Effect of *Solanum dubium* Fruit (*Gubbain*) Extract on the Milk Clotting and Quality of White Cheese (*Gibna Bayda*)

By

Sana Eltahir Osman Khir

B.Sc. Honours (Agric.) University of Gezira (1996)

M.Sc. (Tropical Animal Production) University of Khartoum (2000)

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Supervisor

Dr. Osman Ali Osman El Owni

Co- Supervisor

Dr. Mohamed Osman Mohamed Abdalla

Department of Dairy Production

Faculty of Animal Production

University of Khartoum

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DEDICATION

To my parents

Eltahir and Suaad

My brothers and sisters

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I thank God Almighty for giving me the health, strength and courage to accomplish this work.

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ABSTRACT

Two experiments were carried out to study the effect of *Solanum dubium* fruit extract on weight loss, chemical composition, microbiological quality and sensory characteristics of white cheese (*Gibna Bayda*) during storage. In the first experiment, *Solanum dubium* fruit enzymes were characterized. The result showed that the fruit extract of *Solanum dubium* extract reached its maximum enzyme activity when the fruits were dark yellow in colour and completely dry. Maximum milk clotting activity was obtained by using freeze-dried extract. The loss in activity of the fruit liquid enzyme as well as solid enzyme form increased significantly at room temperature storage compared to refrigerator storage. Subjecting the extract to 70°C for 10 minutes showed no detrimental effect on its milk coagulating activity. The extract activity was completely destroyed when subjected to 80°C for 20 minuets.The saturation with ammonium sulphate (60%) gave high milk- clotting activity (5.03 mg/ml) as well as protein content. The partially purified extract had the highest enzymatic activity at 70°C and pH 10. In the second experiment, the partially purified extract was used in making white cheese. The results indicated that both storage period and salt concentration had significantly affected weight loss and chemical composition of the cheese during storage. Type of milk coagulant (chymosin and *Solanum dubium* fruit extract) had significant effect on weight loss, total solids, fat, protein, soluble protein, salt, tyrosine and tryptophan. However, it had insignificant effect on titratable acidity and ash contents. Addition of calcium chloride has significantly ($P<0.05$) affected weight loss, total solids, titratable acidity, ash, and salt of the cheese, and gave insignificant effect on fat, protein, soluble protein, tyrosine and tryptophan. Microbiological quality of cheese samples was

significantly affected by storage. Type of coagulant and salt level significantly affected flavour, texture, and saltiness of cheese samples. However, there was insignificant effect on colour of cheese samples. The chemical composition (total solids, fat, titratable acidity, protein, salt and ash contents) of whey from cheese making was significantly affected by storage. *Solanum dubium* fruit extract may be used as a substitute for rennet enzyme.

ملخص البحث

أجريت تجربتين لدراسة تأثير مستخلص ثمرة نبات الجبين على نقص الوزن، التركيب الكيميائي، الخصائص الميكروبيولوجية والحسية للجبن الابيض خلال فترة التخزين. في التجربة الأولى، تم دراسة خصائص الإنزيمات المستخلصة من ثمرة نبات الجبين. اوضحت النتائج أن مستخلص ثمرة نبات الجبين أعطى اعلى نشاط أنزيمي عندما كانت الثمار صفراء داكنة اللون وجافة تماماً. مستخلص الثمرة أعطى أعلى نشاط في تجبن اللبن عند إستخدام طريقة التجفيف المجمد في تحضيره. إزداد فقد النشاط الأنزيمي مستخلص ثمرة نبات الجبين الذي تم تخزينه بصورة سائلة وصلبة عند تخزينه في درجة حرارة الغرفة مقارنة بتخزينه في الثلاجة . تعريض المستخلص لدرجة حرارة 70 °م لمدة 10 دقائق لم يكن له أثر واضح على قوة التجبن. نشاط المستخلص توقف عند تعريضه لدرجة حرارة 80°م لمدة 20 دقيقة. التشبع بكبريتات الأمونيوم (60 %) أعطى أعلى فعالية في تجبن اللبن (5.03 مليجرام/مل) وكذلك محتوى البروتين. مستخلص ثمرة نبات الجبين النقي جزئياً أعطى أعلي نشاط أنزيمي في درجة حرارة 70 °م، ودرجة تركيز الهيدروجين 10. في التجربة الثانية، استخدم مستخلص ثمرة نبات الجبين النقي جزئيا في عمل الجبن الابيض. أوضحت النتائج أن كل من فترة التخزين وتركيز الملح لها تأثير معنوي على نقص الوزن والتركيب الكيميائي للجبن خلال التخزين. العامل المجبن (الكيموسين ومستخلص ثمرة الجبين) لها تأثير معنوي على نقص الوزن، المواد الصلبة الكلية، الدهن، البروتين، البروتين الذائب، الملح، التايروسين والتربتوفان.إلا أنها ليس لها تأثير معنوي على محتوى الرطوبة والرماد. إضافة كلوريد الكالسيوم ذات تأثير معنوي على نقص الوزن، المواد الصلبة الكلية، الحموضة، الرماد والملح؛ وأعطت تأثير غير معنوي على الدهن، البروتين، البروتين الذائب، التايروسين والتربتوفان. المحتوى الميكروبيولوجي للجبن تأثر معنوياً بالتخزين. العامل المجبن ومستوى الملح أثر معنوياً على النكهة، القوام والملوحة لعينات الجبن.إلا أنه ليس له تأثير معنوي على اللون في عينات الجبن. التركيب الكيميائي

(المواد الصلبة الكلية، الدهن،الحموضة، البروتين، الملح والرماد) لشرش الجبن المصنعة تأثر معنوياً بالتخزين. مستخلص ثمرة نبات الجبين يمكن أن يستخدم كبديل للمنفحة.

Effect of *Solanum dubium* Fruit (*Gubbain*) Extract on the Milk Clotting and Quality of White Cheese (*Gibna Bayda*) during Storage

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Chapter One

Introduction

Cheese making as means of preserving the most important constituents of milk in highly concentrated form is in vogue all over the world. It provides a palatable milk product of high nutrition value which can be kept fresh for a long time. Cheese is an economical source of milk protein. It is rich in calcium, vitamins, nourishing and easily digestible food (Nir, 2004). Cheese making is aimed to make milk preservation attractive and durable. Its shelflife varies from few days to several years (Walstra *et al*., 1999).

Majority of cheeses around the world are manufactured traditionally and in many cases still are manufactured, using an enzymatic coagulant extracted from the abomasa of milk-fed calves. This extract, known as calf rennet, consists of two proteolytic enzymes: chymosin (EC 3.4.23.4), the major component (88–94% milk clotting activity, MCA) and bovine pepsin (EC 3.4.23.1; 6–12% MCA). The relative proportion of these enzymes varies with individuality and age of calves, the method of rennet separation and pH values at which the milk clotting activity is measured (Guinee and Wilkinson, 1992).

The principal role of chymosin in cheese making is to coagulate milk by specifically hydrolyzing the Phe_{105}-Met_{106} bond of the micelle-stabilizing protein, κ-casein, which is in many times more susceptible to chymosin than any other bond in milk proteins and leads to the coagulation of milk (Fox *et al*., 2000).

In the latter part of the last century, cheese consumption increased while the availability of calf rennet decreased, which led to

rennet shortages and subsequent price increases. In addition, 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 (Guinee and Wilkinson, 1992; Broome and Limsowtin, 1998; Fox *et al*. 2000).

Some plants of the family solanaceae such as *Solanum dubium* (Plate 1) had been tried for the extraction of milk-clotting enzymes (Habbani, 1992; Osman, 1996; Mohamed and Habbani, 1996; Yousif *et al*., 1996; Osman, 2001). Their research showed positive results in using this extract for manufacture of Sudanese white cheese.

Solanum dubium Fresen, "Gubbain", is a well known wild plant that grows widely in Khartoum during the rainy season. It also grows in the west and east shores of the White Nile, South of the Blue Nile, Gezira, Kordfan and Darfour regions (Salih, 1979).

Objectives:

This research aims at using *Solanum dubium* Fresen fruit as alternative source of animal rennet, when animal rennet is not available or slaughter of calves for chymosin is not feasible, in addition, *Solanum dubium* is free of toxins (Osman, 2001), also where restrictions are imposed against the use of animal rennet, particularly in developing countries. Therefore, the objectives are to study:

1. Extraction and partial purification of the enzyme from the fruits of *Solanum dubium* Fresen.
2. Characterization of *Solanum dubium* Fresen fruit extract.
3. Effect of *Solanum dubium* extract on weight loss and chemical composition of white cheese.

4. Effect of *Solanum dubium* extract on sensory evaluation and microbiological quality of white cheese.

Plate 1. *Solanum dubium* plant (Green)

Chapter Two

Literature Review

2.1 History of cheese

In ancient times, in Eastern Europe and Western Asia, the practice of carrying milk in bags made of animal's stomach probably resulted in the first cheeses being made more or less by accident. The Romans first described cheese making in detail, and the Roman legion was instrumental in spreading the art of cheese making throughout Europe. In Roman times, an enzyme preparation from goat, lamb or even hare stomachs was mixed with sheep or goat's milk (cow's milk was not produced on a large scale before the thirteenth century). The curds separated from the whey were salted and stored for consumption later on (Madden, 1995).

O'Conner (1993) reported that the origin of cheese making is lost in unrecorded history. There is evidence to suggest that cheese was made as far back as 7000 BC. There are numerous references to cheesemaking in the Bible while the writings of Homer and Aristotle indicate that cheese was made from the milk of cow, sheep, mare and asses. Around 300 AD trade in cheese between countries especially on sea routes became so great that the Roman emperor Diocletain had to fix maximum prices for the cheese.

By the nineteenth century, some farms were selling rennet extracts in small quantities for the convenience of domestic cheese manufacture. In 1874, a Danish chemist Christin Hansen founded a laboratory in Copenhagen and started the first industrial production of calf rennet extract. This was obtained from the stomachs of unweaned calves that were slaughtered for veal production and not specifically

to obtain the enzyme. World production of rennet now exceeds 25 million litres per year (Madden, 1995).

2.2 Sudanese white cheese

Osman (1987) reported that *Gibna Bayda* is a traditionally fermented, pickled type of cheese (Gibna: cheese, Bayda: colloquial Sudanese Arabic word for white). It is considerd that the art was introduced to Sudan through the Greek immigrans.

Sudanese white cheese (*Gibna Bayda*) is the most common cheese in Sudan. It has strong odour and taste. It is made from raw or pasteurized whole milk, skim milk or reconstituted milk, depending on natural lactic acid bacteria and no starter is used (Khalid and El Owni 1991).

In Sudan cheese processing is a major preservation method of surplus milk in rural areas especially during rainy season when plenty of milk is a vailable (Osman 1987). El Owni and Hamid (2007) stated that the most popular type of cheese produced in Sudan is the white cheese locally known as *Gibna Bayda*. It is generally consumed fresh or matured for a period of several months. It's made from full fat raw milk; high concentrations of sodium chloride are added before renneting (Osman 1987).

Raheem (2006) added that *Gibna Bayda* is white cheese made in Sudan. It is similar to Domiati cheese made in Egypt. 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 followed by salt addition to give 7-10% solution in milk. Rennet or rennet extract is added to obtain a firm coagulum which develops in four to six hours. The coagulum is transferred thereafter to wooden moulds lined with cheese cloth muslin and the whey is

allowed to drain overnight. The curd is cut into 10 cm cubes preserved in the whey in tins or other suitable airtight containers and sealed.

2.3 Mechanisms of milk coagulation

The main purpose of coagulant in cheese making is the conversion of liquid milk to a gel; that can be catalyzed by different proteases (Green, 1984). There are two main phases in the mechanism of milk clotting: the primary or enzymatic phase and the secondary or coagulation phase (Dalgleish, 1982; Payens, 1993).

The coagulation of milk is the result of two processes: the attack on the k-casein of the casein micelles by the proteolytic enzymes contained in rennet and the clotting of the micelles which have been destabilized by this enzymatic attack. Milk-clotting enzymes split bovine k-casein at the phe_{105}- met_{106} bond. The rate of the enzymes reaction has been shown to increase linearly with the enzyme concentration, in agreement with a first-order mechanism (Castle and Wheelock, 1972; Dalgleish, 1979). The aggregation phase occurs by a random, diffusion-controlled Smoluchowski mechanism (Dalgleish *et al.*, 1981; Green, 1984), the rate of micellar aggregation being independent of their size and little affected by doubling rennet concentration (Dalgleish *et al*., 1981). Intermicellar linkages which appear on electron micrographs during micellar aggregation become stronger with time bringing the micelles into contact and, eventually, micelles fuse together (Green and Morant, 1981; McMahon and Brown, 1984).

Several theories have been proposed by different workers on the coagulation of milk by protease enzyme to explain this mechanism. Since 1930, Linderstrom-Lang and Holter developed a theory that casein complex of milk owe its stability to the presence of a

component that acts as a stabilizer. Rennet action start by degrading this component specifically and the modified complex flocculates in a secondary phase (Eck, 1987).

2.4 Rennet and rennet substitutes

Rennet is a natural complex of enzymes produced in mammalian stomach to digest the mother's milk. Rennet contains a proteolytic enzyme protease that coagulates the milk, causing it to separate into solid curd and liquid whey. The active enzyme in rennet is called chymosin but there are also other important enzymes in it, such as pepsin or lipase (from Wikipedia).

Rennet is one of the best known coagulants used widely by cheese makers for many varieties of cheese. A crude rennet extract may be obtained from the fourth stomach (abomasum) of goat kids or calves when they are about four weeks old. Male goat kids or calves that have been fed on milk and that are not required for breeding are usually used (O'Conner, 1993)

In the 1960's the food and Agriculture Organization of the United Nations predicted a severe shortage of calf rennet. It was anticipated that an increased demand for meat would lead to more calves being reared to maturity so that less rennet would be available. Also a growing number of people following vegetarian's diets do not eat cheese made with calf rennet. As a result, over the last thirty years several substitutes for calf rennet have been made available, allowing the supply of enzyme to keep pace with cheese production and providing alternative sources for vegetarians. Today there are two major sources of chymosin for coagulating milk: from animals (veal calves, adult cows and pigs) and from different kinds of fungi. In addition there are now chymosins derived from genetically modified

microbes. In the latter case, copies of the genes responsible for chymosin production are isolated from calf stomach cells and incorporated into the genetic material of yeast cells. These can be grown on an industrial scale and the chymosin isolated (Madden, 1995).

The most common rennet substitutes include bovine, porcine and to a lesser extent, chicken pepsins and microbial proteases from *Rhizomucor miehei*, *R. pusillus* and *Cryphonectria parasitica* (Fox and McSweeney, 1997; Fox *et al*., 2000). The proteolytic activities of chymosin and porcine pepsin were compared on buffalo, cow and goat whole casein by Awad *et al*. (1998) and it was reported that both enzymes attacked α_{s1}- and β-caseins in the same region as calf rennet.

Trujillo *et al*. (2000) compared some milk clotting enzymes (calf and lamb rennets, bovine chymosin and pepsin, and proteases from *R. miehei* and *C. parasitica*) on ovine casein and reported that lamb rennet and *C. parasitica* proteases showed the lowest and the highest degree of proteolysis, respectively. These authors reported that all enzymes hydrolysed ovine casein resulting in the formation of α_{s1}-1 and β-1 (the first breakdown products produced by chymosin) as initial breakdown products of α_{s1}- and β-caseins, respectively, but *C. parasitica* also produced a series of degradation products with lower electrophoretic mobilities than β-casein. *C. parasitica* proteinase cleaves κ-casein at Ser_{104}-Phe_{105} rather than Phe_{105}-Met_{106}, which is cleaved by chymosin and *R. miehei* proteinase (Drohse and Foltmann, 1989). Porcine pepsin tends to be more heat-sensitive, followed by *C. parasitica*, bovine pepsin, chymosin, *R. pusillus* protease and *R. miehei* protease in order of increasing heat stability (Broome and Limsowtin, 1998), although the heat stability of the microbial

coagulants can be reduced after treatment with various chemical agents (Garge and Johri, 1994). The use of coagulants that are more heat stable than calf rennet should be avoided; otherwise excess proteolytic activity may remain in the curd where it may result in excessive proteolysis and bitterness unless ripening times and/or cooking temperatures are changed to compensate for the more rapid rate of proteolysis (Guinee and Wilkinson, 1992).

Microbial rennet was more proteolytic than calf rennet and exhibited specific proteolytic action on k- casein similar to that obtained with calf rennet. Other casein fractions were degraded continuously and non specific, but β- casein was the fraction most susceptible to hydrolysis (Melachouris and Tuckey, 1967). However, Joseph *et al*. (1993) stated that cheese made with chymosin and *Mucor miehei* proteases were similar in functional characteristic in general.

According to Marie and Xiaoshan (2000) the microbial proteinases are generally more proteolytic than chymosin with varying heat stability. These enzymes liberated more non- protein nitrogen from casein and can cleave α and β- casein as well as κ- casein at the natural pH of milk. Acid proteinases from *Cryphonectria paracitica* are more heat labile than those from *Rizomucor miehei*, which are characterized as thermostable.

Mucor rennin from *Mucor pusillus* Var Lint. will be useful for manufacture of cheese, as a chymosin substitute (Arima *et al*., 1968). Mickelsen and Nancy (1969) found that fungal rennet showed greater proteolytic activity than did veal rennet or pepsin on whole, α and β- casein.

Fungal enzyme from *Penicillium funiculosum* E-NRC 629 a rennet substitute was used as milk clotting enzyme in the manufacture of Edam cheese from cow's milk. Obtained results show that, the breakdown of protein content, and total volatile fatty acids was higher in Edam cheese made with fungal enzyme or its mixture with rennet enzyme than in control cheese throughout the ripening period (Degheidi, 1996).

The proteolytic specificity and activity of *Mucor miehei* protease (Rennilase) and *Endothia parasitica* protease (Suparen) on buffalo, cow, and goat whole casein and β-casein (CN) were studied by analyzing the degradation products. The results suggest that Rennilase hydrolyzes casein of the three species in a manner similar to that of chymosin, resulting in the formation of α_{s1}-I and β-I, -II, -III as initial degradation fragments of α_{s1}- and β-CN. α_{s1}-I was also the initial breakdown product of α_{s1}-CN by Suparen. Contrary to Rennilase, Suparen showed a higher affinity toward β-CN and hydrolyzes β-CN, giving rise to degradation products characterized by mobility lower than that of β-CN (Awad *et al*. 1999).

Nouani *et al*. (2009) reported that the extracellular protease from *Mucor pusillus* was purified 18-fold with 7.56% recovery by ion-exchange chromatography and gel filtration. The enzyme was found to be monomeric in nature, having a molecular mass of 49 kDa. These properties- except for temperature- were similar to those of rennet

According to Krishna and Mathur (1979**)** purified bacterial milk clotting enzyme from *Bacillus subtilis* k.26 was used for making cheddar cheese. He found that during cheese manufacture, acid development was relatively faster, protein and fat losses were lower

than when crude bacterial enzymes were used. The α and β- casein fractions were degraded more in bacterial enzyme cheese. Puhan and Irvine (1973) pointed that cheese made with proteases from a mutant of *Bacillus subtilis* revealed more and different proteolytic activity than did different cheese made with calf rennet.

In the last decade, genetically engineered microorganisms have been exploited increasingly for the production of commercial coagulants. The gene for chymosin has been cloned and inserted into microorganisms such as *Kluyveromyces marxianus* var. *lactis, Aspergillus niger* var. *awamori* or *Escherichia coli* which led to the development of recombinant chymosins which are now marketed commercially as Maxiren (DSM Food Specialities, Netherlands) and Chymax (Chr. Hansen, Denmark). Recombinant chymosins have been approved for commercial use in foods in many, but not all countries, and they have been used in ever-increasing quantities in USA and Western Europe and now represent about 35% of the total market (Fox *et al*., 2000).

2.5 Plant proteases

Plant proteases have been investigated as milk coagulants, but only a small number of aspartic proteinases from plant origin have been isolated and partially characterized (Tavaria *et al*., 1997; Sousa, 1998). A unique feature shared by most of these plant proteinases is an extra segment of about 100 amino acid residues which bears no sequence similarity with proteinases of mammalian or microbial origins (Faro *et al*., 1995).

Many aspartic and other proteinases are obtained from plants and some of them have been studied as coagulants, i.e., proteinases from *Benincasa cerifera* (Gupta and Eskin, 1977), *Calotropis procera*

(Ibiama and Griffiths, 1987; Mohamed and O'Connor, 1996), *Dieffenbachia maculate* (Padmanabhan *et al*., 1993), fruit parts of *Solanum dubium* (Yousif *et al.*, 1996), *Centaurea calcitrapa* (Tavaria *et al*., 1997) and flowers of *Cynara cardunculus* (Barbosa, 1983; Sousa, 1993 and Sousa, 1998). Although most plant coagulant preparations were reported to have an excessively low ratio of milk clotting to proteolytic activity, which results in bitter peptides in ripened cheese, or to an excessively low clotting power that gives rise to low cheese yields. The difficulties experienced with these preparations result mainly from the unique composition of the plant extracts, which contain a complex cocktail of enzymes whose activity is difficult to control.

Miniature (20 g) Cheddar-type cheeses were manufactured using blends of *Cynara cardunculus* proteinases and chymosin as coagulant (100: 0, 50: 50, 25: 75 and 0: 100 *C. cardunculus* proteinese: chymosin). There were no substantial differences between the compositions of cheeses made using any of the four coagulant blends. Cheeses manufactured with coagulant blends containing *C. cardunculus* proteinases exhibited higher levels of pH 4.6-soluble nitrogen than cheese made using chymosin as coagulant. The extent of breakdown of α_{s1}-casein, as measured by urea-polyacrylamide gel electrophoresis (urea-PAGE), was greater in cheeses made using coagulant preparations containing *C. cardunculus* proteinases as a constituent than in cheese made using 100% chymosin as coagulant. Different reverse-phase high-performance liquid chromatography (RP-HPLC) peptide profiles of the ethanol-soluble and -insoluble fractions were obtained for cheeses made using either *C. cardunculus* proteinases or chymosin as a coagulant. Principal component analysis

and hierarchical cluster analysis of RP-HPLC data confirmed that the inclusion of even small proportions (25%) of *C. cardunculus* proteinases with chymosin in the coagulant blend greatly altered the pattern and extent of proteolysis in miniature Cheddar-type cheeses (O'Mahony *et al*., 2003).

O'Conner (1993) reported that juice extracts from fruits and plants have long been used as milk coagulants. These include extracts from papaya (papain), pineapple (bromelin), castor oil seeds (ricin) and the latex of the fig tree and the plant *Calotropis procera* which grows abundantly in many parts of Africa. These extracts are suitable for softer crude cheese which is consumed within a few days. The extracts are not suitable for hard cheese with long maturing periods on account of their excessive proteolytic activity which leads to bitter flavours in the ripened cheese.

2.5.1 *Solanum dubium* plant in Sudan

Solanum dubium Fresen is an indigenous plant in northern and central Sudan. It is a woody herb; stem is solid erect, green in colour and about 30 cm in height. The stem and its branches bear numerous sharp spines, white in colour about 1- 3 mm in length and about 1 mm in thickness near the base. The leaves are alternate, long petiole, simple, ovate, acuminate or obtuse at the apex, pale green in colour. The petiole is 2- 6.5 cm long and 1- 3 mm in diameter covered with sharp whitish spines. The lamina bears spines only on the midrib and main veins. The main root is about 5 mm in thickness and 15 cm in length. It bears numerous, verb thin rootlets brown in colour. The inflorescence is composed of 2- 8 pedicellate flowers arranged in moncheasial scorpoid cyme. The flower is a hermaphrodite, actinomorphic with a yellow center core- like structure formed of the

persistent of 5 united green sepals, and bears numerous sharp spines. The corolla is violet in colour, rotate, of five petals united at the base with distance of 4 mm forming a tube which is terminating with 5 oval lanceolate lobes and a very short filament. Anthers open by two apical pores. The fruits are grouped in clusters with exile alternately bent to bring all clusters to one side of the stem or the branch. It's a berry globular in shape being 1 cm in diameter with smooth lustrous surface. Unripened fruits are green and almost enclosed in spiny calyx, while, the ripened fruits are yellow. The seeds are dark brown in colour. The taste is minutely pitted (Andrews, 1956; Salih, 1979).

According to Yousif *et al*. (1996) Jubein (*Solanum dubium*), a major problem for many farmers in Sudan, is a noxious weed belonging to the plant that flourishes during the rainy season (typically starting in June- August in Sudan) and usually bears fruits about January with green fruits which become yellow when fully ripened. Fruits are usually dry on the stem; their thorny surface causes them to adhere to grazing animals and facilitates seed dissemination. Animals do not eat *Solanum dubium* because of its bitter taste and thorny leaves.

