NUTRITIONAL QUALITY AND SAFETY OF PROCESSED *WAGASHI* CHEESE USING *CALOTROPIS PROCERA* FOUND IN BENIN AND KENYA

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DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been submitted to any other college, institution or university for the award of a degree

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DEDICATION

I wish to dedicate this work to my late parents who GOD called to be with. My son Owen GANDONOU, my brothers and sisters, my cousin Stan DEKOUN, my dear companion Ingrid GANDONOU, members of TOSSOU, AKPOMEY, GANDONOU families and to all my friends.

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LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation	Description
CHO	Carbohydrate
DM	Dry Matter
FDA	Food and Drugs Administration
JICA	Japan Iternatonal Corporation Agency (JICA)
JKUAT	Jomo Kenyatta University of Agriculture & technology
PAUSTI	Pan African University Institute of Scienece Technology and Innovation
WFP	World Food Program

ABSTRACT

The Peulh cheese, locally known as *Wagashi*, is widespread and is mainly consumed in local West African communities including Benin. It is a form of cheese mainly made from cow's milk coagulated using fresh *Calotropis procera* plant extracts. The *C. procera* is a traditional medicinal plant found in many parts of the world. It is particularly utilized in West Africa in treatment of many ailments. Several studies have shown the usefulness of the plant. However, it has also been shown to contain toxic substances that pose health risks to humans. The present study investigated nutritional quality and safety of *Wagashi* cheese processed with milk and *Calotropis procera* obtained from Benin and Kenya. This study assessed the toxic substances contained in *Calotropis procera* leaves used for the processing of the milk into cheese. The aim was to obtain information necessary in improving nutritional and sanitary quality of *Wagashi* and to protect consumer health. The milk samples were collected from different cattle breeds and *C. procera* plants used in processing the cheese were obtained from Kenya and Benin. Milk was coagulated using *C. procera* samples from both countries. The resultant cheese and whey were subjected to biochemical analysis and phytochemical screening. The two *Calotropis procera* varieties collected in Benin and in Kenya were subjected to comparative qualitative and quantitative phytochemical analysis. The respective proximate analysis results for milk collected from Girolando breed in Mono and Borgou regions were 89.14% and 86.38% moisture content, 0.69% and 0.62% ash, 1.32% and 2.01% protein, 2.15% and 3.75% fat, 4.38% and 3.60% carbohydrate and 2.53% and 2.52% lactose. Proximate analysis for milk collected from Borgou breed in

Borgou and Collines regions were 81.78% and 86.57% moisture content, ash 0.63% and 0.71%, protein 1.73% and 2.24%, fat 7.5% and 3.95%, carbohydrate 5.38% and 4.96% and 2.37% and 2.83% lactose. The moisture content, ash, protein, fat, carbohydrate and lactose of milk samples collected from Lagunaire breed in Zou region were 84.19%, 0.67%, 2.44%, 5.10%, 6.122% 2.02% respectively. The respective moisture content, ash, protein, fat, carbohydrate and lactose of milk samples from Friesian and Ayrshire breeds collected in Kenya were 89.632% and 88.59%, 0.68% and 0.31% 2.33% and2.17%, 4.25% and 4.90% 3.14% and 4.43%, 2.47% and 1.84%. Biochemical analysis of all the cheese and whey obtained from different milk samples were similar. The *Calotropis procera* collected in Benin and Kenya has shown the presence of phytochemical such as Alkaloids, Flavonoids, Tannins, Cardiac glycosides, Phenols, Saponins, Steroids in both the stem and leaves. No phytochemicals was detected in the whey and cheese after qualitative analysis. However, flavonoids were found in the cheese after quantitative analysis. More results and the implication of the findings are discussed in details in the thesis. At the end of this studies we concluded that the cheese is still safe for consumption since the toxins (cardiac glycosides) were not detected in cheese. However more investigation need to be carry out on quantitative analysis.

Keys words: Milk, cheese, whey, biochemical, *Calotropis procera*, phytochemical analysis.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The supply of healthy and nutritious food is a main element of food security in Africa. Efforts are thus made to ensure that food and nutrition security is guaranteed. This can be achieved through increased production, appropriate processing and preservation. All these approaches are critical in ensuring food and nutrition security in Africa since the continent is characterised by within and between year food supply fluctuations mainly resulting from relying on rainfed agriculture to produce food. Milk like other agricultural commodities has periods of glut and periods of shortage and this could explain the popularity of cheese making in West Africa coupled with the need to satisfy protein needs in rural poor households. Cheese is a nutritious food and one of the numerous products from processing of milk of cows, goats, sheep, buffalos, camels and yaks. It is produced by coagulation of the milk protein known as casein (Scott, 1986; Akinloye et al., 2014). According to Fox *et al.* (2000), cheese is a product processed from milk by acidification and coagulation. The cheese is produced in a wide range of flavours, textures and forms by coagulation of the milk protein. Cheese production serves as a means of preserving essential nutrients in milk, and it has been identified as an excellent source of proteins, fat, minerals and vitamins (O'Conner, 1993; Chikpah et al., 2014). A little studied traditional cheese in Benin, "*Wagashi*", is prepared using cow milk and coagulation is stimulated with fresh *Calotropis procera* plant leaves extract. The plant is widely used in production of cheese in west Africa. According to the study done by Kareen *et al.* (2008), both the leaves and latex of *Calotropis procera* have bactericidal effects on pathogenic

microorganisms. However, the plant has also been shown to have some toxic compounds such as Calotropin, Uscharin, Calotoxin, which need to be identified and their fate following use in making the cheese determined.

Commercial milk coagulants exist but their cost is a limiting factor in poor rural households in Africa making cheap alternatives attractive. However care needs to be taken to ensure that the latter's use does not introduce health risks to the consumers. Use of *Calotropis procera* leaves in making fresh cheese from fresh milk was first reported among the Fulani pastoralists (Abakar, 2012).

1.2 Statement of the Problem

Global population increase has led to an increased demand for food and more specifically protein resulting from an increase in urbanisation, increased household incomes, improved living standards in developing countries. In the absence of response mechanisms to the increased demand for food, there is danger of starvation. For instance, a World Food Program (WFP) report (WFP, 2009) indicated that 12% (nearly one million people) of Benin population was food insecure and vulnerable to nutrition insecurity. Among the many approaches being used to ensure a supply of necessary quality and sufficient nutrients to the increased number of consumers include processing and preservation. During rainy seasons when there is good supply of milk from pastoral and agro-pastoral producers, there is oversupply of milk much of which goes to waste as result of lack of preservation approaches in these production systems more so in Kenya where cheese consumption is low. This is aimed at increasing the shelf life of highly perishable foods such as fresh milk. In good seasons (in periods of better than average rainfall) there is always excess

supply of milk in pastoral and agro-pastoral production systems in West Africa much of which would go to waste due to lack of refrigeration. The potential of producing cheese in West Africa is hampered by the high cost of commercial milk coagulants. This has resulted in use of traditional plant, *Calotropis procera,* but whose (phyto)chemical composition (toxic compounds such as Calotropin, Uscharin, Calotoxin) and its human health risk resulting from using it as a coagulant has not been studied. Previous study has shown that accidental exposure to the plant's latex produces dermatitis, keratitis and toxic iridocyclitis (Sehgal *et al.,* 2005).

1.3 Justification of the study

Fulani cheese "*Wagashi*" is one of Benin's most important livestock products. It represents a form of preservation of milk in the solid state and contains high proteins content. As a result, it can be used to irrigate various forms of malnutrition, particularly protein-energy malnutrition, which is common in developing countries. In Kenya domestic ruminant milk is one of the most accessible sources of protein. It plays an important role not only nutritionally, but also economically and socio-culturally. One of the simplest and most cost effective method of preservation is cheese processing. The Fulani cheese making has potential for use in the pastoral and agro-pastoral production systems in Africa as it uses plants (*Calotropis procera*) which grow in the dry areas where much of the production occurs. It is however critical to determine the fate of the toxic and non-toxic substances found in the plant to ensure that it is safe for use in processing of human food. Egounléty *et al.* (1994) observed that despite the fact that *Calotropis procera* contains some toxic substances (heterosides cardiac Oxic acid, especially calotropin), no case of food poisoning due

to the consumption of *Wagashi* cheese has been reported to date. This observation needs to be elucidated by scientific research which is what this study was set to address that is to assess the nutritional quality and safety of processed *Wagashi* cheeses made using milk and *Calotropis procera* plant collected from Benin and Kenya.

1.4 Objectives

1.4.1 General objective

This study aimed at assessing the nutritional quality and safety of *Wagashi* cheeses processed with milk from different cattle breeds and *Calotropis procera* plant types found in Benin and Kenya.

1.4.2 Specific objectives

The specific objectives of the study were:

i. To determine biochemical composition of milk from different cow breed in Benin and Kenya;
ii. To evaluate phytochemical composition of *Calotropis procera* collected from Benin and Kenya;
iii. To determine nutritional and textural characteristics and phytochemical composition of *Wagashi* cheeses processed with milk from different cattle breeds and *Calotropis procera* plant in Benin and Kenya.

1.5 Hypothesis

The hypotheses being tested were:

H_{a1}: Biochemical composition of milk vary according to cattle breed and country

H_{01}: *Calotropis procera* from Benin and Kenya has similar phytochemical profile;

H_{a1}: Nutritional and phytochemical characteristics of *Wagashi* depend on origin and composition of milk and *Calotropis procera* plant

1.6 Scope and significance of the study

The study is limited to milk and *Calotropis procera* plant collected from specific regions in Benin and Kenya. It is, however, assumed the findings are applicable to other regions where milk production occurs as well as that the phytochemical characteristics of *Calotropis procera* plant from one region to another does not vary. The implications of the findings would therefore be that *Calotropis procera* plant leaves' extract is usable and safe for production of cheese.

CHAPTER TWO: LITERATURE REVIEW

2.1 Milk

2.1.1 Milk definition

Milk is defined as lacteal secretion, practically free from colostrum obtained by the complete milking of healthy cows. Milk that is in the final form for beverage use should be pasteurized, and should not contain less than 8.25% milk solid –not – fat and not less than 3.25% milk fat (FDA, 1998, O'cansey, 2010). It can also be defined as a white fluid secreted by the mammary glands of female mammals for the nourishment of their young and consists of minute globules of fat suspended in a solution of casein, albumin, milk sugar and inorganic salts (Douglas, 2007).

2.1.2 Milk production

2.1.2.1. Milk production in the world

Domestic animal production has proven to be a good source of food all over the world, and a rapid growth in milk and dairy consumption has been seen in many developing countries over the last ten years (FAO, 2002). Milk is one of the oldest foods known to man (Nickerson, 1999) Milk is produced by all mammals; for human consumption, mainly by goats, sheep, cattle, buffaloes and camels and 90% of the milk consumed by humans is from dairy cattle (FAO, 1990). Milk (plant/animal) and its other various products form the major portion of food for infant and adult all over the universe. Total world milk production stood at 453,733 metric tonnes (MT) with

Europe producing the largest amount of 153,392 MT whilst Africa produced the least (Ocansey, 2010).

2.1.2.2 Milk production in Benin and Kenya

For a long time, in Africa, milk activity at the farm and the household level has been confined to a way of diversification or intensification in mixed farming systems or looked upon as the main social component of traditional pastoral systems (Alary *et al.,* 2007). Due to the difficult conservation of fresh cow's milk in developing countries, attempts of technological approaches were developed in order to transform it into value added products (Dossou *et al*., 2006).

2.1.2.2.1 Milk production in Benin

The total milk production in Benin is approximately 82.76 million litres per year, which translates 13 L/capita (DPP/MAEP, 2002). In Benin, the role of milk in the diet and economy of pastoral communities is well established. Milk contributes more than 50% to the annual incomes of Fulani households (Ogodja *et al.,* 1991; Dossou *et al*., 2006).2 The milk comes from a cattle herd estimated at 1,717,900 head in 2004 and an increase of an annual rate of about 3.6% (APRM, 2004). This herd is composed of lagoon breed bulls, Borgou and Somba (31%), M'bororo zebu, Goudali and white Fulani (7.7%), as well as their cross (61.3%) (Dossou *et al*., 2006). The Directorate of Livestock (DE) through the PAFILAV and livestock development projects initiated actions to improve the situation of cattle farming in Benin. These projects operate directly in rural areas. The model is presented in Kora (2005) and is practiced in Kpinnou state farms in Mono, Bétékoukou and Samiodji in Zou and

Okpara in Borgou. The table 1 bellow present result of proximate composition of milk from different breeds in Benin.

Table 1: Physico-chemical characteristics of milk from three different cattle breeds

	Type of breeds		
Parameters	**Borgou**	**Lagunaire**	**Girolando**
Density (g/cm3)	1.03	1.03	1.03
pH	6.60	6.62	6.57
Moisture (%)	80.73	83.64	87.58
Acidity (%lactic acid)	0.21	0.25	0.17
Proteins (% DM)	31.51	29.10	25.67
Lipids (% DM)	34.52	35.13	36.16
Carbohydrates (% DM)	32.82	33.73	32.41
Ash (% DM)	1.13	2.01	5.74

Source: (Kora, 2005)

The Girolando breed has the lowest protein value and the highest water content. This results in a lower cheese yield of 16 to18% against 20 to 25% for other breeds. On the other hand, the milk of this cow is of better microbiological quality that is to say contains less germs than that of the other breeds. This is due to the respect of the rules of hygiene of the trade in the case of this race (Dossou., *et al* 2006)

2.1.2.2.2 Milk production in Kenya

Kenya's dairy industry is dynamic and plays an important economic and nutrition role in the lives of many people ranging from farmers to milk hawkers, processors, and consumers. Kenya has one of the largest dairy industries in sub-Saharan Africa.

