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# Effect of Heat Treatment on Milk Prepared for Cheese Making

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# الآية

# بسم الله الرحمن الرحيم

قال تعالى:

(وَإِنَّ لَكُمْ فِي الأَنْعَامِ لَعِبْرَةً تُسْقِيكُم مِّمَّا فِي بُطُونِتِ مِن بَيْنِ فَرْثٍ وَدَمٍ لَّبَناً خَالِصاً سَآئِغاً لِلشَّارِبِينَ)

صدق الله العظيم

سورة النعل الآيت (66)

# **Dedication**

This work is dedicated

To my father, to the spirit my Mother, Brother,

sisters and my small girl (Gonwan)

To my friends

With my sincere love and respect

Hiba

# Acknowledgment

Firstly I'm grateful to the almighty of god for his guidance without whom I wouldn't have finished this research then I would like to express my appreciation to my supervisor **Dr. Altieyb Ibrahim Ali** who has cheerfully answered my queries provided me with materials checked my examples assisted me in a myriad ways with the writing and helpfully commented on earlier drafts of this project. I wish to express grateful thanks to **Dr. Eman Omer Basheer Mohammed.**, ,**Dr.Amaal and Dr.Ejlal Elkhider**. Also I am very grateful to my friends project and thanks to my family for supporting me to bring out this project.

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## **Abstract**

This study was conducted to evaluate the effect of heat treatments on milk based cheese manufacture. The milk was divided into four groups. Each group had 5litters However, group one (comparative group), had no subjected to any heat treatments, whereas, group two was pasteurized to 72°C for 15 sec, while group three was boiled to 100.17°C for 6 min. Group four was used reconstituted dry milk, the total solids as the same to natural milk. Cheese samples were subjected to physical, chemical, microbial and sensory evaluation analysis. The study showed that, group four occurred the highest yield 1300g, then boling 1000g followed by pasteurized milk group 950g and the lowest raw milk group 680g. The analysis show that, coagulate time was 2hr and 30min for milk powder group, 1hr and 50min boiled milk, 1hr. and 40min pasteurized milk, and 1hr. and 15min raw milk respectively. The analysis showed that, the taste was 8.55 for milk powder, 7.60 for boiled, 6.60 for pasteurized and 5.25 for raw milk respectively. The texture was in acceptance score 8.40 for milk powder, 7.90 for boiled, 6.50 for pasteurized, 6.55 for untreated one respectively. Flavor score was an acceptance score 8.05 for dry milk, 7.15 for boiled, 6.90 for pasteurized and 5.80 for untreated one respectively.

For chemical analysis we observed that the cheese from dry milk was the highest one in all chemical composition except Acidity than all type of cheese under study, on the other side cheese from boiling milk was the lowest one in all chemical parameters except Acidity it was the highest

On the other hand untreated cheese had highest Total Viable Bacterial count TVBC; obesities the cheese making by treated milk with boiling had lowest Total Viable Bacterial count TVBC.

The panelists preferred cheese based milk powder when compared with other different treatments finally the cheese mad by Dry milk was the best one.

# تأثير المعاملات الحرارية على اللبن المعد لصناعه الجبن المستخلص

صممت هذه الدراسة لمعرفة اثر المعاملات الحرارية علي اللبن المعد لصناعة الجبنة .تم تقسيم اللبن إلى أربع مجموعات حيث كانت المجموعة الأولى (مجموعة المقارنة) لم تتعرض لأي معاملة حرارية بينما تمت بسترة المجموعة الثانية على درجة حرارة 72□م لمدة 15 ثانية , أما المجموعة الثالثة فقد تم غلي اللبن لدرجة حرارة 100.17□م لمدة 6 دقائق تقريبا في, المجموعة الرابعة فقد استخدم لبن مجفف أعيد ذوبانه حيث المواد الصلبة الكلية (TS) مشابهة للبن الطبيعي .تم تحليل عينات الجبنة فيزيائيا, كيميائيا ثم الأحياء الدقيقة والاختبارات الحسية .أظهرت الدراسة أن المجموعة الرابعة أعطت اعلى وزن 1.340 جرام ثم المغلي 1000 جرام وتلاها اللبن المبستر .2.30 جرام وأدناها اللبن الخام 680 جرام. أظهرت الدراسة زمن التجبن كان 2.30 ساعة وربع اللبن الخام على التوالي.

من خلال التحليل الكيميائي وجد ان الجبن المصنع من اللبن المجفف اعطي اعلي القيم لكل المكونات الكيميائية عدا الحموضة مقارنة بكل الاجبان تحت الدراسة. من جانب اخر جبن اللبن المغلى سجل اقل القيم لكل القياسات الكيميائية عدا الحموضة كان اعلاها.

من جانب آخر الجبن غير المعامل حراريا كان ذا محتوي عالي من البكتريا عكس الجبن المصنع من اللبن المغلي الذي أظهر أقل محتوي بكتيري

أظهرت الاختبارات الحسية أن الطعم كان درجة مقبولة 8.55 المجفف ،7.60 المغلي ،6.60 المبتر ،5.25 الغير معامل علي التوالي.القوام كان درجة مقبولة 8.40 المجفف 7.90 المغلي 7.15 المبستر 6.55 الغير معامل علي التوالي.النكهة درجة مقبولة 8.05 المجفف المغلي 7.15 المبستر 6.90 الغير معامل 5.80 على التوالي.

وقد كانت أفضلية التذوق للجبن المعد من اللبن المجفف مقارنة ببقية الأجبان، وقد إتضح أن الجبن من اللبن المجفف كان الأفضل.

.

# **CHAPTER ONE**

# **INTRODUCTION**

Cheese is a popular manufactured food product. Cheese making started out as an accidental curdling of milk. It is commonly believed that cheese evolved in the Fertile Crescent between the Tigris and Euphrates rivers, in Iraq, some 8,000 years ago, during Agricultural Revolution (Fox, et al. 2000)

is a nutrient-dense food. Cheese provides a high concentration of nutrients relative to its energy content. The nutritional composition of cheese depends on the type of milk used and the manufacturing and ripening procedures (Üçüncü 2004). When milk is made into cheese, casein and fat are concentrated, because they are retained in the curd during manufacture. Other milk components are mainly removed along with whey. Therefore, cheese contains relatively small amounts of the water-soluble constituents (whey proteins, lactose, and water-soluble vitamins), which partition mainly into the whey. None of the milk components is fully retained in cheese and new substances may be added, notably salt (Walstra *et al.*, 2006, Fox *et al.*, 2000).

