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**Unit Operation Alteration for Developing the
Characteristics of Local White Cheese**

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Unit Operation Alteration for Developing the Characteristics of
Local White Cheese

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Dedication:

First of all, I will dedicate this study to our Almighty Allah, who gave his strength and knowledge for her everyday life.

To my grandmother, Fatima who inspired to be strong despite of many obstacles in life.

To my parents, Mr. Minwer and Mrs. Ma'zoza for their understanding and for their overwhelming, support morally and financially.

To my loving husband and daughter, Ayman Zain and Jana, my eternal gratitude.

To my brothers, Maymoon, Faisal, Yazan, Mohamed, and Laith for their eternal love.

To my supervisor Dr. Ibrahim Afaneh, to my home land Palestine and to my beloved mosque Al- Aqsa.

Declaration:

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed:

Haneen Minwer Younes Saleh

Date:

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Unit Operation Alteration for Developing the Characteristics of Local White Cheese

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Abstract:

Local White Cheese is the mostly consumed in Palestine: this cheese should show meltability and high stretch ability to fit in the production of high quality Kunafa and other popular local sweets and pastries. Different ways are being practiced to improve these characteristics but all of these ways it is not controllable and may produce low quality cheese which suffers from excessive deformation, off-color and off-flavor. Many reserches have been done during last years.

In addition of adjusting PH by using acid and microorganisms, steam bath and steam injection have been used to produce cheese with new characteristics. A well-designed matrix was made and 128 samples were distributed in four groups; the first one coagulated with enzyme, the second with enzyme and startur culture, the third with enzyme and different concentration of acid while the fourth with enzyme, startur culture and different concentration of acid. out of the four groups 10 samples were selected and inspected physically. During the month of December of the year 2016, a cross-sectional study was conducted with school's teachers from 6 schools in Hebron. The examined subjects were 100 teachers (50 female and 50 male) with an average age of 30.68 ± 1.6 years. During the inspection 5 samples have got higher rating than standard one. Four samples reproduced by using the surfactant and the result was different according to there procesures.

The conclusion is that using steam injection can help to produce a softer, smoother and more compact texture for white cheese, while using steam bath can increase the elasticity, stretchability and hardness. The presence of acid in little concentration can increase the elasticity, improve the surface and increase the brightness while found the microorganisms can increase the consistency and decrease the porosity of the cheese. Moreover, acid with microorganism enable to making the added value of these application.

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chapter One

1. Introduction:

1.1 Background:

Food industry plays a major role in people's life. In agricultural countries, food become the top talk subject national wide. Food industry is constructing one of the most important industrial sectors in these countries in term of labor force, size of investment, and the share-part in the national economy.

In general, food processing has a wide spectrum of processing elements, unit operations, characteristics, and requirements. But all kinds of food processing share the same purpose which manifested since existed by four major folds as following:

1. To extend the period during which a food remains wholesome (the shelf life) by preservation techniques which inhibit microbiological or biochemical changes and thus allow time for distribution, sales and home storage.
2. To increase variety in the diet by providing a range of attractive flavors, colors, aromas and textures in food (collectively known as eating quality, sensory characteristics or organoleptic quality); a related aim is to change the form of the food to allow further processing (for example the milling of grains to flour).
3. To provide the nutrients required for health (termed nutritional quality of a food).

4. To generate income for the manufacturing company.

Each of these aims exists to a greater or lesser extent in all food production, but the processing of a given product may emphasize some more than others. For example, frozen vegetables are intended to have sensory and nutritional qualities that are as close as possible to the fresh product, but with a shelf life of several months instead of a few days or weeks. The main purpose of freezing is therefore to preserve the food. In contrast, sugar confectionery and snack foods are intended to provide variety in the diet, and a large number of shapes, flavors, colors and textures are produced from basic raw materials.

All food processing involves a combination of procedures to achieve the intended changes to the raw materials. These are conveniently categorized as *unit operations*, each of which has a specific, identifiable and predictable effect on a food. Unit operations are grouped together in certain pattern to form a process. The combination and sequence of operations determines the nature of the final product.

In industrialized countries, the market for processed foods is changing, and in contrast to earlier years, consumers no longer require a shelf life of several months at ambient temperature for the majority of their foods. Changes in family lifestyle, and increased ownership of freezers and microwave ovens, are reflected in demands for foods that are convenient to prepare, are suitable for frozen or chilled storage, or have a moderate shelf life at ambient temperatures. There is now an increasing demand by consumers for foods that have minimum synthetic additives, or have undergone fewer changes during processing. These foods more closely resemble the original raw materials and have a 'healthy' or 'natural' image.

Correspondingly, growth in demand for organic foods has significantly increased in Europe during the 1990s. These demands have an important influence on changes that took place in the food processing industry. Manufacturers have responded by reducing or eliminating synthetic additives from products (particularly colorants and flavors) and substituting them with natural or 'nature-equivalent' alternatives. They have also introduced new ranges of low-fat, sugar-free or low-salt products in nearly all sub-sectors (Anon, 1999).

New products that are supplemented with vitamins, minerals and probiotic cultures (or ‘functional’ foods) have appeared in recent years, and products containing organic ingredients are now widely available. At the time of writing (2016-2017), a debate over the safety of genetically modified (GM) food ingredients is unresolved. Consumer pressure for more ‘natural’ products has also stimulated development of novel ‘minimal’ processes that reduce the changes to sensory characteristics or nutritional value of foods (Pouliot, Y. and Boulet, M., 1995).

Trends that started during the 1960s and 1970s, and accelerated during the last 10–15 years, have caused food processors to change their operations in four key respects:

- First, there is increasing Improvements to food quality during the last 10–15 years have also been achieved through changes in legislation, including legal requirements on manufacturers and retailers to display ‘due diligence’ in protecting consumers from potentially hazardous foods. This has in part arisen from a series of highly publicized cases of food poisoning and food adulteration in Europe during the 1980s and 1990s, and the outbreak of Bovine Spongiform Encephalitis (BSE) in British cattle, which led to public pressure for improved food safety and quality. Legislation is now increasingly international in its focus and application, and international standards for both specific products and also for methods of achieving quality assurance are in force (Pouliot, Y. and Boulet, M., 1995). Investment in capital intensive, automated processes to reduce labor and energy costs.
- Second, there has been a change in philosophy from quality control, achieved by testing final products, to a more sophisticated approach to quality assurance, which involves all aspects of management.
- Third, high levels of competition and slowing of the growth in the food market in Europe and USA during the 1970s has caused manufacturers to adopt a more proactive approach to creating demand, using sophisticated marketing techniques and large advertising budgets. Mergers or take-overs of competitors have resulted from the increased competition.
- Fourth, there has been a shift in power and control of food markets from manufacturers to large retail companies.

The changes in technology have been influenced by a variety of factors: substantial increases in the costs of both energy and labor, by public pressure and legislation to reduce negative environmental effects of processing, particularly air or water pollution and energy consumption. Food processing equipment now has increasingly sophisticated levels of control to reduce processing costs, enable rapid change-overs between shorter production runs, to improve product quality and to provide improved records for management decisions. Microprocessors are now almost universally used to control food processing equipment. The automation of entire processes, from reception of materials, through processing and packaging to warehousing, has become a reality. This requires a higher capital investment by manufacturers but, together with improved quality assurance, reduces production costs and wastage. It increases production efficiency, uses less energy and often fewer operators, and generates increased revenue and market share from products that have higher quality.

1.2 Food Processing in Palestine:

The food processing industry is considered one of the oldest industries in Palestine. In its early days, this industry was limited to the production of few processed foods and sweets. Currently, this industry is a major contributor to the Palestinian economy and its gross domestic product. In addition, this sector has created job opportunities in the local market, and has been employing 11,400 employees as of the year 2012 (The Palestinian Central Bureau of Statistics (2013)). This sector's importance also lies in its provision of needed processed foods essential for food security in Palestine, through producing 120 different food commodities.

Developing the food processing industry can have spillover effects on all associated sectors and industries, and in particular on the agricultural sector that acts as the main supplier of inputs. It also plays a key role in creating an added value to agroproducts.

One of the main important sub-sectors in Palestine is the dairy sector. The sector consists of 41 factories, and provides around 1,700 direct jobs and approximately double this figure (3,400) as indirect jobs. The sector depends totally on Palestinian fresh milk. Prior to 2001, the amount of

milk production did not increase more than 40% but with the improvement in the Palestinian industry, the percentage has been increased to 70%.

The improvement can also be seen with the increase of the dairy cows owned by Palestinian farms. In 1999, there were 10,826 dairy cows; now, however, the number exceeds 19,000 dairy cows. Additionally, today there are more than 10 Palestinian farms, which contain more than 200 dairy cows each and operating according to best practices and operating high technology equipment. The sector's total investments are 67 million dollars, while the export volume is 5 million dollars annually, which mainly consists of white boiled cheese. The dairy sector has great potentials according to several local and international experts. This encourages further future investment and improvements in the production process of the sector.

Cheese is one of the most versatile foods suitable for all age groups which can be consumed in many different meal occasions. Traditionally, cheese has been regarded by the consumers as a nutritious food because it is a source of high quality proteins, dietary calcium, fat and other nutrients. The basic composition and structure of cheeses are determined by the manufacturing operations like pH at renneting, but it is during ripening that the individual and unique characteristics of each cheese variety develop.

Local white brine cheese is probably the most popular and economically important traditional cheese in Palestine (Messer *et al.*, 1985). As this cheese is usually produced by traditional methods, problems such as non-uniform quality and flavor and texture defects are often encountered. local White cheese is a close textured brined cheese made from cow's milk, sheep's milk or mixtures of them. The main flavor characteristics are milky and saltiness, this cheese is widely consumed all over Palestine as a major diet in breakfast and after partial or complete desalting can be used in the production of some bakery products and Arabic sweets particularly Kunafa and madlokah which is the traditional sweets dishes in Palestine (Basson, 1981).

It is desirable that Local White cheese shows the ability to melt and stretch in order to fit in the production of a high quality Kunafa. However, these characteristics are not always available, especially in cheese made from high quality milk where spontaneous fermentation is minimal in

the milk as well as in the curd before boiling (Basson, 1981). Different ways are being practiced improving these characteristics:

- Firstly, using a mixture of cow, sheep or goat milk, but this method is not reliable.
- Secondly, keeping the curd which is produced from raw milk at room temperature for a period of time till it is boiled. In fact, this period may allow a spontaneous growth of natural microflora. However, this method is practiced in braided cheese Mujadalah or Mushalalah cheese but not practical in Local White cheese and very difficult to control.
- The third one is storing boiled cheese in its cans for a long period, i.e. aging. This long period may allow substantial microbial growth namely halotolerant and halophytic microorganisms or the cheese may be altered by certain proteolytic enzymes that affect milk proteins. Also, this method is neither practical nor reliable (Basson, 1981).

Although, the way of curd fermentation is the most effective characteristic, it is not controllable and may produce low quality cheese which suffers from excessive deformation, off-color and off-flavor during boiling.

The idea of making cheese stretchable, meltable and smooth is to hydrolyze some of the peptide bonds of the more or less intact proteins (casein) or to destruct some covalent cross-linking bonds of the protein matrix, using protease enzymes, particularly coagulation enzymes and emulsifier salts, which called melting salts (Hassan, 2007).

Coagulation enzymes in cheese making have a dual role. The primary function is to coagulation milk in order to produce cheese curds; in addition, a small proportion of the coagulant is carried over into the cheese. This residual coagulant remains proteolytically active in most aged cheeses and plays an important role in the development of texture and flavor by minimizing the cross linkage and by partial hydrolysis of milk proteins (Messer *et al.*, 1985).

However, the objective of this study was to study the effect of unit operation alteration and some additives on the characteristics of Local White brined cheese.

1.3 Local White cheese preservation and deterioration:

Local White cheese is classified as semi-hard cheese (moisture content 45-55%). It is boiled in brine for the sake of preservation (Humeid, 1990) and it is often consumed after removing a part of its salt by soaking in water.

The keeping ability of Local White cheese depends mainly on high salt concentration of storage medium (brine) and boiling of cheese before storage in cans (Humeid, 1990). The boiled cheese may develop bad flavor during storage in cans, slime formation, bitterness gassiness or hardening problem, in spite of high salt concentration of the brine. It was found that the cause of deterioration is halophilic bacteria that are able to grow at high salt concentration. All these defects can affect quality of cheese and cause severe deterioration in cheese. Therefore, in a study to avoid high salts and microbial growth effect, reducing the PH by adding lactic acid. Decreasing the PH of cheese to 4 by the addition of lactic acid permitted the reduction of salt in brine and cheese to 10% concentration, this combination inhibited the growth of bacteria without negatively affecting texture and sensory quality of cheese.

1.4 Importance of Local White cheese in the production of Arabic sweets:

Desalting of Local White cheese make it suitable for consuming at breakfast, as snack or as an ingredient in the preparation of local traditional dishes and sweets, especially Kunafa (Basson, 1981). Kunafa (Kunafeh or Knafeh) is probably the most popular Arab sweets in many Arab Middle Eastern countries. Kunafa is made of vermicelli like threads made of wheat batter formed on heated metal surfaces. The threads are mixed with flesh (samna) and spread over round aluminum baking pan covered with a desalted Local White cheese layer, then covered with a further Kunafa threads layer and baked in both sides. The baked Kunafa is sweetened with a heavy sugar syrup flavored with rose essence. A second way of preparation is to grind the threads after mixing with ghee to fine granules, a layer of these granules, is spread over a baking plate, covered with desalted Local White cheese and sprinkled with a light layer of the dough granules then baked and thoroughly on a special gas flame, then turned over a second plate, heated gently and sweetened with sugar syrup (Yammani *et al.*, 1997).

One of the most important characteristic of the used cheese in Kunafa preparation is meltability and stretchability, the meltability is important to brand cheese to Kunafa crumbs to form a continuous layer facilitating turning over of Kunafa, whereas stretchability is favored by the consumer as in pizza (Yammani *et al.*, 1997).

1.5 Scientific Background:

In order to be able to fully understand what determines cheese quality and texture, it is necessary to have an understanding of the physical and chemical mechanisms that occur during cheese processing.

Generally, the components and processing techniques are basically the same for all cheeses, but the proportions of these components vary, which promote the production of different white cheeses characteristics.

1.5.1. Milk Composition:

The main components of milk are fat, lactose, proteins and minerals. These contribute to the ~13% total solids content in milk. The general composition of raw milk is shown in Table 1.1. The fat component of milk is mainly triglycerides (98%), present as an emulsion of fat globules stabilized by a phospholipid and glycoprotein membrane. The minerals of milk occur either in solution or are associated with the proteins, as either undissolved salts or bound ions. Lactose is a soluble carbohydrate molecule, a disaccharide of glucose and galactose.

Table 1.1: Typical raw cow milk composition.

Component	Level in milk % (w/w)
Water	87.3
Fat	3.9
Protein	3.25
Casein Protein	2.6
Whey Protein	0.75
Lactose	4.6
Minerals	0.65
Organic acids	0.18

From Walstra and Jenness (1984)

The protein content of milk, ~3.5% (w/w), can be divided into whey and casein. Protein precipitated at 20°C from milk adjusted to pH 4.6 is called casein, and comprises ~80% of the total protein. Casein exists mainly as spherical colloidal structures known as micelles. The remaining proteins which remain soluble at pH 4.6 are called the whey or serum proteins.

1.5.1.1. Casein:

Casein proteins can be fractionated into four distinct groups; α_{s1} -casein, α_{s2} -casein, β -casein, and κ -casein. The properties of casein proteins have been reviewed by Whitney (1988), Rollema(1992), and Swaisgood (1992) (see Table 1.2). The casein proteins are amphipathic as they contain both polar and non-polar regions. Casein proteins appear to have some secondary structure as determined by spectral methods and various predictive methods, and a definite unordered tertiary structure (Swaisgood, 1992). κ -Casein is estimated to contain 10-20% α -helix, 20-30% β -structure and 15-25% β -turns (Swaisgood, 1992). α_{s1} -, α_{s2} - and β -caseins appear to have more α -helix and less β -structure, than κ -casein, particularly α_{s1} - and α_{s2} -caseins (Swaisgood, 1992). Holt and Sawyer (1988) showed that the conserved predicted helix content of α_{s1} -, α_{s2} -, β - and κ -casein are 24%, 17% and 13% and 3%, respectively. Approximately 5- 17% of casein protein residues are proline, and this has a marked influence on the protein structure, as proline is often found in the bends of folded protein chains. Proline residues are occasionally

found in both α -helix and β -structures, but are generally not favorable to either (Richardson *et al.*, 1992). The tertiary structure of the caseins has been investigated by three-dimensional modelling, based on the secondary structure developed from spectroscopic data (Richardson *et al.*, 1992).

Caseins undergo post-translational phosphorylation, glycosylation and proteolysis. The seryl and occasionally threonyl residues of all caseins are phosphorylated to varying degrees. Glycosylation of κ -casein occurs mainly in the polar domain and the availability of threonyl and seryl glycosylation sites are determined by the secondary and/or tertiary structures, as none of the threonyl residues in α_{s1} -, α_{s2} - and β -casein are modified (Swaisgood, 1992). β -Casein is particularly susceptible to proteolysis by plasmin, releasing γ -caseins and proteose peptones. Chymosin is noted for its specific hydrolysis of the Phe-Met bond which splits κ -casein into its hydrophobic and hydrophilic domains (Swaisgood, 1992).

The binding of Ca^{2+} to caseins is mainly through the phosphoseryl residues. Binding capacity follows the order $\alpha_{s2} > \alpha_{s1} > \beta$ -casein, where Ca^{2+} -induced precipitation starts in the range of 2 mM to 15 mM of Ca^{2+} . Even at Ca^{2+} concentrations less than 2 mM α_{s2} -casein begins to precipitate (Toma & Nakai, 1973), and at concentrations of 3-8 mM α_{s1} -casein precipitates. At 1 °C, β -casein remains soluble in Ca^{2+} concentrations up to 400 mM, but precipitates in the range of 8- 15 mM Ca^{2+} at 37°C (Farrell *et al.*, 1988). κ -Casein remains relatively soluble due to the low number of phosphoseryl residues (Toma and Nakai, 1973).

Table 1.2-a: Properties of casein protein.

Attribute	Casein protein			
	α_{s1} -casein	α_{s2} -casein	β -casein	κ -casein
Concentration in Milk (g/L)	10	2.6	9.3	3.3
Molecular weight (Da)	23614	25320	23983	19023
Phosphoseryl residues	8,9	10,11,12,13	4,5	1

Table 1.2-b: Properties of casein protein.

Attribute	Casein protein			
	α_{s1} -casein	α_{s2} -casein	β -casein	κ -casein
Proline residues	17	10	35	20
Cysteine residues	0	2	0	2
Hydrophobicity (kJ/residue)	4.9	4.7	5.6	5.1
Distribution of protein in the micellar phase (%)	94	97	87	85
Net charge at pH 6.6	-21.0 to -23.5	-12.2 to -17.1	-9.2 to -13.8	-2.0 to -3.0

Table 1.2-a,b: Adapted from Walstra and Jenness (1984), Swaisgood (1992) and Rollema (1992).

Self-association of caseins is mainly through electrostatic and hydrophobic interactions. α_{s1} -Casein association is initiated by increasing ionic strength; at low ionic strength (0.003-0.01 M) and neutral pH the protein exists as a monomer. The association decreases as the pH increases, and temperature has little effect (Rollema, 1992). The extent of α_{s2} -casein association is dependent on ionic strength, with self-association increasing with increasing ionic strength, but decreasing above 0.2 M.

Payens and Schmidt, 1966 and Snoeren *et al.*, 1980 expressed the polymerization of both α_{s1} - and α_{s2} -caseins proceeds by a series of consecutive, as following:



The self-association mechanism of β - and κ -caseins, with their amphiphilic behavior is micellar-like in its nature, and can be described as a monomer-polymer equilibrium, as following:



Association is driven by hydrophobic interactions between the C-terminal segments, and opposed by repulsion between the charged N-terminal segments. Hydrophobic interactions cause the temperature effect, and the ionic strength effects are a result of the charge repulsion (Rollema, 1992). β -Casein association is strongly dependent on ionic strength and temperature. As the temperature or ionic strength is increased β -casein association increases (Schmidt and Payens, 1972). The degree of β -casein polymerization (n) depends on temperature, ionic strength and probably pH, and ranges between 12-59 (Rollema, 1992). Polymerization of K-casein is relatively independent of temperature (4 and 20°C) and ionic strength (0.1 to 1.0 M), and n is approximately 30 (Vreeman *et al.*, 1981).

1.5.1.1.1. Casein Micelle Structure:

Due to their high hydrophobicity, ~95% of the caseins associate together to form micelles. The micelle is composed of casein protein (92%) and inorganic salts (8%) largely in the form of calcium phosphate (Swaisgood, 1992). The size distribution of the casein micelles is relatively wide, 15-600 nm with an average diameter of 200 nm (McGann *et al.*, 1980). The micelle structure is rather porous, indicated by the high values for voluminosity and hydration that have been found (Rollema, 1992). Compositional studies based on micellar size show that the fractional content of α_s - and β -casein decreases and that of κ -casein increases with decreasing micelle size (Donnelly *et al.*, 1984). The outer surface layer of the micelle is thought to be composed of equimolar amounts of α_s - and κ -caseins with a small amount of β -casein, while the interior contains β - and α_s -caseins in equimolar amounts and only a minor amount of κ -casein (Dalglish *et al.*, 1989). κ -Casein which is found predominantly at the surface is responsible for micelle stabilization. The hydrophilic C-terminal of κ -casein protrudes into the surrounding solution, reducing surface hydrophobicity and providing electrostatic and steric stabilization (Walstra, 1990; Swaisgood, 1992).

Although the structure of the casein micelle is not entirely clear, it is believed to be composed of a number of sub-micelles or sub-units linked together by colloidal calcium phosphate (CCP), with hydrophobic and hydrogen bonds contributing to the relatively stable structure. Electron micrographs have been the basis for the sub-micelle theory, as discrete subunits ~10 nm in

diameter have been observed in casein micelles (Schnidt & Buchheim, 1970; Knoop *et al.*, 1973; Kalab *et al.*, 1982). A number of models based on the sub-micelle structure have been put forward by Slattery and Evard (1973), Schnidt (1982) and Walstra and Jenness (1984). The structural model proposed by Schnidt (1982) (see Figure 1.1).

Sub-micelles of varying casein composition are linked by CCP. Sub-micelles enriched in κ -casein are located at the surface while κ -casein depleted sub-micelles are buried inside the micelle. Walstra and Jenness (1984) developed the model to include the concept of steric stabilization of the micelle by κ -casein (Figure 1.2). The C-terminal end of κ -casein is proposed to stick out into the surrounding solution giving the micelle a hairy surface. A recent model proposed by Holt (1992) suggests that the micelle is composed of macrogranules of calcium phosphate incorporated in a protein matrix, with the micelle surface having a hairy layer to provide steric stabilization. This model is without sub-micelles, and there have been recent objections to a sub-micelle model (Visser, 1992). Visser (1992) claims that changes in the micelle caused by calcium sequestration, cooling and the lowering of pH of skim milk are not effectively explained by the sub-micelle model. Therefore, the casein micelle structure is not fully established, and further work is required to determine the true nature of the protein/mineral complex.

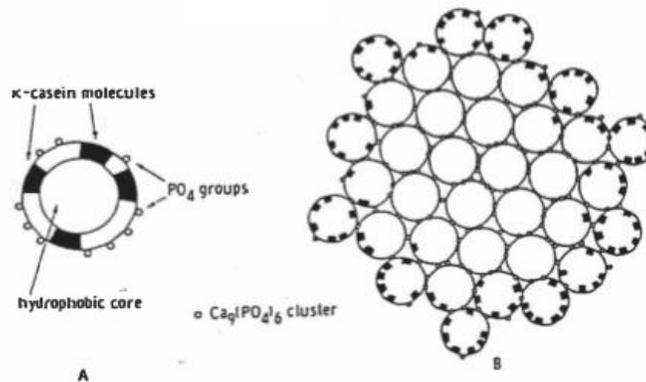


Figure 1.1: Sub-micelle showing A. sub-micelle and B. Casein micelle

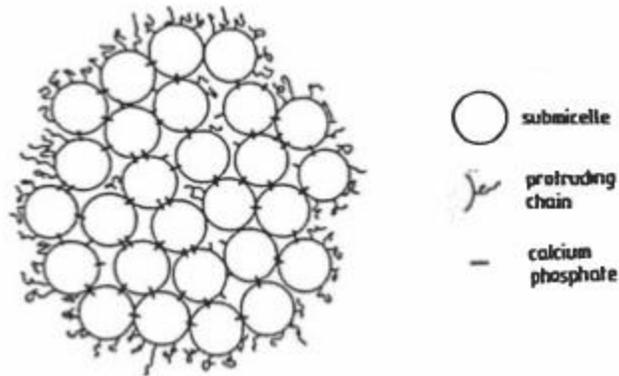


Figure 1.2: The protruding C-terminal portion of κ -casein.

