconds. Volume of 0.1 N NaOH used was recorded to determine acidity of milk in terms of lactic acid by using following expression % Acidity (as lactic acid) = Volume of NaOH used X 0.1

3.3.2.3. Fat

The Gerber method was used to determine fat content in milk (Marshall, 1993). Sulphuric acid (10mL) was poured into butyrometer and then 10.94mL of milk sample was slowly added into butyrometer followed by 1mL of amyl alcohol. The contents of butyrometer were thoroughly mixed and centrifuged at 1100 rpm for 5 minutes. Butyrometer was transferred to a water bath at 65°C for at least 3 minutes and the percent fat was recorded directly from the butyrometer scale.

3.3.2.5. Casein

The casein content in milk was determined by method (998.06) given in AOAC (2000). Casein was precipitated from milk at pH 4.6 using acetic acid and sodium acetate solutions. The acidified solution, which contains the noncasein N components of the test portion, was separated from casein precipitate by filtration. The filter paper along with residue was placed in hot air oven for drying. After drying filter paper wrapped casein was subjected to Kjeldhal's method to determine casein nitrogen and than multiplied with 6.38 to get casein % in the sample.

3.3.2.6. Calcium

The calcium content in milk was determined by using flame photometer.(Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) by following the method described by Kirk and Sawyer (1991). One mL of milk sample was taken in conical flask and added 10mL nitric acid into test sample. The sample was heated at 60-70°C for 15minutes until solution was clear then it was heated at 80 °C for 30 minutes. Later 5mL perchloric acid was added and heated at 80°C for 15 minutes and then boiled vigorously until 1-2 mL volume of sample was left. The sample content was filtered and diluted with deionized water up to 100 mL. The standard solutions of known concentration of calcium were prepared and run on flame photometer followed by sample to determine the calcium content in the milk samples. The standard curve was obtained by plotting absorbance value of standards against appropriate concentrations of calcium.

3.4. Preparation of mother cultures

Indegenous cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus* were propagated and tested for curd formation individually and in different combinations. The best combination (2:1) was selected and used to prepare mother culture using UHT skim milk as a substrate medium. Skim milk was inoculated with the propagated culture and incubated at 37°C for 6 hours to prepare the mother culture. Similarly commercially available cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were propagated in the same combination (2:1) as for indigenous cultures to prepare the mother culture.

3.5. Mozzarella cheese manufacturing

The different cheese samples were prepared according to detail given in Table 3.1.Cow milk which were standardized at 1.5 and 2.5% fat level were first pasteurized at a temperature of 65 °C for 30 minutes. After this the standardized milks weredivided into two parts. Then cooled to 37°C subsequent to this one part of each milk was inoculated with combined culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) @ 2% of indigenous culture and other part with commercial culture. After ripening for 30 minutes, the milk at 37°C was set with

Chymosin (Double Strength Chy-max,) @ 0.077mL/kg of milk. Approximately 40 minutes after rennet addition, the curd was cut with 1.9 cm wire knives and then allowed to heal in the whey for 10 minutes. Then with periodic gentle agitation of curd to prevent matting the curd was heated from 37°C to 42°C over 30 minutes. The temperature was increased gradually to 42°C during cooking of the curd. The whey was drained at pH6.2 and matted curd was turned every 15-20 minutes and milled at pH5.2. Then the salt was added at the rate of 1.5% of the curd. The stretching of salted curd was done by means of hand in water at a temperature of 70°C until the uniform and elastic cheese consistency was achieved. The quantity of water used for stretching was 2.5 times of the weight of curd and had 3% of salt w/w. The salted curd was then molded and vacuums packed and stored at 4°C for ripening.

Figure: 3.1. Flow diagram for manufacturing of Mozzarella cheese

Milk Standardization
↓
Pasteurization
(65°C; 30 min)
↓
Inoculation
(2% mother culture; 37°C)
↓
Incubation
(37°C; 30min)
↓
Renneting
(0.077mL/kg; 37°C; 40min)
↓
Cutting
↓
Healing
10min
↓
Scalding
(37-42°C; 45min)
↓
Whey removal
pH 6.2
↓
Cheddaring
↓
Milling
pH 5.2
↓
Salting
(1.5%)
↓
Stretching
In hot water; 70°C
↓
Molding
↓
Ripening (4°C)

3.6 Compositional analysis of Mozzarella cheese during ripening

3.6.1. Physico-chemical analysis of Mozzarella cheese

The Mozzarella cheese was tested for moisture, protein, fat, ash, pH, acidity and salt after 20 days interval during 60 days of ripening period.