2.5.2 Chemical composition of *Solanum dubium*

Solanum dubium fruit contains 45.10% fiber, 20.15% carbohydrate, 16.63% crude proteins, 5.60% ether extract, 5.90% moisture and 6.0% ash (Habbani, 1992). The x- ray fluorescence analysis showed the presence of potassium, calcium, iron, manganese, sulphur, phosphorous, copper and bromine as the most abundant elements in *Solanum dubium* fruits. *Solanum dubium* is readily soluble at low salt concentrations functionality was determined as nitrogen and at the extremes of pH values (Habbani, 1992).

According to Salih (1979) *Solanum* plants containing steroidal substances are of considerable medicinal and economic importance due to the suitability of these substances as starting materials for the synthesis of steroidal drugs such as corticosteroids and sex hormones. Over 100 species of the genus *Solanum* have been reported to contain steroidal compounds, and the solasodine, the most suitable starting material for the synthesis of steroidal drugs, has been isolated from at least 60 species of *Solanum*.

Thin layer chromatography and gas liquid chromatography revealed the presence of six amino acids in *Solanum*, three of which being present in the hydrolysate were identified as phenylalanine, valine, and alanine (Osman, 1996).

Sulieman *et al*. (1988) studied the aqueous extract of the whole fruit, fruit coat and seed of *Solanum incanum* Lim for their milk coagulating properties. Phytochemical examination indicated that the active principle is glycoside and the whole extract was necessary for coagulation.

2.5.3 Characterization of *Solanum dubium* fruit extract

2.5.3.1 Effect of incubation temperature on milk-clotting activity

Recently Osman (2001) reported that *Solanum* rennet has a maximum activity at 60°C. Osman (1996) showed that the enzyme extract from *Solanum dubium* fruits has a maximum activity at a temperature between 45-50°C, a gradual decrease in activity was observed as the temperature increased reaching its minimum activity at 65°C.

Habbani (1992) reported that the maximum activity of *Solanum dubium* extract was shown at 31°C. Similar results were found by Mohamed and Habbani (1996) who pointed that the "Gubbain" extract

activity was increased up to 38°C and started to decline thereafter. Sidrach *et al*. (2005) found that the milk clotting protease from *Cynara scolymus* express maximum activity at 70°C.

Vieira De So and Barbosa (1972) showed that cardo clotting enzyme is stable at high temperature and shows an increasing clotting activity up to 70°C, above this temperature the activity falls and above 75°C completely disappears.

Bodansky (1924) studied milk-clotting enzyme of *Solanum elaeagnifolium*, and he found that the enzyme has a higher optimum temperature (80- 85°C) and resists heat better than animal rennet. Similar results were reported by Melachouris and Tuckey (1967) that the maximum activity of microbial rennet isolated from a culture of *Bacillus cereus* was obtained between 75-80°C. Milk clotting enzyme extracted from Kesinai (*Streblus asper* Lour) leaf exhibited maximum activity at 65°C. Results were also reported by Aworh and Nakai (2006) that the enzyme from Sodom apple (*Calotropis procera*) leaves was more active at 65°C than 35°C.

Pascaline and Daniel (2006) found that *Mucor miehei* and *Mucor pusillus* protease are much more stable at 53°C for 100 minutes, while bovine pepsin and the *Endothia parasitica* proteases are rapidly inactivated at 53°C in < 100 minutes. Extracellular aspartate protease from *Rizopus oryzae* was purified 91 times with 26% recovery using ammonium sulphate fraction, ion- exchange and size- exclusion chromatographic techniques, which acts optimally at 60°C and was more stable in temperature range of 30- 45°C (Kumer *et al*., 2005). Similar results were also reported by D' Ambrosio *et al*. (2003) who found that proteolysis and milk clotting activity in extract

obtained from the crustacens *Munida* has optimal temperature at 55-60°C.

Raposo and Domingos (2008) showed that, the optimum temperature for proteolytic activity of aspartic proteinases (Aps) from *Centaurea calcitrapa* plant cell suspensions was 52°C. The enzymes remained fully active when exposed for 6 hours at 4°C and 25°C. For all other temperatures, after 1 hour of incubation, activity decreased. At 37°C activity decreased 40%, and at 52°C decreased 60%. After 6 hours of incubation at 70°C, purified Aps extract lost almost all of its activity. However, the purified enzyme from goat (*Capra hircus*) was stable up to 55°C with maximum activity at 30°C (Kumar *et al*., 2006). Campos *et al*. (1990) pointed that the proteolytic activity of the crude extract from wild thistle (*Cynara cardunculus*) was found to be 37°C.

2.5.3.2 Effect of pH on the activity of *Solanum dubium* extract

Osman (2001) reported maximum activity of *Solanum dubium* extract was observed at pH 5.5 and the enzyme activity decreased with increasing pH value. Similar results were obtained by Habbani (1992) who reported that the activity of *Solanum dubium* rennet decreased with increasing pH value and the maximum activity was at pH 4.6 and 4.5. Kumer *et al*. (2005) showed that the purified enzyme from *Rizopus oryzae* is an acid protease with optimum pH of 5.5 and retained 96% of residual activity between pH 5.5 and 7.5. However, Thunell *et al*. (1979) found that the milk clotting activity from *Mucor miehei* protease to be destroyed at 79.5°C, 76.6°C and 73.9°C above pH 5.4, pH 5.8 and pH 6 respectively.

The maximum enzyme activity from *Bacillus sphaericus* was at a wide range of pH 5.7- 7.5 (El-Bendary *et* a*l*., 2007). However, Melachouris and Tuckey (1967) demonstrated that the milk clotting

activity of microbial rennet isolated from a culture of *Bacillus cereus* was found to be less sensitive to pH changes of the substrate than calf rennet.

Aworh and Nakai (2006) reported that the milk clotting activity using enzyme from Sodom apple leaves increased with pH at 65°C with enzyme being almost twice as active at pH 6.4 as at pH 5.4- 5.7. Similar results were obtained by Tavares *et al.* (1997) in their findings that Tuna protease was less sensitive to losses of activity than rennet at pH values above 6.4. Both enzymes became unstable beyond pH 7 and completely lost their activities at pH 8. While D'Ambrosio *et al.* (2003) found the optimal value of pH of the total proteolytic activity of the crustaceans *Munida* to be in the ranges of pH 6.6 – 7.5.

Raposo and Domingos (2008) studied the optimum pH of purified plant aspartic proteinases from C. *calcitrapa* cell suspensions and they detected maximum activity at pH 5.1. Kumar *et al*. (2006) found that the milk clotting activity of a purified enzyme extracted from abdominal tissue of goat kid decreased steadily as pH increased and indicated maximum activity at pH 5.5. The proteolytic activity of the crude extract from wild thistle (*Cynara cardunculus*) was found to be at pH 5.7 (Campos *et al*., 1990). However, extract from kesinai (*Streblus asper* Lour) leaf showed maximum enzyme activities at pH 7.2 (Ishak *et al*., 2006). Milk clotting protease from *Cynara scolymus* expressed maximum activity at pH 5 (Sidrach *et al*., 2005). Heimgartner *et al*. (1989) studied three proteases (Cynara 1, 2 and 3) purified from dried flowers of *Cynara cardunculus*. All three enzymes expressed maximum milk-clotting activity at pH 5.1. Nouani *et al*. (2009) reported that optimum activity falls within the range of the pH

acids, 5 for the fig tree (*Ficus carica*) enzyme and 5.5 for the artichoke protease (*Cynara scolymus*).

2.5.3.3 Effect of calcium chloride concentration on *Solanum dubium* extracts activity

Calcium is an important factor in cheese making, its effect on the activity of *Solanum dubium* extract showed an increase with increasing concentration (Habbani, 1992 and Mohamed and Habbani, 1996). Chazarra *et al*. (2007) found that the rennet strength of artichoke (*Cynara scolymus*) flowers extract increased with increasing concentration of calcium.

Ishak *et al*. (2006) showed that the presence of calcium chloride up to 6 mM decreased the milk clotting time of extract obtained from kesinai (*Streblus asper* Lour) leaf. Study of a milk clotting activity from *Bacillus sphaericus* was done by El-Bendary *et al*. (2007), and the results from their study indicated that the milk clotting activity of the purified enzyme was stimulated with increasing calcium chloride concentration up to 0.25%.

2.5.4 Toxicity of *Solanum dubium*

Although some species of *Solanum* are highly toxic and contain the steroid alkaloid, solanidine, glycoside, solanine, and a variety of other glycoalkaloids, the toxicity of *Solanum dubium* seed was studied by feeding rats the extract enzyme as well as white cheese made with enzyme. The results showed that *Solanum dubium* seed extract and *Solanum* cheese did not significantly affect the total protein and minerals of serum of all fed groups, and no remarkable gross or histopathogical alteration were detected in the liver or kidney of all experiment and control group (Osman, 2001).

2.6 Effect of salting and addition of calcium chloride on cheese

2.6.1 Salting and the role of salt in cheese

Salt has three major functions in cheese: it acts as a preservative, contributes directly to flavour, and is a source of dietary sodium. Together with the desired pH, water activity and redox potential, salt assists in cheese preservation by minimizing spoilage and preventing the growth of pathogens. The dietary intake of sodium in the modern western diet is generally excessive, being two to three times the level recommended for desirable physiological function (2.4 g Na, or 6 g NaCl per day). However, cheese generally makes a relatively small contribution to dietary sodium intake except if high quantities of high-salt cheeses such as Domiati and Feta are consumed (Guinee, 2004). Sodium chloride influences cheese ripening principally through its effect on water activity, control of varios enzyme activities in cheese, synerisis of the curd and physical changes in proteins which influence cheese texture and solubility (Fox *et al*., 1995).

In addition to these functions, salt level has a major effect on cheese composition, microbial growth, enzymatic activities and biochemical changes, such as glycolysis, proteolysis, lipolysis and para-casein hydration, which occur during ripening. Consequently, the salt level markedly influences cheese flavour and aroma, rheology and texture properties, cooking performance and, hence, overall quality. Many factors affect salt uptake and distribution in cheese and precise control of these factors is a vital part of cheese making process to insure consistent, optimum quality (Guinee, 2004)**.**

Cheese is salted by adding sodium chloride directily to milk and packed in tin (Khalid and El Owni 1991). In Sudanese white cheese

the salt is added befor rennetig in amounts varing between 8-12% (Osman, 1987).

According to Mark (2002) salt directly contributes to flavour and it also influences the perception of other flavour compounds. Salt affects enzyme activity, the growth and metabolism of microorganisms, and due to its effect on casein, will decrease proteolytic activity. Without the contribution of enzymes or microorganism capable of metabolic activity at the salt concentration in the cheese, very little flavour will develop. Cheese very low in salt may be more prone to develop bitterness and a softer texture. Kosikowski (1977) added that the purpose of salting is to prevent the growth of spoilage microorganisms and contributes towards the flavour of cheese. During ripening, salt diffuses throughout the cheese and so the differences in salt content of the centre and periphery decrease with ripening time.

Pastorino *et al*. (2003) reported that adding salt to cheese alters protein interactions, such that the protein matrix becomes more hydrated and expands. However, increasing the salt content of cheese did not cause an exchange of calcium with sodium. Therefore, calcium- mediated protein interactions remain a major factor controlling cheese functionality.

Walstra *et al*. (1985) found that the addition of the salt to milk, as in the manufacture of Domiati, rather than the curd might be considered desirable, as it would give uniform S/m in the cheese immediately after manufacture. However, the addition of salt to cheese milk, even at low levels (<1% w/w), is undesirable as it severely impairs the ability of milk to clot and form curd (Abu- El-Nur, 1998), and of the curd to synerese. Creamer (1985) pointed that

the adverse effect of salt on curd formation is probably a consequence of firstly binding of Na^+ ions by the casein and the continued reduction in the level of colloidal calcium, secondly, increase in casein hydration especially as the pH decreases in the range 6.6-5.2.

Adding salt to milk or casein systems promotes dissociation of calcium and phosphate from within casein micelles and into solution (Gaucheron *et al*., 2000). They suggested that adding salt promotes calcium solubilization from paracasein in casein pellets and cheese (Kindstedt *et al*., 1992), thus displacing calcium from the protein matrix and into the serum.

O'Conner (1993) reported that, the salt may be added to cheese curd or it may be incorporated in the finished cheese by immersion in a brine solution. The addition of salt retards the growth of bacteria which may cause flavour and other defects in the cheese.

El Owni and Hamid (2008) found that Salt concentration significantly affected weight loss, chemical composition, and microbial content and sensory characteristics of Sudanese white cheese.

2.6.2 Addition of calcium chloride

Bille *et al. (2001)* reported that the addition of calcium chloride to pasteurized milk for cheese making affected the flavour of the cheese and reduced preference for it. So most of the sensory panelists, however, preferred the cheese without added calcium chloride as it had a better taste and smell and was softer, more pliable and tender to eat. Cheese without added calcium chloride tends to retain more moisture, mature faster and develop greater acidity. Furthermore the addition of calcium chloride to cheese has an influence on the flavour and total nutrient content (Wolfschoon-pombo, 1997).

Calcium chloride is commonly added to milk, especially during winter, to improve coagulation. Addition of 0.02% (about 1.8 mM) calcium chloride to cheese increases curd firmness about 32%. Curd firmness may increase 81% when 10 mM calcium chloride is added, higher concentration results in decreased curd firmness (Jen and Ashworth, 1970)

2.7 Chemical composition and quality characteristics of cheese

Abdalla and Nuser (2009) studied the effect of storage period on chemical and sensory characteristics of white cheese made from pasteurized milk. Results showed that fat, protein and total solids content decreased with advancement of storage period, while ash content and titratable acidity increased throughout storage period. Sensory evaluation 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 (2007) studied twenty four Sudanese *Gibna Bayda* samples manufactured in Zalingei area, Western Sudan. The result showed that *Gibna Bayda* contained 45.17-58.17% total solids, 19.27-23.83% fat, 19.70- 29.73% TN, 0.26-1.28% WSN, 1.87- 7.80% ash, 2.27- 8.77% salt, 0.36- 1.80% titratable acidity, VFA, 2.77- 5.07 mg/100gm, 131.0- 277.50 mg/100gm tryptophan, 6.63- 146.80 mg/100gm tyrosine respectively. The values obtained for ripening indices were 12.33- 18.33 and 93.0- 133.33 for FRI and SRI of cheese respectively.

According to Warsama *et al*. (2006) Sudanese white cheese contained 47.8% total solids, 14.0% fat, 15.9% protein, and 6.2% ash. Sudanese white cheese made with 6% salt had higher total solids, crude protein and fat content (Hamid *et al.*, 2008).

Average total solids, fat, protein, and salt contents were 38.83, 16.66, 15.42, and 3.56% respectively, at the end of 30 days storage of Turkish fresh goat cheese manufacture using controlled process (Kilio *et al*., 2004). Sensory evaluation showed a weak goat milk flavour in cheese by panelists and the most acceptable cheese was at 30th day of storage from flavour point of view. Alalade and Adeneye (2006) found 70.75% moisture, 39.00% fat, 37.08% protein, 2.53% ash, 29.25% total solids, and 4.85 pH in Wara cheese stored at room temperature.

2.8 Organisms associated with cheese

The principal microorganisms that are involved in refrigerated milk spoilage are Psychrotrophic organisms. Most psychrotrophs are destroyed by pasteurization temperatures, however, some bacteria such as *Bacillus cereus*, *B. licheniformis* and *B. sporothermodurans* are able to survive and grow in subsequent processing operations during cheese making (Johnson *et al*., 1990; Patterson *et al*., 1996). It was observed that milk, being the only natural source of the disaccharide lactose, undergoes spoilage in a unique way as the lactose in milk is conspicuously utilized by coliform bacteria (Jay *et al.,* 2005). Pasteurization of milk used in the manufacture of Domiati pickled soft cheese reduced the mean total colony count (cfu/g) by 5 logs during a storage period of 30 days, coliform and staphylococci were completely eliminated from the first day by this treatment (Aly and Galal, 2002).

El Owni and Hamid (2008) studied the effect of storage period on microbiological characteristics of Sudanese white cheese (*Gibna Bayda*). Their result showed that total bacterial count (TBC), coliforms, *E. coli*, *Staphylococcus aureus* and psychrotrophic bacterial

counts significantly ($P<0.05$) decreased when storage period progressed. Investigation was carried out to evaluate the effect of packaging material on the quality of Sudanese white cheese. Results of microbial analysis showed significant difference ($P<0.05$) in total bacterial count, coliform, *E.coli* and yeasts counts of cheese in different packaging materials (El Owni and Hamid, 2009). The highest values were in plastic packages stored at different temperatures, while there was no significant difference ($P>0.05$) in moulds count. The total bacterial count was significantly ($P<0.05$) affected by storage temperature, the highest value was at room temperature. However, yeast, moulds, coliform and *E.coli* counts were not significantly ($P<0.05$) affected. The total bacterial count, yeasts and moulds increased during storage period, while coliform and *E.coli* counts decreased (Alhassan, 2005).

Sulieman (2007) investigated the effect of heat treatment of milk on the quality characteristics of Jibna-beida (white cheese). The heat treatment methods used included pasteurization of cow milk at 65^{o}C for 30 min, and boiling of cow milk at 102^{o}C for 15 min. The microbiological analysis indicated that raw milk contained high counts of total viable microbes, coliforms, staphylococci, yeast and molds. However, all these microbial groups were highly reduced in pasteurized and boiled milks**.** The psychrophilic counts, total bacterial counts, coliform counts and yeasts and molds counts showed significant differences ($P<0.05$) with different ripening times (15 and 30 days) in processed cheese made from white Sudanese cheese by Nour El Diam and El Zubeir (2006).

El Zubeir *et al*. (2006) conducted a survey in Restaurants in Khartoum State, a total of thirty one Enterobacteriaceae were found in

twenty samples (66.7%) out of thirty samples of Sudanese white cheese. The total isolated include *Citrobacter freundii* (46.7%), *E. coli* (20%), *Enterobacter aerogenes* (6.6%), *Pseudomonas aeruginosa* (3%), *P. mirabilis* (16.7%) and *Salmonella ssp*. (10%)

Soft cheeses have higher moisture content when compared to hard cheese and have lower shelf life due to microbial spoilage. Most soft, unripe cheese is microbiologically unstable due to metabolic activity of bacteria, yeast or mould contaminants (Farkye and Verdamuthu, 2002). There have been reports (Ryser, 1998) of some pathogenic microorganisms including *Listeria monocytogenes*, and *Staphlococcus aureus* in soft cheese. *Esherichia coli* serotype 0157:H7, has been associated with the consumption of French Brie and Camembert soft cheeses in the USA and Scandinavia (D'Aoust, 1989).

Many cases of brucellosis food poisoning caused by *Brucella spp*. have been associated with Baledi or Jibnah (Raheem 2006). Another mountain cheese from Greece 'Orinotyri' made from fresh ewe's milk had a microbial count of 10^7 cfu/g for Enterobacteriaceae, 10^7 cfu/g for coliforms and 10^2 cfu/g for yeast after 10 days. This was attributed to the high pH of the cheese before the action of lactic acid bacteria during ripening (Prodromou *et al*., 2001).

The microbiology of the Domiati cheese has been very well investigated (Gilles, 1984; Nielsen, 1984; Abou-Donia, 1986). In the production of this cheese the use of starter is optional but when used it must be salt tolerant. The fresh cheese is rather salty, and as it is aged it develops considerable acidity. The total counts of bacteria in fresh cheese have been reported to be 2×10^4 cfu/g as determined on

standard plate count. After pickling and ripening for 2-5 months, the count was reduced to 2×10^2 (Rakshy and Attia, 1979).

Lactic acid bacteria such as lactococci and lactobacilli have been isolated from the surface slime on Domiati cheese (Abo-Elnaga, 1974). Fahmy and Youssef (1978) found that *Lactococcus lactis* sub-sp. lactis and *lactis biovar diacetylactic* were the dominant lactococci, and that L. casei sub-sp. casei and *L. delbrueckii* sub-sp. bulgaricus were the dominant lactobacilli in ripened Domiati cheese.

When the effect of 3-11% salt on the inhibition of gas forming organisms (coliforms) was studied, there was a positive relationship between the presence of coliforms and poor flavour and texture of cheese (Sadek and Eissa, 1956; Abu-Donia, 1991). El-Molla *et al*. (1981) reported that *S. entericia* sub-sp. entericia serotype typhimurium survived with 5% salt added to the cheese milk but there was more inhibition at salt level of 10% and 15%.

Sousa and Malacta (1997) reported that Enterobacteriaceae and coliform counts were 1.7 and 1.4 log units higher in 60 day vegetable rennet cheese than in 60 day animal rennet cheese. The longer coagulation time of vegetable rennet favoured microbial growth. However, Kilio *et al*. (2004) found that the number of lipolytic and proteolytic bacteria reached a maximum value at 15th day and staphylococci and coliforms were not enumerated at the end of storage period. Coliform bacterial count of Wara cheese was 472.75×10^5 cfu/gm which was significantly ($P< 0.05$) affected by storage period (Alalade and Adeneye, 2006).

Turkoglu *et al*. (2003) studied the microbiological quality of Orgu cheese, and they found that coliform, total aerobic mesophilic bacteria, lactic acid bacteria, lipolytic and proteolytic microorganisms

and yeasts and molds count were 3.73, 6.89, 6.78, 5.29, 4.50 and 5.45 cfu/gm, respectively. However, the coliform, total aerobic mesophilic bacteria, lactic acid bacteria, lipolytic and proteolytic and yeasts and molds counts were 5.99, 7.82, 7.01, 5.73, 4.56 and 5.03 cfu/gm, respectively in Sikma cheese (Ceylan *et al*., 2003)

2.9 Cheese ripening

Cheese ripening is a complex process involving a range of microbiological and biochemical reactions. Microorganisms present in cheese throughout ripening, play a significant role in the ripening process (Cogan and Beresford, 2002).

Cheese ripening is a very complex biochemical process by which the rubbery or elastic crud is converted into a smooth-bodied and fully flavoured cheese (Kheadr *et al*., 2003). Flavour and texture are considered as the two main criteria in determining the acceptability of aged cheese. The time required for developing characteristic flavour and texture varies from a few weeks for soft cheeses up to three years for very hard varieties. During this period, cheese attain their own characteristics through a multitude of chemical, microbiological and biochemical changes whereby protein, fat and residual lactose are broken down to primary products which are further degraded to secondary products (Kheadr *et al*., 2003).

Cheese manufacture and ripening involves the action of enzymes (from rennet and milk) and selected microorganisms, both directly, while growing, and indirectly, through their enzymes after death and lyses (McSweeney, 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 (McSweeney, 2004_b)

Proteolysis is usually regarded as the most important biochemical event during cheese ripening and one of the most important factors for development of typical cheese flavuor and texture. Proteolytic agents in cheese originate from five sources: indigenous milk proteinases (plasmin and cathepsin D); rennet (chymosin) or rennet substitute (e.g., bovine, porcine, and chicken pepsins) or acid proteinases from *Rhizomucor miehei*, *R. pusilus*, and *Cryphonectria parasitica*; proteinases and peptidases from starter microorganisms (e.g., *Lactococcus*, *Streptococcus*); proteinases and peptidases from secondary microorganisms (e.g., *Propionibacterium* spp., *Brevibacterium linens*, yeasts and molds); and enzymes from non-starter bacteria(Sousa and Malcata,1997). Initial hydrolysis of caseins is caused chiefly by residual rennet itself and by enzymes contributed by viable or lysed starter and non-starter microorganisms. The production of small peptides and free amino acids result from the catalystic action of bacterial proteinases and peptidases (Sousa and Malcata, 1997).

Proteolysis is probably the most important biochemical event during the ripening of most cheese varieties with a major impact on flavour and texture (Fox, 1989). However, during cheese ripening, the caseins are broken down by proteolysis. Several proteolytic agents are involved; casein is hydrolysed to large peptides mainly by the coagulant and some indigenous milk enzymes, large peptides are hydrolysed to small peptides by microbial proteinases and small peptides are hydrolysed to amino acid by microbial peptidases. The extent of this degradation process plays an important role in

determining cheese flavour and texture and depends on the activities of rennet and microbial enzymes (Law, 1987). Proteolysis can vary substantially according to cheese variety. Therefore, every type of cheese has its own characteristic proteolytic pattern, resulting from the enzymatic degradation of peptides by various enzymes, and also from amino acid catabolism (Polo *et al*., 1985)

Proteolysis in cheese during ripening plays a vital role in the development of texture as well as flavour and has been the subject of several reviews (Fox *et al*., 1995; Fox and McSweeney, 1996). Proteolysis contributes to textural changes of the cheese matrix, due to break down of the protein network, decrease in a_w through water binding by liberated carboxyl and amino groups and increase in pH (in particular in surface mould-ripened varieties), which facilitates the release of sapid compounds during mastication. It contributes directly to flavour and to off-flavour (e.g., bitterness) of cheese through the formation of peptides and free amino acids as well as liberation of substrates (amino acids) from secondary catabolic changes, i.e., transamination, deamination, decarboxylation, desulphuration, catabolism of aromatic amino acids and reactions of amino acids with other compounds.