Dissociation of casein from the micelle is caused by a number of treatments, such as heating, cooling, pH adjustment, calcium chelating agents. Heating (120°C) and cooling milk (from 30 to 5°C) both causes dissociation of casein proteins from the micelle, in particular κ -casein on heating (Singh and Creamer, 1991b) and β -casein on cooling (Downey & Murphy, 1970). Acidification results in the loss of the micellar calcium phosphate, which is accompanied by a concomitant release of casein proteins from the micelle (Rose, 1968; Roefs *et al.*, 1985; Dalgleish & Law, 1988). At constant pH, gradual removal of calcium from the micelles by chelation with EDTA, initially causes a release of caseins without any major effect on the micelle size, but ultimately leads to complete micellar disintegration (Lin *et al.*, 1972).

1.5.1.2. Whey Proteins:

Raw bovine milk contains ~0.7% whey protein. The whey proteins are globular to ellipsoid in structure, relatively soluble and heat labile, with the exception of the proteose peptones. The five major groups of whey proteins are β -lactoglobulin (β -lg), α -lactalbumin (α -la), bovine serum albumin (BSA), immunoglobulins (Ig) and proteose peptones (PP). The structure and properties of various whey proteins have been reviewed by Swaisgood (1982), Eigel *et al.* (1984), Mulvihill and Donovan (1987) and Whitney (1988) (see Table 1.3).

Table 1.3 General characteristics of whey proteins in milk.

Whey protein	Concentration in milk (g kg ⁻¹)	MW (Da)	Isoelectric point	Disulphide bond
β-Lactoglobulin	3.3	18363	5.13	2
α-Lactalbumin	1.2	14147	4.2-4.5	4
Bovine serum albumin	0.4	66267	4.7-4.9	17
Immunoglobulins	0.7	(1.5-10)*10 ⁵	5.5-8.3	21
PP and minor whey proteins	0.8	(4.1-40.8)*10 ³ of proteose peptones	-	0

1.5.1.2.1. β-Lactoglobulin (β-Lg):

The most abundant whey protein is β-Ig, which represents 50% of the total whey proteins in bovine milk. There are seven known genetic variants of β-Ig; A, B, C, D, E, F and G (Eigel *et al.*, 1984). The A and B genetic variants (β-Ig A and β-Ig B) are the most common and exist in almost the same frequency. These two variants differ at positions 64 and 118, where Asp and Val in β-Ig A are replaced by Gly and Ala in β-Ig B (Braunitzer *et al.*, 1972).

In its natural state, β-Ig is a dimer of two non-covalently linked monomeric subunits. The dimer is stable between pH 5.5 and 7.5, but dissociates due to strong electrostatic repulsions below pH 3.5. In the pH range 3.5 to 5.2, the dimers tetramerize to form octamers. Above pH 7.0 β-Ig undergoes reversible conformational changes, and above pH 8.0, the protein is unstable and forms aggregates of denatured protein (Lyster, 1972).

There are five cysteine residues per molecule of β-Ig, but only four of these are able to form disulphide linkages. The first disulphide bridge occurs between positions 66 and 160, while the second forms between position 106 and one of two positions, 119 and 121. This leaves one free thiol group distributed equally between positions 119 and 121 (McKenzie, 1971; McKenzie *et*

al., 1972). The thiol group is important in the interactions that occur between β -Ig and the other proteins on heating, mainly κ -casein and α -lactalbumin (Walstra and Jenness, 1984).

The secondary structure of β -Ig, determined by circular dichroism, contains 10% α -helix, 43% anti-parallel β -sheet and 47% unordered structure (Townend *et al.*, 1967). The monomer is roughly spherical with a diameter of 3 nm (Green *et al.*, 1979). Four distinct crystal forms of β -Ig (lattices K, X, Y and Z) have been obtained by X-ray crystallography (Green *et al.*, 1979). (Papiz *et al.*, 1986) identified the orthorhombic lattice Y as consisting of nine strands of anti-parallel β -sheet, eight of which wrap around to form a flattened conical barrel. Around this barrel is a three-turn α -helix.

The biological function of β -Ig still remains unknown, although from its amino acid composition the protein is high in nutritional value. Stability to acidic conditions and gastric proteolysis have suggested that β -Ig serves a transport function in the intestine of neonate calves where specific receptor sites have been found (Papiz *et al.*, 1986). There is some indication that retinol and fatty acids are bound to β -Ig in milk (Garrick, 1986; Puyol *et al.*, 1991), and that their uptake in the intestinal tract is enhanced by this association (Said *et al.*, 1989).

1.5.1.2.2. α -Lactalbumin (α -La):

The second most abundant whey protein, α -la, has three known genetic variants, and accounts for 20% of the whey proteins. There are four interchain disulphide bonds, but no sulphhydryl groups are present. The disulphide bridges are located between amino acids 6 and 120, 28 and 111, 61 and 77 and between 73 and 91 (Eigel *et al.*, 1984).

The secondary structure of α -la contains 26% α -helix, 20% β -sheet, and 60% unordered structure (Robbins & Holmes, 1970). The similarity in the primary structure of α -la and lysozyme has led to the development of a three-dimensional structure for α -la based on the main chain conformation of lysozyme (Browne *et al.*, 1969).

The physiological role of α -la is to form part of the enzyme galactosyltransferase, which produces galactose in lactose synthesis. α -La binds two atoms of calcium very closely, and is rendered susceptible to denaturation when these atoms are removed (Walstra and Jenness, 1984).

1.5.1.2.3. Bovine serum albumin (BSA):

BSA is identical to the serum albumin found in the blood stream, and represents ~5% of the total whey proteins (Eigel *et al.*, 1984). The protein is synthesized in the liver and gains entry into the milk through the secretory cells. The secondary structure consists of 55% α -helix, 16% β -sheet and 25% unordered structure (Reed *et al.*, 1975). There is one free thiol group and 17 disulphide bonds which act to form the protein into a multiloop structure. The structure is thought to be ellipsoid in shape with three domains, two large double loops and one small loop (Brown, 1977). BSA appears to function as a carrier of small molecules, such as fatty acids, but any specific role that BSA may play is unknown (Walstra and Jenness, 1984).

1.5.1.2.4. Immunoglobulins (Ig):

Immunoglobulins which make up 10% of the whey proteins are antibodies, that are polymers of two kinds of polypeptide chains, light chains (MW of 22,400 Da) and heavy chains. There are two types of light chains (κ and λ .) which differ in amino acid chain structure but have homologous sequences (Larson, 1992). The heavy chains can be of several different types, γ (MW of 52,000 Da), α (MW of 52,000-56,000 Da) or μ (MW of 69,000 Da).

Both light and heavy chains have a constant region and a variable region, which is associated with immunological specificity. Two light and two heavy chains are joined by disulphide linkages to form a Y shaped structure (Silverton *et al.*, 1977). A disulphide bond connects each of the light chains to a separate heavy chain, and the heavy chains are held together by disulphide bonds (Larson, 1992). Immunoglobulins contain 21 disulphide bonds per molecule (Kumar and Mikolajcik, 1973). The majority of Ig in milk are transported there from the blood, and these blood Ig are overwhelmingly comprised of Immunoglobulin G (IgG). IgG consist of two identical heavy (γ) and two identical light (κ or λ .) chains. Four types of immunoglobulins have been found in bovine milk, IgM, IgA, IgE, IgG (Eigel *et al.*, 1984).

1.5.1.2.5. Proteose peptones (PP):

The whey proteins which remain soluble at pH 4.6 after heating at 95-100°C for 30 min, but which are insoluble in 8-12% trichloro acetic acid are called proteose peptones. There are four groups, named after their electrophoretic mobility; PP-3, PP-5, PP-8-fast and PP-8-slow. PP-3 is probably derived from a fat globule membrane constituent, and components PP-5, PP-8-fast, and PP-8-slow are fragments of β -casein produced from proteolysis by alkaline milk proteinase (Walstra and Jenness, 1984; Mulvihill and Donovan, 1987).

1.5.1.2.6. Minor whey proteins:

β_2 -Microglobulin is a single polypeptide chain consisting of approximately 100 amino acid residues (MW of 11,800 Da). Transferrin is a common blood plasma protein. Lactoferrin and transferrin are both iron binding proteins that exist as large single chain polypeptides of 600-700 residues. Lactoferrin is an inhibitor of bacteria because it deprives them of iron, but the antibacterial effect is not significant because of its very low concentration (Walstra and Jenness, 1984).

1.5.1.3. Minerals:

The salts in milk are divided mainly between the colloidal and soluble phases, with a limited amount bound to the fat globules (Walstra and Jenness, 1984). Milk is supersaturated with calcium and phosphorus in the form of phosphate. This allows the formation of insoluble colloidal calcium phosphate (CCP) complexes which stabilize the micelle. X-ray absorption and infrared spectroscopy indicate that CCP resemble brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (Holt *et al.*, 1982, 1989). The structure of CCP has been shown to be amorphous by X-ray and electron diffraction (Knoop *et al.*, 1979).

Holt in 1995 suggests that, CCP can be viewed as hydrated clusters of calcium and phosphate ions surrounded by casein phosphate clusters. The ion cluster is approximately 2.5 nm in diameter (McGann *et al.*, 1983), indicating a composition of approximately 66 calcium ions, 132 water molecules and 66 phosphate moieties (Holt, 1995). An alternative model for the structure

of CCP have been proposed by van Dijk (1990a, b, 1991). Two organic phosphate groups are linked to a structure combining 4 inorganic phosphates and 8 divalent ions (mostly calcium). The micellar system also contains magnesium and citrate which interact and form part of the CCP structure. The salt balance between the colloidal and soluble phases largely determines the physico-chemical state of milk and hence the thermal behavior of the proteins. The distribution of the main salts between the two phases is shown in Table 1.4.

Table 1.4: Distribution of major salts between the colloidal and Soluble Phases.

Mineral	Concentration in milk (mg per 100g)	Present in serum (%)
Na	48	95
K	143	94
Mg	11	66
Ca	117	32
Inorganic phosphate	203	53
Citrate	175	92

From Walstra and Jenness (1984)

Milk salts fall into three families of correlations. The first involves lactose, K, Na and Cl. Lactose is negatively correlated with K in order for the milk to be osmotically balanced with blood, and Na is positively correlated with Cl. The second correlation involves; (i) diffusible Ca, Mg and citrate and; (ii) Ca^{2+} , HPO_4^{2-} and pH. At constant pH, diffusible Ca and Mg are closely and directly correlated with citrate, and there is a negative correlation between Ca^{2+} and HPO_4^{2-} (Holt, 1985). The third involves colloidal Ca, Mg, P and citrate (constituents of CCP), which are closely related to the casein content.

In the serum phase, the monovalent ions Na^+ , K^+ and Cl^- exist in milk mainly as free ions, whereas the multivalent ions such as Ca^{2+} , Mg^{2+} , PO_4^{3-} and Cit^{3-} form complexes such as HPO_4^{2-} , $CaCit^-$ and $MgHPO_4$ (Holt, 1985). Free ion concentrations in the serum phase determined calorimetrically, by ion-selective electrode, by ion exchange methods and by calculation indicate that 20-30% of Ca and Mg are free ions (Holt, 1985).

Chapter two

2. Literature Review

2.1 Introduction:

On reviewing the available literature, it is evident that extensive research has been carried out to address various aspects of the cheese characteristics. With this in mind, in an attempt to simplify and delimit the subject to some extent, this chapter will focus primarily on the work that has been carried out on the alteration needed to develop and enhance white cheese in term of unit operation and material enhancers addition.

The primary objective of this chapter is to provide an insight into the theoretical and practical aspects of the making cheese process. This will include an examination of the transport properties related to the curd formation phenomena, with major emphasis being placed on the curd development aspects. Thus, the chapter will review and highlight the structural and formation of the curd as well as the curd characteristics in term of cheese merchandize.

2.2 Cheese making:

Milk is composed of water, proteins (primarily casein), lipid, lactose, and minerals. The goal when making cheese is to form a network from the casein that entraps the fat and some of the water and concentrate this by removing excess whey. However, making cheese is a process consists several steps, as shown in Figure 2.1.

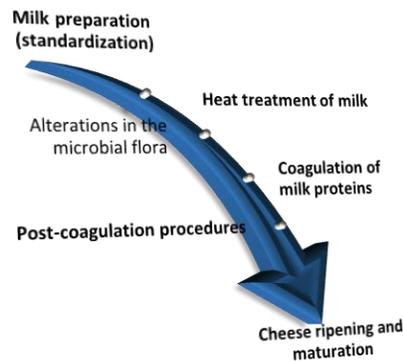


Figure 2.1: schematic flow chart for cheese making

2.2.1. Milk preparation (standardization):

The first stage in the processing of cheese is milk standardization. Milk standardization gives the producer the ability to manipulate the composition of the final cheese by controlling the composition of the starting milk in order to meet the legal definition of the specific variety and to improve yields. There are several ways in which milk is standardized (Lucey and Kelly, 1994). First, the fat content of the milk can be altered. This is done in order to meet legal standards for fat in the dry matter and for labeling considerations. Typically, fat content is increased by adding cream or decreased by adding skim milk. Second, protein content can be altered. The casein content of the original milk ultimately determines the amount of cheese that can be produced since caseins form the structural matrix and can retain moisture (Scott, 1998). Protein content is usually increased by either adding nonfat dry milk or evaporated condensed milk.

Other considerations of the initial milk that affect cheese yield include the source of the milk and whether or not the milk is homogenized. Milk coming from mastitic cows has higher somatic cell counts, indicating an increase in plasmin activity. Plasmins degrade β -caseins, (Fox, 1989) which initially results in less network formation and lower yields. Also, the breed of cow and time of lactation influences the composition of the milk (Lucey and Kelly, 1994). Homogenization is the process in which fat globules in the milk are broken up into smaller pieces. Use of homogenized

milk for cheese-making will produce higher yields since more moisture will ultimately be retained in the protein network. However, such milk produces a finer network of protein, which impairs curd-matting properties and results in cheese with decreased end-use functionality such as stretch and melt (Rowney *et al.*, 1999).

Additionally, the use of ultrafiltration to concentrate the milk has adverse effects on texture and rate of maturation. Ultra-filtered (UF) milk affects the composition, structure, and maturation of cheese, which then affects the texture. In a study comparing cheese milk, cheese curds, and final cheese at five weeks and twenty-four weeks of UF milk cheese and control milk cheeses, it was found that the protein network became coarser throughout the entire processing scheme as the concentration factor of the milk increased. They also found that larger pockets of fat in the curds resulted in greater concentrated milks throughout the entire processing scheme (Green *et al.*, 1981). From a sensory perspective, firmness, crumbliness, granularity, and dryness increased as the concentration factor of the milk increased. Elasticity was affected little by the concentration. Since structurally, the UF and the control milk were similar, they hypothesized that structural differences seen between the curds of each were probably due to differences in the concentration of milk components rather than the structural differences in the initial milk. They hypothesized that the basic structure of the protein network is established during the initial curd formation and is not altered much during later stages of ripening.

2.2.2. Heat treatment of milk:

Heat treatment is one of the major processing steps of milk. Regardless of its final use, the majority of milk is heat treated at least once. The most used heat treatments of milk are pasteurization and sterilization. Heat treatment of milk is performed to limit bacterial load and enzyme activity to secure the safety of the dairy product for human consumption and for extending the shelf life of the final product. Heat treatment also gives rise to different chemical changes in milk, such as non-enzymatic browning reactions involving lactose and especially lysine residues in the protein. This gives rise to off-flavors, change in color and loss of available

lysine which has high nutritive value (Singh and Waungana, 2001). Furthermore, when heating above 100 °C can cause decrease in pH, which is caused by formation of organic acids from lactose degradation and precipitation of calcium phosphate (Martinez-Castro *et al.*, 1986). Pasteurization is a heat treatment which aims to reduce the number of pathogens to such an extent that it does not constitute any health hazard. There are different pasteurization methods but one of the most used is high-temperature, short-time heating (HTST) where the milk is heated to 72-80 °C for 15-30 s. Pasteurization at low temperature, long time (LTLT) at 63 °C for 30 min is still used, but not to the same extent as HTST due to longer processing time and it has been shown that HTST gives less chemical changes than LTLT heat treatment (Lewis and Deeth, 2008). Ultra-high temperature (UHT) heating is a sterilization process of milk which is used to destroy all microorganisms and spores in the milk and many enzymes are also inactivated. This is done by heating temperatures of 135-150 °C for 1-10 seconds (Fox and Kelly, 2006).

The design of heat treatment and combination of temperature and heating time depends on the desired approach with least undesirable changes. High heat treatment and heating time gives most significant changes. Heat treatment causes whey protein denaturation which is an irreversible process. The mineral balance also changes during heat treatment. Calcium and phosphate becomes more insoluble and binds to the casein micellar structure. This is reversible for temperatures below 100°C, while severe heating can cause hydrolysis of phosphoserine of caseins and calcium phosphate can precipitate out of solution which are irreversible processes (Gaucheron, 2011).

2.2.2.1. Heat induced denaturation of milk proteins:

Caseins are very heat resistant due to their loose structure and it is generally accepted that they can withstand heating at 140 °C for 15-20 min as the random coiling of the primary chain is generally hard to destroy compared to secondary and tertiary structures. To some extent both dephosphorylation and hydrolysis of the caseins has been found in heat treated milk (Belitz *et al.*, 2004; Farrell Jr. *et al.*, 2004; Fox, 1980).

Heat treatment above 100°C gives a decrease in micellar size due to increase in colloidal phosphate and dissociation of κ -casein from the micelle surface (Singh and Waungana, 2001).

The globular structure of whey protein makes them heat labile. Heat treatment of the whey proteins above 60°C, results in unfolding of the globular structure and the proteins thereby denature.

The denaturation of whey proteins is generally considered involving two steps. The first step is an unfolding of the native globular structure, which leads to exposure of hydrophobic residues and disulphide bonds. If the heat treatment is minimal, the unfolded protein can refold into native structure. At high temperatures, the unfolded proteins will form new hydrophobic interactions and disulphide bridges which can result in refolding the protein, but this is often disordered and gives rise to a random structure. The increase in the reactivity of the unfolded protein heated at high temperatures can also lead to the second step of the denaturation process. The unfolded whey proteins can form aggregates with other molecules, mostly through disulphide bonding and covalent bonds (Singh and Latham, 1993). Immunoglobulin's and BSA are the least stable whey proteins, β -Lg is intermediate and α -La is the most resistant protein to heat denaturation. These differences in extent of heat denaturation are caused by the differences in structure and strength of intermolecular bonds (Anema, 2008; Corredig and DaLgleish, 1996a).

The dimer of β -Lg dissociates between 30 and 55°C, but these changes are reversible and the monomers can rebound by cooling if the temperature does not exceed 60°C. When heating to temperatures above 60-70°C, the tertiary- and also partly secondary structure of the monomer starts to unfold, leading to exposure of the free thiol group (Cys121) and hydrophobic parts of the residues chain, resulting in a reactive monomer. (Iametti *et al.*, 1995 and Iametti *et al.* 1996). The formation of these monomers is irreversible and they cannot refold to native state. Instead there will be formed non-native monomers, which can form aggregates with other monomers but also aggregates with other types of proteins can be formed (Tolkach and Kulozik, 2007).

α -La is the least heat resistant whey protein with a denaturation temperature around 62°C, but the unfolding at this temperature is reversible. It does not form aggregates or modified monomers at heating temperature below 80°C at neutral pH (pH 6.6-6.8). This is due to α -La having no free thiol groups which can change the reactivity of α -La (Eigel *et al.*, 1984). α -La is capable of refolding to its native state in presence of calcium if the disulphide bonds are still intact (Brew, 2003). The binding of calcium is very pH dependent and calcium dissociates from the α -La binding site at pH below 5 which makes α -La lose the ability to refold to its native structure after heat treatment. Severe heating conditions with temperatures above 100°C for several minutes disrupt the disulphide bonds and formation disulphide linked aggregates of denatured α -La occurs (Singh and Havea, 2003).

The main aggregates formed as a consequence of heat treatment of milk, are complexes formed by aggregation of denatured whey proteins and complexes between β -Lg and κ -casein on the surface of the casein micelles via disulphide bonds and hydrophobic interactions. At temperatures below 70°C the interaction is mostly caused by hydrophobic interactions while at higher temperatures it is mostly caused by disulphide bonds (Corredig and DaLgleish, 1996a; O'Connell and Fox, 2011).

The κ -casein and β -Lg interactions are most pronounced when the κ -casein is placed on the surface of casein micelles as the association between β -Lg and κ -casein is less favorable when κ -casein is dissolved in serum. This can be caused by κ -casein is present in a more compact structure when dissolved in serum whereas placed on the surface of casein micelles the structure of κ -casein is more loose (Donato *et al.*, 2007). The formation of these complexes may be altered by a slow heating rate or heating for a long time at lower temperatures. This gives longer time for the β -Lg to unfold and associate with the casein micelles.

In contrast, a rapid heating rate to the required temperature gives a shorter time for unfolding and it is more likely that β -Lg refolds in a non-native structure or forms aggregates with other unfolded monomers instead of associating with κ -casein (Oldfield *et al.*, 1998b).

α -La does not associate with the casein micelles on its own like β -Lg; it has to form complexes with β -Lg which then associates with the casein micelle and it requires a prolonged heating period to start associating with the casein micelle (Oldfield *et al.*, 1998b; Oldfield *et al.*, 2000).

The rate of denaturation is mainly controlled by heating temperature, heating time and pH but also protein concentration and ionic strength have been proved to have some effect (McSwiney *et al.*, 1994; Oldfield *et al.*, 2000; Qi *et al.*, 1995). At neutral pH the free disulphide group of β -Lg is very reactive and this is the main mechanism for aggregation and gives a faster aggregation of β -Lg. Dissanayake *et al.* (2013b) have shown that the denaturation rate is significantly lower at pH 3 compared to pH 6 and the aggregates formed at pH 3 are caused by non-covalent bonding. This is consistent with free disulphide groups being inactivated at acidic conditions. At neutral pH most whey protein complexes formed by denaturation are soluble. A decrease in pH below 6.2 followed by heating gives a faster formation of whey protein/ κ -casein complexes and they were often associated with the casein micelles. Heating at a pH above 6.8 leads to dissociation of the whey protein/ κ -casein complexes from the micelle surface (Zúñiga *et al.*, 2010).

The reaction kinetics of the denaturation of β -Lg has been widely investigated in order to predict the extent of denaturation according to different heat treatments. Dannenberg and Kessler (1988) determined the reaction order for the denaturation of β -Lg for each temperature by using the model, equation 1.

$$\frac{c_t^{1-n}}{c_0} = 1 + (n - 1)kt$$

where n is reaction order different from 1 and $\frac{c_t}{c_0}$ is the ratio of denatured β -Lg at holding time t and k is the rate constant at a given temperature. They found that a reaction order of 1.5 gave a linear correlation between denaturation of β -Lg and holding time in skim milk for various temperatures having the rate constant defined as the slope of the linear graph.

This has been verified since, i.e. Zúñiga *et al.*, (2010) and it is now widely used to report denaturation of β -Lg in milk. Kessler and Beyer (1991) have shown that the reaction number varies between 1.5 and 2 according to the casein/whey protein ratio in the milk with skim milk having a reaction order of 1.5 while sweet whey has a reaction order of 2.