In Africa, Kenya is the only country, after South Africa that produces enough milk for both domestic consumption and export. According to Muriuki et al. (2004) the dairy industry is the single largest agricultural sub-sector in Kenya, larger even than tea.

Kenyans are amongst the highest milk consumers in the developing world, consuming an estimated at 145 litres per person per year, more than five times milk consumption in other East African countries (SDP, 2005). Among all developing countries, only Mongolians and Mauritanians consume more milk per dollar earned than do Kenyans (ILRI, 2007). On the production side, Kenya is self-sufficient in milk. In 2005, the country produced approximately 3.5 billion litres of milk, against a consumption of about 3 billion litres (Wambugu *et al.,* 2011). Milk production in Kenya is predominantly by small scale farmers, who own one to three dairy animals, and produce about 80 percent of the milk in the country. Smallholder dairy production systems range from stall-fed cut-and-carry systems, supplemented with purchased concentrate feed, to free grazing on unimproved natural pasture in the more marginal areas (Wambugu *et al.,* 2011).

2.1.3 Milk nutritional composition

The major constituents of cow's milk are water, protein, fat and lactose (Table 2). The minor components are vitamins, minerals and salts. Lactose and casein most readily distinguishes milk from other foods (O'cansey, 2010) The composition of milk varies among animals, between breeds, with the feed and health of animals and the stage of lactation among others (Fox, 2003). Milk is a complex mixture of proteins, carbohydrates, vitamins, minerals and other constituents dispersed in water

(Harding, 1999). Raw milk is a highly nutritious product. It is a suspension (micellar casein in equilibrium, somatic and microbial cells), an emulsion of fat globules and lactose with a solution of hundreds of other soluble molecules of which serum proteins high nutritional value, minerals, growth factors, vitamins, hormones (Boudier, 2011). However, the wealth of nutriments and water made him a highly perishable product. Milk is an excellent source of nutrients such as vitamins, amino acids, fats, minerals, proteins and sugar, making it an excellent medium for microbial proliferation (Akinyele *et al.*,1999).

Table 2: Percent 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	16.77	7.45	3.78	3.00	4.88	0.78
Bufallo						
Zebu	13.45	4.97	3.18	2.38	4.59	0.74
Woman	12.57	3.75	1.63		6.98	0.21

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

2.1.3.1 Water

Water is the major component of milk, representing 87% of the total composition. The other components are suspended or dissolved in this medium. A small amount of water is bound to the milk protein and some hydrated to the lactose and salts giving milk a water activity (a_w) of 0.993 (Jenness, 1988). Also according to Fox, (2003)

Water is the main constituent of milk. It serves as solvent for the salts in milk, proteins and lactose. It also controls the activity of many reactions like enzyme activity and microbial growth which affect the stability of milk and milk products. The amount of water can affect their quality, stability and preservation of milk products.

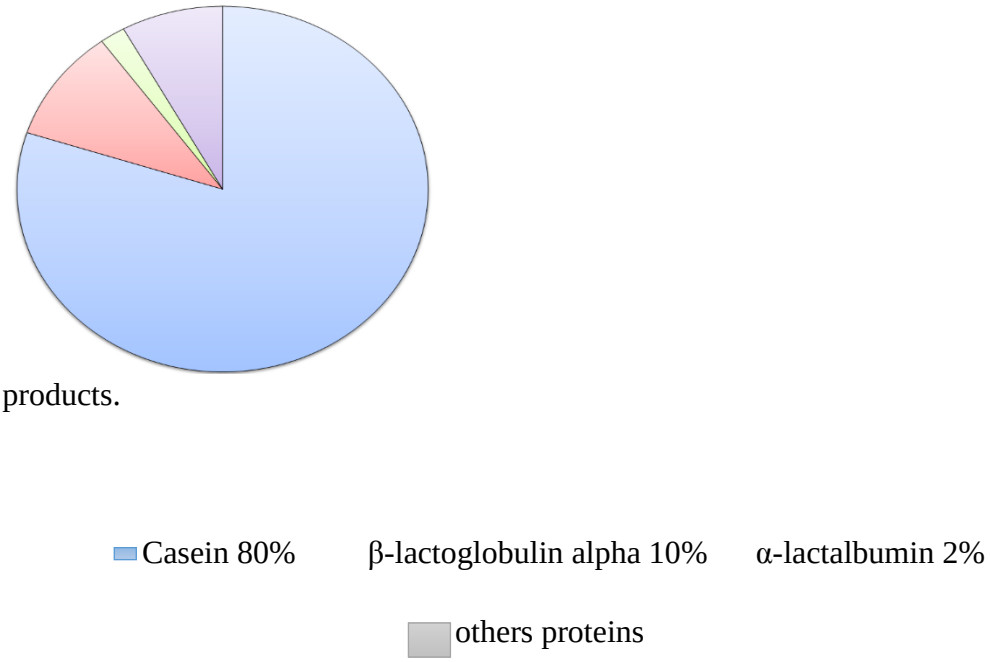

Figure 1: Milk proteins. Source (Benslama, 2016)

2.1.3.2 Protein

The protein content greatly affects the properties of milk and milk products more than any other constituent. Casein and whey proteins are the main groups of proteins (figure1) which make up about 80% and 20% respectively of total milk (Fox, 2003). The acid whey contains two groups of proteins; lactalbumins and lactoglobulins. The fraction of lactoglobulin contains

mainly immunoglobulins whiles the fraction of lactalbumin contains two major proteins which are β-lactoglobulin (β-Lg) and α-lactalbumin and other minor proteins like blood serum albumin and lactoferrin (Fox, 2003).

2.1.3.3 Lipids

Cow's milk contains about 35 g / l of lipid (Cheftel *et al.,* 1977). Lipids constitute the bulk of milk fat (greater than 98%), the rest are polar lipids and lipoidal substances. Fats or oils collectively called lipids are constituents of biological fluids, tissues and foods which are soluble in an apolar solvent (Fox, 2003). Lipids are water-insoluble organic biomolecules that can be extracted from cells and tissues by non-polar solvents (O'cansey, 2010). Lipid in milk varies according to the conditions of breeding. It is the most variable constituent of milk, consisting of a mixture of single lipids (98.5%) which are suspended in milk and forms tiny droplets (fat globules) and forms a emulsion. The lipid concentration ranges from 10 to 500 g / l depending on the species. According to Lehninger (1977), lipids perform important biological functions. These functions include:

1. structural components of membranes
2. storage and transport forms of metabolic fuel
3. protective coating on the surface of many organisms
4. cell surface components concerned in cell recognition**,** species specificity, and tissue immunity.

2.1.3.4 Lactose

Lactose is a disaccharide sugar present in solution in milk, and usually the main solid element of milk. Its power sugar is six times lower than that of sucrose. It is also the major carbohydrate of milk found in cows' milk at levels of approximately 4.8% (Holsinger, 1988). Of all the common sugars, lactose has the lowest relative sweetness, and it is the least soluble (17 g per 100 g at 20^{o}C) (Aurand and Woods, 1973).

2.1.3.5 Salts and Vitamins

The mineral salts of milk constitute less than 1% of the milk. Anionic components include chlorides, phosphates, sulphates-carbonates and citrates and cations in the largest amounts are calcium, potassium, sodium, and magnesium (Jenness, 1988). The vitamins brought by the milk are especially the vitamins B2, B12 (water-soluble) as well as vitamins A, D and E (fat-soluble). The vitamin C, present at 8mg / l in fresh milk, is very fast degraded and its content fall by more than 50% after 36 hours of refrigeration (Benslama., 2016).

2.1.4 Milk Coagulation

Coagulation is the first step in turning milk into cheese. This coagulation results in the formation of a gel, resulting from physicochemical changes occurring at the level of casein micelles (Rutgers *et al.*,1996). The mechanisms proposed in coagulum formation differ totally according to whether these changes are induced by acidification or by the action of coagulating enzymes (Eck, 1984). Milk coagulation is caused by the denaturation of casein, the major protein in milk. Fat and

seroproteins have a passive role. Milk can be coagulated by adding rennet or acidifying it through lactic acid bacteria or by chemical acidification. This results in an aggregation of casein micelles giving a gel (or coagulum). The different caseins are organized in micelles which are aggregates of several casein molecules. (Benslama, 2016)

2.1.4.1 Lactic coagulation or acidic coagulation

In milk, casein micelles and fat globules are negatively charged. This results in an electrostatic repulsion that ensures the stability of the milk. The kappa casein fragments are hydrophilic and lie on the periphery of the micelles, where they create a layer of hydration (retained water prevents the colloids from getting closer together). Lactic acid, resulting from the degradation of lactose by lactic acid bacteria, carries positive charges that neutralize the negative charges of micelles. At pH 4.6 (pHi of casein) we obtain their neutrality. The acid thus dehydrates the micelles, which allows them to get closer. The micelles bind by hydrophobic interactions (weak, reversible bonds) by retaining in their network the fat globules, the microorganisms, the vitamins, all the particles which can be retained in the meshes of the casein network. We obtain a gel: This is the lactic coagulation of the milk (fermented milk) (Benslama, 2016).

2.1.4.2 Coagulation by action of rennet

A large number of proteolytic enzymes of animal, plant or microbial origin have the property of coagulating the casein complex. Rennet, a mixture of chymosin and

pepsin, secreted in the abomasum of young milk-fed ruminants is the best known coagulant enzyme and its mechanism of action is well established. The mechanism of coagulation by rennet has two stages: splitting of lime caseinate into lime Para caseinate and proteose, and in solubilization of lime Para caseinate and formation of a gel thanks to the calcium salts in solution. (Kora, 2005)

The study done by Eck (1987) has confirmed that the coagulation of milk by the action of rennet has two phases, the primary phase or enzymatic phase and the secondary phase or phase of coagulation. The enzymatic phase corresponds to the hydrolysis of *k*-casein at the Phe 105-Met 106 bond. The peptide chain is cut into two unequal segments: paracasein and caseino glycopeptide. With the release of the caseinoglycopeptide, *k*-casein 3loses its stability factors (electrical charge, degree of hydration). As a result, the paracasein formed becomes insoluble. During the secondary phase, the insoluble micelles polymerize. There is gelation, hence the formation of the coagulum.

2.2 *Calotropis procera*

2.2.1 Taxonomy

Calotropis procera (Aiton) also referred to as the sodom apple is a plant belonging to Asclepiadaceae family. Taxonomic description is:

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

Figure 2: Young specimen of *Calotropis procera.* Source: (Dietmar, 2005)

2.2.2 Cultivation and distribution of *Calotropis procera*

Species: *Calotropis procera* (Aiton) W.T. Aiton –Roostertree (Chandrawat and Sharma, 2015)

There are two subspecies of *C. procera*, but only spp. procera is naturalised in Australia (Forster, 1992). In its native range, *C. procera* is polymorphic. However, the population in Australia is quite uniform, suggesting development from one or very few introductions (Forster, 1992). The genus *Calotropis* comprises three species of shrubs found in tropical and subtropical Africa, Asia and India: *C. procera*, C. *gigantea* (L.) WT Aiton and C. *acia BuchHam* (Rahman and Wilcock, 1991). *Calotropis procera* is very widespread in the tropical and subtropical areas of Africa (Somalia, Egypt, Libya, the Algerian South, Morocco, Mauritania, Benin, Chad,

Niger, Mali, Senegal, Togo), in Asia (India, Pakistan, Afghanistan, Iran, Saudi Arabia), in the centre and in the South of America (Benyahia-Krid *et al.,* 2016). *Calotropis procera* is an Ayurvedic plant with important medicinal properties. It is known by various vernacular names like Swallow wort in English, Madar in Hindi and Alarka in Sanskrit. It is found in most parts of the world with a warm climate in dry, sandy and alkaline soils (Shoaib *et al.,* 2013). The *Calotropis procera* is a shrub or small tree up to 2.5 m (max. 6) high, stem usually simple, rarely branched, woody at base and covered with a fissured, corky bark; branches somewhat succulent and densely white tomentose; early glabrescent. All parts of the plant exude a white latex when cut or broken.

