

PRODUCTION OF A BUFFALO MILK CHEESE

By

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DECLARATION

I here by declare the work reported in the project report was exclusively carried out by me, under the supervision of Prof.A. Bamunuarachchi Mr.T.D.W.Siriwardhana and Dr. K.K.D.S. Ranaweera. Any part of this project report has not been submitted earlier or concurrently for same or any other degree.

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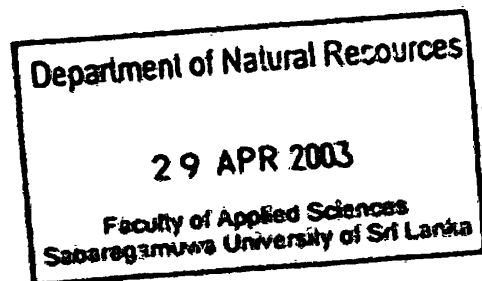
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AFFECTIONATELY DEDICATED TO

MY EVER LOVING PARENTS

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ABSTRACT

In Asia the buffalo contributes over one third of the total milk production. Buffalo milk is rich and creamy because it has a higher proportion of fat, protein, lactose and minerals as compared to cow's milk.

In Sri Lanka there is an abundant of buffalo milk that is about 81 million liters / annum, but no one make an attempt to manufacture cheese from buffalo milk at commercial level except curd and yoghurt.

Therefore, the objective of this study was to produce one of the major types of cheeses, Gouda from buffalo milk that will be marketable in Sri Lanka.

After quality testing of raw buffalo milk the general procedure of cheese manufacturing was proceeded with suitable modifications to obtain required quality buffalo milk Gouda cheese. The production process was scaled- up using the cheese vat.

A sensory evaluation test was carried out for the final product with comparing commercially available cow milk cheese to for the consumer acceptability. The cost of the finished product was also calculated.

The cheese made from buffalo milk contain higher levels of fat, protein, lactose, minerals, and vitamins more than cheese made from cow's milk. Therefore, the nutritional value of the buffalo milk cheese is greater than cow milk cheese.

The moisture content of the final product was in acceptable level, 52.7% , which was in the range of normal semi – hard Gouda cheese from 45 – 55%.

The cost evaluation for the production process without ripening and final processing of the cheese is lower than cow milk cheese available in the market. There high nutritive, low cost buffalo milk cheese can be obtained by this manufacturing process.

According to the results of sensory evaluation, this product got similar consumer acceptability for colour and appearance, but aroma, flavour and texture were differed from the cheese made from cow milk, which are already available in the Sri Lankan market. But the prepared sample was an unripened and unprocessed one, which was not to ripened due to the time limitation for the study. If the product allowed ripening more than 2 months, appearance, texture, aroma and flavor will develop same as commercially available cheese, could be get more accurate results for the sensory evaluation process.

Finally we can conclude that the preparation of cheese can be done using buffalo milk and the products could be obtained very much similar to the product available at the market after the ripening process.

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CHAPTER 1

INTRODUCTION

In addition to being delightful foods that contribute variety and interests to our diets, cheese of various kinds always has been important sources of nutrients to man whenever milk producing animals could be raised. The major ingredient used in cheese manufacturing is the curd of the milk of cows and other mammalian animals. Milk is a nearly perfect natural food contains nutrients such as proteins, fats, carbohydrates, vitamins, and minerals in such proportion that those nutrients could act as a team in the body building process and other physiological functions.

Over one third of total milk production in Asia is contributed by buffaloes. In Sri Lanka there's abundant buffalo milk that is about 81 millions/annum., although the use of buffalo milk for production of milk – based products is very rare, except curd and yoghurt manufacturing. In other countries buffalo milk is processed to produce cheese, ice cream, dried milk powder, UHT cream, casein and butter due to various functional and compositional properties of buffalo milk.. But no one make an attempt to manufacture cheese from buffalo milk available in Sri Lanka. Therefore it is a market requirement that the production of cheese from buffalo milk, which will be able to market in Sri Lanka.

Cheese can be defined as a product made from the curd of the milk of cow and other animals, the curd been obtained by the coagulation of the milk casein with an enzyme (usually rennin) or with an acid (lactic acid) with or without further treatment of the cur by heat, pressure, salt and ripen (usually with selected micro organisms).

Gouda cheese is an important semi-hard variety which requires shorter ripening period than other cheese varieties. Gouda cheese has been prepared from buffalo milk by modifying the production process which was used to produce Gouda cheese from cow's milk. A Gouda type cheese from buffalo milk was successfully made after standardization of the milk to a casein/fat ratio of about 0.7 and dilution with (10-15) % of water prior to pasteurization in India.

OBJECTIVES

- 1. To diversify the processed product range from buffalo milk**
- 2. To expedite the possibilities of the manufacture of Mozzarella and Gouda cheese using buffalo milk.**
- 3. To compare the organoleptic properties of buffalo and cow milk based cheese.**

CHAPTER 2

LITERATURE REVIEW

2.1 Buffalo Milk

Among the farm livestock, buffalo plays a pivotal role in agricultural economy of many Asia, European, African and Latin American countries as an animal for milk, meat and a beast of burden. In tropical and subtropical regions of the world revere buffaloes contribute towards milk production because of their ability to thrive under different ecologies. In Asia the buffalo contributes over one third of the total milk production. In terms of the percentage of world milk from buffaloes, following countries can be ranked.

Table 2.1: Buffalo milk production in the world

Country	Percentage of milk from buffaloes
India	65.44
Pakistan	21.84
China	4.98
Egypt	4.15
Nepal	1.72

2.1.1 Composition of Buffalo Milk

Buffalo milk is rich and creamy because it has a higher proportion of fat, protein, lactose and minerals as compared to cow's milk. In general buffalo milk fat content tends to vary between (7.22-12.60)% , protein vary between (3.60-6.04)% , lactose between (3.70-5.48)% and ash between (0.70-0.86)% .

2.1.2. Factors Affecting the Composition of Buffalo Milk

The milk composition is influenced by a number of factors which can be genetic, physiological and environmental. Nutrition levels, feed stage of lactation, and other animal husbandry practices also effect milk composition. A comparative summary of the gross chemical composition of buffalo milk, milk from Zebu cow (*Bos indicus*) and western cow (*Bos taurus*) is delineated in table 2.3.

Table 2.2: The major constituents of buffalo and cow milk (per 100g milk)

Constituents	Buffalo	Cow
Water (g)	76.05	37.60
Fat (g)	12.46	3.80
Protein (g)	6.03	3.30
Lactose (g)	4.90	4.70
Calcium (g)	0.19	0.12
Retinol (mg)	65	Winter-25 , Summer – 35
Carotene (mg)	-	Winter-12 , Summer – 25
Thiaminc (mg)	50	45
Riboflavin (mg)	100	180
Folic acid (mg)	-	6
Vitamin B ₁₂ (mg)	0.03	0.35
Energy content :		
kJ	430	280
Kcal	102	67
Solid-Not-Fat (g)	11.70	8.70
Total solid (g)	24.16	12.50

In consistency in the composition of milk not only influences the quality of dairy products, but may also influence the adapted processing strategy, seasonal variations in milk composition creates new challenges, peculiar to buffalo milk processing.

2.1.3. Functionality and Physicho-Chemical Properties of Buffalo Milk

Precise and in depth information of the Physicho-chemical and functional attributes of milk, is an essential prerequisite for its automated industrial processing. Most of the prevalent processing technologies, apparently originated in the western world, when cow milk and milk products pre-dominated. Therefore, the processing strategies were essentially based on the knowledge of chemistry and functionality of cow milk. Species related differences in milk composition have become subject of topical interest, as processing of milk from species other than cow, is been adopted by the dairy industry in several countries of the world. With the emergence of buffaloes as a prominent milk species particularly in countries of Asia, Africa and Latin America, been made to develop and adopt appropriate technologies for buffalo milk processing. It becomes essential to understand the unique physicho-chemical and functional

properties of buffalo milk to overcome the various challenges encountered during its processing.

2.1.3.1. Colour and Flavour of Buffalo Milk

White to milky appearance of milk is associated with the scattering phenomenon of light by the fat globules and the colloidal calcium caseinate and Phosphate in milk. Fresh buffalo milk is whiter in colour than cow milk, and exhibited a slight blue, green blue. This has been attributed to the larger size of buffalo fat globules, colloidal casein and the presence of blue green biliverdin in buffalo milk. Buffalo milk and milk products are readily differentiated from that of cow by appearance along as colour difference are often striking. Absence of carotenoids is a unique and distinguishing feature of buffalo milk. The characteristic of white-pale yellow colour typical of cow milk is due to carotenoids.

Buffalo milk has a smooth, pleasant, slightly sweeter and richer flavour than cow milk, apparently due to the higher fat, protein and lactose concentration.

2.1.3.2. Acidity and pH

The titrable acidity and lactic acid content of fresh buffalo milk are 0.16% lactic acid and 1.82mg/100ml respectively. These values are relatively higher than 0.15%, 1.41mg/100ml reported for cow milk. The development of acidity is relatively faster in buffalo milk than, cow milk, possibly due to higher lactose content. Acidity development is faster in raw buffalo milk than in heated milk.

Pasteurization, boiling or sterilization of buffalo milk do not result in a significant change of milk acidity. The pH of fresh buffalo milk varied between 6.63 – 6.08 which is apparently higher than 6.5 – 6.7 reported for cow milk. Heating of milk particularly in the range of (10-70) °C, resulted in a decrease in pH. Dilution of milk with water and separation of fat resulted in pH increase.

2.1.3.3. Buffering Capacity

The presence of protein, phosphates, carbohydrates, citrates and number of other minor constituents, make fresh buffalo milk a complex buffer. The buffalo milk exhibited a higher buffering capacity with a peak value of 0.0417 obtained between acidic pH ranges of 4.8 – 5.2.

2.1.4. The Importance of Buffalo Milk

The compositional and physico-chemical attributes of buffalo milk which make it unique and appropriate for processing have been delineated in the following accord. The important features of milk's composition are its content of fat non-fat- solids (SNF) and total solids.

And also higher total solids in buffalo milk affect the higher yield of milk products, such as cheese or concentrated milk in addition to them.

Table 2.3: Physical characteristics of cow and buffalo milk

Character	Cow	Buffalo
Colour	Yellowish	White
Specific gravity	1.0307	1.0314
Viscosity (cP)	1.236	1.262
Globule size (u)	2.9-3.4	5.4-5.6
Globules / ml (Av.)	1118	828

Inherently high fat content results in better yield of cream, butter buffalo ghee. High protein concentration of buffalo milk than cow's milk enhances the yield of casein and caseinates. Cow's milk generates 89 cal/100g from total solids, protein and fat while buffalo milk produces higher energy per unit weight 109 cal/100g. Although fat contributes high energy from them approximately 9 kilo cal/ g. Milk fat is rich in a significant amount of essential fatty acids such as linoleic and arachnoids which also acts as a carrier of fat soluble vitamins A, D,E and K. It plays a distinctive role as a flavouring agent in dairy products. The rich pleasing flavour of milk fat is not adequately duplicated by any other type of fat. Undoubtedly, the fine body and texture in most dairy products is one factor that makes them so appealing to the consumer. They imparts soft , smooth and rich testing qualities and overcome flat , hard , grainy and watery characteristics which are normally encountered in their absence.

2.1.5. Manufacture of Products from Buffalo Milk.

Buffalo are the second largest source of milk supply in the world. In India half of the milk processed by the organized dairies comes from buffalos as they are better converters of local feeds into milk than cows.

The excess fat in buffalo milk is usually skimmed off to play for the processing. Buffalo milk fat has less cholesterol and more tocopherol, which is a natural antioxidant. Buffalo milk is richer in calcium and phosphorous and lower in sodium and potassium than cow milk. The peroxidase activity in buffalo milk is much higher than in cow milk which accounts for the natural preservability. Buffalo milk, being significantly different in composition from cow milk, has posed a number of technological problems in its processing for the manufacture of dairy products. Until about a decade ago it was felt that buffalo milk was probably not suitable for the manufacture of dairy products, mainly because the existing technology for

processing cow milk proved unsuitable for the buffalo milk. (Dalaya *et al.*, 1971). This called for a new look at the physico-chemical changes that occur in buffalo milk during heat treatment, rennet treatment and microbial fermentation.

As a result new methods had to be found and today milk products such as butter, cheese, condensed milk, skim milk powder, infant food and fermented milk products (cheese, yoghurt) are being successfully manufactured from buffalo milk in addition to a wide range of indigenous milk products.

2.1.6. Problems Encountered with Buffalo Milk Processing

Techniques and dairy equipment designed for the processing of cow milk are often not adequately suited for buffalo milk processing. It has been recognized that processing parameters for manufacturing cow milk products, cannot be applied in to buffalo milk. Dairy processors experienced several technological challenges due to the unique compositional, physico-chemical and functional attributes of buffalo milk. It becomes necessary to introduce several process modifications in the manufacture of buffalo milk products.