In many cheese varieties, the initial hydrolysis of caseins is caused by the coagulant and to a lesser extent by plasmin, which results in the formation of large (water-insoluble) and intermediate-sized (water-soluble) peptides which are degraded subsequently by the coagulant and enzymes from the starter and non-starter microflora of the cheese. The extracellular, cell envelope-associated proteinase of *Lactococccus* (lactocepin, PrtP) contributes to the formation of small peptides in cheese probably by hydrolysing the larger peptides

produced from α_{s1}-casein by chymosin or from β-casein by plasmin, whereas the peptidases (which are intracellular) are released after the cells have lysed and are responsible for the degradation of short peptides and the production of free amino acids. 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 microflora. 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(McSweeney, 1997). Proteolysis contributes to the taste of cheese by the production of peptides and free amino acids and the sapid flavour compounds generally partition into the soluble fraction on extraction of cheese with water. Large peptides do not contribute directly to cheese flavour, 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).

Molina *et al*. (1999) further fractionated the water soluble fraction of cheeses made from cow's, ewe's and goat's milk and assessed the contribution of small peptides, free amino acids and volatile components to cheese flavour. Differences were reported in intensity and predominance of individual tastes in the various fractions of cheeses 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 flavour intensity was found in the fractions with the highest concentration of amino acids and volatile compounds (Molina *et al*., 1999).

The composition of the amino acid fraction and the relative proportions of individual amino acids are thought to be important for the development of the characteristic flavour (Broome *et al*., 1990; Engels and Visser, 1994; Molina *et al*., 1999). However, the relative proportion of individual amino acids appears to be similar in many cheese varieties and increasing the concentration of free amino acids in cheese does not necessarily accelerate ripening nor flavour intensity (Christensen *et al*., 1995). Fox and Wallace (1997) suggested that cheese flavour and the concentration of free amino acids could not be correlated, since different cheeses (e.g., Cheddar, Gouda and Edam) have very different flavours, although the concentration and relative proportions of free amino acids were generally similar.

According to Rossano *et al*. (2005) Miniature (20g) cheddar type cheeses were manufactured using enzyme extract from the crustacean *Munida* or chymosin as coagulant. Cheeses were ripened at 8°C and samples were collected for analysis after 2, 6 and 12 weeks. The results showed that cheese manufacture with *Munida* extracts had a higher extent of degradation of β-casein than cheese made using chymosin as coagulant. In general, the products of proteolysis were more complex in cheese made using the *Munida* extracts than in cheese made by chymosin as coagulant. 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.

2.10 Effect of vegetable rennet on chemical composition, quality and microbiolgical characteristics of cheese

Fernández-Salguero and Sanjuán (1999) Studied enzymes from thistles of the genus *Cynara* in the making of traditional ewe's milk cheese. They found that casein hydrolysis was found to be much more extensive and faster in cheese made using vegetable rennet. The levels of insoluble tyrosine and tryptophan were higher in cheese produced with vegetable rennet. However, the type of rennet had no significant effect on cheese composition in terms of moisture, fat, protein, salt and pH at the center and the surface over the ripening period (Sousa and Malcata, 1997).

Roa *et al*. (1999) reported that, vegetable rennet extract from *Cynara cardunculus* flower was traditionally used in the manufacture of La Serena cheese. When measurements were carried out in 16 different cheeses, vegetable rennet appeared to be highly stable during cheese ripening. Cheese composition (moisture, pH, NaCl, fat and protein) was kept relatively constant during ripening.

Free fatty acids reached lower values in cheese made from milk coagulated with vegetable rennet than in that made from milk coagulated with animal rennet during most of ripening period (Gaya *et al*., 1990).

Moisture content was lower in the interior and on the surface of cheese made with vegetable rennet compared with that made with animal rennet. Higher whey retention in cheese with animal rennet allowed more lactose to be available for microbial fermentation (Fernandez del Pozo *et al*., 1988).

Adetunji and Salawu (2008) claims that the nutritional contents of *Carica papaya* and *Calotropis procera* processed cheese were

evaluated; fat, protein, moisture, sugar, Zn, Mn, Fe, and Cu and their values were 22.3 and 31.45%, 31.60% and 33.84%, 62.5% and 61.70%, 2.05 and 8.10%, 1.19 and 4.14% 2.80 and 2.35%, 4.8 and 4.7%, 4.6 and 7.3% for *Carica papaya* and *C. procera* processed cheese, respectively. The value obtained for fat, protein, sugar, Zn, and Cu were higher in *C. procera* processed cheese while Fe and Mn were higher in *C. papaya* processed cheese.

Yield, chemical composition and texture profile of cheese made with vegetable rennet from Sodom apple leaves were compared with those of a direct acid cheese made with calf rennet. Yield, moisture, fat and protein contents were 14.47%, 49.70%, 26.15% and 20.0% respectively, for cheese made with vegetable rennet and 12.45%, 44.80%, 29.84% and 20.4%, respectively, for the direct acid cheese made with calf rennet. Cheese made with vegetable rennet had less soluble nitrogen than that made with calf rennet despite the fact that vegetable rennet was more proteolytic in casein soluble than calf rennet. Relative to that made with vegetable rennet was harder, less cohesive and gummier, presumably because of differences in chemical composition between the cheeses (Aworh and Muller, 1987).

Roserio *et al*. (2003) pointed that plant proteases are considerd too proteolytic, leading to generation of excessive acid, bitter flavour and texture defect in cheese.

Tejada *et al*. (2007) studied two vegetable coagulants (powdered vegetable and crude aqueous extract) from the cardoon (*Cynara cardunculus*) for ewe milk cheese manufacture and stored for 90 days. They found that cheese made with vegetable coagulant displayed a slightly more bitter taste than those made with rennet. However, Prados *et al*. (2007) studied a powdered vegetable coagulant

from cardoon (*Cynara cardunculus*) compared with calf rennet in batches of Manchego cheese, which were monitored over a 6- month ripening period. They found that, for most chemical parameters (moisture, fat, protein, acidity, NaCl, pH and water activity), no differences were observed between the two types of coagulants. However, higher casein hydrolysis was observed after 2 days of ripening in cheese produced with vegetable coagulant compared with those made with rennet. Soluble nitrogen was significantly higher and the other nitrogen fractions were only slightly higher in cheese made with vegetable rennet. In general, the sensory quality (odaur, colour, taste intensity and creaminess) was higher in cheese obtained with vegetable coagulant than those made with animal rennet.

Chen *et al*. (2003) studied aqueous extract of Australian cardoon (*Cynara cardunculus* L.). Flowers were used to produce Pecorino cheese from ovine milk and the chemical, biochemical and sensory properties of the cheese were compared with cheeses made using two commercial (calf and microbial) rennet preparations. They found that the cheeses contained 49.8–54.6% moisture, 28.2–31.2% fat, 2.1–2.3% NaCl and 2.6–3.1% total nitrogen. Very few differences in chemical composition were observed between cheeses manufactured with the three different coagulants. Biochemically, however, cheese manufactured with cardoon extract contained significantly ($P<0.05$) higher levels of water-soluble nitrogen but lower levels of phosphotungstic acid soluble nitrogen, while the levels of trichloroacetic acid soluble nitrogen were similar among the three types of cheese. Compared with cheeses made with commercial rennet preparations, cheese made with cardoon extract was perceived to be

softer, creamier and less bitter, and was rated significantly ($P<0.05$) higher in overall liking by both trained and consumer panels.

2.11 Whey of cheese

Whey is the liquid that remains after most of the fat and the protein in the milk are removed during cheese making process. Whey contains valuable nutrients, i.e. whey protein, carbohydrate and minerals. The whey from cheese making vary according to the type of cheese made and, therefore, the content of protein, salt and lactose also vary. As whey contains about half of the total solids in the original milk it should not be thrown away as waste but should be used as animal feed or for human nutrition (O'Conner, 1993; Butylina, 2007).

Whey from milk coagulated with vegetable rennet had 10.4% total solids, 2.54% fat and 0.33% total N. High levels of total N in whey from milk coagulated with vegetable rennet may be ascribed mainly to the strong proteolytic activity of the coagulant with formation of soluble N which resulted in heavy fat and total solids in whey than from milk coagulated with vegetable rennet. This break down of casein network resulted in heavy fat and total solids losses in whey from milk coagulated with vegetable rennet (Nuńez *et al*., 1991).

Barbosa *et al*. (1976) reported 1.14% total proteins for whey from vegetable rennet and 0.86% for whey from animal rennet in Camembert cheese making. Significant increases of protein in whey from vegetable rennet were also obtained for Grana, Provolone and Bel Paese cheeses (Barbosa *et al*., 1981). Higher solids, protein and fat contents were found in whey from cheddar cheese made with *Bacillus*

subtilis proteinases as coagulant enzymes (Puhan and Irvine, 1973) which lowered cheese yield by 10% compared with that made with calf rennet.

Chapter Three

Experiment one

3. Extraction, partial purification and characterization of milk clotting proteases from *Solanum dubium* fruit

3.1 Materials and Methods

3.1.1 Plant material

The plant material used in this study was collected from Abu-Naama area, Sinnar State, Sudan. The *Solanum dubium* fruits were collected at different stages of maturity; from first stage in September when the fruits were small, green with white seeds till the last stage of maturity in April when the fruits were yellow, with black and completely dry seed.

The *Solanum dubium* fruits were carefully cleaned and powdered using finger, laboratory mortar and electric grinder. The dry powder (5gm) was macerated with distilled water (30ml) and kept for 15 minutes, 24 hours at 5°C, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours and 168 hours, then filtered through filter paper. In another experiment the crushed material (5gm) was macerated with distilled water (30 ml) and kept for 15 min, 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours and 48 hours at 5°C.

3.1.2 Enzyme preparation

Four extraction methods were tried to select the one which give a reasonably high activity.

1. Extraction with distilled water:

The yellow fruits of *Solanum dubium* plant were powdered using laboratory mortar, then five grams were macerated in a conical

flask for 24 hours at 5°C using distilled water (30ml) with occasional shaking for the first 3 hours and solutions were then filtered through filter paper. The aqueous filtrate was used for testing its milk clotting activity.

2. Drying in a current of warm air:

A measured volume of filtrate (*Solanum* extract) was spread on a shallow glass basin and exposed to a current of air at 45°C (Krisharmurti and Subrahmanyan, 1948_a) till compeletly dry.

3. Soaking in sodium chloride and evaporation in a current of warm air:

Hundred grams of coarsely ground plant powder were soaked in 1%, 2%, 3%, 4% and 5% sodium chloride for 24 hours at 5°C. The operation was carried out at 5-10°C overnight. The solution was filtered and the filtrate was finally spread on a shallow glass basin and exposed to a current of a warm air (45°C), according to the modified method of Krisharmurti and Subrahmanyan (1948_b).

4. Freeze-drying

The powdered yellow fruits (100gm) were macerated in a conical flask for 24 hours using distilled water with occasional shaking for the first 3 hours and the solution was kept in a deep freeze at -25°C then freeze-dried in Stokes freeze-dryer.

3.1.3 Stability of *Solanum dubium* fruit extract

Powdered extracts of *Solanum dubium* fruits (Drying in a current of warm air) were kept in storage at refrigerator (4°C) and room temperature (37°C) for five months, while aqueous extracts of the *Solanum dubium* fruits were kept in refrigerator (4°C) and room temperature (37°C) for three months. Samples from each have been tested every month for activity.

3.1.4 Determination of crude *Solanum dubium* extracts activity

1. The activity of *Solanum dubium* fruit extract was determined according to the method described by Habbani (1992). One ml of the *Solanum* extract was pipetted into glass tubes containing 10 ml skimmed –milk. The tubes were placed in a water bath at 37°C, and continuously examined for the first onset of coagulation. Clotting activity was determined according to the following equation:

Activity (U) = (Volume of extract/ Clotting time (seconds)) ×100

The unit of activity (U) is defined as the number of ml of extract required to clot 10 ml of skimmed milk solution in 100 seconds at 37°C.

2. Enzyme activity assays were performed using the method of Anson (1938). The test substrate used was 2 %(w/v) of casein (Hammersten BDH chemicals, Poole, England), dissolved in a 0.03 M phosphate buffer, the substrate and enzyme were brought to the incubation temperature (usually 37°C unless stated otherwise) before adding the enzyme to the substrate. One ml of the diluted *Solanum dubium* extract was added to a duplicate set of test tubes containing a 1 ml substrate. The mixture was shaken vigorously and incubated for 10 minutes at 37°C in a shaker water bath adjusted to give 100 revolutions per minute. The reaction was stopped by adding 2 ml (w/v) of 5% Trichloroacetic acid (TCA). The mixture was left for 20 minutes prior to filtration through a Whatman filter paper No.42.

The filtrate(1 ml) was transferred into a test tube and 5 ml of Lowry solution C (Lowry *et al.*, 1951) was added, the mixture was then allowed to stand for 20 minutes and 0.5 ml of the Folin and Ciocalteaue (1927) phenol reagent diluted 1:1 in distilled water was added. The test tubes were left at room temperature for 45 minutes for

color development and the absorbance was measured at 660 nm using a PD-303-1-OMA-102 spectrophotometer. Blank was prepared by the same procedure used for the samples except that the casein was added after the addition of the TCA. For interpretation of the results a tyrosine standard curve was prepared and the activity was calculated by reading off the equivalent tyrosine expressed in terms of equivalents of tyrosine per ml.

Preparation of tyrosine standard:

L-tyrosine (0.2 gm) was dissolved in 2.5% TCA solution and made up to one liter to give 0.2 mg/ml. From this, stock solutions were made using 2.5% TCA to give concentrations ranging from 20 µg/ml to 200 µg/ml.

Definition of unit of activity

One unit of protease was defined as the amount of enzyme required to produce 1µg equivalent of 5% TCA soluble product (as tyrosine) per millimeter per minute at 37°C using 2% casein solution at pH 11.

Definition of specific activity unit

One specific activity unit is defined as one enzyme activity unit divided by one milligram protein (enzyme).

N.B.

The above units were recommended by the commission of Enzymes of the IUB (International Union of Biochemistry) 1961.

3.1.5 Properties of *Solanum dubium* extract (raw)

3.1.5.1 Effect of milk temperature on time of coagulation

The milk was brought to different temperatures starting from 35°C to 80°C, then the extract was added and the time taken for the coagulation of milk at such temperature was recorded. In all cases 1 ml of the extract was added to 10 ml of reconstituted skim milk.

3.1.5.2 Effect of preheating of milk on *Solanum dubium* activity

The same volumes of milk were heated to different temperatures; 35°C, 40°C, 50°C, 60°C, 70°C and 80°C and kept at those temperatures for 10 minutes and then cooled. One ml of *Solanum* extract was added to 10 ml of the milk, and the time of coagulation recorded at each temperature.

3.1.5.3 Effect of incubation temperature on activity of *Solanum dubium* extract

The activity of *Solanum dubium* extract was measured at various temperatures by adding 1 ml of *Solanum dubium* extract to 10 ml of reconstituted skim milk and kept at different temperatures, and the time of coagulation at each temperature was recorded.

3.1.5.4 Effect of heating the *Solanum dubium* extract

In order to ascertain the change in activity of enzyme kept at different temperatures the following experiment was performed. A series of small test-tubes each containing about 1.5 ml of *Solanum dubium* extract in water were immersed in a water-bath at the desired temperature. The tubes were gently shaken for half a minute and then stoppered so that no water could evaporate. After varying times of heating, each tube was plunged into an ice-bath which quickly stopped further destruction of the enzyme. The tubes were tilted and rotated horizontally to collect condensed moisture on the walls of the tube.

The residual clotting activity was determined and compared to that of unheated solution.

3.1.5.5 Effect of *Solanum dubium* extracts concentration on its activity

Effect of concentration of *Solanum dubium* extract on activity was studied by two different methods. In the first experiment, different volumes (50 ml, 100 ml, 150 ml, 200 ml, 250 ml, 300 ml, 350 ml and 400 ml) of reconstituted skim milk were used for clotting study using the same concentration (1 ml) of *Solanum* extract. In the other experiment to the same volume of milk (10 ml) different quantities (0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, 1 ml) of *Solanum dubium* extract were added for the curdling study.

3.1.6 Concentration of *Solanum dubium* extract by ammonium sulphate

The crude extract of *Solanum dubium* fruit was precipitated by ammonium sulphate using different concentrations (0-90%). Precipitation was carried out at 5°C, and the precipitate was recovered by centrifugation. The supernatant was discarded and the sediment from each salt concentration was re-suspended in buffer solution (pH 10) and dialyzed against distilled water for 24 hours, while the distilled water was changed six times. The enzyme was then dialyzed against buffer of pH 10 for 12 hours. The volumes of the dialyzed enzyme concentration and protein contents were measured.

Protein content of partially purified *Solanum dubium* extract was determined using proteins (Lowry) protocol (Lowry *et al*., 1951).

3.1.7 Characterization of partially purified enzyme(s)

3.1.7.1 Effect of incubation temperature on enzyme activity

The effect of temperature on the enzyme concentrate activity was determined at 40, 50, 60, 70, 80, 90 and 100°C. Casein solution (2%) in buffer at pH 10 was used as enzyme substrate. The enzyme concentrate was diluted to 100 fold. One ml of the diluted enzyme solution was added to one ml of casein solution and incubated at the required temperature for 10 minutes. Proteolysis was stopped by the addition of 2 ml of 5% TCA and the method of Anson (1938) already described.

3.1.7.2 Effect of pH on enzyme activity

Casein solution (2% w/v) was suspended in 4, 5, 6, 7, 8, 9, 10, 11 and 12 pH buffers. Both enzymes and substrate were allowed to equilibrate with the incubation temperature of 37°C before adding the enzyme to the substrate. Enzyme activity was measured according to Anson (1938).

3.1.7.3 Effect of calcium chloride on coagulation time

Different amounts of calcium chloride (0%, 0.02%, 0.04%, 0.06%, and 0.08%) were added to reconstituted skim milk. One ml of *Solanum dubium* extract was added to each calcium chloride skim milk substrate.

3.2 Results and Discussion

3.2.1 Plant materials

The Highest activity ($P< 0.05$) was obtained when *Solanum dubium* fruit was crushed using laboratory mortar (1.27±0.23 U/ml), followed by fingers (0.90±0.09 U/ml) and electric grinder (0.57±0.08 U/ml). The slight loss of activity from the latter extract may be attributed to the combined effect of the mechanical process and the heat generated by the grinder. Mohamed and Habbani (1996) found that a milk coagulating substance was obtained from dry, finger crushed "Gubbain" berries showed no detectable enzymatic activity.

Fig. 1 (appendix 2) show the milk-clotting activity of *Solanum dubium* fruit soaked in distilled water for 15 minutes to 168 hours. The maximum milk-clotting activities were 2.26±0.05, 1.84±0.04, and 1.27±0.10 obtained in 24, 48, and 72 hours of soaking the crushed plant materials. Analysis of variance showed significant differences ($P<0.001$) amonge time intervals. Similar results were found by Osman (1996) and Osman (2001). Our results disagree with Habbani (1992), Mohamed and Habbani (1996) and Yousef *et al*. (1996).

3.2.2 Effect of stage of maturity of *Solanum dubium* fruit on enzyme activity

Table 3-1 shows the relationship between stage of maturity of *Solanum dubium* fruit and their milk-clotting activity and pH. Activity significantly ($P<0.001$) increased from 0.03±0.01 to 1.87±0.38 U/ml at stage 1 and stage 8 respectively. The *Solanum dubium* fruit reached its maximum activity after the plant was completely dry and the fruit is dark yellow in color (Plate 2) and dry (approximately 8 months after

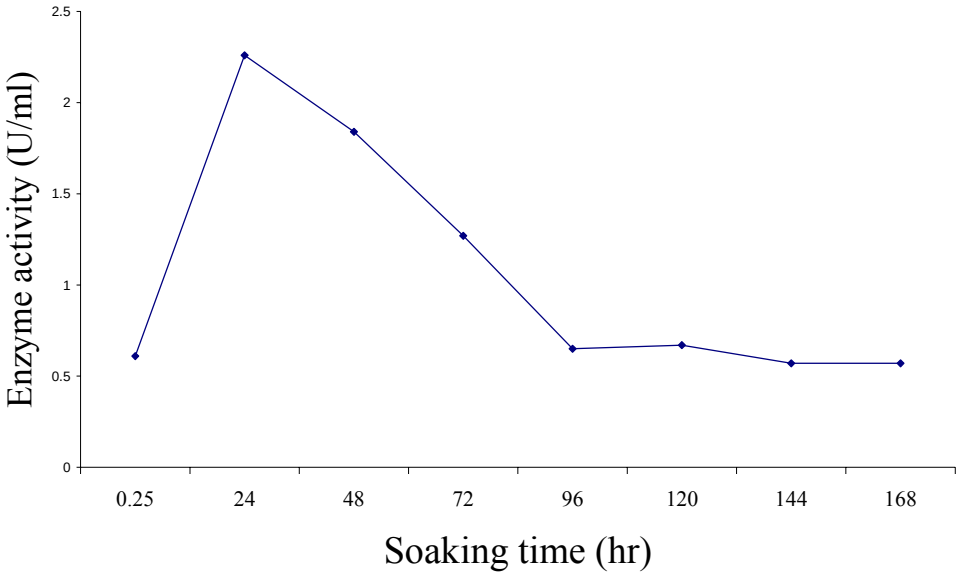

Fig. 1: Effect of soaking time of crushed *Solanum dubium* fruit in distilled water on milk clotting activity

Table (3-1): Effect of maturity stage of *Solanum dubium* fruit on enzyme activity

Stage of maturity	pH	Activity U/ml
Stage 1	4.91^{c} ±0.10	0.03^{a} ±0.01
Stage 2	6.62^{e} ±0.33	0.04^{a} ±0.01
Stage 3	5.52^{d} ±0.08	0.13^{a} ±0.08
Stage 4	4.89^{c} ±0.07	0.25^{b} ±0.09
Stage 5	4.65^{b} ±0.04	0.30^{b} ±0.09
Stage 6	4.54^{ab}±0.04	0.53^{c} ±0.14
Stage 7	4.67^{b} ±0.04	0.71^{d} ±0.11
Stage 8	4.38^{a} ±0.03	1.87^{e} ±0.38
L.S	***	***

L.S= Level of significance

Means in the same column with different superscript are significantly ($P<0.05$) different.

*** = ($p< 0.001$)

Plate 2. *Solanum dubium* plant

growing). This result is in agreement with Rifaat *et al.* (1970) who found that, in general the milk-clotting activity of the latex of the different varieties was affected by the stage of ripening. These activities seemed to be correlated with the degree of maturity of the fruits. Our results disagree with Whitaker (1958) who reported that the enzyme activity of the fruits was at its highest level when they were unripened green.

3.2.3 Extraction of the milk-clotting coagulant

The results show that, maximum milk-clotting activity ($P<0.001$) was obtained from *Solanum dubium* fruit extracted with freeze-drying (3.60±0.43U/ml), and then by *Solanum dubium* fruit extracted with distilled water and evaporated at 45°C (3.39±0.74 U/ml) followed by *Solanum dubium* extracted by distilled water (1.30±0.17 U/ml). The lowest activity (0.05 U/ml) was obtained by extraction with 5% NaCl and this extract was subjected to a current of warm air (45°C).

The results show that milk-clotting activity significantly decreased ($P<0.001$) from 3.65 U/ml when *Solanum dubium* was extracted with 1% NaCl in distilled water to 1.74 when *Solanum dubium* was extracted with 5% NaCl. Our results disagreed with Yousif *et al.* (1996) who found that the 5% NaCl solution extracted more of the compound associated with clotting in *Solanum dubium*. Osman (2001) reported that the higher activity was obtained by *Solanum dubium* fruit extracted with distilled water, followed by *Solanum dubium* fruit extracted with freeze-drying, then *Solanum dubium* fruit extracted with distilled water and evaporated at 40-50°C, the lower activity was obtained by *Solanum dubium* fruit extracted with 5% NaCl in distilled water.

3.2.4 Thermal stability of *Solanum dubium* extract

The effect of storage on activity of *Solanum dubium* extract in liquid and solid forms is presented in Tables 3-2 and 3-3 respectively. Activity of *Solanum dubium* extract (liquid) stored in room temperature decreased from 1.24±0.25 to 0.58±0.11, 0.33±0.07, and 0.07±0.04U/ml in days 30, 60, and 90 respectively, but when stored in refrigerator the activity decreased from 1.85±0.59 in day zero to 1.33±0.12, 1.08±0.26, and 0.20±0.05 U/m in days 30, 60, and 90 respectively ($P<0.001$).

