Sudan University of Science and Technology

College of Graduate Studies

Effect of Using Rennin and Plant Enzymes on Time of Milk Coagulation, Yield, Chemical Composition and Sensory Characteristics of White Soft Cheese

اثر استخدام إنزيم الرنين والإنزيمات النباتية علي زمن تجبن اللبن، والإنتاج، والتركيب الكيمائي والصفات الحسية للجبن الأبيض الطري

By:

Ahmed Abdelrahman Mohamed Ahmed

Supervised by:

Dr. Altayeb Ibrahim Ali

Department of Animal Production,

College of Agricultural Studies,

Sudan University of Science and Technology

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الاستهلال

قَالَ تَعَالَىٰ:

﴿ وَإِنَّ لَكُمْ فِي ٱلْأَنْعَٰمِ لَعِبْرَةً ۖ نُّسْقِيكُم مِّمَّا فِي بُطُونِهِۦ مِنۢ بَيْنِ فَرْثٍ وَدَمٍ لَّبَنًا خَالِصًا سَآئِغًا لِّلشَّٰرِبِينَ ﴿٦٦﴾ ﴾

صدق الله العظيم

سورة النحل (66)

Dedication

This work is dedicated

To my father, Mother, Brother, sisters and to my friend

With love and respects

Acknowledgments

Firstly and lastly thanks to ALLAH who gave me persistence, and patience to complete this work. No words can adequately express my deep gratitude to my supervisor **Dr. Altayeb Ibrahim Ali** for generously providing and for patience, constant support, advices and insight was invaluable to me. He is always available not only for consultation but also to solve any difficulties. Then I wish to express grateful thanks to administration of Sudan University of Science and Technology, College of Agricultural Studies for allowing me to conduct my research and providing any assistance requested.

Gratitude *To* my father, Mother, Brother, sisters and also extended to all people and friends that assisted me in this research.

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Abstract

This research was conducted to study the effect of using rennin enzyme and plant enzymes on the coagulation time of milk, yield, composition and sensory characteristics of the production cheese. The rennin enzyme was used as a control, the plant enzymes were extracted from Solanum dubium (Jibeen) and Calotropis procera (Osher) plant. Plant enzymes were added to the milk in two concentrations (30%, 60%) coagulation time of milk, yield, composition and sensory characteristics. The total number of cheese sample used were five for the different treatment. Average coagulation time when used the rennin, Osher and Jibeen (30%, 60%) enzymes recorded, was120, 123.33±15.27, 130±14.14 minutes respectively. A significance different ($P<0.05$) were detected between average coagulation times. Coagulation time of milk by rennin enzymes required less time, while more time was required by the Osher enzymes compared to Jibeen enzymes. Average total yield of soft cheese obtained from 2 kg cow milk when using rennin, Osher and Jibeen (30%,60%)enzymes were250, 200±10, 252.5±3.53 g respectively. a significant different ($P<0.05$) was detected between average total cheese yield .Jibeen coagulation resulted in good cheese yield, followed by the rennin and Osher. The average moisture continent was found as 61.99±0.52, 62.62±1.49, 61.17±0.64, 45.25±0.09, 64.38±0.01when using Osher, Jibeen (30%60%) and rennin enzymes respectively. The average protein% was found as 15.2±0.17, 11.34±0.05, 20.04±0.12, 20.47±0.52, 19.43±0.49when using Osher, Jibeen (30%60%) and rennin enzymes respectively. Average fat % was found as 13.77±0.09, 9.67±0.23, 11.99±0.04, <11.74±0.26, 16.56±0.23, when using Osher, Jibeen (30%60%) and rennin enzymes respectively. Average ash % was found as 3.69±0.20, 4.46±0.36, 3.88±0.07, 4.15±0.04, 3.05±0.16, when using Osher, Jibeen (30%60%) and rennin enzymes respectively. also a significant variation($P<0.05$) was detected between the means of milk composition (moisture, protein, fat,ash%). The milk composition showed low percentages, when plant enzymes were used compared to rennin enzymes, Jibeen enzymes gave higher average % rather than Osher enzymes. In general, milk coagulation using plant enzymes resulted in a decrease in the total solids of the soft cheese in association with the type of coagulant used. According to

panelists the points scored for flavor were 4.50±2.22, 5.30±2.07, 5.20±1.25, 5.00±1.69, 6.80±2.82 using Osher, Jibeen (30%60%) and rennin enzymes respectively.Concerning the taste the point using Osher, Jibeen (30%60%) and rennin enzymes respectively 5.80±2.05, 5.00 ±1.87, 6.20±1.39, 5.80±1.76, 7.30±2.00. For texture given when using Osher, Jibeen (30%60%) and rennin enzymes respectively 5.80±2.11, 6.20 ±2.82, 5.50±1.71, 6.40±1.42, 7.70±2.16. Also a significant different ($P < 0.05$) was detected between the average point given flavor, taste and texture for all treatments. The Rennin cheese scored the highest points for the sensory characteristics followed by Jibeen cheese. Oshes cheese scored the lowest points.

المستخلص

أجريت هذه الدراسة لمعرفة اثر استخدام إنزيم الرنين والإنزيمات النباتية (العشر الجبين) علي زمن التجبن، الإنتاج، التركيب الكيميائي، الصفات الحسيه للجبنه البيضاء واستخدم إنزيم الرنين للمقارنة واستخدمت الإنزيمات النباتية بتركيزيّن (30،60)% أظهرت الدراسة أن زمن التجبن الكلي لاستخدام إنزيم العشر، الجبين (30،60) % وإنزيم الرنين كانت النتائج 120،15.27 ± 123.33، 130±14.14 علي التوالي. أظهرت الدراسة وجود فروقات معنوية لزمن التجبن للتراكيز المستخدمة حيث تتطلب إنزيم الرنين زمنا اقل مقارنة بالإنزيمات النباتية كما تتطلب العشر زمنا اقل مقارنة مع الجبين.

إنتاج الجبنه الكلي (جرام لكل 2 كجم لبن) عند استخدام الرنين والعشر والجبين بالتراكيز (30،60)% 250،10±200، 252.5±3.53 g علي التوالي. اظهر الإنتاج الكلي للجبنه فرقا معنويا لجميع المعاملات .اظهر تجبن إنزيم الجبين إنتاجا جيدا للجبنه وتلاه إنزيم الرنين.

كانت نسبة الرطوبة عند استخدام إنزيم الرنين،العشر،الجبين بالتراكيز(30،60)% 61.99±0.52، 62.62±1.49،61.17±0.64 ،45.25±0.09 ،64.38±0.01 على التوالي.

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أظهرت الدراسة أن هنالك فروقات معنوية في متوسط نسبة الرطوبة،البروتين الدهن، الرماد لجميع المعاملات، أعطت الإنزيمات النباتية نسب اقل لمكونات اللبن بينما ارتفعت نسبة هذه المكونات باستخدام إنزيم الرنين.

عند إجراء اختبار التذوق أعطت النكهة 4.50±2.22،5.30±2.07 ،5.20±1.25، 5.00±1.69 ، 6.80±2.82 نقطه عند استخدام انزيم العشر، الجبين،الرنين علي التوالي.

بالنسبة الطعم أعطى 5.80±2.05، 5.00 ±1.87، 6.20±1.39، 5.80±1.76 ، 7.30±2.00.
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نقطه عند استخدام إنزيم العشر، الجبين،الرنين علي التوالي.

أظهرت الدراسة أن هنالك فروق معنوية لمتوسطات النقاط التي أعطيت للنكهة، الطعم، القوام لجميع المعاملات. أعطت العينات التي تم تجبينها بواسطة إنزيم الرنين نقاط أعلى عند استخدام الإنزيم المستخلص من العشر والجبين.

CHAPTER ONE

INTRODUCTION

Milk is a global drink that is apolyphasic emulsion having physical ,chemical and biological properties and can be fermented into a wide range of different products flavors, consistencies and structure (Huria,2003).Also milk contains compounds that are essential to human ,such as proteins ,fats carbohydrates ,vitamins ,calcium ,phosphorus and other minerals and it also provides energy(Pauline and Karin,2006)

One of the major milk products known is the cheese Fox, *et.al* (2000) stated that cheese is the generic name for a group of fermented milk based food produced throughout the world in a great diversity of flavors, texture and forms.

The making of cheese as a means of preserving that most important constituent of milk in a highly concentrated form is in vogue all over the world. It provides as a palatable milk products of high food value, which can be kept fresh for long time, (Niir, 2012)

The trend nowadays is produce different new varieties and types of the so-called functional cheese dairy products explained, functional dairy products with a proven healthy benefits are based on milk that enriched with functional component ,or the products are based on ingredients originating from milk and the most common functional dairy products are those with probiotic bacteria, quite frequently enriched with prebiotic carbohydrates .the connection between functional food and cheese is a straight forward one, since cheese is generally a fermented product and potentially an appropriate vehicle for probiotic bacteria, (Donelly,2003)

The conversion of milk from fluid to gel (coagulation) is a basic step common to all types of cheese. The coagulation of milk is a consequence of protein destabilization, which is brought by acid proteinases chymosin, the active component of rennet. (Varnahm and Sutherland, 1994).

According to O،Conner (1993), rennet is a general term that describes a variety of enzymes of animal (especially calves), plant or microbial origin used to coagulate milk during cheese milking. For coagulation of milk in the manufacture of cheese, calf rennet is most wide –spread and desirable and has been dominant in the industry of cheese processing for long time .Gouda (1990) mentioned, the calf rennet is used in cheese making and is important in the formation of casein nerk during coagulation and known to contribute to proteolysis in pickled cheese. Since the past century a shortage in the calf rennet has been noticed due to the decrease in the availability of sucking calve, as they are mainly reared for milk or beef production rather than other purposes. So it cover the supply of the cheese industry with calf rennet, it was to a remarkable extent restricted .Craw (1993) indicated the limited supply of rennet and its resulting high price have necessitated research ,for many decades ,to come up with an alternative milk coagulant. The trend now in order to cover such a problem, plant enzymes are used in some parts of the world in cheese making. Ibiama and Griffiths (1987) and yousif *et.al* (1996) reported, the utilization of milk coagulation enzymes extracted from *calotropis procera* (Sodom apple) and *solanum dubium* (Jibeen) plant are used in traditional cheese production as substitute of calf rennet.

Objectives:

The main objectives of this research are:

1- To study the effects of using rennin and plants coagulants on the coagulation time during white soft cheese manufacturing.

2- To study and assess the effects of using rennin and plants coagulants of yield, composition, and sensory evaluation of produced white soft cheese.

3- To study if the produced cheeses satisfy the international standards and specifications issued for white soft cheese.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of cheese

Cheese has been defined as a product made from milk by coagulation the casein with the help of rennet or similar enzymes in the presence of lactic acid produced by added microorganisms, from which part of moisture has been removed by cutting, cocking and pressing, which has been shaped in mould and then ripened by holding it for same time at suitable temperature and humidity (Kutty and Sheeba 2014)

James (2013) described cheese as a fresh product obtained after coagulation and whey separation of milk, cream or partially skimmed butter milk or a mixture of these products.

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

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

2.2 History of cheese:

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

There is no conclusive evidence indicating where cheese making originated from in Europe , Central Asia or Middle East, but the practice has spread within Europe prior to Roman time, and it had become a sophisticated enterprise by the time the Roman Empire came into being (Arvind, 2010).

According to Simpson (1979), the origin of the word cheese appears to be the Latin ''caseus'', from which the modern word casein is closely derived out the earliest source, is probably from the Porto-Indo –European root, Kwat, which means to ferment, become sour.

2.3 Classification of cheese

The most common classification of cheese is according to the moisture content of the cheese. The FAO/WHO classified the type of cheese according to moisture content as follows:

Table (1) Classification of cheese

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

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

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

2.4 Chemical composition cheese

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

Rennin in cheese compose in average 90 %of the milk fat, 75% of the milk protein, 30% - 40% of the milk salts and 5% of the milk lactose, (Osman, 2007).

Chemical composition of Sudanese white soft cheese (Gibna Beida):

The type of cheese consumed widely by the different socio-economic of Sudanese families is the white soft cheese called Gibna Beida It is not known exactly when Gibna Beida was first introduced in to Sudan, but it is most likely that the Sudan has known this cheese for nearly a century (Dirar 1993).

