Determination of the effect of fat percentage on functionality of Low Moisture Mozzarella Cheese made by cow milk

By

R.A.N.D. Perera.
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Department of Food Science & Technology
Faculty of Applied Sciences
Sabaragamuwa University of Sri Lanka
Buttala (91100)
Sri Lanka.

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DECLARATION

The work describe in this thesis was carried out by me at the Department of Food Science & Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, under the supervisor of Mr. U. Rathnayaka, Lecturer, Department of Food Science & Technology, Faculty of Applied Sciences Sabaragamuwa University of Sri Lanka & Mr. W.G.N. Fernando, Line Manager, MILCO (Pvt) Ltd, Digana. A report on this has not been submitted to any other university for another degree.

.....................Signature

10/09/06.....Date

Certified by,

Internal Supervisor,

Mr. R.M.U.S.K. Rathnayaka.,

Lecturer,

Department of Food Science & Technology,

Faculty of Applied Sciences,

Sabaragamuwa University of Sri Lanka,

Buttala.

Tel: +94 77 7076684.

.....................Signature

10/09/06.....Date

External Supervisor,

Mr. W.G.N. Fernando,

Line Manager,

MILCO (Pvt) Ltd,

Digana.

Tel: +94 81 2374266.

.....................Signature

21/09/06.....Date

Mrs. K.M. Somawathie,

Actg. Head,

Department of Food Science & Technology,

Faculty of Applied Sciences,

Sabaragamuwa University of Sri Lanka.

Buttala.

.....................Signature

.....................Date

Affectionately
Dedicated

To

My Parents
&
Teachers.

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ABSTRACT

Low Moisture Mozzarella cheese is a member of pasta filata, or stretched curd cheese. It is distinguished by a plasticizing and kneading treatment of the fresh curd in hot water, which imparts to the finished cheese its characteristic fibrous structure, and melting and stretching properties.

Low Moisture Mozzarella has much lower water content (typically 47- 48 %), longer shelf life, firmer body, good shredding properties than the other types of mozzarella cheeses, and is used primarily as ingredients for pizza and related foods.

This study was focused on the determination of effect of fat percentage on functionality of Low Moisture Mozzarella cheese made by cow milk. The effect of fat percentage was determined by standardizing cow milk to fat percentage of 2.6 %, 2.9 %, 3.2 % and 3.5 % to achieve four different cheese samples. Cheese manufacturing time, stretching time, concentration of salt, pH and calcium percentage was not changed during the manufacturing process. Cheese moisture, protein, ash and fat contents were evaluated after 7 days of manufacturing. Meltability and stretchability of frozen samples were evaluated after 7 and 14 days respectively.

As the fat percentage was increased, there was a decrease in moisture and protein content of the cheese. However, the moisture & protein did not replace the fat on an equal basis. But, the decrease in the overall amount of moisture is much more less than that of the protein amount. Statistical Analysis revealed that a significant difference in stretch and melt between cheeses with different fat percentages. Stretchability was not significantly different for cheeses and meltability was significantly different during freezing storage for 14 days. Fat percentage of 3.2% can be recommended as the best fat content to produce Low Moisture Mozzarella cheese with optimum quality characteristics such as flavour, colour, sourness, appearance and overall acceptability. But problems associated with sensory characteristic, particularly hardness should be developed.

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LIST OF ABBREVIATIONS

°C	- Centigrade
°F	-Fahrenheit
FDM	-Fat in Dry Matter
g	-gram
Hr	-Hours
Ht	-Height
Kg	-Kilo gram
L	-Liter
LMMC	-Low Moisture Mozzarella Cheese
min	-Minutes
ml	-mili Liter
s	-Second
SLSI	-Sri Lanka Standards Institute

CHAPTER 1

Introduction

The present word "cheese" is derived through the Old English word "cese" and "chiese" from the Latin "caseus". The equivalent word in German, French, Spanish and Italian are "kase", "fromage", "queso" and "formaggio", respectively (Scott, 1998).

Cheese is the generic name for a group of fermented milk based food products produced throughout the world in a great diversity of flavors, textures, and forms. (Fox et al., 2000) There are number of cheese varieties according to the type of cheese, internal characteristics, external characteristics, weight of cheese, fat-in-dry-matter, water content and water-in-fat-free substances (Scott, 1998).

Mozzarella is a prominent member of the pasta filata, or stretched curd (Kindstedt, 1999) which was originally manufactured from high fat buffalo milk in the Battipaglia region of Italy, but now made all over Italy, in other European countries and U.S.A. from cow milk (Ghosh and Singh, 1991).

In the US, Mozzarella cheese is divided into four separate categories defined by standards of identity on the basis of moisture content and fat-in-dry-matter (FDM), namely Mozzarella, low-moisture, low moisture part-skim and part-skim mozzarella.

Mozzarella and part-skim mozzarella are high in moisture (>52%), soft bodied, and often consumed fresh as table cheeses. Low-moisture and low moisture part-skim mozzarella have much lower water content (typically 47-48%), longer shelf life, firmer body, good shredding properties, and are used primarily as ingredients for pizza and related foods (Kindstedt, 1999).

The functionality of mozzarella cheese is important since about 75% of all mozzarella cheese is used as an ingredient for pizza. Low-moisture mozzarella (LMMC) and low moisture part-skim (LMPS) mozzarella cheese, approximately 20% fat on wet basis, are types commonly used for pizza baking because of their desirable functional characteristics.

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. The reduction of fat content leads to poor functionality during pizza baking, the low fat cheeses (5 to 10% fat) display limited shred melts and fusion, followed by scorching of intact shreds, resulting in a pizza with an typical burnt appearance. The high fat cheeses (15 to 25% fat) they are baked under identical conditions exhibit desired functionality. Complete shred melt and fusion, followed by the formation of light brown blisters (Rudan and Barbano, 1998).

This new knowledge, along with the knowledge from prior research, should lead to development of LMMC for use on pizza products that will have acceptable sensory characteristics and functionality over wide range of pizza baking conditions.

1.1. Overall Objective:

Determine the effect of fat percentage on functionality of Low-Moisture Mozzarella Cheese made by cow milk.

1.2. Specific objectives:

1.2.1. Studying the manufacturing process of Low Moisture Mozzarella Cheese.

1.2.2. Identification of most suitable fat percentage for Low Moisture Mozzarella cheese

1.2.3. Analysis of physico-chemical and microbiological properties of Low Moisture Mozzarella.

1.2.4. To compare commercially available mozzarella & Low Moisture Mozzarella for sensory characteristics and functional properties.

CHAPTER 2

Literature Review

2.1 Composition of milk

Milk consists of a complex mixture of lipids, carbohydrates, proteins, and many other organic compounds and inorganic salts dissolved or dispersed in water. Some of these compounds such as carbohydrates, lactose, most of the salts and vitamins are soluble in water. Other components such as lipids, proteins and calcium phosphate are dispersed throughout the water in the colloidal state.

Table 2.1 Typical gross composition (kg/100kg) of cow's milk.

Constituent	Percentage
Fat	3.9 %
Total Protein	3.3 %
Casein	2.6 %
Whey	0.7 %
Lactose	4.6 %
Ash	0.7 %
Total	12.5 %

(Hill, 2006)

2.1.1 Water

Milk is a natural liquid food containing a high percentage of water (87%). Milk is actually a concentrated food designed to produce rapid growth in mammals and contains more solid material than many of our other common foods. Water is the medium in which all the other components of milk (total solids) are dissolved or suspended. A small percentage of the water in milk is hydrated to the lactose and salts, and some is bound in the proteins (Johnson, 1987).

2.1.2 Lipids

The lipids in milk are predominantly Triglycerides (triacylglycerols), which make up about 98% of the total lipid fraction; the remaining 2% comprises diglycerides, monoglycerides, fatty acids, phospholipids, sterol (principally cholesterol), and trace amounts of fat-soluble vitamins (A, D, E and K). Typical values for the concentration of lipids in cow's milk are as follows.

Lipid Class	**Percentage**
Triacylglycerols	97.5 %
Diacylglycerols	0.36 %

Monoacylglycerols	0.027 %
Cholesteryl ester	T %
Cholesterol	0.31 %
Free fatty acids	0.027 %
Phospholipids	0.6 %

*T = trace.

(Fox et al., 2000)

2.1.3 Proteins

The proteins of milk belong to two main categories that can be separated based on their solubility at pH 4.6 at 20 °C. Under these conditions, one of the protein group precipitates; these are known as caseins. The proteins that are remaining soluble under these conditions are known as serum or whey proteins. Approximately 80% of the total proteins are casein and 20% of the total proteins are whey.

a. Casein

Bovine casein consists of four types of protein with substantially different properties: alpha (s1), alpha (s2), ß and kappa; these make up approximately 38%, 10%, 34% and 15%, respectively, of whole casein.

b. Whey Protein

The whey protein fraction of cow's milk contains four main proteins: beta lactoglobulin (beta-lg, 50%), alpha-Lactalbumins (alpha-la, 20%), blood serum albumin (BSA, 10%), and immunoglobulins (Ig, 10%, mainly IgG_1, with lesser amounts of IgG_2, IgA, and IgM).

2.1.4 Lactose

Lactose is the principal carbohydrate in milk, which is the only source of lactose for human. It is a disaccharide (2 sugars) made up of glucose and galactose (which are both monosaccharides). It is not as sweet as sucrose. When lactose is hydrolyzed by ß -D-galactosidase (lactase), an enzyme that splits these monosaccharides, the result is increased sweetness, and depressed freezing point. One of its most important functions is its utilization as a fermentation substrate. Lactic acid bacteria produce lactic acid from lactose, which is the beginning of many fermented dairy products (Hill, 2006).

2.1.5 Milk salts

Some of the salts in milk are present at concentrations below their solubility limit and are therefore fully soluble. However, others, especially calcium phosphate, exceed their solubility and occur partly in solution and partly in the colloidal phase, associated mainly with the casein micelles. These salts are collectively referred to as micellar or colloidal calcium phosphate (CCP), although several other elements or ions are present as well. Several elements are also present in the milk fat globule membrane, mainly as constituents of enzymes.

