Mozzarella cheese is a variety of Pasta-filata group that had its origin in Italy. The term Pasta-filata, which is derived from an Italian phrase means ‘spun paste’ or ‘stretched curd’ that refers to a unique plasticizing and stretching process that is shared by all Pasta-filata cheese. This cheese had remained an ethnic product with a limited market until World War II, when Italian cuisine in general and pizza pie in particular, began its meteoric rise in popularity that continues to the present.

2.1 NATURAL MOZZARELLA CHEESE

Natural Mozzarella cheese is produced from buffaloes’ or cows’ milk by starter culture method or by direct acidification technique using various food grade acids. Recently, the amended FSSA standard has laid down requirements for “Mozzarella” and “Pizza” cheese as presented in Table 2.1A and Table 2.1B (FSSAI, 2011).

Table 2.1A FSSAI requirements for Mozzarella and Pizza cheese

<table>
<thead>
<tr>
<th>Parameters (per cent)</th>
<th>Type of Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mozzarella</td>
</tr>
<tr>
<td>Moisture, Max.</td>
<td>60.0</td>
</tr>
<tr>
<td>Fat on dry matter, Min.</td>
<td>35.0</td>
</tr>
</tbody>
</table>

According to USDA (1980), Mozzarella cheese is classified as (a) Mozzarella cheese, (b) Low-moisture Mozzarella cheese (LMMC), (c) Part-skim Mozzarella cheese (PSMC) and (d) Low-moisture part skim Mozzarella cheese (LMPSM) as shown in Table 2.2.

Traditional Mozzarella manufacturing uses standardized and pasteurized milk that is cultured, coagulated, cut and cooked. After whey drainage, the curd is cheddared to develop further acidity, and then milled. The cheese curd is then subjected to a kneading and plasticizing process in hot water. The hot plastic curd is then molded into desired shape and salted preferably in chilled brine. This thermo-chemical treatment inactivates most residual milk coagulant and reduces starter population, decreasing the
potential for proteolysis in the cheese during refrigerated storage (www.innovatewithdairy.com).

**Table 2.1B Microbiological requirements for Mozzarella and Pizza cheese as per FSSAI**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Permissible counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Plate Count (Processed Cheese) / g</td>
<td>50,000-75000 per g</td>
</tr>
<tr>
<td>Coliform count / g</td>
<td>100-500</td>
</tr>
<tr>
<td><em>E. coli</em> / g</td>
<td>Less than 10</td>
</tr>
<tr>
<td>Salmonella /g</td>
<td>Absent</td>
</tr>
<tr>
<td>Yeast and Mould count / g</td>
<td>10-100</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (Coagulase positive) / g</td>
<td>100-1000</td>
</tr>
<tr>
<td>Anaerobic Spore count (<em>Cl. perfringens</em>) / g</td>
<td>10-100</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em> /25g</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**Table 2.2 Classification of Mozzarella cheese as per USDA (1980)**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moisture</td>
</tr>
<tr>
<td>Mozzarella</td>
<td>52.0-60.0</td>
</tr>
<tr>
<td>Low-moisture Mozzarella</td>
<td>45.0-52.0</td>
</tr>
<tr>
<td>Part-skim Mozzarella</td>
<td>52.0-60.0</td>
</tr>
<tr>
<td>Low-moisture part skim Mozzarella</td>
<td>45.0-52.0</td>
</tr>
</tbody>
</table>

**2.2 APPLICATION OF MOZZARELLA/PIZZA CHEESE**

Mozzarella cheese, especially the LMPSM often referred to as pizza cheese, is consumed fresh or after a brief period (5-15 days) of ageing (USDA, 2007). It is normally used as a topping on pizza pie and its major function is to cling onto all the other ingredients on pizza, as it melts. Mozzarella cheese constitutes one of the most expensive ingredients of a pizza pie. Use of Mozzarella cheese as an ingredient in prepared foods such as cheeseburgers, sandwiches, pasta dishes, macaroni and traditional foods etc. is dramatically increasing world-wide.

Due to the ongoing growth in the food service industry, it is expected that cheese manufactures will be required to produce cheese that offer a variety of functional...
properties (viz. shredability, meltability, stretchability, chewiness, etc.) depending on the end use (Rowney et al., 1999).

2.3 CHEESE ANALOGUES

Cheese analogues, also referred to as cheese substitute or imitations, are the products that partly or wholly substitute or imitate cheese and in which milk fat, milk protein or both are partially or wholly replaced by non-milk based components, principally of vegetable origin (Fox et al., 2000). Cheese analogues are the products made out of dairy, partial dairy or non-dairy ingredients, which tend to resemble the particular natural cheese counterpart. Cheese analogues made by processed cheese technique, are oil in water emulsions wherein the oil phase is stabilized by the protein/aqueous phase (Shimp, 1985; Ennis and Mulvihill, 1997). They are being used increasingly due to their cost-effectiveness, simplicity of their manufacture and storage stability.

Cheese substitutes have USDA approval for use in school lunch programmes when combined with natural or processed cheese at levels up to 50.0 per cent in cooking applications (Taylor and Wilson, 1975).

2.4 PRODUCTION STATISTICS OF NATURAL AND ANALOGUE MOZZARELLA CHEESES

The production statistics of Mozzarella cheese against the total production of cheese will demonstrate the popularity of this variety of cheese, especially since it is very much preferred as a topping on pizza pie among others.

2.4.1 PRODUCTION OF MOZZARELLA CHEESE AND PIZZA

The world milk production has increased from 480 million MT in 1970 to 790 million MT (including all types of milk) in 2012 representing an increase of 64%. A large part of this expansion has been used to increase the cheese production in all regions of the world. The world cheese production reached the 20 million MT in 2011 and the growing trend is continued in 2012-2013 includes all natural cheeses except processed cheese (Annon, 2014). 80% of the natural cheese is produced from cow milk and rest is produced using sheep, goat and buffalo milk. EU and Northern America produce more than 75% of the world cheese production. Europe, with a production of 8.7 million tones per annum (~53.0 per cent of world production) is the largest producing region; North and Central America produces about 28.0 per cent of world cheese (Fox et al., 2004). Mozzarella cheese production in US was over 1.25 million tones in 2002 (IDFA, 2004).
which constitutes 33.0 per cent of the total cheese produced (USDA, 2003). Mozzarella cheese production in Italy was 1,90,000 tons in 2002 (Angelis et al., 2006). Mozzarella cheese accounts for nearly 35.0 per cent of the total cheese produced in Brazil (Andreatta et al., 2007).

Market of cheese in India is 1250 crore in 2013 and forecasted to grow at rate of 20% annually (articles.economicsimes.indiatimes.com). The total consumption of cheese in India is about 7,500 tonnes (www.foodindustryindia.com, www.icfdec.com). The per capita consumption of cheese in India is 2.5 kg per annum as against 16.0 kg per annum in US (www.indiapackagingshow.com).

Anand Milk Union Limited (AMUL), one of the pioneers in introducing Mozzarella cheese commercially in India has a large contribution to the cheese market in India. Amul's annual sale of Mozzarella cheese was about 800 tonnes in 2001 (www.thehindubusinessline.com). Amul has captured about 55.0 per cent share in the Rs. 70 crore industry cheese market. Other players in the Indian market for Mozzarella and pizza cheese are Dynamix Dairy Industries Ltd., Baramati; Parag milk foods (Go Cheese) Pune, Modern Dairies Ltd., Karnal; Vintage Foods and Industries Ltd., Bangalore; Ramsons Dairy Products, New Delhi; G.T. Foods, Ujjain, etc.