Various physico-chemical differences in buffalo milk that contribute to the unique challenges in its processing.

1. Composition of buffalo milk is effected to a much greater extent by factors such as age, stage of lactation and season that in cattle. Compositional inconsistencies results in the lack of uniformity in the composition of buffalo milk products.
2. Buffalo milk has a much higher proportion of fat, protein, lactose and calcium as compared to cow milk. The high fat content in buffalo milk makes it incumbent to standardize fat: SNF ratio of milk by either skimming or adding of non-fat solids as additional step during its processing.
3. Buffalo milk casein micelles are relatively bigger in size (135 μ m) than those of cows (90 μ m) and appear more opaque and aggregated. A large micelle size is likely to influence buffalo milk processing by membrane technologies such as ultra filtration and reverse osmosis. Buffalo milk casein terms of micelle size, voluminosity (solvation), susceptibility to rennet, compositional heterogeneity and mineral constituents.

4. The structural and chemical properties of milk fat greatly influence the melting point crystallization behaviour emulsifying property solubility and surface activity. (Ramamurthy, 1976).
5. Buffalo milk has a higher pH and viscosity.
6. The buffering capacity of buffalo milk is relatively higher due to higher mineral load.
7. The ripening or fermentation process is often slower in buffalo milk.
8. The rennet clotting time of buffalo milk is much lower due to high level of calcium.
9. The curd tension of buffalo milk is higher when compared to cow milk.
10. Heating resulted in more browning in buffalo milk than in cow milk. The higher level of lactose and protein seem to accelerate mellowed browning during buffalo milk processing.
11. Polymerization of casein due to heating is considerably higher in buffalo milk than cow milk.

2.1.7. Other Milk Sources

A number of species of large domesticated animals are used to provide milk for human consumption. Milk of different mammals is consumed as sources of milk on a geographical basis. In Sri Lanka cows, buffalos and goats are the main produces of milk. In Southern Europe goats and sheep, lapps of Northern Europe, water buffaloes in South East Asia, cows in Europe, Asia, Africa, Australia, New-Zealand etc., reindeer in cold climatic regions, camels in Middle-East countries are used as sources of milk. The other animals used for milk production are llama, horse and zebu.

2.2. Cheese

Cheese solid or semisolid food product prepared from the milk of the cows, ewes, goats water buffaloes or other mammals as a means of preserving the food value of milk. Most cheese today is made from cow's milk. Cheese made from the milk of the ewes and the goats is richer in protein than that produced from the milk of the cows.

Cheese has been made since prehistoric times. It is believed that the first cheese was produced inadvertently, probably through the practice of carrying milk in pouches made from animal stomach. The bacteria in the milk and the digestive juices from the stomach work together to form a curd and then a curd cheese. Cheese making artifacts dating from 2000 BC have been found. The Romans developed a large cheese industry, and later, making became a specialty

of monasteries. Many European abbeys developed secret recipes, and particular varieties began to develop in certain regions of Europe. Today, although mechanization has largely replaced the old hand-techniques, the characteristics of hundreds of individual cheese types have been preserved.

According to the definition given in SLS standards cheese can be described as the fresh or matured product obtained draining after coagulation of milk, cream, skimmed or partially skimmed milk, butter milk or a combination of some or all these products. Cheese is one of the oldest and most nutritious food product. It is an important item in the diet of almost all people, because it is relatively easy to make and can be preserved for long period. It may be used as a snack, in salads, as a cooking cheese in casserole dishes, or grated for use in pizza and other food. It may be include as an ingredient in processed cheese.

According to the SLS standards, the finished product should satisfy the following requirements.

- 1. The products shall be cream or yellowish in colour. This requirement will not be applicable to mould ripened cheese.**
- 2. The product shall have a pleasant odour and the flavour characteristic of the particular variety of cheese.**
- 3. The product shall be good texture and of uniform consistency.**
- 4. The product shall be clean and free from dirt and extraneous matter.**
- 5. The product shall also conform to the requirements specified in table.**

2.2.1 Different Varieties of Cheese

The diversity of cheese types is truly breathtaking. Despite the limited range of raw materials (Bovine, Ovine, Caprine, or Buffalo milk.) approximately 500 varieties of cheese recognized by the International Dairy Federation Bank are produced.

A number of attempts have been made to make the classification scheme more discriminating by including factors such as origin of the cheese milk, the moisture content, which is controlled by the extent of cutting and draining of the curd; the types of the microorganisms added the encouraged to grow; the temperatures and acidity employed in the production process in addition to humidity, an length of maturation, or ripening and time are primarily responsible for the character of a cheese. The percentage of fat in the milk is also important. The higher the fat content the more mellow and attractive the cheese. Low – fat cheeses are tough and lack flavour.

Table 2.4: Requirements for Cheese

Characters	Requirements			
	Hard	Semi Hard	Soft	Processed / Spread
Fat % by mass in (on dry basis)	45.00	45.00	45.00	45.00
Moisture % by mass	34 – 45	45 – 55	55 - 80	35 – 80
Dry matter * % by mass	55 – 65	45 – 55	20 - 45	20 – 65
Salt % by mass	3.00	3.00	3.00	3.0

*Dry matter = 100 – Water content

2.2.1.1 Classification of Cheese

It is difficult to make a strict classification of all the various existing types of cheese, because there are many broad – line cause. The following descriptive criteria are normally used.

Method of coagulating casein in curd making;

We distinguish here between rennet and acid cheese. Some cheeses are produced with both lactic acid and rennet, are called acid rennet cheeses, cottage cheese belongs to this category.

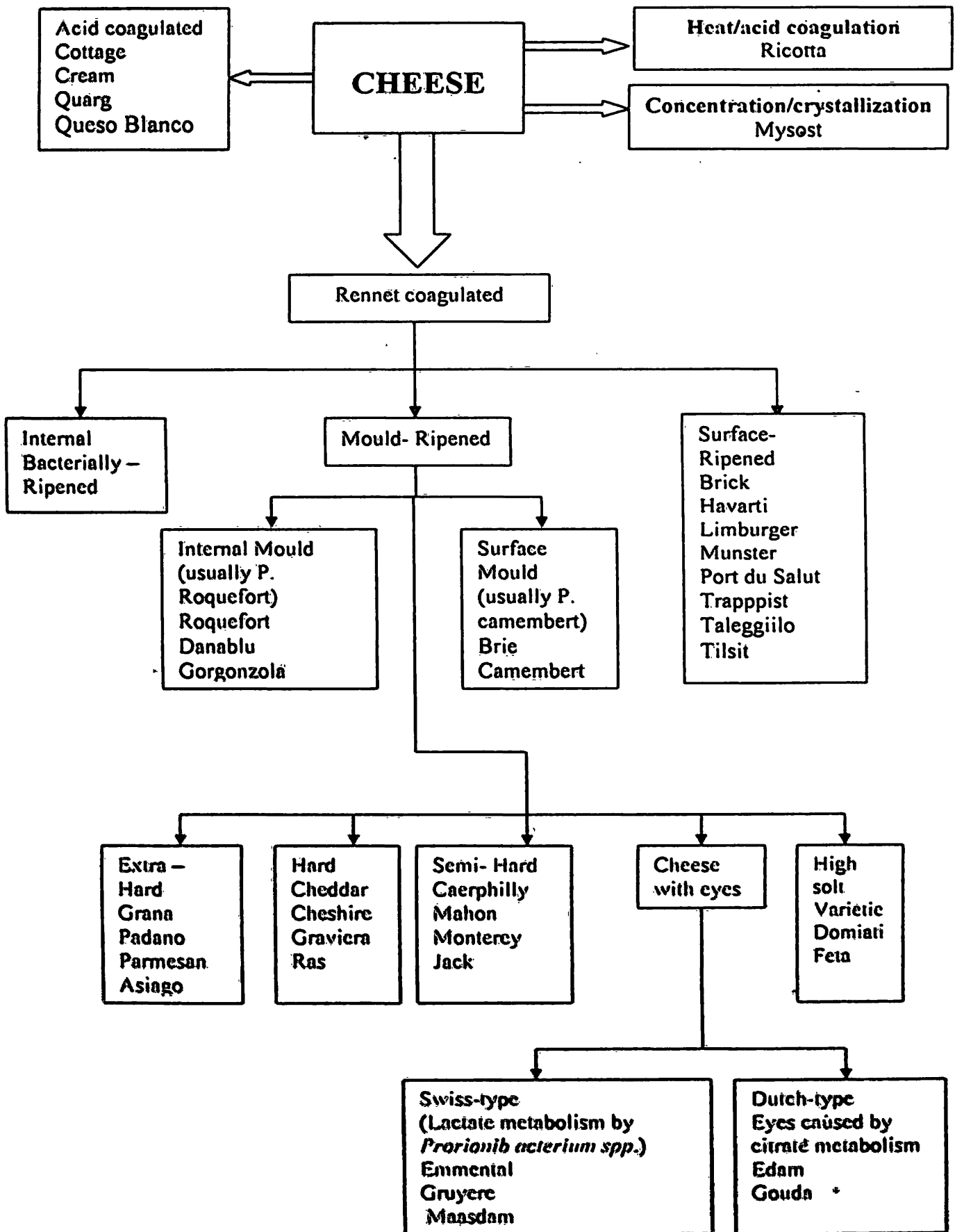
Water content;

We distinguish between hard semi-hard and soft cheeses. In hard cheeses such as Emmenthal and cheddar, a large proportion of moisture is drained from the curd during the process, making the finished cheeses hard and dry. Soft cheeses like camembert and Brie have much higher moisture content in the curd.

Principal microorganisms used for ripening;

There are smear cheeses ripened with bacteria smeared on the surface, such as Tilsit, Port Salut and St. Paulin; blue-veined cheeses like Roquefort and Gorgonzola; and white mould cheeses like Brie and Camembert.

Figure 2.1: The diversity of cheese



Texture of the cheese :

We distinguish between round eyed, granular and close-textured cheese. The holes or eyes in cheese are formed during the ripening process by lactic acid bacteria which involve carbon dioxide as a by product of lactose fermentation.

The carbon dioxide collects in interstices in the curd. If the curd is molded in to cheeses in the atmosphere, air will be trapped between the curd grains. Carbon dioxide will then collect at these, interstices to form "granular" eyes. Tilsit is an example of a granular cheese. If the cheese is molded below the surface of the whey, to exclude air, the interstices will be fewer in number, and the carbon dioxide collecting in them will form round bubbles, eyes, when the curd is pressed. Gouda is an example of a round-eyed cheese. Close-textured hard cheeses are made with starter cultures which involve very little carbon dioxide, and all the lactose is fermented before final forming take place. The only extant hard cheese with a close texture is cheddar cheese, and that name will be used in what follows here to denote close-textured cheeses.

In Sri Lanka, cheeses can be classified in to four major categories, according to SLS standards.

Hard cheese – Ripened cheese with moisture content with in the range of 35% to 45%

— E.g. Cheddar, Cheslire, Cantal, Emmental

Ripened cheese – Varieties of cheese with appropriate microbial growth on the surface, which contributes to the characteristic flower of the cheese. The microbial growth may include yeast, mould and bacteria or combination of these microorganisms.

