Manufacture of White Cheese Using *Solanum dubium* with the addition of *Ziziphus spina-christi* and *Cinnamomum zeylanicum* Oils as Antimicrobial Agents

By

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الآية

قال تعالى:

﴿ فَأَعْرَضُوا فَأَرْسَلْنَا عَلَيْهِمْ سَيْلَ الْعَرِمِ وَبَدَّلْنَاهُم بِجَنَّتَيْهِمْ

جَنَّتَيْنِ ذَوَاتَى أُكُلٍ خَمْطٍ وَأَثْلٍ وَشَيْءٍ مِّن سِدْرٍ قَلِيلٍ ﴾

صدق الله العظيم

(سبأ: 16)

DEDICATION

To my lovely parents

To my dear friends

To my brother and sisters

To my colleagues

To all those whom I love

Acknowledgement

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

I wish to express my deepest and sincere gratitude to my supervisor Professor Mohamed Osman Mohamed Abdalla, Department of Dairy Production, Faculty of Animal Production for his guidance, useful advice and support, and wise guidance and continuous encouragement during the study period.

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Manufacture of White Cheese with *Solanum dubium* Using Ziziphus *spina-christi* and *Cinnamomum zeylanicum* Oils as Antimicrobial Agents

M. S.c. in Dairy Production and Technology

Sharifa Yousif Mohammed Yousif

Abstract: This study was carried out to evaluate the microbiological characteristics of white cheese manufactured with the addition of sidir and cinnamomum oils as antimicrobial agents. Cheese was manufactured from warmed (45^0C) raw cow milk to which starter culture (0.045 gm/L) and sidir and sinnamon oil extracts (0.3% and 0.5%) were added. *Solanum dubium* seeds were coarsely powdered using electric grinder, and 20 gm of the powder were soaked in 100 ml distilled water for 3 hr, filtered and 40 ml of the liquid were used for the coagulation of milk. Salt (6% w/w) was added to the whey used for cheese preservation. Cheese was preserved at 4°C for 21 days, and microbiological characteristics (total viable bacteria [TVB], coliform bacteria, *Staphylococcus aureus*, yeasts and moulds) were determined at 1, 7, 14 and 21-day intervals. The statistical analysis was carried out using Statistical Analysis Systems (SAS, ver. 9), and general linear model (GLM) procedure was used to determine the effect of type and concentration of oil and storage period on the microbiological characteristics of cheese. Mean separation was done by Duncan multiple range test ($P \leq 0.05$). Results showed that the type of oil significantly affected ($P<0.01$) TVB and coliform bacteria counts, with the count of TVB count being higher in the control sample (log 8.73 cfu/gm), while the highest count of coliform bacteria was in

cheese with sidir oil (log 7.50 cfu/gm). The concentration of oil significantly affected TVBC ($P<0.001$) and coliform bacteria counts ($P<0.05$), with the highest TVB count being in the control sample (log 8.73 dfu/gm), while the highest coliform bacteria count was in cheese with 0.5% sidir oil (log 7.56 cfu/gm). The storage period of the control sample significantly ($P<0.01$) affected all microorganisms under study except TVBC, while the storage of cheese made with sidir oil significantly ($P<001$) affected TVBC and *S. aureus* counts. The storage period of cheese made with cinnamomum oil significantly ($P<0.01$) affected all microorganisms except TVB count. It was concluded that the use of cinnamomum oil, at a concentration of 0.5% gave slightly better results in controlling the activity of microorganisms under study. The study recommended further studies on the use of higher concentrations of oil extract in addition to using oils extracted from natural plants.

صناعة الجبن الأبيض باستخدام نبات الجبين (*Solanum dubium*) ومستخلص زيوت السدر (*Ziziphus spina–christi*) والقرفة (*Cinnamomum zeylanicum*) كمضادات للميكروبات