Domino's is buying Mozzarella cheese from domestic companies such as Modern Dairies Ltd., Karnal and Bangalore based Vintage Foods and Industries Limited. Pizza Hut, owned by Tricon Restaurant International, UK has tied up with Dynamix Dairy Industries Ltd., Baramati for sourcing Mozzarella cheese, which they were earlier importing from Australia. Pizza Hut was believed to be close to 50.0 per cent market share of the organized pizza-retailing segment in India. Yums plans Pizza Hut to be in 55 cities in 2006, increasing the number of pizza hut outlets in India to 130 from 95 in the year 2000 (www.hinduonnet.com).

The international prices of Mozzarella hovers around $ 2,300 per ton (US $ 3.3-3.5 per kg); adding basic custom duties to this, the cost of the imported cheese comes to Rs. 150-160 per kg. The domestic bulk prices work out at Rs.135-140 per kg. The current market price of Amul Mozzarella is Rs. 187 per kg. Indian fast food chain Nirula and Co. Pvt. Ltd., Noida, Delhi sells pizzas at price greater than Rs. 590.0 (www.hinduonnet.com).

2.4.2 CHEESE ANALOGUE MARKET

The USA was first to develop imitation cheese in the early 1970s. In 1980, production of imitation cheese based on casein and vegetable fat commenced in the UK
(Shaw, 1984) and in Japan (Akino and Yoshioka, 1982). In Switzerland and Australia, cheese substitute for cottage and processed cheese were sold, which were made entirely from soy oil (Anon., 1989; Mc Carthy, 1990). The annual production of cheese analogues stood at 3,00,000 and 10,000 tonnes respectively in the US and UK in 1990 (Mc Carthy, 1990). Compared to the USA, the European market is relatively small (i.e. 20,000 tonnes). The sales of imitation cheese in the US are stabilizing at 7.0 per cent; in Sweden at 3.0 per cent; in UK at 1.0 per cent and in Europe the corresponding figure is less than 2.0 per cent (Mc Carthy, 1990; Schelhaas, 1993).

### 2.5 NEED FOR DEVELOPING CHEESE ANALOGUES

The factors that led to the development of cheese analogues are discussed under the following headings:

#### 2.5.1 SIMPLICITY OF MANUFACTURE AND ECONOMY

Due to rise in the milk prices, the cost of natural cheese has risen sharply which makes it less accessible to a large segment of middle and lower income group consumers. The cheese analogues can be formulated to make them cheaper by use of lower priced ingredients i.e. certain vegetable oils as compared to milk fat and vegetable protein isolates as compared to milk derived proteins. Some part of protein can be substituted with cheaper starch-based derivatives. Moreover, the relatively low cost of manufacturing equipment, low requirement of man power, less cheese making time compared with that required for natural cheese and absence of maturation period helps in reducing the cost of cheese analogue (Anon., 1982; Shaw, 1984). In USA, the average retail prices for various types of imitation cheeses are 16.0-36.0 per cent lower than natural cheese prices; pizza with imitation cheese averaged 14.0 percent less in price than natural cheese pizza of the same brand (Graf, 1986; Siapantas, 1978).

#### 2.5.2 TACKLING THE PROBLEM OF MILK SHORTAGE

The cheese analogues can solve the problem of short supply of milk, especially in the developing countries through use of indigenously available vegetable protein and fat sources. Even milk based protein ingredients may be imported to cater to the demand of cheese by the consumers, when milk supply is in scarcity.

#### 2.5.3 SUPPLEMENTATION OF THE CHEESE SHORTAGE

Non-dairy cheese can be used as partial or total replacement for natural cheese in products such as pizza, enchiladas, macaroni dishes, fondu, blended dips, cheeseburgers, etc. In developing countries, where dairy products especially cheeses are
expensive and produced in insufficient quantity, cheese substitutes prepared from dairy and/or non-dairy ingredients provides a nutritious alternative and can be used to supplement the shortage of cheese in the market place (Santos et al., 1989).

2.5.4 DIVERSE FUNCTIONALITY AND STABILITY

Cheese substitute offers diversity in functionality (e.g. shredability, flowability, melt resistance) made possible by their tailor-made formulations, coupled with their high functional stability during storage. Many cheese makers produce Mozzarella imitations characterized by their melting and free-running properties, primarily for use in pizzas (Anon., 1993). When used for cooking purpose (e.g. pizza pie, macaroni, sauces for vegetables), the substitute products are stated to be indistinguishable from natural cheese in odour, flavour and physical characteristics (Vernon, 1973; Jana 1998).

2.5.5 NUTRITIONAL AND HEALTH ASPECTS

In recent years, there is an ever-increasing interest among consumers for food products that contain less total fat, saturated fat, cholesterol and calories. Cheese analogues are able to meet special dietary needs and can acts as vehicle for health benefits/supplement, e.g. lactose-free, cholesterol-free and enriched with vitamin, mineral, fiber, etc. (Anon., 1986; Morris, 1986). Cheese substitute can be manufactured to have nutritional equivalence or to have nutritional advantages over the natural counterpart cheese (Shaw, 1984; Holdt, 1992).

2.6 CLASSIFICATION OF CHEESE ANALOGUES

Imitation products are products which attempt to reproduce the functional and organoleptic properties of a milk product, while substitute products are those which have the same uses (Guay and Morisset, 1996).

Imitation/substitute cheese products are classified into three main categories: (a) Tofu-based cheeses, (b) Filled cheeses, and (c) Analogue cheeses, based on the ingredients used and the manufacturing procedures adopted (Fox et al., 2000).

2.6.1 TOFU-BASED CHEESE/SUBSTITUTES

Fermented soybean cheese has been produced using lactic acid fermentation of soybean milk (Jeong and Choi, 1980). Tofu-based cheese substitute is made out of Tofu, casein and soya oil. Tofu also referred to as ‘paneer analogue’ is a soft coagulated product obtained from soy milk by precipitation with calcium sulphate or other suitable coagulants. ‘Sufu’ or ‘furu’ is a cheese-like fermented soybean product with a spreadable creamy consistency and pronounced flavour (Zhong et al., 2001).
2.6.2 FILLED CHEESE

Filled cheese is cheese in which milk fat is partly or fully replaced by vegetable oils, which in turn could be partially hydrogenated to impart eating profile similar to that of milk fat. Filled cheese is made by conventional in-vat cheese making methods (Fox et al., 2000). Filled cheeses can be made to have required quantum of polyunsaturated fat through use of healthful vegetable oils. A directly acidified, low-cholesterol filled-Mozzarella cheese has been made from skim milk emulsified with sunflower oil (Ghosh and Kulkarni, 1996). Reconstitution of the dried milk to solids content higher than normal is reported to yield better quality of filled cheese (Mohamed, 1980).