2.1.6 Enzymes

Enzymes are a group of proteins that have the ability to catalyze chemical reactions and the speed of such reactions. The action of enzymes is very specific. Milk contains both indigenous and exogenous enzymes. Exogenous enzymes mainly consist of heat-stable enzymes produced by psychrotrophic bacteria: lipases, and proteinases. There are many indigenous enzymes that have been isolated from milk. The most significant group is the hydrolases:

- Lipoprotein lipase
- Plasmin
- Alkaline phosphatase (Hill, 2006).

2.1.7 Vitamins

Vitamins are organic substances essential for many life processes. Milk includes fat-soluble vitamins A, D, E, and K. Vitamin A is derived from retinol and ß -carotene. Because milk is an important source of dietary vitamin A, fat reduced products, which have lost vitamin A with the fat, are required to supplement the product with vitamin.

There is also a small amount of vitamin C (ascorbic acid) present in raw milk but is very heat-labile and easily destroyed by pasteurization (Hill, 2006).

2.2 Physical properties of cow's milk

Physically milk is a rather diluted emulsion combined with a colloidal dispersion in with the continuous phase is a solution. Its physically properties are similar to those of water but are modified by the concentration of solutes and by the state of dispersion of the other components (Jenness et al, 1987).

2.2.1 pH and acidity

The pH of cow's milk is commonly stated as falling between 6.5 and 6.7, with 6.6 being the most usual value. It should be emphasized, however, that this value applies only at temperatures of measurement near 25 °C. Many components in milk provide the buffering action. Major buffering groups are caseins and phosphates, while salts like ionic constituents are major buffering groups.

2.2.2 Density and specific gravity

Density refers to the weight per unit volume of product and "Specific gravity" is the ratio of the density of a product to that of water at some specific temperature. The coefficient of thermal expansions the effect of temperature on density, and each substance has its own coefficient. Thus, when speaking of specific gravity, it is desirable to state both the sample and water temperatures; frequently, they are the same. Normally fresh milk's density and specific gravity are 1030kgm^{-3} and 1.0321 respectively at 20 °C.

2.2.3 Viscosity

The viscosity of milk and dairy products depends upon the temperature and on the amount and state of dispersion of the solid components. Caseinate micells and the fat globules are the most important contributors to the viscosity. Therefore, variations of that compound alter the viscosity of milk.

Viscosity of milk is important to determine following,

- The rate of creaming.
- The flow condition of dairy processes.
- Rate of mass and heat transfer.

2.2.4 Freezing point

The freezing point of milk, like that of any aqueous system, depends on the concentration of water-soluble components. The freezing point of bovine milk is usually within the range of -0.512 to -0.550 °C. The average value is close to -0.522 °C.

Freezing point of water will change according to,

- Seasonal effect
- Feed
- Water intake
- Stage of lactation
- Breed of cow
- Heat stress
- Times of day (morning or evening)

2.3 Product from cow's milk

Milk is a dynamic mixture of proteins, fats, carbohydrates, salts and water co-existing as emulsion, colloidal suspension, and true solutions. Milk products are prepared by alteration of these relationships either by removal of, or by changing the ratio of, one or more of the components.

2.3.1 Flavored milk

Flavoured milk is milk that has sugar, colorings and (mostly inexpensive artificial) flavourings added to make it more appetising, especially to children. (Wikipedia, 2006)

2.3.2 Fermented milk products

a. Yoghurts

Yoghurt or yogurt, or less commonly yoghourt, joghurt (in German) or yogourt, is a dairy product produced by bacterial fermentation of milk. Any sort of milk may be used to make yoghurt, but modern production is dominated by cow's milk. It is the fermentation of milk sugar (lactose) into lactic acid that gives yoghurt its gel-like texture and characteristic tang. It is often sold in a fruit, vanila, or chocolate flavour, but can also be unflavored (Wikipedia, 2006).

2.3.3 Butter

Butter is a dairy product made by churning fresh or fermented cream or milk. In many parts of the world, butter is an everyday food. Butter is used as a spread and a condiment, as well as in cooking applications such as baking, sauce making, and frying. Butter consists of butterfat surrounding minuscule droplets consisting mostly of water and milk proteins. The most common form of butter is made from cow's milk, but can also be made from the milk of other mammals, including sheep, goats, buffalo, and yaks. Salt, flavorings, or preservatives are sometimes added to butter (Wikipedia, 2006).

2.3.4 Ghee

Ghee is a type of clarified butter important in Indian cuisine and Pakistani cuisine. Ghee is made by simmering unsalted butter in a large pot, until its water has boiled off and its protein has settled to the bottom. The clarified butter is then spooned off, taking care not to disturb the milk solids on the bottom of the pan. This method of preparation gives ghee a somewhat nutty aroma that Western clarified butter does not have (Wikipedia, 2006).

2.3.5 Concentrated milk products

a. Evaporated milk

Evaporated milk is fresh, homogenized milk from which 60 percent of the water has been removed. It is then chilled, fortified with vitamins and stabilizers, packaged, and finally sterilized. Standards require whole evaporated milk contain at least 7.9 percent milk fat and 25.5 percent milk solids. The high heat process gives it a bit of a caramelized flavor, and it is slightly darker in color than fresh milk. The evaporation process naturally concentrates the nutrients and the calories; so evaporated versions are more calories filled and nutritious than their fresh counterparts. When mixed with an equal amount of water, it can be substituted for fresh milk in recipes (Wikipedia, 2006).

b. Condensed milk

Condensed milk is cow's milk from which water has been removed and to which sugar has been added, yielding a very thick, sweet product that can last on the shelf for years. That is also known as sweetened condensed milk (Wikipedia, 2006).

2.3.6 Dried milk product

Powdered milk is typically made by spray drying nonfat skim milk. Pasteurized milk is first concentrated in an evaporator to about 50% milk solids. The resulting concentrated milk is sprayed into a heated chamber where the water almost instantly evaporates, leaving behind fine particles of powdered milk solids (Wikipedia, 2006).

2.3.7 Cheese

Cheese is a solid food made from the curdled milk of cow's, goats, sheep, water buffala or other mammals. The milk is curdled using some combination of rennet (or rennet substitutes) and acidification Bacteria acidify the milk and play a role in defining the texture and flavor of most cheeses. Some cheeses also feature molds, either on the outer rind or throughout (Wikipedia, 2006).

2.4 Milk as a raw material for cheese making

2.4.1 Nutritional value of fat & cholesterol

Fat plays several important functions in cheese: it affects, for example, cheese firmness, adhesiveness, mouth-feel, and flavor. It also contributes significantly to the nutritional properties of cheese, as most cheeses contain significant amounts of fat. Thus, cheese contributes a significant amount of both saturated and total fat to the diet.

The cholesterol content of cheese varies from approximately 10 to 100mg/100g, depending on the variety. It is well established that dietary cholesterol intake exerts a much smaller influence than the intake of dietary saturated fat on a person's blood cholesterol level, which is a significant risk indicator for coronary heart disease (Fox et al., 2000).

2.4.2 Effect of fat on milk for cheese

a. Flavor

Lipids contributes to cheese flavor three ways,

1. There are sources of fatty acids, especially short chain fatty acids, which have strong and characteristic flavors. Fatty acids are produced through the action of lipases in a process referred to as lipolysis. In some varieties, the fatty acids may be contributes to other sapid and aromatic compounds, especially methyl ketones and lactones.
2. Fatty acids, especially polyunsaturated fatty acids, undergo oxidation, leading to the formation of various unsaturated aldehydes that are strongly flavored and cause a flavor defect referred to as oxidative rancidity. Lipid oxidation appears to be very limited in cheese, probably owing to its low redox potential (-250mv)
3. Lipid function as solvents for sapid and aromatic compound produced not only from lipids but also from protein and lactose. Lipid may also absorb from environment compounds that cause off-flavors of the various possible contributions of lipids to cheese flavor, lipolysis and modification of the resultant fatty acids are most significant (Fox et al., 2000).

b. Texture

The contribution of fat to the rheological properties of cheese depends on its physical state and therefore on the temperature, which controls the ratio of solid fat: liquid fat. At low temperatures (< 5 °C), where the milk fat is predominantly solid, the fat adds to the elasticity of the casein matrix. The solid fat globules limit deformation of the casein matrix, as the deformation of the latter also required deformation of the fat globules enmeshed within its spores. As the proportion of liquid fat increases, the fat behaves more like a fluid and confers viscosity rather than elasticity or rigidity on the cheese. Moreover, liquid fat act as a lubricant on fracture surfaces of the casein matrix and thereby reduces the stress required to fracture the matrix. Generally an increase in the fat content of cheese is accompanied by decrease in the levels of protein and moisture as well as decrease in the firmness (Fox et al., 2000).

c. Yield

The single most important factor effecting cheese is the composition of the milk, particularly the concentration of fat and casein, which together represent around 94% of the dry matter of cheddar cheese. Yield increases linearly as the concentrations of fat and casein are increased.

The contribution of fat and casein to the yield of a particular cheese type, such as cheddar or cream cheese, is critically dependant on the casein: fat ratio to which the milk is standardized. For any cheese variety, increasing the casein: fat ratio (e.g., by reducing the level of fat) results in a higher moisture content (and dissolved solid) and, apart from the acid curd cheese such as Quarg, in higher level of ash. Conversely, reducing casein: fat ratio (e.g., by maintaining the casein level constant and increasing the fat content) increases the level of fat and decreases the level of moisture in the cheese. Similarly, the contributions of fat and casein to the yield of different varieties of cheese depend on the ratio of protein (mainly casein) to the fat in the cheese, which is controlled by standardization to the desired casein: fat ratio in the milk (Fox et al., 2000).