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المستخلص: أجريت هذه الدراسة لتقييم الخصائص الميكروبيولوجية للجبن الأبيض المصنوع بإضافة زيت السدر(***Ziziphus spina–christi***) و زيت القرفة (***Cinnamomum zeylanicum***) كعوامل مضادة للميكروبات. تم تصنيع الجبن من لبن بقر خام دافئ (45°C) أضيف إليه بادئ (0.045 جم/لتر) ومستخلص زيوت السدر والقرفة (0.3٪ و 0.5٪). تم طحن بذور الجبين، وتم نقع 20 جم من المسحوق في 100 مل من الماء المقطر لمدة 3 ساعات، ثم تمت تصفيتها واستخدم 40 مل من السائل لتخثر اللبن. تمت إضافة الملح (6٪ وزن/وزن) إلى الشرش لحفظ الجبن. تم حفظ الجبن علي درجة حرارة 4 مئوية لمدة 21 يوما، وتم تحديد الخصائص الميكروبيولوجية (العد الكلي للبكتيريا الحية، بكتيريا القولون، المكورات العنقودية الذهبية، الخمائر والعفن) على فترات 1 و 7 و 14 و 21 يوما. تم إجراء التحليل الإحصائي باستخدام نظم التحليل الإحصائي (SAS, ver. 9)، وتم استخدام طريقة النموذج الخطي العام (GLM) لتحديد تأثير نوع وتركيز الزيت وفترة التخزين على الخصائص الميكروبية. تم استخدام اختبار دنكان للنطاقات المتعددة للفصل بين المتوسطات ($p\leq0.05$). أظهرت النتائج أن نوع الزيت اثر معنويا ($P<0.01$) على العد الكلي للبكتيريا الحية وبكتيريا القولون مع اعلي عدد للبكتيريا الحيةفي عينة الجبن الضابطة (لو 8.73 مستعمرة/جم)، في حين أن أعلى عدد لبكتيريا القولون كان في الجبن المصنع باضافة زيت السدر (لو 7.50 مستعمرة/جم). أثر تركيز الزيت معنويا علي العد الكلي للبكتريا ($P<0.001$) وبكتريا القولون ($P<0.05$)، حيث كان اعلى عدد للبكتيريا الحية في عينة الجبن الضابطة (لو 8.73 مستعمرة/جم)، بينما كان أعلى عدد لبكتريا القولون في الجبن المصنع باستخدام زيت السدر (0.5٪) (لو 7.56 مستعمرة/جم). أثرت فترة تخزين عينة الجبن الضابطة معنويا ($P<0.01$) على جميع الكائنات الحية الدقيقة قيد الدراسة باستثناء العد الكلي للبكتيريا الحية، في حين أن فترة تخزين الجبن المصنوع من زيت سيدر اثرت معنويا ($P<001$) علي العد الكلي للبكتيريا الحية والمكورات العنقودية. اثرت فترة تخزين الجبن المصنع باضافة زيت القرفة معنويا ($P<0.01$) على جميع الكائنات الحية الدقيقة باستثناء العد الكلي للبكتيريا الحية. خلصت الدراسة إلى أن استخدام زيت القرفة عند تركيز 0.5٪ أعطى نتائج أفضل قليلا في السيطرة على نشاط العد الكلي للبكتريا الحية قيد الدراسة. أوصت الدراسة باجراء مزيد من الدراسات حول استخدام تراكيز أعلى من مستخلص الزيوت بالإضافة إلى استخدام الزيوت المستخلصة من النباتات الطبيعية.

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CHAPTER ONE
INTRODUCTION

Cheese making is the means of preserving the most important constituents of milk in highly concentrated from is. It provides a palatable milk product of high value which can be kept fresh for a long time; Cheese is important source of milk protein. Calcium, and vitamins, its shelf life varies from few days to several years (Wastrel *et al*., 1999).

The majority of cheeses around the world are manufactured traditionally and in many cases still is manufactured using an enzymatic coagulant extracted from the abomasums of milk-fed calves.

This extract, known as calf rennet, consists of two photolytic enzymes: *chymosin* the major component (88-94% milk clotting activity,) and bovine pepsin. 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 Phe105-Met106 bond of the micelle-stabilizing protein k-casein, which is in many times more susceptible to chymosin than any other bond in milk proteins and leads to 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 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* had been tried for the extraction of milk-clotting enzymes (*Habbani*, E.S.

1992; Osman 2001). Their research showed positive results in using this extract for manufacture of Sudanese white cheese.

Solanum dubium (G*ubbain*) is a well known wild plant that grows widely in Sudan during the rainy season (Salih, 1979). Known additives for food as materials that do not use their own food or one of the food ingredients for food and not required to have nutritional value .added to food in order to prevent corruption or prolog the period save or improve some properties, and preservatives is one of important food additives which are added in order to prevent the growth and activity of microorganisms added *Cinnamomum verum* and *Zizyphus chirista-spina* oil concentration 0, 1, 2%. The genus *Zizyphus* is known for its medicinal properties as an antimicrobial, antioxidant, anti-inflammatory and as an immune system stimulant (Said *et al.,* 2006).

The essential oil cinnamon also has antimicrobial properties (Lopez, *et al.,* 2005), and it can aid in the preservation of certain food (George, 2007). Cinnamon has been reported to have remarkable pharmacological and traditionally been used to treat toothache and fight bad breath and its regular use is believed to stave off common cold aid digestion (Alice Hart, 2007).

The objectives:

1. To manufacture white cheese using Gubbain as coagulant.
2. To determine the effect of types and concentrates of oils (sidir and cinnamomum) on the microbiological characteristic of white cheese.
3. To determine the effect of storage period of the microbiological characteristic of white cheese using oils (sidir and cinnamomum).

CHAPTER TWO
LITERATURE REVIEW

2.1. History of cheese:

In ancient times, in Eastern Europe and western Asia, the practices 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 enzymes preparation from goat, lamb or even hare stomachs was mixed with sheep or goat's milk (cow's milk was not produce on a large scale before the thirteenth century). The curds separated from the whey were salted and stored for consumption later (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 cheese making 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 Diocletian 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. In1874, a Danish chemist Christen Hansen founded a laboratory in Copenhagen and started the first industrial production of calf rennet ax tract this was obtained from the stomachs of unwearied calves that are

slaughtered for veal production and not specifically to obtain the enzyme (Madden, 1995).

2.2 Sudanese white cheese:

Osman (1987) reported that (Gibna Bayda) is a traditionally fermented, pickled type of cheese (Gibna: cheese, Bayda: Arabic word for white). It is considered that the art was introduced to Sudan. It is made from raw or pasteurized whole milk, skim or reconstituted milk, depending on natural lactic acid bacteria and no starter is used (Khalid and El Owin1991).