In the case of solid form of *Solanum dubium* extract stored in room temperature, its activity decreased from 3.32±0.65 in day zero to 2.76±0.42, 1.97±0.15, 1.62±0.24, 1.53±0.17, and 1.18±0.19 U/ml in days 30, 60, 90, 120, and 150 respectively. However, the activity of *Solanum dubium* extract stored at refrigerator at day zero was 4.51±1.13 U/ml it decreased to 2.84± 0.32, 2.06±0.16, 1.69±0.14, 1.60±0.12, and 1.12±0.28 U/ml in days 30, 60, 90, 120, and 150 respectively ($P<0.001$).

It is clear that the loss in activity on storage of solid *Solanum dubium* extract was much less as compared to liquid *Solanum dubium* extract. Further, the loss in activity of *Solanum dubium* extract in the liquid as well as solid form was significantly more at room temperature. In the case of storage in refrigerator, the loss in activity was appreciably less. Hence, the storage of solid *Solanum dubium* extract in the refrigerator was favorable for retaining the activity. Similar results were found by Singh *et al.* (1973) who studied vegetable rennet from *Withania coagulans*, and found that the loss in activity on storage of solid rennet was much less at both

Table (3-2): Effect of storage temperature on activity (U/ml) of *Solanum dubium* extract (liquid form)

storage (days)	Room temperature (37°C)	Refrigerator(5°C)
Day one	1.24±0.25	1.85±0.59
30	0.58±0.11	1.33±0.12
60	0.33±0.07	1.08±0.26
90	0.07±0.04	0.20±0.05
L.S	***	

L.S= Level of significance for interaction

***= (P<0.001)

Table (3-3): Effect of storage temperature on activity (U/ml) of *Solanum dubium* extract (solid form)

Storage (days	Room temperature(37°C)	Refrigerator(5°C)
Day one	3.32±0.65	4.51±1.13
30	2.76±0.42	2.84±0.32
60	1.97±0.15	2.06±0.16
90	1.62±0.24	1.69±0.14
120	1.53±0.17	1.60±0.12
150	1.18±0.19	1.21±0.28
L.S	***	

L.S= Level of significance for interaction

***= (P<0.001)

refrigerator and room temperature. Tejada *et al*. (2008) pointed that refrigerator storage of extract from *Cynara cardunculus* L., can not be considered a suitable method for prolonged preservation of aqueous cardoon extract. The frozen storage of aqueous extracts proved ideal for prolonged storage of vegetable coagulant.

3.2.5 Effect of milk temperature on coagulation time

Fig. 2 (appendix 3) illustrates the effect of milk temperature coagulation time. The coagulation time gradually decreased from 51.28±0.08 at 30°C to 10±0.00 seconds at 80°C. It may be observed that, the higher the temperature of milk when the *Solanum dubium* extract is added, the shorter the coagulation time.

3.2.6 Effect of preheating of milk on coagulation time

Temperature at which milk has been kept before addition of *Solanum dubium* extract also exercises a considerable effect on coagulation time. As will be seen from the results in Fig. 3 (appendix 4) the coagulation time (seconds) increased with an increase in temperature at which the milk is preheated. Heating probably denatures the protein and consequently the coagulation time is lengthened. Krishnamurti and Subrahmany (1948_a) found the same results when using milk coagulant enzyme of *Ficus carica* Linn. The effect seems to be more pronounced in the case of animal rennet which is not able to coagulate cow milk heated to 70-80°C.

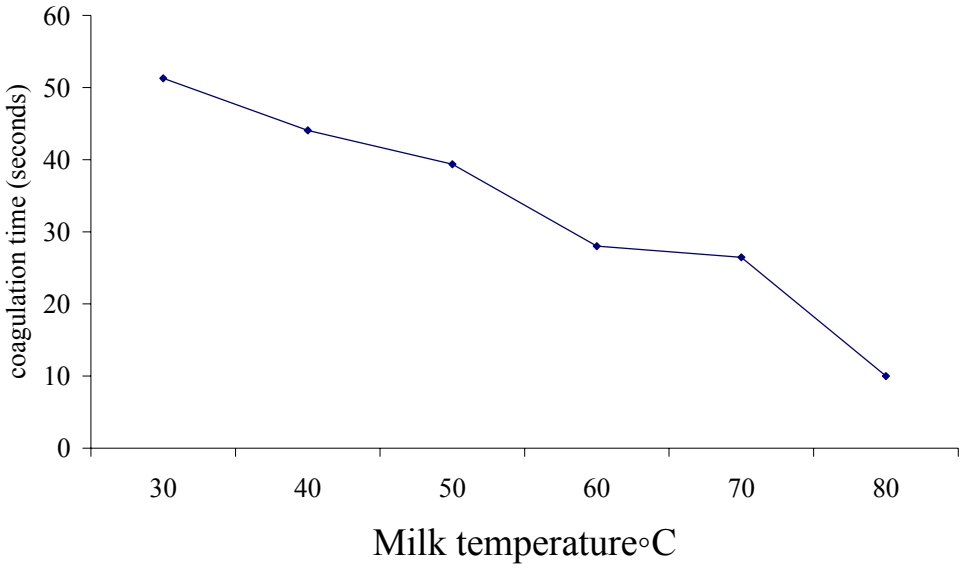

Fig. 2: Coagulation time as affected by temperature of milk treated with *Solanum dubium* extract

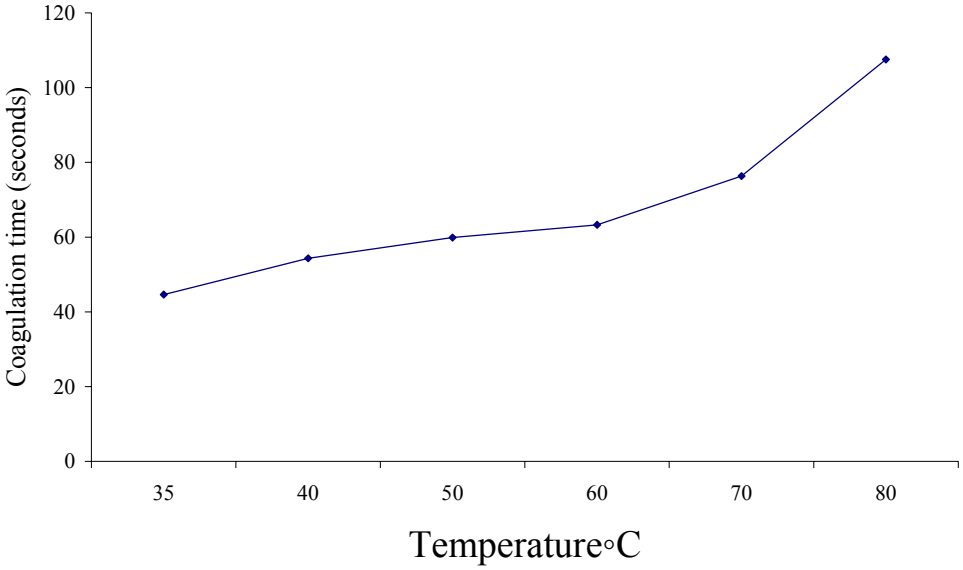

Fig.3: Coagulation time as affected by preheated milk treated with *Solanum dubium* extract

3.2.7 Effect of incubation temperature on coagulation time

Results from Fig. 4 (appendix 5) show that the milk-clotting time decreased with increasing incubation temperature ($P<0.05$). There is a weak curd at 80°C. The findings are similar to Walde *et al.* (1984) who reported that when temperature decreased, the clotting time of a crude extract of sun flowers seeds (*Helianthus annus* L.) increased. However, Angela *et al* (1993) pointed that; the best coagulation temperature is 27 to 29°C for extraction of cardoon flower (*Cynara cardunculus* L.).

3.2.8 Heat stability of the *Solanum dubium* extract

It may be seen from the results in Table 3-4 that keeping the extract at 70°C up to 10 minutes did not have any detrimental effect on its milk coagulantion power. However, at 70°C for up to 20 and 30 minutes, the milk coagulum was weakened and at 80°C when kept for 20 minutes. The activity of *Solanum dubium* fruit extract was completely destroyed at 90 and 100°C. Compared with animal rennet, the enzyme is more stable and resistant to heat, the former being completely destroyed even at 55°C. This result is in accords with Osman (1996) who found that milk-clotting activity was lost after wet-heating of *Solanum dubium* fruit extract at 80°C for 10 minutes, and after dry-heating *Solanum dubium* fruits extract at 100°C for 24 hours.

Kirshnamurit and suberahmanyan (1948_a) studied the milk clotting enzyme of *Ficus carica* Linn. The results showed that, inactivation commences even at 50°C when the enzyme is kept at that temperature for a fairly long time. The enzyme is completely destroyed above 70°C within 5 minutes. However, Habbani (1992) reported that when *Solanum dubium* extract was subjected to various

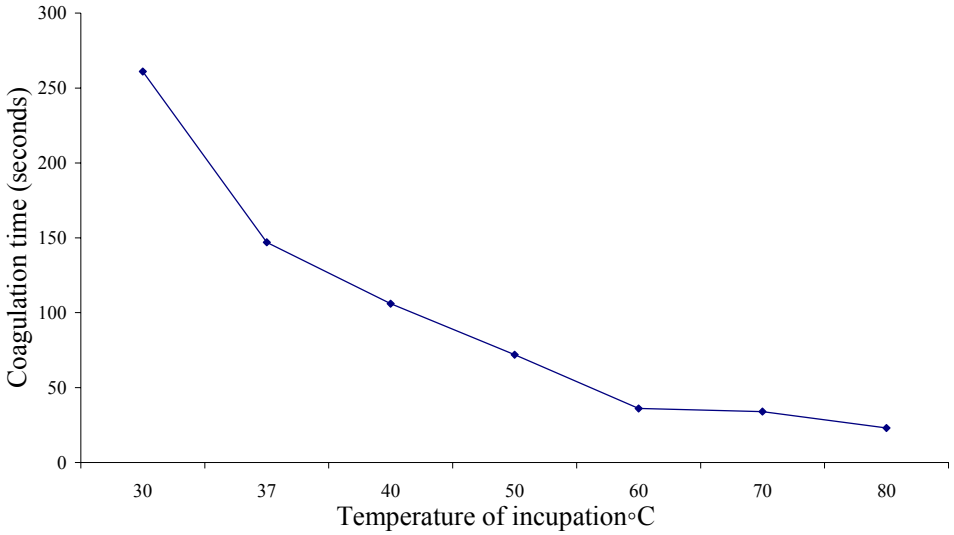

Fig. 4: Effect of incubation temperature on milk coagulation time

Table (3-4): Effect of thermal treatment on inactivation of *Solanum dubium* extract activity (U/ml)

Temperature ˚C	Time/minutes			
	Zero	10	20	30
30	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00
40	0.80±0.05	0.70±0.05	0.61±0.03	1.03±0.02
50	0.75±0.04	0.69±0.06	0.53±0.06	0.59±0.00
60	0.67±0.01	0.65±0.01	0.63±0.03	0.56±0.03
70	0.62±0.01	0.49±0.02	0.32±0.01	0.50±0.03
80	0.33±0.00	0.1±0.00	0.01±0.00	0.26±0.03
90	0.004±0.00	0.004±0.00	0.004±0.00	0.004±0.03
100	0.004±0.00	0.004±0.00	0.004±0.00	0.004±0.03
L.S	***	***	***	***

L.S = Level of significance for interaction

* = (P <0.001)

temperatures for half an hour, the present activity remained decreasing until it reached a very low value at 100°C. Both calf and microbial rennet from a culture of *Bacillus cereus* showed a high degree of inactivation after heating for three minutes above 55 and 65°C, respectively (Melachouris and Tuckey 1967). Pascaline and Daniel (2006) found that the *Mucor miehei* and *Mucor pusillus* proteases are much more stable at 53°C when heated for 100 minutes, while *Endothia parasitica* protease is rapidly inactivated at 53°C in 10 minutes. Nouani *et al*. (2009) found the enzyme extracted from *Mucor pusillus* to be stable at 30-50°C and it was completely inactivated by heating for 30 minutes at 65°C.

3.2.9 Enzyme concentration

Effect of increasing enzyme concentration on time taken to coagulate the same volume of milk (10 ml) is shown in Fig. 5 (appendix 6). The results showed that with increasing crude *Solanum dubium* extract concentration, the time of clotting decreased from 18.53±0.76 to 1.05±0.05 minutes. Similar effects were obtained by Habbani (1992), Osman (1996) and Ahmed *et al*. (2009).

3.2.10 Effect of ammonium sulphate concentration on activity of *Solanum dubium* extract

The crude extract of *Solanum dubium* fruits was precipitated with ammonium sulphate concentrations ranging from 0.00% to 90%. The results in Table 3-8 revealed that the saturation of 60% gave a higher milk-clotting activity (5.03 mg/ml) as well as protein content (14.25mg/ml) with 61.05% yield and 9.74 fold of purification. However, Osman (2001) found that the saturation range of 40-50% gave the highest milk clotting activity as well as milk-clotting/protein content with 27.5% yields and 1.88 fold of purification.

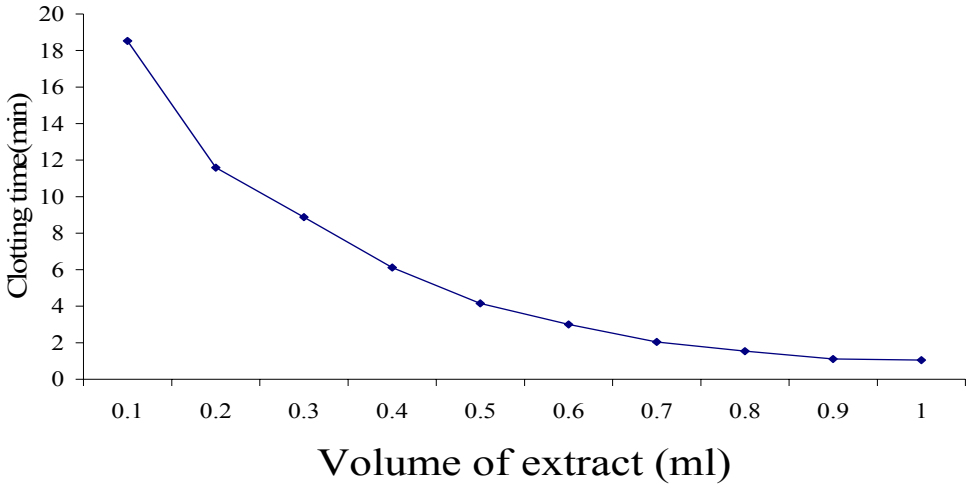

Fig. 5: Effect of *Solanum dubium* extract concentration on milk coagulation time

Table (3-5): Effect of ammonium sulphate saturation (%) on milk clotting activity, yield and fold of purification in *Solanum dubium* fruits

Saturation of ammonium sulphate	Volume (ml)	Enzyme activity mg/ml	Protein content mg/ml	[a] Specific activity (units/ml)	[b] Total activity (units)	[c] Yield (%)	[d] Fold of purification
Crude enzyme	25	3100	85.5	36.26	77500	100	-
10%	5.8	0.08	1.85	45.04	483.31	0.62	1.24
20%	7.2	1.50	2.75	545.45	10800	13.94	15.04
30%	8.8	3.28	6.25	525.33	28893	73.28	14.49
40%	8.2	3.85	9.75	394.87	31570	40.74	10.89
50%	8.6	4.33	10.00	433.33	37266	48.09	11.95
60%	9.4	5.03	14.25	353.21	47313	61.50	9.74
70%	9.8	4.33	13.50	320.99	42466	54.79	8.85
80%	8.8	4.08	7.25	563.21	35933	46.37	15.53
90%	10.3	3.83	9.50	403.51	39483	50.95	11.13

[a] Specific activity= activity /protein content

[b] Total activity = activity ×volume of fraction

[c] Yield = total activity of the fraction / total activity of crud enzyme×100

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

3.2.11 Characterization of partially purified enzymes

3.2.11.1 Effect of incubation temperature on milk-clotting activity

It is obvious from Fig. 6 (appendix 7) that *Solanum dubium* extract exhibited optimum temperature for milk clotting activity at 70°C. The results are similar to Sidrach *et al.* (2005) and Vieira De So and Barbosa (1972) who studied milk clotting activity from *Cynara* scoly*m*us and Cardo, respectively. Our result not similar to Habbani (1992), Osman (1996), Mohamed and Habbani (1996), Osman (2001), D'Ambrosio *et al*. (2003), Kumer *et al*. (2005), Kumar *et al*. (2006), Aworh and Nakia (2006), Pascaline and Daniel (2006), Raposo and Domingos (2008) and Nouani *et al*. (2009). Ahmed *et al*. (2009) found that the enzyme from *Solanum dubium* Fresen seeds stable against a wide range of temperature (20-90°C).

3.2.11.2 Effect of pH on the activity of *Solanum dubium* extract

The activity of *Solanum dubium* extract on a pH range from 4 to 12 was shown in Fig. 7 (appendix 8). Results showed that the *Solanum dubium* extract activity increased with increasing pH value from 4 up to pH 10 which was the maximum activity, and then the activity declined .The findings disagree with Osman (2001) who reported the maximum activity of *Solanum* extract to be at pH 5.5 and the enzyme activity decreased with increasing pH value. Also our results not similar to Habbani (1992), D'Ambrosio *et al.* (2003), Kumer *et al.* (2005), Aworh and Nakai (2006), Ishak *et al*. (2006), Kumar *et al*. (2006), El-Bendary *et a*l. (2007), Raposo and Domingos (2008) and Nouani *et al*. (2009).

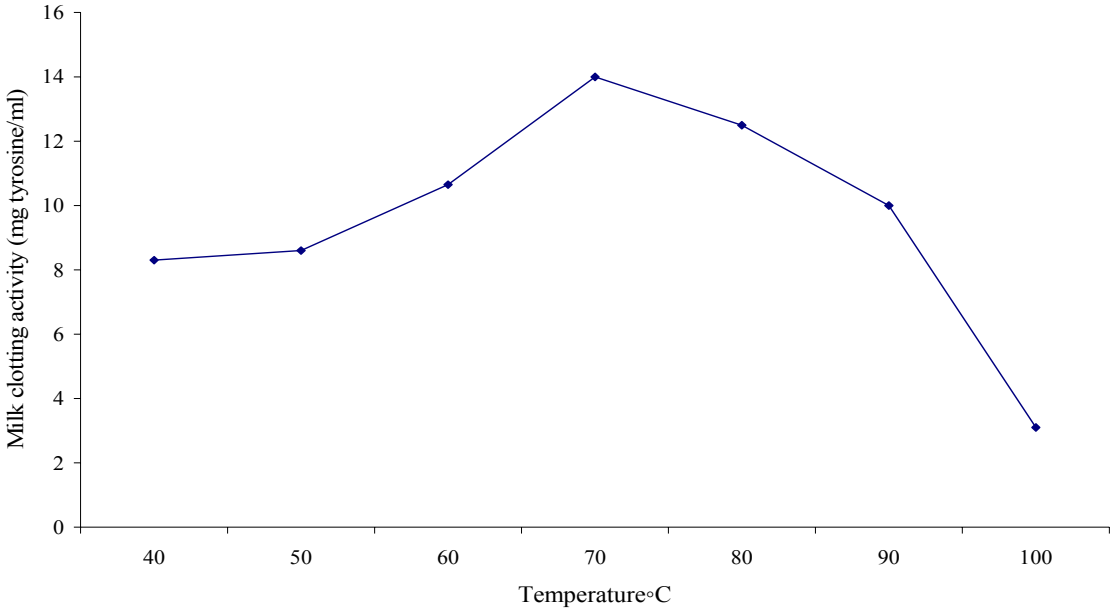

Fig. 6: Effect of incubation temperature on milk clotting activity of partially purified *Solanum dubium* extract

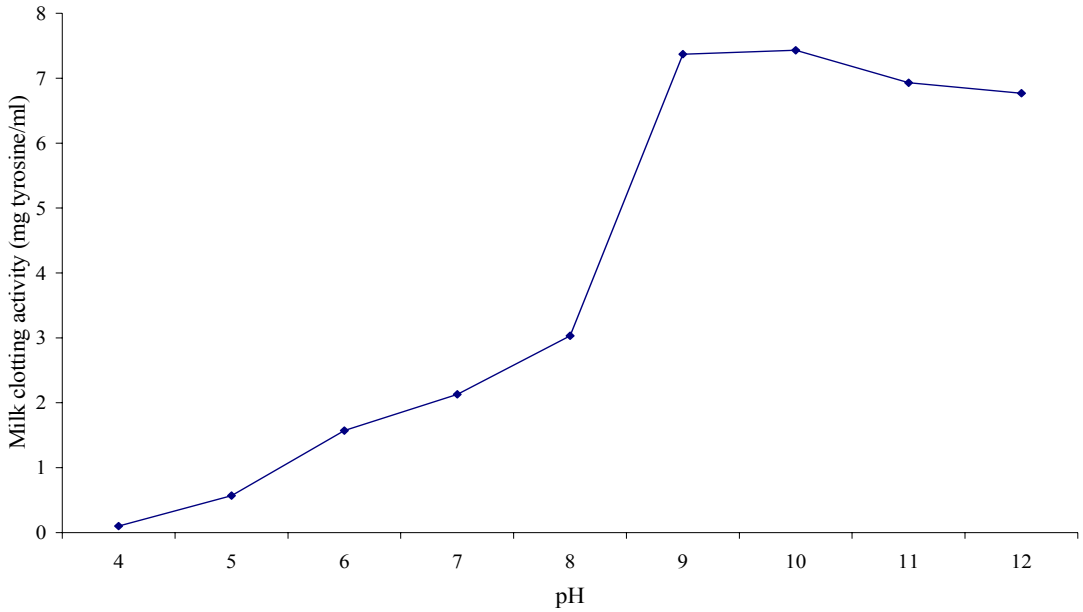

Fig. 7: Effect of pH on milk clotting activity of partially purified *Solanum dubium* extract

3.2.11.3 Effect of $CaCl_2$ on partially purified *Solanum dubium* extract activity

The findings demonstrated that the activity (mg tyrosine/ml) of partially purified *Solanum dubium* extract increased with increasing calcium chloride concentration from 4.30±0.00 at zero% to 5.65±0.07 at 0.08%. Similar results were found by Habbani (1992), Ishak *et al*. (2006), Mohamed and Habbani (1996), Chazarra *et al*. (2007) and El- Bendary *et al.* (2007) .Nouani *et al*. (2009) reported that the optimum activity of purified extract from *Mucor pusillus* was at $CaCl_2$ concentration of 0-20 mM.

3.2.11.4 Effect of substrate quantity on coagulation time

Fig. 8 (appendix 9) showed addition of 0.25 ml of partially purified *Solanum dubium* extract to different volumes of milk which was selected to be the best *Solanum* extract concentration after so many experiments. So later we used (25 ml: 50 litres) *Solanum dubium* extrat concentration for cheese making.

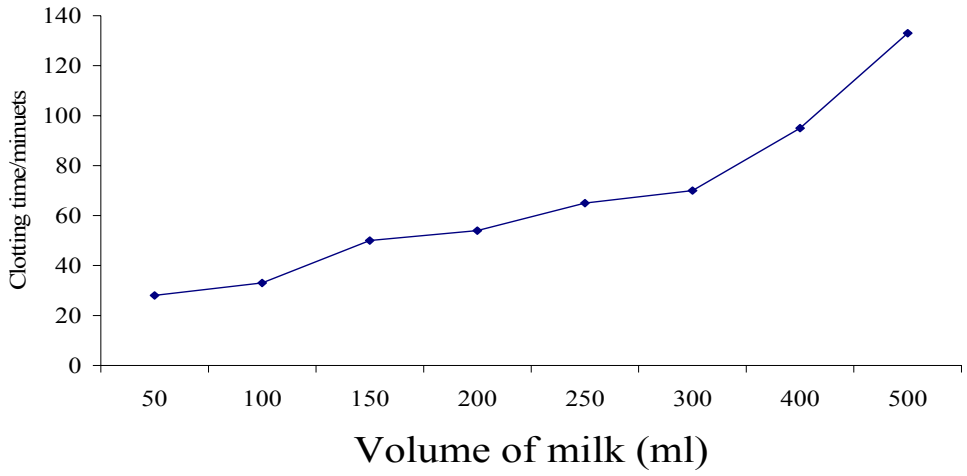

Fig. 8: Effect of substrate quantity on milk coagulation time by *Solanum duobium* extract

Chapter Four
Experiment Two

4. Manufacture of Sudanese white cheese (*Gibna Bayda*) with *Solanum dubium* fruit extract and rennet

4.1 Materials and Methods

Three replicates of cheese were made; in each batch eight cheeses were produced. In cheese one (ch1) animal rennet was used as coagulant with 4% salt without addition of calcium chloride (0.0%). In cheese two (ch2) animal rennet was used with 4% salt and addition of 0.02% calcium chloride. In cheese three (ch3) animal rennet was used as coagulant with 8% salt without added calcium chloride (0.0%). In cheese four (ch4) animal rennet was used as coagulant with 8% salt and with addition of calcium chloride (0.02%). In cheese five (ch5) *Solanum dubium* extract was used as coagulant with 4% salt and without addition of calcium chloride (0.0%). In cheese six (ch6) *Solanum dubium* extract was used as coagulant with 4% salt and with 0.02% calcium chloride. In cheese seven (ch7) *Solanum dubium* extract was used as coagulant with 8% salt and without addition of calcium chloride (0.0%). In cheese eight (ch8) *Solanum dubium* extract was used as coagulant with 8% salt and 0.02% calcium chloride.