3.6.1.1. Moisture content

The moisture content in Mozzarella cheese was determined by drying samples in oven by keeping at 103 ± 5°C till the constant weight of dried cheeses is obtained by the Method No. 926.08 of AOAC (1990). First the enough amount of sand was added to the dish to cover bottom of dish and a glass rod was placed in it. Then dish was placed in oven at 103°C for one hour. After removal from the oven it was placed in desiccator to cool. Weighed the dish and denoted as (W1).

After this 2 g of grated cheese sample was weighed (W2) in the dish and mix cheese and sand carefully using glass rod and placed in oven at 103°C for more than 5 hours. Then removed from oven and allowed for cooling in the desiccator and recorded the weight (W3). Moisture was calculated by using following expression.

$$\%Moisture= W2 - W3 \div W2 - W3 \times 100$$

3.6.6.2. Fat content

The fat content in cheese was determined by Gerber method as described by (Marshall, 1993). Cheese butyrometer bearing the inscription"I.S. 69: 1955" and 3g cheese sample was used. 10 mL sulphuric acid was added into butyrometer followed by 3 mL distilled water at 60°C. Then cheese sample that was in wrapped in grease proof cellophane paper was inserted to butyrometer and again 5 mL distilled water at 60°C and 1 mL of amyl alcohol was added. The butyrometer was shaked vigorously to dissolve cheese particles completely and then centrifuged it at 1300 rpm for 5 minutes. The butyrometer was placed in water bath at 65°C with the stopper downwards for 2 to 3 minutes and recorded the percentage of fat by measuring fat column.

3.6.6.5. pH

The 20 g of shredded Mozzarella cheese was blending with 12 mL water to prepare the cheese slurry and pH was measured by a pH meter (Inolab WTW Series 720) after calibrating it with fresh pH7.0 and 4.0 standard buffers (Ong et al., 2007).

3.6.6.6. Acidity

Acidity in Mozzarella cheese was estimated by titration Method No. 920.124 of AOAC (1990). Oneg of grated and shredded cheese sample was mixed with warm water to make volume 10 mL, it was vigorously shaked and filtered. The phenolphthalein was used as indicator and filtrate was titrated with 0.1N NaOH.

$$\text{Acidity \% (as lactic acid)} = \frac{0.0090 \times \text{Volume of NaOH used} \times 100}{\text{Weight of the sample}}$$

3.6.6.7. Salt content

The salt contents in Mozzarella cheese samples were determined by Volhard method (Method No. 975.20; AOAC, 1990). Two g of grated and shredded cheese sample was weighed in 50 mL beaker, 20 mL warm water was added and stirred to break the particles to make slurry. The slurry was transferred to 250 mL Erlenmeyer flask. The beaker was rinsed with 10 mL warm water and added to the Erlenmeyer flask. 25 mL of 0.1N silver nitrate, 10 mL of nitric acid and mL of water was added to sample in Erlenmeyer flask. Then it was placed on a hot plate for boiling in a hood. When solution boiled, potassium permanganate was added in 5 mL portions until the solution turned brown and remained brown for at least 5 minutes on gentle boiling. Heating was continued until the brown color disappeared and resulted in a straw colored clear solution. There was white curd like particles in the solution indicating the silver chloride aggregates. Then the hot solution was filtered into a clean 250 mL Erlenmeyer flask. Filter paper was washed thoroughly with hot water. Solution was cooled to room temperature and 2 mL ferric ammonium sulfate was added as an indicator. The excess silver nitrate was titrated with 0.1N potassium thiocyanate (KSCN) to the first pale reddish brown color that lasted for 30 seconds. The volume of 0.1N potassium thiocyanate used for titration against sample and blank was recorded. The salt (sodium chloride) content was calculated by using the following formula:

$$\text{Sodium chloride \%} = \frac{[(\text{mL of 0.1 N AgNO3}) - (\text{mL of 0.1N KSCN})] \times 0.0585}{\text{Weight of sample (g)}} \times 100$$

CHAPTER-IV

RESULTS AND DISCUSSION

4.1. Physicochemical composition of milk

The composition of milk is not absolute as many factors influence the end product. These variations in milk composition can be related to genetics, environment, milk production, stage of lactation, diseases, season, locality and age of the animal (Gopalkrishan and Lal, 1994).