Cheese is a biochemically dynamic product and undergoes significant changes during ripening. The major biochemical changes involved during cheese ripening are proteolysis, lipolysis, metabolism of residual lactose, lactate and citrate, and the formation of volatile compounds (Beuvier, E., Buchin, S. 2004). Ripening gives to different cheeses characteristic flavors, textures, and appearances Gunasekaran, S., Ak, M.M., (2003)

Fresh cheeses constitute a major proportion of the cheese consumed in some countries. Most of these cheeses are produced by acid coagulation

Casein is the main protein in cheese, although the water-soluble milk proteins

lactalbumin and  $\beta$ -lactoglobulin may also be present, depending on the amount of whey Entrapped in the cheese. Casein is slightly deficient in sulfurcontaining amino acids, so.

The biological value of cheese protein is slightly less than that of total milk protein ('Üçüncü, Mustafa. 2004, Fox *et al.*, 2000). Cheese contains trace amounts of carbohydrate, primarily lactose. The residual lactose in cheese curd is, normally, fermented to lactic Acid by starter bacteria during manufacture and ripening. Cheese is also a suitable nutrient for patients who have diabetes or lactose mal absorption, because of the low lactose ratio it contains. Since most of the milk fat is retained in the cheese curd, the fat-soluble vitamins in milk also partition into the curd. Most of the vitamin A in milk fat 80-85% is present in cheese fat. Conversely, most of the water-soluble vitamins in milk

Partition into the whey during curd manufacture. The 10- 20 % of vitamin  $B_1$ , 20-30% of vitamin  $B_2$  and biotin, 30-60% of folic acid and vitamin  $B_{12}$  in milk can partition in cheese. Significant quantities of vitamin  $B_{12}$  are produced in Swiss cheeses by prop ionic acid bacteria. Cheese is also an important source of calcium, phosphorus, and magnesium. The amount of salt added during the manufacture of different cheese varies significantly, resulting in large differences in the concentration of sodium in cheese (,Üçüncü, Mustafa. 2004, Fox *et al.*, 2000).

K-casein is the only protein hydrolyzed during the rennet coagulation of milk and it is hydrolyzed specifically at the peptide bond between Phenylalanine 105-106 Methionine. As a result, κ-casein is converted into Para-κ-casein and Glycomacropeptide. Glycomacropeptide is hydrophilic so remain as whey. There are about 10 forms of κ-casein that differ in sugar content; hence, 10 caseinomacropeptide are produced. All the caseinomacropeptides are soluble in 2 % trichloroacetic acid (TCA) but only the glycosylated forms are soluble at higher concentrations of TCA.

Therefore, TCA-soluble nitrogen can be used to monitor the primary phase of rennet coagulation (,Üçüncü, Mustafa. 2004, Fox *et al.*, 2000).

# The main objectives of this topic:

- 1. To determine the optimum heat treatment to milk prepared for cheese making.
- 2. To study and assess the effects of using heat treatment coagulants of yield, composition, and sensory evaluation of produced white soft cheese.
- 3. To study if the produced cheeses satisfy the international standards and specifications issued for white soft cheese.

# **CHAPTER TWO**

# LITERATURE REVIEW

## 2.1 Milk definition

Milk is a global drink that is apolyphasic emulsion having physical, chemical and biological properties and can be fermented into a wide range of different products flavors, consistencies and structure (Huria, 2003).

Also milk contains compounds that are essential to human, such as proteins, fats carbohydrates, vitamins, calcium, phosphorus and other minerals and it olso provides energy (Pauline and Karin 2006).

The trend nowadays is produce different new varieties and types of the so-called functional cheese dairy products explained, functional dairy products with a proven healthy benefits are based on milk that enriched with functional component, or the products are based on ingredients originating from milk and the most common functional dairy products are those with probiotic bacteria, quite frequently enriched with prebiotic carbohydrates. The connection between functional food and cheese is a straight forward one, since cheese is generally a fermented product and potentially an appropriate vehicle for probiotic bacteria (Donelly, 2003). The conversion of milk from fluid to gel (coagulation) is a basic step common to all types of cheese. The coagulation of milk is a consequence of protein destabilization, which is brought by acid proteinases chymosin, the active component of rennet. (Varnam and Sutherland, 1994). According to O'Conner ,(1993) rennet is a general term that describes a variety of enzymes of animal (especially calves), plant or microbial origin used to coagulate milk during cheese milking. For coagulation of milk in the manufacture of cheese, calf rennet is most wide spread and desirable and has been dominant in the industry of cheese

processing for long time. Crow (1993) indicated the limited supply of rennet and its resulting high price have necessitated research, for many decades, to come up with an alternative milk coagulant. The trend now in order to cover such a problem, plant enzymes are used in some parts of the world in cheese making.

## 2.1 Definition of cheese

Cheese has been defined as a product made from milk by coagulation the casein with the help of rennet or similar enzymes in the presence of lactic acid produced by added microorganisms, from which part of moisture has been removed by cutting, cocking and pressing, which has been shaped in mould and then ripened by holding it for same time at suitable temperature and humidity (Kutty and Sheeba, 2014). Described cheese as a fresh product obtained after coagulation and whey separation of milk, cream or partially skimmed butter milk or a mixture of these products (James, 2013).

Cheese is a stabilized curd of milk solids produced by casein coagulation and entrapment of milk fat in the coagulum, where the water content is greatly reduced, in comparison with milk, by the separation and removal of why from curd, with the exemption of same fresh cheeses, the curd is textured, salted, shaped and pressed into moulds before storage or curing or ripening, according to (Fernandes, 2009).

(IDF, 1998) defined cheese as a product made from curd obtained from whole, partly skimmed or skimmed milk of cows, or from milk of other animals, with or without added cream, by coagulating with rennet, lactic acid or other suitable enzymes, and with or without further treatment of the separated curd by heat or pressure or by means of ripening ferments, special mold or seasoning.

# 2.2 History and back ground of cheese

The real beginning of cheese making is unrecorded in history; it must have been occurred within few centuries after the domestication of cows and other mammals about 800B.C (Clarence *et al.*, 2004).

There is no conclusive evidence indicating where cheese making originated from in Europe, Central Asia or Middle East, but the practice has spread within Europe prior to Roman time, and it had become a sophisticated enterprise by the time the Roman Empire came into being (Arvind, 2010). The origin of the word cheese appears to be the Latin "cases", from which the modern word casein is closely derived out the earliest source, is probably from the Porto-Indo-European root, Kwat, which P means to ferment, become sour (Simpson,1979).

# 2.3 Classification of cheese

# The FAO/WHO classified the type of cheese according to moisture content as follows:

**Table.1:** Classification of cheese

Types		Fats	
Very Hard	49-56%	Low fat	10-25%
Hard	54-63%	Medium fat	25-45%
Semi hard	61-69%	Full fat	45-60%
Soft	67-76%	High fat	≥-60%

According to Herrington (2000), four different major factors are responsible for variation in cheese, which are: the differences in the nature of the milk used, method of coagulation, moisture and ripening. Also Biswas and Bahattachary (2006) mentioned, the different classification depend on

origin of utilized milk, type of coagulation, processing standard, geographical region and additives and special operations during manufacturing.