2.6.3 ANALOGUE CHEESE

Analogue cheese products are made by blending various raw materials of dairy or non-dairy origin together, using techniques similar to those for processed cheese manufacture with the aid of heat, mechanical shear and emulsifying salts. Analogue cheese products may be further categorized as (Shaw, 1984):

(a) **Dairy:** It is made using both protein (i.e. casein, caseinates, milk protein concentrates, total milk proteinate) and fat sources (i.e. cream, white butter, anhydrous milk fat) of dairy origin along with other functional ingredients and cheese flavouring.

(b) **Partial dairy:** In this, the fat source is vegetable oil (e.g. soya oil, palm oil, rapeseed oil and their hydrogenated equivalents) and protein source is dairy-based (usually rennet casein and/or caseinate) along with other requisite ingredients.

(c) **Non-dairy/Synthetic:** It is made using both fat and protein of vegetable origin. A formulation for non-dairy analogue may comprise of soy protein, soya oil and artificial colour and flavour.

It is worth mentioning that majority of the work reported in literature on cheese analogues is of partial-dairy type.

2.7 LEGISLATION AND LABELING REQUIREMENTS

The designation and labeling for cheese analogues should clearly distinguish them from cheese or pasteurized processed cheese products (Guinee et al., 2004). In India presently there is no separate defined product category for analogue/imitation cheese. According to FDA regulation, simulated cheese product should bear a label with prominent word ‘imitation’ and immediately thereafter the name of the food imitated (Mc Carthy, 1990). In US, an imitation cheese is a product which is a substitute for, and resembles another
cheese, but can be inferior nutritionally in terms of essential nutrients, but not in fat or calorie content (Shaw, 1984).

French law dictates that Mozzarella substitutes made of fats or proteins of non-milk origin has to be labeled as 'food preparations for pizzas' (Anon., 1993). In Italy, the Imitation Mozzarella cheese (IMC) products can be marketed as 'processed cheese' with a minimum of 35.0 per cent fat-on-dry-matter (FDM) or as a food product to be used as an ingredient for other foods (Toppino et al., 1988 a,b).

2.8 INGREDIENTS USED IN THE FORMULATION OF CHEESE ANALOGUES

Cheese analogue is made out of formulation containing a protein and fat source along with functional additives (viz., acid, emulsifying salts, common salt, emulsifier and stabilizer) along with flavouring, mold inhibitor and colourings. The ingredients utilized in preparing cheese analogues, with special emphasis on Mozzarella cheese analogue are discussed here under appropriate headings:

2.8.1 Protein source

The role of protein component in cheese analogue includes water binding and emulsification of the oil phase. The protein component in a cheese analogue stabilizes the oil-in-water emulsion by reducing the interfacial tension at the aqueous phase-oil droplet interface and by increasing the viscosity of the aqueous phase, thereby decreasing the frequency of collisions between oil droplets (Shimp, 1985; Ennis and Mulvihill, 1997). Various protein sources of dairy origin (viz. casein, caseinate, milk protein concentrate) and of vegetable origin (viz. soy, groundnut) used for the manufacture of MCA are reviewed below.

2.8.1.1 Casein

The major protein source in partial or dairy based analogue cheeses is acid casein or rennet casein (Nishiya et al., 1989; Ennis and Mulvihill, 1999). Powdered rennet casein may be considered as a young dried skim milk cheese consisting of para-κ-casein cross-linked by calcium. Rennet casein is the favoured protein source for semi-hard block products and for MCA (Abou El-Nour et al., 1996; Ennis and Mulvihill, 2001; O’ Sullivan and Mulvihill, 2001), whereas caseinate is preferentially used for spreadable products. Rennet casein contributes to bland flavour, pale colour and superior functional
properties (viz., shredding, melting, stretching) to MCA as compared to one made with proteins from other sources (Aimutis, 1995).

Rennet casein from mid-lactation milk was found to be more beneficial than that made from early or late-lactation milks for manufacture of MCA with regard to the plasmin content of cheese (O’ Malley et al., 2000; Ennis and Mulvihill, 2001). Compositional parameters such as moisture, ash and calcium were found to weakly relate to the performance of rennet casein in MCA manufacture (O’ Sullivan and Mulvihill, 2001). High cooking temperature (up to 75°C) is not advisable in preparing rennet casein for use in MCA. Washing of the rennet curd at temperatures varying from 60 - 86°C did not alter the characteristics of casein. Use of high air-inlet temperature for drying of rennet casein had an adverse influence on hydration time, viscosity, solubility and browning (O’ Sullivan et al., 2002 a,b).

Rennet casein which has a high Ca/casein ratio, the degree of calcium sequestration and para-casein aggregation is easily controlled by using the correct blend of emulsifying salts to give the desired degree of casein hydration/aggregation and fat emulsification in the analogue cheese (Guinee, 2002). MCA has been made using rennet casein @ 23 % in the formulation for partial dairy based cheese analogue preparation (Sharma 2012)

Acid casein based cheese analogue exhibited better emulsification properties and meltability employing disodium phosphate as emulsifying salt than that based on rennet casein (Savelló et al., 1989). Nishiya et al. (1989a,b) reported that IMC made from lactic acid casein had good meltability, even without the use of emulsifying salts; the stringiness of cheese was poor.

MCA has been prepared using buffalo acid casein (@ 21.0 per cent) and vegetable oil blend (@ 12.5 per cent) comprising of corn oil and hydrogenated vegetable oil (45:55). The product had superior melting, stringiness and chewiness as compared to fresh natural Mozzarella cheese (Jana et al., 2005). MCA has been made from acid casein concentrate (44.0 per cent TS) as protein source and mildly soured butter, sunflower oil and partially hydrogenated coconut oil as the fat source. Acid casein based MCA had better taste, softer texture, lower adhesiveness and higher melting than rennet casein based product (Hoffmann et al., 2005).

Cheese analogue has also been made using a combination of acid and rennet casein (1:1); the total casein content in the product was 15.0-30.0 per cent (Galal et al., 1983).
2.8.1.2 Caseinates

The characteristics sought after in caseinates for preparation of cheese analogues include quick hydration and dispersion, adequate swelling properties, optimal protein-protein interaction and retaining the pseudo-plastic behaviour over a range of shear rates (Hokes et al., 1989). The caseinate having higher content of ash, fat, lactose, non-casein protein, calcium, phosphorus and lower water absorption and stability to added calcium were found to be functionally better than their counterparts for preparation of cheese analogues (Fleming et al. 1985).

Ca-caseinates have been widely used in the manufacture of cheese analogues. Na-caseinate yielded a translucent product and tended to give oily appearance to the MCA compared to those made with Ca-caseinate (Sherkat and Walker, 2002). Cheese analogues prepared with Na-caseinate had higher pH, degree of fat emulsification and degree of casein dissociation, but was softer than that prepared using Ca-caseinate (Cavalier-Salou and Cheftel, 1991).

Blends of Na- and Ca-caseinates have been successfully used to produce IMC (Rule and Werstak, 1978; Rule et al., 1980).

2.8.1.3 Milk protein concentrate and total milk proteinate

Dried ultra filtration retentate of skim milk referred to as 'Total Milk Proteinate' (TMP) has gained increased importance as an alternative high milk protein product for various types of cheese analogues. Replacement of up to 40.0 per cent of rennet casein with TMP (85.0 per cent protein) resulted in satisfactory quality processed cheese analogue; an increase in firmness and decrease in flowability was noticed with increasing level (10.0-50.0 percent) of TMP used (Abou-El-Nour et al., 1996). Part replacement of rennet casein with Milk Protein Concentrate (MPC) protein powder (85.0 per cent protein) up to 40.0 per cent increased the firmness of spread-type processed cheese analogues (Abou-El-Nour et al., 1998).

