Sudan University of Science and Technology

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Effect of Sun flower Seeds' Extract and Milk Source on Rennet Coagulation Time, Yield and the Quality of White Cheese during Storage

أثر مستخلص بذور زهرة الشمس ونوع اللبن على زمن التخثر وإنتاج و جودة الجبن البيضاء أثناء فترة التخزين

By

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Supervisor

Prof. Omer Ibrahim Ahmed Hamid

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قال تعالى:

(وفوق كل ذى علم عليماً)

سورة يوسف (76)

(وما أوتيتم من العلم إلا قليلاً)

سورة الإسراء (85)

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DEDICATION

This work is dedicated to my beloved parents*,*
Uncle Mustafa,
My sisters and brothers,
Students,
And Researchers,

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*First and foremost, I would like thank **Allah** Almighty for giving me the health, strength and courage to accomplish this work, and to take this opportunity to express my deepest gratitude and sincerest thankfulness to my advisor Professor **Omer Ibrahim Ahmed Hamid** for his valuable helps and continuous supports,throughout my study.*

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Lastly, and most importantly, my deepest love and appreciation goes to my family for their support not only through my Ph.D study, but through the years which prepared me for this stage. Thank you all now and always.

ABSTRACT

This study was aimed to extract milk-clotting enzyme from sunflower *(Helianthus annuus*) seeds and to determine its potentiality for manufacturing white soft cheese from cows and goats milk during storage. The parameters evaluated included: coagulation time, yield, physicochemical composition, microbiological analyses and sensory characteristics of the manufactured cheese. The sunflower seeds' were blended and extracted using two types of buffers, Na acetate buffer and 5% NaCl acetate buffer. Milk-clotting and proteolytic activities were evaluated. The enzyme was partially purified using ammonium sulfate fractionation techniques. Twenty five (25) liters of fresh cow's full cream milk were purchase from the farm of College of Animal Production Science and Technology, Sudan University of Science and Technology, and (25) liters goats' milk was purchased from a private farm at Khartoum North. Four treatments were carried out in this study as follows: In the first and second treatments cow's milk cheese was made from rennet and sunflower enzymes respectively. In the third and fourth treatments goat's milk cheese was made from rennet sunflower enzymes respectively. And milk pasteurization, 0.02% $CaCl_2$ and 2% starter culture were added then white cheese was made and stored at 4°c for 63 days. Physicochemical composition, microbiological analyses and sensory evaluations was done for the cheese samples at 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 days intervals. Results indicated that sunflower seeds extracted with 5% NaCl in 50 mmol/L acetate buffer, pH 5.0, had the highest milk-clotting activity (MCA) and lowest coagulation time compared to that extracted with only acetate buffer (pH 5.0). Ammonium sulfate at 30–50% saturation purified the enzyme to 4.3 folds with MCA of 241.0 U/mL and final enzyme yield of 10.9%. The partially purified enzyme was characterized by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) that showed two bands with molecular weight of 120 and 62 kDa. When compared with other plant enzymes, the partially purified sunflower enzyme was found to have higher milk-clotting activity and lower proteolytic activity. The results showed that milk source significantly ($p<0.01$) affected the cheese yield, while did not showed significant effect on coagulation time. Coagulant type was significantly ($P<0.05$) and ($p<0.01$) affected the cheese yield and coagulation time, respectively. Cheese made from cow milk using sunflower enzyme had higher yield compared to that obtained using commercial rennet, whereas the opposite was observed when using goat milk. Milk source, coagulant type and storage period significantly ($p<0.01$) affected all the studied physicochemical parameters: total solids contents, ash, fat, protein, acidity, tryptophan and tyrosine. Microbiological analyses showed significant ($P<0.01$) effect of milk source on total viable bacterial count, *coliforms* counts. *E. coli* and *Staphylococcus aureus* counts were not detected in all the cheese samples throughout the study. Coagulant type showed a

significant effect ($P<0.05$) and ($P<0.01$) on *Total viable bacteria* and *coliforms* counts, respectively, while *Lactobacilli* count did not affected. Storage period showed significant ($P<0.01$) effect on all microbial analysis of the manufactured white cheese. Milk source significantly ($p<0.01$) affected the sensory characteristics of the cheese except texture did not showed significant ($p>0.05$) variation, whereas coagulant type and storage period revealed a significant differences ($p<0.01$) and ($p<0.05$) on the sensory characteristics of the manufactured cheese, respectively. It is concluded that *Helianthus annuus* seeds enzyme could be used as a substitute for rennet enzyme.

مستلخص الدراسة

هدفت هذه الدراسة لإستخلاص إنزيم مخثرللبن من بذور زهرة الشمس, وتحديد فعاليته في تصنيع الجبنة البيضاء الطرية ومن لبني الأبقار والماعز خلال التخزين. والمقاييس التي قيمت تشمل: زمن التخثر , والكمية المنتجة , التركيب الفيزيوكيميائي ,التحاليل الميكروبيولوجية والخصائص الحسية من الجبن المصنعة. تم طحن وإستخلاص بذور زهرة الشمس بإستخدام نوعين من المحاليل المنظمة، محلول منظم خلات الصوديوم و5% كلوريد صوديوم مع محلول منظم خلات الصوديوم. ومن ثم تقييم فعاليات تجبن اللبن والتحلل البروتيني. تمت تنقية الإنزيم جزئياً بإستخدام تقنيات تجزئة كبريتات الأمونيوم. تم شراء خمسة وعشرين (25) لتراً من لبن الأبقار الطازج كامل الدسم من مزرعة كلية علوم وتكنولوجيا الإنتاج الحيواني, جامعة السودان للعلوم والتكنولوجيا , وتم شراء لبن الماعز من مزرعة خاصة بالخرطوم بحري. أجريت أربعة معاملات في هذه الدراسة على النحو التالي: في المعاملتين الأولى والثانية تم تصنيع جبن لبن البقرمن إنزيمي الرنين وزهرة الشمس, على التوالي. وفي المعاملتين الثالثة والرابعة تم تصنيع جبن لبن الماعز من إنزيمي الرنين وزهرة الشمس, على التوالى. بعد بسترة الحليب, أضيفت 0.02 % كلوريد كالسيوم و 2٪ بادئ حموضة ومن ثم تصنيع الجبن البيضاء وتخزينها في 4° م لمدة 63 يوماً. وقد أجري تحليل التركيب الكيميائي، و الميكروبيولوجي والتقييم الحسي لعينات الجبن في الفترات 0، 7، 14، 21، 28، 35، 42، 49، 56 و 63 يوماً. وأوضحت النتائج أن بذور زهرة الشمس المستخلصة مع 5٪ كلوريد الصوديوم في 50 مليمول / لتر لمحلول منظم الخلات، وأس هيدروجينى5.0، كانت أعلى فعالية في تجبن اللبن (MCA) وأدنى فى زمن التخثر مقارنة بالبذور المستخلصة بمحلول منظم الخلات فقط (أس هيدروجينى 5.0). التشبع بكبريتات الأمونيوم في 30-50٪ أعطى تنقية الإنزيم إلى 4.3 أضعاف وأعلى فعالية في تجبن اللبن ب. 241.0 وحدة / مل والنتاج النهائي للانزيم ب 10.9٪. وقد تم تمييز الانزيم المنقى جزئياً بعمود الفصل الجهد الكهربى (SDS-PAGE) و التي أظهرت حزمتين بأوزان جزيئة 120 و 62 كيلو دالتون. وبالمقارنة مع الإنزيمات النباتية الأخرى، وجد أن إنزيم زهرة الشمس المنقي جزئياً به أعلى فعالية في تجبن اللبن والتحلل البروتينى. وأظهرت النتائج أن مصدر اللبن له فرق معنوى جداً ($P<0.01$) على كمية الجبن المنتجة، بينما لم يظهر أثر معنوى على زمن التخثر. أثر نوع المخثر معنويا ($P<0.05$) و ($P<0.01$) على كمية الجبن المنتجة و زمن التخثر، على التوالي. الجبن المصنع من لبن البقر و بانزيم زهرة الشمس، ذو أعلى نتاجاً مقارنة بالمتحصل عليها باستخدام المنفحة التجارية، في حين لوحظ العكس عند استخدام لبن الماعز. أثر مصدر الحليب ونوع المخثر و فترة التخزين معنوياً ($P<0.01$) على جميع القياسات الفيزوكيميائية قيد الدارسة: الجوامد الكلية، الرماد، الدهون، البروتين، الحموضة، التربتوفان والتيروسين. وأظهرت التحاليل الميكروبيولوجية تأثير معنوي ($P<0.01$) من مصدر الحليب على العدد الكلي للبكتيريا ، الكوليفورم. لم يتم الكشف عن الإكولاي والمكورات العنقودية الذهبية في جميع عينات الجبن طوال فترة الدراسة. أظهر نوع المخثر تأثير معنوي ($P<0.05$) و ($P<0.01$) في العدد الكلي للبكتريا و الكوليفورم، على التوالي، في حين أن بكتريات حمض اللاكتيك لم تتأثر. وأظهرت فترة التخزين أثر معنوي ($P<0.01$) على جميع التحاليل الميكروبية من أنواع الجبن الأبيض المصنع. مصدر الحليب أثر معنوياً ($P<0.01$) على الخصائص الحسية للجبن باستثناء القوام لم يظهر فرق معنوي (ف> 0.05)، في حين أن نوع المخثر وفترة التخزين أوضحت وجود فروق معنوية ($P<0.01$) و ($P<0.05$) على الخصائص الحسية من الجبن المصنعة، على التوالي. ويستنتج من ذلك أن إنزيم بذور دوار الشمس يمكن أن يستخدم كبديل لأنزيم المنفحة.

CHAPTER ONE

INTRODUCTION

1.1. Back ground:

Sudan is an agricultural country with a large livestock population, it estimated to be 140 million heads: 41 million heads of cattle, 52 million heads of sheep, 43 million heads of goat and 4 million heads of camel, (AOAD, 2009).

Cows are the main source for milk supplies; they produce 90% of the world total supply of milk, followed by goat and sheep (AOAD, 1993) in the past goats were more important for milk. Although goat's milk, like cow's milk and human milk, contains lactose, many people with lactose intolerance can drink goat milk. It was hypothesized that the reason lies in goat milk's superior digestibility. Goat's milk is more completely and easily absorbed than cow's one, leaving less undigested residue behind in the colon to quite literally ferment and cause the uncomfortable symptoms of lactose intolerance (Ceballos *et al.,* 2009).

Cheese is an ancient food and there is no conclusive evidence indicating where cheese-making originated. The date of origin of it ranges from around 800 BC when sheep were first domesticated to around 3000 BC, (Smith, 2005). Alfa-laval Dairy Hand book, (1989) defined cheese as the fresh natural product obtained after coagulation of milk, cream, and skim or partially skimmed, butter milk or a combination of these products. Cheese is another product produced from fresh milk which is allowed to be fermented under certain conditions. It is however, a major milk preservation method in Sudan (Hamid *et al.,* 2008).

Cheese can be made from the milk of cows, sheep, goats and camels (Herrington, 2000); it can also be made from cream milk, skim milk, whey, or mixture of two of these. Each type of milk imparts the characteristics quality of cheese made from it and the resulting cheese will diver in body texture, and flavor (Andrew, 2010).

Milk coagulants such as rennet enzyme are still not available in rural areas of Sudan, where most of the cheese is produced. Rennet is the most desirable enzyme for coagulation of milk to manufacture cheese as well as, it is important in the formation of the casein network during coagulation, (Gouda, 1990). A shortage of this enzyme has occurred as the decrease in general availability of sucking calves as they are left for beef, thus decreasing the rennet availability and increasing its cost, (Craw, 1983).

More restrictive ethical concerns associated with production of such animal rennet led to a search for suitable rennet substitutes for cheese making. Several proteases from animal, microbial and plant sources were investigated as likely substitutes and have been reviewed by Fox *et al*. (2000) and Kheir *et al,* (2011).

There are available sources of milk coagulants in Sudan (Bender and Bender, 1999) the family Solanaceae, include Gubbien *(Solanum dubium), Solanum innacum, Solanum torvaum*, tomatoes *(Solanum esculentum) and* eggplant *(Solanum melongena)*, and many other species. According to Ahmed *et al*, (2009 b) Solanaceae family were found to have a high clotting and proteolytic activity compared with other plants enzymes. Gubbien is one of the commonly used coagulants and dairy farmers in some parts of the Sudan use the berries of *Solanum dubium* to make white soft cheese using goat and sheep milk. Milk coagulation takes about 2 h and the curd is pressed to remove whey. The cheese obtained has a slight bitter taste and a fragile crumbly texture. The bitterness of the cheese is probably a result of the presence of some alkaloids or unspecific

proteolytic activity of the enzymes during processing due to impurities because the farmers used raw seeds. It may be possible to reduce bitterness by using a purified enzyme and by using optimum extract concentration.

Sun flower (*Helianthus annuus*) is a member of the Asteraceae or Compositae family. The Genus *Helianthus annuus* is formed of both annual herbaceous and perennial species.

Various types of cheeses have a short shelf life whereas other cheeses are adapted to extended storage. During storage cheese develops properties that are characteristics of a particularly type of cheese, Fox, (1993). Sensory characteristics of foods play an essential role in consumer behavior, particularly choice and the decision to purchase in the market place,Mohammed, 2009).

Pathogenic organisms in milk and milk products have long been associated with spread of diseases. Dairy products are the major vehicle for transmission of human diseases such as brucellosis, Salmonellosis and tuberculosis, Bryan, (1983). Unless milk used for cheese processing is pasteurized otherwise treated to destroy pathogenic or toxin producing organisms present in raw milk could be found in cheese. These organisms may find their way into cheese as a result of environmental contamination during Processing, (Marshall, 1993).

Although many studies have been carried out to purify and manufacture soft white cheese from (*Solanum dubium)* seed's extract (Talib *et al*. 2006 and Talib *et al*, 2009), relatively few studies have been carried out on the purification of the Sun flower (*Helianthus annuus*) seeds' extract (Egito *et al.,* 2007). Therefore, the extraction of enzymes from the above mentioned sources and manufactured of white soft cheese from cows' and goats' milk needed more investigations.

1.2. Objectives:

This research is initiated in order to investigate:

1) Extraction and purification of sun flower seeds enzyme.

2) Manufacturing white soft cheese from cow's and goat's milk using the extracted seed and rennet enzymes.

3) The effect of storage period on chemical composition, sensory characteristics and micro biology of the manufactured cheese.

4) Determination the effect of coagulating enzymes on RCT (Rennet Coagulation Time) of different milks.

CHAPTER TWO

Literature Review

2.1. Milk in Sudan:

Hassabo (2009) stated that cow milk production to be 7.1 million tons for the Sudan, from the local breeds, where 95% from the total production are produced by the tradition sector, where as the rest 5% are produced in cities. Hassan, (1994) estimated camel milk production to be 1,399,000 tons for the Sudan at an annual production rate of (1500 – 3000) liters per year for a she- camel.

Cows are the main source for milk supplies; they produce 90% of the world total supply of milk, followed by goat and sheep (AOAD, 2003) in the past goats were more important for milk. Although goat's milk, like cow's milk and human milk, contains lactose, many people with lactose intolerance can drink goat milk. It was hypothesized that the reason lies in goat milk's superior digestibility. Goat's milk is more completely and easily absorbed than cow's one, leaving less undigested residue behind in the colon to quite literally ferment and cause the uncomfortable symptoms of lactose intolerance (Ceballos *et al.,* 2009).

The nutrient composition of goat milk is very different than that of cow's milk. In addition to containing 13% more calcium than cow's milk, goat milk is also have 25% more vitamin B_6, 47% more vitamin A, 134% more potassium and 350 % more niacin. Goat milk is also higher in chloride, copper and manganese and contains 27% more of the essential nutrient selenium (Sulieman *et al.*, 2012). Especially in Europe, goat's milk lends itself to use in the same types of

products that are considered for cow's milk, and perhaps surpasses the cow's milk in some categories. Major amounts of goat's milk are processed annually into dried milk, evaporated milk, cheese and yogurt as well as being sold as bottled whole milk. The use of goat's milk in the production of cheese has become very widespread in France. This use is largely based on the capacity of goat milk curd to be frozen and produce a product not only equal but often considered better in flavor than one in which freezing did not take place. This process is normally accomplished without using salt and the curd can be held for up to six months at use 5° C, (Sulieman *et al.*, 2012). Because world cheese production increased by a factor of approximately 3.5, (Jacob *et al*., 2011). Cheese making is a major milk preservation method in Sudan, (Abdel Razig and Babiker, 2009).

2.2. Cheese:

2.2.1. History of cheese:

Smith (2005) defined cheese as the solid food made from the milk of cows, goats, sheep and other mammals and it is lighter weight , more compact and has a longer shelf-life than the milk from which it is made. However, Alfa-laval Dairy Hand book, (1989) defined cheese as the fresh natural product obtained after coagulation of milk, cream, and skim or partially skimmed, butter milk or a combination of these products. Smith, (2005) stated that cheese is an ancient food and there is no conclusive evidence indicating where cheese-making originated. The date of origin of it ranges from around 800 BC when sheep were first domesticated to around 3000 BC. The first cheese may have been made by people in the Middle East or by nomadic Turkish tribes. Also, he wrote the earliest archaeological evidence of cheese making has been found in Egyptian tomb murals dating to about 2000 BC. The earliest cheese was likely to have been quite sour and salty, similar in texture to rustic cottage cheese or Feta.

Many of the best known cheeses today were first recorded in the late middle ages or after, such as Cheddar cheese, which was recorded in 1500 BC, Parmesan in 1597, Gouda in 1967 and Camembert in 1791. Factory made cheese overtook traditional cheese making during the world war II and factories have been the source of most cheeses in America and Europe ever since (Harold, 2004).

2.2.2. Classification of cheese:

There are more than 400 different kinds of cheeses that can be made and the cheese has more than 2000 names since the same cheese may have two or more different names, for example, cheddar cheese is one of the American type cheeses in United States (Donnelly, 2014). And according to them cheese classification falls into one of four main groups mainly; soft, semi-soft, hard and very hard, (Fox *et al*, 2016).

This classification is based on moisture content in the cheese, thus the body and texture of cheese range from soft unripened cheese, (such as Cottage) with 80% moisture to very hard grated shaker cheese (such as Parmesan and Romano cheese). The ripened cheese has 32% - 34% moisture. However, the descriptive criteria normally used in cheese classification depend on; Coagulation method (rennet and acid cheese), Water content (hard, semi hard and soft cheese), Microorganisms used for ripening (bacteria, molds) and Cheese texture (holes or eyes, granular, close texture) (Alpha Laval Dairy Handbook, 1989).

2.2.3. Types of cheese in Sudan:

Cheese making in Sudan is based on small- scale production and cheese is widely consumed by socioeconomic classes of the Sudanese. There are available statics on either side of manufacture or scale consumption of cheese in Sudan that makes it difficult to estimate the amount of cheese produced (Abdel Razig *et al.,* 2002).

The old traditional cheese of Sudan (Kush-Kush) is very simple and very sour product prepared by nomads. While, the dominant cheese manufactured in Sudan is Gibna beida which is the local name for white cheese and is the most popular type. Also, Mudaffara, Romia and recently Mozzarella cheese has been introduced (Khateeb, 1997). Also, Ibrahim (2008) reported that beside white soft cheese, Mudaffara and recently Gouda and Mozzarella cheeses are introduced.

2.2.3.1. White soft cheese:

Sudanese white soft cheese (Gibna beida) falls in the family of soft and semi-soft pickled cheeses of the East European countries, the East Mediterranean Region and North Africa the cheese may be consumed fresh but more commonly after maturation in salt brine or salted whey in sealed containers. The brine solution and salted whey improve the flavor of cheese between this group and other typical soft cheeses of temperate zones. Under warm conditions, cheese deteriorates rapidly before ripening and salting becomes essential for its preservation (AbdelRazig, 1996 and AbdelRazig and Babiker, 2009).

Sudanese white cheese is made from cows, ewe's goats, skim milk powder or mixture of later milk with any other kind of milk from the species mentioned (Jack, 1955). The most traditional cheese type that produced in Sudan is Gibna Bayda (Osman, 1987). Cheese is produced in Sudan throughout the country especially in El-Doiem, White Nile State, El-Obeid, North Kordofan State, Nyala, South Darfur State and other localities in the country (Elowni and Hamid, 2007). However, cheese making in Sudan is the major preservation method for surplus milk in rural areas especially during rainy season when plenty of milk is available (Elowni and Hamid, 2008). The making of Gibna Byda in Sudan varies from one place to the other. The variation is due to the

method of processing, salting before or after renneting or during moulding also due to the way of treating the whey. Sudanese Gibna Bayda is unique among cheese varieties in that high concentrations of table salt (Sodium chloride) is added to the milk before processing (Osman, 2005) it is manufactured from raw or heated milk (Ibrahim, 2003). During processing under tropical conditions cheese deteriorates rapidly before it ripens thus salting before renneting become essential for it preservation (Elsiddig, 2006).

Goat's milk is more easily digested than cow's milk, also goat's milk has 13% less lactose than cow's milk and most people who are allergic to cow's milk tend not to be allergic to goat's milk (Einsiedel, 2005). Goat's milk has been used since ancient time for the manufacture of different types of cheese, throughout the world. Many famous varieties of cheese are made from goat's milk while in Sudan goat's milk products are not highly accepted because fresh goat's milk has a mild "goaty flavour" this flavour is mainly due to the presence of short chain fatty acids, the breed of goats un hygienic milky of the goats and the diet of the animal, has a lot to do with the taste of the milk (Metry *et al*., 2007).

The unique nutritional composition relative to cow's milk should also be emphasized. This reflects especially in cheese yield: ca. 5.5: 1 of ewe's milk is required to produce 1 kg of cheese, whereas ca 11:1 of cow's milk is required for the same amount of cheese. This greater cheese yield is explained by the quantitatively higher content of fat (7.5%) and caseins (4.6%) of ewe's milk than cow's counterpart (3.9% of fat and 2.6% of caseins); a similar realization holds with goat's milk (4.5% of fat and 3.0% of caseins). In addition to the aforementioned higher cheese yield, goat's and ewe's milk have beneficial health properties, because of their mineral and vitamin levels, and their different protein profile; furthermore, goats and ewes can grow in more unfavorable

environmental conditions, from a feeding point of view, than cows. On the other hand, cheese manufactured from milk of ewes or goats possess a much higher added value as gourmet products are in stake (Walstra *et al.*, 2006).

Differences were reported in intensity and predominance of individual tastes in the various fractions of cheese made from milk of the three species; it was suggested that bovine milk cheeses were mainly salty and sour, ovine milk cheeses had predominant umami taste and caprine milk cheese was umami, astringent and bitter and the highest cheese flavor intensity was found in the fractions with the highest concentration of amino acids and volatile compounds (Molina *et al*., 1999 b).

Cheese making is a major milk preservation method in Sudan (Ibrahim, 1971) Sudanese Gibna Bayda is unique among cheese varieties in that high concentrations of table salt (Sodium chloride) are added to the milk before processing (Osman, 2005). It is manufactured from raw or heated milk, (Ibrahim, 2003). During processing under tropical conditions cheese deteriorates rapidly before it ripens thus salting before renneting becomes essential for its preservation (Elsiddig, 2006). The same basic steps are followed for manufacturing cheeses like renneting milk, cutting the curd, salting, moulding and pressing. In some varieties additional steps include ripening or storing in brine. The most preserving agents for cheese are acidity, salt or low moisture (Walstra *et al*., 1999). Fresh soft cheeses are made by using only traditional methods in different geographical locations in the world; the traditional method of manufacture involves renneting for curd formation, fermentation and preparation for markets and these cheeses were consumed in fresh form (Mikulec and Jovanovic, 2005). Gibna Bayda is white cheese made in Sudan it is similar to Domaiti cheese which is made in Egypt. A starter is not used, and the storage life of the cheese may be more than one year. The

procedure for making this cheese includes heating of the fresh milk to 35°C and followed by salt addition to give 7-10% solution in milk. Followed by add rennet or rennet extract to obtained a firm coagulation which develops in four to six hours, the coagulum is transferred to wooden moulds lined with muslin and the whey allow to drain overnight. The curd cut into 10cm cubes, and the cubes put into tins or other suitable airtight containers and the tin or container is filled with whey from the same cheese and sealed (Raheem, 2006).

2.2.3.2. Muddaffra cheese: (Braided cheese)

Muddaffara cheese is a local braided semi-hard unripened cheese with close texture and yellowish colour, slightly acid and salty taste. It is known in the urban communities of Sudan (Elsiddig, 2006).

There are some cheese in the world that resemble Muddaffara cheese Examples are: kash karal (the Balkan region), Roomi cheese (Egypt), Romia (the Middle East), caciocoralla (Italy), Rass cheese (Egypt), Magdoula (Syria), Pasta filata (a group which includes provolone and Mozarella cheese) (Elsheikh, 1997). The Sudanese consumer has always shown, since several generations, a particular favoritism to the taste of braided cheese. The relatively high cost of braided cheese is mainly attributed to its complicated technology that includes scolding, slicing, cooking, manual working of the curd and finally braiding, preparation of braided cheese usually takes quite a long time compared to white cheese. The process needs highly experienced and skilled workers (Abdel Razig and Babiker, 2009). In addition the method of manufacture needs to be improved in order to produce a safe product with good quality (Elsheikh, 1997).

The general practice used in Sudan and in the Middle East countries is that cheese is made from cow's and goat's milk which has a great role in dairy processing although large quantities of milk are produced (Abdel Razig and

Babiker, 2009). However, there was no significant difference between the different types of milk in acidity and pH (Elsheikh, 1997).

2.2.3.3 Mozzarella cheese:

Mozzarella is an Italian cheese traditionally made from high fat milk it melts easily and is used extensively in cooking e.g. lasagna and pizza. It has been manufactured and introduced to the market due to the recent popularity of Italian dishes. The manufacture of Mozzarella was first practiced in Khartoum Dairy Product Factory in 1992 (Khateeb, 1997).