Factors such as the methods used to detect differences in β -Lg denaturation, lack of enough data to make an accurate determination, the heating methods and how the samples are heated, including preheat time, temperature and cooling rate, can have a great impact on the denaturation of β -Lg (Oldfield *et al.*, 1998a).

Dannenberg and Kessler (1988) also investigated the effect of temperature on the rate constant k for denaturation of β -Lg in skim milk. The rate constants for various temperatures can be used to make an Arrhenius plot, equation 2.

$$\ln(k) = \ln(k_0) - \frac{E_a}{R} \cdot \frac{1}{T}$$

This is visualized plotting the logarithm of rate constants against the inverse temperature in kelvin. From linear regression of the data points, it is possible to calculate the activation energy for the denaturation process. The Arrhenius plots are used to detect the effect of temperature on specific chemical reaction.

Dannenberg and Kessler in 1988 modeled the effect of temperature on the rate constant for β -Lg A, β -Lg B and α -La of skim milk. They found to be of Arrhenius, which can be used to determine the rate of denaturation of a given temperature (see Figure 2.2).

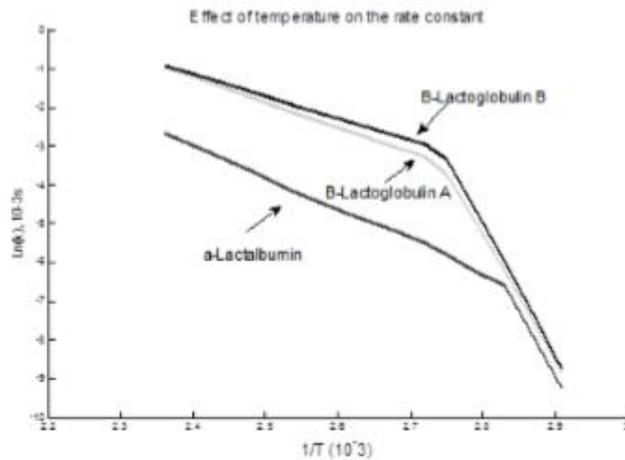


Figure 2.2: The effect of temperature on rate constant for β -Lg A, β -Lg B and α -La of skim milk.

They observed that the relationship is linear, but only for given temperature ranges, namely 70-90°C and 95-150°C. The graph shows a bend around 95°C where the activation energy above 95°C decreases significantly. This change in rate constant and activation energy indicates that two reactions are taking place. At lower temperatures, denaturation and unfolding is the dominating reaction which requires large quantities of energy, while at higher temperatures aggregation dominates which does not require same amount of energy.

The kinetics of α -La has been investigated in similar way and a reaction order of 1 was found and verified since experiments made by Dannenberg and Kessler (1988); Oldfield *et al.*, (1998a). As seen in figure 2.9, α -La also shows a change in activation energy when looking at the temperature effect on the rate constant, but this change occurs around 80°C compared to 95°C for β -Lg. The activation energy for α -La in the temperature range 85-150°C is higher than the activation energy for β -Lg which supports the theory of α -La being less heat stable but less prone to heat denaturation. However, this pattern for α -La is only seen when it is solution with other denatured whey proteins. Calvo *et al.* (1993) found no thermal aggregation of α -La in absence of other whey proteins and heat treated at 90°C for 24 min. Addition of β -Lg caused aggregation of α -La which was depended on the content of free thiol groups present on β -Lg.

2.2.3. Alterations in the microbial flora:

After the milk is pasteurized, a cocktail of starter cultures is added. These serve three purposes. **First**, the starter cultures digest the lactose in the milk and produce lactic acid, which aids in lowering the pH of the milk creating the ideal environment for coagulation. The pH affects the reactivity of the binding sites on the casein molecules and therefore, influences the structure of the matrix (Rowney *et al.*, 1999). **Second**, the browning characteristics of the final cheese are determined by how the starter cultures process the lactose. Lactose is a sugar composed of one molecule of galactose and one molecule of glucose. For example, *Lactobacillus delbrueckii* only digests glucose, leaving some residual galactose, which will brown due to Maillard browning when the final cheese is heated. **Third**, starter cultures produce enzymes that affect cheese flavor and texture.

2.2.4. Coagulation of milk proteins:

The ability of casein micelles to stay in solution at natural milk (pH ~6.7) relies on the net negative charge and hydrophilic character of the C-terminal end of κ -casein at the micelle surface. There are two approaches to induce micelle aggregation; by enzymatic action (cheese) or by acidification (fermented milk products). The outcome of these reactions is to a large extent determined by amounts and proportions of the various components in milk, with the protein composition contributing significantly in this regard. To determine the coagulation properties of a given milk, different traits to describe the process are measured. Coagulation time (CT), defined as the time from addition of coagulant until coagulation starts, and curd firmness at a given time after addition of coagulant, will be used throughout this work.

2.2.4.1. Enzyme-induced coagulation:

Coagulation can be done enzymatic by adding different kinds of enzymes which causes the caseins to clot. One of the most used enzymes is the enzyme Chymosin, also called rennet, which is a milk clotting enzyme originally obtained from calf stomach. Alternatively, pH can be lowered to the isoelectric point for casein which also makes the caseins coagulate.

Chymosin cleaves κ -casein between amino acid residue 105 and 106, resulting in a hydrophilic part, caseinomacropeptide, and a hydrophobic part, para- κ -casein (Belitz *et al.*, 2004). By removing the hydrophilic part of κ -casein, the casein micelles lose their solubility and when approximately 85% of κ -casein is hydrolyzed, the colloidal stability of micelles is reduced in such an extent that they will start coagulate (Fox and McSweeney, 1998).

It is widely known that milk treated at high temperatures has longer coagulation times, reduces the firmness and forms weaker gels when manufacturing cheese (Waungana *et al.*, 1996a). This can be caused by complexes of denatured whey protein and κ -casein, leading to the formation of appendages on the micelle surface which makes the Phenylalanine 105-Metionine 106 bond of κ -casein less susceptible to hydrolysis by rennet and thereby decreases the coagulation abilities according to cheese production (Tolkach and Kulozik, 2007).

Singh and Waungana (2001) observed that heat treatments which resulted in denaturation degrees of less than 60% of β -Lg had little effect on gelation time of milk, while gel strength decreased for all detected denaturation degrees. These impairments in rennet coagulation properties can be restored in some extent after severe heating by addition of CaCl_2 and lowering pH (Hougaard *et al.*, 2010; Waungana *et al.*, 1996b).

Several modifications of milk have been investigated to reduce rennet coagulation time and improve texture. One method is to concentrate milk to achieve higher protein content. This gives firmer gels reduced decrease in coagulation properties when heated at high temperatures, while having the same degree of whey protein denaturation. This is due to the increase of caseins which are placed closer because of reduced volume and thereby can the coagulation be altered (McMahon *et al.*, 1993). Another method is microfiltration of milk to reduce the amount of whey proteins, such as Micellar Casein Isolate (MCI). By removal of the whey proteins, a reduction in coagulation time and an increase in gel firmness can be observed, also after heating (Pierre *et al.*, 1992; Wang *et al.*, 2007). This supports the theory of whey proteins have a negative effect on the rennet coagulation abilities of milk.

2.2.4.2. Acid-induced coagulation:

At acid coagulation of milk, casein micelle properties are altered by a lowered milk pH (Lucey & Singh, 1997). This causes CCP to dissociate from the micelles and the negative charges in the casein micelles are neutralized, with aggregation occurring as the isoelectric point of the casein micelle (pH 4.6) is approached. A porous network of loosely linked aggregates is formed. Milk used in manufacture of fermented milk products is generally subjected to a quite severe heat treatment (90°C, 5-10 min), with a marked effect on the end product. Temperatures above 60°C cause denaturation of whey proteins (mainly β -lg), which via disulphide bonds either associate with κ -casein on the casein micelles (McKenzie *et al.*, 1971; Sawyer, 1969) or form soluble aggregates (Guyomarc'h *et al.*, 2003a; Haque & Kinsella, 1988). This results in increased curd firmness (Dannenberg & Kessler, 1988a) due to an increased number and strength of bonds of the acid gel, as denatured whey proteins associated with casein micelles interact with each other (Lucey & Singh, 1997).

Further, the concentration of protein in the gel network will be increased because of the active participation of denatured whey protein in structure formation.

2.2.5. Post-coagulation procedures:

After the curd has coagulated, it is cut into smaller, cube-shaped pieces in order to expel excess moisture and whey from the protein network. When fresh curd is cut, the curd particles are easily deformed, improving syneresis. The curd particles can then fuse together after expelling some water due to the protein in the curd (van Vliet and Walstra, 1983).

It is crucial that the firmness of the coagulant be optimal before cutting. If the curd is too weak when cut, the network will shatter and fat will be lost, decreasing yields and altering texture; if the curd is too firm when cut, the protein network will break and there will be high loss of casein, again, altering texture. After cutting, the curds are left alone for a short period of time known as a healing period. During the healing period, a "skin" forms on the outside of the curd, which prevents further losses of fat and moisture. After that, whey is drained from the curds. The pH of the whey determines the proportions of chymosin and plasmin retained in the cheese (Fox, 1989). Both of these have the capability to cause breakdown of the protein network during storage, which greatly impacts the final texture of the cheese. The curds are salted and matted together to make the protein network more dense, resulting in a characteristic texture.

It is at this point that the altered curds are typically pressed into molds. If the curds are not salted, the molds may be soaked in brine. Salt influences textural changes as the cheeses age. Such changes are most apparent in brined cheeses, where immediately following molding; cheeses are immersed in a sodium chloride solution. Osmotic pressure differences exist between the cheese and the brine, causing the sodium chloride to migrate into the block; simultaneously, in order to maintain equilibrium osmotic pressure, moisture from the cheese block exits into the brine (Guinee and Fox, 1987).

2.2.6. Cheese ripening and maturation:

The cheese is now ready to be stored. Typically, as they age, a decrease in firmness (or softening) of the cheese body occurs. The degradation of the protein matrix is thought to cause this change since the lipid phase is discontinuous and would, therefore, not contribute as much to

the overall consistency of the body (de Jong, 1976). Two phases of texture development during storage have been identified. Phase one occurs within the first seven to fourteen days after production. During this time, the rubbery texture of the young cheese is converted into the smoother characteristic texture of the specific variety. It is believed that during this phase, proteolysis of the casein network is taking place. Hydrolysis by residual coagulant of about 20% of the α_{s1} -casein, which produces the α_{s1} -I peptide, causes a weakening of the casein network (de Jong, 1976; Lawrence *et al.*, 1987); specifically, the Phe23-Phe24 or Phe24-Val25 bonds are most susceptible to hydrolysis by residual enzyme (Fox, 1989). The α_{s1} -I peptide is present in all cheeses during the early stages of ripening. A more gradual change in cheese texture occurs during phase two of ripening. It is during this time period that the rest of the α_{s1} -casein and the other caseins are hydrolyzed. Unlike phase one, which takes only days, phase two occurs over a period of months (Lawrence *et al.*, 1987). However, it has been shown that the β -casein does not change as much during ripening as α_{s1} -casein (de Jong, 1976; Creamer and Olson, 1982).

It should be noted that in very old cheeses, some varieties (i.e. Cheddar) show highly brittle texture. Several factors could explain such a change. First, as the cheeses age, evaporation of available water on the surface occurs, causing a “drier”, more fragile cheese. Second, as proteolysis occurs, more and more “new” ionic peptides are created; as each “new” group is created, competition for available water increases. Less water is available to solvate the protein chains and the resulting cheese is harder and less deformable (Creamer and Olson, 1982).

2.2.6.1. Effects of proteolysis:

Changes in the texture of cheese can be related to the rate at which proteolysis occurs; these reactions are affected by many things. **First**, though most coagulant is lost in the whey when drained, some is retained in the curd. If no active coagulant is present in the curd, then no α_{s1} -casein degradation can occur, and therefore, cheese softening will not happen (i.e. the cheese will maintain the young, rubbery texture) (Lawrence *et al.*, 1987). The amount of residual coagulant depends upon the pH of the system. Lower curd pH at draining encourages retention of more

rennet resulting in increased hydrolysis of α_s1 -casein. **Second**, the pH at drain determines the amount of plasmin in the curd. Plasmin is a native milk proteinase that is responsible for much of casein breakdown. Plasmins are associated with the casein micelle in fresh milk, but as the pH decreases, they dissociate from the caseins. **Third**, the salt to moisture ratio affects the amount of intact casein; at lower ratios, there is less intact casein than at higher ratios. As discussed earlier, the salt to moisture ratio in the molded cheese also controls the activity of the residual rennet and plasmin in the cheese. **Fourth**, the storage temperature (or ripening temperature) impacts the rate of proteolysis, though the impact on textural characteristics depends upon the type of protein being proteolyzed. It is believed that α_s1 -casein hydrolysis contributes more structurally to the cheese than the other caseins. At temperatures below 6°C, the amount of β -casein hydrolyzed decreases significantly, but the amount of α_s1 -casein hydrolyzed only slightly decreases. Therefore, cheeses of the same variety ripened at different temperatures below 6°C are not very different texturally. However, ripening temperatures above 10°C have significant effects on creating textural differences since more α_s1 -casein is hydrolyzed as the temperature is increased. Cheese proteolysis is negatively correlated with firmness, indicating softening of the cheese as the protein matrix is broken down. **Fifth**, changes in pH during storage affect the rate of proteolysis. Generally, the rate of breakdown of α_s1 -casein is greater at lower storage pH than the rate of breakdown of β -casein. Finally, both dissolved calcium in the cheese serum and calcium bound to the protein network have been shown to affect the rate of proteolysis. However, it is difficult to distinguish the direct effect of calcium since the total amount of calcium retained in the curd is determined by the point at which the whey is drained from the curd. Simultaneously, the drain point also controls the amount of residual rennet and plasmin in the curd, both of which are factors determining cheese texture (Lawrence *et al.*, 1987).

2.2.6.2. Effects of PH:

Texture of cheese is also dependent upon the pH of the finished cheese, which affects the state of the protein aggregates. Cheeses having a low pH (near the isoelectric point of casein) show a granular texture and shatter when deformed; higher pH cheeses are more plastic and elastic. At low pH, strong ionic and hydrophobic intra-aggregate forces hold the casein aggregates in a

compact formation (inter-aggregate forces are weaker). Water in this system is less mobile. At higher pH, casein molecules have a net negative charge. Though the hydrophobic interactions still exist, the ionic interactions change to a repulsive nature. The tight protein aggregates absorb water to solvate the no neutral ionic charges. This effect can be minimized depending upon the extent of ionic calcium bound to the casein in the cheese, which decreases the solubility of the protein (Creamer and Olson, 1982). Additionally, the mineral equilibrium within the cheese influences the texture. Calcium acts to cement the casein micelles together. During maturation, calcium is transported from the center to the outside of the cheese causing the core to have lower calcium content (Adda *et al.*, 1982).

2.2.6.3 Effects of brine migration:

Brine-immersed cheeses show dramatic changes in texture during the early stages of aging. Brine migration patterns and rates have been modeled (Geurts *et al.*, 1974; Geurts *et al.*, 1980); the pseudo diffusion coefficient of sodium chloride through the moisture in Gouda cheese was estimated to be a rate of $0.2 \text{ cm}^2 \text{ day}^{-1}$. Sodium chloride affects both the matrix and the serum phases of the cheese, which, in turn, affects the overall texture. It has been determined that sodium chloride in the serum phase of Mozzarella cheese promotes the microstructural swelling of the para-casein matrix resulting in an increased water-holding capacity and formation of a hydrated gel. Simultaneously, the sodium chloride promotes the solubilization of intact caseins from the para-casein matrix; it is hypothesized that these proteins are able to freely migrate between the matrix and the serum phase (Guo and Kindstedt, 1995; Guo *et al.*, 1997). The calcium phosphate bridges that connect the bare casein micelles in the protein matrix are affected in a process called demineralization. The sodium ions are able to displace the calcium ions in the calcium phosphate bridge. This allows for water in the system to be able to bind to the complex, either increasing the water holding capacity of the matrix, or promoting the protein to become soluble in the serum (Geurts *et al.*, 1972). Additionally, salt changes the appearance of cheeses making them less opaque. As was discussed, salt increases absorption of serum into the matrix making a more homogeneous matrix. This results in fewer discontinuities (surfaces) to cause light to scatter, making the cheese appear more translucent (Paulson *et al.*, 1998).

2.2.6.4 Effects of composition:

Cheese composition also affects final texture. Cheeses having a higher fat content are less firm and more elastic; the recently popular low fat are firmer and less smooth due to the increase in the amount of protein matrix (and lack of lipid filler). Likewise, the level of protein directly affects the firmness of the cheese; the more protein the cheese has initially, the firmer the cheese is. It has also been shown that small variations in water content greatly affect firmness; water content is affected by cheese making conditions and by surface evaporation during ripening (Adda *et al.*, 1982).

Chapter Three

3. Materials and Methods:

3.1 Introduction:

A number of aspects pertaining to cheese making development were investigated within this research. This chapter provides an insight into the experimental procedures and techniques used and present essential information regarding the selected materials.

The development step required in making cheese is dependent on many systems and sample preparation attributes. Therefore, the influence of selected of operating conditions and sample preparation were assessed in a factory cheese production level where samples are prepared according to industrial level. The classification of samples and assortment were evaluated in terms of sample characteristics (e.g consistency, texture, taste, saltiness, etc.), as well, the sample microbial load is also tested in the factory laboratory.

3.2 Materials:

3.2.1. Processing Materials:

Materials used in this investigation are the same materials used in the daily work of the factory except those never been addressed in the industry. However, the following materials involved in the investigation are:

- Row cow's Milk obtained from a dairy farm, Palestine.
- CaCl_2 coarse particles, obtained from Sun Company for food raw materials (Nablous, Palestine).
- Powder Animal Rennet was obtained from CHR-Hansen's Laboratories, (Copenhagen, Denmark)
- Tween 80 Surfactant, Food grade was obtained from Sun Company for food raw materials (Nablous, Palestine).
- NaCl salt was obtained from Sun Company for food raw materials (Nablous, Palestine).

3.2.2. Laboratory Materials:

Since the chemical and physical composition of milk is measured by using the milkoScan, therefore, the only laboratory materials used was limited to microbial count and examination. The materials used are the same materials used in the factory at laboratory daily work. The following materials were used as follow:

- Sterile peptone saline diluents (Merck, Germany)
- Plate count agar (PCA, Merck, Germany)
- Man Rogosa Sharpe agar (MRS, Biolife 401728).
- Mac-Conky agar (DM141D, UK)
- Xylose Lysine Deoxycholate agar (XLD) ager (Merck, 1.05287)
- Baird Parker agar (Biolife, 401116)

- Bactident Coagulase Biolife
- Tryptone Bile X-Glucuronide agar (TBX agar, LAB HAL003)
- PolymyxinPolymyxin-aAcridflavin-Lithium Chloride-Ceft-azidime-Aesculine-Mannitol ager (PALCAM, Biolife 401604)

3.3. Methods:

Methods applied in this investigation included the method of chemical and physical analysis of milk, method of cheese-making, method of sampling, method of testing, and method of sensorial analysis.

3.3.1. Chemical and Physical Milk Analysis Method:

Using the milkoScan (MS 301, DePont,UK) milk sample was taken from raw milk after mixing, the sample was brought to the lab. The sample was well shaken, and the device curvet was filled with milk sample for measuring purposes. The milk results obtained included: PH, added water, Total solid, fat content, freezing point, lactose content, protein content, ...etc.

3.3.2. Cheese-Making Method:

Local white brine cheese was manufactured using fresh raw milk which was obtained from a dairy farm, Palestine. Milk was pasteurized at 75°C for 15 second using a plate pasteurizer and cooled at (39°C - 41°C). Then milk was transported carefully to a cheese vat with the temperature of 37°C. The milk was supplemented with 0.15 g of CaCl₂/kg of milk and held at 35°C until the final pH of milk reached 6, 6.2 and 6.4 before the addition of rennet.

Rennet was diluted 30-fold with cold water then added to each batch of milk. After approximately 55 min, the curd was cut crossways into cubes of 1 cm³ and left for 10 min. After being cut, the curd was allowed to settle for 3-5 min and then gently agitated at a gradually increasing rate for 10 min to avoid fusion of freshly cut curd cubes and facilitate whey expulsion.

The sliced curd was carefully ladled from the vat into a stainless steel mould of under the initial pressure which gradually increased up and held constant to the end of pressing for each lot of cheese. After complete draining, the curd was stored at 16-18°C and covered with 2Q±1 % (w/v) brine (brine was beforehand pasteurized at 80°C for 10 min and filtered through a clean cloth after rapid cooling) for 12 hours. After this stage, the blocks were placed in airtight still containers covered with 10-12% brine and stored at 13-15°C for 4 weeks and kept at 5°C after that.

3.3.3. Sampling Method:

Cheeses were sampled for analysis at the age of 0, 2, 4, 6, 8 weeks. One of the cheese container was randomly selected and the cheese block was removed and allowed to drip for 2 min. Then, one slice of about 100 g was cut from an edge and another one from the middle of the block and placed in a plastic waterproof bag. The two pieces were ground to a homogeneous paste and small portions were taken from it for microbial analyses in duplicate base.

3.3.4. Microbial Analysis Method:

Using the standard method of microbial testing introduced by E-BAM, USA, all samples were examined after 0, 2, 4, 6, 8 weeks of ripening. 25 grams of cheese samples were added aseptically to 225 ml of sterile peptone saline diluents (1.0 g peptone, 8.5 g sodium chloride in 1L distilled water) and homogenized in a stomacher. Total *coliform* was counted on Mac-Conky agar (DM141D, UK) after incubation at 37°C with a double layer of medium. For LAB enumerations, 1ml sample was inoculated into 10 ml of molten de Man Rogosa Sharpe agar (MRS, Biolife 401728). After solidifying 10 ml overlay of the same molten medium was added. The incubation was carried out at 37°C for 72 h (Dave and shah, 1996). *S.aureus* was counted on Baird Parker agar (Biolife, 401116) supplemented with egg yolk incubated at 37°C for 48 h. *S. aureus* colonies were further tested for positive coagulase reaction (Bactident Coagulase Biolife). *E. coli* strain was counted on Tryptone Bile X-Glucuronide agar (TBX agar, LAB HAL003) incubated at 37°C for 24 h. Yeast and Molds was enumerated on Dichloran rose bengal chloramphenicol (DRBC) agar supplemented with 100 mg chloramphenicol/liter after incubation

in the dark at 25°C for 4 days. All plates were examined for typical colony types and morphological characteristics associated to each culture medium. Presumptive colonies of the above bacteria were verified by confirmation tests. For estimated counts below the limit of detection, the most-probable-number (MPN) technique was used. Serial dilutions of three further or buffered peptone water tubes of three successive dilutions were incubated at 37°C for 48 h. Buffered peptone water was used as a nonselective medium for recovering sub-lethally injured bacteria and for minimizing the underestimation of counts. All plates were examined for typical colony types and morphological characteristics associated to each culture medium.

3.3.5. Sensorial analysis method:

Cheese samples were sensorial evaluated during storage for 0, 2, 4, 6, and 8 weeks according to standard method of Panel testing (Papas *et al.*, 1996). The evaluation was carried out by score from 1 to 9 where 9 is the best scoring. Samples were introduced to evaluation without soaking in fresh water. The samples were subjected for several criteria evaluations including, taste, texture, consistency, saltiness, mouth feeling, internal porosity, etc... Two levels of evaluation were carried out, accordingly;

- At Research level: where the total samples produced tested and evaluated by two researchers.
- At Market level: random sample of 100 adults customers were used to carry out this evaluation. Samples were tasted and physically tested. Customers were asked to taste and destructive checking up the samples enable to rank the addressed samples in order of preference. The addressed samples were introduced under coding and one of them was the reference sample that produced by the factory steps and roles.

3.3 Methodology:

The goal of this investigation is to introduce better characteristics in local white cheese enable to enhance the white cheese in term of palatability, shelf-life, and acceptance. This investigation fulfilling its goal through the following methodology:

- First: Highlighting the element of change could be investigated. Those elements came out of several years of experience, which included:
 - At unit operation stage: steam addition,
 - At additive use: to enhance curd formation by using surfactant, acid, and starter culture.
- Second: setting up validating procedure to verify the effect of these elements addressed, as the following:
 - Steam addition as a new unit operation application: the addition of steam was tested in the following determined stages and shaped:
 - Steam bath: the cheese curd was obtained from the cheese vat and subjected for steam bathing. This method is applied for curd prior to molding.
 - Steam injection: the cheese curd was obtained from cheese vat and subjected for steam injection within the curd. This method is applied for curd prior to molding.

