بسم الله الرحمن الرحيم

Sudan University of Science & Technology

College of Graduate Studies

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

أثر استتخدام إنزيم الرنين والأنزيمات النباتية في زمن تجبن اللبن والإنتاج والتركيب الكيميائي والصفات الحسية للجبن الأبيض الطري

A Thesis submitted in fulfillment of the requirements of Sudan University of Science and Technology for the Degree of PhD.
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استهلال

قال تعالى:

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا) **صدق الله العظيم - سورة طه – الآية (114).**

Dedication

To my wife, son and daughters.

To the spirit of my parents.

To my colleagues and friends.

To those who are following the way of animal production.

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Thanks to Allah who helps me to complete this work. I am deeply indebted to my Supervisor Dr. Anas Mohammed Osman for his continuous support, guidance, encourage and ideal supervision to this work. Thanks are extended to my colleagues and all members of department of Animal Production, College of Agricultural Studies, Sudan University of Science and Technology for their support and encouragement. I am also indebted to Professor Mohammed Osman Gaafar for his continuous support, encourage and urging to complete this work.

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Abstract

This research was conducted to study the effect of using renin enzyme and two other plant enzymes on the coagulation time of milk, yield, composition and sensory characteristics of produced cheese. The renin enzyme was used as control. The plant enzymes were extracted from Solanum dubium (Jibeen) and Calotropis procera (Osher) plants. Plant enzymes were added to the milk in 3 concentrations (50%, 75% and 100%). Coagulation time, yield, composition and sensory characteristics were assessed during the lactation period, which was divided into 1st., 2nd. and 3rd. stages. (1st. 2nd. and 3rd. two months after calving) and for the whole lactation period. The total number of cheese samples used was 216 for the different treatments. Average coagulation time for whole lactation period and all treatments when using renin, Osher (50%) and Jibeen (50%) enzymes recorded, was 97.33± 4.62, 124.37± 5.88 and 96.29± 4.96 minutes respectively. Using renin, Osher (75%) and Jibeen (75%) enzymes, coagulation time obtained was 80.37± 3.54, 113.62± 7.29 and 87.75± 2.23 minutes respectively and 79.45± 3.65, 108.83± 5.62 and 85.95± 4.95 min. for renin, Osher (100%) and Jibeen (100%) enzyme respectively.

A significant difference ($p<0.05$) was detected between the average coagulation time for all stages, whole lactation period and all treatments. Coagulation of the milk by renin enzyme requited less time, while much time was required by Osher enzyme compared to Jibeen enzyme. The variation in time required by the different coagulants was related to the type of enzyme used, chemical composition of milk, method of coagulation applied, as well as the clotting activity of the milk.

The average total yield of soft cheese obtained from 5kg cow milk, when using renin, Osher (50%) and Jibeen (50%) enzymes for the whole

lactation period and all treatments was 913.5± 8.66, 635.9± 14.09 and 662.25± 15.58 g respectively. For renin, Osher (75) and Jibeen (75%) enzymes was 944.3± 32.89, 697.8± 16.99 and 729.3± 41.8 g. respectively.

For renin, Osher (100%) and Jibeen (100%) enzymes, the average cheese yield was 916.0± 11.54, 788.7± 16.70 and 804.3± 9.55 g respectively. The average total cheese yield showed a significant difference ($p<0.05$) for all stages, whole lactation period and all treatments. Renin coagulation resulted in good cheese yield, followed by Jibeen and Osher. Differences in cheese yield was affected by many factors, e.g. milk composition, enzymes and type of enzyme used for coagulation, production of weak curd produced by plant enzymes and others.

For whole lactation period and all treatments, the average protein % was found as 14.42± 0.44, 11.96± 0.46 and 13.44± 0.74 when using renin, Osher (50%) and Jibeen (50%) enzymes respectively, 14.26± 0.36, 12.14± 0.35 and 13.61± 0,60 for renin, Osher (75%) and Jibeen (75%) enzymes respectively and 14.36± 0.39, 12.02± 0.26 and 13.55±± 0.42 for renin, Osher (100%) and Jibeen (100%) enzymes respectively.

The average fat % obtained was 20.06± 0.84, 15.99± 0.75, and 16.95± 0.80, for renin, Osher (50%) and Jibeen (50%) enzymes respectively/. An average fat % of 19.97± 0.80, 16.00± 0.59 and 18.35± 0.44 obtained for renin, Osher (75%) and Jibeen (75%) enzymes respectively. Also a content of 20.39±0.57, 15.91± 0.40 and 18.54± 0.31 % for renin, Osher (100%) and Jibeen (100%) enzymes respectively.

The ash % when using renin, Osher (50%) and Jibeen (50%) enzymes obtained, was 4.27± 0.37, 3.27± 0.31, and 3.47± 0.26

respectively. Using renin, Osher (75%) and Jibeen (75%) enzymes, the ash % 4.07± 0.28, 3.04± 0.21, and 3.47± 0.26 respectively, and 4.12± 0.24, 3.04± 0.52 and 3.25± 0.16 for renin, Osher (100%) and Jibeen (100%) enzyme respectively. Also a significant variation ($p<0.05$) was detected betweenthe means of the milk components (protein, fat, ash %) for the different stages, whole lactation period and all treatments. The milk components showed low percentages, when plant enzymes were used compared to renin enzyme, Jibeen enzymes gave higher average % rather than Osher enzyme. In general, coagulating the milk with plant enzymes resulted in a decrease in the total solids % of the soft cheese in association with the type of coagulant used, variations in the contents of the milk components in addition to the method of coagulation used.

According to panelists the points scored for flavor were 9.00± 0.63, 5.87± 0.62 and 6.62± 0.69 using renin, Osher (50%) and Jibeen (50%) enzymes respectively. Using renin, Osher (75%) and Jibeen (75%) as 8.83± 0,86, 5.62± 0.76 and 6.62± 0.76 respectively, and 8.79± 0.77, 5.91± 1.28, and 6.87± 0,74 when renin, Osher (100%) and Jibeen (100%) enzymes were used.

Concerning the taste, the points attained when using renin, Osher (50%) and Jibeen (50%) enzymes, 8.08± 0.71, 6.25± 0.82, and 7.20± 0.88 respectively. 8.54± 0.77, 5.83± 0,76 and 6.58± 0.71 when using renin, Osher (75%) and Jibeen (75%) enzymes respectively, and 8.45± 0.88, 5.79± 0.83 and 6.50± 0,78 for renin, Osher (100%) and Jibeen (100%) enzymes respectively.

For texture, the points given when using renin, Osher (50%) and Jibeen (50%) enzymes were 8.97± 0.77, 5.83± 1.04 and 7.12± 0.97 respectively. Scored points when using renin, Osher (75%) and Jibeen

(75%) enzymes were 8.66± 0.70, 5.91± 0.71, and 8.12± 0.67 respectively, and 8.58± 0.88, 5.97± 0.72, and 8,08± 0.71 for renin, Osher (100%) Jibeen (100%) enzymes respectively. Also a significant difference ($p<0.05$) was detected between the average points given for flavor, taste and texture for all stages, whole lactation period and all treatments. Renin cheese scored the highest points for the sensory characteristics followed by Jibeen cheese. Osher cheese scored the lowest points. Variation in flavor, taste and texture of the soft cheese produced by the different coagulants may be related to the type of enzymes used for coagulation and their concentrations, chemical composition of the cheese milk and also the decrease of proteolytic activity of plant enzymes, due to increase of acidity when starterculture was added.

Based on the results obtained, Jibeen enzyme may be considered as an alternative for other enzymes, when the availability, especially renin enzyme is restricted for one reason or others.

مستخلص

أجريت هذه الدراسة لمعرفة أثر استخدام إنزيم الرنين والأنزيمات النباتية (العشر والجبين) في زمن التجبن، الإنتاج، التركيب الكيميائي، والصفات الحسية للجبنة البيضاء واستخدام أنزيم الرنين للمقارنة واستخدمت الأنزيمات النباتية بثلاثة تركيزات (50%، 75%، 100%). وتمت الدراسة خلال ستة شهور قسمت لثلاثة فترات، (أول، ثاني، ثالث شهرين بعد الولادة).

أظهرت الدراسة أن زمن التجبن الكلي خلال فترة الحليب الكلية لجميع المعاملات عند إستخدام أنزيم الرنين، عشر (50%)وجبين (50%) كانت 4.62 ±97.33، 5.88 ±124.37، و 4.96 ±96.29، على التوالي. وكانت 3.57 ±80.37، 7.29 ±113.62، و 2.23 ±87.75 عند إستخدام إنزيم الرنين، العشر (75%) والجبين (75%) على التوالي. بينما كانت 3.54 ±70.45، 5.62 ±108.83، و 4.95 ±85.95 عند استخدام إنزيم الرنين ، العشر (100%) والجبين (100%) على التوالي. أظهرت الدراسة أن هنالك فرق معنوي لزمن التجبن بين الثلاثة أنزيمات المستخدمة حيث تطلب إنزيم الرنين زمناً أقل مقارنة بالأنزيمات التباتية، كما تطلب الجبين زمناً أقل بمقارنته بالعشرر. وقد يعزى ذلك لنوع الأنزيم المستخدم، التركيب الكيميائي للبن، طريقة التجبن ثم قابلية اللبن المستخدم للتجبن.

إنتاج الجبنة الكلي (جرام لكل 5 كيلوجرام لبن) عند إستخدام الرنين، العشر (50%) والجبين (50%) كان 8.66 ±913.5، 14.09 ±635، و15.58 ±652.25، على التوالي. وكان إنتاج الجبين (جرام لكل 5 كليوجرم لبن) 32.9 ±944.3، 16.99 ±697.8، و 41.8 ±729.3 عند إستخدام انزيم الرنين، عشر وجبين (75%) على التوالي، بينما كان إنتاج الجبنة 11.54 ±916.0، 16.7 ±788.72 و 9.55 ±804.3 عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) على التوالي. أظهر الإنتاج الكلي للجبنة فرقاً معنوياً ($p0<0.05$) خلال دورة الحليب الكلية لجميع المعاملات. أظهر تجبن انزيم الرنين إنتاجاً جيداً للجبنة وتلاه انزيم الجبين. الإحتلافات في إنتاج الجبنة تعزى لتركيب اللبن، الإنزيم المستخدم ونوعه وا نتاج الخثرة الضعيفة بواسطة الأنزيمات النباتية.

خلال دورة الحليب الكلية لجميع المعاملات وعند استخدام انزيم الرنين، عشر (50%) وجبين (50%) كانت نسب البروتين 0.44 ±14.42، 0.46 ±11.96، و 0.74 ±13.44، على التوالي. وكانت نسبة البروتين 0.36 ±14.26، 0.35 ±12.14، و 0.60 ±13.61 عند استخدام انزيم الرنين، العشر والجبين (75%) على التوالي. بينما كانت عند استخدام انزيم الرنين

العشر (100%)، والجبين (100%) 0.39 ±14.36، 0.26 ±12.02، و 0.42 ±13.55 على التوالي.

كانت نسب الدهن عند استخدام انزيم الرنين، العشر (50%) والجبين، 0.84 ±20.06، 0.75 ±15.99، و 0.80 ±16.95 على التوالي. كانت نسبة الدهن 0.8 ±19.97، ±16.0 0.59، و 0.44 ±18.35 عند استخدام انزيم الرنين، عشر (75%) وجبين (75%) على التوالي. بينما كانت نسب الدهن عند استخدام انزيم العشر (100%) والجبين (100%) 0.57 ±20.39، 040 ±15.91، و 0.31 ±18.54 على التوالي.

كانت نسب الرماد عند استخدام انزيم الرنين، العشر (50%) والجبين (50%) ±4.27 0.37، 0.31 ±3.27، و 0.26 ±3.47 على التوالي. وكانت نسبة الرماد عند استخدام انزيم الرنين عشر (75%) والجبين 0.28 ±4.07، 0.21 ±3.04 و 0.26 ±3.47 على التوالي. بينما كانت نسب الرماد عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) 0.24 ±4.12، ±3.04 0.52 و0.16 ±3.25 على التوالي. أظهرت الدراسة أن هنالك فروق معنوية في متوسط نسب البروتين، الدهن، الرماد ($p<0.05$) خلال دورة الحليب الكلية لجميع المعاملات. أعطت الأنزيمات النباتية نسب أقل لمكونات اللبن (البروتين، الدهن، والرماد) بينما ارتفعت نسب هذه المكونات عند استخدام انزيم الرنين. وقد لوحظ ارتفاع نسب هذه المكونات عند استخدام الجبين مقارنة بالعشر. التجبن بواسطة الأنزيمات النباتية يؤدي لإنخفاض نسبة المواد الصلبة الكلية. وقد يعزى ذلك لإختلاف في تركيب اللبن أو طريقة التجبن المستخدمة.

عند إجراء إختبارات التذوق أعطت النكهة 0.63 ±9.00، 0.62 ±5.87 و 0.69 ±6.62 نقطة عند استخدام انزيم الرنين عشر 50% والجبين 50% على التوالي. بينما أعطت 0.86 ±8.83، ±5.62 0.76 و 0.76 ±6.62 نقطة عند استخدام انزيم الرنين عشر (75%) والجبين (75%). بينما أعطت النكهة 0.77 ±8.79، 1.28 ±5.91 و 0.74 ±6.87 عند استخدام انزيم الرنين عشر (100%) والجبين (100%) على التوالي.

بالنسبة للطعم أعطى 0.71 ±8.08، 0.82 ±6.25 و 0.88 ±7.2 نقطة عد استخدام انزيم الرنين، عشر 50% والجبين 50%. وعند استخدام الرنين عشر (75%) والجبين (75%) على التوالي 0.77 ±8.54، 0.76 ±5.83 و 0.71 ±6.58 على التوالي. بينما أعطى الطعم 0.88 ±8.45، 0.83 ±5.79 و ±6.50 0.78 نقطة عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) على التوالي.

بالنسبة للقوام أعطى 0.77 ±8.97، 1.04 ±5.83، و 0.97 ±7.12 نقطة عند استخدام انزيم الرنين، عشر 50% والجبين 50% على التوالي. وأعطى القوام عند استخدام انزيم الرنين، عشر (75%)

والجبين (75%) 0.7 ±8.66، 0.71 ±5.91 و 0.67 ±8.12 نقطة على التوالي. بينما اعطى القوام 0.88 ±8.58، 0.72 ±5.97 و 0.71 ±8.08 نقطة عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) على التوالي.

أظهرت الدراسة أن هنالك فروق معنوية لمتوسطات النقاط التي أعطيت للنكهة، الطعم والقوام خلال دورة الحليب الكلية لجميع المعاملات. أعطيت العينات التي تم تجبينها بواسطة إنزيم الرنين نقاط أعلى عند استخدام الإنزيم المستخلص من العشر. وقد تعزى هذه الإختلافات نتيجة لأنواع الأنزيمات المستخدمة وتركيزاتها، التركيب الكيميائي للبن المعد لصناعة الجبن ولإنخفاض مقدرة الأنزيمات النباتية لتحليل البروتين، وذلك لزيادة الحموضة عند إستخدام البادي في صناعة الجبنة.

بناءاً على هذه النتائج يمكن إستخدام الإنزيم المستخلق من الجبين كبديل لإنزيم الرنين عند عدم توفره.

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Chapter One

1.0 Introduction:

Milk is a global drink that is a polyphasic emulsion having physical, chemical and biological properties and can be fermented into a wide range of different products with different flavors, consistencies and structure (Huria, 2002). 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).

To maintain the maximum use of milk, it should be transferred to milk products as to decrease the percentage of water that leads to the reduction of the activity of microorganisms which make milk spoil, e.g. lactic acid bacterial (Aziz, 1977).

One of the major milk products known is the cheese. Fox, et. al. (2007) stated, cheese is the generic name for a group of fermented milk based food products produced throughout the world in a great diversity of flavors, texture and forms.

The making of cheese as a mean of preserving the most important constituents of milk in 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 a long time, (Niir, 2010).

O'Conner (1993) explained, cheese is an excellent source of protein, fat, and minerals such as calcium, iron, phosphorus, vitamins and essential amino acids.

The trend nowadays is to produce different new varieties and types of the so-called functional cheeses as a functional dairy products Saxelin et. al. (2003) 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 foods 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 a gel (coagulation) is a basic step common to all types of cheeses. The coagulation of milk is a consequence of protein destabilization, which is brought by acid proteinases chymosin, the active component of renin. (Varnam and Sutherland, 1994).

According to O'Conner (1993), renin is a general term that describes a variety of enzymes of animals (specially calves), plant or microbial origin used to coagulate milk during cheese making. For coagulation of milk in the manufacture of cheese, calf renin is the most wide-spread and desirable and has been dominant in the industry of cheese processing for a long time. Gouda (1990) mentioned, calf renin is used in cheese making and is important in the formation of the casein network during coagulation and known to contribute to proteolysis in pickled cheese. Since the past century a shortage in calf renin had been noticed due to the decrease in the availability of sucking calves, as they are mainly reared for milk or beef production rather than other purposes. So, to cover the supply of the cheese industry with calf renin, it was to a remarkable extent restricted. Craw (1993) indicated, the limited supply of renin 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 overcome 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 coagulating enzymes extracted from Calotropis procera (Sodom apple) and Solanum dubium (Jibeen) plants are used in traditional cheese production as substitute of calf renin.

The general objectives of the current research is to study and compare the effects of renin and plant enzymes milk coagulants on cheese quality, i.e. composition, sensory characteristics and yield in association with enzyme activity, time of coagulation, ripening process and period, salting and acceptability of processed cheese by consumers.

1.1 Objectives:

The main objectives of this research are:

- To study the effects of using renin and plants coagulants on the coagulation time during white soft cheese manufacturing.
- To study and assess the effects of using renin and plants coagulants of yield, composition, and sensory evaluation of produced white soft cheese.
- 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 coagulating the casein with the help of renin 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 some 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 whey from curd, with the exemption of some 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 Association (IDFA) (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 renin, 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.

Cheese is a group of fermented milk products made basically for preservation of milk constituents safe for consumption for longer periods as given by Shahata (1997).

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 8000 B.C (Clarence et. al. 2004).

There is no conclusive evidence indicating where cheese making originated from either 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.

Until its modern spread along with European culture, cheese was nearly unheard of in oriental cultures, uninvented in the pre-Colombian Americas and of only limited use in Sub-Mediterranean Africa, mainly being wide spread and popular only in Europe and areas influenced strongly by its culture. But with spread, first of European imperialism, and later of Euro-American culture and food, cheese has gradually become known and increasingly popular worldwide, though still really considered a part of local ethnic cuisines outside Europe, Middle East and Americas. (Mc Gee 2004).

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%

Abu Daood et. al. (2003) divided the types in three major groups, hard, semi-hard and soft with a moisture content of 30-35%, 40-50% and 50-70% for each respectively.

Four major groups are given by Alkholi (1999), which were very hard, hard, semi hard and soft with a moisture content of 30-40%,40%, 40-45%, and 45-75% respectively. A moisture content of less than 40% is given for dried very hard, 40-49%, for very hard, 50-59% for hard, 60-69% for soft and 70-80% for fresh cheese (Shahata, 1997).

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

Spreer (1998) explained, for another classification into groups and types, different aspects and characteristics can be used, such as:

- Types of consistency (hard, semi soft and soft cheese).
- Types of milk (cow, sheep, goat, buffalo).

- Chemical composition (Ca-content in conjunction with pH_1 dry matter, water, fat).
- Ripening process (ripened, non-ripened, fresh cheese).
- Variation in taste.
- Type of whole formation (large, medium and small round holes, cracks, irregulars holes, no holes).
- Surface characteristics (blue fungus or white fungus cheese, smear cheese, skinless cheese).

2.4 Chemical Composition of Cheese:

Cheese contains almost all milk components in concentrated form such as protein, fat, minerals and lactose, which to greater extent is converted into organic salts (Elnimer 2007). Murshidi (1998) explained, cheese contains the undissolved components of milk, e.g. casein, amounts of fat and salt beside water containing few amounts of salts, lactose and albumin.

Renin cheese composes in average of 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 into 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 cheese as given by the Sudanese Standards 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%.

Caric et. al. (1993) indicated, the protein content of Sudanese white soft cheese was found to be 19.76% and 20.12% when the fat % of the milk used ranged between 2.2% and 4.4% respectively. Different fat levels, ripening time of the cheese, different storage periods and types of

packaging showed in most cases highly significant difference ($P < 0.001$) for protein content of the processed cheese (Caric et. al. 1993). The fat content of Sudanese white soft cheese ranges between 15.4% and 23.4%, when the percentage of the fat content of cheese milk ranges between 2.2-4.4% as given by Dimitreli et. al. (2004) and also showed significant differences with different fat levels, ripening time and storage period of produced cheese.

Concerning the ash level of the cheese, it was found to range from 1.75% to 5.35% in association with the storage period and the fat content. The ash % was decreased with long periods of storage and the decrease in the fat levels as explained by Abdalla (1993). The total solids of the white soft cheese range between 41.58% and 50.32%, when the fat of the milk prepared for cheese making was 2.2 and 4.4 respectively and also a high significant variation was reported due to variation in the fat content of the cheese, (Abdel Razige, et. al. 1996).

2.5 Milk Coagulation:

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

either by microorganism or by plant enzymes extracted from some types of plants.

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, must 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 into a gel state (coagulate, gallert), especially into a lyogel.

The coagulation of milk is influenced mainly by the type and concentration of coagulation enzyme, coagulation temperature, properties and concentration of proteins and the pH value, (Storry and Ford, 1982).

2.5.1 Renin Coagulation:

2.5.1.1 Mechanism of Coagulation:

The mechanism of casein precipitation by enzymatic coagulation after addition of the recent enzyme to milk is described by Fredriksen (2011), Spreer (1998) and Daviani et. al (1980) as follows:

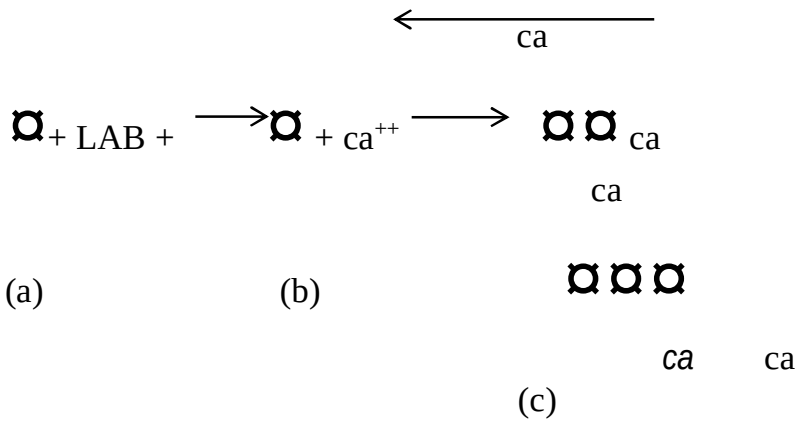
The casein precipitation takes place in two stages:

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 (renin gel, renin gallert) and it is the real cheese material.

Fig. (1) Shows the described mechanism



(a) Casein micelle.

(b) Casein particle without the effect of the protective colloid.

(c) Casein paracasinate (Renin gel).

The milk serum proteins are not affected by the enzymatic reaction, since they remain water insoluble and migrate as whey proteins in the aqueous phase.

2.5.2 Renin coagulation:

Renin or (renin) is a complex of enzymes produced in the stomachs of ruminants mammals. Chymosin is a protease enzyme that curdles the casein in milk. This helps young mammals digest their mother milk. Renin can also be used to separate milk into solid curds for cheese making and liquid whey. In addition, chymosin renin contains other important enzymes such as lipase. Renin is used for the production of most cheeses. The mammal must be slaughtered to obtain its renin. Non-animal alternatives for renin are suitable for consumption by vegetarians (Kopelman et. al. 1975).

2.5.3 Types and Production of Renin

2.5.3.1 Production of Natural Calf-renin:

Natural calf renin is extracted from the inner mucosa of the fourth stomach chamber (the abomasum) of young unweaned calves as part of the livestock butchering. The stomachs are by products of real production. If renin is extracted from calf it is not suitable for lactovegetarians to consume.

2.5.3.2 Vegetable Renin:

Many plants have coagulation properties. Greeks used extract of fig juice for coagulation, also dried caper leaves, nettles, mallow and ground ivy (creeping chartie) were used also. Enzymes from Cynara or thistle were used in some traditional cheese production in the Mediterranean. Phytic acid derived from unfermented soybeans or fermentation produced chymosin (FPC) may also be used (Farkye, 2004).

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

2.5.3.3 Microbial Renin:

Some molds such as Rhizo mucor miehe are able to produce proteolytic enzymes. These molds are produced in a fermenter 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 renin tend towards some bitterness especially after long maturation periods. Cheeses produced by this way are suitable to vegetarians (Lee et. al.. 1990).

2.5.3.4 Fermentation Produced Chymosin:

Because of the above imperfections of microbial and animal renin, many producers sought further replacement of renin. With development of genetic engineering, it became possible to isolate renin genes from animals and introduce them into certain bacterial, fungi or yeast to make them produce chymosin during fermentation (Hayalogllu, et. al. 2004).

The genetically modified micro-organism is destroyed after fermentation and chymosin isolated from fermentation broth, so that the fermentation produced chymosin (FPC) used by cheese producers does not contain any genetically modified microorganism component or ingredient. FPC is identical to chymosin made by animals, but produced in more efficient way. FPC products have been on market since 1990 and have been considered in the last 20 years the ideal milk clotting enzyme, (Singh, et. al. 1989).

Fermentation produced chymosin is used more often in industrial cheese making in North America and Europe today because it is less expensive than animal renin (Uysal, et. al. 1996).

FPC was the first artificially produced enzyme to be registered and allowed by the U.S. Food and Drug Administration, in 1990 about 60%

of United State hard cheese was made with FPC, and it has up to 80% of the global market share for renin.

By 2008, about 80% to 90% of commercially made cheeses in the United State and Britain were made using FPC (Tulay et. al. 2013). Today the most widely used FPC is produced either by the fungus Aspergillas niger and commercialized under the trade mark CMY-MAX® by the Danish company Chr. Hansen or produced by Kluyveromyces lactis and commercialized under the trade mark MAIREN® by Butch company DSM.

FPC is chymosin B, so is more pure compared with animal renin which is a multitude of proteins. FPC can deliver several benefits to cheese producers compared to animal or microbial renin, such as higher production yield, better curd texture and reduced bitterness. (Fox, et. al. 1996).

Cheese produced with FPC can be certified Kosher and Halal and suitable for vegetarians if no animal based alimentation was used during the chymosin production in the fermenter, (Lee, et. al. 1990).

Older calves (grass fed or grain fed) renin contains less or no chymosin, but a high level of pepsin and can only be used for special types of milk and cheeses. As each ruminant produces a special kind of

renin to digest the milk of its own species, milk specific renin are available such as type of goat renin for goats milk and lamp renin for sheep's milk (Van Haoydonk 1987).

2.5.3.5 Traditional Method of Renin 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 crude renin that remains in the filtered solution can then be used to coagulate milk. About 1g, of this solution can normally coagulate 2.4 litre of milk (Cremer1985).

2.5.3.6 Modern Method of Renin Production:

Deep frozen stomachs are milled and put into an enzyme extracting solution. The crude renin extract is then activated by adding acid, the enzymes in the stomach are produced in the active form and are activated by the stomach acid. The acid is then neutralized and the renin extract is filtered in several stages and concentrated until reaching a typical potency of about 1 gram coagulates 15kg of milk. One kg of renin extract has about 0.7g of active enzymes. The rest is water and salt and sometimes sodium benzoate (0.5-1.9%) for preservation and typically 1kg of cheese contain about 0.0003 of renin enzyme (Najera, et. al 2008).

2.5.3.7 Alternative Sources of Renin:

Because of the limited availability of mammalian stomachs for renin 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 renin range from plants and fungi to microbial sources. Cheese could be produced from any of these varieties of renin.

2.5.4 Plant Coagulation:

Plant coagulation was not registered till as commercial methods of coagulation due to a lot of hazards 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). Plants used for coagulating milk are e.g. the following:

2.5.4.1 Solanum dubium (Jibeen) Coagulation:

It is a wild plant considered as weed found in most areas of Sudan. It has not an economic importance. Locally, named Jibeen, and belongs to family Solanaceae.

Enzymes extracted from family Solanaceae were used in several trails for coagulation of milk. The coagulated milk gave different cheese compositions, yield and time required for coagulation (Beeby, 1980), and reported that coagulation took much time compared to renin enzyme coagulation, found significant difference among the same plant and that Solanum dubium was the best among the spp. of Solanum, but the chemical composition of the cheese showed low percentages in all components compared to all other types of enzymes used for coagulation. These results were similar to those of Andren, et. al. (1982), who reported that the plant coagulation resulted in low quality cheese specially Solanum Spp. and Calotropis procera and found low percentages of most cheese components and its total solids compared to cheeses made from milk by renin coagulation.

Talib et. al (2007) studied the coagulating properties of Solanum dubium (Jibeen) seed extracts. The Jjibeen seeds were extracted with both water and citrate phosphate buffer. Effect of enzyme concentration, milk pH, milk temperature and heat inactivation of crude enzyme 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 enzyme was decreased at pH of the milk over 6.2, increasing of the milk temperature above 40^{o}C, decreased the clotting

time and the activity of the enzyme was lost on pH 4.6 and 6.6 and temperature 60^{o}C at pH 3.6 for 10 minutes, but at pH 4.6, 5.6 and 6.6 and temperature 70^{o}C, the enzyme activity was not affected, but it lost its activity at 80^{o}C/10 minutes. Shaw (1980) also noticed that plant enzymes are too proteolytic for cheese making. If proteolytic activity is excessive, cheese yield and retention fat in the curd may be diminished and it has undesirable effects on the body and texture of finished cheese (Yousif et. al 1996).

The quantification of milk activity in solutions containing proteolytic enzymes is a major concern in industrial cheese making, and cheese research as given by (Carlson et. al. 1985).

2.5.4.2 <u>Calotropis procera</u> (Osher) Coagulation:

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

It was known that early Osher plant specially the milk juice found in its fruit and leaves had the power to coagulate milk, but it was tested for coagulation recently by Dalgleish (1985) who explained the potentially of this plant for coagulation, but reported a slow development for coagulation followed by decrease of the percentage of all cheese components, and its 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 cheese with low percentage of protein and fat in addition to the investigation of some toxic materials during the analysis of chemical composition of this plant.

2.5.4.3 Terristris Enzyme Coagulation:

Terrestris is an annual plant belongs to the family Zygophyliaceae widely distributed around the world.

Enzyme extracted from this plant were known to the nomads who used this plant very early (all parts of the plant) for milk coagulation. de Koning, et. al. (1978), reported that some trials were conducted to study the ability of this enzyme to coagulate milk and technological characteristic of some types of white cheese, and mentioned this plant had a good tendency to coagulate milk, after some time coagulation began and completed after 92 minutes, so the enzyme required much time for coagulation compared to renin enzyme but these results showed no significant difference, compared to control enzyme (Renin). The chemical composition was mildly differ and showed also no significant difference. In addition to this, cheese yield and sensory evaluation followed the same trend. These results were parallel to those of Kaye, et. al. (1978) who found that the chemical composition (fat % and protein %) did not show a significant variation, when they tested three

concentrations of the enzyme extracted from the fruit of the plant and explained, there was a significant difference among these three concentrations for milk coagulation, but there was no significant difference between the higher concentrate and renin enzyme for milk coagulation, but the percentages of cheese components and its total solids tend to be higher when milk coagulated by renin enzyme compared to different concentrations of Terristris enzyme.

