

**UNIVERSITY OF GHANA**  
**COLLEGE OF BASIC AND APPLIED SCIENCES**

**COAGULATING POTENTIAL OF FRESH COW MILK FROM DAIRY  
CATTLE IN GHANA AND THE ECONOMIC VALUE OF PROCESSING  
CHEESE**

**BY**

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## **DECLARATION**

I declare that this work was conducted by me under supervision in the Department of Nutrition and Food Science, University of Ghana, Legon.

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## **DEDICATION**

I dedicate this research work to the Almighty Lord for how far He has brought me, my parents:

Mr. John Aboagye and Mrs. Agatha Aboagye and my best friend Mr. Alfred Agbekudzi.

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## **ABSTRACT**

Milk is a nutritious food obtained from ruminants such as cattle, goats and sheep. In Ghana, milk is usually obtained from indigenous cattle. These have low milk yield hence the introduction of hybrids and exotic breeds. Embracing these new breeds has to an extent increased milk yield. The problem is that the yield is still not enough. Also, the milk obtained is usually sold in the fresh state which goes bad within a short time and is also patronized by few Ghanaians. For these reasons it is necessary to add value to fresh milk and one way of doing this is by processing cheese. This would provide farmers with some economic benefit and also bring more variety in processed milk products in the country.

Milks were collected from three different cattle breeds; White Fulani, Jersey and a Cross breed. Physicochemical analyses were done on the milks to compare them. After which each of the milks was coagulated with five different coagulants (Sodom apple, rennet, acid, Sodom apple + acid and rennet + acid) to ascertain their coagulating potential and physicochemical properties. Then, the milks were pooled and coagulated using the different coagulants to observe their cheesing characteristics and protein profile. Finally, economic analysis was done on the different cheeses to find out if it was viable to commercially produce cheese in Ghana. One-way ANOVA was used to determine if differences existed amongst the milks from the breeds and the cheeses made from the different coagulants. Two-way ANOVA was used to determine the interaction between the breeds and coagulants for the various indices measured for the coagulum obtained.

It was observed that the physicochemical properties of the milks from the breeds were similar in fat and protein content. Milk from the White Fulani took the shortest time to coagulate and milks coagulated with Acid first had the least coagulation time. The highest coagulum yield was obtained from milk processed with Sodom apple and milk from the Jersey cow. Coagulum made with Acid

had the highest meltability and coagulum from Sodom apple was highest for 7 out of 10 sensory attributes. Amongst all the coagulum, the ones processed from Rennet + acid and Sodom apple + acid had the hardest texture with cheese from Sodom apple being the softest. For the protein profile, Rennet only had all the caseins being hydrolysed. Acid only and Sodom apple + acid showed similar results. All three caseins were not hydrolysed. The economic analysis showed that the cheeses if processed commercially can be sold at the following prices: Rennet only - GH¢ 8.00, Acid only - GH¢ 16.00, Sodom apple only - GH¢ 6.00, Sodom apple +acid - GH¢ 12.00 and Rennet +acid - GH¢10.00.

Cheese can be obtained from the breeds analyzed using different coagulants and it would be viable and profitable to produce cheese commercially in Ghana.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Cattle Rearing in Ghana

According to the Country's Fact Sheet on Food and Agriculture Policy Trends (n.d), Ghana's economy largely depends on agriculture. This sector encompasses both crops and livestock. Livestock are rich sources of protein in human diet. Examples of livestock reared in Ghana include ruminants like cattle, goats and sheep and non-ruminants like pigs and poultry. Cattle can be bred in any part of the country but are predominantly in the Northern part. There are fewer cattle rearing areas in the Western part of the county due to the higher incidence of tsetsefly in these areas. The three Northern regions are responsible for 75% of the total cattle bred in the country (Adzitey, 2011). The cattle found in Ghana include indigenous breeds (Ghana Sanga, White Fulani, Sokoto Gudali); exotic breeds (Jersey) and cross breeds or hybrids (Friesian-Sanga). The exotic breeds are usually reared for milk and the indigenous for both meat and milk but mostly for meat. The indigenous breeds produce very low quantity of milk as compared to the exotic breeds (Aboagye, 2002; Animal genetic training resource n.d).

In Ghana, the milk produced is usually used to feed the calf, feed the farmer's family and the surplus, which is very little, is sold in the informal market (Aboagye, 2002).

Milk is a nutritious food and can be processed into various products (Pereira, 2014). Locally, milk is processed into various products such as yoghurt, burkina, boiled milk and wagashie. Processing of milk adds value to it and can make the milk market more attractive to farmers, processors and sellers. One lucrative area for adding value to milk is cheese making. Cheese just like its raw material, milk, is nutritious but has a longer shelf life as compared to milk. It has concentrated amount of proteins, fat, and minerals (Pereira, 2014). A study showed that caseins which make up

cheese have antihypertensive effect and help in weight control by influencing intestinal motility. This allows food to move slowly in the gastrointestinal tract, leading to delayed sensation of hunger in the consumer. (De Noni & Cattaneo, 2010). Cheese is used to prepare many foods in the world and these foods are gradually but steadily finding their way on our Ghanaian menus. Examples are pizza, cheese burgers, cheese cake and lasagna. Some cheeses produced around the world include Gouda, cottage cheese, cheddar cheese, mozzarella, feta and Quarg cheese.

## **1.2 Problem statement**

Currently, many Ghanaian dairy farmers are making efforts to scale up their milk production in order to increase their profits. A one on one interview with Mr. Frimpong, the Deputy Director of Amrahia Dairy Farms, revealed that some farmers have been introduced to rearing the exotic breeds while some have hybrids of indigenous and exotic breeds. This is increasing the milk yield hence making milk more available. A visit to some farms in the Greater Accra and Eastern region of Ghana showed that the indigenous breeds produce milk between 1L to 5L a day and those with the hybrids and foreign breeds are able to produce milk between 2L-20L. This clearly shows an increase in the milk yield as compared to the indigenous breeds provided there is good animal rearing management and feeding in particular. There is however, still not enough milk to meet the growing demand for milk and milk products in Ghana. Farmers are also not able to keep the milk for long because of its short life span and lack of chilling equipment. Furthermore, studies have shown that Ghanaians do not patronize fresh milk as compared to other countries (Gate Way to Dairy Production and products (2018); Smallholder Dairy Production and Marketing- Opportunities and Constraints,(2001) and Aidoo, *et al.* (2009) found that consumers preferred processed milk to fresh milk. This means farmers cannot gain much from the sale of fresh milk only.

One way of making fresh milk more beneficial is by adding value to it. Value addition improves the product and makes it more economically beneficial. A case study in Dakota showed that a group of dairy farmers earned more when they processed their raw milk into cheese and ice cream (Coltrain, Barton, & Boland, 2000). There are various ways of adding value to our milk, and processing milk into cheese is one of them. Currently, wagashie, a soft cheese is the only cheese produced in Ghana and it is mostly found on the informal market. Casual one-on-one interviews with wagashie sellers around Accra, revealed that selling wagashie is lucrative for people in the informal market. Those who sell the cheese can make almost 50% profit when it is sold fried, also depending on the type of market and the demand for the product, more profit can be made (unpublished data). According to the UK Dairy Council cheese fact sheet (2017), 10L of fresh milk can produce 1kg of cheese. Informal price checks in some supermarkets in Accra showed that 100g of cheese is sold on average at GH¢14.00 and 1L of fresh unpasteurized milk is sold on the informal market at approximately GH¢4. This suggests that if a farmer makes 1kg of cheese from 10L of milk, it could be sold for GH¢140.00 whereas, that same 10L of milk will be sold for only GH¢40. Apart from being economically beneficial to farmers, the cheeses can now be sold on the formal market attracting even more revenue to the farmer and making dairy products accessible to the middle and upper class who can afford them but because of perceptions of safety avoid local dairy products. Although, the cost of processing has not been factored into these estimations, there seems to be a comparative profitability in processing the milk into cheese as opposed to selling the milk in its fresh form.