The method of manufacturing mozzarella cheese slightly differs from one place to another. In the United State, however, the cheese is produced from whole or partially skimmed milk. Small amounts of starter culture or organic acids followed by rennet extract are added. Forms is not cooked, but simply cut and the whey is drained off. The matted curd are formed into blocks, drained and at worm temperature undergo mild acid ripening at pH 4.4-5.2 At critical pH or acidity the curd is heated in water, stretched and molds, placed in proper forms, and slightly salted. Artificial flavor and flavor producing enzymes normally are not added to mozzarella cheese (Kosikowiski, 1982).

2.2.3.4. Romia cheese:

It is hard type of cheese and probably derived from Romano cheese which is an Italian coated hard cheese; while Romia is not limited little quantities of this type of cheese are produced at Kazagael in the west of the country, (Khateeb, 1997).

2.3. Cheese ripening:

According to Smit (2003) cheese ripening involves a complex series of micro-biological and biochemical events which result in the development of the flavor

and texture characteristics of each variety, and biochemical changes which occur during ripening include metabolism of residual lactose and of lactate and citrate (often although incorrectly, referred to as glycolysis), lipolysis and metabolism of free fatty acids and proteolysis and metabolism of free amino acids, that the proteolysis is the principle biochemical event which occurs during the ripening and the initial breakdown of the caseins is catalyzed by residual coagulant (usually chymosin) and the principle indigenous proteinase in milk, plasmin.

2.3.1. Proteolysis in cheese ripening:

Cheese manufacture and ripening involves the action of enzymes (from rennet and milk) and elected microorganisms, both directly, while growing, and indirectly, through their enzymes after death and lyses (Mc Sweeney, 2004 a). Microbiological changes in cheese during ripening include the death and lyses of starter cells, and the growth of adventitious flora like nonstarter lactic acid bacteria. Cheese texture softens during ripening as a consequence of hydrolysis of the casein micelles during proteolysis, changes to the water – binding ability of the curd and changes in pH (Mc Sweeney and Fox, 2004).

The final products of proteolysis are free amino acids and their concentration in cheese at any stage of ripening is the net result of the liberation of amino acids from casein, their degradation to catabolic products and perhaps some synthesis by the cheese micro flora. This general outline of proteolysis can vary substantially between cheese varieties (Sousa *et al*., 2001)

Compounds which contribute to cheese flavour are added or are produced during manufacture (e.g., lactic acid and NaCl) but are mainly formed as consequence of the many biochemical changes which occur during ripening; cheese taste is an important organoleptic attribute and the correct balance of

sapid compounds is vital to cheese quality (Mc Sweeney, 1997). Proteolysis contributes to the taste of cheese by the production of peptides and free amino acids and the sapid flavor compounds generally partition into the soluble fraction on extraction of cheese with water.

Large peptides do not contribute directly to cheese flavor, but are important for the development of the correct texture; however, large peptides can be hydrolysed by proteinases to shorter peptides that may be sapid (Sousa *et al.*, 2001). Kilio *et al.*, (2004) stated that acidity generally, increases throughout the ripening of cheese. This increase, to a certain degree, is the indication of ripening of cheese.

2.3.2. Lipolysis in cheese ripening:

McSweeney and Sousa, (2000) and McSweeney (2004 a) concluded freshly-made curds of various cheese varieties have bland, and largely similar, flavours and it is during the ripening period that flavour compounds are produced which are characteristic of each variety; Originally it was thought that cheese flavour resulted from a single compound or class of compounds; while this is largely true for blue-mould varieties, it is now generally accepted that the flavour of most cheeses results from the combination of a large number of sapid compounds present in the correct ratios and concentrations; Hundreds of compounds have been implicated in cheese flavour; some of which have been identified in Cheddar cheese. The major biochemical pathways which occur in cheese ripening are the following: the metabolism of residual lactose, lactate and citrate (sometimes, although erroneously, referred to as 'glycolysis'), liberation of free fatty acids, FFA (lipolysis). And McSweeney and Sousa, (2000) concluded his lipolysis results directly in the formation of flavour compounds by liberating free fatty acids (FFA).

McSweeney (2004 a) concluded Lipolysis in cheese is catalysed by lipases from various source, particularly the milk and cheese microflora, and, in varieties where this coagulant is used, by enzymes from rennet paste.

2.3.3. Glycolysis in cheese ripening:

The biochemical changes occurring during ripening include the metabolism of residual lactose and of lactate and citrate (often, though erroneously, referred to collectively as 'glycolysis'), lipolysis and proteolysis, Mc Sweeney (2004 a) and he concluded that residual lactose is metabolized rapidly to lactate during the early stages of ripening. Lactate is an important precursor for a series of reactions including oxidation or microbial metabolism, while citrate metabolism is of great importance in certain varieties, (Parente and Cogan, 2004).

McSweeney and Fox, (2004) reported that cheese is a fermented dairy product, a key feature of its manufacture is the metabolism of lactose to lactate by selected cultures of lactic acid bacteria (LAB) known as starters. The rate and extent of acidification influence the initial texture of the curd by controlling the rate of demineralization. Most of the lactose in milk is lost in the whey as lactose or lactate during cheese manufacture; however, low levels of lactose remain in the curd at the end of manufacture (e.g. 0.8–1.0% for Cheddar at milling (McSweeney, 2004 a). The complete fermentation of lactose is important in cheese to avoid the development of an undesirable secondary microflora, (McSweeney, 2004 a). Lactose that remains unfermented by the starter is probably metabolized by nonstarter lactic acid bacteria (NSLAB) flora (McSweeney and Fox 2004**,** McSweeney and Sousa, 2000).

2.4. Cheese Packaging:

Several factors are involved in selecting a package for a cheese, these are: type of cheese, resistance to mechanical damage, presence of specific flora, whole sale or retail packaging, permeability to water vapor, presence of oxygen and carbon dioxide or both, ammonia, light, labeling facilities, migration of flavour from package material to product and the system for storage (Walstra *et al*.,

1999). Ahmed Idris and Alhassan, (2010) reported that packaging of cheese is to minimize losses of moisture and penetration of oxygen which would help in mould growth; he stated that Sudanese white cheese was packed in tins which proved to be hazardous because they cause reaction with milk acids and that resulted in corrosion; oxygen is an important factor in corrosion occurrence in plain containers, if it is present in small quantities it dissolves some of the packaging tins which result in detinning of the interior and perforation of the can; and he stated that plastic materials were used for packaging many food products during manufacture and handling of cheese, electrostatic charges occur on plastic, these charges attraction borne materials such as dust and microorganisms.

The positive effects of using the foil packaging of Emmental cheese during ripening; included less water loss, attractive eye formation, softer cheese body, absence of hard cheese rind and less need for cheese care, while the negative effects and their causes include mould growth on cheese surface due to penetration of O_2 and H_2O, discoloration and sharp taste near the cheese surface due to insufficient NaCl level in the rind area and restriction escape of CO_2; and cited that cheese packed too young in plastic containers contained too much moisture and had too high pH after ripening, Nuser (2001).

Abdalla *et al.*, (2013) indicated that plastic containers are sterile, but they may become contaminated if not handled in an acceptable manner. Plastic containers are now widely used for packaging of cheese in Sudan.

2.5. Cheese storage and handling:

Various types of cheeses have a short shelf life whereas other cheeses are adapted to extended storage. During storage cheese develops properties that are characteristics of a particularly type of cheese, Fox (1993). Walstra *et al.*, (1999) stated that storage temperature affects growth rate of microbes of a

desirable flora, activity of their enzymes, rennet and starter bacteria. Also they found that high storage temperature cause quick ripening, but it enhances the risk of spoilage, low temperature causes unsatisfactory ripening of cheese. At low temperature the flavour of cheese was not developing.

Altaif (2011) cited the effect of storage on the quality and chemical composition of Sudanese white cheese and she found that the moisture content at one day storage was (57.97% $\pm$ 0.9) and it rapidly dropped to (39.85% $\pm$ 1.6) after 60 days of storage and he stated that increasing the time of cheese storage resulted in decrease of the moisture content of the cheese due to the increase of acidity which affected the concentration of the curd.

Aly and Galal (2002) found that the moisture content decreased with prolonged storage time. Talib *et al.,* (2009) also found that the moisture content decrease rapidly during the first two weeks.

Kur (1992) reported that the fat content of Sudanese white soft cheese increases throughout the storage period, and the increase was attributed to the decrease in soluble consistence of cheese that resulted from the partial degradation of protein.

Nuser (2001) and Hayaloglou *et al.,* (2005) found the total solids decreased during storage period due to protyolitic and lipolytic effect of microorganisms. While Abdel Razig and Babekir (2009), Elowni and Hamid (2008) found that the total solids of Sudanese white soft cheese increased during storage period.

Abdel Razig and Babekir (2009) recorded that the ash content of Sudanese white soft cheese increased with time till the end of storage. And also found

that the lowest ash content (2.03%) at the beginning and the highest ash obtained at end of storage was (3.53%).

Kur (1992) found that the mean acidity value of the 8% salted white cheese at one day storage was 0.114% $\pm$ 0.01 and increased to 0.67 % $\pm$ 0.1 after 30 days of storage.

Bilal (2000) found that the cheese samples stored in plastic containers lost weight less than those stored in cans during storage of Sudanese white soft cheese. Also she stated that fat, total solids contents, ash and titratable acidity were higher in cheese stored in cans, while protein was higher in cheese stored in polyethylene bags.

Mohammed (2009) showed that during storage of cooked cheese, viable bacterial count did not show significant change, while revealed that no significant change in uncooked cheese. He concluded that storage of cooked cheese significantly affected colour ($P<0.05$), flavour ($P<0.01$), taste ($P<0.05$) and overall acceptability ($P<0.001$). While body and saltiness revealed no significant change. Uncooked cheese did not significantly affect colour and flavour, while taste was significantly ($P< 0.05$) affected by storage of uncooked cheese. Body, saltiness and overall acceptability were highly significantly ($P<0.001$) affected by storage period. Colour, flavour, taste, body and saltiness were not significantly affected by cooking, while the overall acceptability showed significant ($P<0.05$) change. The highest acceptability score was in cooked cheese.

2.5. 1. Cold storage:

The shelf life of cheese can be prolonged by cold storage since microorganisms grow at slower rate, (Mohammed, 2009). Cold storage guarantees control of

microbial population in fresh (Pasta filata) cheese produced from pasteurized cow's milk with different technologies, (Coppola *et al,* 1995). Collombo *et al,* (1992) studied the influence of different temperatures (4, 15 and 30.5) ˚C and protective gases (Air or CO_2) on the quality of stored cheese to optimize storage under laboratory conditions and the result showed that low temperature were more effective than protective gases for prolonged cheese storage.

2.6. Cheese salting (NaC1):

Salt is an important component of cheese with respect to preservation, flavour, and consistency, rate of ripening, rind and shape retention. Salt has three major functions in cheese: It acts as preservative, contributes directly to the flavour and is a source of dietary sodium (Guinee, 2004).

Salt application in cheese making involved it's mixing with dry milled curd, rubbing onto the surface of the molded pressed cheese and immersing cheese in a concentrated solution of it until the desired amount is absorbed (Walstra *et al*., 1999). A combination of these methods may also be applied. The way of salting affects the properties of cheese. Salting has an effect on cheese yield because it results in loss of weight (Walstra *et al.*, 1999). Kristiansen *et al,* (1999) reported that free amino acid tyrosine and tryptophan were decreased with increased salt content of cheese.

2.7. Cheese yield and weight losses:

During the conversion of milk into cheese curd, milk constituents are separated in to tow groups, those that are retained in the curd and those that are lost in the whey. cheese curd retains most of the fat and casein in the original milk, while the whey contains mostly water, lactose , proteins (peptides and other nitrogenous compounds) and minerals that are soluble at the pH of cheese

making and the typical yield of cheese ranges from 9 to 15% depending on the chemical composition of the milk , efficient recovery of fat and casein in the cheese, losses of milk constituent in the whey resulting from milk handling and treatment and cheese making procedures , and the final moisture content of the cheese (Farkey, 2004).

Abdel- Razig, (1996) studied the production of white soft cheese from different milk sources and he reported that the cheese Yield for fresh cow's milk with 2% sodium chloride was 19.08%.

Ahmed and Khalifa (1989) studied the manufacture of white soft cheese (Gibna Beyda) from recombined milk and they reported a high yield between (19- 19.2) %. (Khalid and El Owni, 1991) studied the effect of sodium chloride concentration on the yield and chemical composition of Sudanese white soft cheese and concluded that the yield of fresh cheese was 22.9% and 23.8% for milk salted with 6% and 8%, respectively.

Khateeb (1997) reported that the yield of fresh cheese was generally high, with yield ranging between 23.70% and 33.34% with an average of 27.80% However, Altaif (2011) reported the yield of technology of Sudanese white cheese " Gibna Beyda" made from unsalted milk was 8-14%

Hamid and Elowni (2008) studied the processing and properties of Sudanese White soft cheese in South and West Darfur and they found that the average cheese yield ranges between 13.25-17.00%. Kheir *et al,* (2011) indicated that type of coagulant (*Solanum dubium* extract and rennet) had no significant effect on weight loss.

Pappas *et al,* (1996) studied the effect of salting method and storage time on composition and quality of feta cheese and found that the yield of cheese decreases with the increase in storage time.

Abdel Razig and Babiker (2009) concluded that the weight loss of cheese significantly affected by the storage time, and it increased gradually till the end of storage period. Abdalla *et al,* (2011) indicated that storage period significantly increased weight loss of Sudanese white cheese.

2.8. Chemical composition of white soft cheese (Gibna Beyda):

Nuser (2001) studied the effect of the storage period on chemical composition of fresh white cheese, and found fat content to be 25.13% protein 23.26%, total solids 48.47%, ash 3.5% and titratable acidity 0.66%. The values of cheese stored for 45 days were 22.5% for fat content, 20.23% for protein, 47.2% for total solids, 3.73% for ash and 0.74% for titratable acidity. Kheir *et al.* (2011) indicated that type of coagulant (*Solanum dubium* extract and rennet) had no significant effect on titratable acidity, protein, salt and ash content of white cheese. However, there was a significant effect on total solids, fat, soluble protein, tyrosine and tryptophan contents. Also, Sabah El-Kheir *et al.* (2002) studied the chemical and organoleptic properties of Sudanese white cheese obtained from different supermarket in Khartoum State and found that the total solids varied widely from 52.05 to 36.55% and the total protein was 9.24 - 16.10% while the fat content was 18.1 - 27.3% and the ash content was 5.70 - 7.55%.

2.8.1. Moisture content:

The moisture content of cheese could be influenced by the fat, protein and calcium content of the cheese milk, size of curd particle, cooking temperature, and the rate of acid development and added salt (Altaif (2011). The moisture content is estimated to be 60% (as the lowest limit) as given by Sudanese

Standards and Metrology Organization (SSMO, 2002). Abdalla (2005) studied the effect of starter culture on the quality of Gibna Beyda and found that the moisture content of cheese with 2% sodium chloride is 56.15%. Altaif (2011) concluded that moisture was not significantly decreased with increase in salt concentration and the storage period was significantly affected the moisture content.

2.8.2. Fat content:

The fat content is estimated to be 20% (as the lowest limit) as given by Sudanese Standards and Metrology Organization (SSMO, 2002). The fat content of white cheese with 2% sodium chloride is 27.60% as recorded by Abdalla (2005) in addition Mohammed (2007) found it as 29.7± 3.2%. Khalid (1991) reported that the fat content of cheese made from 6% and 8% salted milk cheese ranged between 15-32% and 13-30%, respectively.

Elowni and Hamid (2007), Hamid and Elowni (2008) and Talib *et al*, (2009) recorded the fat content of white cheese is about (22.80%), (23.79%) and 15 - 18%, respectively. And Nuser (2001) reported that the fat content in cheese curd decreased over the time due to leakage of some fat curd into the brine solution.

2.8.3. Total solids content:

It was estimated to be 40% (as the lowest limit) as given by Sudanese Standards and Metrology Organization (SSMO, 2002). Ahmed (1998) found that raw milk cheese with 6% sodium chloride gave the highest total solids content (48.35% ±1.11) in comparison with that of cheese with 4% sodium chloride (44.04% ±4.39) and 2% sodium chloride (43.71% ±3.51), while Ibrahim (2003) reported that the average of the total solids of Gibna Beyda obtained from different factories in Eldueim area was between (39.42% - 45.5). Abdel – Razig, (1996)

recorded 44.59% total solids of white soft cheese with 2% salt. Also a range from (45.17-58.17) % was reported by Elowni and Hamid (2007).

2.8.4. Protein content:

The average protein percent per dry matter weight of white soft cheese is estimated to be 15% (as lowest limit), as given by Sudanese Standards and Metrology Organization (SSMO, 2002). Abdel- Razig (1996) stated that the protein - chloride is about 15%. On the other hand Mohammed (2007) found that the protein content of Gibna Beyda salted with 3% sodium chloride was (21.3% ±5.3). Abdel-Raziq and Babekir (2009) mentioned that the protein content decreases during the storage period, due to the degradation of protein and loss in picking whey. While Tarakci *et al.*, (2004) found that the protein gradually increased during the ripening period and as salt concentration increases, the protein increases with no significant. Talib *et al.*, (2009) reported that the protein content of soft cheese was slightly decreased with time. Abdalla *et al.*, (2011) indicated that storage period significantly increased chemical components except salt content; storage period had a significant effect on protein content of whey.

2.8.5. Ash content:

It was estimated to be 5% (as the lowest limit) as given by Sudanese Standards and Metrology Organization (SSMO, 2002). Abdel-Razig (1996) found that the content of Gibna Beyda was 2.21% while Mohammed (2007) found that the ash content of Gibna Beyda was (0.5%±0.1) Ash content of Gibna Beyda was (1.87-7.80) % and 5.34% as recorded by ElOwni and Hamid, (2007) and Hamid and ElOwni (2008), respectively.

2.8.6. Acidity:

Acid production in cheese during manufacture results in acidic pH. The resulting pH affects synersis, consistency and flavour of the cheese. Walstra *et al.,* (1999) showed that the rate of acid production affected synergic and moisture content of cheese. Also pH of the cheese was decreased as the result of the action of starter culture.

Hamid, (2005) reported that the acidity of fresh cheese from milk with 5, 7.5 and 10% sodium chloride was 0.7, 0.4 and 0.2% respectively, however the acidity increased to 2.4, 1.7 and 1.3% (as lactic acid), respectively after three month of storage. Abdalla (2005) recorded the acidity of Gibna Beyda as 0.22% from milk salted by 2% sodium chloride. And Pappas *et al.*, (1996) mentioned that the acidity of cheese increased significantly period. Abdalla *et al.*, (2011) indicated that storage period significantly increased chemical components; storage period had a significant effect on titratable acidity.

2.9. Spoilage of cheese:

Dairy products such as milk, butter, cream and cheese are all susceptible to microbial spoilage because of their chemical composition, (Jay, 2000).

The most common spoilage microorganisms of milk and dairy products are the Gram- negative rods such as *Pseudomonas spp.,* and coliforms and Gram-positive spore- forming bacteria such as *Bacillus spp*., *Clostridum spp.*, lactic acid bacteria such as *streptococcus spp.*, and yeasts and moulds (IDF, 1994) . The most common general that bring about spoilage in food are *Acetobacter, Acinetobacter, Entrobacter, Flavobacterium, Micrococcus, Maraxella* and *Serratia*, (Jay, 2000).

Ceylan *et al.*, (2003) studied the microbiology of Sikma cheese in Turkey and found that there was a significant negative correlation between lactic acid bacteria and lipolytic bactria and there was a significant positive correlation

between the lipolytic and proteolytic bacteria. They also found that the lipolytic and proteolytic counts were lower than the lactic acid bacterial count. They found that the yeasts and moulds count was higher than the limited (<100 cfu/gm) and there was a significant negative correlation between the yeasts and moulds counts and Coliform counts and that is probably due to acid forming ability of lactic acid bacteria.

2.10. Cheese and food – borne diseases:

Cheese is a highly nutritious medium for many bacteria; it has been shown to be responsible for relatively few food poisoning outbreaks. Cheese is a relatively safe food product, but 28% of the outbreaks involved cheese made from raw milk, and other reasons for these food- poisoning outbreaks were poor starter's activity, poor hygiene and faulty pasteurization (Fox *et al*., 2000).

The organisms most commonly associated with cheese- poisoning outbreaks are *Listeria monocytogenes, Escherichia, Staphylococcus aureus* and *Salmonella*, Elsiddig (2006). *L. monocytogenes* is more important since the outbreaks involved several deaths, Fox *et al.*, (2000).

Araujo *et al.*, (2000) investigated the presence of some pathogenic bacteria in soft cheese and found that *Staphylococcus aureus* was in about 20% of samples and *Escherichia coli* were isolated from 21.1% of the samples.

Ceylan *et al.*, (2003) studied the microbiology of Sikma cheese in Turkey, and suggested that the coliform bacteria found in cheese samples were due to contamination during storage and the variation observed between samples could be related to the storage ripening and production under conditions not assuring good microbiological quality and the coliform counts in cheese are expected to decrease during ripening by low pH and antagonistic action of lactic acid bacteria.

Vaishnavi *et al*., (2001) studied the bacteriology of Indian cheese (Paneer) and suggested that the contamination of cheese with *S.aureus* and faecal bacteria can be attributed to the practice of preparing large-bulks of cheese in advance of requirement, which are brought for long periods at room temperature. They also indicated poor hygienic conditions and faults in manufacturing and / or handling during process of cheese production.

2.11. Cheese microbiology:

Microorganisms are an essential component of all natural cheese varieties and play important roles during both cheese manufacture and ripening, Beresford and Williams (2004). Mohammed (2009) indicated that 38% of the isolates were *Lactococcus*, 17% *Enterococcus*, 14% *Str. thermophilus*, 12% *methophilic lactobacilli*, 10% *Leuconostoc* and 9% *thermophilic lactobacilli*. Ibrahim, (2003) concluded that the total viable bacterial count (TVBC), *coliform* bacterial count and *staphylococcus* count were significantly affected by storage period. On the other hand a recent survey involved the characterization of 4379 bacterial isolates from 35 European artisanal dairy products, including 25 cheeses. Abdalla *et al.*, (2011) concluded that *total viable bacteria*, *str.cocci* and *lactobacilli* counts (the power 10 cfu/gm) decreased as the storage period progressed.

Abdel Razig and Babiker (2009) concluded that the total bacterial count decreased, while coliform, yeast and mould and *S.aureus* recorded nil in all cheese samples during storage of white cheese type's manufactured by lemon, orange and grapefruit juices, respectively. Kheir *et al.*, (2011) indicated that total viable bacteria, streptococci and lactobacilli counts (cfu/gm) were significantly affected by storage period and as the storage period increased their count decreased.

2.11.1 Viable bacterial count:

During storage period, the mean value of viable bacterial count (VBC) increased to maximum of the power 10 is 9.88 $\pm$ 0.34 at day 30 and then slightly decreased to $\log_{10}$ 9.79 $\pm$ 0.5 at the end of the storage period, Ibrahim (2003). Nour El-Daim and Elzubeir (2007) concluded that storage period of processed white soft cheese showed a significant difference ($p< 0.05$) with viable bacterial count (VBC), yeast and molds counts.

2.11.2. Coli forms:

Coliforms are gram-negative aerobic and facultative anaerobic short rod; the term includes *E.coli, Enterobacters, klebseilla* in addition to the species from other general of *Enterobacteriacae* (Hamid, 2005). According to Walstra *et al.*, (1999) stated that coliform bacteria can grow only as long as sugar is available for fermentation because they cannot ferment lactic acid. Some *coliforms* like *Enterobacter aerogenes* ferment citric acid slowly, whereas *E.coli* does so more quickly. Rapid fermentation reduces the risk of blowing because the produced hydrogen reacts with metabolites of citric acid fermentation. Also, he stated that growth of *coliforms* in cheese was prevented by using fast-souring starter culture that rapidly converts lactose to lactic acid, thereby decreasing the pH in a short time to a level that inhibits their growth. A comparison is made by Birollo and Vinderola (2001) between *Enterococcus* and non lactic acids micro flora (*Coliforms, Pseudomonas, S.aureus* and *yeasts*) for their suitability to be used as hygiene indicators.

2.11.3. *Staphylococcus aureus:*

Bashir (1999) reported that total bacterial count, *S.aureus*, yeast and mould from samples collected from various cheese processing plants in El-Dueim were $6.1x10^4$, $4.9x10^2$ and $1.6x10^4$, respectively. Aly and Galal (2002) found

that staphylococcus count was 6.2×10^6 per gram for the fresh cheese and the count was decreased to 1.9×10^6 when Domiati cheese made from raw milk and stored for four months. They also stated that the decrease in growth of staphylococcal count in raw milk cheese could be due to the high salt content and low pH values during the storage period as well as absence of aerobic condition required for their growth. Positive isolation for *E.coli* was isolated from Sudanese white cheese (Warsama *et al*, (2006). In cooked hard cheese staphylococcus *aureus* is usually in-activated during the first day of manufacture due to the high cooking temperature (Zangeral and Ginzinger, 2001).

2.11.4. *E.coli:*

Five outbreaks of food borne disease due to pathogenic *E.coli* have been traced to the consumption of soft cheese; however, *E.coli* strains normally do not tolerate low pH values at or below 5.4 (Fox *et al*., 2000).

2.11.5. *Lactobacilli:*

Growth of thermophilic lactobacilli can induce texture and flavour defects. The organisms involved include the common lactobacilli (*Lactobacillus plantarum, L. Casei* and *L. brevis*) and salt-tolerant lactobacilli. Walstra *et al.*, (1999) demonstrated that if the number of common lactobacilli reached 2×10^7 per gram of cheese in 4 - 6 weeks cause various gassy, putrid flavour and excessive openness. They also stated that salt-tolerant lactobacilli grow in cheese cause texture defects and phenolic, putrid, mealy, fruity and hydrogen sulfide-like flavour in four to six months-old cheeses. Zottola and Smith (1993) reported that gas forming by salt-tolerant lactobacilli over 10^3 colonies per milliliter of brine is considered dangerous.