2.5.4.4 Cynara cardunculus Coagulation:

According to Sofia et. al. (2005), enzymes extracted from plant Cynara Cardunculus can also be used for coagulation, which proved to have a proteolytic activity. Cyprosins (Cunarases) are aspartic proteinases present in the aqueous extract of cynara carunculus L. (Cardoon) flowers used as milk coagulant for the manufacture of some Portuguese and Spanish traditional cheeses. Synprosins have an activity on k-casein similar to that of renin and a pronounced specific activity on other casein fractions (Queiroz et. al. 1993).

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 ca^{++} in milk. So, any factor that affects the content of the ca^{++}

affect the time of coagulation. Heat treatments 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 two cases a source of ca^{++} must be added to milk prepared for cheese processing, e.g. $Cacl_2$ which is added at certain level (0.02% and 0.03%) for sterilized and dried milk respectively (Scancalapore, et. al. 1988). For this reason when coagulation time was compared to different treatments, milk should be identical for each treatment avoiding all factors that affect the levels of ca^{++} in milk.

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

One of the main factors that affects the time of coagulation is the method of coagulation. Natural milk produced from healthy cow required 90-105 minutes for total coagulation. This time is changed when the coagulation enzyme is changed (van Hooydonk, et. al. 1984). This demonstrated that renin coagulation required few time compared to plant enzyme and showed a significant differences ($P<0.05$) among the

different methods of coagulation. However, the time of coagulation also differed between different types of plant enzymes. Hamed (1998), compared two types of plant enzyme (from solanum and terristris) and reported that solanum enzyme required a lot of time for coagulation compared to terristeris enzyme and thus followed by weak milk curd and low percentages of cheese yield.

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

($p > 0.05$) between some spp. of terristris and renin enzyme, but he found significant difference among some plant enzymes and renin enzyme.

Some investigators reported no significant difference between three methods of coagulations (renin enzyme, FPC and plant enzymes).

A close relationship was found between milk clotting activity and time in association with plant enzyme used and milk quantity. The milk clotting activity was calculated by Ibiama and Griffiths, 1987) as follows:

$$X = 100\ D/T$$

Where X = Milk clotting activity (unit/ml)

D = Dilution or quantity of milk containing 1ml of the crude enzyme.

T = Clotting time in seconds.

Sinyth et. al. (1987) revealed a significant difference among three treatments used to compare the length of time of coagulation between renin enzyme and two types of plant coagulation enzymes extracted from Terristeris spp. and Solanum spp.

2.7 Cheese Yield:

The typical yield of cheese ranges from 9-15% depending on the chemical composition of the milk, efficient recovery of fat and casein in the cheese, losses of milk constituents in the whey resulting from milk handling and treatment and cheese making procedure and the final moisture content of the cheese (Frakey, 2004). According to Abel Razig (1996), cheese yield from cow milk (2% salt) was found to be 19.08%. Babiker (1987) gave a range of 8-14% for unsalted white soft cheese and Khateeb (1997) noticed, the yield of fresh cheese between 23.7 – 33.34% with an average of 27.8%.

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

- Milk composition.
- Gentic variation.
- Physiological factors.
- Processing conditions.
- Lactation stage.
- Seasonal variation.
- Type of milk.
- Starter culture used.

- Standardization of milk.
- Heat treatment of milk.
- Homogenization of milk.
- Type of coagulant used.
- Curd firmness.
- Curd handling system.
- Storage of milk.

The cheese yield is directly related to the final moisture content of the finished cheese, in addition to milk composition, degree of recovery of the fat and casein by the curd during cheese making, explained Kosikowshki (1978).

Abdel Razig and Babiker (2009) mentioned the weight loss of cheese significantly affected by storage time and it increases gradually till the end of the storage period

2.7.1 Yield by Renin and Plant Enzymes Coagulation:

Foltman (1987) explained, cheese yield increased when the ca^{++} increased and he recommended addition of $Cac1_2$ to milk exposed to ultra heating. Renin coagulation gave good results for cheese yield when the $Cac1_2$ was added when dry milk or sterilized milk were used.

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

Another results were obtained by Sinyth, et. al. (1987) who demonstrated that plant coagulation resulted in low yield of white cheese due to weak curd produced by plant coagulants.

These results were in line to those given by Merin, (1989) that 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. The renin 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 renin power for coagulation and then cheese texture and cheese yield were progressed, but increasing the temperature above $40^{o}C$ affects the power of the enzyme and altered the coagulation process followed by decrease in cheese yield (Nijera 2003).

Another recent result by Oscan, et. al. (2012) indicated that use of starter culture and its addition to milk prepared for cheese making increases cheese yield; this may due to the maximum renin activity when the acidity of milk increases and also to the protease enzymes of plant origin used and their coagulation power. But all of them showed significant difference compared to renin enzyme; also cheese yield coagulated by Terristeris spp. gave results near to the results of renin enzyme, but some variations in cheese between the two types of 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.

Renin 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 renin enzyme compared to different plant enzymes. Dalgleish et. al. (1989) gave that the ash content of cheese was created by using renin 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 renin 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 used for 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 renin 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 two months up to four months after calving) showed a significant difference ($p<0.05$) for protein and fat percentages and the ash percentage was higher but no significant difference ($p>0.05$) for ash percentages was found when milk was

coagulated by renin enzyme compared to plant enzymes and microbial enzyme. Furthermore, some plant enzymes, e.g. (Terrestiris enzymes) tend to give results near to the renin enzyme and also no significant difference between these enzymes and renin enzyme was detected. The results obtained by Kumosinski, et. al. (1991) showed that most of cheese components and its total solids differ according to the type of enzyme used for coagulation. The percentages of protein and fat showed a significant difference between renin enzyme and two types of plant enzymes (two different concentrations of solanum and terristris enzymes). Terristris enzyme gave results near to that of the control enzyme, which was renin enzyme. Horme, (1990) compared three methods of coagulation enzymes (renin, 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 renin coagulation and lower when milk was coagulated by plant enzymes specially the enzyme extracted from (Calotropis procera plant) due to the losses of most milk components with the drained whey according to weak curd formed when milk was coagulated by these enzymes.

Also a significant difference was detected between renin and plant coagulation (enzymes extracted from Terristeris ssp. and solanum spp),

but comparing the coagulation caused by renin enzyme and terristris spp enzyme no significant difference was recorded ($p>0.05$) despite the increase in yield of renin cheese (Sinyth, et. al. 1987).

2.9 Sensory Evaluation:

Humans have used their senses to evaluate 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. safe 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 the 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 the 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 the 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 free of bitter taste, rancidity and rottening.
- 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, when abnormal change in color, advanced dryness or abnormal rotenning, blowing and abnormal holes are noticed.

Kumosinski et. al. (1991) reported, the taste, texture and flavor of cheese were affected by the method of coagulation and found significant differences 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 evaluation, when different types of plants, microbial or renin enzymes were used. This was clear to taste and flavor, but some differences were found for the texture, even among the same spp. of plant enzyme, although these differences were not statistically significant.

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

The excessive proteolytic activity of the plant enzyme during the ripening process of the cheese has undesirable effects on the body and texture of the finished cheese as noticed by Yousif et. al. (1996).

Cheese contained different amounts 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 preservative effect, favors water binding, promotes formation of the skin and finally, influences the solidification of the cheese, which increases with increasing salt concentration.

Chapter Three

3- Materials and Methods:

Three types of cheese using three different coagulants were manufactured. The three coagulants used were renin enzyme and two plant enzymes extracted from Osher (Calotropis procera) and Jibeen (Solanum dubium).

The renin enzyme and the produced cheese were used as control. The other plant enzymes were used with different concentrations, (50%, 75% and 100%).

3.1 Method of Extraction of Plant Enzymes:

3.1.1 Extraction of Osher Enzyme (Calotropis procera):

The leaves of the plant were dried by sun and air until a constant weight was reached. 100g were socked in distill water and Nacl (5%) for 24 hours. Then the solution was filtered by filter paper and kept in refrigerator at 7^{o}C

Then used as coagulant after three days only. Three concentrations were used (50%, 75% and 100%).

3.1.2 Extraction of Jibeen (Solanum dubium):

The fruit of the plant was used. It was crushed after drying to constant weight by sun. The plant was crushed then 100gm from the

plant was socked in salted distilled water (5% Nacl) for 24 hours and then filtered by filter paper and the solution was preserved in at 4-7^{o}C for three days and then used as coagulant with three different concentrations 50%, 75%, and 100%.

3.1.3 The Renin Enzyme:

The renin enzyme was obtained from a 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 in a solid form (tablets). Each tablet weight two grams and it was sufficient for coagulating 100kg of milk. Each tablet was dissolved into 100ml of distilled water only 5ml from the solution after dissolving the enzyme were added to each 5kg. 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 of Kenana farm which belongs to Kenana Sugar Compay (KSC) at Kenana, White Nile State, Sudan. Milk used during the normal period of lactation

(six months after calving) which was divided into three sub-periods explained as follows:

a. The first two months after calving (1st. stage).
b. The second two months after calving (2d. stage).
c. The third two months after calving (3rd. stage).

3.3 Collection of Samples:

For each treatment eight samples of milk were collected every two months for each concentration and hence 24 samples were collected for each treatment during every period, i.e. for each treatment 3x24 = 72 samples, were collected. The total numbers of the samples collected during the whole period were 72x3 = 216 samples of white cheese.

Samples were collected weekly. For each sample 5kg of milk were used for cheese making. The total amount of milk used during this study was 216x5 = 1080kg during the six months.

3.4 Source of Plant Enzymes:

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

3.5 Procedures of Manufacturing:

3.5.1 Renin Cheese:

1. 5kg of milk were taken from the milk of the dairy herd of Kenana farm.
2. Milk was heated to 72^{o}C , then cooled to 42^{o}C.
3. Starterculture was added (1%) and then temperature was adjusted to 42^{o}C for 45 minutes.
4. Renin enzyme was added, then coagulation of milk was observed.
5. Time of coagulation was recorded from the addition of the renin enzyme till the complete coagulation of milk occurred.
6. When coagulation occurred, the curd was put on wooden trail, surrounded by clothes with harrow orifices to ensure good draining of whey.
7. Cheese was salted by socking it into salty solution, where the concentration of Nacl was 10% for 24 hours.
8. The weight of cheese was determined after the salting was completely done after 24 hours from the beginning of cheese salting.

3.5.2 Osher Enzyme (Calotripis procera) Cheese:

1. 5 kg of cow milk were obtained from the milk of Kenana farm dairy herd.
2. Milk was heated to 72^{o}C and then cooled to 42^{o}C.
3. 1% of starterculture was added and temperature was adjusted to 42^{o}C for 45 minutes.
4. The extracted Osher enzyme was added at three different concentration (50%, 75%, and 100%) respectively during the first, second and third two months after calving respectively.
5. Time of coagulation was recorded from the addition of the enzyme till the complete coagulation of milk.
6. Cheese was salted by socking it into 10% solution of Nacl for 24 hours.
7. Cheese was weighted and its final weight was determined.

3.5.3 Jibeen Cheese (Solanum dubium):

1. 5kg of cow milk from Kenana dairy herd were used for cheese.
2. Milk was heated to 72^{o}C then cooled to 42^{o}C.
3. 1% of the starterculture was added and temperature was adjusted to 42^{o}C for 45 minutes.
4. Jibeen enzyme was added with three different concentrations (50%, 75% and 100%).

5. Time of coagulation was recorded from the addition of the enzyme till the complete coagulation occurred.
6. When coagulation occurred, the cheese was surrounded with clothes with narrow orifices and then put on wooden trails for complete draining whey.
7. Cheese was salted by Nacl by socking it into 10% Nacl solution for 24 hours.
8. Cheese weight was then determined.

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 during different periods of lactation until the complete coagulation occurred which was determined by the change of the watery 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 socking the cheese for 24 hours in 10% Nacl solution. Immediately after the period of socking cheese weight was determined by electrical balance weighing up to three kg and determine the weight to one decimal.

3.6 Laboratory Analysis:

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

Apparatus:

- Gerber tube with cork stoppers.
- Centrifuge (100 Revolution/1minute).
- Water bath.
- Holder

Material:

- Cheese samples.
- H_2SO_4 (90-91%).
- Amylalchol.
- Distilled water.

Procedure:

Ten (10)ml of sulphuric acid wee poured in clean and dry cheese Gerber tube followed by addition of 3grams minced cheese sample. Amyalchol is then added (1ml) to the mixture followed by addition of distilled water, mixture mixed thoroughly till no white particles were seen. The tube was then centrifuged to the water bath at 60-65^{o}C for 3-5 min. the fat % was read immediately from the Gerber tube.

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

Kjeldhal Method:

Apparatus:

- Kjeldhal Flask.
- Heaters
- Volumeteri flask (100nl)
- Conical flask
- Burrets
- Pippets.

Material:

- Cheese saple.
- Conc. H_2S0_4.
- Distilled water
- NaoH
- Boric acid.
- Indicator (promo cresol green + methyl-red
- Hcl
- - kjeldal tabs.

Procedures:

Three (3) gms of cheese and 2 Kjeldhal tablets were brought into the Kjeldhal flask and 25ml of conc. H_2S0_4 were added. The mixture, using a heater, then digested till a clear solution was obtained after 2-3 hours. The digested sample was then poured into volumetric flask and diluted to 100ml with distilled water. The 5ml from the dilution was transferred to Kjeldhal flask and 10ml of Naoh were poured, received in a conical flask containing 25ml of 4% boric acid and 3 drops of the indicator. The distillation was continued until the volume in the flask reaches 75ml. flask is removed and distillate was titrated against 0-14.Hcl, until red color was obtained.

The total protein % is calculated as follows:

Nitrogen % = T x 0.1 x 0.114 x 20/w x 100.

Protein % = Nitrogen x 6.38

Where:

T: Titration figure.

0.1: Normality of Hcl.

0.0114: Atomic weight of Nitrogen.

20: Dilution factor.

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

Apparatus:

- Aluminum dish.
- Dry oven.
- Dissecator.
- Muffle furnace oven.
- Balance.

Materials:

- Cheese sample.

Procedure:

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

  Ash% = $\_\text{Ash \%} = \frac{W1}{W0} \text{x } 100$

Where:

W1: Weight of ash.

W0: weight of samples.

3.7 Sensory Evaluation:

The sensory characteristics of the cheese samples produced by using renin, Solanum dubium and Calotropis procera enzymes during the different stages of lactation were judged by well-trained panelists in terms of flavor, taste and texture. Each property was evaluated by giving certain points and the average was then calculated. the assessment was performed using the evaluation sheet given below:

Evaluation Sheet

Item / Sample No.	**Flavor**	**Taste**	**Texture**
1			
2			
3			
4			
5			
6			
7			
8			
Mean			

Flavor	**Taste**	**Texture**
- High normal 10	- Highly palatable 10	- High consistent 10
- Very normal 7	- Very palatable 7	- Very consistent 7
- Normal 5	- Palatable 5	- Consistent 5
- Less normal 3	- Less palatable 3	- Less consistent 3
- Abnormal 1	- Unpalatable 1	- Not consistent 1

3.8 Statistical Analysis:

The obtained data were subject to statistical analysis by using program Statistical Package for Social Science (SPSS, 2007) program. Data of samples were analyzed statistically by using 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:

The obtained results for all treatments are given in the following tables.

Table (2) Average coagulation time (minutes) by renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	77.25	5.36	126.12	7.62	96.87	6.26	*
(2)	2nd stage	81.37	2.92	123.00	4.27	93.87	5.13	*
(3)	3rd stage	97.37	4.80	124.00	5.63	98.12	2.10	*
Total	Whole period	97.33	4.62	124.37	5.88	96.29	4.96	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

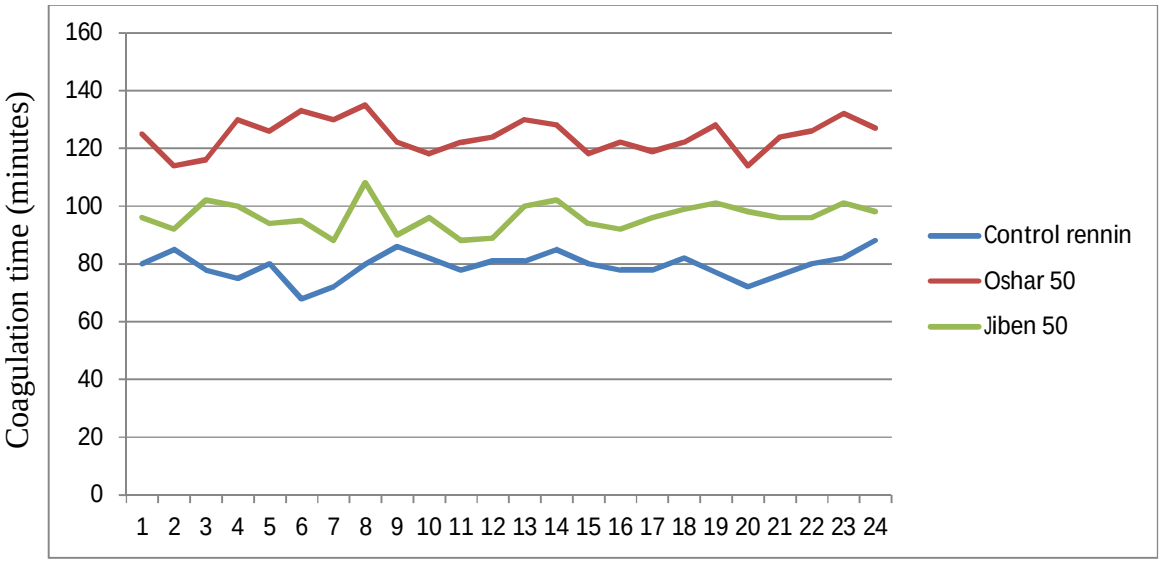
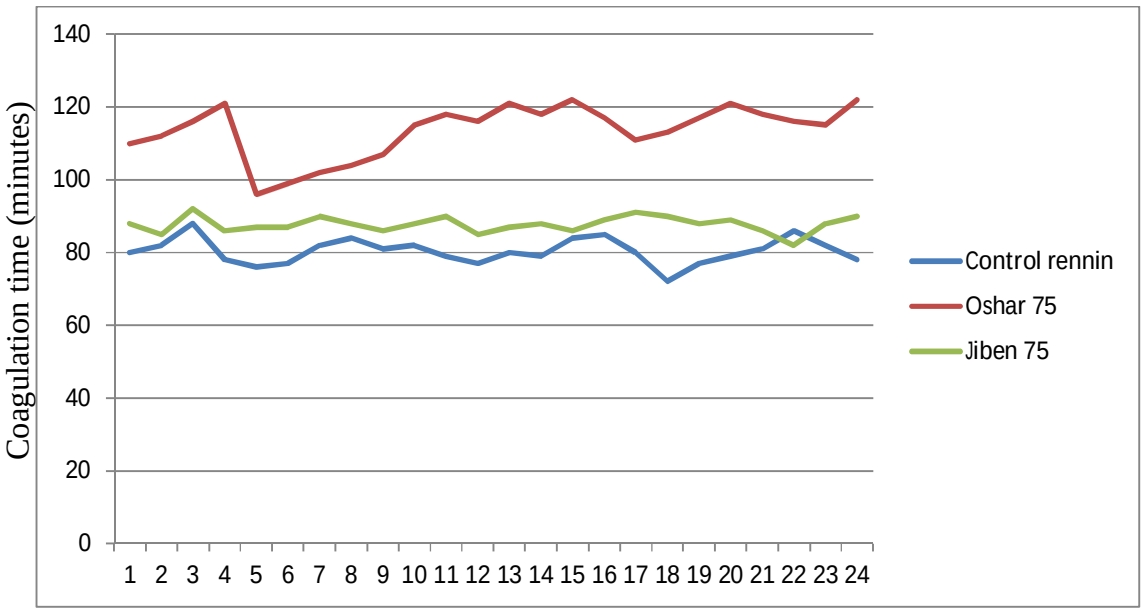


Table (3) Average coagulation time (minutes) by renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	80.87	4.62	107.50	5.88	87.87	4.96	*
(2)	2nd stage	80.87	2.69	116.75	4.59	87.37	1.68	*
(3)	3rd stage	79.37	4.06	116.62	3.73	88.00	2.87	*
Total	Whole period	80.37	3.54	113.62	7.29	87.75	2.23	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (4) Average coagulation time (minutes) by renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	78.87	2.94	111.12	7.39	85.12	5.22	*
(2)	2nd stage	80.12	4.67	109.37	3.85	83.62	4.24	*
(3)	3rd stage	79.37	3.54	106.00	4.34	89.12	4.12	*
Total	Whole period	79.45	3.65	108.83	5.62	85.95	4.95	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

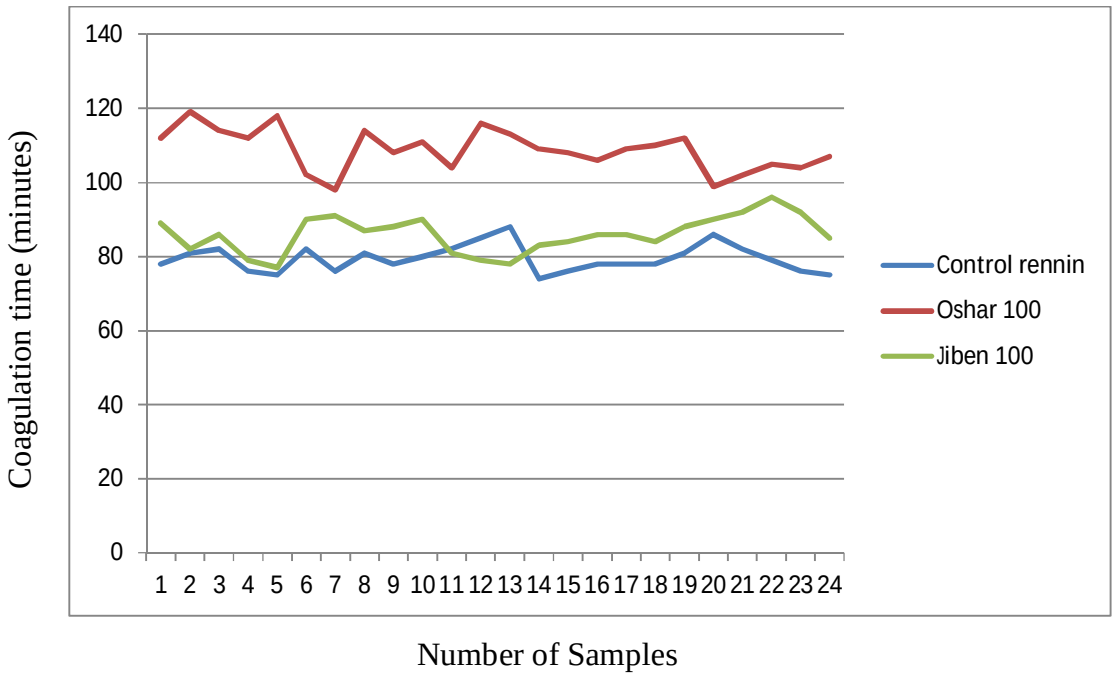


Table (5) Average cheese yield (grams) for each 5kg milk by addition of renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	915.7	5.09	635.2	13.07	656.0	10.91	*
(2)	2nd stage	916.00	9.05	645.8	15.23	672.6	20.30	*
(3)	3rd stage	909.00	10.14	626.8	6.93	659.1	9.37	*
Total	Whole period	913.5	8.66	635.9	14.09	662.5	15.58	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

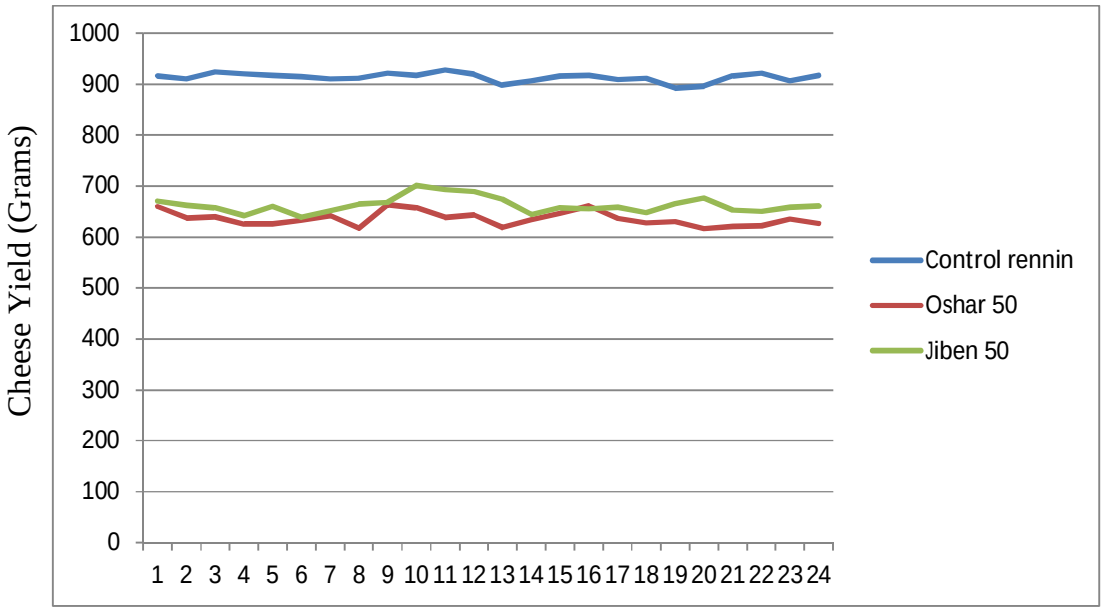
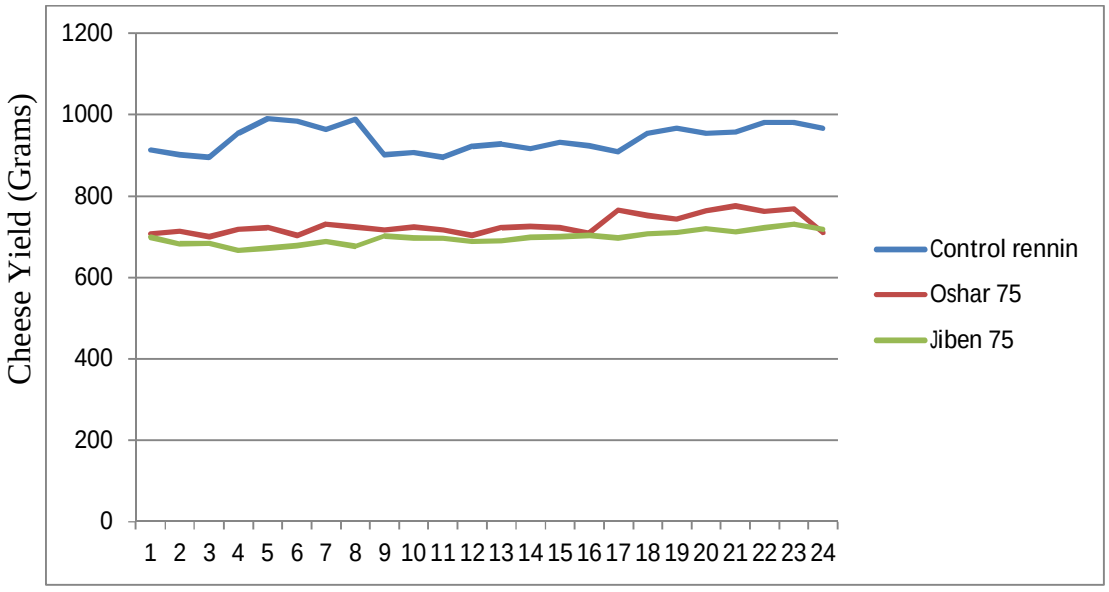


Table (6)Average cheese yield (grams) for each 5kg milk by addition of renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	949.1	39.51	681.1	9.87	715.2	10.67	*
(2)	2nd stage	915.7	13.12	697.2	5.36	717.5	8.01	*
(3)	3rd stage	958.7	22.87	715.1	10.28	755.3	20.44	*
Total	Whole period	944.3	32.89	697.8	16.99	729.3	41.80	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (7) Average cheese yield (grams) for each 5kg milk by addition of renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	912.3	15.06	776.2	21.04	800.6	11.22	*
(2)	2nd stage	916.2	8.17	790.0	10.65	800.0	4.50	*
(3)	3rd stage	919.6	10.70	800.0	10.97	812.7	5.70	*
Total	Whole period	916.0	11.54	788.7	16.70	804.3	9.55	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

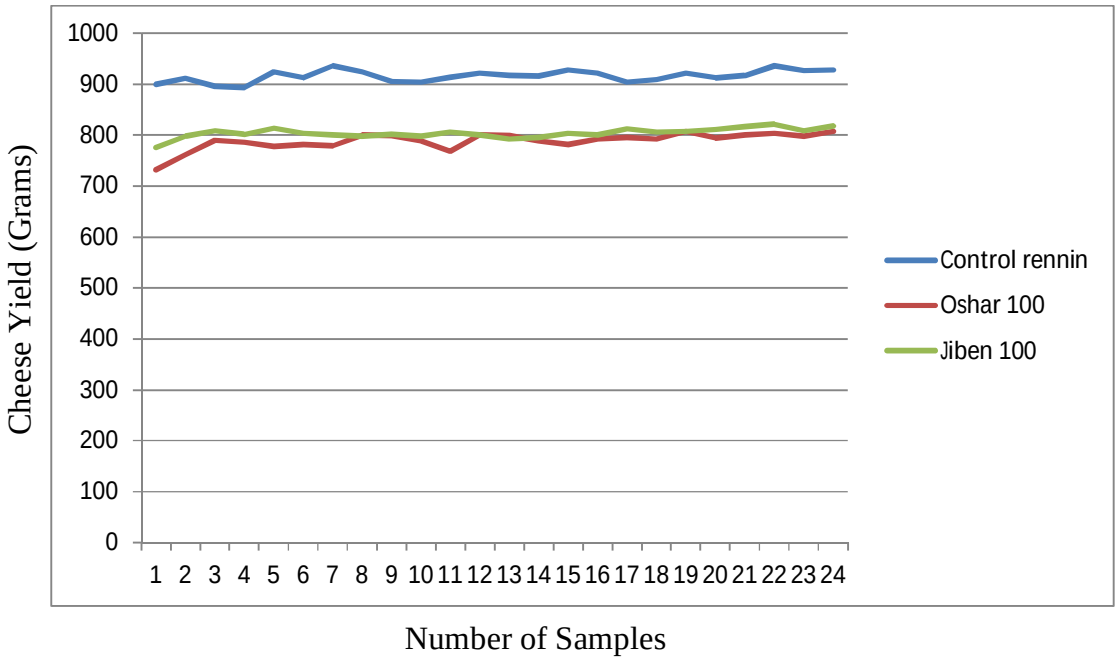


Table (8) average protein % obtained by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	14.46	0.45	11.80	0.45	13.55	0.73	*
(2)	2nd stage	14.30	0.55	11.82	0.51	13.38	1.04	*
(3)	3rd stage	14.50	0.33	12.26	0.26	13.40	0.41	*
Total	Whole period	14.42	0.44	11.96	0.46	13.44	0.74	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