The chemical composition of Sudanese white soft as given by the Sudanese Standars and metrology organization (SSMO) (2002) according to dry matter weight and lowest limit as follows:

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

2.5 Milk coagulation

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

According to Blume (2013), the type of coagulation used depends on the type of cheese desired. The conversion of milk from a fluid to a gel (coagulation) is a basic step common to all types of cheese (Varnam and Sutherland, 1994). Traditional cheese technology requires that the protein, especially the casein most be separated from milk by coagulation., Spreer (1998) explained the colloidal casein particles with a stable and even distribution must be coagulated, which means that the protein is converted from suspended state in to a gel state (coagulate gallert),especially into alyogel. The coagulation of milk influenced mainly by the type and concentration of coagulation enzyme, coagulation temperature, properties and concentration of proteins and the PH value ,(Story and Ford 1982).

2-5-1 Rennin Coagulation

2-5-1-1 Mechanism of Coagulation

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

The casein precipitation takes place in two stages:

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

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

2.5.2 Rennin coagulation

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

2.5.3 Types and production of rennet

2.5.3.1 Production of natural Calf-rennet

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

2.5.3.2 Vegetable rennet

Many plants have coagulation properties Greeks used extract of Fig juice for coagulation, also dried caper levels, nettles, mallow and ground ivy (creeping

chartie) were used also. Enzymes from Cynara or thistile were used in some traditional cheese production in the Mediterranen. Phytic acid derived from un fermented soybeans or fermentation produced chymosin (FPC) may also be used (Farkye, 2004).

Vegetable rennet is also suitable for vegetarians. Vegetable rennet might be used also in production of Kosher and Halal cheese , but nearly all Kosher cheese are produced with either microbial rennet oe vegetable rennet ,usually contain mod (Lee,et.al.1990).

2-5-3-3 Microbial Rennet

Some molds such as Rhizo mucor miehe are able to produce proteolytic enzymes. These molds are produced in afermenter and then specially concentrated and purified to avoid contamination with unpleasant by products of the mold growth (Farkye *et.al*.1990).

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

2.5.3.5 Traditional Method of Rennet production

Dried and cleaned stomachs of young calves are sliced into small pieces and then put in salted water or whey together with vinegar to lower the PH of the solution after some time (over night or several days). The solution filtered. The cured rennet that remains in the filtered solution can then be used to coagulate milk. About 1g,of this solution can normally coagulate 2.4 liter of milk (Cremer1985).

2.5.3.6 Modern Method of Rennet Production

Deep frozen stomachs are milled and put into an enzyme extracting solution. The cured rennet extract is then activated by adding acid; the enzymes in the

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

2.5.3.7 Alternative Sources of Rennet

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

2.5.4 Plant coagulation

Plant coagulation was not registered till as commercial methods of coagulation due to a lot of hazard of using these plants as coagulants for cheese processing. A lot of hazards faced the usage of plant enzymes for coagulation due to the low yield, long time of coagulation and finally the toxicity of these plants (Walstra, *et.al.* 2005).

Plant enzymes are still used in some parts of the world for cheese making as noticed by Shaw (1986).

Example of parts used of milk coagulation

2.5.4.1 *Solanum dubium* (Jobeen):

It is wild plant considered as weed found in most areas in Sudan. It has not an economic important. Locally names Jobeen, and belongs to family Solanaceae.

Talib *et.al.* (2007) studied the coagulation properties of Solanum dubium (Jobeen) used extracts. The jobeen seed were extracted with both water and citrate phosphate buffer. Effect of enzymes concentration, milk PH, milk temperature and heat inactivation of cured enzymes on clotting activity were measured. Results obtained showed that clotting time decreased by increasing concentration of the plant seed extract, the clotting activity of the enzymes was decreased at PH of the milk over 6.2 increasing of the milk temperature above 40C, decreased the clotting time and the activity of the enzymes was lost on PH 4.6 and 6.6 temperature 60C at PH 3.6 for 10 minutes, but at PH 4.6, 5.6 and temperature 70C, the enzymes activity was not affected, but it lost its activity at 80C/10 minutes (Shaw,1980) also noticed the plant enzymes are too proteolytic for cheese making. If proteolytic activity is excessive, cheese yield and retention fat in curd may be diminished and it has undesirable effects on the body and texture of finished cheese (Yousif *et.al.*1996).

2.5.4.2 *Calotropis procera* (osher):

It is small shrub found in fertile and light soils. It has no economic important. It belongs to family Asclepiadoidecae.

It was known that early osher plant specially the milk juice found in it is fruit and levels had the power to coagulate milk, but it was tested for coagulation recently by Dalgleish (1985) who explained the porentiality of the plant coagulation followed by decrease of the percentage of all cheese components, and it is total solids. The same result was obtained by Bines *et.al.* (1989) who demonstrated that coagulation by enzyme extracted from this plant lead to low quality cheeses with low percentage of protein and fat in addition to the investigation of the toxic material during the analysis of chemical composition of the plant.

2.6 Time of coagulation

Time of coagulation is affected by many factors. The most important factor is the chemical composition of milk particularly the content of the Ca^{++} in milk. So, any factor that affects the content of the Ca^{++} affect the time of coagulation. Heat treatment are one of the main arguments that changed the level of Ca^{++} in milk and this was clear when milk is sterilized or dried, in these cases a source of Ca^{++} must be added to milk prepared for cheese processing e.g. ca cl_2 which is added at certain level (0.02%and 0.03%) for sterilized and dried milk respectively. for this reason when coagulation time was compared to different treatments, milk should be identic Lee *et.al.* (2003).for each treatment avoiding all factors that affect the levels of the Ca^{++} in milk.

Another factors which affects time of coagulation is the stage of lactation where the chemical composition of milk greatly changed and this is clear during the first few days after calving and during the last months before calving, during these periods the chemical composition is completely different compared to the normal days of lactation (Cutren–Vapur *et.al.*, 2012).

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

coagulation compared to terristeris enzymes and thus followed by weak milk curd and low percentage of cheese yield.

Another results were obtained by the (Van Hooydonk *et.al.* (1987) who compared the time of coagulation and cheese yield for the different types of milk produced from cow milk during the normal stage of lactation, but at different time. Milk was coagulated by rennin enzymes and two different plant enzymes (Solanum and Terristris) using different concentration from the enzymes. Result obtained showed that milk taken at different time during the normal lactation revealed no significance difference ($p > 0.05$) and explained that types of enzymes influences the time of coagulation, which were high, optimum and lower for solanum, terristris and rennet coagulation respectively; no significant different ($p > 0.05$) between rennet coagulation and terristris coagulation was reported. However, there was clear difference between rennin enzyme and the terristris enzymes. This urged some investigators to do more researches on plant enzymes used for coagulation. These results were similar to the some finding of Lee *et.al.*(2003), who also reported no significant difference ($p > 0.05$) between some species. Of terristris and Rennin enzymes, but he found significant differentce among some plant enzymes and rennin enzyme.

2.7 Cheese Yield:

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

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

- Milk composition
- Genetic variation
- Physiological factors
- Processing condition
- Lactation stage
- Seasonal variation
- Type of milk
- Starter culture used
- Standardization of milk
- Heat treatment of milk
- Type of coagulant of milk
- Type of coagulant used
- Curd firmness
- Curd handling system
- Storage of milk

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2.7.1 Yield by Rennin and Plant Enzymes Coagulation:

Foltman (1987) explained, that cheese yield increased when the Calcium increased and he recommended addition of Ca cl_2 to milk exposed to ultra heating. Rennin coagulation gave good results for cheese yield when the $Cacl_2$ was added when dry milk or sterlized milk were used.

Plant enzymes generally resulted in low cheese yield. A significant difference ($p<0.05$) between rennin coagulation and plant enzyme was recorded, (Nour El Daim *et. al.* 2007).

Merin, (1989) plant coagulation resulted in weak milk curd and high losses of fat and protein with the whey drained; this resulted in low yield of cheese and mentioned significant difference ($p<0.05$) between different methods of

enzymes coagulation. Merin(1989) rennin enzyme gave the highest cheese yield, but no any significant difference among the plant coagulating enzyme was reported however, some of them gave high yield compared to other plant enzymes and usage of terristeris spp. enzyme which gave high yield compared to other plant enzyme, and thus it was recommended.

Cheese yield was affected by milk PH, temperature, enzyme concentration and type of enzyme. The decrease in PH increases rennin power of coagulation and then cheese texture and cheese yield were progressed, but increasing the temperature above 40°C affects the power of the enzyme and altered the coagulation process followed by decrease in cheese yield (Nijera 2003).

Another recent result by Ozcan, *et. al*. (2012) indicated the use of starter culture and its addition to milk prepared for cheese making increases cheese yield; this may due to the maximum rennin activity when the acidity of milk increases and also to the protease enzyme of plant origin used and their coagulation power. But all of them showed significant difference compared to rennin enzyme; also cheese yield coagulated by Terristeris spp. gave results near to the results of rennin enzymes were observed.

2.8 Effect of Method of Coagulation on Cheese Composition:

The chemical composition of cheese was greatly affected by the type of coagulation. Rennin is the most popular enzyme for cheese making; it gives good results since the main cheese components (protein, fat and ash) were higher than the other plant coagulants, (Green, *et. al*. 1987).

Also Rollema *et. al*. (1988) reported that the levels of protein and total solids of white cheese tend to increase when milk coagulated by rennin enzyme compared to different plant enzymes. Dalgleish *et. al*. (1989) gave that the ash content of cheese was created by using rennin enzyme when the fat content of

milk prepared for cheese making was increased. The fat (4.4) % of milk relatively increased, also the ash (4.8) % for white cheese compared to 2.2% of fat of milk where the ash of cheese was 3.6% In addition, the protein % followed the same trend since it was increased due to increase of fat % and rennin coagulation rather than acid coagulation and plant enzyme coagulation.

Pszezola (1989) reported, the type of enzyme used for coagulation and the levels of total solids in milk cheese processing affect most of the cheese components and the cheese yield since the levels of protein, fat and ash% tend to increase, when the total solids of milk used for cheese making were high. The variation was clear and was significantly affected according to the type enzyme used for coagulation and it was found to be higher (protein, fat and ash percentage) when milk was coagulated by rennin enzyme, compared to plant and microbial enzymes.

Reddly *et. al*. (1990) reported that the chemical composition of hard cheese differs greatly due to type of enzyme used for milk coagulation. The protein, fat, ash and total solids percentage of cow milk from the same stage of lactation (after months up to four months after calving) showed a significant ($p<0.05$) for protein and fat percentage and ash percentage was higher but no significant difference ($p<0.05$) for ash percentage was found when milk was coagulated by rennin enzyme compared to plant enzymes and microbial enzyme. Furthermore, some plant enzymes, e.g. (Terrestiris enzymes) tend to give result near to the rennin enzyme and also to significant difference between these enzymes and rennin enzyme was detected. The result obtained by Kumosinski, *et. al*. (1991) showed that most of cheese components and its total solids according to the type of enzyme used for coagulation. The percentage of protein and fat showed a significant difference between rennin enzyme and types of plant enzymes (different concentrations of solanum and terristris enzymes).

Terristris enzyme gave result near to that of the control enzyme, which was rennin enzyme. Horme, (1990) compared three methods of coagulation enzyme (rennin, fermentation produced chymosin (FPC) and plant enzyme), and found that the percentages of protein, fat and ash showed a significant difference ($p<0.05$) and they were highest for rennin coagulation and lower when milk was coagulated by plant enzyme specially the enzyme extracted from (Calotropis procera plant) due to the losses of most milk components with the drained whey according to weak formed when milk was coagulated by these enzymes.

Also a significant difference was detected between rennin and plant coagulation (enzymes extracted from terristeris ssp. And solanum ssp), but comparing the coagulation caused by rennin enzyme and terristris ssp enzyme no significant difference was recorded ($p<0.05$) despite the increase in yield of rennet cheese (Singh *et. al*. 1987).

2.9 Sensory Evaluation:

Humans have used their senses to evaluated food for several thousands of years and individuals can often tell by sight, smell, taste and to lesser extent touch, whether or not given food or beverage items are good or bad, e.g. save or toxic (Drake *et. al*. 2009).

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

Engels *et. al*. (2005) mentioned, the production of lactic acid by organisms used in fermented dairy products determines the flavor of the product, whereby, these microorganisms play a number of major beneficial roles in

food industry, since they transform organic matter in foods and thereby contribute not only to the preservation of food, but also to flavor and texture.

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

The Sudanese Standards and Metrology Organization, SSMO (2002) described the sensory evaluation of white soft cheese as follows:

- Color: normal if the cheese is white or white-yellowish .
- Taste: palatable if the cheese is free of bitter taste, rancidity and rotating.
- Smell: Normal if cheese shows no external or foreign odors.

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

Zidan (2004) explained, cheese should be normal in all its properties and is considered spoiled, abnormal change in color, advanced dryness or abnormal rotenning, blowing and abnormal holes are noticed.