While considering adding value to cow milk in Ghana, it also necessary to find out if the milks have the potential of being made into cheese in the first place. This is because some cows produce milk that cannot clot. Clotting is the first step in cheese making hence, a very important factor in

cheese making milk. In order for milk to be considered as one that can be used for cheese processing, it should be able to clot within 30 minutes after adding the enzyme rennet (Troch *et al.*, 2017).

### **1.3 RATIONALE**

Cheese lasts longer than fresh milk and can be sold at a higher price than fresh milk. In addition, cheese is a versatile ingredient in many ethnic and continental cuisines and Ghanaians have embraced many of these recipes. A visit to various supermarkets in Ghana inevitably shows a range of imported cheeses and cheese-based products on the shelves. Also, popular restaurants like Pizza Inn, Burger King and Baritas always have at least a meal made from cheese featuring on their menus. These observations may be signaling an available and yet unexploited market for production of cheese in Ghana. Adding value to our milks by coagulating them for cheese would be beneficial to farmers, consumers, restaurants and sellers. This cheese would eventually have a longer shelf life, may be more acceptable than fresh milk, bring variety and add economic value to fresh milk.

To this end, this study focused on finding out whether milks from some locally bred cattle can be used to make cheese.

### **1.4 OBJECTIVES**

#### **1.4.1 Main Objective**

To investigate the cheesing potential of milk obtained from locally bred cows and determine the economic value of the process.

### **1.4.2 Specific Objectives**

To compare those physicochemical properties of the milk that influence coagulation.

To determine the yields and speed of coagulation of milks when different coagulants are used

To determine physicochemical properties of the coagulum obtained when different coagulants are used.

To determine some cheesing properties of coagulum processed from pooled milks from different cow breeds.

To explore the economic value of commercial cheese processing for milk obtained from locally bred cows

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Cattle Breeds in Ghana

Cattle are ruminants that are reared domestically for the purposes of milk, beef, hide for leather and meat and draught power on the farm (Felius, Koolmees, & Theunissen, 2011). In Ghana, there are two main types of cattle. They are the *Bos taurus* and *Bos indicus*. *Bos taurus* like the N'dama cattle are humpless and the *Bos indicus* like the White Fulani are humped. The indigenous *Bos taurus* breeds are almost only found in West Africa now. Currently there are six indigenous breeds of cattle in Ghana; West African Shorthorn, N'dama, Sokoto Gudali, White Fulani, Sanga and Muturu. Various hybrids (Friesian-Sanga, Jersey-Sanga) and a few exotic breeds (Jersey) can also be found in Ghana (MOFA, 2016). Almost all these breeds are milk producers except the Muturu. Indigenous cattle in Ghana are for dual purposes. They are generally reared for their meat and some milk is obtained from them too. Cattle in Ghana are low milk producers and compared to the exotic breeds and hybrids, they mature much slower. This flaw is accounted for by the low nutritional composition of their diet, poor management systems (Aboagye, 2002) and the fact that they are not solely dairy cattle.

##### 2.1.1 Types of Cattle

The West African Shorthorn, also called the Ghana Shorthorn or WASH are *Bos Taurus*. They are found all over the country but are mostly concentrated in the Northern and Ashanti region. West African shorthorns are small with long necks and heads. They also have short and thin horns. In terms of coat colour, the most common colour is black and white but there are a few with solid black, white and mottled black and white patterns. As *Bos Taurus*, they have underdeveloped dewlap and umbilical folds (Animal Genetic Training Resource, n.d). They are resistant to

trypanosomosis, tick-borne disease and are heat tolerant. West African Shorthorns produce the least amount of milk as compared to the other indigenous breeds. They are not able to let down milk without their calves and their milk yield ranges between 384L-774L for 182 to 295 days of lactation (Animal Genetic Training Resource, n.d). Rege, Aboagye & Tawah (2004), showed that they are able to produce more milk when provided with a healthier diet.

The N'dama cattle are *Bos taurus* with long horns. They are believed to have originated from Fouta Djallon in Guinea. Currently, they can be found in countries such as Senegal, Ghana, Togo, Nigeria, Cameroon and Gabon (Animal Genetic Training Resource, n.d). N'damas are medium sized cattle with short legs, short and broad heads and long and curvy horns. Their dewlap and umbilical folds are underdeveloped. Their coat colour is usually fawn but it can range from dark fawn, to grey, chestnut and to red with a black head. They are also intolerant to trypanosomiasis (DAGRIS 2005). In Ghana N'dama's provide both meat and milk but the milk produced is quite low (Animal Genetic Training Resource, n.d). In a year, a cow can produce 70L-100L of milk (FAO 1980). The full lactation yield is usually between 500L-600L (Portar, 1991).

Just as the name depicts, the White Fulani has a white coat with the ears, eyes, muzzle, hooves, horn and tail tips being black. They have a well-developed dewlap and hump. They have a long head with slender horns that are curved upwards with an outwards turn at the tips. They are generally tall and have a well-developed udder. White Fulanis' are heat tolerant and have a low mortality rate. Traditional owners usually keep them for milking purposes (Animal Genetic Training Resource, n.d). According to Tawah & Rege (1996), they are able to produce more milk as compared to other *Bos indicus*. Average milk yield per average lactation length of 220days is 627L-1034L (Animal Genetic Training Resource, n.d).

Sokoto Gudali are usually found in Benin, Ghana, Nigeria and Mali. They are one of five breeds from the Gudali family. Because of their hardy nature, they are able to survive in very dry conditions such as those experienced in the northern regions of Ghana (Animal Genetic Training Resource, n.d). The Sokoto Gudali cattle, are of the *Bos indicus* family with long head and long ears that are large and convex and are sometimes observed to be hanging. As their name Gudali implies in Hausa, they have short legs and short horns. Their coat colour is usually multi-coloured but the most dominating colours are black and white. Among all the indigenous cattle found in Ghana, they are the most efficient in milk production. A mean total of 1101.3L of milk can be obtained in 244.8 days of lactation (Animal Genetic Training Resource, n.d).

Sanga is a hybrid obtained by crossing the N'dama and the WASH. The Sanga Cross has been stabilized over the years hence it is now seen as a pure breed. This breed is mostly found in the Northern, Greater Accra and Volta regions of the country. In a personal communication with the Deputy Director at Amrahia Dairy Farms he expressed that the Sanga is one of the most common breeds found throughout the country because of its hardy nature. They have an undeveloped hump with a long head. They have a variety of horn sizes, but majority are small and slender. Their coat colour is usually variegated. A study conducted by Okanta (1992), showed that the average daily yield of milk was  $875 \pm 11$  ml.

Jerseys are exotic dairy cattle that are able to survive in the arid conditions of Ghana because they are heat tolerant. They were introduced into the country by an NGO (Heifer International) in 2008 (MOFA, 2016). Jerseys come in a variety of colours but the generally occurring colour is fawn with or without white marks. They have a small body size as compared to other dairy cattle (Paulson, Endres, & Reneau, 2015). They are able to calf at an early age because of their high fertility rate and produce about 6000L of milk per year. Milks obtained from the Jersey are very

high in protein and butterfat leading to a high solid content. The protein content is about 3.8% and that of the butterfat is 4.8% (Ontario Dairy Facts and Figures, n.d).

The Holstein-Friesians were believed to have originated from the Netherlands. They are a high milk producing cattle providing 90% of milk in the United States of America (Paulson et al., 2015). Their coats are usually black and white or red and white. They are able to produce about 8600L of milk per year (Ontario Dairy Facts and Figures, n.d). They were introduced into Ghana from Europe to improve the milk yield of our indigenous breeds, but they were not able to survive due to the climatic conditions in the country (Aboagye, 2002). They were later crossed with the Sanga via artificial insemination. These hybrids known as the Friesian-Sanga are still in existence in a few farms in the country.

## **2.2 Milk Composition and Physicochemical Properties**

Milk is a nutritious food obtained from the lacteal secretion of mammals such as cows, sheep, goats, buffalos and donkeys (Pereira, 2014).

### **2.2.1 Properties of milk from different dairy ruminants**

Milk from all ruminants have different milk compositions. They vary based on various factors such as genotype, breed, age, diet and stage of lactation (Schwendel et al., 2014). Generally, sheep and buffalo milk have higher dry matter: 18.6% and 17.75% respectively as compared to other ruminants. Goat milk has a dry matter content of 13.3% which is quite similar to that of cow milk at about 12.8%. That of the donkey is lowest at 10.8%. A similar trend is observed for the fat content. That of the buffalo and sheep is highest followed by the goat, cow and finally the donkey.