# 2.4 Chemical composition cheese

Cheese contains almost all milk components in concentrated from such as protein, fat, minerals, lactose, which to greater extent is converted in to organic salts, Murshidi, et, al (1998) explained, cheese contains the un dissolved components of milk, e.g. casein, amounts of fat and salt beside water containing few amounts of salt lactose and albumin.

Rennin in cheese compose in average 90 % of the milk fat, 75% of the milk protein, 30% - 40% of the milk salts and 5% of the milk lactose, (Osman, 2007). Chemical composition of Sudanese white soft cheese (Gibna Beida):

The type of cheese consumed widely by the different socio-economic of Sudanese families is the white soft cheese called Gibna Beida It is not known exactly when Gibna Beida was first introduced in to Sudan, but it is most likely that the Sudan has known this cheese for nearly a century (Dirar 1993). The chemical composition of Sudanese white soft as given by the Sudanese Standers and metrology organization (SSMO) (2008) according to dry matter weight and lowest limit as follows:

**Table.2**: The chemical composition of Sudanese white soft cheese

Moisture content	60%
Fat content	20%
Total solids	40%
Protein content	15 %
Ash	5%

# 2.5 Milk coagulation

Cheese is produced by coagulation of milk by certain of enzymes, which were either of animal origin or extracted from some plant (Miller *et al.*, 2007). Coagulation was done either by precipitation of casein due to the activity of enzyme or by proteolysis activity either by microorganism or by plant enzymes extracted from some type of plant.

According to Blume (2003), the type of coagulation used depends on the type of cheese desired. The conversion of milk from a fluid to a gel (coagulation) is a basic step common to all types of cheese (Varnam and Sutherland, 1994). Traditional cheese technology requires that the protein, especially the casein most be separated from milk by coagulation., Spreer (1998) explained the colloidal casein particles with a stable and even distribution must be coagulated, which means that the protein is converted from suspended state in to a gel state (Coagulate gallert), especially into alyogel. The coagulation of milk influenced mainly by the type and concentration of coagulation enzyme, coagulation temperature, properties and concentration of proteins and the PH value, (Storry and Food 1982).

# 2.5.1 2 Rennin coagulation

Rennet or (rennin) is a complex of enzymes produced in the stomachs of ruminants' mammals. Chymosin is protease enzyme that curdles the casein in milk. This helps young mammals digest there for cheese making and liquid whey. In addition, chymosin rennet contains other important enzymes such as lipase. Rennet is used for the production of must cheeses. Non-animal alternatives for rennet are suitable for consumption by vegetarians (Kopelman *et al.*, 1975).

# 2.5.1.1 Mechanism of Coagulation

The mechanism of casein precipitation by enzymatic coagulation after addition of the recent enzymes to milk is disturbed by Fredriksen *el al* (2011), Spreer (1998) as follows:

The casein precipitation takes place in two stages:

- 1. Enzymatic or primary phase.
- 2. Coagulation or secondary phase

In the enzymatic phase, the k-casein protective colloids fractions of glycomacropeptids (None protein nitrogen) (NPN) and the hydrating sphere of the casein micelle disappears and the protection against a joining disintegrates, while during the coagulation phase (at optimum temperature and pH) salt bridges from between the ca-sensitive micelles, because of the resistance of ca-ions reaching or linking rapidly and causing precipitation. The water insoluble calcium casinate complex formed from the colloidal dissolved ca-casinate complex is called coagulum (rennet gel, rennet gallert) and it is the real cheese material.

# 2.5.2Types and production of rennet

## 2.5.2.1 Production of natural Calf-rennet

Natural calf rennet is extracted from the inner mucosa of the fourth stomach chamber (the abomasums) of young UN weaned calves as part of the livestock butchering. The stomachs are by product of real production. If rennet is extracted from calf it is not suitable for lacto vegetarians to consume.

# 2.5.2.2 Vegetable rennet

Vegetable rennet is also suitable for vegetarians. Vegetable rennet might be used also in production of Kosher and Halal cheese, but nearly all

Kosher cheese are produced with either microbial rennet one vegetable rennet ,usually contain mod (Lee, *et al.* 1990).

## 2.5.2.3 Microbial Rennet

Some molds such as Rhizo mucor miehe are able to produce proteolytic enzymes. These molds are produced in fermented and then specially concentrated and purified to avoid contamination with unpleasant by products of the mold growth The advantages of using plant

proteases is that such natural enzymes can be eaten by vegetarians and also may be certified as Kosher and Halal (pino et al 2009).

The flavor and taste of cheese produced with microbial rennet tend toward some bitterness especially after long maturation periods. Cheese produced by this way are suitable to vegetarians (Lee *et al.*, (1990).

## 2.5.2.4 Alternative Sources of Rennet

Because of the limited availability of mammalian stomachs for rennet production, cheese makers looked for other ways to coagulate the milk. Since the least Roman times the many sources of enzymes that can be substitute for animal rennet range for plant and fungi to microbial sources. Cheese could be produced form any of these varieties of rennet.

# 2.5.2.5 Traditional Method of Rennet production

Dried and cleaned stomachs of young calves are sliced into small pieces and then put in salted water or whey together with vinegar to lower the PH of the solution after some time (over night or several days). The solution filtered. The cured rennet that remains in the filtered solution can then be used to coagulate milk. About 1g, of this solution can normally coagulate 2.4 liter of milk (Cremer, 1985).

## 2.5.2.6 Modern Method of Rennet Production

Deep frozen stomachs are milled and put into an enzyme extracting solution. The cured rennet extract is then activated by adding acid; the enzymes in the stomach are produced in the active from activated by the stomach acid. The acid is then neutralized and the rennet extract is filtered in several stages and concentrated until reaching a typical potency of about 1 gram coagulates 12Kg of milk. One Kg of rennet extract has about 0.7g of active enzymes. The rest in water and sometimes sodium benzoate (0.5-1.9%) for preservation and typically 1Kg of cheese contain about 0.0003 of rennet enzymes (Najera, *et al.*, (2008).

## 2.5.2.7 Pasteurization of Milk

Heat treatment destroys microorganisms and enzymes that can cause damage During ripening. Also, pasteurization kills pathogens that can survive for some time, Especially in soft-type cheeses. To avoid recontamination after pasteurization, strict

Hygienic measures have to be taken (Walstra, et al., 2006).

There are alternatives to heat treatments for reducing the number of Microorganisms in milk. These are:

- 1. Treatment with H<sub>2</sub>O<sub>2</sub>
- 2. Activation of the lactoperoxidase-H<sub>2</sub>O<sub>2</sub>- thiocyanate system
- 3. Bactofugation
- 4. Microfiltration Fox et al.,(2000).