Kumosinski *et. al.* (1991) reported, that taste, texture and flavor of cheese were affected by the method of coagulation and found significant difference for taste and texture ($p<0.05$) and a mild difference for flavor, but these differences were not significant.

According to Jackob *et. al.* (2011) no significant differences were found between sensory evaluations, when different types of plants, microbial or rennin enzymes were used. This was clear to taste and flavor, but some differences were found for the texture, even among the same ssp. Of plant enzyme, although these differences were not statistically significant.

Talib *et. al.* (2006) assessed the organoleptic characteristics of cheese made by using different concentration (5, 10, 15 and 20%) of enzyme extracted from *solnum dubium* (Jibeen seeds). The results obtained, showed that the cheese were scored high in color, texture and flavor, taste and appearance for the first three concentrations, while the cheese produce by the high concentration (20%) of Jibeen seeds was scored least in overall appearance by panelists, because of the bitter taste and nutty flavor associated with it.

The excessive proteolytic activity of plant enzyme during the ripening process of the cheese has noticed by Yousif *et. al.* (1996).

Cheese contained different amount of $NaCl_2$ except fresh cheese.

The salt affects flavor, consistency and durability of the cheese (Walstra *et. al.* 2005).

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

CHAPTER THREE

MATERIAL AND METHODS

Area and time study:

The experiment was conducted in department of animal production college of Agricultural Studies, Sudan University of Science and Technology, Shambat, during the period from 15/5/2017 to20/7/2017.

Three type of cheese using three different coagulants were manufactured. The three coagulants used were rennin enzyme and plant enzyme extracted from Osher (*Calotropis procera*) and jibeen (*Solamum dubium*).

The rennin enzyme and the produced cheese were used with different concentrations, (30%, 60%).

3.1 Method of Extraction of Plant Enzymes

3.1.1 Source of Plant Enzyme

The plant used for extraction were obtained from shambat area during summer to ensure good sun drying for the plant before the extraction of the enzyme.

3.1.2 Extraction of Osher Enzyme (*Calotropis procera*)

The fruits of the plant were dried by sun and air until a constant weight was reached. 50g of dry fruits were soaked 250 ml in distilled water for 24 hours. Then the solution was filtered by filter paper and kept in refrigerator at 7°C

The was using coagulant after as two days only. Two concentrations were used (30%, 60%)

3.1.3 Extraction of jibeen (*Solanum dubium*)

The life plant was used .It was crushed after drying to constant weight by sun. The plant was crushed then 50g from the plant was soaked in 250 ml distilled water for 24 hours and then filtered by filter paper and the solution was preserved in at 4-7°C for days and then used as a coagulant with two different concentrations 30%, 60% .

3.1.4 The rennin enzyme

The rennin enzyme was obtained from Veterinary pharmacy from shambat area -Khartoum North -Sudan. The enzyme was recently prepared only three months were passed from the date of production and it was stored at cool dry place before using for cheese making. The enzyme was a solid farm (tablets). Each tablet wished grams and it was sufficient for coagulating 100g of milk. Each tablet was dissolving the enzyme were added to each 2ml.from the solution after dissolving the enzymes were added to each 2kg of milk used as a sample for cheese making .

3.2 Source of Milk

Milk used for cheese making for the three treatments (control, Osher enzyme and Jibeen enzyme) was obtained from the Dairy herd farm of department of animal production of shambat.

3.4 Manufacturing procedures

3.4.1 Rennin Cheese

1. Milk was heated to 72°C, and then cooled to 42°C.

2. Starter culture was added (1%) and then temperature was adjusted to 42°C for 45 minutes.
3. Rennin enzyme was added, and then coagulation of milk was observed.
4. Time of coagulation was recorded from the addition of the rennin enzyme till the completed coagulation of milk occurred.
5. When coagulation occurred, the curd was put on wooden tray, lined with narrow orifices to ensure good draining of whey.
6. Cheese was salted by soaking it into salty solution; with 8% concentration of $NaCl_2$ was 8 % for 24 hours.
7. The weight of cheese was determined after 24 hours from the beginning of cheese salting.

3.5.2 Osher enzyme (*calotripis procera*) cheese

1. 2 kg of milk were heated to 72°C and then cooled to 42°C.
2. The extracted Osher enzyme was added at two different concentrations (30%, 60%) respectively
3. Time of coagulation was recorded from the addition of the enzyme till the completed coagulation of milk.
4. Cheese was weighted and its final weigh was determined after 24 hours from the beginning of cheese salting.

3.5.3 Jibeen Cheese *(Solanum dubium)*

1. 2 kg of milk were heated to 72°C then cooled to 42°C.
2. Jibeen enzyme was added with two different concentrations (30%, 60)
3. Time of coagulation was recorded from addition of the enzyme till the complete coagulation occurred.
4. When coagulation occurred the cheese was surrounded with clothes with narrow orifices and then put on wooden trails for complete draining whey.

5 .Cheese weight was then determined after 24 hours from the beginning of cheese salting.

3.5.4 Determination of time of coagulation:

This was determined by recording the time immediately after the addition of the different types of enzymes to the complete coagulation occurred which was determined by the change of the watery (liquid state) of milk to hard or semi-hard state due to formation of the milk curd.

3.5.5 Determination of cheese yield:

It was determined by weighing of cheese after soaking the cheese for 24hours in 8% $NaCl_2$ solution. Immediately after the period of soaking cheese weight was determined the weight to one decimal.

3.6 Laboratory analysis

3.6.1 Determination of Fat Content (A.O.A.C, 1990) soxliet Method

Analytical balance
Electrical drying oven
Soxlite extraction unit.
Desiccators .

Reagent:

Petroleum ether
Cotton wool
Sample

Procedure:

5g of cheese was weighed and covered with cotton that was previously extracted with Petroleum ether. Then the sample and a previously dried and weighed extraction flask containing about 100 ml petroleum ether were

attached to the extraction unit. Then, the extraction was conducted for 6hr. At the end of the distillation period the flask was disconnected from the unit and the solvent was recovered. Later, the flask with the remaining cured ether extract was put in on oven at 105^0 C for 10 min, cooled to room temperature in desiccator, re weighed and the dried extract was registered as fat content according to the following formula :

$$\text{Fat percent} = \text{weight of fat} \div \text{weight of sample} \times 10$$

3.6.2 Determination of Protein (A.O.A.C, 1990):

Kjeldhal Method:

Apparatus:

- Kjeldhal Flask.
- Heaters.
- Volumetric Flask (100ml) .
- Burrets .
- Pippets .

Material:

- Cheese sample.
- H_2SO_4.
- Distiller water.
- Noah .
- Boric acid.
- Indicator (promo cresol green+methyl-red)
- HCl
- Kjeldal tabs

Procedure:

The (3) gram of Cheese and 2 kjeldhal tablets were brought in to kjeldhal flask 25 ml of conc. H_2SO_4 were added the mixture , using a heater , then digested till a clear solution was obtained after 2-3 hr . The digested sample was then poured into volumetric flask and diluted to 100ml with distilled water. The 5ml from the dilution was transferred to the kjledhal flask and 10ml of Naoh were poured, received in a conical flask containing 25 ml of 4% boric acid and 3 drops of the indicator. The distillation was continued unit the volume in the flask reaches 75ml. flask is removed and distillate was titrated against 0-14 HCl , until red color was obtained.

Total protein % is calculated as follows:

Nitrogen %=T×0.1×0.114×20÷w×100

Protein%= Nitrogen×6.38

Where:

T: Titration figure.

0.1: Normality of HCl.

0.114: Atomic weight of Nitrogen.

20: Dilution factor.

3.6.3 Determination of Ash Content (A.O.C, 1990):

Apparatus

- Aluminum.
- Dry oven.
- Dissector .
- Muffle furnace oven.
- Balance.

Material:

- Cheese sample.

Procedure:

An empty aluminum dish was weight and 3 g of cheese were weighted and put into the aluminum dish. Then sample and dish were evaporated to dryness using a dry oven, dried by the dissecator till cooled to room temperature. Again the sample was weighted, then put in a muffle furnace dissector and then weighed

$$\text{Ash } \% = \frac{w1}{w0} \times 100$$

Where:

W1: weight of ash.

W0: weight of samples.

3.7 Statistical analysis

Data was analysis by using program Statistical Package for Social Science (SPSS, 2007) program, and one way analysis of variance (ANOVA). Means with a significant difference ($P<0.05$) were compared by the least significant difference (LSD) test.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results:

Table 4.1: Average coagulation time (minutes) by rennin, and different concentrations of Osher and Jibeen (30%, 60%)enzymes :-

Treatment	Mean	Std. deviation	Sig
Osher	123.33	15.27	*
Jibeen	130	14.14	*
Control	120		

NS= Not Significant

* = Significant (P > 0.05)

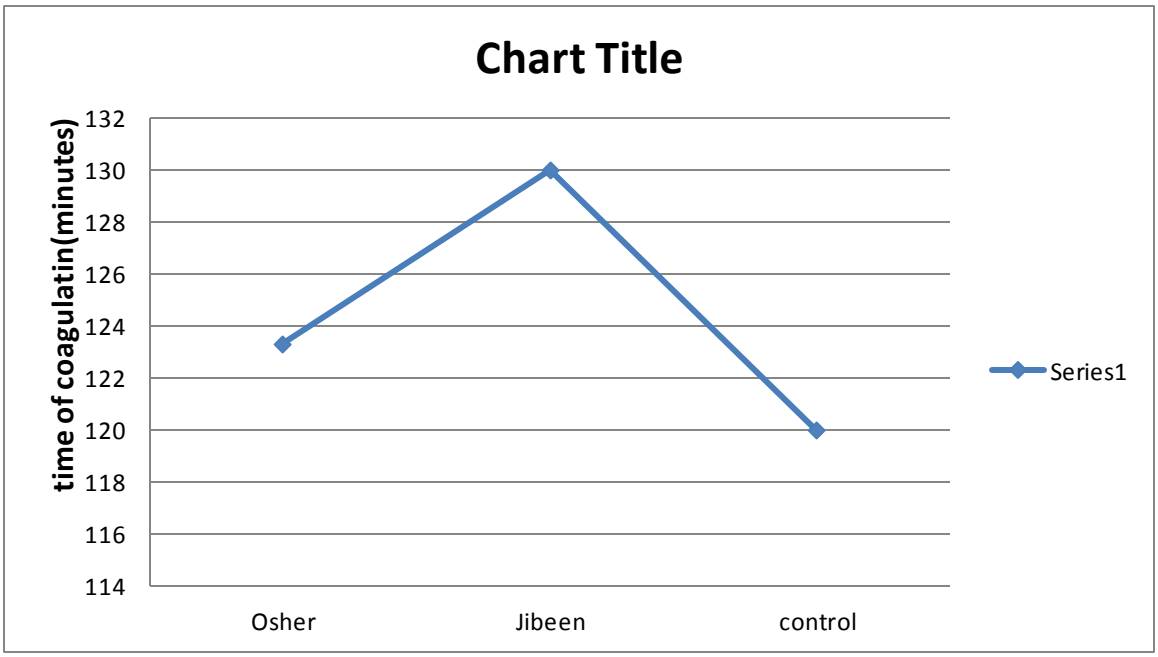


Table4.2: Average cheese yield (germs) for each 2.5 kg by addition rennin, and different concentrations of Osher and Jibee (30%, 60%) extraction

Treatment	Mean	Std. deviation	Sig
Osher	200	10	*
Jibeen	255.5	3.53	*
control	250		

NS= Not Significant

* = Significant (P > 0.05)

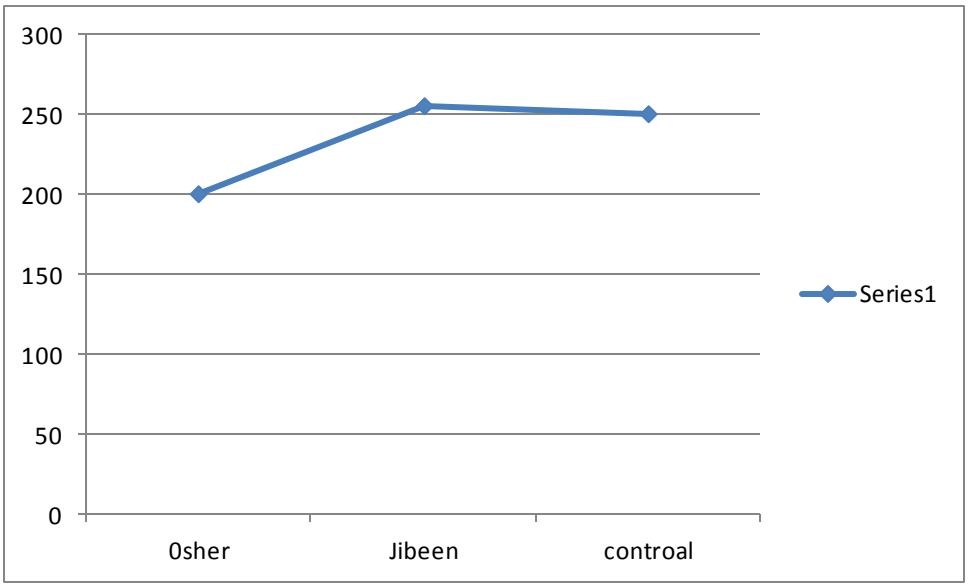


Table 4.3: Average moisture content % obtained by using rennin, and different concentrations of osher (30%,60%) extraction