2.12. Quality of the white soft cheese:

Sensory characteristics of foods play an essential role in consumer behavior, particularly choice and the decision to purchase in the market place (Mohammed, 2009). Sensory evaluation of cheese using descriptive analysis as a research tool has blossomed in last decade; sensory attributes (flavour and texture) are critical to identify cheese and consumer acceptance although, many approaches have been developed to evaluate the sensory attributes of foods (Drake, 2007).

Descriptive analysis is an integral part of identifying differences in ingredients characterizing the aromas and flavours in cheese (Avsar *et al.,* 2004) and evaluating the texture or body of cheese, Drake, (2007). Some characteristics which influence the quality of cheese are: acidity, salt and moisture contents. The acidity influences the moisture, flavour, body and texture of the cheese (Kur, 1992). Hamed *et al.*, (1992) found that the composition and quality of white cheese were affected more by storage temperature than by heat treatment of cheese milk where cheese made from pasteurized milk received the highest organoleptic scores after 90 days at room temperature and refrigerator (92% and 88%) respectively. However, cheese made from raw milk received the highest organoleptic score after 60 days at room temperature and after 90 days at refrigerator temperature. The storage at room temperature for four months of market processes had a more pronounced effect on the quality and rheological properties of processed cheese (Hamed *et al.,* 1992). Also, he reported the score for flavour, texture, colour and general appearance of fresh and stored processed cheese showed a general tendency to decrease throughout the storage period. Mohammed (2009) stated that the sensory characteristics of flavour and texture of processed cheese decreased during storage at 37°C.

Bilal (2000) stated that colour and taste were best in white cheese stored in cans in comparison with those kept in polyethylene bags. However, saltiness and texture were significantly ($P<0.05$) high in cheese packed in polyethylene bags. Cheese stored in cans was more acceptable than those stored in polyethylene bags. He also, concluded that cheese made from cow's milk was the best in all characteristics ($P<0.05$) while cheese made from 5% soy milk ranked second. Nuser (2001) mentioned that the storage period did not affect the colour and body of white cheese, while, saltiness was significantly affected by the storage period, and high scores of saltiness were at day 45 while the lower ones were at day zero. Khalid (1991) found that cheese made with 6% salted milk is better in quality than those made with 8% salted milk. Osman *et al.,* (2008) cited that the cheese shows a superior quality during 30 days of storage and then the quality deteriorated as the acidity continued to decrease. However, cheese was still acceptable after 90 days of storage. Abdalla and Mohamed (2009) indicated that colour and body of cheese did not significantly change during storage period, while flavour, taste, saltiness and overall acceptability gradually improved throughout the storage period.

El Owni and Hamid (2008) concluded that texture, flavour and colour of the Sudanese white soft cheese samples significantly improved during storage until day 120 then decreased in scores thereafter. Also, Hamid *et al.,* (2008) showed that the sensory characteristics of Sudanese white cheese with 6% salt were better when compared with those made with 4% salt. Abdalla *et al.,* (2011) concluded that there was a slight improvement in flavour and a decrease in saltiness during storage period but there was no significant effect on cheese colour and texture. Kheir *et al.,* (2011) indicated that no significant differences were observed in sensory characteristics in terms of colour, flavour, texture and saltiness between the rennet and *Solanum dubium* extract cheeses.

2.13. General characteristics of enzymes

According to Osman (2005) enzymes defined as indispensable compounds that play a key role in metabolism by bringing direction and control to the physiological processes of living cells. Any change in enzyme complement of living cells is immediately reflected in the physiological and biochemical processes of the cell; enzymes are also defined as proteins specialized in catalyzing biological reactions. Another definition of enzymes was stated by Devlin (1986) who claimed that enzyme are protein evolved by the cells of living organisms for the specific function of catalyzing chemical reactions. All enzymes are proteins, but not all proteins are enzymes.

Certain enzymes contain non-protein components such as carbohydrates, lipids, phosphate, metal ions or small organic moieties. The complete enzyme system usually includes both the protein and non- protein parts, called holoenzyme. The protein part is termed the apoenzyme and non- protein part, the prosthetic groups or cofactors; the type of cofactor or coenzymes concerned in the enzymic process aide in classification, (Osman 2005).

Enzymes cause chemical reactions to occur at their fastest rates when the temperature is at an optimum level. For most enzymes, this is in the range of 15.6˚C to 65.5˚C, but some reactions may occur at temperatures above or below the optimum range. Thus, some enzymes are able to react slowly at temperatures well below that of the freezing point of water and other at temperatures above 71.1˚C, because proteins are changed chemically and physically and coagulated by high temperatures, especially when moisture is present, enzymes are usually inactivated at temperature between 71.1and 93.3˚C . Enzymes also have an optimum pH at which they cause reaction to occur at the fastest rates, the optimum pH for most enzymes was found to be in the range of pH 7.0-8.0 (Vieira, 1996).

2.14. Milk clotting enzymes:

2.14.1. Rennet enzyme:

Rennet is the most desirable enzyme for coagulation milk to manufacture cheese as well as, it is important in the formation of the casein network during coagulation. A shortage of this enzyme has occurred as the decrease in general availability of sucking calves as they are left for beef, thus decreasing the rennet availability and increasing its cost (Talib *et al*., 2009).

Goat *Capra hircus* chymosin was found to be more thermo stable than cattle chymosin and equally stable to buffalo chymosin. According to its characteristics goat chymosin might be a suitable rennet substitute for production of quality cheese out of goat buffalo and cow milk (Kumar *et al*., 2005). Increasing world cheese production and consumption together with the increase of calf rennet's price and a reduced supply of calf rennet, has led to a systematic investigation for new rennet substitutes. Much research interest has been directed towards discovering a milk-clotting enzyme which would satisfactory replace calf rennet in cheese manufacture. More recently, the incidence of bovine spongiform encephalopathy (BSE) has reduced both supply and demand for bovine rennet (Roseiro *et al*, 2003).

2.14.2 .Microbial rennet

Microbial rennet produced by genetically engineered bacteria have proven suitable substitutes for animal rennet, but increasing attention has been directed toward natural rennet extracted from plants such as Ananas comosus (Cattaneo *et al.*, 1994), Carica papaya (Cabezas *et al*, 1981), Ficus carica (El-Shibiny *et al*., 1973), Calm viscera (Gupta and Eskin, 1977), Cynara cardunculus (Heimgartner *et al*., 1990), and Cynara scolymus (Sidrach *et al*, 2005). In

America and Canada now 80% of cheese is produced by chymosin from cow genes genetically engineered in *E. coli* (IFT, 2000).

2.14.3. Vegetable coagulants

Moreover, consumer constraints on the use of rennet have led to a growing interest in vegetable coagulants. The use of plant coagulants allows the target for cheese production and hence contributes to improve the nutritional intake of people and whose use of animal rennet's is restricted (Gupta and Eskin, 1977). Also, the increasing attention has been directed towards the use of fruits or flowers as sources of milk-clotting enzymes (Lopes *et al*., 1998).

2.14.3.1 Solanaceae family:

Fruits of some plants have been tried to that subject (Hamdy *et al*., 1976) reported that coagulant enzyme will be extracted from *Solanum torvaum,* using 5% NaCl. *Solanum innacum*, (Suleiman *et al.,* 1988) have been tried as a source of milk-clotting enzymes. Other species of *Solanum* genus such as: *Solanum incanum, Solanum esculentum, Solanum macrocarpon* and *Solanum melongena* have shown the ability to clot milk (Suleiman *et al*., 1988; Guiama *et al*., 2010 a). Also, (Guiama *et al*., 2010 b) studied nine Solanum species (*Solanum aculeastrum, Solanum aethiopicum, Solanum anomalum, Solanum cerasiferum, Solanum dasyphyllum, Solanum indicum, Solanum nigrum, Solanum nodiflorum* and *Solanum terminale*) have shown the ability to clot milk and he concluded that high milk-clotting and low proteolytic activities were found in the coagulant from *S. aethiopicum* obtained after 24 h of soaking dried berries in 5% NaCl solution and extract from *S. cerasiferum* berries shows high milk-clotting and proteolytic activities.

Yousif *et al*., (1996) concluded that there was a linear relationship between the inverse of the extract of *Solanum dubium*'s enzyme concentration and the milk-

clotting activity for all fruit parts. Also, he observed that the water extract of *Solanum dubium* berries had lower milk clotting activity than the 5% NaCl in acetate buffer extract. And he found that the milk clotting activity was higher in the seed and whole berry extracts than in the berry coat extracts.

2.14.3.2. Sun flower seeds

Sun flower (*Helianthus annuus*) is a member of the Asteraceae or Compositae family; the Genus *Helianthus annuus* is formed of both annual herbaceous and perennial species; the sunflower's characteristics of turning toward the sun during the day accounts for both its common name and its botanical name; Greek Helios means the sun and another means flower; sun flower was the second important oil crop in the world during the sixties and seventies of the twentieth century. After the increase of the rape seed production, sun flower became the third oil crop after soybeans and rapeseed (Hamid, 2009).

Sun flower (whole seed) fruit is sold as a snack food, after roasting in ovens, with or without salt added. Sunflowers can be processed into a peanut butter after native, sun better, especially in China, Russia, the United States, the Middle East and Europe. In Germany it is mixed together with rye flower to make *Sonnenblumenkernbrot* (literally: sun flower whole seed which is quite popular in German-speaking Europe. It is also sold as food for birds and can be used directly in cooking and salads (NSA, 2009).

Egito *et al*., (2007) showed that the sunflower extract had a Specific clotting activity and hydrolyzed –casein as does chymosin in bovine casein. Nasr *et al*., (2015) concluded that the partially purified sunflower enzyme has higher milk-clotting activity and lower proteolytic activity and can be used in cheese making.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Experiment area:

The experiment was conducted at Sudan University of Science and Technology, College of Animal Production Science and Technology, Kuku (2012- 2015).

3.2. Experimental design:

In this study two experiments were conducted, in the first one the sun flower seeds' enzyme was extracted using Sodium acetate buffer with NaCl (5%). Then, the extracted enzyme was partially purified using ammonium sulphate.

In the second experiment, four treatments were carried out. In the first, two treatments, cow's milk were made into white soft cheese, using rennet enzyme and sunflower seeds' partially purified extract. While, in the second, two treatments, goats' milk were made into cheese using rennet enzyme, and sun flower seeds' partially purified extract. Cheese samples from all treatments were packed in plastic containers (250 gm) and stored at refrigerator temperature for two months. Rennet coagulation time (RC), Yield, Chemical, microbiological and sensory evaluations were carried out for 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 days.

3.3. Seeds preparation:

The seeds materials used in this study were collected from Shambat central market, Khartoum North, Sudan. The seeds of sun flower (*Helianthus annulus*) were carefully cleaned, manually decorticated, and then coarsely powdered using an electric grinder.

3.4 Enzyme Extraction:

According to), Talib *et al*., (2009) and Guiama *et al*., (2010 a) extracts were prepared in duplicate; 10 g of Sunflower seeds were immersed in (100) ml Na acetate buffer with 5% (w/v) NaCl with stirring. The extraction procedure was continued for 24 h at 4°C. The extracts were filtrated through cheese cloth and centrifuged at 5,000 rpm for 20 min. The supernatants were examined for milk-clotting and protease activities in each extract.

3.5. Determination of milk-clotting activity:

The milk-clotting activity of extract from sun flower seeds was determined following the procedure described by IDF (1992). Sixty gms of low-heat skimmed milk powder (Confectionery skim milk powder, Poland) was reconstituted in 500 mL 0.01 M $CaCl_2$ (pH 6.5); this mixture was stored at 4°C. Extract was added at a proportion of 0.1 ml per ml of milk. These extracts were added to reconstituted milk at final volumes in the range 0.1 to 2 ml. The coagulation point was determined by periodic manual rotating of the test tube, at very short time intervals. The clotting time was recorded when discrete particles were discernible. One milk-coagulating unit (U) was defined as the amount of protein that coagulates 10 mL of reconstituted low-heat skimmed milk powder at 30°C in 100 sec (Berridge, 1952). The milk clotting activity (MCA) of the extract was measured, assuming that all the soluble proteins are enzymes which coagulate milk at 30°C.

$$\text{MCA (U/ml)} = \frac{100 \times S}{CT \times E} \times 100$$

Where:

100: dilution factor.

S: volume of milk.

CT: clotting time.

E: volume of enzyme.

3.6. Determination of Protease Activity

The method of (Sarath, 1989) was followed to determine the protease activity of *Helianthus annulus* extract:

One % casein was prepared in 50 mM Na phosphate buffer, pH, 6.5. To start the assay, 0.5 ml of temperature-equilibrated substrate was added to 0.1 ml of the enzyme solution (in centrifugal tube) and mixed gently. The reaction mixture was incubated at 30°C for 30 min, and then the reaction was terminated by the addition of 1.0 ml of 5% trichloroacetic acid (TCA). The blank was prepared in the same way by adding TCA prior to the addition of the substrate. After standing for 30 min at room temperature, the reaction mixtures were centrifuged at 12,000 rpm for 20 min to separate the TCA-soluble products. The absorbance was read at 280 nm with reference to the blank. One unit of the enzyme activity was defined as the amount of the enzyme required to cause a unit increase in absorbance at 280 nm across a 1 cm path length, under the assay conditions.

3.7. Determination of the Protein contents:

The protein content of the crude extract and fractions obtained after the partial purified enzyme was estimated by measuring the extinction at 280 nm. Quantitative determination of the protein content was estimated according to Bradford method (1976). 2.5 ml of Bradford reagent were added to 0.25 ml of the samples and BSA (Bovine serum albumin) solution as standard (both at different dilutions) and incubated for10 min, then the absorbance was read at 595 nm. The protein content was calculated from the BSA standard curve.

3.8. Partial Purification of the enzyme:

All purification steps were performed at 4°C unless specified.

3.8.1 Ammonium sulfate fractionation:

The supernatant of the seeds' extraction was used for enzyme purification by ammonium sulfate fraction method according to Ahmed *et al*. (2009a) and Ahmed *et al*. (2009 b). Initially, solid ammonium sulfate was slowly added with stirring to the crude extract (600 ml) preparations to 30% saturation. During the addition of solid ammonium sulfate, the pH of the enzyme solution was kept at pH 5.0 by a dropwise addition of either 7% NH_4OH or 0.1 M H_2SO_4, and the mixture was kept on ice for 30 min. The precipitates were separated from the supernatant by centrifugation at 8000 rpm (Centurion Scientific Co., Ltd, west Sussex, UK) for 20 min at 4°C. Solid ammonium sulfate was further slowly added to the supernatant to 55% saturation, and the solution was kept on ice for another 30 min before being centrifuged at 8000 rpm for 20 min at 4°C. Solid ammonium sulfate was again further slowly added to the supernatant to 80 % saturation of ammonium sulfate, and the solution was kept on ice for another 30 min before being centrifuged at 8000 rpm for 20 min at 4°C. The precipitates collected were dissolved in a small volume (about 2 ml) of 50 mM sodium acetate buffer, pH 5.0, and then dialyzed overnight at 4°C against the same buffer (10 liter) with several change of the dialysis buffer. The extract was then centrifuged, and the supernatants were examined for milk-clotting and protease activities as well as protein concentration. The partially purified enzyme either directly used enzyme characterization and cheese making or freeze-dried at -50°C. The latter step was repeated until the quantity of the freeze-dried partially purified enzyme reaches 2 grams and stored at -20°C until used for cheese making and enzyme characterization

3.9. SDS-PAGE and Zymography of Sunflower enzyme.

The lyophilized samples (5 mg) were reconstituted in 1 ml of 125 mMTris-HCl buffer pH 6.8 containing 2.5% SDS, 1% sucrose, and 0.05% bromophenol blue (Egito *et al.,* 2007). Then, 25 µl (60 µg protein) was loaded onto SDS-PAGE containing 0.1% gelatin or casein. The gel without substrate was used for SDS-PAGE analysis. The electrophoresis was carried out using 4% staking gel and 12 % resolving gel at 20 mA/gel for 90 min at 4°C. After the electrophoresis, the gels were either directly stained with CBB for 1 h at room temperature (SDS-PAGE) or washed twice with the renaturing solution (100 mL of 2.5% of Triton X-100 containing 15 mM $CaCl_2$) for 30 min (Zymography). The gel was then washed several times with distilled water to remove the renaturing solution. The hydrolysis reaction was then proceeded inside the gel by the incubation of the gel in the developing solution (50 mMtris-HCl buffer pH 7.5 containing 15 mM $CaCl_2$) at 37°C for 30 hrs. Thereafter, the gel was washed twice with distilled water and immediately stained with CBB solution (0.5% Coomassiae blue R-250, 5% Methanol, and 10% acetic acid in distilled water) for 1 h at room temperature. The gel was then destained with destaining solution of 10% methanol and 5% acetic acid in dH_2O for several hours. The active bands were appeared as translucent bands in blue background.

3.10. Materials:

Fresh whole cow milk (50 liters) was brought from College of Animal Production Science and Technology farm, Sudan University of Science and Technology, while fresh Saanen goats' milk was brought from local farm at Hillat Kuku, Khartoum North. Rennet powder was obtained from Chr. Hansen's lab, Denmark. Clean and fine Sodium chloride was purchased from local market. Both milk was filtered and divided into 2 equal volumes of (25 liters) each and kept into separate 4 containers.

3.11. Cheese Making:

The cheese was produced according to the following procedure of (Talib *et al*, 2006: Talib *et al*., 2009). Fifty liters of each cow and goats' milk were pasteurized at 72°C for 15 sec and then cooled to 45°C and $CaCl_2$ was added at the rate of 0.02% of milk. Starter culture of lactic acid (*Lactobacillus bulgaricus and Lactobacillus thermophilus*) were added at the rate of 2.0%, and left for 30 minutes to develop acidity.

Rennet tablets (one tablet / 45kg milk) and the partially purified freeze-dried enzymes of the Sunflower seeds were added to the milk at the rate of 2 gm /50 liter of milk. The milk were mixed and left until coagulation completed.

Rennet coagulation time (RCT) was recorded for each treatment. After coagulation the curd were cut vertically and horizontally into 5 cm^3 with a sharp knife.

The whey obtained from the cheese curd was drained and the curd was poured into small wooden boxes lined with cloth accompanied with addition of 3 % NaCl for each treatment and pressed overnight. The curd were removed from the wooden boxes and cut into cubes of 10×5×5 cm^3 then the yield were recorded for each treatment and 200 grams of each cheese sample were placed in plastic containers (of 400g capacity) in triplicates , then sealed. The cheese samples were stored for 63 days at 4 °c. Chemical, microbiological and organoleptic evaluation of the cheese samples were carried out after 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 days in duplicates.

3.12 . Cheese yield percentage:

The cheese yield was determined according to Paolo *et al*., (2008) and Abdel Moneim *et al.,* (2012) as follow:

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$$\text{Yield (\%)} = \frac{\text{Weight of cheese}}{\text{Weight of milk (kg)}} \times 100$$

3.13. Chemical analysis:

3.13.1 Chemical analysis of milk and cheese:

3.13.1.1 Total solid:

Total solids content was determined according to the modified method of AOAC (2003). Two millimeters of milk samples and 10 gms of cheese samples were weighed and placed in a clean dry aluminum dish and heated on a steam bath for 30 minutes. The dishes were then placed in an oven at 100 ^{o}C for three hours (then cooled and weighed quickly. Heating, cooling and weighing were repeated until the difference between two successive readings was less than 0.1mg)

The total solids content was calculated from the following equation:

$$T.S\ (\%) = \frac{W_1}{W_2} X \quad 100$$

Where:

W_1 = weight of sample after drying.

W_2 = weight of sample before drying.

3.13.1.2 Protein content:

The Protein content was determined by kjeldahal method as described by AOAC (1980). Three ml of milk samples and one gram of cheese were weight followed by addition of two kjeldahal tablets (1 mg Na_2 SO_4 and equivalent of 0.1 gm Hg) and digested in Kjeldhal tube (volume 75 ml) then 10 ml of concentrated sulfuric acid (Density1.815 gm/ml at 20^{o} C). The mixture was then digested on a heater until a clean solution was obtained (3 hours) and the flasks were removed and left to cool. The digested sample was diluted up to 75 ml of Kjeldhal tube with distilled water. 3 ml of the distillated sample were poured in a distillator and 3 ml of NaOH was added followed by 3 ml of distilled water and left to boil for 3 minutes. The ammonia in the distillate was

received in a conical flask containing 5 ml of 4% boric acid plus three drops of indicator (bromocresol green plus methyl red). The distillation was continued for three minutes, and then titrated with HCL until the end point was obtained (red color), then read HCL and were recorded.

The Protein content was calculated as follows:

$$C.P\% = \frac{T - BL}{STD - BL} \times \frac{1}{3} \times \frac{75}{3} \times \frac{1}{1000} \times 6.38 \times 100$$

Where:

T: Tested sample read.

BL: Blank read**.**

STD: Standard read.

3.13.1.3 Fat determination:

The fat content was determined by Gerber method according to AOAC (2003) as follows:

In a clean dry Gerber tube, 10 ml of sulfuric acid (density 1.815 gm/ml at 20^{o} C) were poured, and then 10.94 ml of milk sample and three grams of cheese samples were added. Amy1 alcohol 1.0 ml (density 0.815) was added to the mixture followed by addition of water to raise the level of fat in the column. The contents were thoroughly mixed till no white particles could be seen. The Gerber tubes were centrifuged at 1100 revolutions per minute (rpm) for 4-5 minutes and the tubes were then transferred to a water bath at 65^{o} C for three minutes. The fat percentage was then read out directly from the fat column.

3.13.1.4 Ash content:

The ash content was determined according to the method described in AOAC (2009). Ten millimeters of milk samples and two gms of cheese samples were

weighed in a suitable crucible and evaporated to dryness on a steam bath. The sample was placed in a muffle furnace (550^{o} C) for 1.5 hours, then cooled in desiccators and weighed.

The ash content was calculated using the following equation:

$$\text{Ash (\%)} = \frac{W_1}{W_2} \times 100$$

Where:

W_1 = weight of ash

W_2 = weight of sample

3.13.1.5 Titratable acidity:

Titratable acidity was determined according to AOAC (2003). Ten mls of milk and 10 gms of cheese were weighed and placed in a conical flask. For the milk 3 drops of phenolphthalein indicator were added. The samples were titrated against 0.1N NaOH until a faint pink color appeared.

The acidity was calculated as follows:

$$\text{Acidity \%} = \frac{T}{W} \times 100$$

Where:

T: Titration figure

W: Weight of sample

For the cheese Ten grams of cheese were weighed and placed in a conical flask and distilled water at 40 ^{o} C was added until the volume in the flask was 105 ml. The sample was then vigorously agitated and filtered through filter paper (Whatman No. 41) Twenty five milliliters of the filtrated were placed in a conical flask and 3 drops of phenolphthalein indicator were added. The samples were titrated against 0.1N NaOH until a faint pink color appeared.

The acidity was calculated as follows:

$$\text{Acidity \%} = \frac{T \times 4}{W} \times 100$$

Where:

T: Titration figure

W: Weight of sample

3.13.1.6. Tyrosine and tryptophan contents:

The method of Vakaleris and Price (1959) was used to determine tyrosine and tryptophan contents of cheese. Ten grams of each type of cheese were weight in a beaker, dissolved in 40 ml of 0.5 N sodium citrate solution and slightly warmed. The beaker contents were transferred to 200 ml volumetric flask and made up to the volume with distilled water and mixed. Hundred ml of the cheese solution were then transferred to 200 ml conical flask followed by 25 ml of distilled water then 10 ml of 1.41 N HCL was added, mixed well and filtered through Whatman filter paper NO. 42. The absorbance of the clear filtrate was measured at wave length 270 and 290 nm using spectrophotometer (Shimatozu spectrophotometer, Model 02) the tyrosine and tryptophan contents were then calculated from the following equations:

$$\text{mg/tyrosine/100gm cheese} = (0.95\ E_{270} - 1.31_{290}) \times 906$$

$$\text{mg/tryptophan/100gm cheese} = (0.307\ E_{290} - 0.02_{270}) \times 1021$$

Where:

E_{270} and E_{290} were the absorbance of cheese filtrate at 270 and 290 nm, respectively. Nine hundred and six and 1021 were factors associated with the molecular weight (MW×5) of tyrosine and tryptophan, respectively.

3.14. Microbiological analysis:

3.14.1. Preparation of media and glassware:

Culture media were prepared according to the manufacturer's instructions. The media were sterilized using autoclave at pressure 15 (115 - 121 ^{o}C) for 15 – 20

minutes. Plastic containers were washed in running tap water, rinsed with distilled water and sterilized in the autoclave at 115 ^{o}C for 15 minutes. The glassware were soaked in soap water overnight, washed with running tap water many times, finally rinsed with distilled water and allowed to dry. All the glassware was sterilized in an oven at 160 ^{o}C for one hour (Marshall, 1993).

3.14.2. Preparation of samples and dilutions:

Samples of different kinds of white cheese stored under the same conditions were taken in sterile plastic containers. Two hundred grams of cheese were taken for microbiological examination. Samples were taken aseptically from containers. Eleven grams of cheese were added to 99 ml of sterile 2% aqueous sodium citrates solution in a flask and shaken well (making 25 complete up and down or back and forth movements of about 30 cm in 7 seconds) to make 10^{-1} dilution then 1 ml from the above mentioned dilution (10^{-1}) was aseptically transferred to 9 ml sterile peptone water. This procedure was repeated to make serial dilutions of 10^{-3},10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} from suitable dilutions , 1 ml was transferred to Petri-dishes (duplicate) and the culture medium was poured aseptically into each Petri- dish , mixed gently , left to solidity and incubated (in an inverted position) (Houghtby *et al*., 1992).

3.14.3 Total bacterial count (TBC):

Total bacterial count using nutrient agar medium was determined according to Houghtby *et al*., (1993).

3.14.3.1 Preparation of the media:

The manufacturer's instructions were followed by dissolving 2.8 gram of powder in 100 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes, (Frank *et al*., 1992).