# 2.5.2.8 Boiling of milk

Raw milk may contain pathogenic microorganisms from the farm environment, including vegetative bacteria, such as *Staphylococcus aurous*, *Campylobacter jejuni*, *Salmonella* spp., *Escherichia coli*, *Yerisinia enterocolitica*, and spore formers, such as *Bacillus* and *Clostridium* species. These major vegetative pathogens can be effectively controlled by

pasteurization, and are not the main determinants of keeping quality. The heat treatments kill and reduction pathogen bacteria. *Bacillus cereus* spores are relevant here, being the main pathogen which will survive pasteurization and grow at low temperature. *Bacillus* can cause defects in heat-treated milk, for example bitty cream, and produce an intense bitter flavor, but it rarely causes food poisoning because infected products are so unacceptable. Boiling milk to 100 °c was effective procedure to reduction bacterial count and keep milk quality (Lewes and Deeth, 2008).

## 2.5.2.9 Full milk Powder

Milk powder is dehydrated milk solids. Its manufacture involves the gentle removal of water From liquid milk at the lowest possible cost under stringent hygiene conditions while still Retaining all the desirable properties of liquid milk; color, flavor and nutritional value (Pearce, 1995). Whole (full cream) milk contains typically about 87% water, while skim (Defatted) milk contains about 91%. During milk powder manufacture, this water is removed By boiling at a low temperature under reduced pressure in a process known as evaporation.

The resulting concentrated milk is then sprayed as a fine mist into hot air to further remove Moisture and give a powder.

The main reasons for converting milk into a powder form are to reduce bulk for storage and Transport, to extend shelf-life by reducing water activity and thus preventing or slowing Spoilage reactions and to change the product chemically, physically and/or functionally for

Particular end uses (Pearce, 1995)

# 2.6 The Time of coagulation

Time of coagulation is affected by many factors. The most important factor is the chemical composition of milk particularly the content of the Ca<sup>++</sup> in milk. So, any factor that affects the content of the Ca<sup>++</sup> affect the time of

coagulation. Heat treatment are one of the main arguments that changed the level of Ca<sup>++</sup> in milk and this was clear when milk is sterilized or dried, in these cases a source of Ca<sup>++</sup> must be added to milk prepared for cheese processing e.g. ca cl<sub>2</sub> which is added at certain level (0.02% and 0.03%) for sterilized and dried milk respectively. for this reason when coagulation time was compared to different treatments, milk should be identic (Lee *et al.*, 2003).

One the main factors that affects the time of coagulation is the method of coagulation. Natural milk produce from healthy cow required 90-150 minutes for total coagulation. This time is change when the coagulation enzyme is change (Van Hooydonk, *et al.*, 1984). This demonstrated that rennin coagulation required few time compared to plant and showed a significant differences (p<0.05) among the different methods of coagulation. However, the time of coagulation also different types of plant enzymes. Hamed (1998), compared two types of plant enzymes (from solanum and terristris) and reported that solanum enzymes required a lot of time for coagulation compared to terristeris enzymes and thus followed by weak milk curd and low percentage of cheese yield.

Another results were obtained by the Van Hooydonk *et al.*, 1987) who compared the time of coagulation and cheese yield for the different types of milk produced from cow milk during the normal stage of lactation, but at different time. Milk was coagulated by rennin enzymes and two different plant enzymes (*Solanum* and *Terristris*) using different concentration from the enzymes. Result obtained showed that milk taken at different time during the normal lactation revealed no significance difference (p> 0.05) and explained that types of enzymes influences the time of coagulation, which were high, optimum and lower for solanum, terristris and rennet coagulation respectively; no significant different (p> 0.05) between rennet coagulation

and terristris coagulation was reported. However, there was clear difference between rennin enzyme and the terristris enzymes. This urged some investigators to do more researches on plant enzymes used for coagulation. These results were similar to the some finding of Lee *et al.*,(2003), who also reported no significant difference (p>0.05) between some species. Of terristris and Rennin enzymes, but he found significant difference among some plant enzymes and rennin enzyme.

## 2.7 Cheese Yield

Typical yield cheese ranges from 9-15% depending on the chemical composition of the milk, efficient of fat casein in the cheese, losses of the milk constituents in the whey resulting from milk handling and treatment and cheese making procedure and final moisture content of the cheese (Frakey, 2004).

Everett and *et al.*, (2003) and Paolo *et al.* (2008) gave a number of factors affecting cheese yield such as:

- Milk composition.
- Genetic variation.
- Physiological factors.
- Processing condition.
- Lactation stage.
- Seasonal variation.

# Type of milk:

- Starter culture used.
- Standardization of milk.

- Heat treatment of milk.
- Type of coagulant of milk.
- Type of coagulant Storage of milk

## Used:

- Curd firmness.
- Curd handling system.

# 2.8 Sensory Evaluation

Humans have used their senses to evaluated food for several thousands of years and individuals can often tell by sight, smell, taste and to lesser extent touch, whether or not given food or beverage items are good or bad, e.g. save or toxic Paolo *et al.*, (2008).

According to Farell *et al.*, (1990), sensory evaluation of cheeses were affected by so many factors, such as quality of milk its chemical composition, methods of coagulation and experiences of evaluators, and significant differences (p<0.05) for flavor, taste and texture for cheese processed by different types of enzymes were detected.

Engels *et al.*, 2005) mentioned, the production of lactic acid by organisms used in fermented dairy products determines the flavor of the product, whereby, these microorganisms play a number of major beneficial roles in food industry, since they transform organic matter in foods and thereby contribute not only to the preservation of food, but also to flavor and texture.

Furthermore, Takala 1990) mentioned that sensory evaluation in general was also affected by types of animals, chemical composition of animal feeds, period of storage and enzymes.

SSMO (2008) described the sensory evaluation of white soft cheese as follows:

**Color:** normal if the cheese is white or white-yellowish.

**Taste:** palatable if the cheese is free of bitter taste, rancidity and rotating.

**Smell:** Normal if cheese shows no external or foreign odors.

Consistency: Texture firm, homogenous all over the mass and easily to cut.

Kumosinski *et al.*, (1991) reported, that taste, texture and flavor of cheese were affected by the method of coagulation and found significant difference for taste and texture (p<0.05) and The salt affects flavor, consistency and durability of the cheese (Walstra e al., (2005).

According to (Spreer, 1998) the main purpose of salting is to influence the taste of the cheese and it also regulates the acid content and has a preservative effect, flavors water binding, promotes formation of the skin and finally, influences the solidification of the cheese, which increases with increasing salt concentration.

# **CHAPTER THREE**

# **MATERIALS AND METHODS**

# 3.1 Area and time study

The experiment was conducted in the department of animal production college of Agricultural Studies, Sudan University of Science and Technology, Shambat, Four type of cheese using raw milk, pasteurized milk boiling milk and dry milk.