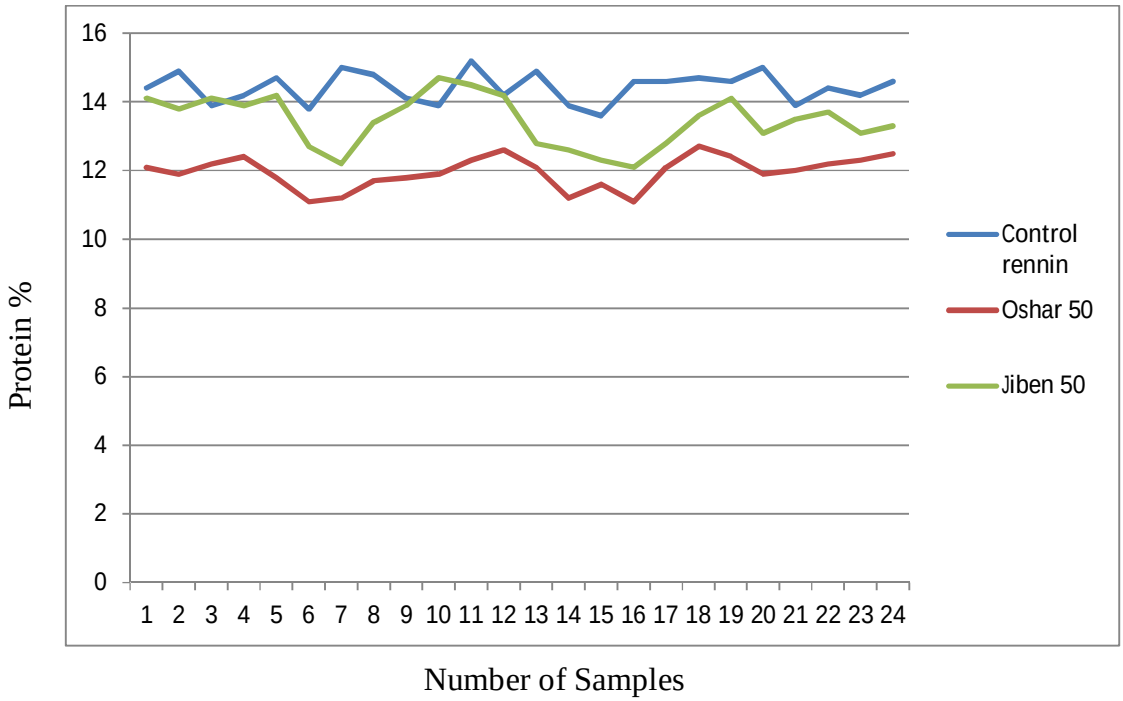


Table (9)average protein % obtained by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	14.31	0.39	12.25	0.50	13.45	0.50	*
(2)	2nd stage	14.22	0.33	12.17	0.27	13.16	0.62	*
(3)	3rd stage	14.28	0.41	12.00	0.22	13.87	0.45	*
Total	Whole period	14.26	0.36	12.14	0.35	13.61	0.60	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

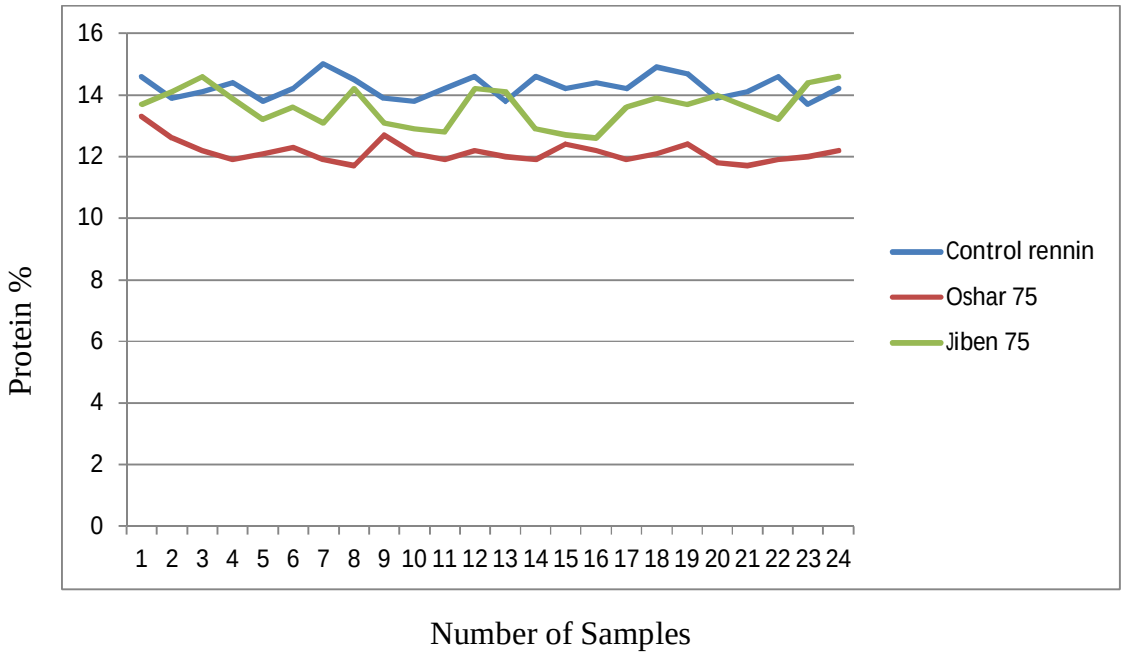


Table (10)average protein % obtained by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	14.51	0.37	12.02	0.31	13.35	0.38	*
(2)	2nd stage	14.49	0.39	11.97	0.31	13.96	0.19	*
(3)	3rd stage	14.11	0.33	12.06	0.17	11.68	0.45	*
Total	Whole period	14.36	0.39	12.02	0.26	13.55	0.42	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

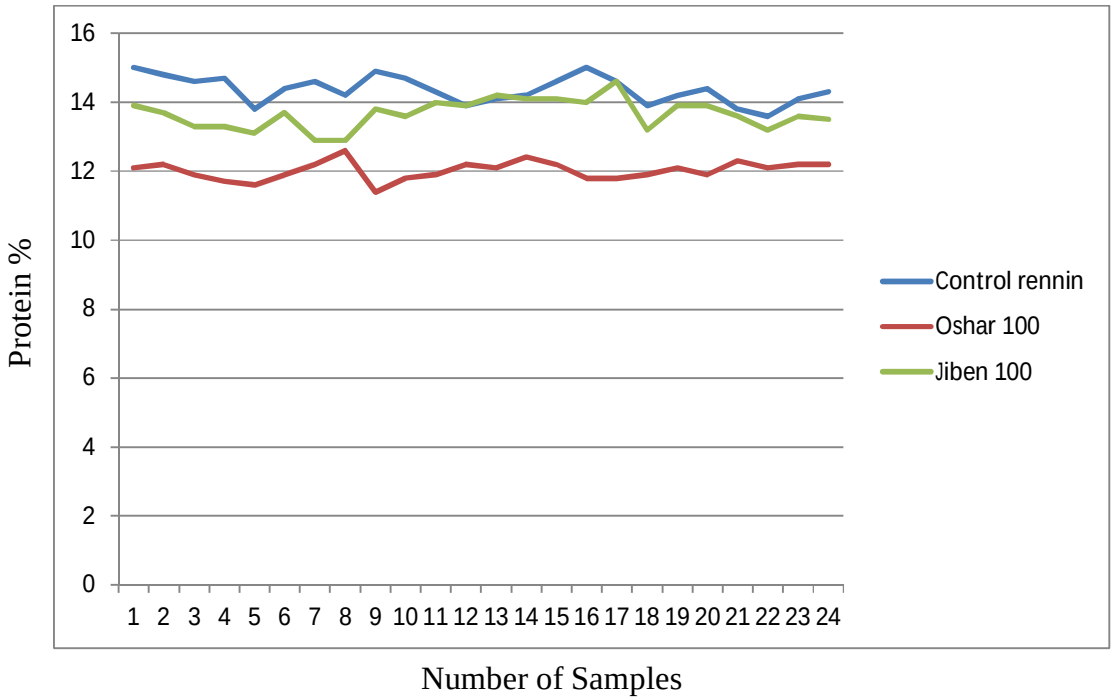
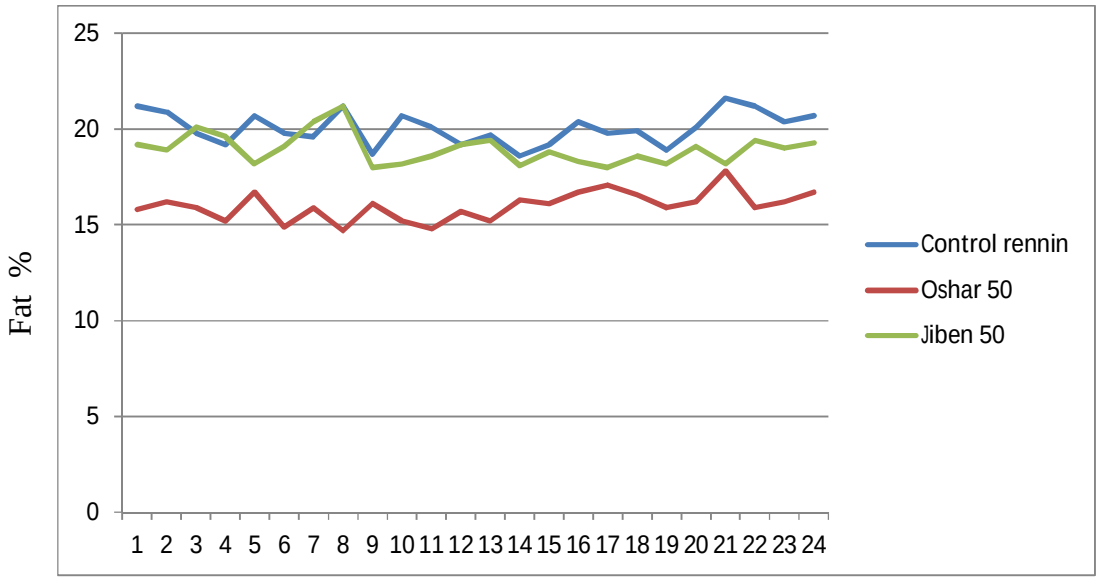


Table (11) average Fat % obtained by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	20.30	0.78	15.66	0.67	19.58	0.94	*
(2)	2nd stage	19.57	0.77	15.76	0.65	17.97	0.52	*
(3)	3rd stage	20.32	0.85	16.55	0.65	18.72	0.54	*
Total	Whole period	20.06	0.84	15.99	0.75	16.95	0.80	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (12) average Fat % obtained by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	20.12	0.75	16.15	0.75	18.50	0.37	*
(2)	2nd stage	19.40	0.52	16.00	0.46	17.81	0.45	*
(3)	3rd stage	20.38	0.83	15.85	0.57	18.51	0.38	*
Total	Whole period	19.97	0.80	16.00	0.59	18.35	0.44	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

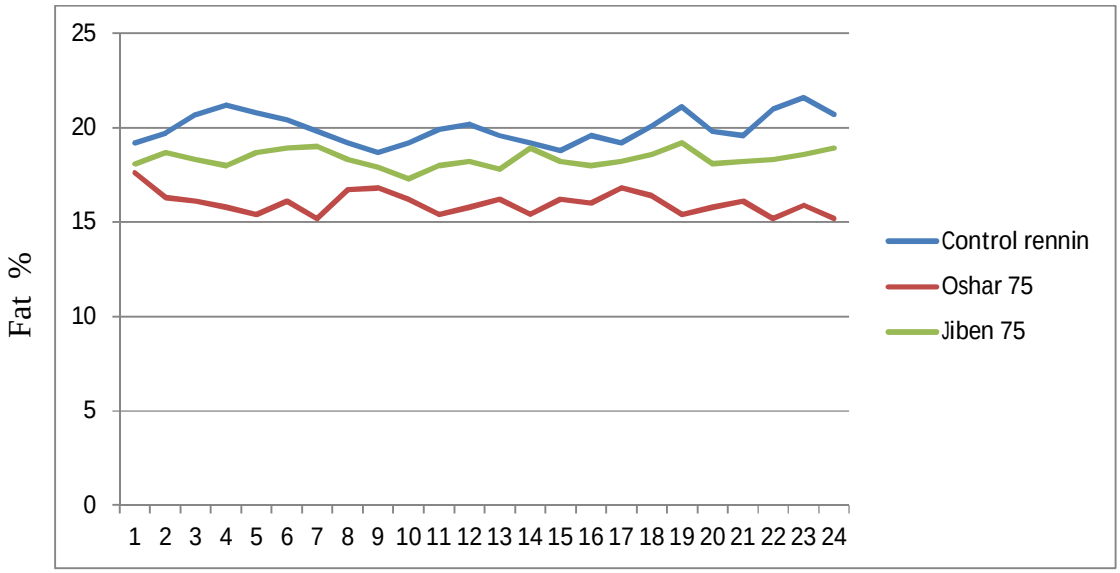
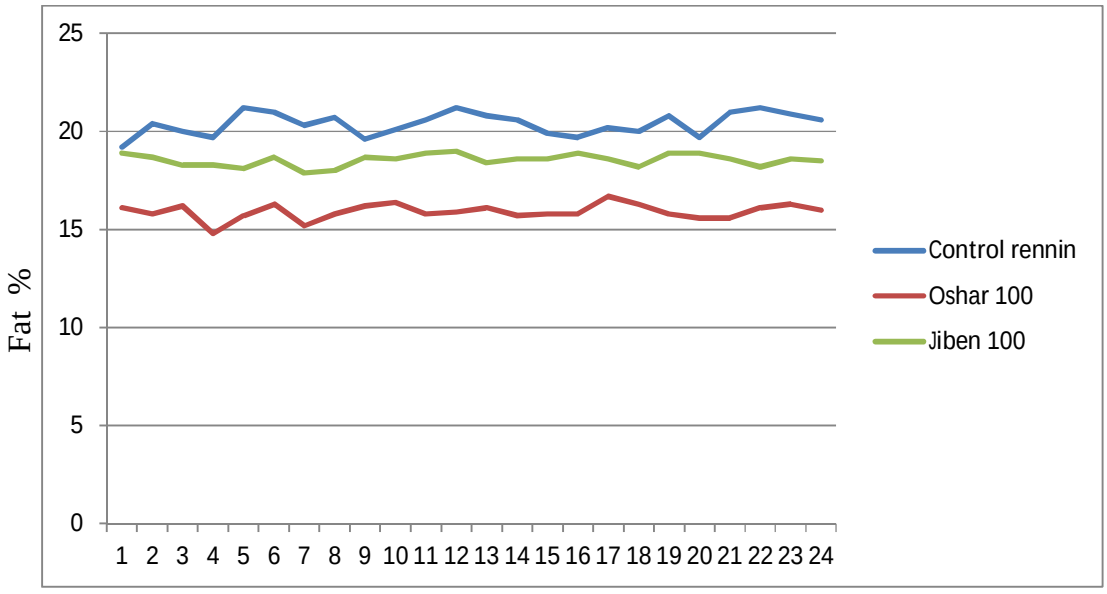


Table (13)average Fat % obtained by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	20.31	0.66	15.73	0.51	18.13	0.36	*
(2)	2nd stage	20.31	0.57	15.76	0.24	18.71	0.20	*
(3)	3rd stage	20.55	0.52	16.05	0.38	18.56	0.26	*
Total	Whole period	20.39	0.57	15.91	0.40	18.54	0.31	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



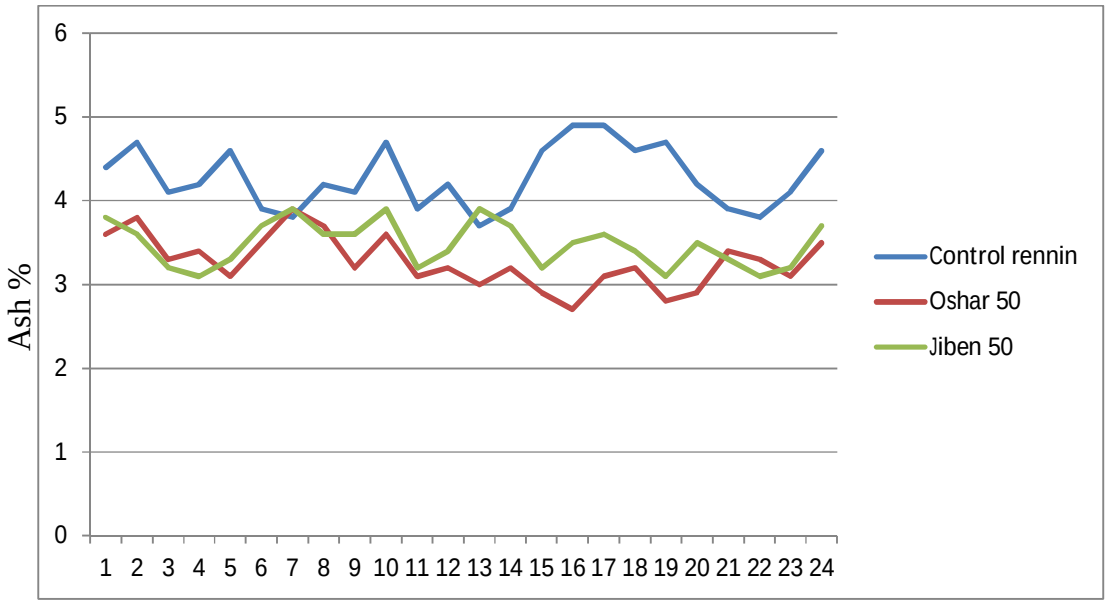
Number of Samples

Table (14) average ash % obtained by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	4.23	0.31	3.53	0.26	3.52	0.29	*
(2)	2nd stage	4.25	0.43	3.11	0.26	3.55	0.27	*
(3)	3rd stage	4.35	0.40	3.16	0.23	3.36	0.22	*
Total	Whole period	4.27	0.37	3.27	0.31	3.47	0.26	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



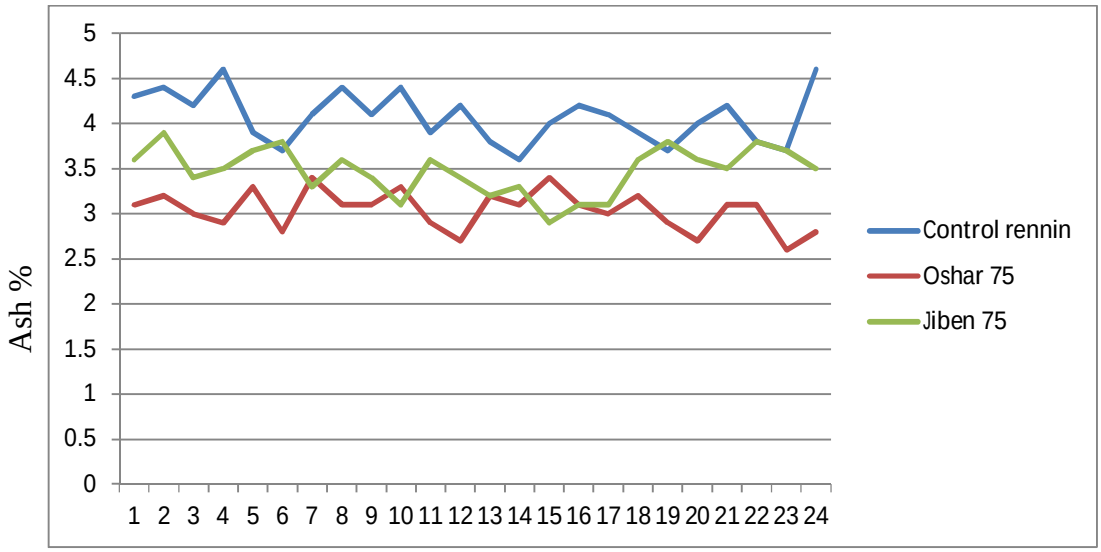
Number of Samples

Table (15) average ash % obtained by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	4.20	0.29	3.10	0.20	3.60	0.20	*
(2)	2nd stage	4.02	0.25	3.10	0.22	3.25	0.22	*
(3)	3rd stage	4.00	0.30	2.92	0.21	3.57	0.22	*
Total	Whole period	4.07	0.28	3.04	0.21	3.47	0.26	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (16) average ash % obtained by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	4.05	0.28	4.03	0.21	3.21	0.26	*
(2)	2nd stage	4.11	0.25	2.092	0.19	3.21	0.14	*
(3)	3rd stage	4.21	0.25	3.10	0.16	3.35	0.16	*
Total	Whole period	4.12	0.24	3.04	0.52	3.25	0.16	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

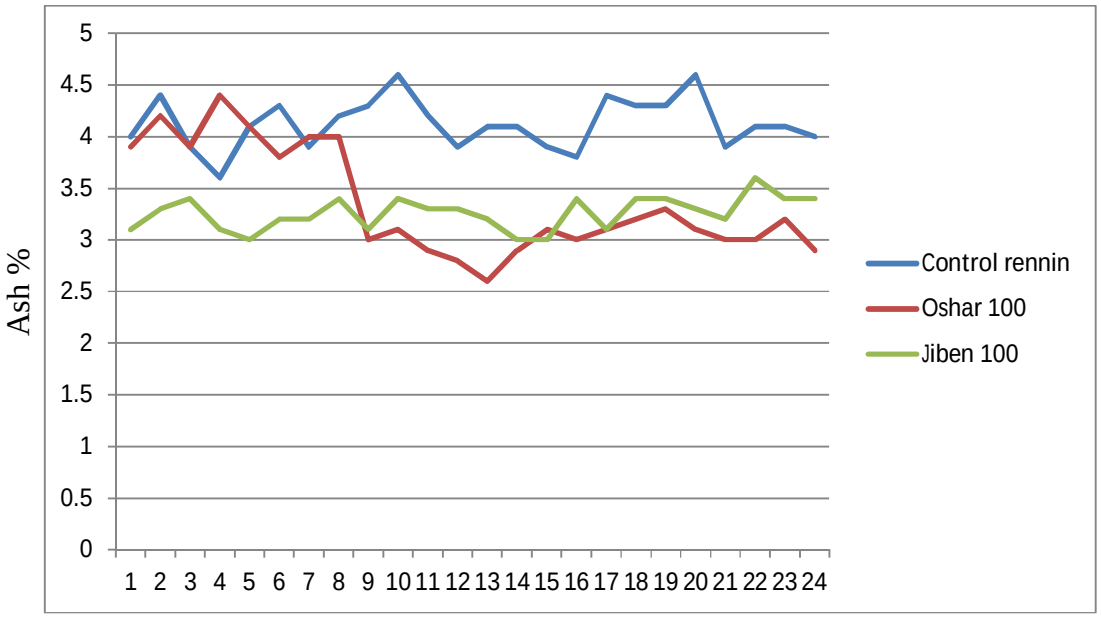
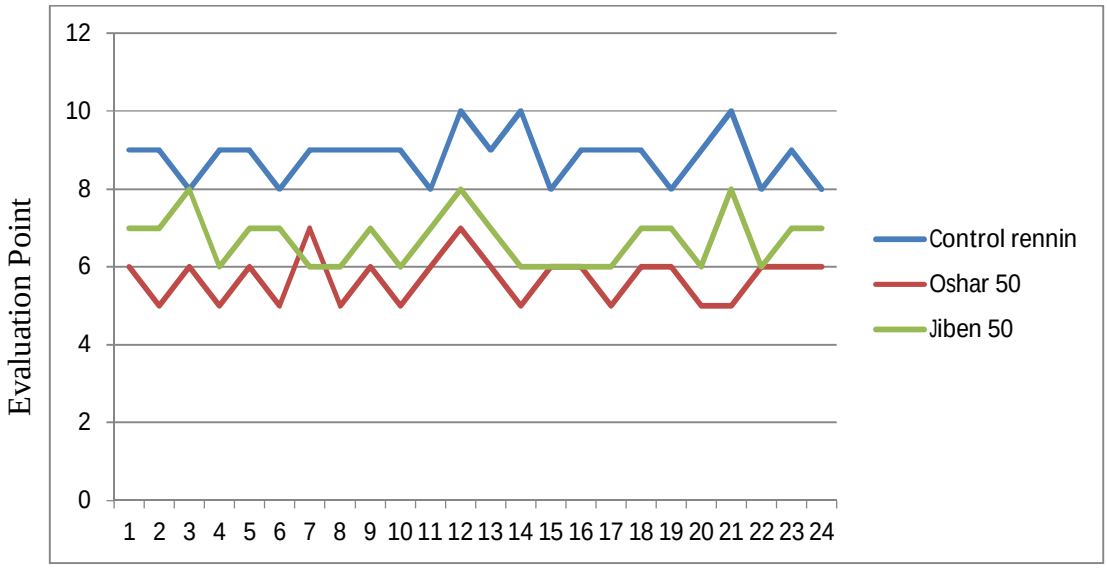


Table (17) average evaluation points given for flavor by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.75	0.46	5.62	0.74	6.75	0.70	*
(2)	2nd stage	9.00	0.75	5.87	0.64	6.62	0.74	*
(3)	3rd stage	8.75	0.70	5.62	0.51	6.75	0.70	*
Total	Whole period	9.00	0.63	5.87	0.62	6.62	0.69	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (18) average evaluation points given for flavor by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.78	0.64	5.62	0.70	6.75	0.91	*
(2)	2nd stage	8.83	0.70	5.70	0.74	6.70	0.74	*
(3)	3rd stage	7.87	0.64	5.50	0.92	6.62	0.74	*
Total	Whole period	8.83	0.86	5.62	0.76	6.62	0.76	*

NS ≡ not significant

*** ≡ significant (p < 0.05)**

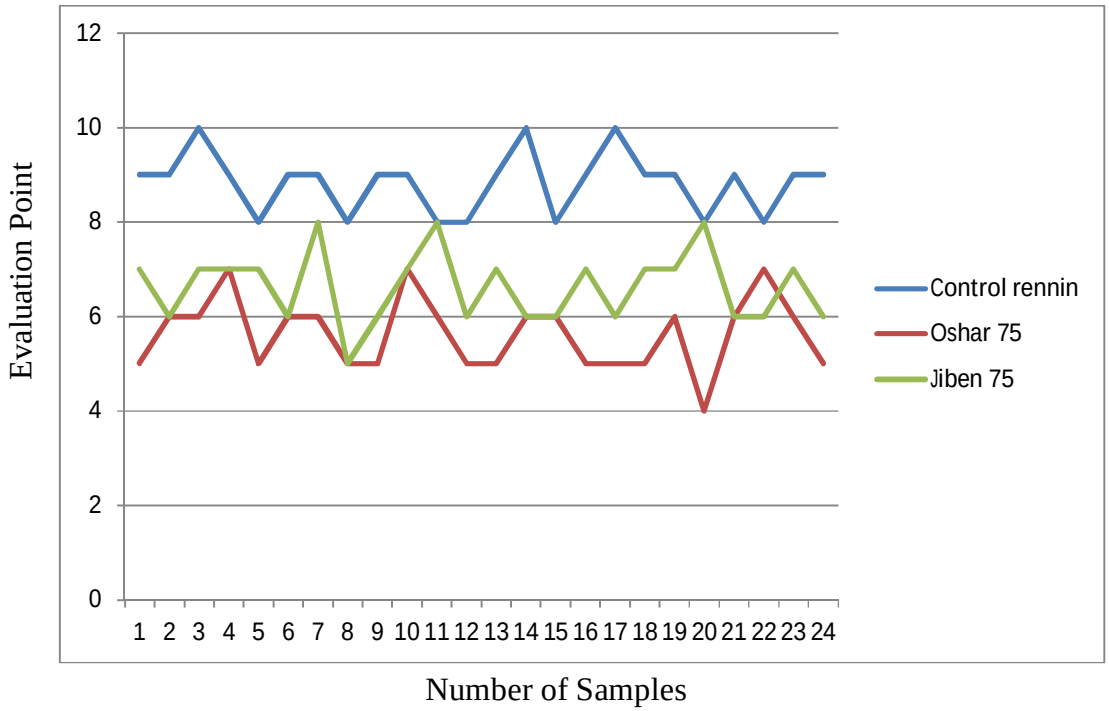


Table (19)average evaluation points given for flavor by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	9.00	0.75	6.75	1.58	6.87	0.83	*
(2)	2nd stage	8.62	0.91	5.50	0.92	6.95	0.70	*
(3)	3rd stage	8.75	0.70	5.50	0.92	7.00	0.75	*
Total	Whole period	8.79	0.77	5.91	1.28	6.87	0.74	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

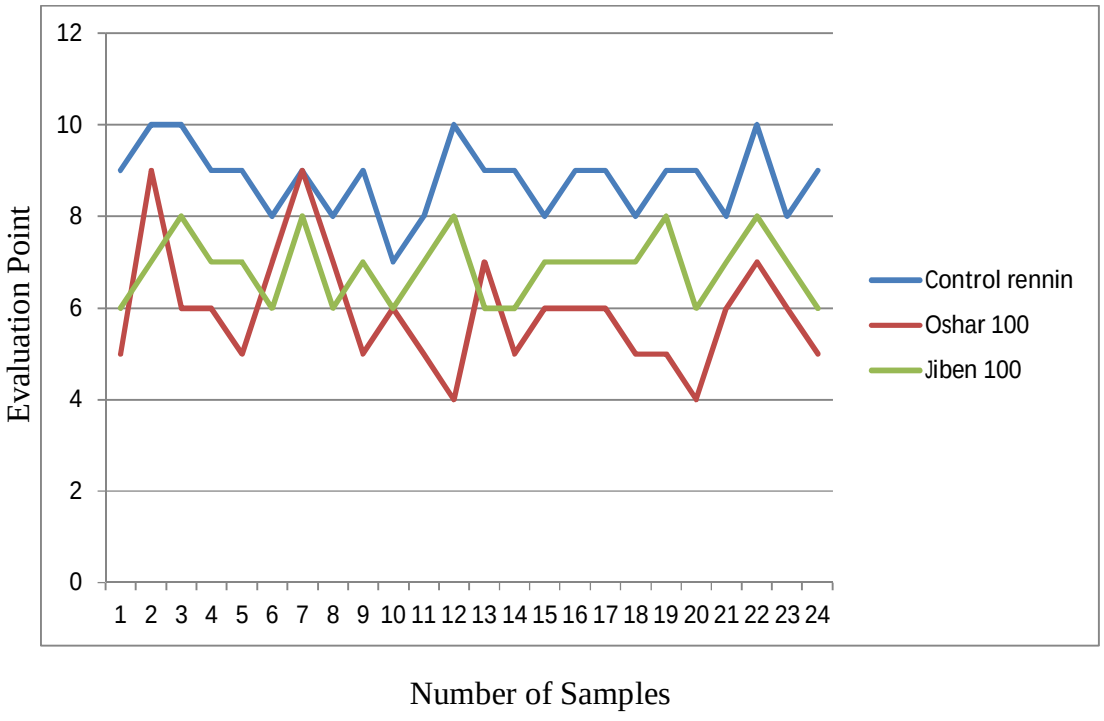


Table (20) average evaluation points given for taste by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.25	0.70	6.75	0.70	8.00	0.75	*
(2)	2nd stage	8.62	0.74	6.00	0.75	7.12	0.64	*
(3)	3rd stage	8.37	0.74	6.00	0.75	6.50	0.53	*
Total	Whole period	8.08	0.71	6.25	0.82	7.20	0.88	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