4.1.1 Source of milk and salt

Cow's milk was obtained from Wad Elnial town (Sinnar State). Seventy litres of milk were used for each batch. The milk was analyzed for titratable acidity, total solids, fat, protein, and ash contents. The salt was obtained from the local market.

4.1.2 Source of animal rennet

Rennet tablets were obtained from Christen Hansens Laboratory (Denmark).

4.1.3 Source of *Solanum dubium* extract

Solanum dubium extract was prepared as described in section 3.1.6. `Twenty five milliliters (25 ml: 50 litres of milk) of the extract were used for coagulating milk in cheese making.

4.1.4 Cheese manufacture

The milk was strained by a clean cloth, heated to 62°C for 30 min., and then cooled to 40°C. The salt was dissolved in small quantity of milk and then added to the whole raw milk in the required concentration. Calcium chloride was added (0.02%) to the milk in cheese (ch2, ch4, ch6, and ch8). Rennet tablets were added at a rate of one tablet per 50 litres of milk in cheese (ch1, ch2, ch3, and ch4). However, in cheese ch5, ch6, ch7, and ch8, *Solanum dubium* extract was added (25 ml: 50 liters milk). The milk was then stirred for 10 minutes and left for coagulation.

The coagulated milk was cut for whey separation, and then the curd of each cheese was poured into a wooden mould lined with a clean cloth and pressed by a heavy weight overnight. Next day the cheese was removed from the mould and cut into small cubes. The whey of each cheese was collected in a separate container, boiled for five minutes, cooled and used for preservation of the particular cheese. The different cheeses were stored at room temperature (35-37°C) for 90 days.

The cheeses were analyzed at day one, 15, 30, 45, 60, 75, and 90 intervals for chemical composition and sensory characteristics. However, cheese samples ch1, ch3, ch5, and ch7 were analyzed for microbiological analysis.

4.1.5 Chemical analysis of milk, whey and cheese

4.1.5.1 Titratable acidity of milk, whey and cheese

Titratable acidity of the milk used for preparation of the two types of cheese manufacture and their whey were determined according to the method described by AOAC (1990).

4.1.5.1.1 Titratable acidity of milk and whey

Ten ml of milk and the whey each were pipette into clean porcelain dish and five drops of phenolphthalein indicator were added and titrated against 0.1 N NaOH. Then the titratable acidity of each sample was calculated as follows:

$$\text{Titratable acidity} = \frac{T}{W}$$

Where T = Titration figure W = Weight of sample

4.5.2.2 Titratable acidity of cheese

For the titratable acidity of cheese, 10 grams of each cheese type were weighed and placed in a conical flask. Distilled water at a 40°C was added to the sample until the volume in the flask rose up to 105 ml. The flask was vigorously agitated and filtered through Whatman filter paper No.43. Twenty five ml of the filtrate were pipetted in a 75 ml beaker. Five drops of phenolphthalein indicator were added to the filtrate and titrated against 0.1 N NaOH, till a faint pink colour that lasted for about 30 seconds was obtained. The titratable acidity was then calculated as follows (AOAC 1990):

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

Where T= Titration figure W = Weight of sample

4.1.5.2 Determination of total solids content

Total solids contents of milk, whey and cheese were determined according to AOAC (1990). Five ml of each milk and whey, and two grams of cheese, were weighed into three pairs of clean dried pre-weighed aluminum dishes. The weight of each sample and the dish was recorded. The dishes were put in an air oven at 100°C for three hours, then placed in desiccators to cool for 30 minutes and weighed. Heating, cooling and weighing were repeated several times until the difference between weightings were less than 0.5 mg. the total solids content of each of the three samples was calculated as follows:

$$\text{Total solids} = \frac{W_1 \times 100}{W_0}$$

Where W_1 = Weight of sample after drying

W_0 = Weight of sample before drying

4.1.5.3 Determination of fat content

Fat contents of the milk, cheese and whey were determined according to Foley *et al*. (1974). For the determination 10 ml quantities of sulfuric acid (1.815 gm/ml density at 20°C) were added to three pairs of Gerber tubes, the first contained three grams of ground cheese and the other two 10.95 ml quantities of both milk and whey each separately. To each tube one ml of amyl alcohol was added. The contents of the three tubes were mixed thoroughly till no white particles were seen. They were then centrifuged at 1100 rpm for five minutes and transferred to a water bath at 65°C for three minutes.

4.1.5.4 Determination of protein contents

The protein content of the milk, cheese and whey were determined according to AOAC (1990) using Kjeldahl method. Three empty and dried porcelain dishes were weighed. Three grams of cheese, 10 ml amounts of milk and whey samples were weighed each separately then transferred to Kjeldahl flasks. Twenty five ml of concentrated free nitrogen sulfuric acid (1.86 densities) were added to each of milk, whey and cheese followed by two kjeldahl tablets then they were digested on a heater until clean solutions were obtained. The flasks were removed and left to cool. Each digested sample was poured in a 100 ml volumetric flask, diluted to 100 ml with distilled water and allowed to cool. Five ml of each diluted sample were transferred to a distillatory followed by 10 ml of 40% NaOH. The distillate of each of the three samples was received in a conical flask of 100 ml capacity containing 25 ml of 2% boric acid and three drops of bromo-cresol green plus methyl red indicator then the distillation was continued until the volume in the flask reach 75 ml. The flasks were then removed titrated against 0.1 N HCl until the end points were reached (red colour). The protein contents were then calculated as follows:

$$\text{Nitrogen (\%)} = \frac{T \times 0.1 \times 20 \times 0.014}{\text{Weight of the sample}} \times 100$$

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.38$$

Where:

T= Titration figure

N = Normality of HCl

0.014 = The atomic weight of nitrogen/ 1000

20 = Dilution factor

4.1.5.5 Determination of Ash content

Ash contents of the milk, cheese and whey were determined according to AOAC (1990). Two grams of cheese, 10 ml of milk and 10 ml of whey were placed in clean dry pre-weighed crucibles each separately. The containers were put on a steam bath for 30 minutes sand placed in a muffle furnace at 550°C for 2.5 to 3 hours. They were then removed, placed in desiccators and left to cool, re-weighed and the ash content of each of the three samples were determined as follows:

$$\text{Ash \%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

4.1.5.6 Determination of Soluble protein

Soluble protein content of each type of cheese under storage were determined according to Ling (1963) by weighing five grams of cheese in a beaker, followed by addition of 25 ml of warm distilled water (50°C). The samples were ground perfectly. The mixture was well stirred in 250 ml graduated flask, filtered through filter paper, washed with distilled water and the volume was made up to 250 ml. Twenty five ml of the filtrate were digested and distilled. Ammonia (NH_3) was received in 20 ml of 2 % boric acid with, bromo-cresol green plus methyl red indicator. The contents were titrated with 0.02 N HCl solutions then the soluble protein was calculated as follows:

$$\text{Soluble protein (\%)} = T \frac{\times 0.02 \times 20 \times 0.014 \times 100 \times 6.38}{\text{Weight of the sample}}$$

4.1.5.7 Determination of salt contents

Salt contents of the cheese samples and their whey were determined by titration according to Breene and Price (1961). For the determination 10 grams of cheese and 10 ml of whey were weighed each in a conical flask. Fifty ml of warm distilled water at 50-60°C were added to each flask and the contents werre stirred until a homogenous suspension was obtained. The suspension of each sample was transferred to a 250 ml graduated cylinder, diluted to 250 ml with distilled water, mixed thoroughly and allowed to stand for 5 to 10 minutes. When the suspended cheese was settled out, 2 ml of 2% solution of potassium chromate in distilled water was added to a 25 ml aliquot of the supernatant of each sample. The mixture was then titrated with 0.01711 N solution of silver nitrate. The first discernible colour change due to the red colour of precipitated sliver chromate was taken to be the end point. Knowing that each ml of silver nitrate solution is equivalent to 0.1% salt, the percentage of salt was then calculated.

4.1.5.8 Determination of tyrosine and tryptophan contents

The method of Vakaleris and Price (1959) was used to determine tyrosine and tryptophan contents of eight cheese samples. Ten grams of each type of cheese were weighed in a beaker, dissolved in 40 ml of 0.5% N sodium citrate solution and slightly warmed. The contents in the beaker 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 were added and mixed well and filtered through Whatman filter paper No. 42. The absorbance of the clear filtrate was measured at a wavelength 270 and 290 nm using spectrophotometer. The tyrosine and tryptophan contents were then calculated from the equation:

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

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

Where:

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

4.1.6 Microbiological examination

4.1.6.1 Preparation of media and glassware

All media were obtained in a dehydrated form and stored in a hygroscopic environment in a cool dry place away from light and prepared according to the manufacturer's instructions. The media were sterilized using autoclave at 15 lbs pressure (121˚C) for 15 minutes. Plastic containers were washed in running tap water rinsed with distilled water and sterilized in the autoclave at 115˚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. Graduated pipettes were plugged with cotton wool and Petri-dishes put into canisters and sterilized. All glassware was sterilized in an oven at 160˚C for one hour (Marshall, 1992).

4.1.6.2 Preparation of sample dilutions

Samples of different kinds of cheese (ch1, ch3, ch5, ch7) were taken in sterile plastic containers. Eleven grams of each cheese were added to 99 ml of distilled water warmed at 45˚C in a clean sterile flask, then shaken until a homogenous solution was obtained to make 10^{-1} dilution. One ml from the above- mentioned dilution (10^{-1}) was assertively transferred to 9 ml sterile distilled water (in a dilution bottle). This procedure was repeated to make serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and10^{-8}. From each dilution, I ml was transferred to

duplicate Petri-dish, mixed gently, left to solidify and incubated in an inverted position. The typical colonies in each Petri- dish were counted (Houghtby *et al*., 1992).

4.1.6.3 Total viable bacterial count

This bacterial count was determined according to Houghtby *et al*., (1992) using standard plate count agar for USA. The plates were incubated at 37°C for 48 hours. Typical colonies in the selected dilution were counted (25-250 colonies in each dilution).

4.1.6.4 Streptococci count

The M17 agar (Merck-15108) medium was used to determine streptococci count. The plates were incubated at 37°C for 48 hours (Oksuztepe, 2005).

4.6.5 Lactobacilli count

Total lactobacilli were determined according to Frank *et al*. (1992) using MRS agar medium. The plates were incubated at 37 for 48 hours. Typical colonies in the selected dilutions were counted.

4.1.7 Sensory evaluation

A panel of 10 untrained panelists were chosen to judge on the quality of cheese (colour, flavour, texture, and saltiness) using a sensory evaluation sheet (Appendix 1).

4.1.8 Statistical analyses

Statistical analyses were carried out using SPSS programmed (1998). Completely Randomized Design and General linear models were used. Duncan's multiple range tests were used for mean separation between the treatments. The level of significance $P< 0.05$ was used in this study.

4.2 Results and discussion

The average chemical composition of the milk used for cheese making in this study was as follows: titratable acidity 0.20 %, total solids 12.12 %, protein 3.26 %, fat 3.85%, and ash 0.73 %.

4.2.1 Chemical composition of white cheese

4.2.1.1 Effect of the storage period on weight loss and chemical composition of white cheese

4.2.1.1.1 Weight loss

Results in Table (4-1) show the effect of storage period on weight loss of the white cheese. Storage period significantly ($P< 0.001$) affected the weight loss of the cheese. The weight loss of the cheese increased gradually till the end of the storage period. The lowest weight loss (7.05 %) was obtained after 15 days of storage, while the highest (24.16) was obtained after 90 days.

The findings in this study agreed with those of Bilal (2000), Nuser (2001) and El Owni and Hamid (2009) who reported that weight loss of the Sudanese white cheese increased as the storage period progressed.

4.2.1.1.2 Chemical composition

The chemical composition of the white cheese samples studied is presented in Table (4-1). As shown in the table the titratable acidity of the white cheese increased significantly ($P< 0.001$) with advancing storage period. The titratable acidity increased from 0.76 % at day zero to 1.03, 1.10, 1.31, and 1.49 % at day 15, 30, 45 and 60, respectively and then decreased to 1.19 and 1.13 % at day 75 and 90 respectively. These results were in agreement with those reported by Bilal (2000), Nuser (2001), Kilio *et al*. (2004), Sulieman (2007), El Owni and Hamid (2008), and El Owni and Hamid (2009). The development of titratable acidity during storage till day 60 could be attributed to growth of lactic acid bacteria microbial enzymes.

Table (4-1): Effect of storage period on weight loss and chemical composition of the white cheese

Storage period(days)	Weight loss%	Total solids %	Fat %	Acidity %	Protein %	Soluble protein %	Salt %	Ash %	Tyrosine mg/100g cheese	Tryptophan mg/100g cheese
Day one	0	47.19^{a}	22.98^{a}	0.76^{a}	16.75^{bc}	0.67^{b}	3.70^{b}	3.15^{d}	1.64^{c}	0.47^{b}
15	7.05^{a}	48.47^{a}	24.70^{b}	1.03^{b}	18.67^{d}	0.60^{ab}	3.62^{ab}	2.76^{c}	1.68^{c}	0.46^{b}
30	10.56^{b}	51.32^{b}	25.98^{c}	1.10^{bc}	17.43^{cd}	0.65^{b}	3.79^{b}	2.60^{bc}	1.44^{b}	0.43^{a}
45	14.97^{c}	48.66^{a}	25.33^{bc}	1.31^{d}	17.22^{c}	0.78^{c}	3.68^{b}	3.32^{d}	1.64^{c}	0.45^{b}
60	18.28^{d}	48.75^{a}	25.10^{bc}	1.49^{e}	15.54^{b}	0.66^{b}	3.72^{b}	2.19^{a}	1.37^{ab}	0.40^{a}
75	20.64^{e}	47.44^{a}	26.14^{c}	1.19^{c}	17.55^{cd}	0.59^{ab}	3.48^{a}	2.13^{a}	1.47^{b}	0.40^{a}
90	24.16^{f}	47.70^{a}	25.79^{bc}	1.13^{c}	11.03^{a}	0.51^{a}	3.73^{b}	2.39^{ab}	1.30^{a}	0.37^{a}
S.E	0.32	0.52	0.39	0.04	0.44	0.04	0.05	0.12	0.04	0.01
L.S	***	***	***	***	***	***	NS	***	***	***

S.E = standard error

* **= (P< 0.001)

L.S = level of significance

Means within columns bearing the same letter are in significantly (P>0.05) different

which increased the level of lactic acid in the cheese (Walstra *et al*., 1999). On the other hand the decrease in titratable acidity at day 75 and 90 might be due to utilization of lactic acid by other micro flora during storage.

The total solids content increased significantly ($P< 0.001$) from 47.19 % at day zero to 48.47 and 51.32 % at days 15 and 30, respectively, and then decreased to 48.66, 48.75, 47.44, and 47.70 % at day 45, 60, 75, and 90 respectively. The findings were in agreement with Bilal (2000) and Hamid (2005), who reported that the total solids content of the white soft cheese increased during storage period. The increase was due to continuous loss of moisture from the curd as a result of lactic acid development which caused curd contraction (El Owni and Hamid, 2008). However, the decrease in total solids content from day 45 to day 60 was possibly due to proteolytic effect of microorganisms on the protein and dissolution of fat and salt into the pickling solution (Nuser, 2001).

The fat content of the white cheese was significantly ($P< 0.001$) affected by the storage time. The values were 22.98, 24.70, 25.98, 25.33, 25.10, 26.14, and 25.79 % at day zero, 15, 30, 45, 60, 75, and 90. As will be seen from this result the fat content increased with progress in storage time from day zero to day 75. The increase in fat content could be attributed to breakdown of the cheese proteins and their loss in the whey (El Owni and Hamid, 2008; Hamid, 2005; Bilal, 2000; Babiker, 1987 and Salama *et al*., 1982). However, the decrease in fat contents during storage at day 90 was probably due to the lipolytic activity of microorganisms on fats resulting in leakage of some fat from curd into the pickling whey (Khalid, 1991; Nuser, 2001and Hamid, 2005).

The storage time significantly ($P< 0.0001$) affected the protein content of the white cheese. The highest value was 18.67 % at day 15 while the lowest protein content of 15.45 % was at day 60. The increase

of crude protein content during ripening was due to decrease in moisture contents (Kalid, 1991; Abdel Razig, 1996; and Hamid, 2005). Also the decrease in protein content of cheese during storage may be due to protein degradation leading to formation of water soluble compounds (Nuser, 2001).

The soluble protein content of the white cheese increased significantly ($P< 0.001$) from 0.67% at day zero to 0.78 % at day 45, and then gradually decreased to 0.66, 0.59, and 0.51 % at day 60, 75, and 90. These results agreed with those of Abdel Razig (1996); Hayaloglu *et al*. (2002) and Hamid (2005) who reported that proteolysis in pickled cheeses such as Feta, Domiati and White cheese occur during storage. However, the decrease in soluble protein at day 60, 75, and 90 may be due to the stop of the decrease in proteolysis.

The salt content of the white cheese was not significantly affected by storage period (Table 4-1). The salt content decreased from 3.79 at day 30 to 3.86, 3.72, 3.48, and 3.73 % at days 45, 60, 75, and 90.

The ash contents of the cheese were significantly ($P< 0.001$) affected by the storage time. Their values decreased from 3.15 % at day zero to 2.19, 2.13, and 2.39 % at day 60, 75, and 90. The highest ash content was at day 45. Hamid (2005) found the ash contents of the cheese samples to increase from day 120 to day 240. The decrease in ash content was possibly due to diffusion of salt from the curd into the pickling whey (Abdalla, 1992).

The tyrosine and tryptophan contents of the cheese significantly ($P< 0.001$) increased at the beginning of the storage till day 45, and then decreased at the end of storage period. The lowest tyrosine and tryptophan was 1.30 and 0.37 mg/100g cheese at day 90. 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. Abdel Razig

(1996) and Hamid (2005) reported that amino acid tyrosine and tryptophan contents increased as the storage period progressed.

4.2.1.2 Effect of coagulant type on weight loss and chemical composition of white cheese

4.2.1.2.1 Weight loss

Data in Table 4-2 show that cheese weight loss was found to be significantly ($P< 0.001$) affected by type of coagulant. The results indicated that the weight loss of cheese made with *Solanum dubium* extract was higher (16.71%) than that of cheese made with rennet (15.17%). This may be due to high proteolytic activity of the *Solanum dubium* extract.

4.2.1.2.2 Chemical composition

Table 4-2 shows the effect of coagulant type on chemical composition of white cheese. Statistical analysis revealed that type of coagulant had no significant effect ($P>0.05$) on titratable acidity of white soft cheese. However, a slightl increase in titratable acidity was observed in cheese produced by rennet (1.15%) compared to cheese produced with *Solanum dubium* extract (1.14%). This was attributed to high whey retention in cheese with animal rennet which allowed more lactose to be available for microbial fermentation, with a more pronounced increase in acidity than in vegetable rennet cheese. Our results are similar to Núnez *et al*. (1991). However, this result not similar to Abu-Zeid (1994) who found that higher acidity value in cheese made with vegetable rennet from *Sonchus olerceus* L. that the longer coagulation time of vegetable rennet possibly favored microbial growth and consequently, a higher acidity was reached in curd from vegetable rennet. Total solids and fat contents of white soft cheese was significantly ($P< 0.001$) higher in cheese made with *Solanum dubium* extract

Table (4-2): Effect of coagulant type on weight loss and chemical composition of white cheese

Weight loss and chemical composition	Coagulant		S.E	L.S
	Rennet	*Solanum*		
Weight loss%	15.17	16.71	0.19	***
Total solids %	47.33	49.68	0.28	***
Fat%	24.21	26.08	0.21	***
Acidity%	1.15	1.14	0.02	NS
Protein%	16.88	17.32	0.23	*
Soluble protein%	0.57	0.71	0.02	*
Salt%	3.62	3.72	0.03	NS
Ash%	2.65	2.65	0.64	NS
Tyrosin mg/100gcheese	1.40	1.61	0.02	***
Tryptophan mg/100gcheese	0.39	0.46	0.01	***

S.E = standard error

L.S = level of significance

* = ($P<0.05$)

***= ($P<0.001$)

NS = Not significant

compared to the cheese made with rennet. The values of total solids and fat were 49.68% and 26.08% in cheese made with *Solanum dubium* extract compared with 47.33% and 24.21% in cheese made with rennet, respectively. Mohamed and O'Conner (1999) found values of 40- 44.32% for total solids and 15- 18.78% for fat in cheese made with by *Calotropis procera* as coagulant. Our results agreed with Núnez *et al.* (1991), who reported that the total solids and fat content were higher for vegetable rennet cheese compared to cheese with animal rennet. Also results were in agreement with Abou Zeid (1994).

The protein and soluble protein content of cheese were significantly ($P< 0.05$) affected by type of coagulant. Cheese made with *Solanum dubium* extract showed the highest protein and soluble protein contents (17.32 % and 0.71%), while cheese made with rennet had the lowest protein and soluble protein (16.88% and 0.57%). These findings were similar to Núnez *et al*. (1991) who reported 14% protein content for vegetable rennet cheese and 13.3% for animal rennet cheese. Also our results were similar to Fernández-Salguero and Sanjuán (1999) who reported that the high levels of soluble nitrogen was found in cheese produced using vegetable rennet.

The salt content of cheese was not significantly affected by type of coagulant. However, cheese made with *Solanum dubium* extract secured the highest salt content (3.72%), while that made with rennet had the lowest (3.62%). This finding agreed with Núnez *et al*. (1991) who reported that type of coagulant had no significant effect on salt content of cheese.

Coagulant type did not significantly affect the ash content of cheese made with rennet and *Solanum dubium* extract was 2.65%. Mohamed and O'Connor (1999) reported 1.97- 2.59% ash for white soft cheese made with *Calotropis procera* juices.

Tyrosine and tryptophan (mg/100g cheese) were significantly higher in cheese made with *Solanum dubium* extract (1.61 and 0.46) than cheese made with rennet (1.40 and 0.39). These results are in agreement with Abu-zeid (1994) and Fernández-Salguero and Sanjuán (1999).

4.2.1.3 Effect of coagulant type and storage period on weight loss and chemical composition of white cheese

Results in Table 4-3 show no significant differences (P> 0.05) in weight loss, titratable acidity, total solids, fat, protein, soluble protein and salt contents of the white soft cheese as affected by storage period and coagulant type.

The weight loss gradually increased with progress in storage period, the maximum weight loss being reached at the end of storage (90 days) of both rennet and *Solanum dubium* extract cheese. The higher weight loss was 24.57% in cheese made with *Solanum dubium* extract at day 90. However, the lowest weight loss was 6.49% at day 15 in cheese made with rennet.

The titratable acidity increased from 0.74 at day zero to 1.49% at day 60 then gradually decreased to 1.12% at day 90 in rennet cheese. However, in cheese made with *Solanum dubium* extract, the titratable acidity increased from 0.78 at day zero to 1.49% at day 60 then gradually decreased to 1.15% at day 90.

The total solids and fat contents were higher (52.76% and 27.04%) in cheese made with *Solanum dubium* extract at day 30. The lower total solids and fat contents were 45.45% and 21.17% in cheese made with animal rennet at day zero. Similar results were found by 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. Our results disagreed with El-Shibiny *et al*. (1973) for Domiati cheese made with

Table (4-3): Effect of interaction between storage period and type of coagulants on weight loss and chemical composition of white cheese

Storage period(days)	Weight loss%		Total solids%		Fat%		Acidity%		Protein%	
	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*
Day one	0	0	45.45	48.92	21.17	24.79	0.74	0.78	16.04	17.47
15	6.49	7.61	47.16	49.79	24.10	25.29	1.02	1.03	18.68	18.67
30	9.16	11.96	49.88	52.76	24.92	27.04	1.12	1.08	17.14	17.71
45	13.66	16.29	48.17	49.14	24.92	25.75	1.36	1.03	17.59	16.85
60	17.65	18.91	47.54	49.96	23.88	26.33	1.49	1.49	15.23	15.86
75	20.33	20.93	46.62	48.26	25.52	26.75	1.20	1.18	17.23	17.86
90	23.76	24.57	46.47	48.92	24.98	26.59	1.12	1.15	16.25	16.85
S.E	0.45		0.74		0.55		0.05		0.36	
L.S	NS		NS		NS		NS		NS	

S.E = Standard error

L.S = Level of significance for interaction

NS =Not significant

Contd.