The samples tested were group number one, and coded in sample matrix shown in Table 3.1.

- Enhancing curd formation:
 - By using rennet enzyme and starter culture: This investigation is targeting the curd enhancement by introducing the starter culture to the milk and allowed to develop for 30 min prior to rennet enzyme application. The sample matrix of this group (No. 2) is shown in Table 3.2.
 - By using rennet enzyme and citric acid: This investigation is targeting the curd enhancement by introducing the citric acid in three concentrations,

0.035, 0.05, and 0.1% (wt/wt) to the milk post to rennet enzyme application. The sample matrix of this group (No. 3) and samples coded with a, b, and c letter, respectively, according to the acid concentration as shown in Table 3.3-3.5.

- By using rennet enzyme, Starter culture, and citric acid: This investigation is targeting the curd enhancement by introducing the citric acid in three concentrations, 0.035, 0.05, and 0.1% (wt/wt) and starter culture to the milk post to rennet enzyme application. The sample matrix of this group (No. 4) and samples coded with a, b, and c letter, respectively, according to the acid concentration as shown in Table 3.6-3.8.
- Third: placing the samples produced in 50 ml capped container and stored under refrigeration condition. Each sample for each storage time inspection was 10 containers to avoid open-close procedure. Thus, all samples never been reclosed.
- Samples subjected to microbial analysis
- Samples were evaluated by two researchers and short listed to 10 samples.
- The short-listed samples were reproduced and used for market evaluation.

Table 3.1: Sample matrix for first group investigating effect of steam bath and injection.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G1 S1	x	x	N/A			
2	G1 S2	X	x		X		
3	G1 S3	X	x			X	
4	G1 S4	X	x				X
5	G1 S5	x		N/A			
6	G1 S6	x			x		
7	G1 S7	X				x	
8	G1 S8	X					x

9	G1 S9		X	N/A			
10	G1 S10		X		x		
11	G1 S11		X			x	
12	G1 S12		X				x
13	G1 S13			N/A			
14	G1 S14				X		
15	G1 S15					X	
16	G1 S16						X

Table 3.2: Sample matrix for second group investigating the effect of enzyme and starter culture.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G2 S1	x	x	N/A			
2	G2 S2	X	x		X		
3	G2 S3	X	x			X	
4	G2 S4	X	x				X
5	G2 S5	x		N/A			
6	G2 S6	x			x		
7	G2 S7	X				x	
8	G2 S8	X					x
9	G2 S9		X	N/A			
10	G2 S10		X		x		
11	G2 S11		X			x	
12	G2 S12		X				x
13	G2 S13			N/A			

14	G2 S14				X		
15	G2 S15					X	
16	G2 S16						X

Table 3.3: Sample matrix for third group investigating the effect of enzyme and 0.035% citric acid.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G3 S1 a	x	x	N/A			
2	G3 S2 a	X	x		X		
3	G3 S3 a	X	x			X	
4	G3 S4 a	X	x				X
5	G3 S5 a	x		N/A			
6	G3 S6 a	x			x		
7	G3 S7 a	X				x	
8	G3 S8 a	X					x
9	G3 S9 a		X	N/A			
10	G3 S10 a		X		x		
11	G3 S11 a		X			x	
12	G3 S12 a		X				x
13	G3 S13 a			N/A			
14	G3 S14 a				X		
15	G3 S15 a					X	
16	G3 S16 a						X

Table 3.4: Sample matrix for third group investigating the effect of enzyme and 0.05% citric acid.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G3 S1 b	x	x	N/A			
2	G3 S2 b	X	x		X		
3	G3 S3 b	X	x			X	
4	G3 S4 b	X	x				X
5	G3 S5 b	x		N/A			
6	G3 S6 b	x			x		
7	G3 S7 b	X				x	
8	G3 S8 b	X					x
9	G3 S9 b		X	N/A			
10	G3 S10 b		X		x		
11	G3 S11 b		X			x	
12	G3 S12 b		X				x
13	G3 S13 b			N/A			
14	G3 S14 b				X		
15	G3 S15 b					X	
16	G3 S16 b						X

Table 3.5: Sample matrix for third group investigating the effect of enzyme and 0.1% citric acid.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G3 S1 c	x	x	N/A			

2	G3 S2 c	X	x		X		
3	G3 S3 c	X	x			X	
4	G3 S4 c	X	x				X
5	G3 S5 c	x		N/A			
6	G3 S6 c	x			x		
7	G3 S7 c	X				x	
8	G3 S8 c	X					x
9	G3 S9 c		X	N/A			
10	G3 S10 c		X		x		
11	G3 S11 c		X			x	
12	G3 S12 c		X				x
13	G3 S13 c			N/A			
14	G3 S14 c				X		
15	G3 S15 c					X	
16	G3 S16 c						X

Table 3.6: Sample matrix for third group investigating the effect of enzyme, starter culture, and 0.035% citric acid.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G4 S1 a	x	x	N/A			
2	G4 S2 a	X	x		X		
3	G4 S3 a	X	x			X	
4	G4 S4 a	X	x				X
5	G4 S5 a	x		N/A			

6	G4 S6 a	x			x		
7	G4 S7 a	X				x	
8	G4 S8 a	X					x
9	G4 S9 a		X	N/A			
10	G4 S10 a		X		x		
11	G4 S11 a		X			x	
12	G4 S12 a		X				x
13	G4 S13 a			N/A			
14	G4 S14 a				X		
15	G4 S15 a					X	
16	G4 S16 a						X

Table 3.7: Sample matrix for third group investigating the effect of enzyme, starter culture, and 0.05% citric acid.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G4 S1 b	x	x	N/A			
2	G4 S2 b	X	x		X		
3	G4 S3 b	X	x			X	
4	G4 S4 b	X	x				X
5	G4 S5 b	x		N/A			
6	G4 S6 b	x			x		
7	G4 S7 b	X				x	
8	G4 S8 b	X					x
9	G4 S9 b		X	N/A			

10	G4 S10 b		X		x		
11	G4 S11 b		X			x	
12	G4 S12 b		X				x
13	G4 S13 b			N/A			
14	G4 S14 b				X		
15	G4 S15 b					X	
16	G4 S16 b						X

Table 3.8: Sample matrix for third group investigating the effect of enzyme, starter culture, and 0.1% citric acid.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G4 S1 c	x	x	N/A			
2	G4 S2 c	X	x		X		
3	G4 S3 c	X	x			X	
4	G4 S4 c	X	x				X
5	G4 S5 c	x		N/A			
6	G4 S6 c	x			x		
7	G4 S7 c	X				x	
8	G4 S8 c	X					x
9	G4 S9 c		X	N/A			
10	G4 S10 c		X		x		
11	G4 S11 c		X			x	
12	G4 S12 c		X				x
13	G4 S13 c			N/A			

14	G4 S14 c				X		
15	G4 S15 c					X	
16	G4 S16 c						X

Chapter Four

4. Results and Discussion:

4.1 Introduction:

This chapter is focusing on showing the results obtained with introducing a required discussion for the results enable to introduce better understanding for the mechanism and the goal and purpose of this investigation. The results introduced are classified according to the stage of alteration, as following:

- At processing stage: which included:
 - Unit operation level
 - Additives level
- At microbial status stage, which included:
 - Bacterial level
 - Fungi level
- At sensorial stage, which included:
 - At laboratory level
 - At market level

4.2 At Processing Stage:

The focal point of alteration at processing stage is to study the effect of addition of a unit operation. In this field the steam effect was highlighted as a suitable physical agent that could create the development required in the local white cheese. On the other hand the investigation highlighted the effect of certain additives that could create an added value to the investigated local white cheese. These additives are limited to be acid, microorganism, and surfactant.

4.2.1. At Unit Operation Level:

4.2.1.1. Incorporating steam:

The steam incorporation was designed to introduce several effects at the matrix of cheese as well as cheese characteristics. The three forms of steam incorporation were investigated, namely;

- Steam injection and steam bath
- Steam injection alone
- Steam bath alone

4.2.1.1.1. Incorporating steam injection and bath:

As shown in the sample design matrix 32 samples were prepared to study the effect of steam injection with steam bath. The results showed high effect of steam injection and bath at different levels. To facilitate the result discussion the following table shows the sample matrix in correlation with steam injection and steam bath within this investigation. The steam injection was investigated in the four groups. The results varied according to the other elements involved.

- The first set of experiments: Involved implementing the steam injection to samples produced by using enzyme. Wide spectrum of packaging style were implemented, namely; brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.1.

Table 4.1: Application of steam injection and bathing to samples produced enzyme stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G1 S2	8	8	8	7	6	7	7	3	5	6
2	G1 S3	7	7	7	6	7	7	7	3	5	5
3	G1 S4	9	9	9	8	8	8	7	5	6	7

As shown in the table, the samples produced in this group was solid, the internal layers of curd is remarkably arranged in smooth way. The taste was very acceptable with noticeable a low level of elasticity and producing a gentle squawking upon ingestion. It was so clear that the effect of brine solution was in term of decreasing the hardness of the samples with decreasing the strengthening of brine solution. The most appealing sample among this group was “G1 S4” sample (samples stored in brine solution 10%). This results comes in accordance with L.R was found that

- The second set of experiments: Involved implementing the steam injection and bath to samples produced by using enzyme and Microorganism. Wide spectrum of stored brine solution was used, namely; 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.2

Table 4.2: Application of steam injection and bathing to samples produced enzyme and microorganism stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G2 S2	9	9	7	7	8	7	7	2	7	7
2	G2 S3	9	8	7	7	8	7	7	3	7	7
3	G2 S4	9	8	8	8	8	8	7	3	7	8

As shown in the table, the samples produced in this group was solid, the internal layers of curd is remarkably arranged in clear way specially in sample “G2 S3” and in compact way as appeared in sample “G2 S2”. However, sample “G2 S4” revealed un arranged layers arrangement. The taste was acceptable with noticeable a low level of elasticity in “G2 S2” with little increased in “G2 S4”. It was so clear that the “G2 S2” has more cracks than “G2 S4”. The brine solution was not having a high effect in this group at over all the saltiness was accepted by decreasing the percent of porosity.

- The third set of experiments: Involved implementing the steam injection and bath to samples produced by using enzyme and different concentration of acid (0.035%, 0.05%, and 0.1%). The same spectrum of packaging style was implemented. The results are shown in Table 4.3.

Table 4.3: Application of steam injection and bathing to samples produced by enzyme and different concentration of acids and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G3 S2 a	5	6	5	5	6	5	6	3	3	7
2	G3 S3 a	5	6	5	5	6	5	6	3	3	7
3	G3 S4 a	5	6	5	5	6	5	6	3	3	7
4	G3 S2 b	7	6	7	7	6	7	7	7	8	7
5	G3 S3 b	7	6	7	6	6	7	7	7	8	7
6	G3 S4 b	7	7	7	6	6	6	7	6	7	6
7	G3 S2 c	6	5	4	4	4	4	6	3	3	5
8	G3 S3 c	6	5	4	4	4	4	6	3	3	5
9	G3 S4 c	7	6	6	6	4	6	7	6	7	6

* Where a, b, and c represents acid conc. 0.035%, 0.05%, and 0.1%, respectively.

AS shown in Table 4.3, the variation among samples produced by using enzyme with different acid concentration. The group is hard and the curd ordered in clear layers in samples with low acid concentration and disappeared with high acid concentration. The coherent increase with decreasing the concentration of acid and the sandy teats appeared. The elasticity rose with low acidity while the high acid concentration has not elasticity effect. At over all the saltiness is normal and the teats was accepted.

- The fourth set of experiments: Involved implementing the steam injection and bath to samples produced by using enzyme, Microorganism, and different concentration of acid

(0.035%, 0.05%, 0.1%). The same spectrum of packaging style were implemented, namely; brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.4.

Table 4.4: Application of steam injection and bathing to samples produced by enzyme, microorganism, and different concentration of acid and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G4 S2 a	7	7	6	4	5	7	7	3	4	2
2	G4 S3 a	7	6	6	7	7	7	7	4	5	5
3	G4 S4 a	7	8	6	6	7	7	7	5	5	6
4	G4 S2 b	7	6	6	4	5	7	7	3	4	2
5	G4 S3 b	7	5	5	7	7	7	7	5	6	6
6	G4 S4 b	8	8	6	6	7	8	7	5	5	8
7	G4 S2 c	7	6	6	4	5	7	7	3	4	2
8	G4 S3 c	7	4	4	6	7	8	8	6	7	7
9	G4 S4 c	8	6	7	7	6	7	7	6	6	7

From Table 4.4, it can be noted that the group have a moderate hardness and the curd ordered in clear layers in samples with low acid concentration and disappeared in with high acid concentration. The consistency increase with decreasing the concentration of acid and the sandy taste appeared. The elasticity rose with low acidity while the high acid concentration has not

elasticity effect. At over all the saltiness is normal and the teats was accepted. The groups have high similarity with third group but with less hardness.

4.2.1.1.2. Incorporating steam injection:

As shown in the sample design matrix 32 samples were prepared to study the effect of steam injection. The results showed high effect of steam injection at different levels. To facilitate the result discussion the following table shows the sample matrix in correlation with steam injection within this investigation. The steam injection was investigated in the four groups. The results varied according to the other elements involved.

- The first set of experiments: Involved implementing the steam injection to samples produced by using enzyme. Wide spectrum of packaging style were implemented, namely; brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.5.

Table 4.5: Application of steam injection to samples produced by using enzyme and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G1 S6	8	7	4	3	6	7	7	7	7	3
2	G1 S7	8	8	6	7	7	7	7	7	7	5
3	G1 S8	9	9	9	8	8	8	7	7	7	8

As shown in Table 4.5, the samples produced in this group were solid and the hardness decrease with decrease the concentration of salt, the internal layers of curd did not appear and the sandy teats is dominant while the internal pours appeared with high cracking. The taste was acceptable with low level of elasticity and producing a gentle squawking upon ingestion. “G1 S8” sample the most accepted (samples stored in brine solution 10%).

- The second set of experiments: Involved implementing the steam injection to samples produced by using enzyme and Microorganism. Wide spectrum of packaging style were implemented, namely; brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.6.

Table 4.6: Application of steam injection to samples produced by using enzyme and microorganism and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G2 S6	8	9	6	6	7	7	7	8	8	7
2	G2 S7	8	8	7	7	7	7	7	8	8	7
3	G2 S8	8	7	7	7	7	7	7	8	8	8

As shown in the Table 4.6, the samples produced in this group have a moderate hardness decreased gradually from high salt concentration to low salt concentration; the internal layers of curd are remarkably arranged in clear way especially in sample “G2 S6” decreased slightly in sample “G2 S8”. Compact texture in sample “G2 S8” so that have normal salty taste in addition that have slight elasticity taste in contrast with “G2 S6” sample which have sandy teats. “G2 S8” sample have the acceptance between the group samples.

- The third set of experiments: Involved implementing the steam injection to samples produced by using enzyme and different concentration of acid (0.035%, 0.05%, 0.1%). Wide spectrum of packaging style were implemented, namely; brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.7

Table 4.7: Application of steam injection to samples produced by using enzyme and different concentration of acid and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G3 S6 a	9	7	6	5	5	9	8	9	9	8
2	G3 S7 a	4	6	7	5	6	9	8	9	9	8
3	G3 S8 a	9	6	6	6	6	9	8	9	9	9
4	G3 S6 b	9	7	6	5	5	9	8	9	9	8
5	G3 S7 b	9	6	7	5	6	9	8	9	9	8
6	G3 S8 b	9	6	7	7	6	9	8	9	9	9
7	G3 S6 b	9	8	7	6	5	9	8	9	9	8
8	G3 S7 b	9	7	7	5	6	9	8	9	9	8
9	G3 S8 b	9	6	6	6	6	9	8	9	9	9

Table 4.7 shows the variation among samples produced by using enzyme with different acid concentration. The group is soft and has a smooth surface, compact curd with notable external gelatinization. the curd ordered in clear layers in more solid samples which consist low acid concentration and high salt concentration in brine solution and disappeared in samples with high

acid concentration and low salt concentration in brine solution. at over all the consistency is not appeared character in this group and the saltiness is high except “G3 S8 a” which have normal salt concentration.

- The fourth set of experiments: Involved implementing the steam injection to samples produced by using enzyme, microorganism, and different concentration of acid (0.035%, 0.05%, 0.1%). Wide spectrum of packaging style was implemented, namely; vacuum package, brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.8.

Table 4.8: Application of steam injection to samples produced by using enzyme, microorganism, and different concentration of acid and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G4 S6 a	7	7	3	3	6	6	6	5	5	6
2	G4 S7 a	6	6	4	4	5	6	7	4	4	6
3	G4 S8 a	6	6	4	3	6	6	7	4	4	6
4	G4 S6 b	7	7	4	4	7	7	6	5	5	7
5	G4 S7 b	6	6	3	3	4	6	7	4	4	7
6	G4 S8 b	6	6	3	3	7	6	7	4	4	7
7	G4 S6 c	6	6	5	5	5	5	7	6	6	5
8	G4 S7 c	5	4	3	3	3	5	7	4	4	6
9	G4 S8 c	5	4	3	3	3	5	7	4	4	6

As shown in Table 4.8, the variation among samples produced by using enzyme with microorganism and different acid concentration. The group is soft as in sample “G4 S8 c” to little hardness as in sample “G4 S6 a” the curd ordered in clear layers in more solid samples which consist low acid concentration as in sample “G4 S8 a” and high salt concentration in brine solution and disappeared in samples with high acid concentration and low salt concentration in brine solution. The microorganisms increase hardness with clear layers and increase cracking percent at over all the color between G4s samples more dark and the softness is more high than that in G3 samples with the same conditions.

4.2.1.1.3. Incorporation of steam bath:

As shown in the sample design matrix 32 samples were prepared to study the effect of steam bath. The results showed high effect of steam bath at different levels. To facilitate the result discussion the following table shows the sample matrix in correlation with steam bath within this investigation. The steam bath was investigated in four groups. The results were vary according to the other elements involved.

- The first set of experiments: Involved implementing the steam bath to samples produced by using enzyme. Wide spectrum of packaging style were implemented, namely; vacuum package, brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.9.

Table 4.9: Application of steam injection to samples produced by using enzyme and different concentration of acid and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G1 S10	7	6	8	8	6	7	7	3	3	4
2	G1 S11	8	7	8	8	7	8	8	3	3	6
3	G1 S12	9	9	9	7	8	7	7	3	3	7

As shown in the Table 4.9, the samples produced in this group were highly elastic in cleared way special in “G1 S12” which preserved in low salt concentration (10%) and that caused produce more tenderness texture while the “G1 S10” which package in 20% brine solution have a compact texture with cleared layers. There is lovely little cracking in “G1 S12” and in the form of layers in “G1 S10”.

- The second set of experiments: Involved implementing the steam bath to samples produced by using enzyme and microorganism. Wide spectrum of packaging style were implemented, namely; brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.10.

Table 4.10: Application of steam bath to samples produced by using enzyme and microorganism and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G2 S10	7	6	8	8	7	8	7	4	4	6
2	G2 S11	8	7	8	8	7	8	8	4	4	7
3	G2 S12	9	9	9	7	8	8	8	4	4	8

Samples produced in this group have a moderate elasticity increased slightly from high salt concentration to low salt concentration, the internal layers of curd are remarkably arranged in clear way with low salt concentration as in sample “G2 S12” decreased slightly with high salt concentration as in sample “G2 S10”, as shown in Table 4.10. Even more, all the samples in this group have a moderate saltiness in acceptable way especially in “G2 S12” which have a lovely soft texture in contrast with another samples in the group. In addition, “G2 S12” has a light cracking more than other samples which give it together with previous reasons the preference to be the best.

- The third set of experiments: Involved implementing the steam bathing to samples produced by using enzyme and different concentration of acid (0.035%, 0.05%, 0.1%). Wide spectrum of packaging style were implemented, namely; vacuum package, brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.11.

Table 4.11: Application of steam injection to samples produced by using enzyme and different concentration of acid and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G3 S10 a	7	8	6	6	6	7	7	6	6	6
2	G3 S11 a	7	8	7	7	6	7	7	6	6	6
3	G3 S12 a	7	7	7	6	7	7	7	6	7	7
4	G3 S10 b	7	7	7	7	6	6	8	7	7	7
5	G3 S11 b	7	7	7	7	6	7	8	6	6	7
6	G3 S12 b	7	6	6	6	6	6	6	8	7	7
7	G3 S10 c	7	7	7	6	6	6	9	5	6	8
8	G3 S11 c	7	6	6	6	6	6	9	5	6	6
9	G3 S12 c	7	6	6	6	6	6	6	9	5	6

Table 4.11 shows the variation among samples produced by using enzyme with different acid concentration. the group is soft and have a smooth service, compact curd with notable external gelatinization. the curd ordered in clear layers with light elasticity in more solid samples which consist low acid, high salt concentration in brine solution and disappeared in samples with high acid, low salt concentration in brine solution.at over all the consistency is not appeared character in this group specially with high acid concentration, in addition, the saltiness is high as well as the cracking. “G4 S12 a” the best in the group.

- The fourth set of experiments: Involved implementing the steam bathing to samples produced by using enzyme and microorganism and different concentration of acid

(0.035%, 0.05%, 0.1%). Wide spectrum of packaging style was implemented, namely; brine solution 20%, brine solution 16%, brine solution 10%. The results are shown in Table 4.12.

Table 4.12: Application of steam bathing to samples produced by using enzyme, microorganism, and different concentration of acid and stored with different brine solution concentration.

No.	Sample	Texture	Hardness	Consistency	Cracking	Salty	General appearance	Color	Internal porosity	External porosity	Gross acceptance
1	G4 S10 a	6	6	5	5	5	6	7	3	3	5
2	G4 S11 a	6	6	5	5	5	6	7	3	3	5
3	G4 S12 a	5	5	4	4	5	6	7	3	3	6
4	G4 S10 b	6	7	5	5	5	6	7	3	3	5
5	G4 S11 b	6	7	5	5	6	6	7	3	3	6
6	G4 S12 b	5	6	4	4	6	6	7	3	3	6
7	G4 S10 c	5	5	5	5	6	6	7	3	3	6
8	G4 S11 c	5	5	4	4	7	6	7	3	3	7
9	G4 S12 c	5	4	3	3	7	6	7	3	3	7

Table 4.12 shows the variation among samples produced by using enzyme with microorganism and different acid concentration. the group is harder than the same conditions between samples in group 3 the layers unordered in clear way and have high internal and external porosity, high saltiness and cracking, on the other hands, the taste in “G4 samples” better than that in “G3”

under the same conditions, as well as in “G3” the sample number 14 in group 4 have the best characteristic with decrease in consistency by increase in the acid concentration used.

From all of the last results, may conclude that the presence of acid in little concentration can increase the elasticity and increase the brightness.

While the use of microorganisms can increase the consistency and decrease the porosity; moreover, acid with microorganism can make very great combination with great texture.

4.2.2 At Additives level:

As the coagulation and aggregation of cheese curd is highly affecting the curd characteristics; curd formation enhancers were studied in term of using surfactant to decrease the repulsion among molecules.

4.2.2.1 Using Surfactant:

Surfactant was suggested as a catalyst to study its impact on the order state of cheese molecules. Different concentrations of food grade surfactant were investigated (0.5, 0.25, 0.05%). it is important to discuss the results rather than choosing the best result thus selected samples provides specific characteristics, according to panel test. However, the best selected samples resulted in this investigation was chosen for the purpose of surfactant application. The addition of surfactant was designed to be either:

- Before coagulation:

The surfactant added to the milk directly then mixed carefully with sanitized mixer before the rennet added. This procedure is done for four selected samples in all chosen concentrations.

- After coagulation:

The surfactant added to the curd directly then mixed carefully as possible as we can with hands before the coagulant is pressed. This procedure is done for four selected samples in all chosen concentrations.