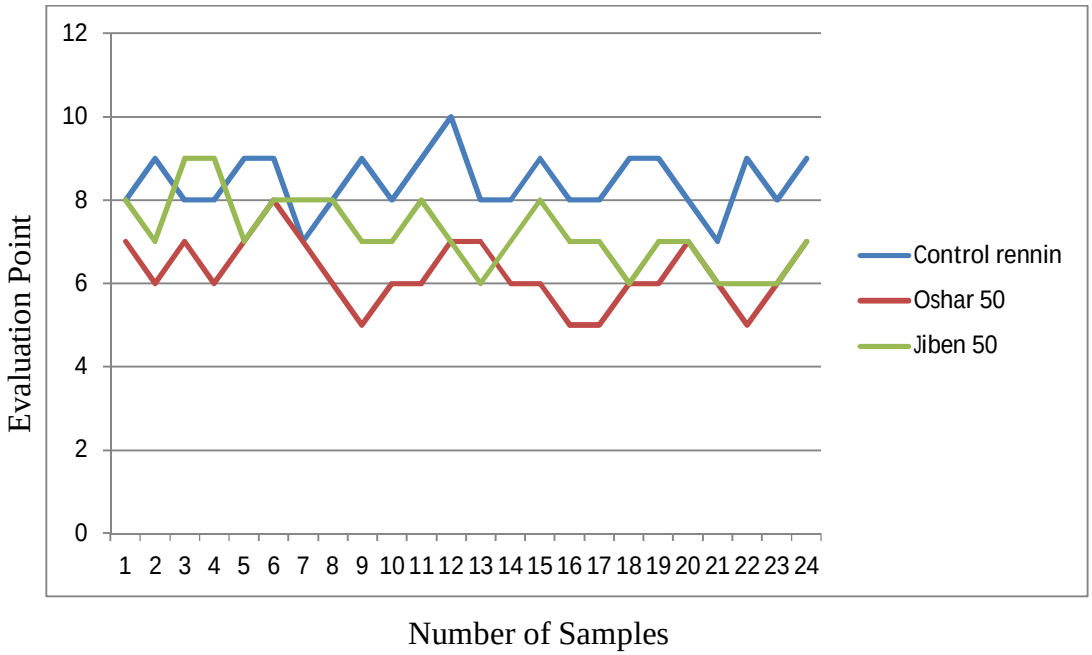


Table (21)average evaluation points given for taste by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.12	0.83	5.62	0.74	6.50	0.53	*
(2)	2nd stage	8.50	0.53	5.75	0.70	6.75	0.70	*
(3)	3rd stage	9.00	0.75	6.12	0.83	6.50	0.92	*
Total	Whole period	8.54	0.77	5.83	0.76	6.58	0.71	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

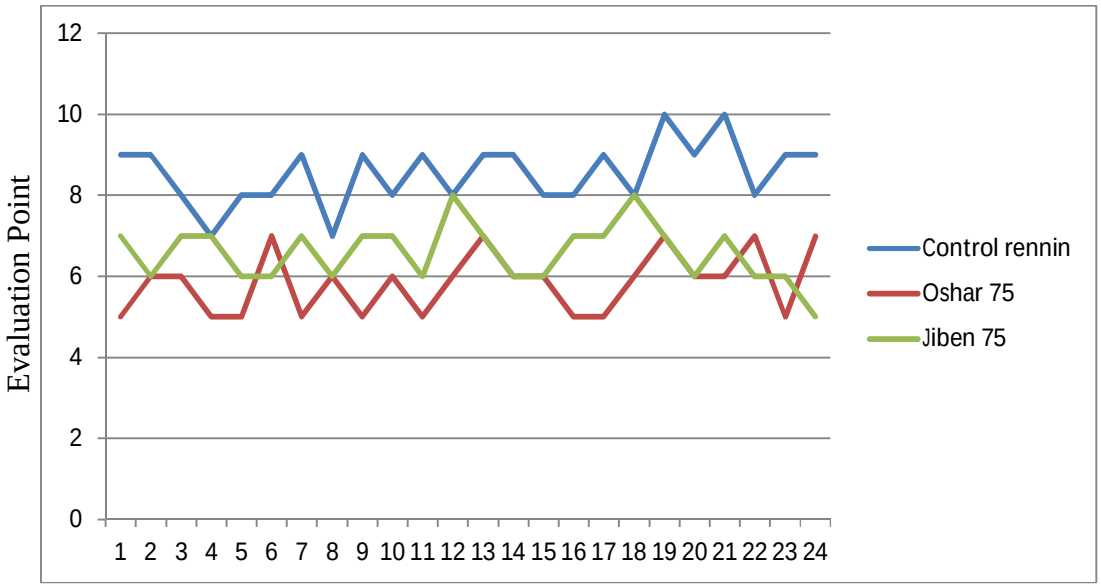


Table (22)average evaluation points given for taste by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.00	0.75	5.50	0.92	6.37	0.74	*
(2)	2nd stage	8.50	0.92	6.25	0.70	6.75	0.70	*
(3)	3rd stage	8.87	0.83	5.62	0.74	6.37	0.91	*
Total	Whole period	8.45	0.88	5.79	0.83	6.50	0.78	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

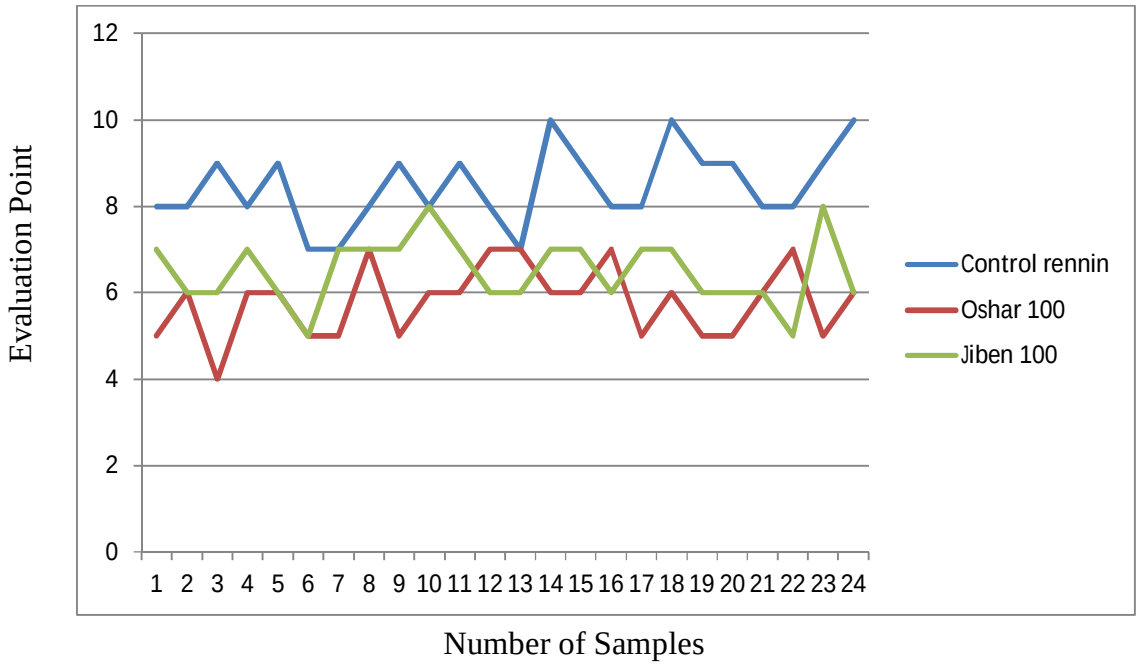


Table (23) average evaluation points given for texture by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	9.00	0.75	5.87	0.83	6.87	0.64	*
(2)	2nd stage	8.75	0.70	5.62	0.74	6.75	0.70	*
(3)	3rd stage	8.62	0.91	6.00	1.51	7.75	0.70	*
Total	Whole period	8.97	0.77	5.83	1.04	7.12	0.97	*

NS ≡ not significant

*** ≡ significant (p < 0.05)**

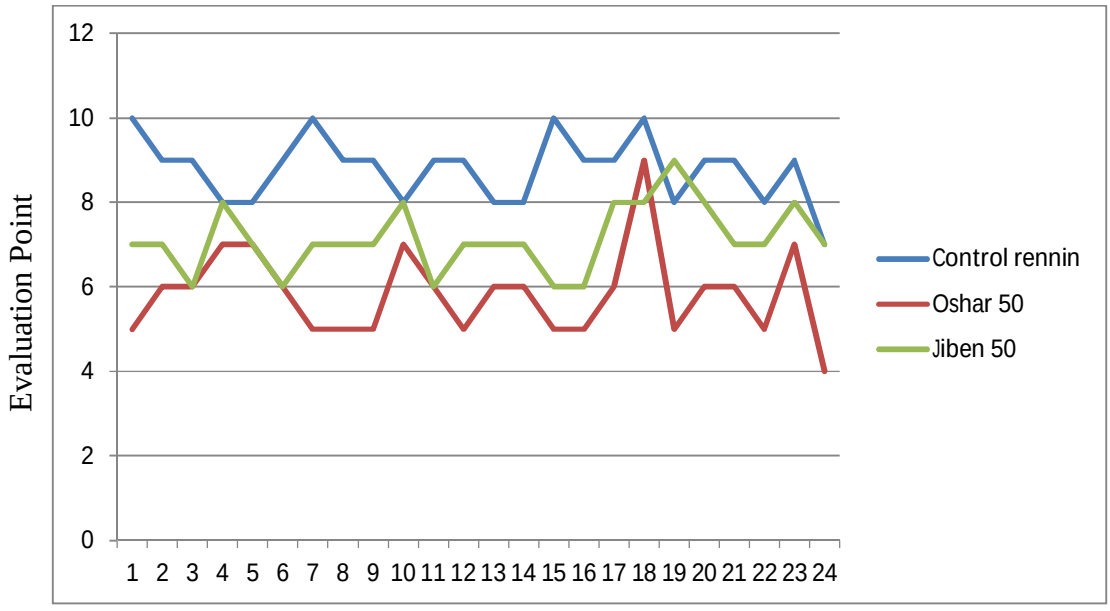


Table (24)average evaluation points given for texture by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.62	0.74	6.00	0.75	8.12	0.64	*
(2)	2nd stage	8.75	0.70	5.75	0.70	8.25	0.70	*
(3)	3rd stage	8.62	0.74	6.00	0.75	8.00	0.75	*
Total	Whole period	8.66	0.70	5.91	0.71	8.12	0.67	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

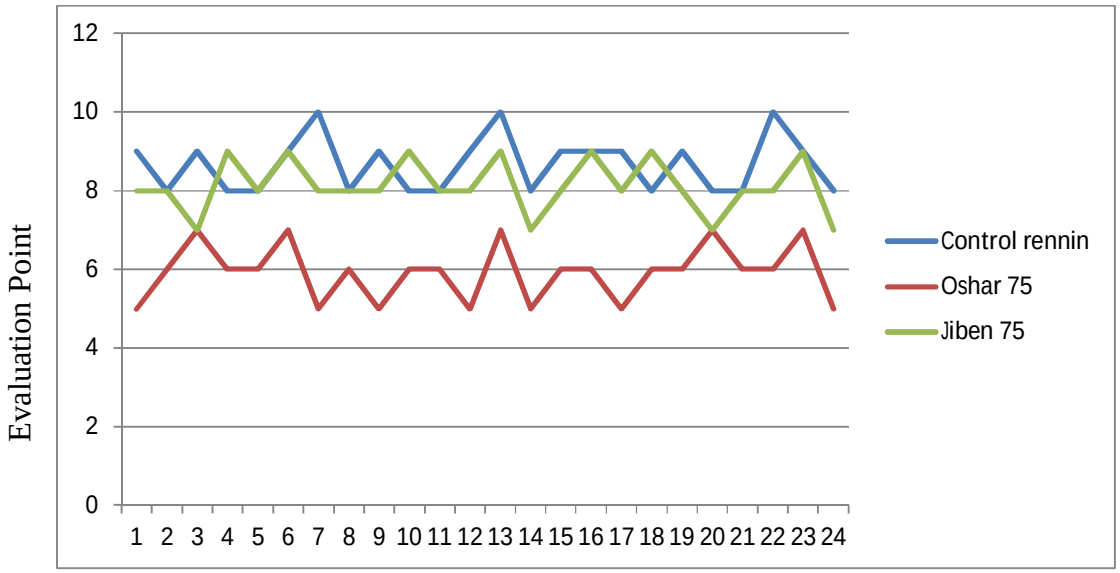
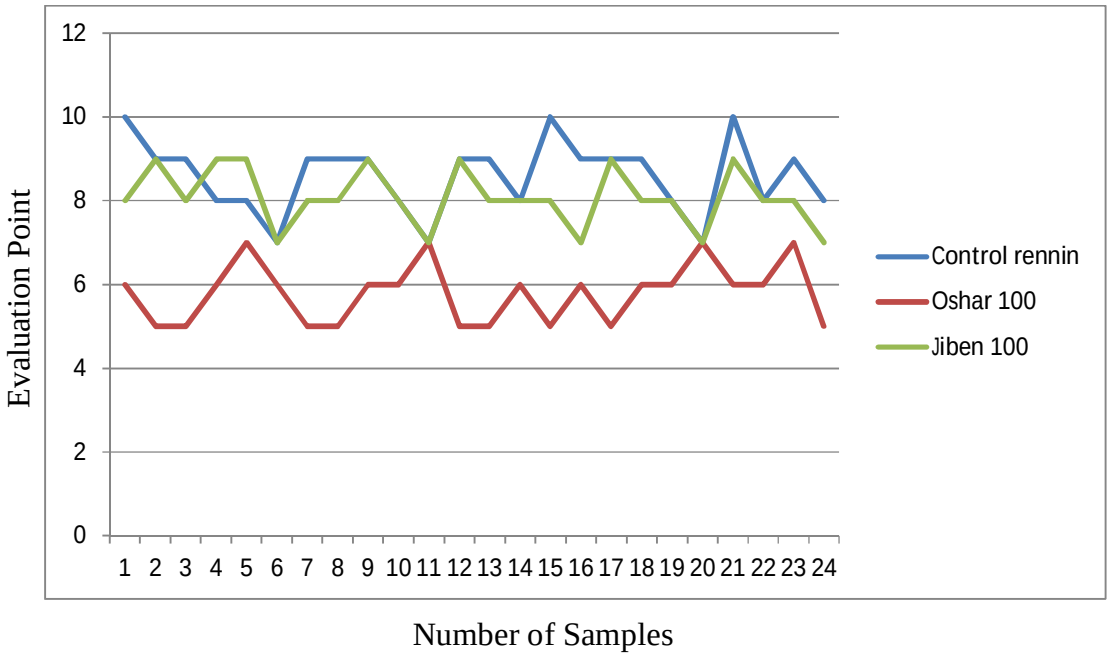


Table (25)average evaluation points given for texture by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.62	0.91	5.62	0.74	8.25	0.70	*
(2)	2nd stage	8.62	0.91	5.75	0.70	8.00	0.75	*
(3)	3rd stage	8.50	0.92	6.00	0.75	8.00	0.75	*
Total	Whole period	8.58	0.88	5.97	0.72	8.08	0.71	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



4.2 Discussion:

4.2.1 Time of Coagulation:

Table (2) shows the average coagulation time (minutes), by using renin Osher (50%) and Jibeen (50%) enzymes for the lactation stages (1^{st}. 2^{nd}. and 3^{rd}.), and for the whole lactation period for all treatments. The average time for the whole lactation period for renin, Osher (50%) and Jibeen (50%) was 97.33± 4.62, 124.37± 5.88 and 96.29 ± 4.96 minutes respectively.

Table (3) shows the average coagulation time by using renin, Osher (75%) and Jibeen (75%) for the different stages and lactation period. Average time for the whole period of treats obtain was 80.37 ± 3.54, 113.62 ± 7.29 and 87.75 ± 2.23 minutes respectively.

Table (4) shows the average coagulation time by using renin, Osher (100%) and Jibeen (100%) for the 3 stages and lactation period. The average for the whole lactation period and all treatments obtained was 79.45 ± 3.65, 108.83 ± 5.62 and 85.95 ± 4.95 for renin Osher (100%) and Jibeen (100%) respectively.

Results indicated a significant difference between the means of the coagulation time for all stages and whole lactation period for all treatments, and for renin and the different concentrations of Osher and

Jibeen enzymes (50%, 75% and 100%) ($p<0.05$). Similar results revealing significant differences were given by (van Hooydonk et. al. 1984), Synth et. al. (1937), Lee et. al. (2003).

According to the results obtained, renin enzyme required less time to coagulate the milk compared to Osher and Jibeen enzymes, and this agreed with that reported by the above mentioned authors. The Colatropis procera plant enzyme (Osher) required much time for coagulation than Jibeen enzyme which was in conformity with the results obtained by Hamed (1998).

The variation in the coagulation time required by the different coagulants is depending on the type of the enzyme used (van Hooydonk et. al. 1987), the chemical composition of milk, particularly the Ca^{++} (Scancalpore et. al. 1983) stage of lactation period where the chemical composition of milk greatly changed (Curten – Vapuretal 2012), menthod of coagulation applied (Van Hoydon et. al. 1987) and finally the clotting activity of the milk (Ibiama and Griffiths, 1987).

4.2.2 Cheese Yield:

Table (5) shows the average total yield of white soft cheese (g) per 5 kg milk obtained by adding renin Osher (50%) and Jibeen (50%) enzymes for the three stages of the lactation period and the whole lactation period.

The average yield during the whole lactation period for all treatments was 913.5 ± 8.66, 635.9 ± 14.09 and 662.5 ± 15.58 for renin Osher (50%) and Jibeen (50%) respectively.

Table (6) shows the average cheese yield (g) per 5kg milk for the different stages and whole lactation period for renin, Osher (75%) and (75%) respectively. The average yield for the whole lactation period obtained was 944.3 ± 32.89, 697.8 ± 16.99 and 729.3 ± 41.8 for renin, Osher (75%) and Jibeen (75%) respectively.

Table (7) shows the average yield (g) per 5 kg milk for the different stages and whole lactation period for renin, Osher (100%) and Jibeen (100%). The average yield for the overall lactation period for all treatments obtained was 916.0 ± 11.54, 788.7 ± 16.70 and 804.3 ± 9.55 g for renin, Osher (100%) and Jibeen (100%) enzymes respectively. The average yield showed a significant difference ($p<0.05$) for all stages, whole lactation period, all treatments for renin, Osher and Jibeen enzymes concentrations. Similar results indicating the significance between the means of the yield were given by Everett et. al. (2003), Synth et. al. (1987), Nijera (2003), Nur Eldaim et. al. (2007) and (Osman et. al. 2012). According to the results obtained, renin coagulation resulted in a good chees yield followed by Jibeen and Osher coagulation, which gave low cheese yield. The variation in the cheese yield is affected by many

factors of which the milk composition of vital importance, (Faltman,1987). The low yield of white soft cheese obtained by plant coagulation enzymes is due to the weak curd produced by such coagulants Synth, et. al. (2007), high losses of fat and protein drained with the whey (Merin, 1989) and enzyme and type of enzyme used Nijera, (2003). Other factors associated with cheese yield are demonstrated in chapter two according to Everett et (2003) and Paolo et. al. (2008).

4.2.3 Chemical Composition of Cheese:

4.2.3.1 Protein Content:

Table (8) shows the average protein % obtained by using renin, Osher (50%) and Jibeen (50%) for lactation stages and whole lactation period. The average protein % for the whole lactation period was 14.42 ± 0.44, 11.96 ± 0.46 and 13.44±0.74 for renin, Osher (50%) and Jibeen (50%) enzymes for all treatments..

Table (9) shows average protein % obtained using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period. The average protein % for the whole lactation period was 14.26 ± 0.36, 12.14 ± 0.35 and 13.61 ± 0.60 for renin, Osher (75%), and Jibeen (75%) respectively and for all treatments.

Table (10) shows the average protein % obtained using renin, Osher (100%) and Jibeen (100%) for different lactation stages and whole lactation period and all treatments was 14.36 ± 0.39, 12.02 ± 0.26 and 13.55 ± 0.42 for renin, Osher (100%) and Jibeen (100%) respectively. The results obtained showed a significant difference ($p<0.05$) between the average of protein % when using renin and different concentrations of Osher and Jibeen coagulants for all lactation stages, whole lactation period and all treatments. Similar results were also given by Caric et. al. (1995), Green et. al. (1987) and Bradly et. al. (1990). The differences in the protein content of the white soft cheese were associated with type of coagulant used for processing. Coagulating the milk with renin enzyme, the levels of protein tend to increase compared to plant enzymes (Rollman et. al. 1988), Dimitreli et. al. (2004) and (Kumosinski et. al. 1991).

4.2.3.2 Fat Content:

Table (11) shows the average fat % obtained by using renin Osher (50%) and Jibeen (50%) enzymes for the stages of lactation and whole lactation period. The average fat % for whole lactation period for all treatments was 20.06±0.84, 15.99±0.75 and 16.95±0.80 for renin, Osher (50%) and Jibeen (50%) respectively.

Table (12) shows average fat % using renin, Osher (75%) and Jibeen (75%) enzymes for the 3 stages and whole lactation period. The average fat % for whole lactation period for all treatments obtained was 19.97±0.80, 16.00±0.59 and 18.35±0.44 for renin, Osher (75) and Jibeen (75%) respectively.

Table (13) shows average fat % using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period.

The average fat % obtained for the whole lactation period and all treatments was 20.39±0.57, 15.91±0.40 and 18.54±0.31 for renin, Osher (100%) and Jibeen (100%) enzymes respectively. Also, results showed a significant difference ($p<0.05$) between the average fat % for all stages, whole lactation period and all treatments. The average fat % obtained when using renin enzyme was higher compared to that of the plant enzymes. This indicated, the type of coagulant used affected the fat component of the milk used. Also differences in the fat content of the raw milk prepared for white cheese processing and method of coagulation used cause variation in the final fat content of the finished cheese. This comes in agreement with that explained by Abdalla (1993) (Home 1990) and (Cari et. al. 1993).

4.2.3.3 Ash Content:

Table (14) shows average ash % obtained using renin, Osher (50%) and Jibeen (50%) enzymes for the 1st. 2nd. and 3rd. stage of lactation and whole lactation period. The average ash % for the whole lactation period for all treatments obtained was 4.27±0.37, 3.27±0.31, and 3.47±0.26 for renin, Osher (50%) and Jibeen (50%) enzymes respectively.

Table (15) shows average ash % obtained using renin, Osher (75%) and Jibeen (75%) enzymes for different lactation stages and whole lactation period. The average ash % for the whole lactation period obtained was 4.07±0.28, 3.04±0.21 and 3.47±0.26 for renin, Osher (75%) and Jibeen (75%) enzymes respectively.

Table (16) shows the average ash % obtained using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period. The average ash % for the whole lactation period and all 0treatments obtained was 4.12±0.24, 3.04±0.52 and 3.25±0.16 for renin, Osher (100%) and Jibeen (100%) enzymes respectively. A significant difference ($p<0.05$) was detected between the average of the ash % for the different stages, lactation period, renin enzyme and plant enzyme with different concentrations for all treatments. Ash content showed low percentages, when plants coagulants were used compared to renin enzyme. The chemical composition of cheese showed low cheese

components, when the cheese milk was coagulated by plant enzymes (Bebe, 1980). In general, coagulating the milk with plant enzymes resulted in a decrease in the percentages of the total solids of white soft cheese. This agreed with that reported by Andren et. al. (1982), Dalglish (1985), Bines (1989) and Psozola (1989).

4.2.4 Sensory Evaluation:

(Refer to evaluation Sheet)

4.2.4.1 Flavor of Cheese:

Table (17) shows the evaluation points given for flavor of cheese using renin, Osher (50%) and Jibeen (50%) enzymes for different lactation stages and whole lactation period. The average evaluation points the flavor scored, for the whole lactation period were 9.00±0.63, 5.87±0.62 and 6.62±0.69 for renin, Osher (50%) and Jibeen (50%) enzymes for all treatments respectively.

Table (18) shows evaluation points for flavor using renin, Osher (75%) and Jibeen (75%) enzymes for the stages and whole lactation period. The evaluation points scored for the whole lactation period for all treatments obtained were 8.83±0.86, 5.62±0.76 and 6.62±0.76 for renin, Osher (75%) and Jibeen (75%) enzymes respectively.

Table (19) shows average evaluation points given for flavor using renin, Osher (100%) and Jibeen (100) enzymes respectively. The average given for the whole lactation period was 8.79±0.77, 5.91±1.28 and 6.87±0.74 for renin Osher (100%) and Jibeen (100%) enzymes and for all treatments respectively. A significant difference ($p<0.05$) was detected between the average of scored points for flavor, when renin and different concentrations of Osher and Jibeen enzymes for all stages, whole lactation period and all treatments were used. Similar results were obtained by Farell et. al. (1990) and Kumosinski et. al. (1999). It was noticed that renin cheese scored the higher points followed by Jibeen and Osher cheeses. This indicated, the type of enzyme used for coagulation determined the level of flavor intensity, (Takala, 1993). Other factors that affect the flavor of cheese is the concentration of extracted plant enzymes when used in cheese making, (Talib et. al. 2006); other factors influencing the flavor are the chemical composition of milk, types of animals and chemical composition of feed, (Englels et. al. 2005), (Takala 1993).

4.2.4.2 Taste of Cheese:

Table (20) shows the points given for taste of cheese using renin, Osher (50%) and Jibeen (50%) enzymes for the stages and whole lactation period for all treatments. Average points given for the whole

lactation period were 8.08±0.71, 6.25±0.82 and 7.20±0.88 for renin, Osher (50%) and Jibeen (50%) enzymes respectively.

Table (21) shows the points given for taste using renin, Osher (75%) and Jibeen (75%) enzyme for the stages of lactation and whole lactation period for all treatments. The average points for the whole lactation period obtained were 8.54±0.77, 5.83±0.76 and 6.58±0.71 for renin, Osher (75%) and Jibeen (75%) enzymes cheese respectively.

Table (22) shows average evaluation points for taste using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments. The average points for the whole lactation period were 8.45±0.88, 5.79±0.83 and 6.50±0.78 for renin, Osher (100%) and Jibeen (100%) enzymes cheese respectively. The scored points revealed a significant difference ($p<0.05$) between the means given for taste of the cheeses prepared by renin enzyme and different coagulation of Osher and Jibeen enzymes for all stages, overall lactation period and all treatments. Renin cheese scored the highest points for taste compared to other plant enzymes. Jibeen cheese showed a relatively high scores raher than Osher cheeses. This indicated that the taste of the soft cheese was affected by the type of coagulant used, beside the factors affecting flavor previously mentioned, in addition to the concentration of the plant enzymes used. This agreed with that reported

by Talib, et. al. (2006), Takela (1990) and Farell, et. al. (1990), but were in contrast with the results given by Jacob, et. al. (2011).

4.2.4.3 Texture of Cheese:

Table (23) shows the average points given for texture of cheese using renin, Osher (50%) and Jibeen (50%) enzymes for the different stages and whole lactation period. The average points given for the whole lactation period and all treatments were 8.97±0.77, 5.83±1.04 and 7.12±0.97 for renin, Osher (50%) and Jibeen (50%) cheeses respectively.

Table (24) shows the average scored points for cheese texture using renin, Osher (75%) and Jibeen (75%) enzymes for all stages, whole lactation period and all treatments. The average points obtained for the whole lactation period for cheese texture were 8.66±0.70, 5.91±0.71 and 8.12±0.67 when renin, Osher (75%) and Jibeen (75%) enzymes were used.

Table (25) shows average evaluation points given for cheese texture using renin, Osher (100%) and Jibeen (100%) enzymes for all stages, whole lactation period and all treatments. The average scored points for cheese texture for whole lactation period were 8.58±0.88, 5.97±0.72 and 8.08±0.71 for renin, Osher (100%) and Jibeen (100%) enzyme cheeses respectively. Also a significant difference was detected hereby ($p<0.05$). The texture of renin cheese was firm followed by

Jibeen and Osher cheeses. This cleared the effect of the type of enzyme used for coagulation on cheese texture. Plant enzymes have an excessive proteolytic activity that affect the texture of the finished cheese (especially during the ripening process) and this might be the reason for non-firmness of some cheeses produced using plant enzymes e.g. Calotropis procera (Osher) plant, (Yousif, et. al. 1996). Also results given by (Talib et. al. 2006) and Kumosinski et. al. (1990), followed the same trend.

Chapter Five

Conclusion and Recommendations

5.1 Conclusion

Renin enzyme especially calf renin, is considered as one of the most wide spread, desirable and dominant milk coagulant in cheese processing. In the past few years, the limited supply and the high prices of renin necessitated the need of finding other milk coagulants. To overcome such a problem plant enzymes were extracted from various plants, e.g. Solanum dubium (Jibeen) and Colatropis procera (Osher) and others. Comparing renin to plant enzymes, it gave the best results for all studied parameters subject of the current research (Time of milk coagulation, cheese yield, composition and sensory characteristics) and significant differences ($p<0.05$) were detected between the averages of the parameters for the stages of lactation and whole lactation period and for all treatments. Yet, Jibeen enzyme showed an acceptable standard levels when used. Enzyme extracted from Osher gave the lowest results when compared to renin and Jibeen enzymes. Hence plant enzymes may also be used in the future as milk coagulants in the cheese industry of Sudan under certain conditions.

5.2 Recommendations:

Based on obtained results, following recommendations might be given:

- Jibeen enzyme can be used as an alternative for renin and other plants enzymes, since the plant grows in most areas of the Sudan, in addition to its effectiveness.
- More attention should be paid when using plant enzymes, particularly, chemical composition and suitable concentrations to be used to avoid health hazards by consumers, e.g. toxicity.
- More research studies should be carried out on plant enzymes, as well as the economic impact linked with the utilization.

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بسم الله الرحمن الرحيم

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College of Graduate Studies

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

أثر استتخدام إنزيم الرنين والأنزيمات النباتية في زمن تجبن اللبن والإنتاج والتركيب الكيميائي والصفات الحسية للجبن الأبيض الطري

A Thesis submitted in fulfillment of the requirements of Sudan University of Science and Technology for the Degree of PhD.
In Animal Production (Dairy Technology)

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April 2016

استهلال

قال تعالى:

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَى إِلَيْكَ وَحْيُهُ وَقُلْ رَبِّ زِدْنِي عِلْمًا) **صدق الله العظيم - سورة طه – الآية (114).**

Dedication

To my wife, son and daughters.