High total solids in sheep milk makes it very appropriate for cheese making. The same can be said for buffalo, cow milk and goat milk. Donkey milk is usually not used in cheese production because of its low dry matter. It generally has very low protein especially in caseins content (www.fao.org).

### **2.2.2 Properties of milk from different breeds of cows**

Breed of cattle has an influence on milk produced (De Marchi et al., 2008). Milk from *Bos indicus* have higher fat content as compared to *Bos Taurus*. This means that cattle breeds such as Sokoto Gudali and White Fulani should have higher fat content as compared to N'dama, Jersey, and WASH (beef2live.com). This is not always the case though. The genetic make-up of the breed also has an influence on the milk composition (De Marchi et al., 2008).

Most of the cattle bred in Ghana are for dual purpose, that is beef and milk, but some of the exotic breeds like the Jersey are purposely for milk. This means that the genetic make-up of the dairy cattle provides it with the added advantage of producing milk of better quality (Schwendel et al., 2014). Therefore, milk from the dairy cow would be slightly different from that of the dual purpose cow. For instance, the fat content of milk from White Fulani is 3.6% (Adesina, 2012) as compared to that of a Jersey which is around 4.6% (Heinrichs, Jones, & Bailey, 2016). Jerseys are known to have higher fat content as compared to their other dairy cow counter parts like the Holstein and Guernsey. The fat content of Jersey milk is 4.6%, Guernsey 4.51% and Holstein 3.65% (Heinrichs et al., 2016). There is usually a lot of variation in the fat content of cow milk as compared to the protein and ash content hence, the comparisons of the breeds based on fat content (Walstra, Wouters, & Geurts, 2006).

Milk obtained from cattle contains proteins, fat, minerals, vitamins, carbohydrates and water in different proportions based on the type and breed of animal as well as the management practices for rearing the animal (FAO, 2013).

### **2.2.3 Composition of cow milk**

Cow milk contains proteins, lactose, fat, vitamins and minerals. Protein content of milk is ranges between 3.2-3.8%. Milk proteins are very important to human diet and nutrition because they contain all the essential amino acids needed by the body. The proteins are also easily digestible and are bioavailable (Pereira, 2014). Lactose, which accounts for about 4-5% of milk is the sugar or carbohydrate found in milk. Lactose is made up of galactose and glucose (Pereira, 2014). It is not as sweet as sucrose hence the almost bland taste of milk. Depending on the breed of cow, the fat content of milk ranges from 2.5-5.5% ( Walstra, Wouters, & Geurts, 2006). This fat is made up of both saturated and unsaturated fatty acids. Averagely, the saturated fatty acids such as myristic and palmitic acid make up 70% of the fat content whereas unsaturated fatty acids like linolenic acid make up 30% (Lindmark Månsson, 2008). The vitamins present in milk include fat soluble vitamins A, D, E and water-soluble vitamins B complex and C. The quantity of fat soluble vitamins is dependent on the amount of fat that is in the milk. Therefore, skimmed milk would have a lower quantity of A, D and E as compared to whole milk since it has less fat content. (Pereira, 2014). Milk contains various minerals. Some of these minerals include calcium, phosphorus, zinc, potassium and selenium. The most abundant mineral found in milk is calcium. This explains why milk is said to be a very rich source of calcium. The phosphorous present comes in organic and inorganic forms (Gaucheron, 2011). Milk is mostly made up of water. Milk has about 87% water with the remaining 13% consisting of the other nutrients (Pereira, 2014). The high amount of water is responsible for the quick spoilage of milk.

One physical property that helps distinguish cow milk from other dairy milks is the colour. Cow milk is usually yellowish white in colour. The colour intensity varies based on the type of breed. Cow milk as compared to buffalo milk is yellower because of the higher  $\beta$  carotene content in it (Ullah et al., 2017).

Milk pH is a very important chemical property that enables milk remain in its liquid state. Milk pH ranges between 6.6-6.8 (Tsioulpas, Lewis, & Grandison, 2007). When the pH of milk is lowered, the milk proteins begin to denature making the milk change from its liquid state to a solid state (Phadungath, 2005a). Higher milk pH gives an indication that the cow is suffering from mastitis; a disease that affects the udder of the cow hence affecting the quality of milk (Contreras & Rodríguez, 2011).

### **2.2.3.1 Milk Proteins**

The solid content of milk which is approximately 13% is made up of 3.2-3.8% proteins (Pereira, 2014). The proteins in milk endow it with unique properties that are not observed in many protein-containing foods. The proteins in milk are in two forms. The soluble proteins called caseins and the insoluble proteins called whey. Caseins make up 80% of milk proteins and the remaining 20% is whey (Phadungath, 2005a).

There are four types of caseins in milk. They are  $\alpha_{s1}$ ,  $\alpha_{s2}$ ,  $\beta$  and  $\kappa$  casein (Walstra et al., 1999). The  $\alpha_s$  proteins contain the highest number of phosphoserine. This is followed by  $\beta$  casein with  $\kappa$  casein having only one phosphoserine group. The phosphoserine groups of the  $\alpha$  and  $\beta$  caseins make them sensitive to calcium salts hence, precipitating out in the presence of calcium.  $\kappa$  caseins on the other hand prevent the other caseins from precipitating because of their lack of phosphoserine groups (Phadungath, 2005a). This process of precipitation also denatures the proteins, changing the state of the milk from liquid to solid. The side chain of the caseins also influences their hydrophilic and

hydrophobic nature.  $\beta$  caseins are the most hydrophobic and  $\kappa$  caseins the most hydrophilic due to the glycoproteins present. This determines their position on the micelle (Walstra et al., 1999). Caseins, unlike other proteins that are denatured at high temperatures, remain stable above 100°C. Denaturation begins above 120°C, however, they are denatured by low pH at an isoelectric point of 4.6 (Phadungath, 2005a). Some studies done on caseins showed that they have antihypertensive effect and help in weight control by influencing intestinal motility. This allows food to move slowly in the gastrointestinal tract preventing one from getting hungry at a faster rate. (De Noni & Cattaneo, 2010).

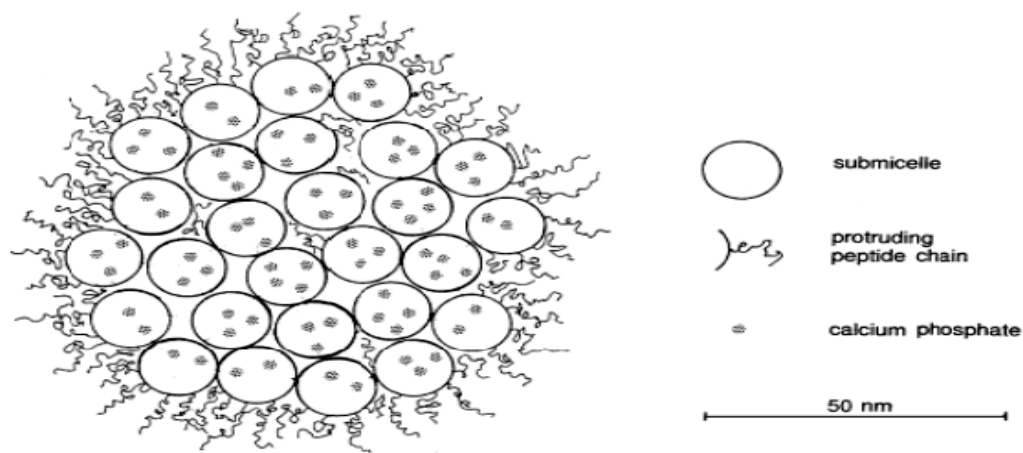
The other proteins in milk known as whey are the insoluble components of milk proteins obtained from the removal of caseins. It is mainly made up of  $\alpha$  lactalbumin and  $\beta$  lactoglobulin. It is made up of globular proteins which have high densely folded peptide chains with high hydrophobicity. Whey also known as serum denatures when heat is applied to it (Walstra et al., 1999). It is usually lost during cheese making through the process of dewatering.