1. **Sample “G1 S12”:** Sample number 12 from Group 1 coagulated by Enzyme and exposed to steam bath then preserved in 10% brine solution concentrate. It was impressive sample with high elasticity, layered texture with a small amount of cracking, acceptance saltiness and very acceptable taste.

- Before coagulation:

In 0.5% concentrate: the elasticity increased greatly and the Layered arrangement was clear considerably and the fibres more Consistence. Moreover, removed the cloth after the steam bath easier. However, the taste was unacceptable with high concentration from surfactant.

In 0.25% concentrate: the elasticity increased and the layers become more ordered and Consistence. They become less attached to cloth but the taste still unacceptable but in less negatively with reducing the concentration of surfactant.

In 0.05% concentrate: the surfactant has not great effect in elasticity or consistency and does not turn its propose. The concentration was not enough to do different.

In conclusion, the 0.25% concentration of surfactant have the best effect on cheese characterization but with reduced it about 0.05% to get rid of the bitter taste.

- After coagulation:

Adding the surfactant to the “G1 S12” after coagulation has a negative effect on the texture with three concentrations which were used. They lost their elasticity and the fibres does not exist, Moreover the taste become sandy and unacceptable with effective concentrations 0.5% and 0.25%.

All over finding: sample “G1 S12” is better without surfactant.

2. **Sample “G3 S8 a”**: Sample number 8 from group 3 in type “a” coagulated with enzyme and 0.035% of acid. Exposed to steam injection and preserve in 10% concentrate of brine solution. It was in golden group as it’s describe by the researcher with nice texture, shiny surface, compact layered without internal or external pores or fibers, external gelatinization and clear softness like pudding.

- Before coagulation:

In 0.5% concentrate: adding high concentrate of surfactant to the milk before coagulation have a negative effect on “G3 S8 a” by increasing the softness and loss of the texture by destroying the linkages.

In 0.25% concentrate: the texture look like the standard one with little effect of less consistent and more softness in good way. The result was not bad but the taste was not the best.

In 0.05% concentrate: using the surfactant with small concentration have no effect on the texture or the taste. It was non-effective concentrate.

In conclusion, adding the surfactant to the “G3 S8 a” before coagulation have a positive effect with using 0.25% concentrate except the negative effect to the taste, which can be removed by reducing about 0.05% from the percentage of concentration.

- After coagulation:

In 0.5% concentrate: adding high concentration of surfactant after coagulation has a negative effect on the texture of “G3 S8 a”. The texture becomes high compact, firm and tough and the layers highly cleared.

In 0.25% concentrate: with this concentration, the texture become more compact, more firm and the layers become clear. In contrast, there was no significant effect in the taste. The sample loses its special characters and the concentration do not make a perfect effect.

In 0.05% concentrate: using the surfactant with small concentration has no effect on the texture or the taste. It was not an effective concentration.

To conclude, using the surfactant after coagulation has no positive effect even if it is used with different concentrations.

3. **Sample “G2 S2”**: Sample number 2 from group 2, coagulated with enzyme and Microorganisms. Exposed to steam injection and steam bath. Finally preserved in 20% concentration of brine solution. The texture was tough, highly compact, layered and elastic with acceptable cracking and saltiness.

- Before coagulation:

In 0.5% concentration: the texture becomes much less compact and the layers more arranged but the taste highly saltier. The result was not satisfactory.

In 0.25% concentration: with this concentration, the effect is still the same but with less strength. And the final result still not satisfactory.

In 0.05% concentration: using small concentration still has no effect.

In conclusion, using surfactant in “G2 S2” before coagulation has a good effect on texture but increases the saltiness taste.

- After coagulation:

The effect of adding surfactant to the “G2 S2” after coagulation have approximately the same effect when it is added before coagulation with just one difference which is the way of fibers order, it is more acceptable and the taste is not salty

4. **Sample “G3 S16 b”**: sample number 16 from group 3, coagulated with enzyme and 0.05% acid. Not exposed to steam treatment and preserved in 10% concentrate of brine solution. The texture was soft, bright, compact, consistent with light layers and elasticity and with acceptable saltiness.

- Before coagulation:

In 0.5% concentration: the texture becomes more compact and the layers more arranged but the taste is highly bitter. The result was not satisfactory.

In 0.25% concentration: with this concentration, the effect on the texture was not notable but the bitter taste still exists and the final result still not satisfactory.

In 0.05% concentration: using small concentration still has no effect.

In conclusion, using surfactant in “G3 S16 b” before coagulation has not a different effect on texture, in contrast, it makes the taste not acceptable.

- After coagulation:

Adding the surfactant to the “G3 S16 b” after coagulation makes a difference and the texture becomes more consist with more cleared layers and nuts color but the effect on the taste still the same, specially with high concentrations. The result is not bad when used good concentration which is less than 0.25% and more than 0.05%.

All over finding, using surfactant with “G3 S16 b” have an interested effect on the texture but still need a control on the concentration.

The application of surfactant was never been stated in the available literature review. This investigation was the first to investigate surfactant application in cheese.

4.3 At Microbial Examination Stage:

This investigation as mentioned before is aiming to create alteration in the processing of local white cheese enable to enhance the local white cheese and introduces certain characteristics. Microbial examination is playing major role in cheese evaluation, cheese acceptability, cheese shelf-life, and cheese characteristics. Therefore, this investigation will discuss the effect of alteration on the microbial status which is one of the major aspects to evaluate cheese.

4.3.1 At Bacterial Level:

The Microbial tests were chosen according to the Palestinian white cheese specifications declared in the Palestinian standard number PS302 (Appendix 2). The national standard for white cheese stated the importance to evaluate three different bacteria as a matter of quality and shelf-life evaluation, namely;

- *Escherichia coli*
- *Total coliform*
- *Staphylococcus aureus*

Samples produced with different applications were stored for two months. A reference sample from the factory was stored with the same condition and produced in the same date of investigation samples' date.

4.3.1.1 *Escherichia coli*:

Investigated for the *E. coli* testing were carried out for all samples. Different samples according to the testing group were compared to the standard sample produced according to the factory regular method. Factory sample is produced with addition of Natamycin as a natural preservative, while the investigated samples contained no preservative.

Results obtained for *E. coli* testing for the eight groups of this investigation were shown in Tables 4.13-4.20.

Table 4.13: *E. coli* status examined for first samples' group that was studied for two months.

Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	50	200	350
G1 S1	Nil	Nil	Nil	Nil	Nil
G1 S2	Nil	Nil	Nil	Nil	Nil
G1 S3	Nil	Nil	Nil	Nil	Nil
G1 S4	Nil	Nil	Nil	Nil	Nil
G1 S5	Nil	Nil	Nil	Nil	Nil
G1 S6	Nil	Nil	Nil	Nil	Nil
G1 S7	Nil	Nil	Nil	Nil	Nil
G1 S8	Nil	Nil	Nil	100	Nil
G1 S9	Nil	Nil	Nil	Nil	Nil
G1 S10	Nil	Nil	Nil	Nil	Nil
G1 S11	Nil	Nil	Nil	Nil	Nil
G1 S12	Nil	Nil	Nil	Nil	Nil
G1 S13	Nil	Nil	300	TNTC	TNTC
G1 S14	Nil	Nil	200	TNTC	TNTC
G1 S15	Nil	Nil	Nil	TNTC	TNTC
G1 S16	Nil	Nil	Nil	TNTC	TNTC

Table 4.14: *E. coli* status examined for second samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	200	500	600
G2 S1	Nil	Nil	Nil	Nil	Nil
G2 S2	Nil	Nil	Nil	Nil	Nil
G2 S3	Nil	Nil	Nil	Nil	Nil
G2 S4	Nil	Nil	Nil	Nil	Nil
G2 S5	Nil	Nil	Nil	Nil	Nil
G2 S6	Nil	Nil	Nil	Nil	Nil
G2 S7	Nil	Nil	Nil	Nil	Nil
G2 S8	Nil	Nil	Nil	Nil	Nil
G2 S9	Nil	Nil	Nil	Nil	Nil
G2 S10	Nil	Nil	Nil	Nil	Nil
G2 S11	Nil	Nil	Nil	Nil	Nil
G2 S12	Nil	Nil	Nil	Nil	Nil
G2 S13	Nil	Nil	Nil	Nil	Nil
G2 S14	Nil	Nil	200	TNTC	TNTC
G2 S15	Nil	Nil	20	30	100
G2 S16	Nil	Nil	Nil	TNTC	TNTC

Table 4.15: *E. coli* status examined for third samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	200	500	600
G3 S1 a	Nil	Nil	Nil	Nil	Nil
G3 S2 a	Nil	Nil	Nil	20	Nil
G3 S3 a	Nil	Nil	Nil	Nil	Nil
G3 S4 a	Nil	Nil	Nil	Nil	Nil
G3 S5 a	Nil	Nil	Nil	Nil	Nil
G3 S6 a	Nil	Nil	Nil	Nil	Nil
G3 S7 a	Nil	Nil	Nil	Nil	Nil
G3 S8 a	Nil	Nil	Nil	10	Nil
G3 S9 a	Nil	Nil	Nil	Nil	Nil
G3 S10 a	Nil	Nil	Nil	Nil	Nil
G3 S11 a	Nil	Nil	Nil	Nil	Nil
G3 S12 a	Nil	Nil	Nil	Nil	Nil
G3 S13 a	Nil	Nil	Nil	Nil	Nil
G3 S14 a	Nil	Nil	Nil	Nil	Nil
G3 S15 a	Nil	Nil	Nil	Nil	Nil
G3 S16 a	Nil	Nil	Nil	Nil	TNTC

Table 4.16: *E. coli* status examined for fourth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	200	500	600
G3 S1 b	Nil	Nil	Nil	Nil	Nil
G3 S2 b	Nil	Nil	Nil	Nil	Nil
G3 S3 b	Nil	Nil	Nil	Nil	Nil
G3 S4 b	Nil	Nil	Nil	Nil	Nil
G3 S5 b	Nil	Nil	Nil	Nil	Nil
G3 S6 b	Nil	Nil	Nil	Nil	Nil
G3 S7 b	Nil	Nil	Nil	Nil	Nil
G3 S8 b	Nil	Nil	Nil	Nil	Nil
G3 S9 b	Nil	Nil	Nil	Nil	Nil
G3 S10 b	Nil	Nil	Nil	Nil	Nil
G3 S11 b	Nil	Nil	Nil	Nil	Nil
G3 S12 b	Nil	Nil	Nil	Nil	Nil
G3 S13 b	Nil	Nil	Nil	Nil	Nil
G3 S14 b	Nil	Nil	Nil	Nil	Nil
G3 S15 b	Nil	Nil	Nil	Nil	Nil
G3 S16 b	Nil	Nil	Nil	Nil	Nil

Table 4.17: *E. coli* status examined for Fifth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	200	500	600
G3 S1 c	Nil	Nil	Nil	Nil	Nil
G3 S2 c	Nil	Nil	Nil	Nil	Nil
G3 S3 c	Nil	Nil	Nil	Nil	Nil
G3 S4 c	Nil	Nil	Nil	Nil	Nil
G3 S5 c	Nil	Nil	Nil	Nil	Nil
G3 S6 c	Nil	Nil	Nil	Nil	Nil
G3 S7 c	Nil	Nil	Nil	Nil	110
G3 S8 c	Nil	Nil	Nil	Nil	30
G3 S9 c	Nil	Nil	Nil	Nil	Nil
G3 S10 c	Nil	Nil	Nil	Nil	Nil
G3 S11 c	Nil	Nil	Nil	Nil	Nil
G3 S12 c	Nil	Nil	Nil	Nil	Nil
G3 S13 c	Nil	Nil	Nil	Nil	150
G3 S14 c	Nil	Nil	Nil	Nil	120
G3 S15 c	Nil	Nil	Nil	Nil	TNTC
G3 S16 c	Nil	Nil	Nil	Nil	100

Table 4.18: *E. coli* status examined for sixth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	200	500	600
G4 S1 a	Nil	Nil	Nil	Nil	Nil
G4 S2 a	Nil	Nil	Nil	Nil	20
G4 S3 a	Nil	Nil	Nil	Nil	Nil
G4 S4 a	Nil	Nil	Nil	Nil	Nil
G4 S5 a	Nil	Nil	Nil	Nil	Nil
G4 S6 a	Nil	Nil	Nil	Nil	Nil
G4 S7 a	Nil	Nil	Nil	Nil	Nil
G4 S8 a	Nil	Nil	Nil	Nil	Nil
G4 S9 a	Nil	Nil	Nil	Nil	Nil
G4 S10 a	Nil	Nil	Nil	Nil	Nil
G4 S11 a	Nil	Nil	Nil	Nil	Nil
G4 S12 a	Nil	Nil	Nil	Nil	Nil
G4 S13 a	Nil	Nil	Nil	50	TNTC
G4 S14 a	Nil	Nil	Nil	40	200
G4 S15 a	Nil	Nil	Nil	TNTC	TNTC
G4 S16 a	Nil	Nil	Nil	10	100

Table 4.19: *E. coli* status examined for seventh samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	200	500	600
G4 S1 b	Nil	Nil	Nil	Nil	Nil
G4 S2 b	Nil	Nil	Nil	Nil	20
G4 S3 b	Nil	Nil	Nil	Nil	Nil
G4 S4 b	Nil	Nil	Nil	Nil	Nil
G4 S5 b	Nil	Nil	Nil	Nil	Nil
G4 S6 b	Nil	Nil	Nil	Nil	Nil
G4 S7 b	Nil	Nil	Nil	Nil	Nil
G4 S8 b	Nil	Nil	Nil	Nil	Nil
G4 S9 b	Nil	Nil	Nil	Nil	Nil
G4 S10 b	Nil	Nil	Nil	Nil	Nil
G4 S11 b	Nil	Nil	Nil	Nil	Nil
G4 S12 b	Nil	Nil	Nil	Nil	Nil
G4 S13 b	Nil	Nil	Nil	10	300
G4 S14 b	Nil	Nil	100	TNTC	TNTC
G4 S15 b	Nil	Nil	10	200	400
G4 S16 b	Nil	Nil	10	400	TNTC

Table 4.20: *E. coli* status examined for eighth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	200	500	600
G4 S1 c	Nil	Nil	Nil	Nil	Nil
G4 S2 c	Nil	Nil	Nil	Nil	Nil
G4 S3 c	Nil	Nil	Nil	Nil	Nil
G4 S4 c	Nil	Nil	Nil	Nil	Nil
G4 S5 c	Nil	Nil	Nil	Nil	Nil
G4 S6 c	Nil	Nil	Nil	Nil	Nil
G4 S7 c	Nil	Nil	Nil	Nil	Nil
G4 S8 c	Nil	Nil	Nil	Nil	Nil
G4 S9 c	Nil	Nil	Nil	Nil	Nil
G4 S10 c	Nil	Nil	Nil	Nil	Nil
G4 S11 c	Nil	Nil	Nil	Nil	Nil
G4 S12 c	Nil	Nil	Nil	Nil	Nil
G4 S13 c	Nil	Nil	Nil	Nil	Nil
G4 S14 c	Nil	Nil	20	400	TNTC
G4 S15 c	Nil	Nil	500	TNTC	TNTC
G4 S16 c	Nil	Nil	Nil	200	TNTC

Results obtained in Table 4.13-4.20 showed an obvious *E.coli* growth after one month of storage for samples produced without steam treatment, while samples treated with steam showed great stability of shelf-life and *E. coli* growth does not exist. Investigation samples are not just introduced better stability for the *E. coli* growth, but it produced longer shelf-life without preservative. It is obvious that the introduction of steam for the process helped in developing

better shelf-life. This is due to the high sterilization effect of steam on *E. coli* which helped in sterilizing milk after the curd formation, as well known that cheese raw milk is not subjected for high temperature treatment more than early 70s °C.

The two-month shelf-life refrigerated white cheese has relatively short life in comparison to the pasteurized cheese that boiled with brine solution. As shown in the obtained results, the factory regular cheese was unable to exceed the two month storage time successfully; while the investigation cheese easily exceeded the shelf life and were able to reach 4 months as shown for the selected samples.

4.3.1.2 Total Coliform:

The same as for *E. coli*, all samples were tested for Total *Coliform* situation. The investigation's samples were tested and compared with the factory sample which produced according to the standard factory method for producing white cheese. The factory samples are fortified with natural antioxidant of NataMycin type. However, the investigation's samples contained no preservative.

Results obtained for Total *Coliform* testing for the eight groups of this investigation were shown in Tables 4.21-4.28.

Table 4.21: Total *Coliform* status examined for first samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G1 S1	Nil	Nil	Nil	Nil	Nil
G1 S2	Nil	Nil	Nil	Nil	Nil
G1 S3	Nil	Nil	Nil	Nil	Nil
G1 S4	Nil	Nil	Nil	Nil	Nil
G1 S5	Nil	Nil	Nil	Nil	Nil
G1 S6	Nil	Nil	Nil	Nil	Nil
G1 S7	Nil	Nil	Nil	Nil	Nil
G1 S8	Nil	Nil	Nil	Nil	Nil
G1 S9	Nil	Nil	Nil	Nil	Nil
G1 S10	Nil	Nil	Nil	Nil	Nil
G1 S11	Nil	Nil	Nil	Nil	Nil
G1 S12	Nil	Nil	Nil	60	60
G1 S13	Nil	Nil	Nil	Nil	Nil
G1 S14	Nil	Nil	200	TNTC	TNTC
G1 S15	Nil	Nil	Nil	TNTC	TNTC
G1 S16	20	40	60	TNTC	TNTC

Table 4.22: Total *Coliform* status examined for second samples' group that was studied for two months

	Storage Time				
Sample Code	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G2 S1	Nil	Nil	Nil	Nil	Nil
G2 S2	Nil	Nil	Nil	Nil	Nil
G2 S3	Nil	Nil	Nil	Nil	Nil
G2 S4	Nil	Nil	Nil	Nil	Nil
G2 S5	Nil	Nil	Nil	Nil	Nil
G2 S6	Nil	Nil	Nil	Nil	Nil
G2 S7	Nil	Nil	Nil	50	50
G2 S8	Nil	Nil	Nil	Nil	Nil
G2 S9	Nil	Nil	Nil	Nil	Nil
G2 S10	Nil	Nil	Nil	Nil	Nil
G2 S11	Nil	Nil	Nil	Nil	Nil
G2 S12	Nil	Nil	Nil	Nil	Nil
G2 S13	Nil	Nil	Nil	Nil	Nil
G2 S14	Nil	Nil	200	TNTC	TNTC
G2 S15	Nil	Nil	50	100	100
G2 S16	Nil	Nil	Nil	Nil	Nil

Table 4.23: Total *Coliform* status examined for third samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 a	Nil	Nil	Nil	Nil	Nil
G3 S2 a	Nil	Nil	Nil	20	20
G3 S3 a	Nil	Nil	Nil	Nil	40
G3 S4 a	Nil	Nil	Nil	Nil	Nil
G3 S5 a	Nil	Nil	Nil	Nil	Nil
G3 S6 a	Nil	Nil	Nil	Nil	Nil
G3 S7 a	Nil	Nil	Nil	Nil	Nil
G3 S8 a	Nil	Nil	Nil	TNTC	TNTC
G3 S9 a	Nil	Nil	Nil	Nil	Nil
G3 S10 a	Nil	Nil	Nil	Nil	Nil
G3 S11 a	Nil	Nil	Nil	Nil	Nil
G3 S12 a	Nil	Nil	Nil	Nil	Nil
G3 S13 a	Nil	Nil	Nil	Nil	Nil
G3 S14 a	Nil	Nil	Nil	Nil	Nil
G3 S15 a	Nil	Nil	Nil	Nil	Nil
G3 S16 a	Nil	Nil	Nil	Nil	Nil

Table 4.24: Total *Coliform* status examined for fourth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 b	Nil	Nil	Nil	Nil	Nil
G3 S2 b	Nil	Nil	Nil	Nil	Nil
G3 S3 b	Nil	Nil	Nil	Nil	TNTC
G3 S4 b	Nil	Nil	Nil	Nil	Nil
G3 S5 b	Nil	Nil	Nil	Nil	Nil
G3 S6 b	Nil	Nil	Nil	Nil	TNTC
G3 S7 b	Nil	Nil	Nil	Nil	Nil
G3 S8 b	Nil	Nil	Nil	Nil	TNTC
G3 S9 b	Nil	Nil	Nil	Nil	Nil
G3 S10 b	Nil	Nil	Nil	Nil	Nil
G3 S11 b	Nil	Nil	Nil	Nil	Nil
G3 S12 b	Nil	Nil	Nil	Nil	Nil
G3 S13 b	Nil	Nil	Nil	Nil	Nil
G3 S14 b	Nil	Nil	Nil	Nil	Nil
G3 S15 b	Nil	Nil	Nil	Nil	Nil
G3 S16 b	Nil	Nil	Nil	Nil	Nil

Table 4.25: Total *Coliform* status examined for fifth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 c	Nil	Nil	Nil	Nil	Nil
G3 S2 c	Nil	Nil	Nil	Nil	Nil
G3 S3 c	Nil	Nil	Nil	Nil	Nil
G3 S4 c	Nil	Nil	Nil	Nil	TNTC
G3 S5 c	Nil	Nil	Nil	Nil	Nil
G3 S6 c	Nil	Nil	Nil	Nil	Nil
G3 S7 c	Nil	Nil	Nil	Nil	Nil
G3 S8 c	Nil	Nil	Nil	Nil	Nil
G3 S9 c	Nil	Nil	Nil	Nil	Nil
G3 S10 c	Nil	Nil	Nil	Nil	Nil
G3 S11 c	Nil	Nil	Nil	Nil	Nil
G3 S12 c	Nil	Nil	Nil	Nil	Nil
G3 S13 c	Nil	Nil	Nil	Nil	Nil
G3 S14 c	Nil	Nil	Nil	Nil	Nil
G3 S15 c	Nil	Nil	Nil	Nil	Nil
G3 S16 c	Nil	Nil	Nil	Nil	Nil

Table 4.26: Total *Coliform* status examined for sixth samples' group that was studied for two months.

	Storage Time				
Sample Code	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 a	Nil	Nil	Nil	Nil	Nil
G4 S2 a	Nil	Nil	Nil	Nil	Nil
G4 S3 a	Nil	Nil	Nil	Nil	Nil
G4 S4 a	Nil	Nil	Nil	Nil	Nil
G4 S5 a	Nil	Nil	Nil	Nil	Nil
G4 S6 a	Nil	Nil	Nil	Nil	Nil
G4 S7 a	Nil	Nil	Nil	Nil	Nil
G4 S8 a	Nil	Nil	Nil	Nil	Nil
G4 S9 a	Nil	Nil	Nil	Nil	Nil
G4 S10 a	Nil	Nil	Nil	Nil	Nil
G4 S11 a	Nil	Nil	Nil	Nil	Nil
G4 S12 a	Nil	Nil	Nil	Nil	Nil
G4 S13 a	Nil	Nil	Nil	Nil	Nil
G4 S14 a	Nil	Nil	Nil	Nil	Nil
G4 S15 a	Nil	Nil	Nil	10	50
G4 S16 a	Nil	Nil	Nil	20	10

Table 4.27: Total *Coliform* status examined for seventh samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 b	Nil	Nil	Nil	Nil	Nil
G4 S2 b	Nil	Nil	Nil	60	100
G4 S3 b	Nil	Nil	Nil	Nil	Nil
G4 S4 b	Nil	Nil	Nil	Nil	Nil
G4 S5 b	Nil	Nil	Nil	Nil	Nil
G4 S6 b	Nil	Nil	Nil	Nil	Nil
G4 S7 b	Nil	Nil	Nil	Nil	Nil
G4 S8 b	Nil	Nil	Nil	10	Nil
G4 S9 b	Nil	Nil	Nil	Nil	Nil
G4 S10 b	Nil	Nil	Nil	Nil	Nil
G4 S11 b	Nil	Nil	Nil	Nil	Nil
G4 S12 b	Nil	Nil	Nil	Nil	Nil
G4 S13 b	Nil	Nil	Nil	Nil	Nil
G4 S14 b	Nil	Nil	Nil	Nil	Nil
G4 S15 b	Nil	Nil	Nil	TNTC	TNTC
G4 S16 b	Nil	400	400	400	TNTC

Table 4.28: Total *Coliform* status examined for eighth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 c	Nil	Nil	Nil	Nil	Nil
G4 S2 c	Nil	Nil	Nil	Nil	Nil
G4 S3 c	Nil	Nil	Nil	Nil	Nil
G4 S4 c	Nil	Nil	Nil	40	60
G4 S5 c	Nil	Nil	Nil	Nil	Nil
G4 S6 c	Nil	Nil	Nil	Nil	Nil
G4 S7 c	Nil	Nil	Nil	Nil	Nil
G4 S8 c	Nil	Nil	Nil	Nil	Nil
G4 S9 c	Nil	Nil	Nil	Nil	Nil
G4 S10 c	Nil	Nil	Nil	Nil	Nil
G4 S11 c	Nil	Nil	Nil	Nil	Nil
G4 S12 c	Nil	Nil	Nil	Nil	Nil
G4 S13 c	Nil	300	TNTC	TNTC	TNTC
G4 S14 c	Nil	200	500	TNTC	TNTC
G4 S15 c	Nil	200	500	TNTC	TNTC
G4 S16 c	Nil	Nil	200	TNTC	TNTC

As shown in Table 4.21-4.28, reasonable number of Total *Coliform* were manifested for samples without steam treatment after one month of storage, while samples treated with steam showed great stability of shelf-life and the growth of Total *Coliform* was not reported.