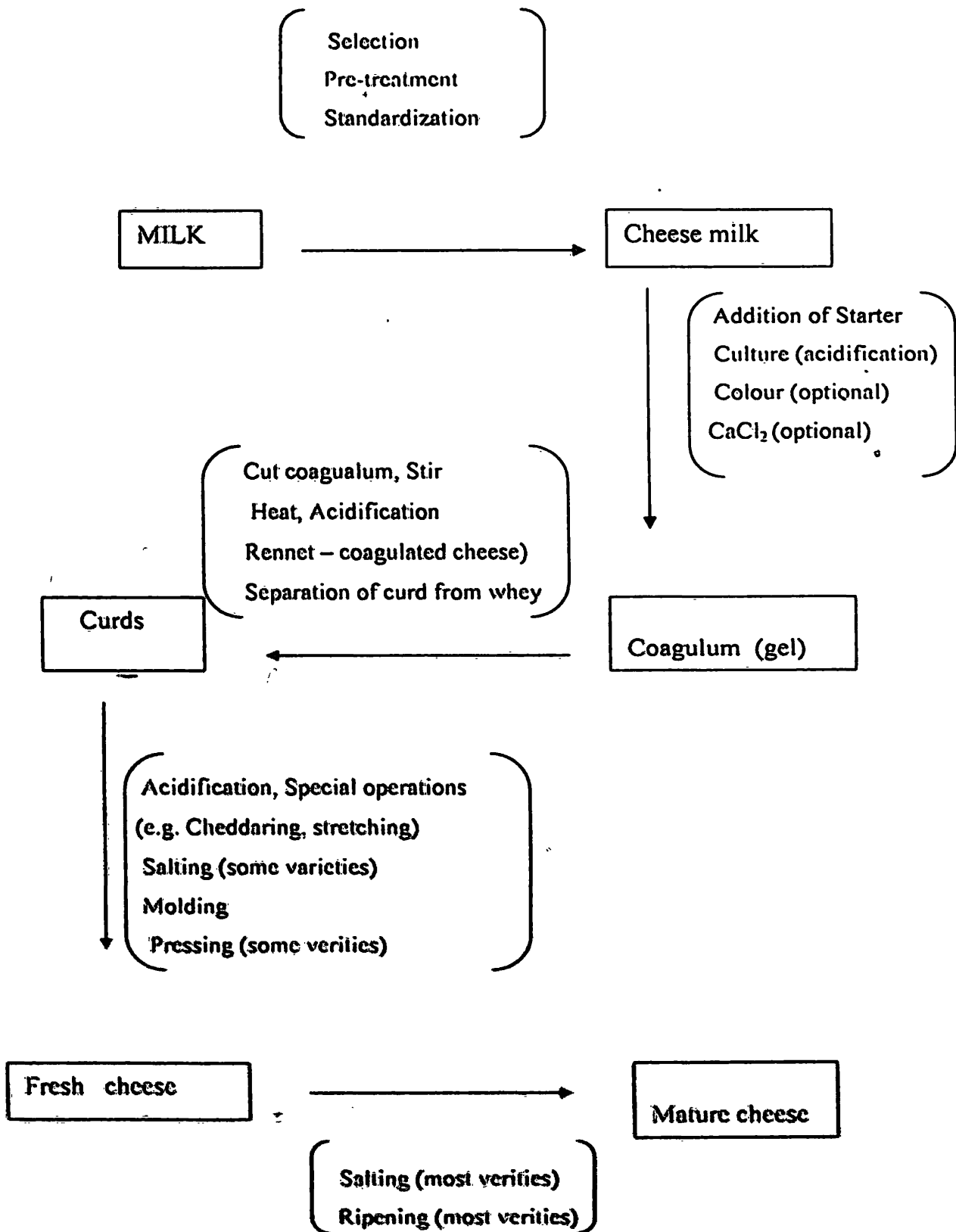
Semi Hard Cheese – Ripened cheese with moisture content is with in the range of 45% to 55%. E.g. Gouda, Mozzarella, ,Caerphilly, Taleggio, Liederkranz

Soft Cheese - Ripened or unripened cheese with moisture content with in the range of 55% to 80%. E.g. Cambridge, Coulommiers, Quarg, Cottage

Processed Cheese or Cheese Spread - The product obtained by heating cheese with permitted emulsifiers and / or stabilizers or with or without buffering agents.

2.3. Process of Manufacture of Cheese

Figure 2.2: General protocol for cheese manufacture



2.3.1. Modern Cheese Making

Until about 1850 most cheese was produced in small dairies. The first cheese making factories were merely enlarged forms of the farm house dairy, but gradually the equipment became large and mechanized. Since 1945 cheese making equipments and methods have changed radically. Today factories can handle up to 1,000,000 liters (about 264,000 U.S. gal) of milk a day. Equipments covered rather than open and aseptic techniques are used to produce starter. Further improvements include ever-increasing automation, controlled temperatures for maturing, intensive pressing, cutting in to retail-size film pressed to form blocks are either dipped in wax or wrapped in plastic film and left to cure at temperatures of about 10 °C (50 °F).

Although it is considered mature at 3 months, well matured cheddar should be kept for 12 months or longer. A good cheddar should be homogeneous (without holes or cracks), creamy white or yellow in colour, and have a mild cheesy odour. Cheddar is sometimes coloured in red orange by adding Annatto, a vegetable dye to the milk. Processed cheese is a mixture of ground cheese (usually cheddar and other hard varieties), emulsifying salts such as phosphates, other ingredients such as milk powder, whey powder, and coloring and flavoring materials, and sometimes, spices. Vegetable gum is often added to produce a chewy texture. Steam is blown into the mixture to raise the temperature to 80 °C (150 °F) or higher. This yields a molten plastic mass that is then poured into a metal or ceramic container, or into portion-containers for foil wrapping.

2.3.2. Cheese Ingredients and Functions

Starter - The cheese industry understands that the term 'starter' means a culture of desirable microorganisms usually bacteria in some nutrient materials, which is usually milk. This bacteria has the function of fermenting the lactose of milk to produce lactic acid, which in turn, curdles and coagulates the milk. They also contribute to the flavour of the cheese. Most starters used in cheese making are controlled cultures of *Streptococcus lactis*, *Streptococcus cremoris* and *Streptococcus diacetylactis*. The acid conditions created by the starter also help to inhibit the growth of undesirable bacteria, which may otherwise cause spoilage in the finished product.

Colour - Normally β carotene and annatto dyes are used. β -carotene and annatto are natural vegetable colouring, which can be used if required to give a yellow and orange / red colour respectively.

Coagulant- The coagulation is usually assisted by the addition of rennet. This is a natural extract from the stomach of the calf and it contains the enzyme rennin that acts upon the protein in the milk in such a way to cause its coagulation. Rennin assists in cheese making in two ways. Firstly, it coagulates the milk. Secondly, if rennin serves the heating to which it may be exposed after it is added to milk, its action continues in the curd after removal of the whey and during holding and curing of the cheese. This action helps in the development of a good, sharp, cheese flavour, an improved texture and smooth mellow body in curd cheese.

Additives- Additives of calcium chloride to milk influence the clotting activity of rennet. The more calcium added the higher the activity. Salt removes excessive whey trapped in the curd and helps in the development of the improved texture and a smooth body in curd cheese.

Salt – Addition of salt helps to preserve the cheese by inhibiting the action of bacteria and brings out the flavour.

2.3.3. Nutritional Value

Hard cheese such as cheddar is one of the most concentrated of common foods. Hundred grams (about 3.5 oz) supplies about 36 percent of protein, 80 percent of calcium, and 34 percent of the fat in the recommended daily allowance. Cheese is also a good source of some vitamins and minerals. Nutritionally, milk is best preserved by condensing or drying, as no part of it is lost. Conversion to cheese is an excellent method, because virtually all the fat and most of the protein are retained, and the latter is partially digested.

The curd form in the first stage of the cheese making is composed of about the half the total solids of whole milk. It contains virtually all the fat and casein, about two thirds of the calcium, most of the vitamin A, one quarter of the riboflavin and about one sixth of the thiamin in the original milk. The lactose, whey proteins, and the rest of the minerals and vitamins remain in the whey. The curd formed during the making of skim milk cheese contains only about one third of the solids of the original milk. The losses of lactose, minerals and vitamins in the whey are very much of the same as those during cheese making from whole milk.

The fate of the nutrients in the curd during ripening depends on the type of the cheese. Hard and the semi hard cheese may lose small amounts of nutrients, in the whey expelled during pressing, but the activity of bacteria during ripening leads to the synthesis of several water soluble vitamins. Blue-veined cheese receive an additional contribution to their content of water soluble vitamins from the growth of *Penicillium spp.* mould. Much of the vitamin

content synthesized during the ripening of soft cheese remain in the rind and may contribute little nutritionally since the rind is often not eaten.(see table 2.6)

Table 2.5: Percentage of nutrients in curd and whey from whole milk

Nutrient	Curd	Whey
Fat	94	06
Protein	75	23
• Casein	96	04
• Whey	04	96
Lactose	06	94
Calcium	62	38
Vitamin A	94	06
Thiamin	15	85
Riboflavin	26	74
Folic Acid	05	95
Vitamin B ₁₂	25	75
Vitamin C*	06	84
Total Solids	48	52

*Some Vitamin C is destroyed by the action of light.

2.3.4. Whey

Whey is the liquid remaining after the separation of curd in cheese making. It contains the whey proteins, lactose, and much of the minerals from the original milk. Today cheese whey is condensed or dried and used for animal feeds. For human nutrition, dried whey is used in the baking industry, in the manufacture of whey soups and sauces and of fruit whips. An increasing industrial use of whey is as a source of lactose, which is used by the pharmaceutical industry to dilute drugs in pills. (See table 2.7)

Because of cheese is a high protein food, it is an ideal nutritional replacement for meat in a vegetarian diet. It is rich in the essential amino acids, calcium, phosphorous. other minerals, and vitamins and has a high caloric value. It is a particularly good food for children.

2.4. Buffalo Milk Cheese

Cheese made from buffalo milk tends to have a hard and dry body and a short and crumbly texture, combined with slow ripening capacity. Buffalo caseins micelles, being larger in size retain less water than do cow caseins micelles during curd formation. The voluminosity of

Table 2.6: Composition of cheese of various types (per 100 g)

Constituent	Hard cheddar	Semi-Hard Edam	Blue-Veined Roquefort	Soft Camembert	Cottage Cheese
Water (g)	35	43	40	51	79
Fat (g)	33	24	31	23	0.4
Protein (g)	26	26	21	19	16.9
Calcium (g)	0.83	0.76	0.32	0.38	0.09
Vitamin A (retinol eq. µg)	380	250	300	240	3
Thiamin(µg)	50	60	30	50	30
Riboflavin(mg)	0.50	0.35	0.70	0.45	0.28
Energy Content					
KJ	1670	1330	1500	1180	340
Kcal	400	320	360	280	82

the caseins micelles from buffalo milk is less than that from cow milk and it rapidly decreases with temperature range (35-45) °C. Hence, it is necessary either to drain the whey earlier than is the case with cow or add Sodium Chloride while cheddaring the cheese cubes to retain moisture.

Dr. J.Czulak of Australia has standardized a method of producing cheddar cheese from buffalo milk. In India both the Kaim District Milk Producer's Union (commonly known as AMUL) and scientists at NDRI have successfully prepared Sumti cheese

Table 2.7: Composition of cheddar cheese whey

Constituents	Percentages
Water (g)	93.3
Fat (g)	0.3
Protein (g)	0.9
Lactose (g)	4.7
Calcium (g)	0.05
Retinol (µg)	2.0
Thiamine(µg)	40.0
Riboflavin (µg)	80.0
Folic Acid (µg)	5.0
Vitamin B₁₂ (µg)	0.15
Vitamin C (mg)	1.0

and Karnal cheese from buffalo milk (Bhattacharya et.al, 1969, 1970, 1972). In Italy a typical local cheese prepared from buffalo milk is mozzarella cheese. In a pilot study conducted in Iraq under the auspices of FAO, a method suitable for the manufacture of mozzarella cheese from buffalo milk in the country has been developed. AMUL has also stated production of such cheese.

2.4.1. Buffalo Milk Cheese Varieties

1. Buffalo milk Mozzarella Cheese

Mozzarella is a well known variety of Italian cheese which has gained popularity throughout the world. In Italy, the name 'Mozzarella' is exclusively used for the cheese prepared from buffalo milk without the admixture of milk from the species. The term "Mozzarella di bufala" in Italy now enjoys a legal production as a product that is made strictly from buffalo milk. Mozzarella is used on Pizza toppings, which is undoubtedly a very popular product in United State and Europe.

Two approaches for manufacture of buffalo milk Mozzarella cheese have been standardized. The conventional approach involves fermentation of milk by starter cultures rennet coagulation separation of curd, stretching and brining of the product. The other procedure often referred as direct acid Mozzarella, involves addition of acids instead of starter culture fermentation before renneting.

The quality of buffalo milk Mozzarella cheese was affected by a number of factors such as pasteurization of milk , level of fat in milk, type of starter culture used , type of acids used,

extent of thermal treatment during manufacturing and type of enzyme used for clotting the milk.

The stretchability of Mozzarella cheese is an important character which determines its acceptability level of fat in milk, did not affect cheese stretchability significantly. However the cheese from low fat (3% fat) course and hard. Excessively high fat (5% fat) in milk resulted in a soft product which exhibited excessive fat leakage on a pizza. Mozzarella cheese prepared from milk with 4% fat was suitable for pizza topping.

It was suggested that Mozzarella prepared using a culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in 1:1 ratio, produced cheese which had the flavour, textural and rheological quality suitable for use as a pizza topping.

The influences of producing variables such as preheating of milk, pH of acidification, on the cheese texture have been investigated.

2. Buffalo Ricotta Cheese

Buffalo Ricotta is prepared from the whey left after production of mozzarella di bufala cheese. Pure white in colour, Buffalo Ricotta has a fine, slightly moist texture and a bland sweetish flavor. Like fresh, unripened cheeses, Ricotta does not have a long life and must be consumed within a short time.