2.2.3 Utilization of *Calotropis procera*

Calotropis procera is primarily harvested because of its distinctive medicinal properties (Shoaib *et al.,* 2013). Odugbemi, (2006) study has reported that *Calotropis procera* has been used traditionally to treat diseases like fever, eczema, diarrhea, leprosy, ringworm, cough, asthma and convulsion in Nigeria. Traditional doctors in West Africa have claimed to have successfully used the plant to cure many diseases. In the traditional Asian medical system, it has been used for bronchitis, pain, asthma and tumors (Muzammal, 2014). The latex of *Calotropis procera* extract is easily available and is used in medicine for treatment of many diseases. It is used as wound healing agent, anti-diarrheas, anti-inflammatory, and anti- rheumatism agent. It is also used against malaria and skin infection (Qari *et al.,* 2008). The milky latex and flowers were considered to improve digestion, Catarrh and increases appetite (Oudhia, 2001). Kuta, (2008) stated that *Calotropis procera* is

also used by traditional medicine practitioners in Gwari communities for the treatment of ring worms. The plant Sodom apple (*Calotropis procera*) has been given much attention since it has the potential to completely substitute animal and microbial rennet used for commercial cheese production according to Chikpah *et al.,* (2014). The extracts from the *Calotropis* plant has been used traditionally as the sole coagulant of milk in a number of African countries (Benin, Togo, Ghana, Burkina Faso, Chad, Nigeria) for the production of a soft cheese called '*wagashi*' (Ashaye *et al.*, 2006; Chikpah *et al.* 2014)

2.2.4 Composition and toxicity in *Calotropis procera*

According to Shaker *et al.* (2010), *Calotropis procera* has biologically active substances such as flavonoids, cardioactive glycosides, triterpenoids, alkaloids, resins, anthocyanins, tannins, saponins and proteolytic enzymes. *Calotropis procera* R. Br. (Asclepiadaceae) is a plant widely distributed in tropical and subtropical regions of Africa and Asia with a long history of use in traditional medicine. A wide range of chemical compounds including cardiac glycosides, flavonoids, phenolic compounds, terpenoides have been isolated from this species (Mueen Ahmed *et al.*, 2005; Elimam *et al.*, 2009). 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.

The latex of *C. procera* contains several alkaloids (such as calotropin, catotoxin, calcilin, gigantin etc.) which are caustic and considered poisonous in nature. Basak *et al.* (2009) reported 29 cases of patients who presented with accidental ocular contact

or injury with the latex of *C. procera*. All patients presented with sudden painless vision with photophobia. All eyes had conjunctively congestions and mild to severe corned edema with descemet's folds. However, the significant ocular morbidity caused by *C. procera* latex may be preventable by simple health education (Márcia *et al*., 2010). Maia de Lima *et al.* (2011) demonstrated that *C. procera* is a cardiotoxic plant. The plant is also known for its toxic properties that include dermatitis, iridocyclites and acts like a poison and produces lethal effects (Vadlapudi *et al.,* 2009). The latex of *Calotropis procera* contains compounds of high toxicity. In addition to calactin, a calatoxin from usharine and usharidine, it contains calotropin a glycoside with effects similar to digitalis (Desheesh *et al*., 2000). The glycosides contained in the sap of *Calotropis procera* have toxic effects on the heart as well as known calotropin and usharin in medicine (Kees, 1996). It is possible to consider the risk of toxicity following prolonged consumption of Fulani cheese, but no case of food poisoning due to the consumption of Fulani cheese has been reported to date (Egounléty *et al.,* 1994). From cow's, goat's or sheep's milk cheese is obtained by coagulation with an animal or plant coagulant (*Calotropis procera*) (Kora, 2005).

2.2.5 Coagulating properties of *Calotropis procera*

The *Calotropis procera* has been shown to possess some coagulating properties and has been used as a coagulant in cheese making by Fulani farmers, particularly in Benin and Nigeria. (Dossou *et al.,* 2006). According to Aïsso *et al.* (2015) the coagulation, a key operation in the production of Peulh cheese, is traditionally done in Benin using the fresh leaves and stems of *Calotropis procera*. The coagulation test

carried out by (Capo-chichi, 2004) on the different parts of *Calotropis procera* proved that all the parts (leaves, stems, fruits and latex) have a coagulating activity on milk casein. The leaves and the stem give about the same results, the fruits are less effective, the latex on the other hand has a more marked activity and the dilutions in the 10th and 100th give the best results (Capo-chichi, 2004).

2.3 Cheese

2.3.1 Definition of Cheese

Cheese is the fresh or matured product obtained after coagulation and draining of milk, cream, skimmed or partly skimmed milk, buttermilk or a combination of some or all of these products (FAO/ WHO, 1973). It is also a general term used to describe curdled milk. According to Codex Stan 283 (1978) cheese is a matured or not fully matured soft, semi-hard, hard, or extra-hard dairy product, which may be coated, and in which the whey protein to casein ratio does not exceed that of milk by coagulating wholly or partly the protein of milk, skimmed milk, partly skimmed milk, cream, whey cream or buttermilk, or any combination of these materials, through the action of rennet or other suitable coagulating agents, and by partly draining the whey resulting from the coagulation, while respecting the principle that cheese making results in a concentration of milk protein and that therefore, the protein content of the cheese will be definitely higher than the protein level of the blend of the milk supplies from which the cheese was made and by processing techniques involving coagulation of the protein of milk and products obtained from milk which give an end-product with the same physical, chemical and organoleptic characteristics.

2.3.2 Origin of *wagashi*

Wagashi according to Malomo *et al.* (2015) is a traditional soft cheese consumed in several parts of West Africa which originates from the Fulani cattle herdsmen from northern Nigeria who refer to the liquid cow's milk as *Wara* and the curd-like texture of the cheese as ‘Kashi’ (Ogundiwin, 1978). According to oral tradition reported by D’Olivera (1985), Fulani women would have noticed that during the harmattan period the coagulation of the milk was very slow. To speed it up, these women put the milk near the fire. However, they noted an acceleration of coagulation milk in which the leaves of *Calotropis procera* were immersed, serving to protect the milk during its transport in the calabash. These women assumed that this phenomenon was due to the leaves they put in the milk to stabilize it during transportation to the house. According to the same source, the pioneer of Peulh cheese technology would be the first breeder to get the curd seeds by applying the of leaves *Calotropis procera* would process the milk. The name *Wagashi* is presumed to have originated from a peasant Baatonou who advanced the idea that the seeds of the curd could be grouped together and put in shape; hence the name "Gassarou Babarou" in Baatonu, which means the ball of cheese. The prefix "Wara" would mean cake in Baatonu. The authentic name of the Fulani cheese would be "Waragassarou Babarou". What ethnolinguistic mutations have transformed today in waragashi in Baatonu, *Wagashi* in Dendi, Woagashi or Gassiigué in Peulh, among other terms. It can therefore concluded that Fulani cheese technology was undertaken in Benin even though the origin has not been extensively studied (Dossou *et al* 2006). The production method of coagulation milk with *Calotropis procera* is practiced throughout the Beninese

territory. The practice is also carried out in west and northern Nigeria (Awohr and Egounlety, 1986; Waters - Bayer, 1988) and in the north of Togo.

2.3.3 Cheese production

Cheese making in Africa is largely dictated by traditions. Due to shortage of milk, cheese production is expensive and powdered milk and cheese may have to be imported. The production of cheese in African countries has been increasing throughout the 21st Century from 430,000 metric tonnes in 1990 to 743,000 metric tonnes in 2002 (FAO, 2002). This increase is expected to continue as more diversification takes place and more food processing is encouraged. Egypt has the highest cheese production of African countries and accounts for about 67 % of African cheese production (Raheem, 2006). In Benin the cheese remains the most widespread and the most consumed, both in rural and urban areas. It stands out as the best form of milk preservation (Dossou *et al.,* 2006). In Kenya the Maasai tribesmen who herd large numbers of Zebu cattle and consume large quantities of milk have not adopted the cheese making tradition. Nomads in developing arid countries have suffered hunger in recent years. It would be nutritionally advantageous if the idea of producing cheese, a stable and nutritious food which can be stored for longer period of time, were introduced to tribal chiefs (Kosikowski and Mistry, 1997). Increase in the production of milk and dairy products would be a major step in improving the protein intake and ensuring a more balanced diet (Raheem, 2006). There is very little scientific information available on the cheeses made in Africa. Recipes and

processes are passed from generation to generation by observation and practical experience.

2.3.4 Nutritional quality of *Wagashi*

In countries with a dairy industry, cheese provides an ideal vehicle for preserving the valuable nutrients of milk. Cheese is an excellent source of protein, fat and minerals such as calcium, iron and phosphorous, vitamins and essential amino acids, making it an important food in the diet of both young and old. In Benin, the milk is processed into various products such as yoghurt, curd and *Wagashi*, a traditional cheese from an artisanal process developed by the Fulani ethnic group. The *Wagashi* is the most popular and most consumed milk derivate products (Aïssi *et al.*, 2009). *Wagashi* is an important source of animal protein, especially for people with low incomes and could efficaciously contribute to solving problems related to proteins deficiency in the diets in Africa (Kèkè *et al.*, 2008). Figure 3 shows samples of two common types of *Wagashi* produced in Benin.

Figure 3: *Wagashi* : (a) freshly prepared (b) soaked in sorghum water for coloration

Cheese is a nutrient dense food which provides fat, high quality proteins, oligopeptides, amino acids, vitamins and minerals. Table 3 presents the proximate analysis results for different types of cheese found in Benin.

Table 3: Proximate composition of cheese from three different cattle breed.

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

DM – Dry matter. Source: (Kora, 2005)

2.3.5 Typology of cheese and production technology

2.3.5.1 Typology of cheese

A summary classification of the different categories of cheese made by Sanogo (1994) is presented in Table 4. In this classification, Peulh cheese can be inserted at two levels, namely, among fresh cheeses, soft cheese when it is in the unripened raw state and among cooked pressed cheeses when it is Semi-hard texture after a refining process by heat treatment with plant extracts (Dossou *et al.*, 2006).

Table 4: Typology of cheese

Types of Cheese	Characteristics	Examples
Fresh cheese	Little dripping Significant humidity Without ripening	Petit Suisse, Chevre frais, minas, raw peulh cheese
Mature cheese	Refinement	
Soft pasta	No pressing	Camembert, Feta
Uncooked pressed pasta	Mixed curd, pressing	Saint paulin, Tekeme
Cooked pressed pasta	Curd rennet, brewing and heating the curd, pressing	Gruyere Coloured peulh cheese
Pasta spun	Curd thread	oaxaca
Very dry cheese	Dehydration pushed	Fromage sec de chevre Frommage de poutour
Melted cheese	Cheese fusion	Cancoillotte, cheese

Source: (Sanogo.,1994; Dossou., *et al* 2006)

According to Dossou *et al.* (2006), the typology criteria used in the literature for the classification of Fulani cheeses are colour (red or white) and shape (oval or flat). But the thickness and diameter of the cheese should be taken into account.

2.3.5.1 Technology of production

The method of production of Fulani cheese uses cow's milk and *Calotropis procera* as the main raw materials. Fresh cow's milk is heated slightly and coagulated with *Calotropis procera*. The coagulum obtained, cooked, drained and molded is presented on the market in different shapes and sizes (Dossou *et al.*, 2006).

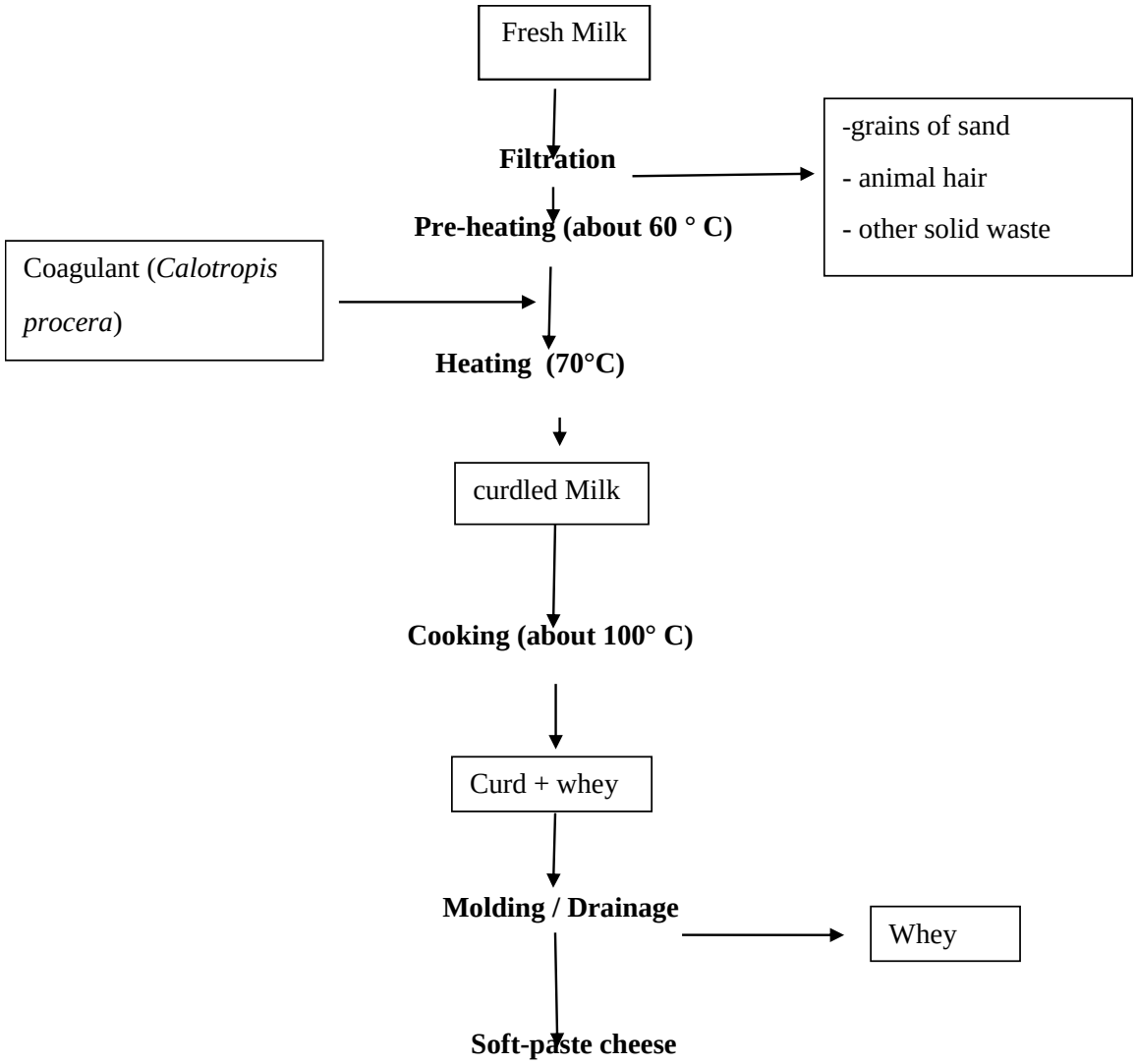

Figure 4: Flow chart of processing of Peul cheese

Source: (Dossou et al., 2006)