The investigation's samples showed longer shelf-life without using a preservative. It is obvious that the introduction of steam for the process helped in developing better shelf-life. As Total *Coliform* is heat sensitive, sterilization effect of steam on Total *Coliform* helped in sterilizing milk after the curd formation. This added value of steam was not being able by using the traditional method of producing long shelf-life local white cheese since the texture and taste will be highly affected. At the same time, raw milk proposed for making cheese is not subjected for high temperature treatment.

The two-month shelf-life refrigerated white cheese has relatively short life in comparison to the pasteurized cheese that boiled with brine solution. As shown in the obtained results, the factory regular cheese was unable to exceed the two-month storage time successfully, while the investigation's samples were easily exceeded the shelf life and able to reach 4 months as shown for the selected samples and showed no growth of Total *Coliform*.

4.3.1.3 *Staphylococcus aureus*:

Staphylococcus aureus species is important to be tested for considering the legibility of white cheese. Investigation's samples were tested for *Staphylococcus aureus* count. As well the tested samples were compared with the factory sample which produced according to the standard factory method for producing white cheese. The factory samples are produced with adding natural preservative of very common type called NataMycin. At the same time the investigation's samples produced without using any preservative.

Tables 4.29-4.36 represent the obtained results for the investigation's samples in comparison with the factory sample.

Table 4.29: *Staphylococcus aureus* status examined for first samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G1 S1	Nil	Nil	Nil	Nil	Nil
G1 S2	Nil	Nil	Nil	TNTC	TNTC
G1 S3	Nil	Nil	Nil	TMT	TNTC
G1 S4	Nil	Nil	Nil	TNTC	TNTC
G1 S5	Nil	Nil	Nil	Nil	Nil
G1 S6	Nil	Nil	Nil	Nil	Nil
G1 S7	Nil	Nil	Nil	Nil	Nil
G1 S8	Nil	10	30	140	140
G1 S9	Nil	Nil	Nil	Nil	Nil
G1 S10	Nil	Nil	Nil	Nil	Nil
G1 S11	Nil	Nil	Nil	Nil	Nil
G1 S12	Nil	Nil	Nil	Nil	Nil
G1 S13	Nil	Nil	Nil	Nil	Nil
G1 S14	Nil	Nil	100	TNTC	TNTC
G1 S15	Nil	Nil	TNTC	TNTC	TNTC
G1 S16	Nil	20	300	TNTC	TNTC

Table 4.30: *Staphylococcus aureus* status examined for second samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G2 S1	Nil	Nil	Nil	Nil	Nil
G2 S2	Nil	Nil	Nil	Nil	Nil
G2 S3	Nil	Nil	Nil	Nil	Nil
G2 S4	Nil	Nil	TNTC	TNTC	TNTC
G2 S5	Nil	Nil	Nil	Nil	Nil
G2 S6	Nil	Nil	Nil	Nil	Nil
G2 S7	Nil	Nil	Nil	Nil	Nil
G2 S8	Nil	Nil	100	TNTC	TNTC
G2 S9	Nil	Nil	Nil	Nil	Nil
G2 S10	Nil	Nil	Nil	TNTC	TNTC
G2 S11	Nil	Nil	Nil	TNTC	TNTC
G2 S12	Nil	Nil	200	TNTC	TNTC
G2 S13	Nil	Nil	Nil	Nil	Nil
G2 S14	Nil	Nil	Nil	Nil	Nil
G2 S15	Nil	Nil	Nil	Nil	Nil
G2 S16	Nil	Nil	500	TNTC	TNTC

Table 4.31: *Staphylococcus aureus* status examined for third samples' group that was studied sorted for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 a	Nil	Nil	Nil	Nil	Nil
G3 S2 a	Nil	Nil	Nil	TNTC	TNTC
G3 S3 a	Nil	Nil	Nil	TNTC	TNTC
G3 S4 a	Nil	Nil	Nil	TNTC	TNTC
G3 S5 a	Nil	Nil	Nil	Nil	Nil
G3 S6 a	Nil	Nil	Nil	TNTC	TNTC
G3 S7 a	Nil	Nil	Nil	TNTC	TNTC
G3 S8 a	Nil	Nil	Nil	TNTC	TNTC
G3 S9 a	Nil	Nil	Nil	Nil	Nil
G3 S10 a	Nil	Nil	Nil	TNTC	TNTC
G3 S11 a	Nil	Nil	Nil	TNTC	TNTC
G3 S12 a	Nil	Nil	Nil	Nil	Nil
G3 S13 a	Nil	Nil	Nil	Nil	Nil
G3 S14 a	Nil	Nil	Nil	TNTC	TNTC
G3 S15 a	Nil	Nil	Nil	Nil	TNTC
G3 S16 a	Nil	Nil	Nil	TNTC	TNTC

Table 4.32: *Staphylococcus aureus* status examined for fourth samples' group that was studied for two months

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 b	Nil	Nil	Nil	Nil	Nil
G3 S2 b	Nil	Nil	Nil	TNTC	TNTC
G3 S3 b	Nil	Nil	Nil	TNTC	TNTC
G3 S4 b	Nil	Nil	Nil	TNTC	TNTC
G3 S5 b	Nil	Nil	Nil	Nil	Nil
G3 S6 b	Nil	Nil	Nil	TNTC	TNTC
G3 S7 b	Nil	Nil	Nil	TNTC	TNTC
G3 S8 b	Nil	Nil	Nil	TNTC	TNTC
G3 S9 b	Nil	Nil	Nil	Nil	Nil
G3 S10 b	Nil	Nil	Nil	Nil	TNTC
G3 S11 b	Nil	Nil	Nil	Nil	TNTC
G3 S12 b	Nil	Nil	Nil	Nil	TNTC
G3 S13 b	Nil	Nil	Nil	Nil	Nil
G3 S14 b	Nil	Nil	Nil	Nil	TNTC
G3 S15 b	Nil	Nil	Nil	TNTC	TNTC
G3 S16 b	Nil	Nil	Nil	Nil	TNTC

Table 4.33: *Staphylococcus aureus* status examined for fifth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 c	Nil	Nil	Nil	Nil	Nil
G3 S2 c	Nil	Nil	Nil	Nil	TNTC
G3 S3 c	Nil	Nil	Nil	Nil	TNTC
G3 S4 c	Nil	Nil	Nil	Nil	TNTC
G3 S5 c	Nil	Nil	Nil	Nil	Nil
G3 S6 c	Nil	Nil	Nil	Nil	TNTC
G3 S7 c	Nil	Nil	Nil	Nil	TNTC
G3 S8 c	Nil	Nil	Nil	TNTC	TNTC
G3 S9 c	Nil	Nil	Nil	Nil	Nil
G3 S10 c	Nil	Nil	Nil	Nil	Nil
G3 S11 c	Nil	Nil	Nil	Nil	TNTC
G3 S12 c	Nil	Nil	Nil	Nil	Nil
G3 S13 c	Nil	Nil	Nil	Nil	Nil
G3 S14 c	Nil	Nil	Nil	Nil	TNTC
G3 S15 c	Nil	Nil	Nil	TNTC	TNTC
G3 S16 c	Nil	Nil	Nil	TNTC	TNTC

Table 4.34: *Staphylococcus aureus* status examined for sixth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 a	Nil	Nil	Nil	Nil	Nil
G4 S2 a	Nil	Nil	Nil	Nil	Nil
G4 S3 a	Nil	Nil	Nil	Nil	Nil
G4 S4 a	Nil	10	10	10	10
G4 S5 a	Nil	Nil	Nil	Nil	Nil
G4 S6 a	Nil	Nil	Nil	Nil	Nil
G4 S7 a	Nil	Nil	200	TNTC	TNTC
G4 S8 a	Nil	100	500	TNTC	TNTC
G4 S9 a	Nil	Nil	Nil	Nil	Nil
G4 S10 a	Nil	Nil	Nil	Nil	Nil
G4 S11 a	Nil	Nil	Nil	Nil	Nil
G4 S12 a	Nil	Nil	Nil	Nil	Nil
G4 S13 a	Nil	Nil	Nil	Nil	Nil
G4 S14 a	Nil	Nil	Nil	Nil	Nil
G4 S15 a	Nil	20	200	TNTC	TNTC
G4 S16 a	Nil	Nil	Nil	Nil	Nil

Table 4.35: *Staphylococcus aureus* status examined for seventh samples' group that was studied sorted for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 b	Nil	Nil	Nil	Nil	Nil
G4 S2 b	Nil	Nil	Nil	Nil	Nil
G4 S3 b	Nil	Nil	Nil	400	TNTC
G4 S4 b	Nil	Nil	TNTC	TNTC	TNTC
G4 S5 b	Nil	Nil	Nil	Nil	Nil
G4 S6 b	Nil	Nil	Nil	Nil	Nil
G4 S7 b	Nil	Nil	Nil	Nil	Nil
G4 S8 b	Nil	Nil	100	600	TNTC
G4 S9 b	Nil	Nil	Nil	Nil	Nil
G4 S10 b	Nil	Nil	Nil	Nil	Nil
G4 S11 b	Nil	Nil	300	300	300
G4 S12 b	Nil	Nil	Nil	300	TNTC
G4 S13 b	Nil	Nil	Nil	Nil	Nil
G4 S14 b	Nil	Nil	Nil	400	TNTC
G4 S15 b	Nil	Nil	600	TNTC	TNTC
G4 S16 b	Nil	Nil	TNTC	TNTC	TNTC

Table 4.36: *Staphylococcus aureus* status examined for eighth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 c	Nil	Nil	Nil	Nil	Nil
G4 S2 c	Nil	Nil	Nil	Nil	TNTC
G4 S3 c	Nil	Nil	Nil	Nil	Nil
G4 S4 c	Nil	Nil	TNTC	TNTC	TNTC
G4 S5 c	Nil	Nil	Nil	Nil	Nil
G4 S6 c	Nil	Nil	Nil	Nil	Nil
G4 S7 c	Nil	Nil	Nil	Nil	TNTC
G4 S8 c	Nil	Nil	Nil	Nil	Nil
G4 S9 c	Nil	Nil	Nil	Nil	Nil
G4 S10 c	Nil	Nil	Nil	Nil	Nil
G4 S11 c	Nil	Nil	Nil	Nil	Nil
G4 S12 c	Nil	Nil	Nil	Nil	TNTC
G4 S13 c	Nil	Nil	Nil	Nil	Nil
G4 S14 c	Nil	Nil	30	500	TNTC
G4 S15 c	Nil	Nil	500	500	TNTC
G4 S16 c	Nil	Nil	30	200	TNTC

As shown in Table 4.29-4.36, an expected growth for *Staphylococcus aureus* after one month of storage for samples produced without steam treatment. While samples treated with steam showed great stability of shelf-life, due to absence of *Staphylococcus aureus* growth.

Investigation's samples are not just introduced better stability for the *Staphylococcus aureus* growth, but it produced longer shelf-life without preservative. It is obvious that the introduction of steam for the process helped in developing better shelf-life. This is due to the high sterilization effect of steam on *Staphylococcus aureus* which helped in sterilizing milk after the curd formation, as well known that cheese raw milk is not subjected for high temperature treatment more than early 70s °C.

As shown in the obtained results, the factory regular cheese was unable to exceed the two month storage time successfully; while the investigation cheese easily exceeded the shelf life and able to reach 4 months as shown for the selected samples. This is due to the fact that the two month shelf-life refrigerated white cheese has relatively short life in comparison to the pasteurized cheese that boiled with brine solution.

4.3.2 Yeast and Mold:

Yeast and Mold are common problem that caused several failing for good number of cheese confiscated cheese products in Palestinian market. Therefore, the situation of Yeast and Mold is highly recommended enable to gauge if the sample is edible or not. Therefore, all samples were tested for Yeast and Mold statue. The investigation's samples were tested and compared with the factory sample which produced according to the standard factory method for producing white cheese. The factory samples are manufactured with using natural preservative. NataMycin type is one of very effective preservative and working as antibacterial and antifungals agent. However, the investigation's samples contained no preservative.

Results obtained for Yeast and Mold testing for the eight groups of this investigation were shown in Tables 4.37-4.44.

Table 4.37: Yeast and Mold status examined for first samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G1 S1	Nil	Nil	Nil	Nil	Nil
G1 S2	Nil	Nil	Nil	Nil	Nil
G1 S3	Nil	Nil	Nil	Nil	Nil
G1 S4	Nil	Nil	Nil	Nil	Nil
G1 S5	Nil	Nil	Nil	Nil	Nil
G1 S6	Nil	Nil	Nil	Nil	Nil
G1 S7	Nil	Nil	Nil	Nil	Nil
G1 S8	Nil	Nil	Nil	Nil	Nil
G1 S9	Nil	Nil	Nil	Nil	Nil
G1 S10	Nil	Nil	Nil	Nil	Nil
G1 S11	Nil	Nil	Nil	Nil	Nil
G1 S12	Nil	Nil	Nil	Nil	Nil
G1 S13	Nil	Nil	Nil	Nil	Nil
G1 S14	Nil	Nil	70	70	70
G1 S15	Nil	Nil	20	20	20
G1 S16	Nil	Nil	Nil	Nil	Nil

Table 4.38: Yeast and Mold status examined for second samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G2 S1	Nil	Nil	Nil	Nil	Nil
G2 S2	Nil	Nil	Nil	Nil	Nil
G2 S3	Nil	Nil	Nil	Nil	Nil
G2 S4	Nil	Nil	20	20	20
G2 S5	Nil	Nil	Nil	Nil	Nil
G2 S6	Nil	Nil	Nil	Nil	Nil
G2 S7	Nil	Nil	Nil	Nil	Nil
G2 S8	Nil	Nil	20	20	20
G2 S9	Nil	Nil	Nil	Nil	Nil
G2 S10	Nil	Nil	Nil	Nil	TNTC
G2 S11	Nil	Nil	Nil	Nil	TNTC
G2 S12	Nil	Nil	Nil	Nil	TNTC
G2 S13	Nil	Nil	Nil	Nil	Nil
G2 S14	Nil	Nil	Nil	Nil	60
G2 S15	Nil	Nil	210	TNTC	TNTC
G2 S16	Nil	Nil	TNTC	TNTC	TNTC

Table 4.39: Yeast and Mold status examined for third samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 a	Nil	Nil	Nil	Nil	Nil
G3 S2 a	Nil	Nil	Nil	Nil	TNTC
G3 S3 a	Nil	Nil	Nil	Nil	TNTC
G3 S4 a	Nil	Nil	Nil	Nil	TNTC
G3 S5 a	Nil	Nil	Nil	Nil	Nil
G3 S6 a	Nil	Nil	Nil	Nil	Nil
G3 S7 a	Nil	Nil	Nil	Nil	TNTC
G3 S8 a	Nil	Nil	Nil	Nil	TNTC
G3 S9 a	Nil	Nil	Nil	Nil	Nil
G3 S10 a	Nil	Nil	TNTC	TNTC	TNTC
G3 S11 a	Nil	Nil	TNTC	TNTC	TNTC
G3 S12 a	Nil	Nil	Nil	Nil	Nil
G3 S13 a	Nil	Nil	Nil	Nil	Nil
G3 S14 a	Nil	Nil	TNTC	TNTC	TNTC
G3 S15 a	Nil	Nil	Nil	Nil	Nil
G3 S16 a	Nil	Nil	Nil	Nil	90

Table 4.40: Yeast and Mold status examined for fourth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 b	Nil	Nil	Nil	Nil	Nil
G3 S2 b	Nil	Nil	TNTC	TNTC	TNTC
G3 S3 b	Nil	Nil	TNTC	TNTC	TNTC
G3 S4 b	Nil	Nil	80	80	80
G3 S5 b	Nil	Nil	Nil	Nil	Nil
G3 S6 b	Nil	Nil	Nil	Nil	TNTC
G3 S7 b	Nil	Nil	Nil	Nil	Nil
G3 S8 b	Nil	Nil	TNTC	TNTC	TNTC
G3 S9 b	Nil	Nil	Nil	Nil	Nil
G3 S10 b	Nil	Nil	TNTC	TNTC	TNTC
G3 S11 b	Nil	Nil	TNTC	TNTC	TNTC
G3 S12 b	Nil	Nil	Nil	Nil	Nil
G3 S13 b	Nil	Nil	Nil	Nil	Nil
G3 S14 b	Nil	Nil	TNTC	TNTC	TNTC
G3 S15 b	Nil	Nil	190	190	TNTC
G3 S16 b	Nil	Nil	Nil	Nil	TNTC

Table 4.41: Yeast and Mold status examined for fifth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G3 S1 c	Nil	Nil	Nil	Nil	Nil
G3 S2 c	Nil	Nil	180	180	TNTC
G3 S3 c	Nil	Nil	TNTC	TNTC	TNTC
G3 S4 c	Nil	Nil	200	200	TNTC
G3 S5 c	Nil	Nil	Nil	Nil	Nil
G3 S6 c	Nil	Nil	Nil	Nil	TNTC
G3 S7 c	Nil	Nil	Nil	Nil	Nil
G3 S8 c	Nil	Nil	Nil	Nil	TNTC
G3 S9 c	Nil	Nil	Nil	Nil	Nil
G3 S10 c	Nil	Nil	TNTC	TNTC	TNTC
G3 S11 c	Nil	Nil	30	30	TNTC
G3 S12 c	Nil	Nil	150	150	TNTC
G3 S13 c	Nil	Nil	Nil	Nil	Nil
G3 S14 c	Nil	Nil	TNTC	TNTC	TNTC
G3 S15 c	Nil	Nil	Nil	Nil	TNTC
G3 S16 c	Nil	Nil	30	30	TNTC

Table 4.42: Yeast and Mold status examined for sixth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 a	Nil	Nil	Nil	Nil	Nil
G4 S2 a	Nil	Nil	Nil	Nil	Nil
G4 S3 a	Nil	Nil	Nil	Nil	Nil
G4 S4 a	Nil	Nil	Nil	TNTC	TNTC
G4 S5 a	Nil	Nil	Nil	Nil	Nil
G4 S6 a	Nil	TNTC	TNTC	TNTC	TNTC
G4 S7 a	Nil	Nil	30	110	208
G4 S8 a	Nil	Nil	Nil	10	100
G4 S9 a	Nil	Nil	Nil	Nil	Nil
G4 S10 a	Nil	Nil	Nil	Nil	Nil
G4 S11 a	Nil	Nil	Nil	Nil	Nil
G4 S12 a	Nil	Nil	Nil	Nil	Nil
G4 S13 a	Nil	Nil	Nil	Nil	Nil
G4 S14 a	Nil	Nil	Nil	Nil	Nil
G4 S15 a	Nil	30	30	190	210
G4 S16 a	Nil	10	10	10	100

Table 4.43: Yeast and Mold status examined for seventh samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 b	Nil	Nil	Nil	Nil	Nil
G4 S2 b	Nil	Nil	Nil	Nil	Nil
G4 S3 b	Nil	Nil	Nil	Nil	Nil
G4 S4 b	Nil	40	40	40	40
G4 S5 b	Nil	Nil	Nil	Nil	Nil
G4 S6 b	Nil	Nil	Nil	Nil	Nil
G4 S7 b	Nil	Nil	Nil	Nil	Nil
G4 S8 b	Nil	30	30	30	30
G4 S9 b	Nil	Nil	Nil	Nil	Nil
G4 S10 b	Nil	Nil	Nil	Nil	Nil
G4 S11 b	Nil	Nil	Nil	Nil	Nil
G4 S12 b	Nil	TNTC	TNTC	TNTC	TNTC
G4 S13 b	Nil	Nil	Nil	Nil	Nil
G4 S14 b	Nil	TNTC	TNTC	TNTC	TNTC
G4 S15 b	Nil	Nil	Nil	Nil	Nil
G4 S16 b	Nil	Nil	Nil	Nil	Nil

Table 4.44: Yeast and Mold status examined for eighth samples' group that was studied for two months.

Sample Code	Storage Time				
	0 time	2 weeks	4 weeks	6 weeks	8 weeks
Standard Sample	Nil	Nil	Nil	50	100
G4 S1 c	Nil	Nil	Nil	Nil	Nil
G4 S2 c	Nil	Nil	Nil	Nil	Nil
G4 S3 c	Nil	Nil	Nil	Nil	Nil
G4 S4 c	Nil	10	20	50	100
G4 S5 c	Nil	Nil	Nil	Nil	Nil
G4 S6 c	Nil	Nil	Nil	Nil	Nil
G4 S7 c	Nil	Nil	Nil	Nil	Nil
G4 S8 c	Nil	20	20	20	35
G4 S9 c	Nil	Nil	Nil	Nil	Nil
G4 S10 c	Nil	Nil	Nil	Nil	Nil
G4 S11 c	Nil	Nil	Nil	Nil	Nil
G4 S12 c	Nil	20	20	Nil	Nil
G4 S13 c	Nil	Nil	100	700	700
G4 S14 c	Nil	Nil	30	500	500
G4 S15 c	Nil	300	500	TNTC	TNTC
G4 S16 c	Nil	TNTC	TNTC	TNTC	TNTC

As shown in Table 4.37- 4.44, Yeast and Mold status is relatively varied from the status of examined bacteria. Some investigation's samples showed small rate of Yeast and Mold growth in some steam treated samples of each eight groups. This could appear as the effect of steam is not as same as on the bacteria. But still it can be declared that the effect of steam still very strong on

the Yeast and Mold due to two reasons, namely; from the growth pattern during the storage time of the investigation it showed that the samples passed the investigation period with relatively zero number of Yeast and Mold and this is for most samples.

At the same time the second reason is the Yeast and Mold status for samples produced without steam treatment. A huge number of growth were observed. Therefore, samples treated with steam showed great stability of shelf-life, due to absence of relatively absence of Yeast and Mold.

On the other hand as the refrigerated white cheese is subjected for fungi growth at most time, it is a great success to produce white cheese with good Yeast and Mold status.

Again as in the bacterial investigation, as shown in the obtained results, the factory regular cheese was unable to exceed the two month storage time successfully; while the investigated cheese easily exceeded the shelf life and able to reach 4 months as shown for the selected samples. This is due to the fact that the two-month shelf-life refrigerated white cheese has relatively short life in comparison to the pasteurized cheese that boiled with brine solution.

4.4 At sensorial evaluation level:

As the shelf-life testing and microbial growth plays a major role in introducing an acceptable white cheese to the market.

On the other hand, it is very important regardless the microbial status to have the cheese accepted at different level of classification and assortment. Therefore, it is very important to produce developed white cheese product as well as to have the new modified product accepted at two levels:

- At research level
- At the market level

4.4.1. At research level:

Classifying the produced cheese at research level is very important and needed to be focused on studying if the required characteristics exist or not. This is very important that enables to scan samples with most acceptable characteristics to consumers.

132 samples were produced in this investigation. Around 28 samples were excluded due to false applications and found to be non-conform with the production elements.

104 samples were involved in all evaluating steps, as shown in tables (3.1-3.8). The criteria used to evaluate samples were highly recognized by several occasions of evaluating new products performed for food companies (Meat, Dairy, Soft drinks companies). The short listed best samples resulted from the evaluation process is concluded ten samples, as shown in Table 4.45.