3. Requeson Cheese from Buffalo Milk Whey

Requeson is a whey cheese, which has been traditionally made from buffalo cheese whey. Two methods of manufacturing Requeson were standardized. In the first method, Calcium Chloride was added to the milk along with heating to precipitate the whey protein. Better product yields were observed when Calcium Chloride was used for Requeson manufacture. However, the cheese manufactured using citric acid received higher overall sensory scores than the cheese manufactured using Calcium Chloride. The product obtained by using citric acid had a higher fat content of about 20%. The shelf life of Requeson cheese was about 6 days at a storage temperature of about 4°C.

4. Stracchino Type Buffalo milk Cheese

Stracchino is a generic term used for a range of Italian cheese. These cheeses are generally characterized by a rich soft texture and a white appearance. Stracchino cheeses are usually rindless and are consumed fresh. Stracchino type cheeses may be either mild (dolce) or sharp

(piccants). Most Stracchino cheeses are ripened for a short period of ten to fifteen days. These cheeses are often so delicate, that they are rarely exported. Surplus buffalo milk is often converted to cheeses of this variety particularly to obtain a rich high fat product. The buffalo milk Stracchino type cheeses were characterized by a high fat content of about 59% (on dry matter basis) and a total nitrogen content of about 5.4%. The product exhibited a titratable acidity (owing) between (1.0-1.2) % (lactic acid equivalent) and calcium to phosphorous ratio of 1.7.

5. Buffalo milk Telemea cheese

Telemea or Teleme is a popular cheese produced in several Balkar countries. It is believed to have originated in Greece. Teleme cheese was usually made from sheep milk, however buffalo milk was who used obtain a creamy and rich product. Sometimes a mixture of buffalo and cow milk was used to produce the cheese. Telemea cheese was characterized by a soft, moist texture and a creamy white colour. It was ripened for about ten days in brine. It is often classified as a pickled cheese because of its typical sharp flavor and salty taste.

6. Surti Cheese

Surti cheese is one of the best known varieties of Indian buffalo milk cheese. It is a popular product in the western states in India. The cheese apparently originated in the town of Surat, where it was traditionally prepared from buffalo milk by the addition of a curd rennet preparation. Relatively few rennet coagulated products are traditionally produced in India as heat and acid coagulated traditional products such as 'Khoa' and 'Paneer' are preferred. Surti cheese was prepared from buffalo whole milk.

6. Domiati Cheese

Domiati is a soft, white Egyptian cheese named after the town of Domiati (Domiatta) in northern Egypt. Best quality Domiati is traditionally prepared from Buffalo milk, however, cow milk or a mixture of cow and buffalo milk, may also be used. The cheese is either consumed fresh or after pickling in salted whey, which imparts a sharp salty tang. Fresh buffalo milk with about 7% fat was preferred as it gave a rich and a superior grade Domiati cheese. Salt was added at usually high levels varying between 5 to 15 percent.

8. Karish Cheese

Karish, Kariesh or Kareish cheese is one of the most popular soft fresh lactic cheeses of Egypt. It contains all the skim milk constituents, most of the calcium and phosphorous and a typical sharp flavor. In southern part of Egypt, fresh buffalo milk is often allowed to ferment in earthenware called "Matrad" or "Zeer" to prepare a curd traditionally known as Labun Rayeb a Labun Matrad (Abou- Donia and Donia, 1984). Buffalo milk in a Matrad, is left undisturbed in a warm dark place until the cream rises and the milk coagulates due to natural fermentation. The cream is removed from the top and beaten into butter and the curd is either consumed fresh as is made into Karish cheese. It is estimated that about 30% of the milk in Egypt is converted to Karish cheese. Karish cheese is traditionally made from defatted

9. Mish cheese

Mish cheese is an important variety of Egyptian cheese, which is often consumed as an appetizer before meals. Mish has a characteristic yellowish brown colour, a sharp distinct flavor and high salt content. It is prepared by pickling Karish cheese in a pickling medium traditionally in earthenware jars called "zallaa" or "Ballass" and stored for ripening for a period of more than one year.

10. Ras cheese

Ras cheese locally known as Kefalotyri is a popular variety of hard Egyptian cheese. It is consumed after ripening for about 3 to 6 months. Traditionally whole buffalo milk was used for Ras manufacture, however sometimes, cow milk or mixture of buffalo and cow milk are used.

11. Buffalo milk Brinza Cheese

Brinza or Bryndza is a Hungarian pickled cheese, matured in brine or whey. The cheese is traditionally made from sheep milk, however in Egypt buffalo milk or sometimes from a mixture of cow, goat and buffalo milk.

12. Buffalo milk cheddar cheese

Some compositional and functional characteristics of unripened and ripened buffalo milk cheddar cheese manufactured by the standardized process have been presented in table.

Table 2.8: Compositional and functional characteristics of unripened and ripened cheddar made from buffalo milk.

Characteristics	Ripening period (months)		
	0	6	12
Moisture (%)	39.32	34.95	32.09
Fat (%)	29.32	31.50	32.93
pH	5.00	5.55	5.80
Non protein nitrogen/ total nitrogen	4.10	20.65	22.44
Total volatile fatty acids (ml .1N NaOH/10g cheese)	3.51	6.53	8.47
Free fatty acids (μ Mol/g fat)	3.47	9.22	13.91

Buffalo milk cheddar cheese manufactured by the modified process, almost similar moisture and fat content as in cheese made from cow's milk. However, the ratio of non protein nitrogen concentration of total volatile fatty acids and free fatty acids in buffalo milk cheddar were significantly lower when compared with cow milk cheese. Slow ripening of buffalo milk cheddar cheese, was a critical limiting factor impeding the utilization.

13. Gouda cheese from buffalo milk.

Gouda cheese was originally prepared in Holland and Utrecht. It is an important semi-hard variety prepared from cow milk. By suitably modifying the production process, acceptable quality Gouda cheese has been prepared from buffalo milk.

14. Buffalo milk Brick Cheese

A procedure to manufacture brick cheese using buffalo milk was standardized (Satia, 1969). Compositional characteristics of brick cheese manufactured by the standardized process from buffalo milk have been described in the table (Satia, 1969).

15. Blue Veined Cheeses from Buffalo milk

The blue veined cheeses are one of the finest varieties of cheeses. The best known cheese of this type is the Roquefort cheese, which is primarily made from ewe's milk. A distinct feature

of these cheeses is that ripening is done by mould *Penicillium roqueforti* and *Penicillium glaucum*.

Gross composition of blue veined cheese prepared from buffalo milk as well as cow milk has been given in the table. Loss of fat in whey was higher during manufacture of cheese from buffalo milk. The total yield of cheese was, however more from buffalo milk.

16. Buffalo Milk Swiss Cheeses

Swiss cheeses are characterized by the presence of shiny eyes slightly woody texture, sweet fragrance and a hazelnut like flavor. These cheeses are popular due to cheddar cheese. Swiss cheese was prepared from buffalo milk after modifying the production process.

Buffalo milk Swiss cheese ripened for about 6 months had lower moisture content than cow milk Swiss. However the product had higher level of fat and salt content. The average composition of Swiss cheese made from buffalo and cow milk has been delineated in the table.

17. Cream Cheese from Buffalo Milk

A standardized process was developed for the manufacture of cream cheese from buffalo milk. Cream cheese prepared by the standardized process, exhibited a shelf life of about 25 days, when stored at 5°C.

18. Buffalo Milk Cottage Cheese

Cottage cheese made from buffalo milk exhibited a rather hard and coarse texture with poor absorption of cream dressing. The following modifications were introduced in the manufacturing protocol to produce cottage cheese of acceptable quality.

1. Addition of 0.1% sodium chloride to pasteurized and cooled skim milk (31°C).
2. Cutting the coagulum with ½ inch knife at an acidity of 0.7% lactic acid.
3. Cooking the curd at 60°C in 75min.

2.4.2 Problems Associated with Buffalo Milk Cheese Manufacture

The problems involved in cheese manufacture from buffalo milk have been recently reviewed by Ganguli (1978) from the standpoint of the defect physico-chemical properties cited above.

The major defects, probable reasons for these and techniques for overcoming them are summarized below.

Major defects:-

- Slow development of acidity.
- Faster renneting time than in cow milk.
- Low retention of moisture by micelles.
- Slow flavor development.
- Slower proteolysis and lipolysis than in cow milk.

Probable reasons for defects :-

- High buffer capacity due to high mineral content.
- High level of Calcium ions (Ca^{++}) in the micelles.
- Low voluminosity at (35-40)°C and large micelles size.
- Slow primary action of rennet and low susceptibility of casein to proteolysis.

Modified techniques:-

- Add more starter culture to enhance acidity development.
- Lower the rennet concentration to prolong the milk clotting time.
- Reduce the (cheddaring) heating period to retain more water in cheese as use a salting process.
- Standardized casein to fat ratio to 0.7:1.0 by using cow milk powder to improve ripening process.

2.5. Dutch – Type Cheeses

A group of cheese originated in Holland are sometimes referred to as Dutch cheeses. The best known and popular of these are Edam and Gouda. Both are semi-soft to hard. Cheeses having a characteristic mild flavour that varies in intensity with age. The basic manufacturing procedure is similar to cheddar with modifications to obtain higher moisture content and the curd is not generally salted prior to hooping.

The Edam cheese is made in to round ball-shaped cheeses weighing from about ½-1+½ lb in the United States and is covered with a red wax.

Gouda is made in to a shape of a flattened sphere and may weighing from less than 1 lb to 50 lb. most common are the smaller sizes of less than 1 lb and having the red wax coating similar to Edam.

The Dutch-Type Varieties of Cheeses are Defined as those that ;

Are made of fresh cows' milk, the milk being at most partly skimmed (generally leading to at least 40% fat in the dry matter of the cheese.)

Are clotted by means of rennet (usually extracted from calves' stomachs)

Use starter consisting of *Mesophilic lactococci* and usually *Leuconostocs* that generally produces CO₂.

Have a water content of the fat-free cheese below 63% (ratio of water to solids-not-fat < 1.70).

Are pressed to obtain a closed rind.

Are salted after pressing, usually in brine.

Have no essential surface flora

Are at least somewhat matured (a few weeks) and thus have undergone significant proteolysis.

Consequently the cheese usually has a semi- hard to hard consistency and a smooth texture, usually with small holes, the flavour intensity varies widely.

So defined, Dutch-type varieties constitute one of the most important type of cheeses like Gouda and Edam produced in the world comparable in that respect to cheddar and the group of white, fresh cheeses.

Variation within the type is considerable;

Loaf shape may be sphere (Edam) a flat cylinder with bulging sides (Gouda)

Fat content in the dry matter ranges from 40 to over 50%

Water content in the dry matter ranges from 40 to 50%

Salt content in the cheese ranges from 2 to 7%

pH may be anywhere from 5.00 to 5.6

Maturation may be taken from 2 weeks to 2 years

2.5.1. Composition of Buffalo Milk Gouda Cheese

Gouda cheese prepared from buffalo milk, had a higher level of fat, moisture, lactose and ash as compared to cow milk cheese. The various compositional and functional characteristics of Gouda cheese prepared from buffalo as well as cow milk have been described in the table 2.9.

2.6. Cheese Plant Equipment

One of the widely used operations in the food industry is the manufacture of cheese. Many plants still used the old style rectangular tank system; however the new semi continuous process, which was developed in Australia, is also making progress. One of the principal developments in the cheese – manufacturing business has been increased emphasis upon sanitary precautions and more exact biological control.

Cheese Vats

The vat consists of a metal or wood outer box and an inner metal tank, supported on a wooden beam, which allows space water between the two tanks. The typical cheese tank has an H – shape steam pipe in the bottom of the outer tank to distribute heat evenly throughout the

Table 2.9: Gross Composition of Gouda Cheese Manufactured from Buffalo and Cow Milk

Constituents	Buffalo Milk	Cow Milk
Moisture (%)	45.91	43.74
Fat (%)	28.05	27.67
Total nitrogen (%)	3.53	3.775
Soluble nitrogen (%)	0.0846	0.1978
Non protein nitrogen (%)	0.0604	0.0808
Lactose (%)	1.07	0.84
pH	5.68	5.39
Ash (%)	3.50	3.05

vessel. It is important that the vat and the outer jacket be provided with the proper drainage for the both the product and the condensate from the steam. Cheese vats are obtainable in sizes in 100 to 2500 gal capacity.

The cheese vat is used for making for wide variety of cheeses. However, in the basic process system, it is necessary to;

1. Set the curd by means of suitable starter or enzyme and
2. To cook the curd , the system usually includes a holding period at constant temperature during the setting operation. Cooking and agitation of the curd, draining of the whey and washing of the finished curd .

In the manufacture of cottage cheese , it is frequently found advisable to add salt and creamed dressing , as well as do a thorough job of mixing in the vat, before the finished product is packed.

The Agitator

Since proper agitation of the curd is very important in the manufacture of cheese, it is important that some mechanical means be provided. This is usually accomplished by what is known as a forking type agitator. The agitator moves and cools the curd after it is cut. As it moves back and forth from one end to the other or between the location of an adjustable stop, the entire contents of the vat are agitated and mixed. The machine is a greater labour server and helps to obtain uniformity of the product.