#### 3.2 Materials

## 3.2.1 Food materials

- Fresh milk was obtained from Animal Production Farm/ Sudan University and transferred immediately in sterilized container to the Animal Production Lab at Shammbat, Bahri City.
- Coagulant tablets were purchased from local veterinary pharmacy at Omdurman city and stored at room temperature.
- Dry Milk was purchased from local market at Omdurman city.
- Salt (Sodium Chloride NaCl<sub>2</sub>) was purchased from local super market at Omdurman city and stored at room temperature.

# 3.2.2 Chemical and reagents

Chemicals and reagents used were obtained from the lab line company-Khartoum. All the chemical and reagents were of analytical grades.

# 3.2.3 Other materials

- Face mask and gloves were purchased from local pharmacy at Omdurman city.
- Backing materials were purchased from local market at Omdurman city.

# 3.2.4 Raw material preparation

# 3.2.4.1 Milk preparation

Fresh milk was milked manually and filtrated by sterilized gauze. Raw fresh milk was analyzed for protein, fat, ash, moisture and total solids, in addition pH and acidity were determined and the results are shown in Table. Then the quantity of milk was divided to four groups.

# 3.2.4.2 Cheese processing

**Table(3): Product formula** 

Ingredient	Amount
Fresh milk	4.5liter
Starter culture	2%
Rennet	1%

## 3.3 Methods

## **3.3.1 Raw Milk**

Ten liters of milk were filtered through clean and sterilized cloth sheet then the starter culture was added at rate of 1%, incubated at 45° for 45°C m. Rennet enzyme was added at rate of 2gm/50 letter percent and incubated once again until coagulation was occurred. Curd was cut in to pieces placed in Sid wooden mold covered in the gauze, and left to drain for 12 hrs.

# 3.3.2 Milk pasteurized

Ten liters of milk were filtered through clean and sterilized cloth sheet. Then the milk was pasteurized at 72°C for 15 sec, cooled to 45 °C then the starter culture was added at rate of 1%, incubated at 45 °C for 45ments. Rennet enzyme was added at rate of 2gm/50 letter percent and incubated once

again until coagulation was occurred. Curd cut in to pieces placed in wooden mold covered in the gauze, and left to drain for 12 hrs

# 3.3.3 boiled Milk

Ten liters of milk were filtered through clean and sterilized cloth sheet. then the milk was heated at 100.17 for 45sec, cooled to 45 °C then the starter culture was added at rate of 1%, incubated at 45 °C for 45 ments. Rennet enzyme was added at rate of 2gm 50 letter percent and incubated once again until coagulation was curd cut in to pieces placed inside occurred. Wooden mold, covered in the gauze and left to drain for 12 hrs.

## 3.3.4 Full milk Powder

650 gm of milk powder were reconstituted in 5 liters' distilled water then the milk was heated at 72 °C for 15sec, cooled to 45 °C then the starter culture was added at rate of 1%, incubated at 45 °C for 45 m. Rennet enzyme was added at rate of 2gm 50 letter percent and incubated once again until coagulation was curd cut in to pieces placed in obtained. Wooden mold, covered in the gauze and left to drain for 12 hrs.

# 3.3.5 Analytical methods

The physicochemical analyses were determined according to (AOAC, 2005).

## 3.3.5.1 Moisture content

# **Principle:**

The moisture of a weighed sample is removed by heating the sample in an oven (under atmospheric pressure) oven (Kat-NR.2851, Elektrohelios, Sweden) at  $105 \pm 10^{\circ}$  until a constant weight was obtained. After drying, the covered sample was transferred into a desicator and cooled to room

temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

## **Calculation:**

**Moisture content %** 

Moisture content % = 
$$\frac{(W_1-W_2)}{W_1} \times 100$$

Where:

 $W_1$ = Original weight of sample.

 $W_2$ = Weight of sample after drying.

## 3.3.5.2 Determination of total solids

Total solids (TS) content was determined according AOAC (2005). A clean aluminum moisture dishes were dried. Five gm of the sample were weighed in a dry clean flat bottomed aluminum dish and heated on a steam bath for 15 minutes. The dishes were placed into a forced draft oven at 100°C for 3 hrs. The dishes were transferred to desiccators to cool and weighted. Heating, cooling and weighting were repeated several times until the difference between successive weighting was less than 0.1mg .the total solids (T.S) content were calculated as follows:

$$T.S\% = \frac{W_1}{W_2} \times 100$$

Where:

W₁= Weight of sample after drying

W<sub>2</sub>=Weight of sample before drying

# 3.3.5.3 Crude protein determination

The crude protein content was determined in all samples by micro-Kjeldahl method using a copper sulphate or sodium sulphate catalyst according to the Official Method of the AOAC,(2005).

# **Principle:**

The principle of the method consists of sample oxidation and conversion of nitrogen to ammonia, which reacts with the excess amount of sulphuric acid forming ammonium sulphate. The solution is made alkaline and the ammonia is distilled into a standard solution of boric acid (2%) to form the ammonia-boric acid complex, which is titrated against a standard solution of HCL (0.1N). Accordingly, the crude protein content is calculated by multiplying the total N % by 6.25 as a conversion factor for protein.

Procedure: 0.5 gm sample was accurately weighed and transferred together with 2-3 glass pellets, kjeldahl catalyst (No 33064, BDH, England) and 20ml concentrated sulphuric acid (No 18474420, Mark AG, Germany) into kjeldahl digestion flask. After that, the flask was placed into a kjeldahl digestion unit (Tecator, Sweden) for about 3hours, until a colorless digest was obtained. Following, the flask was left to cool to room temperature. The distillation of ammonia was carried out in 30 ml boric acid (2%) by using 40 ml distilled water and 60 ml sodium hydroxide solution (33 %). Finally, the distillate was titrated with standard solution of 0.1N HCL in the presence of 2-3 drops of indicator (Bromocreasol green and methyl red) until a brown reddish color was observed. The total nitrogen and protein were calculated using the following formula:

$$N\% = \frac{\text{volum of HCL} \times N \times 14}{\text{weight of sample} \times 1000} \times 100$$

 $P\% = N\% \times 6.38$  (factor)

Where:

N% = crude nitrogen.

P% = crude protein.

N = normality of HCL.

14 = equivalent weight of nitrogen.

## 3.3.5.4 Determination of fat content

Fat content was determined by Gerber method as described by AOAC (2005). Ten milliter of Sulphuric acid (specific gravity 1.820 gm/ml at 155°C) were measured into Gerber butyrometers, and mixed well, 10.94 mL of sample was slowly added into butyrometers tube. One milliter of amyl alcohol was added and lock stopper was inserted securely with the stoppers end up. Gerber tube was grasped and shacked with precaution until the sample was completed digested, the Gerber tube were centrifuged at 1100 rpm for 4minutes. Butyrometer was then placed in a water bath at 65°C for at least 3 minutes. The fat percent was finally reading directly from the Gerber tube Colum.