2.8.2 Milk protein replacer

Owing to the relatively high cost of casein, much effort has been vested in its partial replacement by cheaper substitutes. Replacement of casein with various vegetable proteins, e.g. soybean (Lee, 1981; Taranto and Yang, 1981; Anon., 1982; Yang and Taranto, 1982; Yang et al., 1983), peanut protein (Chen et al., 1979; Guirguis et al., 1985; Santos et al., 1989), pea protein (El-Sayed, 1997; Verma et al., 2005) has been attempted in cheese analogue preparation. Substitution level greater than 20.0 per cent w/w of the total protein as vegetable protein is found to give inferior quality MCA
compared to that made using exclusively casein. The problems encountered when utilizing vegetable proteins at higher levels include lack of elasticity, lower hardness, adhesive/sticky body, impaired flow and stretch and poor flavour (Ahmed et al., 1995; Guinee et al., 2004).

All caseinate analogues have been reported to yield unacceptable strong, rubbery products. Therefore, some manufacturers opt using a blend of caseinate with other protein sources. A mixture of soy protein isolate (SPI), Na-caseinate and corn starch has been used to prepare imitation cheese (Chen et al., 1979; Lee and Son, 1985). Polymerized whey protein concentrate (WPC) with 5.5 per cent protein has been used to substitute casein up to 13.0 per cent, without adversely affecting the yield stress and meltability of the cheese analogue (Dees and Foegeding, 2002).

2.8.2.1 Soybean protein

Lee and Marshall (1981) replaced Na-caseinate with native or boiled soy protein concentrate (SPC) in preparation of cheese analogue. Cheese curd containing boiled SPC was more porous than control curd or that containing native soy protein. Alcalase or trypsin treated SPI were more suitable for cheese analogues than the untreated ones (Kim et al., 1992). Yang and Taranto (1982) prepared Mozzarella cheese analogue using SPI, gelatin and gum arabic, which exhibited both textural and stretching properties similar to natural Mozzarella cheese.

2.8.2.2 Peanut protein

Among various vegetable sources, peanut shows potential as a source of both protein and oil due to its bland flavor and light color. However, most of the peanut cheese-like products have been prepared by ripening methods using microbial inoculation. A quick and simple non-fermentation method was reported for production of a cheese-like matrix from peanut protein isolate (PPI) (Krishnaswamy and Patel, 1968; Krishnaswamy et al., 1971). Curd made from peanut milk has been used in the preparation of processed cheese-like spread (Santos et al., 1989). PPI has been used to replace caseinate at levels of 40.0-50.0 per cent in preparation of MCA. Melted peanut cheese analogue had stretch characteristics similar to that of natural Mozzarella cheese (Chen et al., 1979b).

2.8.3 Starch products as filler or replacer of protein and fat

Starch has been incorporated in imitation cheese mainly to replace the more expensive casein (Zwiercan et al., 1987; Mounsey and O’ Riordan, 2001). Native maize starch is preferentially used while starches from other sources or the modified ones
pre-gelatinized, chemically or enzymatically modified) being used less frequently. Native starch can be used at levels of 3.0 per cent to replace about 15.0 per cent of total casein in cheese analogue (Mounsey and O’ Riordan, 2001).

Rice starch showed potential as a low-cost partial casein replacer in imitation cheese due to its low amylose content and limited swelling capacity; it yielded good rheological and melting properties to resultant product (Mounsey and O’ Riordan, 2001). Pre-gelatinized starches from rice and/or waxy-maize resulted in enhanced viscosity of the heated dispersion of starch and casein, compared to that of casein alone. The imitation cheese containing such starches were softer, less cohesive and had reduced melting compared to the one made using casein alone (Mounsey and O’ Riordan, 2008b).

Wheat starch has been successfully used at 3.0-9.0 per cent level to replace part of casein in manufacture of analogue cheese. It led to longer processing time and reduced its meltability (Mounsey and O’ Riordan, 1999). The meltability of imitation cheese was adversely affected when pre-gelatinized starch was used at the rate of \( \geq 15.0 \) per cent of rennet casein (i.e. 3 per cent of product).

### 2.8.4 Fat source

The role of fat in cheese is to contribute to its physical characteristics by giving opacity and influences rheology of cheese. Fat in Mozzarella cheese contributes to stretch and flow by providing a lubricating effect when melted (Zisu and Shah, 2005). Fat acts as precursor of many flavour compounds and it modifies their perception and volatility, especially in the mouth. Fat globules normally act as filler between protein fibers, reducing the interactions among proteins within the cheese matrix (Eric, 1993). The level of fat used in cheese has a direct impact on product acceptability (Olson and Johnson, 1990). The fat ingredients that have been used in MCA formulation include the following:

#### 2.8.4.1 Milk fat

Processed cheese analogue based on rennet casein has been prepared using Anhydrous milk fat (AMF) (27.0 per cent in the formulation) and/or white butter as the fat source. A casein/butter ratio of 77:23 was recommended (Bowland and Foegeding, 2001; Varghese and Sachdeva, 2002). Processed cheese analogues based on high protein skimmed milk powder and ultrafiltrated retentate can be made to possess more creamy and buttery flavour by using AMF as fat source (Muir et al., 1999; Anon., 2007).
2.8.4.2 Vegetable oils

Partial dairy cheese analogue has been produced using cheaper vegetable oil/fat (Bachmann, 2001; Sharma 2012). Due to increased awareness of the people about the dangers of cholesterol in milk fat, healthful vegetable oils have been prominently used as fat source in partial dairy cheese analogues. These vegetable oils may preferably be partially hydrogenated so as to have desirable melting point (Wiley melting point of 30-43°C). Vegetable margarine has also been employed in the formulation of IMC (Bell et al., 1980).

Use of vegetable fat can give the cheese analogue a consistency that makes it more suitable for certain applications (e.g. in deep freeze products) and for longer shelf life products (Anon, 1989).

Different formulations for MCA have been developed using vegetable oils such as soy, palm, palm kernel, cotton seed, coconut, corn, etc (Chen et al., 1979; Lobato-Calleros et al., 1997; Ahmed et al., 2001; Bennett et al., 2006; Sharma 2012). Vegetable oil blends viz. corn oil:Hydrogenated vegetable oil (HVO) or soy oil:HVO, 45:55 has been successfully used as fat source in the formulation of MCA (Jana et al., 2005). Corn oil: HVO was superior to soy oil:HVO as fat source in the preparation of acid casein based MCA.

2.8.5 Fat replacers

Granular resistant starch and retrograded resistant starch have been used as fat replacer and as a source of fiber in imitation cheese. Over 50.0 per cent of the fat content of imitation cheese could be replaced with resistant starches, without any adverse effect on meltability. Incorporation of starch led to an increase in hardness of cheese analogue; the effect was marked in case of retrograded starch. The cohesiveness of imitation cheese was increased by use of 5.0 per cent granular resistant starch (Montesinos-Herrero et al., 2006). Cheese analogue has been prepared using WPC or low-methoxyl pectin, both at level of 0.0-4.0 per cent as fat replacer (Lobato-calleros et al., 1998).