The Cheese Curd Mills

In the manufacture of cheddar and some other types of cheese, it is necessary to cut the slab of curd into relatively small pieces or chunks so that they can properly drain out of the curd and also to enable it to be washed thoroughly and to provide an opportunity for salt to become more thoroughly mixed with the curd.

The basic principle of the curd mill is that of a rotating cylinder on which sharp discs are mounted in such a manner that when the livery slab cheese run through it, the cheese is cutting to strips.

The Cheese Press

One of the final processes in the manufacture of cheese is the placing of the cheese in hoops, which are then putting a press and pressure applied. The newer type of pressures utilizes a hydraulic control system which is labour saving and quite effective.



Plate: 3.1. The Cheese Vat

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1. Quality Testing of Raw Buffalo Milk

Quality and quality control are very important aspects in the milk and milk processing industry because it has a great influence on the quality of the final product. Following test were carried out to assess the quality of the raw buffalo milk.

3.1.1 Organoleptic Tests

That is the assessment of quality by the evidence of the senses. As far as milk is concerned, this term is limited to smell and colour although appearance is strictly speaking an organoleptic property. Taste, odour and colour of raw buffalo milk were tested.

Materials

Bottle of raw buffalo milk

Beaker - 250ml

Method

About 150 – ml of milk was transferred to a 250 ml beaker, tested for taste, smell and colour.

3.1.2. Physical Properties

Each physical property of milk is, of course, a resultant determination by the contributions of its constituents. Obviously, physical properties vary with composition. Furthermore, the process to which milk is subjected in manufacturing and utilization induce changes in the constituents that are reflected in physical properties.

1. Specific Gravity

Materials

Specific gravity bottle (50ml) with a well fitted ground glass stopper.

Milk sample

Water bath maintained at 30 °C

Thermometer

Electronic precision balance

Method

Previously weight – W_1 specific gravity bottle was filled with distilled water at 30 °C until overflowing and by holding the bottle in such a manner to prevent the entrapment of air bubbles.

The stopper was inserted and the bottle was immersed in a bath maintained at 30 °C. After 30 minutes the bottle was removed from the water bath and allowed to dry completely.

The weight of the bottle and its content was measured – W_2 . Then the specific gravity bottle after cleaning and drying was filled with milk and the weight – W_3 was taken as described earlier.

3.1.3 Chemical tests

1. Titrable Acidity

Materials

Milk sample

Burette – with 0.1ml graduation

Beakers

Electronic precision balance

Conical flasks – 100ml

Wash bottle

Stirring rod with policeman

Phenolphthalein 1% - 1g in 100ml of 95% ethanol

Sodium Hydroxide 0.1 N – 0.49 g in 100ml

Method

5ml of milk thoroughly stirred was transferred to a 100ml conical flask using a pipette. The pipette was rinsed using two volumes of water and transferred the rinse to the flask containing milk and thoroughly mixed. 0.5ml of phenolphthalein indicator was added and titrated with 0.1N sodium hydroxide to obtain the first permanent (30sec) colour change, to pink. The volume of 0.1N sodium hydroxide required for the titration was recorded.

3.1.4. Microbiological Tests

1. Clot on Boiling

Materials

A milk sample

Beaker – 100ml

Thermometer

Bunsen burner with tripod stand

Method

A small quantity of milk was boiled for five minutes whilst maintaining the temperature at 100 °C. The beaker was examined for any signs of coagulation or fine particles of curd on the inside surface of the beaker.

3.2. Preparation of Cheese Using Buffalo Milk as the Raw Material

The method used for the production of hard and semi-hard cheese can be regarded as modification of the method used in the production of the cheddar cheese. For each variety the cheese maker has selected the treatment conditions that will allow the curd to give the required acidity, moisture content and allow the development of the texture and flavour during ripening. Almost every stage of the cheese making process can be modified. Most of the different characteristics of varieties of cheese results from differences in fineness of cutting the curd during milling, the temperature to which the

curd is heated and the pressure applied during pressing. In this method, whole milk was used for the preparation. The milk used is of high fat content. The procedure used for the manufacture of Gouda – type cheese was adopted.

Scale up the production process of buffalo milk Gouda cheese

Mechanization has been extensively introduced into cheese plants, many difficult and labourious operations have now been taken over by machines.

The cheese tank is equipped with a combined cutting and stirring tools; the heating and cooling processes are accomplished by circulation of the media through the jacket or by direct addition of hot water through a sprinkle system. The hole curd making process is controlled from a panel according to a present program in which all times, temperature and operations are specified.

Materials

Cheese vat and other instruments.

Weighing scale

Muslin cloth

Cheese mould and press

Plastic colander

Ingredients

Milk 25liters

Freeze-dried lactic (Mesophilic Homeofermentation) culture for direct vat set – 0.75g

Rennet (100% Chyomin as the active enzyme) – 1g

Salt

β - Carotene.

Method

The milk was tested for acidity after filtering and pasteurized in cheese making vats by LTLT (at 63 °C for 30 minutes) and then cooled to 37 °C. Freeze-dried culture was then added after mixed thoroughly. Rennet was added after an interval of 20 minutes (1g of rennet was dissolved in chlorine free water) and stirred well. The mixture was

allowed to curdle by allowing standing for 40 minutes in a water bath whose temperature was maintained at 37 °C. The curd formed was cut in to pieces and placed in a colander lined with the muslin cloth and the whey was drained to a separate bowl. Draining of whey was followed by addition of equal amount of distilled water at a temperature of 40 °C. The rest of the whey was removed and the corners of the muslin bag were tied form a bag. Then the bag of curd was put in to the mould and mechanical pressure applied for 12 hours. The pressed curd was immersed in 7% brine solution for 12 hours. The cheese was then exposed to a cool and a dry atmosphere for rind formation. The finished product was placed in a plastic container and refrigerated. A hedonic test between the prepared sample and a commercially available product was carried out.

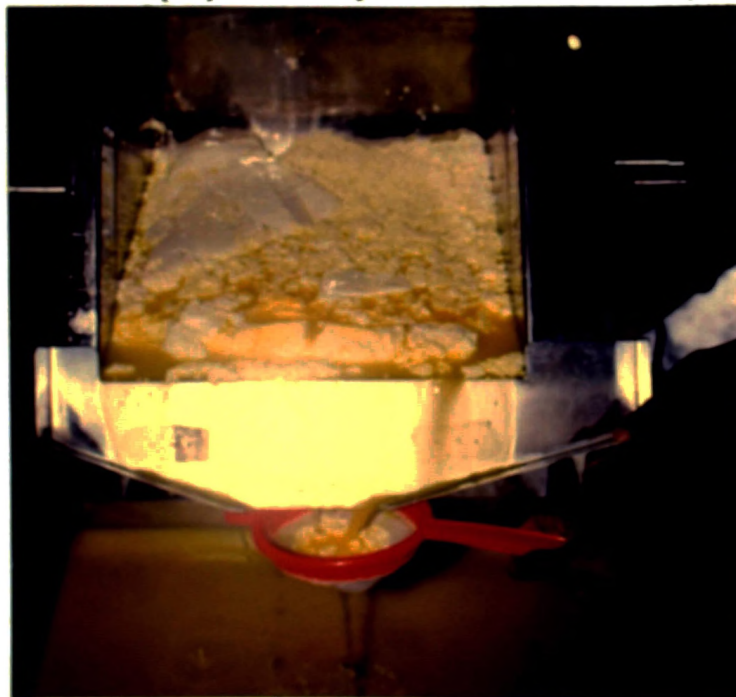


Plate: 3.2. Processing Steps of Cheese

3.3. Quality Testing of Cheese

3.3.1. Organoleptic Tests

Taste, odour and colour were tested in the prepared unripened cheese.

Materials

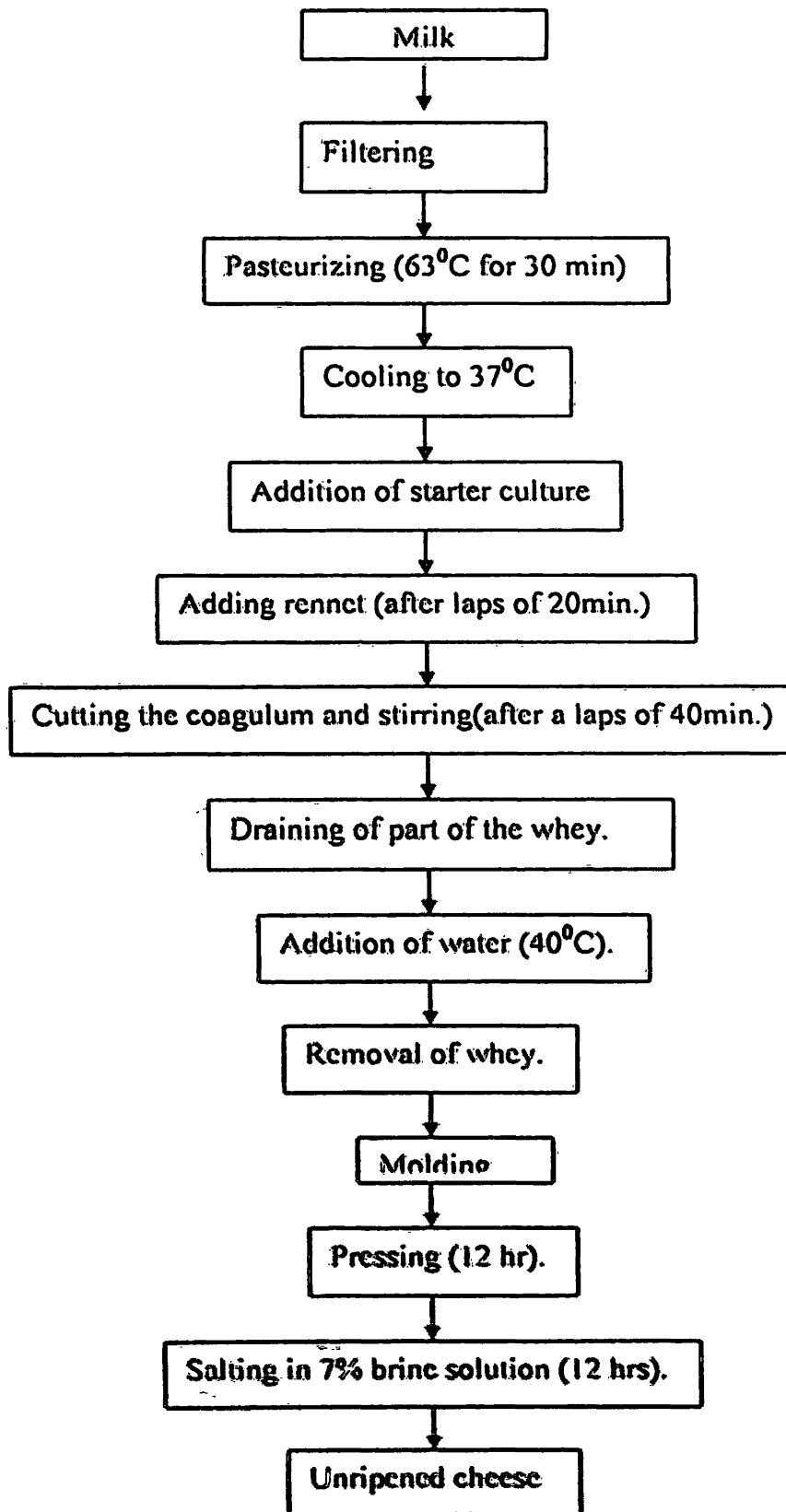
Cut cheese cubes of 2cm approximately.

White plates

Method

Cheese was tested for its taste, odour and colour.

Figure 3.1: Production Process of Gouda Cheese



3.3.2. Microbiological Tests for Cheese

1. Yeast and Moulds

Materials

Prepared sample (cheese)

Precision electronic balance

Apparatus for sterilization (Autoclave and oven)

Incubator controlled at 25 °C

Water bath controlled at 45 °C (to melt the sterile culture media)

Sterile Petri dishes

Sterile pipettes – 1 and 10 ml

pH meter

Screw capped bottles containing 9- ml of sterile diluents

Diluents	-	Peptone	1.0g
		NaCl	8.5g
		Water	1000g

Test tube plugged with cotton wool containing 15- ml of sterile yeast extract dextrose chloramphenicol agar medium.

Composition of Yeast extract dextrose chloramphenicol agar medium:

Yeast extract	5.0g
Dextrose	20.0g
Chloramphenicol	0.1g
Agar	15.0g
Water	1000ml

Method

Making the dilution:

A sterile 1ml pipette was introduced into the well-mixed test sample with its reaching not more than ½ inch from the surface of the well-mixed test sample.