In Sudan cheese processing is a major preservation method of surplus milk is available (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 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 reuniting (Osman1987).

Raheem (2006) reported that (Gibna Bayda) is white cheese made in Sudan, being similar to Domiati cheese of in Egypt, the starter is not used, and the Shelf life of the cheese may be more than one year. The procedure for making this Cheese includes heating of the fresh milk to35ºC 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 in the whey in tins or other suitable airtight containers and sealed.

2.3. History of cheese making:

Cheese making began about 8000 years ago and has spread throughout the world leading to remarkable diversity of cheese (I linger and Mounier, 2011).In Eastern Europe and Western Asia the practice of

carrying milk in bags made of animal's stomach probably resulted in the first cheese being made 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 lamb stomachs was mixed with sheep or goats.

2.4. Cheese manufacture:

The changes in cheese manufacturing protocols have resulted in the reduction of manufacturing time and the necessity for consistent and reliable starter activity (Johnson and Lucey, 2006). A required step in cheese manufacture is separating the milk into solid curds (casein and liquid whey) at the isoelectric point of casein (pH 6.4), and this is done by acidifying the milk and adding chymosin The acidification can be accomplished directly by the addition of acid such as vinegar, but usually starter bacteria are employed instead while converting milk sugar into lactic acid. The same bacteria and enzyme play a role in the eventual flavor of aged cheese. Most cheeses are made with starter bacteria from the genera Lactococccus, Lactobacillus or Streptococcus. Swiss starter cultures also include *Propionibacterum* shermanii which produces carbon dioxide during giving Swiss or Emmental holes (called "eyes") (Fox *et al.*, 2011).

2.5. Mechanism of milk coagulation:

The main purpose of coagulant in cheese making is the conversion of liquid milk to 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 non-enzymatic phase (Dalgleish, 1982; Payens, 1993).

The coagulation of milk is the result of two processes: the attack on the k-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 spilt bovine k-casein at the phe105-met106 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 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 micelles 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 helter) developed a theory that casein complex of milk owe its stability to the presence of a component that acts a stabilizer. Rennet action starts by degrading this component specifically and the modified complex flocculates in a secondary phase (Eck, 1987).

2.6. Plant proteases:

Plant proteases have been investigated as milk coagulants, but only a small number of aspartic pretenses from plant origin have been isolated and partially characterized (Tavaria *et al*., 1997; Sousa, 1998). A unique feature shared by most of these plant pretenses is an extra segment of about 100 amino acid residues which bears no sequence similarity with pretenses 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 rom ***Benincasa cerifera*** **(**Gupta and Eskin 1977), *Calotrpis procera* (Ibiama

and Griffiths, 1987; Mohamed and O'Connor ,1996), Dieffenbachia maculate (Padmanabhan *et al.*, 1993), fruit parts of *Solanumdubium (*Yousif *et al.*, 1996), *Centaurea calcitrapa* (Tavaria *et al.*, 1997) and flowers of cynara *cardunclus* (Barbosa 1983; Soua,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 give rise to low cheese yields. The difficulties experienced with these preparation result mainly from the unique composition of the plant extracts, which contain a complex cocktail of enzymes whose activity is difficult to control.

There were no substantial differences between the compositions of cheese made using any of the four coagulant blends. Cheese manufacture with coagulant blends containing proteinases exhibited higher levels of Ph 4.6-soluble nitrogen than cheese made using chymosin as coagulant. The extent of breakdown of ∞_{s1}-casein, as measured by urea-polyacrylamide gel electrophoresis (urea-PAGE), was greater 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 fraction were obtained for cheeses made using either proteinases or chymosin a coagulant principal component analysis and hierarchical cluster analysis of Rp -HPLC data confirmed that the inclusion of even small proportions (25%) of proteinases with chymosin in the coagulant blend greatly altered the pattern and extent of proteolysis in miniature cheddar-type cheese (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 plant 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 flavors in the ripened cheese.

2.7. *Solanum dubium* plant in Sudan:

Solanum dubium Freshen is an indigenous plant in northern and central Sudan. It is a woody herb; stem is solid erect, green in color and about 30 cm in height. The stem and its branches bear numerous sharp spines, white in color about 1-3mm in length and about 1mm in thickness near the base. The leaves are alternate, long petiole, simple, ovate, acuminate or obtuse at the apex, pale green in color 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 color. The inflorescence is composed of 2-8 pedicellate flowers arranged in the flower is a hermaphrodite, 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 color, rotate, of five petals united at the base with distance of 4mm 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 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 color. The taste is minutely pitted (Andrews, 1956; Salih, 1979).

According to (Yousif *et al.*, 1996), *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.

2.7.1.Characterization of Solanum dubium fruit extract:

2.7.1.1. Effect of incubation temperature on milk –clotting activity:

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

Habbani (1992) reported that the maximum activity of *Solanumdubium* extract was shown at 31ºC.Similar results were reported by Mohamed and Habbani (1996) who pointed that "*Gubbain*" extract activity was increased up to 38ºC and started to decline thereafter.

Vieira de Sa and *Barbosa* (1972) showed that cardo clotting enzyme is stable at high temperature showing an increasing clotting activity up to 70ºC, above these temperatures the activity falls with the activity completely disappears above 75ºC.