To the spirit of my parents.

To my colleagues and friends.

To those who are following the way of animal production.

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Thanks to Allah who helps me to complete this work. I am deeply indebted to my Supervisor Dr. Anas Mohammed Osman for his continuous support, guidance, encourage and ideal supervision to this work. Thanks are extended to my colleagues and all members of department of Animal Production, College of Agricultural Studies, Sudan University of Science and Technology for their support and encouragement. I am also indebted to Professor Mohammed Osman Gaafar for his continuous support, encourage and urging to complete this work.

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Abstract

This research was conducted to study the effect of using renin enzyme and two other plant enzymes on the coagulation time of milk, yield, composition and sensory characteristics of produced cheese. The renin enzyme was used as control. The plant enzymes were extracted from Solanum dubium (Jibeen) and Calotropis procera (Osher) plants. Plant enzymes were added to the milk in 3 concentrations (50%, 75% and 100%). Coagulation time, yield, composition and sensory characteristics were assessed during the lactation period, which was divided into 1st., 2nd. and 3rd. stages. (1st. 2nd. and 3rd. two months after calving) and for the whole lactation period. The total number of cheese samples used was 216 for the different treatments. Average coagulation time for whole lactation period and all treatments when using renin, Osher (50%) and Jibeen (50%) enzymes recorded, was 97.33± 4.62, 124.37± 5.88 and 96.29± 4.96 minutes respectively. Using renin, Osher (75%) and Jibeen (75%) enzymes, coagulation time obtained was 80.37± 3.54, 113.62± 7.29 and 87.75± 2.23 minutes respectively and 79.45± 3.65, 108.83± 5.62 and 85.95± 4.95 min. for renin, Osher (100%) and Jibeen (100%) enzyme respectively.

A significant difference ($p<0.05$) was detected between the average coagulation time for all stages, whole lactation period and all treatments. Coagulation of the milk by renin enzyme requited less time, while much time was required by Osher enzyme compared to Jibeen enzyme. The variation in time required by the different coagulants was related to the type of enzyme used, chemical composition of milk, method of coagulation applied, as well as the clotting activity of the milk.

The average total yield of soft cheese obtained from 5kg cow milk, when using renin, Osher (50%) and Jibeen (50%) enzymes for the whole

lactation period and all treatments was 913.5± 8.66, 635.9± 14.09 and 662.25± 15.58 g respectively. For renin, Osher (75) and Jibeen (75%) enzymes was 944.3± 32.89, 697.8± 16.99 and 729.3± 41.8 g. respectively.

For renin, Osher (100%) and Jibeen (100%) enzymes, the average cheese yield was 916.0± 11.54, 788.7± 16.70 and 804.3± 9.55 g respectively. The average total cheese yield showed a significant difference ($p<0.05$) for all stages, whole lactation period and all treatments. Renin coagulation resulted in good cheese yield, followed by Jibeen and Osher. Differences in cheese yield was affected by many factors, e.g. milk composition, enzymes and type of enzyme used for coagulation, production of weak curd produced by plant enzymes and others.

For whole lactation period and all treatments, the average protein % was found as 14.42± 0.44, 11.96± 0.46 and 13.44± 0.74 when using renin, Osher (50%) and Jibeen (50%) enzymes respectively, 14.26± 0.36, 12.14± 0.35 and 13.61± 0,60 for renin, Osher (75%) and Jibeen (75%) enzymes respectively and 14.36± 0.39, 12.02± 0.26 and 13.55±± 0.42 for renin, Osher (100%) and Jibeen (100%) enzymes respectively.

The average fat % obtained was 20.06± 0.84, 15.99± 0.75, and 16.95± 0.80, for renin, Osher (50%) and Jibeen (50%) enzymes respectively/. An average fat % of 19.97± 0.80, 16.00± 0.59 and 18.35± 0.44 obtained for renin, Osher (75%) and Jibeen (75%) enzymes respectively. Also a content of 20.39±0.57, 15.91± 0.40 and 18.54± 0.31 % for renin, Osher (100%) and Jibeen (100%) enzymes respectively.

The ash % when using renin, Osher (50%) and Jibeen (50%) enzymes obtained, was 4.27± 0.37, 3.27± 0.31, and 3.47± 0.26

respectively. Using renin, Osher (75%) and Jibeen (75%) enzymes, the ash % 4.07± 0.28, 3.04± 0.21, and 3.47± 0.26 respectively, and 4.12± 0.24, 3.04± 0.52 and 3.25± 0.16 for renin, Osher (100%) and Jibeen (100%) enzyme respectively. Also a significant variation ($p<0.05$) was detected betweenthe means of the milk components (protein, fat, ash %) for the different stages, whole lactation period and all treatments. The milk components showed low percentages, when plant enzymes were used compared to renin enzyme, Jibeen enzymes gave higher average % rather than Osher enzyme. In general, coagulating the milk with plant enzymes resulted in a decrease in the total solids % of the soft cheese in association with the type of coagulant used, variations in the contents of the milk components in addition to the method of coagulation used.

According to panelists the points scored for flavor were 9.00± 0.63, 5.87± 0.62 and 6.62± 0.69 using renin, Osher (50%) and Jibeen (50%) enzymes respectively. Using renin, Osher (75%) and Jibeen (75%) as 8.83± 0,86, 5.62± 0.76 and 6.62± 0.76 respectively, and 8.79± 0.77, 5.91± 1.28, and 6.87± 0,74 when renin, Osher (100%) and Jibeen (100%) enzymes were used.

Concerning the taste, the points attained when using renin, Osher (50%) and Jibeen (50%) enzymes, 8.08± 0.71, 6.25± 0.82, and 7.20± 0.88 respectively. 8.54± 0.77, 5.83± 0,76 and 6.58± 0.71 when using renin, Osher (75%) and Jibeen (75%) enzymes respectively, and 8.45± 0.88, 5.79± 0.83 and 6.50± 0,78 for renin, Osher (100%) and Jibeen (100%) enzymes respectively.

For texture, the points given when using renin, Osher (50%) and Jibeen (50%) enzymes were 8.97± 0.77, 5.83± 1.04 and 7.12± 0.97 respectively. Scored points when using renin, Osher (75%) and Jibeen

(75%) enzymes were 8.66± 0.70, 5.91± 0.71, and 8.12± 0.67 respectively, and 8.58± 0.88, 5.97± 0.72, and 8,08± 0.71 for renin, Osher (100%) Jibeen (100%) enzymes respectively. Also a significant difference ($p<0.05$) was detected between the average points given for flavor, taste and texture for all stages, whole lactation period and all treatments. Renin cheese scored the highest points for the sensory characteristics followed by Jibeen cheese. Osher cheese scored the lowest points. Variation in flavor, taste and texture of the soft cheese produced by the different coagulants may be related to the type of enzymes used for coagulation and their concentrations, chemical composition of the cheese milk and also the decrease of proteolytic activity of plant enzymes, due to increase of acidity when starterculture was added.

Based on the results obtained, Jibeen enzyme may be considered as an alternative for other enzymes, when the availability, especially renin enzyme is restricted for one reason or others.

مستخلص

أجريت هذه الدراسة لمعرفة أثر استخدام إنزيم الرنين والأنزيمات النباتية (العشر والجبين) في زمن التجبن، الإنتاج، التركيب الكيميائي، والصفات الحسية للجبنة البيضاء واستخدام أنزيم الرنين للمقارنة واستخدمت الأنزيمات النباتية بثلاثة تركيزات (50%، 75%، 100%). وتمت الدراسة خلال ستة شهور قسمت لثلاثة فترات، (أول، ثاني، ثالث شهرين بعد الولادة).

أظهرت الدراسة أن زمن التجبن الكلي خلال فترة الحليب الكلية لجميع المعاملات عند إستخدام أنزيم الرنين، عشر (50%)وجبين (50%) كانت 4.62 ±97.33، 5.88 ±124.37، و 4.96 ±96.29، على التوالي. وكانت 3.57 ±80.37، 7.29 ±113.62، و 2.23 ±87.75 عند إستخدام إنزيم الرنين، العشر (75%) والجبين (75%) على التوالي. بينما كانت 3.54 ±70.45، 5.62 ±108.83، و 4.95 ±85.95 عند استخدام إنزيم الرنين ، العشر (100%) والجبين (100%) على التوالي. أظهرت الدراسة أن هنالك فرق معنوي لزمن التجبن بين الثلاثة أنزيمات المستخدمة حيث تطلب إنزيم الرنين زمناً أقل مقارنة بالأنزيمات التباتية، كما تطلب الجبين زمناً أقل بمقارنته بالعشرر. وقد يعزى ذلك لنوع الأنزيم المستخدم، التركيب الكيميائي للبن، طريقة التجبن ثم قابلية اللبن المستخدم للتجبن.

إنتاج الجبنة الكلي (جرام لكل 5 كيلوجرام لبن) عند إستخدام الرنين، العشر (50%) والجبين (50%) كان 8.66 ±913.5، 14.09 ±635، و15.58 ±652.25، على التوالي. وكان إنتاج الجبين (جرام لكل 5 كليوجرم لبن) 32.9 ±944.3، 16.99 ±697.8، و 41.8 ±729.3 عند إستخدام انزيم الرنين، عشر وجبين (75%) على التوالي، بينما كان إنتاج الجبنة 11.54 ±916.0، 16.7 ±788.72 و 9.55 ±804.3 عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) على التوالي. أظهر الإنتاج الكلي للجبنة فرقاً معنوياً ($p0<0.05$) خلال دورة الحليب الكلية لجميع المعاملات. أظهر تجبن انزيم الرنين إنتاجاً جيداً للجبنة وتلاه انزيم الجبين. الإحتلافات في إنتاج الجبنة تعزى لتركيب اللبن، الإنزيم المستخدم ونوعه وا نتاج الخثرة الضعيفة بواسطة الأنزيمات النباتية.

خلال دورة الحليب الكلية لجميع المعاملات وعند استخدام انزيم الرنين، عشر (50%) وجبين (50%) كانت نسب البروتين 0.44 ±14.42، 0.46 ±11.96، و 0.74 ±13.44، على التوالي. وكانت نسبة البروتين 0.36 ±14.26، 0.35 ±12.14، و 0.60 ±13.61 عند استخدام انزيم الرنين، العشر والجبين (75%) على التوالي. بينما كانت عند استخدام انزيم الرنين

العشر (100%)، والجبين (100%) 0.39 ±14.36، 0.26 ±12.02، و 0.42 ±13.55 على التوالي.

كانت نسب الدهن عند استخدام انزيم الرنين، العشر (50%) والجبين، 0.84 ±20.06، 0.75 ±15.99، و 0.80 ±16.95 على التوالي. كانت نسبة الدهن 0.8 ±19.97، ±16.0 0.59، و 0.44 ±18.35 عند استخدام انزيم الرنين، عشر (75%) وجبين (75%) على التوالي. بينما كانت نسب الدهن عند استخدام انزيم العشر (100%) والجبين (100%) 0.57 ±20.39، 040 ±15.91، و 0.31 ±18.54 على التوالي.

كانت نسب الرماد عند استخدام انزيم الرنين، العشر (50%) والجبين (50%) ±4.27 0.37، 0.31 ±3.27، و 0.26 ±3.47 على التوالي. وكانت نسبة الرماد عند استخدام انزيم الرنين عشر (75%) والجبين 0.28 ±4.07، 0.21 ±3.04 و 0.26 ±3.47 على التوالي. بينما كانت نسب الرماد عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) 0.24 ±4.12، ±3.04 0.52 و0.16 ±3.25 على التوالي. أظهرت الدراسة أن هنالك فروق معنوية في متوسط نسب البروتين، الدهن، الرماد ($p<0.05$) خلال دورة الحليب الكلية لجميع المعاملات. أعطت الأنزيمات النباتية نسب أقل لمكونات اللبن (البروتين، الدهن، والرماد) بينما ارتفعت نسب هذه المكونات عند استخدام انزيم الرنين. وقد لوحظ ارتفاع نسب هذه المكونات عند استخدام الجبين مقارنة بالعشر. التجبن بواسطة الأنزيمات النباتية يؤدي لإنخفاض نسبة المواد الصلبة الكلية. وقد يعزى ذلك لإختلاف في تركيب اللبن أو طريقة التجبن المستخدمة.

عند إجراء إختبارات التذوق أعطت النكهة 0.63 ±9.00، 0.62 ±5.87 و 0.69 ±6.62 نقطة عند استخدام انزيم الرنين عشر 50% والجبين 50% على التوالي. بينما أعطت 0.86 ±8.83، ±5.62 0.76 و 0.76 ±6.62 نقطة عند استخدام انزيم الرنين عشر (75%) والجبين (75%). بينما أعطت النكهة 0.77 ±8.79، 1.28 ±5.91 و 0.74 ±6.87 عند استخدام انزيم الرنين عشر (100%) والجبين (100%) على التوالي.

بالنسبة للطعم أعطى 0.71 ±8.08، 0.82 ±6.25 و 0.88 ±7.2 نقطة عد استخدام انزيم الرنين، عشر 50% والجبين 50%. وعند استخدام الرنين عشر (75%) والجبين (75%) على التوالي 0.77 ±8.54، 0.76 ±5.83 و 0.71 ±6.58 على التوالي. بينما أعطى الطعم 0.88 ±8.45، 0.83 ±5.79 و ±6.50 0.78 نقطة عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) على التوالي.

بالنسبة للقوام أعطى 0.77 ±8.97، 1.04 ±5.83، و 0.97 ±7.12 نقطة عند استخدام انزيم الرنين، عشر 50% والجبين 50% على التوالي. وأعطى القوام عند استخدام انزيم الرنين، عشر (75%)

والجبين (75%) 0.7 ±8.66، 0.71 ±5.91 و 0.67 ±8.12 نقطة على التوالي. بينما اعطى القوام 0.88 ±8.58، 0.72 ±5.97 و 0.71 ±8.08 نقطة عند استخدام انزيم الرنين، عشر (100%) والجبين (100%) على التوالي.

أظهرت الدراسة أن هنالك فروق معنوية لمتوسطات النقاط التي أعطيت للنكهة، الطعم والقوام خلال دورة الحليب الكلية لجميع المعاملات. أعطيت العينات التي تم تجبينها بواسطة إنزيم الرنين نقاط أعلى عند استخدام الإنزيم المستخلص من العشر. وقد تعزى هذه الإختلافات نتيجة لأنواع الأنزيمات المستخدمة وتركيزاتها، التركيب الكيميائي للبن المعد لصناعة الجبن ولإنخفاض مقدرة الأنزيمات النباتية لتحليل البروتين، وذلك لزيادة الحموضة عند إستخدام البادي في صناعة الجبنة.

بناءاً على هذه النتائج يمكن إستخدام الإنزيم المستخلق من الجبين كبديل لإنزيم الرنين عند عدم توفره.

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Chapter One

1.0 Introduction:

Milk is a global drink that is a polyphasic emulsion having physical, chemical and biological properties and can be fermented into a wide range of different products with different flavors, consistencies and structure (Huria, 2002). 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).

To maintain the maximum use of milk, it should be transferred to milk products as to decrease the percentage of water that leads to the reduction of the activity of microorganisms which make milk spoil, e.g. lactic acid bacterial (Aziz, 1977).

One of the major milk products known is the cheese. Fox, et. al. (2007) stated, cheese is the generic name for a group of fermented milk based food products produced throughout the world in a great diversity of flavors, texture and forms.

The making of cheese as a mean of preserving the most important constituents of milk in 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 a long time, (Niir, 2010).

O'Conner (1993) explained, cheese is an excellent source of protein, fat, and minerals such as calcium, iron, phosphorus, vitamins and essential amino acids.

The trend nowadays is to produce different new varieties and types of the so-called functional cheeses as a functional dairy products Saxelin et. al. (2003) 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 foods 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 a gel (coagulation) is a basic step common to all types of cheeses. The coagulation of milk is a consequence of protein destabilization, which is brought by acid proteinases chymosin, the active component of renin. (Varnam and Sutherland, 1994).

According to O'Conner (1993), renin is a general term that describes a variety of enzymes of animals (specially calves), plant or microbial origin used to coagulate milk during cheese making. For coagulation of milk in the manufacture of cheese, calf renin is the most wide-spread and desirable and has been dominant in the industry of cheese processing for a long time. Gouda (1990) mentioned, calf renin is used in cheese making and is important in the formation of the casein network during coagulation and known to contribute to proteolysis in pickled cheese. Since the past century a shortage in calf renin had been noticed due to the decrease in the availability of sucking calves, as they are mainly reared for milk or beef production rather than other purposes. So, to cover the supply of the cheese industry with calf renin, it was to a remarkable extent restricted. Craw (1993) indicated, the limited supply of renin 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 overcome 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 coagulating enzymes extracted from Calotropis procera (Sodom apple) and Solanum dubium (Jibeen) plants are used in traditional cheese production as substitute of calf renin.

The general objectives of the current research is to study and compare the effects of renin and plant enzymes milk coagulants on cheese quality, i.e. composition, sensory characteristics and yield in association with enzyme activity, time of coagulation, ripening process and period, salting and acceptability of processed cheese by consumers.

1.1 Objectives:

The main objectives of this research are:

- To study the effects of using renin and plants coagulants on the coagulation time during white soft cheese manufacturing.
- To study and assess the effects of using renin and plants coagulants of yield, composition, and sensory evaluation of produced white soft cheese.
- 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 coagulating the casein with the help of renin 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 some 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 whey from curd, with the exemption of some 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 Association (IDFA) (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 renin, 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.

Cheese is a group of fermented milk products made basically for preservation of milk constituents safe for consumption for longer periods as given by Shahata (1997).

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 8000 B.C (Clarence et. al. 2004).

There is no conclusive evidence indicating where cheese making originated from either 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.

Until its modern spread along with European culture, cheese was nearly unheard of in oriental cultures, uninvented in the pre-Colombian Americas and of only limited use in Sub-Mediterranean Africa, mainly being wide spread and popular only in Europe and areas influenced strongly by its culture. But with spread, first of European imperialism, and later of Euro-American culture and food, cheese has gradually become known and increasingly popular worldwide, though still really considered a part of local ethnic cuisines outside Europe, Middle East and Americas. (Mc Gee 2004).

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%

Abu Daood et. al. (2003) divided the types in three major groups, hard, semi-hard and soft with a moisture content of 30-35%, 40-50% and 50-70% for each respectively.

Four major groups are given by Alkholi (1999), which were very hard, hard, semi hard and soft with a moisture content of 30-40%,40%, 40-45%, and 45-75% respectively. A moisture content of less than 40% is given for dried very hard, 40-49%, for very hard, 50-59% for hard, 60-69% for soft and 70-80% for fresh cheese (Shahata, 1997).

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

Spreer (1998) explained, for another classification into groups and types, different aspects and characteristics can be used, such as:

- Types of consistency (hard, semi soft and soft cheese).
- Types of milk (cow, sheep, goat, buffalo).

- Chemical composition (Ca-content in conjunction with pH_1 dry matter, water, fat).
- Ripening process (ripened, non-ripened, fresh cheese).
- Variation in taste.
- Type of whole formation (large, medium and small round holes, cracks, irregulars holes, no holes).
- Surface characteristics (blue fungus or white fungus cheese, smear cheese, skinless cheese).

2.4 Chemical Composition of Cheese:

Cheese contains almost all milk components in concentrated form such as protein, fat, minerals and lactose, which to greater extent is converted into organic salts (Elnimer 2007). Murshidi (1998) explained, cheese contains the undissolved components of milk, e.g. casein, amounts of fat and salt beside water containing few amounts of salts, lactose and albumin.

Renin cheese composes in average of 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 into 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 cheese as given by the Sudanese Standards 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%.

Caric et. al. (1993) indicated, the protein content of Sudanese white soft cheese was found to be 19.76% and 20.12% when the fat % of the milk used ranged between 2.2% and 4.4% respectively. Different fat levels, ripening time of the cheese, different storage periods and types of

packaging showed in most cases highly significant difference ($P < 0.001$) for protein content of the processed cheese (Caric et. al. 1993). The fat content of Sudanese white soft cheese ranges between 15.4% and 23.4%, when the percentage of the fat content of cheese milk ranges between 2.2-4.4% as given by Dimitreli et. al. (2004) and also showed significant differences with different fat levels, ripening time and storage period of produced cheese.

Concerning the ash level of the cheese, it was found to range from 1.75% to 5.35% in association with the storage period and the fat content. The ash % was decreased with long periods of storage and the decrease in the fat levels as explained by Abdalla (1993). The total solids of the white soft cheese range between 41.58% and 50.32%, when the fat of the milk prepared for cheese making was 2.2 and 4.4 respectively and also a high significant variation was reported due to variation in the fat content of the cheese, (Abdel Razige, et. al. 1996).

2.5 Milk Coagulation:

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

either by microorganism or by plant enzymes extracted from some types of plants.

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, must 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 into a gel state (coagulate, gallert), especially into a lyogel.

The coagulation of milk is influenced mainly by the type and concentration of coagulation enzyme, coagulation temperature, properties and concentration of proteins and the pH value, (Storry and Ford, 1982).

2.5.1 Renin Coagulation:

2.5.1.1 Mechanism of Coagulation:

The mechanism of casein precipitation by enzymatic coagulation after addition of the recent enzyme to milk is described by Fredriksen (2011), Spreer (1998) and Daviani et. al (1980) as follows:

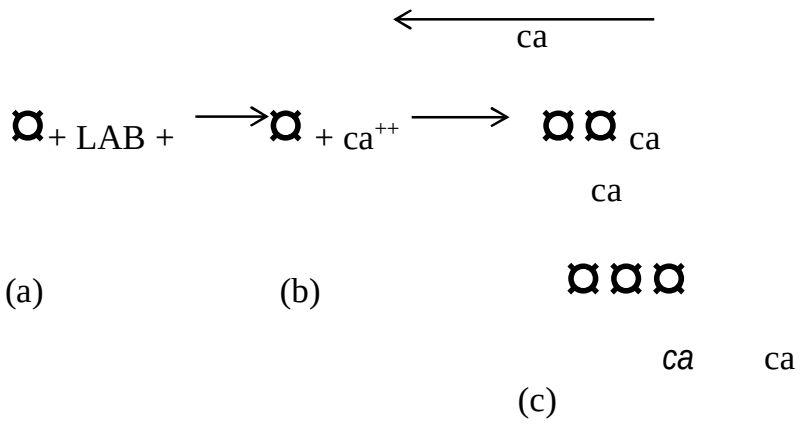
The casein precipitation takes place in two stages:

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 (renin gel, renin gallert) and it is the real cheese material.

Fig. (1) Shows the described mechanism



(a) Casein micelle.

(b) Casein particle without the effect of the protective colloid.

(c) Casein paracasinate (Renin gel).

The milk serum proteins are not affected by the enzymatic reaction, since they remain water insoluble and migrate as whey proteins in the aqueous phase.

2.5.2 Renin coagulation:

Renin or (renin) is a complex of enzymes produced in the stomachs of ruminants mammals. Chymosin is a protease enzyme that curdles the casein in milk. This helps young mammals digest their mother milk. Renin can also be used to separate milk into solid curds for cheese making and liquid whey. In addition, chymosin renin contains other important enzymes such as lipase. Renin is used for the production of most cheeses. The mammal must be slaughtered to obtain its renin. Non-animal alternatives for renin are suitable for consumption by vegetarians (Kopelman et. al. 1975).

2.5.3 Types and Production of Renin

2.5.3.1 Production of Natural Calf-renin:

Natural calf renin is extracted from the inner mucosa of the fourth stomach chamber (the abomasum) of young unweaned calves as part of the livestock butchering. The stomachs are by products of real production. If renin is extracted from calf it is not suitable for lactovegetarians to consume.

2.5.3.2 Vegetable Renin:

Many plants have coagulation properties. Greeks used extract of fig juice for coagulation, also dried caper leaves, nettles, mallow and ground ivy (creeping chartie) were used also. Enzymes from Cynara or thistle were used in some traditional cheese production in the Mediterranean. Phytic acid derived from unfermented soybeans or fermentation produced chymosin (FPC) may also be used (Farkye, 2004).

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

2.5.3.3 Microbial Renin:

Some molds such as Rhizo mucor miehe are able to produce proteolytic enzymes. These molds are produced in a fermenter 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 renin tend towards some bitterness especially after long maturation periods. Cheeses produced by this way are suitable to vegetarians (Lee et. al.. 1990).

2.5.3.4 Fermentation Produced Chymosin:

Because of the above imperfections of microbial and animal renin, many producers sought further replacement of renin. With development of genetic engineering, it became possible to isolate renin genes from animals and introduce them into certain bacterial, fungi or yeast to make them produce chymosin during fermentation (Hayalogllu, et. al. 2004).

The genetically modified micro-organism is destroyed after fermentation and chymosin isolated from fermentation broth, so that the fermentation produced chymosin (FPC) used by cheese producers does not contain any genetically modified microorganism component or ingredient. FPC is identical to chymosin made by animals, but produced in more efficient way. FPC products have been on market since 1990 and have been considered in the last 20 years the ideal milk clotting enzyme, (Singh, et. al. 1989).

Fermentation produced chymosin is used more often in industrial cheese making in North America and Europe today because it is less expensive than animal renin (Uysal, et. al. 1996).

FPC was the first artificially produced enzyme to be registered and allowed by the U.S. Food and Drug Administration, in 1990 about 60%

of United State hard cheese was made with FPC, and it has up to 80% of the global market share for renin.

By 2008, about 80% to 90% of commercially made cheeses in the United State and Britain were made using FPC (Tulay et. al. 2013). Today the most widely used FPC is produced either by the fungus Aspergillas niger and commercialized under the trade mark CMY-MAX® by the Danish company Chr. Hansen or produced by Kluyveromyces lactis and commercialized under the trade mark MAIREN® by Butch company DSM.

FPC is chymosin B, so is more pure compared with animal renin which is a multitude of proteins. FPC can deliver several benefits to cheese producers compared to animal or microbial renin, such as higher production yield, better curd texture and reduced bitterness. (Fox, et. al. 1996).

Cheese produced with FPC can be certified Kosher and Halal and suitable for vegetarians if no animal based alimentation was used during the chymosin production in the fermenter, (Lee, et. al. 1990).

Older calves (grass fed or grain fed) renin contains less or no chymosin, but a high level of pepsin and can only be used for special types of milk and cheeses. As each ruminant produces a special kind of

renin to digest the milk of its own species, milk specific renin are available such as type of goat renin for goats milk and lamp renin for sheep's milk (Van Haoydonk 1987).

2.5.3.5 Traditional Method of Renin 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 crude renin that remains in the filtered solution can then be used to coagulate milk. About 1g, of this solution can normally coagulate 2.4 litre of milk (Cremer1985).

2.5.3.6 Modern Method of Renin Production:

Deep frozen stomachs are milled and put into an enzyme extracting solution. The crude renin extract is then activated by adding acid, the enzymes in the stomach are produced in the active form and are activated by the stomach acid. The acid is then neutralized and the renin extract is filtered in several stages and concentrated until reaching a typical potency of about 1 gram coagulates 15kg of milk. One kg of renin extract has about 0.7g of active enzymes. The rest is water and salt and sometimes sodium benzoate (0.5-1.9%) for preservation and typically 1kg of cheese contain about 0.0003 of renin enzyme (Najera, et. al 2008).

2.5.3.7 Alternative Sources of Renin:

Because of the limited availability of mammalian stomachs for renin 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 renin range from plants and fungi to microbial sources. Cheese could be produced from any of these varieties of renin.

2.5.4 Plant Coagulation:

Plant coagulation was not registered till as commercial methods of coagulation due to a lot of hazards 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). Plants used for coagulating milk are e.g. the following:

2.5.4.1 Solanum dubium (Jibeen) Coagulation:

It is a wild plant considered as weed found in most areas of Sudan. It has not an economic importance. Locally, named Jibeen, and belongs to family Solanaceae.

Enzymes extracted from family Solanaceae were used in several trails for coagulation of milk. The coagulated milk gave different cheese compositions, yield and time required for coagulation (Beeby, 1980), and reported that coagulation took much time compared to renin enzyme coagulation, found significant difference among the same plant and that Solanum dubium was the best among the spp. of Solanum, but the chemical composition of the cheese showed low percentages in all components compared to all other types of enzymes used for coagulation. These results were similar to those of Andren, et. al. (1982), who reported that the plant coagulation resulted in low quality cheese specially Solanum Spp. and Calotropis procera and found low percentages of most cheese components and its total solids compared to cheeses made from milk by renin coagulation.

Talib et. al (2007) studied the coagulating properties of Solanum dubium (Jibeen) seed extracts. The Jjibeen seeds were extracted with both water and citrate phosphate buffer. Effect of enzyme concentration, milk pH, milk temperature and heat inactivation of crude enzyme 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 enzyme was decreased at pH of the milk over 6.2, increasing of the milk temperature above 40^{o}C, decreased the clotting

time and the activity of the enzyme was lost on pH 4.6 and 6.6 and temperature 60^{o}C at pH 3.6 for 10 minutes, but at pH 4.6, 5.6 and 6.6 and temperature 70^{o}C, the enzyme activity was not affected, but it lost its activity at 80^{o}C/10 minutes. Shaw (1980) also noticed that plant enzymes are too proteolytic for cheese making. If proteolytic activity is excessive, cheese yield and retention fat in the curd may be diminished and it has undesirable effects on the body and texture of finished cheese (Yousif et. al 1996).

The quantification of milk activity in solutions containing proteolytic enzymes is a major concern in industrial cheese making, and cheese research as given by (Carlson et. al. 1985).

2.5.4.2 <u>Calotropis procera</u> (Osher) Coagulation:

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

It was known that early Osher plant specially the milk juice found in its fruit and leaves had the power to coagulate milk, but it was tested for coagulation recently by Dalgleish (1985) who explained the potentially of this plant for coagulation, but reported a slow development for coagulation followed by decrease of the percentage of all cheese components, and its 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 cheese with low percentage of protein and fat in addition to the investigation of some toxic materials during the analysis of chemical composition of this plant.

2.5.4.3 Terristris Enzyme Coagulation:

Terrestris is an annual plant belongs to the family Zygophyliaceae widely distributed around the world.

Enzyme extracted from this plant were known to the nomads who used this plant very early (all parts of the plant) for milk coagulation. de Koning, et. al. (1978), reported that some trials were conducted to study the ability of this enzyme to coagulate milk and technological characteristic of some types of white cheese, and mentioned this plant had a good tendency to coagulate milk, after some time coagulation began and completed after 92 minutes, so the enzyme required much time for coagulation compared to renin enzyme but these results showed no significant difference, compared to control enzyme (Renin). The chemical composition was mildly differ and showed also no significant difference. In addition to this, cheese yield and sensory evaluation followed the same trend. These results were parallel to those of Kaye, et. al. (1978) who found that the chemical composition (fat % and protein %) did not show a significant variation, when they tested three

concentrations of the enzyme extracted from the fruit of the plant and explained, there was a significant difference among these three concentrations for milk coagulation, but there was no significant difference between the higher concentrate and renin enzyme for milk coagulation, but the percentages of cheese components and its total solids tend to be higher when milk coagulated by renin enzyme compared to different concentrations of Terristris enzyme.

