JULY 2019

M.Sc. in Food Engineering

MOHAMED C. D. ABOUDOULAYE

REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

STANDARDIZATION AND CHARACTERIZATION OF WANGASHI CHEESE

M.Sc. THESIS

IN

FOOD ENGINEERING

BY

MOHAMED CHERIFOU DINE ABOUDOULAYE

JULY 2019

STANDARDIZATON AND CHARACTERIZATION OF WANGASHI CHEESE

M.Sc. Thesis

in

Food Engineering

Gaziantep University

Supervisor

Prof. Dr. Sevim KAYA

by

Mohamed Cherifou Dine ABOUDOULAYE

July 2019

REPUBLIC OF TURKEY
GAZİANTEP UNIVERSITY
GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES
FOOD ENGINEERING

Name of the Thesis : Standardization and Characterization of Wangashi cheese

Name of the Student : Mohamed Cherifou Dine ABOUDOULAYE

Exam Date : 17.07.2019

Approval of the Graduate School of Natural and Applied Sciences

Prof. Dr. A. Necmeddin YAZICI
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Maskan MEDENI
Head of Department

This is to certify that we have read this thesis and that in our consensus/majority opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

Prof. Dr. Sevim KAYA
Supervisor

Examining Committee Members: Signature

Prof. Dr. Sevim KAYA ……..

Prof. Dr. Hüseyin BOZKURT ……..

Assoc. Prof. Dr. Ahmet Levent INANÇ ……..

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Mohamed Cherifou Dine ABOUDOULAYE

ABSTRACT

STANDARDIZATION AND CHARACTERIZATION OF WANGASHI CHEESE

ABOUDOULAYE, Mohamed Cherifou Dine
M.Sc. in Food Engineering
Supervisor: Prof. Dr. Sevim KAYA
July 2019
77 pages

Wangashi cheese processing based on the use of *Calotropis procera* plant stems as a coagulant was characterized and standardized. Crude extract obtained from *Calotropis* plant stems was used to set solution of milk powder at various pH (4-8) and temperature (35-80^{o}C) in order to examine effect of pH and temperature on milk clotting and proteolytic activities. The pH 5.5 and temperature of 70^{o}C were recorded as optimum pH and temperature. After that concentration of the crude extract enzyme was assayed to purify using ammonium sulfate precipitation at various percentage of saturation (20%-80%) at determined optimum pH and temperature, whereby the saturation of 70% was detected to be the best because of its high specific activity, yield and purification fold. Two different of Wangashi cheese were produced in laboratory, one using directly the crude extract and the other the purified crude extract from *Calotropis procera* at optimum condition. Their chemical, textural and thermal properties were determined using standard methods. A significant difference between parameters tested was observed ($p < 0.05$). A decreasing in moisture content, increasing in protein content and also an improvement of color and textural parameters were recorded in cheese obtained using purified crude extract *Calotropis procera* stems.

Key Words: Wangashi cheese, *Calotropis procera*, milk clotting activity, proteolytic activity, Standardization.

ÖZET

WANGASHI PEYNIRININ STANDARDIZASYONU VE ÜRETIMININ KARAKTERIZASYONU

ABOUDOULAYE, Mohamed Cherifou Dine
Yüksek Lisans Tezi, Gıda Mühendisliği
Danışman: Prof. Dr. Sevim KAYA
Temmuz 2019
77 sayfa

Calotropis procera bitkisinin kullanımına dayalı Wangashi peynir işleme pıhtılaştırıcı olarak kaynaklanıyor ve standardize edildi. *Calotropis* bitki saplarından elde edilen 100 mL ekstrakt süt pıhtılaşmasını ve proteolitik aktivitelerin pH ve sıcaklığını optimize etmek için çeşitli pH (4-8) ve sıcaklıkta (35-80^{o}C) analiz edildi. pH 5.5 ve 70'lik sıcaklık, optimum pH ve sıcaklık olarak kaydedildi. Daha sonra, *Calotropis procera*'dan elde edilen ham ekstrakt enzimi, çeşitli optimum doygunluk yüzdesinde (% 20 - 80) amonyum sülfat çökeltmesi kullanılarak, optimum pH ve sıcaklıkta amonyum sülfat çökeltmesi kullanılarak saflaştırmaya tabi tutuldu; yüksek spesifik aktivite, verim ve saflaştırma katlaması. Laboratuvarda, biri doğrudan ham özü, diğeri ise Calotropis procera'dan elde edilen arıtılmış ham özüt kullanılarak iki çeşit Wangashi peyniri üretildi. Kimyasal, dokusal ve termal özellikleri standart yöntemler kullanılarak belirlenmiştir. Test edilen parametreler arasında anlamlı bir fark olduğu gözlendi ($p < 0.05$). Arıtılmış ham özüt *Calotropis procera* sapları kullanılarak elde edilen peynirde nem içeriğinde azalma, protein içeriğinde artış ve ayrıca renkte dokusal parametrelerin aynı zamanda bir iyileşme kaydedilmiştir. Bu bulgu, termal özellik analizinde kaydedilenlerle tutarlıydı.

Anahtar Kelimeler: Wangashi peyniri, *Calotropis procera*, süt pıhtılaşma aktivitesi, proteolitik aktivite, Standardizasyon.

"Dedicated to my mother"

ACKNOWLEDGEMENTS

I would like to thank:

The Turkish Government for it support for awarding a scholarship which allowed to complete my Master Thesis.

My supervisor, Prof. Dr. Sevim KAYA for her guidance and support throughout the study. I am thankful for her encouragement and motivation.

Prof. Dr. Sibel FADILOĞLU for helping in the enzyme treatment part and Prof. Dr. Ahmet KAYA for helping in analysis of thermal properties and for his continuous advices and support.

My mother for her unshakeable support while being separated from her; my father and uncles for being their son and theirs limitless love; my brother for his advices and encouragements.

All of my friends especially Oscar ZANNOU and Jerry Dehouegnon AGOSSOU and the support staff of the Food Engineering Department for providing help and support for the completion of my thesis.

This work is supported by Coordination Unit of Scientific Research Projects (BAP) of Gaziantep University, through project number MF.YLT.19.07.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

TGA	Thermogravimetric Analysis
DCS	Differential Scanning Calorimetry
TPA	Texture Profile Analysis
FFA	Free Fat Acid
SNF	Solid Non Fat
TCA	Trichloroacetic Acid
PA	Proteolytic Activity
PS	Phase Separator
rpm	Revolutions per minute
O/W	Oil in Water
MCU	Milk Clotting Unity
MFFB	Moisture Free Fat Base
FDB	Fat Dry Base
ASP	Ammonium Sulphate Precipitation
MCA	Milk Clotting Activity

CHAPTER 1

INTRODUCTION

Assurance of food safety for consumers involves process of production, environment and respect of good hygienic practice including different performed analysis on the products during, before and after production also the final product packaging. Based on these principles it is necessary to engage studies about traditional foods to rank them into the class of standard or safe foods because those traditionally foods through their degree of consumption appear important in the economy of most African country. In this precise case agriculture and livestock are ranged into first scale in Benin's economies (Sessou et al., 2013). Among livestock products, cow's milk has a non-negligible socioeconomic impact. Indeed, in Benin, milk plays a part more than 50% of yearly household revenue of Fulani ethnic group (Dossou et al., 2006). Talking about traditionally food, some foods are produced from raw milks (cow, cheep, goat's milk) in Benin could be included. Among these foods such as Degue, yogurt and cheese which is known under various appellations and writing. It called Waranga, Wagashi or Wangashi, it is the most popular and most consumed milk derivate products.

Wangashi is an important pool of animal protein, a highly nutritious food with a considerable source of protein, fat, vitamins and minerals such as calcium, iron and phosphorus. It is used instead of meat or fish, or in combination with them in miscellaneous food recipes especially for people with low incomes and could efficaciously provide solution to problems related to proteins deficiency in the diets in Africa (Mohamed et al., 2018). Its inconsiderable lactose content makes it an appreciable and acceptable food to many people who suffer from lactose intolerance associated with milk consumption in Africa and Asia due to low levels of intestinal β-galactosidase (lactase).

Wangashi manufacturing process was developed by the nomadic Fulani as a means strategy of preserving excess milk and is based on the milk coagulating properties of the juice from the leaves and stems of the Sodom apple plant (*Calotropis procera*). The juice which is recovered by crushing Sodom apple leaves or stems is mixed with raw cow milk gently heated in a pot over fire wood or water. The leaves and stems of the Sodom apple, due to contained an organic acid which called calotropin, it has the ability to coagulate milk. Following coagulation, the loose curd pieces are poured into small raffia baskets which allow to drain from the whey. Wangashi is assigned to the category of soft unripened cheese therefore it has a high moisture content ranged about 50-60% which makes it highly perishable. Wangashi under ambient temperature undergo considerable chemical changes, it has been observed that the shelf life does not exceed three days, after the second day of storage. Moisture change, proteolysis and lipolysis are given rise to by increased activity of the resident lactic acid bacteria and adventitious microbes. The moisture content reduces giving place to hardening. Proteolysis sets in causing sourness and lipolysis occurs imparting a rancid aroma to the product. The change in the composition is squired by changes in the sensory quality of the product (Ashaye et al., 2006). Traditional Wangashi is known to have a bland taste as well as a bitter after taste. The bitter taste results from the high non-specific proteolytic activity of Sodom apple which also affects the yield of Wangashi and it causes the generation of excessive acid, bitter flavors and green coloration in the product (Mahami et al., 2012). The product has a bland taste mainly because the production process has not been standardized. The shelf life of the product may be affected and it also makes the product unsafe for the consumer.

The scientific study of traditional cheese making offers a growing understanding of the inherent nature, strength and limitations of traditional food processing and preservation techniques. Cheese-making is one of the oldest methods of preserving excess milk and is a major business worth billions of dollars in many industrialized countries. Cheeses are now unique products in their own right and cheese-making has advanced beyond being merely a food preservation technique. Wangashi production prospers mainly in the peri-urban milk producing areas where it provides employment mainly to women and increases the income of fresh cow milk sellers. A higher demand for traditional soft cheese (Wangashi) increased income of milk sellers in Benin.

Moreover, Aworh and Egounlety (1985) and Kees (1996) reported the processing technology of Wangashi and its stabilization by heat treatment and using chemical additives such as propionic acid and sorbates. In addition, Kèkè et al. (2008) reported a method of conservation of Wangashi using strains of *Lactobacillus plantarum*. Furthermore, the conservation of Wangashi by chemical method has a negative effect on the sensorial quality of the product.

Wangashi cheese is produced traditionally in North Benin. Steps of production have not been standardized and some of important quality parameters have not been determined yet. The aims of the study are analyzing the enzyme that will be extracted from *Calotropis procera* plant used for coagulation of milk to form the cheese and standardization of the cheese production steps (formulate the recipe; carry the recipe into industrial scale to commercialize it). The local cheese types should be standardized and commercialized in order to carry the cheese to new generations. Commercial production of the cheese needs integration of traditional production techniques into industrial scale. The cheese will be produced in laboratory with caring traditional method and then steps will be specified to be applied in industry. In order to classify the cheese, textural, structural, melt-ability and chemical analysis will be performed.

The steps of this study could be defined as:

Primary step: The enzyme of the plant was extracted and the milk coagulation capacity and proteolytic activity were examined. The determined ratio was further tested in cheese production and compared with the traditional method.

Second step: Plant ratios, processing times and temperatures were clarified in order to standardize the cheese production process. About ten students studying at the university (who knowing the original cheese were identified and they tested the products sensibly.

Third step: Structural, textural and chemical properties of cheese were determined. Study was done to determine the effect of storage time and temperature change on the textural parameters during 2 weeks at 4ºC and 25ºC.

CHAPTER 2

LITTERATURE SURVEY

2.1. Milk

2.1.1. Definition of Milk

Milk, a precious liquid, is referred as the key base raw material for all dairy products; it can be expressed in term of a significant reservoir of water, proteins, carbohydrates, lipids, vitamins, milk sugar and minerals. Beside these principal constituents, milk also is a source of other non-negligible substances like vitamins, pigments, enzymes, gases and phospholipids (substances with fatlike properties). It has an essential role to provide high level nourishment to the offspring of the mammalian species from which it was originated. However, milk from a various variety of animals such as cows, goats, sheep and buffalo has become an important and considerable part of the human diet. A lacteal secretion, collected from colostrum through a complete milking of healthy cows is also considered as milk. A amount of solid- non- fat and milk fat at least and not less than the limit respectively 8.25% and 3.25% are the main characteristics of a beverage use milk after undergoes the pasteurization process (FDA, 1998, O'cansey, 2010). As an exceptional feeding of the new born mammal during its first days, milk is also well known as a white fluid As an uncommon bolstering of the new conceived vertebrate during its first days, milk is likewise notable as a white liquid discharged by the mammary organs of female warm blooded animals. Its constituents (substances) are responsible for growth and providing energy building the materials destined to these functions (Douglas, 2007).

2.1.2. Milk production

2.1.2.1. Milk Production in the World

Milk has become an essential source of food because of its major in protein, fat, minerals and nutrients substance contents. In most of developing countries, the preservation technics are based on the processing of milk in order to convert it into value added to avoid its deterioration. Worldwide, some animal production especially production coming from domestic animal, has proved to be a best and interesting source of food; an increase or advance in milk production and consumption of dairy production has been noticed in many countries manly the one developing areas over the last two cinquena (FAO, 2017). Milk is ranged into the class of the oldest foods known to humanity. Almost the total human consumption (82%) in term of milk, is from mammals; principally from main sources such as goats, sheep, cattle, buffaloes and camels (FAO, 2017). Total world milk production has increased; India leading the ranking (21%) of global production followed by Asian countries and Brazil while Africa occupied the last place in the ranking. Global milk production is extended to rate of 2% (2017 to 2019) with a production from 811.9 to 828 million tonnes. (Anonymous, 2019).

2.1.2.2. Milk Production and Consumption in Benin

In Benin, livestock is the second most important agricultural activity after crop production. Livestock is plays a main role in Benin's economic, it is an interpretation to reduce the unemployment problem of the juvenile layer living in zone and socio-social reconciliation of the country network. Livestock production takes an important place in the horticultural Gross Domestic Product in Benin, with a rate of representation approximately 25% (INSAE, 2015; Agossou et al., 2017). According to Vissoh (2015), cattle mainly exotic cattle and goat breeds are the high source of milk production in Benin. The local cattle breed is made from different breeds like a crossed breed form which represents 61.3% followed by trypanotolerant cattle (Borgou, Somba and Lagunaire) and zebus (M'bororo, Goudali and White Fulani) respectively 31% and 7.7% as representative rate (Alkoiret et al 2011; Kassa et al., 2016; Hervé 2017; Agossou et al., 2017a). Due to the low out come and in order to hence the level and diversify the level of local milk production a foreign breed called Girolando

originate from Brazil, which producing daily 7.22 ± 0.15 kg of milk, has been inserted into the local system throughout the Livestock Development Project (Phase III) in November 2004 (Doko et al., 2012; Adambi et al., 2018) and Alpine goats in 2005 (Doko et al., 2012). The study of Kassa et al. (2016) showed off that going with the idea of improving the production, a performance of 4.5 liter/day has been reached throughout adequate programs ensuring the crossing over between the local breeds and Azawak (high productive breed). Socio-ethnical groups, the animals' rearing practices and ecological zones are the main factors characterizing the local production system (pastoral, agro-pastoral and sedentary system). Cows are the predominance of the composition of cattle herds which confer them the feature of raw material in dairy production (ANOPER, 2014). Through the programs implemented of improving the dairy animal sector, Benin's dairy herd has been increased from 1.6 to 2.3 million (1996-2016), which may allow affirming that the dairy herd has increased approximately 5% each year. Currently local milk production over the last decade is conservatively estimated at 92001 thousand tones (2007) and at 112302 thousand tonnes (2017), which are mostly from agropastorial producers (FAO, 2017); the production is predominantly by small scale farmers, who own one to three dairy animals. Milk is collected by small holder herdsmen for home consumption and for sale. Milking is done often in the morning in the presence of the calf to induce milk let down. The total cow's milk production in Benin represents approximately 3.14% of global production in West Africa meanwhile the cow milk production represents almost 90.64% of the total milk production in Turkey, including buffalo, cow, goat and sheep milk production with a global production estimated at 20699894 tones (2017), which is largely greater than Benin production. The Figure 2.1 presents the trend of milk production in Benin. In Benin, pasteurized raw milk is consumed by few people whiles the major part of the population consumes the products obtained from milk transformation. Some technological hypotheses have been evaluated to convert these products into products of first necessity to avoid milk spoilage since the preservation of raw milk (fresh cow's milk) has become difficult, (Dossou et al., 2006).

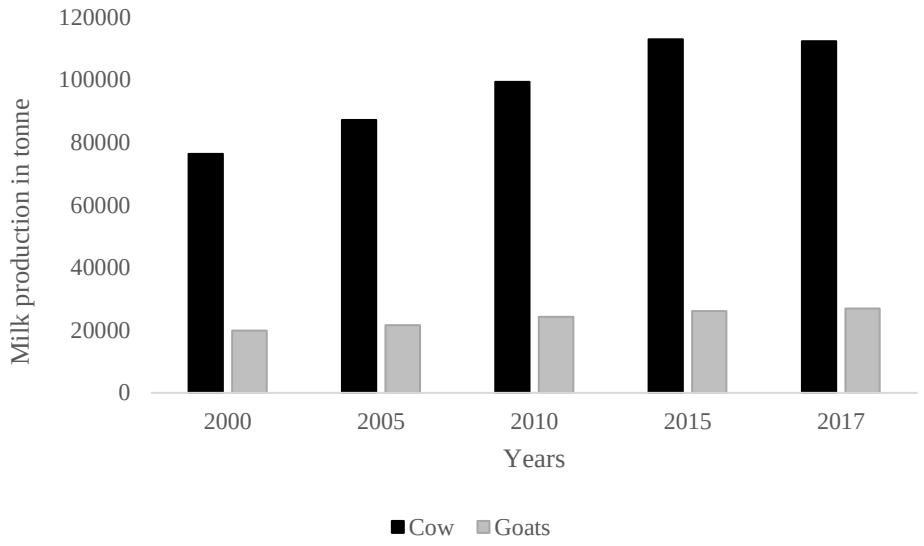

Figure 2.1 Milk production in Republic of Benin

2.1.3. Milk Nutritional Composition

Milk contains important source of nutrients for human well-being. It is easily distinguished from others food by the presence of its constituents such as lactose and casein.

Table 2.1 Composition of milk used for human food

Mammal	Total Solids	Fat	Protein	Casein	Lactose	Ash
Cow	12.60	3.80	3.35	2.78	4.75	0.70
Goat	13.18	4.24	3.70	2.80	2.80	0.70
Sheep	17.00	5.30	6.30	4.60	4.60	0.80
Water Bufallo	16.77	7.45	3.78	3.00	4.88	0.78
Zebu	13.45	4.97	3.18	2.38	4.59	0.74
Human	12.57	3.75	1.63	6.98	0.21	

Source: (Potter and Hotchkiss 1995, O'cansey, 2010)

Beside these constituents; proteins, carbohydrates, vitamins, minerals are also found and other category of constituents which are dissipated in water (Table 2.1). Protein, water, fat and lactose are major source of cow's milk while Vitamins, minerals and salts are the minor components. The variations observed in the composition of milk are related to the species (source of milk) and it composition depends on feeding

system and general treatment afflicted to the animals. The ecological conditions, stage of lactation, the breed, age are also the main factors affecting the composition of milk (O'cansey, 2010). Raw milk is an excellent nutritious product. Urea, protease-peptones, peptides are non-protein nitrogen, constituents of the nitrogenous fraction of cows' milk. They estimated in total nitrogen at 78, 18 and 4 g per 100g, respectively (Law et al., 2010). However, milk is considered as a product that rapidly perishes because of its wealth of nutriments and water.