Osher	Mean	Std. deviation	Sig of (LSD)
30%	61.99	0.52	*
60%	62.62	1.49	*
Control	64.38	0.01	

NS= Not Significant

* = Significant (P > 0.05)

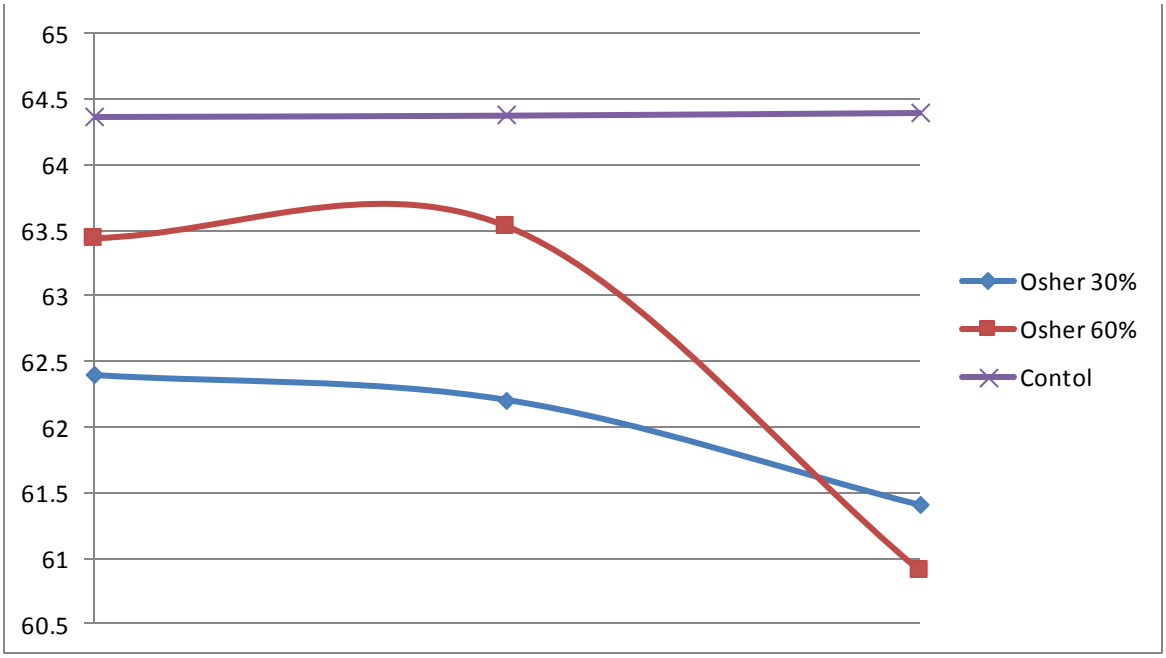


Table 4.4: Average moisture content % obtained by using rennin, and different concentrations of Jibeen (30%, 60%) enzymes

Jibeen	Mean	Std. deviation	Sig of (LSD)
30%	61.17	0.64	*
60%	65.25	0.09	*
Control	64.38	0.01	

NS= Not Significant

* = Significant (P > 0.05)

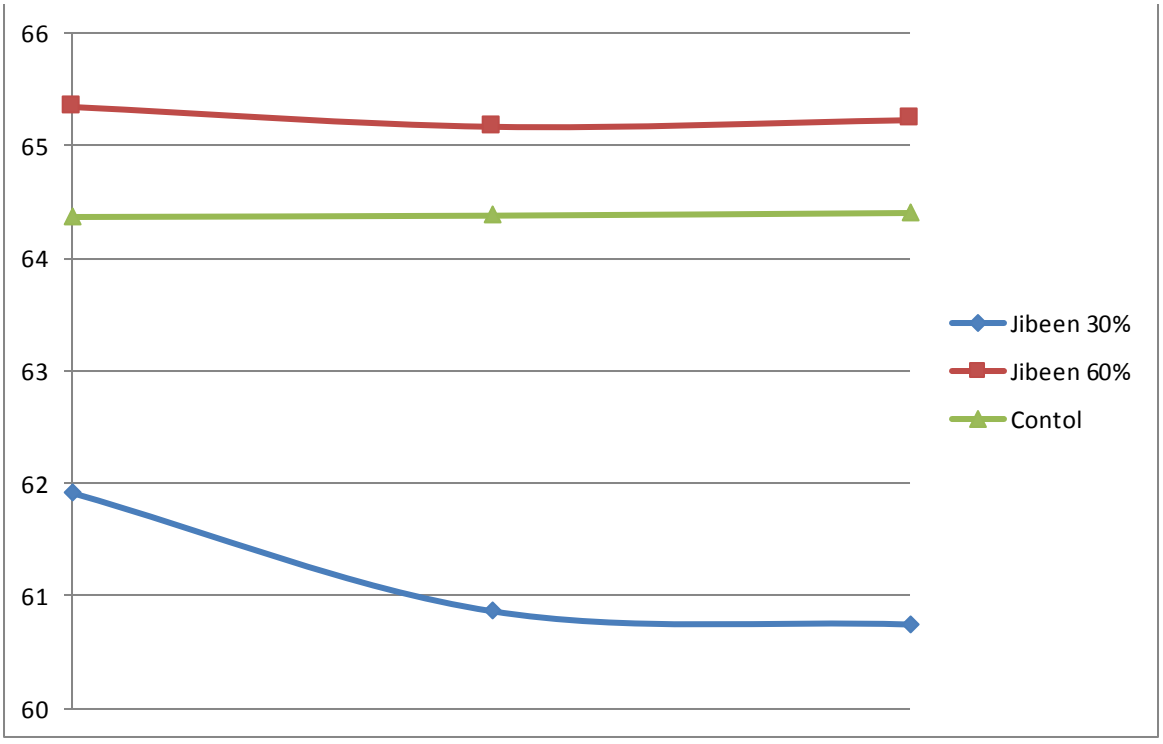


Table 4.5: Average protein % obtained by using rennin, and different concentrations of Osher (30%, 60%) extraction

Osher	Mean	Std. deviation	Sig of (LSD)
30%	15.2	0.17	*
60%	11.34	0.05	*
Control	19.43	0.49	

NS= Not Significant

* = Significant (P > 0.05)

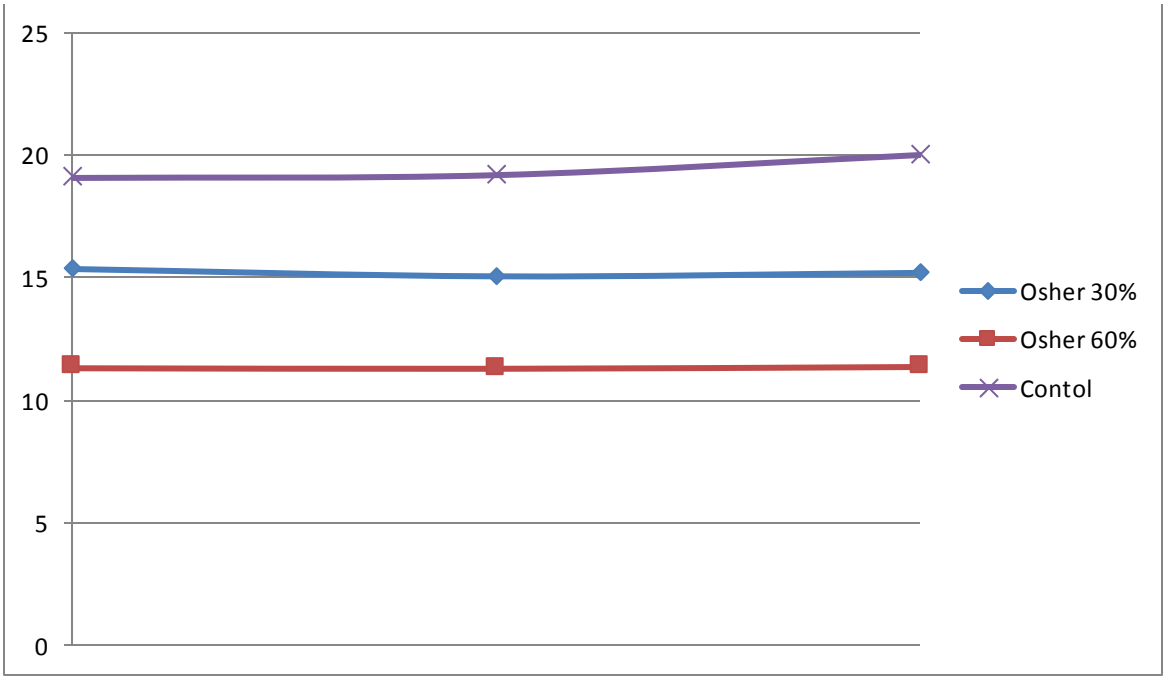


Table 4.6: Average protein % obtained by using rennin, and different concentrations of Jibeen (30%,60%) enzymes

Jibeen	Mean	Std. deviation	Sig of (LSD)
30%	20.04	0.12	*
60%	20.47	0.52	*
Control	19.43	0.49	

NS= Not Significant

* = Significant (P > 0.05)

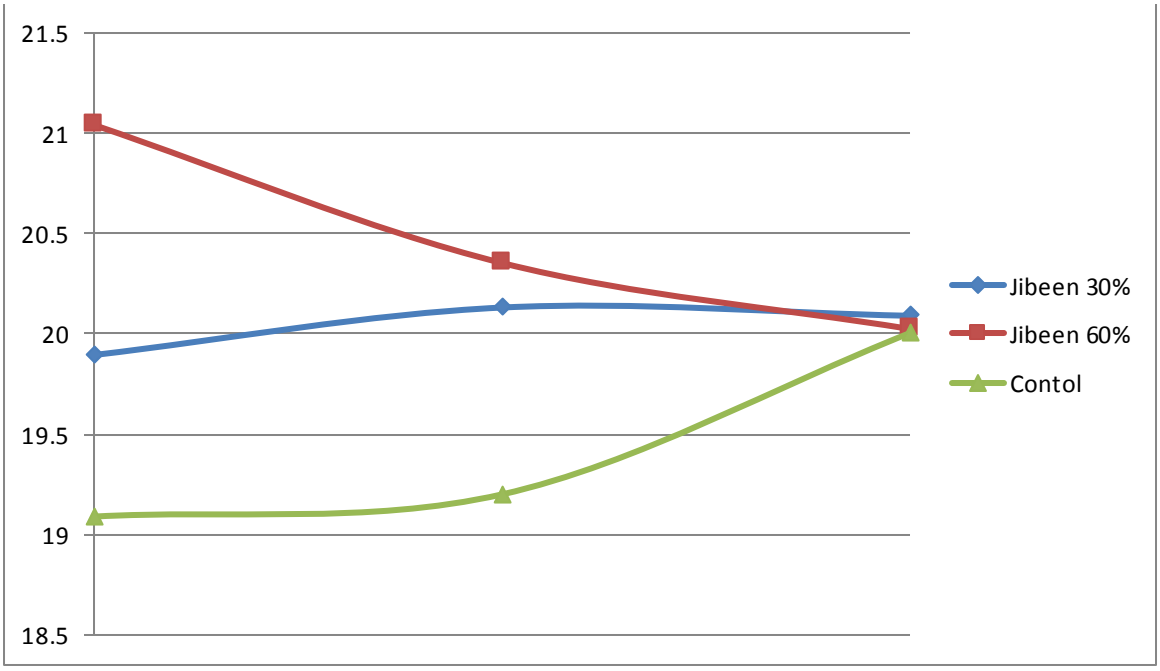


Table 4.7: Average fat % obtained by using rennin, and different concentrations of Osher (30%,60%) enzymes

Osher	Mean	Std. deviation	Sig of (LSD)
30%	13.77	0.09	*
60%	9.67	0.23	*
Control	16.43	0.49	

NS= Not Significant

* = Significant (P > 0.05)