3.14.3.2 Plating:

From each dilution 1 ml was transferred into sterile Petri dishes (duplicate) followed by addition of 15 – 18 ml melted, cooled (45 - 46 ^{o}C) nutrient agar, mixed thoroughly by rotating the dishes first in one direction and then in the opposite direction. When medium was solidified, the dishes were incubated in an inverted position at 35 ± 2 °C for 24 hours.

3.14.3.3 Counting:

Plates contain 25 – 250 colony were selected and manually counted. The number of colony forming units (cfu) in the dilution was obtained by multiplying the reciprocal of the dilution.

3.14.4. **Coliforms count:**

The count was performed according to (Christen *et al*., 1992 and Marshall, 1993) using MaConkey agar media.

3.14.4.1. Preparation of the media:

The manufacturer's instructions were followed by dissolving 5.5 grams of powder in 100 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes, (Christen *et al*., 1992).

3.14.4.2 Plating and Counting:

One ml quantities of each sample dilutions 10^5, 10^6 and 10^7 were streaked in dried plate of MaConkey agar media. The culture was incubated at 35 °C for 24 hours, and then colonies were manually counted.

3.14.5. *E.coli* counts:

The count was performed according to (William and Dennis, 1998) using MaConkey agar media and Eosin methylene blue agar (EMB) for identification.

3.14.5.1 Preparation of the media:

The manufacturer's instructions were followed by dissolving 5.5 grams of powder in 100 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes, (Christen *et al.*, 1992).

3.14.5.2 Plating and Counting:

One ml quantities of each sample dilutions 10^5, 10^6 and 10^7 were streaked in dried plate of Maconkey agar media. The culture was incubated at 35 °C for 24 hours, and then colonies were used for further confirmation of the presence of E. coli by streaking a loopful from each colony on Eosin methylene blue agar (EMB) for identification of colonies which show brilliant green, which are characteristic features of growth of *E. Coli* in the medium . The isolates were further characterized by biochemical tests according to Cowan and steel (1993) Special attention was paid to the pattern of reactions of the organism in IMVIC tests, and the positive tests were recorded for a positive *E. coli* count.

3.14.6. *Staphylococcus aureus* counts:

The count was performed according to (Christen *et al*., 1992 and Marshall, 1993) using Mannitol salt agar.

3.14.6.1. Preparation of the media:

The manufacturer's instructions were followed by dissolving 11.1 grams of powder in 100 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes, (Christen *et al*., 1992).

3.14.6.2. Plating and Counting:

One ml quantities of each sample dilutions 10^5, 10^6 and 10^7 were streaked in dried plate of Mannitol salt agar. The culture was incubated at 35 ± 2 °C for 24 hours, where colonies of *staphylococcus aureus* were recognized by bright yellow zones formation in Mannitol salt agar (Jawez and Adel, 1990). And then colonies were manually counted.

3.14.7. *Lactobacillus* count:

The count was performed according to (Murray *et al*., 1995) using Mann-Rogosa-Sharpe (MRS).

3.14.7.1 Preparation of the media:

The manufacturer's instructions were followed by dissolving 7 grams of powder in 100 ml of distilled water, heated to boiling point and sterilized in an autoclave at 121 ^{o}C for 15 minutes, (Murray *et al*., 1995).

3.14.7.2 Plating and Counting:

One ml quantities of each sample dilutions 10^5, 10^6 and 10^7 were streaked in dried plate of MRS. The culture was incubated at 35 ± 2 °C for 24 hours. And then colonies were manually counted.

3.15. Sensory evaluation:

The quality of cheese stored in refrigerator were Judged by 10 untrained panelists for colour, flavor, texture and general appearance using sensory evaluation sheet (Appendix I) as describes by Larmond, (1977).

3.16 .Statistical analysis:

Statistical analysis programme (SPSS, 1998) Social Package for Statistical Science (version. 17) was used. General Linear Model was used to determine the effect of coagulant types, milk source and storage period on the physiochemical, microbiological and organoleptic properties of white cheese. Least Significant Difference test was used for the mean separation between the treatments. Level of Significance ($P<0.05$) was used.

CHAPTER FOUR

RESULTS

4.1. Purification of Milk-Clotting Enzyme from *Helianthus annuus* seeds:

4.1.1. Extraction:

The experiments in (Table 1) showed that *Helianthus annuus* seeds extracted with 5% NaCl in 50 mM sodium acetate buffer (pH 5.0) had higher milk-clotting activity compared to that extracted with the same buffer without adding 5% NaCl. Therefore, 5% NaCl in sodium acetate buffer (pH 5.0) was used as an extracting buffer throughout the study. The extraction period for the enzyme varied from 24 to 120 h, and it was found that the extraction time had a high significant effect on the clotting time and the best results of coagulation time (13.50±3.11 seconds) were in 24 hrs (Table 2).

4.1.2 Milk clotting activity:

Table 3 summarized the milk clotting and proteolytic activity of the partially purified enzyme compared to other coagulants. As shown *Helianthus annuus* had higher clotting and lower proteolytic activities compared to other plant enzymes. It was found that clotting and proteolytic activities of the partial purified enzyme were 241.6 units/ml and 0.07 (OD 660 nm), respectively, and that of rennet were 249.6 units/ml and 0.05 (OD 660 nm), respectively. The results obtained indicated that *Helianthus annuus* was highly active compared to rennet.

Table (2): Efficiency of extractants to extract milk-clotting activity and clotting time from *Helianthus annuus* seeds:

Extractant	**Milk clotting activity (U/ml)**	**Clotting time (sec)**	**L.S**
50 mM Na acetate buffer (pH 5.0)	161.00 ± 4.59	26.00 ± 5.16	*
5%NaCl in acetate buffer (pH 5.0)	185.33 ± 14.50	21.13 ± 7.38	*

*: significant ($p \leq 0.05$). *Helianthus annuus* seeds (10 g) were grounded in mortar, and then extracted with 100 ml of 5% NaCl in acetate buffer at 4°C for 24 h.

Table (2): The effect of extraction time on the clotting time:

Extraction time (hrs)	Clotting time (sec) M ± SD	Level of significant
6	30.00 ± 2.94^{ab}	**
12	20.00 ± 1.63^{bcefg}	**
24	13.50 ± 3.11^{cdfgh}	**
36	21.00 ± 5.89^{bcdef}	**
48	22.00 ± 5.89^{bcde}	**
72	23.50 ± 5.32^{bcd}	**
96	26.00 ± 3.65^{abc}	**
120	32.50 ± 1.91^{a}	**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

Table (3): Ratio of Milk clotting activity/ proteolytic activity of *Helianthus annuus* seeds enzyme and rennet:

Enzyme	**Clotting activity (units/ml)**	**Proteolytic activity (OD 660 nm)**	**Ratio (units/OD660 nm)**
Rennet	249.6	0.05	4992
Partial purified ***Helianthus annuus***	241.6	0.07	3451.4

4.1.3 Ammonium Sulfate Fractionation:

Ammonium sulfate precipitation was performed as one purification step of *Helianthus annuus* milk-clotting protease. The crude extract (600 mL in 50 mM sodium acetate buffer, pH 5.0) was precipitated with ammonium sulfate (0-80% saturation) as described in Material and Methods. The results showed that the (30-50%) saturation fraction had the highest milk-clotting activity. This fraction was dialyzed overnight against 50 mM sodium acetate buffer (pH 5.0). The partially purified enzyme obtained from the fractionation was subjected to further fractionation with ammonium sulfate (in the saturation range of 30-50%). Separate ammonium sulfate fraction with higher specific activity, were collected at 5% saturation intervals over the range of 30-50% saturation. The results showed that ammonium sulfate fractions of 30-35%, 35- 40%, 40-45% and 45-50% saturation had the same milk clotting activity; therefore these four fractions were pooled together as one fraction (30-50%). The combined fraction was dialyzed overnight against 50 mM sodium acetate buffer (pH 5.0) with several changes of the buffer, and then used for further study.

Table (4) summarized the purification results of milk-clotting enzyme from *Helianthus annuus* seeds. The partial purification step of the enzyme resulted in 4.31 folds purity with a yield of 10.87 % and specific activity of 0.2886 (U/mg protein).

Table (4): Summary of the partial purification of milk-clotting enzyme from *Helianthus annuus* seeds

Purification Steps	Volume (ml)	MCA (units/ml)	Protein content (mg/ml)	Total activity (Units)	Specific activity (Units/mg)	Yield (%)	Purification (Fold)
Crude extract	30	185.3	2767	5559	0.0670	100.0	1.0
$AmSO_4$ *(30–50%)	2.5	241.6	837	604	0.2886	10.87	4.31

a Specific activity= MCA /protein content

b Total activity = MCA x volume of fraction

c Yield = $\frac{\text{total activity of the fraction}}{\text{total activity of crude enzyme}} \times 100$

d Fold of purification =Sp. activity of fraction/ Sp. of the crude fraction

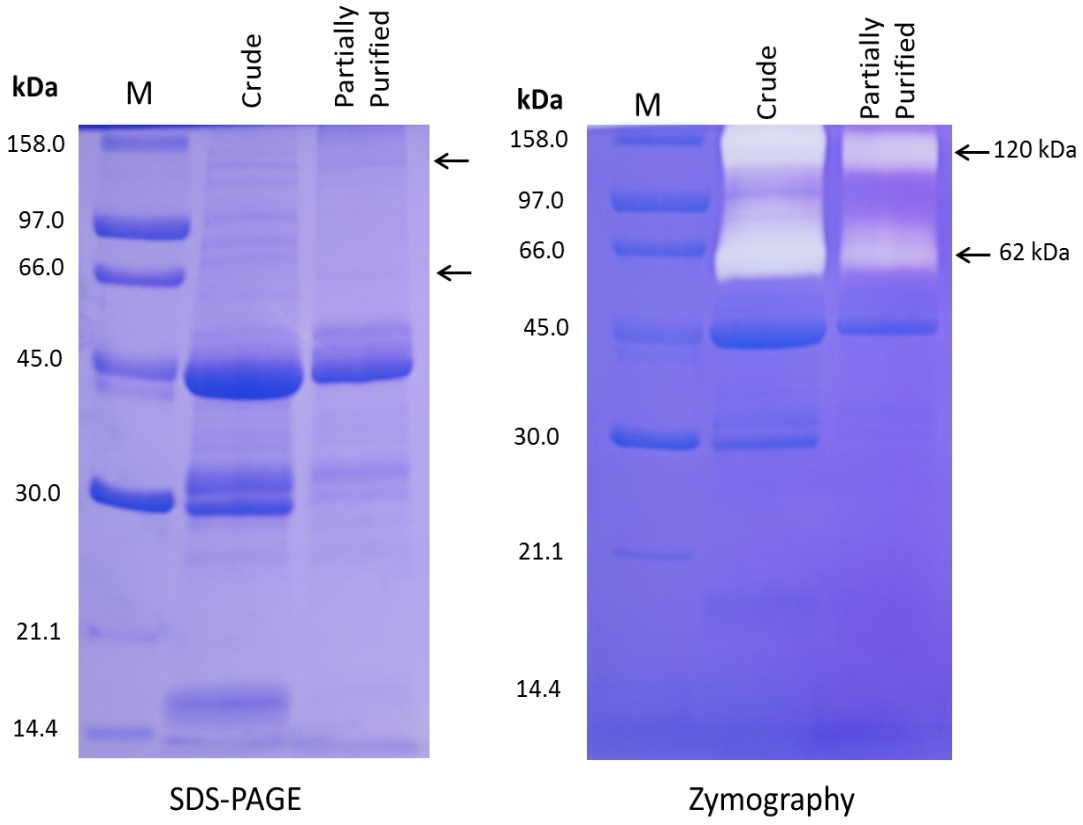
* Combined fraction.

4.1.4. Characterization of milk-clotting enzyme from *Helianthus annuus* seeds:

4.1.4.1. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Zymogarphy

Sodium dodecyl sulphate polyacrylamide gel electrophoresis under reducing conditions (SDS-PAGE) was used to check the purity and to determine the molecular mass of the enzyme. As shown in Figure 1(a), the enzyme was electrophoressed as two bands which suggested the monomeric nature of the enzyme.

As shown in Figure 1(b), Zymogram activity staining on 12% SDS non reduced polyacrylamide gel using casein as substrate showed that a well resolved band on reduced SDS-PAGE corresponding to the protease activity. The molecular mass of the protease was determined by comparison of the migration distance of the protease to that of standard marker proteins as shown in Figure 2. The molecular mass of the partial purified enzyme was calculated to be 120 and 62 kDa.



(a) (b)

Figure (1): (a) SDS-PAGE, and (b) Activity staining (Zymography) of partial purified milk-clotting enzyme from *Helianthus annuus* seeds. In both, Lane 1 molecular weight standards from high molecular weight descending; MBP-β- galactosidase, Phosphorylase b, Bovine Serum Albumin, Ovalbumin, Carbonic anhydrase, Soybean trypsin inhibitor, and α-Lactalbumin. Lane 2, (60 µg) crude extract of *Helianthus annuus*. Lane 3 (60 µg) active partial purified *Helianthus annuus* enzyme.

4.2. Cheese yield percentage and coagulation time of white soft cheese using the partial purified *Helianthus annuus* enzyme:

Table (5) showed the effect of milk source on the yield and coagulation time of the manufactured white soft cheese. The results indicated that high significant differences (P>0.01) in the yield percentage of cow's milk cheese compared to goats' one: 21.60 ± 4.43% and 17.90 ± 2.03%, respectively. And there are not significant differences in the coagulation time: 2.12 ± 1.57 hrs and 2.13 ± 1.87 hrs for cows' and goats' milk, respectively.

Table (6) demonstrated the effect of coagulant type on the yield percentage and coagulation time of white soft cheese. The results showed there is a significant difference in the yield percentage of cheese produced by rennet and (Sunflower extract / H) (18.73 ± 1.10% and 20.78 ± 5.37%, respectively). The results of *sunflower* cows' cheese had higher yield percentage compared to those from rennet. On the other hand, the results indicated that high significant differences were found in coagulation time of rennet and Sunflower (0.65 ± 0.24 and 3.60 ± 0.27 hrs, respectively).

Table (7) revealed the effect of interaction between milk source and coagulant type on the yield percentage and coagulation time of the white soft cheese. The results showed high significant differences in the interaction between milk source and coagulant type on the yield percentage; 17.80±0.39% and 25.40±0.39% of cows' rennet and Sunflower coagulant cheeses, respectively. Whereas, the yields of goats' rennet and Sunflower cheeses are: 19.65±0.39% and 16.15±0.39%, respectively.

Table (5) Effect of milk source on the yield and coagulation time of white soft cheese:

Parameters	Milk source		Level of significant
	Cow	Goat	
Yield (%)	21.60 ± 4.43^{a}	17.90 ± 2.03^{b}	**
Coagulation time (hrs)	2.21± 1.45	2.31± 1.67	NS

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.01$).

NS: Not significant

Table (6): Effect of coagulant type on the yield and coagulation time of white soft cheese:

Parameters	Coagulant type		Level of significant
	Rennet	***H***	
Yield (%)	18.73 ± 1.10^{b}	20.78 ± 5.37^{a}	*
Coagulation time (hrs)	0.91 ± 0.07^{b}	3.60 ± 0.27^{a}	**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

H: *Helianthus annuus*

Table (7) Effect of milk source and coagulant type on the yield and coagulation time of the manufactured white soft cheese:

Item	Milk source				L.S
	Cow		Goat		
	Rennet	*H*	Rennet	*H*	
Yield (%)	17.80±0.3[b]	25.40±0.39[a]	19.65±0.35[a]	16.15 ± 0.07[b]	**
Coagulation time (hrs)	0.95 ± 0.07	3.47 ± 0.02	0.88± 0.06	3.74 ± 0.37	**NS**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

H: *Helianthus annuus*

4.3. Milk analysis:

4.3- 1. Chemical composition of milks:

The average chemical composition of the milk used for cheese making in this study were as follows: Titratable acidity 0.16% and 0.17%; total solids 11.85% and 12.15%; protein 5.136% and 6.357%; fat 4.3% and 3.9% ; ash contents 0.65% and 0.7%, for cow and goat, respectively.

4.3-2. Microbiological analysis of milks:

The average microbiological counts of cow and goat milk's used for cheese processing in this study were as follows: 9×10^6, 2.5×10^3, 2×10^2 and 8×10^{10} ; and 10×10^6, 2×10^3, 1.8×10^2 and 6×10^{10} CFU/ml for total bacterial, *coliform*, *E.coli*, and *lactobacillus* counts, respectively.

4.4 Chemical composition of white soft cheese:

4.4.1 Effect of milk source on chemical composition of white soft cheese:

Table (8) showed the effect of milk source on chemical composition of white cheese. Total solids and ash contents of white soft cheese were significantly ($P< 0.01$) higher in cheese made with goats' milk compared to those made with cows' one (table 4-8). The values of total solids and ash were 39.32 ± 3.48 % and 2.60 ± 0.31 % in cheese made with goats' milk compared with 38.04 ± 3.93 % and 1.85 ± 0.26 % in cheese made with cows' one, respectively.

The protein content of cheese was significantly ($P< 0.01$) affected by the type of milk (table 4-8). Cheese made with goats' milk showed the highest protein content (17.67 ± 2.35 %), while those made with cows' milk, had the lowest value (16.16 ± 2.17 %).

The results demonstrated that fat and acidity contents of white soft cheese were significantly (P< 0.01) higher in cheese made with cows' milk (19.36 ± 3.45 % and 0.86 ± 0.06 %) compared to those made with goats' milk (16.98±2.26 % and 0.82 ± 0.14 %), respectively, (table 8).

Tyrosine and tryptophan contents (mg/100g cheese) were significantly (P< 0.01) higher in cheese made with goats' milk (319 ± 68.99 and 175 ± 78.39 mg/100g cheese) than those made with cows' milk (109±53.66 and 158±47.68 mg/100g cheese), respectively, (table 8).

Table (8): Effect of milk source on chemical composition of white soft cheese:

Chemical composition	Milk source		Level of significant
	Cow	Goat	
Total solids (%)	38.04 ± 3.93^{b}	39.32 ± 3.48^{a}	**
Ash (%)	1.85 ± 0.26^{b}	2.60 ± 0.31^{a}	**
Protein (%)	16.16 ± 2.17^{b}	17.67 ± 2.35^{a}	**
Fat (%)	19.36 ± 3.45^{a}	16.98 ± 2.26^{b}	**
Acidity (%)	0.86 ± 0.06^{a}	0.82 ± 0.14^{b}	**
Tyrosine (mg/100 gm cheese)	109 ± 53.66^{b}	319 ± 68.99^{a}	**
Tryptophan (mg/100 gm cheese)	158 ± 47.68^{b}	175 ± 78.39^{a}	**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

4.4.2. Effect of coagulant on chemical composition of white soft cheese:

Table (9) shows the effect of coagulant type on chemical composition of white cheese:

Total solids and ash contents of white soft cheese were significantly (P< 0.01) higher in cheese made with rennet compared to those made with *Helianthus annuus* (table 9). The values of total solids and ash were 41.49±2.19 % and 2.38 ± 0.45 % in cheese made with rennet compared with 35.87±2.70 % and 2.07 ± 0.46 % in those made with *Helianthus annuus*, respectively.

The protein content of cheese were significantly (P< 0.01) affected by type of coagulant (table 9). Cheese made with rennet showed the highest protein content (18.14 ± 1.79 %); while those made with *Helianthus annuus* had the lowest protein (15.70±2.27 %**).**

Fat contents of white soft cheese was significantly (P< 0.01) higher in cheese made with rennet (20.41±2.70 %) compared to that made with *Helianthus annuus* (15.93 ± 1.56 %), (table 9).

Acidity was significantly (P<0.01) higher in cheese made with *Helianthus annuus* 0.85±0.13 % compared to the cheese made with rennet 0.83 ± 0.09 %, (table 9).

Tyrosine (mg/100g cheese) was significantly (P< 0.01) higher in cheese made with rennet (233±106.84 mg/100g cheese) than that made with *Helianthus annuus* (196 ± 134.75 mg/100g cheese), (table 9). Whereas tryptophan were significantly (P<0.01) higher in cheese made with *Helianthus annuus* 216 ±

54.02 (mg/100g cheese) compared to the cheese made with rennet 117 ± 24.97(mg/100g cheese), respectively, (table 9).

4.4.3. Effect of the storage period on chemical composition of white cheese:

The chemical composition of the white soft cheese samples was presented in Table (10). The total solids content decreased significantly ($P<0.05$) (38.61±4.81%) at day zero until week3 then increased from week 4 until week 6 then decreased at week 7 thereafter decreased at week 9 (38.68±5.18%).

The ash contents of the cheese were significantly ($P< 0.01$) affected by the storage time (table 10). The lowest ash contents was at day one (2.01±0.41%) and the highest value was at week8 (2.46±0.40%). As the storage period progressed the ash content significantly increased until week 2 then decreased at week 3, 4 and increased till week 8 and at the last week decreased.

The storage time significantly ($P< 0.01$) affected the protein content of the white cheese (table 10). The protein content increased till week 7 then decreased at week 9. The highest value was 19.44±2.40 % at week 7 while the lowest protein content (14.57±1.36 %) was recorded at zero time.

The titratable acidity of the white soft cheese increased significantly ($P< 0.01$) with advanced storage period. The acidity developed from 0.79±0.04% at zero time up to 0.93±0.08% at week, 3, and then decreased to 0.92±0.08 at week 4 up to 0.72±0.14% at week 9.

Table (9) Effect of coagulant on chemical composition of white soft cheese:

Chemical Composition	Coagulant		Level of significant
	Rennet	***H***	
Total solids (%)	41.49±2.19[a]	35.87±2.70[b]	**
Ash (%)	2.38 ± 0.45[a]	2.07 ± 0.46[b]	**
Protein (%)	18.14 ± 1.79[a]	15.70±2.27[b]	**
Fat (%)	20.41±2.70[a]	15.93 ± 1.56[b]	**
Acidity (%)	0.83 ± 0.09[b]	0.85 ± 0.13[a]	**
Tyrosine(mg/100 gm cheese)	233±106.84[a]	196 ± 134.7[b]	**
Tryptophan (mg/100 gm cheese)	117 ± 24.97[b]	216 ± 54.02[a]	**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

NS: Not significant

H: *Helianthus annuus*

The fat content of the white cheese was significantly (P< 0.01) affected by the storage time (table 10). The highest value was (22.75±1.06%) at zero time while the lowest value (17.38±2.56%) was at week 8. As the storage period progressed the fat contents decreased.

The tyrosine contents of the cheese significantly (P< 0.01) increased at the beginning of the storage (166 ± 81.60 mg/100 gm cheese) till week 5 which recorded 275 ± 148.36 mg/100 gm cheese, and then decreased at the end of storage period (226 ± 141.53 mg/100 gm cheese) at week 9. However, tryptophan contents significantly (P< 0.01) decreased gradually, at zero time recorded the highest value (206±84.71 mg/100 gm cheese) while the lowest values (137±85.16; 146±56.23 mg/100 gm cheese) were at week 2 and 3 table, (10).

Table (10): Effect of Storage period on chemical composition of white soft cheese:

Storage period/ wks	TS (%)	Ash (%)	Protein (%)	Fat (%)	Acidity (%)	Tyrosine (mg/100 gm cheese)	Tryptophan (mg/100 gm cheese)
Day 0	38.61 ± 4.8^{abcd}	2.01 ± 0.41^{efghi}	14.57 ± 1.36^{ij}	22.75 ± 1.0^{abc}	0.79 ± 0.04^{ef}	166 ± 81.60^{fghi}	206 ± 84.71^{a}
wk 1	38.55 ± 4.20^{abcd}	2.20 ± 0.42^{cde}	15.42 ± 1.91^{hi}	22.50 ± 0.71^{abce}	0.88 ± 0.02^{cd}	156 ± 104.02^{ghi}	170 ± 87.41^{bcf}
wk 2	38.54 ± 3.65^{abcde}	2.19 ± 0.39^{cdef}	15.61 ± 1.62^{gh}	22.00 ± 1.41^{abc}	0.89 ± 0.03^{bc}	215 ± 100.37^{cde}	137 ± 85.16^{fghj}
wk 3	37.83 ± 3.19^{bcdg}	2.09 ± 0.42^{defgh}	17.95 ± 2.28^{c}	17.75 ± 1.3^{abcdeg}	0.93 ± 0.08^{a}	273 ± 141.31^{ab}	146 ± 56.23^{efghi}
wk 4	38.20 ± 2.75^{abcdf}	2.05 ± 0.56^{efgh}	18.40 ± 0.60^{b}	17.75 ± 3.73^{abcdeg}	0.92 ± 0.08^{ab}	194 ± 102.89^{defg}	174 ± 63.77^{bce}
wk 5	38.54 ± 3.21^{abcde}	2.27 ± 0.76^{bcd}	16.97 ± 1.45^{e}	17.88 ± 3.35^{abcef}	0.89 ± 0.10^{bc}	275 ± 148.36^{a}	150 ± 38.25^{cdg}
wk 6	39.36 ± 4.84^{ab}	2.47 ± 0.57^{a}	16.88 ± 2.39^{ef}	19.75 ± 3.37^{abcd}	0.83 ± 0.12^{de}	188 ± 127.19^{efgh}	177 ± 58.70^{bc}
wk 7	38.85 ± 3.48^{abc}	2.35 ± 0.43^{abc}	19.44 ± 2.40^{a}	18.25 ± 2.71^{abcef}	0.79 ± 0.12^{ef}	252 ± 139.85^{ac}	176 ± 45.63^{bcd}
wk 8	39.70 ± 3.23^{a}	2.46 ± 0.40^{ab}	17.36 ± 2.36^{d}	17.38 ± 2.56^{bcdeh}	0.77 ± 0.11^{fg}	197 ± 120.67^{def}	184 ± 70.54^{b}
wk 9	38.68 ± 5.18^{abcd}	2.15 ± 0.26^{defg}	16.56 ± 2.95^{fg}	19.00 ± 4.20^{abcde}	0.72 ± 0.14^{gh}	2.26 ± 141.53^{bd}	147 ± 41.57^{degh}
Level of significant	*	**	**	**	**	**	**

Mean values bearing different superscripts within rows are significantly different ($p\leq0.05$)

4.4.4 Effect of interaction between milk source and coagulant type on chemical composition of white soft cheese:

Type of milk source (cow and goat) and type of coagulant (rennet and partially purified *H. annuus* enzyme /H) had a highly significant ($P<0.01$) effect on total solids content of white cheese (Table 11). Total solids contents were higher in cheese made of cow's milk with rennet (41.66%) compared to total solids contents in goats' milk cheese with the partially purified *H. annuus* enzyme (37.31%).