2.1.3.1. Water

A representing of 87% of the total composition classifies water as the major component of milk. It is a principal constituent of milk which plays the role of substrate in charge of dissolving the salts, proteins and lactose in milk. As for the other components they are dissolved in the medium. The water activity (aw) of milk is estimated at 0.993 by completing a reaction in which a small amount of water is bound to the milk protein and some hydrated to the lactose and salts (Jenness, 1988). The quality, stability and preservation of milk can be affected by the amount of water which is responsible for the control of enzyme activity and microbial growth reactions Fox, (2003).

2.1.3.2. Protein

Protein is the main cause affecting the properties of milk and its derivatives products. With an estimated representing about 20% and 80% respectively, whey and casein protein are the considerable groups of protein which make up the total milk (Fox, 2003). Lactalbumins and lacto globulins are the two constituents of the acid whey. These constituents found as β and α forms such as: β-lacto globulin (β-Lg) and α-lactalbumins associated to blood serum albumin and lactoferrin considered as a fractional part of protein, are the constituents of Lactalbumins while the fraction of lacto globulin contains mainly immunoglobulins (Fox, 2003).

2.1.3.3. Lipid

Lipids, the most variable constituents of milk are considered as an extracted water-insoluble organic biomolecules from cells and tissues using non-polar dissolving agents (O'cansey, 2010). The bulk of milk fat, polar lipids and lipoidal substances are

the constituents of lipids. Lipids in milk varies according to the conditions of breeding, they are responsible for some biological function which include: Protection of coating on the surface of many organisms, structuration of components of membranes, storage and transport forms of metabolic fuel (Lehninger, 1977). Lipids are composed of fats or oils commonly constituted from tissues, biological fluid and food which are non-soluble in a polar solvent (Fox, 2003). With a fat globules that varies from 0.1 to 22 μm in diameter, O/W (oil-in-water) is a type of emulsion conferred to lipids. Milk fat is characterized by some constituents such as: lipid, triacylglycerol, and phospholipids with a rate respectively ranging in 97–98%, 2–1%, 0.2–0.4%. Beside these constituents the presence of sterols, traces of fatty acids, vitamins A, D, E, and K are also remarkable in milk fat while whole milk and skimmed milk detain respectively an amount of cholesterol ranging between 14 mg and 2 mg per 100 mL. Milk fat globule membrane is responsible for the fat present in milk generally found as a dispersed globules enfolded (Keenan et al., 2006). Excessive shearing and turbulence considered as involuntary damage of the membrane, are strongly undesirable in cheese processing. For instance, in Emmental, Gouda and Cheddar, these aspects are responsible for lipolysis, excessive rate of FFA, bitter, and soapiness and metallic as undesirable flavor.

2.1.3.4. Lactose

Lactose can be defined a disaccharide present in milk composed of glucose and galactose. It is a milk sugar, known as a principal carbohydrate of milk found at levels of approximately 4.8% (Holsinger, 1988). In most of the manufacture and within the composition of some dairy ingredients, lactose appears as an important constituent because of its capacity of crystallization. It is also the most abundant of the milk solid. Calcium known as a main source of energy, its absorption is promoted by lactose. However, some diseases such as gastrointestinal results from the lack of the enzyme lactase. The availability of certain dairy products depends on the lactase aseptically incorporated into the fresh product before packaging process. Lactose is the one least soluble (17 g per 100 g at 20^{o}C) and it is ranged among other sugars which has the lowest relative sweetness (Aurand et al., 1973).

2.1.3.5. Salts and Vitamins

Cow milk is a source of certain number of minerals which are Potassium, Calcium, Sodium, Magnesium, Sulphate found in the largest amounts (Jenness, 1988). The minerals content of milk is expressed as the white cinder obtained after an extra heating process at a temperature over 500^{o}C of a given weight of used milk. It is called Ash. Due to heat which leads to the decomposition and volatilization of some minerals, it is not typically identic to milk mineral level (Law et al., 2010).The minerals are found at small concentration less than 1 percent in milk, which are affected within heat stability and coagulation process using rennin. The firmness of curd during cheese making are influences by the calcium level of milk. The water-soluble vitamins such as vitamins B2, B12 as well as fat-soluble vitamins (A, D and E) are especially brought by the milk. With a very fast degraded and its content which fall by more than 50% after 36 hours of refrigeration, the vitamin C, is present at 8mg / l in fresh milk (Benslama et al., 2016).

2.2. Enzymes Used in Cheese Production

Enzyme utilization is a main step in cheese processing which leads to milk clotting. As mentioned Harboe et al. (2010) and Jacob et al. (2011), generally, the stomachs of ruminants are the main source of most coagulant agents used for cheese manufacturing, besides that, the use of coagulants from plants and microbes source was also known in the first days. However the utilization of microbes and plant enzymes source is perceived as an alternative solution since the animal source has been encountering some problems or is subject of some critics such as: the limited availability of ruminant stomachs (Jacob et al. 2011), high price of rennet, religious concerns (e.g., Islam and Judaism), diet (vegetarianism) or ban on recombinant calf rennet (in France, Germany and The Netherlands). It is important to emphasize that almost all the milk coagulants used as enzymes draw their source from aspartic proteases, while other groups of enzymes such as cysteine and serine proteases were also used (Jacob et al. 2011). As seen in (Figure 2.2) there is a various types of coagulant.

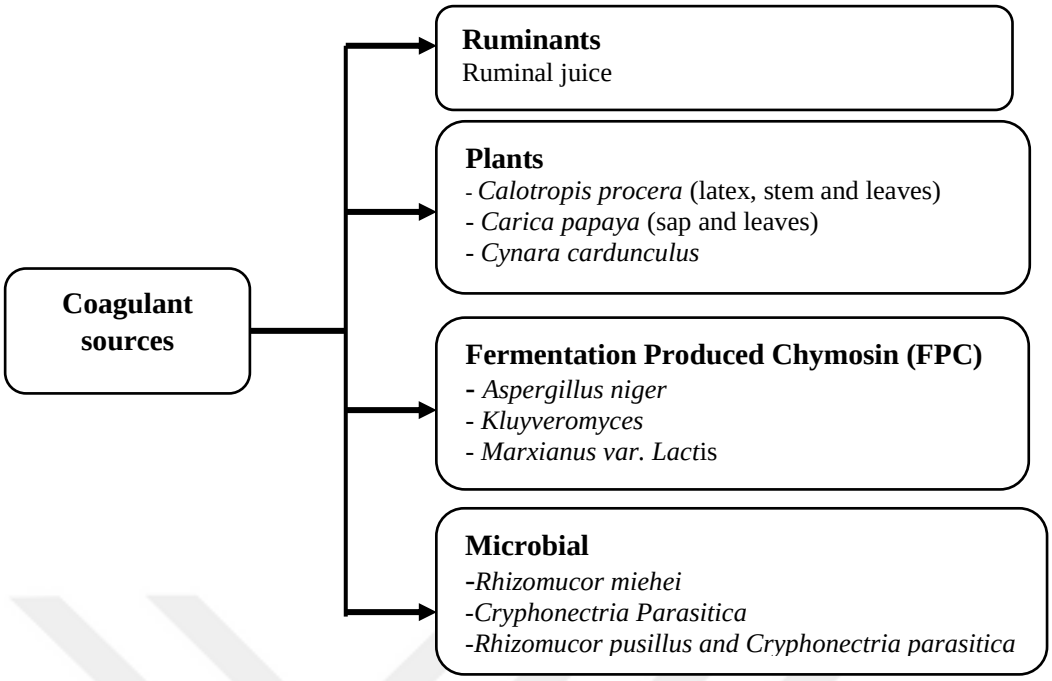

Figure 2.2 Shows the origin of different coagulants used for cheese making

A large number of authors such as (Harboe, 1992b; Guinee et al., 1992; Garg et al., 1994) investigated on different category of coagulant, their properties and applications; these researches revealed that the bitter flavors in some various cheese like the ripened cheese results from the excessive proteolytic activity, and therefore the use of these extracts are not recommended for hard cheese with long maturing periods but appropriate for softer type curd cheese that the self-life is not long (O'Connor, 1993). The classification and identification of rennet and coagulants are most based on their source.

2.2.1. Animal Origin Enzymes

Chymosin and pepsin enzymes are the main principal constituents for the most commonly used rennet. It is found also as the form of recombinant products. The principal area of innovation in cheese making relates of improvement and development of sensory and textural characteristics of products (García, et al., 2012). Calf rennet is the most known in term of enzyme used in cheese processing and used as reference to measure alternative products. A higher general proteolytic activity and a considerable sensitivity to pH are attributed to high pepsin that detains Bovine rennet. Lamb ovine and kid-caprine or caprinae rennet and calf or adult bovine rennet have a bond of

similarity; so adult bovine can valuably replace the calf rennet due to their equilibrated active enzyme (Foltman, 1992). To reach the products of some level with a certain number of characteristic flavors, in South Italy, the animal rennet used is generally composed with lipase during the cheese manufacturing. These combinations are known under the appellation of rennet past, was obtained by macerating and drying the stomachs of suckling lambs, calves, whose stomachs are filled with milk. Thus, the mixing of rennet and lipase enzymes (pregastric and possibly stomach) in a non-standardized ratio is part of the rennet paste (Law et al., 2010).

2.2.2. Microbial Origin Enzymes

The fungal origins are the source of the most popular microbial coagulants used in cheese processing. Due to their proteolytic activity, most bacterial proteases identified principally as milk clotting are less suitable. However, for almost some period ranging 30-40 years, certain microbial coagulant derived from source such as: *Rhizomucor miehei, Rhizomucor pusillus* and *Cryphonectria parasitica, R. Miehei* known as proteases are mostly used (Jacob et al., 2011). The excessive proteolytic, with a reduced ripening time and bitter cheese is cause of the higher heat stability of the derivatives obtained from R. miehei. For the control of excessive proteolysis, coagulants that have high heat stability capacity than calf rennet should be avoided and there should be differences in the coagulation temperature (Sousa et al., 2001).

2.2.3. Plant Origin Enzymes

The vegetable coagulant is widely used in cheese processing around the world (West Africa, in the Mediterranean and Southern European). Cynara sp is used in the largest variety and production of cheeses in Spain and Portugal. Plant origin is a substitute of rennet, which has been experienced in cheese making. Almost every part of plant (leaf, stem, latex etc...) has been involved as milk coagulants (O'Connor, 1993). In some most case, the use of plant as a coagulant leads or allows obtaining a desired final product, and therefore contributing to enhance the nutritional values of those whom the use of animal rennet has become a prohibition (Gupta et al, 1997). The clotting activity has been identified from many plant preparations (Aworth et al., 1987; Edwards et al., 1983; Padmanabhan et al., 1993; Pozsar et al., 1969; Tamer, 1993). However due to their the excessive proteolytic activity observed in these plants, which

is responsible for reducing the yield of cheese and cause undesired flavors in the product; makes the large number of plant coagulants as unsuitable for cheese processing (Lo Piero et al., 2002). For instance, traditional Portuguese and Spanish are made using the cardoon extract since some centuries. In West Africa especially in the Republic of Benin, Nigeria, and Ghana, Calotropis procera (Sodom apple) has been used for traditional cheese making. Besides that, other types of plant or vegetable extracts used as cheese coagulants are lemon juice (Adetunji et al., 2007), also the leaves and sap of Carica papaya are identified to be used as a coagulant (Martinez-Ruiz et al., 2013).

2.2.3.1. *Calotropis Procera*

C. procera is identified as a polymorphic, it is exists in the form of two subspecies. One of that specie is known under the appellation of spp. procera that is recognized and naturalized in some countries; for instance the Australia (Forster, 1992). In tropical and subtropical Africa, Asia and India, three species of shrubs belong to the genus Calotropis; C. procera, C. gigantea and C. acia BuchHam (Rahman et al., 1991). Its propagation is considerable in some Asia countries (India, Pakistan, Afghanistan, Iran, Saudi Arabia), also in the center and in the South of America but the most in Africa (Somalia, Egypt, Libya, the Algerian South, Morocco, Mauritania, Benin, Chad, Niger, Mali, Senegal, Togo) due to the ecological and climate condition which characterize these tropical and subtropical areas (Benyahia-Krid et al., 2016). According to (Shoaib et al., 2013), the warm climate in dry is a prerequisite for the plant to produce a best breeding. Zenger et al. (2008) study has shown that the milky sap of this plant contains three toxic glycosides: calotropin, uscharin, and calotoxin as well as steroidal heart poisons, known as cardiac aglycones. Some accidental cases when contact with the latex of Calotropis have been also presented by Maia de Lima et al. (2011). The possibility of toxicity risk should be taken account following prolonged consumption of traditional Fulani cheese, but no case of food intoxication relate to the uptake of that cheese has been reported so far (Egounlety et al., 1994).

2.2.3.1. 1. Taxonomy

Calotropis procera (Figure 2.3) known also under other appellation as Sodom apple, it is classified among the Asclepiadaceae plant family, recognized through its size

about 6m height and its small trees up to 2.5 ± 6m height, stem simple, almost without branch, covered by tiny little blossom and small green fruits; it is largely widespread most in West Africa and other parts of the world.

Figure 2.3 Specimen of *Calotropis procera*

Every part of the plant detains white latex used as coagulant for cheese processing. It belongs to the **Kingdom**: Plantae – Plants, **Subkingdom**: Tracheobionta – Vascular plants, **Superdivision**: Spermatophyta – Seed plants, **Division**: Magnoliophyta – Flowering plants, **Class**: Magnoliopsida – Dicotyledons, **Subclass**: Asteridae, **Order**: Gentianales, **Family**: Asclepiadaceae – Milkweed family, **Genus**: Calotropis R. Br. – calotropis.

2.2.3.1. 2. Utilization of *Calotropis Procera*

Calotropis procera, is a plant largely known due to its utilization in the medical part. Concerning its capacities or properties to bring satisfaction to some diseases, several authors have reported its traditional utilization which is appreciated in some area of the world. It was involved in traditional medicine by traditional doctors in West Africa, with the aim of relieving certain pains such as: diarrhea, leprosy, fever, ringworm, cough, asthma and convulsion. Due to its antibiotic property, it is applied in the treatment of wounds, also as anti-diarrheas, anti-inflammatory agent, and a way to treat the rheumatism. Its application against malaria and skin infection has also reported by (Qari et al., 2008). For the improvement of digestion, Catarrh and increases appetite, some part of *Calotropis procera* especially the sap and flowers were often also used (Oudhia, 2001). According to Kuta, (2008), the use of *Calotropis procera* as traditional

medicine generally treatment agent of ring worms, is widespread in Gwari communities for the treatment of ring worms. Its application in the traditional Asian medical system against bronchitis, pain, asthma and tumors has been demonstrated by (Muzammal, 2014). Besides its medical properties, *C.procera* leaves are used in the treatment of water *C.procera* leaves. According to (Chikpah et al., 2014), since the use of animal source coagulant (rennet) and microbial application for commercial dairy production (cheese) is become critical almost a prohibition for some population, the plant source for instance, Sodom apple (*Calotropis procera*) has drawn much attention by its capacity to be used as an alternative. The traditional utilization of the extracts from the *Calotropis* plant as the coagulant of milk in some of African countries (Benin, Ghana, Togo, Burkina Faso, Chad, Nigeria) for the cheese manufacturing has been demonstrated (Ashaye et al., 2006; Chikpah et al. 2014).

2.2.3.1. 3. Coagulating Properties of *Calotropis Procera*

Used around the world, particularly in west Africa (Benin, Nigeria, Ghana etc…) by Fulani farmers; many articles reviewed that Calotropis procera has been shown to possess some coagulating properties and has been used as a base material for milk coagulation in cheese production (Dossou et al., 2006). In Benin, traditionally the fresh leaves and stems of Calotropis procera are used for milk clotting, which is a crucial step in the Peulh cheese processing (Aïsso et al. 2015). The study of (Capo-chichi, 2004) presented that almost all the parts of Calotropis procera such as: leaves, stems, fruits and latex detain a coagulating capacity on milk casein at different rate of coagulation. He brought out that the leaves and the stem has similar coagulating activity, while the fruits are less effective. According to the results, the latex is the part that marked more activity and its dilutions approximately in 10th and 100th show best results compared to the other parts (Capo-chichi, 2004). In Ghana, a local cheese is also prepared using the latex , the extract collected from *Calotropis procera* leaves and stems; but a bitter taste in the cheese noticed is related to the high proteolytic activity that carried by the extract (Mahami et al., 2012).

2.2.4. Enzyme Treatment

2.2.4.1. Extraction of Plant Enzyme

For the most part, these compounds (enzymes) have been extricated from their regular source by watery maceration of different plant organs, for example, blossoms, seeds, roots and leaves. There are a few distinct methods for setting up the fluid concentrate of the plant material. The dried entire or squashed cardoon blooms are absorbed water at room temperature for a variable timespan. At that point, the filtrate is gathered and this rough concentrate is utilized as coagulant (Roseiro et al. 2003). An elective technique for extraction is pounding the dried blooms with unrefined kitchen salt, laying the glue on a cotton fabric (which goes about as a strainer) and solubilizing the chemicals by permeation with warm milk (Sousa et al., 2002). The unrefined concentrate can likewise be additionally purged to get halfway refined protein or unadulterated chemical relying on the level of cleaning. Precipitation with ammonium sulfate is a successful method to deliver significant measures of dynamic proteases from the blooms of C. cardunculus (Barros et al. 2001). Cardosin A and B were separated from the stigmae and stylets of dried blooms of C. cardunculus (Silva et al., 2003). Proteases were extricated from marks of shame of C. scolymus (Sidrach et al. 2005), dried blooms of Moringa oleifera (Pontual et al. 2012), new blossoms Silybum marianum (L.) Gaertn. (Vairo-Cavalli et al. 2005, 2008). A halfway cleansed chemical concentrate, named onopordosin was gotten from the upper bits (marks of disgrace and styles) of new blooms from Onopordum acanthium (Brutti et al. 2012). Protein removes were gotten from Bromelia hieronymi organic products (Bruno et al. 2002) and the extract was named hieronymain (Bruno et al. 2010). Stripped ginger rhizomes were utilized to acquire the catalyst extricate (Hashim et al. 2011). Seeds of various plants have additionally been utilized to get ready plant extricates for cheddar making. Protease separates were gotten from the seeds of Solanum dubium (Ahmed et al. 2009a, 2010), and stripped seeds of sunflower (Helianthus annuus) and entire (Albizia lebbeck) seeds (Egito et al. 2007). Nestor et al. (2012) got the enzyme concentrate from the berries of Solanum elaeagnifolium. Likewise, proteases were gotten from the latex of fig tree and assessed for the milk-coagulating properties (Kumari et al. 2012; Sharma et al. 2012)

2.2.4.2. Milk Clotting Activity

Milk clotting activity is the most significant property of enzymes utilized in cheese making. It is the capacity of the catalyst for explicit κ-casein hydrolysis (Jacob et al. 2011). It very well may be estimated by various techniques, for example, Soxhlet, Berridge, and universal standard strategy and the units utilized are Soxhlet units, Berridge, or Rennet units and International Milk Clotting Units, individually (Harboe et al. 2010). The enormous number of techniques, various units at various conditions utilized by various scientists has made it hard to think about the units for milk-clotting activity. Notwithstanding, a few creators have analyzed the milk-thickening action of plant proteases with calf rennet in similar conditions. Nestor et al. (2012) thought about the milk-thickening movement of chemical concentrate from Solanum elaeagnifolium berries with calf rennet and found that the milk-coagulating action was 39.4 and 2,474 milk clotting unit (MCU) at 32 °C individually. Ahmed et al. (2009) looked at the milk-coagulating action of compound concentrate from Solanum dubium with calf rennet and found that the milk-thickening movement was 880 and 2,496 MCU at 37 °C, individually. Kumari et al. (2012) looked at the milk-thickening movement of chemicals Religiosin A and Religiosin B from Ficus religiosa with rennet and found that the milk coagulating action was 387, 803, and 4,989 MCU at 37 °C separately. A decent milk-coagulating enzyme is portrayed by a high explicit caseinolytic movement and a low broad proteolytic action, since the proteolysis emphatically influences the tactile properties of cheese. At the point when a potential rennet substitute is contemplated, it is especially essential to assess enough the debasement samples of the caseins in light of their impacts on yield, consistency and kind of the last cheese (Fox 1989). Coagulation thinks about are significant in the assembling and development of cheeses and a decent milk clotting agent is portrayed by a high explicit caseinolytic action (Manzoor et al., 2013).