Inulin is a fructose polymer synthesized from sucrose or extracted from chicory roots that finds application as a food ingredient (Niness, 1999). Inulin, in the form of a heated (80oC), 25.0 per cent solution when used at the rate of 3.50 per cent in rennet casein based MCA formulation, could replace up to 63.0 per cent of the fat, without adversely affecting its melting behaviour (Hennelly et al., 2006).
2.8.6 Stabilizers

Use of carrageenan, which interacts strongly with the casein helps in enhancing the body, texture and emulsification of the analogue product, especially having low content of cheese proteins (Morirano, 1977). Gum arabic when used at the rate of 19.0 per cent induced a pseudoplastic flow behaviour in the melted MCA, culminating in stretchability. Replacement of gum arabic with xanthan gum (XG)-locust bean gum (LBG) mixture overcame the surface stickiness problem encountered with use of gum arabic alone (Yang et al., 1983). XG considerably improved the stability of starch to low pH and freeze-thaw, which are important for the storage stability of frozen MCA (Yang et al., 1983).

Jana (1998) evaluated the effects of XG, Carageenan (CAR) and LBG alone and in combination (viz., XG-LBG, CAR-LBG, XG-CAR in 1:1 proportion) at the rate of 0.42 per cent in MCA based on acid casein and vegetable oils. They recommended use of either XG alone or XG-LBG blend (1:1) as stabilizer for acid casein based MCA taking into consideration the fat emulsification, flavour and stretch characteristics.

Several workers have also taken help of stabilizers viz., starch @ 2.0-5.0 per cent (Fox et al., 2000; Montesinos-Herrero et al., 2006), starch plus guar gum @ 0.86 per cent (O’Malley et al., 2000) in the manufacture of MCA.

2.8.7 Emulsifier

In cheese analogue emulsification plays a key role in deciding the functionality of the product. Several emulsifiers such as lecithin, tween-80, decaglycerol decaoleate, mixture of mono- and di-glycerides, lactylated monoglycerides, polyoxyethylene mono- and di-glycerides of fatty acids, etc. have been used in the preparation of imitation cheese for emulsification and dispersion of fat/oil in the protein matrix (Motoki et al., 1982; Hansen et al., 1985; Jana et al., 2005). Emulsifiers having high hydrophilic-lipophilic balance and that are fluid at ambient temperature are the preferred ones (Rule et al., 1980). The inclusion of such an additive during processing resulted in improved mixing and consequently less oiling-off during baking. Octaglycerol monooleate has been used at the rate of 1.0 per cent in the formulation of IMC (Rule et al., 1978). Lecithin performed better as an emulsifier in preparation of MCA based on acid casein and vegetable oil compared to Tween-80, when used at the rate of 0.15 per cent (Jana et al., 2001).
2.8.8 **Emulsifying salts**

The role of emulsifying salt is to improve the emulsifying capacity of the protein present in casein and in cheese. Emulsifying salts generally comprise of a monovalent cation (i.e. sodium) and a polyvalent anion (e.g. phosphate). The most commonly used emulsifying salts include sodium citrates, sodium orthophosphates, sodium polyphosphates, di-sodium phosphates and blends of phosphates and citrates or of several phosphates only (Guinee et al., 2004).

They promote a series of physico-chemical changes within the cheese matrix with the aid of heat and shear, which aids in rehydration of the insoluble aggregated para-casein and converts them to an active emulsifying agent. The changes exerted by an emulsifying salt include calcium sequestration, para-casein hydration and dispersal, pH adjustment and stabilization, emulsification and structure formation (Lee et al., 1986; Eymery and Pangborn, 1988; Cavalier-Salou and Cheftel, 1991).

The incorporation of an emulsifying salt is necessary to impart melting properties to cheese product (Shimp, 1985). MCA has been prepared utilizing tri-sodium citrate (Yang et al., 1983; Lobato-Caleros et al., 1997; Bennett et al., 2006), disodium phosphate (O’Malley et al., 2000) and their admixtures (Jana, 1998; Montesinos-Herrero et al., 2006; Mounsey and O’Riordan, 2008a), at levels ranging from 0.6 to 2.3 per cent. The salient characteristics of some important emulsifying salts used in cheese analogue are reviewed below.

### 2.8.8.1 Citrates

Out of various citrates, trisodium citrate (TSC) is used most commonly due to its high solubility, higher buffering action and fairly good calcium sequestering power. However, it exhibits low calcium sequestration, fat emulsification and para-casein hydration properties as compared to polyphosphates and pyrophosphates. Mono sodium citrate, when used alone, gives over-acid processed cheese which are mealy, acidic and crumbly with poor emulsification of fat (Gupta et al., 1984).

### 2.8.8.2 Phosphates

The various types of phosphates used in preparation of processed cheeses and cheese analogues include sodium monophosphate, linear condensed phosphates and polyphosphates. Amongst the orthophosphates, disodium hydrogen orthophosphate is preferentially used. When used alone, mono- and tri-sodium phosphate (TSP) salts tend to produce over acidic and under-acidic product respectively. TSP among other phosphate salts is added in cheese analogues to exchange calcium with sodium or bind to
the colloidal calcium phosphate (Fox et al., 2000). Calcium sequestering property of polyphosphate is higher compared to that of pyrophosphate, orthophosphate and citrates; such property helps in protein hydration and dispersion properties in MCA preparation. Orthophosphates such as di-sodium phosphate (DSP) is the most commonly used in cheese analogues. Use of DSP stabilizes the milk casein against protein coagulation and prevents development of cooked flavour in cheese products (Ennis and Mulvihill, 1999). The para-casein hydration and dispersion increases with an increase in chain length of phosphates (Cavalier-salau and Cheftel, 1991).

2.8.9 Acidulants

A food grade acid may be used in the cheese analogue formulation to adjust the pH in the range 5.8 to 6.5 for attaining desirable flavour and functionality for use as pizza topping. Suitable food grade acids include lactic acid, adipic, citric, acetic, phosphoric and blends of these; malic and hydrochloric acids have also been used (Chen et al., 1991). Lactic and citric acids have been used more frequently than others in cheese analogues. Acidic components may be incorporated into or may be inherent in the ingredients used in the manufacture of cheese analogues (Kratochvil, 1986).

Lactic acid has been used at the rate of 0.13-0.78 percent in the formulation by various researchers (Lobato-Caleros et al., 1997; Jana, 1998; O’ Malley et al. 2000). Citric acid has been the acid of choice in the formulation at the rate of 0.6-0.80 percent in MCA based on TSC chelating system (Bennett et al., 2006; Motesinos-Herrero et al., 2006; Mounsey and O’ Riordan, 2008a).

2.8.9.1 Lactic acid

Lactic acid is listed as GRAS additive; its mild acidic (acrid) taste reportedly does not mask weaker aromatic flavours. When added to a variety of food systems, it imparts acidity, flavour and exerts microbial inhibition. Food-grade lactic acid is available as 50.0 and 88.0 per cent aqueous solutions and as powder (Dziezak, 1990).