Bodansky(1924) studied milk-clotting enzyme of *Solanumelaeagnifolium*, and found that the enzyme has a higher optimum temperature (80-85ºC) resistiy 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 was obtained between 75-80ºC.Milk clotting enzyme extracted from *Kesinai* leaf exhibited maximum activity at 65ºC.

Results were also reported by Mullerand Nakai (2006) that the enzyme from Sodom apple (*Calotropisprocera)* leaves was more active at 65ºCThan 35ºC.

Pascaline and Daniel (2006) found that Mucor miehei and mucor pusillus proteases are much more stable at 53ºC for 100 minutes, while bovine pepsin and the *Endothia parasitica* proteases are rapidly inactivated at 53 in <100 minutes. Extracellular aspartate protease from *Rizopusoryzae* was purified 91 times with 26% recovery using ammonium sulphate fraction, ion-exchange and size-exclusion chromatographic techniques, which act optimally at 60ºC and was more stable in temperature range of 30-45ºC(*Kumer etel;2005*). Similar result were also reported by D' *Ambrosioet al.(2003)* who found that proteolysis and milk clotting activity in extract obtained from the crustaceans Munida has optimal temperature at 55-60ºC.

Raposo and *Dimingos* (2008) showed that, the optimum temperature for proteolytic activity of aspartic proteases from *Centaurea* calcitrapa plant cell suspensions was 52ºC.of the enzymes remained fully active when exposed for 6 hours at 4ºCand 25ºC.For all other temperature, after 1 hour of incubation activity decreased.

However, the purified enzyme from goat (*Caprahircus*) was stable up to 55ºCwith maximum activity at being 30ºC(Kumar *et al.*, 2006). (Campos *et al,. 1990)* pointed that the proteolytic activity of crude extract from wild thistle (*Cynara carunculou*) was found to be 37ºC.

2.7.1.2. **Effect of PH on activity of *Solanum dubium* extract:**

Osman (2001) reported that the 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 activity of *Solanum dubium* rennet decreased with increasing PH value and maximum activity was at pH 4.6 and 4.5

(Kumer *et al.*, 2005) showed that the purified enzyme from *Rizopusoryzae* is an acid protease with optimum pH of 5.5 and retained 96% of residual activity between pH 5.5 and 7.5.

2.7.1.3.Effect of calcium chloride concentration on*Solanum Dubium* extracts activity:

Calcium is an important factor in cheese making, it's and effect on the activity of *Solanum dubium* extract showed an increase with increasing concentration (Habbani, 1992; Mohamed and Habbani, 1996). (Chazarra*et al.*,2007) found that the rennet strength of artichoke (*Cynara scolymus*). Flowers extract increased with incresing concentration of calcium.Study of the 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 to 0.25%.

2.8. 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 cheese made with *solanum* 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 experimentals and control groups (Osman, 2001).

2.9. **Proximate composition of *ziziphus spina-christi* (botanic):**

The proximate composition of plant material consist of determining the major classes of chemical component, the proximate composition

provides good initial impression of relative nutritive value and utility of agricultural product and allows basis of composition between different species, plant parts and cultivation conditions (Abdel-Rahim,2004) and include:-

2.9.1. Oil content:

Fat (lipids) include free fatty acids, mono-glycerides, di-glycerides, tri-glycerides, phospholipids (Abdel- Rahim, 2004). Duke (1985) reported that the fat content of *Zizphus* spina-Christi fruits was 0.9%. Abdelmuti (2002) found that fruits 0.6% fat in pulp.

2.9.1.1. Crude fiber:

Nour *et al*. (1987) reported that the crude fiber content in *Ziziphus spina-christi* between 5.3 and 7.4 in the pulp. Abdelmuti (2002) and Anthony (2005) found that fruits contain 4.1% fiber in flesh pulp.

2.9.1.2. Mineral content:

Abdelmuti (2002) and Anthony (2005) found that the fruit contains 0.01%sodium, 1.91% potassium, 0.61% calcium, 0.12% magnesium, 20mg\100g iron and 13mg/100g Manganese on dry matter basis, and Abdelmuti (2002) found that the fruit contain 0.13% phosphorus on dry matter of basis.

2.9.1.3. Sugar content:

Nour *et al* (1987) and Abdelmuti (1991) reported that the flesh Zi-spina-christi fruits is rich in carbohydrates (80.6% in dry matter) notably starch (21.8%), sucrose (21.8), glucose (9.6%) and fructose (16%).

2.9.1.4. **Medicinal uses:**

Ziziphus species are used in folk medicine for the treatment of some diseases in the world (Abdel-zaher *et al.*, 2005).

The decoction of fresh fruit is used to prompt the healing of fresh wounds and also used as body wash while fruits are used for dysentery(Blatter,1978). The fruits are also used for bronchitis, coughs, and tuberculosis (Duke, 1985). Some of the fruits and fruit trees are also important sources of traditional medicine as low-income groups in rural arease frequently use them (Mander *et al*., 1996). Its bark is used to heal ulcer, wounds, scabies, throat problems and burning sensation of the body. Fruit is purified toenrich blood, treat bronchitis, fever, and enlargement of the liver. *Ziziphus spina-christi* fruits are eaten to treat diarrhea and malaria and as an antispasmodic. *Cinnamomum* spp. bark contains, 0.5-4% essential oil containing 80% cinnamaldehyde, up to 10% eugenol 5-10% transcinnamic acid 4-10% phenolic compound .