# 3.3.5.5 Determination of Ash

The ash content was determined by gravimetric method AOAC (2005). Five gm of the samples were weighed in crucibles, and then placed in a muffle furnace at 550-600 C° for 3 hrs until ashes were carbon free. The crucibles were then cooled in desiccators and weighed. The ash content was calculated using the following equation:

$$Ash\% = \frac{W_1}{W_2} x 100$$

Where:

W₁= Weight of ash

W<sub>2</sub>=Weight of sample

# 3.3.5.6 Determination of pH

The pH value was determined according to AOAC (2005).

Ten gm of samples were weighed and placed in a conical flask and distillate water at 40°C was added until the volume in the flask was 105 ml. The sample was then vigorously agitated and filtered. Then pH of the filtrate was measured by using a recalibrated pH meter model (HI 8521 microprocessor bench pH / MV /°C meter). This has been calibrated with two standard buffers pH 4 and 7, the pH meter was placed into the sample, then it was directly read.

# 3.3.5.7 Titrable acidity

Ten gm of samples were weighed and placed in a conical flask and distillate water at 40 °C was added until the volume in the flask was 105 ml. The sample was then vigorously agitated and filtered. 25 ml of the filtrate were pipette into porcelain dish and 3-4 drops of phenolphthalein indicator were added. The sample was titrated against 0.1N NaOH until a faint pink color. The acidity calculated from the following equation:

Acidity 
$$\% = T \times 4/W$$

Where:

T= Titration figure.

W=Weight of sample.

## 3.4 Microbial tests

# 3.4.1 Collection of samples

Samples of cheese were withdrawn from each treatment, kept in sterile containers in ice and transferred immediately to the microbiology laboratory, Faculty of Agric, University of Khartoum for microbial analysis.

# 3.4.2 Sterilization of glassware

Glassware was washed thoroughly, left to dry and sterilized in a hot air oven at 160 C<sup>0</sup> for at least 3 hours (Harrigan and McCance, 1976). Instruments such as loops, needles, forceps, spoons and knives were sterilized by flaming directly after dipping in spirit.

## 3.4.3 Culture media used

# 3.4.3.1 Plate count agar (oxoid)

The plate count agar medium was used to determine total bacterial count. Seventeen and half grams of this media were suspended in a liter of distilled water, dissolved by bringing to boiling with frequent stirring, mixed and distributed into conical flasks sterilized by autoclaving at 121°C for 15 minutes (Harrigan and McCance, 1976).

# 3.5.1 Preparation of serial dilution

Thirty grams from each treatment were weighed aseptically in a sterile bottle and then blended with 270 ml sterile pepton water by using an electric blender (Homogenizer MSE). The emulsion was blended for 3 minutes to give dilution as described by Harrigan and McCance, (1976).

# 3.5.2 Microbial parameters studies

Total viable count was carried out by using the standard plate count method as described by Harrigan and McCance (1976). One milliliter from the suitable dilution was transferred aseptically into sterile Petri dishes. To each dilution 10-15 ml of (melted and cooled 45°C) plate count agar were added. The inoculums was mixed with the medium and allowed to solidify. The plates were then incubated at 37°C for 48 hours. A colony counter (Quebec colony Counter and Hand Tally) was used to count the viable bacteria.

# 3.6 Sensory evaluation

The panels consisted of researcher from the National Food Research Center (NFRC)semi-trained according to the procedure of Cross *et al.*, (1978). The panel evaluated the prepared cheese samples for color, taste, flavor, texture, over all acceptability, using a hedonic scale of 7 points (7 extremely like, 1 extremely dislike). The sample used for sensory evaluation were randomly selected, separately and kept warm for evaluation. Every treatment was given a code number. From each treatment a sample of about 13 samples were placed in a dish having a code number of each sample under natural light. Water at room temperature was made available for the panel to be used between sample testing.

# 3.7 Statistical analysis

The data collected from the different treatments were subjected to analysis of variance (ANOVA) and whenever appropriate the mean separation procedure of Duncan was employed (Steel and Torrie, 1980). The SAS program (SAS, 2002), was used to perform the general liner model (GLM). analysis

# **CHAPTER FOUR**

# **RESULTS AND DESCCUION**

# 4. Chemical composition of white cheese as affected by heat treatment

Table 4. illustrated that, there were significant (p<0.05) difference were detected among cheese samples. It is obvious that, Total solids T.S content significant affect by heat treatment. Cheese proceed from milk powder had T.S content of 69.38%, while, cheese proceeds by boiling milk had T.S content of 44.42%. Similar result was obtained by Khalid (2007) who found that; total solid of cheese made from milk powder was 69.14%. The results supporting with that reported by Haug *et al.*, (2007) who stated that, heat treatments significantly affected on total solids of white cheese. The current result of total solids for cheese treated by pasteurized milk was higher than that result obtained by Abdalrazig and Ahmed (2009) who found that, total solid of cheese treated by pasteurized milk was 53.32%. Furthermore, Ahmed (2009) reported that, total solid is the measure of the water content of white cheese.

Table (4.): Total solids of cheese as affected by heat treatments

Treatment	Mean	SD	Sig
Boiling	44.42 <sup>d</sup>	0.32	
Pasteurize	50.97 <sup>c</sup>	0.32	**
Raw	54.77 <sup>b</sup>	0.22	
Powder	69.38 <sup>a</sup>	0.07	

Values are means  $\pm$  SD.

Means in the same column bearing the superscript small letters are significantly different

Table5. demonstrated that, there were significant (p<0.05) difference were detected among protein content of cheese samples. It is clear that, protein content significant affect by heat treatment. Dry milk based cheese had the highest protein content 30.18%, whereas, cheese proceeds by boiling milk had protein content of 20.67%. Khalid (2007) who found that; protein content of cheese made from dry milk was 27.24%. The results supporting with that reported by Haug *et al.*, (2007) who stated that, heat treatments significantly affected on protein total. The protein content of untreated sample was similar for that one occurred by Khalid (2007) who found that, protein content of untreated cheese was 26.01%.

Furthermore, Haug *et al.*, (2007) stated that, cheese technology requires that the protein, especially the casein, must be separated from milk by coagulation.

Table (5): Protein of different types of cheese

Treatment	Mean	SD	Sig
Boiling	$20.67^{\rm d}$	0.33	
Pasteurize	24.83°	0.18	**
Raw	26.00 <sup>b</sup>	0.30	
Powder	30.18 <sup>a</sup>	0.01	

Values are means  $\pm$  SD.