2.8.9.2 Citric acid

Citric acid is a weak organic acid listed as GRAS additive for use in food. It is available as a white crystalline powder. It imparts acidic taste to food (used as flavouring) and also acts as a preservative. The buffering properties of citrates are used to control pH in various cheese products (www.tateandlyle.com). As it has goodmetal chelating ability, it is commonly used in combination with citrate emulsifying salt (Mounsey and O’ Riordan, 2008a).
2.8.10 SALT

Salt (NaCl) is added to enhance the flavour characteristics and it may influence functional characteristics of cheese as the sodium may displace calcium from casein and caseinates by influencing their aqueous phase solubility and thus affecting the emulsification characteristics of the system (Jana and Upadhyay, 2003). The salt had been used in the formulation of MCA ranging from 0.3 to 1.9 per cent levels (Jana, 1998; Budiman et al., 2002; Bennett et al., 2006; Hoyer and Kirkeby, 2007).

Ahmad et al. (2001) observed reduction in the meltability of MCA based on casein and palm/palm kernal oil, containing 2.0 per cent salt as compared to its counterpart made without any salt.

2.8.11 PRESERVATIVES

The preservatives that have been employed in few cheese analogue formulation includes sorbic acid and potassium sorbate at the rate of 0.09-0.15 per cent as a mycostatic agent (Motoki et al. 1982; Fox et al., 2000; O’ Malley et al., 2000; Mounsey and O’ Riordan, 2008a).

2.8.12 FLAVOURINGS

Flavouring compounds available in the form of liquid, dry (powder) or oil based have been used in cheese analogues, some being artificial whereas others may be of natural origin such as Enzyme modified cheese (EMC) flavour. EMC is defined as concentrated cheese flavour, produced enzymatically from cheeses of various ages and are principally used as an ingredient in processed foods, analogue cheese and cheese spreads. Cheese flavouring have been used at levels of 1.0 to 5.0 per cent. Lower pH (i.e. pH of 5.5) enhances the flavour intensity of imitation cheese flavoured with EMC; the extent of lipolysis in EMC also affected its flavour profile (Noronha et al., 2008). Starter distillate at the rate of 0.04 per cent and yogurt has been successfully used as flavouring for MCA (O’ Malley et al., 2000; Sherkat and Walker, 2002).

Oil-based cheese flavouring @ 0.27 per cent, with or without use of 10.0 percent of natural Mozzarella cheese, has been used in the MCA formulation to impart flavour to the resultant product (Jana, 1998).

2.8.13 MISCELLANEOUS ADDITIVES

Various colorants have been used in the MCA formulation to improve the appearance and acceptability of the cheese analogue. Vitamin mineral premix and color has been used at the rate of 0.10 and 0.03 per cent respectively in the formulation of IMC (Rule et al., 1978). Calcium chloride has been used at the rate of 0.97 and 0.36
per cent level in MCA based on Na-caseinate and acid casein respectively (Rule et al., 1978; Jana and Upadhyay, 2001).

2.9 FORMULATION OF MOZZARELLA CHEESE ANALOGUE

In order to obtain the desired composition and functionality, the chosen ingredients have to be utilized in proper proportion and are required to be added in a proper sequence. The formulation for cheese analogue is one of the determinants of its quality, over and above the processing method which is to be adopted. Cheese analogue formulation is mostly affected by the type of protein and fat sources selected. For instance, use of acid casein as protein source will require addition of lower amount of acid to attain the desired pH compared to use of rennet casein. Additionally, calcium may be required to be added in the form of calcium chloride or tri-calcium phosphate in MCA formulation to tone up the required calcium content (Jana, 1998; Hoffmann et al., 2005). When vegetable oil or white butter or AMF is used as fat source, an emulsifier may be needed in the formulation to aid emulsification. The various formulations adopted by several workers for obtaining MCA are presented in Table 2.3.

2.10 CHEESE MAKING EQUIPMENTS AND METHODOLOGY

The cheese analogues are made by blending various raw materials together (i.e. direct fabrication) using techniques similar to those for processed cheese manufacture. Processed cheese equipments such as batch cooker, mixer, continuous scraped surface cookers, Stephan kettle, twin-screw extruder have been used for manufacture of MCA.

2.10.1 BATCH PRODUCTION OF MOZZARELLA CHEESE ANALOGUE

High speed (1500 rpm) Stephan cutter (Cavalier Salou and Cheftel, 1991), Stephan kettle (www.gchahn.com, Ahmed et al., 2001) and horizontal twin-screw cooker (e.g. Damrow, Blentech) operating at screw speed of 40 rpm has been employed in the manufacture of cheese analogue (Montesinos-Herrero et al., 2006). Horizontal twin-screw cooker design ensures adequate blending and a relatively low degree of mechanical shear compared to the homogenizing effect exerted by the processed cheese cookers (Guinee et al. 2004).

A typical manufacturing procedure involves blending required quantities of water and dry ingredients (e.g. casein, emulsifying salt, starch, salt, etc.) followed by addition of oil and cooking to 80-85°C, preferably in a Stephan kettle having provision for direct steam...
injection, with continuous stirring. A uniform homogeneous molten mass is obtained in about 5-8 min. Flavouring material and pH regulator are then added and the mixture is blended for further 1-2 min followed by hot packaging (Guinee et al., 2004).

2.10.2 EXTRUSION PROCESSING FOR MOZZARELLA CHEESE ANALOGUE

In the production of cheese analogues extruder are being widely used. Cheese analogues have been prepared by extrusion-cooking of Ca-caseinate and butter oil, with or without emulsifying salts (Cavalier Salou et al., 1991). Compared to batch-prepared cheeses of the same composition, the extruded cheese strips displayed a similar texture, but a lower degree of fat emulsification and higher degree of casein dissociation due to the lower intensity of thermal and mechanical processing during extrusion. Cheftel et al. (1992) produced emulsified and gelled cheese analogues, with texture ranging from hard blocks to soft spreads, with a single extruder pass from caseinate and various fats.

2.10.3 CONTINUOUS MOZZARELLA CHEESE ANALOGUE PRODUCTION

In line with continuous production of natural cheese, various processes have been developed for continuous production of few varieties of cheese analogues (Hoyer and Kirkeby, 2007).

In continuous production of MCA, the first step includes dosing of dry ingredients (casein/caseinate, starch, salt, stabilizer, flavourings, emulsifying salts) through a tri blender, into tanks equipped with dissolver (high shear mixtures) which gives a homogeneous blend. This blend is pumped to a ‘consistator’ plant (specialized type of scraped surface heat exchanger), wherein the product is heated from about 30°C to 85°C and held at that temperature for certain period. The shear applied in scraped surface heat exchanger affects the texture and stringiness of the resultant analogue.

After heating, the product goes to a buffer tank for hot filling. The MCA produced is packaged in sausage shaped bag or bag-in-box. During filling, air is removed from the cheese product. Thereafter, rapid cooling of cheese mass is accomplished to 10°C prior to its refrigerated storage. The capacity of such machines ranges from 800-1300 kg/hr/cylinder, which utilizes steam at 120°C as heating medium. Use of more number of cylinders increases its production capacity (Hoyer and Kirkeby, 2007).