The protein content was 3.68 to 3.71%. Similarly, the casein content in milk (2.82 to 2.85%) was 2.69 to 2.79%. The concentration of lactose in milk was 4.88%. The pH and acidity of all milk samples were found in the range of 6.62-6.64 and 0.13-0.14%, respectively. The calcium content of milk was 1120 mg/100g.

The protein, fat, lactose, casein and calcium are dependable variables. The species and breed of animals, stage of lactation, season, time and sequence of milking are the factors responsible for the differences in these constituents. The results of the present investigation are in agreement with the findings of various researchers (Ganguli, 1992; Patino, 2004; Ahmed *et al.*, 2008).

4.2. Compositional analysis of Mozzarella cheese during ripening

The standardized milk samples were used for the preparation of Mozzarella cheese. The samples of Mozzarella cheese were subjected to chemical analysis and the results are presented and discussed here in after.

4.2.1. Physicochemical composition of Mozzarella cheese

4.2.1.1. Moisture content of cheese

Moisture is the major component of cheese which acts as a plasticizer in the protein matrix thereby making it less elastic and more susceptible to fracture upon compression (Fox *et al.*, 2000). Therefore moisture analysis is one of the important assessments of quality of the finished product not only because it is important to abide by the set product specifications but also because moisture variation in moisture can affect the textural properties and shelf-life of the cheese (Lee *et al.*, 2004).

The result regarding the mean squares of the moisture content of different Mozzarella cheese is given in Table 4.2. The variables such as ripening ($P<0.05$), milk sources ($P<0.01$), fat levels ($P<0.01$) and culture ($P<0.05$) significantly affected the moisture content of different cheese. The interactions among all these variables exhibited non-significant influence on the moisture content of Mozzarella cheese.

The result with respect to moisture content revealed that the moisture content of Mozzarella cheese decreased significantly from 48.63% (0 day) to 47.63% after 60 days of ripening. The significantly ($P<0.01$) higher moisture content 48.76% was observed in cheese prepared from mixture of cow milk cheese (47.49%). The fat levels of milk also have significant effect on the moisture content of Mozzarella cheese. The cheese prepared from milk having 2.5% fat possessed less moisture content (46.30%) as compared to the cheese prepared with milk that contained 1.5% fat. The cheese prepared from indigenous culture (Table 4.4) got higher value for moisture content (48.40%) as compared to cheese prepared from commercial culture . This variation may be related to the faster acid production rate (Table 4.17) in the indigenous cultured cheese as compared to the commercial culture cheese which contained less moisture content (Dave *et al.*, 2003).

The moisture content of Mozzarella cheese found in present study are in accordance with the previous findings of Sheehan *et al.* (2004) and Catherine *et al.* (1998) who reported that the moisture content of Mozzarella cheese varied between 45% to 50%. The decrease in moisture content during the ripening period is supported by the findings of Mallatou and Pappa (2005) who reported that moisture and moisture in non fat substance (MNFS) decrease sharply during first 20 days which might be due to reduced hydration of casein at this stage.

4.1.2.2 Stretchability, meltability, browning and yield

Parameter	Cheese trial 1	Trial 2	Trial 3
Stretchability (cm)	15	16	16
Meltability			
Browning	++	+++	+++
Yield (g/L milk)	90	97	100

4.1.2.3 Chemical composition of Mozzarella cheese

The chemical composition of fresh Mozzarella cheese made using non-homogenized and homogenized cow milk standardized with 3 and 1.5% fat are presented in Table 2. The same table shows that, the fat/DM of Mozzarella cheese slightly increased when homogenized cow milk was used. On the contrast TP/DM decreased when pre-cheese milk was homogenized. Furthermore, Table 2 shows that, the Ca and P/DM also, decreased when homogenized cow milk was used.