The test sample was sucked up and released ten times to the 1-ml mark and 1-ml of milk was then measured out holding the pipette in the vertical position.

1-ml of test sample was introduced into the first bottle of the diluting series with the tip touching the side of the bottle at a point about ½ inch above the surface of the diluent.

A fresh pipette was introduced to the 1 in 10 dilution with its tip reaching not more than 1 inch below the surface of the diluent and the fluid sucked and released ten times to the 1ml mark.

1ml of the fluid was introduced to the second bottle of the diluting series with the tip touching the side of the bottle at a point about ½ inch above the surface of the diluents. Similarly using separate pipettes other dilutions of 10^{-3} , 10^{-4} and 10^{-5} were also prepared.

Pour plate method for yeast and mold count:

Using a sterile pipette 1 ml each of the first decimal dilution was transferred to two sterile Petri dishes. 15 ml of sterile plate count medium at 45 °C was poured into each Petri dishes containing the first decimal dilution and mixed the inoculums carefully with the medium.

Petri dishes were placed on a clean horizontal surface and allowed to solidify.

Inverted dishes were then incubated at 25 °C for 5 days.

The above procedure was repeated with other dilutions using a fresh sterile pipette for each dilution.

Plates containing not more than 500 colonies were retained and the number of moulds colonies present were counted.

2. Coli forms

Materials

Prepared sample (cheese)

Precision electronic balance

Apparatus for sterilization (Autoclave and oven)

Incubator controlled at 25 °C

Water bath controlled at 45 °C (to melt the sterile culture media)

Sterile Petri dishes

Sterile pipettes – 1 and 10 ml

pH meter

Screw capped bottles containing 9- ml of sterile diluents

Diluents	-	Peptone	1.0g
		NaCl	8.5g
		Water	1000g

Test tube plugged with cotton wool containing sterile MacConkey broth (single strength)

MacConkey broth (single strength):

Peptone	20.0g
Lactose	10.0g
Bile salt	5.0g
Bromocresol purple	0.01g
Water	1000g

Method

Making the dilution:

The process of making the dilution was same as the Test of yeast and moulds.

Examination for Presumptive Coli form;

Using a sterile pipette 1ml each of the first decimal dilution was transferred to three culture tubes containing MacConkey broth using the same technique described above. The above procedure was repeated with other dilutions using a fresh sterile pipette for each dilution.

Cultures were incubated at $\pm 37^{\circ}\text{C}$ for 24 hours.

After 24 hours, cultures were examined for acid and gas production.

Re- incubation the culture at $\pm 37^{\circ}\text{C}$ for 24 hours, and examined for acid and gas production.

3.3.3 Sensory Evaluations

Difference Test between Prepared Sample and Commercial Product :

Hedonic tests are designed to measure the degree of liking for a product. Category scale ranging from like extremely, through neither like nor dislike, to dislike extremely with varying members of categories, are used. Panelist indicate their degree of liking for each sample by choosing the appropriate category.

A hedonic test between the prepared sample and a commercial product – highland cheese, was carried out.

Materials

Identical Petri dishes with lids containing a piece of laboratory prepared cheese: -25

Identical Petri dishes with lids containing a piece of cheese commercially made : -25

Disposable plastic spoons

Questionnaire for hedonic test (Figure 3. Appendix 111)

Method

A hedonic test was conducted to determine whether a significant difference existed between different characters of two varieties (prepared and commercial) of cheese, using a 5 point category scale. (Appendix 1)

Twenty – five untrained panelists (in house) were selected from the university and the importance of the sensory testing, testing method and procedures were explained. A demonstration was carried out to show the manner in which the questionnaire should be marked.

Two varieties are presented in two identical containers to three panelists. The sample containers were coded with three digit randomly selected numbers. Each sample was given a different number.

The code numbers were;

342 - Prepared sample

468 – Commercial product – ‘Highland’

All the samples were simultaneously presented to each panelist in balanced order with questionnaire (Appendix 1) and re-tasting of the sample was allowed. Panelists were instructed to evaluate the coded sample for degree of liking. They evaluated by checking a category on the scale that ranged from like extremely to dislike extremely. For data analysis, the categories were converted to numerical scores ranging from 1 to 5 where 1 represented dislike extremely and 5 represented like extremely. The numerical scores for each sample were tabulated and analyzed by the analysis of variance (ANOVA) to determine whether any significant differences in mean degree of liking scores exist among the samples.

3.3.4 Cost Calculation

The cost of producing one (1kg) kilogram of cheese was calculated.

3.3.5. Chemical Tests

1. Moisture Content

The moisture content of cheese may be determined by gravimetric method by evaporating the water and weighing the residue.

Materials

Flat bottomed dishes and glass rod

Analytical balance

Drying oven

Desiccators

Water bath

Sea sand

Grater

A sample of cheese

Method

The dish containing 20g of sand and the glass rod was placed inside the drying oven, dried for one hour, allowed to cool in a desiccator, and weighed accurately to the nearest 0.1 mg. Few drops of distilled water was added and mixed thoroughly. The dish was placed on a boiling water bath for thirty minutes and then placed in an oven preheated to 100 °C. After four hours the sample was moved into a desiccator, allowed to cool and weighed to the nearest 0.1 mg. The drying, cooling and weighing process was repeated at hourly intervals until there was no difference in two successive weighings, greater than 0.1 mg. The lowest weight was recorded.

2. Dry Matter

The solid constituent of cheese are often grouped and referred to as "Dry matter". The percentage of which is calculated as the difference between 100 and moisture content.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Quality Tests for Raw Buffalo Milk

4.1.1. Organoleptic Tests

1. Taste

Taste - Sweet

2. Odour

Odour - Pleasant

3. Colour

Colour - Creamy white

The sweet taste of buffalo milk is due to lactose content that is slightly higher than cow milk. Freshly drawn buffalo milk tastes slightly sweet and has a characteristic odour. In cow milk there is a yellowish tinge due to milk at the presence of carotene in fat. The fat in buffalo milk is creamy white due to absence of carotene.

4.1.2. Physical Properties

1. Specific Gravity

Weight of the empty specific gravity bottle (W1) = 13.703 g

Weight of the empty specific gravity bottle + water (W2) = 24.432 g

Weight of the empty specific gravity bottle + milk (W3) = 24.787 g

$$\text{Specific Gravity} = \frac{W3 - W1}{W2 - W1}$$

$$\text{Specific Gravity} = \frac{24.787 - 13.703}{24.432 - 13.703} = \frac{11.084}{10.729}$$

$$\text{Specific Gravity} = 1.033$$

Each physical property of milk is, of course, a resultant determination by the contributions of its constituents. Obviously, physical properties vary with composition. Furthermore, the process to which milk is subjected in manufacturing and utilization induce changes in the constituents that are reflected in physical properties.

Milk is a complex colloidal system in which the dispersion medium water contains salts and sugar in solution. It is therefore heavier than water. The specific gravity of milk is influenced by the relation of its constituents, each of which has a different specific gravity.

They are approximately as follows.

Fat	0.93
Lactose	1.666
Protein	1.346
Casein	1.310
Salts	4.210

The specific gravity of milk may be determined by either finding the weight of a known volume or by measuring the volume of a known weight. Here specific gravity was determined by using a specific bottle. The method determines the ratio of weight of a unit of milk at 30°C.

Specific gravity may be used as an indicator of adulteration of milk with water. It has been determined that average specific gravity of milk is 1.032. Specific gravity of buffalo milk used for the preparation was 1.033.

4.1.3. Chemical Tests

1. Titrable Acidity

Table 4.1: Tabulated values for acid determination

Volume of milk /cm ³	Weight of 5.0ml/g	Volume of 0.1N NaOH used /cm ³		
		Sample 1	Sample 2	Sample 3
5.00	5.157	1.05	1.05	1.05

Acidity is expressed as percentage of lactic acid (1ml of 0.1 N NaOH = 0.009g of lactic acid)

$$\begin{aligned}
 \text{Percentage of Acidity} &= \frac{(\text{ml of NaOH}) \times (\text{N NaOH}) \times 9}{\text{Weight of sample}} \\
 &= \frac{(1.05\text{cm}^3) \times (0.1 \text{ N}) \times 9}{5.1} \\
 &= 0.183 \%
 \end{aligned}$$

Normal fresh milk has a hydrogen ion concentration of approximately pH 6.5 to 6.6 which is an indication that milk is slightly acidic. Fresh milk however does not contain any lactic acid and the acidity is due to certain constituents of milk some of which cause an acid reaction and other actually combine with the alkali. Acidity of fresh milk is known to be due to the phosphates, proteins (casein and albumin) and to a slight degree due to the presence of carbon dioxide and citrates in the milk.

Titration acidity test should reveal no developed acidity in good quality milk. Both the flavour and the odour are adversely affected as acidity develops and also the acidity of the milk to survive several processes, during the processing in a plant. The titration acidity of fresh milk usually shows less than 0.18%. Buffalo milk used for the preparation of cheese gave a value of 0.183% for titration acidity.

4.1.4. Microbiological Tests

1. Clot on Boiling

There was no clot in the milk sample. This test reveals milk used for the preparation of cheese was in good quality

This test is essentially in holding the milk sample under defined conditions until it clots when held at 100 °C for five minutes. It is universally accepted that if milk clots on boiling it cannot be marketed. This test measures in effect the commercial life of milk. The clot on boiling test is the most important. At times this is the only test carried out to determine the quality. Acidity test together with the above test on fresh milk will usually indicate the milk which is likely to coagulate during processing.

4.2. Quality Tests for Cheese

4.2.1. Organoleptic Tests

1. Taste - Both sour and salty
2. Odour - Pleasant
3. Colour - Cream

Buffalo milk Gouda cheese should contain pH 5.68 acidity level was in the final product. A cream yellowish colour was obtained in the final product by adding beta carotene 4g/25 l during the processing.

4.2.2. Microbiological Test

1. Yeast and Molds

Molds presented in large numbers that is higher than SLS standards, which may be due to poor sanitary condition in the laboratory.

2. Coli forms

Coli form test was negative in the final product.

4.3.Sensory Evaluation

468 - Prepared sample.

342 - Commercial Product.

Table 4.2: Category Scores for the Hedonic Test

Panelists	Character									
	Appearance		Colour		Aroma		Flavour		Texture	
	468	342	468	342	468	342	468	342	468	342
1	4	2	5	5	3	4	3	5	4	3
2	1	3	4	3	3	3	1	5	3	2
3	3	2	3	2	4	3	5	4	4	2
4	5	4	5	5	3	4	4	4	4	3
5	3	3	4	3	4	3	4	3	4	3
6	3	4	3	4	3	3	5	4	5	1
7	5	4	4	5	2	3	2	5	2	4
8	4	4	3	4	2	3	3	4	5	4
9	3	4	4	3	4	5	2	3	3	2
10	5	4	3	4	2	3	4	5	4	3
11	4	4	4	3	3	4	3	2	4	4
12	3	4	3	2	3	3	5	4	4	3
13	3	4	5	3	3	2	5	3	5	3
14	5	3	4	3	5	2	4	4	3	2
15	3	4	4	4	4	3	2	4	4	3
16	5	2	4	3	5	3	5	4	2	4
17	4	2	3	2	5	3	3	4	4	3
18	3	4	3	4	4	2	5	4	3	4
19	1	2	3	4	4	3	4	3	1	5
20	4	2	5	2	1	5	1	5	2	4
21	4	5	4	3	4	2	4	2	4	3
22	5	4	4	3	4	2	5	3	5	5
23	1	3	4	3	5	3	2	3	4	2
24	5	4	3	2	4	3	5	4	4	2
25	3	2	4	3	4	4	3	3	5	3

Table 4.3 : Results of sensory evaluation

Character	F ratio
Appearance	Calculated < Tabular ($p \leq 0.05$) – no significant difference in appearance.
Colour	Calculated < Tabular ($p \leq 0.05$) – no significant difference in in colour.
Aroma	Calculated > Tabular ($p \leq 0.05$) – There was a significant difference in aroma.
Flavour	Calculated > Tabular ($p \leq 0.05$) – There was a significant difference in flavour.
Texture	Calculated > Tabular ($p \leq 0.05$) – There was a significant difference in texture.

The ANOVA indicated that there was a significant difference in aroma, texture and flavour of the prepared and commercially available samples of cheeses (see appendix III). There were no significant differences in other characters of the two cheese samples.

Sensory evaluation is a multi disciplinary science that uses human panelist and their senses of light, smell, touch and having to evaluate the sensory characteristics and the acceptability of the food products, as well as the other materials. There is no known instrument that can replicate or replace human response, making sensory evaluation component of any food study essential. Sensory analysis is applicable to a variety of areas such as product development, quality control, storage studies and process development.