2.2.4. 3. Proteolytic Activity

Proteolytic catalysts from the plant sources have gotten exceptional consideration in view of their expansive substrate explicitness, movement in a wide scope of pH esteems, temperature, and nearness of natural mixes just as different added substances. Numerous activities in food industry are completed at high temperature, for example,

hydrolysis of proteins at high temperature, enzymatic processing of aspartame and different peptides, preparing and blending (Sharma et al., 2009). In this way, the look for important proteases with unmistakable movement and particularity is dependably a ceaseless test for fluctuated modern applications. As stated, the proteolytic action of dairy culture is significant overseeing factor assuming a noteworthy job in different cell and physiological procedures. Nature of milk and dairy items might be impacted by measures of proteolytic catalysts, for example, plasmin and variables influencing them The degranulation of somatic cells is by all accounts the central cause of the proteolytic exercises with for the most part unbiased proteases, for example, elastase, collagenase, cathepsin G, proteinase 3, and so forth. (Owen et al., 1999; Jain et al., 1993). Several studies were showed that proteolytic activity can be influenced by some factors; for instance pressure treatments at 20°C reduce micellar dimensions either by disruption of linkages among caseins and inorganic constituents, hydrophobic bonds, or both, and increases the dimensions of Ca, P, and Mg in the serum The study of Garcı´a-Risco et al. (2000) showed off that pressurization (400 MPa, 15 min) from 40 to 60°C decreased the proteolytic movement and improved the organoleptic properties of milk, contrasted and similar processing at room temperature room temperature. Proteolytic activity is determined using many method involving the use of casein as a substrate and buffers and can be calculated as the difference in percentage of nitrogen in the fraction soluble at optimum pH, determined by the Kjeldahl method before and after incubation. Higher temperatures of pressurization (40 to 60°C) led to a progressively lower proteolytic degradation (Garcı´a-Risco et al., 2000). The proteolysis firmly influences the textural and tactile properties of cheese (Manzoor et al., 2013).

2.2.4.4. Ammonium Sulfate Precipitation

Ammonium sulfate precipitation is one of the classical methods of protein fractionation. The technique is generally utilized for the underlying partition of unrefined concentrates since it is reasonable for preparative scale work and in light of the fact that it can have selectivity not the same as those of particle trade or gel filtration chromatography. The basic salt immersion required to hasten a protein relies upon the nature and the centralization of the protein just as on the pH and the temperature of the arrangement. The salt precipitation method can likewise be done in the turnaround

way. In particular, the protein blend is first totally encouraged within the sight of a transporter and afterward solubilized with a diminishing salt slope (Bayliss et al., 2002). In a solitary task, the proteins are isolated into a progression of portions with little contrasts of salt immersion in this manner making it conceivable to pick the best recuperation of the ideal protein with minimal measure of contaminants. The use of buffers is necessary in order to obtain a clean precipitate. Ammonium sulfate is exceedingly hydrated, and a concentrated ammonium sulfate arrangement lessens the accessible water in all respects impressively. The outline on the correct shows two proteins (Figure 2.4), with their hydrophilic districts hued blue. The protein on the left has moderately couple of hydrophilic locales, and thus will total and hasten at a generally low convergence of ammonium sulfate - maybe around 20 - 30% immersion. Conversely, the protein on the privilege has impressively progressively hydrophilic districts, and subsequently will stay in arrangement until the convergence of ammonium sulfate is significantly higher - maybe around 50 - 60% immersion.

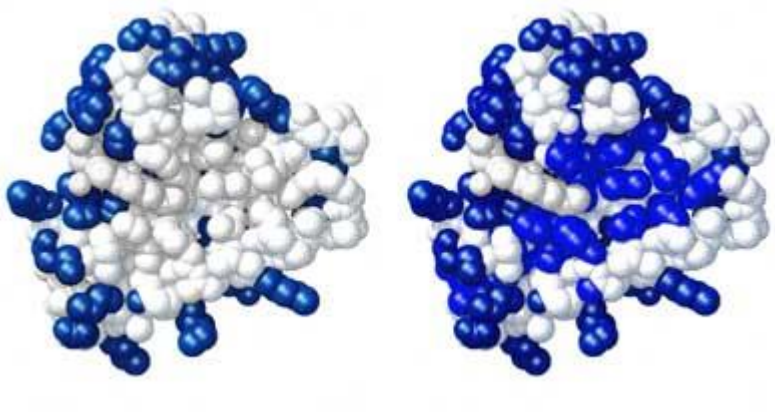

Figure 2.4 Protein with their hydrophilic districts hued blue

This implies it is conceivable to isolate proteins from a blend based on their relative hydrophobicity by bit by bit expanding the grouping of ammonium sulfate (Camara-Artigas, 2018).

2.3. Cheese

2.3.1. Definition of Cheese

In many industrialized countries cheese is related to a big pole of business (Aworh, 2008). According to Belewu et al. (2012), it is perceived as one of a commonest dairy

product around the world. Cheese is attributed to the end product resultant from the coagulation and draining of raw milk. It is also defined as a fermented food made from the pressed cruds of milk of various animals, firm and elastic or soft and semi-liquid in texture. Protein, fat and casein are the main constituents of the basic solids of a milk concentrate which is considered as a cheese. Codex Stan 283 (1978), likewise characterized cheese as a developed or not completely inferred dairy item that can take different textural angle (delicate, semi-hard, hard or additional hard), which might be experience covering step, and in which the extent of whey to casein protein is constrained in milk through the coagulation procedure of complete or fractional milk protein and other piece of fixings, for example, skimmed milk, halfway skimmed milk, cream, buttermilk or any blend of these materials, utilizing rennet or other fitting material for coagulation; the fluid got after milk thickening movement is depleted agreeing standard cheese producing process. Be that as it may, the measure of protein contained in the cheddar will be surely higher than the protein rate of the blending base crude material from which the cheese that was readied and by cheese manufacturing technology which includes coagulation of milk protein content with a last item normally safe in term of substance structure, organoleptic highlights and physical viewpoint (Zhou et al., 2014).

2.3.2. Classification of cheese

There are a lot of varieties of cheese based on certain well defined criterion, however their principles of proteins coagulation in milk to obtain coagulum and then decant them from the serum are similar.

Table 2.2 Classification of cheese

MFFB %	Designation (Texture)	FDB %	Designation (Fat)
<41	Extra hard	>60	High fat
49-56	Hard	45-60	Full fat
54-63	Semi-hard	25-45	Medium fat
61-69	Semi-soft	10-25	Low fat
>67	Soft	<10	Skim

Source: Dairy processing Handbook

According (Coker et al., 2005) many types of cheese present nowadays; these cheese are classified regarding their compositions such as: the percentage of water present in cheese, the microorganism used in ripening, and the length of the maturing period of the cheese etc. Table 2.2 shows the classification of cheese based on their Moisture Fat Free Basis (MFFD) and Fat Dry Basis (FDB) equals' percentage moisture on fat-free basis. The study of (Fellows, 2014) brought out some classification of cheese base of their textural properties. For instance , cheeses might be broadly took the appellations of 'delicate', 'semi-delicate', 'separate hard', 'semi-hard' and 'hard' cheeses.

2.3.3. Cheese Manufacture

Cheese manufacture is one of old practice that dated to some centuries. It is basically a procedure of drying out and fermentation whereby the fat and protein (casein) of milk are focused somewhere in the range of 6 and 12 crease, and the pH is diminished from 6.6 in milk to pH ranging in 4.6 -5.4 in fleshly made crude (J.F. Martin et al., 2017). It preserves the most significant constituents of milk (for example fat and protein) which are determinant variables of cheese yield, (Banks et al., 1981), according to (Coker et al., 2005), as cheese utilizes two of the great standards of sustenance conservation, to be specific maturation with lactic corrosive and lessening water movement by taking out water and including salt (NaCl) Potential redox, because of development bacterium that adds to the capacity safely of cheese. Its handling remained a workmanship instead of a science until moderately as of late. Milk for cheese production must not be contained any trace of antibiotics, visible impurities and any abnormal taste or odor. The high microbiological and chemical quality of milk is the main criteria of selection of milk for cheese manufacturing. So in order to produce a cheese of better quality and progressively reliable with the impressive advancement of information about the science and microbiology of milk and cheese, it was conceivable to control the progressions associated with cheese making in an increasingly controlled way, which include the implement of some steps of development such as: Pasteurization, Acidification, Coagulation, Synerises, Salting, Ripening to help the cheese makers.

- **Pasteurization**

At this stage, almost all microorganisms, yeasts, and types of coli present can cause the deterioration of cheese characteristics by generating carbon dioxide and an unwanted proteolysis; harmful pathogenic bacteria that are in the origin of illnesses, for example, tuberculosis and leptospirosis should be eliminated by heating milk at 73^{o}C for nearly15 seconds (O'Connor, 1993; Coker et al., 2005). After the milk treatment, it seem necessary to standardize it to allow the complete occurring of the fat ratio adjustment (O'Connor, 1993).

- **Acidification**

Acidification in most of case starts before and transcends the other processing operations. It is appears as a one of the fundamental tasks in the production of most cheese varieties amid whey is properly released from the coagulum and the control of the development of numerous unwanted microscopic organisms is happened (Coker et al., 2005). Pathogenic food contamination and gas delivering microorganisms as types of non-starter microbes in cheese, their growth are also controlled by acidification process. For a cheese to be considered as safe item from the general wellbeing perspective, it should be properly handled. In some case the additional production of acid is required, which involves the use of starters bacteria that produce probiotics that are likewise limit or restrain the development of nonstarter microorganisms (Fox, 1993). The application of starters is usually interpreted by the transformation of lactose to lactic acid addicting lactic acid bacteria responsible for this conversion. According to O'Connor, (1993), normal aging or whey from the past parcel of cheddar normal aging or whey from the past parcel of cheese is preferred instead of commercial starter preparations for some cheese varieties. The enormous volumes of starter required for cheese making are made in unique mass starter aging pots in which the milk is heat treated to wreck undesirable microorganisms, spores and phages and cooled to about 22°C, a temperature appropriate for starter development. The solidified starter is blended and maturation proceeds for around 6 to 16 hours. The measure of starter required fluctuates for the diverse cheese assortments (Coker et al., 2005).

- **Coagulation**

Coagulation is an important operation during cheese manufacturing; during which under characterized states of temperature and by activity of a coagulant specialist, the

physical part of milk changes from fluid to a jam like mass through the modification of milk protein. Lemon juice, plant rennet and proteolytic enzyme, for example, Chymosin (rennin) and other enzymes from the mold Rhizomucor miehei acquired by biotechnology are the common used coagulants. The pH and temperature are the factors affecting the coagulation, therefore to creates a suitable situation for ideal movement of rennin and to a pH of 4.5 as the preparing continues, making an unacceptable domain for undesirable microbes, subsequently expanding the safety of the finished product, these enzymes that possess an acidic characteristic, should be used under an adequate condition (Kongo et al., 2013). The entrapment of microorganisms are the resultant of a constant grid of strands of casein micelles, which consolidate fat globules, water, minerals and lactose based on the utilization of a rennet coagulum (Coker et al., 2005).

- **Syneresis**

The investigation of (Law et al., 2010) demonstrated that, syneresis is the reworking of casein atoms, which results in a fixing of the casein arrange and the final product is that dampness is crushed out of the casein organize. Syneresis, otherwise called contracting of the coagulum, is to a great extent the consequence of constant rennet activity. It causes loss of whey, and is quickened by cutting, mixing, cooking, salting or squeezing the curd, just as the expanding measure of acid created by the starter, and bit by bit increments amid cheese making. Subsequently, the cheese curd contracts and dampness is ceaselessly removed amid the cooking stages (Coker et al., 2005). The milk composition such as Calcium and casein, pH of the whey, cooking temperature, rate of blending of the curd-whey blend and time impact the rate and degree of syneresis. For some characterized cheeses, the degree of syneresis is identified with the composition of the finished product (cheese) (Fox, 1993).

- **Salting**

Salting is used as preservation method, however it influences the textural characteristic and flavor properties of the end product by controlling microbial development and compound action. It very well may be likewise impacts the pH in the cheese as per the dimension and technique for salting. To improve the pH of the curd for these varieties must be near a definitive esteem (pH 5.1) at salting, a few varieties, for the most part

of British root, are salted by blending dry salt with the curd (Fox, 1993). The basic utilized is NaCl that its focus in cheese is extending between (0.7- 4% and 2-10%). It is likewise imperative to see that salt in the dampness stage is adequate to end the development of starter bacteria. The salt can be included at various strides; for example either straightforwardly to the curd after the whey is kept running off and before embellishment or squeezing into shape, or by drenching the formed cheddar hinder in saline solution for a few days following production (O'Connor, 1993). Expansion of salt to the cut curd draws more whey from the cheese curd and a portion of the salt diffuses into the curd. The assurance of how much salt is consumed by the curd, is identified with the pH of the curd, the contact time and the salt molecule size and structure.

- **Ripening or Maturation**

Ripening, also called maturation can be defined as a period of curing in which some cheese varieties are undergo after its consumption. This period can be varied from about three weeks to more than two years. Cheese ripening involves the essential debasement of milk components by glycolysis, lypolysis and proteolysis (Marilley et al., 2004). The aging procedure of cheddar is extremely perplexing and includes microbiological, biochemical, structural, physical and sensory changes during storage amid capacity to the curd bringing about the flavor and surface trademark in the specific variety (Mcsweeny, 2004). Cheddar surface mellows amid maturing as a result of hydrolysis of the casein micelle by proteolysis and changes to the water-restricting capacity of the curd and changes in pH which may cause different changes, for example, the movement and precipitation of calcium phosphate (Mcsweeny, 2004). It majorly affects the nature of most cheese varieties with the exception of unripened cheeses including fresh acid curd cheeses (Quark and Cream cheese) and some fixing cheeses (Law et al., 2010). The primary degradation of milk constituents caused by glycolysis, lipolysis and proteolysis occurs during cheese ripening which can be designed by a metabolized completely to lactic acid and catabolized to frame acidic and propionic acids, carbon dioxide, esters and liquor by the catalysts of the starter cultures or secondary cultures in the milk (Marilley et al., 2004).

2.3.4. Origin of Wangashi Cheese

The traditional Peulh cheese would have owed its originality from the Fulani cattle herdsmen that their principal activity is based on traditional milk collect and its transformation. It is realized that during harmattan the milk coagulation is not rapid due to the temperature and the loss of some amount of raw during transportation from the far to site of transformation were the problems encountered by the local processors. The Fulani women after applied many technique to avoid the loss and the spoilage of raw milk, which methods that did not work so the idea to use the leave of calotropis procera which were immersed, serving to protect the milk during its transport in the calabash. This taught would pass beyond the idea of stabilization to other capacity of milk coagulation. These women assumed that this phenomenon was due to the leaves they put in the milk to stabilize it during transportation to the house. Whereby the idea of the first production of cheese is placed firstly in Baatonu called "Gassarou Babarou", which means the ball of cheese. The authentic name of the Fulani cheese is "Waragassarou Babarou". What ethno linguistic mutations have transformed today in different pronunciation such as Wangashi, Waragashi, Woagashi or Wara respectively in Benin, Nigeria and Ghana. Even though the origin has not been extensively studied, according to (Dossou et al 2006), it can be assumed that Fulani cheese technology was undertaken in Benin and according to (Awohr and Egounlety, 1986; Waters - Bayer, 1988) the production method of milk coagulation using Calotropis procera is carried out in west and northern Nigeria and in the north of Togo. Wangashi is known as the most consumed dairy product a part of degue and yogurt in Benin (Mohamed et al., 2018). The study of Malomo et al. (2015) showed off that Wangashi is also a traditional soft cheese consumed almost in every part of West Africa.

2.3.5. Nutritional quality of Wangashi Cheese

Cheese contents the valuable nutrients of milk (protein, fat and minerals, for example, calcium, iron and phosphorous, nutrients and fundamental amino acids), which confers it the normal for a key nourishment in term of sustenance in the eating regimen of both youthful and old. In Benin milk is used for different dairy items handling, for example, yogurt, degue and Wangashi, a traditional value added developed by the nomad Fulani ethnic group. Based on its source of animal protein, Wangashi is classified and

consider as a reference used to solving problems related to proteins deficiency in the diets in Africa especially for people with low incomes (Kèkè et al., 2008). The traditional processing, which is found in pastoral households, involves using the remaining milk after direct consumption as drinking milk to make some dairy products, particularly cheese, which is sometimes colored (Figure 2.5) using colored water with dried sorghum leaves to extend it shelf life in order safeguard its nutritional values (Sessou et al., 2013).

Figure 2.5 Fresh Wangashi cheese and soaked Wangashi cheese

The traditional processing, which is found in pastoral households, involves using the remaining milk after direct consumption as drinking milk to make some dairy products, particularly cheese, which is sometimes colored (Figure 2.5) using colored water with dried sorghum leaves to extend it shelf life in order safeguard its nutritional values (Sessou et al., 2013). Table 2.3 presents the proximate analysis results for different types of cheese found in Benin (Kora, 2005).

Table 2.3 Proximate composition of cheese from three different cattle breeds

Type of Cheese	Borgou	Lagunaire	Girolando
pH	6.4	6.4	6.5
Moisture (%)	65.23	65.73	66.13
Acidity (% lactic acid)	0.17	0.17	0.14
Proteins (% DM)	36.26	36.03	33.65
Lipids (% DM)	43.30	44. 44	45.60
Carbohydrates (% DM)	15.08	15.27	13.44
Ash (% DM)	5.33	4.23	7.29

It has been seen that cheese is a supplement thick sustenance pool which gives fat, great proteins, oligopeptides, amino acids, nutrients and minerals.

2.3.6. Traditional manufacturing of Wangashi

According to several authors, the Fulani cheese is generally processed using the raw milk and the *Calotropis procera* (leaves, stem, sap …) as the base raw materials. Fresh cow's milk is heated slightly up to temperature around 70°C and extracted coagulant from *Calotropis procera* is added to milk (Figure 2.6). The curd formed, cooked, drained and molded is brought on the market under different designs for selling (Dossou et al., 2006).