Table - Chemical composition of standardized fresh cow milk

FAT(%)	DM	Fat\DM	Ca	Ca\DM	P	P\DM	Acidity	ph	
3%	12.58	23.86	0.170	1.34	0.162	1.30	0.17	6.7	
1.5%	10.65	14.08	0.172	1.62	0.163	1.52	0.18	6.6	

DM – dry matter, TP – total protein, Ca – calcium, P – phosphorus.

LITERATURE CITED

Abd El-Salam M. H., Alichanidis E. 2004.Cheese varieties ripened in brine, in: Fox P.F., McSweeney P.L.H., Cogan T.M., Guinee T.P. (Eds.), Cheese: Chemistry, physics and microbiology, 3rd edn., Elsevier Academic Press, Amsterdam, The Netherlands, pp. 227-249.

Adda, J., J. C. Gripon, and L. Vassal. 1982. The chemistry of flavor and texture generation in cheese. Food Chem. 9:115–129.

Ahlgren, J. 1966. Mechanism of mastication: a quantitative cinematographic and electromyographic study of masticatory movements in children, with specialreference to occlusion of the teeth. Acta Odontologia Scandinavia 24(supplement 44):1.

Ahmed, S.I., F. Gaucher, E. Rousseau, Beaucher, J.F. Grongnet and F. Gaucheron. 2008. Effects of acidification on physico-chemical characteristics of buffalo milk: A comparison with cow's milk. Food Chem. 106:11-17.

Akalin, A.S., S. Gonc and Y. Akbas. 2002. Variation in organic acids content during ripening of pickled white cheese. J. Dairy Sci., 85:1670-1676.

Alicia, N.C., and A.E. Bevilacqua. 1999. Freezing low moisture Mozzarella cheese: Changes in organic acid Content. Food Chem.64:193-198.

Amarita, F., M. de la Plaza, P.F. de Palencia, T. Requena, C. Pelaez. 2006. Cooperation between wild lactococcal strains for cheese aroma formation. Food Chem. 94:240-246.

Antoniou, K.D., R. Petridis, S. Raphaelides, Z. Ben Omar and R. Kesteloot. 2000. Texture assessment of French cheeses. J. Food Sci. 65:168-172.

AOAC. 1990. Official Methods of Analysis. The Association of Official Analytical Chemists. 15th Ed. Arlington, USA.

AOAC. 2000. Official Method Of Analysis Of The Association Of Analytical Chemists. 17th Rev. Ed., Association of Official Analytical Chemists, Washington, DC.

Apostolopoulos, C., V. E. Bines, and R. J. Marshall. 1994. Effect of post-cheddaring manufacturing parameters on the meltability and free oil of Mozzarella cheese. J Soc. Dairy Technol. 47(3): 84-87.

Arnott, D., H. Morris and W. Combs. 1957. Effect of certain chemical factors on the melting quality of processed cheese. J. Dairy Sci. 40: 957–963. attributes. Food Technology 17: 74–77.

Auldist, M. J., K. A. Johnston, N. J. White, W. P. Fitzsimons, and M. J. Boland. 2004. Acomparison of the composition, coagulation characteristics and cheese makingcapacity of milk from Friesian and Jersey dairy cows. J. Dairy Res. 71:51–57.

Axelsson, L.T. 1993. Lactic acid bacteria: Classification and physiology, In: Salminen, S. and A. Von Wright. (Eds.) Lactic Acid Bactuis. Marcel Deker, New York. Pp:1-69.

Azarnia, S., N. Robert and B. Lee. 2006. Biotechnological methods to accelerate Cheddar cheese ripening. Crit. Rev. Biotechnol. 26:121-143.

Banks, J.M. 1998. Cheese. In: Early, R. (Ed.) The Technology of Dairy Products. 2nd Ed. C.H.I.P.S. Mazoch Road, Weimar, Texas, USA. pp. 81-122.

Banks, J.M. 2004. The technology of low fat cheese manufacturing. Int. J. Dairy Technol. 57(4):199-207.

Barbano, D. M., Y. Hong, J. J. Yun, K. L. Larose and P. S. Kindstedt. 1995.Mozzarella cheese: impact of three commercial culture strains on composition, yield, proteolysis, and functional properties, Proceedings of 31st Annual Marschall Invitational Italian Cheese Seminar, Madison, Wisconsin, USA.