Storage period(days)	Soluble protein %		Salt %		Ash %		Tyrosine (mg/100gcheese)		Tryptophan (mg/100gcheese)	
	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*
Day one	0.52	0.81	3.70	3.69	3.00	3.29	1.49	1.79	0.43	0.51
15	0.50	0.69	3.67	3.77	2.69	2.83	1.49	1.88	0.41	0.53
30	0.64	0.65	3.75	3.83	2.31	2.88	1.29	1.59	0.36	0.44
45	0.72	0.85	3.57	3.79	3.41	3.24	1.65	1.63	0.45	0.46
60	0.56	0.75	3.64	3.79	2.13	2.25	1.33	1.42	0.38	0.41
75	0.55	0.64	3.40	3.55	2.26	1.99	1.36	1.58	0.37	0.44
90	0.47	0.54	3.81	3.65	2.73	2.06	1.19	1.41	0.34	0.40
S.E	0.05		0.08		0.17		0.06		0.02	
L.S	NS		NS		*		*		*	

S.E = Standard error

L.S = Level of significance for interaction

* = ($P<0.05$)

NS = Not significant

enzyme preparation from fig latex. Alalade and Adeneye (2006) found that the total solids content of Wara cheese was significantly affected by storage period.

Protein content was 18.68% at day 15 in rennet cheese and decreased to 16.25% at day 90. However, the protein content in cheese made using *Solanum dubium* extract was 18.67% at day 15 and decreased to 16.85% at day 90. The lowest protein content was 15.23% in rennet cheese at day 60. The differencec in protein content in cheese prepared with rennet or *Solanum dubium* extract during storage period were small. Our results were similar to those found by Mohamed *et al*. (2009) who 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. Also our results were similar to Sousa and Malcata (1997) who reported that aqueous extract of flowers of *Cynara cardunculus* had no significant effect on protein content of cheese over the ripening period. Slight decrease in protein content was reported by Krishnaswamy *et al*. (1961) in cheddar cheese produce with vegetable rennet from *Ficus carica* during ripening.

The highest soluble protein content (0.85%) was in cheese made with *Solanum dubium* extract at day 45, compared to lowest soluble protein in rennet cheese at day 90. Similar results were found by Fernández-Salguero and Sanjuán (1999) who reported that soluble nitrogen of cheese made with thistles of the genus *Cynara* at 60, 80, and 100 days of ripening was greater than that in cheese produced using animal rennet. El-Shibiny (1973) reported that during storage the soluble nitrogen content of cheese made with the enzyme preparation from fig latex gradually increased reaching a value of 0.57%. The increase in

soluble nitrogen content during pickling may be due to the proteolytic action of microbial proteinases on the curd.

The salt content was 3.69, 3.77, 3.83, 3.79, 3.79, 3.55, and 3.65% in cheese made with *Solanum dubium* extract at day zero, 15, 30, 45, 60, 75, and 90, respectively, while the salt content in rennet cheese at day zero, 15, 30, 45, 60, 75, and 90 was 3.70, 3.67, 3.75, 3.57, 3.64, 3.40, and 3.81%, respectively. Storage period and coagulant type had no significant ($P > 0.05$) effect of salt content of white cheese. Similar results were reported by Sousa and Malacta (1997) and Núnez *et al*. (1991).

The ash content was highest in rennet cheese (3.41%) at day 45. The lowest ash content was 1.99% in cheese made with *Solanum dubium* extract at day 75 with no significant difference. Similar result was found by Krishnaswamy *et al*., (1961) who found that ash content was 3.39% and 3.47% at 3 months and 6 month of storage for vegetable rennet cheese, and 3.46% and 3.53% at 3 month and 6 months of storage for animal rennet cheese.

The tyrosine and tryptophan contents (mg/ 100g cheese) were significantly ($P < 0.05$) affected by type of coagulant during storage period. The highest tyrosine and tryptophan values were 1.88 and 0.53 at day 30 in cheese made by *Solanum dubium* extract, while the lowest tyrosine and tryptophan were 1.19 and 0.34 in rennet cheese. Our findings were not similar to Chen and Zall (1986), Abu-Zeid (1994) and Fernández-Salguero and Sanjuán (1999) who reported 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. Higher contents in both soluble amino acids in the cheese samples made with vegetable rennet.

4.2.1.4 Effect of salt concentration on weight loss and chemical composition of white cheese

Table 4-4 shows that salt concentration significantly affected weight loss, titratable acidity, total solids, fat, protein, soluble protein, salt, ash, tyrosine, and tryptophan contents of white soft cheese.

The highest weight loss was found in cheese salting with 8% salt and this could be due to continuous loss of moisture as a result of curd contraction and water expulsion (Hamid, 2005). Our findings were in agreement with the results reported by Bilal (2000), El Owni and Hamid (2008) and Kalid and El Owni (1991), who found that weight loss from raw milk cheese with 8% salt was significantly higher than that with 6% salt during storage.

The highest (1.38%) titratable acidity was in cheese with 4% salt, while, the lowest (0.91%) titratable acidity was in cheese with 8% salt. The results were similar to the finding of Hamid (1998), El Owni and Hamid (2008), and Kalid (1991) who reported that the acidity of the cheese with low level of salt was slightly higher than that of cheese with high level of salt. The low acidity of cheese with high level of salt was possible due to slow lactic acid development because of inactivation of lactic acid forming bacteria by high level of salt (8%). High acidity of cheese samples with 4% salt could be attributed to lactic acid produced by natural lactic acid bacteria.

The total solids content was 46.07% and 50.93% in cheese made with 4% and 8% salt. Our results were similar to those of Hamid (1998) who found that total solids content of the white cheese made from raw milk with 6% salt were higher (48.35±1.11%) than those with 4% salt (44.08± 4.39%). Similar results were reported by Khalid (1991) and El Owni and Hamid (2008) and. Salting reduces moisture, thus serving to control it in the final cheese (Kosikowski, 1982).

Table (4-4): Effect of salt concentration on weight loss and chemical composition of white cheese

Weight loss and chemical composition	Salt concentration		S.E	L.S
	4%	8%		
Weight loss%	13.20	16.55	0.19	***
Total solids %	46.07	50.93	0.28	***
Fat%	24.29	26.00	0.21	***
Acidity%	1.38	0.91	0.02	***
Protein%	16.82	17.38	0.23	*
Soluble protein%	0.57	0.70	0.02	***
Salt%	2.79	4.56	0.03	***
Ash%	2..02	3.27	0.64	***
Tyrosine mg/100gcheese	1.56	1.45	0.02	***
Tryptophanmg/100gcheese	0.43	0.41	0.01	*

S.E = Standard error

L.S = Level of significance

* = ($P<0.05$)

***= ($p<0.001$)

Fat content of cheese samples with 8% salt were higher (26%) than those with 4% salt (24.29%). The increase in fat content of cheese with 8% salt might be attributed to inactivation of lipolytic bacteria by high salt concentration or may be due to water loss from the cheese into the whey. Similar results were obtained by Hamid (2005).Our findings disagree with the results of Khalid (1991) and Babiker (1987) who reported that fat content of the white soft cheese decreased with increasing salt concentration.

Protein content was higher (17.38%) in cheese with 8% salt compared to 16.82% in cheese with 4% salt. These results were consistant with the results of Hamid (2005) who stated that the protein content of cheese with high level of salt was higher than that of cheese with 4% salt. Khalid (1991) found that protein content of white soft cheese with 8% salt was higher than that with 6% salt. The increase in protein content might be due to low moisture content in the curd as a result of high salt concentration (Kosikowski, 1982).

Soluble protein content of cheese samples with 8% salt was higher than those with 4% salt. These results disagree with Hamid (2005) who reported that the soluble protein content of the cheese samples with 4% salt were higher than those with 6% salt. Elzayat *et al*. (1989) reported that soluble protein contents of the cheese increased during storage as a result of increase in salt but at slower rate.

Salt content of cheese made with high level of salt (8%) was higher (4.56%) compared to cheese made with 4% salt (2.79%).

Ash content of the cheese with 8% salt was higher (3.27%) in comparison with those with 4% salt (2.02%). These findings were in line with the results of Khalid (1991), Abdel Razig (1996), Hamid (1998) and Hamid (2005) who reported that ash content of the white cheese with 6% salt was significantly higher than those with 4% salt. The high ash

content of the cheese with 8% salt was possible due to increases level of salt in the cheese samples.

Tyrosine and tryptophan contents were higher in the cheese samples with 4% salt (1.56 and 0.43 mg/100g cheese). Similar results were found by Hamid (2005) who reported that tyrosine and tryptophan content were higher in the cheese samples with 4% salt. Also similar to Madadlou *et al*. (2007) who found that tyrosin-tryptophan decreased as salt-in-moisture of cheese increased. The increased in tyrosine and tryptophan contents of the cheese with 4% salt might be attributed to rapid degradation of proteins by proteolytic agents at low salt concentration (Abdel-Salam, 1987).

4.2.1.5 Effect of storage period and salt concentration on weight loss and chemical composition of white cheese

Table 4-5 shows the effect of storage period and salt concentration on weight loss and chemical composition of white cheese. The weight loss was significantly ($P< 0.001$) higher in cheese made with 8% salt than cheese made with 4% salt. The weight loss increased gradually from 5.87% at day 15 to 20.90% at day 90 in cheese made using 4% salt, and from 8.24% at day 15 to 27.42% at day 90 in cheese made with 8% salt. Similar resuls were found by Hamid (2005) who reported that weight loss of cheese with 6% salt was higher from day zero to day 180 when compared with 4% salt. Also our results coincide with Khalid and El Owni (1991). The increase in weight loss of the cheese with 8% salt during storage was probably due to continuous loss of moisture from the curd. However, the low weight loss in cheese with 4% salt could be attributed to absorption of the whey by the curd which decreased weight loss.

Table (4-5): Effect of storage period and salt concentration on weight loss and chemical composition of white cheese.

Storage period(days)	Weight loss%		Total solids%		Fat %		Acidity %		Protein %	
	4%	8%	4%	8%	4%	8%	4%	8%	4%	8%
Day one	0	0	47.85	46.52	24.63	21.33	1.01	0.52	18.03	15.47
15	5.87	8.24	46.92	50.03	24.96	24.44	1.23	0.82	18.69	18.66
30	8.27	12.85	49.26	53.38	24.96	27.00	1.34	0.86	17.53	17.53
45	12.67	17.27	45.66	51.65	24.15	26.52	1.63	0.98	17.02	17.41
60	14.46	22.10	45.15	52.34	23.06	27.15	1.72	1.26	14.37	16.72
75	17.02	24.25	44.15	50.73	24.65	27.63	1.38	1.00	16.56	18.53
90	20.90	27.42	43.53	51.86	23.62	27.96	1.34	0.92	15.78	17.32
S.E	0.51		0.74		0.55		0.05		0.62	
L.S	***		***		***		NS		***	

S.E = Standard error

L.S = Level of significance for interaction

*** = ($P<0.001$)

NS = Not significat

Contd.

Storage period(days)	Soluble protein%		Salt%		Ash%		Tyrosine (mg/100gchees)e		Tryptophan (mg/100gcheese)	
	4%	8%	4%	8%	4%	8%	4%	8%	4%	8%
Day one	0.64	0.69	2.60	4.80	2.17	4.12	1.80	1.48	0.51	0.43
15	0.58	0.61	2.78	4.46	2.02	3.50	1.66	1.71	0.47	0.47
30	0.61	0.68	2.95	4.63	1.90	3.29	1.47	1.41	0.39	0.41
45	0.56	1.01	2.82	4.53	2.58	4.07	1.66	1.62	0.46	0.46
60	0.62	0.69	2.78	4.65	1.78	2.60	1.48	1.27	0.42	0.38
75	0.49	0.67	2.67	4.29	1.76	2.49	1.52	1.42	0.41	0.39
90	0.47	0.55	2.91	4.55	1.90	2.86	1.34	1.26	0.38	0.36
S.E	0.05		0.08		0.17		0.06		0.02	
L.S	***		*		*		NS		NS	

S.E = Standard error

L.S = Level of significance for interaction

* = (P<0.05)

***= (P<00.01)

NS = Not significat

Salt concentration during storage period had no significant (P> 0.05) effect on titratable acidity. The titratable acidity of cheese made with 4% salt gradually increased from 1.01% at day zero to 1.72 % at day 60 and then gradually decreased to 1.34% at day 90. However, the titratable acidity increased gradually from 0.52% at day zero to 1.26% at day 60 in cheese made with 8% salt and then decreased to 0.92% at day 90. In general the titratable acidity of the cheese with 4% salt was higher in comparison with that with 8% salt from day zero to day 90. Similar results were found by Hamid (2005) who reported that the low titratable acidity of the samples with 6% salt during storage period was possibly due to retardation effect of high level of salt on the growth of lactic bacteria, while the high titratable acidity of the cheese with 4% salt from day zero to day 180 could be due to increase in activity of lactic bacteria during storage.

The total solids content was significantly (P< 0.001) affected by storage period and salt concentration. The total solids content was 49.26% at day 30 in cheese made using 4% salt and then gradually decreased to 43.53% at day 90. However, in cheese made with 8% salt, the total solids increased from 46.52% at days zero to 53.38% at day 30 and then decreased to 51.65, 52.34, 50.37, and 51.86% at days 45, 60, 75, and 90. These total solids contents were higher in cheese made with 8% salt compared to cheese made with low level of salt (4%). Similar findings were reported by Hamid (2005) and Khalid (1991) who stated that cheese with 8% salt had higher total solids content than that with 6% salt during storage of the white soft cheese.

The fat content of cheese during storage was significantly (P< 0.001) affected by salt concentration. The fat content of cheese with 8% salt increased from 21.33% at day zero to 24.44, 27.0, 26.52, 27.15, 27.63, and 27.96% at days 15, 30, 45, 60, 75, and 90 respectively, while

those with 4% salt decreased from 24.63% at day zero to 23.62% at day 90. The lower fat content of cheese with 4% salt from day 30 to day 90 could be due to continuous loss of moisture and degradation products from the curd (Hamid, 2005).

Protein content was significantly ($P<0.001$) affected by salt concentration during storage. The protein content was 18.03, 18.69, 17.53, 17.02, 14.37, 16.56, and 15.78% at days zero, 15, 30, 45, 60, 75, and 90 days respectively, in cheese made with 4% salt, while the protein content increased from 15.47% at day zero to 17.32% at day 90 in cheese made with 8% salt. These results disagreed with Hamid (2005) who reported that protein content of cheese samples with 6% salt was higher than that with 4% salt. Nuser (2001) reported that the increase in protein content of cheese during initial stage of storage followed by a decrease at latter stage might be due to low moisture content in the cheese with high salt concentration whilst the lower protein contents might be attributed to degradation effect of proteolytic agents on protein of the cheese.

The soluble protein was significantly ($P< 0.001$) higher (1.01%) at day 45 in cheese made with 8% salt compared to 0.56% at day 45 in cheese made with 4% salt at day 90. These results were in disagreement with Hamid (2005) and Kosikowski (1982) who stated that the low soluble protein content was attributed to the inhibition of proteolytic agents by high salt concentration.

The highest salt content was 4.80% at day zero in cheese made with 8% salt. However, the lowest salt content was 2.60% at day zero in cheese made with 4% salt. The higher salt content of cheese with 8% compared to cheese with 4% salt was possibly due to the high level of salt concentration in the former cheese (Khalid, 1991).

The salt concentration significantly ($P< 0.05$) affected the ash content during storage. The ash content increased from 2.17% and 2.02%

at day zero and day 15 to 2.58% at day 45, then decreased to 1.90% at day 90 in cheese made with 4% salt. However, the ash content decreased from 4.12, 3.50, 3.29, and 4.07% at days zero, 15, 30, and 45 to 2.86% at day 90 in cheese with 8% salt. Similar results were found by Hamid (2005) who reported higher ash content of cheese with 6% salt throughout the storage period in comparison with those with 4% salt.

The tyrosine and tryptophan contents (mg/ 100g cheese) of white soft cheese were not significantly affected by salt concentration during storage. The highest tyrosine and tryptophan values were 1.80 and 0.51 in cheese made using 4% salts at day zero. The lowest tyrosine and tryptophan was 1.26 and 0.36 in cheese made with 8% salt at day 90. The result was in agreement with Hamid (2005) who found that the tyrosine and tryptophan contents of cheese samples with 6% were lower in comparison with those with 4% salt from day zero to day 240. The higher tyrosine and tryptophan contents of cheese with 4% salt might be explained by the fact that at low salt concentration protein degradation increases, hence soluble amino acid content increases (Abdel-Salam, 1987).

4.2.1.6 Effect of calcium chloride levels on weight loss and chemical composition of white cheese

Table 4-6 shows the effect of calcium chloride level on weight loss and chemical composition of the white cheese. Addition of calcium chloride significantly ($P< 0.001$) increased weight loss from 15.34% in cheese without addition of calcium chloride (0%) to 16.55% in cheese made with addition of 0.02% calcium chloride.

Calcium chloride level significantly ($P< 0.05$) affected the titratable acidity. Cheese without calcium chloride secured higher titratable acidity (1.17%), while cheese made with 0.02% calcium

Table (4-6): Effect of addition of $CaCl_2$ on weight loss and chemical composition of white cheese

Weight loss and chemical composition	Calcium chloride		S.E	L.S
	Zero%	0.02%		
Weight loss%	15.34	16.55	0.19	***
Total solids %	48.08	48.92	0.28	NS
Fat%	25.04	25.25	0.21	NS
Acidity%	1.17	1.11	0.02	*
Protein%	17.17	17.03	0.14	NS
Soluble protein%	0.63	0.64	0.02	NS
Salt%	3.61	3.73	0.03	***
Ash%	2.56	2.73	0.06	*
Tyrosine mg/100gcheese	1.52	1.50	0.02	NS
Tryptophan mg/100gcheese	0.43	0.42	0.01	NS

S.E = Standard error

L.S = Level of significance

* = ($P<0.05$)

*** = ($P<0.001$)

NS = Not significat

chloride secured the lowest titratable acidity (1.11%). This result was similar to Hamid (1998) and Bille *et al*. (2001) who reported that cheese with no added calcium chloride developed higher final acidity than cheese with calcium chloride.

Calcium chloride level significantly ($P< 0.05$) affected the salt and ash contents. Cheese made with 0.02% calcium chloride cheese showed higher salt (3.73%), and ash (2.73%), while cheese without addition of calcium chloride (0%) showed the lowest salt (3.61%), and ash (2.56%). This result was not similar to Hamid (1998) who reported that total solids and ash contents were higher in raw milk cheese (0% calcium chloride). Lucy and Fox (1993) stated that the addition of calcium chloride to milk significantly decreased the ash content. Our results are similar to Bille *et al*. (2001) who reported higher total solids and ash content in Gouda cheese made with added calcium chloride. They stated that calcium chloride accelerated moisture loss from the cheese during its processing almost to the lowest recommended level. Thus cheese with calcium chloride had a drier appearance and hard–feel than the cheese without calcium chloride.

Fat and total solids content of cheese was not significantly affected by the addition of calcium chloride. However, the highest fat content was in cheese made with 0.02% calcium chloride (25.25%).These results were in agreement with Lucy and Fox (1993), Hamid (1998) Bille *et al*. (2001). Cheese made with 0.02% calcium chloride showed higher total solids content (48.92%), while cheese without addition of calcium chloride (0%) showed the lowest total solids content (48.08%).

Calcium chloride level did not significantly ($P> 0.05$) affect the protein and soluble protein contents of cheese. However, there was a slight increase in soluble protein with addition of calcium chloride. This

finding was not similar to Cakmakci and Kurt (1993) who reported that the addition of calcium chloride decreased soluble protein in cheese.

Calcium chloride level did not significantly (P> 0.05) affect the tyrosine and tryptophan contents (mg/ 100g cheese). However, there was a slight decrease in tyrosine and tryptophan of cheese with addition of calcium chloride.

4.2.1.7 Effect of calcium chloride and storage period on weight loss and chemical composition of white cheese

Calcium chloride level significantly (P< 0.05) affected the weight loss of white cheese during storage period (Table 4-7). The weight loss of cheese was higher in cheese made with addition of calcium chloride (0.02%). It increased from 7.66% at day 15 to 24.77% at day 90 of storage. However, the weight loss in cheese made without addition of calcium chloride (0%) increased from 6.45% at day 15 to 9.95, 14.36, 17.67, 20.03, and 23.56% at day 30, 45, 60, 75, and 90.

The addition of calcium chloride did not significantly (P> 0.05) affect the titratable acidity of the white cheese during storage period (Table 4-7). However, slightly higher titratable acidity was found in cheese made without addition of calcium chloride (0%). The titratable acidity gradually increased from 0.77% at day zero to 1.04, 1.14, 1.35, and 1.53% at days 15, 30, 45, and 60 then graduall decreased to 1.15% at day 90 in cheese made with no calcium chloride (0%). However, with addition of 0.02% calcium chloride, the titratable acidity of cheese gradually increased from 0.75% at day zero to 1.45% at day 60, and then decreased to 1.14% and 1.11% at days 75 and 90, respectively.

The total solids content (Table 4-7) gradually increased from 46.65% at day zero to 50.36% at day 30, and then decreased to 47.66% in cheese made without addition of calcium chloride (0%). Also the total solids content increased gradually from 47.72% at day zero to 52.29% at

Table (4-7): Effect of storage period and addition of $CaCl_2$ on weight loss and chemical composition of white cheese

Storage period(days)	Weight loss %		Total solids %		Fat %		Acidity %		Protein %	
	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%
Day one	0	0	46.65	47.72	23.42	22.54	0.77	0.75	16.77	16.73
15	6.45	7.66	48.22	48.73	24.13	25.27	1.04	1.01	19.01	18.34
30	9.95	11.17	50.36	5229	25.71	26.25	1.14	1.06	17.55	17.30
45	14.36	15.58	48.82	48.49	25.29	25.38	1.35	1.27	17.56	16.87
60	17.67	18.89	47.99	49.51	2475	25.46	1.53	1.45	15.06	16.03
75	20.03	21.24	46.87	48.01	25.69	26.58	1.23	1.14	17.43	17.66
90	23.56	24.77	47.66	47.73	26.28	25.29	1.15	1.11	16.80	16.30
S.E	0.39		0.74		0.55		0.05		0.36	
L.S	*		NS		NS		NS		NS	

S.E = Standard error

L.S = Level of significance for interaction

* = Significance ($P<0.05$)

NS = not significat

Contd.

Storage period(days)	Soluble protein%		Salt%		Ash%		Tyrosine (mg/100gcheese)		Tryptophan (mg/100gcheese)	
	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%
Day one	0.68	0.65	3.68	3.72	3.18	3.11	1.66	1.62	0.48	0.46
15	059	0.60	3.60	3.65	2.59	2.93	1.75	1.62	0.48	0.46
30	0.66	0.64	3.66	3.92	2.46	2.73	1.57	1.32	0.43	0.38
45	0.74	0.83	3.62	3.73	2.93	3.71	1.62	1.66	0.45	0.45
60	0.69	0.62	3.61	3.82	2.18	2.20	1.35	1.40	0.39	0.40
75	0.59	0.60	3.50	3.95	2.11	2.14	1.41	1.53	0.38	0.42
90	0.48	0.53	3.63	3.83	2.45	2.34	1.27	1.32	0.38	0.36
S.E	0.05		0.08		0.17		0.06		0.02	
L.S	NS		NS		NS		NS		NS	

S.E = Standard error

L.S = Level of significance for interaction

NS = Not significat

day 30, and then decreased to 47.73% at day 90 in cheese made with addition of calcium chloride (0.02%). The addition of calcium chloride had no significant (P> 0.05) effect on total solids of white soft cheese during storage.

The fat content of cheese was not significantly (P> 0.05) affected by addition of calcium chloride during storage (Table 4-7). The fat content in cheese with no calcium chloride increased from 23.42% at day zero to 26.28% at day 90, while in 0.02% calcium chloride cheese the fat content increased from 22.54% at days zero to 25.29% at day 90.

The protein content of cheese was not significantly (P>0.05) affected by addition of calcium chloride during storage (Table 4-7). Higher protein content (19.01%) was found at day 15 in cheese made with addition of calcium chloride (0.02%). The lowest protein content was 15.06% at day 60 in zero % calcium chloride cheese.

The addition of calcium chloride had no significant (P>0.05) effect on soluble protein of white cheese (Table 4-7). However, higher soluble protein content was found in 0.02% calcium chloride cheese which was 0.83%, while the lowest soluble protein content was 0.48% in zero% calcium chloride cheese.

Salt content in cheese was not significantly (P>0.05) affected by the addition of calcium chloride during storage period (Table 4-7). The highest salt content (3.95%) was in 0.02% calcium chloride cheese at day 75. However, the lowest salt content (3.50%) was in 0% calcium chloride cheese at day 75

Ash content decreased from 3.18% at day zero to 2.45% at day 90 in cheese made without addition of calcium chloride (0%), while in cheese made with addition of 0.02% calcium chloride, the ash content was higher (3.71%) at day 45 (Table 4-7). As will be seen from the table

there was no significant (P>0.05) effect of calcium chloride on ash content of cheese during storage period.