2.3.6 Texture

According to Phadungath (2002), texture is quite difficult to define as it means different things to different people. Texture is derived from the word textura in Latin, which means a weave. Texture was formerly used to refer to the structure, feel and appearance of fabrics (Rosenthal, 1999). Food technologist attempted to define

texture in terms of food because the meaning of texture did not cover the food aspect (Phadungath, 2002). International Organization for Standardization (ISO) (1992) defines texture as a sensory characteristic perceived largely by way of the senses of touch and movement. Texture is the primary quality characteristic of cheese products. Texture analysis refers to the mechanical testing of food. Texture of products plays a very important aspect in the preference and acceptance of food products. Texture Profile Analysis (TPA) and penetration test are some types of texture analysis that can be done on cheese products. Instrumental measurement of the textural properties of cheese is frequently used to understand consumers' perception on both cheese quality and the influence of the processing technology on cheese quality (Tudoreanu and Dumitrean, 2009).

2.4 Whey

Whey is a by-product of the dairy industry, which for years was thought to be insignificant and was either used as an animal feed or was disposed of as waste. The whey is the watery and thin liquid, which is obtained during cheese making by coagulating and separating casein proteins from milk. Whey's composition and sensory characteristics vary depending on the kind of the whey (acid or sweet), the source of the milk (cow, sheep, bovine milk) and the feed taken by the animal which produced the milk used in the cheese making process, the time of the year and the stage of lactation (Tsakali *et al.,* 2010). In the case of sweet whey rennet type enzymes are used at a min pH of 5,6 to induce coagulum and in the case of acid whey coagulum is created when milk is acidified by lactobacillus culture or mineral acid at a max pH of 5.1.

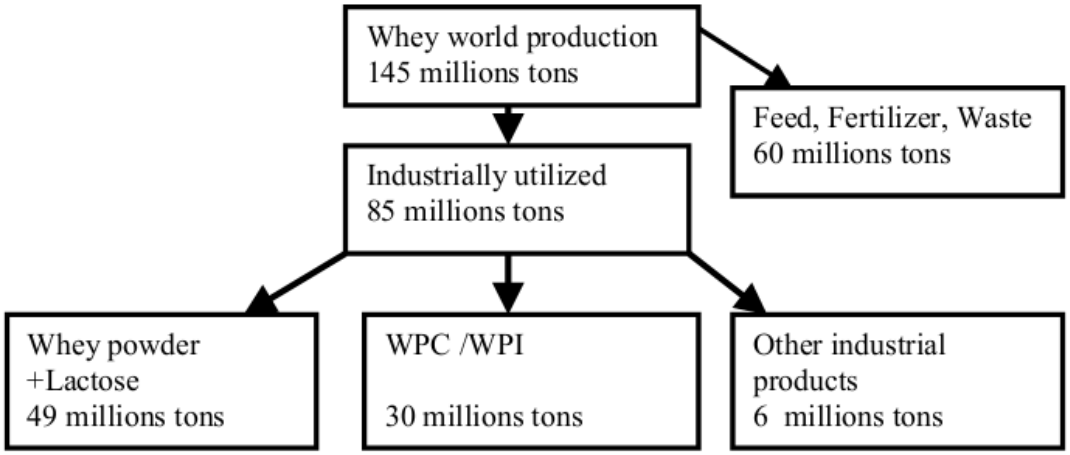

Figure 5: Global production and Utilization of whey Source: (Tsakali *et al*., 2010)

Whey contains various bioactive components that demonstrate a range of immune-enhancing properties. Several studies have shown that whey-derived components can reduce the risk of metabolic syndrome, which can lead to various chronic diseases, such as cardiovascular disease and diabetes (Xóchitl *et al.*, 2012). According to Baldasso *et al.* (2011), whey is the by-product of cheese or casein production, it is of relative importance in the dairy industry due to the large volumes produced and the nutritional composition. Worldwide whey production is estimated at around 180 to 190×10^6ton/year; of this amount, only 50% is processed. Whey contains more than half of the solids present in the original whole milk, including whey proteins (20% of the total protein) and most of the lactose, water-soluble vitamins and minerals. Consequently, whey can be considered a valuable by-product with several applications in the food and pharmaceutical industries. Table 6 presents the chemical composition of sweet and acid whey.

Table 5: Composition of milk and cheese whey

% by Weight	Milk	Cheese Whey
Fat	4.7	0.05
Lactose (milk sugar)	4.5	5.0
Casein Protein	2.7	0.10
Whey Protein	0.55	0.65
Minerals	0.85	0.50
Minor Components	0.20	0.30
Water	86.5	93.4
Total	**100.00**	**100.00**

Source: www.dairyforall.com, Tsakali *et al.* (2010).

Table 6: The sweet and acid whey composition

Constituent	Sweet Whey	Acid Whey
Water	93-94	94-95
Dry Matter	6-6.5	5-6
Lactose	4.5-5	3.8-4.3
Lactic Acid	Traces	Up to 0.8
Total Protein	0.8-1.0	0.8-1.0
Whey Protein	0.6-0.65	0.6-0.65
Citric Acid	0.1	0.1
Minerals	0.5-0.7	0.5-0.7
pH	6.4-6.2	5.0-4.6
SH Value	About 4	20-25

Source:(III-Dairy-G-WheyProducts-2.,vhttps://nzic.org.nz/ChemProcesses/dairy/3G.

2.5 Phytochemical screening analysis

Phytochemicals are the chemicals that present naturally in plants. Some phytochemicals are:

Alkaloids

These are basic (alkali-like), nitrogen-containing organic constituents found in some plants. Alkaloids are organic bases. Many alkaloids are poisonous; others are addictive such as cocaine, and some are used clinically such as morphine. Alkaloids are produced by secondary metabolism of primary metabolites, usually amino acids. Alkaloids of the pyrrolizidine and indolizidine types cause serious toxicity (and death) in livestock, mainly horses, cattle and sheep that graze on. Pyrrolizidines, such as heliotridine, cause chronic liver damage and malignant tumours (Woolley, 2001).

Saponins

These are high-molecular-weight glycosides, consisting of a sugar unit(s) linked to a triterpene or a steroid aglycone. Many saponins have detergent properties. They lower the surface tension of aqueous solutions and therefore give stable foams when in contact with water. In fact, the name “saponin” stems from the latin word sapo (soap). Saponins are also known to cause haemolysis (lysis of erythrocytes with the release of hemoglobin), have a bitter taste, and be toxic to cold-blooded animals (Madland, 2013).

Tannins

These are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides including cellulose, hemicellulose, pectin, alkaloids, nucleic acids and minerals, among others (Vansoest.,1994)

Flavonoids

These are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham.,1960). They play a major role in successful medical treatments of ancient times, and their use has persisted up to now.

Phenols

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Rao, 2012).

Cardiac glycosides

They are named so, due to their action on the heart muscle. The aglycone part here is steroid, which is chemically cyclopenta phenanthrene. The steroidal aglycones are of

two types: cardenolide and bufadienolide. The more prevalent in nature is the cardenolide type. Digitalis is plant containing cardio active glycosides, but the plant used in our laboratory is Nerium oleander of the family Apocyanaceae. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal (Rao.,2012)

Steroids

These are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Firn, 2010). According to Madziga et al., 2010, steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animal with wasting illness.

CHAPTER THREE: MATERIALS AND METHODS

Standard laboratory materials including sample preparation ingredients, reagents, apparatus, equipment and tools as specified in the standard methods were used in carrying out the analyses of samples. These materials are specified within the description of methods respectively.

3.1 Survey

A survey involving 40 cheese producers was conducted using a semi-structured questionnaire (Appendix 1). The 40 cheese producers were selected randomly from north to south according to the areas where cheese production is high (figure 6). Data collected include socio-demographical background of the respondents, the different raw materials used for *Wagashi* production and detailed process of *Wagashi* production.

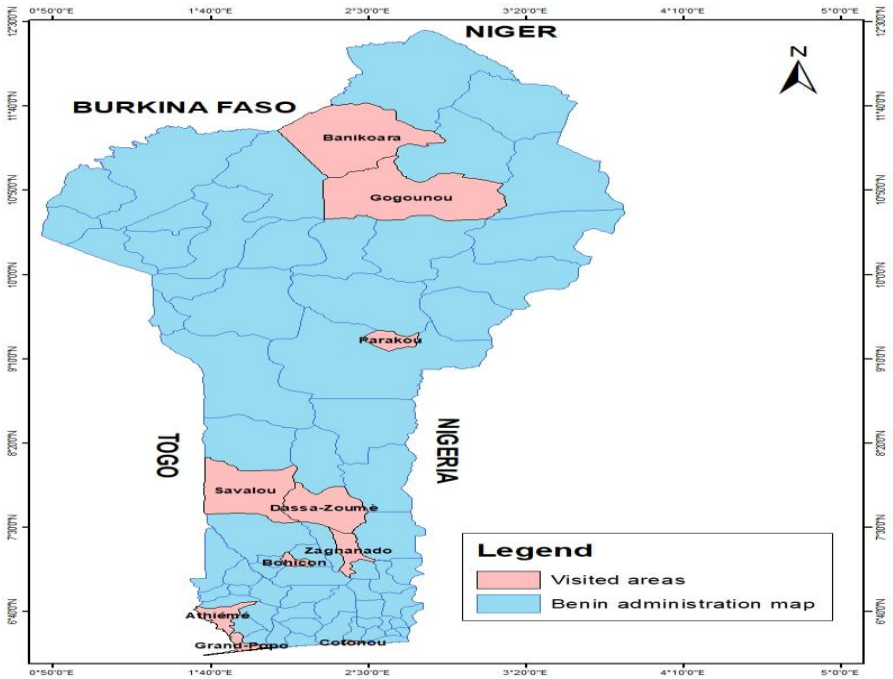

Figure 6: Map showing areas visited in Benin for survey

3.2 Samples collection

Milk and *Calotropis procera* plant samples were collected from Benin and Kenya. The milk samples were collected in 50 ml falcon tubes and about 10 kg of the plant *Calotropis procera* were collected in Benin and in Kenya. Milk samples from Benin were obtained from Borgou, Girolando, Lagunaire breeds in different regions (Appendix 2) in the country. In Kenya milk was mainly obtained from Friesian and Ayrshire breeds reared at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) Farm at Juja Campus, Kenya. The *Calotropis procera* plant samples were sourced in areas where they were found to grow. In Kenya they were collected from the dry areas of Makueni County. The cheese was made following traditional methods as describe in Section 3.3 below.

3.3 Cheese production

Approximately 5 litres of milk sample was filtrated into the pot on fire for heating. The milk was heated to between 60 ºC -70 ºC. The *Calotropis procera* leaves and stem (50 g) extracts which were previously mixed with some small volume(0.5 L) of fresh unheated milk were filtrated into the warm milk. The mixture was left on fire and maintained at temperatures between 60 ºC -70 ºC until coagulation was achieved. The heating was stopped following the separation of curd and whey (appendix 3). The sign of coagulation was observed within the range of 8-12 min. It was transferred into a small rafia basket to facilitate whey drainage and characteristic shape, when the cheese was firm enough it was removed from the raffia basket and

placed inside a covered plastic container or zip bags for analysis. The following flow diagram describe the above procedure.