Protein content of white soft cheese was found to be significantly ($p<0.01$) affected by milk source and coagulant type (table 11). The protein content was higher (18.81%) in cheese made of goats' milk with rennet, and lower (14.86%) in cheese made of cow's milk with the partially purified *H. annuus* enzyme.

The fat content of white cheese was significantly ($P<0.01$) higher in cheese made with cow's milk and rennet (22.28%), compared to (18.55%) and (15.40%) in cheese made of goats' milk with rennet and *H. annuus* enzyme, respectively (table 11).

Titratable acidity was significantly ($P<0.01$) affected by coagulant type and milk source (table 11). Highest titratable acidity was found in cheese made of cow's milk with *H. annuus* enzyme which was 0.89%. However, lowest titratable acidity was 0.81% in *H. annuus* cheese with goats' milk.

Tryptophan content (mg/ 100g cheese) was significantly ($P<0.01$) higher in cheese made with goats' milk with *H. annuus* enzyme 247.31 (mg/ 100g cheese). The lowest tryptophan content was 102.96 in rennet cheese with

goats' milk. While tyrosine content (mg/ 100g cheese) was significantly (P<0. 05) affected by coagulant type and milk source (table 11). Highest value was 329.46 (mg/ 100g cheese) recorded in cheese of goats' milk with rennet. The lowest one was 82.91 (mg/ 100g cheese) in cheese of cow's milk with *H. annuus* enzyme.

Coagulant type and milk source was not significantly (P>0.05) affect ash content of the white cheese (table 11). However, ash was higher (2.76%) in goats' milk cheese with rennet, and lower (1.70%) in cow's milk with partially purified *H. annuus* enzyme.

Table (11): Effect of interaction between milk source and coagulant type on chemical composition of white soft cheese:

chemical composition	Milk source				S.E	L.S
	Cow		Goat			
	Coagulant type		Coagulant type			
	Rennet	*H*	Rennet	*H*		
TS (%)	41.66±	34.42±	41.33±	37.31±	0.21	**
Ash (%)	2.0±	1.70±	2.76±	2.45±	0.03	NS
CP (%)	17.46±	14.86±	18.81±	16.53±	0.05	**
Fat (%)	22.28±	16.45±	18.55±	15.40±	0.21	**
Acidity (%)	0.84±	0.89±	0.82±	0.81±	0.01	**
Tyrosine (mg/100 gm cheese)	135.69 ±	82.91±	329.46 ±	309.17±	5.87	*
Tryptophan (mg/100 gm cheese)	131.41 ±	185.01±	102.96 ±	247.31±	2.51	**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

NS: Not significant.

L.S = Level of significance for interaction

H: *Helianthus annuus*

4.4.5. Effect of interaction between milk source and storage period on chemical composition of white soft cheese:

Milk source significantly (P< 0.01) affected the total solids contents of white cheese during storage period (Table 12). The total solids content of cheese was higher in cheese made with goats' milk. It decreased gradually from 40.20% at zero time to 38.15% at week 4 and then increased with the increase of storage. However, in cheese made with cow's milk, the total solids increased from 37.02% at zero time to 38.80% at week 2 and then decreased to 37.33% at week 3 and then fluctuated until at the end of storage (36.53%) in week 9.

The ash content significantly (P< 0.01) affected by the milk source during storage period (table 12). Cow's milk cheese ash increased from 1.65% at zero time to 1.88 and 1.89% at weeks 1 and 2, respectively, then decreased gradually till reached 1.61% at week 5, then fluctuated and finally increased to 2.04% in week 9. While ash contents of goats' milk cheese increased from 2.38% at zero time to 2.53% at week 1 then showed decreased and increased until reached 2.26% at week 9.

Protein content was significantly (P<0.01) affected by milk source during storage (table 12). The protein content was 14.25% at zero time then fluctuated until reached the highest value 19.47% at week 3 and decreased to 14.30% at week 9, in cheese made with cow's milk, while the protein content increased gradually from 14.89% at zero time to 21.38% at week 7 then decreased gradually to 18.82% at week 9, in cheese made with goats' milk.

The fat content of cheese was significantly ($P<0.01$) affected by milk source during storage (table 12). The fat content in cheese with cow's milk increased from 19.63% at zero time to 22.00% at week 9, while in goats' milk cheese the fat content increased from 17.25% at zero time then increased and decreased to 16.00% at weeks 8 and 9.

The milk source significantly ($P<0.01$) affected the titratable acidity of the white cheese during storage period (table 12). The titratable acidity gradually increased from 0.78% at zero time to 0.95% at week 3 then gradually decreased to 0.85% at week 9 in cheese made with cow's milk. However, with goats' milk cheese, the titratable acidity of cheese gradually increased from 0.81% at zero time to 0.96% at week 4, and then decreased to 0.59% at week 9.

The tyrosine content (mg/100g cheese) was significantly ($P<0.01$) affected by the milk source during storage period of cheese (table 12). Tyrosine content of cheese made with cow's milk increased from 94.62 (mg/ 100g cheese) at zero time to 146.26 (mg/100g cheese) at week 3 then decreased to 94.31 (mg/ 100g cheese) at week 9. The highest tyrosine content was 405.52 (mg/ 100g cheese) at week 5 in goats' milk cheese and the lowest tyrosine content was 236.81 (mg/ 100g cheese) at zero time.

The milk source significantly ($P<0.01$) affected tryptophan content (mg/100g cheese) of cheese during storage period (table 12). The tryptophan content was 196.77 and 215.39 (mg/100g cheese) at zero time and then fluctuating to 145.63 and 148.08 (mg/100g cheese) at week 9, in cheeses made with cow's and goats' milks, respectively.

Table (12): Effect of interaction between milk source and storage period on chemical composition of white soft cheese:

Storage period (wks)	TS (%)		Ash (%)		Protein (%)		Fat (%)		Acidity (%)		Tyrosine (mg/100 gm cheese)		Tryptophan (mg/100 gm cheese)	
	Cow	Goat	Cow	Goat	Cow	Goat	Cow	Goat	Cow	Goat	Cow	Goat	Cow	Goat
0 Time	37.02	40.20	1.65	2.38	14.25	14.89	19.63	17.25	0.78	0.81	94.62	236.81	196.77	215.39
wk 1	38.20	38.83	1.88	2.53	15.63	15.20	18.25	15.75	0.87	0.89	66.92	246.05	173.40	165.91
wk 2	38.80	38.28	1.89	2.50	15.48	15.74	19.75	17.25	0.91	0.87	127.40	302.92	90.70	182.39
wk 3	37.33	38.33	1.75	2.43	19.47	16.42	18.00	17.50	0.95	0.91	146.26	399.88	128.34	162.76
wk 4	38.25	38.15	1.63	2.48	18.36	18.45	17.00	18.50	0.88	0.96	114.02	273.35	179.88	168.11
wk 5	38.10	38.98	1.61	2.93	15.68	18.27	20.00	15.75	0.85	0.93	145.43	405.52	152.60	148.22
wk 6	38.33	40.40	1.98	2.96	15.15	18.61	20.50	19.00	0.84	0.82	72.297	304.62	165.61	188.20
wk 7	38.63	39.08	1.95	2.75	17.50	21.38	19.75	16.75	0.85	0.74	141.07	363.71	172.05	180.66
wk 8	39.28	40.13	2.10	2.83	15.79	18.94	18.75	16.00	0.87	0.66	90.66	303.29	177.15	191.65
wk 9	36.53	40.83	2.04	2.26	14.30	18.82	22.00	16.00	0.85	0.59	94.31	357.02	145.63	148.08
S.E	0.48		0.06		0.11		0.47		0.01		13.12		5.62	
L.S	**		**		**		**		**		**		**	

S.E = Standard error

L.S = Level of significance

4.4.6. Effect of interaction between coagulant type and storage period on chemical composition of white soft cheese:

Coagulant type significantly (P<0.01) affected the total solids contents of white cheese during storage period (Table 13). The total solids content of cheese with rennet decreased from 42.37% at zero time to 38.23% at week 9. While, cheese with the partially purified *H. annuus* enzyme (H) decreased from 34.85% at zero time, then fluctuating until reached 38.15% at week 9.

Ash contents was affected significantly (P<0.01) by coagulant type during storage period of white cheese (Table 13). The ash content of cheese with rennet increased from 2.03% at zero time to 2.61% at week 6 then decreased to 2.27% at week 9. Whereas, cheese with partially purified *H.* enzyme, had the lowest value (2.00%) at zero time and the highest one (2.38%) at week 8.

Protein contents of white cheese were significantly (P<0.01) increased due to coagulant type during storage period (Table 13). The lowest protein contents of cheese were 15.79 and 13.34% at zero time and the highest values were 20.27 and 18.61% at week 7, for rennet and partially purified *H.* enzyme, respectively.

Coagulant type had significant (P<0.01) effect on the fat contents of white cheese during storage period (Table 13). The fat content of cheese with rennet and the partially purified *H.* enzyme were 21.63 and 15.25% respectively at zero time therefore the values fluctuated until the end of storage.

The titratable acidity of white cheese was significantly (P<0.01) affected by the coagulant type during storage period (Table 13). The titratable acidity of the cheese with rennet increased from 0.77% at zero time and fluctuating to 0.90% at week 6 then decreasing to 0.73% at week 9. While, cheese with partially purified *H.* enzyme increased from 0.82% at zero time, gradually increased to 0.99% at week 3 then gradually decreased to 0.71% at week 9.

The tyrosine content (mg/100g cheese) was significantly (P<0.01) affected by the coagulant type during storage period of cheese (Table 13). Tyrosine content of cheese made with rennet increased gradually from 173.20 (mg/ 100g cheese) at zero time to 264.22 (mg/ 100g cheese) at week 5 then decreased to 236.92 (mg/ 100g cheese) at week 9. While, tyrosine content of cheese made with partially purified *H* enzyme was 158.24 (mg/ 100g cheese) at zero time and then fluctuating till tyrosine content was 214.41 (mg/ 100g cheese) at week 9.

Tryptophan content (mg/100g cheese) significantly (P<0.01) decreased with coagulant type during storage period of cheese (Table 13). The tryptophan content was 136.10 and 276.06 (mg/100g cheese) at zero time and then fluctuating to 108.43 and 185.28 (mg/100g cheese) at week 9, in cheeses made with rennet and partially purified *H.* enzyme, respectively.

Table (13): Effect of interaction between coagulant type and storage period on chemical composition of white soft cheese:

Storage period (wks)	TS (%)		Ash (%)		Protein (%)		Fat (%)		Acidity (%)		Tyrosine (mg/100 gm cheese)		Tryptophan (mg/100 gm cheese)	
	Rennet	H	Rennet	H	Rennet	H	Rennet	H	Rennet	H	Rennet	H	Rennet	H
Day 0	42.37	34.85	2.03	2.00	15.79	13.34	21.63	15.25	0.77	0.82	173.20	158.24	136.10	276.06
wk 1	42.40	34.63	2.40	2.00	17.17	13.67	19.75	14.25	0.89	0.86	194.08	118.88	88.19	251.12
wk 2	41.88	35.20	2.39	2.00	17.11	14.11	20.50	16.50	0.87	0.91	238.67	191.64	90.48	182.60
wk 3	40.48	35.18	2.27	1.90	19.42	16.48	18.50	17.00	0.87	0.99	246.78	299.36	110.41	180.69
wk 4	40.63	35.78	2.35	1.75	18.96	17.85	21.00	14.50	0.86	0.98	247.07	140.30	116.81	231.18
wk 5	41.35	35.73	2.50	2.03	17.35	16.60	19.75	16.00	0.81	0.97	264.22	286.73	114.77	186.06
wk 6	43.45	35.28	2.61	2.33	18.29	15.47	22.75	16.75	0.90	0.77	206.09	170.83	134.62	219.19
wk 7	41.60	36.10	2.40	2.30	20.27	18.61	20.00	16.50	0.85	0.74	289.12	215.65	144.43	208.28
wk 8	42.58	36.83	2.55	2.38	18.89	15.84	19.25	15.50	0.77	0.76	229.57	164.38	127.64	241.16
wk 9	38.23	39.13	2.27	2.03	18.13	14.99	21.00	17.00	0.73	0.71	236.92	214.41	108.43	185.28
S.E	0.48		0.06		0.11		0.47		0.01		13.12		5.62	
L.S	**		**		**		**		**		**		**	

S.E: Standard error L.S: Level of significance for interaction **: ($P<0.01$) H: *Helianthus annuus*

4.4.7 Effect of interaction between milk source, coagulant type and storage period on chemical composition of white soft cheese:

Milk source, coagulant type and storage period showed a highly significant ($P<0.01$) effect on total solids, protein, fat, acidity, tyrosine, tryptophan and a significant ($P<0.05$) effect was found only on ash contents of white soft cheese, (table 14).

The highest total solids content was (45.80%) in goats' milk cheese made using rennet enzyme at zero time of storage period (table 14). At week 1 and 5, the highest total solids content were (42.85 and 41.50) % in goats' milk cheese made using rennet enzyme, respectively, and the lowest ones were (34.45 and 35.00) % in cows' milk cheese made with the partially purified *H. annuus* enzyme (H), whereas, the highest total solids contents were (42.70, 40.70, 41.15, 43.90, 43.00 and 42.75) % in cows' milk cheese made with rennet enzyme at weeks 2, 3, 4, 6, 7 and 8, respectively, and the lowest total solids contents were (33.95 and 34.90 %) in cows' milk cheese made with *H.* enzyme at weeks 3 and 7, respectively. The highest total solids contents were 45.55% in goats' milk cheese made with *H* enzyme and the lowest one was (32.70 %) in cows' milk cheese made with the *H* enzyme, at week 9 of storage period.

The highest ash content was (3.23%) in goats' milk cheese made using rennet enzyme at week 6 of storage period, while the lowest content value was (1.30%) in cows' milk cheese made with *H.* enzyme at week 4 of storage period (table 14). The results showed higher ash contents in both cheeses made with rennet enzyme with cows' and goats' milks than those made with *H.* enzyme, except in goats' milk cheese made with the *H.*enzyme at zero time were slightly higher compare to rennet one (2.45

and 2.30)%, respectively. And were slightly equals in cows' milk cheese made with both rennet enzyme and *H.*enzyme at the end of storage period, week 9 (2.04 and 2.05) %, respectively.

The highest protein content was 21.49% in goats' milk cheese made with rennet enzyme at week 7, whereas, the lowest content was 12.55% in cows' milk cheese made with *H.* enzyme at week 9 (table 14). Cheeses made with rennet from all source of milk were higher in protein content than those made with *H.* enzyme during storage period.

The cheese made with rennet from all milk sources were higher in fat contents in comparison with those made with *H.* enzyme during storage period (table 14). The highest and lowest fat contents were recorded in cows' milk cheese. The highest value (25.50%) was in cheese made with rennet enzyme at week 9 and the lowest one (13.00%) was in cheese made with *H.* enzyme at week 4.

Cheese made with *H.* enzyme of cows' milk was higher in titratable acidity content in comparison with that made with rennet during storage (table 14). Whereas, the cheese made with *H.* enzyme from goats' milk was higher in titratable acidity content in comparison with that made with rennet until week 5 then became lower than the rennet one until the end of storage period. Both the highest and lowest titratable acidity contents were recorded in goats' milk cheese made with *H.* enzyme. The highest titratable acidity content was 1.03% at week 5 while, the lowest one was 0.57% at week 9 of storage period.

The highest tyrosine content was 452.46 (mg/100 gm cheese) in goats' milk cheese made with *H.* enzyme at week 3 and the lowest one (25.29 mg/100

gm cheese) was in cows' milk cheese made with the same enzyme at week 1 of storage period (table 14).

The tryptophan contents were significantly ($P<0.01$) higher in goats' milk cheese (table 14). The highest value (311.17 mg/100 gm cheese) was in cheese made with *H.* enzyme at zero time and the lowest value (78.53 (mg/100 gm cheese) was in cheese made with rennet at week 1.

Table (14): Effect of interaction between milk source, coagulant type and storage period on chemical composition of white soft cheese:

Storage period (wks)	TS (%)				Ash (%)				Protein (%)			
	Cow		Goat		Cow		Goat		Cow		Goat	
	Rennet	H	Rennet	H	Rennet	H	Rennet	H	Rennet	H	Rennet	H
Day 0	38.94	35.10	45.80	34.60	1.75	1.55	2.30	2.45	15.53	12.97	16.06	13.72
wk 1	41.95	34.45	42.85	34.80	2.10	1.65	2.70	2.35	17.12	14.15	17.21	13.19
wk 2	42.70	34.90	41.05	35.50	2.08	1.70	2.70	2.30	16.79	14.17	17.42	14.06
wk 3	40.70	33.95	40.25	36.40	1.94	1.55	2.60	2.25	21.10	17.84	17.74	15.11
wk 4	41.15	35.35	40.10	36.20	1.95	1.30	2.75	2.20	18.90	17.81	19.01	17.89
wk 5	41.20	35.00	41.50	36.45	1.90	1.31	3.10	2.75	15.95	15.41	18.75	17.78
wk 6	43.90	32.75	43.00	37.80	2.00	1.95	3.23	2.70	16.59	13.72	19.99	17.23
wk 7	43.00	34.25	40.20	37.95	2.00	1.90	2.80	2.70	19.05	15.95	21.49	21.27
wk 8	42.75	35.80	42.40	37.85	2.20	2.00	2.90	2.75	17.50	14.07	20.27	17.61
wk 9	40.35	32.70	36.10	45.55	2.04	2.05	2.50	2.01	16.06	12.55	20.20	17.44
S.E	0.68				0.08				0.16			
L.S	**				*				**			

S.E = Standard error L.S = Level of significance for interaction H: *Helianthus annuus*

Contd table (14):

Storage period (wks)	Fat (%)				Acidity (%)			
	Cow		Goat		Cow		Goat	
	Rennet	H	Rennet	H	Rennet	H	Rennet	H
0 Time	22.75	16.50	20.50	14.00	0.74	0.81	0.79	0.82
wk 1	22.50	14.00	17.00	14.50	0.89	0.84	0.89	0.88
wk 2	22.00	17.50	19.00	15.50	0.88	0.93	0.85	0.89
wk 3	19.00	17.00	18.00	17.00	0.92	0.98	0.82	1.00
wk 4	21.00	13.00	21.00	16.00	0.82	0.94	0.90	1.01
wk 5	23.00	17.00	16.50	15.00	0.80	0.90	0.82	1.03
wk 6	24.00	17.00	21.50	16.50	0.81	0.87	0.98	0.66
wk 7	22.50	17.00	17.50	16.00	0.82	0.88	0.87	0.61
wk 8	20.50	17.00	18.00	14.00	0.88	0.86	0.67	0.66
wk 9	25.50	18.50	16.50	15.50	0.85	0.86	0.60	0.57
S.E	0.66				0.02			
L.S	**				**			

S.E = Standard error L.S = Level of significance for interaction N.S = not significant H: *Helianthus annuus*

Contd table (14):

Storage period (wks)	Tyrosine (mg/100 gm cheese)				Tryptophan (mg/100 gm cheese)			
	Cow		Goat		Cow		Goat	
	Rennet	H	Rennet	H	Rennet	H	Rennet	H
Day 0	80.74	108.51	265.66	207.70	152.58	240.96	119.62	311.17
Wk 1	108.54	25.29	279.63	212.48	97.85	248.95	78.53	253.29
Wk 2	127.40	127.40	349.94	255.89	90.70	90.70	90.27	274.51
Wk 3	146.26	146.26	347.30	452.46	128.34	128.34	92.48	233.05
Wk 4	175.45	52.60	318.69	228.00	138.63	221.14	95.00	241.22
Wk 5	123.61	167.25	404.83	406.22	115.18	190.03	114.36	182.08
Wk 6	108.66	35.94	303.53	305.71	156.47	174.75	112.76	263.63
Wk 7	226.20	55.94	352.05	375.36	168.12	175.98	120.74	240.58
Wk 8	142.41	38.91	316.73	289.85	153.25	201.06	102.04	281.27
Wk 9	117.61	71.01	356.23	289.85	113.04	178.21	103.81	192.35
S.E	18.55				7.95			
L.S	**				**			

S.E = Standard error L.S = Level of significance for interaction H: *Helianthus annuus*

4.5. Microbiological quality of white soft cheese:

4.5.1 Effect of milk source on microbiological quality of white soft cheese:

Results in table (15) show the effect of milk source on microbiological quality of white cheese samples during storage.

4.5.1.1 Total viable bacterial count

Total viable bacterial count (TBC) of the white cheese were significantly ($P<0.01$) affected by milk source (table 15). However, cheese made of goats' milk had higher (7.87 ± 0.55Log cfu/ml) TBC in comparison with that made of cows' milk (7.71± 0.60Log cfu/ml), respectively.

4.5.1.2 Coliforms count:

Coliforms counts of the white cheese were significantly ($P<0.01$) affected by milk source (table 15). Although, cheese made using cows' milk were higher (7.22 ± 1.61Log cfu/ml) coliforms counts than those made of goats' milk (6.92 ±1.95Log cfu/ml).

4.5.1.3 *E. coli* count:

E. coli count was not detected in all the cheese samples throughout the storage period.

4.5.1.4 *Staphylococcus aureus* count:

Staphylococcus aureus count was not detected in all the cheese samples throughout the storage period.

4.5.1.5 *Lactobacilli* count:

Lactobacilli counts of the white cheese (table 15) was not found to be affected significantly ($P>0.05$) by milk source. Therefore the cheese made

with goats' milk was slightly higher (1.57±0.36Log cfu/ml) in *lactobacilli* counts than those made with cows' milk (1.51±0.31Log cfu/ml).

Table (15): Effect of milk source on microbiological quality of white soft cheese:

Microbiological analysis (Log cfu/ml)	**Milk source**		**L.S**
	Cow	**Goat**	
TVBC	7.71 ± 0.60^{b}	7.87 ± 0.55^{a}	**
Coliforms	7.22 ± 1.61^{a}	6.92 ± 1.95^{b}	**
E. coli	N.D	N.D	-
Sta. aureus	N.D	N.D	-
Lactobacilli	1.51±0.31	1.57±0.36	NS

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

ND: not detected.

L.S: level of significant

4.5.2 Effect of coagulant types on microbiological quality of white soft cheese:

Table (16) illustrates the effect of coagulant type on microbiological quality of white cheese.

Total viable bacterial count (TVBC) of white soft cheese was significantly ($P<0.01$) higher in cheese made with the partially purified *Helianthus annuus* enzyme (H) 7.83±0.53 (Log cfu/ml) compared to the cheese made with rennet 7.74±0.63 (Log cfu/ml), (table 16).

Coliforms of the white cheese was significantly ($P<0.01$) affected by coagulant type (table 16). Cheese made with rennet had higher counts 7.56±0.69 (cfu/ml) than those of *Helianthus annuus* cheese (H. cheese) 6.57±2.33 (Log cfu/ml), respectively.

E. coli and *Staphylococcus aureus* counts were not detected in all the cheese samples throughout the storage period (table 16).

Coagulant type did not significantly ($P>0.05$) affect *Lactobacilli* counts of the white cheese (table 16). However, cheeses made using *H.* enzyme was slightly higher 10.67±2.55 (Log cfu/ml) in comparison with that made with rennet 10.66±2.30 (Log cfu/ml), respectively.

Table (16): Effect of coagulant on microbiological quality of white soft cheese:

microbiological quality (Log cfu/ml)	**Coagulant**		**Level of significant**
	Rennet	***H***	
TVBC	7.74±0.63[b]	7.83±0.53[a]	*
Coliforms	7.56±0.69[a]	6.57±2.33[b]	**
E. coli	N.D	N.D	-
Sta. *aureus*	N.D	N.D	-
Lactobacilli	10.66±2.30	10.67±2.55	NS

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

N.D: not detected.

H: *Helianthus annuus*

4.5.3. Effect of Storage period on microbiological quality of white soft cheese:

Results in table (17) show the effect of storage period on microbiological quality of the white cheese during ripening.

Total viable bacterial and Coliforms counts of cheese were significantly ($P<0.01$) decreased with an increase in storage time, (table 17). They were decreased from 8.38 ± 0.03 and 7.71±0.23 (Log cfu/ml) at zero time to 7.13 ± 0.19 and 3.97±2.41 Log (Log cfu/ml) at week 9, respectively.

E. coli and *Staphylococcus aureus* counts were not detected in all the cheese samples throughout the storage period, (table 17).

Lactobacilli count had significantly ($P<0.01$) affected by the storage period (table 17). The results indicated that lactobacilli count decreased from 12.06±0.20 (Log cfu/ml) at day zero to 4.73±0.66 (Log cfu/ml) at week 9.