2.5 Cheese

2.5.1 Definition of cheese

The expansion in the number of types of cheese makes a simple definition of cheese difficult, and a description, such as "the curd of milk produced by enzyme activity and subsequent separation of the whey from the coagulation to give a more solid curd which is cheese" does not cover whey cheese, lactic cheese, cream cheese and some of the cheeses produced by the newer techniques (e.g. ultra- filtration or reverse osmosis). The definition is not, therefore, universally acceptable.

2.5.2 A Brief History of cheese

Most authorities consider that cheese was first made in the Middle East. The earliest type was a form of sour milk, which came into being when it was discovered that domesticated animals could be milked. A legendary story has it that cheese was 'discovered' by an unknown Arab nomad. He is said to have filled a saddlebag with milk to sustain him on a journey across the desert by horse. After several hours riding he stopped to quench his thirst, only to find that the milk had separated into pale watery liquid and solid white lumps. Because the saddlebag, which was made from the stomach of a young animal, contained a coagulating enzyme known as rennin, the combination of the rennin, the hot sun and the galloping motions of the horse had effectively separated the milk into curds and whey. The nomad, unconcerned with technical details, found the whey drinkable and the curds edible.

2.5.3 Classification of cheese

The great range of cheese varieties, excluding minor local variants, makes classification of cheese extremely complicated. It is obvious that a well-known variety has certain distinctive characteristics, under the following heading;

1. Country of origin
2. Source of raw material- cow, goat, buffalo
3. Types of cheese- hard, semi hard, soft, fresh, acid coagulated or whey cheese
4. Internal characters- close or open texture, large, medium or small eyeholes, slit openings in curd, ripened with blue or white moulds, colour of curd, presence of herbs or spices.
5. External characters- rind that is hard or soft, smooth or rough, smear or mould-coated, dusted with spices, herbs or ash, type of final coating (plastic, wax, leaves).
6. Weight of cheese- shapes and size
7. Fat-in-dry-matter- percentage maximum
8. Water content- percentage maximum
9. Water-in-fat-free substances (WFF) (Scott, 1998).

A simple classification of cheese

Table 2.2 Classification of cheese

SI NO	Characteristics	Requirement for cheese			
		Hard	Semi hard	Soft	Processed cheese or cheese spread
1.	Fat, percent by mass, (on dry basis) min.	45.0	45.0	45.0	45.0
2.	Moisture, percent by mass	35 to 45	45 to 55	55 to 80	35 to 80
3.	Dry matter*, percent by mass	55 to 65	45 to 55	20 to 45	20 to 65
4.	Salt, percent by mass, max	3.0	3.0	3.0	3.0

* Dry matter = 100-water content

SLS 773: 1987

2.5.4 Health and nutrition

In general, cheese supplies a great deal of calcium, protein, and phosphorus. A 30 gram (one ounce) serving of cheddar cheese contains about seven grams of protein and 200 milligrams of calcium. Nutritionally, cheese is essentially concentrated milk: it takes about 200 grams (seven ounces) of milk to provide that much protein and 150 grams to equal the calcium.cheese's highly saturated fat actually leads to an increased risk of heart disease. Therefore a number of food safety agencies around the world have warned of the risks of raw-milk cheeses. The U.S. Food and Drug Administration states that soft raw-milk cheeses can cause "serious infectious diseases including listeriosis, brucellosis, salmonellosis and tuberculosis". It is U.S. law since 1944 that all raw-milk cheeses (including imports since 1951) must be aged at least 60 days.

Cheese is often avoided by those who are lactose intolerant, but ripened cheeses like Cheddar contain only about 5% of the lactose found in whole milk, and aged cheeses contain almost none. Some people suffer reactions to amines found in cheese, particularly histamine and tyramine. Some aged cheeses contain significant concentrations of these amines, which can trigger symptoms mimicking an allergic reaction: headaches, rashes, and blood pressure elevations.

2.5.5 Overview of cheese manufacturing process

General protocol for cheese manufacture.

Milk

↓ Selection
Pre-treatment
Standardization

Cheese milk

↓ Addition of:
Starter culture (acidification)
Color (optional)
$CaCl_2$
Coagulation (rennet or acid or heat/acid)

Coagulum (gel)

↓ Cut coagulum
Stir
Heat
Acidification (rennet or coagulated)
Separation of curds from whey

Curds

↓ Acidification
Special operation (e.g., cheddaring, stretching)
Salting (some varieties)
Molding
Pressing (some varieties)

Fresh cheese

↓ Salting (most varieties)
Ripening (most rennet-coagulated)

Mature cheese

Fig 2.1 General protocol for cheese manufacture

2.5.6 Selection of milk

The composition of cheese is strongly influence by the composition of the cheese milk, especially the content of fat, protein, calcium, and pH. The constituents of milk are influenced by several factors, including the species, breed, individually, nutritional status, health, and stage of lactation of the producing animal. Owing to major compositional abnormalities, milk from cows in the very early or late stage of lactation and those suffering from mastitis should be excluded. The milk should be free of chemical taints and free fatty acids, which cause off-flavors in the cheese, and antibiotics, which inhibit bacterial cultures. The milk should be of good microbiological quality, as contaminating bacteria will be concentrated in the cheese curd and may cause defects or public health problem (Fox et al., 2000).

2.5.7 Standardization of milk composition

Different cheese varieties have a certain fat-in-dry-matter content, in effect, a certain fat: protein ratio, and this content has legal status in the "standard of identity" for many cheese varieties. While the moisture content of cheese, and hence the level of fat plus protein, is determined mainly by the manufacturing protocol, the fat: protein ratio is determined mainly by the fat: casein ratio in cheese milk. Depending on the ratio required, it can be modified by,

- Removing some fat by natural creaming, as in the manufacture of parmigiano-reggiano, or centrifugation.
- Adding skim milk.
- Adding cream
- Adding milk powder, evaporated milk, or ultrafiltration retentate.

(Fox et al., 2000).

2.5.8 Pasteurization

Most (>99.9%) of bacteria found in raw milk are heat labile and are killed by pasteurization at 72 °C for 15 S; most milk for cheese making is subjected to this heat treatment. pasteurization killed all potential pathogens that might be presented in milk, but spores of Clostridium and Bacillus are not killed by this treatment. Pasteurization also inactivates several enzymes in the milk, including lipase and alkaline phosphate. Lack of alkaline phosphatase activity in milk indicates that the milk has been properly pasteurized. While pasteurization, reduces the risk of producing low-quality cheese resulting from the growth of undesirable bacteria and kills pathogens and food-poisoning microorganisms (Fox et al., 2000).

2.5.9 Calcium

Calcium plays a major role in the coagulation of milk by rennet and the subsequent processing of the coagulum, and hence it is common practice to add $Cacl_2$ (e.g., 0.01%) to cheese milk.

2.5.10 pH

The p^H of milk is a critical factor in cheese making. The pH is inadvertently adjusted by the addition of 1.5-2% starter culture, which reduces the pH of the milk immediately by about 0.1 units. States concentrates (sometimes called direct to vat starters), which are sometimes used, have no immediate acidifying effect.

2.5.11 Starter

The starter adds to the cheese milk 30-60 min before rennet addition. During this period, the starter began to grow and produce acid, a process referred to as ripening. Ripening serves a number of functions. It allowed the starter bacteria to enter their exponential growth phase and hence to be highly active during cheese making; this is not necessary with modern high quality starters. The lower pH was more favorable for rennet action and gel formation.

2.5.12 Cheese colour

Colour is a very important attribute of foods and serves as an index of quality, although in some cases, it is merely cosmetic. The principle indigenous pigments in milk are carotenoids, which are obtained from the animal's diet, especially from fresh grass and cloves. In additionally cheese colours may be obtained by adding carotenoids (synthetic or natural extracts). Initially, annatto may have been used in cheese manufacture to give the impression of a high fat content in partially skimmed cheese (Fox et al., 2000).

2.5.13 Coagulation

The essential step in the manufacture of all cheese varieties involves coagulation of the casein components of the milk protein system to form a gel that entraps the fat, if present. Coagulation may be achieved by,

- Limited proteolysis by selected proteinases (rennets)
- Acidification to PH 4.6
- Acidification to a PH value grater than 4.6 in combination with heating to roughly 90 °C

(Fox et al., 2000).

2.5.14 Postcoagulation Operations

Rennet or acid coagulated milk gels are quite stable if maintained under quiescent conditions, but if cut or broken, they rapidly undergo syneresis, expelling whey. Syneresis essential concentrates the fat and casein of the milk by a fat of 6-12, depending on the variety. In the dairy industry, concentration is normally achieved through thermal evaporation of water and more recently by removing water through semi permeable membranes. The syneresis of rennet or acid coagulate milk gels is thus an unusual method of dehydration. The rate and extent of syneresis are influenced, inter alia, by the milk composition, specially the concentrations of Ca2+ and casein; the P^H of the whey; the cooking temperature; the rate of stirring of the curd-whey mixture; and, of course, time (Fox et al., 2000).

2.5.15 Salting culture

Salting is the last manufacturing operation. Salting promotes syneresis, but it is not a satisfactory method for controlling the moisture content of cheese curd, which is best achieved by ensuring that the degree of acidification, heating, and stirring in the cheese vat are appropriate to the particular variety. Although salting should be a very simple operation, quite frequently it is not performed properly, with adverse effects (Fox et al., 2000).

2.5.16 Ripening

The changes that occur during ripening and hence the flavor, aroma, and the texture of the mature cheese are largely predetermined by the manufacturing process, that is, by the composition (especially moisture, NaCl, and $P^{H)}$, by the level of residual coagulant activity, by the type of starter, and in many cases by secondary inocula added to or gaining access to the milk or curd. One or more of the following agents causes the biochemicals changes occur during ripening.

- The coagulant
- Indigenous milk enzymes, especially proteinase and lipase, which are particularly important in cheese made from raw milk
- Starter bacteria and their enzymes
- Secondary microorganisms and their enzymes.

(Fox et al., 2000).