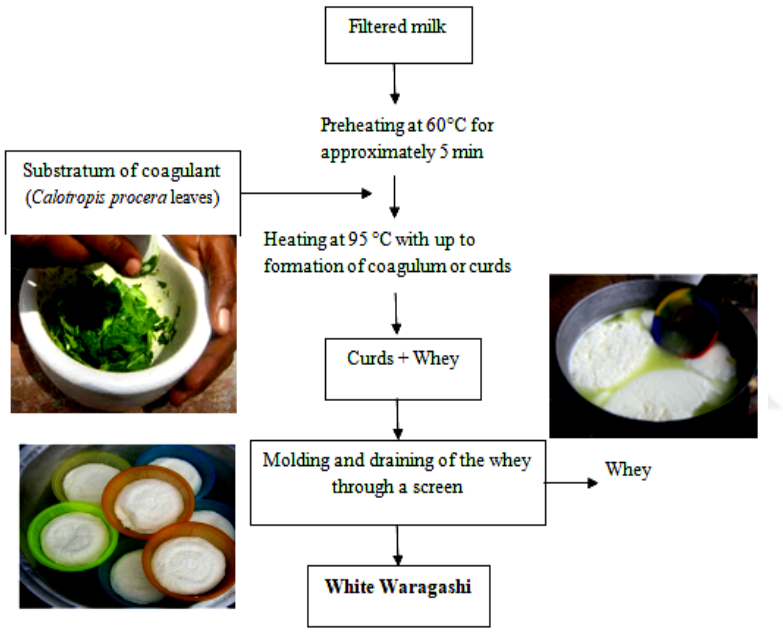

Figure 2.6 Flow chart of processing of Peulh cheese

In West Africa, extra fresh milk is transformed into different stable items such as peulh delicate cheese (Wangashi), Nono (aged skimmed milk), nyarmie and yoghurt by the Fulani pastoralists. Wangashi is described as soft white unripened cheese by (Ogundiwin, 1978); it has also been described as soft, wet, feta-like cottage cheese made from whole milk (Jansen, 1990). Because of its high moisture content, it can be classified in the same category with the cottage cheese, which involves the application of starter culture during its manufacturing while Wangashi does not use. They do not both undergo ripening and they have a curd like texture. (Ashaye et al., 2006). Wangashi is mainly devoured at home or sold available broadly in the Northern of

Benin. Many leaves and stem extracts of various plant such as pawpaw (Carica papaya) can also be used as a coagulant, however the concentrates from *Calotropis procera* are wanted to the concentrates of pawpaw since cheese prepared with *Calotropis procera* has a better flavor contrasted with the cheese handled with pawpaw leaf extricates (O'Connor, 1993). Wangashi production includes the utilization of simple hardware, and its handling conditions are not ordinarily standardized neither optimized (Belewu et al., 2005).

2.4. Standardization

The standardization is a method or process of implementing and developing technical standards based on the maximizing of safety. It likewise brings advancement, first since it gives organized strategies and dependable information that spare time in the development procedure and, second, since it makes it simpler to disperse historic thoughts and learning about driving edge system. Models encourage regular day to day existence. They increment safety and can be utilized to support activities. Standardization guarantees that items and techniques are fitting for their expected use. It guarantees that items and frameworks are good and interoperable (Lincke et al., 2018). It can be applied on traditional food in order to carry out the ancient receipt into the industrial scale allowing the product to be known by the future generation. At this step for some traditional food their processing conditions should be fixed for instance by optimizing the pH and temperature (Maiti, 2017). The pH of cheese is a significant physicochemical parameter influencing the texture, taste and microbiological quality of cheese. The expansion of starter culture for milk aging (fermentation), the de-fermentation of certain assortments of cheese during development and the capacity of the curd framed to oppose changes in pH decide the last pH of cheese. The fundamental parts of cheese that influences the pH of cheese are the caseins. The degradation of casein produces inorganic phosphates and natural acids and their dimensions in cheese are impacted by milk nutrients, curd treatment and its impact on syneresis (Lucey et al., 1992). Lipolysis additionally builds the causticity of cheese by the generation of free unsaturated fats (Law et al., 2009).Decrease in pH of cheeses during development is because of the proceeded with creation of lactic corrosive lactic corrosive microbes and the freedom of amino acids, for example, aspartic and glutamic acids during

proteolysis (Sallami et al., 2004). The pH of delicate soft fresh cheeses ranges from roughly 4.1 to 5.4 (ICMSF, 1996), nonetheless, the support greatest which is around pH of 5.0 is significant in cheese making since the ideal pH for most cheese ranges from 5.0 - 5.2. As the pH of cheese is decreased towards pH 5.0 by lactic acid aging, the cradle limit is likewise expanding.

2.5. Characterization of Cheese

The variety in the biochemical composition and innovative properties of milk and dairy items has different roots: the animal's feeding routine, hereditary variables and cold stockpiling of the milk. In spite of the fact that the impact of an expansion in cell fixation on the well-being of the animal is known from a pathological perspective, the outcomes on the biochemical synthesis and innovative properties of milk and dairy products stay misty (Snyder, nd).

2.5.1. Chemical Analysis

Characterizing the dietary benefit of a food production is progressively troublesome in light of the fact that such a definition must consider each one of those properties of a food, for example, visual intrigue, smell, taste and surface, which collaborate with the faculties. These properties can be impacted by an enormous number of mixes which to some extent have not been distinguished. Other than their dietary benefits, sustenance are progressively being made a decision as per properties which decide their taking care of (Ortiz, 2017). Cheese is exposed to compound investigation for a variety of reasons, for example, to discover its synthesis for wholesome purposes, to guarantee its consistence with models of identity, to survey the effectiveness of production or as a record of value. Including not just in illustrating the synthesis of the crude materials and finished products, yet in addition with the progressions which happen in food amid its generation, handling, stockpiling and cooking (HACCP, 2009). The exceptional mind boggling nature of food results in a huge number of wanted and undesired responses constrained by assortment of parameters. To pick up an important understanding into these responses, it is important to separate the production into model frameworks. A thorough assessment of food necessitates that explanatory procedures keep pace with the accessible innovation. The pH and moisture

content of cheese are very important parameters affecting the texture, flavor and microbiological safety of cheese (Fox et al., 2004).

2.5.2. Color measurement

Color is the discernment that outcomes from the location of light after it have collaborated with a product. The apparent shade of an item is influenced by three elements: the physical and compound organization of the article, the phantom synthesis of the light source enlightening the article, and the ghastly affectability of the watcher's eye(s). The color of any product can shift in three measurements, to be specific: tint, commonly what the purchaser alludes to as the "shading" of the article (green); softness, additionally called the brilliance of the item (light versus dim green); and immersion, likewise called the immaculateness of the shading (unadulterated green versus grayish green). It is a significant quality factor in buyer item acknowledgment. Shade of an item is impacted by how it reflects, retains or transmits light, which thusly is identified with the physical structure and synthetic nature of the nourishment (Rudan et al., 1998). The color contrasts from every assortment of cheese, and the whiteness is considered as a significant quality parameter for Gaziantep cheese (Kaya, 2002). Fat bestows whiteness to the cheese by dissipating light (Rudan et al., 1998). It is a significant proportion of value in the food commercialization since it is considered by customers to be identified with product freshness, readiness, attractive quality and sanitation (McCraig, 2002; Jeli'nski et al., 2007). Color estimation instruments, as per the gauges created by the Commission Internationale de l'Eclairage, change or channel reflected spectra to deliver reproducible shading space facilitates, to be specific, L* (record of whiteness), a* (file of redness), and b* (file of yellowness) (Commission Internationale de l'Eclairage, 1986; MacDougall, 2001). Its estimations are regularly done in a research center based instrument (Hunter Lab meter or Minolta Chroma meter) however they can likewise be procured by online instruments. Inferable from maturing impacts of light sources and finder frameworks, customary alignment of colorimetric gear against color gauges is basic. Colorimetry is utilized routinely in quality control and item improvement to evaluate the shade of curd and cheese. Shading is identified with feeding regimen of bovine, expansion of color and cheese variety. (Dufoss'e et al., 2005; Olson et al., 2006).

2.5.3. Texture Profile Analysis

Texture is gotten from the word textura in Latin, which implies a weave. Texture was some time ago used to allude to the structure, feel and presence of textures (Rosenthal, 1999).Texture of nourishment characterizes the "eating nature of sustenance". The textural properties of food product are examined with a wide scope of rheological instruments. The International Organization for Standardization (ISO, 1992) characterizes textural aspect of an item as, all the rheological and basic (geometric and surface) qualities of the item distinguishable by methods for mechanical, material, and, where proper, visual and sound-related receptors. These days rheology is a science with applications in numerous ventures including particularly physicists and specialists which are keen on the useful and hypothetical parts of this science. As expressed the meaning of rheology goes for estimating those properties of materials that control disfigurement and stream conduct when subject to outside powers (Gunasekaran et al., 2003). Cheese texture might be characterized as a 'composite tangible quality' coming about because of a mix of physical properties that are seen by the faculties of touch (counting kinaesthesis and mouth-feel), sight and hearing. It remains the essential quality property of cheese. Instrumental estimation of the textural properties of cheese is normally used to comprehend buyers' discernment on cheese quality just as the impact of the handling innovation on cheese quality. The TPA investigation of cheese according to Brown, (2002) is utilized for the estimation of properties, for example, hardness, fracturability, cohesiveness, springiness, chewiness, stickiness, adhesion and strength. As per Phadungath, (2002), Texture is very hard to characterize as it implies various things to various individuals. Food technologist endeavored to characterize texture as far as food product in light of the fact that the importance of surface did not cover the product angle. Texture is the essential quality normal for cheese items. Its investigation alludes to the mechanical testing of product. Texture properties of any product play a significant perspective in the inclination and acknowledgment of sustenance items (Tudoreanu et al., 2009).

2.5.4. Thermal Properties Analysis

Thermal properties are currently utilized in an extremely enormous scope of logical assessments. Other than the more concoction zones, for example, polymers, fine

natural synthetic concoctions and pharmaceuticals, they have applications to gadgets, in development, geography and building, in materials science and in food quality control. They frequently give data difficult to get by other analytical technics. All the time, a mind boggling material, for example, a polymer composite, will demonstrate unequivocal and trademark impacts on warming which identify with its tendency, composition and history (Blowey, 2010). Thermal properties are used to describe the analytical techniques that measure the physical and chemical properties of a sample as a function of temperature or time. Warm investigation called Thermal Analyses (TA) is characterized as: A gathering of methods wherein a property of the example is observed against time or temperature while the temperature of the sample, in a predetermined air, is modified (Gaviln, 2010).

A DSC analyzer estimates the vitality changes that happen as a sample is warmed, cooled or held isothermally, together with the temperature at which these progressions occur. The vitality changes empower the user to discover and gauge the advances that happen in the sample quantitatively, and to take note of the temperature where they occur, thus to describe a material for dissolving forms, estimation of glass advances and a scope of progressively complex occasions. The fundamental property that is estimated by DSC is heat stream, the progression of vitality into or out of the sample as an element of temperature or time, and typically appeared in units of mW on the y-axis. Since an mW is an mJ/s this is truly the progression of vitality in unit time (Holba et al., 2017). The genuine estimation of warmth stream estimated relies on the impact of the reference and isn't outright. What is important is that a stable instrumental reaction or pattern is delivered against which any progressions can be estimated. The beginning stage of the bend on the y-axis might be picked as one of the beginning parameters, and it ought to be set at or near zero. It is at some point used to think about the wonder of cheese dissolve capacity in term of melt-ability. For most varieties of the cheeses, dissolve capacity is one of the properties of cheese at raised temperatures and it might be characterized as the simplicity with which cheese streams or spreads after warming (Muthukumarappan et al., 1999).

Thermogravimetric analysis (TGA) is an exploratory procedure wherein the weight or, carefully, the mass of a sample is estimated as an element of test temperature or time. The sample is regularly warmed at a steady warming rate (alleged unique estimation)

or held at a consistent temperature (isothermal estimation), yet may likewise be exposed to non-straight temperature projects, for example, those utilized in test controlled TGA (purported SCTA) tests. The decision of temperature program will rely on the kind of data required about the sample (Basosidik et al., 2012). Also, the environment utilized in the TGA test assumes a significant job and can be receptive, oxidizing or latent. Changes in the air amid estimation may likewise be made. Wangashi with its high moisture content will be investigated I term of thermal stability to establish its evaporation and desorption capacities.

CHAPTER 3
METHODOLOGY

3.1. Materials

Standard laboratory materials including samples preparation, ingredients that were purchased on a local market, analytical grade reagents obtained from Sigma-Aldrich were used in analysis. Apparatus, equipment and tools as specified in the standard methods were used to carry out the analyses of the samples. These materials are specified within the description of methods respectively.

3.2. Experimental Studies

3.2.1. Extraction of Enzyme

Calotropis procera stems were collected from Botanic garden of University of Abomey Calavi in Republic of Benin and were brought to Gaziantep during 24 hours with care to protect them. These stems were washed, cut into small portions and crushed using blender. An amount of 40 g of *Calotropis procera* stems were mixed with 100 mL of triple distilled water. The obtained mixing was also filtered using whatman one phase separator (1ps) filter paper/185 mm filter paper and centrifuged 3000 rpm at room temperature, for 15 min. The final solution was decanted and the clean separated liquid was stored at refrigerator (4±1°C) and used until all experiments had been completed.

3.2.2. Milk Clotting Activity

Milk clotting activity of the enzyme at various pH and process temperature were determined using 0.25 g of powdered skim milk obtained from a Turkish local market that measured into a clean test tube accompanied by 0.75 mL of 0.05 M Sodium acetate

buffer pH 5.5. The test tube was shaken until the milk dissolved and was heated at 50^{o}C using water bath for 15 min. Later the tubes were collected and 1 mL of crude extract or purified enzyme were added and the coagulation time were determined to estimate the enzyme's clotting activity.

3.2.2.1. Effect of pH on the Milk Clotting Activity

As described above the same amount of powdered milk was taken into a clean tube test followed by 0.75 mL of 0.05 M Sodium acetate buffer that the pH concentration was adjusted from 5.0 to 8.0. The test tubes were shaken until the milk powder was dissolved, after which tubes were undergo the heating process for 15 min at 50^{o}C. In order to get the milk clotting activity, 1 ml of crude extract or purified extract from *Calotropis procera* was added and the time milk was taken to be clotted by enzyme was recorded as coagulation time value.

3.2.2.2. Effect of Temperature on the Milk Clotting Activity

Precisely 0.25 g of powdered milk was weighted into clean test tubes containing 0.05 M Sodium acetate buffer (pH= 5.5), these test tubes were placed in water bath and shaken for 15 min at different temperature raging between 35^{o}C-80^{o}C until the dissolution of milk completely. After that, 1 mL of crude extract or precipitate enzyme extract from *Calotropis procera* was added into each test tube and the coagulation time was recorded as enzyme milk clotting activity.

3.2.3. Proteolytic Enzyme Activity

Protease activity was assayed using crude enzyme and casein as a substrate through the modified method of Lad and Butler (1972). The casein (1%) solution was prepared by heating up to 100^{o}C in water bath 1 g of casein in 99 mL of 0.1 M of potassium phosphate buffer at pH 5.5 for 12 min without boiling the solution. Once the casein dissolved into the buffer, the solution was cooled and used substrate. 5 mL of 1% of casein solution was carried into tube to which 1 mL of crude enzyme or precipitated fraction was added, well mixed by swirling and let equilibrate in incubator for 10 min at 50^{o}C. After incubation, 5 mL of 5% of Trichloroacetic Acid (TCA) was added to stop the reaction and tube was again incubated for 30 min. Before measuring the

absorbance at 660 nm, the mixture were centrifuged at 3000 rpm for 30 min using eppendorf Centrifuge 5810 R and to this mixture were added 5 mL and 1 mL respectively of 0.05 M of sodium carbonate solution and Folin's reagent. The activity of enzyme was obtained in term of µmoL tyrosine liberated time total volume of assay according to volume of enzyme assay, time and volume used in colorimetric determination.

3.2.3.1. Effect of pH on the Proteolytic Enzyme Activity

Two different types of buffer solutions used to arrange the pH of the substrate were adjusted at around pH 5 and 8. Citrate and potassium phosphate buffers were used respectively for pH ranging between 5-6 and 7-8. The reaction mixture contains 1% of casein solution, 1 mL of crude enzyme and other reagents based on the process described above. To the tubes, 0.005 M sodium carbonate was added to regulate any pH drop created by the addiction of Folin's reagent, the mixing into test tubes were thoroughly mixed and incubated at 50^{o}C. After which the proteolytic activities were recorded as µmoL tyrosine liberated and converted in term of (µmoL/min/mL) enzyme.

3.2.3.2. Effect of Temperature on the Proteolytic Enzyme Activity

The reaction mixture contains 1% of casein solution, 1 mL of crude enzyme and other reagents such 5 mL of 0.05 M sodium carbonate solution and 1 mL of Folin's reagent based on the method described above. This solution was assayed at various temperatures (35, 40, 50, 60, 70 and 80^{o}C) and constant pH of 5.5. The test tubes contained the substrate at constant pH were incubate at different temperatures and the absorbance values read were inserted in the standard curve in order to get the enzyme activity in term of proteolytic which was calculated according to enzyme concentration with total volume solution assayed by time of incubation and volume of enzyme assayed and expressed in µmoL/min/mL.

3.2.4. Ammonium Sulfate Precipitation

Ammonium sulfate powdered obtained from Sigma-Aldrich was prepared at various percentage of saturation, from 20 to 80% by adding the amount of gram necessary for each percentage of saturation. 3 mL of the extract of crude enzyme was carried into 6 plastics centrifuge tubes and tubes were labeled from number 1 to 6. Each amount of ammonium sulfate previously weighted according to the saturation was added to the tube 2 through 6, while tube to 1, same amount of crude extract enzyme was added instead of ammonium sulfate and used as a reference. It stored at 4^{o}C for subsequent assays. Once the salt added, tubes were slowly and gently stirred to dissolve rapidly them in order to avoid forming the solutions and get desired saturation. After dissolving all salts, tubes were allowed to stand in ice for 15 min to obtain a best precipitation and then the solutions were centrifuged at 10000*g for 15 min too. Each precipitate fraction was decanted and dissolved in 3 mL of 0.1 M Potassium phosphate buffer, pH 7.4. Purified enzyme's and reference solutions obtained were assayed for protein content determination which allowed us to calculate the enzyme activities (milk clotting, proteolytic and specific activities) using the optimum pH and temperature that are respectively 5.5 and 70^{o}C in order to identify the best saturation.

3.3. Milk Analyses

Milk was brought from a farm located in Gaziantep, Republic of Turkey. The standard procedures were used to assess the quality of this milk in the Laboratories of Food Engineering department of Gaziantep University.

3.3.1. PH Measurement

The pH value of raw milk was measured using pH meter electronic, that 10ml of milk was placed into a small beaker and the pH was determined by dipping the probe of a calibrated Mettle Toledo pH meter into the sample. While the pH values of cheeses were measured according to the method of Kaya et al, (1996) by using the same instrument. 5 gr samples were dispersed in 5 mL water distilled water prior to measuring pH. The procedure was repeated three times and average reading calculated.

3.3.2. Protein Determination

The protein content was determined by formal titration method (AOCA 2006). This method depends on the fact that when formaldehyde is added to neutralize milk, free acid (which can be titrated by alkali) is produced in proportion to the amount of protein present. To 10 mL was added 0.5% phenolphthalein indicator and 0.4 mL of neutral saturated potassium oxalate. The solution was mixed and allowed to stand for a few min, also neutralized with 0.1 M NaOH to standard pink color. 2 mL formalin was added and the solution was anew mixed and allowed to stand for a few min. After which the new acidity produced was titrated with 0.1 M NaOH to the same pink color (a). The mixture of 2 mL and 10 mL of water was titrated with the same alkali as a blank (b). Then the protein content was calculated using this following equation (Eq. 3.1):

$$\%\boldsymbol{Protein} = \mathbf{1.7} * (\boldsymbol{a} - \boldsymbol{b}) \quad \textbf{(3. 1)}$$

3.3.3. Fat Determination

Gerber method described by Kirk (1991) was used to determine the fat in milk samples. About 10 mL of sulfuric acid (H_2SO_4) were put in a butyrometer. Then 10.94 mL of milk sample and 1ml of fat-free amyl alcohol (density 0.809-0.813 at 20ºC) were added, tubes were closed with stopper and the contents were mixed thoroughly until the no white particles were seen. Each tube (stopper downwards) was transferred to water bath at 65ºC until a set ready for centrifuging. The samples were centrifuged at 1100 rpm for 5 min then transferred into a water bath at 65ºC for 3 min. The corks were screwed into the butyrometers until the lower meniscus of the fat was at 0.5 mark. The percentage fat was read off of 0.05% directly from the scale.