Table 4.45: Short listed samples chosen to be the best results, as classified by research level.

No.	Sample Code	Steam Injection	Steam Bath	Refrigerated Stored			
				Vacuum	20% B°	16% B°	10% B°
1	G2 S4	X	x				X
2	G3 S10 c		X		x		
3	G1 S12		X				x
4	G1 S8	X					x
5	G3 S16 b						X
6	G2 S16						X
7	G4 S15 b					X	
8	G4 S4 b	X					X
9	G3 S8 a	X					x
10	G3 S14 a				X		

The results obtained in this investigation is justifying the critical role for introducing steam application to the new invented white cheese making, the addition of citric acid and starter culture prior to curd formation. The concentration of brine solution used to store samples played a major role to accept and reject samples especially the panel taste were carried out to samples that came out of brine solution directly without soaking in fresh water.

4.4.2. At Market level:

It is very critical to have the developed samples accepted at market level. The market is highly controlled by consumer demand. In general, the most concern classification at market level is the all over acceptance, rather than certain criteria, since customers are usually attracted to the taste and consistency.

However, the total eleven samples (ten investigated samples and one factory sample) were together introduced under coding number without referring to any sample identification or way of processing (Appendices A). The customer preference of the introduced samples was shown in Table 4.46.

Table 4.46: Ranking of the investigated samples in comparison to the reference sample produced by the factory.

Sample coding	Rank (percentage)							
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th
G3 S10 c	73%	18%	6%	3%	0%	0%	0%	0%
G3 S8 a	10%	36%	24%	18%	9%	3%	0%	0%
G3 S16 b	10%	18%	36%	27%	6%	3%	0%	0%

G4 S4 b	6%	13%	12%	13%	11%	23%	18%	4%
G3 S14 a	2%	6%	2%	2%	17%	23%	38%	10%
*G5 S1	1%	0%	7%	10%	15%	22%	10%	20%
G1 S8	1%	2%	6%	6%	10%	35%	27%	13%
G2 S16	0%	3%	3%	9%	18%	4%	19%	6%
G2 S4	0%	3%	2%	5%	9%	30%	18%	22%
G1 S12	0%	3%	1%	5%	10%	33%	11%	37%
G4 S15 b	0%	0%	1%	2%	1%	3%	21%	42%

* Factory sample

As shown in Table 4.46 the best result was obtained to sample number “G3 S10 c” which produced by steam application. This is proving the idea of the investigation that is the incorporation of steam with white cheese manufacturing has huge impact not just in increasing the shelf-life as shown in previous results but also customer who tasted these samples without addressing the shelf-life have chosen samples were produced with steam effect without knowing that. At the same time the percentage of customers who choose theses samples were huge and highly recognized the unique characteristics of these samples. As well the first top five accepted samples were samples with certain modification, namely; steam application, using acid and/or starter culture.

4.5 All over discussion for the investigation:

4.5.1. Effect of Steam:

Caseins are very heat resistant due to their loose structure and it is generally accepted that they can withstand heating at 140°C for 15-20 min as the random coiling of the primary chain is generally hard to destroy compared to secondary and tertiary structures. To some extent both

dephosphorylation and hydrolysis of the caseins has been found in heat treated milk (Belitz *et al.*, 2004; Farrell Jr. *et al.*, 2004; Fox, 1980).

Heat treatment above 100°C gives a decrease in micellar size due to increase in colloidal phosphate and dissociation of κ -casein from the micelle surface (Singh and Waungana, 2001).

The globular structure of whey protein makes them heat labile. Heat treatment of the whey proteins above 60°C, results in unfolding of the globular structure and the proteins thereby denature.

The denaturation of whey proteins is generally considered involving two steps. The first step is an unfolding of the native globular structure, which leads to exposure of hydrophobic residues and disulphide bonds. If the heat treatment is minimal, the unfolded protein can refold into native structure. At high temperatures, the unfolded proteins will form new hydrophobic interactions and disulphide bridges which can result in refolding the protein, but this is often disordered and gives rise to a random structure. The increase in the reactivity of the unfolded protein heated at high temperatures can also lead to the second step of the denaturation process. The unfolded whey proteins can form aggregates with other molecules, mostly through disulphide bonding and covalent bonds (Singh and Latham, 1993). Immunoglobulin's and BSA are the least stable whey proteins, β -Lg is intermediate and α -La is the most resistant protein to heat denaturation. These differences in extent of heat denaturation are caused by the differences in structure and strength of intermolecular bonds (Anema, 2008; Corredig and DaLgleish, 1996a).

The dimer of β -Lg dissociates between 30 and 55°C, but these changes are reversible and the monomers can rebound by cooling if the temperature does not exceed 60°C. When heating to temperatures above 60-70°C, the tertiary- and also partly secondary structure of the monomer starts to unfold, leading to exposure of the free thiol group (Cys121) and hydrophobic parts of the residues chain, resulting in a reactive monomer. (Iametti *et al.*, 1995 and Iametti *et al.* 1996). The formation of these monomers is irreversible and they cannot refold to native state. Instead

there will be formed non-native monomers, which can form aggregates with other monomers but also aggregates with other types of proteins can be formed (Tolkach and Kulozik, 2007).

α -La is the least heat resistant whey protein with a denaturation temperature around 62°C, but the unfolding at this temperature is reversible. It does not form aggregates or modified monomers at heating temperature below 80°C, at neutral pH (pH 6.6-6.8). This is due to α -La having no free thiol groups which can change the reactivity of α -La (Eigel *et al.*, 1984). α -La is capable of refolding to its native state in presence of calcium if the disulphide bonds are still intact (Brew, 2003). The binding of calcium is very pH dependent and calcium dissociates from the α -La binding site at pH below 5 which makes α -La lose the ability to refold to its native structure after heat treatment. Severe heating conditions with temperatures above 100°C for several minutes disrupt the disulphide bonds and formation disulphide linked aggregates of denatured α -La occurs (Singh and Havea, 2003).

The main aggregates formed as a consequence of heat treatment of milk, are complexes formed by aggregation of denatured whey proteins and complexes between β -Lg and κ -casein on the surface of the casein micelles via disulphide bonds and hydrophobic interactions. At temperatures below 70°C the interaction is mostly caused by hydrophobic interactions while at higher temperatures it is mostly caused by disulphide bonds (Corredig and DaLgleish, 1996a; O'Connell and Fox, 2011). The κ -casein and β -Lg interactions are most pronounced when the κ -casein is placed on the surface of casein micelles as the association between β -Lg and κ -casein is less favourable when κ -casein is dissolved in serum. This can be caused by κ -casein is present in a more compact structure when dissolved in serum whereas placed on the surface of casein micelles the structure of κ -casein is more loose (Donato *et al.*, 2007). The formation of these complexes may be altered by a slow heating rate or heating for a long time at lower temperatures. This gives longer time for the β -Lg to unfold and associate with the casein micelles. In contrast, a rapid heating rate to the required temperature gives a shorter time for unfolding and it is more likely that β -Lg refolds in a non-native structure or forms aggregates with other unfolded monomers instead of associating with κ -casein (Oldfield *et al.*, 1998b).

α -La does not associate with the casein micelles on its own like β -Lg; it has to form complexes with β -Lg which then associates with the casein micelle and it requires a prolonged heating period to start associating with the casein micelle (Oldfield *et al.*, 1998b; Oldfield *et al.*, 2000).

The rate of denaturation is mainly controlled by heating temperature, heating time and pH but also protein concentration and ionic strength have been proved to have some effect (McSwiney *et al.*, 1994; Oldfield *et al.*, 2000; Qi *et al.*, 1995). At neutral pH the free disulphide group of β -Lg is very reactive and this is the main mechanism for aggregation and gives a faster aggregation of β -Lg. Dissanayake *et al.* (2013b) have shown that the denaturation rate is significantly lower at pH 3 compared to pH 6 and the aggregates formed at pH 3 are caused by non-covalent bonding. This is consistent with free disulphide groups being inactivated at acidic conditions. At neutral pH most whey protein complexes formed by denaturation are soluble. A decrease in pH below 6.2 followed by heating gives a faster formation of whey protein/ κ -casein complexes and they were often associated with the casein micelles. Heating at a pH above 6.8 leads to dissociation of the whey protein/ κ -casein complexes from the micelle surface (Zúñiga *et al.*, 2010).

The motion of steam during injection in the cheese helped in arranging fibers in parallel and gave the cheese homogeneous textures and contributed to reduce internal and external porosity.

4.5.2. Effects of proteolysis:

Changes in the texture of cheese can be related to the rate at which proteolysis occurs; these reactions are affected by many things. First, though most coagulant is lost in the whey when drained, some is retained in the curd. If no active coagulant is present in the curd, then no α ₁-casein degradation can occur, and therefore, cheese softening will not happen (i.e. the cheese will maintain the young, rubbery texture) (Lawrence *et al.*, 1987). The amount of residual coagulant depends upon the pH of the system. Lower curd pH at draining encourages retention of more rennet resulting in increased hydrolysis of α ₁-casein. Second, the pH at drain determines the

amount of plasmin in the curd. Plasmin is a native milk proteinase that is responsible for much of casein breakdown. Plasmins are associated with the casein micelle in fresh milk, but as the pH decreases, they dissociate from the caseins. Third, the salt to moisture ratio affects the amount of intact casein; at lower ratios, there is less intact casein than at higher ratios. As discussed earlier, the salt to moisture ratio in the molded cheese also controls the activity of the residual rennet and plasmin in the cheese. Fourth, the storage temperature (or ripening temperature) impacts the rate of proteolysis, though the impact on textural characteristics depends upon the type of protein being proteolyzed. It is believed that α 1-casein hydrolysis contributes more structurally to the cheese than the other caseins. At temperatures below 6°C, the amount of β -casein hydrolyzed decreases significantly, but the amount of α 1-casein hydrolyzed only slightly decreases. Therefore, cheeses of the same variety ripened at different temperatures below 6°C are not very different texturally. However, ripening temperatures above 10°C have significant effects on creating textural differences since more α 1-casein is hydrolyzed as the temperature is increased. Cheese proteolysis is negatively correlated with firmness, indicating softening of the cheese as the protein matrix is broken down. Fifth, changes in pH during storage affect the rate of proteolysis. Generally, the rate of breakdown of α 1-casein is greater at lower storage pH than the rate of breakdown of β -casein. Finally, both dissolved calcium in the cheese serum and calcium bound to the protein network have been shown to affect the rate of proteolysis. However, it is difficult to distinguish the direct effect of calcium since the total amount of calcium retained in the curd is determined by the point at which the whey is drained from the curd. Simultaneously, the drain point also controls the amount of residual rennet and plasmin in the curd, both of which are factors determining cheese texture (Lawrence *et al.*, 1987).

4.5.3. Effects of Acidity and PH:

Cheeses having a low pH (near the isoelectric point of casein) show a granular texture and shatter when deformed; higher pH cheeses are more plastic and elastic. At low pH, strong ionic and hydrophobic intraaggregate forces hold the casein aggregates in a compact formation (inter-aggregate forces are weaker). Water in this system is less mobile. At higher pH, casein molecules have a net negative charge. Though the hydrophobic interactions still exist, the ionic interactions

change to a repulsive nature. The tight protein aggregates absorb water to solvate the non-neutral ionic charges. This effect can be mineralized depending upon the extent of ionic calcium bound to the casein in the cheese, which decreases the solubility of the protein (Creamer and Olson, 1982). Additionally, the mineral equilibrium within the cheese influences the texture. Calcium acts to cement the casein micelles together. During maturation, calcium is transported from the center to the outside of the cheese causing the core to have a lower calcium content (Adda *et al.*, 1982).

4.5.4. Effect of Salt and Brine solution:

Brine-immersed cheeses show dramatic changes in texture during the early stages of aging. Brine migration patterns and rates have been modeled (Geurts *et al.*, 1974; Geurts *et al.*, 1980); Sodium chloride affects both the matrix and the serum phases of the cheese, which, in turn, affects the overall texture. It has been determined that sodium chloride in the serum phase of cheese promotes the microstructural swelling of the para-casein matrix resulting in an increased water-holding capacity and formation of a hydrated gel. Simultaneously, the sodium chloride promotes the solubilization of intact caseins from the para-casein matrix; it is hypothesized that these proteins are able to freely migrate between the matrix and the serum phase (Guo and Kindstedt, 1995; Guo *et al.*, 1997). The calcium phosphate bridges that connect the bare casein micelles in the protein matrix are affected in a process called demineralization. The sodium ions are able to displace the calcium ions in the calcium phosphate bridge. This allows for water in the system to be able to bind to the complex, either increasing the water holding capacity of the matrix, or promoting the protein to become soluble in the serum (Geurts *et al.*, 1972). Additionally, salt changes the appearance of cheeses making them less opaque. As was discussed, salt increases absorption of serum into the matrix making a more homogeneous matrix. This results in fewer discontinuities (surfaces) to cause light to scatter, making the cheese appear more translucent (Paulson *et al.*, 1998).

4.5.5. Effect of Microorganisms (starter culture):

Starter cultures can serve three purposes. First, the starter cultures digest the lactose in the milk and produce lactic acid, which aids in lowering the pH of the milk creating the ideal environment for coagulation. The pH affects the reactivity of the binding sites on the casein molecules and therefore, influences the structure of the matrix (Rowney *et al.*, 1999) and that cleared in the results previous. Second, the browning characteristics of the final cheese are determined by how the starter cultures process the lactose. Lactose is a sugar composed of one molecule of galactose and one molecule of glucose. For example, *Lactobacillus delbrueckii* only digests glucose, leaving some residual galactose, which will brown due to Maillard browning when the final cheese is heated and that the cause of changing in color which is become more darky. Third, starter cultures produce enzymes that affect cheese flavor and texture and that cleared in the result compared to taste for samples from G3 and G4 produced under the same conditions with the same procedure that appeared in the G4s samples which have better taste than others in G3s samples.

Chapter Five

5. Conclusion and Recommendation

5.1 Introduction:

Due to heavy results obtained in this investigation, numerous conclusions were highlighted. However, the major conclusions, on the basis of the results and discussion presented within this research, can be drawn:

5.2 Cheese characteristics conclusion:

- Elasticity was increased whenever citric acid used.
- Viscosity was increased by the integration of starter culture in curd formation.
- Sample turbidity was increased shapely by increasing the brine solution concentration.
- Sample consistency decreased by increasing the citric acid concentration, higher effect was obtained when acid companied with starter culture.
- Shining texture was obtained by application of steam through bathing.
- Internal impact structure was manifested for the application of steam by injection.

5.3 Alteration in Unit Operation:

- Steam was very important in introducing new required characteristics in white cheese.
- Steam bath effect is highly different than steam injection effect.
- Both steam applications were very important in producing white cheese with long shelf-life.
- Steam introduced new method of sterilization without having bad effect on cheese yield and curd formation.

5.4 Additives and brine solution:

- Surfactant played major role in consistency development.
- Surfactant decreased number of internal porosity and the impact structure of white cheese.
- Acid and starter culture used prior to curd formation played major role in developing cheese characteristics.
- Brine solution helped in strengthening the structure of white cheese
- Brine solution concentration would cause turbidity of the bottled sample.

5.5 Recommendations:

It is very obvious through the results obtained that the cheese processing line needs to be developed. The manifested effect of steam must be granted through the change in the process and the addition of the steam unit operation step.

It is highly recommended to introduce the high effect of steam in increasing the shelf-life of white cheese and even more substituting the use of antimicrobial agents and other preservative.

It is recommended as well to carry one further investigation studying additional materials that could incorporate steam, surfactant, starter cultures, and acids enable to maximize the added value of these applications.

References:

- Anema, S.G. (2008). Chapter 8 - The whey proteins in milk: thermal denaturation, physical interactions and effects on the functional properties of milk. In *Milk Proteins*, A. Thompson, M. Boland, and H. Singh, eds. (San Diego: Academic Press), pp. 239–281.
- Basson, P.(1981). Women and Traditional food technologies: Changes in rural Jordan. Preparation of food at home. *Ecol. Food Nutr.*, 11: 17-23.
- Belitz, H.D., Grosch, W., and Schieberle, P. (2004). *Food Chemistry* (Springer).
- Boxler, C., Augustin, W., and Scholl, S. (2014). Composition of milk fouling deposits in a plate heat exchanger under pulsed flow conditions. *J. Food Eng.* 121, 1–8.
- Braunitzer, G., Chen, R., Schrank, B. & Strange!, A. (1972). Automatische sequenzanalyse eines proteins (β -Iactoglobulin AB). *Hoppe-Seyler's S. Physiol. Chem.* , 353, 832-834.
- Brew, K. (2003). α -lactalbumin. In *Advanced Dairy Chemistry, Volume 1, Proteins*, P.F. Fox, and P.L.H. McSweeney, eds. (Kluwer Academic/Plenum Publishers),.
- Brown, J. R. (1977). Structure and evolution of serum albumin. In *Albumin, Biosynthesis, Functions*. Proceedings of the 11th FEBS Meeting, 50, 11-21 .
- Browne, W. J., North, A. C . T., Phillips, D .C., Brew, K., Vanarnan, T. C .and Hill, R. L. (1969). A possible three-dimensional structure of bovine α -lactalbumin based on that of hen's egg-white lysozyme. *J. Mol. Bioi.* , 42, 65-86.
- Calvo, M.M., Leaver, J., and Banks, J.M. (1993). Influence of other whey proteins on the heat-induced aggregation of α -lactalbumin. *Int. Dairy J.* 3, 719–727.
- Campell, M. (2013). Heating methods for Ultra-High temperature pasteurization (http://www.foodprocessing.com/assets/wp_downloads/PickHeaters_eHandbook_02-13.pdf).
- Corredig, M., and Dalgleish, D.G. (1996a). Effect of temperature and pH on the interactions of whey proteins with casein micelles in skim milk. *Food Res. Int.* 29, 49–55.

- Corredig, M., and Dalgleish, D.G. (1996b). Effect of temperature and pH on the interactions of whey proteins with casein micelles in skim milk. *Food Res. Int.* 29, 49–55.
- Dalgleish, D.G. (1990). Denaturation and aggregation of serum proteins and caseins in heated milk. *J. Agric. Food Chem.* 38, 1995–1999.
- Dalgleish, D.G. (1993). Bovine milk protein properties and the manufacturing quality of milk. *Proc. Jt. EAAPASAS Workshop* 35, 75–93.
- Dalgleish, D. G., Home, D. S. and Law, A. J. R. (1989). Size-related differences in bovine casein micelles. *Biochim. Biophys. Acta.*, 991, 3 83-387.
- Dalgleish, D. G. and Law, A J. R. (1988). PH-Induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. *J. Dairy Res.* , 55, 529-538.
- Dannenberg, F., and Kessler, H.G. (1986). Quantitative densitometry of heat-induced changes in whey proteins following ultrathin-layer isoelectric focusing. *ELECTROPHORESIS* 7, 67–72.
- Dannenberg, F., and Kessler, H.-G. (1988). Reaction Kinetics of the Denaturation of Whey Proteins in Milk. *J. Food Sci.* 53, 258–263.
- Davies, D. T. and Law, A J . R. (1980). The content and composition of protein in creamery milks in south-west Scotland. *J. Dairy Res.*, 47, 83-90.
- Deeth, H.C., and Datta, N. (2002). ULTRA-HIGH TEMPERATURE TREATMENT (UHT) | Heating Systems. In *Encyclopedia of Dairy Sciences*, H. Roginski, ed. (Oxford: Elsevier), pp. 2642–2652.
- Dellamonica, E. S., Holden, T. F., Calhoun, M. J. and Aceto, N. C. (1965). Effect of season on the whey protein nitrogen distribution of pooled milk. *J. Dairy Sci.*, 48, 1585-1590.
- de Koning, P. J., Koops, J. and van Rooijen, P. J. (1974). Some features of the heat stability of concentrated milk m. Seasonal effects on the amounts of casein, individual whey proteins and NPN and their relation to variations in heat stability. *Neth. Milk Dairy J.*, 27, 186-202.
- Dissanayake, M., Ramchandran, L., Donkor, O.N., and Vasiljevic, T. (2013b). Denaturation of whey proteins as a function of heat, pH and protein concentration. *Int. Dairy J.* 31, 93–99.

- Ditchfield, C., Tadini, C.C., Singh, R.K., and Toledo, R.T. (2007). Heat transfer during thermal processing of a temperature dependent non-Newtonian fluid in a tubular heat exchanger. *Adv. Appl. Chem. Eng. Princ. Food Ind.* 46, 472–476.
- Donato, L., Guyomarc'h, F., Amiot, S., and Dalgleish, D.G. (2007). Formation of whey protein/ κ -casein complexes in heated milk: Preferential reaction of whey protein with κ -casein in the casein micelles. *Int. Dairy J.* 17, 1161–1167.
- Donnelly, W. J., McNeill, G. P., Buchheim, W. and McGann, T. C. A. (1984). A comprehensive study of the relationship between size and protein composition in natural bovine casein micelles. *Biochim. Biophys. Acta*, 789, 136- 143 .
- Downey, W. K. and Murphy, R. F. (1970). The temperature-dependent dissociation of β -casein from bovine casein micelles and complexes. *J. Dairy Res.*, 37, 361-372.
- Edgerton, E.R., Jones, V.A., and Warren, J.A. (1970). Effect of Process Variables on the Holding Time in an Ultrahigh-Temperature Steam Injection System1. *J. Dairy Sci.* 53, 1353–1357.
- Edmond, S.. (2001). Continious heat processing. In *Thermal Technologies in Food Processing*, P. Richardson, ed. (Elsevier), pp. 29–48.
- Eigel, W.N., Butler, J.E., Ernstrom, C.A., Farrell, H.M., Jr., Harwalkar, V.R., Jenness, R., and Whitney, R.M. (1984). Nomenclature of Proteins of Cow's Milk: Fifth Revision1. *J. Dairy Sci.* 67, 1599–1631.
- Farrell Jr., H.M., Jimenez-Flores, R., Bleck, G.T., Brown, E.M., Butler, J.E., Creamer, L.K., Hicks, C.L., Hollar, C.M., Ng-Kwai-Hang, K.F., and Swaisgood, H.E. (2004). Nomenclature of the Proteins of Cows' Milk—Sixth Revision. *J. Dairy Sci.* 87, 1641–1674.
- Farrell Jr., H. M., Kumosinski, T. F., Pulaski, P. and Thompson, M. P. (1988). Calcium-induced associations of the caseins: a thermodynamic linkage approach to precipitation and resolubilization. *Arch. Biochem. Biophys.* , 265, 146- 158.
- Fox, P.F. (1980). Heat-Induced Changes in Milk Preceding Coagulation. *J. Dairy Sci.* 64, 2127–2137.
- Fox, P.F., and Kelly, A.L. (2006). Chemistry and Biochemistry of Milk constituents. In *Food Biochemistry and Food Processing*, (Blackwell Publishing).