Table (17): Effect of Storage period on microbiological quality of white soft cheese:

Storage period/wks	Microbiological quality		
	TVBC (Log cfu/ml)	**Coliforms** (Log cfu/ml)	**Lactobacilli** (Log cfu/ml)
Day 0	8.38 ± 0.03^{ab}	7.71 ± 0.23^{abe}	12.06 ± 0.20^{abc}
Wk 1	8.39 ± 0.16^{a}	7.68 ± 0.11^{abcef}	12.27 ± 0.04^{a}
Wk 2	8.17 ± 0.26^{abcd}	8.04 ± 0.15^{ab}	12.20 ± 0.30^{ab}
Wk 3	8.31 ± 0.11^{ac}	8.26 ± 0.08^{a}	11.77 ± 0.22^{abcd}
Wk 4	7.16 ± 0.22^{fghi}	7.38 ± 0.20^{bdeg}	11.21 ± 0.18^{abch}
Wk 5	7.91 ± 0.48^{cdef}	8.01 ± 0.08^{abd}	11.38 ± 0.33^{abcf}
Wk 6	7.97 ± 0.40^{bcde}	8.02 ± 0.17^{abc}	11.62 ± 0.11^{abce}
Wk 7	7.28 ± 0.35^{defg}	6.77 ± 1.22^{cefh}	11.28 ± 0.25^{abcg}
Wk 8	7.20 ± 0.30^{efgh}	4.82 ± 2.39^{dfgi}	8.11 ± 2.32^{bcdi}
Wk 9	7.13 ± 0.19^{ghi}	3.97 ± 2.41^{eghj}	4.73 ± 0.66^{cdej}
L. significant	**	**	**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

4.5.4 Effect of interaction between milk source and coagulant type on microbiological quality of white soft cheese:

Types of milk source (cows and goats) and coagulant (rennet and the partially purified *H. annuus* enzyme/ H) showed significant ($P<0.05$) effect on Total viable bacterial count of white cheese (table 18). The count was higher (7.81 Log cfu/ml) in cheese made with cows' milk with *H.* enzyme compared to rennet cheese (7.61 Log cfu/ml), and in goats' milk cheese made with rennet was slightly higher 7.88 (Log cfu/ml) than those of *H.* enzyme.

E. coli and *Staphylococcus aureus* counts were not found at zero time and the organisms were not detected in all the cheese samples throughout the storage period (table 18).

Coagulant type and milk source had not significant ($P>0.05$) effect on Coliforms and Lactobacilli counts of the white cheese (table 18). Coliforms was higher 7.68 (Log cfu/ml) in cow's milk cheese with rennet and lower 6.75 (cfu/ml) in the same milk with *H.* enzyme (table 18). However, Lactobacilli count revealed higher 10.77 (Log cfu/ml) in cow's milk cheese with *H.* enzyme compared to rennet (10.59 (Log cfu/ml). While cheese made of goats' milk with rennet showed higher Coliforms and Lactobacilli counts (7.44 and 10.72 Log cfu/ml) than those of *H.* enzyme (6.39 and 10.56 (Log cfu/ml), respectively.

Table (18): Effect of interaction between milk source and coagulant type on microbiological quality of white soft cheese:

Microbiological quality (Log cfu/ml)	**Milk source**				**S.E**	**L.S**
	Cow		**Goat**			
	Coagulant type		**Coagulant type**			
	Rennet	***H***	**Rennet**	***H***		
TVBC	7.61	7.81	7.88	7.86	0.04	*
Coliforms	7.68	6.75	7.44	6.39	0.09	NS
E. coli	N.D	N.D	N.D	N.D	-	-
S. aureus	N.D	N.D	N.D	N.D	-	-
Lactobacilli	10.59	10.77	10.72	10.56	0.21	NS

S.E = Standard error

L.S = Level of significance for interaction

ND = Not Detected

H: *Helianthus annuus*

4.5.5 Effect of interaction between milk source and storage period on microbiological quality of white soft cheese:

Results in table (19) illustrate the effect of interaction between milk source and storage period on microbiological quality of white soft cheese.

Milk source significantly ($P<0.01$) affected the total viable bacterial counts (table 19). Both cows' and goats' milk counts decreased with increase in storage time. It decreased from 8.41 and 8.35 Logcfu/ml at zero time to 7.15 and 7.12 Log cfu/ml) at week 9, respectively.

Coliforms counts of cheese was significantly ($P<0.05$) decreased by milk source during storage time (table 19). The results indicated that coliforms count decreased from 7.52 and 7.90 Log cfu/ml at day zero to 4.60 and 3.34 (Log cfu/ml) at week 9 for cows' and goats' milks, respectively.

E. coli and *Staphylococcus aureus* counts were not found at zero time and the organisms were not detected in all the cheese samples throughout the storage period (table 19).

Milk source had no significant ($P>0.05$) effect on Lactobacilli count of the white cheese during storage period (table 19). The Lactobacilli count of cheese samples at zero time were 12.01 and 12.11 Log cfu/ml reduced to Log 4.88 and 4.58 (cfu/ml) at week 9 of storage, respectively.

Table (19): Effect of interaction between milk source and storage period on microbiological quality of white soft cheese:

Storage period (wks)	TVBC (Log cfu/ml)		Coliforms (Log cfu/ml)		Lactobacilli (Log cfu/ml)	
	Cow	**Goat**	**Cow**	**Goat**	**Cow**	**Goat**
Day 0	8.41	8.35	7.52	7.90	12.01	12.11
Wk 1	8.28	8.50	7.73	7.62	12.24	12.30
Wk 2	8.24	8.10	8.10	7.97	12.46	11.93
Wk 3	8.38	8.23	8.31	8.21	11.68	11.86
Wk 4	7.12	7.20	7.54	7.23	11.27	11.15
Wk 5	7.62	8.19	8.03	7.99	11.62	11.15
Wk 6	7.72	8.22	8.13	7.92	11.64	11.60
Wk 7	7.08	7.48	7.15	6.39	11.35	11.21
Wk 8	7.08	7.32	5.06	4.58	7.68	8.53
Wk 9	7.15	7.12	4.60	3.34	4.88	4.58
S.E	0.09		0.20		0.48	
L.S	**		*		**N.S**	

S.E = Standard error L.S = Level of significance for interaction **: ($P<0.01$)

* :($P<0.05$) N.S = Not significant

4.5.6 Effect of interaction between coagulant type and storage period on microbiological quality of white soft cheese:

Table (20) illustrates the effect of coagulant type on microbiological quality of white cheese during storage.

Total viable bacterial count (TVBC) of the white cheese were significantly ($P<0.05$) affected by coagulant type during storage period (table 20). The TVBC counts, decreased with increase in storage time, It decreased from 8.37 and 8.39 (Log cfu/ml) at zero time to 7.20 and 7.08 (cfu/ml) at week 9 for the TVBC counts of the cheese with rennet and *H.* enzyme, respectively.

Coliforms counts of cheese samples was significantly ($P<0.01$) decreased by coagulant type during storage time (table 20). The results indicated that Coliforms count decreased from 7.79 and 7.63 (Log cfu/ml) at zero time to 6.12 and 1.83 (Log cfu/ml) at week 9 for rennet and *H.* enzyme, respectively.

E. coli and *Staphylococcus aureus* counts were not found in all the cheese samples throughout the storage period (table 20).

Coagulant type did not significantly ($P>0.05$) affected lactobacilli count during storage period (table 20). Although, results indicated that lactobacilli count decreased from 11.90 and 12.22 (Log cfu/ml) at zero time to 4.86 and 4.60 (Log cfu/ml) at week 9, for rennet and *H.*enzyme, respectively.

Table (20): Effect of interaction between coagulant type and storage period on microbiological quality of white soft cheese:

Storage period (wks)	TVBC (Log cfu/ml)		Coliforms (Log cfu/ml)		*Lactobacilli* (Log cfu/ml)	
	Rennet	**H**	**Rennet**	**H**	**Rennet**	**H**
Day 0	8.37	8.39	7.79	7.63	11.90	12.22
Wk 1	8.38	8.40	7.70	7.65	12.29	12.25
Wk 2	8.30	8.04	8.14	7.94	12.27	12.12
Wk 3	8.30	8.32	8.23	8.29	11.62	11.93
Wk 4	7.08	7.24	7.42	7.35	11.15	11.27
Wk 5	7.83	7.98	7.98	8.05	11.27	11.50
Wk 6	7.83	8.11	7.97	8.08	11.60	11.64
Wk 7	7.08	7.48	7.27	6.27	11.15	11.40
Wk 8	7.08	7.32	7.00	2.63	8.46	7.76
Wk 9	7.20	7.08	6.12	1.83	4.86	4.60
S.E	0.09		0.20		0.48	
L.S	*		**		**N.S**	

S.E = Standard error L.S = Level of significance ** = (P<0.01)

H: *Helianthus annuus*

4.5.7. Effect of interaction between milk source, coagulant type and storage period on microbiological quality of white soft cheese:

Milk source, coagulant type and storage period showed a highly significant ($P<0.01$) effect on total viable bacterial counts (TVBC) and no significant ($P>0.05$) variation were found on coliforms and lactobacilli counts of white soft cheese, (table 21).

Total viable bacterial count (TVBC) reached its maximum count (8.59 Log cfu/ml) at week 1 in cheese made of goats' milks with rennet enzyme and the minimum counts (7.00 Log cfu/ml) was at weeks 7, 8 and 9 in all manufactured cheese types, (table 21).

The highest coliforms counts (8.33 Log cfu/ml) was at week 3 in cheese made of cows' milk with rennet enzyme and the lowest counts (1.45 Log cfu/ml) was at week 9 in cheese made of goats' milk with *H.*enzyme (table 21)**.** At week 4 and 7 Coliforms counts were 7.54, 7.54, 7.15 and 7.15 Log cfu/ml for cheese made of cows' milk with rennet and with *H.*enzymes, respectively.

E. coli and *Staphylococcus aureus* counts were not detected in all the cheese samples throughout the storage period, (table 21).

The maximum count of lactobacilli was 12.49 (Log cfu/ml) at week 2 in cheese made of cows' milk with rennet enzyme and the minimum count was 4.28 (Log cfu/ml) at week 9 in cheese made of goats' milk with *H.* enzyme (table 21)**.** Cheese made of cows' milk with *H.* enzyme had the higher counts than that of rennet except in week 1 and 2 which were in reverse.

Table (21): Effect of interaction between milk source, coagulant type and storage period on microbiological quality of white soft cheese:

Storage period (wks)	TVBC (Log cfu/ml)				Coliforms (Log cfu/ml)				*Lactobacilli* (cfu/ml)			
	Cow		Goat		Cow		Goat		Cow		Goat	
	Rennet	H	Rennet	H	Rennet	H	Rennet	H	Rennet	H	Rennet	H
0 Time	8.39	8.42	8.35	8.35	7.65	7.39	7.93	7.87	11.93	12.10	11.87	12.35
1 wk	8.18	8.39	8.59	8.41	7.81	7.65	7.59	7.65	12.27	12.22	12.31	12.29
2 wk	8.25	8.22	8.34	7.87	8.14	8.06	8.13	7.81	12.49	12.42	12.06	11.81
3 wk	8.32	8.45	8.28	8.19	8.33	8.29	8.13	8.29	11.59	11.77	11.65	12.08
4 wk	7.00	7.24	7.15	7.24	7.54	7.54	7.30	7.15	11.15	11.39	11.15	11.15
5 wk	7.24	8.01	8.43	7.95	8.00	8.06	7.95	8.03	11.39	11.84	11.15	11.15
6 wk	7.39	8.05	8.27	8.17	8.18	8.08	7.77	8.07	11.54	11.74	11.65	11.54
7 wk	7.00	7.15	7.15	7.80	7.15	7.15	7.39	5.39	11.15	11.54	11.15	11.26
8 wk	7.15	7.00	7.00	7.64	7.00	3.11	7.00	2.16	7.56	7.81	9.36	7.70
9 wk	7.15	7.15	7.24	7.00	7.00	2.21	5.24	1.45	4.84	4.92	4.88	4.28
S.E	0.12				0.29				0.68			
L.S	**				N.S				N.S			

H: *Helianthus annuus*

4.6. Sensory characteristics of white soft cheese:

4.6.1. Effect of milk source on Sensory characteristics of white soft cheese:

Results in (table 22) show the effect of milk source on sensory characteristics of white soft cheese.

4.6.1.1 Colour:

The colour of the white cheese was significantly ($P<0.01$) affected by milk source, (table 22). However, cheese made from cows' milk had higher (6.55 ± 1.07) scores in comparison with that made from goats' milk (6.05 ±1.63.

4.6.1.2 Flavour:

Flavour of the white cheese had significantly ($P<0.01$) affected by milk source, (table 22). Although, cheese made using cows' milk were higher (6.34± 2.09) than those made with goats' one (5.57±2.32).

4.6.1.3 Texture:

Milk source was not significantly ($P>0.05$) affected the texture of white cheese, (table 22). However, cheese made with cows' milk secured higher scores (6.67±1.75) compared to that made with goats' milk (6.60±1.98).

4.6.1.4 Saltiness:

Saltiness of the white cheese was significantly ($P<0.05$) affected by milk source (table 24). The cheese made using goats' milk was higher scores (5.13±1.93) than that made with cows' milk (4.71±1.76).

Table (22) Effect of milk source on Sensory characteristics of white soft cheese:

Sensory characteristics	Milk source		Level of significant
	Cow	Goat	
Colour	6.55 ± 1.07^{a}	6.05 ± 1.63^{b}	**
Flavour	6.34 ± 2.09^{a}	5.57 ± 2.32^{b}	**
Texture	6.67 ± 1.75^{a}	6.60 ± 1.98^{b}	N.S
Saltiness	4.71 ± 1.76^{b}	5.13 ± 1.93^{a}	*

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

N.S: Not significant

4.6.2. Effect of coagulant on Sensory characteristics of white soft cheese:

Table (23) shows the effect of coagulant type on sensory characteristics of white cheese. All sensory characteristics of white soft cheese illustrated high significant ($P<0.01$) differences in cheese made with the partially purified *Helianthus annuus* enzyme (H enzyme) compared to rennet one.

The value of colour scores was 5.94 ± 1.60 in cheese made with rennet compared with that 6.66 ± 1.04 in cheeses made with the partially purified *Helianthus annuus* (table 23).

The flavour of cheese were significantly ($P<0.01$) affected by the type of coagulant (table 23). Cheese made with the partially purified *Helianthus annuus*, secured the highest flavour scores (6.98 ± 1.91), while cheese made with rennet had the lowest scores (4.93 ± 2.08%).

The texture of white soft cheese was significantly ($P< 0.01$) higher in cheese made with the partially purified *Helianthus annuus* (5.96 ± 1.45) compared to that made with rennet (5.38 ± 1.49) (table 23).

The result indicated that type of coagulant had a high significant effect ($P<0.01$) on the saltiness of white soft cheese (table 23). Saltiness was higher in cheese made with the partially purified *Helianthus annuus* (5.96 ± 1.45) compared to that produced with rennet (3.88 ± 1.64).

Table (23): Effect of coagulant type on sensory characteristics of white soft cheese:

Sensory characteristics	**Coagulant type**		**Level of significant**
	Rennet	***Helianthus annuus***	
Colour	5.94 ± 1.60^{b}	6.66 ± 1.04^{a}	**
Flavour	4.93 ± 2.08^{b}	6.98 ± 1.91^{a}	**
Texture	5.38 ± 1.49^{b}	7.89 ± 1.26^{a}	**
Saltiness	3.88 ± 1.64^{b}	5.96 ± 1.45^{a}	**

Mean values bearing different superscripts within rows are significantly different ($p \leq 0.05$).

4.6.3. Effect of storage period on Sensory characteristics of white soft cheese:

Results in table (24) show the effect of storage period on sensory characteristics of the white cheese during storage:

The storage period affected the colour of the white cheese significantly ($P< 0.05$) (table 24).The highest value colour scores (6.80 ± 0.76) was obtained at week 1, and the lowest ones (5.85 ± 1.92 and 5.85 ± 1.97) were at weeks 2 and 4, respectively.

The flavour of the white cheese samples significantly ($P< 0.05$) affected by the storage period (table 24). The highest value (6.95 ± 1.72) was recorded at week 3, and the lowest ones (5.50±2.47 and 5.50 ± 2.30) were at weeks 1 and 8, respectively.

The texture of the white cheese samples was significantly ($P< 0.01$) affected by the storage period (table 24). The highest value (7.50 ± 1.55) was obtained at zero time, and the lowest one (6.10 ± 1.69) was at week 5.

The saltiness of the manufactured white cheese samples were significantly ($P<0.05$) affected by the storage period (table 24). The highest score (5.45±1.32) was obtained at week 3, and the lowest one (4.45 ± 1.92) was at week 4.

Table (24): Effect of storage period on Sensory characteristics of white soft cheese:

Storage period (wks)	Sensory characteristics			
	Colour	**Flavour**	**Texture**	**Saltiness**
Day 0	6.70 ± 0.97^{ab}	6.75 ± 1.88^{ab}	7.50 ± 1.55^{a}	5.40 ± 1.58^{ab}
Wk 1	6.80 ± 0.76^{a}	5.50 ± 2.47^{bcfg}	6.60 ± 1.98^{abcde}	5.00 ± 2.22^{abcd}
Wk 2	5.85 ± 1.92^{acd}	6.00 ± 1.97^{abc}	6.70 ± 2.15^{abcd}	4.55 ± 2.10^{bcde}
Wk 3	6.50 ± 1.09^{ac}	6.95 ± 1.72^{a}	7.00 ± 1.63^{ab}	5.45 ± 1.32^{a}
Wk 4	5.85 ± 1.97^{abcd}	5.60 ± 2.69^{bcef}	6.85 ± 1.7^{abc}	4.45 ± 1.92^{bcdef}
Wk 5	6.50 ± 1.26^{ac}	6.00 ± 2.17^{abc}	6.10 ± 1.69^{bcdeh}	5.30 ± 1.60^{abc}
Wk 6	6.20 ± 1.42^{acd}	5.70 ± 2.20^{bcd}	6.40 ± 1.7^{bcde}	4.70 ± 1.47^{abcd}
Wk 7	6.10 ± 1.43^{abcd}	5.65 ± 2.37^{bcde}	6.35 ± 1.83^{bcdef}	4.75 ± 2.08^{abcd}
Wk 8	6.25 ± 1.41^{acd}	5.50 ± 2.30^{bcfg}	6.15 ± 2.21^{bcdeg}	4.65 ± 2.35^{abcd}
Wk 9	6.05 ± 1.43^{acd}	5.80 ± 2.34^{bc}	6.70 ± 1.79^{abcd}	4.95 ± 1.54^{abcd}
L.S	*	*	**	*

Means bearing different superscripts with column are significantly ($P<0.05$).

*: significant ($p\leq 0.05$).

**: significant ($p\leq 0.001$).

L.S = Level of significance

4.6.4. Effect of interaction between milk source and coagulant type on Sensory characteristics of white soft cheese:

Milk source (cows and goats) and coagulant (rennet and partial purified *H. annuus* enzyme) did not showed a significant (P>0.05) effect on sensory characteristics of white cheese, although the partially purified *H. annuus* enzyme showed higher results compared to rennet enzyme (table 25).

The higher (6.80 and 6.52) colour scores were in cheese made with cows' and goats' milks using the partially purified *H. annuus* enzyme compared to those of rennet ones (6.31 and 5.58).

The higher flavour scores (7.20 and 6.76) were in cheese made with cows' and goats' milks using the partially purified *H. annuus* enzyme compared to those of rennet ones (5.49 and 4.38, respectively) (table 25).

The texture scores of the cheese samples from cows and goat milk with the partially purified *H. annuus* enzyme were higher (7.82 and 7.96) while those of the cheese made with rennet were lower 5.52 and 5.24 (table 25).

The cheese samples made from cows and goats milk with the partially purified *H.annuus* enzyme showed higher 5.64 and 6.28 saltiness scores in comparison with those made with rennet enzyme 3.78 and 3.98 (table 25).

Table (25): Effect of interaction between milk source and coagulant type on Sensory characteristics of white soft cheese:

Sensory characteristics	Milk source				S.E	L.S
	Cow		Goat			
	Coagulant type		Coagulant type			
	Rennet	H	Rennet	H		
Colour	6.31	6.80	5.58	6.52	0.13	N.S
Flavour	5.49	7.20	4.38	6.76	0.19	N.S
Texture	5.52	7.82	5.24	7.96	0.14	N.S
Saltiness	3.78	5.64	3.98	6.28	0.15	N.S

S.E = Standard error

L.S = Level of significance for interaction

N.S: Not significant

H: *Helianthus annuus*

4.6.5. Effect of interaction between milk source and storage period on Sensory characteristics of white soft cheese:

Results in table (26) showed the changes in sensory characteristics of the white cheese as affected by milk source during storage.

The results showed that no significant (P>0.05) differences in colour, texture, and saltiness except flavour) of the cheese samples. The colour scores in cheese made with cows' milk decreased from 7.00 at zero time and week 1 to 5.90, the lower colour scores was at week 2, while in cheese made with goats' milk, the higher colour scores (6.60) was at week 1 and the lower (5.50) colour score was at week 4, (table 26).

The flavour scores increase from 7.00 at zero time to 7.20 at week 9 in cheese made with cows' milk. While the highest flavour scores 7.10 at week 3 in cheese made with goats' milk and the lowest flavour scores (4.40) appeared at week 9 in cheese made with goats' milk. There was no bitter flavour or taste at the end of storage (table 26).

Higher texture scores were found (7.60 and 7.40) at zero time in cows' and goats' milk cheeses then decreased with the improvement of storage period, (table 26).

The saltiness scores of the cows and goats milk cheese samples were 5.00 and 5.90 at zero time and week 3, respectively (table 26), then decreased with the progress of storage period in both cheeses.

Table (26): Effect of interaction between milk source and storage period on Sensory characteristics of white soft cheese:

Storage period (wks)	Sensory characteristics							
	Colour		Flavour		Texture		Saltiness	
	Cow	Goat	Cow	Goat	Cow	Goat	Cow	Goat
Day 0	7.00	6.40	7.00	6.50	7.60	7.40	5.00	5.80
Wk 1	7.00	6.60	6.00	5.00	6.70	6.50	4.90	5.10
Wk 2	5.90	5.80	6.30	5.70	6.60	6.80	4.40	4.70
Wk 3	6.50	6.50	6.80	7.10	6.90	7.10	5.00	5.90
Wk 4	6.20	5.50	6.30	4.90	6.70	7.00	4.20	4.70
Wk 5	6.70	6.30	6.40	5.60	6.50	5.70	5.00	5.60
Wk 6	6.20	6.20	6.00	5.40	6.60	6.20	4.60	4.80
Wk 7	6.60	5.60	5.80	5.50	6.60	6.10	4.70	4.80
Wk 8	6.40	6.10	5.40	5.60	5.90	6.40	4.60	4.70
Wk 9	6.60	5.50	7.20	4.40	6.60	6.80	4.70	5.20
S.E	0.30		0.43		0.30		0.34	
L.S	N.S		*		N.S		N.S	

S.E = Standard error

L.S = Level of significance for interaction

N.S: Not significant

4.6.6. Effect of interaction between coagulant type and storage period on Sensory characteristics of white soft cheese:

Table (27) shows the changes in sensory characteristics of the white cheese as affected by coagulant type during storage. The cheese samples of the two coagulants showed no significant ($P>0.05$) variations in the flavour, texture, and saltiness except the colour had significant ($P<0.05$) difference.

In cheese made with rennet, the colour scores decrease from 6.70 at zero time to 5.70 at week 9 while in cheese made using *Helianthus annuus* coagulant, the higher colour score 7.00 at week 2 and the lower one was 6.30 at week 7, table (27).

The flavour scores decrease from 6.20 at zero time to 4.10 at week 7 in cheese made with rennet whereas *Helianthus annuus* coagulant cheese decrease from 7.90 at week 3 to 6.30 at week 1, (table 27).

However, a slight improvement in texture scores with increasing storage period in both cheeses with rennet and *Helianthus annuus* enzymes. Higher texture scores were 6.30 and 8.70 at zero time. While the lower texture scores 4.70 and 7.10 at weeks 8 and 5, were in rennet and *Helianthus annuus* coagulant cheeses, respectively (table 27).

The saltiness scores decreased with increase as the storage period progressed, in both cheese with rennet and *Helianthus annuus*. The highest saltiness score 6.40 was in cheese with *Helianthus annuus* coagulant at zero time whereas the lowest score 3.10 was in rennet cheese at week 2, (table 27).

Table (27): Effect of interaction between coagulant type and storage period on Sensory characteristics of white soft cheese:

Storage period (wks)	Colour		Flavour		Texture		Saltiness	
	Rennet	H	Rennet	H	Rennet	H	Rennet	H
Day 0	6.70	6.70	6.20	7.30	6.30	8.70	4.40	6.40
Wk 1	6.60	7.00	4.70	6.30	5.20	8.00	3.80	6.20
Wk 2	4.98	6.90	5.10	6.90	5.20	8.20	3.10	6.00
Wk 3	6.20	6.80	6.00	7.90	5.90	8.10	4.70	6.20
Wk 4	5.15	6.70	4.20	7.00	5.50	8.20	3.30	5.60
Wk 5	6.30	6.70	4.93	7.30	5.10	7.10	4.70	5.90
Wk 6	5.90	6.60	4.60	6.80	5.30	7.50	3.90	5.50
Wk 7	5.90	6.30	4.10	7.20	5.10	7.60	3.30	6.20
Wk 8	6.00	6.50	4.30	6.70	4.70	7.60	3.60	5.70
Wk 9	5.70	6.40	5.20	6.40	5.50	7.90	4.00	5.90
S.E		0.29		0.43		0.30		0.34
L.S		*		**N.S**		**N.S**		**N.S**

S.E = Standard error

L.S = Level of significance for interaction

N.S: Not significant

*: significant ($p \leq 0.05$).

H: *Helianthus annuus*

4.6.7. Effect of interaction between milk source, coagulant type and storage period on Sensory characteristics of white soft cheese:

Table (28) shows the changes in sensory characteristics of the white cheese as affected by milk source and coagulant type during storage. The results demonstrated that there were no significant (P>0.05) differences in colour, flavour, texture, and saltiness of the manufactured cheese. Results also showed that higher sensory characteristics scores of the white cheese were obtained in cheese samples of cows' and goats' milks using *Helianthus annuus* coagulant compared to rennet ones.

The highest colour scores (7.00) was in cows' milk cheese for both rennet and *Helianthus annuus* coagulants at zero time and week 1 and the lowest colour score 4.00 was in goats' milk cheese with rennet at week 4, table (28).

The highest flavour scores (8.20) was in goats' milk cheese with *Helianthus annuus* enzyme at week 3, while the lowest scores (3.00) was in goats' milk cheese with rennet at week 4 (table 28).

The highest texture scores (9.00) was in goats' milk cheese with *Helianthus annuus* enzyme at zero time, whereas the lowest texture scores (4.40) was in cows' milk cheese with rennet at week 8 (table 28).