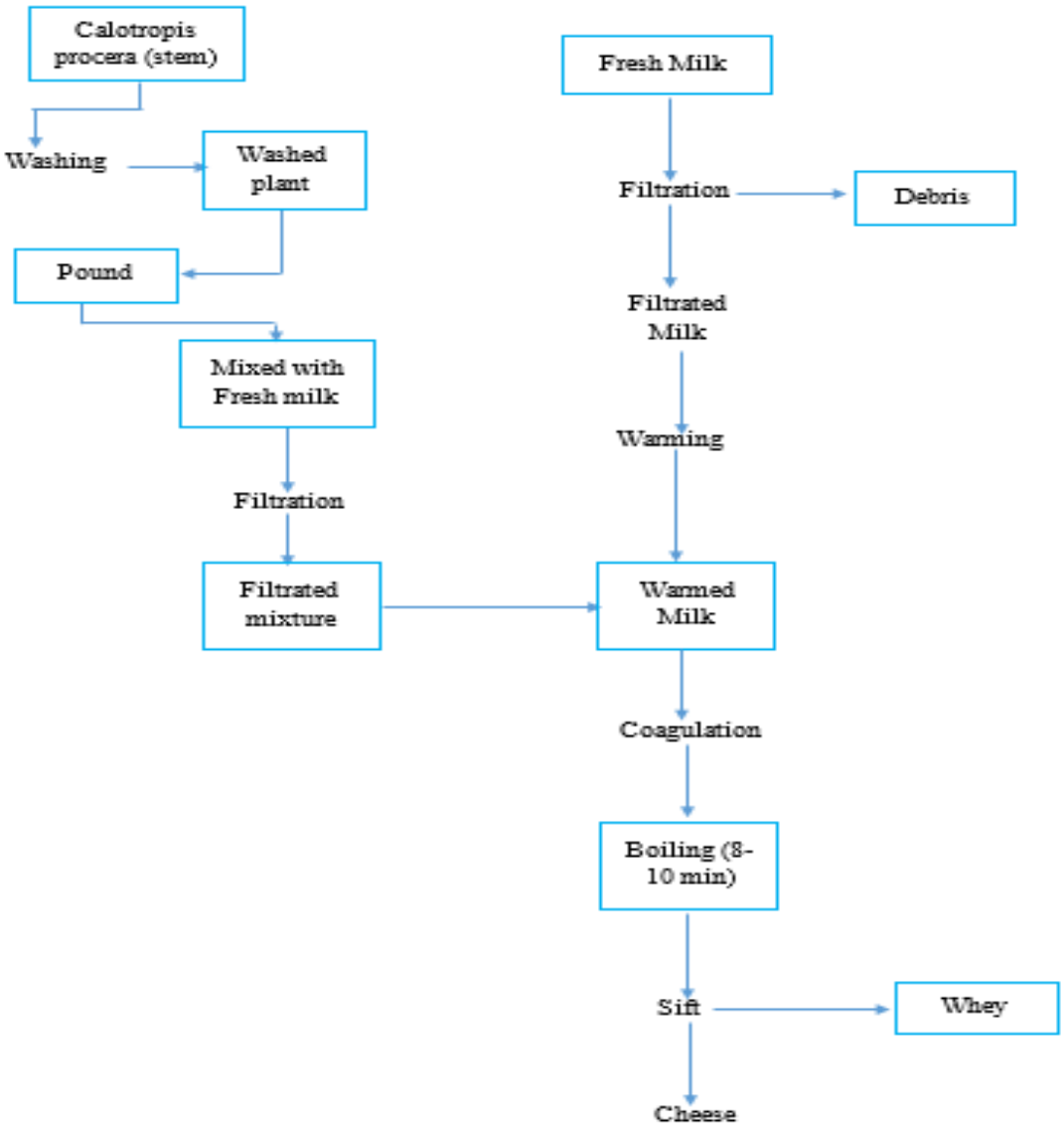

Figure 7: Flow chart of cheese production

3.4 Laboratory analysis

3.4.1 Proximate analysis

3.4.1.1 Moisture

The moisture content was determined according to the methods of analysis described in AOAC (1990). About 5 g of fresh sample (of milk, cheese and whey) was weighed and placed in a clean dry moisture dish and the weight of the sample and dish taken. These were placed in a moisture oven and the temperatures adjusted to 105°C. The samples were dried for 3 hours and removed, cooled and weighed. The amount of moisture in the samples was calculated using the formula:

$$\%\text{Moisture} = \left(\frac{(\text{wt before drying} - \text{wt after drying})}{\text{sample wt}} \times 100 \right)$$

3.4.1.2 Ash

The ash contents of the samples were determined using muffle furnace according to the method of AOAC (1984). About 5 g of fresh sample (of milk, cheese and whey) 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°C. The samples were then allowed to ash for about 5 hours. Temperature was reduced, samples removed and cooled in a desiccator before weighing. The amount of ash was calculated using the formula:

$$\% \text{ ash} = \frac{(\text{wt of crucible with ash} - \text{wt of empty crucible})}{\text{Sample wt}} \times 100$$

3.4.1.3 Fat

Milk

Geber butterfat method described by Kirk (1991) was used to determine the fat in milk samples. About 10 ml of 87% sulfuric acid (H_2SO_4) were put in a butyrometer. Then 11 ml of milk sample and 1ml of amyl alcohol were added, corked and shaken until the no white particles were seen. Each tube was transferred to water bath at 65ºC until a set was ready for centrifuging. The samples were centrifuged at 1100 rpm for 5 min then transferred into a water bath at 65ºC for 3min. The corks were screwed into the butyrometers until the lower meniscus of the fat was at 0 mark.

Cheese and whey

The fat in cheese and the whey were determined by the method of Bligh and Dyer (1959). About 2 g of each sample was weighed in a centrifuge tube, 20ml of 2:1 methanol: chloroform was added, covered with foil and shaken for 20 min. The samples were then centrifuged at 100 rpm for 10min and the supernatant was transferred into a new tube where 10 ml of chloroform was added and vortexed for 1min. Finally 5ml of cold water was added and vortexed for 1min. This was followed by the sample being centrifuged at for 10min at 100 rpm. The chloroform layer (lower) was carefully picked with a pipette and transferred into a weighed flask. The chloroform layer was evaporated to dryness and put in the oven at 70ºC

for 30min. The flask was weighed under desiccator. The percent of fat was calculated using the formula:

$$\% \text{ fat} = \frac{(w_2 - w_1 t)}{w_0} \times 100$$

where w_2 is the weight of flask after, w_1 the weight of flask before and w_0 the weight of sample used.

3.4.1.4 Protein

Protein was determined using the semi micro Kjeldal method (AOAC, 1980). Approximately 2 g of sample was weighed into a digestion flask. It was combined with a catalyst of 5 g potassium sulphate and 0.5 g of copper sulphate and 15 mL of sulphuric acid. The mixture was heated in a fume hood till the digest colour turned blue. This signified the end of the digestion process. The digest was cooled, transferred to 100 mL volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalyst was also made. Ten mL of diluted digest was transferred into the distilling flask and washed with distilled water. Then 15 mL of 40% NaOH was added and this also washed with distilled water. Distillation was done to a volume of about 60 mL distillate. The distillate was titrated using 0.02 N HCL to an orange colour of the mixed indicator, which signified the end point.

$$\% \text{ Nitrogen} = \left(\frac{(V_1 - V_2) \times N \times F \times 100}{\frac{(V \times 100)}{S}} \right)$$

where: $V_1$is the titre for sample in ml, V_2 is titre for blank in mL; N= normality of standard HCL (0.02); f= factor of std HCL solution; V= volume of

diluted digest taken for distillation (10 mL); S= weight of sample taken for distillation (1 g).

$$\% \text{ Protein} = \%\text{Nitrogen} \times \text{Protein factor}(6.25)$$

3.4.1.5 Carbohydrate

The carbohydrate content was determined using the anthrone method described by Hedge and Hofreiter (1962). Approximately 1g of each sample was weighed into a boiling test tube and hydrolyzed by keeping it in boiling water bath for 3 hours with 5mL of 2.5 N-HCl. It was then cooled to room temperature. The samples were neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 mL and centrifuged. The supernatant was collected and 0.5 and 1mL aliquots were taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1mL of the working standard. '0' served as blank. The volume was made up to 1mL in all the tubes including the sample tubes by adding distilled water. Then 4mL of anthrone reagent were added and heated for eight minutes in a boiling water bath, cooled rapidly and the green to dark green colour were read at 630nm. A standard graph (Appendix 4) was drawn by plotting concentration of the standard on the *X*-axis versus absorbance on the *Y*-axis. From the graph the amount of carbohydrate present in the sample tube was calculated as follow:

$$\text{Carbohydrate in 1 g of the sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

3.4.1.6 Lactose

Lactose in milk and whey

Lactose in milk and whey was determined using a lactose scan machine, about 5-10ml of each sample were put into a test tube and placed into the lactose scan. The results of the lactose content were obtained by the readings obtained for each sample. The procedure was repeated three times and average reading calculated.

Lactose in cheese

The lactose in cheese were determined according to AOAC (1996). Approximately 3g of each cheese sample was weighed into 100 ml conical flask (QF) and 50 ml of 96% ethanol added and mixed well. The content was refluxed at 100^{o}C for 1 hour, stirring occasionally. The slurry was filtered and the filtrate collected. The conical flask was rinsed 3 times with 5 ml of 96% alcohol and the content was transferred to 100ml pear-shaped flask and all the solvent evaporated at 60^{o}C to dryness. Then (10) ml of 50% acetonitrile was added to the dried sample and the content shaken vigorously. The content was microfiltered and injected into HPLC to determine the amount of Lactose.

3.4.2 Texture analysis

The firmness of the cheese was determined by using a penetrometer (Model CR-100D, Sun Scientific Co. Ltd, Japan) fitted with a probe, as by described by Jiang *et al.* (1999). The probe was allowed to penetrate the cheese to a depth of 2.5cm and the corresponding force required to penetrate this depth was determined. The cheese samples were all put in a small cylinder of 2 cm of diameter. Firmness was then expressed as Newton (N). The same action was done in triplicate then the mean was calculated.

3.4.3 Phytochemical Analysis

3.4.3.1 Extraction

Fresh leaves and stem of *Calotropis procera* were washed using distilled water and dried in the oven at 55ºC for about 72h. The leaves and the stem were ground separately to obtain powder. 1g of the powder was homogenized in 20ml of methanol and shaken for 72h. The extract was then filtrated. The same procedure was followed in the case of the cheese. The extracts were used for phytochemical screening and quantitative analysis.

3.4.3.2 Qualitative analysis

Tannins

To 3ml Methanolic extract was added 3ml of ferric chloride at 10% ($FeCl_3$). Formation of blue/ black colour was a positive indicator of tannins (Trease and Evans, 2002).

Phenols

The Ferric Chloride test was used where to 1ml of the extract, 2ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium ferro cyanide were added. Formation of blue or green colour showed the presence of polyphenols (Tiwari *et al.,* 2011)

Flavonoids

This was done according to the method of Harbone (1973). Five ml of dilute ammonia solution was added to a portion of extract followed by addition of

concentrated H_2SO_4. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Alkaloids

Alkaloid was determined using Mayer's test as describe by Tiwari *et al.* (2011). The mayer's test was prepared by dissolving a mixture of mercuric chloride (1.36g) and potassium iodide (5g) in water (100ml). To determine the presence of the alkaloids, 1ml of Mayer's test was mixed with 1ml of the samples extract. Formation of yellowish buff indicated the presence of alkaloids.

Cardiac glycosides

Five mL of the solution were placed in a test tube, 5mL of Kedde's reagent, and 5mL of 2 N sodium hydroxide solution were added. The appearance of purple colour indicated a positive test for cardiac glycosides. (Morsy *et al.*, 2016)

Saponins

The Foam test was used in detecting the saponins where to 1 ml of the extracts, 5ml distilled water was added and shaken vigorously. Formation of foam that persisted for over 10 minutes indicated presence of saponins. (Sofowora, 1993).

Steroids

The steroids were determined using Liebermann Burchard test. About 5 mL of the solution were evaporated to dryness. The residue was dissolved in 2mL chloroform and transferred to a small test tube. Acetic anhydride (0.3 mL) was added and mixed gently, then, a drop of concentrated sulphuric acid was added. The appearance of

blue-green colour, observed during 60 min, indicated presence of steroids (Rohit, 2015).

3.4.3.3 Quantitative analysis

Polyphenols

Phenolic compounds in the sample extracts were estimated by a colorimetric assay, following the procedures described by Barros *et al*. (2007) with minor modifications. To 5ml distilled water was added 0.5ml Folin Ciocalteu reagent. After 3min, 1 ml 7.5% sodium carbonate solution, 1ml extract were added to the mixture and made to 10ml using distilled water. The mixture was kept in water bath maintained at 50^{o}C for 16 minutes. UV-Vis detector (ShimadzuLC-10A) was used to read the absorbance at 765nm. Gallic acid was prepared in different concentrations and the absorbance also read at 765nm. The data obtained was used to generate the standard curve (Appendix 5) against which polyphenols in the samples were calculated and expressed as Gallic Acid Equivalent (GAEs)/100gdwb.