2.5.4.4 Cynara cardunculus Coagulation:

According to Sofia et. al. (2005), enzymes extracted from plant Cynara Cardunculus can also be used for coagulation, which proved to have a proteolytic activity. Cyprosins (Cunarases) are aspartic proteinases present in the aqueous extract of cynara carunculus L. (Cardoon) flowers used as milk coagulant for the manufacture of some Portuguese and Spanish traditional cheeses. Synprosins have an activity on k-casein similar to that of renin and a pronounced specific activity on other casein fractions (Queiroz et. al. 1993).

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 ca^{++} in milk. So, any factor that affects the content of the ca^{++}

affect the time of coagulation. Heat treatments 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 two cases a source of ca^{++} must be added to milk prepared for cheese processing, e.g. $Cacl_2$ which is added at certain level (0.02% and 0.03%) for sterilized and dried milk respectively (Scancalapore, et. al. 1988). For this reason when coagulation time was compared to different treatments, milk should be identical for each treatment avoiding all factors that affect the levels of ca^{++} in milk.

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

One of the main factors that affects the time of coagulation is the method of coagulation. Natural milk produced from healthy cow required 90-105 minutes for total coagulation. This time is changed when the coagulation enzyme is changed (van Hooydonk, et. al. 1984). This demonstrated that renin coagulation required few time compared to plant enzyme and showed a significant differences ($P<0.05$) among the

different methods of coagulation. However, the time of coagulation also differed between different types of plant enzymes. Hamed (1998), compared two types of plant enzyme (from solanum and terristris) and reported that solanum enzyme required a lot of time for coagulation compared to terristeris enzyme and thus followed by weak milk curd and low percentages of cheese yield.

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

($p > 0.05$) between some spp. of terristris and renin enzyme, but he found significant difference among some plant enzymes and renin enzyme.

Some investigators reported no significant difference between three methods of coagulations (renin enzyme, FPC and plant enzymes).

A close relationship was found between milk clotting activity and time in association with plant enzyme used and milk quantity. The milk clotting activity was calculated by Ibiama and Griffiths, 1987) as follows:

$$X = 100\ D/T$$

Where X = Milk clotting activity (unit/ml)

D = Dilution or quantity of milk containing 1ml of the crude enzyme.

T = Clotting time in seconds.

Sinyth et. al. (1987) revealed a significant difference among three treatments used to compare the length of time of coagulation between renin enzyme and two types of plant coagulation enzymes extracted from Terristeris spp. and Solanum spp.

2.7 Cheese Yield:

The typical yield of cheese ranges from 9-15% depending on the chemical composition of the milk, efficient recovery of fat and casein in the cheese, losses of milk constituents in the whey resulting from milk handling and treatment and cheese making procedure and the final moisture content of the cheese (Frakey, 2004). According to Abel Razig (1996), cheese yield from cow milk (2% salt) was found to be 19.08%. Babiker (1987) gave a range of 8-14% for unsalted white soft cheese and Khateeb (1997) noticed, the yield of fresh cheese between 23.7 – 33.34% with an average of 27.8%.

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

- Milk composition.
- Gentic variation.
- Physiological factors.
- Processing conditions.
- Lactation stage.
- Seasonal variation.
- Type of milk.
- Starter culture used.

- Standardization of milk.
- Heat treatment of milk.
- Homogenization of milk.
- Type of coagulant used.
- Curd firmness.
- Curd handling system.
- Storage of milk.

The cheese yield is directly related to the final moisture content of the finished cheese, in addition to milk composition, degree of recovery of the fat and casein by the curd during cheese making, explained Kosikowshki (1978).

Abdel Razig and Babiker (2009) mentioned the weight loss of cheese significantly affected by storage time and it increases gradually till the end of the storage period

2.7.1 Yield by Renin and Plant Enzymes Coagulation:

Foltman (1987) explained, cheese yield increased when the ca^{++} increased and he recommended addition of $Cac1_2$ to milk exposed to ultra heating. Renin coagulation gave good results for cheese yield when the $Cac1_2$ was added when dry milk or sterilized milk were used.

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

Another results were obtained by Sinyth, et. al. (1987) who demonstrated that plant coagulation resulted in low yield of white cheese due to weak curd produced by plant coagulants.

These results were in line to those given by Merin, (1989) that 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. The renin 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 renin power for coagulation and then cheese texture and cheese yield were progressed, but increasing the temperature above $40^{o}C$ affects the power of the enzyme and altered the coagulation process followed by decrease in cheese yield (Nijera 2003).

Another recent result by Oscan, et. al. (2012) indicated that use of starter culture and its addition to milk prepared for cheese making increases cheese yield; this may due to the maximum renin activity when the acidity of milk increases and also to the protease enzymes of plant origin used and their coagulation power. But all of them showed significant difference compared to renin enzyme; also cheese yield coagulated by Terristeris spp. gave results near to the results of renin enzyme, but some variations in cheese between the two types of 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.

Renin 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 renin enzyme compared to different plant enzymes. Dalgleish et. al. (1989) gave that the ash content of cheese was created by using renin 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 renin 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 used for 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 renin 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 two months up to four months after calving) showed a significant difference ($p<0.05$) for protein and fat percentages and the ash percentage was higher but no significant difference ($p>0.05$) for ash percentages was found when milk was

coagulated by renin enzyme compared to plant enzymes and microbial enzyme. Furthermore, some plant enzymes, e.g. (Terrestiris enzymes) tend to give results near to the renin enzyme and also no significant difference between these enzymes and renin enzyme was detected. The results obtained by Kumosinski, et. al. (1991) showed that most of cheese components and its total solids differ according to the type of enzyme used for coagulation. The percentages of protein and fat showed a significant difference between renin enzyme and two types of plant enzymes (two different concentrations of solanum and terristris enzymes). Terristris enzyme gave results near to that of the control enzyme, which was renin enzyme. Horme, (1990) compared three methods of coagulation enzymes (renin, 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 renin coagulation and lower when milk was coagulated by plant enzymes specially the enzyme extracted from (Calotropis procera plant) due to the losses of most milk components with the drained whey according to weak curd formed when milk was coagulated by these enzymes.

Also a significant difference was detected between renin and plant coagulation (enzymes extracted from Terristeris ssp. and solanum spp),

but comparing the coagulation caused by renin enzyme and terristris spp enzyme no significant difference was recorded ($p>0.05$) despite the increase in yield of renin cheese (Sinyth, et. al. 1987).

2.9 Sensory Evaluation:

Humans have used their senses to evaluate 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. safe 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 the 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 the 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 the 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 free of bitter taste, rancidity and rottening.
- 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, when abnormal change in color, advanced dryness or abnormal rotenning, blowing and abnormal holes are noticed.

Kumosinski et. al. (1991) reported, the taste, texture and flavor of cheese were affected by the method of coagulation and found significant differences 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 evaluation, when different types of plants, microbial or renin enzymes were used. This was clear to taste and flavor, but some differences were found for the texture, even among the same spp. of plant enzyme, although these differences were not statistically significant.

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

The excessive proteolytic activity of the plant enzyme during the ripening process of the cheese has undesirable effects on the body and texture of the finished cheese as noticed by Yousif et. al. (1996).

Cheese contained different amounts 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 preservative effect, favors water binding, promotes formation of the skin and finally, influences the solidification of the cheese, which increases with increasing salt concentration.

Chapter Three

3- Materials and Methods:

Three types of cheese using three different coagulants were manufactured. The three coagulants used were renin enzyme and two plant enzymes extracted from Osher (Calotropis procera) and Jibeen (Solanum dubium).

The renin enzyme and the produced cheese were used as control. The other plant enzymes were used with different concentrations, (50%, 75% and 100%).

3.1 Method of Extraction of Plant Enzymes:

3.1.1 Extraction of Osher Enzyme (Calotropis procera):

The leaves of the plant were dried by sun and air until a constant weight was reached. 100g were socked in distill water and Nacl (5%) for 24 hours. Then the solution was filtered by filter paper and kept in refrigerator at 7^{o}C

Then used as coagulant after three days only. Three concentrations were used (50%, 75% and 100%).

3.1.2 Extraction of Jibeen (Solanum dubium):

The fruit of the plant was used. It was crushed after drying to constant weight by sun. The plant was crushed then 100gm from the

plant was socked in salted distilled water (5% Nacl) for 24 hours and then filtered by filter paper and the solution was preserved in at 4-7^{o}C for three days and then used as coagulant with three different concentrations 50%, 75%, and 100%.

3.1.3 The Renin Enzyme:

The renin enzyme was obtained from a 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 in a solid form (tablets). Each tablet weight two grams and it was sufficient for coagulating 100kg of milk. Each tablet was dissolved into 100ml of distilled water only 5ml from the solution after dissolving the enzyme were added to each 5kg. 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 of Kenana farm which belongs to Kenana Sugar Compay (KSC) at Kenana, White Nile State, Sudan. Milk used during the normal period of lactation

(six months after calving) which was divided into three sub-periods explained as follows:

a. The first two months after calving (1st. stage).
b. The second two months after calving (2d. stage).
c. The third two months after calving (3rd. stage).

3.3 Collection of Samples:

For each treatment eight samples of milk were collected every two months for each concentration and hence 24 samples were collected for each treatment during every period, i.e. for each treatment 3x24 = 72 samples, were collected. The total numbers of the samples collected during the whole period were 72x3 = 216 samples of white cheese.

Samples were collected weekly. For each sample 5kg of milk were used for cheese making. The total amount of milk used during this study was 216x5 = 1080kg during the six months.

3.4 Source of Plant Enzymes:

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

3.5 Procedures of Manufacturing:

3.5.1 Renin Cheese:

1. 5kg of milk were taken from the milk of the dairy herd of Kenana farm.
2. Milk was heated to 72^{o}C , then cooled to 42^{o}C.
3. Starterculture was added (1%) and then temperature was adjusted to 42^{o}C for 45 minutes.
4. Renin enzyme was added, then coagulation of milk was observed.
5. Time of coagulation was recorded from the addition of the renin enzyme till the complete coagulation of milk occurred.
6. When coagulation occurred, the curd was put on wooden trail, surrounded by clothes with harrow orifices to ensure good draining of whey.
7. Cheese was salted by socking it into salty solution, where the concentration of Nacl was 10% for 24 hours.
8. The weight of cheese was determined after the salting was completely done after 24 hours from the beginning of cheese salting.

3.5.2 Osher Enzyme (Calotripis procera) Cheese:

1. 5 kg of cow milk were obtained from the milk of Kenana farm dairy herd.
2. Milk was heated to 72^{o}C and then cooled to 42^{o}C.
3. 1% of starterculture was added and temperature was adjusted to 42^{o}C for 45 minutes.
4. The extracted Osher enzyme was added at three different concentration (50%, 75%, and 100%) respectively during the first, second and third two months after calving respectively.
5. Time of coagulation was recorded from the addition of the enzyme till the complete coagulation of milk.
6. Cheese was salted by socking it into 10% solution of Nacl for 24 hours.
7. Cheese was weighted and its final weight was determined.

3.5.3 Jibeen Cheese (Solanum dubium):

1. 5kg of cow milk from Kenana dairy herd were used for cheese.
2. Milk was heated to 72^{o}C then cooled to 42^{o}C.
3. 1% of the starterculture was added and temperature was adjusted to 42^{o}C for 45 minutes.
4. Jibeen enzyme was added with three different concentrations (50%, 75% and 100%).

5. Time of coagulation was recorded from the addition of the enzyme till the complete coagulation occurred.
6. When coagulation occurred, the cheese was surrounded with clothes with narrow orifices and then put on wooden trails for complete draining whey.
7. Cheese was salted by Nacl by socking it into 10% Nacl solution for 24 hours.
8. Cheese weight was then determined.

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 during different periods of lactation until the complete coagulation occurred which was determined by the change of the watery 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 socking the cheese for 24 hours in 10% Nacl solution. Immediately after the period of socking cheese weight was determined by electrical balance weighing up to three kg and determine the weight to one decimal.

3.6 Laboratory Analysis:

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

Apparatus:

- Gerber tube with cork stoppers.
- Centrifuge (100 Revolution/1minute).
- Water bath.
- Holder

Material:

- Cheese samples.
- H_2SO_4 (90-91%).
- Amylalchol.
- Distilled water.

Procedure:

Ten (10)ml of sulphuric acid wee poured in clean and dry cheese Gerber tube followed by addition of 3grams minced cheese sample. Amyalchol is then added (1ml) to the mixture followed by addition of distilled water, mixture mixed thoroughly till no white particles were seen. The tube was then centrifuged to the water bath at 60-65^{o}C for 3-5 min. the fat % was read immediately from the Gerber tube.

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

Kjeldhal Method:

Apparatus:

- Kjeldhal Flask.
- Heaters
- Volumeteri flask (100nl)
- Conical flask
- Burrets
- Pippets.

Material:

- Cheese saple.
- Conc. H_2S0_4.
- Distilled water
- NaoH
- Boric acid.
- Indicator (promo cresol green + methyl-red
- Hcl
- - kjeldal tabs.

Procedures:

Three (3) gms of cheese and 2 Kjeldhal tablets were brought into the Kjeldhal flask and 25ml of conc. H_2S0_4 were added. The mixture, using a heater, then digested till a clear solution was obtained after 2-3 hours. The digested sample was then poured into volumetric flask and diluted to 100ml with distilled water. The 5ml from the dilution was transferred to Kjeldhal flask and 10ml of Naoh were poured, received in a conical flask containing 25ml of 4% boric acid and 3 drops of the indicator. The distillation was continued until the volume in the flask reaches 75ml. flask is removed and distillate was titrated against 0-14.Hcl, until red color was obtained.

The total protein % is calculated as follows:

Nitrogen % = T x 0.1 x 0.114 x 20/w x 100.

Protein % = Nitrogen x 6.38

Where:

T: Titration figure.

0.1: Normality of Hcl.

0.0114: Atomic weight of Nitrogen.

20: Dilution factor.

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

Apparatus:

- Aluminum dish.
- Dry oven.
- Dissecator.
- Muffle furnace oven.
- Balance.

Materials:

- Cheese sample.

Procedure:

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

  Ash% = $\_\text{Ash \%} = \frac{W1}{W0} \text{x } 100$

Where:

W1: Weight of ash.

W0: weight of samples.

3.7 Sensory Evaluation:

The sensory characteristics of the cheese samples produced by using renin, Solanum dubium and Calotropis procera enzymes during the different stages of lactation were judged by well-trained panelists in terms of flavor, taste and texture. Each property was evaluated by giving certain points and the average was then calculated. the assessment was performed using the evaluation sheet given below:

Evaluation Sheet

Item / Sample No.	**Flavor**	**Taste**	**Texture**
1			
2			
3			
4			
5			
6			
7			
8			
Mean			

Flavor	**Taste**	**Texture**
- High normal 10	- Highly palatable 10	- High consistent 10
- Very normal 7	- Very palatable 7	- Very consistent 7
- Normal 5	- Palatable 5	- Consistent 5
- Less normal 3	- Less palatable 3	- Less consistent 3
- Abnormal 1	- Unpalatable 1	- Not consistent 1

3.8 Statistical Analysis:

The obtained data were subject to statistical analysis by using program Statistical Package for Social Science (SPSS, 2007) program. Data of samples were analyzed statistically by using 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:

The obtained results for all treatments are given in the following tables.

Table (2) Average coagulation time (minutes) by renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	77.25	5.36	126.12	7.62	96.87	6.26	*
(2)	2nd stage	81.37	2.92	123.00	4.27	93.87	5.13	*
(3)	3rd stage	97.37	4.80	124.00	5.63	98.12	2.10	*
Total	Whole period	97.33	4.62	124.37	5.88	96.29	4.96	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

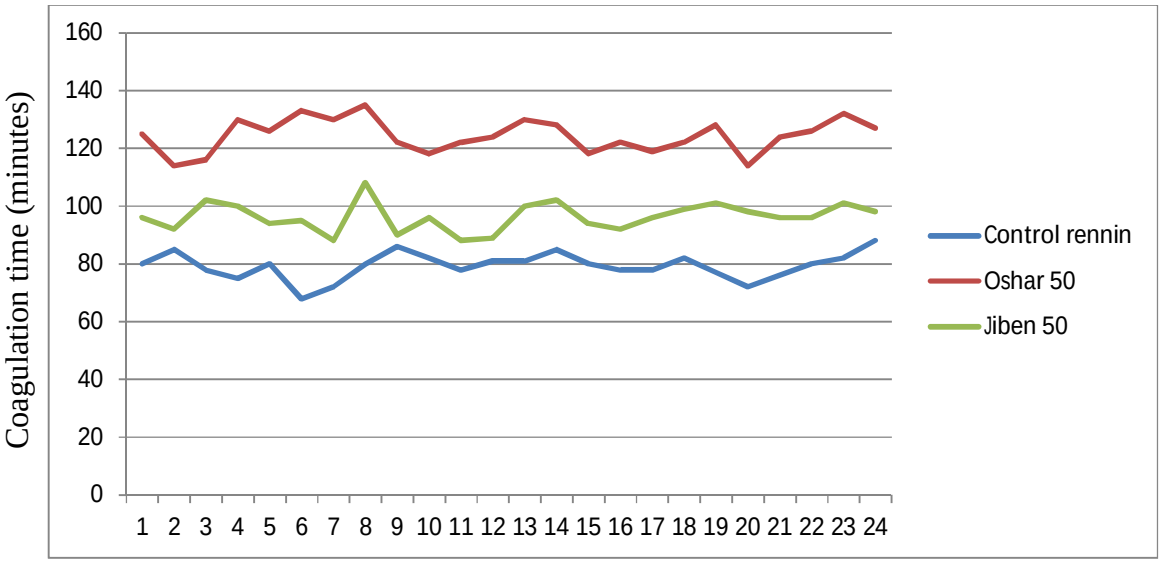
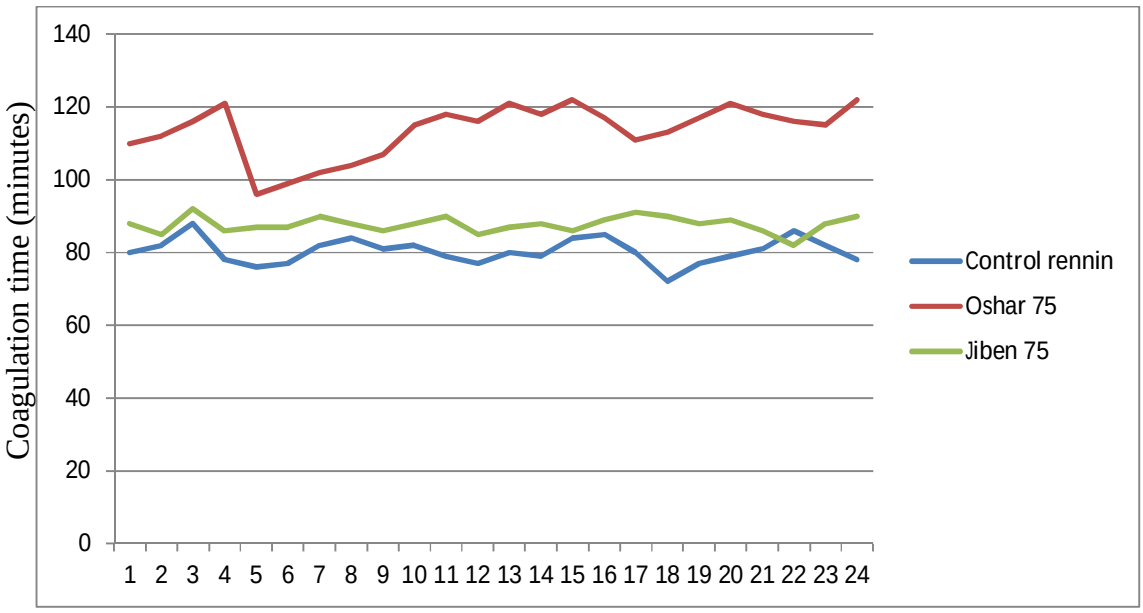


Table (3) Average coagulation time (minutes) by renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	80.87	4.62	107.50	5.88	87.87	4.96	*
(2)	2nd stage	80.87	2.69	116.75	4.59	87.37	1.68	*
(3)	3rd stage	79.37	4.06	116.62	3.73	88.00	2.87	*
Total	Whole period	80.37	3.54	113.62	7.29	87.75	2.23	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (4) Average coagulation time (minutes) by renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	78.87	2.94	111.12	7.39	85.12	5.22	*
(2)	2nd stage	80.12	4.67	109.37	3.85	83.62	4.24	*
(3)	3rd stage	79.37	3.54	106.00	4.34	89.12	4.12	*
Total	Whole period	79.45	3.65	108.83	5.62	85.95	4.95	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

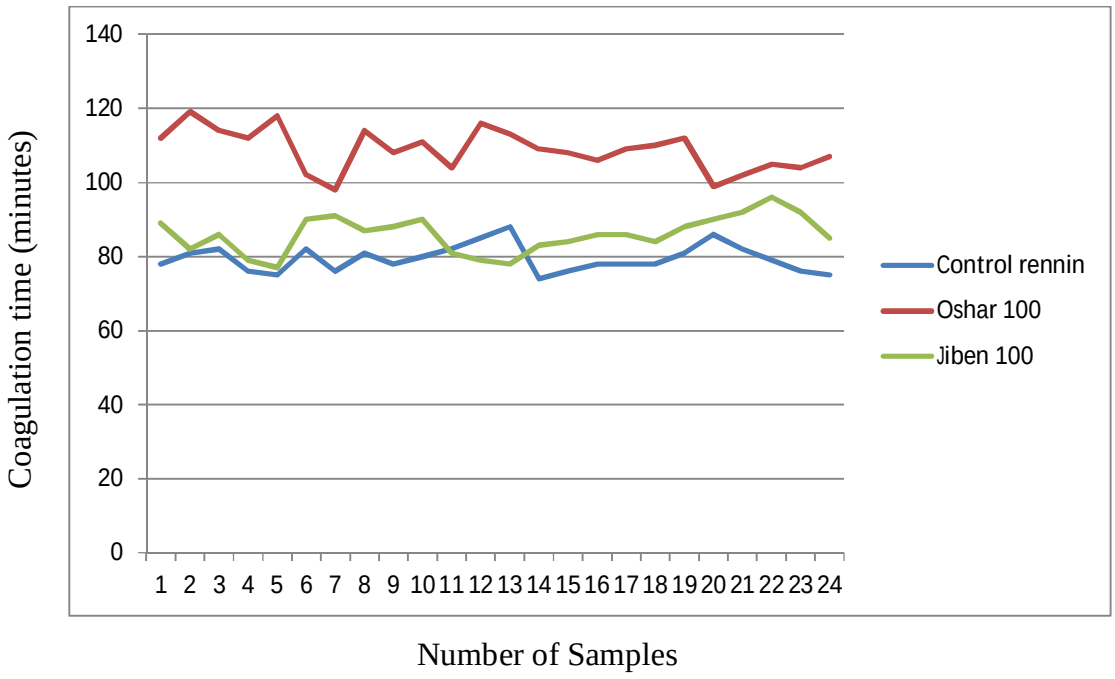


Table (5) Average cheese yield (grams) for each 5kg milk by addition of renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	915.7	5.09	635.2	13.07	656.0	10.91	*
(2)	2nd stage	916.00	9.05	645.8	15.23	672.6	20.30	*
(3)	3rd stage	909.00	10.14	626.8	6.93	659.1	9.37	*
Total	Whole period	913.5	8.66	635.9	14.09	662.5	15.58	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

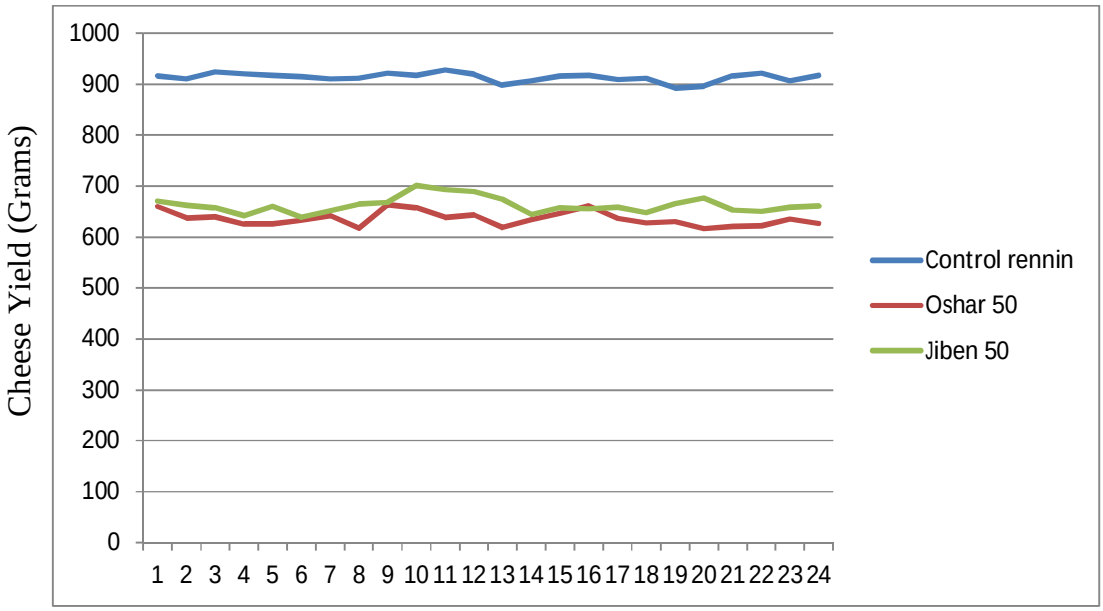
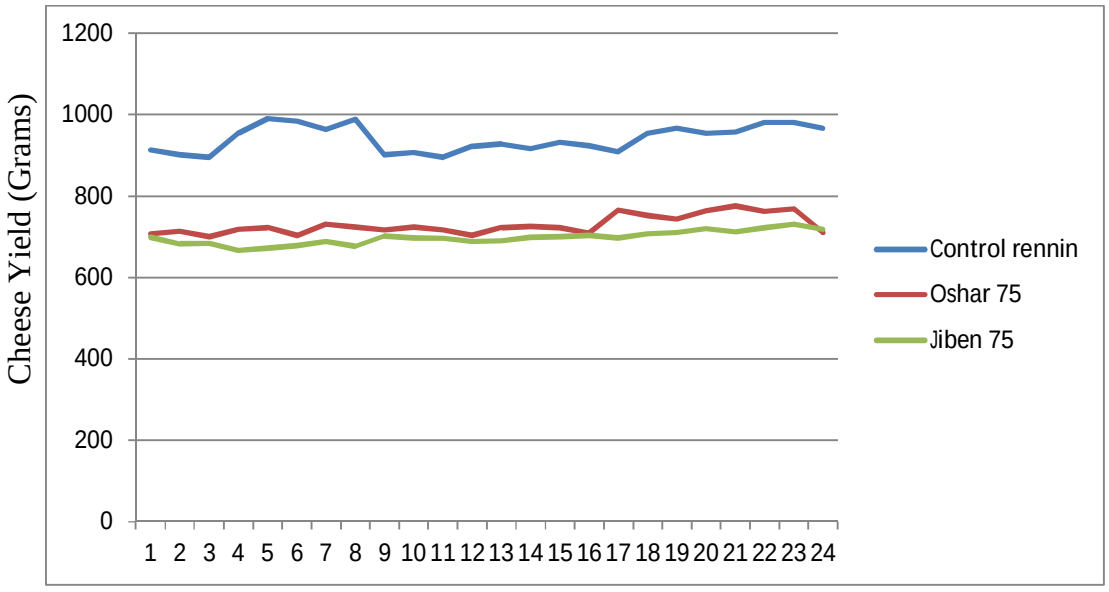


Table (6)Average cheese yield (grams) for each 5kg milk by addition of renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	949.1	39.51	681.1	9.87	715.2	10.67	*
(2)	2nd stage	915.7	13.12	697.2	5.36	717.5	8.01	*
(3)	3rd stage	958.7	22.87	715.1	10.28	755.3	20.44	*
Total	Whole period	944.3	32.89	697.8	16.99	729.3	41.80	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (7) Average cheese yield (grams) for each 5kg milk by addition of renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	912.3	15.06	776.2	21.04	800.6	11.22	*
(2)	2nd stage	916.2	8.17	790.0	10.65	800.0	4.50	*
(3)	3rd stage	919.6	10.70	800.0	10.97	812.7	5.70	*
Total	Whole period	916.0	11.54	788.7	16.70	804.3	9.55	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

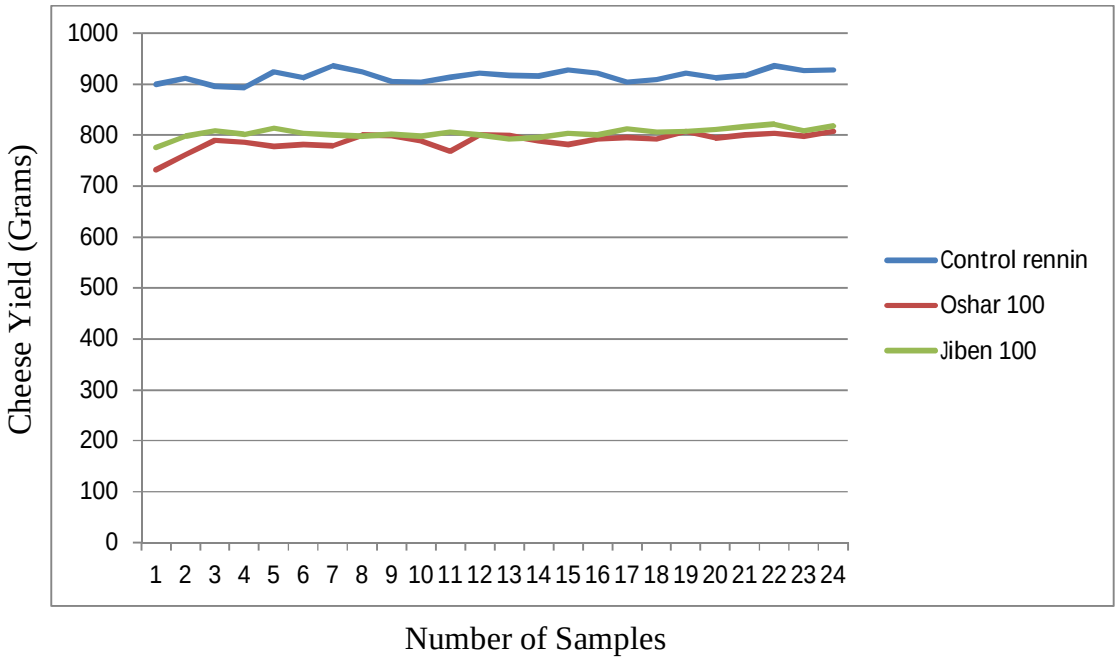


Table (8) average protein % obtained by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	14.46	0.45	11.80	0.45	13.55	0.73	*
(2)	2nd stage	14.30	0.55	11.82	0.51	13.38	1.04	*
(3)	3rd stage	14.50	0.33	12.26	0.26	13.40	0.41	*
Total	Whole period	14.42	0.44	11.96	0.46	13.44	0.74	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