Preference, acceptance and hedonic (degree of liking) test are considered to be consumer tests since they should be conducted using untrained, unsuspecting people selected from the consumer public. Although panelists can be asked to indicate their degree of liking, preference or acceptability of a product directly, hedonic tests are often as indirect measures of preference or acceptance. In this research, hedonic test was carried out to find whether there is a significant difference between the prepared sample and commercially available products obtained from the market.

4.4. Cost Calculation

4kg of buffalo cheese was produced out of 25 liters of milk.

Table 4.4 : Cost evaluation

Description	Amount	Unit Price (Rs.)	Total (Rs.)
Liquid Buffalo milk	25 liters	30.00	750.00
Rennet	1g	-	12.50
Culture	0.75g	-	8.00
β - Carotene	4g	-	35.00
Salt	350g	-	10.00
Electricity	-	-	40.00
Water	-	-	10.00
Labour	-	-	150.00
Total cost (per 4kg of cheese)			1015.50

Cost for production of 1 kg of cheese except the ripening process was Rs.253.88. The ripened cheese available in market is Rs.1200 /1 kg. There with out following the ripening process accurate cost calculation can not be obtained.

4.5. Chemical Test

1. Moisture content

Table 4.5: Tabulated values for moisture determination

Sample	M ₀ (g)	M ₁ (g)	M ₂ (g)
1	35.922	37.033	36.446
2	42.670	44.099	43.321
3	45.770	47.426	46.585

M₀. Mass of dish + sand

M₁. Mass of dish + sand +sample before drying

M₂. Mass of dish + sand +sample after drying

Calculations:

$$\text{Total solids content percentage by mass} = \frac{(M_1 - M_2)}{(M_1 - M_0)} \times 100$$

The moisture content in cow milk cheese should be between the range from 45%-55%. Therefore 52.7% is in this acceptable range. Moisture content is very important factor in cheese because it directly affects its texture. During the pressing step moisture content in the product should be controlled which depended on the time duration and the pressure applying on the product.

Table 4.6: Tabulated Results of the moisture content.

Sample	Moisture percentage by mass	Average
1	52.83	52.7
2	54.44	
3	50.78	

2. Dry Matter

Percentage moisture content of cheese = 52.7 %

Dry matter = 100 – moisture content
= 100 – 52.7
= 47.3%

The dry matter content contributes higher yield of cheese made from buffalo milk due to higher fat content.

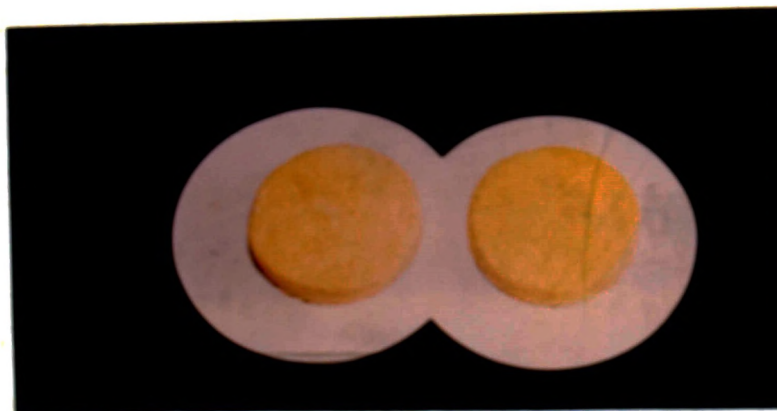
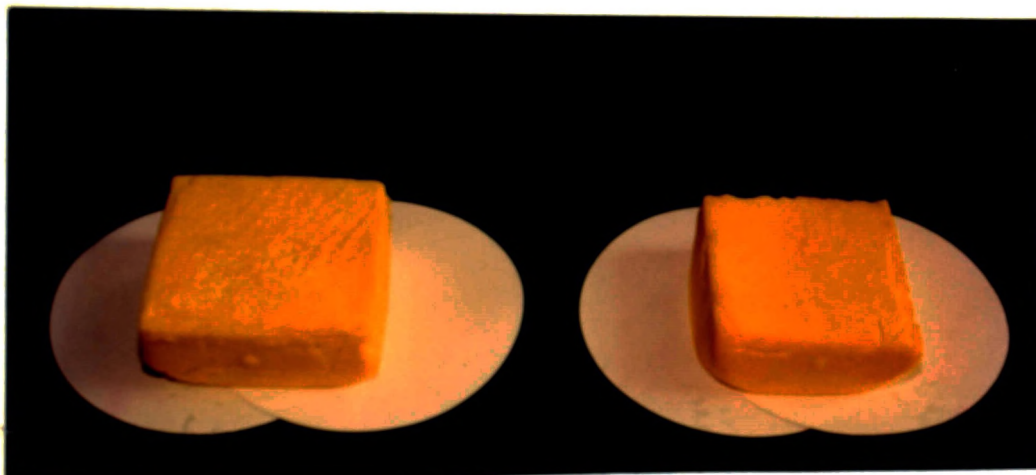


Plate: 4.1. Buffalo Milk Gouda Cheese

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Conclusion

Cheese can be successfully made from buffalo milk with high fat yield.

This unripen cheese had colour and appearance same as commercially available cow milk cheese, but aroma, flavour and texture were differed.

Recommendations for Further Studies

- 1. Ripening process should be carried out and the ripened product should be subject to final processing.**
- 2. Flavour, texture and aroma can be improved by allowing the final product for ripening process.**
- 3. Shelf life studies should be carried out.**
- 4. After ripening and the final processing of the product a market research should be carried out for consumer acceptability of the product.**

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Appendix 1

The questionnaire given for Hedonic Test for cheese

Name:

Date :

Please evaluate the two cheese samples (342 & 468) and put the relevant number for your option for each character as given below.

Eg:

	Aroma	
	342	468
Like extremely	5	5
Like	4	4
Neither like nor dislike	3	3
Dislike	2	2
Dislike extremely	1	1

	Appearan -ce		Colour		Flavour		Aroma		Texture	
	342		342		342		342		342	
Like extremely										
Like										
Neither like nor dislike										
Dislike										
Dislike extremely										

Appendix 11

Tabulated Category Scores for the Hedonic Test.

Panelist Total –p

A – 468

B – 342

Trt – treatment total

G. T.- grand total

For the ANOVA the following calculations were carried out (where, N = The total number of individual responses).

$$\begin{aligned} \text{Correction Factor (CF)} &= \frac{(\text{Grand Total})^2}{N} \\ &= 170^2 / 50 \\ &= 578 \end{aligned}$$

Total sum of squares (measure of the total variance for the test).

$$\begin{aligned} SS(T) &= \sum (\text{each individual responses})^2 - CF \\ &= \sum (4^2 + 1^2 + 3^2 + \dots + 4^2 + 3^2 + 2^2) - 578 \\ &= 62 \end{aligned}$$

Treatment sum of squares (Measured variance among the sample means)

$$\begin{aligned} SS (Tr) &= \frac{\sum (\text{Each treatment total})^2}{\text{Number of responses per treatment}} - CF \\ &= \frac{(89^2 + 81^2)}{25} - 578 \\ &= 1.28 \end{aligned}$$

Panelist sum of squares (measure of the variance among Panelists' mean).

$$\begin{aligned}
 SS(P) &= \frac{\sum (\text{each panelist total})^2}{\text{Number of responses per panelist}} - CF \\
 &= \frac{\sum (6^2+4^2+5^2+ \dots + 9^2+5^2)}{2} - 578 \\
 &= 38
 \end{aligned}$$

Error sum of squares (measure of the variance due to experimental or random error)

$$\begin{aligned}
 SS(E) &= SS(T) - SS(Tr) - SS(P) \\
 &= 62 - 1.28 - 38 \\
 &= 22.72
 \end{aligned}$$

The mean square (MS) values were calculated by dividing the SS values by their respective degree of freedom as follows.

$$\begin{aligned}
 \text{Total degree of freedom, } df(T) &= \text{the total number of responses} - 1 \\
 &= 50 - 1 \\
 &= 49
 \end{aligned}$$

$$\begin{aligned}
 \text{Treatment degree of freedom, } df(Tr) &= \text{the total number of treatments} - 1 \\
 &= 2 - 1 \\
 &= 1
 \end{aligned}$$

$$\begin{aligned}
 \text{Panelist degree of freedom, } df(P) &= \text{the total number of panelists} - 1 \\
 &= 25 - 1 \\
 &= 24
 \end{aligned}$$

$$\text{Error degree of freedom, } df(E) = df(T) - df(Tr) - df(P)$$

$$= 49 - 1 - 24$$

$$= 24$$

$$\text{Treatment mean square, MS (Tr)} = \text{SS (Tr)} / \text{df (Tr)}$$

$$= 1.28 / 1$$

$$= 1.28$$

$$\text{Panelist mean square, MS (P)} = \text{SS(P)} / \text{df(P)}$$

$$= 38 / 24$$

$$= 1.58$$

$$\text{Error mean square, MS(E)} = \text{SS(E)} / \text{df(E)}$$

$$= 22.72 / 24$$

$$= 0.94$$

The F ratios, for the treatments and panelists were calculated by dividing the respective values by MS for error. The tabular F ratios were obtained from statistical tables of the F distribution (see Appendix I I I). Calculated F ratios were compared to tabulated F ratios to determine whether there was any significant difference among the treatment or panelists' means.

ANOVA Table scoring for appearance test

Sources of Variation	df	SS	MS	F Ratio	
				Calculated	Tabular ($p \leq 0.05$)
Total(T)	49	62.00			
Treatment(Tr)	01	01.28	1.28	1.36	4.259
Panelist(P)	24	38.00	1.58	1.68	1.983
Error(E)	24	22.72	0.94		

The tabulated F ratio for treatments with one degree of freedom (df) in the numerator and 24 df in the denominator, at $p \leq 0.05$, is 4.259. The F ratio for panelists with 24 df in the numerator and 24 in the denominator at $p \leq 0.05$, is 1.985.

If the calculated F ratio exceeds the tabulated F ratio for the same number of degree of freedom, then there is evidence of significant differences.

Since the calculated treatments, F ratio of 1.36 did not exceed the tabulated F ratio of 4.259 it was concluded that there was no significant ($p \leq 0.05$) difference in the appearance of the two cheese samples. The calculated panelists' F ratio of 1.68 did not exceed the tabular F ratio of 1.983. Thus, no significant panelist effect was present.

Similarly, the results obtained for other characters were analyzed by ANOVA.

ANOVA Table scoring for colour test

Sources of Variation	df	SS	MS	F Ratio	
				Calculated	Tabular ($p \leq 0.05$)
Total(T)	49	36.42			
Treatment(Tr)	01	03.38	3.38	6.25	4.259
Panelist(P)	24	19.92	0.83	1.53	1.983
Error(E)	24	13.12	0.54		

Table :ANOVA Table scoring for aroma test

Sources of Variation	df	SS	MS	F Ratio	
				Calculated	Tabular ($p \leq 0.05$)
Total(T)	49	44.88			
Treatment(Tr)	01	02.00	2.000	1.66	4.259
Panelist(P)	24	13.88	0.578	0.48	1.983
Error(E)	24	29.00	1.200		

Table : ANOVA Table scoring for flavour test

Sources of Variation	df	SS	MS	F Ratio	
				Calculated	Tabular ($p \leq 0.05$)
Total(T)	49	58.50			
Treatment(Tr)	01	00.18	0.180	0.122	4.259
Panelist(P)	24	23.00	0.958	0.651	1.983
Error(E)	24	35.32	1.471		

Table : ANOVA Table scoring for texture test.

Sources of Variation	df	SS	MS	F Ratio	
				Calculated	Tabular ($p \leq 0.05$)
Total(T)	49	55.78			
Treatment(Tr)	01	04.50	4.500	3.170	4.259
Panelist(P)	24	17.28	0.720	0.508	1.983
Error(E)	24	34.00	1.416		

APPENDIX III

Critical Absolute Rank Sum Differences for "All Treatments" Comparisons at 5% Level of Significance.