#### **2.2.3.2 Micelle Structure of Milk Proteins**

Milk proteins are bound to some minerals collectively called Colloidal Calcium Phosphates (CCP) to form casein micelles. CCP is made up of calcium, citrate, phosphorous and magnesium (Phadungath, 2005a). The structure of the casein micelle is described by various scientists but the most commonly accepted one is that proposed by Walstra in 1984 (Rollema, 1992).

The casein micelle is made up of sub-micelles. Each sub-micelle differs in size; 12-15nm in diameter with each containing 20-25 casein molecules. Micelle sizes can be measured using dynamic light scattering. These sub-micelles are of two types. Those consisting of only  $\alpha$  and  $\beta$  caseins are hydrophobic; and are found in the central part of the micelle. The second type is made up of  $\alpha$  and  $\kappa$  caseins. These are more hydrophilic and usually found on the peripheral of the

micelle. The 'C' terminal of the hydrophilic part, protrudes to form a 'hairy-like' layer. This helps avoid aggregation of the sub-micelles by using steric and electrostatic repulsion thereby maintaining milk in its liquid state (Walstra et al., 1999).



**Figure 2.1: The Structure of Casein Micelle in the Sub-Micelles. Model Showing the Protruding C-Terminal Parts of K-Casein as Proposed by Walstra. (Source:(Walstra, 1999)**

### 2.3 Coagulation of Milk

Milk is transformed into cheese by three major processes. These are coagulation, dewatering and refining. Without denaturation or coagulation occurring cheese cannot be made. The curd that forms is known as coagulum (Troch et al., 2017). In order for the caseins to remain in solution, they form micelles as stated earlier. Acids and enzymes are able to destabilize the micelle causing it to precipitate. They both have different mechanisms of operating, hence produce different coagulum.

Milks coagulate when the pH is reduced. Coagulation of milk occurs at an isoelectric point of 4.6 at 30°C (Phadungath, 2005a). At this point the colloidal suspension precipitates out to form a coagulum. Milk can be acidified via various processes such as direct acidification by adding acids such as hydrochloric acid, acetic acid, citric acid and glucono- $\delta$ -lactone or indirectly by using lactic acid bacteria. Lactic acid bacteria can be used to reduce the pH of milk. They accomplish this by utilizing the lactose in the milk to produce lactic acid (Phadungath, 2005b).

Coagulation via acidification occurs after CCP dissociates from the micelles. The negative charges in the casein micelles become neutralised by the hydrogen ion from the acid. This results in aggregation of the casein micelle at the isoelectric point. The final cheese produced at the end of coagulation becomes acidic (Troch et al., 2017).

Apart from acids, milks can also be coagulated using enzymes. These enzymes are called rennet (Pezeshki et al., 2011). Rennet can be obtained from plants, animals, or microorganisms. Generally, they cleave the  $\kappa$  casein of the milk micelle at Phenylalanine<sub>105</sub> and Methionine<sub>106</sub>. The proteinase found in rennet that helps in hydrolyzing caseins are cysteine, serine or aspartic acid proteinases (Shah, Mir, & Paray, 2014). Cysteine proteinases, also known as thiol proteinases have a cysteine group in their active site. They are well noted for their ability to be active in a wide range of pH and temperatures. Serine proteinases also have a serine residue in their active site just like cysteine proteinases (Shah et al., 2014). Aspartic proteinases have two aspartic residues which have a specificity for cleaving peptide bonds between hydrophobic amino acid residues. They are also very active at an acidic pH (Domingos et al., 2000). After  $\kappa$  casein is cleaved by rennet, it becomes incapable of protecting the hydrophobic components of the micelle structure. The phosphorylated inner  $\alpha$  and  $\beta$  caseins, then start to interact with the calcium present to form paracaseinate (Lucey, 2002). This begins the coagulation process.

### 2.3.1 Types of Coagulating Enzymes

There are generally two sources of enzyme coagulants. They include animal and plant sources. Chymosin an animal source is a type of rennet found in the abomasum (fourth stomach) of a calf before it is weaned (Çakmakçi and Boroğlu, 2004). It is important for digesting milk in the calf. Years ago, cheese makers cleaned and dried the stomach and when the enzyme was needed they soaked it in whey to extract it. As time went on, the stomach was kept frozen instead of dried, and extraction done when necessary (Jacob, Jaros, & Rohm, 2011). Now, chymosin is being produced via genetic engineering where microorganisms are engineered to produce the enzyme. It is obtained by fermentation in microorganisms such as *Escherichia coli*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* (Jacob et al., 2011). This has become necessary because of the number of calves that have to be sacrificed in order to obtain the enzyme. Chymosin can produce firmer gels than plant enzymes and gives higher yields (Ben Amira et al., 2017).

It is active at temperatures between 30-42<sup>0</sup>C and a pH range of 6.2-6.8 and 5.1 - 5.5 Chymosin cleaves the κ casein of the milk micelle at Phenylalanine<sub>105</sub> and Methionine<sub>106</sub>. The proteases in Chymosin are specific in their reaction (Ben Amira, et al., 2017).

There are various plants that have the ability to coagulate milk and these include Pawpaw (carica papaya), Sodom apple (*Calotropis procera*) (Akinloye & Adewumi, 2014), Moringa (*Moringa oleifera*) (Orhevba & Taiwo, 2016) and Cadoon flowers (*Cynara cardunculus*) (Ben Amira et al., 2017). Due to availability, affordability and the ease to extract the enzymes, cheese processors are gradually introducing plant rennet into their production (Freitas et al., 2016).

Just like chymosin, most of the proteases cleave the bonds of κ-casein at phenylalanine<sub>105</sub> and methionine<sub>106</sub>. A few of these proteases are not specific hence cleave other bonds apart from the phenylalanine<sub>105</sub> and methionine<sub>106</sub> bond (Ben Amira et al., 2017). These proteases are distributed

in the plants in different parts (leaves, flowers, stems, roots, fruits) and at different concentrations. Plant rennet usually have very high proteolysis. This can affect the texture and yield of the final cheese obtained. The higher the activity of the enzyme, the lower the yield and the less firm the cheese. This may be due to the extra hydrolysis of other bonds in the casein and the rearrangement of the coagulum structure (Ben Amira et al., 2017). When using plant rennet, the crude extract usually imparts colour to the product. This is usually seen for the leaves of sodom apple and pawpaw which impart a green coloration to the final cheese (Akinloye & Adewumi, 2014). To control this, some researchers have extracted the enzymes by centrifuging and using the supernatant.

#### **2.3.1.1 Calotropis procera**

*Calotropis procera* is a shrub from the family Asclepiadaceae (Upadhyay, 2014). In Ghana, they are known as Sodom apple but are identified in other places as Rubber bush, Sodom's milkweed, Swallowwort, Akud and Giant milkweed (Upadhyay, 2014). They are mostly found in the tropical and subtropical areas of the world (Upadhyay, 2014). *C. procera* has gray green broad leaves with a fleshy and waxy appearance. The stem has a dry corky bark and is generally curved (Orwa et al., 2011). Its flowers are small and clustered. The flowers are cream or greenish white in colour at the base and purple or violet at the extremities of the lobes. It has green fruits that are inflated and usually arranged laterally on the branch (Orwa et al., 2011). It is resistant to drought and heat and tolerant to salty soils (Orhevba & Taiwo, 2016). It can grow at deserted areas such as road sides and over used lands where most plants cannot survive. It also grows in all manner of soil types and climates (EcoPort, 2007). *C. procera* is a very important shrub. Every part of it is very useful. Generally it can be used as a source of fuel (EcoPort, 2007), for medicinal purposes (Upadhyay, 2014) and as a coagulant for coagulating milk (Akinloye & Adewumi, 2014) .