2.6. Mozzarella cheese

2.6.1 History

Mozzarella originated from the Naples-Salerno region of southern Italy where it was made from water buffalo milk. Although some still is, most of that made in the U.S. is produced with cow's milk. Initially Italian-made mozzarella was a soft, fresh cheese that aged quickly and had to be used within a week of manufacture. A product of such short shelf life did not fit into the American distribution system. So a firmer (lower moisture) version that would last at least a month was created which is what's used by pizzerias today.

2.6.2 Types of Mozzarella

The U.S. Department of Agriculture (USDA) specifies four types of mozzarella, divided according to moisture (i.e., water) and milkfat content. Note that the percent of milkfat is not based on weight of the cheese but on the weight of solids in the cheese. The solids portion of cheese is what's left after all moisture is evaporated off and, so, is sometimes called dry weight As an example, a cheese that is 45 percent moisture would be a 55 percent solid.
So when a cheese is said to have, say, 50 percent milk fat, it does not mean that the cheese consists of 50 percent fat but, rather, that the solids portion of the cheese is 50 percent fat. This percent is referred to as milkfat-in-solids. It's also called FDB, meaning Fat Dry Basis, and also FTS, meaning Fat in Total Solids.

Four Types of Mozzarella

	Moisture %	Milkfat-in-solids %
Whole milk mozzarella	52-60%	45% or more
Part-skim mozzarella	52-60%	30-45%
Low-moist. whole milk mozz.	45-52%	45%or more
Low-moist. part-skim mozz.	45-52%	30-45%

(Correll, 2002)

2.6.3 Low moisture mozzarella

Low moisture mozzarella (LMMC) has much lower water content (typically 47-48%), longer shelf life, firmer body, good shredding properties, and is used primarily as ingredients for pizza and related foods (Kindstedt, 1999).

2.6.4 Production of low moisture mozzarella cheese

Traditional manufacture of LMMC, as outlined in fig. 1, is quite similar to that of cheddar, with some notable exceptions. Usually, a mixed culture consisting of *Streptococcus salavarius* ssp. *Thermophilus* (formerly *Streptococcus thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (formerly *Lactobacillus bulgaricus*) or *Lactobacillus helveticus* is used. These thermophilic bacteria thrive at higher scalding and cheddaring temperatures (e.g. 42 °C) used compared to cheddar cheese manufacture. The curd is cheddared to pH 5.2, and then subjected to a kneading and plasticizing process in hot water at approximately 70 °C. The hot plastic curd is moulded into desired shaped, cooled briefly in chilled water and salted in chilled brine (Kindstedt, 2000).

2.6.5 Pasteurization temperature

Higher pasteurization temperatures can be used to aid in moisture retention in the manufacture of lower-fat mozzarella. The higher temperatures cause more whey proteins to denature, trapping them in the curd matrix. Whey proteins bind water, thus increasing the moisture content of the cheese. Increasing the moisture content of lower-fat mozzarella improves its body and mouthfeel. When fat is removed from mozzarella, the protein content increases, resulting in a cheese that is too tough to melt and stretch properly. Cheesemakers can overcome this by increasing the moisture content, which leads to a softer and more pliable cheese (Watson, 2006).

2.6.6 Homogenizing of cream

To decrease milk fat particle size, and thereby increase total surface area of fat. This simulates a higher fat content because of the increased surface area, resulting in a cheese with desirable full-fat attributes, such as improved mouthfeel, melt, etc.

The process requires blending skim milk with homogenized cream to a composition that will produce a pre-determined lower-fat mozzarella. Because increased fat globule surface area increases water binding, cheesemaking procedures such as cook temperature usually need to be modified. When milkfat particle size is reduced, cheese whiteness improves. Whiteness influences the consumer's point-of-purchase decision for lower-fat mozzarella because of the association of whiteness with mozzarella quality and freshness. Thus, whiteness is a critical success factor in mozzarella manufacture (Watson, 2006).

2.6.7 Modifying cook temperature

Cooking temperature in the cheese vat influences moisture removal from curd during cheesemaking. Because differences in moisture content have an impact on mozzarella functionality, determining the appropriate cook temperature and maintaining it is critical in manufacturing high-quality, functional mozzarella. A higher cooking temperature produces cheese with lower moisture content and decreases proteolysis during refrigerated storage. Differences in cooking temperature do not significantly change meltability and free-oil properties; however, apparent viscosity of melted cheese is higher with higher cooking temperature. Increased refrigerated storage time decreases hardness of unmelted cheese, increases meltability, decreases apparent viscosity and increases free-oil formation (Watson, 2006).

2.6.8 Variation of pH

Whey pH has an effect on coagulant inactivation, which ultimately affects unmelted cheese texture. Mozzarella texture is important because of its influence on shredding properties. As the pH of mozzarella curd and whey decreases prior to draining, more calcium is transferred from the curd into the whey. Generally, this transfer increases the cheeses moisture content, resulting in a softer cheese texture, providing all other factors are equal. Draining pH does not affect protein, fat or salt content. However, texture profile analysis, hardness, springiness and apparent viscosity are lower with lower draining pH throughout storage. Variation in draining pH may have a larger or smaller impact on cheese texture during refrigerated storage, depending on the coagulant used. The amount of coagulant inactivation resulting at different cooking temperatures (in the cheese vat prior to draining) may be different at different draining pH. In general, all coagulants become more resistant to thermal inactivation at lower pH. An increase in cooking temperature to inactivate the coagulant and achieve a firmer cheese texture during refrigerated storage is less effective when draining pH is low. Selecting the appropriate whey draw pH and continually controlling to that point will produce mozzarella with more consistent functional characteristics. Cheese pH is important because of its influence on proteolytic changes, which are considered to be the most important biochemical event during the ripening of cheese. Differences in mill pH do not significantly affect moisture, fat, protein or salt content of the cheese or the indices of proteolysis and meltability. Hardness of unmelted mozzarella decreases, apparent viscosity of melted cheese decreases and free-oil formation increases. Proteolysis of casein may be the cause of the significant cheese functionality changes during refrigerated storage. The practical significance of these results is that mill pH can probably vary over a range of 0.2 pH units without significantly affecting the chemical composition or functional properties of mozzarella cheese. (Target milling pH is 5.25.) However, because cheese pH has some influence on the growth of undesirable non-starter bacteria in cheese, it is best to keep cheese pH low and use other parameters to control the firmness and functional properties of mozzarella (Watson, 2006).

2.6.9 Matting / Cheddaring

In the manufacture of lower-fat mozzarella, maintaining moisture content is of utmost importance. Slow acid development during the cheddaring step can assist in this effort. Slower acid development decreases the rate of whey expulsion, thereby retaining moisture in the curd. This can be achieved by less frequent turning during cheddaring, which keeps the curd on top cool, slowing acid development (Watson, 2006).

2.6.10 Cooking / Stretching

Stretching temperature and residence time profoundly influence aging in mozzarella. Control of temperature and mechanical treatment of the cheese during stretching could allow cheesemakers to improve control of the composition and functionality of mozzarella cheese. Differences in stretching temperature cause differences in the amount of coagulant that remains active in the cheese after stretching. Differences in free-oil release are probably due to differences in characteristics of the physical dispersion of fat in the cheese. The viscosity of melted cheese decreases and free-oil formation increases. Proteolysis of casein may be the cause of the significant cheese functionality changes during refrigerated storage. Mill pH can vary over a range of 0.2 pH units without significantly affecting the chemical composition or functional properties of mozzarella cheese. (Target milling pH is 5.25.) However, because cheese pH has some influence on the growth of undesirable non-starter bacteria in cheese, it is best to keep cheese pH low and use other parameters to control the firmness and functional properties of mozzarella (Watson, 2006).

2.6.11 Defects of mozzarella cheese

It is possible that mozzarella cheese may develop several specific rheological defects that lead to poor shredding properties, such as soft body defect and soft ring defect (Kindstedt, 1999).

a. **Soft body defect**

A soft pasty body and poor shredding properties that develop during ageing, especially in the cheese center, characterize it. This defect was associated with high levels of *Lactobacillis casei* ssp. *casei*, a common contaminant of raw milk (Kindstedt, 1999).

b. **Soft ring defect**

It is common to brine-salted cheeses, including mozzarella. A soft, moist, sometimes slimy, surface layer that is evident immediately after the cheese has been salted in freshly prepared brine characterizes the defect. This defect is caused by the migration of calcium from the cheese into the brine, which results in partial solubilization of casein. Such calcium leaching of can be prevented by calcifying the brine before its first use, which prevent soft ring defect in mozzarella.

Mozzarella sometimes develops a. soft, pastry, and high moisture surface during aging. This defect has been linked to the practice of salting warm curd (e.g. 54 °C) in cold (e.g. 4 °C) brine, which results in the retention of higher moisture levels at the cheese surface than occurs when brining is performed at higher temperatures. During ageing, diffusion of water from the low salt centre of the cheese to the high salt surface can lead to the high moisture, soft surface (Kindstedt, 1999).

CHAPTER 3
Materials and Methodology

3.1 Quality Testing of Milk

3.1.1 Organoleptic Quality

Materials

Fresh cow's milk

Methodology

Fresh cow's milk was examined for its organoleptic properties, such as taste, smell and color.

3.1.2 Microbial Quality

Materials

Fresh cow's milk

Test tube

Water Bath

Thermometer

Methodology

3 ml of fresh cow's milk was obtained to a test tube and 3 ml of alcohol was added to it. After that formation of flocculation on the test tube was examined.

3.2 Production process of Low Moisture Mozzarella Cheese

Materials

Fresh cow's milk

Thermophilic starter 1.5-2%

Rennet

Salt

pH Meter

Water

Thermometer

Spoon

Gas Cooker

Electronic Balance

Knife

Watch

Methodology

Manufacturing process of Low-Moisture Mozzarella Cheese.