3.3.4. Total Solid in Milk

3.5 g of milk was mixed into a dish and placed on boiling water for 30 min. Then it was transferred to a well-ventilated oven 100ºC and dried for 2 ½ hours following by a cooling for 30 min in desiccator. The weight of residue in the dish was considered as a percentage of total solid of the sample. After which solid non-fat (SNF) value was

determined by taking the difference between the total solid and the fat content in the milk.

3.3.5. Titration Acidity

For the estimation of percentage titration acidity in terms of lactic acid, 20 g of milk was weighted into a flask then diluted with twice of its volume of CO_2 free water obtained by heating distilled water up boiling point when the flask was hermetically covered. Titration was made with 0.1 N NaOH to the first pink color using 1% in alcohol of phenolphthalein. While Titratable acidity values of cheese samples were determined using titration method (Kurt et al., 1999). 10 gr of grated cheese samples and 105 mL distilled water were mixed for 5 minutes. 25 mL of the mixture was filtered. The mixture was also titrated with 0.1 N NaOH in the presence of phenolphthalein indicator.

3.4. Manufacturing and Analyses of Cheese

3.4.1. Production Steps of Wangashi Cheese

Two types of cheese were produced with Cow's milk supplied from local farm and analyzed. Milk was heated up to temperature 70^{o}C and pH 5.5 then 40 mL of crude enzyme or 6 mL of purified extract enzyme was added and heated at about 100^{o}C for 15 min. After cooking the curd whey mixture was poured into specific baskets and left to drain of its whey about 160 min. Samples for analyses were cut into pieces and stored at different temperature (4^{o}C and 25^{o}C) in order to perform the texture analyses during two weeks. The following flows charts describes the step of processing.

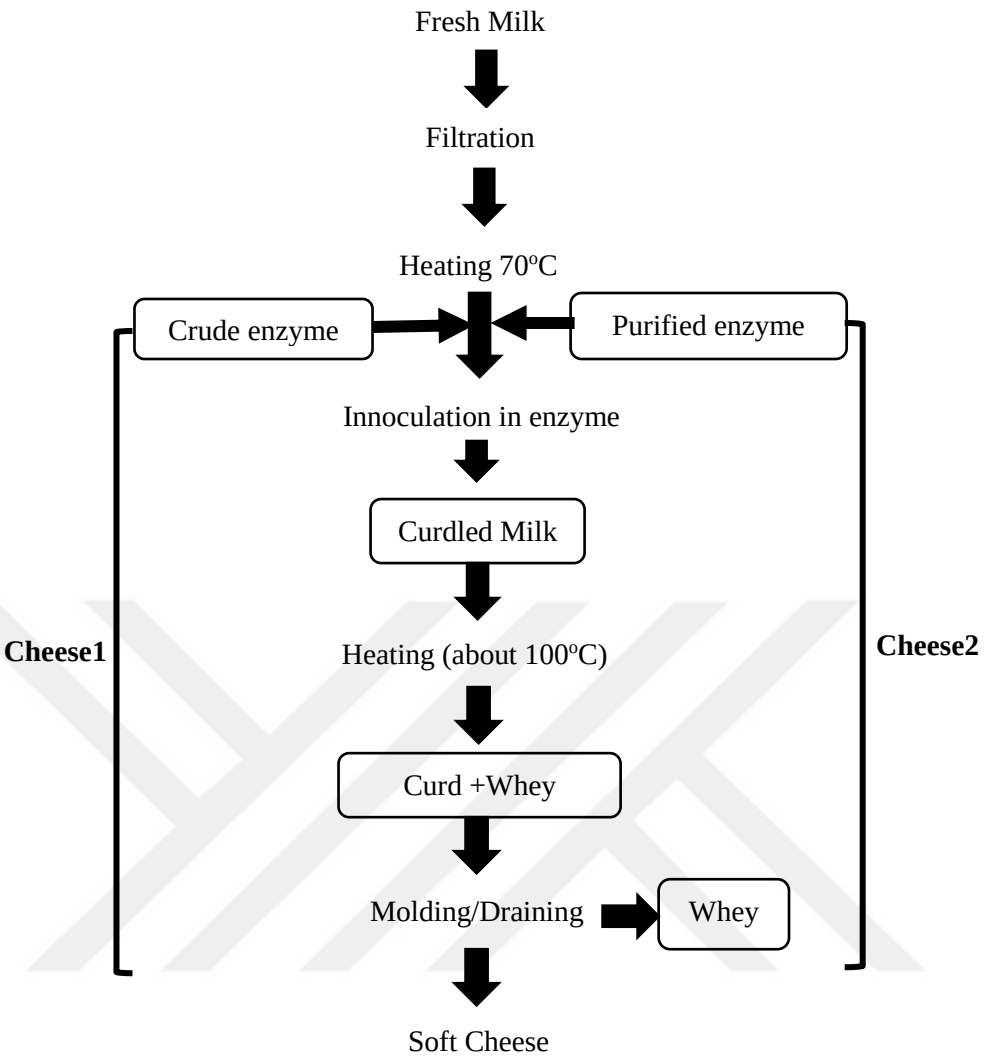

Figure 3.1 Modified Wangashi processing

3.4.2. Moisture Content Determination

Moisture content was determined by modifying Dean and Stark method. 5.00 g of cheeses were weighted into flask followed by 200 mL of toluene. After which the flasks containing the cheese samples and the less dense solvent was fixed to a condenser using a side arm and the mixture was heated about 90 min. The water in cheese samples was evaporated and moved up into the condenser where it was cooled and converted into liquid water, which then trickled into the graduated tube. When no more water is collected in the graduated tube, distillation is stopped and the volume of water is read from the tube. Thereafter the amount of water read was multiple by hundred and divided by the weight of sample in order to get the moisture content value.

3.4.3. Fat Determination

The fat contents were determined by using automatic Soxhlet extraction method for which 10.00 g of cheese samples were previously dried into oven dry at 105 during 30 -45 min to reduce the water content and cooled in the desiccator. After which samples were poured into white cartouches combined with glass that the weights were known followed by 75 mL of hexane. Samples were undergo to system and after immersion, washing and recovering which took 95 min; samples were collected and cooled again before weighting in order to know the amount of oil. The amounts of fat were calculated using the following formula (Eq. 3.2):

$$\%\boldsymbol{Ash} = \frac{\boldsymbol{W_2 - W_1}}{\boldsymbol{W_s}} * \mathbf{100} \quad \textbf{(3. 2)}$$

where W_1, W and W_0 are respectively the weight of flask after, the weight of flask before and the weight of sample used.

3.4.4. Protein Determination

Protein was determined using the Kjeldahl method (AOAC, 2005). 1±0.5 g of sample was weighed into a digestion flask. It was followed by a catalyst of 7.00 g potassium sulphate and approximately 0.5 g of copper sulphate and 12 mL of sulfuric acid. The digester was set at 400°C and the mixture was heated in a fume hood for 40 min till the digest color turned blue. This signified the end of the digestion process. The samples were cooled for 25 min, transferred to Kjeldahl apparatus which is automatic. So after the system was watched pressing button 2, the analyses were performed by pressing button 1. After the liquid obtained in 250 mL volumetric flask was titrated with 0.0902 N of HCl solutions to an orange color of the mixed indicator, which signified the end point. Protein was determined following this formula (Eq. 3.3):

$$\%\boldsymbol{Protein} = (\boldsymbol{V}) * \mathbf{0.0902} * \frac{\mathbf{14.007}}{\boldsymbol{W} * \mathbf{1000}} * \mathbf{100} * \mathbf{6.38} \qquad \textbf{(3. 3)}$$

where V and W, are respectively Volume of Titration and Weight of sample.

3.4.5. Ash Determination

The ash contents of the samples were determined using muffle furnace according to the method of AOAC (2005). About 5 g of fresh sample was weighed into a clean and weighed crucible, and charred by heating in a fume hood till smoking ceased. The charred samples were then transferred to a muffle furnace and temperature increased gradually to 550^{o}C. The samples were then allowed to burn for about 2 ½ hours. After reaching constant weight of samples they were removed and cooled in a desiccator for approximately 45 min before weighing. The amount of ash was calculated using the formula (Eq. 3.4):

$$\%\mathbf{Ash} = \frac{\mathbf{W_2 - W_1}}{\mathbf{W_s}} * \mathbf{100} \quad \textbf{(3. 4)}$$

where W2, W1 and Ws designate respectively the total weight of dish with Ash, the empty dish and sample weight.

3.4.6. Free Fat Acid Measurement

Free Fat Acid (FFA) was determined by neutralized 40 mL of ethanol prepared in presence of three drop of phenolphthalein as an indicator and 0.1 N sodium hydroxide. To this solution, 5.00 g of each cheese samples cut into small part were added and mixed. The blend was bubbled on a hot plate and titrated with 0.1N NaOH. The titres were collected and the FFA were determined as oleic acid using the following formula (Eq. 3.5):

$$\%\boldsymbol{FFA} = \frac{\boldsymbol{T * N * F}}{\boldsymbol{10 * W_s}} * \mathbf{100} \quad \textbf{(3. 5)}$$

where T, N, F and W_s represent respectively Titre, Normality of NaOH (0.1), Factor of dominant FFA (Oleic) and sample weight.

3.4.7. Color Measurement

The color of Wangashi was estimated by utilizing the L, a, b and YI color documentation framework. The hardware utilized was the colorimeter (Hunter Lab Color Flex, Hunter Associates Laboratory Inc., Reston, VA, USA). The Wangashi cheese samples were cut and placed in a Petri dish and secured. The color was estimated by setting on the outside of the gear the petri dish containing the Wangashi

samples. The readings were taken haphazardly from three spots and the mean perusing was determined. Prior to estimating, the chromameter was adjusted with a high contrast circle and checked for recalibration in the middle of estimations, albeit no alterations were required. Color esteems were recorded as L* = darkness/lightness (0= dark, 100 = white), a* (– a* = greenness and +a* = redness), and b*(– b* = blueness, +b* = yellowness).

3.4.8. Texture Profile Analyses Measurement

Texture properties of Wangashi cheese samples were checked by a Texture Analyzer TA-XT2 (Stable Micro Systems Ltd., Surrey, UK). The samples were molded consistently with a rectangle probe. Three estimations were taken on each Wangashi sample. TPA parameters estimated were hardness, adhesiveness which is the level of stickiness by mouth feel when bitten multiple times, springiness (the power with which the example comes back to its unique shape or size after fractional pressure it is otherwise called versatility of cheese), gumminess (which is the thickness during biting time required to separate a semi strong sustenance until it is appropriate for biting) and resilience (which is the quantity of bites expected to chew the sample to a consistency reasonable for gulping). These parameters were estimated by the software. Test conditions were a pressure strain 25%, with pre-test speed of 3 mm/sec, test speed 1 mm/sec, post-test speed 1 mm/sec, remove 15mm, time 5 sec and a contact power of 5.0g. The samples were consistently molded with a size of 1cm width, length and height.

3.4.9. Thermal Properties Determination

Thermal properties of cheeses were determined using a Differential Scanning Calorimetry (Pyris-1 DSC-7 Perkin Elmer Ltd. DSC 8000. Norwalk, CT). Previous to the experiment, the DSC was calibrated using standard samples that the phase transition temperatures are known. The sample weight ranged between 8.8-9 mg were weighted into aluminum sample pans (inner volume: 50µL), and pans were hermetically sealed. The cheese samples were heated from temperature 0 to 200^{o}C, 5^{o}C/min as a heating rate and 40 mL/min of N_2 gas. The thermal transitions such as melting and protein denaturation of cheese samples were defined from the peaks or heat of fusion. As for the thermogravimetric analyses, it was performed on a TGA

4000 (Perkin Elmer Ltd.). 20 mg of each cheese samples was weighted and placed in the thermo balance alumina sample pan. These samples were heated from the temperature rang to 25^{o}C to 800^{o}C at 10.00^{o}C/min under Nitrogen at 20.0 mL/min where the weight protein loss and fat derivation in samples were determined.

3.4.10. Statistical Analysis

All of the experiments were done in triplet; all data were collected and analyzed using a statistical program SPSS V. 22. The test of different between cheese samples treatment and ANOVA were performed at $\alpha = 0.05$ and the results were presented within respective tables.

CHAPTER 4
RESULTS AND DISCUSSION

4.1. Enzyme treatment

This study was took into account a pre-study based on the assessment on the different concentration of crude extract stems (15, 20 and 25g) of *Calotropis procera* were crushed and diluted with a constant volume 50 mL of triple distilled water. These three batches of crude extract were used to prepare three types of Wangashi cheese using cow's milk and their chemical characteristics also were checked. It was observed that the chemical properties in term of protein, fat, moisture and pH content of the cheese samples made using 20 g of stems dissolved in 50 mL of triple distilled water were similar to Wangashi properties prepared by Kora, (2005) using cow's milk from various Benin bovine breed (Borgou, Lagunaire and Girolando) and Ferial et al., (2016) as for them used combined leaved and stems of *Calotropis procera*. The parameters of Wangashi cheese obtained by Kora and others were presented in the sequence of introduction. Due to the similarities recorded in cheese samples obtained using 20 g/50 mL of triple distillated water when compared to what other mentioned authors obtained, the crude extract containing 20 g and 50 mL of triple distilled water was used to complete the study. This work was followed by determination of the optimum pH and temperature through the milk clotting and proteolytic activities assessment of *Calotropis procera*.

4.1.1. Optimum temperature and pH

The results are summarized in graphs showing the proteolytic and milk clotting activities values at different pH and temperatures. Figure 4.1 shows the effect on temperature of

milk clotting and proteolytic activities to determine the optimum pH when a constant pH 5.5 was applied and temperature or pH of substrate was adjusted from 35^{o}C to 80^{o}C using 0.1 N NaOH or 0.1 N HCl and citrate or phosphate buffers respectively for milk clotting and proteolytic activities. The obtained results showed that at different and various temperatures studies. *Calotropis procera* stems possessed proteolytic activity which ranged from 18-40% in term of relative activity, while milk clotting activity ranged from 14-53%; the results showed that the enzymes activities were optimal at acid pH 5.5 for milk clotting and proteolytic activities. However there was a complete loss of milk clotting activity at pH 8 while the proteolytic activity was observed almost all the pH but lost from pH 7 to 8; while optimum temperatures were obtained at 70^{o}C for proteolytic and milk clotting activities respectively, though activity was still observed at 80^{o}C.

As reported by Oseni et al. (2013), all the part of Calotropis produced the milk clotting activity and after the latex and root, the stems are known as higher producer of milk clotting activity than the leaves which however had been used as clotting agent in the production of the local soft cheese Wangashi by Ihekoronye et al. (1985). The report of Oseni et al. (2013) showed that the stems have not a strong proteolytic activity which was confirmed by our finding and it will be important to emphasis that this part of *Calotropis procera* plant compared to other parts (latex, root) of plant with higher proteolytic activity, could be standardized and used for the formation of a stable cheese with neither bitter nor off flavor by application of some purification methods. According to Ilany et al. (1967), it is necessary that the enzyme preparation generally should have a low proteolytic activity for a compatible and stable dairy product. The ethno medicinal importance of various parts of this plant which might not unconnected with some of these proteases have also studied and reported by other researchers, (Verma et al., 2010; Behl et al., 1996) also reported the latex to be irritant, caustic and depilatory when applied to the skin. The optimum pH and temperature obtained in this study were little bite different from what recorded by Oseni et al. (2013), who reported the milk clotting and proteolytic activities at 70^{o}C and 60^{o}C respectively while pH 5 and pH 4 for milk clotting and proteolytic activities respectively too. These differences could be probably attributed to the different substrate and concentration of buffers used.

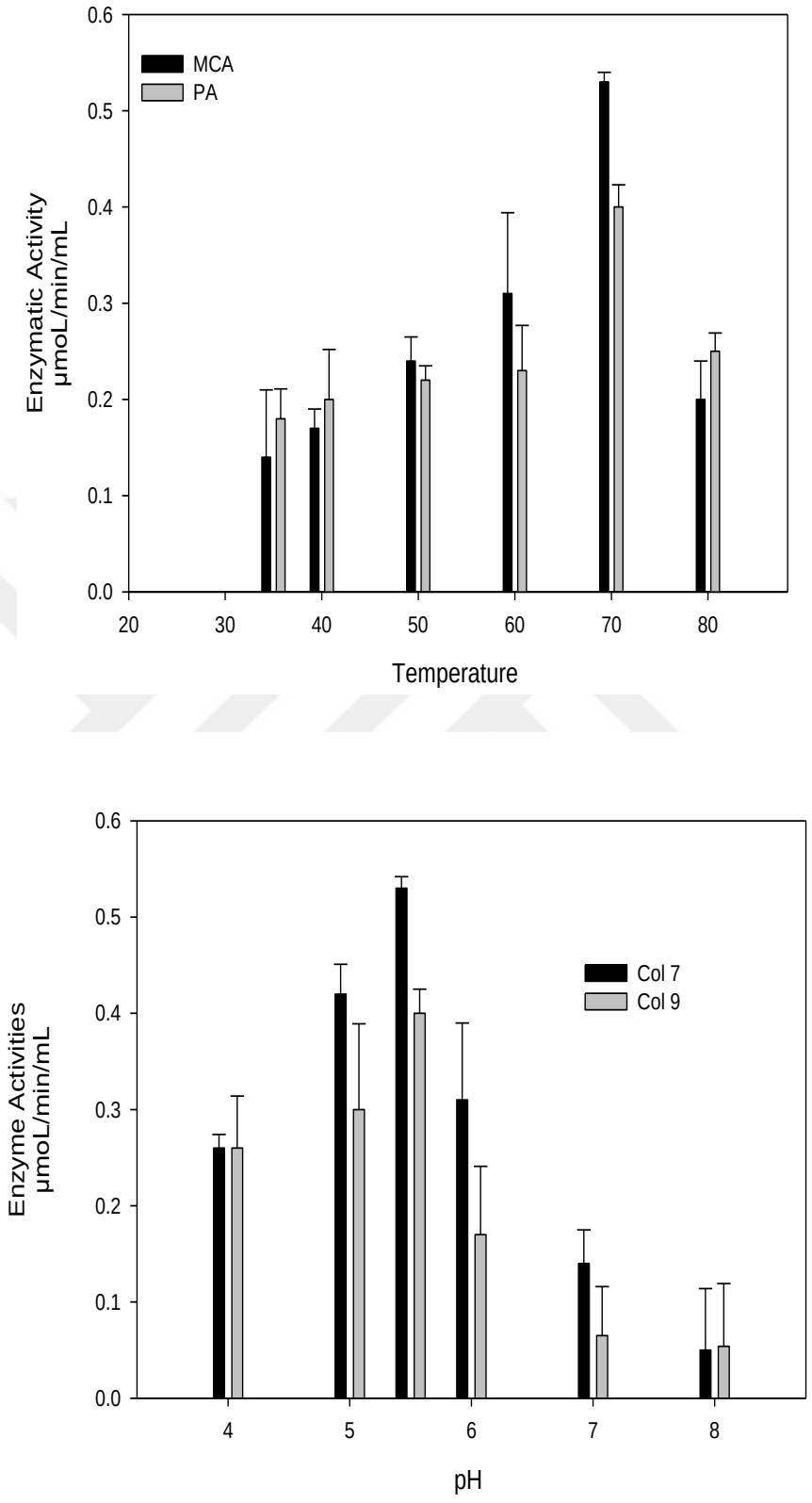

Figure 4.1 Effect of temperature and pH on milk clotting and proteolytic activities

The observed pH optimum 5.5 for milk clotting and proteolytic activities could be resulted from the casein used as substrate for proteolytic and milk powdered as substrate for milk clotting activity. This optimum pH 5.5 for proteolytic activity observed was similar to that reported for *Calotropis procera* latex and papain using gelatin as substrate, while the pH optimum 7.5 was reported by Vanos (1972), for proteolytic activity using albumin as substrate. Hence optimum pH is dependent of the substrate used. The temperature optimum for the proteolytic and milk clotting enzymes activities in this study was obtained to be 70^{o}C.