Table 6 .shows that, there were significant differences (p<0.05) were detected among cheese samples. Clearly, heat treatments significantly affect fat content. Cheese based Dry milk had fat content of 29.19%, whereas, cheese produce by boiling milk had fat content of 19.89%. Ahmed (2009) found that, fat content of cheese was 20.24%. the fat content of cheese based pasteurized milk 20.57% was similar to Khalid (2007) who found that, fat content of cheese was 23.82%. In addition, SSMO (2009) reported that, fat content of white cheese must be < 20%.

Table (6): Fat content of different types of cheese

Treatment	Mean	SD	Sig
Boiling	19.89 <sup>d</sup>	0.05	
Pasteurize	20.57°	0.03	**
Raw	23.17 <sup>b</sup>	0.03	
Powder	29.19 <sup>a</sup>	0.04	

Values are means  $\pm$  SD.

Table(7), shows that, there were significant (P<0.05) difference were detected among different samples. It is obvious that, ash content significant affect by heat treatment. Milk powder based cheese had ash content of 9.75%, while boiled cheese had ash content of 3.90%. The current results supporting with that stated by Haug *et al.*,(2007) who mentioning that, ash content affected by heat treatments. Abdelrazig and Ahmed (2010) found that, the average of ash content for pasteurized cheese was 5.24 %, these variations may be attributes to some factors such as; feeding system, stage of lactation, location and genetic factors as stated by Ahmed (2009).

Ahmed (2009) reported that, cheese based milk powder had ash content of 8.34 %.

Table (7): Ash content of different types of cheese

tretment	Mean	SD	Sig
Boiling	$3.90^{c}$	0.06	
Pasteurize	$4.40^{c}$	0.10	**
Raw	$5.50^{b}$	0.06	
Powder	9.75 <sup>a</sup>	0.30	

Values are means  $\pm$  SD.

Table (8) demonstrated that, significant (P<0.05) differences were detected among cheese samples. The highest pH value was 4.98, recorded when cheese made from untreated milk, while, the lowest one was 4.78, recorded when cheese made from milk powder. Similar result was obtained by Ahmed (2009) who found that, pH value of cheese made from milk powder was 4.76. The pH value of pasteurized cheese was lower than that result obtained by Abdalrazig and Ahmed (2009) who obtained that, pasteurized cheese had pH value of 3.28. The results one line with that reported by Ahmed (2009) who stated that, the drop of pH value for white cheese may be attributed to the presence of lactic acid bacteria by lactic acid culture during fermentation. It is worth mentioning that, all cheese samples within SSMO (2008).

Table (8): pH of different types of cheese:

tretment	Mean	SD	Sig
Boiling	4.93 <sup>a</sup>	0.02	
Pasteurize	4.84 <sup>b</sup>	0.02	**
Raw	4.98 <sup>a</sup>	0.00	
Powder	4.78°	0.00	

Values are means  $\pm$  SD.

Table (9):demonstrated that, significant (P>0.05) differences wer detected among cheese samples. The highest acidity content recorded for cheese made by boiling milk 1.11%, while, the lowest one recorded for untreated cheese 1.01%. Abdalrazig and Ahmed (2009) reported that, acidity content of cheese was 1.22%. This result was not far from that result obtained by Ahmed (2009) who found that, acidity content cheese was1.20%. Haug *et al.*, (2007) stated that, lactose sugar covert to lactic acid caused by acid bacteria.

Table (9): Acidity of different types of cheese

tretment	Mean	SD	Sig
Boiling	1.11 <sup>a</sup>	0.01	
Pasteurize	$1.08^{b}$	0.01	**
Raw	1.01 <sup>c</sup>	0.00	
Powder	1.08 <sup>b</sup>	0.00	

Values are means  $\pm$  SD.

Table (10): shows that there were significant differences (P< 0.05) in TVBC among the different cheese samples. Untreated cheese sample had the highest (P<0.05) TVBC 2.38 cfu/g compared to treated samples. While, boiled milk based cheese was recorded the lowest TVBC 2.09 log<sub>10</sub>cfu/g, this may be differences could be attributed to effect of heat treatments to kill bacteria. Higher total bacterial viable count was recorded by Khalid (2007) who found that, TVBC for untreated cheese was 3.30. This difference may be duo to the effect of different factors such as; hygiene condition of farm and milking, processing hygiene condition. Ahmed and Abdarazig (2010) found that, pasteurized milk based cheese had TVBC of 3.67cfu/g.

Table (10): of TVBC different types of cheese

tretment	Mean	SD	Sig
Boiling	$2.09 \times 10^{3c}$	0.00	
Pasteurize	$2.18 \times 10^{3b}$	0.01	**
Raw	$2.38x10^{3a}$	0.01	
Powder	$1.98 \times 10^{3d}$	0.01	

Values are means  $\pm$  SD.

Means in the same column bearing the superscript small letters are significantly different \*\*Means in the same column bearing different superscript capital letters are significantly different ( $P \le 0.01$ ).

### 4.2 Sensory attributes of white cheese as affected by heat treatment

Table 4.8, demonstrated that, significant (P<0.05) differences were detected among cheese samples. The highest taste score was 6.55 recorded when cheese made from Dry milk, whereas, the lowest taste score was 5.25 recorded in untreated milk. This variation may be attributed to the effect of heat treatment. The current result of cheese based milk powder was lower than that result obtained by Ahmed (2008). The result supporting with that mentioned by Ahmed (2006) who stated that the taste is contributed by chemical composition.

Data in Table, indicated that, there were significant (P>0.05) differences were detected among different treatments of cheese samples. The highest texture score recorded for skim milk powder 6.40, whereas, the lowest texture score recorded for 6.5 pasteurized milk cheese sample. These differences may be attributed to the effect of heat treatment as stated. The result was higher than that result obtained by Mohamed (2013). The result on line with that mentioned by Ahmed (2006) who stated that, the taste is contributed by chemical composition. Furthermore, texture score of cheese affected by different factors; milk source, incubation time, Table, indicated that, significant (P>0.05) variation were detected among different treatment cheese samples. The highest flavor score was 8.5 recorded when cheese made from skim milk powder, while, the lowest flavor score was 5.80 recorded in control sample. This variation may be attributed to the effect of heat treatment. The result was lower than that result obtained by Mohamed (2013). The result on line with that mentioned by Ahmed (2006) who stated that, the taste is contributed by chemical composition.

Table (11): Sensory evaluation of different types of cheese

Cheese types	Mean ± SE		
	Taste	Texture	Flavor
Boiling	7.60±0.29 b	7.90±0.35 <sup>a</sup>	7.15±0.43 ab
Pasteurize	6.60±0.31 °	6.05±0.32 b	$6.9\pm0.36^{b}$
Raw	5.25±0.28 <sup>d</sup>	6.55±0.29 b	$5.8\pm0.29^{c}$
Powder	8.55±0.26 <sup>a</sup>	8.40±0.25 a	8.05±0.41 a
Sig	0.000***	0.000***	0.001**

Values are means  $\pm$  SD.