Addition	Manufacturing step	pH	Time (min)
Milk →	Standardize		
	Pasteurize 72°C – 16 Sec	6.65	
Thermophilic Starter 1.5-2% →	Mixing	6.55	0
Rennet →	Setting	6.45	30
	Cutting	6.40	55
	Scalding		70
	Draining	6.10	130
	Cheddaring 42 °C	5.90	150
	Milling	5.25	210
Water 70 °C →	Stretching		
	Moulding		
	Brining 4 °C		

Fig 2.2 Manufacturing process for Low-Moisture Mozzarella Cheese

Milk was standardized to obtain 2.6%, 2.9%, 3.2% and 3.5% of fat percentage of samples by using skimmed milk and full cream milk, to prepare four different fat levels of LMMC. After that it was pasteurized at 72 °C for 16 s. It was then allowed to cool to 34 °C. After cooling, 1.5-2% thermophilic starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were added and kept on stirring for 30 min. After the pH was reached to 6.45, 0.01% rennet was added and allowed to set in the incubator for 25 min at 34 °C. After the curd was formed and pH was reached to 6.40, it was cut into small pieces with the knife and kept on scalding for 1 hour. After the pH was reached to 6.10, whey was removed. The curd was cheddared at 45 °C for 1 hour. After the pH was reached to 5.25, the curd was milled. Then the curd was stretched by hand, at 70 °C in hot water for 10 min. after stretching the curd was moulded into a desired shape and cooled in chilled water and brining was done under concentrated NaCl solution for 4 hours at 4 °C. After brining the cheese was dried using the tissue papers and packed in polythene bags and stored at 4 °C.

3.3 Quality testing of Mozzarella

3.3.1 Determination of functional properties

3.3.1.1 Determination of meltability

Materials

Schreiber melt-test score sheet

Circular copper tube

Electronic Balance

Petri dish

Ruler

Methodology

A circular copper tube with 39.5 mm was used to obtain a 5 mm thick dish of cheese. Then it was placed in the center area of the covered petri dish, and it was placed in the oven at 232 °C for exactly 5 min. Afterwards the melted cheese was cooled for 30 min after removing from the oven. Then meltability was measured using Schreiber melt-test score sheet.

3.3.1.2 Determination of stretchability

Materials

Ruler

Cheese Sample

Methodology

Cylindrical shaped cheese weighing 1.00g from the five samples were placed in five test tubes and were kept in a boiling water bath to heat up to 65 °C for three minutes. Stretchability was measured by applying constant weight for five cheese samples. Stretch length was measured by decreasing melt length from maximum stretch length.

3.3.2 Determination of chemical properties

3.3.2.1 Determination of Moisture

Materials

Oven

Electronic balance

Porcelain crucibles

Dessicator

Methodology

5 g of sample was weighed in to a moisture dish and it was placed in the oven for 4 hours at 105 °C. Then it was cooled in a dessicator and weighed. This procedure was followed at 30 min intervals until the differences between the two consecutive weighing was not exceeded 1mg.

$$\text{Moisture \%} = \frac{M_1 - M_o}{M_o - M} * 100$$

M1 = mass in grams, of the dish and cheese sample before drying

Mo = mass in grams, of the dish and cheese sample after drying

M = mass of the dish

3.3.2.2 Determination of Fat

Materials

Majoinner flask

Beaker

Chemicals

Hydrochloric acid

Ether

95% Ethanol

Methodology

2.00 g of the sample was weighed into the beaker. Then 2 ml of 95% ethanol and 10 ml of HCl was added and mixed the content thoroughly. HCl was prepared by adding 25 ml of concentrated HCl and 1.0 ml of water. Then the beaker was placed in (70 °C to 80 °C) water bath and stirred for 30-50 minutes frequently. Then the beaker was removed from the water bath and cooled in the atmosphere. 10 ml of ethanol was added to it and mixture was transferred to the separation funnel. Then the beaker was washed with 25 ml of ether in 3 portion of washing and was added to the flask. After that the flask was stopped with a cork and shaken vigorously for about few minutes and 25 ml of petroleum ether was added and shaken again. Then the flask was kept until clear layer of petroleum ether was prepared. Then the upper ether layer was taken into the clean previously weighed dried flask. Then it was dried in the water bath (90 °C) until the constant weighed was obtained.

Calculation,

$$\% \text{ Total fat} = \frac{\text{Weight of fat} * 100}{\text{Weight of sample}}$$

3.3.2.3 Determination of protein

Materials

Distillation Unit

Kjeldhal flask

Digestion Unit

Burette

Gas cooker

Electronic balance

Filter paper

Chemicals

30% NaOH solution

4% Boric solution

Bromocresol green indicator

$CuSo_4$, Se_2O_4 catalyst

Methodology

Approximately 0.5 g of ground cheese sample was weighed. Then 2 g of catalyst was put into the kjeldhal flask with the sample. Then it was digested for 4 hours in the kjeldhal digestion unit. The digest was diluted to 100 ml with distilled water. From that diluted solution 5 ml was taken into the distillation flask. 10 ml of 30% NaOH was added and flask was fixed to the kjeldhal unit. Steam was used to distill off the sample and the distilled-off was collected to the flask containing the 4% boric acid solution (20 ml) where the tip of the condenser was dipped under solution and was collected until no more air bubbles were produced.

A blank was also run and the collected solution was then titrated with the 0.02 M HCl solution.

Calculation,

$$\% \text{ Nitrogen} = \text{M. HCl} * \frac{(V1 - V_0)}{W} * M_N$$

$$\text{Protein } \% = 6.38 * \text{Nitrogen } \%$$

M. HCL	= Concentration of HCl acid.
V_1	= Titrate volume of cheese sample test
V_0	= Titrate volume of blank test.
M_N	= Molecular weight of nitrogen.
W	= Weight of sample.

3.3.2.4 Determination of Ash

Materials

Muffle furnace

Platinum dish

Electronic balance

Desiccator

Methodology

Accurately 5 g of sample was weighed into a clean and dried silica dish. Then it was ignited slowly over the Bunsen flame (inside a fume cupboard) until no more fumes were evolved. The dish was then transferred to the muffle furnace set at 500 °C. Then it was incinerated until the sample was free from black carbon particles (approximately 3 to 4 hours). This process of igniting, cooling and weighing was repeated at 30 minutes intervals until the difference between two successive weighing was less than 1 mg and the lowest mass was recorded.

Calculation,

$$\text{Total ash percentage} = \frac{(Wn - W) * 100}{W_i}$$

W_n	= mass in g of the dish with the ash
W_i	= mass in g of the sample
W	= mass in g of the empty dish

3.3.2.5 Determination of Microbial quality

3.3.2.5.1 Enumeration of yeast & molds

Materials

Auto clave

Graduated pipettes

Petri dish

Test tube

Electronic balance

Blender

Diluent

Peptone	1.0 g
Sodium Chloride	8.5 g
Water	1000 ml

Diluent was sterilized at 121 °C for 20 min

Yeast Extract-Dextrose-Chloramphenicol Agar

Yeast Extract	5 g
Dextrose ($C_6H_{12}O_6$)	20 g
Chloramphenicol	0.1 g
Agar	12 g to 15 g
Water	1000 ml

Medium was sterilized at 121 °C for 15 min

Methodology

Initially all Petri dishes, pipettes and beakers were sterilized at 121 °C for 40 min. Then 10 g of mozzarella cheese sample was blended with 90 ml of diluent in a blender to prepare the 10^0 dilutions. Then six serial dilution ranging from $10^{-1} - 10^{-7}$ was prepared by mixing 1 ml of test sample with 9 ml of diluent. Then 15 ml of culture medium was poured into fifteen petri dishes. After that 1 ml of serial dilution from $10^{-1} - 10^{-7}$ was transferred with seven pipettes. Control plate was prepared with 15 ml of the medium to check the sterility. Dishes were then inverted and incubated at 25 °C. Colonies were counted on each plate after 3, 4, and 5 days on incubation. This procedure was carried out for four different cheese samples (SLS 516: part 2: 1991).

$$N = \frac{\sum C}{(n_1 + 0.01\, n_2)\, d}$$

$\sum C$	= sum of colonies counted on all plates
n_1	= number of plates retained in the first dilution
n_2	= number of plates retained in the second dilution
d	= dilution factor corresponding to the first dilution

3.3.3 Sensory Evaluation

Materials

Sensory evaluation ballet paper

Coded samples

White glasses

Serviette

Glass of portable water

Methodology

Sensory evaluation was done with a participation of 32 untrained panelists of Faculty of Applied Sciences, Sabaragamuwa University with use of ballot papers. Acceptability of four samples was evaluated using 9- point hedonic scale subjectively. Four samples were coded as three-digit number (see App. 01). Coded samples, ballot papers and water glasses were given for each and every panelists and suitable environment was provided for them to do their evaluation unbiasely.

Result were analyzed using computer aided MINITAB 14 statistical Analysis Package According to Kruscal Wallis test at 95% level of significant level (see. App. i).

CHAPTER 04
Results and Discussion

4.1 Quality testing of milk
4.1.1 Organoleptic test

Taste	= Slightly sweet and salty taste
Smell	= Pleasant smell
Colour	= Yellowish white

To improve organoleptic quality of final product, receiving milk should have good organoleptic quality, such as pleasant sweet taste, flavour, smell and colour.
Slightly sweet and salty taste results from the balance between lactose and milk minerals. Flavour and aroma of milk are derived from the fat and the milk fat globule membrane. Compounds involved in determining flavour include carbonyls, alkanals, lactose, ester, sulphur- compounds, nitrogen- compounds and both aliphatic and aromatic hydrocarbons. Carotene pigment, which present in milk fat is responsible for yellowish white colour (Harding, 1995).

4.1.2 Microbial test
The absence of flocculation after addition of alcohol shows milk to have an acceptably low developed acidity. The alcohol acts by dehydrating and denaturing milk proteins. Positive results are obtained with colostrums or near-sour milks. The test can give false positives where mineral imbalance of the milk rather than developed acidity can be caused of flocculation (Varnam and Sutherland, 1991).

4.2 Mozzarella cheese
Mozzarella cheeses according to fat content are as follows.

Table 4.1 Fat percentage of Standardized milk samples

Cheese sample	Fat percentage
382	2.6 %
425	2.9 %
326	3.2 %
557	3.5 %

Four types of mozzarella cheese were produced by raw milk where fat percentages were 2.6 %, 2.9 %, 3.2 % and 3.5 %. They were coded as 382, 425, 326 and 557 with market sample was coded as 601.