The addition of calcium chloride did not significantly (P>0.05) affect the tyrosine content (mg/ 100g cheese) of cheese (Table 4-7). The highest tyrosine content was 1.75 at day 15 in zero% calcium chloride cheese while the lowest tyrosine content was 1.27 in zero% calcium chloride cheese at day 90.

Tryptophan content (mg/100g cheese) was not significantly (P>0.05) affected by addition of calcium chloride during storage period (Table 4-7). The tryptophan content was 0.48, 0.48, 0.43, 0.45, 0.39, 0.38, and 0.38 at day zero, 15, 30, 45, 60, 75, and 90 in zero % calcium chloride cheese. In 0.02% calcium chloride the tryptophan content was 0.46, 0.46, 0.38, 0.45, 0.40, 0.42, and 0.36 at day zero, 15, 30, 45, 60, 75 and 90.

4.2.1.8 Effect of coagulant type and salt concentration on weight loss and chemical composition of white cheese

Type of coagulant (rennet and *Solanum dubium* extract) and salt concentration (4% and 8%) had no significant (P>0.05) effect on weight loss of white cheese (Table 4-8). Weight loss was higher (19.55%) in cheese made with *Solanum dubium* extract and 8% salt compared to 12.52% weight loss in rennet cheese with 4%salt.

Titratable acidity was not significantly (P>0.05) affected by coagulant type and salt concentration (Table 4-8). Highest titratable acidity was found in cheese made with rennet and *Solanum dubium* extract with 4% salt which was 1.39%. Also lowest titratable acidity was 0.91% in rennet and *Solanum* cheese with 8% salt.

Coagulant type and salt concentration had significant (p< 0.001) effect on protein content of white soft cheese. The protein content was higher (17.68%) in rennet cheese made with 8% salt.

Table (4-8): Effect of coagulant type and salt concentration on weight loss and chemical composition of white cheese

Weight loss and chemical composition	Rennet		*Solanum*		S.E	L.S
	Salt concentration					
	4%	8%	4%	8%		
Weight loss%	12.52	17.83	13.88	19.55	0.26	NS
Total solids %	43.53	51.12	48.61	50.74	0.39	***
Fat%	22.69	25.74	25.89	26.27	0.29	***
Acidity%	1.39	0.91	1.36	0.91	0.03	NS
Protein%	16.08	17.68	17.57	17.03	0.19	***
Soluble protein%	0.51	0.63	0.63	0.78	0.03	NS
Salt%	2.80	4.44	2.77	4.67	0.04	*
Ash%	1.97	3.33	2.07	3.22	0.09	NS
Tyrosine mg/100gcheese	1.44	1.36	1.69	1.54	0.03	NS
Tryptophan mg/100gcheese	0.40	0.38	0.47	0.44	0.01	NS

S.E = Standard error

L.S = Level of significance for interaction

* = ($P<0.05$)

***= ($P<0.001$)

NS = Not significat

Soluble protein content was not significantly (P> 0.05) affected by coagulant type and salt concentration. However, higher soluble protein content (0.78%) was in cheese made with *Solanum dubium* extract and 8% salt while the lowest soluble protein content was 0.51% in rennet cheese with 4% salt.

The salt content of white cheese was significantly (P< 0.05) higher in cheese made with *Solanum dubium* extract and 8% salt (4.67%), compared to 4.44% and 2.80% in rennet cheese with 8% and 4% salt, respectively (Table 4-8).

Coagulant type and salt concentration did not significantly affect ash, tyrosine and tryptophan contens of the white cheese (Table 4-8). However, ash was higher (3.33%) in rennet cheese with 8% salt, while tyrosine and tryptophan (mg/ 100g cheese) were 1.19 and 0.47 in cheese made of *Solanum dubium* extract with 4% salt. The lowest tyrosine and tryptophan contents were 1.36 and 0.38 in rennet cheese with 8% salt.

4.2.1.9 Effect of coagulant type and addition of calcium chloride on weight loss and chemical composition of white cheese

Coagulant type and addition of calcium chloride did not significantly affect weight loss of white cheese (Table 4-9). However, the weight loss of cheese was higher (17.60%) in cheese produced using *Solanum dubium* extract with 0.02% calcium chloride and lower weight loss (14.84%) in rennet cheese without added calcium chloride (0%).

The titratable acidity was 1.19% and 1.12% in rennet cheese with zero% and 0.02% calcium chloride, respectively, while 1.16% and 1.11% in cheese made using *Solanum dubium* extract with zero% and 0.02% calcium chloride, respectively, (P< 0.05). Total solids and fat contents of cheese were significantly (p< 0.05) affected by type of coagulant and calcium chloride level (Table 4-9). The higher total solids and fat content

Table (4-9): Effect of coagulant type and $CaCl_2$ on weight loss and chemical composition of the white cheese

Weight loss and chemical composition	Rennet		*Solanum*		S.E	L.S
	Zero%	0.02 %	Zero%	0.02%		
Weight loss%	14.84	15.51	15.83	17.60	0.26	NS
Total solids %	46.16	48.49	50.00	49.36	0.39	*
Fat%	23.74	24.69	26.34	25.82	0.29	*
Acidity%	1.19	1.12	1.16	1.11	0.03	NS
Protein%	16.63	17.13	17.71	16.94	0.19	*
Soluble protein%	0.56	0.58	0.71	0.70	0.03	NS
Salt%	3.59	3.65	3.64	3.81	0.04	NS
Ash%	2.50	2.79	2.61	2.68	0.91	NS
Tyrosine mg/100gcheese	1.40	1.40	1.64	1.59	0.03	NS
Tryptophan mg/100gcheese	0.39	0.39	0.47	0.44	0.01	NS

S.E = Standard error

L.S = Level of significance for interaction

* = Significance ($P<0.05$)

NS = Not significat

were 50.0% and 26.34% in cheese made with *Solanum dubium* extract without addition of calcium chloride (0%).

Coagulant type and addition of calcium chloride had a significant effect ($P< 0.05$) on protein content of white cheese (Table 4-9). Highest protein content was 17.71% in zero% calcium chloride cheese using *Solanum dubium* extract. However, the lowest protein content was 16.63% in rennet cheese without addition of calcium chloride.

Soluble protein content of cheese was not significantly ($P> 0.05$) affected by type of coagulants and addition of calcium chloride (Table 4-9). Higher soluble protein content (0.71%) was in cheese made with *Solanum dubium* extract without addition of calcium chloride compared to lower (0.56%) soluble protein content in rennet cheese without addition of calcium chloride (0%).

Salt and ash contents was not significantly ($P> 0.05$) affected by coagulant type and addition of calcium chloride (Table 4-9). Salt contents were 3.59% and 3.65% in rennet cheese with zero% and 0.02% calcium chloride, and 3.64% and 3.81% salt content in cheese made with *Solanum dubium* extract with zero% and 0.02% calcium chloride, respectively.

Type of coagulant and addition of calcium chloride did not significantly ($P> 0.05$) affect tyrosine and tryptophan (mg/ 100g cheese) contents of white cheese (Table 4-9). Higher tyrosine and tryptophan contents were 1.64 and 0.47 in cheese made using *Solanum dubium* extract without addition of calcium chloride, and lower tyrosine and tryptophan contents were 1.40 and 0.39 in rennet cheese with addition of 0.02% calcium chloride.

4.2.1.10 Effect of coagulant type, salt concentration and addition of calcium chloride on weight loss and chemical composition of white cheese

Coagulant type, salt concentration and addition of calcium chloride showed a significant effect on weight loss, total solids, fat and salt content of white soft cheese (Table 4-10). However, no significant (P> 0.05) effect was found on tittratable acidity, protein, soluble protein, ash, tyrosine and tryptophan content of cheese (Table 4-10).

Weight loss was higher (19.63%) in cheese made using *Solanum dubium* extract with 8% salt without addition of calcium chloride (0%). However, lower (13.03%) weight loss was in *Solanum* cheese with 4% salt without addition of calcium chloride (0%).

The titratable acidity was slightly higher (1.42%) in rennet cheese using 4% salt without addition of calcium chloride (0%).

Total solids content were higher (52.61%) in rennet cheese made using 8% salt and 0.02% calcium chloride.

The fat content was highest (27.12%) in cheese made using *Solanum dubium* extract coagulant and 8% salt without addition of calcium chloride (0%). However, lowest fat content was 22.68% in rennet cheese with 4% salt and without addition of calcium chloride (0%).

Protein content was slightly higher (17.87%) in rennet cheese made using 8% salts with addition of 0.02% calcium chloride.

Soluble protein and salt contents were higher (0.80% and 4.45%) in *Solanum* cheese with 8% salts without addition of calcium chloride (0%).

Ash content was higher (3.58%) in rennet cheese produced using 8% salt with addition of 0.02% calcium chloride.

Tyrosine (mg/ 100g cheese) was higher (1.71) in *Solanum* cheese produced using 4% salt and 0.02% calcium chloride.

Table (4-10): Effect of type of coagulants, salt concentration and addition of $CaCl_2$ on weight loss and chemical composition of the white cheese

Weight loss and chemical composition	Rennet				*Solanum*				S.E	L.S
	4%		8%		4%		8%			
	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%		
Weight loss%	12.53	12.51	17.16	18.50	12.03	15.73	19.63	19.46	0.37	***
Total solids %	42.69	44.38	49.64	52.09	49.64	49.32	52.09	49.39	0.56	*
Fat%	22.68	22.69	24.80	26.68	25.55	26.23	27.12	25.42	0.42	*
Acidity%	1.42	1.37	0.95	0.86	1.37	1.36	0.96	0.87	0.04	NS
Protein%	15.77	16.39	17.49	17.87	17.91	17.23	17.50	16.65	0.27	NS
Soluble protein%	0.50	0.51	0.61	0.65	0.62	0.64	0.80	0.76	0.04	NS
Salt%	2.79	2.81	4.38	4.50	2.83	2.72	4.45	4.90	0.06	***
Ash%	1.94	2.00	3.07	3.58	2.11	2.04	3.12	3.33	1.13	NS
Tyrosine mg/100gcheese	1.45	1.42	1.34	1.39	1.67	1.71	1.62	1.47	0.04	NS
Tryptophan mg/100gcheese	0.40	0.40	0.38	0.38	0.47	0.47	0.47	0.42	0.01	NS

S.E = Standard error

L.S = Level of significance for interaction

* = Significance ($P<0.05$)

NS = Not significat

Higher (0.47) tryptophan (mg/ 100g cheese) was in *Solanum* cheese made using 4% salt with zero% and 0.02% calcium chloride, also with 8% salt without addition of calcium chloride (0%).

4.2.2 Microbiological quality of white soft cheese

4.2.2.1 Effect of storage period on Microbiological quality of white cheese

Results in Table 4-11 show the effect of storage period on microbiological quality of the white cheese during ripening.

4.2.2.1.1 Total viable bacterial count (TBC)

Total viable bacterial count of cheese significantly ($P< 0.001$) decreased with increase in storage time. It decreased from 5.1×10^7 at day zero to 5.9×10^5 at day 90. Increase in TBC during early stage of ripening could be attributed to rapid growth of microorganism. However, decrease in TBC from day 60 to day 90 may be attributed to lactic acid production. Similar results were found by El Owni and Hamid (2008). Storage periods showed significant differences ($p<0.05$) in total bacterial counts (Nour El Diam and El Zubeir, 2006).

4.2.2.1.2 Streptococci count

Streptococci count was significantly ($P< 0.001$) affected by storage period. The count of cheese samples at zero days was 5.8×10^7 reduced to 4.1×10^5 at day 90. Therefore, as the storage period progressed streptococci count decreased.

4.2.2.1.3 Lactobacilli count

Lactobacilli count was significantly ($P< 0.001$) affected by storage period. The results indicated that lactobacilli count decreased from 2.9×10^7 at day zero to 5.4×10^5 at day 90.

Table (4-11): Effect of storage period on the microbiological quality of white cheese

Storage period(days)	Total viable bacterial count (cfu/gm)	Streptococci count (cfu/gm)	Lactobacilli count (cfu/gm)
Day one	5.1×10^{7b}	5.8×10^{7c}	2.9×10^{7c}
15	1.3×10^{7a}	2.1×10^{7b}	1.8×10^{7b}
30	1.1×10^{7a}	9.8×10^{6ab}	9.2×10^{6ab}
45	1.4×10^{7a}	4.1×10^{6a}	1.2×10^{7b}
60	2.9×10^{6a}	1.7×10^{6a}	2.3×10^{6a}
75	2.3×10^{5a}	1.7×10^{5a}	2.3×10^{5a}
90	5.9×10^{5a}	4.1×10^{5a}	5.4×10^{5a}
S.E	4.7×10^{6}	5.5×10^{6}	3.5×10^{6}
L.S	***	***	***

S.E = Standard error

L.S = Level of significance

*** = ($P<0.05$)

Means values bearing different superscripts within the same columns are significantly ($p<0.05$) different.

4.2.2.2 Microbiological quality change of white cheese during storage

4.2.2.2.1 Total viable bacterial count (TBC)

Result in Table 4-12 illustrates changes in TBC of the different cheese samples during storage. Type of coagulant and salt concentration (4% and 8%) had no significant effect on TBC during storage period.

TBC of the cheese with 4% salt was higher (7.210^7 and 6.310^7) than that with 8% salt (4.6×10^7 and 2.4×10^7) at day zero. The TBC decreased from 7.2×10^7 at day zero to 3.6×10^5 at day 75, and then increased to 2.0×10^6 at the end of storage (day 90) in rennet cheese with 4% salt. In rennet cheese with 8% salt the TBC reduced gradually from 4.6×10^7 at day zero to 3.0×10^4 at day 90.

In cheese made using *Solanum dubium* extract as coagulant, higher TBC (6.3×10^7) was at day zero using 4% salt. However, the lower TBC (1.4×10^4) was at day 90 using 8% salt.

4.2.2.2.2 Streptococci count

Result in Table 4-13 show change in streptococci count of the white soft cheese samples during storage with no significant difference ($P> 0.05$) during storage. The cheese samples with 4% salt using rennet and *Solanum* coagulant had higher streptococci count in comparison with that with 8% during storage period. As the storage period progressed streptococci count decreased in values. The higher streptococci count was 8.3×10^7 in *Solanum* cheese with 4% while the lowest streptococci count was 8.9×10^3 in *Solanum* cheese with 8%.

4.2.2.2.3 Lactobacilli count

Results in Table 4-14 demonstrate changes in lactobacilli count of the white soft cheese samples during storage period. Lactobacilli count was not significantly affected by coagulant type and salt concentration. The cheese samples with 4% salt using rennet and *Solanum* coagulant had higher lactobacilli count in comparison with 8% during storage

Table (4-12): Change in TBC (cfu/gm) of white cheese during storage

Type of coagulant	Rennet		*Solanum*	
Salt concentration	4%	8%	4%	8%
Day one	7.2×10^{7}	4.6×10^{7}	6.3×10^{7}	2.4×10^{7}
15 days	1.6×10^{7}	8.9×10^{6}	2.3×10^{7}	4.4×10^{7}
30 days	8.0×10^{6}	6.7×10^{6}	2.2×10^{7}	4.9×10^{6}
45 days	4.8×10^{7}	1.6×10^{6}	6.0×10^{6}	3.5×10^{5}
60 days	3.0×10^{6}	4.6×10^{6}	2.6×10^{6}	1.6×10^{6}
75 days	3.6×10^{5}	1.4×10^{5}	4.0×10^{5}	2.6×10^{4}
90 days	2.0×10^{6}	3.0×10^{4}	3.0×10^{5}	1.4×10^{4}
S.E	9.4×10^{6}	9.4×10^{6}	9.4×10^{6}	9.4×10^{6}
L.S	NS			

S.E = Standard error

L.S = Level of significance for interactin

NS = Not significant

Table (4-13): Change in streptococci of white cheese during storage

Type of coagulant	Rennet		*Solanum*	
Salt concentration	4%	8%	4%	8%
Day one	5.6×10^{7}	1.3×10^{7}	8.3×10^{7}	8.1×10^{7}
15 days	3.2×10^{7}	2.0×10^{7}	3.0×10^{7}	4.2×10^{6}
30 days	7.5×10^{6}	6.1×10^{6}	2.1×10^{7}	4.7×10^{6}
45 days	1.1×10^{7}	1.1×10^{6}	4.5×10^{6}	3.4×10^{5}
60 days	5.2×10^{5}	3.4×10^{6}	1.8×10^{6}	1.0×10^{6}
75 days	3.0×10^{5}	9.0×10^{4}	1.9×10^{5}	1.2×10^{5}
90 days	1.4×10^{6}	2.9×10^{4}	2.5×10^{5}	8.9×10^{3}
S.E	1.1×10^{7}	1.1×10^{7}	1.1×10^{7}	1.1×10^{7}
L.S	NS	NS	NS	NS

S.E = Standard error

L.S = Level of significance

NS = Not significant

Table (4-14): Change in lactobacilli (cfu/gm) of white cheese during storage

Type of coagulant	Rennet		*Solanum*	
Salt concentration	4%	8%	4%	8%
Day one	4.4×10^{7}	1.4×10^{7}	4.7×10^{7}	9.2×10^{6}
15 days	3.1×10^{7}	1.7×10^{7}	2.0×10^{7}	4.5×10^{6}
30 days	9.9×10^{6}	8.0×10^{6}	1.4×10^{7}	5.3×10^{6}
45 days	4.1×10^{7}	2.2×10^{6}	5.9×10^{6}	4.0×10^{5}
60 days	2.1×10^{6}	2.1×10^{6}	3.2×10^{6}	1.7×10^{6}
75 days	4.9×10^{5}	1.4×10^{5}	2.4×10^{6}	3.1×10^{4}
90 days	1.8×10^{6}	4.0×10^{4}	3.0×10^{5}	6.2×10^{3}
S.E	6.9×10^{6}	6.9×10^{6}	6.9×10^{6}	6.9×10^{6}
L.S	NS			

S.E = Standard error

L.S = Level of significance for interaction

NS = Not significant

period. The lactobacilli count was 4.4×10^7, 3.1×10^7, 9.9×10^6, and 4.1×10^7 at day zero, 15, 30, and 45, and then decreased to 2.1×10^7 and 4.9×10^5 at day 60 and 75 in rennet cheese with 4% salt. In *Solanum* cheese with 4% salt, the higher lactobacilli count was 4.7×10^7 at day zero. While the lower lactobacilli count (6.2×10^3) at day 90 in *Solanum* cheese with 8% salt.

4.2.2.3 Effect of coagulant type on microbiological quality of white cheese during storage

Results in Table 4-15 show the effect of coagulant type on microbiological quality of white cheese samples during storage.

4.2.2.3.1 Total viable bacterial count

Total viable bacterial count (TBC) of the white cheese samples during storage were not significantly ($P> 0.05$) affected by type of coagulant. However, cheese made using rennet had higher (5.9×10^7) TBC in comparison with that made using *Solanum dubium* extract as coagulant at zero days. The lowest (1.6×10^7) TBC was in cheese made using *Solanum* coagulant at day 90.

4.2.2.3.2 Streptococci count

Streptococci count in the cheese samples during storage were found to be significantly ($P< 0.001$) affected by coagulant type (Table 4-15). The cheese samples made using *Solanum* coagulant had higher streptococci count (8.2×10^7) when compared with those made using rennet as a coagulant (3.4×10^7) at day zero. As the storage period progressed the streptococci count decreased.

Table (4-15): Effect of type of coagulant during storage period on the microbiological quality of white cheese

Storage period(days)	TBC (cfu/gm)		Streptococci count (cfu/gm)		Lactobacilli count (cfu/gm)	
	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*
Day one	5.9×10^{7}	4.4×10^{7}	3.4×10^{7}	8.2×10^{7}	2.9×10^{7}	2.8×10^{7}
15	1.3×10^{7}	1.4×10^{7}	2.6×10^{7}	1.7×10^{7}	2.4×10^{7}	1.2×10^{7}
30	7.4×10^{6}	1.4×10^{7}	6.8×10^{6}	1.3×10^{7}	8.9×10^{6}	9.5×10^{6}
45	2.5×10^{7}	3.1×10^{6}	5.9×10^{6}	2.3×10^{6}	2.2×10^{7}	3.2×10^{6}
60	3.8×10^{6}	2.1×10^{6}	2.0×10^{6}	1.4×10^{6}	2.1×10^{6}	2.4×10^{6}
75	2.5×105	2.1×105	2.0×105	1.5×105	3.2×105	1.4×105
90	1.0×10^{6}	1.6×105	6.9×105	1.3×105	9.4×105	15×105
S.E	6.6×10^{6}	6.6×10^{6}	7.8×10^{6}	7.8×10^{6}	4.9×10^{6}	4.9×10^{6}
L.S	NS		***		NS	

S.E = Standard error

L.S = Level of significance for interaction

*** = ($P<0.001$)

NS = Not significant

4.2.2.3.3 Lactobacilli count

Coagulant type did not significantly (P> 0.05) affect lactobacilli count in cheese samples (Table 4-15). The result show that as storage time increased lactobacilli count decreased. The lactobacilli count was 2.9×10^7 and 2.8×10^7 at day zero in cheese made using rennet and *Solanum* coagulant, respectively. The lowest count was 1.4×10^5 and 1.5×10^5 at day 75 and 90, respectively in cheese made using *Solanum* as coagulants. Similar results were found by Sousa and Malcata (1997) who reported that lower microbiological count of lactobacilli were obtained for cheese manufactured with plant rennet until 28 days of the ripening.

4.2.2.4 Effect of salt concentration on microbiological quality of white cheese during storage

Result in Table 4-16 shows the effect of salt concentration on the microbiological quality of the white cheese during storage

4.2.2.4.1 Total viable bacterial count

Total viable bacterial count (TBC) of white cheese during storage was not significantly (P> 0.05) affected by salt concentration. The cheese samples with 4% salt had higher TBC (6.8×10^7) in comparison with that with 8% salt (3.5×10^7) at day zero. The high TBC of the cheese with 4% salt was possibly due to the high microbial content of raw milk used for cheese, while the low count of that with 8% salt might be due to the inhibition of microorganisms by the higher salt concentration (Kosikowski, 1977; Walstera, 1999).The TBC decreased with increasing storage time in cheese made with 4% and 8%.

Table (4-16): Effect of salt concentration on the microbiological quality of white cheese during storage

Storage period(days)	TBC (cfu/gm)		Streptococci count (cfu/gm)		Lactobacilli count (cfu/gm)	
	4%	8%	4%	8%	4%	8%
Day one	6.8×10^7	3.5×10^7	6.9×10^7	4.7×10^7	4.6×10^7	1.2×10^7
15	2.0×10^7	6.6×10^6	3.1×10^7	1.2×10^7	2.6×10^7	1.1×10^7
30	1.5×10^7	5.8×10^6	1.4×10^7	5.4×10^6	1.2×10^7	6.6×10^6
45	2.7×10^7	9.7×10^5	7.5×10^6	7.2×10^5	2.4×10^7	1.3×10^6
60	2.8×10^6	3.1×10^6	1.2×10^6	2.2×10^6	2.6×10^6	1.9×10^6
75	3.8×10^6	8.5×10^4	2.5×10^5	1.0×10^5	3.7×10^5	8.5×10^4
90	1.2×10^6	2.2×10^4	8.0×10^5	1.9×10^4	1.1×10^6	2.3×10^4
S.E	6.6×10^6		7.8×10^6		4.9×10^6	
l.S	NS		NS		***	

S.E = Standard error

L.S = Level of significance for interaction

*** = ($P<0.001$)

NS = Not significant

4.2.2.4.2 Streptococci count

Streptococci count was not significantly (P> 0.05) affected by salt concentration. Slightly higher (6.9×10^7) streptococci count was found in cheese with 4% salt compared to 4.7×10^7 in cheese with 8% salt at day zero. However, the lowest streptococci count was 1.9×10^4 at day 90 in cheese made with 8%.

4.2.2.4.3 Lactobacilli count

Lactobacilli count in the cheese during storage was found to be significantly (P< 0.001) affected by salt concentration. The cheese with 4% salt had higher lactobacilli count (4.6×10^7) when compared with cheese with 8% salt (1.2×10^7) at day zero. The lactobacilli count decreased from 4.6×10^7 at zero days to 2.6×10^7, 1.2×10^7, 2.4×10^7, 2.6×10^6, 3.7×10^5, and 1.1×10^6 at day 15, 30, 45, 60, 75, and 90 in cheese with 4% salt. In 8% salt cheese the lactobacilli count decreased from 1.2×10^7 at day zero to 2.3×10^4 at day 90.

4.2.2.5 Effect of coagulant type and salt concentration on the microbiological quality of white cheese

Result in Table 4-17 illustrates the effect of coagulant type and salt concentration on the microbiological quality of white soft cheese. Type of coagulant and salt concentration had no significant (P> 0.05) effect on total bacterial count, streptococci count, and lactobacilli count.