Tannins

Vanillin-hydrochloric acid method (Burns, 1963; Price *et al*., 1978) was used to determine tannins. Approximately about 0.5g of dry ground samples of plant and 1g of cheese and 10ml of whey were put into100ml Erlenmeyer flasks. 10 ml of 4% HCl in methanol were added into each of the flasks and closed with parafilm shake gently for 20min in a shaker. The shaken mixture was then centrifuged at 4500 rpm for 10 minutes. The supernatant aliquots were transferred into 25ml volumetric

flasks. The second extractions were performed by adding 5ml of 1% HCL in methanol to the residue, shaken and centrifuged. The second supernatant was added to the first one in the 25ml volume flasked and up to 25 ml using methanol followed by thorough mixing. A set of catechin standard solutions ranging 0, 10, 20, 40, 60, 80, 100 ppm was prepared using methanol as the solvent. 1ml of each respective standard and sample extract was pipetted into tests tube. Five ml of freshly prepared vanillin-HCl reagent was added into the standard and the samples in the test tubes. Sample blanks of 1ml into another set of the test tubes was prepared and 5ml of 4% HCl in methanol was added to each. The absorbance of the standard solutions, sample extracts and blanks were read in a UV-VIS spectrophotometer at 500nm, 20min after adding Vanillin-HCl reagent to the samples and standards. A standard curve (Appendix 6) from the readings of the catechin standard solutions was plotted. The absorbance for sample blanks were subtracted from the samples absorbance and calculation of the concentration of the sample extracts done.

Flavonoids

Flavonoids content in the extracts were determined by colorimetric method as described by Barros *et al*. (2007) with minor modifications. To 1ml extract 0.3 ml of 5% sodium nitrite and 4 ml distilled water were added and held for 5 minutes. To the mixture 0.3 ml of 10% aluminum chloride was added and held for 6 minutes. Finally, 2ml of 1M sodium hydroxide was added and the content made to 10ml with distilled water. Using UV Visible spectrophotometer, the intensity of pink color was measured at 415 nm. Pure quercetin was prepared in different concentrations and absorbance read at same wave length. The readings were used to make standard

curve (Appendix 7), against which flavonoids in the sample were calculated and expressed as mg of quercetin equivalent (QE)/100gdwb. The same procedure was repeated but with catechin as standard in place of quercetin. Flavonoids in the sample were similarly calculated and expressed as mg catechin equivalent (CE)/100gdwb.

3.5 Statistical analyses

The information collected during survey was analyzed using the Microsoft Excel. The information were put in Excel and analyzed using descriptive statistics. One way ANOVA followed by Newman and Keuls test (in case of significant difference) were used to compare values obtained from different breeds. Data analysis was done using SPSS 16 software.

CHAPTER FOUR: RESULTS

4.1. Survey

The survey took place in different Communes shown in Figure 9. A total of six regions were covered in the survey. Demographic results (table 7) indicate that 93% of the respondents were women implying that much of the cheese making is carried out by women. The age of the respondents clustered around 35 years with 75% of the respondents falling in this category. Cheese making is energy intensive activity and therefore requires energetic persons to accomplish the task. Besides, most of the respondents have no schooling history. The cheese making is mainly carried out by resource poor persons for home consumption and sale of the excess cheese. The most of preferred part of the of *Calotropis procera* plant in cheese production are the leaves and the stem (table 8) according to the survey findings. Besides, using the plant to coagulate the milk in cheese making process many respondents also used the plant for medicinal purposes.

Table 7: Socio-demographic information of the respondent

Socio-demographic	**Sex**		**Age**		**Education**			
	F	M	20-35years	≥35 years	No level	primary	secondary	university
	93%	7%	25%	75%	70%	15%	7.5%	7.5%

Table 8: Different part and type of the *Calotropis procera* used

part of *Calotropis procera* Used	**Fruit**		**Leaves**		**Stem**		**Rout**	
	Yes	No	Yes	No	Yes	No	Yes	No
	2	38	22	18	39	1	1	39
type of *Calotropis procera* used	**Old specimen**		**Young specimen**		**Both specimen**			
	22.5%		57.5%		20%			

4.2 Proximate composition

All the results of proximate analysis were reported on wet basis

4.2.1 Milk proximate composition

Proximate composition of milk collected from different breeds of cattle in Kenya and Benin is presented in Table 9. The milk moisture content was significantly different ($P \leq 0.05\%$) from one breed to another. The Friesian breed had the highest moisture content at 89.63% followed by Girolando breed collected from Mono region in Benin at 89.14%. This was followed by milk from Ayrshire breed from Juja Kenya whose milk water content was at and 88.60%. Milk from the Borgou breed from Borgou region in Benin had the lowest milk water content at 81.78%.

The ash content from different breeds and regions differed significantly ($p \leq 0.05$). It was highest (0.70%) for Borgou breed from Collines region in Benin, then came in

the Girolando breed (0.69%) from Mono region in Benin. The lowest ash content (0.31%) was obtained from the breed Ayrshire collected in Juja in Kenya.

The milk protein content varied amongst breeds and was highest for the Lagunaire breed from Zou region in Benin at 2.44%. This was followed by Friesian breed at 2.33% with the lowest protein content being observed in Girolando breed from Mono region in Benin. Significant difference was observed between and amongst all milk samples except between Borgou breed from Collines region in Benin and Ayrshire breed from Kenya.

Borgou breed from Borgou region in Benin had the highest milk fat content at 7.5% and was significantly different ($p \leq 0.05$) from all other milk samples in the study. The lowest milk fat content was obtained from Girolando breed in Mono region in Benin. The highest milk carbohydrate content of 6.12% was obtained from Lagunaire breed. The lowest carbohydrate content was obtained from the Friesian breed at 3.21%. The highest milk lactose content 2.83% was registered from Borgou breed while the lowest was recorded from Ayrshire breed.

Table 9: Proximate and Lactose composition of milk from all breeds of cows

Breeds	%M.C	%ASH	%Protein	%Fat	%CHO	%Lactose
Girolando Mono	89.14±0.30^{d}	0.69±0.00^{cd}	1.32±0.01^{a}	2.15±0.05^{a}	4.38±0.12^{b}	2.53±0.03^{d}
Borgou Girolando	86.38±0.65^{c}	0.62±0.00^{b}	2.01±0.03^{c}	3.75±0.05^{b}	3.61±0.29^{a}	2.52±0.09^{d}
Borgou-Borgou	81.78±1.73^{a}	0.63±0.00^{bc}	1.73±0.04^{b}	7.5±0.0^{e}	5.38±0.24^{c}	2.37±0.04^{c}
Collines-Borgou	86.57±1.34^{c}	0.70±0.01^{d}	2.25±0.24^{d}	3.95±0.25^{b}	4.91±0.38^{bc}	2.83±0.03^{e}
Zou-Lagunaire	84.19±0.41^{b}	0.67±0.03^{bcd}	2.44±0.03^{e}	5.1±0.2^{d}	6.12±0.55^{d}	2.02±0.03^{b}
Friesian	89.63±0.23^{d}	0.68±0.05^{bcd}	2.33±0.01^{de}	4.25±0.25^{c}	3.21±0.20^{a}	2.47±0.03^{cd}
Ayrshire	88.60±0.77^{d}	0.31±0.05^{a}	2.17±0.05^{d}	4.93±0.12^{d}	4.42±0.09^{b}	1.84±0.07^{a}

M.C: moisture content; CHO: Carbohydrate; Values are mean ± Standard deviation; mean followed by the same letter in in the same column are not significantly different at P value ≤ 0.05

4.2.2 Cheese proximate composition

There was significant variation between the moisture content of the different cheeses $p \leq 0.001$ (Table 10). The cheese obtained from the milk of breed Girolando had the highest moisture content (68.36%) while the lowest moisture contents is obtained from the cheese obtained from milk of breed Ayrshire (56. 25%). There was no significant variation between the ash composition of the cheeses from milk of breed Girolando collected in Mono and Borgou, milk of breed Lagunaire and milk from breed Borgou collected in department of Borgou. A significantly higher ash content composition (2.36%) was obtained in the cheese from milk of breed Borgou

collected in Department of Collines in comparison to that obtained from milk from other breeds.

The analysis of the results also indicates significant difference between the protein composition of cheeses obtained from all breeds ($p \leq 0.001$). Protein level significantly higher was obtained in cheese from the milk of breed Ayrshire (13.67%) in comparison to cheese from the milk of other breeds. Results also showed that significantly higher fat content was obtained from the cheese of the breed Ayrshire's milk (13.67%) followed by breed Friesian's milk (12.81%) both collected in Kenya. while the lowest fat content (3.02%) was obtained in cheese from breed Girolando's milk collected in department of Mono in Benin. The carbohydrate content (5.17%) obtained from the cheese of breeds Girolando's milk collected in Department of Borgou was significantly higher than that of other four breeds. Lactose content from the breed Lagunaire collected in Department of Zou and breed Borgou collected in department of Borgou was significantly lower than that from the milk of the others breeds.

Table 10: Proximate and lactose composition of cheese from milk of all breeds of cows from Benin and Kenya

Breeds	%M.C	%ASH	%Protein	%Fat	%CHO	%Lactose
Mono-Girolando	68.36±1.03^{d}	1.58±0.08^{a}	9.11±0.08^{c}	3.02±0.31^{a}	4.61±1.22^{bc}	4.26±0.09^{b}
Borgou-Girolando	62.89±1.40^{b}	1.50±0.06^{a}	8.59±0.66^{ab}	4.54±0.11^{b}	5.17±0.03^{c}	4.12±0.08^{b}
Borgou-Borgou	57.34±0.09^{a}	1.46±0.30^{a}	8.03±0.77^{a}	4.52±0.09^{b}	3.77±0.29^{b}	2.90±0.08^{a}
Collines-Borgou	64.59±0.70^{c}	2.36±0.01^{c}	8.79±0.80^{bc}	4.22±0.21^{b}	4.79±0.44^{bc}	4.16±0.10^{b}
Zou-Lagunaire	62.41±1.21^{b}	1.41±0.09^{a}	8.18±0.09^{a}	7.11±0.46^{c}	4.01±0.79^{b}	3.00±0.02^{a}
Friesian	57.20±0.61^{a}	2.01±0.04^{b}	11.89±0.16^{d}	12.81±0.47^{d}	2.27±0.03^{a}	4.37±0.54^{b}
Ayrshire	56.25±0.74^{a}	2.04±0.02^{b}	13.00±0.38^{e}	13.67±0.41^{e}	2.34±0.03^{b}	4.49±0.30`^{b}

M.C: moisture content; CHO: Carbohydrate; Values are mean ± Standard deviation; mean followed by the same letter in in the same column are not significantly different at P value =0.05

4.2.3 Whey proximate composition

Whey has a high moisture content than milk and cheese (Table 11). The moisture content (96.17%) obtained in whey from the breed Girolando's milk collected in department of Mono was significantly higher in comparison to the other breeds

The highest ash content (1.03%) was obtained in the whey from the breed Borgou's milk collected in department of Collines while the lowest value (0.30%) was obtained in whey from the breed Girolando's milk collected in department of Mono this is justified by the fact that this had the higher moisture content.

The highest protein content (1.22%) was obtained in the whey from the breed Girolando's milk collected in department of Borgou while the lowest protein content (0.44) was obtained in whey from the breed Girolando's milk collected in department of Mono.

Fat content vary from one breed to another. The highest fat content (1.58%) was obtained in whey from the breed Ayrshire's milk while the lowest value (0.33%) was obtained in whey from the breed Girolando's milk collected in department of Mono.

Table 11: Proximate and lactose composition of whey

Breeds	%M.C	%ASH	%Protein	%Fat	%CHO	%Lactose
Mono Girolando	96.17±0.18^{e}	0.30±0.00^{a}	0.44±0.02^{a}	0.33±0.00^{a}	3.74±0.57^{ab}	0.24±0.00^{a}
Borgou Girolando	91.68±0.20^{a}	0.52±0.01^{ab}	1.22±0.06^{f}	1.36±0.26^{d}	6.18±0.07^{d}	2.06±0.00^{e}
Borgou-Borgou	93.94±0.22 ^{d}	0.52±0.45^{ab}	1.14±0.06^{e}	0.96±0.24^{c}	4.27±0.34^{b}	0.99±0.00^{b}
Collines-Borgou	92.76±0.02^{bc}	1.03±0.02^{c}	0.66±0.08^{cd}	0.59±0.02^{b}	5.45±0.36^{c}	2.48±0.00^{f}
Zou-Lagunaire	93.00±0.09^{c}	0.37±0.02^{ab}	0.70±0.03^{d}	0.93±0.30^{c}	3.48±0.16^{a}	1.36±0.00^{cd}
Friesian	92.57±0.11^{b}	0.50±0.00^{ab}	0.53±0.09^{b}	0.74±0.00^{b}	3.42±0.36^{a}	1.41±0.03^{d}
Ayrshire	92.70±0.38^{bc}	0.54±0.00^{b}	0.60±0.02^{bc}	1.58±0.11^{e}	4.01±0.64^{ab}	1.27±0.07^{c}

M.C: moisture content; CHO: Carbohydrate; Values are mean ± Standard deviation; mean followed by the same letter in in the same column are not significantly different at P value =0.05

4.3 Texture analysis

The cheeses from the breed Girolando milk had significantly higher extrusion forces 0.2N and 0.17N than cheeses from the Breed Borgou 0.15N and 0.13N and then breed Lagunaire 0.007N collected in Benin. This confirms the fact that the cheeses produced from the milks of the breed Lagunaire are not more consistent than the cheeses produced from the milk of breed Girolando and breed Borgou.