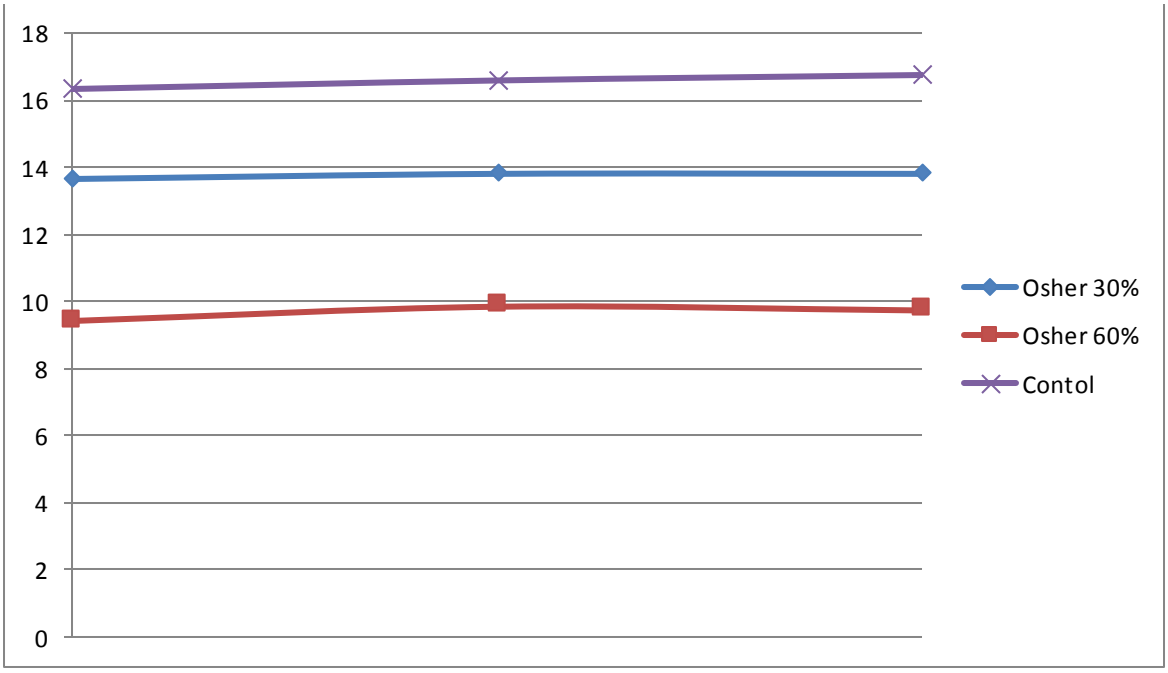


Table 4.8: Average fat % obtained by using rennin, and different concentrations of Jibeen (30%,60%) enzymes

Jibeen	Mean	Std. deviation	Sig of (LSD)
30%	11.99	0.04	*
60%	11.74	0.26	*
Control	16.56	0.23	

NS= Not Significant

* = Significant (P > 0.05)

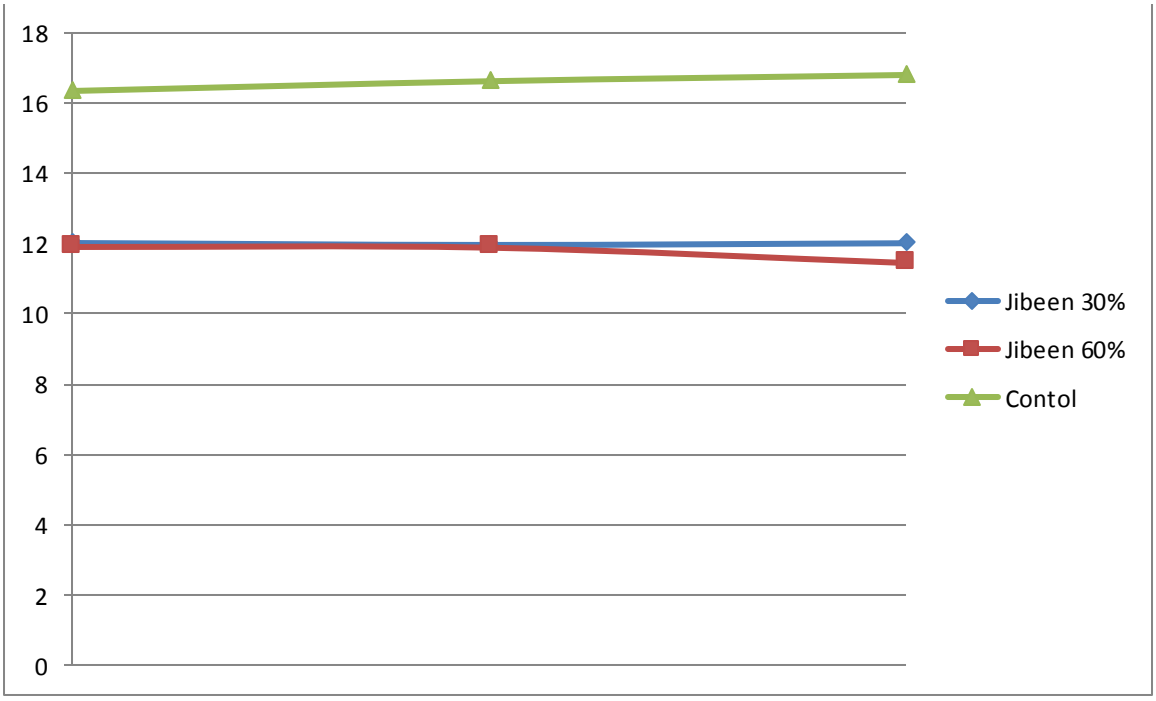


Table 4.9: Average ash % obtained by using rennin, and different concentrations of Osher (30%,60%) enzymes

Osher	Mean	Std. deviation	Sig of (LSD)
30%	3.69	0.20	*
60%	4.46	0.36	*
Control	3.03	0.16	

NS= Not Significant

* = Significant (P > 0.05)

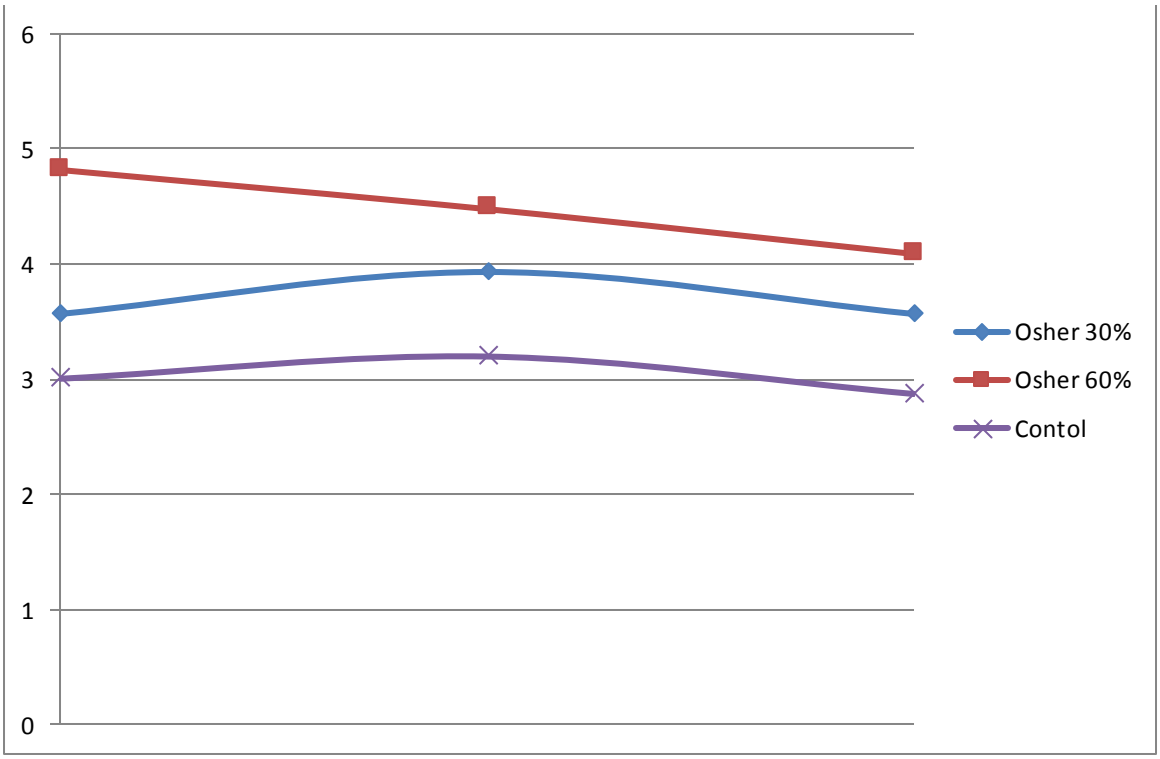


Table 4.10: Average ash % obtained by using rennin, and different concentrations of Jibeen (30%,60%) enzymes

Jibeen	Mean	Std. deviation	Sig of (LSD)
30%	3.88	0.07	*
60%	4.15	0.04	*
Control	3.05	0.16	

NS= Not Significant

* = Significant (P > 0.05)

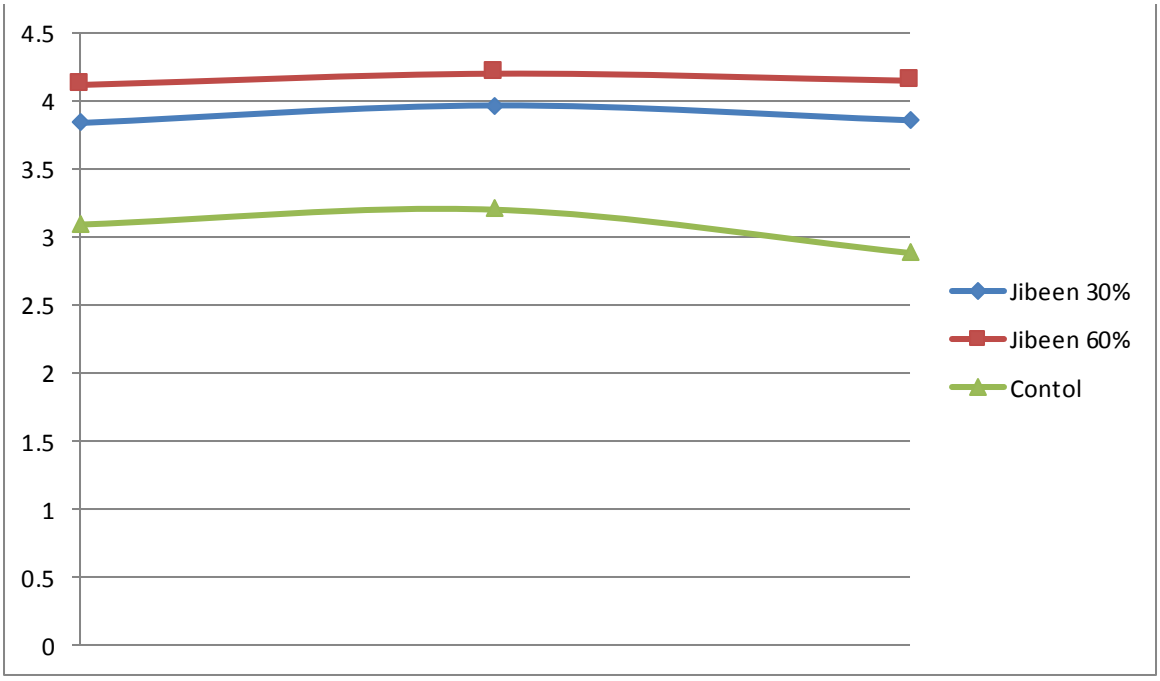


Table 4.11: Average evaluation point give for flavor by using rennin, and different concentrations of osher (30%,60%) enzymes

Osher	Mean	Std. deviation	Sig of (LSD)
30%	4.50	2.22	*
60%	5.30	2.07	*
Control	6.80	2.82	

NS= Not Significant

* = Significant (P > 0.05)