**Figure 2.1: Sodom Apple (*Calotropis procera*) Plant**

### **2.3.1.2 *Calotropis procera* as a Coagulant**

In many places in West Africa, *C. procera* is used for coagulating milk to produce a soft cheese. This cheese is called wagashie in Ghana and Wara or warankasi in Nigeria (Orhevba & Taiwo, 2016). *C. procera* is used to coagulate milks at high temperatures. A study showed that the rate of coagulation of milk was highest at 70<sup>0</sup>C at an optimum pH of 5.6 (Raheem, Suri, & Saris, 2007). Cysteine protease is responsible for the coagulation of the milk (Freitas et al., 2016). Cysteine protease causes proteolysis of the caseins by using the thiol group of a cysteine residue as a

nucleophile. The two main enzymes found in the *C. procera* responsible for proteolysis are procerain and procerainB. They differ in their iso-elcectric point, molecular weight and optimal pH for activity. Procerain, has an optimal working pH of 8 and the cysteine present in it has a molecular weight of 28.8KDa but little is known about procerainB. According to Rayanatou et al. (2017), the hydrolysis is not specific as that of chymosin. Chymosin specifically cleaves  $\kappa$  casein whereas there is proteolysis of all the caseins for *C. procera*. However, proteins found in the whey component obtained after coagulation with *C. procera* are similar to those in chymosin. The coagulum formed by *C. procera* is slightly different from that of chymosin. Coagulum from *C. procera* is more compact than that of chymosin (Rayanatou et al., 2017) and *C. procera* influences the colour of coagulum obtained by imparting a green colour to it. .Coagulum from *C. procera* has higher yield than some plant coagulants like lemon juice (Akinloye & Adewumi, 2014).

## **2.4 Cheese**

Cheese is a compact nutrient dense food mainly made from dairy milk (Singh, Drake, & Cadwallader, 2003). After coagulating the milk, whey is drained out and allowed to ripen for days and sometimes, years. There are different kinds of cheeses found all over the world. They are generally grouped into soft, hard, fresh and ripened cheese (Budreckiene & Struzeckiene, 2014). The textures and flavours are influenced by the ripening process and type of coagulant used (Gutiérrez-Méndez, et al., 2013). Acid addition makes cheese less firm as compared to milk with higher pH at about 6.0-6.2. Also, acid induced cheeses have a higher moisture content as compared to rennet induced cheeses (Lucey, Johnson, & Horne, 2003). Usually, fresh cheese is bland and retains the aroma and flavor of the milk used in preparing it. The flavours developed during ripening are also specific to the type of cheese being produced (Murtaza, et al., 2014) .

### **2.4.1 Soft and Hard Cheese**

Soft cheese has a very limited life span because of the high moisture content hence, consumed within a short period after production. Moisture on a fat free basis of hard cheese ranges from 61% - >67% (Dairy Processing Hand Book, n.d). Hard cheeses are obtained from the combination of rennet and acid. They have less moisture as compared to soft cheese. Ripening duration and moulding process plays a role in the final moisture content of hard cheese (Buckley, 2017). Hard cheeses due to their lower moisture content have a longer shelf life. Moisture on a fat free basis of hard cheese ranges from <41%-56% (Dairy Processing Hand Book, n.d). Some examples of hard cheeses include cheddar, gouda and parmesan Some examples of ripened cheese are cheddar, camembert and gouda.

Cottage cheese is made by only acidification of milk. Mesophilic microbes are added to reduce the pH for coagulation. One peculiar feature of cottage cheese is that it is made up of individual grains of relatively similar sizes. It has low calories and is quite low in fat due to the use of skimmed milk for its production (Tratnik et al., 2000).

### **2.4.2 Ripened or Matured and Fresh Cheese**

Ripening is an important step that is done when processing some types of cheeses. It is done at lower temperatures as compared to that needed for coagulation and at controlled humidity. This is done to provide the right conditions for the biochemical, microbiological and chemical reactions that are needed to take place (Singh, Drake, & Cadwallader, 2003). The temperature and humidity also depend on the type of cheese being made. For instance, cheddar is ripened at 8<sup>0</sup>C at a relative humidity of 80% (Singh, Drake, & Cadwallader, 2003) and camembert, 12<sup>0</sup>C at a relative humidity of 93% (Leclercq-Perlat et al., 2013). Ripened cheese as compared to fresh cheese, have different flavour and aroma profiles. This is due to various biochemical reactions in the cheese. These

reactions range from the breakdown of residual lactose, lactate and citrate via glycolysis, proteolysis and lipolysis (McSween, 2004). Other compounds that also influence the flavor profile include alcohols, organic acids, phenolic compounds and sulphur compounds ( Murtaza et al., 2014). Cheddar cheese is a typical example of ripened cheese. It is made by coagulating milk using both acid (lactic acid bacteria) and rennet (Murtaza et al., 2014). After draining, it is usually salted and dyed with annatto to improve upon its colour (orange colour) and flavour. The ripening process influences the texture and flavor. It loses more moisture during the ripening process hence becomes drier and crumblier. The ripening process can range from weeks to years; the longest usually being 5 years (International Dairy Foods Association, 2018)

Fresh cheeses usually do not undergo any form of ripening. Cottage cheese, cream cheese and wagashie are all typical examples of fresh cheese. Usually, Soft cheeses are processed fresh but there are a few soft cheeses that are ripened for a few days or weeks. A typical example of soft ripened cheese is brie. Brie undergoes ripening for a few weeks before consumption (Budreckiene & Struzeckiene, 2014).

Brie cheese has a soft texture and is not crumbly. It is ripened for a few weeks and the final product has a white to cream colour. It is coagulated with rennet and lactic acid bacteria as the starters for the required pH (CODEX, 1973).

## **2.5 Factors Influencing Coagulation**

There are numerous factors that influence the rate of coagulation of milk, the texture and yield of coagulum obtained. The main factors include calcium content, protein content, temperature and

pH of the milk used. Apart from these four factors, the presence of non-coagulating milk can also influence the coagulation process.

### **2.5.1 Calcium Content**

Calcium exists in milk in soluble and colloidal forms. Calcium interacts with  $\alpha$  and  $\beta$  caseins to form a mass known as coagulum (Phadungath, 2005a). Some manufacturers speed up the coagulation process by adding calcium in the form of  $\text{CaCl}_2$ . This increases the yield and reduces the coagulation time. The higher the level of calcium in the milk the lower the pH of the milk. And lower pH influences the rate of coagulation. Calcium generally affects the coagulation time and the texture of the cheese obtained making the resulting cheese firmer (Troch et al., 2017) . It also enhances the retention of minerals, the rate at which protein and fat is retained and also improves the dry weight (Santos et al., 2013).

### **2.5.2 Protein Content**

The protein content is an important factor that influences both acid and enzyme coagulation. The caseins either react with calcium or get denatured due to change in pH to precipitate out or form coagulum. Variation in milk coagulation is largely influenced by protein. The type of casein present is very important.

According to (Abeykoon et al., 2016) high coagulum yield, curd firmness and high meltability was observed from milk with high total casein,  $\kappa$ -casein and  $\beta$ -casein. The concentration of caseins in the milk influences the texture of the coagulum and the firming time. Casein micelle size also influences the texture of the coagulum. Smaller micelles produce firmer coagulum (Walstra, Wouters, & Geurts, 2006).

### **2.5.3 pH**

Lowering the pH changes the size of the casein micelle and causes the hairy layers to collapse. This reduces the stability of the casein micelle, leading to coagulation. This influences the coagulating time and rate of firming (Sinaga, Bansal, & Bhandari, 2017).

The optimum working pH of animal rennet is between 5.1-5.5 and its activity increases with increasing pH (Troch et al., 2017). For acidulation, coagulation can occur between a pH range of 4.6- 6.6. This is possible because at a pH region between 5.0-6.6 there is solubilization of calcium phosphate; then, there is a dissociation of calcium complexes by phosphoserines at a pH range of 4.6- 5.0. These lead to a reduction in both electrostatic repulsion and steric stabilization of the micelle causing coagulation (Phadungath, 2005b)..