2.10. The uses of C. verum:

Cinnamon bark is widely used as a spice. It is principally employed in cooking as condiment and flavoring material (Encyclopedia, 2008).

Cinnamon has rationally been used to treat toothache and fight bad breath and its regular use is believed to stave off common cold and aid digestion (Alice Hart, 2007). Cinnamon is also used as an insect repellent (Beck, 2006).

2.11. Biological activities:

2.11.1. Antibacterial effect of cinnamon:

Mainly composed of the active ingredient cinnamaldehyde was tested on isolated strain of bacteria, including Gram positive Staphylococcus auras', Gram negative E.coli, Enterobacer aerogenes, Pseudomonas aeruginosa (Oo *et al*., 2006).

2.11.2. Antifungal:

Cinnamon oil has been reported as an antifungal agent. Experimentally, promising result on its antifungal effect was reported in two in vitro studies of cinnamon oil Cryptococcus neoformans and *Aspergillus niger* (Lee *et al*., 2005)

2.11.3. Anti-inflammatory:

Although cinnamon historically had not been to treat inflammatory disorders, its anti-inflammatory effect was demonstrated experimentally. Specifically, *Cinnamomum* cassia was used to investigate the anti-inflammatory effect on nitric oxide (Thomas and Kamwell, 2004)

CHAPTER THREE
MATERIALS AND METHODS

This experiment was carried out at the Department of Dairy Production, Faculty of Animal production, University of Khartoum during the period February to April, 2016.

3.1. Materials:

The starter culture, and salt were obtained from the local market, while the *solanum dubium* plants were collected from Shambat area, Khartoum North. Sid r and cinnamoum oil extract were obtained from the Khartoum north market Fresh cow's milk was obtained from the University of Khartoum dairy farm.

3.2. Preparation of *Solanum dubium* extract:

The whole seeds were coarsely powdered using electric grinder, and 20 gm of the powder were soaked in 100 ml distilled water for 3 hours, followed by filtering and 40 ml of the liquid were used for the coagulation of milk.

3.3. Cheese manufacture:

Fresh raw milk (25 L) heated to 62°C for 30min, and then cooled to 40°C., followed by addition of *Gubbain* enzyme extract (2 ml/L). The milk was stirred and left to develop a curd after 5minutes the cured was tested by knife for coagulation. The coagulated milk was cut for separation, and then the curd of each cheese was poured into a wooden mould lined with a clean cloth. After 12 hours was removed from the mould and cut into small cubes. The whey of each cheese was collected in a separate container; The curd was divided evenly into 5 batches:

1) Control, to which no oil is added[T1]
2) 0.3% (v/v) of *Ziziphusspina-christi* oil [T2]
3) 0.5% (v/v) of *Ziziphusspina-christi* oil [T3]

4)0.3% (v/v) of *Cinnamomumzeylanicom* oil [T4]

5)0.5% (v/v) of *Cinnamomum zeylanicom* oil [T5]

The five batches were put in the incubator for two hours, followed by elasticity test, and the curd was cooked at 70°C for 15 min. The curd was preserved in pasteurized (62°C/ 30 min)cooled and salted (1% w/w) water at ≤5°C.

3.4. Microbiological examination:

3.4.1. Preparation of media and glassware:

All media were obtained in dehydrated from and stored in hygroscobic 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 Ibs pressure (121ºC) for 15 minutes. Plastic containers were washed in running tap water rinsed with alcohol and then with distilled water. All glassware was sterilized in an oven at (160 °C) for one hour (Marshall, 1992).

3.4.1.1. Plate Count Agar medium:

The medium consisted of 5 gm casein peptone, 2.5 gm yeast extract, 1.0 gm dextrose and 15 gm agar. The medium was prepared by suspending 28 gm of the powder in one liter of distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121ºC for 15min.

3.4.1.2. MacConky agar medium:

The medium consisted of 17 gm pancreatic digest of gelatin, 3 gm peptones (meat and casein), 10 gm lactose monohydrate, 5 gm NaCl, 1.5 gm dehydrated bile, 1 gm crystal red, 30 gm neutral red and 13.5 gm agar. The medium was prepared by suspending 51.5 gm of the powder in one liter of distilled water, then boiled until dissolved completely and sterilized by autoclaving at 121ºC for 15 minutes.

3.4.1.3. Mannitol salt agar medium:

The medium consisted of 10 gm proteose peptone, 1 gm beef extract, 75 gm sodium chloride, 10 gm D- mannitol, 0.025 gm phenol red and 15 gm agar.

The medium was prepared by suspending 111 gm of the powder in one liter of distilled water, then boiled until dissolved completely and Sterilized by autoclaving at 121ºC for 15 minutes.

3.5. Preparation of serial dilutions:

Samples of different kinds of treatments of cheese (A, B, C, D, and E) were taken in sterile plastic containers. Eleven gm of cheese were dissolved in 99 milliliter of peptone water warmed at 45ºC in a clean sterile flask, and then shaken until a homogenous solution to make 10^{-1} dilutions. Then one ml from the above mentioned dilution was specially transferred to 9 milliliter sterile peptone 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} and 10^{-8}. From each dilution 1ml 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).

3.6. Examination of culture:

Growth on solid media was examined visually with naked eyes forcolonies appearance and change in media.