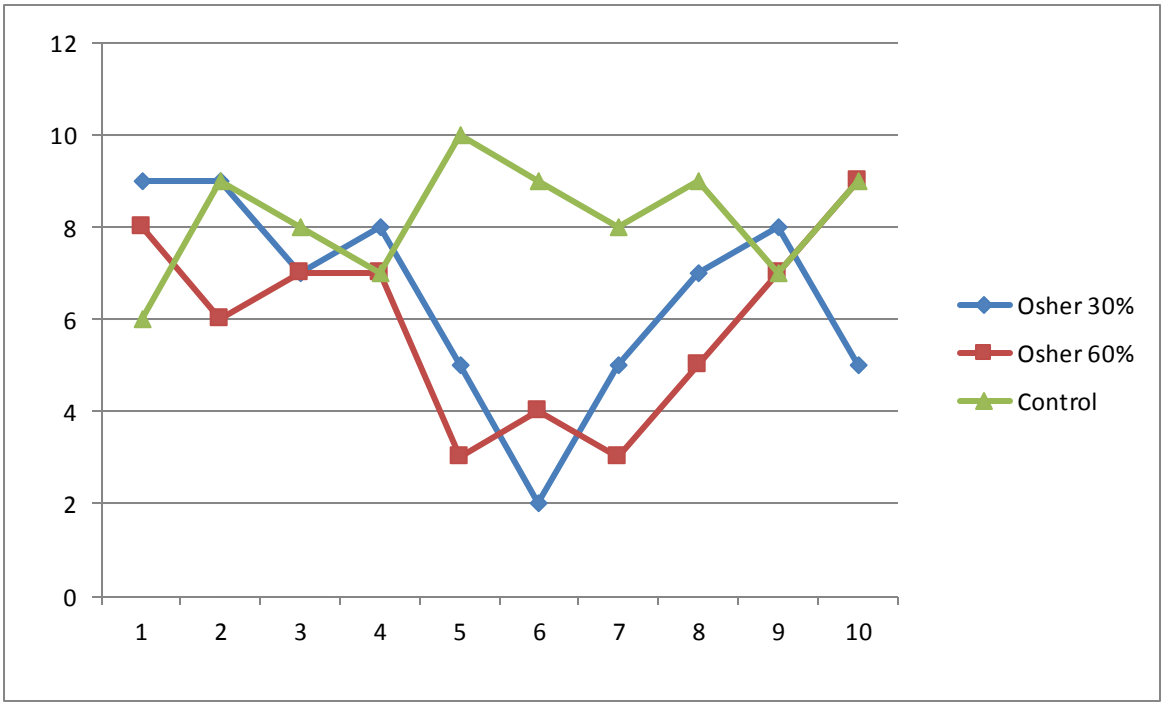


Table 4.12: Average evaluation point give for taste by using rennin, and different concentrations of osher (30%,60%) enzymes

Osher	Mean	Std. deviation	Sig of (LSD)
30%	5.80	2.05	*
60%	5.00	1.87	*
Control	7.30	2.00	

NS= Not Significant

* = Significant (P > 0.05)

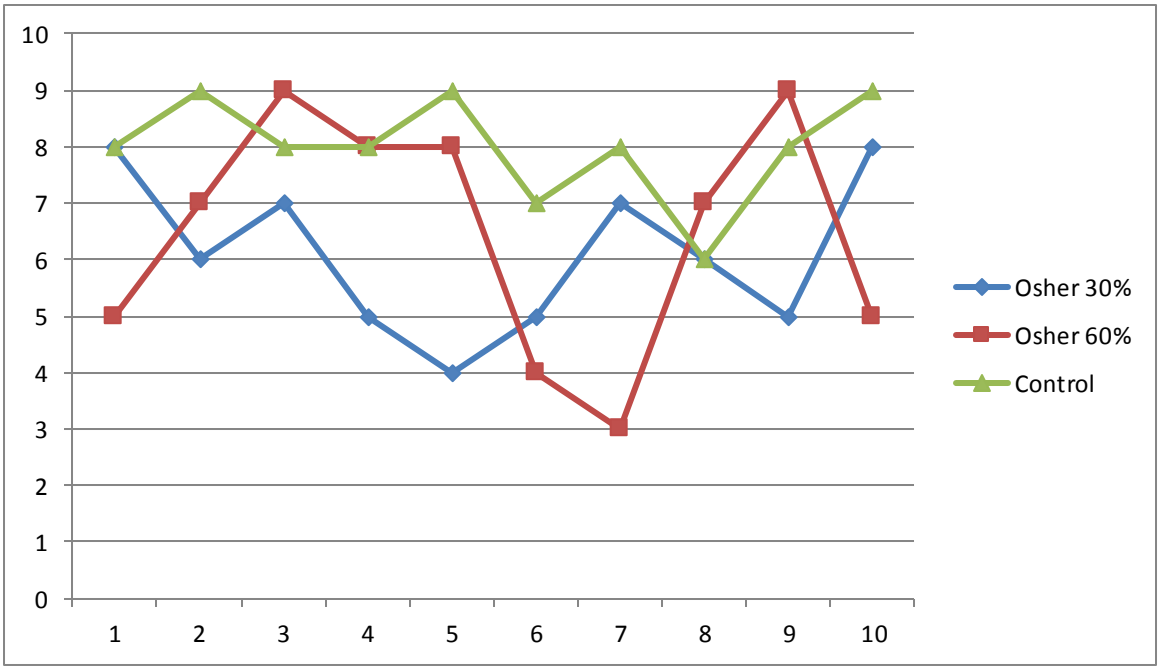


Table 4.13: Average evaluation point gives for texture by using rennin, and different concentrations of osher (30%, 60%) enzymes

Osher	Mean	Std. deviation	Sig of (LSD)
30%	5.80	2.11	*
60%	6.20	2.82	*
Control	7.70	2.16	

NS= Not Significant

* = Significant (P > 0.05)

Table 4.14: Average evaluation point give for flavor by using rennin, and different concentrations of Jibeen (30%,60%) enzymes

Jibeen	Mean	Std. deviation	Sig of (LSD)
30%	5.20	1.25	*
60%	5.00	1.69	*
Control	6.80	2.82	

NS= Not Significant

* = Significant (P > 0.05)

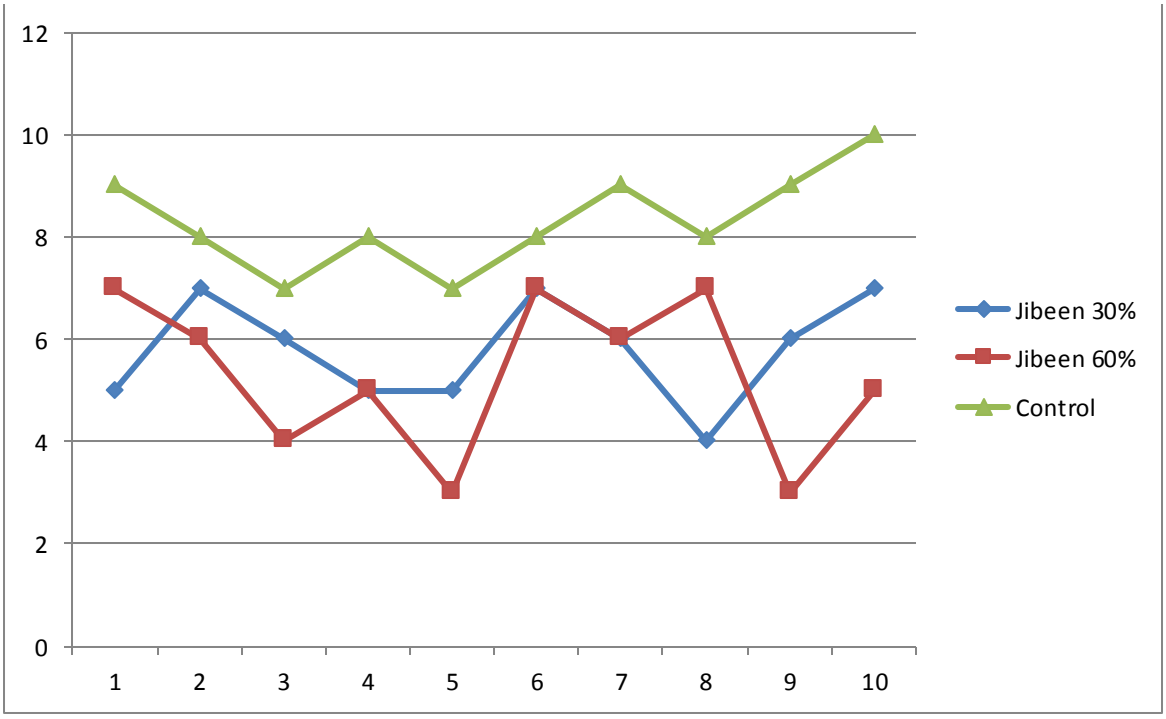


Table 4.15: Average evaluation point give for Taste by using rennin, and different concentrations of Jibeen (30%,60%) enzymes:

Jibeen	Mean	Std. deviation	Sig of (LSD)
30%	6.20	1.93	*
60%	5.80	1.76	*
Control	.730	2.00	

NS= Not Significant

* = Significant (P > 0.05)

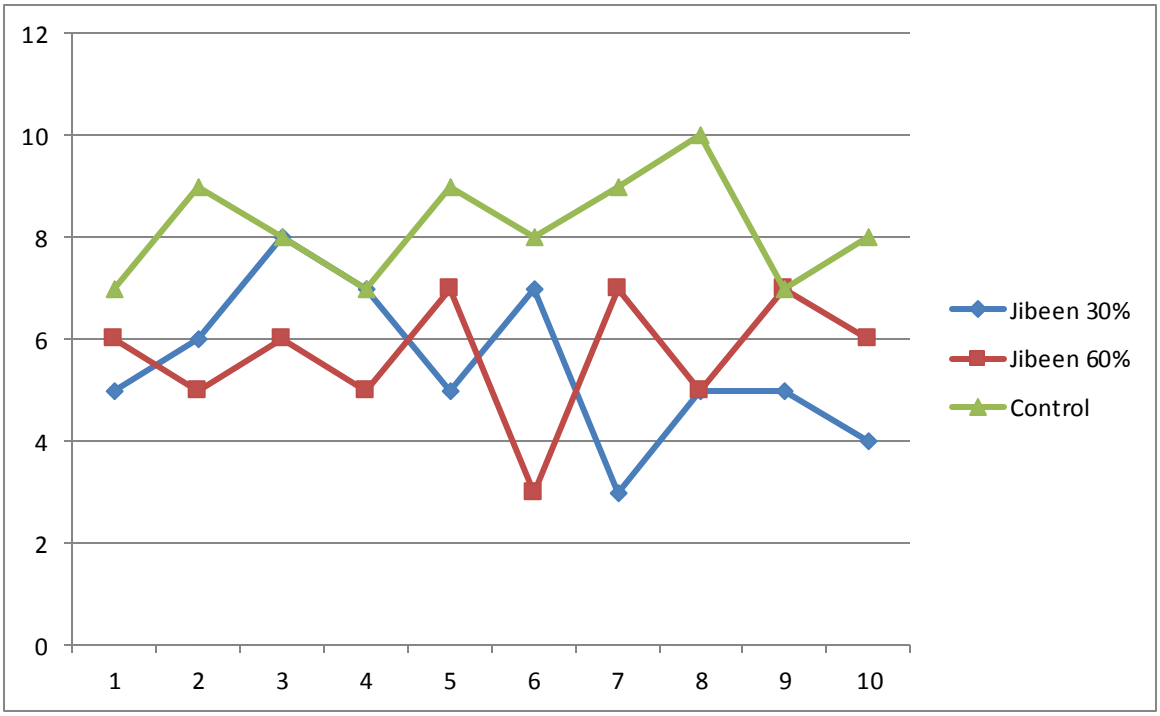
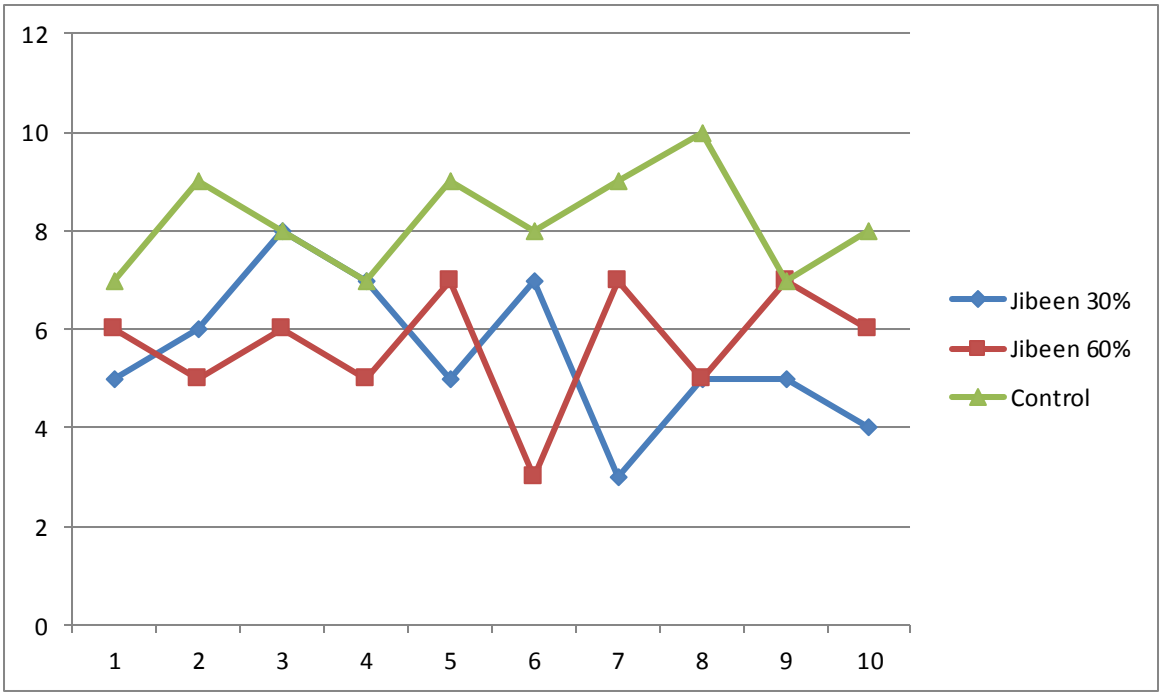