- Fox, P.F., and McSweeney, P.L.. (1998). Dairy Chemistry and Biochemistry (London: Blackie Academic and Professional).
- Garrick, J. D. (1986). Is the Role of B Lactoglobulin to Serve A s A Retinol Carrier in Milk? B.Sc. Honour Project Thesis, University of Aberdeen.
- Gaucheron, F. (2011). MILK SALTS | Distribution and Analysis. In Encyclopedia of Dairy Sciences (Second Edition), J.W. Fuquay, ed. (San Diego: Academic Press), pp. 908–916.
- Green, D. W., Aschaffenburg, R., Camerman, A., Coppola, J. C., Diamand, R. D., Dunnill, P., Simmons, R. M., Komorowski, E. S., Sawyer, L., Turner, E. M. C. & Woods, K. F. (1979). Structure of bovine β -lactoglobulin at 6 Å resolution. *J. Mol. Biol.* , 131, 375-397.
- Gut, J.A.W., and Pinto, J.M. (2003). Modeling of plate heat exchangers with generalized configurations. *Int. J. Heat Mass Transf.* 46, 2571–2585.
- Gutierrez, C.G.C.C., Dias, E.F.T.S., and Gut, J.A.W. (2011). Investigation of the residence time distribution in a plate heat exchanger with series and parallel arrangements using a non-ideal tracer detection technique. *Appl. Therm. Eng.* 31, 1725–1733.
- Harding, F. & Royal, L. (1974). Variations in the nitrogen-containing fractions of bulked milk in England and Wales during the period 1 947 to 1 970. *Dairy Ind.* , 39, 372-378.
- Hassan, A.N., 2007. Structure development in cheese. Proceeding of the 10th Egyptian Dairy Science and Technology Conference, Nov. 19-21, Cairo, Egypt.
- Holt, C. (1985). The milk salts: their secretion, concentration and physical chemistry. In *Developments in Dairy Chemistry-3, Lactose and minor constituents.* ed. P. F. Fox, Elsevier Applied Science Publishers, London. pp. 143- 181 .
- Holt, C. (1992). Structure and stability of bovine casein micelles. *Advances in Protein Chemistry.*, 43, 63- 151 .
- Holt, C. (1995). Effect of heating and cooling on the milk salts and their interaction with casein. In *Heat Induced Changes in Milk.* 2nd Edn., ed. P. F. Fox, International Dairy Federation, Brussels. pp. 105- 133 .
- Holt, C. , Hasnain, S. S. and Hukins, D. W. L. (1982). Structure of bovine milk calcium phosphate determined X-ray. *Biochim. Biophys. Acta*, 719, 299-303 .

- Holt, C., van Kemenade, M. J. J. M., Nelson Jr., L. S., Sawyer, L., Harries, J. E., Bailey, R. T. & Hukins, D. W. L. (1989). Composition and structure of micellar calcium phosphate. *J. Dairy Res.* , 53, 557-572.
- Holt, C. and Sawyer, L. (1988). Primary and predicted secondary structures of caseins in relation to their biological functions. *Protein engr.*, 2, 251-259.
- Hougaard, A.B., Ardö, Y., and Ipsen, R.H. (2010). Cheese made from instant infusion pasteurized milk: Rennet coagulation, cheese composition, texture and ripening. *Int. Dairy J.* 20, 449–458.
- Humeid, H.A., H.A. Tukan, S.K. and M.I. Yamani, 1990. In bag steaming of white brined cheese as a method of preservation. *Michwissenschaft*, 45: 513-516.
<http://www.fao.org/agris/search/display.do?f=/1991/v17/17/DE91V0008.xml;DE91V0008>.
- Iametti, S., Cairoli, S., De Gregori, B., and Bonomi, F. (1995). Modifications of High-Order Structures upon Heating of β -Lactoglobulin: Dependence on the Protein Concentration. *J. Agric. Food Chem.* 43, 53–58.
- Iametti, S., De Gregori, B., Vecchio, G., and Bonomi, F. (1996). Modifications Occur at Different Structural Levels During the Heat Denaturation of β -Lactoglobulin. *Eur. J. Biochem.* 237, 106–112.
- Jang, H. D., and Swaisgood, H. E. (1990). Disulphide bond formation between thermally denatured β -lactoglobulin and κ -casein in casein micelles. *J. Dairy Sci.*, 73, 900-904.
- Kalab, M., Phipps-Todd, B. E. and Allan-Wojtas, P. (1982). Milk gel structure. XIII. Rotary shadowing of casein micelles for electron microscopy. *Milchwissenschaft*, 37, 513-518.
- Keogh, M. K., Kelly, P. M., O'Keefe, A. M. and Phelan, J. A. (1982). Studies of milk composition and its relationship to some processing criteria II. seasonal variations in the mineral levels of milk. *Jr. J. Food. Sci. Technol.* , 6, 13-27.
- Kessler, H.-G., and Beyer, H.-J. (1991). Thermal denaturation of whey proteins and its effect in dairy technology. *Int. J. Biol. Macromol.* 13, 165–173.
- Abu-Khader, M.M. (2012). Plate heat exchangers: Recent advances. *Renew. Sustain. Energy Rev.* 16, 1883– 1891.

- Knoop, A. M., Knoop, E. and Wiechen, A. (1979). Sub-structure of synthetic casein micelles. *J. Dairy Res.*, 46, 347-350.
- Knoop, A. M., Knoop, E. and Wiechen, A. (1973). Electron microscopical investigations on the structure of the casein micelles. *Neth. Milk Dairy J.*, 27, 121-127 .
- Kumar, S. and Mikolajcik, E. M. (1973). Selected physio-chemical characteristics of bovine colostrum immunoglobulin. *J. Dairy Sci.* , 56, 255-258.
- Laemmli, U.K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* 227, 680–685.
- Larson, B. L. (1992). Immunoglobulins of the mammary secretions. In *Advanced Dairy Chemistry-1: Proteins*, 2nd Edn., ed. P. F. Fox. Elsevier Science Publishers Ltd., England, pp. 231 -254.
- Lewis, M.J., and Deeth, H.C. (2008). Heat Treatment of Milk. In *Milk Processing and Quality Management*, A.Y. Tamime, ed. pp. 168–204.
- Lorenzen, P.C., Clawin-Rädecker, I., Einhoff, K., Hammer, P., Hartmann, R., Hoffmann, W., Martin, D., Molkentin, J., Walte, H.G., and Devrese, M. (2011). A survey of the quality of extended shelf life (ESL) milk in relation to HTST and UHT milk. *Int. J. Dairy Technol.* 64, 166–178.
- Lyster, R. L. J. (1972). Review of the progress of dairy science, section C. Chemistry of milk proteins. *J. Dairy Sci.* , 39, 279-318 .
- Martinez-Castro, I., Olano, A., and Corzo, N. (1986). Modifications and interactions of lactose with mineral components of milk during heating processes. *Food Chem.* 21, 211–221.
- Messer., J.W., H.M. Behirey and L.O. Leudecke, (1985). *Standard Methods for Examination of Dairy Products*. 15th Edn., American Public Health Association, Washington, DC., ISBN 08-75531326, pp: 412.
- McDowell, A. K R. (1972a). Seasonal variations in the total nitrogen, non-protein nitrogen and urea nitrogen contents of Friesian and Jersey milk. *J. Dairy Res.* , 39, 27-33.
- McGann, T. C. A., Buchheim, W., Keamey, R. D. and Richardson, T. (1983). Composition and ultrastructure of calcium phosphate-citrate complexes in bovine milk systems. *Biochim. Biophys. Acta*, 760, 415-420.

- McGann, T. C. A., Donnelly, W. J., Keamey, R. D. and Buchheim, W. (1980). Composition and size of distribution of bovine casein micelles. *Biochim. Biophys. Acta*, 630, 261-270.
- McKenzie, H. A. (1971). β -lactoglobulin. In *Milk Proteins*. Vol II, ed. H. A. McKenzie, Academic Press, New York, pp. 257-330.
- McKenzie, H. A., Ralston, G. B. and Shaw, D. C. (1972). Location of sulphhydryl and disulfide groups in bovine β -lactoglobulins and effects of urea. *Biochemistry*, 11, 4539-4547.
- McMahon, D.J., Yousif, B.H., and Kaláb, M. (1993). Effect of whey protein denaturation on structure of casein micelles and their rennetability after ultra-high temperature processing of milk with or without ultrafiltration. *Int. Dairy J.* 3, 239–256.
- McSwiney, M., Singh, H., and Campanella, O.H. (1994). Thermal aggregation and gelation of bovine β -lactoglobulin. *Food Hydrocoll.* 8, 441–453.
- Mulvihill, D. M. and Donovan, M. (1987). Whey proteins and their thermal denaturation – a review. *Irish J. Food Sci. Technol.* , 11, 43-75.
- O’Connell, J.E., and Fox, P.F. (2011). Heat Treatment of Milk | Heat Stability of Milk. In *Encyclopedia of Dairy Sciences (Second Edition)*, J.W. Fuquay, ed. (San Diego: Academic Press), pp. 744–749.
- Oldfield, D.J., Singh, H., Taylor, M.W., and Pearce, K.N. (1998a). Kinetics of Denaturation and Aggregation of Whey Proteins in Skim Milk Heated in an Ultra-high Temperature (UHT) Pilot Plant. *Int. Dairy J.* 8, 311–318.
- Oldfield, D.J., Singh, H., and Taylor, M.W. (1998b). Association of β -Lactoglobulin and α -Lactalbumin with the Casein Micelles in Skim Milk Heated in an Ultra-high Temperature Plant. *Int. Dairy J.* 8, 765–770.
- Oldfield, D.J., Singh, H., Taylor, M.W., and Pearce, K.N. (2000). Heat-induced interactions of β -lactoglobulin and α -lactalbumin with the casein micelle in pH-adjusted skim milk. *Int. Dairy J.* 10, 509–518.
- Papiz, M.Z., Sawyer, L., Eliopoulos, E.E., North, A.C.T., Findlay, J.B.C., Sivaprasadarao, R., Jones, T.A., Newcomer, M.E., and Kraulis, P.J. (1986). The structure of β -lactoglobulin and its similarity to plasma retinol-binding protein. *Nature* 324, 383–385.

- Payens, T. A. J. and Schmidt, D. G. (1966). Boundary spreading of rapidly polymerizing α_{s1} -casein B and C during sedimentation. *Arch. Biochem. Biophys.*, 115, 136- 145.
- Pierre, A., Frauquant, J., Le Gaët, Y., Poit, M., and Maubois, J.L. (1992). Préparation de phosphocaseinate natif par microfiltration sur membrane . *Le Lait* 72, 461–474.
- Phelan, J. A., O'Keeffe, A. M., Keogh, M. K. and Kelly, P. M. (1982). Studies of milk composition and its relationship to some processing criteria 1. Seasonal changes in the composition of Irish milk. *Jr. J. Fd Sci. Technol.*, 6, 1-11.
- Pouliot, Y. and Boulet, M. (1995). Observations on the seasonal variations in the salt balance of concentrated milk. *Int. Dairy J.* , 5, 75-85.
- Puyol, P., Perez, M. D., Ena, J. M. and Calve, M. (1991). Interaction of bovine β -lactoglobulin and other bovine and human whey proteins with retinol and fatty acids. *Agric. Biol. Chem.* , 55, 25 15-2520.
- Qi, X.L., Brownlow, S., Holt, C., and Sellers, P. (1995). Thermal denaturation of β -lactoglobulin: effect of protein concentration at pH 6.75 and 8.05. *Biochim. Biophys. Acta BBA - Protein Struct. Mol. Enzymol.* 1248, 43–49.
- Reed, R. G., Feldhoff, R. C., Clute, O. L. & Peters, T. (1975). Fragments of bovine serumalbumin produced by limited proteolysis. Conformation and ligand binding. *Biochemistry*,14, 4578-4584.
- Regester, G. O. and Smithers, G. W. (1991). Seasonal Changes in the β -lactoglobulin, α -lactalbumin, glycomacropeptide, and casein content of whey protein concentrate. *J. DairySci.* , 74, 796-802.
- Richardson, T., Oh, S. and Jimenez, R. (1992). Molecular modelling and genetic engineering of milk proteins. In *Developments in Dairy Chemistry-1*. ed. P. F. Fox, Applied Science Publishers, London, pp. 545-577.
- Robbins, R. M. & Holmes, L. G. (1970). Circular dichroism spectra of α -lactalbumin. *Biochim. Biophys. Acta*, 221, 234-240.
- Roefs, S . P. F. M., Walstra, P., Dalgleish, D. G. and Home, D. S. (1985). Preliminary note on the changes in casein micelles caused by acidification. *Neth. Milk Dairy J.* , 39, 199- 122.

- Rollema, H. S. (1992) Casein association and micelle formation. In *Advanced Dairy Chemistry-1: Proteins*, ed. P. F. Fox. Elsevier Science Publishers Ltd, England, pp.111-140.
- Rose, D. (1968). Relationship between micellar and serum casein in bovine milk. *J. Dairy Sci.* , 51, 1897- 1902.
- Said, H. M., Ong, D. E. and Shingleton, J. L. (1989). Intestinal uptake of retinol: enhancement by bovine milk β -lactoglobulin. *Am. J. Clin. Nutr.*, 46, 690-694.
- Sanderson, W. B. (1970b). Seasonal variations of some milk protein components. *Proc. XVIII Int. Dairy Congr.*, Vol. 1E., 28.
- Schmidt, D. G. (1982). Association of casein and casein micelle structure. In *Developments in Dairy Chemistry-I* .ed. P. F. Fox, Applied Science Publishers, London, pp. 61 -86.
- Schmidt, D. G. and Buchheim, W. (1970). Elektronenmikroskopische untersuchung der feinstruktur von caseinmicellen in kuhmilch. *Milchwissenschaft*, 25, 596-600.
- Schmidt, D. G. and Payens, T. A. J. (1972). The evaluation of positive and negative contributions to the second virial coefficient of some milk proteins. *J. Colloid Interface Sci.*,39, 655-662.
- Schroyer, J.A. (1997). Understand the Basis of Steam Injection Heating. *Chem. Eng. Process*.
- Shah, R.K., and Sekulić, D.P. (2007). Classification of Heat Exchangers. In *Fundamentals of Heat Exchanger Design*, (John Wiley & Sons, Inc.), pp. 1–77.
- Silverton, E. W., Navia, M. A. and Davies, D. R. (1977). Three-dimensional structure of an intact human immunoglobulin. *Proc. Nat. Acad. Sci. (USA)*, 74, 5140-5144.
- Singh, H. and Creamer, L. K. (1991b). Aggregation and dissociation of milk protein complexes in heated reconstituted skim milks. *J. Food Sci.* , 56, 238-246.
- Singh, H., and Havea, P. (2003). Thermal denaturation, aggregation and gelation of whey proteins. In *Advanced Dairy Chemistry, Volume 1, Proteins*, P.F. Fox, and P.L.H. McSweeney, eds. (Kluwer Academic/Plenum Publishers),.
- Singh, H., and Latham, J.M. (1993). Heat stability of milk: Aggregation and dissociation of protein at ultrahigh temperatures. *Int. Dairy J.* 3, 225–237.

- Singh, H and Newstead; D. F. (1992). Aspects of proteins in milk powder manufacture. In *Advanced Dairy Chemistry-1: Proteins*, ed. P. F. Fox. Elsevier Science Publishers Ltd., England, pp. 735-765.
- Singh, H., and Waungana, A. (2001). Influence of heat treatment of milk on cheesemaking properties. *Cheese Ripening Technol.* 11, 543–551.
- Slattery, C. W. and Evard, R. (1973). A model for the formation and structure of casein micelles from subunits of variable composition. *Biochim. Biophys. Acta*, 317, 529-538.
- Smits, P. & van Brouwershaven, J. H. (1980). Heat-induced association of β -lactoglobulin and casein micelles. *J. Dairy Res.* , 47, 313-325.
- Snoeren, T. H. M., van Markwijk, B. and van Montfoort, R. (1980). Some physicochemical properties of bovine α_{s2} -casein. *Biochim Biophys. Acta.* , 622, 268-276.
- Swaisgood, H. E. (1992). Chemistry of the caseins. In *Advanced Dairy Chemistry-1: Proteins*. 2nd Edn., ed. P. F. Fox, Elsevier Applied Science Publishers, London, pp. 63-110.
- Thulukkanam, K. (2013). *Heat Exchanger Design Handbook* (Taylor & Francis Inc.).
- Tolkach, A., and Kulozik, U. (2007). Reaction kinetic pathway of reversible and irreversible thermal denaturation of β -lactoglobulin. *Lait* 87, 301–315.
- Toma, S. J. and Nakai, S. (1973). Calcium sensitivity and molecular weight of α_{s2} -casein. *J. Dairy Sci.* , 56, 1559-1562.
- Townend, R., Kumosinski, T. F. and Timasheff, S. N. (1967). The circular dichroism of variants of β -lactoglobulin. *J. Biol. Chem.*, 242, 4538-4545.
- van Dijk, H. J. M. (1990a) The properties of casein micelles. 1. The nature of the micellar calcium phosphate. *Neth. Milk Dairy J.* , 44, 65-81.
- van Dijk, H. J. M. (1990b). The properties of casein micelles. 2. Formation and degradation of the micellar calcium phosphate. *Neth. Milk Dairy J.*, 44, 111-124.
- van Dijk, H. J. M. (1991). The properties of casein micelles. 4. The effect of the addition of NaCl, MgCl₂, or NaOH on the partition of Ca, Mg and PO₄ in cows' milk. *Neth. Milk Dairy J.*, 45, 241 -251 .
- Visser, H. (1992). A new casein micelle model and its consequence for pH and temperature effects on the properties of milk. In *Protein Interactions*, ed. H. Visser. VCH Publishers, New York, pp. 135-165.

- Visser, J., and Jeurink, T.J.M. (1997). Fouling of heat exchangers in the dairy industry. *Heat Exch. Fouling* 14, 407–424.
- Vreeman, H. J., Brinkhuis, J. A. and van der Spek, C. (1981). Some association properties of bovine SH- κ -casein. *Biophys. Chem.* , 14, 185-193.
- Walstra, P. (1990). On the Stability of Casein Micelles. *J. Dairy Sci.* 73, 1965–1979.
- Walstra, P. and Jenness, R. (1984). *Dairy Chemistry and Physics*. John Wiley & Sons, New York.
- Wang, Q., Bulca, S., and Kulozik, U. (2007). A comparison of low-intensity ultrasound and oscillating rheology to assess the renneting properties of casein solutions after UHT heat pre-treatment. *Int. Dairy J.* 17, 50– 58.
- Waungana, A., Singh, H., and Bennett, R.J. (1996a). Influence of denaturation and aggregation of β lactoglobulin on rennet coagulation properties of skim milk and ultrafiltered milk. *Food Res. Int.* 29, 715– 721.
- Waungana, A., Singh, H., and Bennett, R.J. (1996b). Influence of denaturation and aggregation of β -lactoglobulin on rennet coagulation properties of skim milk and ultrafiltered milk. *Food Res. Int.* 29, 715– 721.
- White, J. C. D. and Davies, D. T. (1958). The relation between the chemical composition of milk and the stability of the caseinate complex. I. General introduction, description of samples, methods and chemical composition of samples. *J. Dairy Res.* , 25, 236-255.
- Whitney, R. McL. (1988). Proteins of milk. In *Fundamentals of Dairy Chemistry*, ed. N . P. Wong, R . Jenness, M. Keeney & E .H . Marth. Van Nostrand Reinhold Company, New York, pp. 81 - 169.
- De Wit, J.N. (1998). Nutritional and Functional Characteristics of Whey Proteins in Food Products. *J. Dairy Sci.* 81, 597–608.
- Zúñiga, R.N., Tolkach, A., Kulozik, U., and Aguilera, J.M. (2010). Kinetics of Formation and Physicochemical Characterization of Thermally-Induced β -Lactoglobulin Aggregates. *J. Food Sci.* 75, E261–E268.
- Yammani, M.I., J.K. Tukan and S.J. Abu-Tayeh, (1997). Microbiological quality of kunafa and the development of a Hazard Analysis Critical Control Point (HACCP) plan for its production. *Dairy Food Environ. Sanitat.*, 17: 638-643.

7. Appendices:

A:

بسم الله الرحمن الرحيم



تجرى هذه الاستبانة استكمالاً لمتطلبات بحث الماجستير من جامعة القدس/ كلية الدراسات العليا برنامج العلوم التطبيقية والصناعية والذي كان بعنوان:

Unit Operation Alteration for Developing the Characteristics of Local White Cheese

الباحثة حنين منور صالح (21410773)

اسم المدرسة:

الجنس:

• أنثى • ذكر

العمر:

- 22 - 30 سنة
- 30 - 40 سنة
- 40 فما فوق

تقييم عينة رقم 1 (G2 S4)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 2 (G3 S10 c)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 3 (G1 S12)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 4 (G1 S8)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 5 (G3 S16 b)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 6 (G2 S16)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 7 (G4 S15 b)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 8 (G4 S4 b)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 9 (G5 S1)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 10 (G3 S8 a)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تقييم عينة رقم 11 (G3 S14 a)

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	الصفة
																				الطعم
																				القوام
																				التماسك
																				الرائحة
																				المظهر العام

تعديل الطرق والعمليات التصنيعية من أجل تطوير خصائص الجبن الأبيض المحلي

إعداد الباحثة: حنين منور يونس صالح

إشراف: د. إبراهيم عفانة

ملخص:

الجبنة المحلية البيضاء تعد من أشهر أنواع الجبنة المستهلكة في فلسطين والتي يتم استخدامها في صناعة الكنافة والحلويات والمعجنات المحلية الأخرى وتعتبر قابليتها للتمدد وتكوين ألياف مطاطية من أهم المواصفات التي تعطيها الأفضلية ولذلك على مدى السنوات الماضية تم استخدام طرق مختلفة لتحسين خصائص الجبنة المحلية سواءً على المكونات أو على الطريقة التصنيعية ورغم نجاح بعضها في الوصول للهدف المنشود إلا أنه كان على حساب انخفاض القيمة الغذائية وجودة المنتج أو ارتفاع كلفة إنتاجه أو عدم فاعليته في حال الإنتاج بكميات كبيرة وكجزء من منظومة الأبحاث التي تمت في هذا الصدد كانت هذه الدراسة والتي اعتمد الباحث فيها على إنتاج الجبنة باستخدام معاملة جديدة تعتمد على استخدام البخار بطريقتين إما بالحقن أو كحمام بالإضافة للتحكم بمقياس الحموضة (PH) عن طريق استخدام الحمض بتركيز مختلفة والكائنات الحية الدقيقة (البادئة) كما وقام الباحث باستخدام مادة surfactant مصرح بها من منظمة الدواء والغذاء العالمية للاستهلاك البشري لدراسة تأثيرها على الجبنة.

تم تصميم التجربة لتشمل 128 عينة موزعة على 4 مجموعات فكانت كالتالي: المجموعة الأولى تم إنتاجها باستخدام إنزيم الرنين في حين تم إنتاج المجموعة الثانية باستخدام إنزيم الرنين بالإضافة إلى الكائنات الحية الدقيقة (البادئة) أما المجموعة الثالثة فقد تم إنتاجها باستخدام الإنزيم والحمض الذي تم اختياره على ثلاث تراكيز (0.035، 0.05، 0.1)، وأنتجت المجموعة الرابعة باستخدام الإنزيم إلى جانب الحمض بتركيزه الثلاث والبادئة. وبعد سلسله من الفحوصات الكيميائية والفيزيائية تم اختيار 10 عينات لتخضع لتقييم السوق

فكانت النتائج حصول 5 عينات على قبول واستحسان فاق العينة المرجعية التقليدية. 4 عينات تم اختيارها بالاعتماد على معايير خاصة وإعادة انتاجها باستخدام surfactant حيث تمت إضافته على طريقتين: الأولى قبل التخثر، والثانية بعد التخثر وكانت النتائج متفاوتة وتعتمد على الإضافات المستخدمة والطريقة التصنيعية لكل عينة، لكنها أظهرت قدرة عالية على إنتاج جبنة بمواصفات مميزه صماء وخالية من الفراغات الداخلية والخارجية ومضغوطة بشكل لافت في بعض العينات لكنها لم تتناسب مع الطريقة التصنيعية او الإضافات لعينات أخرى.

خلاصة القول استخدام تقنية البخار في تصنيع الجبنة كان له بالغ الأثر على المنتج النهائي وكانت النتائج متفاوتة وتعتمد على المنظومة المستخدمة لكل منتج فالعينات التي خضعت لحقن البخار خلال عملية تكوين الخثرة في صناعة الجبن تحسن قوامها لتصبح أكثر نعومة وطراوة ومضغوطة بشكل خالٍ من الفراغات الداخلية والخارجية وطبقاتها مرتبة بشكل متوازٍ الأمر الذي أعطاهما الشكل المتناسق في حين استخدام حمام البخار ساهم في زيادة قدرة خيوط الخثرة على التمدد والحصول على المذاق البلاستيكي المضغوط على شكل ألياف مطاطية. إن استخدام الأحماض بتراكيز منخفضة يساعد في زيادة المطاطية وإنتاج جبنة بسطح أملس ولامع في حين أن استخدام الكائنات الحية الدقيقة ساهم في زيادة التماسك وتقليل الفراغات الداخلية والخارجية. في حين إن عملية استخدام خليط من الأحماض والكائنات الحية الدقيقة أنتج جبنة بمواصفات فخمة وذات قوام مميز.