4.1.2. Ammonium sulfate precipitation

The optimum pH and temperature (5.5 and 70^{o}C) respectively were used for the rest of the work as it was mentioned above. Part of crude extract was than fractionated using the ammonium sulfate precipitation method. The pellet resulting from each precipitation step was re-dissolved in the phosphate buffer pH 7.4 and analyzed for protein concentration and protease activity. At this step, the majority of enzyme activity was found in the pellet of the precipitation of 70% saturation of ammonium sulfate sulphate, giving not only the highest specific activity (thus, purification fold), but also the highest yield among all fractions (Table 4.1). This finding was consistent with what reported Maria, (2015) who evaluated the role and efficiency of ammonium sulphate precipitation in purification process of Papain crude extract. The results showed that the enzyme activities increased from 20 to 50% but at 80% decreased. The milk clotting activities were observed at 60, 70 and 80% at suitable time whilst for the saturation ranging between 20-50% the milk clotting was taken too long time (a day) before their apparitions and also the protease activities ranging between 20-50% saturation were lower when compared to the crude enzyme; what can be explained by the fact that the amounts of salt at this level were not sufficient to precipitate all the protein content in the solution so that to reach the desired saturation. As for the milk clotting activity, although it is still unclear, evidence indicates that milk clotting might be related to its proteolytic activity (Mocquet et al., 1965). The higher milk clotting activity time was recorded at 70% saturation (150 sec), although it is still unclear, evidence indicates that milk clotting might be related to its proteolytic activity (Mocquet et al., 1965).

Table 4.1 Monitoring of fractionation using ammonium sulfate

Step	Activity (µmoL/min/mL)	Specific Activity (µmoL/min/mL/µg)	Purification Fold	%Yield
Crude extract	0.40	0.005	1	100
20	0.01	0.0009	0.18	3.00
30	0.02	0.0008	0.16	4.50
40	0.05	0.002	0.40	7.40
50	0.30	0.019	3.80	12.25
60	0.77	0.025	5.00	19.20
70	**0.99**	**0.043**	**8.60**	**25**
80	0.88	0.027	5.40	22

The finding in this study was also consistent with the statement of Baraka et al., (2017), who reported the lower enzyme activity and milk clotting activities with crude extract from Artichoke using Ammonium Sulfate Precipitation (ASP) as purification process at a saturation ranging between 20-40% but contradicted Assia et al (2015), who reported an increase milk clotting activity and decrease proteolytic activity using ASP as a purification technique on sunflower seeds. This contradiction can probably due to solvent used for the extraction of crude enzyme; sodium acetate buffer with or without 5% NaCl (w/v) was used while triple distillated water was used in this study. It can be conclude that the milk clotting activity might be related to the percentage of saturation when ASP is used as a purification technique. In this manner, fractional sanitization of the protein utilizing ammonium sulfate is suggested as it prompted clear chemical arrangements with magnificent milk-coagulating properties and cheese making potential.

4.2. Manufacturing and Analyses of Cheese

In order to further confirm the suitability of the partially purified enzyme as rennet substitute in cheese making, the crude extract and purified enzyme were used for the preparation of two types of cheese samples from cow's milk for each of them following the process described above in Figure 3.1 as Cheese 1 and Cheese 2. The produced

cheese was evaluated for the yield and curd formation time and other parameters in term of comparison. This information is indispensable to evaluate the potentiality of the purified enzyme. The cheese yield was recorded for every treatment. The level of cheese yield was determined as percentage of weight of cheese (kg) divided by the percentage of weight of milk (kg). Some part of the samples produced were wrapped with aluminum and stored in refrigerator and a temperature controlled oven to provide normal storage conditions of the cheese since the cheese traditionally has been produced and consumed within a week at room temperature which is 15-25^{o}C. The chemical, thermal and textural properties of the samples were analyzed and compared in the respective sections given below.

4.2.1. Chemical analyses

The parameters of this fresh milk at temperature ranging between 15-17^{o}C were assessed in three replications and average of them were listed in Table 4.2.

Table 4.2 Chemical composition of cow's milk used in the cheese manufacture

Parameters	**Cow's milk**
pH	6.78±0.02
%Protein by weight	2.09±0.55
%Lactic acidy by weight	9.54±1.13
%Fat by weight	3.19±0.03
%Total solid by weight	36.67±2.08

(Arthur, 2006; Kora, 2005, Ferial et al., 2016), however other were completely slightly higher or lower (Dossou, 2006). These different observed may due to the bovine race, the condition applied for milk collection, the feeding of animal and also the weather.

There was a significant difference between moisture content of both cheese samples ($p < 0.05$), (Table 4.3). The cheese obtained using crude enzyme (Cheese 1) had a highest moisture content (59.70%) whilst the lower moisture content was obtained in the cheese made with purified enzyme (Cheese 2). This difference observed in moisture content demonstrated a variation in the percentage yield of cheese samples. The highest yield value was recorded with cheese 1. It may be explained by the way

that the coagulant type influences the cheese yield and the yield of cheese expanded because of the joining of whey proteins (Abdel Razig, 1996; Zaki et al. 1974; Ustunnol et al, 1985). This could be clarified by higher retention of water and the cheese shaped by crude extract enzyme contrasted with that framed by purified enzyme. Likewise, Abdul-Rahaman, (2013) expressed that high rate of water in cheese straightforwardly influence the yield rate, since moisture is considered as one of the major parameter affecting the expansion or diminishing in yield rate. There was a significant difference between the types of cheese in terms of protein, fat, pH, FFA, ash and lactic acidity ($p < 0.05$).

Table 4.3 Chemical compositions in percentage of Wangashi cheeses

Parameters	**Cheese 1**	**Cheese 2**
pH	5.01 ± 0.04^{b}	5.49 ± 0.06^{a}
Moisture	59.70 ± 2.14^{a}	46.67 ± 3.05^{b}
Protein	20.58 ± 0.95^{b}	26.01 ± 1.81^{a}
Fat	13.23 ± 1.69^{b}	15.28 ± 0.07^{a}
Lactic acidity	0.44 ± 0.06^{b}	0.55 ± 0.01^{a}
FFA	7.05 ± 0.08^{a}	1.42 ± 0.01^{b}
Ash	4.22 ± 0.01^{b}	5.02 ± 0.02^{a}

The Cheese 1 had a lower protein content (20.58%) compared to Cheese 2 obtained using purified enzymes with 26.01% as protein percentage. Lower protein recorded in Cheese 1 might be because of the conversion of protein into soluble nitrogen and henceforth, loss of some water soluble nitrogen from the degraded protein. As for the rest of parameters listed above the highest values were observed with the cheese produced using purified enzyme such as 5.49; 15.25%; 5.02%; 0.55% respectively for pH, fat, Ash and lactic acidity percentage. The lactic acidity percentage was recorded higher in cheese 2 opposite to low pH value obtained from cheese 1 can probably be attributed to the increase of proteolytic activity observed in purified enzyme compared to crude enzyme. The analyses of results also showed off that a significantly highest FFA content (7.03%) was observed with the cheese made prepared using crude enzyme. The moisture content (60.21%) obtained for the Cheese 1 samples were similar to the results obtained in previous studies in various type of breeds of cow's

milk. Indeed, the highest moisture content (60.21%) obtained during this study is very similar to that obtained by Aisso et al. (2013) and Kora, (2005). The lowest moisture content (51.25%) obtained in Cheese 2 samples were similar to that obtained by Sacramento (2008). Cheese with low water content has a high shelf life due to limiting the microbial growth. It could then be summarized that the cheese from purified enzyme can be preserved for a longer period of time due to its relatively low water content compared to the Cheese 1 which obtained using crude extract enzyme. The result of ash content (4.22% to 5.02%); are higher to that obtained by Kora, (2005); Sacramento, (2008) and Aisso et al. (2013) who found respectively 1.85%, 2.08% and 1.59% for the ash content in the cheese. The rate of ash was considered as mineral content in cheese, whereby it could conclude that the Cheese 2 had a higher mineral content than Cheese 2. The cheese from purified extract enzyme had the highest protein content respectively 26.01% which was also higher to that obtained by Aisso et al. (2013) and 12.56% obtained by Sacramento (2008). This increasing of protein rate observed might be related to the enzyme treatment using the precipitation technique which allowed the transfer of precipitated protein into the cheese and also the raw milk used for cheeses preparation. It was also observed that the protein content in milk was lower than the protein content in both of cheese samples which could be attributed to the concentration during coagulation. The better yield in cheese depends on casein content in milk, Abakar (2012). The yields in term of weight observed in both of cheese were higher than the results obtained in previous studies but for the Cheese 2 which made using purified enzyme, its high yield might be related to the better coagulation environment that the precipitated salt created for the protein present in the cheese. The cheese with high protein could be useful for the population living in certain areas where it was very difficult to access to food with considerable protein content. There was no significant difference between fat content, however the highest fat content (15.28%) was observed with Cheese 2 which produced using purified extract enzyme. Its fat value was greater than the results found in the previous studies from various breed such as Ayrshire‘s milk (13.67%) and Friesian‘s milk (12.81%), while the fat content (12.93%) of cheese obtained from crude extract enzyme was similar to the value obtained by Mazou (2011) which was 11.44%. It was important to emphasize that the cheeses in these previous studies were produced using the latex of

Calotropis procera which had a high proteolytic and milk clotting activities while the stems were used in this study.

The results of approximate analyses brought out that the cheese produced with crude enzyme had highest FFA content (7.05%) while that recorded in Cheese 2 was 1.42%. The high FFA rate could be originated with natural fermentation due to possible contamination to set in through the action of lipolysis or due to presence of some lipolytic enzymes in unpurified extract.

4.2.2. Color determination

The results of color determination for both cheese samples showed that the color of the Wangashi sample were basically white in color because of their high L* values. However there was a significant different between L*, a* and b* values ($p < 0.05$) of the samples. The whiteness of cheese 2 is higher than the other cheese, which may be explained by the types of enzyme used for the cheese making. For the cheese, the crude extract was purified which made clear the coagulant from its initial color. The sample 1 (cheese 1) had a higher a* negative value (-1.10) when compared to cheese 2 value (Table 4.4). This increasing in a* value can be expressed by the direct transfer of high green color characteristic that the crude enzyme is responsible because this cheese was directly made using the crude enzyme while the Cheese 2 was made from the purified extract where the greenness was reduced by the purification method used and which also reduced the green color of the cheese sample.

Table 4.4 Color parameters of two types of Wangashi cheese

Parameters	Cheese 1	Cheese 2
Color L*	74.85 ± 0.30^{b}	78.87 ± 0.40^{a}
Color a*	-1.10 ± 0.60^{a}	-1.46 ± 0.05^{b}
Color b*	14.99 ± 0.22^{b}	15.96 ± 0.26^{a}

This basic decontamination method (ammonium sulfate precipitation) not just brought about the powerful expulsion of the halfway proteases and shaded materials which existing in the rough concentrate (Barros et al. 2001) yet it likewise focused the protein

arrangement to a serviceable volume that could be utilized for enzyme characterization and cheese making. However, for Cheese 2, b* value (15.96) is slightly higher than that observed with the Cheese 1. This slightly different could be explained by the fact that the salt was used for the enzyme precipitation, the colors of protein precipitated might be transferred to cheese.

4.2.3. Texture Profile Analysis

Textural properties and pH values in relation with storage time, temperature and types of cheese were evaluated. Table 4.5 shows the variation of pH according to temperature, time of storage, type of cheese and their interaction.

Table 4.5 pH of two types of Wangashi cheese at different storage time and temperature

T (°C)	Storage Time (d)	Cheese 1	Cheese 2
4	0	5.01 ± 0.42^{b}	5.49 ± 0.06^{a}
	7	6.40 ± 0.10^{b}	6.74 ± 0.01^{a}
	14	6.65 ± 0.01^{b}	6.86 ± 0.01^{a}
25	0	5.01 ± 0.42^{b}	5.49 ± 0.06^{a}
	7	6.41 ± 0.01^{b}	6.57 ± 0.01^{a}
	14	6.72 ± 0.01^{a}	6.55 ± 0.01^{b}

T is the storage temperature, Different capital letters indicate a statistical difference at a 0.05 level in each row.

Based on the data, there was a significant difference between pH values of cheese samples according to type of cheese, storage temperatures, and time ($P < 0.05$). For both of cheese types the pH values were the same in day 7 but in day 14, it was observed the decreasing of pH in Cheese 2. However there was no significant effect observed concerning temperature-time interaction ($p > 0.05$).

The collected data for hardness of both cheese samples are tabulated in Table 4.6. Hardness was expressed as the maximum peak force during the first compression in TPA. It was the force needed to compress to reach the first deformation. It was

observed that there was a significant effect of storage temperature on cheese hardness ($p < 0.05$).

Table 4.6 Hardness of two types of Wangashi cheese at different storage time and temperature

T (°C)	Storage Time (d)	Cheese 1	Cheese 2
4	0	271.32±11.84[b]	635.84±106.26[a]
	7	392.65±21.91[b]	706.37±25.21[a]
	14	486.71±318.93[b]	916.95±88.64[a]
25	0	271.32±11.84[b]	635.84±106.26[a]
	7	34440.22±995.67[a]	32985.88±6056.38[b]
	14	27454.84±5072.96[b]	34132.56±1050.26[a]

T is the storage temperature, Different capital letters indicate a statistical difference at a 0.05 level in each row.

It could be interpreted as the variation of storage temperature affected the both types of Cheese 1and 2. In contrary, it could also be noticed that there was no a significant effect of the types of cheese, time of storage and their interactions on cheese hardness ($p > 0.05$). However the data of hardness demonstrated that the hardness values of Cheese 2 were increased when time and temperature storage increased while at 25^{o}C in day 14 it was recorded a reducing of hardness in Cheese 1. The higher hardness cheese was recorded for Cheese 2.That difference might be justified by the high protein and low moisture content values recorded in the Cheese 2, which compared to those recorded in cheese 1. This finding is supported by the result of (Yuanrong et al., 2016). The observed low hardness values might be due to the higher rate of protein breakdown occurred in cheese. It could be seen also that the average hardness values were increased by approximately 98% when the temperature lift from initial temperature to 25^{o}C storage temperature.

Adhesiveness was expressed as the work required overcoming the attractive force binding the surface of food and which of other components with which the food takes contact. It is defined as the negative force area for the first bite. The adhesiveness

varied between Wangashi samples evaluated when initial temperature was took into account and also rose from 4 to 25ºC (Table 4.7).

Table 4.7 Adhesiveness of two types of Wangashi cheese at different storage time and temperature

T (°C)	Storage Time (d)	Cheese 1	Cheese 2
4	0	-5.75±1.32^{a}	-0.87±0.51^{b}
	7	**-0.28±0.00^{b}**	**-0.22±0.00^{a}**
	14	-0.48±0.00^{a}	-0.83±0.47^{b}
25	0	-5.75±1.32^{a}	-0.87±0.51^{b}
	7	0.00±0.00^{a}	-0.99±0.00^{b}
	14	-2.33±0.00^{b}	-0.14±0.00^{a}

T is the storage temperature, Different capital letters indicate a statistical difference at a 0.05 level in each row.

The finding results showed off that cheese type, time, temperature and their interactions had significantly affected on adhesiveness cheese ($P < 0.05$). The adhesiveness recorded in both of cheese in day 0 and 7 at 4ºC demonstrated that the adhesiveness in Cheese 2 was higher than that obtained from Cheese 1. It was observed that the adhesiveness increased in day 7 and decreased in day 14 at 4ºC for both of cheese sample, but an inverse reaction was recorded at 25ºC especially was observed in Cheese 2 when in day 7 at 25ºC the adhesiveness was decreased and increased in day 14 while for cheese adhesiveness was 0 in day 7 and decreased to -2.33 in day 14 at 25ºC. However it could be observed that the initial adhesiveness value in Cheese 2 is higher that recorded in Cheese 1. That difference in increasing data observed may be attributed to the use of salt to precipitate the crude enzyme applied in the production of cheese 2. These finding are supported by El-Bakry et al., 2011 who demonstrated that when using potassium salts in cheese making process was found to increase the adhesiveness also, and this may be lead to the increasing in fat globule size and pH value. Yuanrong et al. (2016) reported that when the temperature rose the structure of the full-fat cheese changed and the fat turned soft and increased the adhesiveness whereas the fat of the low-fat cheese was blocked and that reduced the adhesiveness. In the case of our study the cheese 2 had a high fat content when compared to Cheese

1 what may be caused an increasing adhesiveness. In term of cheese consumption, suitable adhesiveness was perceived as a good taste and flavor discharging. However the negative effect of an excessive adhesiveness was reported by (Juan et al., 2007).

The springiness of two type of cheese were collected and presented in Table 4.8. Related to sensory analyses, it is defined as the height recovers during the time elapses between the end and the first bite and the start of the second bite. It is also known as elasticity in term of return of sample to its original form after compression occurred between tongue and hard palate. The results collected showed that temperature, time, cheese type and their interactions ($p < 0.05$), excepted cheese-temperature-time interaction ($p > 0.05$) had significantly affected the springiness of chesses.

Table 4.8 Springiness of two types of Wangashi cheese at different storage time and temperature

T (°C)	Storage Time (d)	Cheese 1	Cheese 2
4	0	0.87 ± 0.03^{b}	0.94 ± 0.03^{a}
	7	0.85 ± 0.02^{b}	0.91 ± 0.00^{a}
	14	0.41 ± 00^{b}	0.59 ± 0.09^{a}
25	0	0.87 ± 0.03^{b}	0.94 ± 0.03^{a}
	7	0.84 ± 0.05^{b}	0.91 ± 0.04^{a}
	14	0.14 ± 0.00^{b}	0.45 ± 0.02^{a}

T is the storage temperature, Different capital letters indicate a statistical difference at a 0.05 level in each row.

In previous studies, no change of springiness was observed in cheddar cheese made with high calcium when temperature was increased from 4 to 25°C (Yuanrong et al., 2016). In this study, it was noticed that springiness for both types of cheese samples were decreased when temperature and time rose from 4 to 25°C Day 0 to Day 14. However the springiness recorded data in cheese 2 were found higher than those collected in cheese. That difference might be explained by the less cross-linking and more proteolysis in the treatment.

The cohesiveness is defined as the ratio of positive force area during the second compression to that during the first compression. The results showed that there was

not a significant difference according to type of cheese, temperature and their interactions ($p > 0.05$), except the storage time and temperature-time interaction which had significantly affected the cohesiveness of cheese ($p < 0.05$) (Table 4.9). It was found that the recorded values in cheese 2 were higher than those collected in cheese 1. These values in case of both cheese increased during the storage time excepted the day 7 at 25°C that it was observed a decreasing of cohesiveness in both of type of cheese when compared to the cohesiveness obtained in day 0, but an increasing in day 14 at same temperature which even exceed the initial values recorded.

Table 4.9 Cohesiveness of two types of Wangashi cheese at different storage time and temperature

T (°C)	Storage Time (d)	Cheese 1	Cheese 2
4	0	0.76±0.02^{b}	0.81±0.00^{a}
	7	0.79±0.01^{b}	0.80±0.01^{a}
	14	0.80±0.06^{b}	0.81±0.01^{a}
25	0	0.76±0.02^{b}	0.81±0.00^{a}
	7	0.75±0.06^{a}	0.74±0.07^{b}
	14	0.79±0.06^{b}	0.87±0.00^{a}

T is the storage temperature, Different capital letters indicate a statistical difference at a 0.05 level in each row.

The chewiness is referred to outcome of hardness, springiness and cohesiveness. Thus it may be influenced by any change of the aspects. The results collected were presented in Table 4.10. A part of type of cheese and some interaction that had not affected the chewiness values; there was a significant effect according time, temperature and time-temperature interaction on chewiness cheese. It was observed that in day 14 at both temperature 4 and 25°C the chewiness cheese were decreased whereas in day 7 at 4 and 25°C the values were increased almost by 99.16%. However the chewiness in cheese 2 were found higher than those collected from cheese 1 by 1.26%. The obtained chewiness for the cheese samples were also higher than recorded by other authors (Kumar et al., 2011) who studied at similar condition but on cheddar cheese. This difference may be justified by the composition of milk used for the cheese

manufacturing and also the pre-treatment applied for the enzyme which involved in the production process.