4.3 Quality testing of mozzarella cheese

4.3.1 Determination of functional properties

4.3.1.1 Determination of meltability

Meltability was obtained for market sample and other four samples.

Following results were obtained by meltability test.

Table 4.2 Meltability of cheese samples

Sample code	Mean melt Score
After one week	
382	3.250 ± 0.463, 3.313 ± 0.458, 3.313 ± 0.458, 3.313 ± 0.458
425	3.563 ± 0.729, 3.438 ± 0.496, 3.438 ± 0.623, 3.313 ± 0.372
326	3.563 ± 0.623, 3.563 ± 0.496, 3.625 ± 0.582, 3.500 ± 0.463
557	4.000 ± 0.756, 3.938 ± 0.729, 3.813 ± 0.651, 3.938 ± 0.678
601	5.313 ± 0.884, 5.125 ± 0.582, 5.313 ± 0.372, 5.125 ± 0.582
After two week	
382	3.313 ± 0.458, 3.500 ± 0.463, 3.438 ± 0.563, 3.375 ± 0.443
425	3.625 ± 0.582, 3.625 ± 0.582, 3.563 ± 0.821, 3.625 ± 0.744
326	3.875 ± 0.518, 3.813 ± 0.594, 3.750 ± 0.598, 3.813 ± 0.594
557	4.250 ± 0.655, 4.250 ± 0.535, 4.313 ± 0.530, 4.313 ± 0.530
601	5.250 ± 0.756, 5.250 ± 0.463, 5.313 ± 0.530, 5.375 ± 0.876

Mean melt Score was obtained by the Melt sheet score results (see. App. ii)

Meltability is most important functional property to evaluate the quality of mozzarella cheese. Initially mozzarella cheese used as an ingredient for pizza, the most meaningful way to assess the melting property of pizza cheese is to subjectively evaluate its performance on pizza baked under commercial conditions. Meltability is expressed as a function of either decreased sample height or increased sample area. At this experiment the Schreiber melt test was performed to quantify the meltability of four types of mozzarella cheese.

According to the statistical analysis (See. App. iii) there was an interaction effect between sample code and storage time at a significant level of 0.05 ($p < 0.05$) in meltability of five cheese samples which are measured 1 week after production and 2 week after production.

Statistical analysis revealed that there is a difference between the mean of meltability and sample code at a significant level of 0.05 ($p < 0.05$). According to the literature, increasing the level of fat, which results in lower value of textural responses and higher values of meltability.

When two- way ANOVA was conducted for 14 days storage time, meltability was statistically difference ($p < 0.05$) among cheese samples with different storage times. The casein matrix in cheese became softer and less elastic during storage because of the breakdown of alpha (s1)-casein, which provides the major contribution to the structure of casein curd. If the alpha (s1)-casein in mozzarella is degraded by proteolytic cleavage, it loses the ability to link with other caseins, causing the protein matrix to lose strength and elasticity causing expected increasing meltability with the storage time (Tunick et al, 1992).

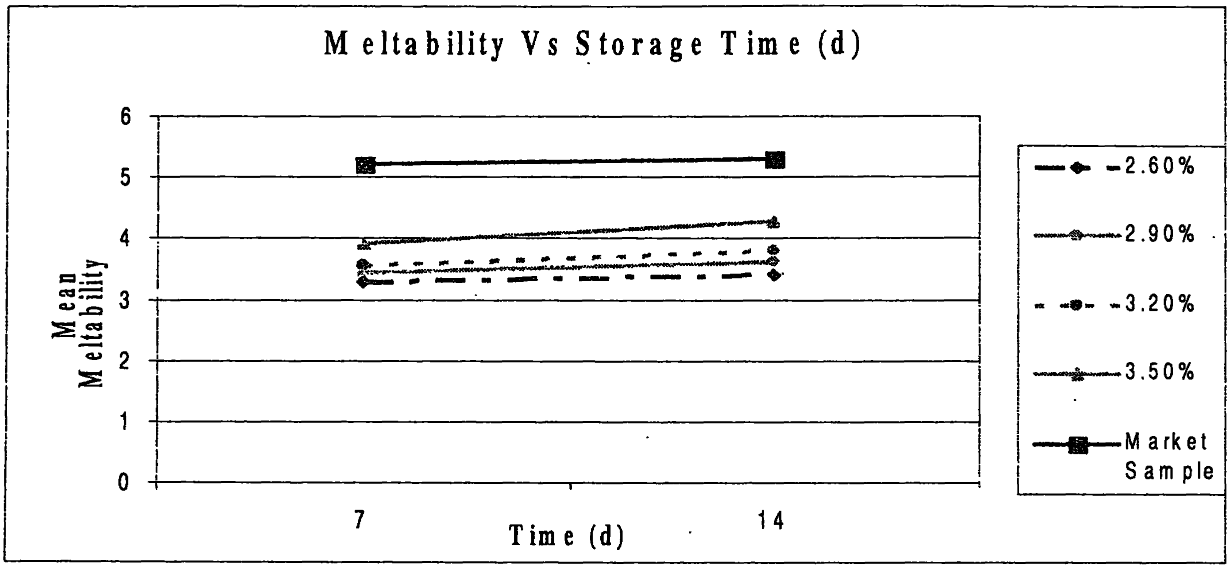

Fig 4.1 Meltability of the cheese sample.

4.3.1.2 Determination of Stretchability

Following results were obtained from stretchability test

Table 4.3 Stretchability of the cheese samples

Sample number	Ht before stretching (cm)	Ht after stretching (cm)	Increased Ht (cm)
After One week production			
382	1, 1, 1	7.7, 5.3, 6.4	6.7, 4.3, 5.4
425	1, 1, 1	7.1, 6.8, 6.9,	6.1, 5.8, 5.9
326	1, 1, 1	6.2; 7.9, 7.3	5.2, 6.9, 6.3
557	1, 1, 1	6.8, 6.7, 8.2	5.8, 5.7, 7.2
601	1, 1, 1	7.5, 8.1, 7.8	6.5, 7.1, 6.8
Two week after produce			
382	1, 1, 1	7.2, 6.4, 7.0	6.2, 5.4, 6.0
425	1, 1, 1	6.8, 7.6, 6.8	5.8, 6.6, 5.8
326	1, 1, 1	7.9, 7.1, 6.9	6.9, 6.1, 5.9
557	1, 1, 1	7.0, 7.7, 8.3	6.0, 6.7, 7.3
601	1, 1, 1	8.5, 8.1, 7.6	7.5, 7.1, 6.6

Stretchability is also most important functional property to evaluate the quality of LMMC. Stretchability is the ability of the melted cheese particles to form fibrous strands that elongate without breaking under tension (Kindstedt, 1999). In this experiment, Stretch length was measured by decreasing melt length from maximum stretch length According to statistical analysis (see.App. iv) there was no interaction effect between sample code and storage time at a significant level of 0.05 ($p > 0.05$) in the stretchability of five cheese samples.

Statistical analysis indicates that there was no difference between mean stretchability and storage time at a significant level of 0.05 ($p > 0.05$). But according to literature, there should be inverse relationship between stretchability and storage time. Change in physical properties of mozzarella cheese during storage might be attributed to the breakdown of (s1)-casein and beta-casein in cheese by residual coagulant and milk plasmin, the changes in the state of water in cheese and the increase in protein hydration sphere of cheese may be the cause for relationship.

When the two way ANOVA was conducted for mean stretchability and sample code, there was a statistically difference at a significant level of 0.05 ($p < 0.05$). Literature reviews that low fat cheese exhibit higher hardness than high fat cheese of comparable age. Fat globules are physically entrapped in the protein matrix of cheese and limit its deformation, with the decreasing fat in cheese thus cause increase in stretchability.

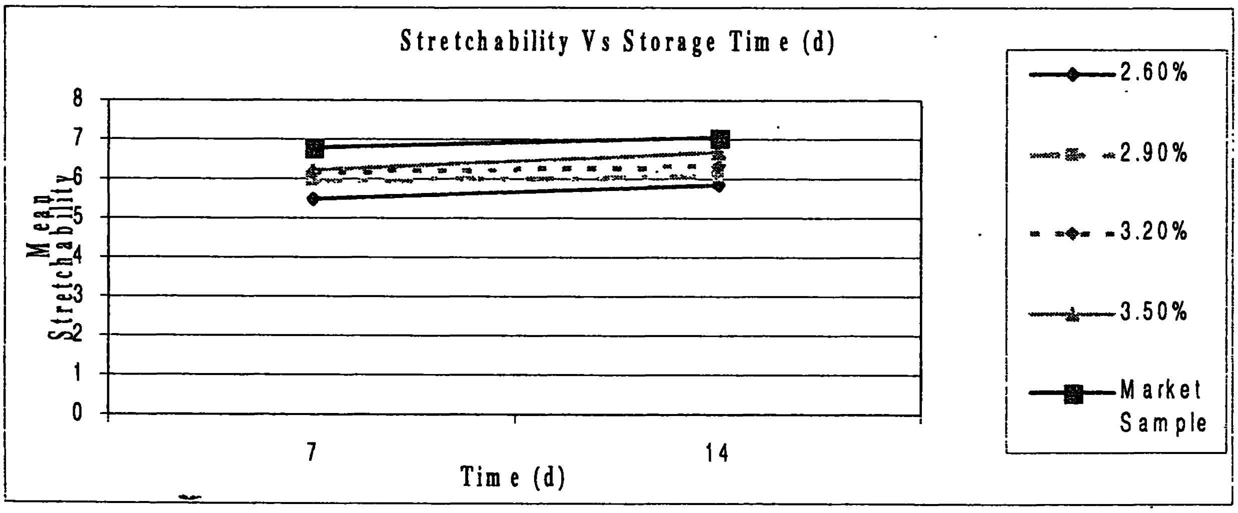

Fig 4.2 Stretchability of the cheese sample

4.3.2 Determination of Chemical properties

4.3.2.1 Determination of Moisture

Following results were obtained from the moisture analysis.