Table 4.16: Average evaluation point give for texture by using rennin, and different concentrations of Jibeen (30%,60%) enzymes:

Jibeen	Mean	Std. deviation	Sig of (LSD)
30%	5.50	1.71	*
60%	6.40	1.42	*
Control	.770	2.16	

NS= Not Significant

* = Significant (P > 0.05)



Time of coagulation table (4.1) shows the average coagulation time (minutes) by rennin, and different concentrations of Osher and Jibee (30%,60%)enzymes The average coagulation time for the whole concentration Osher, Jibeen and rennin was 123.33± 15.27,130±14.14,120, minutes respectively

Result s indicated significant difference between the means of the of the coagulation time for rennin and the different concentration of osher and Jibeen (30% 60%) ($p<0.05$). Similar results revealing significant different were given by (Van Hooydonk *et. al*. 1984), Lee et.al. (2003).

According to the results obtained, Osher enzyme required the less time to coagulate the milk compared to rennin and Jibeen enzymes, this agreed with that reported by the above mentioned authors.

The variation in the coagulation time required by the different coagulation depends on the type of the enzymes used (Van Hooydonk *et. al.*1987).

Cheese yield table (4.2) shows the average total yield of white soft cheese g/ 2kg milk obtained by adding rennin, and different concentrations of Osher and Jibeen (30%,60%)enzymes The average yield during the whole concentration Osher, Jibeen and rennin was 200±10,252.5 ±3.53,250, gram respectively**.**

The average yield showed a significance different for all treatments, and rennin and the different concentration of osher and Jibeen(30 %60%) ($p<0.05$). Similar results revealing significant difference between the means of the yield were given by (Everett *et. al*.2003), Nijera (2003), Nur Eldaim *et.al*. (2007) . According to the result obtained, Jibeen c oagulation resulted in a good cheese yield followed by rennin and Osher coagulation, which by many low cheese yield.

The variation in the cheese yield is affected by many factors of the milk composition of vital importance (Faltman, 1987). Other factors associated with yield are demonstrated in chapter two according to Everett et.al (2003) and Paolo et.al. (2008).

4.2.3 Chemical Composition of Cheese

Moisture content tables (4.3, 4.4) shows the average moisture continent% obtained by rennin, and different concentrations of Osher, Jibeen (30%,60%) enzymes The average moisture continent% for the whole concentration Osher, Jibeen and rennin were 61.99±0.52 ,62.62 ±1.49 , 61.17±0.64 ,62.25 ±0.09 64.38±0.01 for Osher, Jibeen and rennin respectively. The results obtained showed a significant different ($p<0.05$) between the average of moisture continent% when using rennin and different concentration of Osher for all treatment. Similar results were given by (SSMO, (2002)).the difference in moisture content of the white soft cheese was associated with the type of the coagulation used for processing.

Protein Content tables (4.5, 4.6) shows the average Protein % obtained by rennin , and different concentrations of Osher (30%,60%)enzymes The average Protein % for the whole concentration Osher,Jibeen and rennin was15.2 ±0.17,11.34 ±0.05, 20.04±0.12,20.47±0.52 19.43±0.49 for Osher, Jibeen and rennin respectively. The results obtained showed a significant different ($p<0.05$) between the average of protein % when using rennin and different concentration of Osher,Jibeen .

Similar results were given by Caric *et.al* (1995), Green *et.al* the different of protein content of the white soft cheese were associated with type of the coagulation used for processing. Coagulation the milk with rennin enzymes the levels of protein tend to increase compared to plant enzymes, Dimitreli *et. al.*(2004).

Fat Content tables (4.7, 4.8) shows the average Fat % obtained by rennin , and different concentrations of Osher,Jibeen (30%,60%)enzymes The average fat% for the whole concentration Osher,Jibeen and rennin was13.77±0.09,9.67 ±0.23 ,11.99±0.04,11.74 ±0.26 16.56±0.23 for Osher,Jibeen and rennin respectively.The results obtained showed a significant different ($p<0.05$) between the average of fat % when using rennin and different concentration of Osher for all treatment. Similar results were given by Cari et.al (1993); the difference of fat in content of the white soft cheese was associated with the type of the coagulation used for processing.

The average fat %obtained when using rennin compared to the plant enzymes. Also differences in the fat of the raw milk prepared for white cheese processing and method of coagulation used caused variation in the final fat content of the finished cheese. This comes in agreement with the explained by (Home 1990).

Ash Content tables (4.9, 4.10) shows the average ash % obtained by rennin , and different concentration of Osher,Jibeen (30%,60%)enzymes The average % for the whole concentration Osher, and rennin was3.69±0.20,4.46 ±0.36,3.88 ±0.07,4.15±0.04, 3.03±0.16 for Osher, Jibeen and rennin respectively and for all treatment. Ash content showed low percentages, when plant coagulants were used compared to rennin enzyme. This agreed with reported by Dalglish (1985), Psozola (1989) .

4.2.4 Sensory Evaluation

Flavor of cheese tables (4.11, 4.14) show the evaluation points given for flavor of cheese using rennin, and different concentrations of Osher, Jibeen(30%, 60%)The average evaluation points the Flavor scored, were 4.50±2.22, 5.30±2.07, 5.20±1.25, 5.00±1.69, 6.80±2.82 for Osher, Jibeen and rennin enzymes for all treatment respectively. A significant difference ($P<0.05$) was detected between the averages of scored points for flavor, when

rennin and different concentration of Osher, Jibeen enzymes for all treatment were used. The results agreed with Farell *et.al.* (1990).same factors the affect the flavor of cheese is the concentration of extracted plant enzymes when used in cheese making, (Talib *et.al*.2005). Other factors influencing the flavors are the chemical composition of milk, types of animals and chemical composition of feed (Englels *et.al* 2005).

Taste of cheese tables **(**4.12, 4.15) show the evaluation points given for taste of cheese using rennin, and different concentration of Osher, Jibeen (30%, 60%)The average evaluation points the texture scored, were 5.80±2.05, 5.00±1.87, 6.20±1.93, 5.80±1.76, 7.30±2.00 for Osher , Jibeen and rennin enzymes for all treatment respectively. A significant difference ($P<0.05$) was detected between the averages of scored points for taste, when rennin and different concentration of Osher enzymes for all treatment were used. The results agreed with Talib, *et.al.* (2006).same factors the affect the taste of cheese is the concentration of extracted plant enzymes when used in cheese making, (Talib *et.al*.2005).

Texture of Cheese tables (4.13, 4.16) show the evaluation points given for texture of cheese using rennin, and different concentrations of Osher, Jibeen (30%, 60%)The average evaluation points the texture scored, were 5.80±2.11, 6.20±2.82, 5.50±1, 71,6.40±1.42, 7.70±2.16 for Osher ,Jibeen and rennin enzymes for all treatment respectively. A significant difference ($P<0.05$) was detected between the averages of scored points for texture, when rennin and different concentration of Osher, Jibeen enzymes for all treatment were used. The results agreed with Yousif, *et.al*. (1996).same factors the affect the flavor of cheese is the concentration of extracted plant enzymes when used in cheese making, (Talib *et.al*.2005). Other factors influencing the flavors are the chemical composition of milk, types of animals and chemical composition of feed,(Englels *e.al* 2005).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

To overcome such problem plant enzymes were extracted from various plant e.g. *Solnum dubium* (Jibeen) and *Cloatropis procera* (Osher) and other. Comparing rennin to plant enzymes, it give the best resulted for all studied parameters subject of the current research (Time of coagulation, cheese yield, composition and sensory characteristics)

A significant different ($P<0.05$), were detected between the average of the parameters for all treatments. Yet, Jibeen enzymes showed an acceptable stander levels when used. Enzymes extracted from Osher gave the lowest results when compared to rennin and Jibeen enzymes. Hence plant enzymes may also be used in the future as milk coagulation in the cheese industry of Sudan under certain conditions.

5.2 Recommendation

Based on obtained results, following the recommendation might be given:

1-Jibeen enzymes can be used as an alternative for rennin and other plant enzymes, since the plant grows in most areas of the Sudan, in addition to its effectiveness.

2-More attention should be paid when using plant enzymes, particularly, chemical composition and suitable concentrations to be used to avoid health by consumer, e.g. toxicity.

3-More research studies should be carried out on plant enzymes, as well the economic impact linked with the utilization.

References

Arvind, N.S. (2010), Textbook of dairy chemistry, 1st. ed.,Discovery Publishing House PVT Ltd.

Association of Analytical Chemists (AOAC), (1990), Official Methods of Analysis, 15_{th}.ed, Washington, USA.

Bines, V. E. Young, P. and Law, B.A (1989), Coagulation of Milk by clostropis procera plant enzymes, Dairy Res., 56-65

Biswas, S. and S. S. Bahattachary(2006), Milk and Milk product, Japeer Brother production Technology Medical Publishers(P)Ltd, India.

Blueme, J.Y.(2003), Hand book of dairy products, random export, New Delhi, India. by Calotropis procera plant enzyme, Dairy Res., 56,657.

Caric, M. and Khatab M, (1993), Processed cheese products. In Cheese chemistry, physic and microbiology. Major cheese groups 21ed.,14.Chapman and Hall,London .

Carlson, A; C. Hill and N. Olson (1985), Improved Assay procedure for determination of Milk Clotting Enzymes, Journal Dairy,SCi, 68:290-296.

Center of Advanced Food Research (2006), Research Department of Human Nutrition, Dairy Products and Cardio Vascular Diseases, The Royal Vet. Agric. University, Fredric Berg. Denmark.

Clarence, H.E. Wilies, B.C. and M. Harold, (2004). Milk and milk products 4th Edition.J, of Diary R.S.19,622.

Creamer, L.K., Lawrence, R.C. and Gilles, J. (1985) Effect of acidification of cheese milk on the resultant Cheddar cheese. N.Z. J. Dairy Sci. Technol. (20), 185–203.

Crow, V.L., Martl ey, F.G., Coolbear, T. and Roundhill, S. (1993) Influence of phage-assisted lysis of Lactococcus lactis subsp. lactis ML8 on Cheddar cheese ripening. Intern. Dairy J. 5, 451–72

Daigleish, D.G., Horne, D.S. and Law, A.J.R., (1989), Biochim , Biophys. Acta,991,383.

Dalgleish, D.G, Coagulation of Milk by Using Plant Enzymes, Neth. Milk Dairy J., 42,341.

Dimitrel, G and Thmaries A.S (2004), "Temperature and chemical composition on processed cheese apparent viscosity journal of food engineering ,(64):265-271.

Dirar, H.A. (1993), the Indigenous fermented foods of the sudan , A study in African food and Nutrition CAB ,International center for Agriculture and Bioscience , chapter 6 pp:333-338.

Donelly, L (2003), Functional International food : Cheese Goliath Dairy Industries International, Sweden.

Drake, M.A, D. Stephenic, B. Floyd, Stephenic and Michael (2009), History of Sensory Analysis , the Sensory Evaluation of Dairy products, 2nd Ed. Springer Science & Business Media LLC. USA.

Engels, W.J.J.E.T. Van Hyickman Vliey , and Smit, G(2005), Flavor Formation in cheese , In : Dairy processing Improlog Quality , Ed. Smit G. wood Head publishing Ltd. Cambridge , London.

Enzyme Used for Coagulation on Yield of White Cheese, J. Dairy Res. ,56, 79.