3.6.1. Total viable bacterial count:

Plate count agar was used for the enumeration of total bacterial count, 1 ml quantities of each sample decimal dilute 10^{6} was streaked in dried Plate with plate count agar and incubated at 32 ±1ºC for 48 ±3 h for enumeration of total plate count. They were counted by colony counter(Houghtby *et al.*, 1992).

3.6.2. *Staphylococcus aureus count*

Mannitol salt agar (micro master) was used for enumeration for coagulase positive staphylococci (Hougttby *etal*., 1992). Sample decimal dilutions (0.1 ml) were transferred into pre-solidified medium and spread plated using sterile glass rod. The plates were incubated in an inverted position at 37ºC for 48 hr. bright yellow colonies were recognized as*Staphylococcus aureus*. Colonies were counted with manual colony counter and regarded as colony forming unites per gram (cfu/gm).

3.6.3.Yeast and moulds count

Yeast extract agar was used for enumeration of yeast and moulds according to (Frank *et al.* 1992). Sample decimal dilutions (0.1 ml) were transferred into pre-solidified medium and spread plated using sterile glass rod. The plates were incubated at 25ºC for 5 days. Colonies of yeast and moulds were counted by colony counter and recorded as colony forming unites per gram samples (cfu/gm) (Harrigan and McCance, 1976).

3.6.4. Coliform count

MacConkey agar was used to determine the coliform count according to (christen *el al.* 1992). The plates were incubated at 37ºC for 48 hours. Typical colonies were counted (20-200 colonies in each dish).

3.7 Statistical analysis

The statistical analysis was carried out using Statistical Analysis Systems (SAS, ver.9). General linear model (GLM) was used to determine the effect of oil type, oil concentration and storage period on microbiological characteristics of cheese. Mean separation was done by Duncan multiple range tests ($p \leq 0$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Table 1. Microbiological characteristics of white cheese manufactured with sidir and cinnamon oils.

Table (1) presents the microbiological characteristic of cheese manufactured with the addition of sidir and cinnamon oils extract as antimicrobials in addition to the control. All microorganisms under study were significantly affected by oil extract except *S. aureus* and yeast and moulds. TVB and coliform bacteria counts were significantly ($P<0.01$) higher in the control sample (log 8.73 cfu/gm and log 3.04 cfu/gm, respectively), compared to other treatments, while *S. aureus* and yeast and moulds counts were higher in the control sample and low in cheese made with the addition of oils (log 7.25 cfu/gm, log 8.30 cfu/gm). Vural *et al.* (2010) reported that orga cheese (bread cheese) had a contamination rate (cfu/gm) of 1.6×10^8 cfu/gm, 7.8×10^4 cfu/gm, 1.4×10^3 cfu/gm, 2.6×10^4 cfu/gm and 3.7×10^5 cfu/gm of total mesophilic aerobic bacteria, counts coliform bacteria, *Staphylococcus* – micrococcus *spp*., aerobic mould and yeast counts, respectively. These results agree with Nkafamiy *et al.* (2013) who reported that *Zizphusspina-christi* extracts had inhibitory activity against various germination stages of *Staphylococcus aureus* and Korji (2012) who found that the bacteria are sensitive to *Zizphus* spina-christi oil extract. Goyal *et al.* (2012) and Najafi (2013) found *Zizphus* species with broad spectrum of antibacterial activity except yeast and moulds. Motamedi *et al.* (2014) showed a significant antibacterial activity against *S. aureus*. Abalaka *et al.* (2010) found that yeast and moulds were resistant. These results are disagreement with Omer (2012) who reported that the oil extracts are not effective enough to prevent the growth of bacteria. Nasir *et al.* (2015) found that *Cinnamonzelanicem* oil showed an antimicrobial affect against *Candidia*

albicans, *Aspegilusniger*, and streptococci. On the other hand, Motamedi *et al.* (2014) showed that *Zizphus* extracts had no activity against coliform bacteria.

Table 1. Microbiological characteristics (cfu/gm) of white cheese manufactured with sider and cinnamon oils

Microbiological characteristic	Type of oil			SE	SL	P
	Control	1	2			
TVBC	8.73^{a}	7.81^{b}	8.58^{a}	0.241	**	0.0158
Coliform	6.98^{b}	7.50^{a}	7.19^{ab}	0.196	**	0.0194
S. aureus	7.25^{a}	7.25^{a}	7.35^{a}	0.129	N.S	0.7622
Yeasts and moulds	8.30^{a}	8.47^{a}	8.29^{a}	0.127	N.S	0.2504

Means in each row bearing similar superscripts are not significantly different ($p>0.05$)
**=$P<0.01$
NS= Not significant
SL= Significance level
SE=Standard error of means