Table 4.4 Moisture content of the cheese samples.

Cheese Sample	Moisture %
382	50.89 %
425	50.10 %
326	49.60 %
557	49.34 %

As the fat percentage was increased, there was a decrease in moisture and protein content of the cheese. However, the moisture & protein did not replace the fat on an equal basis. But, the decrease in the overall amount of moisture is much more less than that of the protein amount. Reduced moisture levels resulted in greater values for hardness, gumminess, springiness, and chewiness; lower values resulted for cohesiveness and meltability. Water in cheese is either free or bound to the protein since fat; the other major constituent is hydroscopic. Decreasing moisture in cheese results in a firmer texture due to alterations in the casein matrix. In addition, water can act as either a lubricant or a plasticizer between the different proteins. Lowering the moisture therefore increases the hardness and springiness, along with gumminess and chewiness, while making the cheese less meltable. The lack of water also causes the cheese to be crumblier, which reduces its cohesiveness (Tunick et al, 1992).

4.3.2.2 Determination of Protein

Following results were obtained from the protein analysis.

Table 4. 5 Protein content of cheese samples.

Cheese Sample	Protein %
382	22.78 %
425	21.00 %
326	20.10 %
557	19.20 %

Protein percentages of four cheese samples were gradually decreased with the increasing fat percentage of cheese. The reason for this is the total protein and casein contents of milk gradually decreased as the fat content of the milk increased.

4.3.2.3 Determination of Fat

Following results were obtained from the fat analysis.

Table 4. 6 Fat content of cheese samples.

Cheese Sample	Fat %
382	19.70 %
425	23.04 %
326	25.84 %
557	29.35 %

Fat percentage of cheese samples were increased with the increasing of standardize fat percentage in milk. Fat performs many important functions within mozzarella cheese. It contributes to the taste, texture, functionality, and appearance. Because about 75 % of all mozzarella cheese is used as an ingredient for pizza, proper melt and appearance (browning and blistering upon heating) are important characteristics. When fat is removed from cheese, the overall quality of the cheese decreased.

4.3.2.4 Determination of Ash

Following results were obtained from the ash analysis.

Table 4.7 Ash content of the cheese samples.

Cheese Sample	Ash %
382	2.59%
425	2.58 %
326	2.50 %
557	2.97 %

The content of ash in four cheese samples were similar, because the rate of acid production and whey pH at draining were similar for all cheese samples. Cheese calcium and phosphorus level decreased with decreasing coagulation pH, resulting in a contaminant increase in meltability.

4.3.3 Enumeration of yeast and Molds

Following results were obtained from the Yeast and Molds test.

Table 4.8 Yeast and Molds content of the cheese samples.

Cheese Sample	Yeast and Molds g^{-1}
382	28 g^{-1}
425	32 g^{-1}
326	23 g^{-1}
557	29 g^{-1}

All the four samples were contaminated with yeast and moulds. The amount of yeast and mold present were lower than SLS specification for microbial limits in cheese. These amounts of yeast and mold presence indicate that samples were less contaminated.

4.3.4 Sensory evaluation

4.3.4.1 Effect of fat percentage on Flavour of the mozzarella cheese

The results of effect on the flavour of mozzarella cheese show the highest rank for the product, which contain 3.2 % of fat standardize in the milk and the cheese sample code 326. According to the data analysis there is significant difference between the samples at a significant level of 0.05 ($p < 0.05$). According to data sample, sample code 326 the highest sum of rank values with the highest estimated median for flavour. Therefore the samples come under the category of "Like Moderately" according to the 9-point hedonic scale (See.App. v).

4.3.4.2 Effect of fat percentage on Sourness of the mozzarella cheese

The highest rank for the sourness of the product, gained by the sample, which contained 3.2 % fat standardize in the milk and the cheese sample code 326. According to the data analysis there is a significant difference between samples at a significant level of 0.05 ($p < 0.05$). According to data sample, sample code 326 the highest sum of rank values with the highest estimated median for sourness. Therefore the samples come under the category of "Like Moderately" according to the 9-point hedonic scale (See.App. v).

4.3.4.3 Effect of fat percentage on Hardness of the mozzarella cheese

The results show the highest rank for the product, which was Market sample. According to the data analysis there is a significant difference between samples at a significant level of 0.05 ($p < 0.05$). According to the data Market sample gained the highest sum of rank values with the highest estimated median for hardness. Therefore the samples come under the category of "Like Moderately" according to the 9-point hedonic scale (See.App. v).

4.3.4.4 Effect of fat percentage on Colour of the mozzarella cheese

The results of effect on the colour of mozzarella cheese show the highest rank for the product, which contain 3.2 % of fat standardize in the milk and the cheese sample code 326. According to the data analysis there is significant difference between the samples at a significant level of 0.05 ($p < 0.05$). According to data sample, sample code 326 the highest sum of rank values with the highest estimated median for colour. Therefore the samples come under the category of "Like Moderately" according to the 9-point hedonic scale (See.App. v).

4.3.4.5 Effect of fat percentage on Appearance of the mozzarella cheese

The product, which contained 3.2 % of fat standardize in the milk and the cheese sample code 326 achieved the highest rank for the appearance of the mozzarella cheese. According to analyzed data there is significant difference between the samples at a significant level of 0.05 ($p < 0.05$). According to data sample, sample code 326 the highest sum of rank values with the highest estimated median for appearance. Therefore the samples come under the category of "Like Moderately" according to the 9-point hedonic scale (See.App. v).

4.3.4.6 Effect of fat percentage on Overall Appearance of the mozzarella cheese

The results obtained for overall acceptability of the mozzarella cheese show the highest rank for the product, which contained 3.2 % of fat standardize in the milk and the cheese sample code 326. According to analyzed data there is significant difference between the samples at a significant level of 0.05 ($p < 0.05$). The sample with 3.2 % of fat standardize in the milk gained the highest sum of rank value with the highest estimated median for overall acceptability. Therefore this sample comes under the category of "Like Moderately" according to the 9-point hedonic scale (See.App. v).

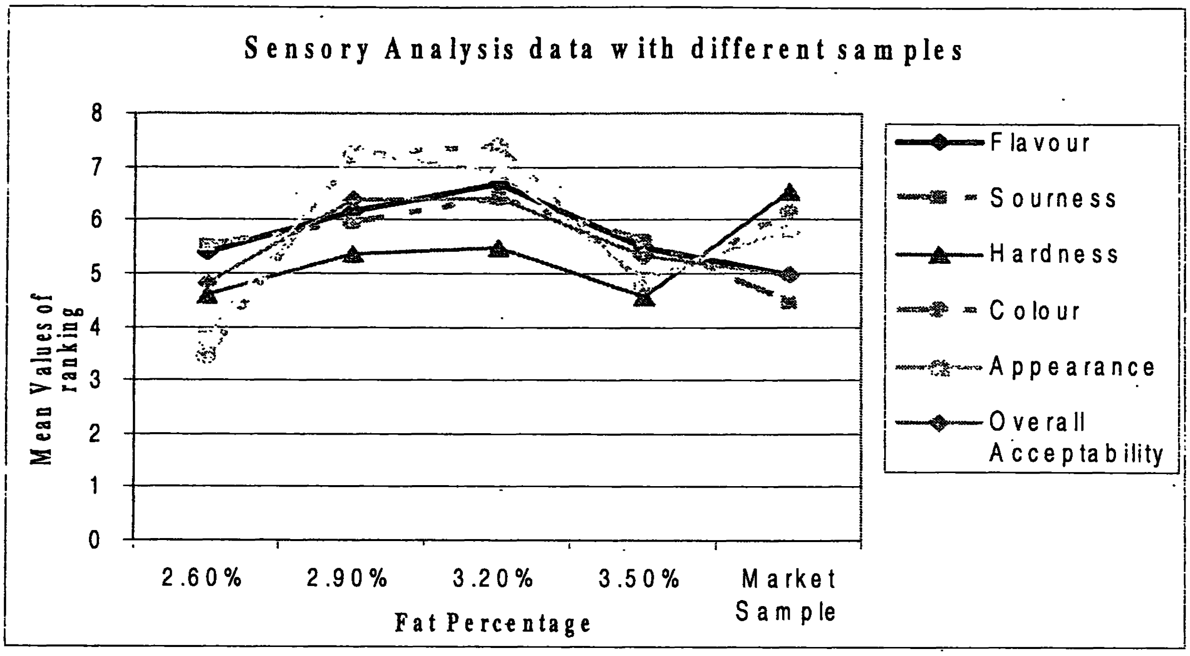

Fig 4.3 Sensory Characteristics of cheese samples.

CHAPTER 5

Conclusions and Recommendations

Increasing fat percentage of standardized milk brought several desirable changes in functional properties of LMMC. Meltability and stretchability increased with increasing fat content. Meltability of cheese increased with storage time. Recent findings reveal that there should be a interaction between strechability and storage time. But according to this finding there is no effect of storage time on the stretchability.

Fat percentage 3.2% can be recommended as the best fat content to produce LMMC with optimum quality characteristics.

The problems associated with sensory characteristics as hardness and functional characteristics as stretchability and meltability of the product should be developed.

Shelf life evaluation and suitable packaging material selection need to be further studied. Shreddability, formation of free oil, surface drying, burning and cooked colour must be examined to determine suitability of the cheese for commercial pizza manufacture.

Further work is required to produce Low fat mozzarella cheese hence there is a growing demand for low calorie, low fat cheese products in market. This Low fat mozzarella cheese should be developed to have acceptable functionalities and may require addition fat mimetics that entrap water and also provide lubricant properties typically provided by fat globules.

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Appendix i

Sensory Evaluation Data

Name : .... Date : ....

Product Description : ....

You are kindly requested to assess each food sample presented, with reference to under mentioned sensory attributes according to your preference considering the following scale.