### **2.5.4 Temperature**

Temperature is an environmental factor that influences the rate at which coagulation occurs. For animal rennet there is no coagulation below 10<sup>0</sup>C (Troch et al., 2017). Coagulation can occur between 10<sup>0</sup>-20<sup>0</sup>C but it is at a very slow rate. Then between 30<sup>0</sup>C and 42<sup>0</sup>C coagulation becomes gradual, falls above 42<sup>0</sup>C and stops completely at 55<sup>0</sup>C (Walstra, Wouters, & Geurts, 2006). Sodom apple however coagulates best at an optimum temperature of 70<sup>0</sup>C (Raheem, Suri, & Saris, 2007). Acid induced coagulation also depends on temperature and pH (Troch et al., 2017). It has been shown that increasing coagulation temperature, increases the rate of enzymatic reaction such as cleaving  $\kappa$ - casein by rennet. Increase in enzymatic activity and the rate of aggregation at higher temperature reduces the coagulation time. However, if the temperature of milk is too high that is, above 36<sup>0</sup>C coagulation time will increase due to heat induced inactivation of rennet (Ong, et al, 2011) .

### **2.5.5 Non-coagulating Milk**

Milk that does not coagulate after 30 minutes after the addition of rennet or acid is known as non-coagulating milk. This milk is not suitable for the production of cheese. The addition of non-coagulating milk to coagulating milk especially effects the firmness of the final coagulum and eventually the cheese obtained. The cause of this problem is not known but the composition of the milk could be of great influence (Troch et al., 2017).

### **2.5.5 Conclusion**

Different breeds of cattle are reared in the country. They provide farmers with additional benefit in the form of milk. Milk is very nutritious and high in protein, fat and minerals. The proteins in milk are very important in the formation of cheese. This is possible via some specific proteins known as caseins. Caseins coagulate in the presence of an acid or enzyme to precipitate out. This precipitate is known as coagulum. This coagulum begins the process of cheese making. Cheese is usually made by coagulating milk, dewatering the coagulum and ripening it. There are different types based on texture and level of maturity. Cheese production in Ghana can further add value to milk production. In order to find out if this is even feasible, this study aims to determine if milk from Ghanaian cattle breeds can coagulate by using different types of coagulants.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Materials

Rennet (Chymosin) - Chymax, *chr. Hansen Standard, Denmark*

Citric acid – Sigma-Aldrich Life Science

Sodom apple (*Calotropis procera*) branches

Cow breeds – White Fulani, Jersey and Cross breed (50% Jersey:25% Sanga:25% Friesian)

Milk from the White Fulani was from Nsawam, the Crosses from Malejor and the Jerseys from Suhum and Afienya.

#### 3.1.1 Rearing practices

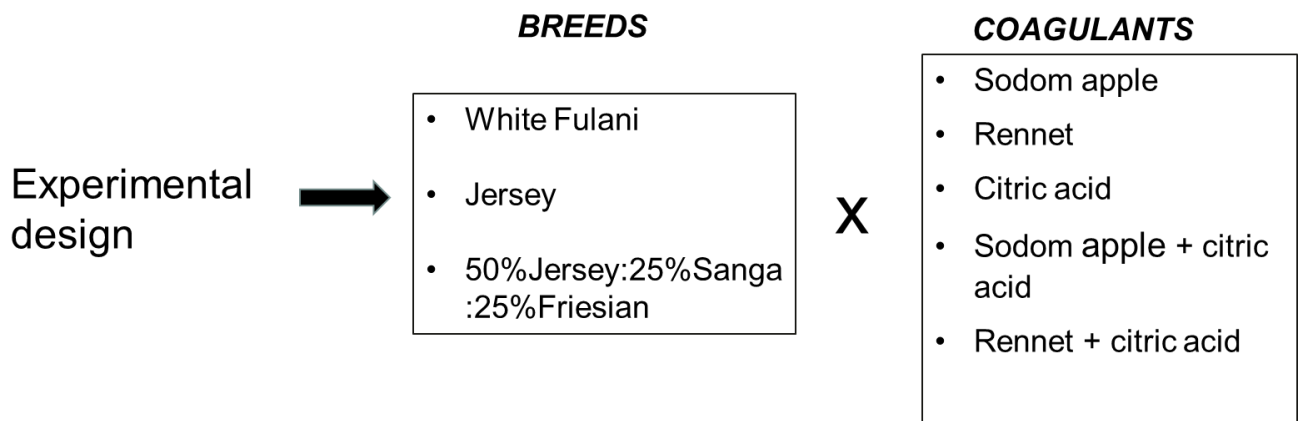
Apart from the White Fulani, the Cross and Jersey cows were reared under the same management system. The White Fulani was reared via open range and the Cross and Jersey via zero Grazing. From time to time, the White Fulani cows were provided with cassava peels. Those managed via zero grazing were provided with brewers spent malt, cassava peels and cut grass like elephant grass. The cows used for the analysis were also at different lactation periods. Some were at the beginning of the lactation cycle, others were at in their mid-lactation period and some at the end of lactation. All the Crosses were pregnant during sample collection.

#### 3.2 Experimental Design

Milk was collected from a total of 6 cows; two of each breed. Milk samples were collected three different times from each cow (at least 2 weeks apart) except for one Cross. The Cross had some health problems after two times of sampling. Analyses were done to compare between the breeds,

the physicochemical properties of the milk that influence coagulation of milk obtained from each breed. Physicochemical analysis was done on the milk from each cow.

Based on a 3 x 5 factorial design (breed x coagulant), milk from each cow was coagulated using each of the following coagulants: Sodom apple (enzyme), rennet (enzyme), citric acid (acid), Sodom apple + acid and rennet + acid (a combination of acid and enzyme). Coagulation potential (coagulation time and coagulum yield) and the physicochemical properties of the dewatered coagulum were analyzed.



**Figure 3.1: Summary of Experimental Design**

All 6 fresh milk samples from the different breeds were then pooled together and dewatered coagulum produced using the various coagulants mentioned previously. The procedure used for processing the dewatered coagulum was replicated. Sensory, texture analysis, meltability and a protein profile were done on the resulting dewatered coagulum to determine their characteristics in relation to cheese. Finally, an economic analysis of the process was evaluated.

### 3.2.1 Sample collection

Fresh milk samples were collected early in the morning from the selected farms in clean 1L plastic bottles with lids. Milk samples were collected right after the cows were milked. Collected milk samples were then transported to the Department of Nutrition and Food Science, where all analyses were done, in an insulated cool box with ice packs. On arrival at the department, the samples were batch pasteurized at 65<sup>0</sup>C for 30 minutes and stored in a fridge at 4<sup>0</sup>C for analyses and processing. Pasteurized milk was stored for at least 4 days before discarding.



**Figure 3.2: Cow Being Milked by a Herdsman.**

### **3.2.2 Coagulant Preparation - Concentrates**

#### **3.2.2.1 Citric acid**

Citric acid (6.4g) was dissolved in 100ml of distilled water at  $27 \pm 2^{\circ}\text{C}$ . Fresh citric acid concentrate was prepared for every batch of milk collected.

#### **3.2.2.2 Sodom apple**

Sodom apple branches (fresh and green) were obtained from University of Ghana campus behind the Balme Library. They were washed thoroughly with portable water, cut into tiny pieces and blended using a dry mill blender. 10g of the blended Sodom apple branches was added to 20ml of milk ( $27 \pm 2^{\circ}\text{C}$ ) and allowed to coagulate at  $70^{\circ}\text{C}$  for 5-6 minutes. Coagulation was done in a water bath. The mixture was stirred, and the whey filtered using a sieve and collected as the concentrate.

#### **3.2.2.3 Rennet**

A gram of granular rennet was dissolved in 10ml of tepid distilled water. Fresh rennet concentrate was prepared for every batch of milk collected.

### **3.2.3 Coagulation Process**

Coagulation was done at different temperatures for the different coagulants. This was based on the optimum temperature at which they are able to coagulate milk. For rennet, the temperature used was based on the manufacturer's instructions. All coagulation was done in a water bath, where the temperature was maintained using a hot plate.

#### **3.2.3.1 Citric acid coagulation**

Pasteurized milk (100ml) was heated to  $32^{\circ}\text{C}$ , then 5ml of citric acid solution was added and stirred for a minute. The sample was allowed to stand for 30 minutes at  $32^{\circ}\text{C}$  and drained for another 30 minutes using a cheese cloth with a 150g weight sitting on it.

### **3.2.3.2 Sodom apple coagulation**

Pasteurized milk (100ml) was heated to 70<sup>0</sup>C. 6ml of Sodom apple concentrate was added to the milk and allowed to stand for an hour at 70<sup>0</sup>C without agitating. The curds were cut into cubes 5 minutes before time and then transferred to a cheese cloth, where it was drained for 30 minutes with a 150g weight sitting on it.