4.2. Effect of concentration of oil on the microbiological characteristics of white cheese

All microorganisms under study except *S. aureus* and yeast and moulds were significantly affected by the concentration of oil extract added (Table 2). TVBC was significantly ($P<0.001$) higher in all concentrations except in 0.3% which was the lowest (log 6.91 cfu/gm), while caliform bacteria count was significantly ($P<0.01$) lower (log 6.98 cfu/gm) in the control sample and higher (log 7.56 cfu/gm) in cheese manufactured with the addition of 0.5% of sidir oil, although *S.aureus* and yeasts moulds counts were not significantly affected by the concentration of oil, *S. aureus* the highest count in cheese manufactured with 0.5% cinnamon of oil (log 7.56 cfu/gm) and yeasts mould the highest count was highest in cheese manufactured with 0.5% cinnamon oil (log 8.64 cfu/gm). These results are not in line with Al-Samory (2007) who studied the effect of *Zizyphus* leaf extract on *Staphylococcusaureus* and found that 100 mg/mL and 250 mg/mL have no effect on the growth of *Staphylococcusaureus*, while higher concentration (500 mg/mL and 750 mg/mL) inhibited the growth. Omer (2012) reported that much lower concentrations of leaf extract (50 mg/mL and 100 mg/m) inhibited the growth of *Staphylococcusaureus*. Mohammed *et al.* (2012) found that the stem barks (aqueous) extract of sidir showed no inhibition of *C. albicans* at low dose but was susceptible above 600 mg/mL. These results are in accord with Nasir *et al.* (2015) who reported that all test strains showed sensitivity to the action of cinnamon oil, and concentration of 625.0 mg/mL was able to inhibit all microorganisms.

Table 2. Effect of concentration of oil on the microbiological Characteristics (log cfu/mg) of white cheese

Type of oil	Concentration (%)	Microbiological characteristics			
		TVBC	**Coliform**	***S. aureus***	**Yeast and moulds**
Control	0	8.73±0.93[a]	6.98±1.06[b]	7.25±0379[a]	8.31±0.68[a]
Sidir	0.3	6.91±4.07[a]	7.45±1.06[a]	7.32±0.79[a]	8.30±0.68[a]
	0.5	8.72±0.74[a]	7.56±0.44[a]	7.18±0.85[a]	8.64±0.54[a]
Cinnamon	0.3	8.61±1.04[a]	7.25±1.02[a]	7.15±0.75[a]	8.28±0.69[a]
	0.5	8.55±0.73[a]	7.13±1.22[a]	7.56±1.16[a]	8.30±0.74[a]
		<0.0001	0.0404	0.7256	0.9556
SL		***	*	N.S	N.S

Means in each row bearing similar superscripts are not significantly different (p>0.05)

NS = Not significant

SL= Significance level

**=P<0.01

***=P<0.001

4.3. Effect of the storage period on the microbiological characteristics of control cheese

Table 3 revealed that all microorganisms under study were significantly affected by the storage period of control cheese sample except TVBC. TVBC steadily decreased during the storage period from (log 8.89 cfu/gm at day 1 to log 7.95 cfu/gm at day 21), while coliform bacteria decreased from log 7.67 cfu/mg at day 1 to log 6.42±0.03 cfu/gm at day 14, then increased towards the end (log 7.20±1.49 cfu/gm). Before decreasing toward the end of the storage period, and *S. aureus* count steadily increased from(log 6.89 cfu/gm at the beginning to log 8.10 cfu/gm at the end of storage period. Yeast and moulds steadily decreased during the storage period from log 8.17 cfu/gm at days 1 to log 7.88 cfu/gm. The result of TVBC is in line with that of Cetinkaya and Soyutemiz (2006) who reported an increasing trend of T.V.B.C in Kaskar cheese during ripening. The decreasing pattern of yeast and mould content is in disagreement with Cetinkaya and Soyutemiz (2006) who formed increasing pattern of yeast and mould content during ripening.

Table 3.Effect of the storage period on the microbiological characteristics of control cheese

Microbiological characteristic	Storage period (days)				SL	P
	1	7	14	21		
TVBC	8.89±4.15[a]	8.84±0.50[a]	9.25±0.05[a]	7.95±1.50[a]	N.S	0.9396
Coliform	7.67±1.20[a]	6.64±0.21[b]	6.42±0.03[b]	7.20±1.49[a]	***	0.0005
S.aureus	6.89±0.10[c]	6.49±0.13[c]	7.54±0.04[b]	8.10±0.08[a]	**	0.0035
Yeast and moulds	8.17±0.45[b]	8.31±0.20[b]	8.89±0.05[a]	7.88±0.46	***	0.0006

Means in each row bearing similar superscripts are not significantly different ($p>0.05$)

NS = Not significant

SL= Significance level

**=$P<0.01$

***=$P<0.001$

Table 4. Microbiological characteristics of white cheese manufactured with the addition of sider oil as antimicrobial agent during the storage period

For cheese made with the addition of sidir oil extracts, TVBC count significantly decreased from log 8.83 cfu/gm at day 1 to log 8.56 cfu/gm at day 21.*Coliform* bacteria insignificantly decreased from log 7.80±cfu/gm at day1 to log 7.38± cfu/gm at day 21, while yeasts and moulds count insignificantly increased from log 8.55±cfu/gm at day 1 to log 8.79±cfu/gm at day 14, before decreasing to log 8.40±cfu/gm at the end and *S. aureus* count significantly ($P<0.001$) increased to a maximum of(log 7.99 cfu/gm at day 7, followed by a gradual decease to log 6.5/cfu/gm at day 14 then increased towards the end of storage period. These results agree with Nkafamiy *et al.* (2013) who found that *S. aureus* was sensitive to the sidir oil.