Panc -lists	Number of Samples									
	3	4	5	6	7	8	9	10	11	12
2	6	8	11	13	15	18	20	23	25	28
3	7	10	13	16	19	21	24	27	30	33
4	8	12	15	19	22	26	29	32	36	39
5	9	13	17	21	25	29	32	36	40	44
6	10	14	18	23	27	31	35	39	43	47
7	10	14	18	23	27	31	35	39	43	47
8	11	15	19	24	28	32	36	40	44	48
9	11	15	19	24	28	32	36	40	44	48
10	11	15	19	24	28	32	36	40	44	48
11	12	16	20	25	29	33	37	41	45	49
12	12	16	20	25	29	33	37	41	45	49
13	13	17	21	26	30	34	38	42	46	50
14	13	17	21	26	30	34	38	42	46	50
15	13	17	21	26	30	34	38	42	46	50
16	14	18	22	27	31	35	39	43	47	51
17	14	18	22	27	31	35	39	43	47	51
18	14	18	22	27	31	35	39	43	47	51
19	15	19	23	28	32	36	40	44	48	52
20	15	19	23	28	32	36	40	44	48	52
21	15	19	23	28	32	36	40	44	48	52
22	16	20	24	29	33	37	41	45	49	53
23	16	20	24	29	33	37	41	45	49	53
24	16	20	24	29	33	37	41	45	49	53
25	16	20	24	29	33	37	41	45	49	53
26	17	21	25	30	34	38	42	46	50	54
27	17	21	25	30	34	38	42	46	50	54
28	17	21	25	30	34	38	42	46	50	54
29	17	21	25	30	34	38	42	46	50	54
30	18	22	26	31	35	39	43	47	51	55
31	18	22	26	31	35	39	43	47	51	55
32	18	22	26	31	35	39	43	47	51	55
33	18	22	26	31	35	39	43	47	51	55
34	18	22	26	31	35	39	43	47	51	55
35	19	23	27	32	36	40	44	48	52	56
36	19	23	27	32	36	40	44	48	52	56
37	19	23	27	32	36	40	44	48	52	56
38	19	23	27	32	36	40	44	48	52	56
39	19	23	27	32	36	40	44	48	52	56
40	20	24	28	33	37	41	45	49	53	57
41	20	24	28	33	37	41	45	49	53	57
42	20	24	28	33	37	41	45	49	53	57
43	20	24	28	33	37	41	45	49	53	57
44	20	24	28	33	37	41	45	49	53	57
45	20	24	28	33	37	41	45	49	53	57
46	21	25	29	34	38	42	46	50	54	58
47	21	25	29	34	38	42	46	50	54	58
48	21	25	29	34	38	42	46	50	54	58
49	21	25	29	34	38	42	46	50	54	58
50	21	25	29	34	38	42	46	50	54	58
51	22	26	30	35	39	43	47	51	55	59
52	22	26	30	35	39	43	47	51	55	59
53	22	26	30	35	39	43	47	51	55	59
54	22	26	30	35	39	43	47	51	55	59
55	22	26	30	35	39	43	47	51	55	59
56	22	26	30	35	39	43	47	51	55	59
57	23	27	31	36	40	44	48	52	56	60
58	23	27	31	36	40	44	48	52	56	60
59	23	27	31	36	40	44	48	52	56	60
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61	23	27	31	36	40	44	48	52	56	60
62	23	27	31	36	40	44	48	52	56	60
63	24	28	32	37	41	45	49	53	57	61
64	24	28	32	37	41	45	49	53	57	61
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66	24	28	32	37	41	45	49	53	57	61
67	24	28	32	37	41	45	49	53	57	61
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72	24	28	32	37	41	45	49	53	57	61
73	24	28	32	37	41	45	49	53	57	61
74	24	28	32	37	41	45	49	53	57	61
75	24	28	32	37	41	45	49	53	57	61
76	24	28	32	37	41	45	49	53	57	61
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78	24	28	32	37	41	45	49	53	57	61
79	24	28	32	37	41	45	49	53	57	61
80	24	28	32	37	41	45	49	53	57	61
81	24	28	32	37	41	45	49	53	57	61
82	24	28	32	37	41	45	49	53	57	61
83	24	28	32	37	41	45	49	53	57	61
84	24	28	32	37	41	45	49	53	57	61
85	24	28	32	37	41	45	49	53	57	61
86	24	28	32	37	41	45	49	53	57	61
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88	24	28	32	37	41	45	49	53	57	61
89	24	28	32	37	41	45	49	53	57	61
90	24	28	32	37	41	45	49	53	57	61
91	24	28	32	37	41	45	49	53	57	61
92	24	28	32	37	41	45	49	53	57	61
93	24	28	32	37	41	45	49	53	57	61
94	24	28	32	37	41	45	49	53	57	61
95	24	28	32	37	41	45	49	53	57	61
96	24	28	32	37	41	45	49	53	57	61
97	24	28	32	37	41	45	49	53	57	61
98	24	28	32	37	41	45	49	53	57	61
99	24	28	32	37	41	45	49	53	57	61
100	24	28	32	37	41	45	49	53	57	61

F Distribution 5% Level of Significance

v ₁ \ v ₂	1	2	3	4	5	6	7	8	9
1	161.45	109.60	213.71	324.58	230.16	237.99	236.77	238.88	240.54
2	18.513	19.000	19.164	19.247	19.296	19.330	19.353	19.371	19.385
3	10.128	9.5521	9.2766	9.1172	9.0135	8.9408	8.8868	8.8452	8.8123
4	7.7088	6.9443	6.5914	6.3883	6.2560	6.1631	6.0942	6.0410	6.9988
5	6.0079	6.7861	5.4095	6.1922	6.0503	4.0503	4.8750	4.8183	4.7725
6	5.9874	6.1433	4.7571	4.6337	4.5874	4.5330	4.5008	4.4688	4.4390
7	5.5914	4.7374	4.3468	4.1203	3.9715	3.8600	3.7870	3.7257	3.6767
8	5.3177	4.4500	4.0602	3.8378	3.6876	3.5806	3.5005	3.4381	3.3881
9	6.1174	4.2665	3.8626	3.6331	3.4817	3.3733	3.2927	3.2290	3.1769
10	4.9646	4.1078	3.7083	3.4780	3.3258	3.2172	3.1355	3.0717	3.0204
11	4.8443	3.9823	3.6874	3.4567	3.3030	3.1946	3.1123	3.0480	2.9962
12	4.7472	3.8853	3.4903	3.2592	3.1050	2.9961	2.9124	2.8480	2.7964
13	4.6672	3.8056	3.4105	3.1791	3.0254	2.9153	2.8321	2.7669	2.7144
14	4.6001	3.7389	3.3439	3.1122	2.9583	2.8477	2.7642	2.6987	2.6458
15	4.5431	3.6823	3.2874	3.0556	2.9013	2.7905	2.7060	2.6406	2.5876
16	4.4940	3.6337	3.2389	3.0069	2.8521	2.7413	2.6572	2.5911	2.5377
17	4.4513	3.5915	3.1968	2.9647	2.8100	2.6987	2.6143	2.5480	2.4943
18	4.4139	3.5546	3.1599	2.9277	2.7729	2.6613	2.5767	2.5102	2.4563
19	4.3808	3.5219	3.1274	2.8961	2.7404	2.6283	2.5435	2.4768	2.4227
20	4.3513	3.4928	3.0984	2.8681	2.7109	2.5990	2.5140	2.4471	2.3928
21	4.3248	3.4668	3.0726	2.8441	2.6848	2.5727	2.4876	2.4205	2.3661
22	4.3009	3.4434	3.0491	2.8167	2.6613	2.5491	2.4638	2.3965	2.3419
23	4.2792	3.4221	3.0280	2.7963	2.6400	2.5277	2.4422	2.3745	2.3201
24	4.2607	3.4028	3.0088	2.7763	2.6207	2.5082	2.4226	2.3551	2.3003
25	4.2447	3.3852	2.9912	2.7667	2.6030	2.4904	2.4047	2.3371	2.2821
26	4.2302	3.3690	2.9751	2.7486	2.5868	2.4741	2.3883	2.3203	2.2653
27	4.2169	3.3541	2.9604	2.7328	2.5719	2.4591	2.3732	2.3053	2.2501
28	4.1980	3.3404	2.9467	2.7181	2.5581	2.4453	2.3593	2.2913	2.2360
29	4.1830	3.3277	2.9340	2.7044	2.5454	2.4324	2.3463	2.2782	2.2229
30	4.1700	3.3163	2.9223	2.6906	2.5338	2.4205	2.3343	2.2662	2.2107
40	4.0848	3.2317	2.8387	2.6060	2.4498	2.3359	2.2490	2.1802	2.1240
60	4.0012	3.1484	2.7561	2.5233	2.3683	2.2540	2.1665	2.0970	2.0401
120	3.9201	3.0716	2.6892	2.4473	2.2900	2.1760	2.0867	2.0164	1.9588
∞	3.8416	3.0057	2.6040	2.3710	2.2141	2.0986	2.0056	1.9354	1.8799

F Distribution 5% Level of Significance

$v_1 \backslash v_2$	10	12	15	20	24	30	40	60	120	∞
1	241.88	243.91	245.95	248.01	249.03	250.09	251.14	252.20	253.23	254.32
2	19.395	19.413	19.429	19.446	19.454	19.462	19.471	19.479	19.487	19.496
3	8.7853	8.7446	8.7029	8.6602	8.6185	8.5768	8.5351	8.4934	8.4517	8.4100
4	5.0814	5.0117	4.9578	4.9025	4.8474	4.7923	4.7372	4.6821	4.6270	4.5719
5	4.7331	4.6777	4.6188	4.5581	4.4972	4.4357	4.3738	4.3114	4.2484	4.1850
6	4.0800	4.0222	3.9591	3.8942	3.8285	3.7621	3.6951	3.6274	3.5591	3.4900
7	3.6265	3.5717	3.5108	3.4445	3.3775	3.3098	3.2414	3.1724	3.1027	3.0323
8	3.3473	3.2840	3.2184	3.1503	3.0805	3.0091	2.9361	2.8624	2.7880	2.7129
9	3.1373	3.0729	3.0061	2.9365	2.8643	2.7904	2.7158	2.6404	2.5643	2.4875
10	2.9782	2.9130	2.8450	2.7740	2.7007	2.6260	2.5508	2.4751	2.3989	2.3221
11	2.8536	2.7878	2.7180	2.6464	2.5721	2.4963	2.4199	2.3430	2.2656	2.1877
12	2.7534	2.6866	2.6165	2.5438	2.4685	2.3917	2.3143	2.2364	2.1580	2.0791
13	2.6710	2.6037	2.5331	2.4589	2.3821	2.3047	2.2268	2.1484	2.0695	1.9901
14	2.6021	2.5342	2.4630	2.3878	2.3101	2.2318	2.1530	2.0737	1.9939	1.9136
15	2.5437	2.4753	2.4035	2.3273	2.2485	2.1691	2.0892	2.0088	1.9280	1.8467
16	2.4930	2.4247	2.3521	2.2750	2.1951	2.1147	2.0338	1.9524	1.8706	1.7883
17	2.4480	2.3797	2.3071	2.2304	2.1495	2.0681	1.9862	1.9038	1.8210	1.7377
18	2.4117	2.3431	2.2706	2.1940	2.1121	2.0297	1.9468	1.8634	1.7795	1.6951
19	2.3770	2.3080	2.2351	2.1585	2.0755	1.9921	1.9082	1.8238	1.7390	1.6536
20	2.3470	2.2770	2.2033	2.1267	2.0425	1.9578	1.8726	1.7869	1.7007	1.6140
21	2.3210	2.2504	2.1767	2.0990	2.0137	1.9278	1.8414	1.7545	1.6671	1.5792
22	2.2967	2.2250	2.1508	2.0721	1.9857	1.8987	1.8112	1.7232	1.6348	1.5460
23	2.2747	2.2020	2.1273	2.0485	1.9611	1.8731	1.7846	1.6956	1.6062	1.5164
24	2.2547	2.1814	2.1077	2.0289	1.9405	1.8515	1.7620	1.6720	1.5816	1.4908
25	2.2365	2.1620	2.0879	2.0090	1.9205	1.8305	1.7400	1.6490	1.5576	1.4658
26	2.2197	2.1440	2.0700	1.9910	1.9015	1.8105	1.7190	1.6270	1.5346	1.4418
27	2.2043	2.1280	2.0530	1.9740	1.8835	1.7915	1.6990	1.6060	1.5126	1.4188
28	2.1900	2.1130	2.0380	1.9590	1.8675	1.7745	1.6810	1.5870	1.4926	1.3978
29	2.1768	2.1000	2.0250	1.9460	1.8535	1.7595	1.6650	1.5700	1.4746	1.3788
30	2.1646	2.0880	2.0130	1.9340	1.8405	1.7455	1.6500	1.5540	1.4576	1.3608
40	2.0772	2.0010	1.9260	1.8470	1.7525	1.6565	1.5590	1.4610	1.3626	1.2638
60	1.9926	1.9170	1.8420	1.7630	1.6675	1.5705	1.4720	1.3730	1.2736	1.1738
120	1.9105	1.8350	1.7600	1.6810	1.5845	1.4865	1.3870	1.2870	1.1866	1.0858
∞	1.8307	1.7550	1.6800	1.6010	1.5035	1.4045	1.3040	1.2030	1.1016	1.0000

$$F = \frac{s_1^2}{s_2^2} = \frac{v_2}{v_1} F_{v_1, v_2}$$

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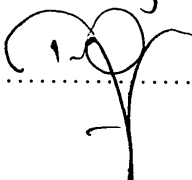
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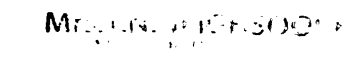
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