The highest saltiness score was 7.00 in goats' milk cheese with *Helianthus annuus* enzyme at zero time and the lowest texture score 4.40 in goats' milk cheese with rennet at week 2, table (28).

Table (28): Effect of interaction between milk source, coagulant type and storage period on Sensory characteristics of white soft cheese:

Storage period (wks)	Colour				Flavour				Texture				Saltiness			
	Cow		Goat		Cow		Goat		Cow		Goat		Cow		Goat	
	R	H	R	H	R	H	R	H	R	H	R	H	R	H	R	H
0 Time	7.00	7.00	6.40	6.40	6.60	7.40	5.80	7.20	6.80	8.40	5.80	9.00	4.20	5.80	4.60	7.00
Wk 1	7.00	7.00	6.20	7.00	5.40	6.60	4.00	6.00	5.60	7.80	4.80	8.20	4.00	5.80	3.60	6.60
Wk 2	5.00	6.80	4.60	7.00	5.60	7.00	4.60	6.80	5.20	8.00	5.20	8.40	3.20	5.60	3.00	6.40
Wk 3	6.40	6.60	6.00	7.00	6.00	7.60	6.00	8.20	6.00	7.80	5.80	8.40	4.20	5.80	5.20	6.60
Wk 4	6.00	6.40	4.00	7.00	5.40	7.20	3.00	6.80	5.40	8.00	5.60	8.40	3.20	5.20	3.40	6.00
Wk 5	6.40	7.00	6.20	6.40	5.40	7.40	4.00	7.20	5.60	7.40	4.60	6.80	4.20	5.80	5.20	6.00
Wk 6	5.80	6.60	6.00	6.40	4.80	7.20	4.40	6.40	5.40	7.80	5.20	7.20	3.80	5.40	4.00	5.60
Wk 7	6.40	6.80	5.40	5.80	4.40	7.20	3.80	7.20	5.40	7.80	4.80	7.40	3.40	6.00	3.20	6.40
Wk 8	6.20	6.60	5.80	6.40	4.20	6.60	4.40	6.80	4.40	7.40	5.00	7.80	3.80	5.40	3.40	6.00
Wk 9	6.20	7.00	5.20	5.80	6.60	7.80	3.80	5.00	5.40	7.80	5.60	8.00	3.80	5.60	4.20	6.20
L.S	N.S				N.S				N.S				N.S			

R: Rennet H: *Helianthus annuus*

CHAPTER FIVE

DISCUSSION

5.1. Extraction and partial purification of milk-clotting enzyme from *Helianthus annuus* seeds:

Coagulation using rennet-like enzymes is the major procedure representing about 75% of cheese production (Fox *et al*., 2015). However, the increased demand for cheese as a convenience food, dessert or snack, a sandwich filler, and food ingredient, has resulted in increased requirements for milk coagulants with good coagulation properties and cheap production cost. Thus, the search for milk-clotting enzyme from various sources has been on the raise, and accordingly in the current study the seeds of sunflower were used to partially purify milk-clotting enzyme.

Application of various extracting systems, in terms of extracting buffers and time, and found that using 5% NaCl in 50 mM sodium acetate buffer, pH 5.0, for 24 hour were effective in extracting the milk-clotting enzyme from sunflower seeds. Our results in table 1 and 2 are in agreement with the observation of Ahmed *et al.,* (2009a) and Talib *et al.,* (2009) who reported that buffer extract of *Solanum dubium* berries had less milk-clotting activity than extract with 5% NaCl sodium acetate buffer. An increase in the buffer salts strength of the extracting solution increases the milk-clotting enzyme, and therefore allowed the extraction increase.

5.2. Characterization of milk-clotting partial purified enzyme from *Helianthus annuus* seeds (SDS-PAGE and zymogram activity):

In addition, by using as simple purification procedure (ammonium sulfate precipitation), some of the total protease in the crude extract was removed out. This simple purification procedure not only resulted in the effective removal of the partial proteases and colored materials which existing in the crude extract (Barros *et al*., 2001) but it also concentrated the enzyme preparation to a workable volume that could be used for enzyme characterization and cheese making.

The partially purification procedure developed in this study resulted in two active bands with a molecular mass of 120 and 62 kDa as judged by SDS-PAGE and zymogram activity staining (Table 4, figure 1). SDS-PAGE gel still showing other contaminating proteins revealing the needs for further purification steps, however, this will increase the production cost of the enzyme. Thus, partial purification of the enzyme using ammonium sulphate is recommended as it led to clear enzyme preparations with excellent milk-clotting properties and cheese making potential.

The cheese making potential of the partially purified milk clotting enzyme from sunflower seeds was apparent from its high ratio of milk clotting/proteolytic activity as well as the firm curd formation, which shown in (table 3). The ratio of milk-clotting activity to proteolytic activity is a useful indicator of the protease efficiency to be used as a coagulant for cheese making (Arima *et al*., 1970).

The presence of two active bands of partially purified enzyme might be indicator of either a heterodimer protease with two enzyme subunits or of two different enzymes. The latter assumption could explain the two bands in the current study as the molecular masses of these bands are comparable to those reported for other milk clotting enzymes. For instance, the molecular weight of the 62 kDa band of partially purified milk-clotting enzyme is similar other known milk clotting enzymes of plant sources, (Kumari *et al.* 2012 and Ahmed *et al*. 2009b). Whereas, the other band of 120 kDa molecular weight is closed to that reported by Egito *et al*, (2007) for milk clotting enzyme of *Helianthus annuus* seeds, however; it slightly disagreed with him in resulting in two bands instead of one band of molecular weight 110 kDa. This difference might be due to the difference in the purification procedures between the two studies. Moreover, the zymogram activity staining also showed two clear zone of proteolytic activity against a blue background. Although the sample in the zymogram gel was treated with 2.5% SDS, the enzyme still displayed activity, indicating that the partially purified milk-clotting enzyme of sunflower seeds is resistance to SDS denaturing, and similar observation has been reported previously (Egito *et al*., 2007). This properity might pave the way of the partially purified milk clotting enzyme of sunflower seeds to be used in other biotechnological applications under harch conditions, beside its potential uses in cheese making industry.

5.3 The coagulation time and cheese yield percentage of the white soft cheese using partial purified *Helianthus annuus* enzyme:

In order to further confirm the suitability of the partially purified enzyme for as rennet substitute in cheese making, the enzyme was used for production of cheese from two types of milks (cow and goats). The produced cheeses were evaluated for the yield and curd formation time in comparison with that of commercial calf rennet. This information is really important to evaluate the potentiality of the purified enzyme from both industrial and commercial stand points.

The findings in this study (Table 5) demonstrated that high significant differences in the percentage yield of cow's milk cheese compared to goats' one with no significant differences in the coagulation time. The differences in cheese yield between the two types of milk might be due to the difference in the biochemical composition mainly fats, proteins and total solids of both milks. Cows' milk has higher total solids and protein than goat milk, while the later milk has higher fats compared to cow milk (Fox *et al*., 2015). This might explain the higher yield of cheese made from cows' milk compared to that made from goat milk. On the other hand, the coagulant type also affect the cheese yield and the results in table 6 and 7 showed a significant difference in the yield percentage of cheese produced by commercial calf rennet and partially purified sunflower milk clotting enzyme.

The results of sunflower cows' cheese had higher yield percentage compared to those from rennet, this might be attributed to the fact that the

yield of cheese increase due to the incorporation of whey proteins (Abdel Razig, 1996). This could be explained by higher retention of water and the softer cheese formed by sunflower enzyme compared to that formed by rennet. In addition, Abdul-Rahman (2013) stated that high content of moisture in cheese directly affect the yield percentage, since moisture considers one of the fundamental factors affecting the increase or decrease of yield percentage. In contrast, our results were in disagreement with those of Abdul-Rahman (2013) who concluded that the yield of cheese made using safflower (12.73%) enzyme extract was lower than that made using calf rennet (13.8 %). This divergence could be attributed to the using of crude extracts compared to our partial purified enzyme in cheese processing besides the variation in the seeds extracts between sunflower and safflower, the clotting efficiency and the source of milk and variation in its composition (Altaif, 2011). On the other hand, the results showed high significant differences in coagulation time of rennet and sunflower enzymes (table 6). This findings were in accordance with those of Talib *et al*, (2009), El Owni *et al.*, (2011) and Abdul-Rahman (2013) who stated that cheese processed from vegetable extract had higher coagulation time compared to rennet ones. This might be attributed to the using of crude and partial purified extracts in cheese processing compared to commercial rennet.

5.4. Chemical composition of white soft cheese:

5.4.1. Effect of milk source on chemical composition of white soft cheese:

Chemical composition of the cheese samples (table 8) were significantly ($P<0.01$) affected by milk source. Total solids contents, ash, protein, Tryptophan and tyrosine) contents were significantly ($P< 0.01$) higher in cheese made with goats' milk compared to those made with cows' one

except fat and acidity. Our findings were in agreements with Bilal (2000) who concluded that fat, acidity and ash were higher in cheese made of cows' milk. Pappa *et al.*, (2006) studied some chemical analysis of Teleme cheese made from ovine, caprine and bovine milks; and he demonstrated that moisture was higher in cheese made from cow's milk than in cheeses made from the other two kinds of milk, while highest fat content was in cheese made from ewe's milk and the highest protein content was in cheeses made from goat's milk; And Queiroga *et al*., (2013) concluded that cows' and goats' milks had significant differences ($P<0.05$) in the moisture, fat and salt contents of Coalho cheese; where the author, previously observed that fresh cheese made from cows' milk displayed a higher moisture content and a lower fat content compared to cheese made with goats milk or a mixture of both, suggesting that manufacturing differences can influence the final cheese composition, independent of the milk source and this probably due to milk type variation in its chemical composition (Altaif, 2011); Also, our results were agreed with the findings of Mustafa *et al.*, (2013); Alizadeh and Lavasani (2013); Ama and Iem (2014) and Kabsoun (2016), who concluded that significant differences in total solids contents, ash, protein and fat of cheese samples using ewes' and camel or mixture of milks compared to cows' one.

Whereas, these results were in disagreement with those of Abdalla, and Abdel Razig (1997) who studied the chemical characteristics of white soft cheese made from cows', goats' and mixed milks, and found that the fat, protein and total solids contents were higher in cows' milk cheese, while ash content and pH values were higher in cheese made from goats' milk, although our findings were similar to those of Abdel Razig (1996) and Sant'Ana *et al*., (2013) who showed that the chemical composition of

Minas cheese made with goat milk, cow milk, or a mixture of the two type were not affected by the type of milk.

5.4.2 Effect of coagulant type on chemical composition of white soft cheese:

Results in table (9) showed significant ($P<0.01$) effect on the chemical composition of white soft cheese (total solids contents, ash, fat, protein, acidity, Tryptophan and tyrosine). The findings were in harmony with those of Núnez *et al*., (1991); Abu-Zeid (1994) and Kheir *et al.*, (2011).

The higher acidity value in cheese made with vegetable coagulant (*Helianthus annuus*) compared to rennet, in these results, may be attributed to the longer coagulation time of vegetable coagulant possibly favored microbial growth and consequently, a higher acidity was reached in curd from vegetable coagulant (Abu-Zeid,1994);

Although our results were not in accordance with those of Abdul-Rahaman (2013) who indicated that there were no significant differences in moisture, fat and titratable acidity in white cheese making while, the type of coagulant had significant effect on percentage of protein and ash, which is similar to our results. Prados *et al*., (2007) who studied the chemical characteristics of Manchego cheese using powdered vegetable coagulant (PVC) obtained from the cardoon (*Cynara cardunculus*) compared with calf rennet, showed no differences were observed between the two types of coagulants for most chemical parameters (moisture, fat, protein and acidity). And Alves *et al.,* (2013) showed no differences were observed of using protease from the fungus *Thermomucor indicae-seudaticae* N31 and commercial coagulant from *Rhizomucor* spp. as clotting agents on moisture content while had significant differences in protein content.

5.4.3. Effect of storage period on chemical composition of white soft cheese:

Findings showed significant ($P<0.05$) effect on total solids contents of white cheese (Table 10). These results agreed to those obtained by Bilal (2000), Hamid (2005) and Kheir *et al.*, (2011), who reported that the total solids content of the white soft cheese increased during storage period. The increase of total solids contents during pickling, a phenomenon attributed to curd contraction and expulsion of whey as a result of acid development (El Owni and Hamid, 2008). While the decrease of total solids contents during storage period was attributed to the degradation of total protein, dissolution of salt and fat in to pickling solution or absorption of pickling whey by curd (Abdalla, 1992; Nuser, 2001).

Results showed significant ($P<0.01$) effect on ash content of white cheese (Table 10). These results were coincided with those of Kheir *et al.*, (2011); Abdel Razig and Babiker (2009) and Hamid (2005) who found the ash contents of the cheese samples increase from day 120 to day 240. The increase in ash contents during pickling may be due to the decrease in moisture content and / or absorption of salt by curd, whereas, the decrease of ash contents may attribute to the diffusion of salt from curd into whey (Abdalla,1992; Abdel Razig, 1996).

Findings showed significant ($P<0.01$) effect on fat of white cheese (Table 10). The fat content decreased with progress in storage time. Similar results were obtained by (Abdel Razig and Babiker 2009; Kheir *et al.*, 2011; and Altaif, 2011). The decrease of cheese fat during pickling might be due to the lipolytic activity of microorganisms on fats resulting in leakage of some fat from curd into the pickling whey. On the other hand, the increase in fat during pickling could be attributed to the breakdown of the cheese proteins

and their loss in the whey (Bilal, 2000; Hamid, 2005 and El Owni and Hamid, 2008).

Significant variations ($P<0.01$) were found in the protein contents of white soft cheese samples (Table 10) during storage. The decrease of protein content probably due to the degradation of protein and loss in the pickling whey leading to the formation of water soluble compound some of which were loss in the pickling solution, (Nuser, 2001). The findings were in harmony with those of (Abdel Razig and Babiker 2009; Kheir *et al.*, 2011; and Altaif, 2011). However, the increase of protein content during storage period could be due to the moisture loss of the cheese, (Kalid, 1991; Abdel Razig, 1996; and Hamid, 2005).

The increase in the acidity of cheese samples during advanced storage period (Table 10) might be mainly due to lactic acid formed by the usually predominating lactic acid forming bacteria (Nofal *et al*., 1981). These results were in agreement with those reported by Bilal (2000), Nuser (2001), El Owni and Hamid (2008), El Owni and Hamid (2009) and Kheir *et al.,* (2011). On the other hand the decrease in the acidity of cheese samples during storage might be due to utilization of lactic acid by other microflora throughout the storage.

Tryptophan and tyrosine contents of white soft cheese samples showed significant ($P<0.01$) variations (Table 10). The results were in line of (Abdel Razig, 1996; Hamid 2005 and Kheir *et al.*, 2011). The increase in tyrosine and tryptophan content of the cheese at the beginning of storage time may be due to excessive proteolysis of cheese protein (Nofal *et al*., 1981 and Abdel Razig, 1996) and Hamid (2005) who reported that amino

acid tyrosine and tryptophan contents increased as the storage period progressed.

5.4.4. Effect of milk source and coagulant type on chemical composition of white soft cheese:

Results (Table 11) showed significant ($P<0.01$) effect on chemical composition (total solids contents, ash, fat, protein, acidity, Tryptophan and tyrosine) of white soft cheese.

Results were similar to Pereira *et al.*, (2010) who concluded significant differences for moisture, pH values, total protein, fat, and salt contents.However, these results were not similar to those obtained by Fernández-Salguero *et al*., (2002) and Galán *et al*., (2012) who revealed no significant differences in moisture, fat, protein and acidity in ewe's cheese using rennet calf and plant coagulant from cardoon *Cynara cardunculus*; except in ash content, which showed significant differences; and this probably due to milk type variation in its chemical composition and to the diversity in coagulant type (Altaif, 2011).

5.4.5. Effect of milk source and storage period on chemical composition of white soft cheese.

The milk source was significantly ($P<0.01$) affected the chemical parameters of the white cheese during storage period (table 12). These results were in the line with those of Abdalla and Abdel Razig (1997) who showed that total solids, ash, fat, acidity of white soft cheese made from cows', goats' and mixed milks increased during storage period, while

protein decreased and tyrosine and tryptophan fluctuated during storage; Pappa *et al*., (2006) concluded that moisture was higher in cheese made from cow's milk than in cheeses made from ovine and caprine milks, where the highest fat content was in cheese made from ewe's milk, the highest protein content was in cheese made from goat's milk; and Queiroga *et al*., (2013) concluded that type of milk (cows' and goats' milks) used in Coalho cheese only influenced ($P<0.05$) the moisture, fat and salt contents of the cheeses during cold storage for 28 days; Also, with Sant'Ana *et al*., (2013) showed significant difference on the chemical composition of Minas cheese made with goat milk, cow milk, or a mixture of the two type, stored in cold conditions for 21 days.

5.4.6. Effect of coagulant type and storage period on chemical composition of white soft cheese:

Results in table (13) showed that coagulant type and storage period had significant ($P<0.01$) effect on chemical parameters (total solids contents, ash, fat, protein, acidity, tryptophan and tyrosine of white cheese). Our results were similar to El-Shibiny *et al*., (1973) findings who stated that total solids contents, ash, fat, protein, acidity of Domiati cheese which was significantly affected by storage period using enzyme preparation from fig latex; Alalade and Adeneye (2006) found that the total solids content of Wara cheese was significantly affected by storage period; Elhaseen *et al*., (2014) concluded that the storage period has significant effect on the chemical composition of white cheese made with lime and grape fruit extracts; and Abdel Razig and Babiker (2009) showed significant differences on the chemical composition of white cheese using lemon, orange and grapefruit juices as coagulants during ripening period of 60 days.

However, these results were not similar with Sousa and Malcata (1997) who reported that type of rennet (aqueous extract of flowers of *Cynara cardunculus*) had no significant effect on total solids and fat contents of the cheese over the ripening period; Kheir *et al.*, (2011) showed no significant differences (P>0.05) in titratable acidity, total solids, fat, protein, soluble protein and salt contents of the white soft cheese as affected by storage period and coagulant type; Prados *et al*., (2007) studied the chemical characteristics of Manchego cheese using powdered vegetable coagulant (PVC) obtained from the cardoon (*Cynara cardunculus*) compared to calf rennet and monitored over a 6-month ripening period, and resulted in no differences between the two types of coagulants for most chemical parameters (moisture, fat, protein and acidity); and results of Pezeshki *et al*., (2011) indicated that, except for pH which was significantly (P< 0.05) lower in cheeses made with *Withania coagulans*, there was no significant difference observed among the cheeses produced with different rennet preparations as in moisture, fat and salt contents during ripening; Talib *et al*., (2009) reported that the decrease in protein content of soft white cheese manufactured using *Solanum dubium* seeds extract during pickling as a result of protein degradation leading to the formation of water soluble compound and some of which lost in the pickling solution leading to increase of nitrogen content in whey; and Fernández-Salguero and Sanjuán (1999) stated that the levels of soluble amino acids gradually increased with ripening. The lower tryptophan values compared to tyrosine values throughout ripening may be as a result, not only of the lower original casein content, but also of the fact that this amino acid is more readily hydrolyzed by microorganisms.

5.4.7. Effect of milk source, coagulant type and storage period on chemical composition of white soft cheese:

Results in (table 14) showed that milk source, coagulant type and storage period showed highly significant (P<0.01) differences on total solids, protein, fat, acidity, tyrosine and tryptophan, while the ash contents significant at (P<0.05) of white soft cheese.

Results in this study were coincided with those of Pereira *et al.,* (2010) and Altaif (2011) who showed significant differences in moisture, fat, protein and acidity, except the latter author, showed no significant differences in the ash content, this probably owing to milk type variation in its chemical composition and to the diversity in coagulant type. However, the results were not in accordance with those obtained by Fernández-Salguero *et al*., (2002) and Galán *et al*., (2012) who revealed no significant differences in moisture, fat, protein and acidity in ewe's cheese using rennet calf and plant coagulant from cardoon (*Cynara cardunculus*) during ripening.

5.5. Microbiological quality of white soft cheese:

4.5.1. Effect of milk source on microbiological quality of white soft cheese:

Total viable bacterial count (TVBC) and Coliforms of the white cheese were significantly (P<0.01) affected by milk source while *lactobacilli* were not affected, (table 15). These results were in the line of Izarig (2011) who concluded that the type of milk had a significant effect on Coliforms count in *mish* made of fresh cows' milk and skim milk. In contrast Galán *et al*., (2012) who revealed that no significant differences were observed in total viable bacterial count**,** Coliforms and *lactobacilli* in ewe's cheese, this

could be probably due to difference in milk sanitation and hygienic treatments between the experiments .

5.5.2. Effect of coagulant type on microbiological quality of white soft cheese:

Total viable bacterial counts (TVBC), Coliforms of white soft cheese were significantly affected by coagulant type; however lactobacilli were not affected (table 16)**.** These results were not in the line of Fernández-Salguero *et al.*, (2002) who studied the microbial parameters of using powdered vegetable coagulant, of cardoon (*Cynara cardunculus*) compared to its crude extract and Galán *et al.*, (2012) who revealed no significant differences were observed in total viable bacterial count**,** Coliforms and lactobacilli in cheese using rennet calf and plant coagulant from cardoon (*Cynara cardunculus*).Moreover, the results of Kheir *et al*., (2011) showed no significant differences in total viable bacterial and lactobacilli counts . This could be due to the diver in coagulant type and heat treatment which suppress the growth of microorganisms (Abdel Razig and Babiker, 2009).

5.5.3. Effect of storage period on microbiological quality of white soft cheese:

Total viable bacterial count, which decreased from day zero to week 9 throughout the storage period (table 17). These results were in agreement with the findings of El Owni and Hamid (2008) and El-Owni *et al.*, (2011). Storage periods showed significant differences ($p>0.05$) in total bacterial counts (Nour El Diam and El Zubeir, 2006). On the other hand the results were in disagreement with those of Ahmed and Alhassan (2010) who

showed increased in TBC during storage. The decrease in TBC may be attributed to lactic acid production in the cheese samples that causes suppression in growth of microorganisms (Ur-Rahman *et al.*, 2000).

The results showed that Coliforms counts decreased with the progress of storage period (table 17). These findings were in harmony with the work of El Owni and Hamid (2008) who reported that Coliforms counts decreased till day 180 and disappeared in 240 day of storage. And with the results of Abdalla *et al*., (2012); who studied the Coliforms counts of white soft cheese stored in five different containers and found Coliforms counts decreased from day zero up to day 180 of storage period and not detected in some containers from the day 120 of storage period. Also, the results of Dhuol and Hamid (2014) concluded the decrease of Coliforms counts during storage period. The decrease in Coliforms counts was possibly due to increased acidity of the cheese as a result of the activity of lactic acid bacteria (Aly and Galal, 2002).

The *E. coli* counts were not detected throughout the study. These findings were coincided with those of Ahmed and Alhassan (2010) who studied the effect of different packaging materials during storage and no *E. coli* was found. Also similar results (Warsama *et al*., 2006) were reported. The presence of *E. coli* in raw milk and their absent after the milk pasteurized and throughout cheese processing and storage period may be due to good hygienic conditions.

The results of *E. coli* counts were not detected throughout the study. The results were in accordance with those of Abdel Razig and Babiker (2009). Whereas, our findings were not in accordance with those found by Warsama *et al.*, (2006), Dhuol and Hamid (2014) *staphylococcus aureus*

decreased significantly with the storage period; Barakat *et al*., (2009) studied and who showed the *staphylococcus aureus* of Domiati cheese made with the Cells of *Lactobacillus reuteri*) and he found that the log of *staphylococcus aureus* was 4.51±0.095, 4.78±0.016, 3.84±0.078 and 3.42±0.085 on the day zero, 30, 60 and 90 of the storage period; the results of Abdalla *et al.*, (2013) found that *Staphylococcus* is detected only in the day zero of the storage period. *Staphylococcus aureus* was detected at day zero as 2.8×10^5 then it disappeared after 60 days of storage, their results was supported by the results of El-Owni and Hamid, (2009) who stated that *Staphylococcus aureus* count in Sudanese white cheese was detected at zero time before storage and completely disappeared after 60 days of storage; and with Ahmed (1985) who showed that 60% of the cheese samples collected from Khartoum market were contaminated with *S. aureus*. Aly and Galal, (2002) attributed the disappearance of *Staphylococcus aureus* to the increase acidity of the cheese and the high salt level. The presence of *Staphylococcus aureus* in the cheese from the day zero up to the end of storage period might be due to the poor sanitary conditions and contamination of cheese during processing and storage.

The *lactobacilli* counts decreased significantly throughout the storage period, (table 17). These results were in accordance with those obtained by Kheir *et al*., (2011) who found the *lactobacillus* of white cheese decreased with the storage period from the day 1 up to the end of the storage period (90) days. These results was also in line with those found by Sousa and Malcata, (1997) who reported that lower *lactobacilli* count was obtained for cheese until 28 days of the ripening. And Dhuol and Hamid (2014) concluded that *lactobacilli* decreased significantly with the storage period and this could be attributed to the increase of the acidity of cheese as a

result of the activity of lactic acid bacteria, (Nour El Diam and El Zubeir, 2006).

5.5.4. Effect of milk source and coagulant type on microbiological quality of white soft cheese:

Results showed significant ($P<0.05$) differences on Total viable bacterial count of white cheese, whereas Coliforms and Lactobacilli counts illustrated no significant differences, (table 18). These findings were in agreement with those of Pereira *et al.*, (2010) who studied the two types of coagulant (animal and plant) and three types of cheese milk (cow, sheep, and goat) and their influence on the microbial analyses of the model Portuguese cheeses and showed significant differences. However, these results were not in agreement with those of Fernández-Salguero *et al.*, (2002) and Galán *et al*., (2012) who revealed no significant differences were observed in total viable bacterial count**,** Coliforms and *lactobacilli* in ewe's cheese using rennet calf and plant coagulant from cardoon (*Cynara cardunculus*).