### **3.2.3.3 Rennet coagulation**

Pasteurized milk (100ml) was heated to 38<sup>0</sup>C. 0.5ml of rennet concentrate was added to the milk and allowed to stand for 30min at 38<sup>0</sup>C without agitating it. The curds were cut into cubes 5 minutes before time and then transferred to a cheese cloth, where it was drained for 30 minutes with a 150g weight sitting on it.

### **3.3.2.4 Acid +Sodom apple coagulation**

Pasteurized milk (100ml) was heated to 32<sup>0</sup>C. 5ml of citric acid concentrate was added to the milk. Sodom apple (6ml) was added to the acidified milk and the temperature raised to 70<sup>0</sup>C. The sample was allowed to stand for an hour at 70<sup>0</sup>C and drained with a cheese cloth for 30 minutes with a 150g weight sitting on it.

### **3.3.2.5 Acid + Rennet coagulation**

Pasteurized milk (100ml) was heated to 32<sup>0</sup>C. 5ml of citric acid concentrate was added to the milk. The temperature was raised to 38<sup>0</sup>C and 0.5ml of Rennet was added to the acidified milk. The sample was allowed to stand for 30min at 38<sup>0</sup>C and drained with a cheese cloth for 30 minutes with a 150g weight sitting on it.

### **3.3 Objective 1 – Physicochemical analyses of milk samples**

#### **3.3.1 pH**

An HI 2211 pH/ORP meter (HANNA instruments) was used to analyze the pH. Milk (60ml) was measured into a 100ml beaker. The probe was dipped into the milk and the pH measured. pH was measured in triplicates for each milk sample. Mean pH values were used for interpretation.

#### **3.3.2 Colour**

The Hunter lab colorimeter (Chroma meter CR-410, Monica Minolta) was used for measuring the colour of the milk samples. The calibrated calorimeter was placed on the fresh milk sample and the colour measurements were taken at  $*L*a*b$ . Values were taken in triplicates for each sample

#### **3.3.3 Protein – Biuret test**

Standards were prepared using a stock of 10mg/ml of bovine serum albumin to prepare a calibration curve. The absorbance was taken at a wave length of 540nm.

Milk samples (0.02ml) were pipetted into test tubes. Distilled water (0.98ml) was added and topped up with 2ml of biuret reagent. The samples were vortexed and allowed to stand for 20 minutes.

The samples were carefully transferred into a cuvette and the absorbance taken at a wave length of 540nm using a Shimadzu UV spectrophotometer (UV-1800). Measurements were taken in triplicates.

The protein concentration was calculated using the calibration curve with the protein being the unknown variable.

#### **3.3.4 Fat - Rose Gottlieb method**

An empty flask was weighed. Milk (10g) was weighed and transferred into the extraction tube. Ammonia (2ml) was added and the sample shaken thoroughly. Ethyl alcohol (10ml) was added and mixed again. Then 25 ml of diethyl ether (peroxide free) was added, stoppered and

shaken vigorously for about a minute. An additional 25ml petroleum ether was added and shaken vigorously for about half a minute. The solution was allowed to stand until the upper ethereal layer separated completely and was clear. The clear ethereal layer was decanted into the weighed flask. Then 15 ml of diethyl ether (peroxide free) was added, stoppered and shaken vigorously for about a minute. Then an additional 15ml petroleum ether was added and shaken again vigorously for about half a minute. The solution was allowed to stand until the upper ethereal layer separated completely and was clear again. The delivery end of the extraction tube was rinsed with a little ether and added to the flask. The content of the flask was evaporated and placed in an air oven at 105°C for 30 mins, cooled in a desiccator and weighed.

### Calculations

$$\% \text{ Fat} = \frac{M1 - M2}{MS} \times 100$$

Where M1- mass of flask with fat

M2- mass of empty flask

MS – mass of sample only

### 3.3.5 Total solids

An empty evaporating dish and lid was dried in an air oven at 105°C for 3 hours and transferred to a desiccator to cool. The empty dish and lid were weighed. Two grams (2g) of the milk sample was weighed into the dried dish, spread uniformly, partially covered with the lid and placed in the oven. It was dried for 3 hours at 105°C. After drying, it was transferred into a desiccator to cool. After cooling it was weighed with the lid.

### Calculations

$$\% \text{ Total solids} = \frac{M1 - M2}{MS} \times 100$$

Where M1- mass of dried can +lid + sample

M2- mass of empty can +lid

MS – mass of sample only

### **3.3.6 Calcium content**

Milk (1g) was weighed into a khedjal flask. Concentrated nitric acid (25ml) was added and heated for about 30mins till a pale-yellow coloration was observed. The sample was cooled and 1ml of perchloric acid was added and heated again till the sample was colourless. It was cooled again and 30ml of distilled water was added and boiled for 10mins more minutes. The sample was cooled, and the calcium content measured using a Perkinelmer Atomic Absorption Spectrometer (PinAAcle 900T). Calcium content was measured in triplicates.

## **3.4 Objective 2- Potential of the milks to coagulate using different coagulants**

### **3.4.1 Coagulation time**

This was observed immediately the coagulant was added. The time in seconds for the first curd to form was recorded. This was noted using a stop watch in seconds.

### **3.4.1 Coagulum yield**

After whey was drained out of the coagulum, the weight of the coagulum was determined gravimetrically, and the % coagulum yield calculated.

$$\% \text{ Coagulum yield} = \frac{\text{weight of coagulum}}{\text{weight of milk used}} \times 100$$

## **3.5 Objective 3- Physicochemical Analysis on dewatered coagulum obtained from different coagulants and milks**

### **3.5.1 Moisture Content**

An empty evaporating dish and lid was dried in an air oven at 105°C for 3 hours and transferred to a desiccator to cool. The empty dish and lid were weighed. Two grams (2g) of the milk sample

was weighed into the dried dish, spread uniformly, partially covered with the lid and placed in the oven. It was dried for 3 hours at 105°C. After drying, it was transferred into a desiccator to cool. After cooling it was weighed with the lid.

### **Calculations**

$$\% \text{ Moisture content} = \frac{M1 - M2}{MS} \times 100$$

Where M1- mass of can +lid+ sample

M2- mass of dried can +lid + sample

MS – mass of sample only

### **3.5.2 pH**

Same as 3.3.1

### **3.5.3 pH**

Same as 3.3.2

### **3.5.4 Change in Colour**

Change in Lab was obtained by subtracting the value of the coagulum from that of the milk. This was to determine how much the samples had changed in comparison to the milk which was the starting point or raw material. When  $\Delta L$  is positive, the sample is lighter than the milk and a negative  $\Delta L$  indicates that the sample is darker than the milk. Negative  $\Delta b$  means the sample is greener than the milk and positive  $\Delta b$  indicates that the sample is redder than the milk. Negative  $\Delta a$  means the sample is more yellow than the milk and a positive  $\Delta a$  indicates that the sample is more blue than the milk.

### **3.4.5 Fat content**

This was calculated using the principle of mass balance

Mass of cheese x fat of cheese = 0.9(mass of milk used for coagulation) x fat of milk

$$\% \text{ fat of coagulum} = \frac{0.9(\text{mass of milk used for coagulation}) \times \text{fat of milk}}{\text{mass of coagulum}}$$

It is assumed that 90% of the fat is retained in coagulum.

(Morison, 1997)

### **3.4.6 Protein Content**

This was also calculated using the principle of mass balance. This is based on the assumption that minerals, water soluble vitamins and lactose are lost with the whey since they are water soluble (Morison, 1997) and (Walstra, Wouters, & Geurts, 2006)

$$\% \text{ moisture} + \% \text{ fat} + \% \text{ protein} = 100$$

$$\% \text{ protein} = 100 - \% \text{ moisture} - \% \text{ fat}$$

## **3.6 Objective 4 – Sensory and Physicochemical properties of the dewatered coagulum**

### **3.6.1 Sensory Analysis- Quantitative Descriptive Analysis (QDA)**

A descriptive panel made up of 12 trained assessors analysed the dewatered coagulum samples. The samples were made from Rennet, Sodom apple, acid, Sodom apple + acid and Rennet +acid coagulation of pooled milk using the same process as described for obtaining the coagulum from individual animals. The assessors met three times in a week for 3 hours each day. The training and final evaluation happened in 9 sessions which was a total of 27 hours.