## **CHAPTER FIVE**

## CONCLUSION AND RECOMMENDATIONS

#### **5.1 Conclusion**

Based on the results obtained in the present study, the following conclusions can be drawn:

Milk powder based cheese had highest total solids T.S, protein, fat and ash contents when compared to other treatments such as; pasteurized and boiling ones. In addition, yield percentage was higher when cheese made from milk powder with took more time to coagulate.

Untreated cheese had highest Total Viable Bacterial count TVBC, on the other hand, cheese making by treated milk with boiling had lowest Total Viable Bacterial count TVBC.

The panelists preferred cheese based milk powder when comparison with other different treatments in terms of; Color, taste, flavor, texture and.

Generally, heat treatments significantly affected cheese quality attributes.

#### **5.2 Recommendations**

It is recommended that, milk powder based cheese could enhance their quality characteristic.

More attention should be paid when cheese made by untreated milk particularly, total viable bacterial count.

Further research is needed to make heat treated cheese more acceptable to consumer.

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# **APPENDICES**









**Equepment of Cheese making** 

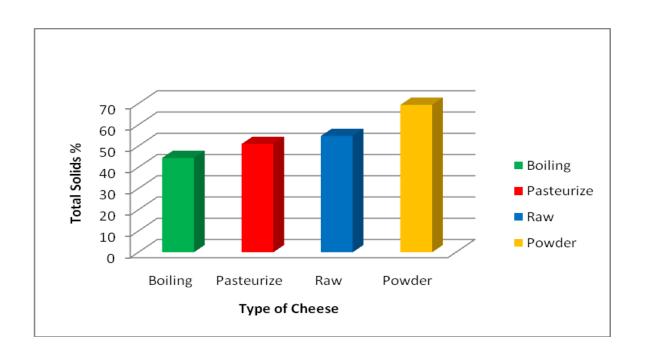




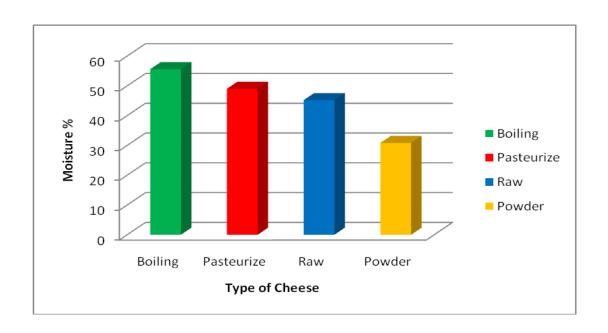


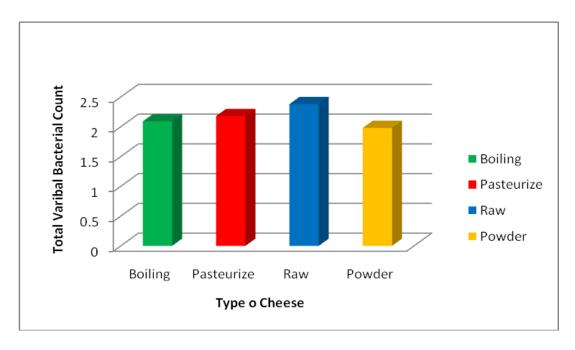


**Stages of Cheese making** 

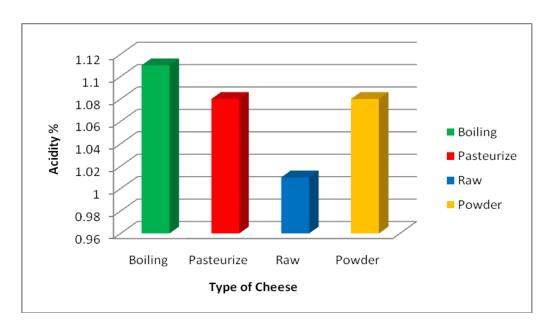


Total solids T.S of cheese as affected by heat treatments

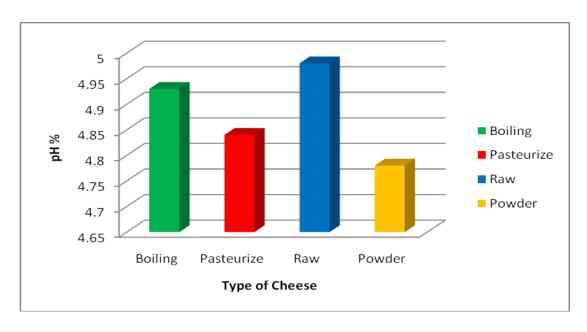




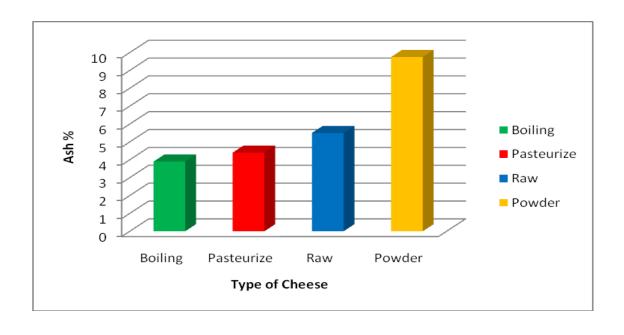
of TVBC different types of cheese



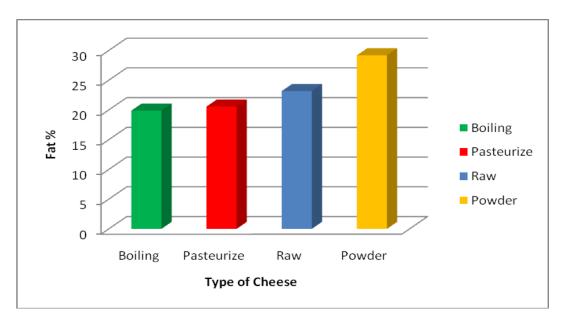
Acidity of different types of cheese



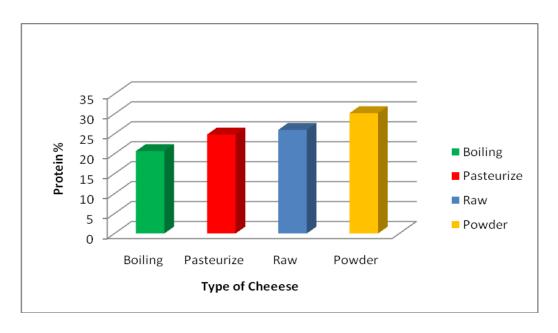
pH of different types of cheese:



Ash content of different types of cheese



Fat content of different types of cheese



Protein of different types of cheese