5.5.5. Effect of milk source and storage period on microbiological quality of white soft cheese:

Total viable bacterial count (TVBC) and Coliforms of the white cheese were significantly ($P<0.01$) and ($P<0.05$) affected by milk source and storage period. While *lactobacilli* were not affected, (table 19).These results were agreed with Izarig (2011) who concluded that the type of milk had a significant effect on Coliforms count in *mish* made of fresh cows' milk, mixed with skim milk and skim milk. Whereas not agreed with Galán

et al., (2012) who revealed no significant differences were observed in total viable bacterial count**,** coliforms and *lactobacilli* in ewe's cheese.

5.5.6. Effect of coagulant type and storage period on microbiological quality of white soft cheese:

Total viable bacterial count (TVBC) and coliforms of the white cheese were significantly decreased by coagulant during storage period and *lactobacilli* were not affected although there was a decrease, (table 20). The results were in harmony with those obtained by Kheir *et al*., (2011) who found the *lactobacilli* of white cheese decreased with the storage period from the day 1 up to the end of the storage period (90) days. And with that found by Sousa and Malcata, (1997) who reported that lower lactobacilli count was obtained for cheese manufactured with plant rennet until 28 days of the ripening. Also Abdel Razig and Babiker (2009) showed total bacterial count decreased during storage of cheese using lemon, orange and grapefruit juices, as coagulants, while the coliforms and *Staphylococcus aureus* were not found in all cheese samples during storage.

However, reverse results were obtained by Galán *et al*., (2012) who revealed no significant differences were observed in total viable bacterial count**,** coliforms and *lactobacilli* during storage of cheese using rennet calf and plant coagulant from cardoon (*Cynara cardunculus*). This may be due to the diver in coagulant type and hygienic conditions through processing and handling (Abdel Razig and Babiker, 2009).

5.5.7. Effect of milk source, coagulant type and storage period on microbiological quality of white soft cheese:

Results (table 21) demonstrated significant (P<0.01) effect on total viable bacterial counts (TVBC) and no significant (P>0.05) variations were found in Coliforms and *lactobacilli* counts of white soft cheese, Similar results were obtained by Pereira *et al.,* (2010) and Fernández-Salguero *et al*., (2002) who studied the microbial parameters of using powdered vegetable coagulant, the cardoon (*Cynara cardunculus*) compared to crude extract, for manufacturing of ewe's milk cheese during 3 months of ripening period. While, Galán *et al*., (2012) revealed no significant differences were observed in total viable bacterial counts, Coliforms and lactobacilli counts in ewe's cheese using rennet calf and plant coagulant from cardoon (*Cynara cardunculus*) during storage.

5.6. Sensory characteristics of white soft cheese:

5.6.1 Effect of milk source on sensory characteristics of white soft cheese:

Results (table 22) showed significant differences in colour, flavour, saltiness of white soft cheese while, milk source had no effect on the texture of the cheese. The results were in harmony with Abdalla, and Abdel Razig (1997); Bilal (2000) who concluded that the highest organoleptic evaluations were in cheese made of cows' milk compared to the mixed and with pure soy milk; Queiroga *et al*., (2013) concluded cows' and goats' milks had significant differences in sensory characteristics of Coalho cheese; Sant'Ana *et al*., (2013) showed significant differences in the sensory characteristics of Minas cheese made with goat, cow, or a mixture of the two types of milk; and with Izarig (2011) concluded that the type of milk had a significant effect on sensory characteristics in *mish* made of fresh cows' milk, skim milk and mixed with both milks. The variation of

colour and flavor of cheese may be attributed to the variation of fat of milk (Carpino *et al.*, 2004).

5.6.2. Effect of coagulant type on Sensory characteristics of white soft cheese:

The effect of coagulant type showed significant differences on sensory characteristics of white soft cheese (table 23). These results were in accordance with those of Nunez *et al*., (1991) who reported that the use of vegetable rennet resulted in a cheese with a more pleasant and pronounced flavour, and the effect of coagulant type on flavour quality and intensity being highly significant ($P<0.001$), Prados *et al*., (2007) reported that the colour of cheese made with a powdered vegetable coagulant from cardoon (*Cynara carnculus*) score was higher compared to the cheese with animal rennet; Omotosho *et al*., (2011) studied the effect of local Nigerian different coagulants: *Calotropis procera,* steep water and alum on sensory evaluation of Wara cheese and the results showed that *Calotropis procera* gave the best coagulum; and Kheir *et al*., (2011) concluded significant differences in colour, flavor, texture and saltiness.

The results were in disagreement with those of Abdul-Rahman (2013) who showed no significant differences in sensory characteristics and Alves *et al*., (2013) showed no differences were observed of using protease from the fungus *Thermomucor indicae-seudaticae* N31 and commercial coagulant from *Rhizomucor spp*. as clotting agents on sensory characteristics of Prato cheese; also Chen *et al.*, (2003) and Roseiro *et al*., (2003) who reported that plant proteases are consider too proteolytic, leading to bitter flavor and texture defect.

5.6.3. Effect of storage on sensory characteristics of white soft cheese:

The results (table 24) showed significant differences in colour, flavor, texture and saltiness of the cheese during storage. These findings were in the line of Elowni and Hamid (2008), Dhuol and Hamid (2014) and Hamid (2014); this might be attributed to fact that the sensory characteristics scores increased generally during ripening of cheese (Tarakci and Kuckoner, 2006). However, the results were not in line with those of Abdalla and Mohamed, (2010)

5.6.4. Effect of milk source and coagulant on sensory characteristics of white soft cheese:

Milk source and coagulant type did not showed a significant difference in sensory characteristics of white cheese, although the partial purified *H. annuus* enzyme showed higher results compared to rennet enzyme (table 25). Our findings were agreed with those of Fernández-Salguero *et al*., (2002). However, these results were not in line of Galán *et al*., (2012) who revealed significant differences were observed in flavor and texture and similar in saltiness while, no significant in ewe's cheese using rennet calf and plant coagulant from cardoon (Cynara cardunculus). This might be due to the fact of milk type variation in its chemical composition and the variation in coagulant type (Altaif, 2011).

5.6.5. Effect of milk source and storage on Sensory characteristics of white soft cheese:

Results (table 26) showed not significant differences in sensory characteristics of white soft cheese except in the flavour. Similar results

were obtained by Abdalla, and Abdel Razig (1997). Bilal (2000) who concluded that the highest organoleptic evaluations were in cheese made of cows' milk, followed by mixed with soy milk and the lowest with soy milk where storage period improved the quality of cheese.

5.6.6. Effect of coagulant and storage on Sensory characteristics of white soft cheese:

Results in table (27) showed no significant differences of coagulant and storage period on sensory characteristics of white soft cheese. These results were in accordance with those of Talib *et al*., (2009) who concluded that cheese prepared using Jiben (*Solanum dubium* seeds extract) has high quality with a very small variations as well as it has a long storage time. Kheir *et al*., (2011) indicated no significant different in colour, flavour, texture, and saltiness of the cheese. However, a slight improvement in colour scores with progressing storage period in both cheeses with rennet and *Solanum dubium* extracts. And with Abdul-Rahman (2013) who showed no significant differences were observed in sensory characteristics between rennet and safflower cheeses.

Whereas the results were not in accordance with Prados *et al.*, (2007) who concluded that the sensory quality (odour, colour, taste intensity and creaminess) of Manchego cheese was higher in cheeses obtained with vegetal coagulant cardoon (*Cynara cardunculus*), than those made with animal rennet, which were monitored over a 6-month ripening period.

Abdalla and Mohamed (2009) reported cheese colour did not significantly change during storage period; and there was no bitter flavour or taste at the end of storage and the decrease in flavour score may be due to growth of

fungus at the surface of the cheese. However, Tejada *et al.*, (2007) reported that cheese made with vegetable coagulant displayed a slightly bitterer taste than those made with rennet. Abdalla and Mohamed (2009) reported that cheese flavour gradually improved throughout the storage period. Also Degheidi (1996) used fungal enzyme from *Pencillium funiculsum* as rennet substitute for manufacture Edam cheese, the experimental cheese gained acceptable body with a good clean flavour during ripening period.

El-Shibiny *et al.*, (1973) reported that the body and texture of cheese made from fig latex were smooth and firm, and the flavour was good and free from defects even after 4 months of ripening.

5.6.7. Effect of milk source, coagulant and storage on Sensory characteristics of white soft cheese:

Table (28) illustrated no significant differences were found on sensory characteristics of the manufactured cheese. Our findings were similar to Fernández-Salguero *et al.*, (2002) who studied the sensory characteristics of using powdered vegetable coagulant, the cardoon (*Cynara cardunc ulus*) compared to *crude extract* to manufacture ewe's milk cheese during 3 months of ripening period.

Our findings were not similar to Galán *et al.*, (2012) who revealed significant differences were observed in flavour and texture while similar with him in saltiness, which were not significant in ewe's cheese using rennet calf and plant coagulant from cardoon (*Cynara cardunculus*) during storage. This might be attributed to the fact of milk type variation in its chemical composition and coagulant type may reflect on the sensory characteristics of cheese (Altaif, 2011).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion:

Based on the results of this study the followings are recorded:

- The ratio of clotting activity (units/ml) to proteolytic activity (OD 660 nm) of partially purified *Helianthus annuus* seeds extract was 3451.4 (units/OD 660 nm) compared to 4992 (units/OD 660 nm) of rennet enzyme.
- Thirty to fifty percent saturation by ammonium sulphate resulted in higher milk clotting activity of *Helianthus annuus* seeds extract and partially purified enzyme to 4.3 folds with MCA of 241.0 U/mL and final enzyme cheese yield of 10.9%.
- *Helianthus annuus* seeds extract showed two bands on SDS-PAGE and the molecular mass of the partial purified enzyme was calculated to be 120 and 62 kDa.
- Two grams (2 gm: 50 litres milk) of a partially purified *Helianthus annuus* seeds extract is recommended for cheese making.
- Both milk sources and enzyme types significantly affected the cheese yield and curd formation time.
- Chemical composition has significantly affected by milk source, coagulant type and storage period.
- *E. coli* and *Staphylococcus aureus* counts were not detected in all the cheese samples throughout the study.
- Sensory characteristics were significantly affected by milk source, coagulant type and storage period, while cheese texture was affected by milk source.

6.2. Recommendations:

The recommendations of this study are:

1. The result therefore, would conclusively recommended the possibility of using partially purified *Helianthus annuus* enzyme as a coagulant as an available and rich source of milk-clotting enzyme in cheese making as a rennet substitute.
2. Further studies in purification and characterization of *Helianthus annuus* enzyme extract.
3. Further research in the vitamin and mineral contents of cheese made with *Helianthus annuus* enzyme during storage is requires.
4**.** Identification of volatile fatty acids and free fatty acid of cheese made with *Helianthus annuus* enzyme during storage in order to evaluate the lipolytic process.

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APPENDICES

APPENDIX 1:

— Sensory evaluation sheet for white soft cheese,

— Name: …….Date:.....

Sample No.	Colour	Flavour	Texture	Saltiness	Remarks

—

— **Colour:**

— Very acceptable 9
— Acceptable 7
— Slightly accept. 5
— Moderately accept. 3
— Not acceptable 1

Flavour:

Extremely intense 9
Intense 7
Moderately intense 5
Slightly intense 3
Poor 1

Texture:

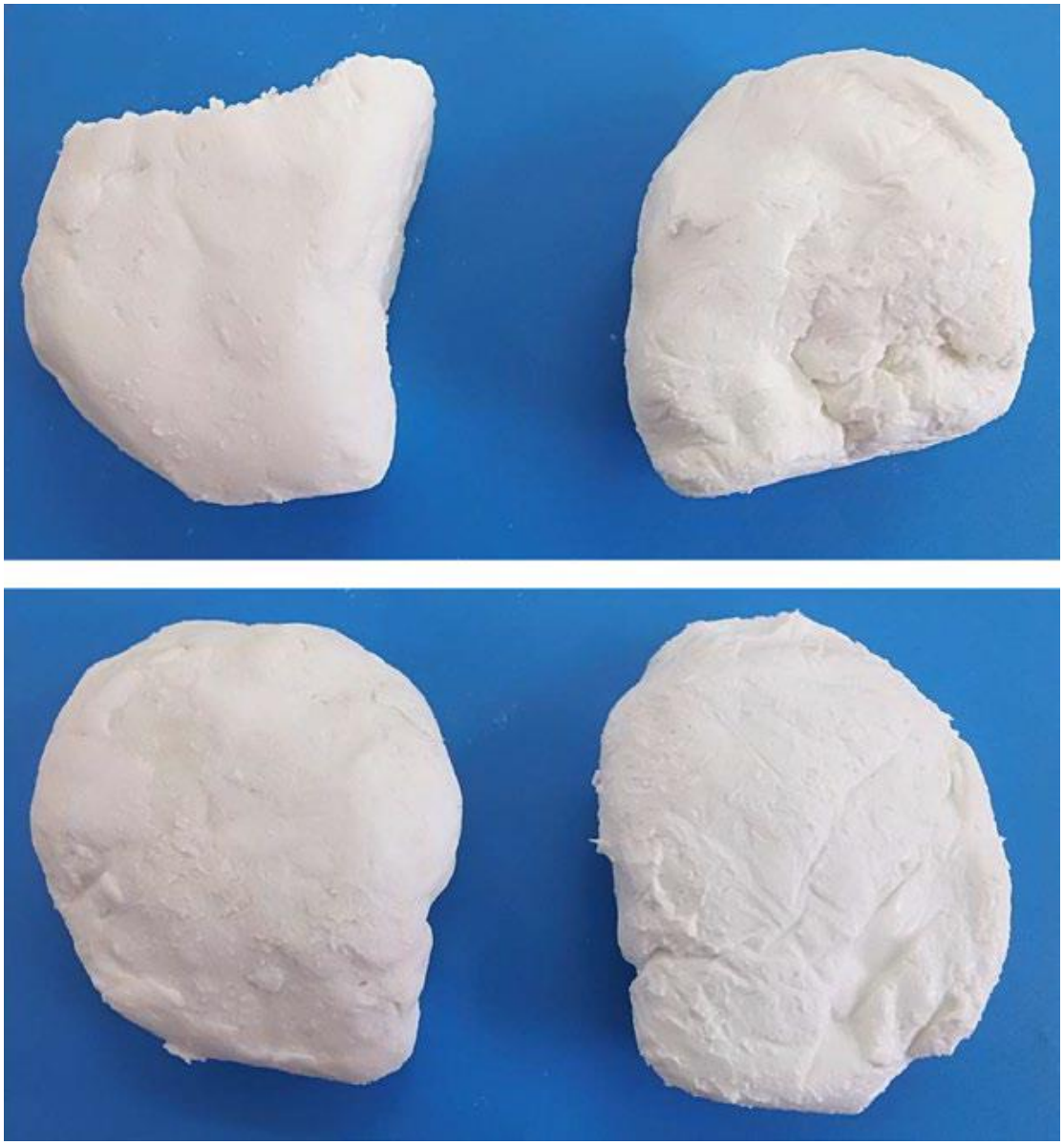
Very soft 9
Soft 7
Slightly soft 5
Tough 3
Very tough 1

Saltiness:

Over salted 9
Salted 7
Moderately salted 5
Slightly salted 3
Poor 1

—APPENDIX 2:

— A B



— C D

— **Figure 2.** Cheese produced using different milk types and enzyme source. The upper photos showed cow milk cheese processed using calf rennet (A) and the partially purified enzyme of sunflower seeds (B).While the lower photos showed, goat milk cheese produced using calf rennet (C) and the partially purified enzyme of sunflower seeds (D).

Appendix (3): Intellectual property

جمهورية السودان

وزارة العدل

المسجل العام للملكية الفكرية

وثيقة براءة

صادرة بموجب أحكام المادة ١٩ (١) من قانون براءات الاختراع لسنة ١٩٧١م، والمادة ٦(١) من اللائحة (الأنموذج رقم ٧)

هذه البراءة منحت تحت مسئولية من منحت إليه دون ضمان لصحتها

المادة ١٨ (٣) من القانون

بناء على الطلب المقدم بتاريخ 2014/12/24م تحت الرقم 2014/3789م نشهد بأن المذكور أدناه قد منح براءة اختراع تحت الرقم 3299

١- اسم صاحب البـــــراءة : *آسيا إبراهيم عبد الرحيم* محمد – عمر إبراهيم احمد حامد

٢ – عنوان صاحب البراءة : جامعة السودان للعلوم والتكنولوجيا – كلية الإنتاج الحيواني – قسم الألبان

ت-0906483549

٣- اسم الاختـــــراع : استخلاص إنزيم لتجبين اللبن من بذور زهرة الشمس .

٤- وصف الاختراع :-

تم استخلاص الإنزيم من بذور زهرة الشمس Helianthus annus وتمت تنقيته جزئيا باستخدام طريقة كبريتات الامونيوم وتم إنتاجه وحفظه في صورة بدرة من خلال استخدام طريقة التجفيف بالتجميد (LYOPhilization) وتم تطبيقه واستخدامه في صناعة الجبنة البيضاء السودانية وكانت نتائجه ممتازة من حيث قوة التجبن على أساس استخدام (١جم/50 لتر لبن) وكانت جودة الجبن المنتج بواسطته عالية الجودة .

٥- حقوق الامتيـــــاز المراد حمايتها :

١/حماية طريقة استخلاص الإنزيم.

٢/حماية الإنتاج والتصنيع.

٦- اسم المخترع الحقيقي : *آسيا إبراهيم عبد الرحيم* محمد – عمر إبراهيم احمد حامد

٧- تجدد البراءة سنوياً .

صدرت هذه الوثيقة تحت توقيعي في اليوم التاسع عشر من شهر يناير سنة ألفين وخمسة عشر ومنح براءة اختراع تحت مسئوليته .

سعاد الامين محمد النور

ع/مسجل عام الملكية الفكرية

SUST Intellectual property workshop

Appendix (4): Photos

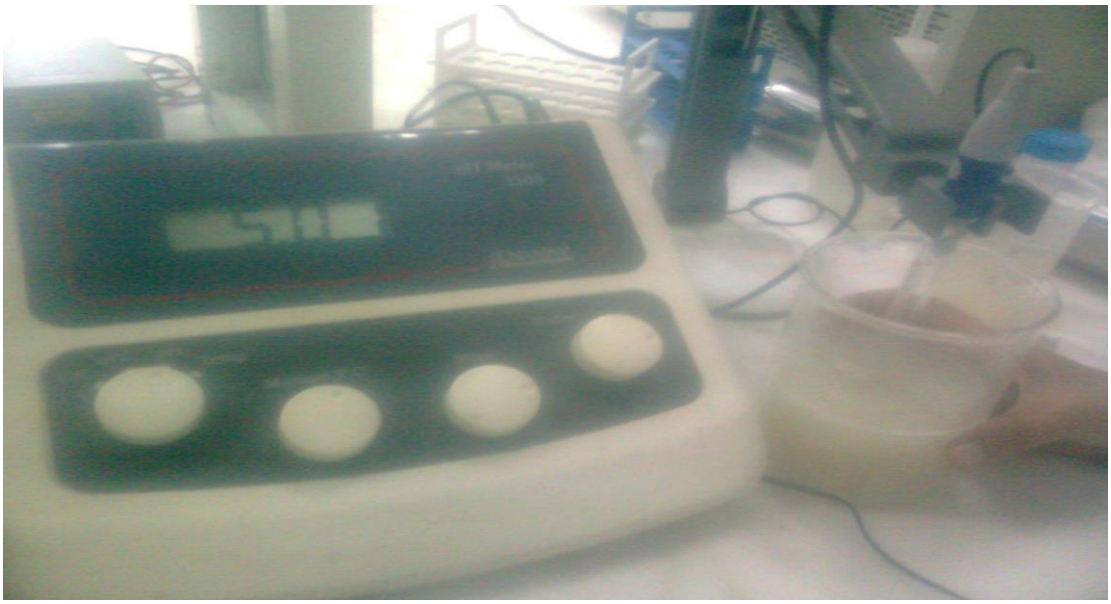
Sunflower seeds

Enzyme Extraction

B **A**

Determination of milk-clotting activity (MCA)

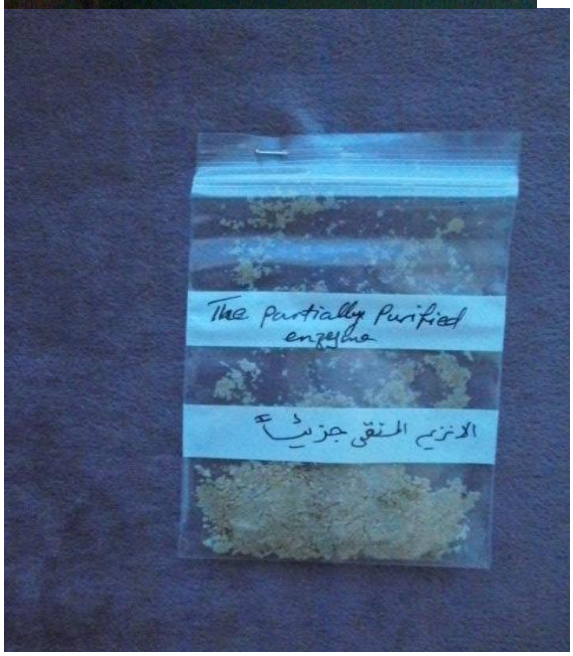
Precipitated enzyme with $AmSO_4$ (30–50%)



Adjust the PH

Precipitation by cold centrifugation stage

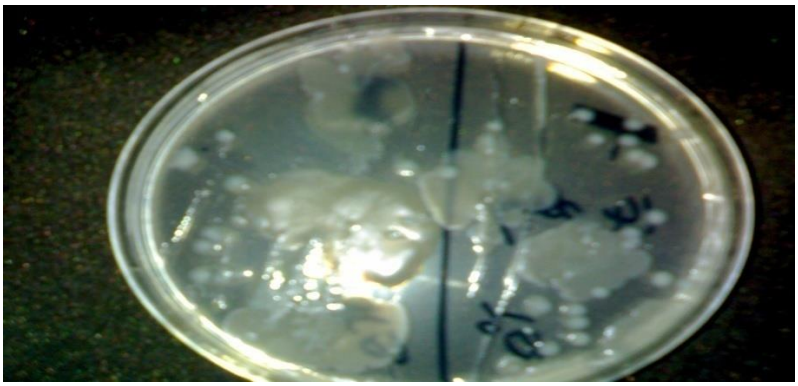
De $(NH_4)_2 SO_4$ stage



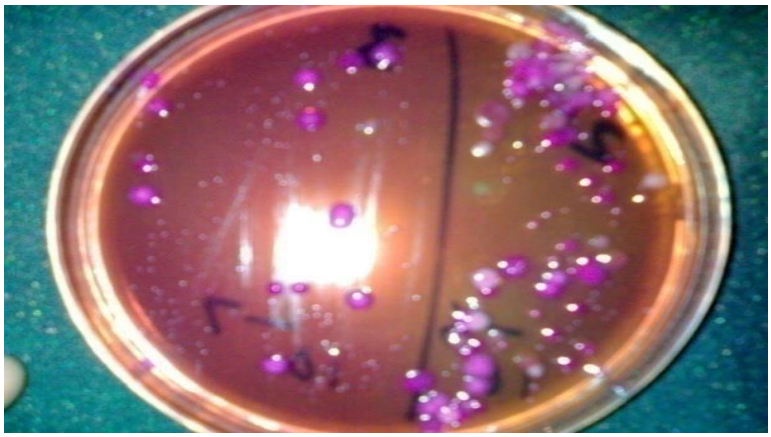
Dry freezing stage **The partially purified enzyme**

Microbial analysis:

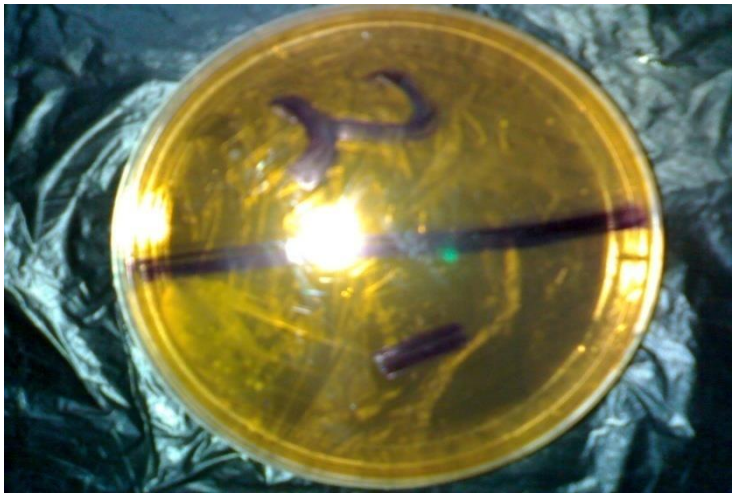
Preparations and streaking in medias



TVBC

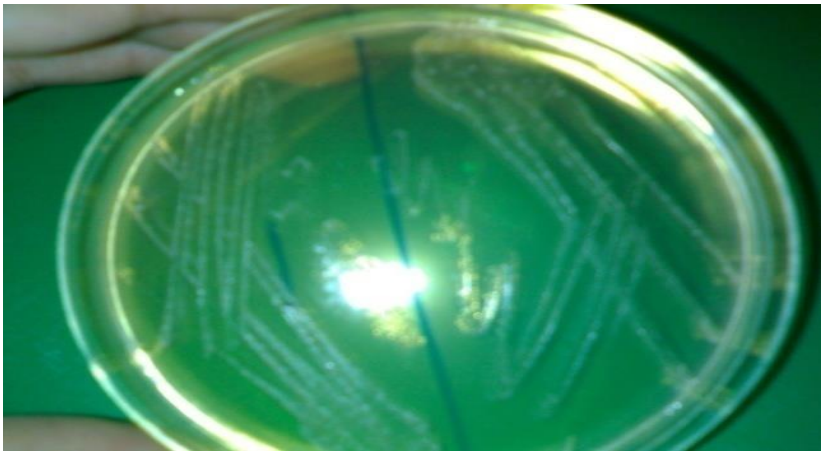


Coliform



Sta. aureus

E.coli



L.A.B