Gergeir (2009) found that the aqueous leaves extract of *Ziziphus spina –christi* showed low activity against yeasts. On the other hand, the results disagree with Coopoosamy *et al.* (2011) who reported that water extracts of leaves, roots and stem bark of *Ziziphus mucronata* showed no activity against all tested Gram negative and Gram positive bacteria. Al-Mutairi*et al*. (2016) reported that sider leaves extract had an inhibitory effect against all tested bacterial species. Alomari *et al.* (2016) reported that the ethanolic and butanol extract were bactericidal and bacteriostatic against *E. coli, S. aureus* and *C. albicans*. Bukar *et al*. (2015) found that the sidir oil had antibacterial activity against *Staphylococcus aureus* and *E. coli.*

Table 4. Microbiological characteristics (log cfu/gm) of white cheese manufactured with the addition of sidir oil as antimicrobial agent during the storage period

Microbiological characteristic	Storage period (days)				SL	P
	1	7	14	21		
TVBC	8.83 ± 0.60^{a}	9.24 ± 0.58^{a}	4.61 ± 4.93^{b}	8.56 ± 8.56^{a}	***	<0.0001
*Colif*orm	7.80 ± 0.91^{a}	7.39 ± 1.02^{a}	7.45 ± 0.43^{a}	7.38 ± 1.47^{a}	N.S	0.2438
S. aureus	6.73 ± 0.53^{b}	7.99 ± 1.36^{a}	6.80 ± 0.43^{b}	7.77 ± 0.34^{a}	***	<0.0001
Yeast and moulds	8.55 ± 8.55^{a}	8.16 ± 0.74^{a}	8.79 ± 0.22^{a}	8.40 ± 0.48^{a}	N.S	0.2469

Means in each row bearing similar superscripts are not significantly different ($p>0.05$)
NS = Not significant
SL=significance level
**=P<0.01
***=P<0.001

4.5. Table 5. Microbiological characteristic of white cheese manufacture with of addiction ofcinnamon oil during the storage period

Table 5 presents the microbiological characteristics of white cheese manufactured with the addition of cinnamon oil extract as antimicrobial. TVBC insignificantly decreased from log 8.70±/ at day 1 to log 8.80± cfu/mg at the end of storage period. Coliform bacteria count significantly (P<0.01),decreased from log 7.87± cfu/gm at day 1 to log 7.11± cfu/gm at day 21, while *S. aureus* count significantly (P<0.01) increased to log 8.12 cfu/gm at day 7, then decreased to log 6.90± cfu/gm at day 14 before increasing again to log 8.51± at day 21, and yeast and moulds count significantly (P<0.01) decreased from log 8.37 cfu/gm at the beginning of the storage period to log 7.72 cfu/gm at the end.

These results are not in line with those of Jana Teplá1 *et al.* (2016) who reported that cinnamon extend the shelf life up to 21 days, and in line with Thabet *et al*. (2014) who reported that coliform bacteria were not detected, while yeasts and moulds and *S.aureus* were detected at insignificant numbers in some treated labneh containing 0.3% cinnamon oil. Liu *et al*., (2017) found that cinnamon possessed significant antibacterial and antifungal activities against Staphylococcus *aureus* and harmful fungi. Urbaniak A. *et al*. (2014) reported that*Staphylococcus, Enterococcus, Enterobacter* genera were susceptible to essential oil obtained from *Cinnamomum zeylanicum*. Cui *et al*. (2016) reported that cinnamon oil exhibited satisfactory antimicrobial activity against coliform bacteria.

Table5. Microbiological characteristics (cfu/gm) of white cheese manufacture with of addition of cinnamon oil during the storage period

Microbiological characteristic	Storage period (days)				SL	P
	1	7	14	21		
TVBC	8.69±0.86^{a}	8.82±0.92^{a}	8.81±0.45^{a}	8.0±1.32^{a}	N.S	0.8289
Coliform	7.78±1.40^{a}	6.92±0.54^{b}	6.88±1.04^{b}	7.11±1.24^{b}	**	0.0077
S.aureus	6.24±0.43^{c}	8.12±0.67^{a}	6.90±0.40^{b}	8.15±0.60^{a}	**	0.0025
Yeast and moulds	8.37±0.73^{b}	8.11±0.55^{b}	8.97±1.07^{a}	7.72±0.60^{c}	**	0.0041

Means in each row bearing similar superscripts are not significantly different ($p>0.05$)

NS = Not significant

SL=significance level

**=$P<0.01$

***=$P<0.001$

CHAPTER FIVE
CONCUSION AND RECOMMENDATION

5.1 Conclusion

1. The *Solanum dubium* coat extract is shown too suitable the preparation white cheese, and resembles *chymosin* fairly.
2. The significant variation in the *Cinnamomumverum* more than *Zizyphus chirista-spina* oil, the better concentration in *Cinnamomum* oil (0.5).
3. The storage period significantly affected all microbiological characteristic under study for cheese made by *solanum dubium* extract and added to type of oil (sidir and cinnamoum).

5.2 Recommendation:

According to the data obtained following recommendation are suggested:

1. Encouragement of use of *Solanum dubium coat* extract for other cheese this Economic value of using Gubbain to target business enterprises.
2. The study of the effect of coagulants on the microbiological characteristic of cheese during storage period.
3. Recommended that many studies of different proportion of oils and their effect on white cheese.
4. Further studies should be done for making white cheese by adding oils (sidir and cinnamomum) to enrich nutritional and health benefits and the extending The shelf life.

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