2.11 TEXTURAL PROPERTIES OF MCA

Texture plays an important role in the quality of the cheese. Various textural properties can be estimated directly by subjective sensory means, but instrumental measurements are preferable as they are easier to standardize and to reproduce (Bachmann, 2001).
The texture of the cheese analogues is related to the manner in which the fat and protein are distributed in cheese matrix. Mulvihill and Mc Carthy (1993) observed that moisture-in-fat-free substances (MFFS) and FDM content were correlated with the textural properties of MCA based on rennet casein. The analogue cheese containing groundnut oil and hydrogenated vegetable oil were softer and tougher, respectively than those containing butter (Chen et al., 1979). The moisture in the cheese protein network acts as plasticizer making it more elastic and less easily fractured (Marshall, 1990). The use of dephosphorylated Na-caseinate resulted in a simulated cheese with greater firmness and restricted meltability (Strandholm, 1991). Use of enzyme modified SPI significantly lowered the hardness and fracturability of MCA and brought about adhesiveness (Lee et al., 1992). The hardness, stress at compression, elasticity, gumminess and chewiness were negatively correlated, whereas cohesiveness was positively correlated with pH 4.6 soluble nitrogen (Mulvihill and Mc Carthy, 1994).

A high fat content yielded softer, less springy, more cohesive and adhesive cheese analogue than that containing lower fat (Stampanoni and Noble, 1991b). Emery and Pangborn (1988) witnessed an increase in hardness, springiness and crumbliness with increasing fat content of cheese analogue, whereas Chen et al. (1979) observed increased firmness but decreased cohesiveness, springiness and adhesiveness in cheese analogues containing higher fat content. Increasing the amount of citric acid or sodium chloride caused a significant decrease in cohesiveness and springiness and an increase in firmness of cheese analogues (Stampanoni and Noble, 1991a). In a caseinate based MCA, the springiness was inversely proportional to the amount of citric acid, while crumbliness first decreased and then increased as a function of the amount of citric acid. Salt influences texture of cheese analogue through ionic strength and Na+/Ca++ competition (Emery and Pangborn, 1988).

An increase in the hardness of MCA was noticed with increase in the percentage of resistant starch, used as partial replacement for casein. The cohesiveness of the cheese analogue was not affected by use of retrograded resistant starch, but increased with increasing concentration of resistant starch (Montesinos-Herrero et al., 2006). Part replacement of casein with pre-gelatinized starch reduced the hardness and cohesiveness of cheese analogue (Mounsey and O’ Riordan 2008b). Partial replacement of casein with TMP increased the hardness and gumminess of cheese analogues (Abou-El-Nour et al., 1996).
2.12 FUNCTIONAL PROPERTIES OF CHEESE ANALOGUES

By changing the proportions of raw materials and adjusting the processing parameters, it is possible to engineer a wide range of functional properties in the resultant analogue (Shaw, 1984). The composition of cheese analogues largely determines its texture, which influences their functionality (Lobato-Calleros et al., 1997). The functional attributes of importance for pizza cheese include ability to shred cleanly, melt sufficiently rapidly, exhibit the desired degree of flow, stretchability, chewiness, oiling-off and/or browning on baking. These parameters are discussed at length here under:

2.12.1 Shredding

Shredability and resistance to clumping at shredding are the major determinants of overall functional characteristics of cheese. The unmelted cheese should be firm enough to allow shredding, without clogging of cheese. Sufficient hardness is required to enable successful shredding of the cheese (Sherkat and Walker, 2002). The grated and shredded particles should be firm enough to prevent matting under pressure. The MCA is reported to exhibit superior shredding characteristics than natural Mozzarella cheese. The shredability of natural Mozzarella cheese deteriorated from 20th day of refrigerated storage, whereas that of MCA remained consistently good throughout one month of refrigerated storage (Jana, 1998). The analogue cheeses tended to have a comparatively higher proportion of fines (particles < 2 mm) upon shredding (Guinee et al., 2000).

2.12.2 Meltability

Meltability refers to the capacity of cheese particles to coalesce to a uniform continuous layer of melted cheese upon heating. Generally, a fused cheese mass is required, with little evidence of the individual shreds.

Among protein, fat and emulsifying salts, protein is the most important one, which affects the melting properties. The melting property of cheese analogue is dependent on the type of casein used (viz., acid or rennet) and on the type and concentration of emulsifying salt (Cavalier-Salau and Cheftel, 1991; Savello et al., 1989). According to Chen et al. (1979), the specific melting of MCA reflects directly the melting behavior of
the constituent caseinate. Cheese analogues made using Na-caseinate and Ca-caseinate loses its melting, when the Ca:Na molar ratio exceeds 0.95 and 0.83 respectively (Aimutis, 1995). Higher pH tended to improve the meltability of caseinate based analogue (Cavalier-Salau and Cheftel, 1991). Replacement of rennet casein with TMP at 10.0-50.0 per cent level reduced its meltability by 5.50-24.6 per cent (Abou-El-Nour et al., 1996). The meltability of MCA made using pre-gelatinized starch decreased with increasing levels of starch used (Mounsey and O’ Riordan, 1999). Increasing the level of sodium chloride decreases the meltability of MCA (Ahmed et al., 2001).

MCA based on acid casein and vegetable fat blend, had superior meltability (Schreiber value of 1.82 vs. 1.05 for natural cheese) over natural Mozzarella cheese (NMC), when fresh. However, upon one month of refrigerated storage, the NMC showed superior meltability (Schreiber value of 4.90 vs. 2.84 for MCA) over MCA (Jana, 1998).

2.12.3 Stretchability

Stretchability often referred to as stringiness, is the ability of the melted cheese to form fibrous strands that elongate, without breaking under tension. According to USDA (2007), Mozzarella cheese should stretch at least 3 inches from the surface of the pizza upon baking.

MCA made from lactic acid casein had poorer stringiness. Unlike this, the one based on Ca-caseinate curd or rennet casein had good stringiness; the later one was superior in this regard (Nishiya et al. 1989).

According to Koide (1983), use of low fat (fat/casein < 1) having melting point of about 32°C, casein/water ratio of 0.5 and calcium content in the range of 15-25 mg/g protein helped in obtaining good stretchability in MCA based on Na-caseinate and hydrolysed starch. Increasing the amount of SPI in the analogue formulation led to an improvement in stretchability. The stretch of MCA was governed by the relative amounts of gelatin, gum and soy protein in the formulation (Yang and Taranto, 1982).

MCA based on acid casein and vegetable fat blend exhibited greater stretch when fresh (i.e. 23.0 vs.17.0 cm for natural Mozzarella), but upon storage of 1 month, the stretch of MCA remained same whereas that of natural cheese increased to 41.0 cm (Jana, 1998).

Stretch values of 15.0-28.0 cm and 78.0-112.0 cm has been reported for commercial samples of MCA and NMC respectively (Guinee et al., 1999, 2000). Rule et al. (1978) reported stretch value of 90.0 cm in MCA on 14th day of refrigerated storage.
2.12.4 Oiling off

The presence of some free fat is desirable in MCA, however, excessive amount detracts from its appearance. A fat leakage area of 0.64 and 0.45 square inches is reported to be desirable for Mozzarella cheese made by starter culture and direct acidification techniques respectively, for use as pizza topping (Breene et al., 1964). The acid casein based MCA showed lower fat leakage than natural Mozzarella, when fresh. However, upon refrigerated storage of one month, there was a decline (from 0.53 to 0.08 cm²) in the oiling-off in case of MCA, whereas NMC showed a progressive increase (from 0.82 to 4.81 cm²) in fat leakage (Jana, 1998).