9- Like Extremely
8- Like Very Much
7- Like Moderately
6-Like Slightly
5- Neither Like nor Dislike
4- Dislike Slightly
3- Dislike Moderately
2- Dislike Very Much
1- Dislike Extremely

Sample Code	Flavor	Sourness	Hardness	Color	Appearance	Overall Acceptability

Comments : ....

....

....

....

....

....

....

....

Thank you

Appendix ii

Mean melt score data for finding meltability

Sample number	Melt test score	Mean Melt test score
After one week product		
382	3, 3, 3, 3, 3, 4, 4, 3	3.250 ± 0.463
	3, 3, 4, 3.5, 3, 4, 3, 3	3.313 ± 0.458
	3, 4, 4, 3.5, 3, 3, 3, 3	3.313 ± 0.458
	3, 3, 3, 4, 4, 3, 3.5, 3	3.313 ± 0.458
425	3, 3, 3, 3.5, 3, 4, 5, 4	3.563 ± 0.729
	3, 4, 4, 4, 3.5, 3, 3, 3	3.438 ± 0.496
	3, 3, 3, 3, 3, 4, 4, 4.5	3.438 ± 0.623
	3.5, 3, 3.5, 3, 3, 3, 4, 3.5	3.313 ± 0.372
326	3, 3, 3, 3, 4, 4, 4, 4.5	3.563 ± 0.623
	4, 4, 3, 3, 3, 3.5, 4, 4	3.563 ± 0.496
	4, 4, 4, 4.5, 3, 3, 3, 3.5	3.625 ± 0.582
	3.5, 3.5, 4, 4, 4, 3, 3, 3	3.500 ± 0.463
557	3, 3, 5, 4, 4, 4, 4, 5	4.000 ± 0.756
	3.5, 3, 3, 4.5, 4, 5, 4, 4.5	3.938 ± 0.729
	5, 4, 4, 3, 3.5, 3, 4, 4	3.813 ± 0.651
	4, 5, 4, 4.5, 4, 3, 3, 4	3.938 ± 0.678
Market sample	4, 5, 5, 5, 5, 7, 6, 5.5	5.313 ± 0.884
	5, 5, 5, 5.5, 5.5, 6, 4, 5	5.125 ± 0.582
	5.5, 5.5, 5.5, 5, 5, 5, 5, 6	5.313 ± 0.372
	5.5, 6, 4, 5, 5, 5, 5, 5.5	5.125 ± 0.582

Mean melt score data for finding meltability

After two week product		
382	3, 4, 4, 3.5, 3, 3, 3, 3	3.313 ± 0.458
	3.5, 3, 3, 4, 4, 3, 3.5, 4	3.500 ± 0.463
	3, 3, 3.5, 4, 4.5, 3, 3.5, 3	3.438 ± 0.563
	3, 3, 3, 3.5, 4, 3, 3.5, 4	3.375 ± 0.443
425	4, 3, 3.5, 3, 3, 4, 4, 4.5	3.625 ± 0.582
	3, 3, 4, 3.5, 3, 4, 4, 4.5	3.625 ± 0.582
	3, 3, 3, 3, 3, 4, 5, 4.5	3.563 ± 0.821
	4.5, 3, 3, 3, 3, 3.5, 4.5, 4.5	3.625 ± 0.744
326	4.5, 4, 4, 4.5, 3, 3.5, 4, 3.5	3.875 ± 0.518
	4, 4, 4.5, 4.5, 3, 3, 4, 3.5	3.813 ± 0.594
	4.5, 4, 4, 4.5, 3, 3.5, 3, 3.5	3.750 ± 0.598
	4.5, 4, 3.5, 4.5, 3, 3, 4, 4	3.813 ± 0.594
557	4, 4, 3, 4.5, 5, 5, 4, 4.5	4.250 ± 0.655
	3.5, 4, 4, 4.5, 4, 5, 4, 5	4.250 ± 0.535
	3.5, 4, 4, 4.5, 5, 5, 4, 4.5	4.313 ± 0.530
	3.5, 4, 4, 4.5, 4.5, 5, 4, 5	4.313 ± 0.530
Market sample	6, 5, 5, 5.5, 5, 6.5, 4, 5	5.250 ± 0.756
	5, 5, 5, 5.5, 5.5, 6, 4.5, 5.5	5.250 ± 0.463
	5, 5, 5, 6, 5.5, 6, 4.5, 5.5	5.313 ± 0.530
	5, 5, 5, 6.5, 6.5, 6, 4, 5	5.375 ± 0.876

Appendix iii

Statistically Analysis data for finding meltability

Two-way ANOVA: Mean Melt versus Time (d), Sample code

```
Source        DF       SS       MS       F      P
Time (d)       1   0.3748  0.37481   79.89  0.000
Sample code    4  18.6892  4.67229  995.86  0.000
Interaction    4   0.1032  0.02579    5.50  0.002
Error         30   0.1408  0.00469
Total         39  19.3079

S = 0.06850   R-Sq = 99.27%   R-Sq(adj) = 99.05%


                Individual 95% CIs For Mean Based on
Time            Pooled StDev
(d)      Mean   ---------+---------+---------+---------+
 7    3.88785   (---*----)
14    4.08145                            (---*----)
                ---------+---------+---------+---------+
                     3.920     3.990     4.060     4.130


                  Individual 95% CIs For Mean Based on
Sample            Pooled StDev
code       Mean   -----+---------+---------+---------+----
326     3.68775         *)
382     3.35188   (*)
425     3.52375      (*)
557     4.10188               *)
601     5.25800                                 (*
                  -----+---------+---------+---------+----
                     3.60      4.20      4.80      5.40
```

Appendix iv

Statistically Analysis data for finding strtchability

Two-way ANOVA: Stretch (cm) versus Time (d), Sample Code

Source	DF	SS	MS	F	P
Time (d)	1	0.5880	0.58800	1.37	0.256
Sample Code	4	5.4647	1.36617	3.18	0.036
Interaction	4	0.1087	0.02717	0.06	0.992
Error	20	8.5933	0.42967		
Total	29	14.7547			

S = 0.6555   R-Sq = 41.76%   R-Sq(adj) = 15.55%

```
                  Individual 95% CIs For Mean Based on Pooled
Time              StDev
(d)       Mean    +---------+---------+---------+---------
 7     6.11333    (-------------*-------------)
14     6.39333              (-------------*-------------)
                  +---------+---------+---------+---------
                  5.75      6.00      6.25      6.50
```

```
                    Individual 95% CIs For Mean Based on
Sample              Pooled StDev
Code        Mean    -----+---------+---------+---------+----
326      6.21667            (---------*--------)
382      5.66667    (---------*---------)
425      6.00000          (--------*--------)
557      6.45000                (---------*--------)
601      6.93333                       (---------*--------)
                    -----+---------+---------+---------+----
                       5.40      6.00      6.60      7.20
```

Chart of Mean(Stretch (cm)) vs Time (d), Sample Code

Appendix v

Statistically Analysis data for finding sensory evaluation parameters

Kruskal-Wallis Test: Flavour versus Sample Code

Kruskal-Wallis Test on Flavour

Sample Code	N	Median	Ave Rank	Z
326	32	7.000	105.5	3.41
382	32	6.000	69.5	-1.51
425	32	6.500	87.8	1.00
557	32	6.000	73.1	-1.02
601	32	5.000	66.7	-1.89
Overall	160		80.5	

H = 15.57 DF = 4 P = 0.004
H = 16.12 DF = 4 P = 0.003 (adjusted for ties)

Kruskal-Wallis Test: Sourness versus Sample Code

Kruskal-Wallis Test on Sourness

Sample Code	N	Median	Ave Rank	Z
326	32	7.000	100.6	2.74
382	32	6.000	80.5	-0.00
425	32	6.000	88.3	1.06
557	32	6.000	79.3	-0.17
601	32	4.000	53.9	-3.63
Overall	160		80.5	

H = 17.48 DF = 4 P = 0.002
H = 17.97 DF = 4 P = 0.001 (adjusted for ties)

Kruskal-Wallis Test: Hardness versus Sample Code

155 cases were used
5 cases contained missing values

Kruskal-Wallis Test on Hardness

Sample Code	N	Median	Ave Rank	Z
326	31	6.000	81.4	0.47
382	31	5.000	63.9	-1.96
425	31	6.000	77.6	-0.05
557	31	5.000	60.0	-2.49
601	31	7.000	107.1	4.03
Overall	155		78.0	

H = 21.24 DF = 4 P = 0.000
H = 21.74 DF = 4 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Colour versus Sample Code

Kruskal-Wallis Test on Colour

Sample Code	N	Median	Ave Rank	Z
326	32	7.000	117.7	5.08
382	32	3.000	32.4	-6.57
425	32	7.000	112.5	4.37
557	32	5.000	54.5	-3.54
601	32	6.000	85.4	0.67
Overall	160		80.5	

H = 80.81 DF = 4 P = 0.000
H = 82.69 DF = 4 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Appearance versus Sample Code

Kruskal-Wallis Test on Appearance

Sample Code	N	Median	Ave Rank	Z
326	32	7.000	106.4	3.53
382	32	3.000	40.1	-5.52
425	32	7.000	115.8	4.82
557	32	5.000	58.0	-3.07
601	32	6.500	82.2	0.23
Overall	160		80.5	

H = 60.48 DF = 4 P = 0.000
H = 62.12 DF = 4 P = 0.000 (adjusted for ties)

Kruskal-Wallis Test: Overall Acceptability versus Sample Code

155 cases were used
5 cases contained missing values

Kruskal-Wallis Test on Overall Acceptability

Sample Code	N	Median	Ave Rank	Z
326	31	7.000	98.2	2.80
382	31	5.000	58.5	-2.70
425	31	7.000	95.7	2.46
557	31	6.000	70.7	-1.01
601	31	5.000	66.8	-1.55
Overall	155		78.0	

H = 19.67 DF = 4 P = 0.001
H = 20.18 DF = 4 P = 0.000 (adjusted for ties)

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