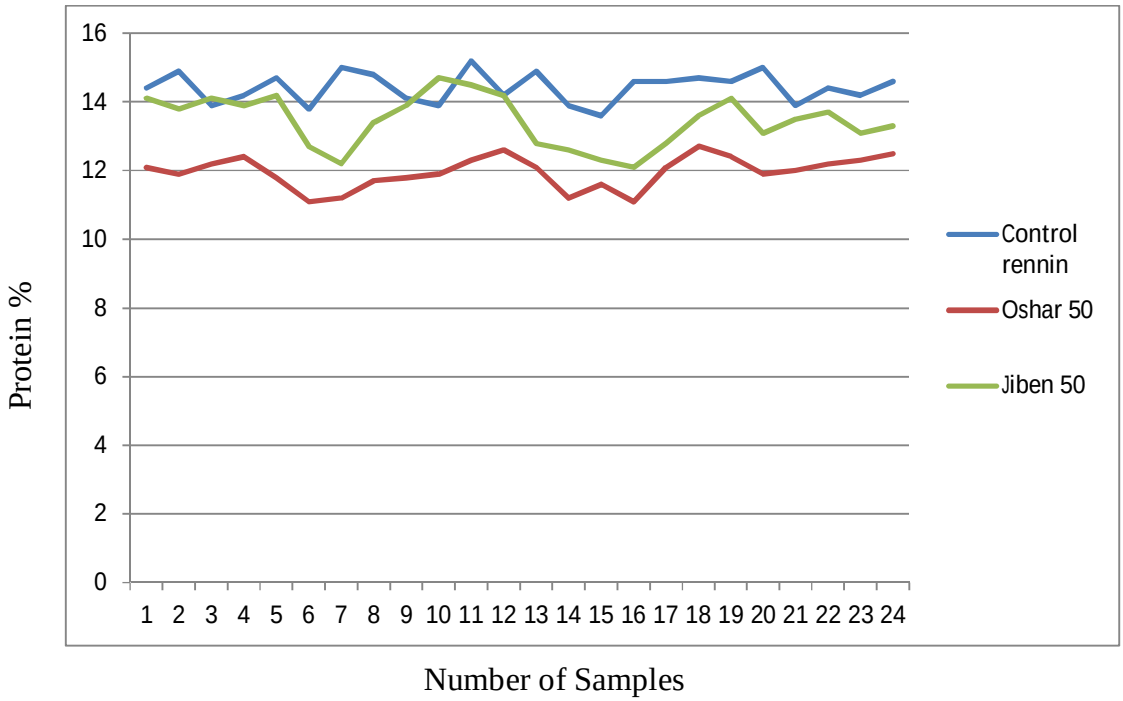


Table (9)average protein % obtained by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	14.31	0.39	12.25	0.50	13.45	0.50	*
(2)	2nd stage	14.22	0.33	12.17	0.27	13.16	0.62	*
(3)	3rd stage	14.28	0.41	12.00	0.22	13.87	0.45	*
Total	Whole period	14.26	0.36	12.14	0.35	13.61	0.60	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

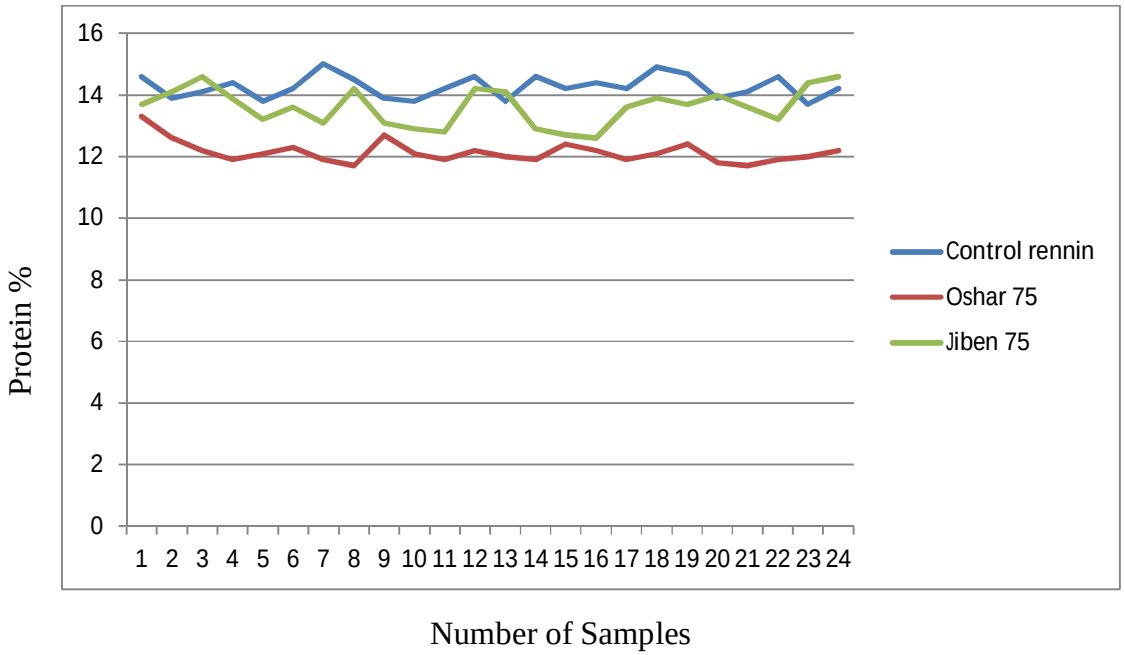


Table (10)average protein % obtained by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	14.51	0.37	12.02	0.31	13.35	0.38	*
(2)	2nd stage	14.49	0.39	11.97	0.31	13.96	0.19	*
(3)	3rd stage	14.11	0.33	12.06	0.17	11.68	0.45	*
Total	Whole period	14.36	0.39	12.02	0.26	13.55	0.42	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

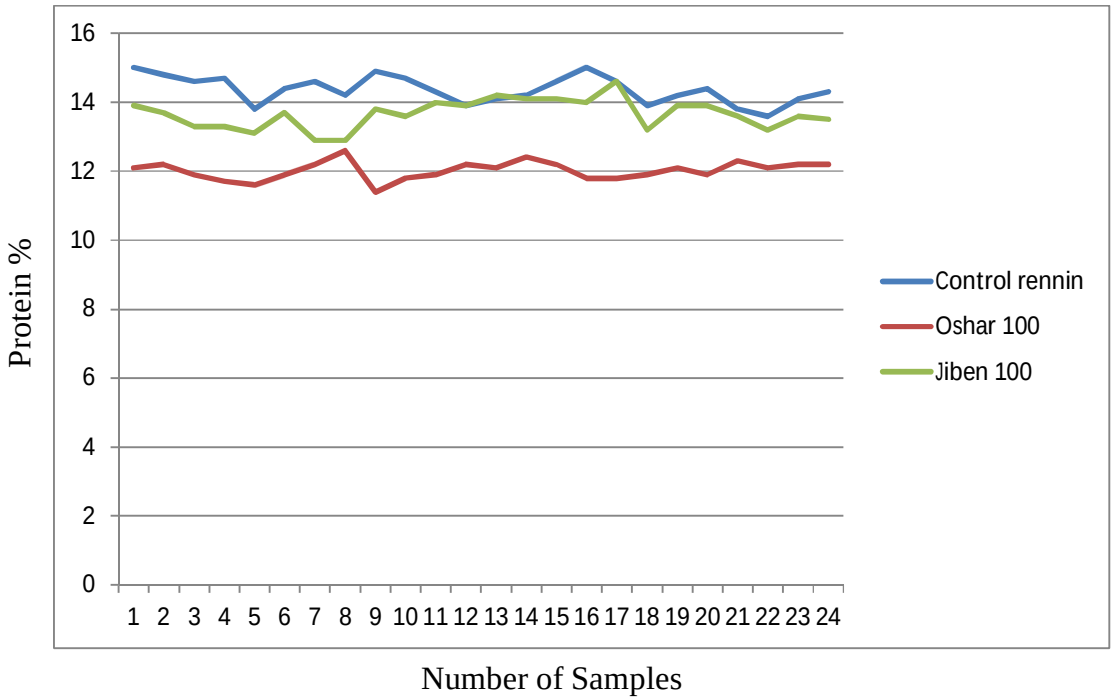
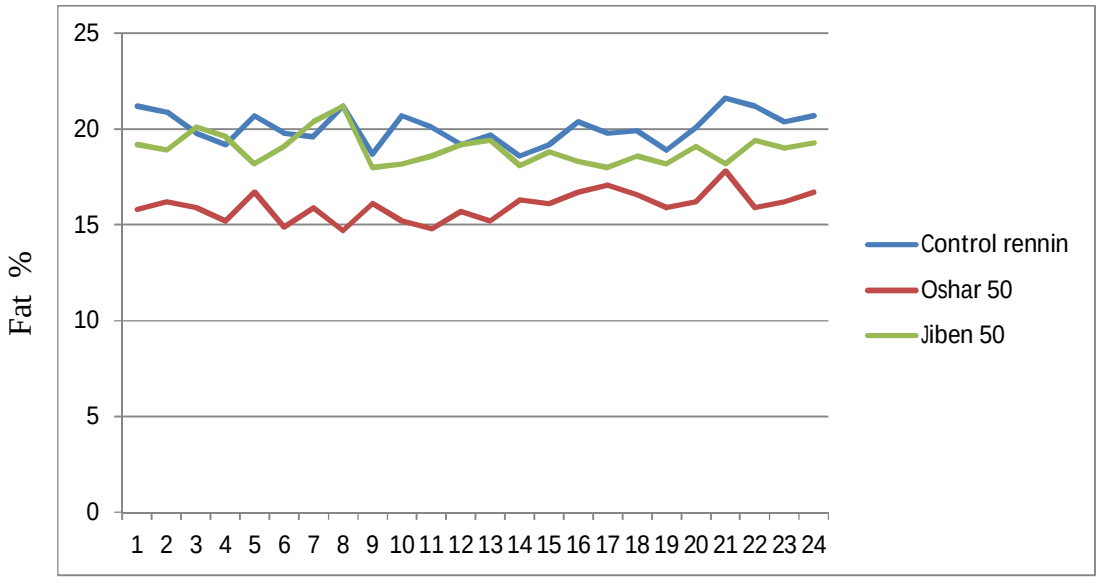


Table (11) average Fat % obtained by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	20.30	0.78	15.66	0.67	19.58	0.94	*
(2)	2nd stage	19.57	0.77	15.76	0.65	17.97	0.52	*
(3)	3rd stage	20.32	0.85	16.55	0.65	18.72	0.54	*
Total	Whole period	20.06	0.84	15.99	0.75	16.95	0.80	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (12) average Fat % obtained by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	20.12	0.75	16.15	0.75	18.50	0.37	*
(2)	2nd stage	19.40	0.52	16.00	0.46	17.81	0.45	*
(3)	3rd stage	20.38	0.83	15.85	0.57	18.51	0.38	*
Total	Whole period	19.97	0.80	16.00	0.59	18.35	0.44	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

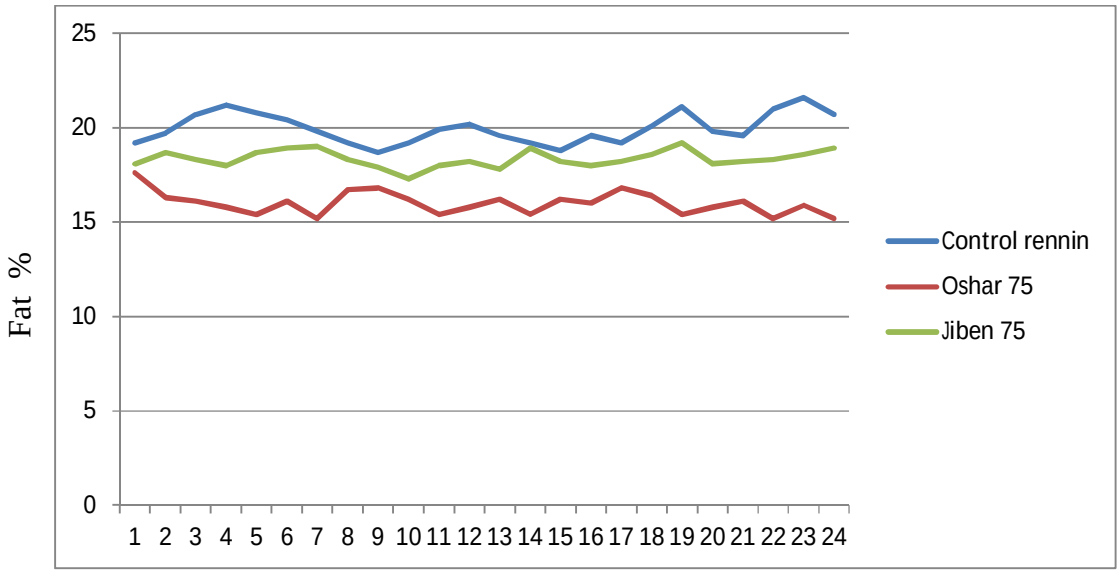
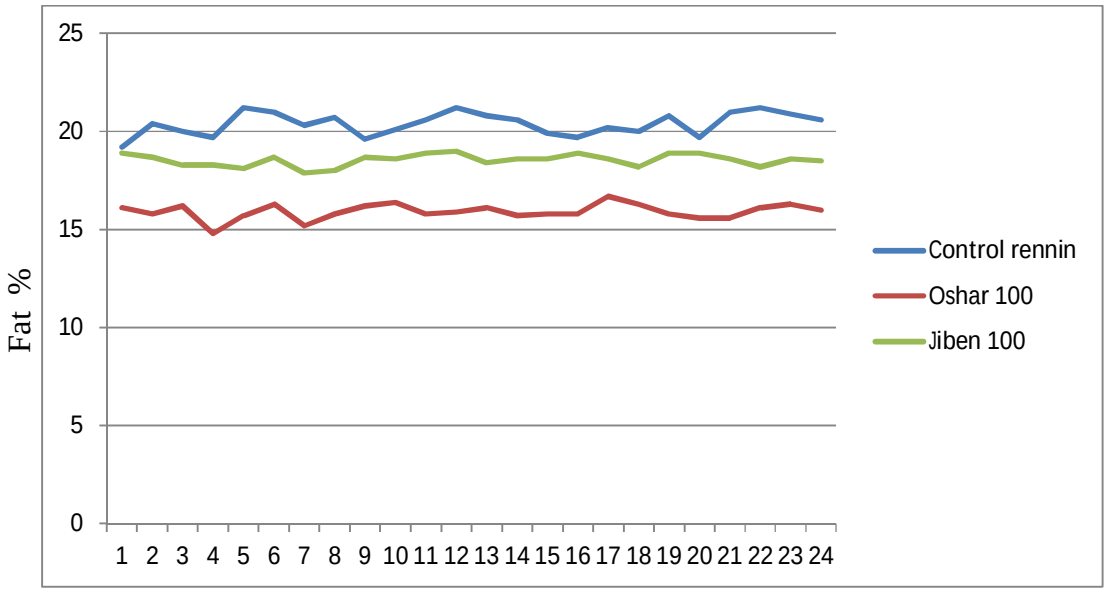


Table (13)average Fat % obtained by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	20.31	0.66	15.73	0.51	18.13	0.36	*
(2)	2nd stage	20.31	0.57	15.76	0.24	18.71	0.20	*
(3)	3rd stage	20.55	0.52	16.05	0.38	18.56	0.26	*
Total	Whole period	20.39	0.57	15.91	0.40	18.54	0.31	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



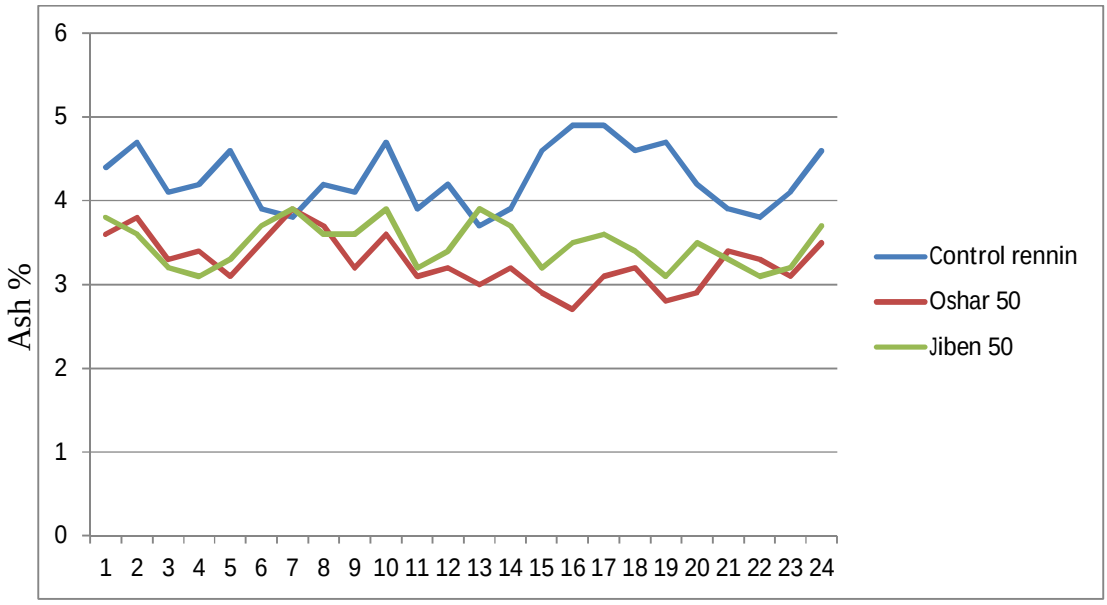
Number of Samples

Table (14) average ash % obtained by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	4.23	0.31	3.53	0.26	3.52	0.29	*
(2)	2nd stage	4.25	0.43	3.11	0.26	3.55	0.27	*
(3)	3rd stage	4.35	0.40	3.16	0.23	3.36	0.22	*
Total	Whole period	4.27	0.37	3.27	0.31	3.47	0.26	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



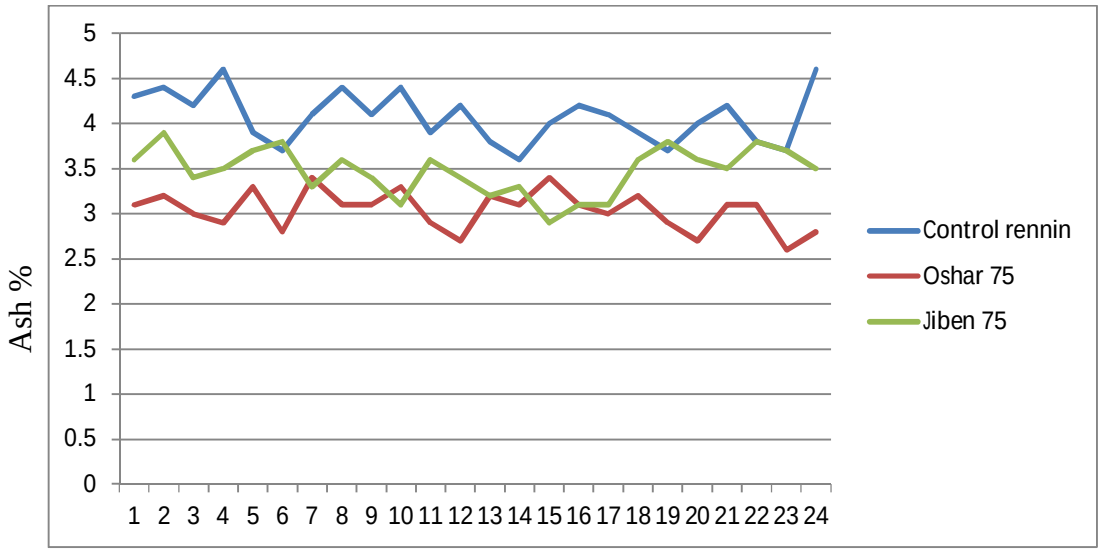
Number of Samples

Table (15) average ash % obtained by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	4.20	0.29	3.10	0.20	3.60	0.20	*
(2)	2nd stage	4.02	0.25	3.10	0.22	3.25	0.22	*
(3)	3rd stage	4.00	0.30	2.92	0.21	3.57	0.22	*
Total	Whole period	4.07	0.28	3.04	0.21	3.47	0.26	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (16) average ash % obtained by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	4.05	0.28	4.03	0.21	3.21	0.26	*
(2)	2nd stage	4.11	0.25	2.092	0.19	3.21	0.14	*
(3)	3rd stage	4.21	0.25	3.10	0.16	3.35	0.16	*
Total	Whole period	4.12	0.24	3.04	0.52	3.25	0.16	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

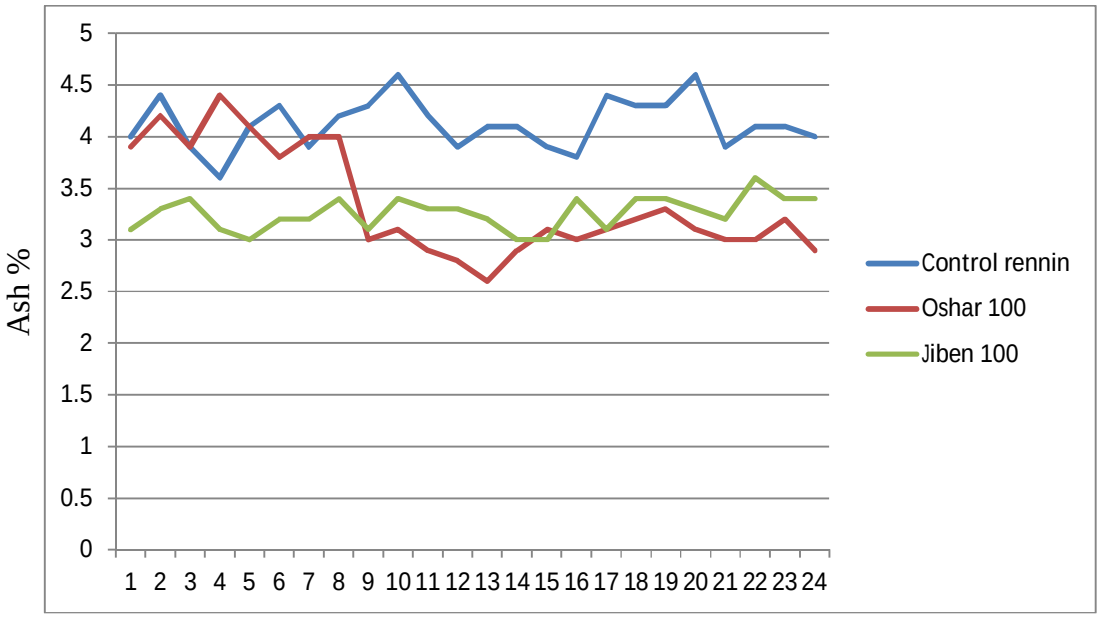
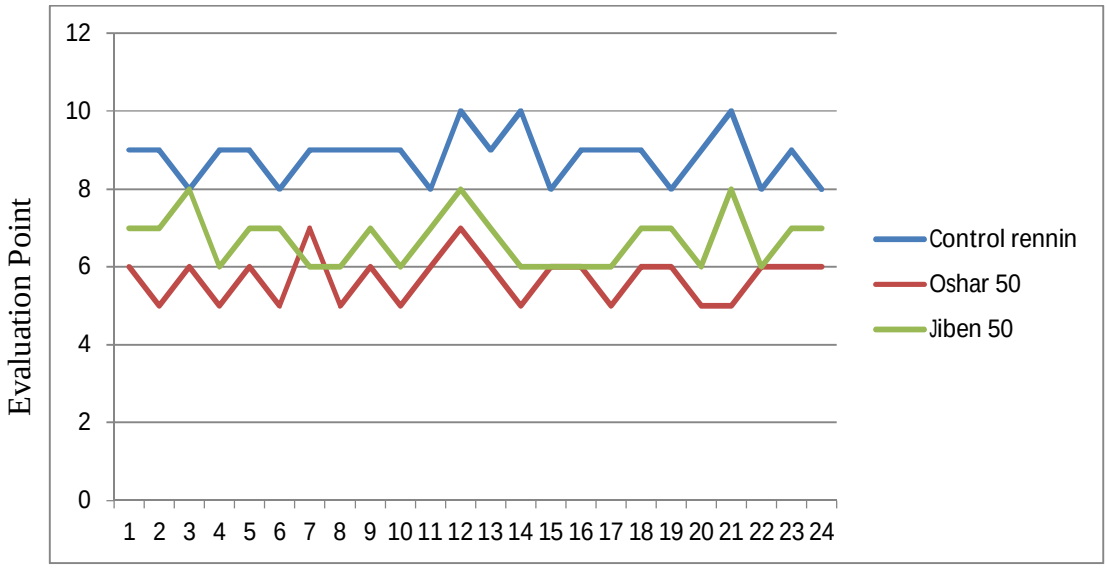


Table (17) average evaluation points given for flavor by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.75	0.46	5.62	0.74	6.75	0.70	*
(2)	2nd stage	9.00	0.75	5.87	0.64	6.62	0.74	*
(3)	3rd stage	8.75	0.70	5.62	0.51	6.75	0.70	*
Total	Whole period	9.00	0.63	5.87	0.62	6.62	0.69	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



Number of Samples

Table (18) average evaluation points given for flavor by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.78	0.64	5.62	0.70	6.75	0.91	*
(2)	2nd stage	8.83	0.70	5.70	0.74	6.70	0.74	*
(3)	3rd stage	7.87	0.64	5.50	0.92	6.62	0.74	*
Total	Whole period	8.83	0.86	5.62	0.76	6.62	0.76	*

NS ≡ not significant

*** ≡ significant (p < 0.05)**

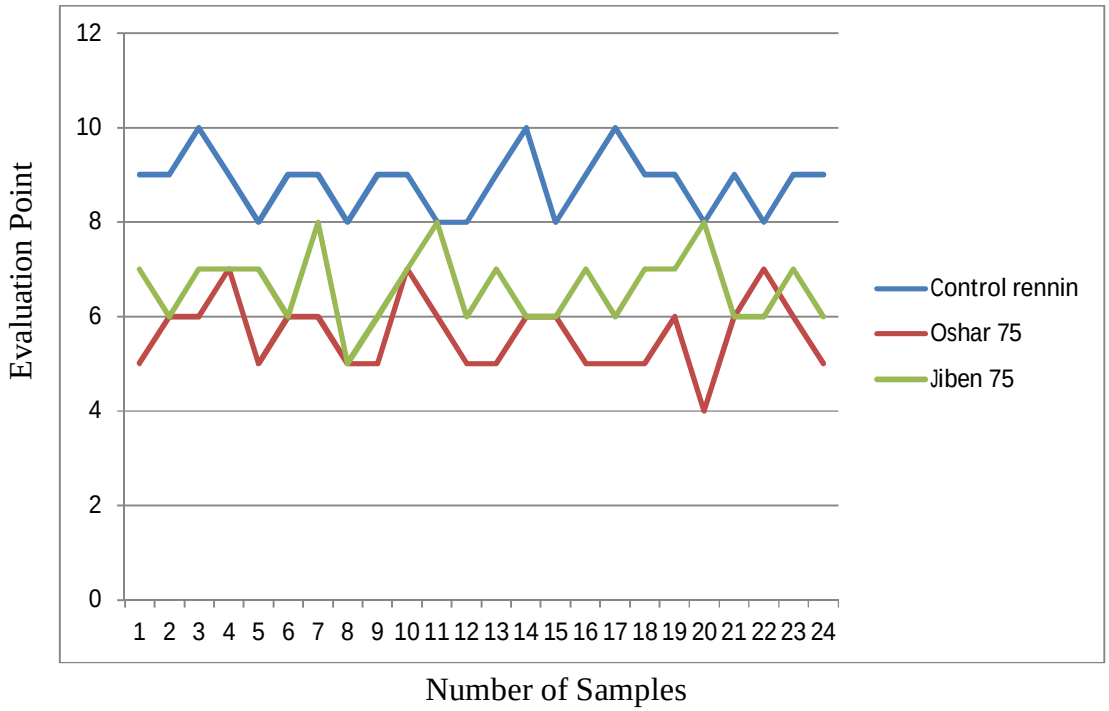


Table (19)average evaluation points given for flavor by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	9.00	0.75	6.75	1.58	6.87	0.83	*
(2)	2nd stage	8.62	0.91	5.50	0.92	6.95	0.70	*
(3)	3rd stage	8.75	0.70	5.50	0.92	7.00	0.75	*
Total	Whole period	8.79	0.77	5.91	1.28	6.87	0.74	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

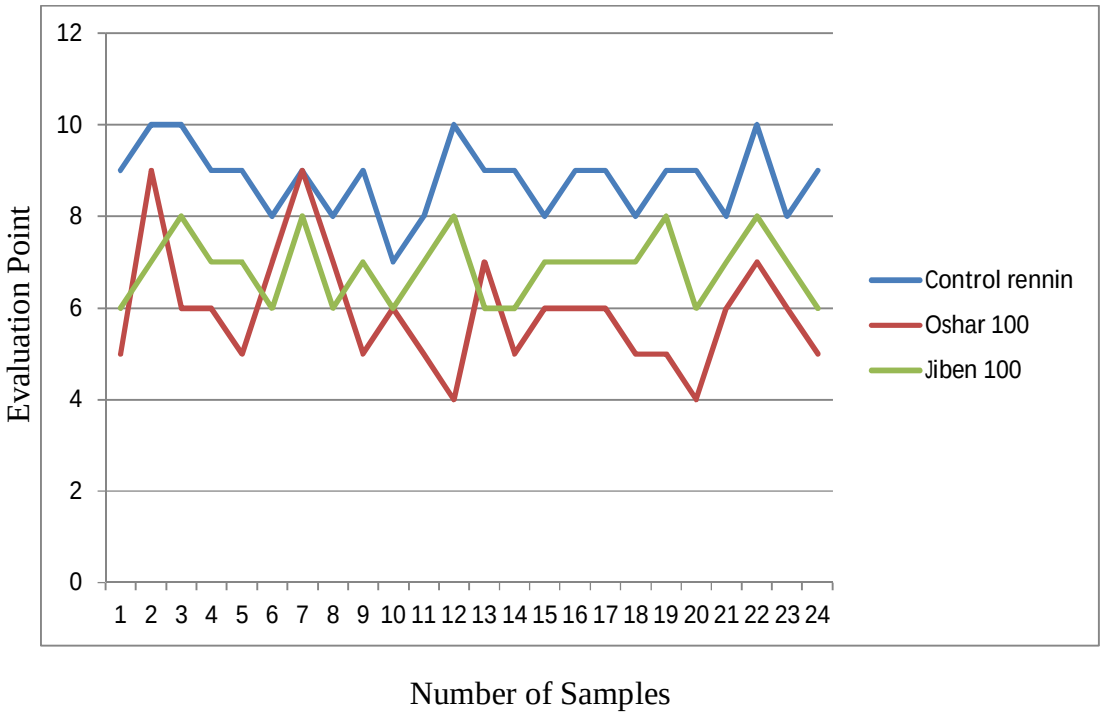


Table (20) average evaluation points given for taste by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.25	0.70	6.75	0.70	8.00	0.75	*
(2)	2nd stage	8.62	0.74	6.00	0.75	7.12	0.64	*
(3)	3rd stage	8.37	0.74	6.00	0.75	6.50	0.53	*
Total	Whole period	8.08	0.71	6.25	0.82	7.20	0.88	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