4.2.2.5.1 Total viable bacterial count (TBC)

As shown in Table 4-17 the TBC was slightly higher (2.1×10^7) in rennet cheese with 4% salt. However, the lowest TBC (5.0×10^6) was found in *Solanum* cheese with 8% salt.

Table (4-17): Effect of coagulant type and salt concentration on the microbiological quality of white cheese

Coagulants type	Rennet		*Solanum*		S.E	L.S
Salt concentration	4%	8%	4%	8%		
TBC count (cfu/gm)	2.1×10^7	9.7×10^6	1.7×10^7	5.0×10^6	3.5×10^6	NS
Streptococci count (cfu/gm)	1.5×10^7	6.2×10^6	2.0×10^7	1.3×10^7	4.2×10^6	NS
Lactobacilli count (cfu/gm)	1.9×10^7	6.2×10^6	1.3×10^7	3.0×10^6	2.6×10^6	NS

S.E = Standard error

L.S = Level of significance for interaction

NS = Not significant

4.2.2.5.2 Streptococci count

Streptococci count was 1.5×10^7 and 6.2×10^6 in rennet cheese with 4% and 8%, respectively, and 2.0×10^7 and 1.3×10^7 in *Solanum* cheese with 4% and 8% respectively.

4.2.2.5.3 Lactobacilli count

Lactobacilli count was 1.9×10^7 and 6.2×10^6 in rennet cheese with 4% and 8% salt. However, the lactobacilli count in *Solanum* cheese with 4% and 8% salt was 3.0×10^6 and 2.6×10^6, respectively.

4.2.3 Sensory characteristics of the white cheese

4.2.3.1 Effect of storage period on sensory characteristics of white cheese

Result in Table 4-18 presente the effect of the storage period on sensory characteristics of cheese during storage.

The storage period affected the colour of the cheese significantly ($P< 0.05$).The highest value (6.42) was obtained at day 30, and the lowest was 5.83 at day 45. This finding was in agreement with Abdel Razig (1996) and El Owni and Hamid (2008). However, the present study disagreed with Nuser (2001) who reported that storage period did not affect the colour of Sudanese white cheese during storage for 45 days. Tarakci and Kuckoner (2006) reported that the appearance and colour scores increased generally during ripening in Turkish Kashar cheese.

The flavour of the cheese was significantly ($P< 0.001$) affected by storage period. The flavour scores of the cheese increased from 5.63 at day zero to 6.50 at day 15 then decreased to 5.97at day 90. Similar result were found by Abdel Razig (1996) and El Owni and Hamid (2008).

The texture of the cheese samples was not significantly ($P> 0.05$) affected by storage period. However, the texture of the cheese increased

Table (4-18): Effect of storage period on sensory characteristic of white cheese

Storage(days)	Colour	Flavour	Texture	Salt
Day one	5.93^{a}	5.63^{b}	5.84^{ab}	4.97^{a}
15	6.20^{ab}	6.50^{c}	5.79^{ab}	5.03^{a}
30	642^{b}	5.47^{ab}	5.73^{ab}	5.06^{a}
45	5.83^{a}	5.38^{ab}	5.70^{a}	5.05^{a}
60	6.18^{ab}	5.64^{b}	5.90^{ab}	5.03^{a}
75	6.14^{ab}	5.78^{b}	5.98^{ab}	5.64^{b}
90	5.96^{a}	5.07^{a}	6.16^{b}	5.25^{a}
S.E	0.13	0.15	0.24	0.14
L.S	*	***	NS	***

S.E = Standard error

L.S = Level of significance

* = ($P<0.005$)

***= ($P<0.001$)

NS = Not significant

Means values bearing different superscripts within the columns are significantly ($p<0.05$) different.

slightly as the storage period progressed. The highest texture scores were 6.16 recoded at day 90, while the lowest (5.70) were obtained at day 45. Our results are in disagreement with those of Abdel Razig (1996) and El Owni and Hamid (2008) who found that storage period had significant effect on texture of cheese.

The saltiness of the cheese was significantly (P< 0.001) affected by storage period. The saltiness of the cheese improved as storage period progresed. The highest saltiness scores (5.64) were recorded after 75 days of storage, and then decreased to 5.25 at day 90. Similar result was found by Abdel Razig (1996) and El Owni and Hamid (2008).

Kur (1992) found that the cheese showed a superior quality during 30 days of storage then the quality deteriorated as the acidity continued to increase, however, was still acceptable after 90 days of storage.

4.2.3.2 Effect of coagulant type on sensory characteristics of white cheese

Result in Table 4-19 presented the effect of coagulant type on sensory characteristics of white soft cheese.

The coagulant type did not significantly affect the colour of the cheese. However, cheese made with rennet scored higher (6.14) colour compared to cheese made using *Solanm dubium* extract as coagulant (6.05). The finding not similar to Prados *et al*. (2007) who reported that the colour of cheese made with a powdered vegetable coagulant from cardoon *Cynara carnculus* score higher compared to cheese with animal rennet.

The flavour of the cheese made with rennet was significantly (P< 0.001) better (5.99) when compared with that made with *Solanum* coagulant (5.32). Similar result was found by Nunez e*t 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

Table (4-19): Effect of coagulant type on sensory characteristics of white cheese

Sensory characteristic	Type of coagulants		S.E	L.S
	Rennet	*Solanum*		
Colour	6.14	6.05	0.07	NS
Flavour	5.95	5.32	0.08	***
Texture	6.52	5.69	0.08	***
Saltiness	5.28	5.01	0.07	*

S.E = Standard error

L.S = Level of significance

* = ($P<0.05$)

*** = ($P<0.001$)

NS = Not significant

flavour quality and intensity being highly significant (P< 0.001). however our result was in disagreement with Roseiro *et al.* (2003) who reported that plant proteases are consider too proteolytic, leading to bitter flavour. The coagulant type was significantly (P<0.001) affected the texture of the cheese. The cheese made with rennet scores higher texture (6.52) than cheese made with *Solanum dubium* extract. This result was not similar to Chen *et al*. (2003). Roseiro *et al*. (2003) reported that plant proteases leading to texture defect in cheese according to proteolytic activity.

The saltiness of the cheese were significantly (P< 0.05) affected by type of coagulant. The cheese made using *Solanum dubium* extract as coagulant had higher saltiness scores (5.28) than that made using rennet coagulant (5.01).

4.2.3.3 Effect of coagulant type on sensory characteristics of white cheese during storage

Table 4-20 shows the changes in sensory characteristics of the white cheese as affected by coagulant type during storage. As will be seen from the result there was no significant different in colour, flavour, texture, and saltiness of the cheese. However, a slightl improvement in colour scores with increasing storage period in both cheeses with rennet and *Solanum dubium* extracts. In cheese made with rennet the colour scores increase from 6.03 at day zero to 6.07 at day 90 while in cheese made using *Solanum* coagulant, the higher colour score 6.35 at day 30 and the lower (5.82) colour score at day zero. Similar result was found by Abdalla and Nuser (2009) who reported cheese colour did not significantly change during storage period. Also our result was not similar to Krishnaswamy *et al*. (1961) who reported that no significant differences in the organoleptic qualities of cheese with vegetable and

Table (4-20): Effect of coagulant type on sensory characteristics of white cheese during storage

Storage periods(days)	Colour		Flavour		Texture		Saltiness	
	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*	Rennet	*Solanum*
Day one	6.03	5.82	6.03	5.23	5.99	5.83	5.21	4.73
15	6.27	6.13	6.63	6.37	6.21	5.38	5.15	4.92
30	6.48	6.35	5.89	5.05	5.87	5.59	5.20	4.92
45	5.82	5.85	5.62	5.14	5.73	5.67	5.06	5.05
60	6.25	6.10	5.83	5.45	6.02	5.87	5.20	4.85
75	6.05	6.23	6.13	5.42	6.09	5.88	5.73	5.55
90	6.07	5.88	5.53	4.61	6.47	5.86	5.41	5.08
S.E	0.18		0.21		0.20		0.19	
L.S	NS		NS		NS		NS	

S.E = Standard error

L.S = Level of significance for interaction

NS = Not significant

animal rennet. The organoleptic qualities of cheese improved slightly during ripening.

The flavour scores decrease from 6.03 and 6.63 at day zero and 15 to 5.53 at day 90 in cheese made with rennet. The lowest flavour score 4.61 appears at day 90 in cheese made with *Solanum* coagulant. There was no bitter flavour or taste at the end of storage and the decrease in flavor 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 Nuser (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. Higher texture score 6.47 at day 90 in rennet cheese. While the lower texture (5.38) was in *Solanum* cheese at day 15. 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.

The saltiness scores increased with the progress of storage period in both cheese with rennet and *Solanum*. The saltiness scores 5.21, 5.15, 5.20, 5.06, 5.20, 5.73, and 5.41 at day zero, 15, 30, 45, 60, 75, and 90 in rennet cheese. However, in cheese made with *Solanum* coagulant scores 4.73, 4.92, 4.92, 5.05, 4.85, 5.55, and 5.08 at day zero, 15, 30, 45, 60, 75, and 90. Same result found by Abdalla and Nuser (2009) who stated that cheese saltiness improved throughout the storage period.

4.2.3.4 Effect of salt concentration on sensory characteristics of white cheese

Table 4-21 shows the main effect of salt level on the sensory characteristics of the white cheese.

The salt level did not significantly (P> 0.05) affect the colour of white soft cheese. However, the colour of cheese with 4% scores higher (6.13) than cheese with 8% (6.07). This result was disagreed with Hamid (1998) who reported that cheese colour was significantly (P< 0.05) affected by salt levels. Also our result was not similar to Hamid (2005) who reported cheese with 6% salt had higher colour scores than that with 4% salt.

The flavour and saltiness of the cheese with 8% salt were higher (5.81) and (5.69) in comparison with that with 4% salt. This might be attributed to the effect of high level of salt in the control of undesirable bacteria which cause flavour development (Kosikowski, 1977). Our finding was in agreement with Hamid (2005). Similar result was found by Khalid (1991) and Hamid (1998) who reported that level of salt significantly (p< 0.05) affected saltiness of white soft cheese.

The texture was significantly (p< 0.001) higher (6.25) in cheese with 4% salt compared to cheese with 8% salt. Similar result was found by Khalid (1991) who stated that cheese from 6% salt milk was of better quality than cheese from 8% salt milk. Our result was disagreeing with Hamid (2005) who reported that texture of cheese samples with 6% salt was better than those with 4% salt.

Table (4-21): Effect of salt concentration on sensory characteristic of white cheese

Sensory characteristic	Salt concentration		S.E	L.S
	4%	8%		
Colour	6.13	6.07	0.07	NS
Flavour	5.47	5.81	0.08	***
Texture	6.25	5.50	0.08	***
Saltiness	4.60	5.69	0.07	***

S.E = Standard error

L.S = Level of significance

*** = ($P<0.001$)

NS = Not significant

4.2.3.5 Effect of coagulant type, salt concentration and addition of calcium chloride on sensory characteristics of white cheese

As will be seen from the result in Table 4-22 the colour was not significantly affected by type of coagulant, salt concentration, and calcium chloride level. However, the flavour, texture, and saltinessof white soft cheese were significantly ($P<0.001$) affected by type of coagulant, salt concentration, and calcium chloride level.

The highest colour scores 6.32 in rennet cheese with 4% salt and zero % calcium chloride and the lowest colour scores 5.90 in *Solanum* cheese with 4% salt and 0.02% calcium chloride.

The flavour was better (6.14) in rennet cheese with 4% salt and 0.02% calcium chloride. Compared to 4.46 in cheese produced using *Solanum dubium* extract, with 4% and salt without calcium chloride (0%) added.

Higher texture scores were 6.81 in rennet cheese without addition of calcium chloride. However, the texture scores lower in rennet cheese with 8% salt and 0.02% calcium chloride. This result is attributed to the fact that cheese without addition of calcium chloride tended to retain more moisture. Similar result was obtained by Bille *et al* (2001) who reported that cheese was softer without added calcium chloride and preferred by sensory panelists.

The saltiness was highest (5.87) in *Solanum* cheese with 8% salt and 0.02% calcium chloride, while the lowest saltiness was 4.37 in *Solanum* cheese with 4% salt without addition of calcium chloride.

Table (4-22): Effect of coagulant type, salt concentration, and addition of $CaCl_2$ on sensory characteristics of white cheese

Sensory characteristic	Rennet				*Solanum*				S.E	L.S
	4%		8%		4%		8%			
	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%	Zero%	0.02%		
Colour	6.32	6.19	5.97	6.08	6.09	5.90	6.11	6.10	0.14	NS
Flavour	5.90	6.14	5.95	5.83	4.46	5.37	5.82	5.64	0.64	***
Texture	6.81	6.58	5.47	5.35	5.83	5.76	5.56	5.61	0.15	***
Saltiness	4.87	4.97	5.47	5.53	4.37	4.19	5.63	5.87	0.15	***

S.E = Standard error

L.S = Level of significance for interaction

*** = ($P<0.001$)

NS = Not significant

4.2.4 Chemical composition of whey

4.2.4.1 Effect of storage time on chemical composition of whey from white cheese

4.2.4.1.1 Titratable acidity

Storage period significantly ($p<0.001$) affected the titratable acidity of whey (Table 4-23). The titratable acidity gradually increased from 0.34% at day zero to 1.86% at day 45 then gradually decreases to 1.65% at day 90.

4.2.4.1.2 Total solids content

Storage period had no significant effect on total solids of whey (Table 4-23). Highest total solids were 13.17% at day 60 and the lowest was 12.40% at day zero. Our result was lower from those found by Hamid (2005) who reported average total solids content of whey to be 15.99% which could be explained by the loss of fat and salt from curd into the whey.

4.2.4.1.3 Fat content

Fat content of whey was not significantly affected by storage period (Table 4-23).The fat content was 0.33, 0.48, 0.54, 0.52, 0.47, 0.40, and 0.45% at day zero, 15, 30, 45, 60, 75, and 90, respectively.

4.2.4.1.5 Salt content

Salt content of whey was significantly ($P< 0.001$) affected by storage period (Table 4-23). As storage period progressed the salt content of whey increased**.** It increased from 4.45% at day zero to 6.67% at day 90.

4.2.4.1.6 Ash content

Storage period did not significantly ($P> 0.05$) affect the ash content of whey (Table 4-23).The ash content increased gradually from 4.52% at day zero to 5.75% at day 45 then decreased to 5.31, 5.21, and 5.26% at day 60, 75, and 90.

Table (4-23): Effect of storage time on chemical composition of whey from white cheese

Storage period(days)	Total solids %	Fat %	Acidity %	Protein %	Salt %	Ash %
Day one	12.40[a]	0.33[a]	0.34[a]	1.31[a]	4.45[a]	4.52[a]
15	13.01[bc]	0.48[bc]	1.49[b]	2.13[c]	6.12[b]	5.17[ab]
30	12.94[bc]	0.54[c]	1.68[c]	1.83[b]	6.24[b]	5.27[ab]
45	13.14[c]	0.52[bc]	1.86[d]	1.93[bc]	6.41[b]	5.75[b]
60	13.17[c]	0.47[bc]	1.78[cd]	1.97[bc]	6.43[b]	5.31[ab]
75	12.70[b]	0.40[ab]	1.77[c]	1.92[bc]	6.34[b]	5.21[ab]
90	12.99[bc]	0.45[bc]	1.65[b c]	1.93[bc]	6.67[b]	5.26[a b]
S.E	0.27	0.04	0.06	0.09	0.32	0.26
L.S	NS	NS	***	***	***	NS

S.E = Standard error

NS = Not significance

L.S = Level of significance

*** = ($P<0.001$)

Means values bearing different superscripts within the same columns are significantly ($p<0.05$) different

4.2.4.2 Effect of coagulant type on chemical composition of whey from white cheese

Result in Table 4-24 shows the effect of coagulant type on chemical composition of whey from white soft cheese.

The titratable acidity of whey was not significantly affected by coagulant type. Higher (1.55%) acidity was recorded in whey of cheese made with rennet coagulant.

Coagulant type did not significantly affect total solids content of whey. Higher total solids were 13.03% in whey from cheese made with *Solanum* coagulant. Similar result was obtained by Barbosa *et al*. (1981) and Nunez *et al*. (1991). This increase of total solids in whey from milk coagulant with vegetable coagulant, may be attributed to strong proteolytic activity of the coagulant leading to breakdown of the casein network resulted in a heavy total solids losses in whey.

Fat content was not significantly ($P > 0.05$) affected by coagulant type. However, slightly increased (0.47%) in fat content of whey from cheese made with rennet was observed. This finding disagreed with Nunez *et al*. (1991) and Puhan and Irvine (1973).

Protein content of whey was not significantly ($P > 0.05$) affected by type of coagulant. The protein content was 1.90% and 1.82% in whey from cheese made with rennet and *Solanum*, respectively. This result was not similar Vieira and Barbosa, (1972), Puhan and Irvine (1973) and Nunez *et al*., 1991 who reported that higher level of total N in whey from milk coagulated with vegetable rennet may be ascribed mainly to the storage proteolytic activity of the coagulant with the formation of soluble N which was released into whey.

The salt and ash content of whey was not significantly affected by type of coagulant.

Table (4-24): Effect of type of coagulants on chemical composition of whey from white cheese

Chemical composition %	Rennet	*Solanum*	S.E	L.S
Total solids	12.78	13.03	0.14	NS
Fat	0.47	0.44	0.02	NS
Acidity	1.55	1.47	0.03	NS
Protein	1.90	1.82	0.06	NS
Salt	6.01	6.18	0.17	NS
Ash	5.14	5.29	0.14	NS

S.E = Standard error

L.S = Level of significance

NS = Not significant

Chapter Five

Conclusions and Recommendations

5.1 Conclusions

From the results of this study the following conclusions can be drawn:

1. Completely dry *Solanum dubium* fruit, crushed using laboratory mortar, soacked for 24 hours in distilled water gave the highest milk-clotting enzymes activity.
2. Maximum milk-clotting enzymes activity was obtained from *Solanum dubium* fruit extracted with freeze-drying.
3. *Solanum dubium* fruit extract reached its maximum milk-clotting activity at 70°C and pH 10.
4. The loss in clotting activity of *Solanum dubium* fruit extract stored in a solid form was much less as compared to liquid form.
5. Sixty percent saturation by ammonium sulphate gave the higher milk clotting activity of *Solanum dubium* fruit extract.
6. The clotting activity of partially purified *Solanum dubium* fruit extract improved with increasing level of calcium chloride concentration to 0.6%.
7. Twenty five mililitres (25 ml: 50 litres milk) of a partially purified *Solanum dubium* fruit extract is recommended for cheese making.
8. Storage period has significantly affected weight loss and chemical composition of white cheese
9. Coagulant type has significantly ($P< 0.05$) affected the weight loss, total solids, fat, protein, soluble protein, and salt, tyrosine, and tryptophan contents of white cheese.

10. Salt concentration has significantly (P< 0.05) affected weight loss, titratable acidity, total solids, fat, protein, soluble protein, salt, ash, tyrosine and tryptophan levels of white cheese.
11. Total viable bacterial count, streptococci and lactobacilli count decreased during storage of white cheese.
12. Cheese made with partially purified *Solanum dubium* fruit extract has lower total viable bacterial, streptococci and lactobacilli count.
13. Cheeseswith 8% NaCl have lower total viable bacterial, streptococci and lactobacilli count than those with 4% NaCl.
14.

5.2 Recommendations

This study recommends:

1. The result therefore, would conclusively recommended the possibility of using partially purified *Solanum dubium* fruit coagulant as a cheap and rich souce of milk-clotting enzyme in cheese making as a rennet substitute.
2. Further purification and Characterization of *Solanum dubium* fruit enzyme extract.
3. Studying the activity and toxicity of *Solanum dubium* fruit extract from different States of Sudan.
4. Introduction of *Solanum dubium* as coagulant in cheese making.

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http://en-wikipedia.org/wiki/Rennet

Appendices

Appendix 1

Sensory evaluation sheet for White soft cheese

Name:……

Sample No.	Colour	Flavour	Texture	Saltiness	Remmarks
1					
2					
3					
4					
5					
6					
7					
8					

Colour		Flavour		Texture		Saltiness	
1. Acceptable	7	1. Extremely intese	9	1. Very soft	9	1. Over Salted	9
2. Slightly Acceptable	5	2. Intense	7	2. Soft	7	2. Salted	7
3. Moderately accep.	3	3. Moderately intense	5	3. Slightly soft	5	3. Moderately salted	5
4. Not acceptable	1	4. Slightly intense	3	4. Tough	3	4. Slightly salted	3
		5. Bland	1	5. Very tough	1	5. Poor	1

Appendix 2

Table (1): Effect of soaking time of crushed materials in distilled water in milk clotting activity

Soaking times	Activity U/ml
15 minutes	0.61^{ab}±0.06
24 hours	2.26^{e}±0.05
48 hours	1.84^{d}±0.04
72 hours	1.27^{c}±0.10
96 hours	0.65^{b}±0.03
120 hours	0.67^{b}± 0.02
144 hours	0.57^{a} ±0.03
168 hours	0.57^{a}±0.02
L.S	***

L.S= level of significance

Means in the column with different superscript are significantly ($P<0.05$) different.

***= ($P<0.001$)

Appendix 3

Table (2): Effect of milk temperature on coagulation time (seconds)

emperature ◦C	Coagulation time
30	51.28^{d}±0.08
40	44.05^{d}±0.09
50	39.37^{c}±0.79
60	28.01^{b}±0.62
70	26.46^{b}±0.11
80	10.00^{a}±0.00
L.S	***

L.S = level of significance

Means within column bearing the same letter are not significantly ($P>0.05$) different.

* = ($P<0.001$)

Appendix 4

Table (3): Effect of preheating of milk on coagulation time (seconds)

Milk temperature °C	Coagulation time (seconds)
35	44.64^{a}±0.23
40	54.35^{a}±0.02
50	59.88^{b}±0.06
60	63.29^{c}±0.05
70	76.34^{d}±0.12
80	107.53^{de}± 0.06
L.S	***

L.S = level of significance

Means within column bearing the same letter are not significantly ($p>0.05$) different.

* **= ($P<0.001$)

Appendix 5

Table (4): Effect of milk temperature on coagulation time (seconds).

Temperature °C	Coagulation time (seconds)
30	261.00^{f}±27.32
37	147.00^{e}±33.29
40	106.00^{d}±4.79
50	72.00^{c}±22.79
60	36.00^{b}±8.41
70	34.00^{b}±7.48
80	23.00^{ab}±5.01
90	15.00^{ab}± 3.54
100	11.00^{a}±2.88
L.S	***

L.S = level of significance

Means within column bearing the same letter are not significantly ($p>0.05$) different.

*** = ($P<0.001$)

Appendix 6

Table (5): Effect of *Solanum dubium* extract concentration on coagulation time

Volume of extract(ml)	Clotting time(min)
0.1	18.53^{h}±0.76
0.2	11.59^{g}±1.13
0.3	8.88^{f}±0.08
0.4	6.12^{e}±0.17
0.5	4.16^{d}±0.05
0.6	3.00^{c}±0.14
0.7	2.04^{b}±0.20
0.8	1.54^{ab}± 0.25
0.9	1.11^{a}± 0.10
1.0	1.05^{a}± 0.05
L.S	***

L.S = Level of significance

Means within column bearing the same letter are not significantly ($p>0.05$) different.

*** = ($P<0.001$)

Appendix 7

Table (6): Effect of incubation temperature on milk clotting activity of partially purified *Solanum dubium* extract

Temperature °C	Milk clotting activity (mg tyrosine/ml)
40	8.30^{b}±0.71
50	8.60^{b}±0.14
60	10.65^{c}±0.21
70	14.00^{e}±0.99
80	12.50^{d}±0.00
90	10.00^{c}±0.00
100	3.10^{a} ±0.28
L.S	*

L.S = Level of significance

Means within column bearing the same letter are not significantly (P>0.05) different.

* = (P <0.05)

Appendix 8

Table (7): Effect of pH on milk clotting activity of partially purified *Solanum dubium* extract.

pH	Milk clotting activity (mg tyrosine/ml)
4	0.10^{a}±0.00
5	0.57^{a}±0.21
6	1.57^{b}±0.06
7	2.13^{b}±0.25
8	3.03^{c}± 0.25
9	7.37^{de}±0.21
10	7.43^{e}±0.38
11	6.93^{de} ± 0.87
12	6.77^{d}±0.58
L.S	***

L.S = Level of significance

Means within column bearing the same letter are not significantly (P>0.05) different.

* = (P <0.001)

Appendix 9

Table (8): Effect of substrate quantity on coagulation time by *Solanum dubium* extract

Volume of milk/ml	Clotting time/minuets
50	28
100	33
150	50
200	54
250	65
300	70
400	95
500	133