Eren –Vapore U, and Ozcan T,(2012) ,Determination of free amino acids for whole – fat Turkish White Brined cheese produced by animal and microbial milk – clotting enzymes with and without the addition of starter culture "Mijekarstov .(vol .62, on 4), pp 241-250.

Everett, D.W. and M.A. E. Auty (2003) , Cheese Structure and current Methods of Analysis, Int. Dairy Journal, 18:759-773.

Farell, H.M., Jr., Brown E.M and kumosinki, T.F.(1990), producing , 23 Int. Dairy Congr.Mohtreal, Vol.,2,1526.

Farell, N.Y ,(2004), Cheese technology, International . Journal of Dairy Technology Ltd., UK.

Farell, N.Y. and Fox P.F.(1990)Objective indices of cheese ripening ,Trends Food Sci Tech., pp37-40.

Fernandes, R. (2009), Dairy products, BSc. Publishing, Leathes Food International Ltd., U.K.

Foltmann, B. (1987) General and molecular aspects of rennets, in Cheese: Chemistry, Physics and Microbiology Vol. 1, 2nd edn (ed. P.F. Fox), Chapman & Hall, London, pp. 37–68

Food and Agriculture organization, World Health program, Milk and milk products , Codex Alim Entarius C0mmission,. Vo. 12.

Food Sci. Technol., 10: 16-19.

Fox, P F ., O Connor T.P., McSweeney P.L.H., Guinee T.P., and O Brien N M, (1996), Cheese physical. Biochemical and nutritional aspects,Adv Food Nut. Res. Vol.(39).Pp, 36-328.

Fox, P.E, Guinee T.P., T.M. Cogan and P.L.H, Mc Sweeny (2000), Fundamental of Cheese Science , Aspen Publisher.

Frederiksen, PD., Hammershoj , M., Barkman , M., Adersen, P.N., Adersen, J.B,. Quist, K.B,. and larsenL.B,. (2011). Variations in coagulation properties of cheese milk from three Danish dairy breeds as

determined by a new free isolation rheometry- based method. Dairy Sci Technol (91), 309-321.

Green, M.I.Hobbs, D.G Morant,S.V and Hill V.A. (1987), Effect of Milk composition on composition of processed White Cheese, J Dairy Res.,45,432.

Hamed, O.I. (1998), The effect of processing conditions on yield, chemical composition and sensory characteristics of white soft 91 cheese. M.Sc.Thesis, University of Khartoum, Sudan.

Herrington, B.L.(2000), Milk and milk processing, Green World Publishers .

Hooydonk, A.C.M., De Koster, P.G., and Boerrigter, I.J.(1987), Different Methods of Coagulation, Neth. Milk Dairy J.,41,3.

Hrone, D.S. and Davidson, C.,(1990), Chemical Composition of Processed White Cheese Coagulated by Three Different Enzymes, Milchwissenschaft 45,712.

Huria, V.K. (2003),Milk, the classic food of modern civilization, Ind. food industry,21(4):24-32.

Ibiama, E. and Griffiths, M.W. (1987), Studies on milk Coagulating Enzyme Calotropain Obtained from Sodom Apple (Calotropis process), Jour. Of Food Agriculture 1(3):157-167.

International Dairy Food Association (DFA)(1998),Cheese Facts. National Cheese Institute, Washington D,C.,USA.

International Journal of Environmental Science and Development (2013), Advance in cheese Technology, J, al. NO.I, February 2013.

Jacob M., D Joros, and Rohm H., (2011) " Recent advances in milk clotting enzymes" int.J. Dairy Technol., vol. 64, no.I,pp14-33.

James ,Y.B.(2013), Handbook of dairy products, Blume Random Exports, India.

Kopelman, I.J: Cogan, U.1975Determanation of clotting power of milk clotting enzmes. Journal of Dairy Science 59 196-199.

Kumosinski, T.F. Brown, E.M. and FarrelH.M., (1991), Factors Affecting Cheese Yield, J. Dairy SCI., 74-2879.

Kutty, I .C. and K. Sheeba(2014), milk production and processing, Daya Publishing House, India.

Lee , S.K,. Buwalda R,J., Euston S.R., Foegeding E.A., McKenna A.B., (2003),Changes in the rheology and microstructure of processed cheese during cooking. Food Science and Technology, 36:33 9-3 45.

Lee, B. H., laleye L. C., Simared R,E., Holley R A., Emmons DB., and R N Giroux, (1990), " Influence of homofermentative Lactobacilli Oil physicochemical and sensory properties of Cheddar, " J. Food Sci.Vol.55.102. pp 386-390.

Merin. U., Talpaz, Ii. & Fishiman, S., (1989), Effect of Types of

Miller, G H., Jarvis J.K and MeBean L.D, (2007) Handbook of dairy food and nutrition, 3rd ed. National Dairy Council, Boca Raton(FL). CRC Press.

Murshidi . U., Talpaz, li. and Fishiman, S.,(1998), Effect of enzymes Used for Coagulation on yield of white Cheese, J. Dairy Res.,56,79.

Najera . A.I.:Renobales. M.D. and Barron.L.J.R. (2008), Effects of pH temperature. Cacl2 and enzymes concentrations on the rennet clotting properties of milk :a multifactorial study. Food Chemistry 80,345-342.

Niir (National Institute of Industrial Research (2012), Modern technology of milk processing and dairy products.(2nd . ed), published and printed by Ajyarkr Gupta for Niir, Delhi India.

Nour ElDiam , M.S.A. and Ibtisam E.M. and El Zubeir(2007) yield and sensory evaluation of the processed cheese from Sudanese white cheese, Research Journal of Animal and Veterinary 2(1):47-52.

O·Conner, C.B. (1993), Traditional Cheese Making Manual, International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia.

Osman, A.M. (2007), Milk Technology Notes for Graduate Studies, Department of Milk Technology, College of Animal Production Science and Technology ,Sudan University of Science and Technology .

Ozcan T., and Kurdal E (2012).,"The effects of using a starter culture, lipase, and protease enzymes on ripening of Mihalic Cheese " Int. J. Dairy Technol.,Vol.(65),no.4 ,pp.

Paolo,F.,S., Andrea ;F. Piero ;N. Massimo and M. Primo (2008),Cheese Yield :Factors of Variation and Predictive formulas , Ann.Fac, Med .Vet. Para,(28:211-232).

Pauline ,E. and R. Karin (2006), preparation of dairy product, Agrobook, 36, Printed by :Digigrafi and Nageningem, Netherlands.

Psczola, D. E. (1989), Effect of method of Coagulation on Percentages of Total Solids (TS) of White Cheese ,Food Technol .43,84.

Reddy, I.M. and Kinsella, J.E.,(1990), Effect of Type of Enzyme used for Coagulation on Chemical Composition of White Cheese,Agric. Food Chem.,,38,50.

Rollema, H.S., Brinkhris ,J.A and Vreeman ,H.J.,(1988),Effect of type of Enzyme on protein content of White Cheese, Milk Dairy J.,42,233.

Shaw, M.B. (1986), Rennet and rennet substitutes , in: modern dairy technology, Vol. 2 Edi Robinson, R.K. (1986). Elsevier Applied Science Publishers, London, New York.

Simpson, D.P. (1979),Cassel·s Latin Dictionary, 5th.Ed.London:Cadel Ltd.,pp 883 ,ISBN 0-304-52257-0.

Singh, J., Chander, H., Bhalerao, V. R. and Dastur, N. N. 1987. Studies on Vegetable Rennet from Withania coagulans. Indian J.

Spreer, E.(1998),Milk and dairy products technology, Marce Dekker, INC, New York,Basel.

Storry, J.E. and G.D Food (1982),Some Factors affecting the post Clotting Development of Coagulum Strength in Rennet Milk ,Jour. Of Dairy Researsh 49 (3):469-477.

Takala, A. G. (1990) Evaluation of Cheese Processed by Citric Acid and Rennin Coagulation, M.Sc. Thesis, University of Khartoum, Sudan.

Talib , A.M., M.M. Abubakre and I.A., Jideani, (2007), Studies on Enzymatic and Coagulation Properties of Jibeen (solanum dubium) Seed Extracts, J. Agric.,SC. Mansoura University, 32(9):1711-7183.

Van Hooydonk, A.C.M., Olieman , C. and Hagedoorn , H.G. (1984), Factors Affecting Time of coagulation of milk , Neth. Milk Dairy J., 22, 118.

Varnam, H. and J. P. Sutherland (1994). Milk and milk products Technology, Chemistry and Microbiology, Chapman and Hall, London, U.K.

Walstra , P;T.J,Geurt, A. Nomen ,A. Gelma and M.A.J.S. Van Boehel (2005), Dairy technology: principles of milk properties and processes, Marcel Deker, INC, New York Basel.

Yousif , B. H; D.J. McMahon and K.M. Shammet (1996) milk Clotting Enzyme from Solanum dubium plant, Int. Dairy Jor.,6:637-644.

المراجع العربية:

زيدان إبراهيم عبد الله (2004)، المواصفات الفنية لمنتجات الألبان الغذائية،إصدار جامعة الإسكندرية ،جمهورية مصر العربية .

عزيز كبرو حنا (1996)، مبادئ تصنيع الألبان ،الطبعة الثانية ، إصدار جامعة بغداد، الجمهورية العربية العراقية .

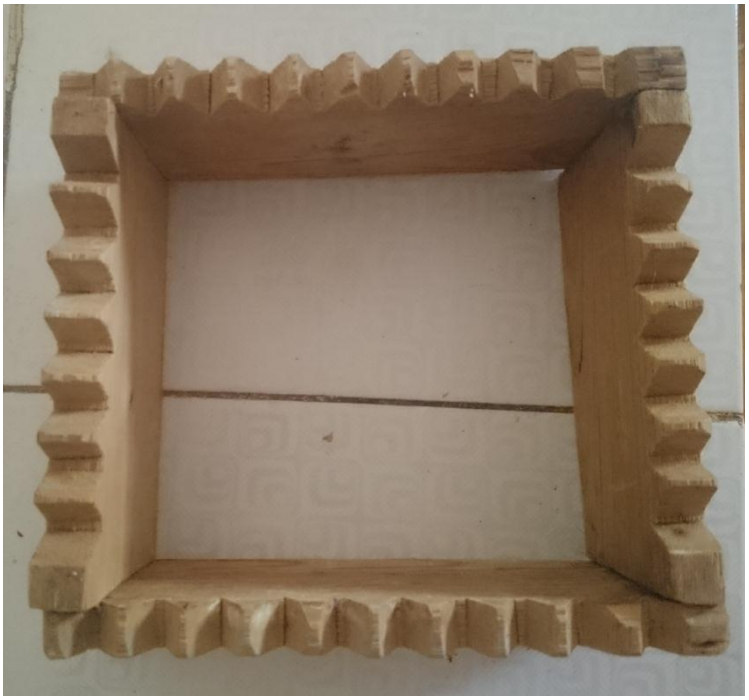
النمر طارق مراد (2007)، الموسوعة في تقنيات صناعة الألبان ومنتجاتها، مكتبة بستان المعرفة، طبع ونشر وتوزيع الكتب جمهورية مصر العربية.

الهيئة السودانية للمواصفات والمقاييس (2002)، مواصفات الجبن الأبيض ،س.د.ق، 1428 ه، اللجنة الفنية للألبان ومنتجاتها، الخرطوم ،السودان.

Appendices

	Osher 30%	Osher 60%	Control	Osher 30%	Osher 60%	Control	Osher 30%	Osher 60%	Control
1	9	8	4	8	6	5	7	6	4
2	9	6	9	10	7	9	10	9	9
3	7	7	8	7	9	8	7	9	8
4	8	7	7	5	8	8	9	3	8
5	5	3	10	4	8	9	6	2	5
6	2	4	3	5	4	3	4	2	6
7	5	3	2	7	10	8	9	8	10
8	7	5	9	6	7	6	9	8	10
9	8	7	7	10	9	8	10	7	10
10	5	9	9	8	10	9	5	8	7

	Jibeen 30%	Jibeen 60%	Control	Jibeen 30%	Jibeen 60%	Control	Jibeen 30%	Jibeen 60%	Control
1	7	8	9	8	6	7	6	6	8
2	7	6	8	6	5	9	8	6	9
3	9	8	7	8	6	5	7	6	6
4	8	9	8	7	5	6	6	4	4
5	5	3	7	7	10	9	9	8	7
6	7	7	8	7	9	8	7	6	8
7	9	6	9	10	7	9	10	9	9
8	8	7	5	10	9	7	10	7	9
9	6	8	9	5	7	7	7	5	6
10	7	8	9	4	6	8	5	7	5



Wooden tray

Gauze

Weights

Thermometer

Incubator

Pasteurization milk

Coagulation milk

Cheese