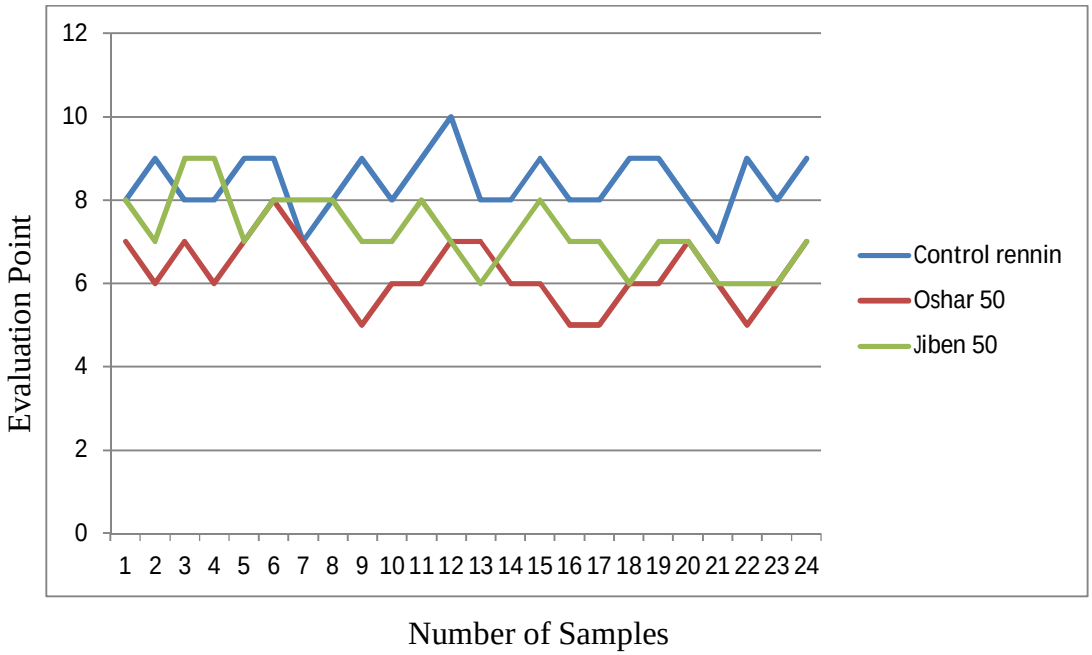


Table (21)average evaluation points given for taste by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.12	0.83	5.62	0.74	6.50	0.53	*
(2)	2nd stage	8.50	0.53	5.75	0.70	6.75	0.70	*
(3)	3rd stage	9.00	0.75	6.12	0.83	6.50	0.92	*
Total	Whole period	8.54	0.77	5.83	0.76	6.58	0.71	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

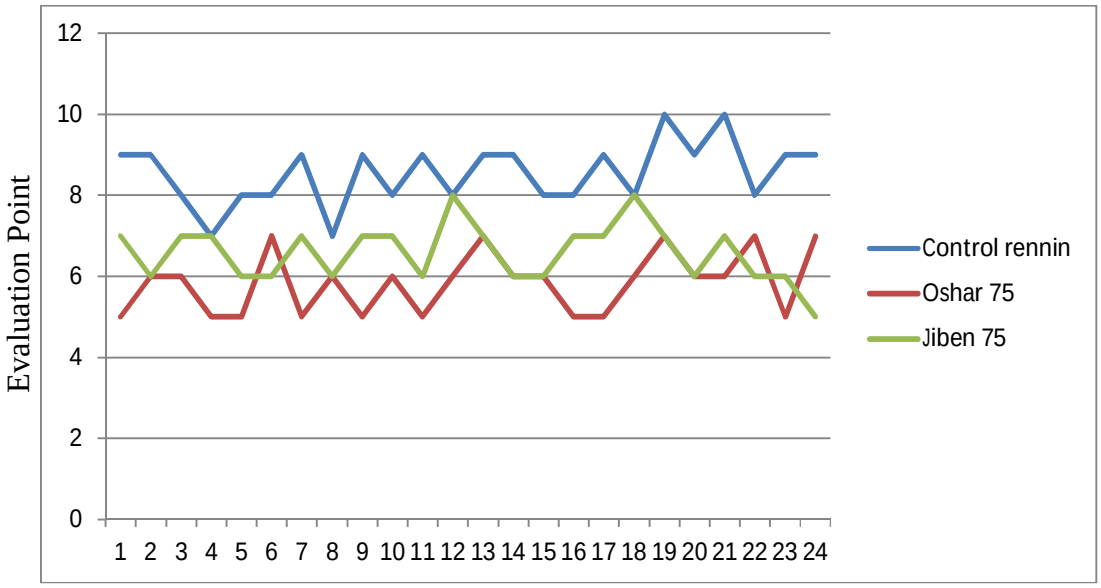


Table (22)average evaluation points given for taste by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.00	0.75	5.50	0.92	6.37	0.74	*
(2)	2nd stage	8.50	0.92	6.25	0.70	6.75	0.70	*
(3)	3rd stage	8.87	0.83	5.62	0.74	6.37	0.91	*
Total	Whole period	8.45	0.88	5.79	0.83	6.50	0.78	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

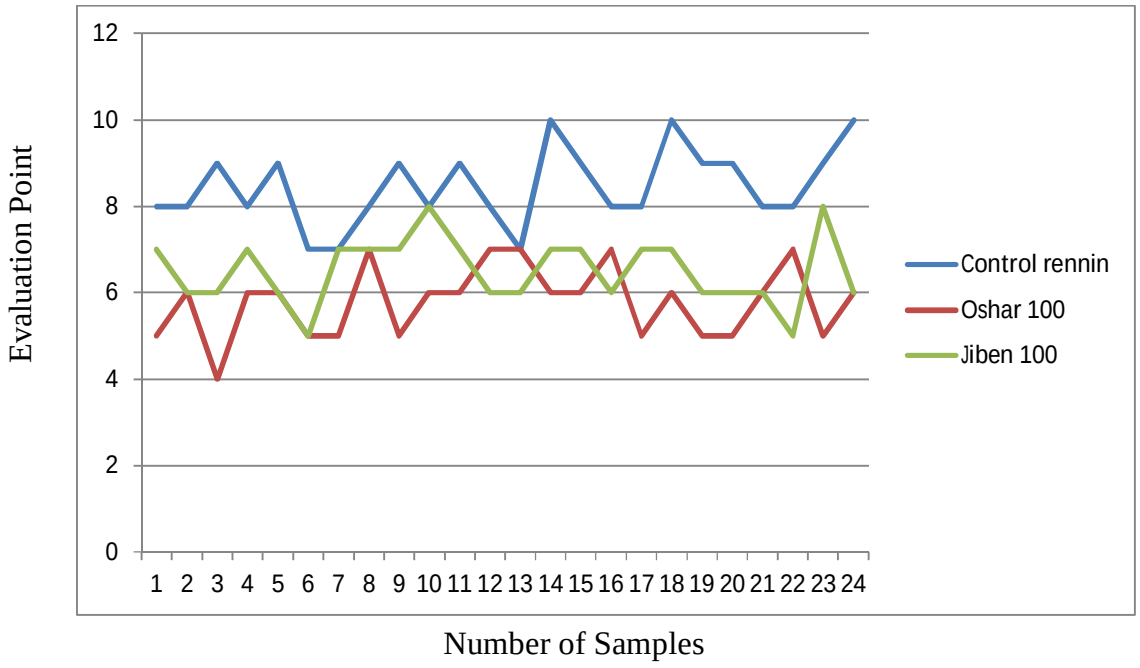


Table (23) average evaluation points given for texture by using renin, Osher (50%) and Jibeen (50%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (50%)		Jibeen (50%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	9.00	0.75	5.87	0.83	6.87	0.64	*
(2)	2nd stage	8.75	0.70	5.62	0.74	6.75	0.70	*
(3)	3rd stage	8.62	0.91	6.00	1.51	7.75	0.70	*
Total	Whole period	8.97	0.77	5.83	1.04	7.12	0.97	*

NS ≡ not significant

*** ≡ significant (p < 0.05)**

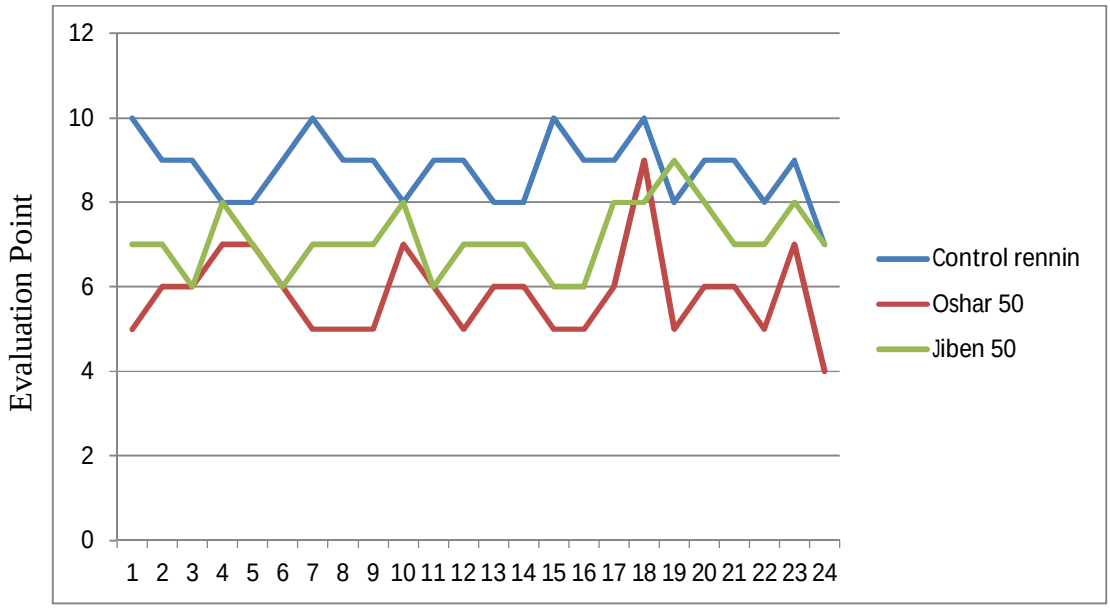


Table (24)average evaluation points given for texture by using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (75%)		Jibeen (75%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.62	0.74	6.00	0.75	8.12	0.64	*
(2)	2nd stage	8.75	0.70	5.75	0.70	8.25	0.70	*
(3)	3rd stage	8.62	0.74	6.00	0.75	8.00	0.75	*
Total	Whole period	8.66	0.70	5.91	0.71	8.12	0.67	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**

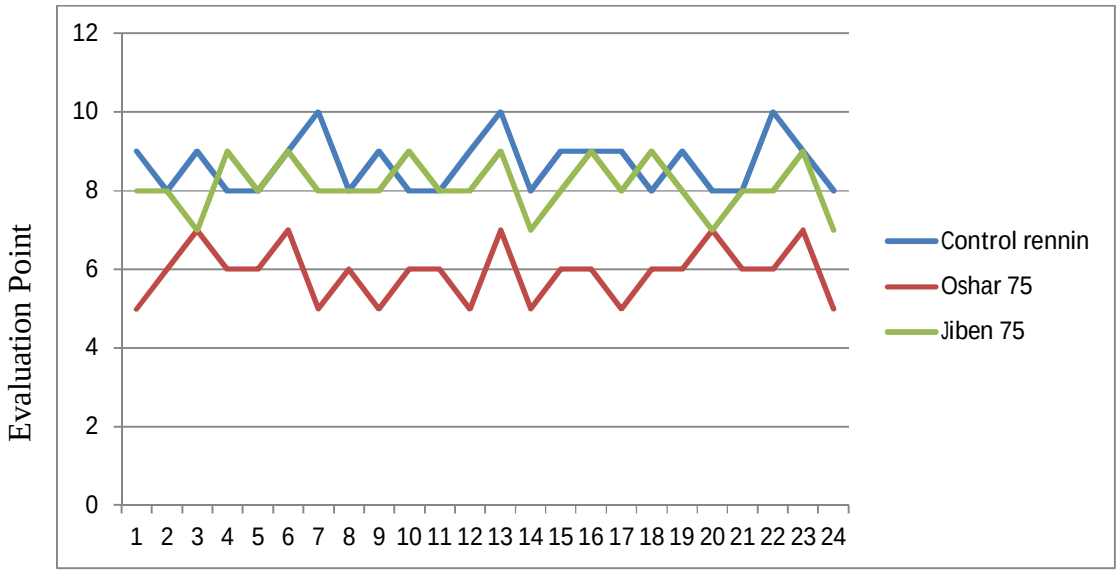
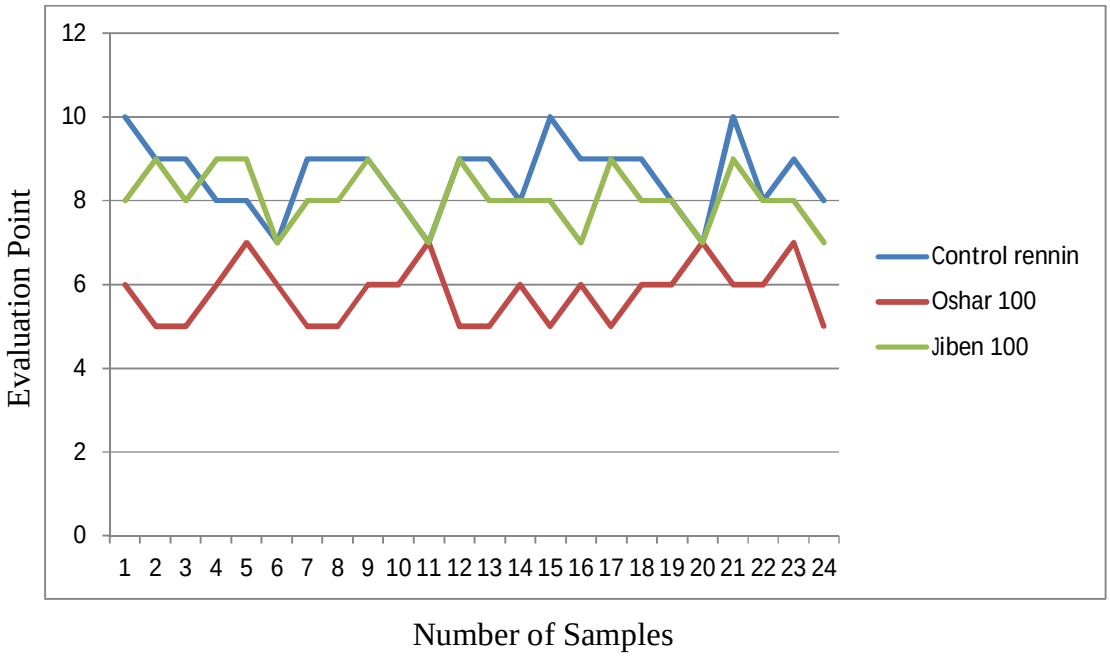


Table (25)average evaluation points given for texture by using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments

Treatment		Renin Control		Osher (100%)		Jibeen (100%)		Significance
Lactation Stage		Mean	SD	Mean	SD	Mean	SD	
(1)	1st stage	8.62	0.91	5.62	0.74	8.25	0.70	*
(2)	2nd stage	8.62	0.91	5.75	0.70	8.00	0.75	*
(3)	3rd stage	8.50	0.92	6.00	0.75	8.00	0.75	*
Total	Whole period	8.58	0.88	5.97	0.72	8.08	0.71	*

NS ≡ not significant

*** ≡ significant ($p < 0.05$)**



4.2 Discussion:

4.2.1 Time of Coagulation:

Table (2) shows the average coagulation time (minutes), by using renin Osher (50%) and Jibeen (50%) enzymes for the lactation stages (1^{st}. 2^{nd}. and 3^{rd}.), and for the whole lactation period for all treatments. The average time for the whole lactation period for renin, Osher (50%) and Jibeen (50%) was 97.33± 4.62, 124.37± 5.88 and 96.29 ± 4.96 minutes respectively.

Table (3) shows the average coagulation time by using renin, Osher (75%) and Jibeen (75%) for the different stages and lactation period. Average time for the whole period of treats obtain was 80.37 ± 3.54, 113.62 ± 7.29 and 87.75 ± 2.23 minutes respectively.

Table (4) shows the average coagulation time by using renin, Osher (100%) and Jibeen (100%) for the 3 stages and lactation period. The average for the whole lactation period and all treatments obtained was 79.45 ± 3.65, 108.83 ± 5.62 and 85.95 ± 4.95 for renin Osher (100%) and Jibeen (100%) respectively.

Results indicated a significant difference between the means of the coagulation time for all stages and whole lactation period for all treatments, and for renin and the different concentrations of Osher and

Jibeen enzymes (50%, 75% and 100%) ($p<0.05$). Similar results revealing significant differences were given by (van Hooydonk et. al. 1984), Synth et. al. (1937), Lee et. al. (2003).

According to the results obtained, renin enzyme required less time to coagulate the milk compared to Osher and Jibeen enzymes, and this agreed with that reported by the above mentioned authors. The Colatropis procera plant enzyme (Osher) required much time for coagulation than Jibeen enzyme which was in conformity with the results obtained by Hamed (1998).

The variation in the coagulation time required by the different coagulants is depending on the type of the enzyme used (van Hooydonk et. al. 1987), the chemical composition of milk, particularly the Ca^{++} (Scancalpore et. al. 1983) stage of lactation period where the chemical composition of milk greatly changed (Curten – Vapuretal 2012), menthod of coagulation applied (Van Hoydon et. al. 1987) and finally the clotting activity of the milk (Ibiama and Griffiths, 1987).

4.2.2 Cheese Yield:

Table (5) shows the average total yield of white soft cheese (g) per 5 kg milk obtained by adding renin Osher (50%) and Jibeen (50%) enzymes for the three stages of the lactation period and the whole lactation period.

The average yield during the whole lactation period for all treatments was 913.5 ± 8.66, 635.9 ± 14.09 and 662.5 ± 15.58 for renin Osher (50%) and Jibeen (50%) respectively.

Table (6) shows the average cheese yield (g) per 5kg milk for the different stages and whole lactation period for renin, Osher (75%) and (75%) respectively. The average yield for the whole lactation period obtained was 944.3 ± 32.89, 697.8 ± 16.99 and 729.3 ± 41.8 for renin, Osher (75%) and Jibeen (75%) respectively.

Table (7) shows the average yield (g) per 5 kg milk for the different stages and whole lactation period for renin, Osher (100%) and Jibeen (100%). The average yield for the overall lactation period for all treatments obtained was 916.0 ± 11.54, 788.7 ± 16.70 and 804.3 ± 9.55 g for renin, Osher (100%) and Jibeen (100%) enzymes respectively. The average yield showed a significant difference ($p<0.05$) for all stages, whole lactation period, all treatments for renin, Osher and Jibeen enzymes concentrations. Similar results indicating the significance between the means of the yield were given by Everett et. al. (2003), Synth et. al. (1987), Nijera (2003), Nur Eldaim et. al. (2007) and (Osman et. al. 2012). According to the results obtained, renin coagulation resulted in a good chees yield followed by Jibeen and Osher coagulation, which gave low cheese yield. The variation in the cheese yield is affected by many

factors of which the milk composition of vital importance, (Faltman,1987). The low yield of white soft cheese obtained by plant coagulation enzymes is due to the weak curd produced by such coagulants Synth, et. al. (2007), high losses of fat and protein drained with the whey (Merin, 1989) and enzyme and type of enzyme used Nijera, (2003). Other factors associated with cheese yield are demonstrated in chapter two according to Everett et (2003) and Paolo et. al. (2008).

4.2.3 Chemical Composition of Cheese:

4.2.3.1 Protein Content:

Table (8) shows the average protein % obtained by using renin, Osher (50%) and Jibeen (50%) for lactation stages and whole lactation period. The average protein % for the whole lactation period was 14.42 ± 0.44, 11.96 ± 0.46 and 13.44±0.74 for renin, Osher (50%) and Jibeen (50%) enzymes for all treatments..

Table (9) shows average protein % obtained using renin, Osher (75%) and Jibeen (75%) enzymes for lactation stages and whole lactation period. The average protein % for the whole lactation period was 14.26 ± 0.36, 12.14 ± 0.35 and 13.61 ± 0.60 for renin, Osher (75%), and Jibeen (75%) respectively and for all treatments.

Table (10) shows the average protein % obtained using renin, Osher (100%) and Jibeen (100%) for different lactation stages and whole lactation period and all treatments was 14.36 ± 0.39, 12.02 ± 0.26 and 13.55 ± 0.42 for renin, Osher (100%) and Jibeen (100%) respectively. The results obtained showed a significant difference ($p<0.05$) between the average of protein % when using renin and different concentrations of Osher and Jibeen coagulants for all lactation stages, whole lactation period and all treatments. Similar results were also given by Caric et. al. (1995), Green et. al. (1987) and Bradly et. al. (1990). The differences in the protein content of the white soft cheese were associated with type of coagulant used for processing. Coagulating the milk with renin enzyme, the levels of protein tend to increase compared to plant enzymes (Rollman et. al. 1988), Dimitreli et. al. (2004) and (Kumosinski et. al. 1991).

4.2.3.2 Fat Content:

Table (11) shows the average fat % obtained by using renin Osher (50%) and Jibeen (50%) enzymes for the stages of lactation and whole lactation period. The average fat % for whole lactation period for all treatments was 20.06±0.84, 15.99±0.75 and 16.95±0.80 for renin, Osher (50%) and Jibeen (50%) respectively.

Table (12) shows average fat % using renin, Osher (75%) and Jibeen (75%) enzymes for the 3 stages and whole lactation period. The average fat % for whole lactation period for all treatments obtained was 19.97±0.80, 16.00±0.59 and 18.35±0.44 for renin, Osher (75) and Jibeen (75%) respectively.

Table (13) shows average fat % using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period.

The average fat % obtained for the whole lactation period and all treatments was 20.39±0.57, 15.91±0.40 and 18.54±0.31 for renin, Osher (100%) and Jibeen (100%) enzymes respectively. Also, results showed a significant difference ($p<0.05$) between the average fat % for all stages, whole lactation period and all treatments. The average fat % obtained when using renin enzyme was higher compared to that of the plant enzymes. This indicated, the type of coagulant used affected the fat component of the milk used. Also differences in the fat content of the raw milk prepared for white cheese processing and method of coagulation used cause variation in the final fat content of the finished cheese. This comes in agreement with that explained by Abdalla (1993) (Home 1990) and (Cari et. al. 1993).

4.2.3.3 Ash Content:

Table (14) shows average ash % obtained using renin, Osher (50%) and Jibeen (50%) enzymes for the 1st. 2nd. and 3rd. stage of lactation and whole lactation period. The average ash % for the whole lactation period for all treatments obtained was 4.27±0.37, 3.27±0.31, and 3.47±0.26 for renin, Osher (50%) and Jibeen (50%) enzymes respectively.

Table (15) shows average ash % obtained using renin, Osher (75%) and Jibeen (75%) enzymes for different lactation stages and whole lactation period. The average ash % for the whole lactation period obtained was 4.07±0.28, 3.04±0.21 and 3.47±0.26 for renin, Osher (75%) and Jibeen (75%) enzymes respectively.

Table (16) shows the average ash % obtained using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period. The average ash % for the whole lactation period and all 0treatments obtained was 4.12±0.24, 3.04±0.52 and 3.25±0.16 for renin, Osher (100%) and Jibeen (100%) enzymes respectively. A significant difference ($p<0.05$) was detected between the average of the ash % for the different stages, lactation period, renin enzyme and plant enzyme with different concentrations for all treatments. Ash content showed low percentages, when plants coagulants were used compared to renin enzyme. The chemical composition of cheese showed low cheese

components, when the cheese milk was coagulated by plant enzymes (Bebe, 1980). In general, coagulating the milk with plant enzymes resulted in a decrease in the percentages of the total solids of white soft cheese. This agreed with that reported by Andren et. al. (1982), Dalglish (1985), Bines (1989) and Psozola (1989).

4.2.4 Sensory Evaluation:

(Refer to evaluation Sheet)

4.2.4.1 Flavor of Cheese:

Table (17) shows the evaluation points given for flavor of cheese using renin, Osher (50%) and Jibeen (50%) enzymes for different lactation stages and whole lactation period. The average evaluation points the flavor scored, for the whole lactation period were 9.00±0.63, 5.87±0.62 and 6.62±0.69 for renin, Osher (50%) and Jibeen (50%) enzymes for all treatments respectively.

Table (18) shows evaluation points for flavor using renin, Osher (75%) and Jibeen (75%) enzymes for the stages and whole lactation period. The evaluation points scored for the whole lactation period for all treatments obtained were 8.83±0.86, 5.62±0.76 and 6.62±0.76 for renin, Osher (75%) and Jibeen (75%) enzymes respectively.

Table (19) shows average evaluation points given for flavor using renin, Osher (100%) and Jibeen (100) enzymes respectively. The average given for the whole lactation period was 8.79±0.77, 5.91±1.28 and 6.87±0.74 for renin Osher (100%) and Jibeen (100%) enzymes and for all treatments respectively. A significant difference ($p<0.05$) was detected between the average of scored points for flavor, when renin and different concentrations of Osher and Jibeen enzymes for all stages, whole lactation period and all treatments were used. Similar results were obtained by Farell et. al. (1990) and Kumosinski et. al. (1999). It was noticed that renin cheese scored the higher points followed by Jibeen and Osher cheeses. This indicated, the type of enzyme used for coagulation determined the level of flavor intensity, (Takala, 1993). Other factors that affect the flavor of cheese is the concentration of extracted plant enzymes when used in cheese making, (Talib et. al. 2006); other factors influencing the flavor are the chemical composition of milk, types of animals and chemical composition of feed, (Englels et. al. 2005), (Takala 1993).

4.2.4.2 Taste of Cheese:

Table (20) shows the points given for taste of cheese using renin, Osher (50%) and Jibeen (50%) enzymes for the stages and whole lactation period for all treatments. Average points given for the whole

lactation period were 8.08±0.71, 6.25±0.82 and 7.20±0.88 for renin, Osher (50%) and Jibeen (50%) enzymes respectively.

Table (21) shows the points given for taste using renin, Osher (75%) and Jibeen (75%) enzyme for the stages of lactation and whole lactation period for all treatments. The average points for the whole lactation period obtained were 8.54±0.77, 5.83±0.76 and 6.58±0.71 for renin, Osher (75%) and Jibeen (75%) enzymes cheese respectively.

Table (22) shows average evaluation points for taste using renin, Osher (100%) and Jibeen (100%) enzymes for lactation stages and whole lactation period for all treatments. The average points for the whole lactation period were 8.45±0.88, 5.79±0.83 and 6.50±0.78 for renin, Osher (100%) and Jibeen (100%) enzymes cheese respectively. The scored points revealed a significant difference ($p<0.05$) between the means given for taste of the cheeses prepared by renin enzyme and different coagulation of Osher and Jibeen enzymes for all stages, overall lactation period and all treatments. Renin cheese scored the highest points for taste compared to other plant enzymes. Jibeen cheese showed a relatively high scores raher than Osher cheeses. This indicated that the taste of the soft cheese was affected by the type of coagulant used, beside the factors affecting flavor previously mentioned, in addition to the concentration of the plant enzymes used. This agreed with that reported

by Talib, et. al. (2006), Takela (1990) and Farell, et. al. (1990), but were in contrast with the results given by Jacob, et. al. (2011).

4.2.4.3 Texture of Cheese:

Table (23) shows the average points given for texture of cheese using renin, Osher (50%) and Jibeen (50%) enzymes for the different stages and whole lactation period. The average points given for the whole lactation period and all treatments were 8.97±0.77, 5.83±1.04 and 7.12±0.97 for renin, Osher (50%) and Jibeen (50%) cheeses respectively.

Table (24) shows the average scored points for cheese texture using renin, Osher (75%) and Jibeen (75%) enzymes for all stages, whole lactation period and all treatments. The average points obtained for the whole lactation period for cheese texture were 8.66±0.70, 5.91±0.71 and 8.12±0.67 when renin, Osher (75%) and Jibeen (75%) enzymes were used.

Table (25) shows average evaluation points given for cheese texture using renin, Osher (100%) and Jibeen (100%) enzymes for all stages, whole lactation period and all treatments. The average scored points for cheese texture for whole lactation period were 8.58±0.88, 5.97±0.72 and 8.08±0.71 for renin, Osher (100%) and Jibeen (100%) enzyme cheeses respectively. Also a significant difference was detected hereby ($p<0.05$). The texture of renin cheese was firm followed by

Jibeen and Osher cheeses. This cleared the effect of the type of enzyme used for coagulation on cheese texture. Plant enzymes have an excessive proteolytic activity that affect the texture of the finished cheese (especially during the ripening process) and this might be the reason for non-firmness of some cheeses produced using plant enzymes e.g. Calotropis procera (Osher) plant, (Yousif, et. al. 1996). Also results given by (Talib et. al. 2006) and Kumosinski et. al. (1990), followed the same trend.

Chapter Five

Conclusion and Recommendations

5.1 Conclusion

Renin enzyme especially calf renin, is considered as one of the most wide spread, desirable and dominant milk coagulant in cheese processing. In the past few years, the limited supply and the high prices of renin necessitated the need of finding other milk coagulants. To overcome such a problem plant enzymes were extracted from various plants, e.g. Solanum dubium (Jibeen) and Colatropis procera (Osher) and others. Comparing renin to plant enzymes, it gave the best results for all studied parameters subject of the current research (Time of milk coagulation, cheese yield, composition and sensory characteristics) and significant differences ($p<0.05$) were detected between the averages of the parameters for the stages of lactation and whole lactation period and for all treatments. Yet, Jibeen enzyme showed an acceptable standard levels when used. Enzyme extracted from Osher gave the lowest results when compared to renin and Jibeen enzymes. Hence plant enzymes may also be used in the future as milk coagulants in the cheese industry of Sudan under certain conditions.

5.2 Recommendations:

Based on obtained results, following recommendations might be given:

- Jibeen enzyme can be used as an alternative for renin and other plants enzymes, since the plant grows in most areas of the Sudan, in addition to its effectiveness.
- More attention should be paid when using plant enzymes, particularly, chemical composition and suitable concentrations to be used to avoid health hazards by consumers, e.g. toxicity.
- More research studies should be carried out on plant enzymes, as well as the economic impact linked with the utilization.

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