Table 12: Firmness of cheese sample

Samples	Firmness (N)
Mono girolando	0.2±0.01
Borgou girolando	0.17±0.005
Borgou Borgou	0.15±0.01
Collines Borgou	0.13±0.01
Zou Lagunaire	0.07±0.01
Friesian	0.18±0.01
Ayrshire	0.17±0.005

Values are mean± standard deviation, N: Newton

4.4 Phytochemical analysis

4.4.1 Phytochemical screening

Phytochemical screening Calotropis procera (Stem and Leaves)

The results of the phytochemical screening on the *Calotropis procera* (Leaves and Stem) are presented in Table 13.

Table 13: Phytochemical screening of the stem and leaves of *Calotropis procera* collected from Benin and Kenya

Phytochemicals	Leaves from Benin plant	Stem from Benin plant	Leaves from Kenya plant	Stem from Kenya plant
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Phenols	++	+	+++	+
Alkaloids	+++	+++	++++	++++
Cardiac glycosides	+	++	++	+++
Saponins	++	+	++	+
Steroids	++	+	++	+

- not detected + detected ++ more colored +++ more intensify ++++ more intensify colored

Phytochemical screening of *Cheese and whey*

The same phytochemical screening analysis was done for the cheese and the whey and any phytochemical was detected.

4.4.2 Quantitative phytochemical analysis

Quantitative phytochemical analysis Plant *(leave and stem)*

The quantitative phytochemical analysis of the plant (leaves and stem). are presented in Table 14. Tannins levels were higher in the leaves (0.030-0.034g/100g) than in the stem (0.016-0.019g/100g). Phenols were also higher in the leaves both from Kenya (0.337g/100g) and Benin (0.3g/100g) than the stem from both Kenya (0.111g/100g) and Benin (0.122g/100g). However, the flavonoids levels were higher in the leaves from the Benin (3.67g/100g) than the Kenya leaves (2.97g/100g). The Flavonoids level was also higher in Benin stem (1.01g/100g) than the Kenya stem (0.67g/100g).

Table 14: Quantitative phytochemical analysis results for *Calotropis procera* (leaves and stem)

Phytochemicals	Leaves from Benin plant	Stem from Benin plant	Leaves from Kenya plant	Stem from Kenya plant

Tanins (g/100g)	0.030±0.013	0.016±0.002	0.034±0.012	0.019±0.010
Phenols (g/100g)	0.3±0.00	0.122±0.001	0.337±0.001	0.111±0.001
Flavonoids (g/100g)	3.67±0.26	1.01±0.08	2.97±0.04	0.67±0.071

Mean± Stdv

Quantitative phytochemical analysis Cheese

The quantitative phytochemical analysis of the cheese showed that the cheese samples contain only flavonoids. The cheese obtained from the breed Borgou collected from the department of Borgou has the highest content of flavonoids, followed by the breed Borgou collected from department of Borgou.

Table 15: Quantitative phytochemical analysis results for cheese

Phytochemicals	**Friesian cheese**	**Ayrshire cheese**	**Mono Girolando**	**Borgou Girolando**	**Borgou Borgou**	**Collines Borgou**
Flavonoids	0.56±0.023		0.49±0.012	0.86±0.0142	0.64±0.012	0.59±0.033

(g/100g)	
Tannins	Not detected with the machines
Phenols	Not detected with the machines

Mean± Stdv

CHAPTER FIVE: DISCUSSION

5.1 Cheese production in Benin

Cheese production is common among the Peulh ethnic group in Benin. During the survey, most of the respondents were women. This suggests that traditional cheese making in Benin is conducted by women. The cheesemakers prefered to use leaves and stem of *Calotropis procera* to make the cheese (because their coagulant effect is

higher). This is similar to what the study of Cakpo-Chichi (2004) who found that the leaves and the stem of the *Calotrpis procera* have higher coagulant effect than the fruits and the roots. More than 57% of the respondents preferred the use of the young specimen of the plant because according to them it yield to high percent of cheese and the sap which is the base of coagulation is higher in the young specimen of the plant. Some of the respondents were not aware about the toxicity of the plant *Calotropis procera.* Rather, they used the plant in traditional medicine to cure some disease such as cough, fever. Some reported that it had been used as protection against witchcraft or to chase any evil or bad spirits. Some respondents were aware of the toxicity and reported that the latex is very dangerous for the eyes and it can cause the skin inflammation.

5.2 Proximate composition in milk, cheese, and whey

The present study investigated the proximate composition of milk from different breeds and geographical provenances, cheese and whey processed from the milk samples. Proximate composition of the samples varied from one breed to another. Several factors (breeds, diet, geographical) may influence these differences levels of moisture content in milk of breeds studied. The high values (81.78% to 89.63%) of water content obtained for the breeds Girolando, Friesian and Ayrshire are similar those found by Vignola (2002), that is 85.5 to 89.5%. Indeed, a high water content could deteriorate the quality of the dairy product by promoting a rapid growth of microorganisms and a reduction in shelf life of the product (Aisso et al 2013).

The ash content of all breeds range from 0.31% to 0.70% which is slightly lower than that found by Bourgeois (1990) which was 0.90%. This might be due to the

breeds, the sampling and samples storage condition. The ash content is an indicator of the total mineral content of the milk. So it can be concluded that the breed Borgou located in Department of Collines and the breed Girolando located in Department of Borgou had the higher minerals content.

The protein content of all the breeds was lower than that obtained by Vignola (2002). This differences may also be due to samples collection, or storage (freezing) methodology or the breed. Fat content in some breed milk was higher than the fat content of all breeds was relatively higher what was obtained in other breeds from other countries. Indeed, Abakar (2012) obtained a fat content of 2.89% while Bourgeois *et al.* (1990) obtained 3.90%.

The carbohydrate content of 6.12% obtained in breed Lagunaire is higher from the one (5.51%) obtained by Kora (2005) for the same breed. All the remaining breeds' carbohydrate content that was within the range found by Vignola (2002) which is 3.6-5.5%. The concentration of carbohydrate varies during the lactation period (Amel *et al*., 2009; Aisso et al., 2013). So,this observed difference could be explained by the lactation period of the cows studied for each breeds. All the lactose content from milk of all breeds is lower than that obtained by Bourgeois *et al.* (1990).

The moisture content (68.36%) obtained for the different cheese samples was similar to the results obtained in previous studies in different breeds. For example, the highest moisture content (68.36%) obtained in breed Girolando during this study is very similar to that obtained by Aisso *et al*. (2013) and Kora, (2005). The lowest moisture content (56.25%) obtained in Ayrshire cheese is similar to that obtained by

Sacramento (2008). Cheeses with low water content will have a high shelf life due to the reduction of the microbial load. It can then be concluded that the cheese from the milk of breed Ayrshire is the best one Compare to the other cheese because it can be preserve for a longer period of time due to its relatively low water content.

The result of ash content (1.41% to 2.36%) are much similar 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 cheese. The cheese from the milk of breeds Friesian and Ayrshire breeds (both collected in Juja in Kenya) had the highest protein content respectively 11.89% and 13% which is similar to that obtained by Aisso *et al.* (2013) and 12.56% obtained by Sacramento (2008). The low values obtained in other breeds milk' cheese could be due to the fact that those cheese were stored for long before analysis. Protein content in cheese was higher than the content in milk. This is due to the concentration during coagulation. The behavior of a milk during coagulation varies essentially with the level of protein (Macheboeuf *et al*., 1993; Aisso *et al*. 2013). Abakar (2012) revealed that cheese yield depends on casein content in milk. The protein content obtained for the milk of the breeds Friesian and Ayrshire could contribute to a better cheese yield and help to improve protein supply in the diets of pastoral populations mostly in rural areas where it is difficult to get food rich in protein.

The highest fat content obtained from the cheese obtained from the breed Ayrshire's milk (13.67%) followed by breed Friesian's milk (12.81%) was similar to the value obtained by Mazou (2011) which was 11.44%. Mazou (2011) stated that there is a decrease in lipid levels in the cold-preserved wagashi (cheese) because there is a

leaching of the cheese during its passage at room temperature and by the migration of fat to the superficial layer of the cheese.

. The lower fat content observed in other breed could be due to the fact that they were frozen for long than the one from the Kenya.

Carbohydrate content of cheese collected in Benin was similar to that obtained by Kora (2005); Sacramento (2008) and Aisso *et al.* (2013) who found respectively 5.23%; 6.90% and 4.92% in cheese. The lower carbohydrate content (2.27%) obtained in cheese from the Friesian' milk and cheese from Ayrshire's milk (both collected in Kenya) might be due to the geographical location of the breeds or to the diet of the animal. Cheese from those two breeds can therefore be recommended for people requiring low carbohydrate diet

The values obtained for moisture content in whey are similar to those obtained by Tsakali *et al.* (2010) and Benslama (2016) who found respectively (93-94%) and 94%. The significant variation obtained in the moisture content from all breeds show that the breed, the animal diet and the geographical situation can affect the composition of the whey. The highest ash content (1.03%) was obtained in the whey from the breed Borgou's milk collected in department of Collines while the lowest value (0.30%) was obtained in whey from the breed Girolando's milk collected in department of Mono. This may be explain by the fact that the milk from that breed had the higher moisture content. Since the ash content determines the mineral content, it can then conclude that the whey from the breed Borgou's milk collected in department of Collines has the higher moisture content. The protein content values from 0.44% to 1.22% obtained are similar to those obtained by Tsakali *et al.* (2010) (0.8-1%).

The results also showed that the composition of the protein in whey vary according to the breeds. The highest fat content (1.58%) was obtained in whey from the Ayrshire's milk while the lowest value (0.33%) was obtained in whey from the breed Girolando's milk collected in department of Mono. In general, it was noted that whey has a very low fat content. This could be explained by the fact that almost all of the milk fat is retained in the curd. This confirm what Benslama (2016) stated that the fat in whey represent only 0.7% of whey dry matter. Carbohydrate and lactose level vary from one breed to another. According to Benslama (2016), after water, the second major component of whey is lactose. Our results confirm that state since after moisture content, carbohydrate and lactose are the highest parameters obtained in the whey from each breed expect for the breed Girolando collected in department of Mono which has lower lactose content. In general, whey is a product rich in nutrients and therefore should be valorized since there is still much to shoot from it. The whey has the nutritive value which can be exploited in food technology to have another by-product from it instead of throwing it away

5.3 Texture of cheese samples

Cheese obtained from the breeds Girolando, has the highest firmness of 0.20 N (Table 10). The cheese obtained from the breeds Lagunaire has the lowest firmness of 0.07N. This is contrary to AISSO *et al.* (20013) observation who found that the cheeses from breed Borgou are more firm than cheese from breed Girolando. This difference could be due to the source and composition of the milk but also the technology of production of the cheese.

This result is different from the one obtained from the breeds in Kenya. The cheese obtained from Friesian breeds has high firmness 0.18N than the Ayrshire breed 0.17N. These differences in firmness can be explained by the denaturation of the texture of Wagashi during the formation of ice since the samples were kept at -20ºC. Mazou (2001) stated the solidification of the water causes an expansion of its volume. This expansion favors the denaturation of the protein molecules of wagashi (cheese). The change in the structure of protein molecules causes friable wagashi (cheese) resulting in a reduction of their resistance to extrusion.

5.4 Phytochemical analysis

5.4.1 Phytochemical screening

Phytochemical screening in *Calotropis procera*

Table 13 shows the results of the qualitative analysis of the plant collected from Benin and Kenya. Tannins and flavonoids presence were just detected (+) in all the plant sample (stem and leaves). The results are different from what was found by Morsy *et al.* (2016) who found that the flavonoids and tannins are not present in the leaves and stem of *Calotropis procera.* This could be due to the geographical localization of the plant or to the extraction methods. The content of phenols detection was higher (++) in Benin leaves and in Kenya leaves(+++) than in both Benin and Kenya stem (+). However, the alkaloids were more intense (+++) in both leaves and stem from Benin, and also the leaves and stem from Kenya plant (++++). The Cardiac glycosides were detected in leaves from Benin, in both stem from Benin and leaves from Kenya (++), and also in stems of Kenya plant (+++). The saponins

and steroids were detected in stems from Benin and Kenya plant (+) and also in both leaves from Benin and Kenya plant (++). Those results confirm the literature and some study done by many authors included shaker et al (2010), Ahmed et al (2005) and Elimam *et al.* (2005) who found that *Calotropis procera* has biologically active substances such as flavonoids, cardioactive glycosides, alkaloids, tannins and saponins.

Phytochemical screening in Cheese and the whey

The absence of phytochemicals especially the cardiac glycosides (toxic component) in cheese and whey could be due to the methodology used for screening or maybe the cheese and the whey does not contain the phytochemical due to the fact of the temperature of cheese production (≥100ºC), the toxins may have decomposed during the cheese's boiling. This make the cheese and the whey safe for consumption. However more investigation need to be done. Otherwise it was deduced that the process of cheese and/or whey processing denatures the phytochemicals, thus assuring the safety of the products. However, further studies exploiting more sensitive analytical methods are required to confirm these findings especially on those phytochemical to understand well their behavior at high temperature.