2.12.5 Browning

The Browning of Mozzarella cheese associated with its cooking, results from the Maillard reaction, primarily between reducing sugars such as lactose and galactose and the amino acid moiety of the proteins (Johnson and Olson, 1985). Browning properties of MCA made using starch was more comparable to that of NMC than the one devoid of starch (Ahmad et al., 2001). Jana (1998) observed browning in both natural and analogue Mozzarella cheeses. However, the intensity of brown colour was more in natural than in analogue cheese.

2.13 MICROSTRUCTURE OF MOZZARELLA CHEESE ANALOGUE

The study of microstructure of the cheese system is important to understand the arrangement of various constituents in cheese matrix as affected by the processing parameters used and to relate them to their rheology and functionality viz., meltability, fat leakage, etc.

The paracasein in NMC was in the form of longitudinally aligned fibers with entrapped columns of fat, consisting of discrete and coalesced fat globules of similar orientation. On the other hand, microstructure of MCA showed a uniform distribution of fat droplets (2-16 µm), with larger globules being predominant in the continuous protein matrix. The fat had a smooth surface and was mostly spherical in shape. In cheese analogue containing native starch, the starch remained as discrete entities in the protein matrix due to its insolubility (Mounsey and O’ Riordan, 2001). Incorporation of pregelatinized starch caused reduction in the emulsification of fat globules, whereas use of native starch in rennet casein based analogue cheese resulted in better emulsification of fat (Mounsey and O’ Riordan, 2008a).
The cheese analogue containing resistant starch showed relatively uniform sized and homogeneous, spherical shaped starch granule; such starch granule boundaries showed little diffusion into the protein matrix (Montesinos-Herrero et al., 2006).

2.14 MICROBIOLOGICAL QUALITY OF MOZZARELLA CHEESE

Recently, PFA (2006) has laid down microbial standards for Mozzarella and Pizza cheese as detailed in Section 2.1 which makes it for the cheese maker to adhere to strict hygienic conditions during its production, packaging and handling. Presence of pathogens (viz., *Staphylococcus aureus*) and/or coliforms in natural or analogue cheese indicates unhygienic production practices and maybe hazardous for human health. High count of spoilage microbes in cheese system is detrimental to its shelf life.

Owing to variety of microorganisms found in the product, Mozzarella cheese has a rather short shelf life. Moreover, due to the action of enzymes elaborated by the spoilage microorganisms, there is a marked change in the functionality of the cheese for use on pizza pie, especially in stored cheeses.

The microbial species that have been isolated from natural Mozzarella cheese are enterococci such as *Enterococcus faecium*, *E. faecalis*, Enterobacteriaceae such as *Escherichia. coli*, some yeasts such as Debaryomyces hansenii and Kluyveromyces marxianus and various spoilage psychrophilic microflora (Cantoni et al., 2003a,b; Parisi, 2003a,b). The microbiological aspects of Mozzarella cheese during its manufacture as well as of market samples have been reported in literature (Catellani and Giordano, 1962; Siano and Cantoni, 1968; Tanetta, 1969; Nilson and La Clair, 1976; Caserio et al., 1977; Nieradka et al., 1979).

Asperger and Brandl (1982) and Sharma (1988) did not find any coliform in fresh NMC. Canned processed LMPSM had Standard plate count (SPC), coliform, yeast and mould and Staphylococcus count of 440, < 1, < 1 and < 10 per g of cheese respectively (Rizvi et al., 1999). An increase in SPC of NMC during its refrigerated storage has been observed by several workers (Aperger and Brandl, 1982; Sharma, 1988).

Total viable count in the vicinity of 103 cfu/g in 1 week old cold stored (4 °C) MCA was considered acceptable (O’ Malley et al., 2000). Non starter lactic acid bacteria, which accounted for 50.0-60.0 per cent of the total count, survived the high temperature processing (85 °C) during MCA manufacture; the SPC at 1 week and 32 weeks was 104 and 108 per g of cheese respectively (O’ Malley et al., 2000). The SPC of fresh and 1
month old refrigerated (6 °C) NMC was reported to be $4.87 \times 10^3$ and $28.75 \times 10^3$ per g of cheese respectively; the same count for MCA was reported to be $4.12 \times 10^3$ and $30.70 \times 10^3$ per g of cheese respectively. Likewise, the yeast and mold count of fresh as well as 1 month old NMC was reported to be 21 and 100 per g respectively; the same count for MCA was 17 and 122 per g of cheese respectively (Jana, 1998).

Toxin production by Cl. botulinum may be a possibility, if cheese analogue is contaminated with it (Kautter et al., 1981).

### 2.15 PROPERTIES OF CHEESE ANALOGUE VIS-À-VIS NATURAL MOZZARELLA CHEESE

Plasticizing of cheese curd in natural Mozzarella cheese promotes limited degree of aggregation and contraction of the paracasein gel matrix, culminating in formation of para-casein fibers which physically entraps cheese fat. In contrast, the conditions used in the manufacture of MCA are designed to disaggregate and hydrate the paracasein aggregates of rennet casein and/or caseinates. The hydrated para-caseinate immobilizes large quantities of water along with emulsifiers and vegetable oil, thereby contributing to physico-chemical stability of the product. Hence, unlike LMPSM the casein in MCA is in the form of a partially hydrated dispersion rather than para-casein fibers (Fox et al., 2000).

Many of the compositional factors (viz., moisture, protein, fat, ash and sodium chloride) that influence natural cheese quality by their influence on proteolysis and rheology did not seem to influence such properties of MCA (Mulvihill and Mc Carthy, 1993). The relatively large fat globule size (e.g. 5-25 µm) found in analogue cheese ensures sufficient degree of oiling-off from the pizza cheese topping upon baking. This in turn, limits dehydration of cheese topping and is conducive to satisfactory flow and succulence characteristics when used on pizza pie (Guinee et al., 2000).

### 2.16 STORAGE CHARACTERISTICS AND STABILITY OF MCA

The rheological changes that occur during cheese ageing/maturation are due to changes in pH, moisture and salt content and mainly to proteolysis of the casein matrix by rennet, indigenous milk proteinases and starter enzymes (Fox, 1989). The extent of proteolysis and protein hydration is the major determinants for the change in functional property during storage of the LMPSM (Guinee, 2002). MCA based on casein were functionally more stable with respect to apparent viscosity and free-oil during refrigerated storage than the natural LMPSM counterparts (Kiely et
al., 1991). The stretch characteristics of MCA remained similar (i.e. 21.0 to 23.0 cm) throughout the refrigerated storage period of 1 month, whereas that of natural Mozzarella cheese improved (i.e. 16.0-41.0 cm) upon storage (Jana, 1998). On the other hand, casein-based cheese analogues containing high level of starch (> 40 g/kg) tends to lose their functionality relatively rapidly (e.g. after 4 weeks) during refrigerated storage. Such loss of functionality is reflected by an increase in loose moisture upon shredding, loss of meltability and flowability and burning or crusting upon baking (Fox et al., 2000). Plasmin mediated proteolysis was observed during storage of MCA; β-casein was hydrolyzed more extensively than αS1-casein (O’Malley et al., 2000). Mulvihill and McCarthy (1994) reported a progressive increase in proteolysis which led to decrease in elasticity and chewiness of MCA during 51.0 weeks of refrigerated (4 °C) storage.

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