Table 4.10 Chewiness of two types of Wangashi cheese at different storage time and temperature

T (°C)	Storage Time (d)	Cheese 1	Cheese 2
4	0	180495.20±8827.88^{b}	481386.73±91153.83^{a}
	7	291753.30±5337.58^{b}	530420.87±26384.50^{a}
	14	3419.85±0.00^{a}	2807.93±154.15^{b}
25	0	180495.20±8827.88^{b}	481386.73±91153.83^{a}
	7	22193978.67±3946644.61^{a}	21914872.33±5106649.85^{b}
	14	3422.2510±0.00^{a}	23101±0.00^{b}

T is the storage temperature, Different capital letters indicate a statistical difference at a 0.05

Simoes et al. (2013) reported that chewiness value decreased when decreasing in the concentration of added cow milk. The increase and the decrease observed in the collected chewiness values at 25^{o}C for both of two types of cheese might be attributed to some denaturation occurred during storage time.

Resilience, in term of description of elasticity, it shared some link of similarity with springiness. The previous was a proportion of capacity that the twisted cheese came back to unique position after expulsion of power quickly, while the last was a proportion of capacity that the disfigured cheese came back to the underlying position after evacuation of power gradually. The resilience result of both of type of cheese was presented in (Table 4.11). These results showed that only the storage time had a significant effect on resilience cheeses as for the rest such as chees type, storage temperature and their interaction there was no significant effect ($P > 0.05$). (Yuanrong et al., 2016) stated the decreasing in all variety of cheese tested of resilience. However in this study the recorded resilience values were increased for both of cheese in day 7 and day 14 at both storage temperature. It was observed that the resilience in cheese 2 were higher than those recorded in cheese 1. That difference may be conclude that cheese had developed more closely elastic recovery compared to cheese 1 (Table 4.11).

Base on the results of different texture parameters tested for two type of Wangashi cheese, it can be concluded that the texture of cheese 2 was improved using the purified enzyme.

Table 4.11 Resilience of two types of Wangashi cheese at different storage time and temperature

T (°C)	Storage Time (d)	Cheese 1	Cheese 2
4	0	$0.38\pm0.02_b$	0.44 ± 0.01^a
	7	0.42 ± 0.02^b	0.45 ± 0.03^a
	14	0.75 ± 0.10^b	0.87 ± 0.01^a
25	0	0.38 ± 0.02^b	0.44 ± 0.01^a
	7	0.50 ± 0.07^b	0.51 ± 0.09^a
	14	0.79 ± 0.05^b	0.82 ± 0.00^a

T is the storage temperature, Different capital letters indicate a statistical difference at a 0.05 level in each row.

4.2.3. Thermal Properties

The thermal properties of both types of Wangashi cheese were performed through DSC and TGA. During these analyses, samples were subjected to a temperature program, in which they were heated according to the range of these temperatures to determine thermal effect which are materialized by enthalpy change and temperature range expressed in term of melting ability, crystallization, weight loss and some chemical reactions.

Differential Scanning Calorimeter (DSC) was applied to characterize the protein stability of the Wangashi cheese samples at temperature ranging between 0-200^{o}C. That characterization was performed in endotherm where melting of cheeses and protein denaturation where displayed. Figure 4.2 and 4.3 show typical DSC melting curves of fresh wangachi cheeses (made with crude extract and purified enzymes). The results showed that the both of cheese had a high end medium temperature melting regions at the difference that the medium temperature melting region of Cheese 2 was separated by a high valley whereas no valley a small one was observed with Cheese 1. The Cheese 1 sample had a temperature melting region from about 8^{o}C to 90^{o}C without

a peak. It also observed that a large melting region at about 123^{o}C to 198.61^{o}C, followed by a medium temperature melting region, mentioned by a big peak at about 117.0^{o}C which not developed any shoulder. It could be seen a small inflection point about 108^{o}C which separated the medium and high temperature melting. The temperature melting region in Cheese 2 was appeared at about 11^{o}C to 147^{o}C with a peak around 165.35^{o}C. It was noticed a similarity between the medium temperatures regions of both of cheese sample described by two shoulders observed between temperature 0-50^{o}C. The second region after high peak started about 175^{o}C and end at about 198.65^{o}C. The heat of fusion for Cheese1 and Cheese 2 was respectively -898.54 J/g and -217.56 J/g. Their onset and end peak temperatures also were respectively 114.46^{o}C and 122.08^{o}C; 163.09^{o}C and 169.79^{o}C. According to the graphs, a significant difference was observed between types of the cheese based on the peak temperature values, which represents the height melting fraction. The high value was recorded for Cheese 2 and also the area value of -1936.352 mJ when compared to Cheese 1 with -88.6.935 mJ as a total area value. These values might probably be attributed to the melting capacity of Cheese 2 and its non-rapid crystallization compared to Cheese 1. When protein is exposed to increasing temperature, losses of solubility or enzymatic activity occurs over a fairly narrow range. As peak temperature was increased in Cheese 2, a number of bonds in the protein molecule were weakened. It might be occurred due to a damage of the long range interactions that was necessary for the tertiary structure.

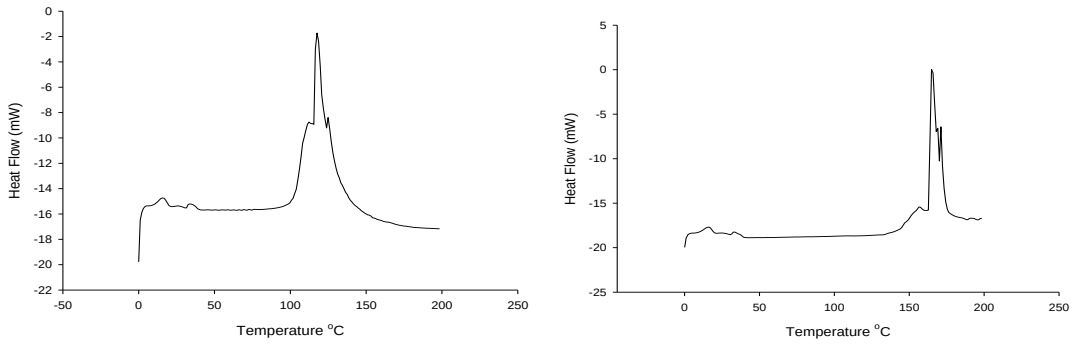

Figure 4.2 DSC curve showing milliwatts (mW) of endothermic heat flow vs. temperature of Wangashi cheese 1 and 2

Once these bonds were first weakened and were broken, the protein obtained would have more flexible structure.

Thermogravimetric analyses was performed to evaluate change in water binding in Wangashi cheese samples. The evaluation of the thermogravimetric curves, and their first appearance in term of derivative demonstrated three scales of weight loss mentioned by the valleys observed on the both TG (weight %) curves (Figure 4.3 and 4.4). It was observed that the presence of water over was about 30-190oC. Approximately this presence of range of water has been reported by Curtis et al. (1999) through means of IR analysis toward the first step of weight loss. The third was attributed to the sample decomposition and the liberation of their pyrolysis product. The free water is corresponded to the first valley thermogravimetric while the second step corresponded the bound water. The first derivative of the weight loss curve is responsible of the interpretation of this process. It was observed from the curves the significant difference between the first peaks which express the behavior of water. The first peak (83.74oC) for Cheese 1 was obtained higher than that recorded in Cheese 2 (78.28oC) which confirmed that the moisture content in Cheese 1 was high compared to Cheese 2.

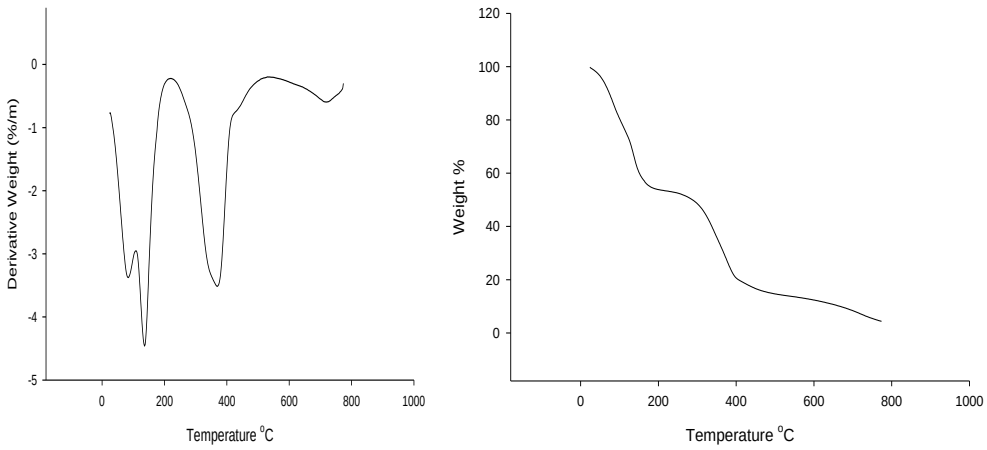

Figure 4.3 Weight-loss and Derivative curves of Wangashi Cheese 1.

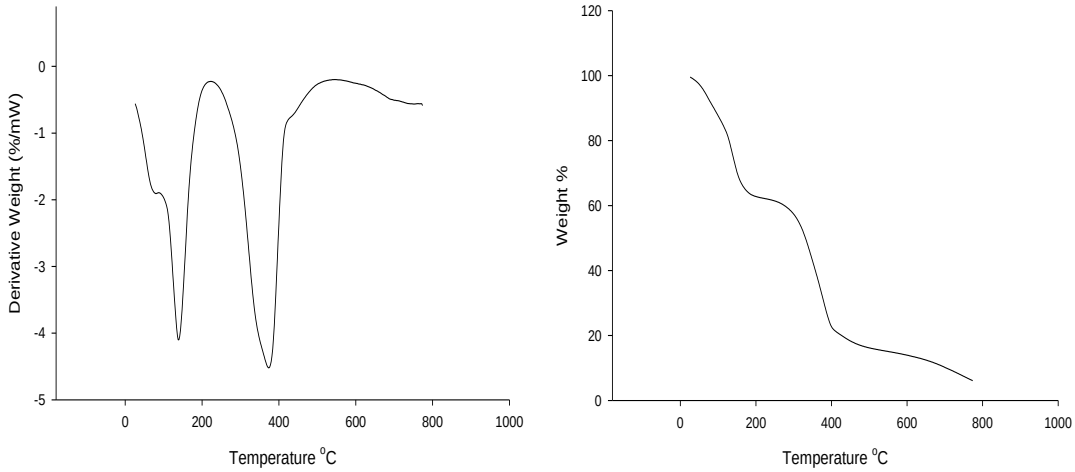

Figure 4.4 Weight-loss and Derivative curves of Wangashi Cheese 2.

The second and third peaks could probably be interpreted respectively as fat and mineral components behaviors. The high two other peak temperatures results was observed in Cheese 2 which were respectively 138.44^{o}C and 373.37^{o}C when compared to those (136.44^{o}C and 369.74^{o}C) collected from Cheese 1. The results were consistent with those obtained by chemical analysis which showed a slightly significant between fat and ash content in cheeses samples.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

Following the main objective of this study, which was to standardize and characterize Wangashi cheese. Wangashi cheese step of production was settled and its chemical, textural parameters were characterized; from the results carried out during this assessment, it could be summarized:

1- Two different coagulants were used to produce Wangashi cheeses; raw crude extract and purified crude extract of *Calotropis procera*. It was found that the purified crude extract had a higher milk clothing, enzymatic and specific activities than raw crude extract of *Calotropis procera*.

2- The low moisture content (43%) was recorded in cheese made with the purified enzyme while the high value observed in cheese produced with the non-treated extract of Calotropis procera. This finding demonstrated that the treatment of crude extract of *Calotropis procera* reduced the moisture content from 21.83% in Wangashi cheese production.

3- The high protein content which is a main characteristic looking by consumers of Wangashi cheese was recorded in cheese produced using purified crude extract of *Calotropis procera*. FFA high value was observed in cheese prepared with the non-treated crude extract of *Calotropis procera* while the low was recorded in cheese obtained using purified crude extract of *Calotropis procera*. The formation of high free fatty acid was possibly due to presence of some lipolytic enzymes in unpurified extract. These results showed that the use of treated crude extract of *Calotropis* improved the quality nutritional of Wangashi cheese.

4- It was observed that there was difference in color parameters between the two types of Wangashi cheese produced. The higher L* (lightness) and lower a* (greenness) were recorded in Cheese 1. The results showed that the use of purified extract crude from *Calotropis procera* increased the whiteness and decreased the greenness color in Wangashi cheese.

5- The differences according the textural parameters tested in both types of Wangashi cheese were observed when various times and different storage temperatures were applied. The high values were recorded in Cheese 2, produced using purified crude extract from Calotropis procera. These results demonstrated once again that the setting of step of production and the use of treated crude extract of Calotropis improved the textural parameters of Wangashi cheese.

The higher proteolytic activity was responsible for bitterness taste in Wangashi cheese, no matter purified or crude extract used. However a higher proteolytic activity recorded in purified enzyme might be due to the extraction method used and also the purification technique. As for a slightly high value of b* (yellowness) was recorded in cheese made using the purified crude extract of *Calotropis procera* compared to value recorded in the other cheese, might also be related to the purification technique which should be improved or others method should be applied in the future studies in order to understand more clear the effect of milk clotting and proteolytic activities of this cheese coagulant. On other hand, as a recommendation, a packaging technique should be designed to increase the shelf life of Wagashi cheese which has limited shelf life and due to high nutritional value it is important to keep it edible.

REFERENCES

Adambi, B. Z. F., Alkoiret, T. I., Toleba, S. S., Ahissou, A., Torre, F. Z., Yacoubou, A. M., Bonou, G. A., Dotche, I. O., Akpaki, V., Youssao A. K. I. (2018). Reproductive Performances of the Borgou cow Inseminated on Natural or Induced estrus with Semen from Gir and Girolando at the Okpara Breeding Farm, *Veterinary World*, **11(5)**: 693-699.

Adetunji, V. O., Babalobi, O. O. (2011). Comparative Assessment of the Nutritional contents of 'Wara' A West African soft cheese using Calotropis procera and Cymbopogon citrates as coagulants. *African Journal of food, Agriculture, Nutrition and Development*, **41,** 331–336

Adegoke, G. O. (2006). Effect of Local Preservative (Aframomum danielli) on the Chemical and Sensory properties of Stored warakanshi. *African Journal of Agricultural Research*, **1(1)**, 010-016.

Agossou, D. J., Toukourou, Y., Koluman, N. (2017a). Livestock Resources and Meat Sector in Republic of Benin*: Perspectives for a Sustainable Development. International Congress of the New Approaches and Technologies for Sustainable Development*. 21-24.

Agossou, D. J., Dougba, T. D., Koluman, N. (2017b). Recent Developments in Goat Farming and Perspectives for a Sustainable Production in Western Africa. *International Journal of Environment, Agriculture and Biotechnology* **2(4)**: 2047-2051.

Ahmed, I. A. M., Morishima, I., Babiker, E. E., Mori, N. (2009). Characterization of partially purified milk-clotting enzyme from Solanum dubium Fresen seeds. *Food Chemistry* **116**:395–400.

Ahmed I. A. M., Babiker, E. E., Mori, N. (2010). pH stability and influence of salts on activity of a milk-clotting enzyme from Solanum dubium seeds and its enzymatic action on bovine caseins. *LWT Food Science Technology* **43**:759–764

AFNOR, (2014). Lait Détermination de la teneur en matière Grasse Méthode acido-butyrique. *Norme NF* -**04**-210.

Aurand, L. W., Woods, A. E. (1973). *Food Chemistry*. AVI Publishing Company, Connecticut. 76-77.

Aïsso, R. C., Aïssi, M. V., Youssao, A. I., Soumanou, M. M. (2016). Caractéristiques physico-chimiques du fromage Peulh produit dans les conditions optimales de coagulation à partir du lait de deux races de vaches du Bénin. *Nature & Technology*, **(14)**, 33.

Aurand, L. W., Woods, A. E., Wells, M. R. (1987). *Food Composition and Analysis*. AVI Books. Van Nostrand Reinhold. New York. 76-77.

Alkoiret, T. I., Radji, M., Babatoundé, S. (2011). *Typologie des élevages bovins installés dans la commune de Ouaké au nord-ouest du Bénin*. Livestock Research for Rural Development, 23: 3.

Ashaye, O. A., Taiwo, O. O., Adegoke, G. O. (2006). Effect of local preservative (Aframomum danielli) on the chemical and sensory properties of stored warakanshi. *African Journal of Agricultural Research*, **1 (1)**. 010-016.

AOAC International, Official Methods of Analysis of AOAC International, AOAC International, 2005.

Association of Official Analytical Chemists (AOAC) (2006). *Official Methods of the Association of Official Analytical Chemists, Association of Official Analytical Chemists*, Inc., Virginia, USA. 69-84.

Aworh, O. C. (2008). *The Role of Traditional Food Processing Technologies In National Development: the West African Experience*. Chapter 3 from Using Food Science and Technology to Improve Nutrition and Promote National Development, Robertson, G.L. & Lupien, J.R. (Eds), © International Union of Food Science & Technology

Aworh, O. C., Muller, H. G. (1987). “Cheese making properties of vegetable rennet from Sodom apple (*Calotropis procera*)”, *Food Chemistry journal*, 26. 71-79

Aworh, O. C., Egounlety, M. (1985). Preservation of West African soft cheese by chemical treatment. J. *Dairy Res*, **52**, 189-195.

Banks, J. M., Banks W., Muir D. D., Wilson A. G., (1981). Cheese yield: composition does matter. *Dairy Ind. Int*. **46 (5)**, 15, 17, 19, 21-22.

Barros, R. M., Ferreira, C. A., Silva, S. V., Malcata, F. X. (2001). Quantitative studies on the enzymatic hydrolysis of milk proteins brought about by cardosins precipitated by ammonium sulfate. *Enzyme Microb Technology* **29**:541–547

Bayliss, R., Stewart, M. (2002). NTF2 from rat, ammonium sulphate conditions.

Basosidik, S., Pankaew, P., Hoonnivathana, E., Limsuwan, P., Naemchanthara, K. (2012). Characterization of Chitin Extracted from Waste Sources via XRD, FTIR, and TGA Techniques. *Advanced Materials Research*, **488-489**, 1404-1408.

Benslama, A. (2016). Le lait et Le lactosérum Université Mohamed Khider-Biskra Faculté des sciences exactes et des sciences de la nature et de la vie Département des sciences de la nature et de la vie.

Benyahia-Krid, F. A., Aissaoui-Zitoun, O., Boughellout, H., Adoui, F., Harkati, A., Bakou, C. R., Zidoune, M. N. (2016). Fresh Cheese “Peulh Type”: Characterization and Sensory Aspects. *J Adv Dairy Res*, **4(163)**, 2.

Belewu, M. A (2001). Nutritional and rheological evaluation of West African soft cheese made from plant coagulant (Calotropis procera) during storage. *J. Food Technol. Afr*. **6(3)**: 93-95.

Blowey, R. (2010). Digital dermatitis ‘mastitis of the foot’: A fresh concept in control. *Livestock*, **15(6)**, 27-30.

Bruno, M. A., Lazza, C. M., Errasti, M. E, López, L. M. I., Caffini, N. O., Pardo, M. F. (2010). Milk clotting and proteolytic activity of an enzyme preparation from Bromelia hieronymi fruits. *LWT Food Sci Technol* **43**:695–701.