5.4.2 Quantitative analysis *Calotropis procera* Cheese and the whey

The Tables 14 and 15 show the result of the quantitative phytochemical analysis in the plant (*Calotropis procera*) and cheese. From Table 12 tannins levels are higher in the leaves (0.030-0.034g/100g) than in the stem (0.016-0.019g/100g). Those values

are lower than those obtained by Doshi *et al.* (2011) who found respectively 0.8g/100g and 0.6g/100g in leaves and in stem of *Calotropis procera* in India

Phenols were also higher in the leaves both from Kenya (0.337g/100g) and Benin (0.3g/100g) than the stem from both Kenya(0.111g/100g) and Benin (0.122g/100g). However, those values are still lower than those obtained by Doshi *et al.* (2011) who found respectively 1.3g/100g and 1.8g/100g in leaves and in stem of *Calotropis procera* tested in India1. Chemically, phenolics are polyphenols which are important secondary metabolites present in plants having various beneficial effects including antioxidant potential.

Regarding to the flavonoids levels, they were higher in the leaves from the Benin (3.67g/100g) than Kenya leaves (2.97g/100g). The Flavonoids level were also high in Benin stem (1.01g/100g) than the Kenya stem (0.67g/100g). However, those values are still lower than those obtained by Kumar *et al*. (2013) who found 1.62g/100g in *Calotrpis procera.*

From the table 15, it is only the flavonoids which were detected in all cheese except cheese from the breeds Ayrshire, this could be due to the amount of *Calotropis procera* used in production of that cheese (50-100g) for 5L. But when doing the phytochemical screening those flavonoids were not detected in both cheese and whey.

CHAPTER SIX: CONCLUSIONS

6. Conclusions

From all this result it can be concluded that milk is very important source nutrients and its composition vary sometimes from one breed to another and from one country to another

The general objective of this study aimed at assessing nutritional quality and safety of processed *Wagashi* cheeses with milk from different cow breed and *Calotropis procera* in Benin and Kenya in order to improve *Wagashi* quality and to protect consumer health, the following conclusions can be drawn:

i. Milk samples collected from Benin and Kenya contained biochemical components such as Protein, Fat, Carbohydrates, Lactose, Water and Ash which are very important for human daily ration. The biochemical constituents of milk varied from one breed to another.

ii. *Calotropis procera* is plant contains many phytochemicals such as tannins, phenols, alkaloids, steroids, flavonoids and cardiac glycosides (toxins). Results showed that C. procera collected from Kenya contained higher phytochemical levels as compared to the ones from Benin.

iii. The *Calotropis procera* is a plant that is widely used in traditional medicine to cure some diseases such as cough, fever, or as protection in Benin by the Fulani people. It has a very strong coagulation effect therefore it brings an added value to the constituents of milk into cheese. The cheese can be there used to replace fish, chicken in especially in Kenya were the milk production

is higher. The whey obtained after the production of Fulani cheese also contained very rich constituents, therefore it can be used for other purposes, such as for instance in animal feed, use in the food industry to obtain other by-products.

iv. Even if the plant (*Calotropis procera*) contains toxic substances (cardiac glycosydes), results showed in this study that toxins were not detected in cheese and in whey which might be due to heat reaction during production process.

CHAPTER SEVEN: RECOMMENDATIONS

7. Recommendations

From this work and its findings, the following recommendations will be suggested for future studies for the nutritional quality and safety of processed *Wagashi* cheeses with milk from different cow breed and *Calotropis procera* to improve *Wagashi* quality and to protect consumer health:

i. To carry out a quantitative study according to toxin standards on the phytochemical composition of the *Calotropis procera* and on the cheese and whey to ascertain their safety level therefore Kenyan population can be sensitized to the utilization of *Calotropis procera* in cheese production which is a sustainable way of milk preservation

ii. To develop a method of coagulant extraction from the plant instead of using the stem and leaves in cheese production.

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APPENDIXES

Appendix 1: Questionnaire

This questionnaire should be completed by cheese makers in different department in Benin in order to complete research work.

Date and time of interview _____ at _____

date time

Department :_____

township: _____

Village : _____

Section 1 – Socio-demographical background of the respondent

1. Sex

☐ F ☐ M

2. Age :

☐ Below 20 yrs ☐ From 20-35yrs ☐ Above 35yrs

3. Ethnic group:

☐Fon ☐ Min☐ Adj☐ Ditar☐i La☐ba Na☐nba M☐rmè Wama

☐ Peulh ☐Others(Specify)

4. Level of education of the interviewer:

☐No formal education ☐ Primary school ☐Secondary school ☐Tertiary education

5. Average literacy of the interviewer:

☐ Illiterate ☐ Literate

Section 2 –Used raw materials for *wagashi* production

6. Which kind of milk do you use for wagashi?

☐Fresh milk ☐ Powder milk ☐ Mixture of the two

7. Justify your answer

8.From which animal comes the fresh milk used ?

☐ Cow ☐ Ewe ☐ Goat

9. Do you buy the milk?

☐ Yes ☐ No

10. If yes where do you buy the fresh milk?

11. If No how do you obtained it?

12.Which plant do you use to coagulate milk during wagashi production?

☐ *Calotropis procera* ☐ Others (Specify)

13. Do you use other raw materials in the production of wagashi?

☐ Yes ☐ No

14. If yes which ?

15.Are you aware about some case of toxicity from the *Calotropis procera*

☐ Yes ☐ No

16.If yes, give some cases

17. How do you collect the plant? ☐ Directly ☐ gloves ☐ Equipment of gathering ☐ other (specify)

18. At what time of the day do you gather the plant?

☐ Morning ☐ Afternoon ☐ Evening whenever

19. Which part of the plant do you use?

☐ fruit ☐ leaves ☐ stem ☐ root ☐ All

20.Which kind of plant do you prefer?

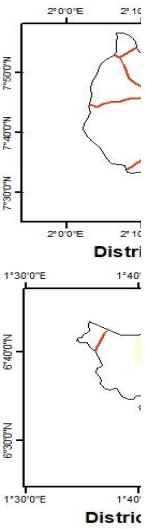
☐ Young ☐ Ancient ☐ Anyone

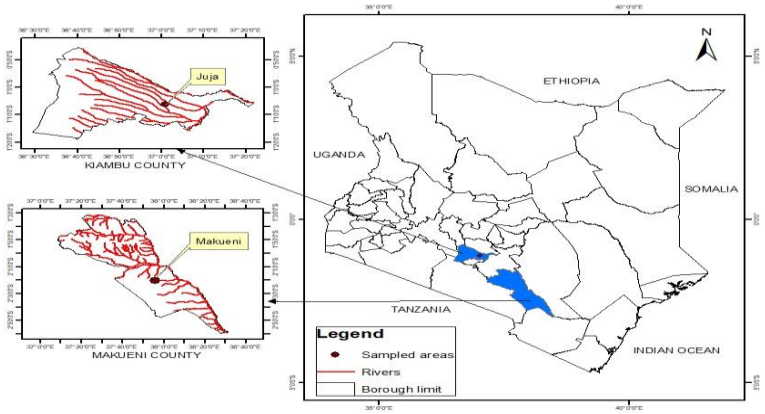
21. What are the other uses of *calotropis procera*

☐ Traditional medicine ☐ others (specify) ☐ don't know

22. For which types of diseases is *Calotropis procera* used in traditional medicine

Appendix 2: Sampling areas in Kenya and Benin





Appendix 3: Different steps of cheeses making.

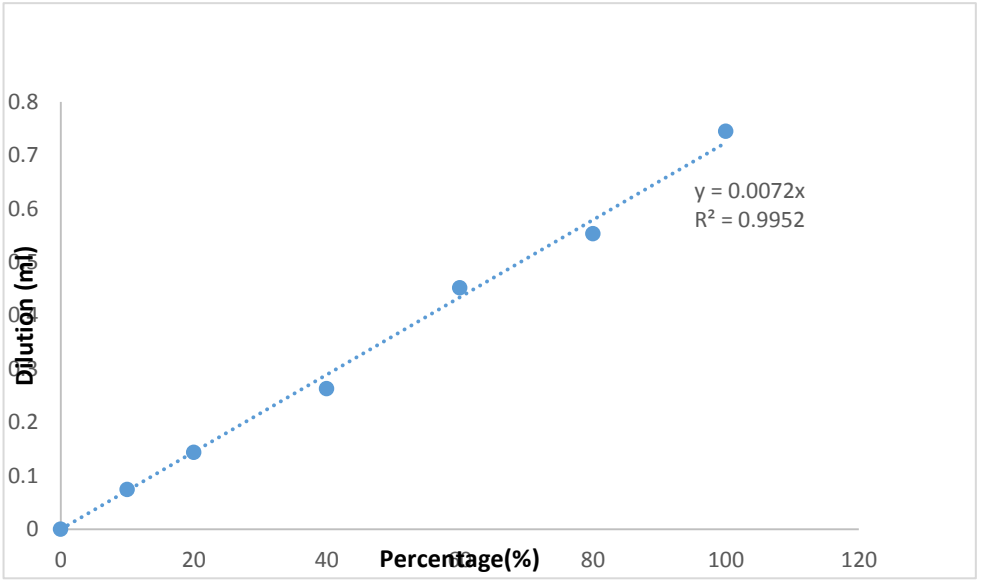
Milk for cheese making

The stem and leaves of C. procera

Adding C. procera to the milk

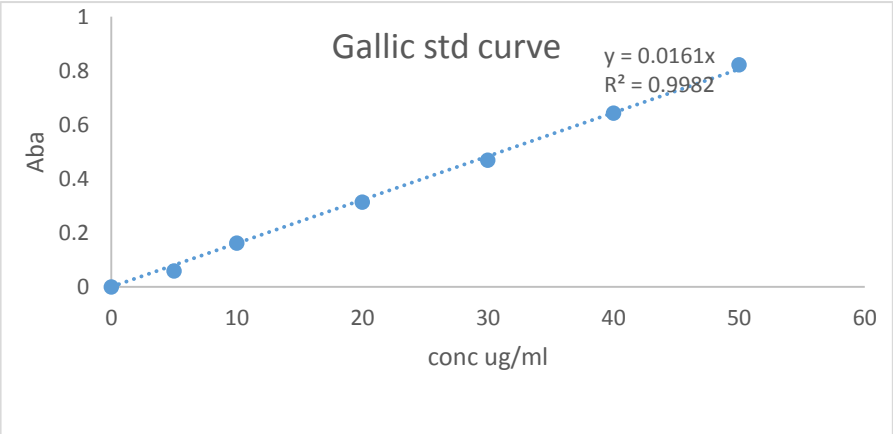
Coagulating step

Sieving *Wagashi* (cheese)

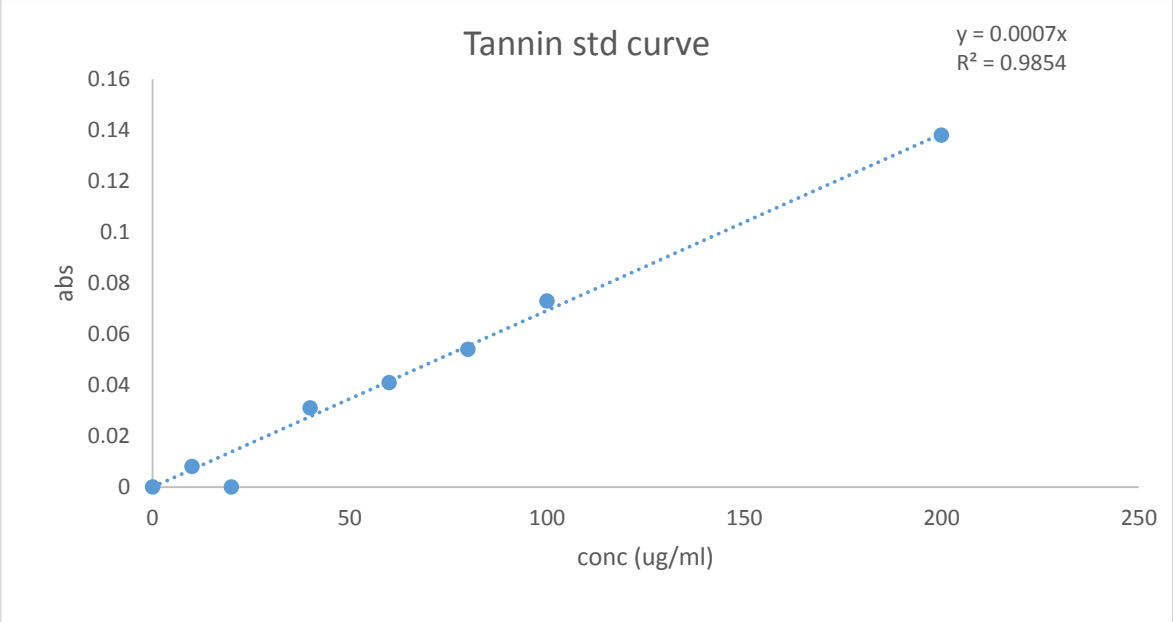


Appendix 4: Carbohydrate standard curve

Appendix 5: Phenols standard curve



Appendix 6: Standard curve for tannin



Appendix 7: Standard curve for Flavonoids