Brutti, C. B., Pardo, M. F., Caffini, N. O., Natalucci, C. L., (2012). Onopordum acanthium L. (Asteraceae) flowers as coagulating agent for cheese making. *LWT Food Sci Technol* **45**:172–179

Camara-Artigas, A. (2018). Lysozyme crystallized in presence of 100 mM ammonium sulphate at pH 4.5.

Codex Alimentarius cheese standard 283-(1978). http://www.codexalimentarius.net. Accessed 15.03.2019.

Coker, C., Craig, H., Keith, J., Lawrie, C. (2005). *Food Science Section and Cheese and Milk fat Technology Section*, New Zealand Dairy Research Institute

Commission Internationale de l'Eclairage (1986). *Colorimetry, 2nd edn, Bulletin No. 15.2, Commission Internationale de l'Eclairage (CIE),* Central Bureau Kegel gasse 27A-1030, Vienna.

Chikpah, S. K., Teye, G. A., Teye, M., Mawuli, F. F. (2014). Effects of different concentrations of fresh and dried Calotropis procera (Sodom apple) extract on cow milk coagulating time, cheese yield and organoleptic properties of West African soft cheese (Wagashie). *European Scientific Journal, ESJ,* **10(27)**.

De Angelis Curtis, S., Curini, R., D'Ascenzo, F., Sagone, F., Fachin, S., Bocca, A. (1999). Use of thermo-analytical techniques to study the repening of Grana Padano cheese. *Food Chemistry***, 66(3)**, 375-380.

Dossou, J., Adote, S. (2006). Fiche technique de production et transformation du lait frais en fromage peulh au Bénin DNSA. FSA/UAC.; 33p.

Douglas, K. M. (2007). Milk as a white fluid secreted by the mammary glands of female mammals for the nourishment of the young. *British Jour. Butr. Res*, **11**, 885-93.

Doko, A. S., Gbégo Tossa I., Tobada P., Mama Yari H., Lokossou R., Tchobo A., Alkoiret, T. I., 2012. *Productive and reproductive performance of dairy cattle Girolando at Kpinnou ranch in southwest of Benin*. Bulletin de la Recherche Agronomique du Bénin, 2012: 35-47.

Dufosse, L., Galaup, P., Carlet, E., Flamin, C., Valla, A. (2005). Spectrocolorimetry in the CIE L*a*b* colour space as useful tool for monitoring the ripening process and the quality of PDO red-smear soft cheeses. *Food Research International*, **38**, 919–924.

Egito, A. S., Girardet, J. M., Laguna, L. E, Poirson, C., Mollé, D., Miclo, L., Humbert, G., Gaillard, J. L. (2007). Milk Clotting Activity of Enzyme Extracts from Sunflower and Albizia seeds and specific hydrolysis of bovine κ-casein. *International Dairy Journal* **17**:816–825

Egounléty, M., Edema, M., Yehouessi, B., Ahouansou, E. A. (1994). *Production et qualité du fromage Peulh (waragashi) en République du Bénin, Université Nationale du Bénin, DNSA*. Rapport de Recherche, Abomey-Calavi, 30-33.

El-Bakry, M., Duggan, E., O'Riordan, E. D., O'Sullivan. M. (2011). Effect of cation, sodium or potassium, on casein hydration and fat emulsification during imitation cheese manufacture and post-manufacture functionality, *LWT Food Science and Technology*, vol. **44(10)**, 2012–2018.

FAO, (2017). Food outlook biannual report on global food markets. FAO Intl.: www.fao.org.tr, 17.06.2019.

Food and Drugs Administration (FDA) (1998). *Milk. In: Code of Federal Regulations. Title 21. Section 131.110. U.S*. Govt. Printing Office. Washington.

Fellows, P. (2014). *Food Processing Technology*, Woodhead Publishing, Cambridge, UK.

Foltmann, B. F. (1992). Chymosin: a short review on foetal and neonatal gastric proteases. *Scandinavian Journal of Clinical and Laboratory Investigations*. **52**, 65–79.

Fox, P. F. (1989). Proteolysis during cheese manufacture and ripening. *Journal Dairy Science* **72**:1379–1400

Fox, P. F., McSweeney, P. L. H., Cogan, T. M., Guinee, T. P. (2004). *Cheese: Chemistry, Physics and Microbiology*, Elsevier.

Fox, P., Mcsweeney, P. (2004). Cheese: *An Overview. Cheese: Chemistry, Physics and Microbiology General Aspects*, 1-18.

Fox, P. F., T. P., Guinee, T. M., Cogan P. L. H., McSweeney. (2000). *Cheese rheology and texture, in: Fundamentals of cheese science* (Fox P.F. et al., editor: Aspen Publisher Inc,).

Fox, P. F. (2003). Milk proteins: general and historical aspects. In: Fox, P.F., McSweeney, P.L.H. (Eds.), *Advanced Dairy Chemistry*, Vol. **1**, Proteins Part A, (3rd Edn.). Kluwer/Plenum, New York, 1-48.

Fox, P. F., Guinee, T. P., Cogan, T. M., McSweeney, P. L. (2017). *Fundamentals of cheese science*. Boston, MA, USA: Springer. 185-229

Gaviln, C. (2010). Turbulence, Vibrations, Noise and Fluid Instabilities. Practical Approach. Computational Fluid Dynamics.

Garg S. K., Johri, B. N., (1994). Rennet: current trends and future research. *Food Review International*, **10**, 313-355.

Gonzalez-Rabade, N., Badillo-Corona, J. A., Aranda-Barradas, J. S., Oliver-Salvador, M. C. (2011). Production of plant proteases in vivo and in vitro a review. *Biotechnol Advanced* **29**:983–996.

Guinee, T. M., Wilkinson, M. G. (1992). Rennet coagulation and coagulants in cheese manufacture. *Journal of the Society of Dairy Technology*, **45**, 94–104.

Gupta, C. B., Eskin, N. A. (1997). Potential use of vegetable rennet in the production of cheese, *Food Technology*, **5,** 62-66.

HACCP and ISO 22000. (2009). www.haccp.org.tr, 17.06.2019

Harboe, M. K. (1992). Chymogen, chymosin rennet manufactured by fermentation of Aspergillus niger. Fermentation-Produced Enzymes and Accelerated Ripening in Cheese making, *International Dairy Federation, Brussels*, **269**, 3-7

Harboe, M., Broe, M. L., Qvist, K. B. (2010). In: Law BA, Tamime AY (eds) *Technology of cheese making*. Wiley-Blackwell, London

Hervé, N. (2017). Insémination artificielle et l'embouche bovine pour un élevage rentable-AGROBENIN. In: Agrobenin.com. Accessed 31 Mar 2019.

Holba, P., Šesták, J. (2017). *The Role of Heat Transfer and Analysis Ensuing Heat Inertia in Thermal Measurements and Its Impact to Non-isothermal Kinetics*. Hot Topics in Thermal Analysis and Calorimetry Thermal Physics and Thermal Analysis, 319-344.

Holsinger, V. H. (1988). Lactose. *In: 'Fundamentals of Dairy Chemistry*. 3rd edn. Wong, N. P. Jenness, R. Keeney, M. and Marth, E. H. (Eds.) Van Nostrand Reinhold. New York. 63-65.

Ihekoronye, A. I., Ngoddy, P. O. (1985). Integrated food science and technology for the tropics. Macmillan.

Institut National de la Statistique et de l'Analyse Economique (INSAE), (2015). RGPH4: Que Retenir Des Effectifs De Population En 2013? (Report in french).

Jacob, M., Jaros, D., Rohm, H. (2011). Recent advances in milk clotting activity. *International Journal of Dairy Technology*, **64**, 14–33.

Jenness, R. (1988). Composition of milk. *In Fundamentals of dairy chemistry*. Springer, Boston, MA. 1-38

Jelinski, T., Du, C. J., Sun, D. W., Fornal, J. (2007) Inspection of the distribution and amount of ingredients in pasteurised cheese by computer vision. *Journal of Food Engineering*, **83**, 3–9.

Juan, B., Trujillo, A. J., Guamis, V., Buffa, M., Ferragut, V. (2007). Rheological, textural and sensory characteristics of high pressure treated semi-hard ewes' milk cheese, *International Dairy Journal*, **17(3)**, 248–254.

Kassa, S. K., Ahounou, G. S., Dayo, G. K., Salifou, C. F. A., Dotché, O. I., Issifou, T. M., Youssao, A. I. (2016). Évaluation et modélisation de la production de lait des vaches Girolando, Borgou, Lagunaire et croisées Azawak× Lagunaire, élevées dans le système semiamélioré au Bénin. *Journal of Applied Biosciences*, **103(1)**, 9829-9840.

Kaya, S., Oner, M. D. (1996). Water activity and moisture sorption isotherms activity of Gaziantep cheese. *Journal of Food Quality* **19**: 121-132.

Kaya, S. (2002). Effect of salt on hardness and whiteness of Gaziantep cheese during short-term bringing. *Journal of Food Engineering* **55**: 155-159.

Keenan, T. W., Maher, I. H. (2006). Intracellular origin of milk fat globules and the nature of the milk fat globule membrane. *Advanced Dairy Chemistry*, **2**,137–171.

Kees, M. (1996). Le fromage Peulh: facile a produire et bien apprecie. Une technologie a vulgariser?

Kèkè, M., Yčhouénou, B., Dahouénon, E., Dossou, J., Sohounhloué, D. C. K. (2008). Contribution ā l'amélioration de la technologie de fabrication et de conservation du fromage peulh waragashi par injection de Lactobacillus plantarum. *Ann Sci Agronom Bénin*, **10(1)**, 73-86.

Kora, S. (2005). Contribution à l'amélioration de la technologie de production du fromage peulh au Bénin. These d'ingénieur agronome. Bénin: Université d'Abomey-Calavi.

Kumar, S., Kanawjia, S. K., Khatkar, S., (2011). Effect of rate of addition of starter culture on textural characteristics of buffalo milk Feta type cheese during ripening. *Journal of Food Science and Technology* (**on line**), 1-5.

Kumari, M., Sharma A., Jagannadham, M. V. (2012). Religiosin B, a milk-clotting serine protease from Ficus religiosa. *Food Chemistry* **131**:1295–1303.

Kirk, R. S. (1991). *Pearson composition and analysis of food*. Longman Scientific and Technical, Newyork, USA. pp537.

Law, A. B., Tamime, A.Y. (2010). *Technology of cheese making second edition*. John Wiley & Sons Ltd, the Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom.

Lincke, D., Hinkel, J. (2018). Economically robust protection against 21st century sea-level rise. *Global Environmental Change*, **51**, 67-73.

Lo Piero, A. R., Puglisi I., Petrone, G. (2002). Characterization of "Lettucine", a serine-like protease from Lactuca sativa leaves, as a novel enzyme for milk clotting. *Journal of Agricutural Food Chemistry*, **50**, 2439–2443

Lucey, J. A., Fox, P. F. (1992). Rennet coagulation properties of late-lactation milk: effect of pH adjustment, addition of CaCl2, variation in rennet level and blending with mid lactation milk. *Irish Journal of Agricultural and Food Research*, **31**, 173–184.

Kuta, F. A. (2008). Antifungal effect of Calotropis procera stem on Epidermo phytonflocosum. *African journal of biotechnology*.**7 (13)**; 2116-2118.

MacDougall, D. B. (2001). Principles of color measurement for food. *Instrumentation and Sensors for the Food Industry* (eds E. Kress-Rogers & C.J.B. Brimelow), pp. 63–84, Woodhead Publishing, Cambridge, UK.

Maiti, S. (2017). Nanoemulsification Technology in Improving Bioavailability of Lipophilic Functional Food-Grade Ingredients and Quality of Food Products. *Nanotechnology Applications in Food*, 203-221.

Maria de Lima, J. M., de Freitas, F. J. C., Amorim, R. N. L., Câmara, A. C. L., Batista, J. S., Soto-Blanco, B. (2011). Clinical and pathological effects of Calotropis procera exposure in sheep and rats. *Toxicon*, **57(1)**, 183-185.

Malomo O. F. Oduola O. Adekoyeni O. O and Jimoh M. O (2013) *International Journal of Food Chemistry*. **I (1)**, 01-13.

Mahami, T., Ocloo, F. C. K., Odonkor, S. T., Owulah, C., Coffie, S. A. (2012). Preliminary study on the influence of moringa seed extracts supplementation on the yield and quality of cottage cheese, **2(1)**: 4-8.

Martínez-Ruiz, N. R., López-Díaz, J. A., Rodrí- guez-Torres K. I. (2010). Physicochemical Characteristics and Sensory Properties of Asadero Cheese manufactured with Plant Coagulant from Solanum elaeagnifolium. *Food and Nutrition Sciences*, **4**, 75-81

Marilley, L., Casey, M. G. (2004). Flavors of cheese products: metabolic pathways. Analytical tools and identification of producing strains. *International Journal of Food Microbiology*, **90**: 139-159.

Mazou, M. (2011). Mise au point d‘un procédé de conservation du Waragashi par le froid; Mémoire d‘ingénieur de conception en Technologie Alimentaire; Ecole polytechnique d‘Abomey- Calavi, 80p.

Mazou, M., Tchobo, F. P., Degnon, R. G., Mensah, G. A., Mohamed, M. (2012). Effect of temperature and salt on the quality of waragashi cheese during storage in Benin Republic. *African Journal of Food Science*, **6(20)**, 494–499.

McCraig, T. N. (2002). Extending the use of visible/near-infrared reflectance spectrophotometers to measure colour of food and agricultural products. *Food Research International*, 731–736.

McSweeney, P. L. H. (2004). Overview of cheese ripening: introduction and overview. *Cheese: Chemistry, Physics and Microbiology, Volume 1: General Aspects* (eds P.F. Fox, P.L.H. McSweeney, T.M. Cogan and T.P. Guinee), 3rd edn, 348–360.

Muehlenkamp-Ulate, M. R., Warthesen, J. J. (1999). Evaluation of several non-starter lactobacilli for their influence on Cheddar cheese slurry. *Journal of Dairy Science*, **82**, 1370–1378.

Moumouni, I., Nouatin, G. S., Baco, M. N. (2011). Du système formation et visites au conseil à l'exploitation agricole familiale au Bénin: rupture ou continuité? *Cahiers Agricultures*, **20(5)**, 376-381.

Néstor G. M., Rubí C. G. D., Héctor J. C. (2012). Exploring the milk-clotting properties of a plant coagulant from the berries of S. elaeagnifoliun var. Cavanillies. *Journal Food Science* **71**:89–94

O'Connor C. B. (1993). Traditional cheese making manual. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia.

O'cansey, K. A. (2010). Development of cheese product from coconut milk. MSc. Thesis. Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Ogundiwin, J. O., Oke, O. L. (1983). Factors affecting the processing of wara Nigerian white cheese. *Food chemistry*, **11(1)**, 1-13.

Ortiz, R. (2017). Leveraging Agricultural Biodiversity for Crop Improvement and Food Security. Routledge Handbook of Agricultural Biodiversity, 285-297.

Padmanabhan, S., Chitre, A., Shastri, N. V. (1993). Milk clotting protease isolated from Dieffenbachia maculate. *Die Nahrung*, **37**, 99-101.

Pontual, E. V., Carvalho, B. E. A., Bezerra, R, S., Coelho, L.C.B.B., Napoleão, T. H., Paiva, P. M. G. (2012). Caseinolytic and milk-clotting activities from Moringa oleifera flowers. *Food Chemistry* **135**:1848–1854

Pozsar, H. K., Vamos, V. L., Nonn, S. H., Hegedues, V. E. (1969). Investigation of milk clotting enzymes from the point of view of cheese production -Selective inhibition of the proteolytic activity of plant proteases. *Elelmis zertudomany*, **3**, 55-70.

Rosenthal, A. J. (1999). Relation between instrumental and sensory measures of food texture. Food texture: Measurement and perception, 1-17.

Roseiro, L. B., Barbosa, M., Ames, J. M., Wilbey, R. A. (2003). Cheese making with vegetable coagulants—the use of Cynara L. for the production of ovine milk cheeses. *International Journal of Dairy Technology*, **56(2)**, 76-85.

Rudan, M. A., Barbano, D. M., Guo, M. R., Kindstedt, P. S. (1998). Effect of the modification of fat particle size by homogenization on composition, proteolysis, functionality, and appearance of reduced fat Mozzarella cheese. *Journal of Dairy Science*, **81(8)**, 2065–2076.

Sallami, L., Kheadr, E. E., Fliss, I., Vuillemard, C. J. (2004). Impact of autolytic, proteolytic, and nisin-producing adjunct cultures on biochemical and textural properties of Cheddar cheese. *Journal of Dairy Science*, 87, 1585–1594.

Simões, M. G., Rabelo, J. G., Portal R. E., Domingues, A. F. N., Oliveira, E. B., Fortes, F. C. L. L. (2013). Physicochemical properties of Butter cheese from Marajó manufactured with buffalo milk and cow milk. *IOSR Journal of Environmental Science, Toxicology and Food Technology,* **5(3)**, 2319-2402

Sessou, P., Farougou, S., Azokpota, P., Youssao, I., Yèhouenou, B., Ahounou, S., Sohounhloué, D. (2013). Endogenous methods for preservation of Wagashi, a Beninese traditional cheese. *African Journal of Agricultural Research,* **8(31)**, 4254-4261.

Sharma, P., Singh L., Gilbaghi, N. (2009). Optimization of process variables for decolorization of Disperse Yellow 211 by Bacillus subtilis using Box-Behnken design. *Journal of Harzadous materials*, **164 (2-3)**, 1024-1029.

Sousa, M. J, Malcata, F. X. (2002). Advances in the role of a plant coagulant (Cynara cardunculus) in vitro and during ripening of cheese from several milk species. *Lait* **82**:151–170

Sidrach, L., Garcia-Canova, F., Tudela, J., Rodríguez-López, J.N. (2005). Purification of cynarases fromtichoke (Cynara scolymus L.): enzymatic properties of cynarase A. *Phytochemistry*, **66**:41–49

Silva, S. V., Allmere T., Malcata F.X., Andrén A. (2003). Comparative studies on the gelling properties of cardosins extracted from Cynara cardunculus and chymosin on cow's skim milk. *International Dairy Journal* **13**:558–564.

Snyder, F. (n.d.). *International Food Safety Standards in WTO Case Law*. Food Safety Law in China, 334-393.

Tamer, I. M. (1993), "Identification and partial purification of a novel milk clotting enzyme from Onopordum turcicum", *Biotechnology Letters*, **15**, 427-432.

Vairo-Cavalli, S., Claver, S., Priolo, N., Natalucci, C. (2005). Extraction and partial characterization of a coagulant preparation from Silybum marianum flowers. Its action on bovine caseinate. *Journal Dairy Research* **72**:271–275

Vairo-Cavalli, S., Silva, S. V., Cimino, C., Malcata, F. X., Priolo, N. (2008). Hydrolysis of caprine and ovine milk proteins, brought about by aspartic peptidases from Silybum marianum flowers. *Food Chemistry* **106**:997–1003

Verma, R., Satsangi, G. P., Shrivastava, J. N. (2010). Ethno-Medicinal Profile of Different Plant Parts of Clotropis procera (AIt.) R. Br. *Ethnobotanical Leaflets*, **2010(7)**, 3.

Vissoh, D., Gbangboche, A. B., Padonou, E., (2015). The Alpine goat's milk production and cheese yield in Benin. *International Journal of Current Research*, **7**: 22108-22112.

Yuanrong Z., Zhenmin L., Beihong, M. (2016). Texture Profile Analysis of Sliced Cheese in relation to Chemical Composition and Storage Temperature. *Journal of Chemistry*, **2016**, 10

Zeng, R. S., Mallik, A. U., Luo, S. M. (Eds.). (2008). *Allelopathy in sustainable agriculture and forestry* 189-282. New York: Springer.

Zhou, W., Therdthai, N., Hui, Y. H. (2014). Introduction to Baking and Bakery Products. Bakery Products Science and Technology, 1-16.