The samples were analyzed in the Sensory Evaluation Laboratory at the Department of Nutrition and Food Science, University of Ghana under controlled environmental conditions. Assessors gave a profile of the modalities; appearance, aroma and texture in hand.

Samples were cut into cubes of 1x1x1cm. They were served in 80cc transparent cups at  $27 \pm 2^{\circ}\text{C}$  and presented to the assessors in a monadic sequential order. Assessors were provided with spoons

to assist with the analysis. The test involved panel training and final evaluation. For training, panelists were made to generate terms, build consensus, rate, rank and do panel performance. Final evaluation was done in booths where the assessors worked individually. The scale used was a 150mm intensity line scale and the samples were evaluated in triplicates using Compusense 5 by Compusense®, Canada.

### **3.6.2 Meltability**

The method described by Poduval and Mistry (1999) was used with some modifications.

Dewatered coagulum (3g) was grated and placed in a test tube. The grated dewatered coagulum in the test tube, was compressed and the height marked. The tube was covered with perforated aluminium foil to allow the escape of gas during heating. The tube was placed vertically in a fridge at 4°C for 30min then horizontally in a preheated air oven at 105°C for 10 min. The flow distance of the melted dewatered coagulum was measured in cm. Meltability for each cheese sample was done in triplicates.

### **Calculation**

Meltability = final distance of cheese – initial distance of cheese

### **3.6.3 Texture Analysis**

The method described by Zheng et al. (2016) was used with some modifications. The texture profile analysis (TPA) was done using a Stable Micro System Texture Analyzer. A double-bite compression cycle using a cylinder with a flat ended probe of diameter (75mm) was used for compressing the dewatered coagulum. A pre-test speed of 2mm/sec, test speed 1mm/sec, post-test speed 5mm/sec and a 75% strain were applied. The samples were cut into cubes of 1x1x1cm, thawed and analyzed at room temperature (27±2°C). Each sample was run 15 times and the four

most consistent values were selected for further statistical analysis. The indices measured were hardness, adhesiveness, springiness, chewiness and cohesiveness.

#### **3.6.4 Protein profile- SDS PAGE**

Proteins were extracted from the 5 dewatered coagulums and the pooled milk used for coagulation. This was done to identify the caseins present in the samples using SDS PAGE. A casting plate of 1.5mm was used. A concentration of 12% separating gel and 3.9% stacking gel were prepared into the casting plate for the separation. A gram of the cheese and milk samples were used in sample preparation. Sample buffer and sodium dodecyl sulfate (SDS) were added to the samples and sonicated. Dithiothreitol (DTT) was added to the sonicated samples and they were centrifuged at 5000rpm for 10 minutes. The supernatant was collected into a falcon tube and stored in the freezer. 100µl each of the supernatant were pipetted into eppendorf tubes. Sample buffer and loading buffer were added and boiled for 10 minutes. 0.5µl each of the samples were then pipetted into the wells of the gel with 0.5µl of BSA and 0.5µl of molecular protein marker. The gel was allowed to run at a voltage of 100V until the samples reached the bottom of the gel. The gel was removed and transferred into a clean container. Staining buffer (Coomassie blue) was added and allowed to stand for about two hours. The staining buffer was discarded, and de-staining buffer was added and allowed to stand for a couple of hours till the bands were clear and visible. The image of the gel was captured using an Amersham Imager 600.

#### **3.7 Objective 5 – Setting up, running and producing cheese in a cheese factory (Economic analysis)**

This economic analysis was to explore the economic potential of commercially processing cheese in Ghana. The analysis was done for cheese made from each of the coagulants: Rennet only, Acid only, Sodom apple only, Rennet + acid and Sodom apple + acid. Even though farmers

have a higher yield from some of the crosses and exotic breeds, the current quantities produced by the few dairy cows available would be insufficient to run a cheese factory every single day, thus an assumption has been made that such a factory if existing would operate 3 times a day each week thus a total of 144 days in the year. The economic analysis, was done based on the method developed by Peters & Timmerhaus (1991). Modifications and/or assumptions are shown in the calculations.

### **3.7.1 Total Capital Investment (TCI)**

This involves the summation of the working capital and the fixed capital. The working capital is the amount of money needed for the start and operation of a plant whereas the fixed capital is the capital that is needed to supply the necessary manufacturing and plant facilities and their installation too.

### **3.7.2 Total Production Cost (TPC)**

This refers to the costs that would be incurred in the operation of a plant as well as the sale of the products. The estimation of costs for operating the plant and selling 100g of cheese can be grouped under the general heading of total production cost. The Total Production cost is generally divided into manufacturing costs and general expenses. Manufacturing costs are also known as operating or production costs (Peters and Timmerhaus, 1991).

Total product cost is usually done on annual cost basis. This is because:

- The effect of seasonal variations is smoothed out,

- Plant on-stream time or equipment operating factor is considered,

- It permits more-rapid calculation of operating costs at less than full capacity, and

- It provides a convenient way of considering infrequently occurring but large expenses such as annual turnaround costs.

### **3.7.3 Profitability Analysis (PA)**

This gives a measure of the amount of profit that can be obtained from a given situation.

### **3.8 Statistical analyses**

Minitab software version 17 was used to analyze data obtained from the physicochemical analyses. One-way ANOVA was done to determine if differences existed amongst the milk samples and the cheese samples. Two-way ANOVA was used to determine the interaction between the breeds and coagulants for the various coagulum obtained. Excel-Stats (Addinsoft, France) was used to analyse Sensory data using two-way ANOVA. Tukey's HSD post hoc analysis was further carried out to explain sample differences.

## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

#### 4.1 Physicochemical properties of milk that influence coagulation

Physicochemical analysis was done on the milks from the different breeds to determine their composition.

**Table 4.1: Physicochemical Properties of Milks from Different Breeds of Cattle**

	pH	FAT	PROTEIN	TOTAL	ASH	CALCIUM
BREED		(%)	(mg/ml)	SOLIDS (%)	(%)	(mg/100g)
WHITE						
FULANI	6.784±0.03 <sup>a</sup>	2.46±0.25 <sup>a</sup>	13.36±0.67 <sup>a</sup>	12.29±0.24 <sup>b</sup>	0.76±0.09 <sup>a</sup>	289.05±25.88 <sup>a</sup>
CROSS	6.707±0.06 <sup>b</sup>	3.26±1.0 <sup>a</sup>	13.15±2.02 <sup>a</sup>	13.81±0.89 <sup>a</sup>	0.64±0.08 <sup>b</sup>	165.69±23.38 <sup>c</sup>
JERSEY	6.808±0.11 <sup>a</sup>	3.46±1.95 <sup>a</sup>	13.65±1.74 <sup>a</sup>	14.61±2.76 <sup>a</sup>	0.79±0.11 <sup>a</sup>	194.63±41.53 <sup>b</sup>

Values with different alphabets are statistically different for each physicochemical property at p<0.05.

##### 4.1.1 pH of milk samples

pH of the breeds showed a statistical significant difference between the Cross and the other two breeds. Although there was a significant difference, the values obtained were in the range required for pH of fresh milk. Fresh milk pH ranges between 6.6-6.8.; at this pH the milk is able to stay in the liquid form (Tsioulpas et al., 2007). Lactation period has an influence on the pH of milk. The differences in the pH could be due to the different lactation periods of the cows. At early lactation, the pH is low. It increases slightly during mid-lactation and further increases during late lactation

(Tsioulpas et al., 2007). Four out of six of the cows were in their mid-lactation period. They included a Jersey, a Cross and both White Fulani. The other Jersey was in her early lactation period and the other Cross in her late lactation period. Since the Cross had the least pH amongst the breeds it would have been expected that the cows would be in their early lactation period, but this was not so. The deviation may be due to the differences in lactation period within the Cross breeds.

#### **4.1.2 Fat and protein content of milk samples**

Fat and protein content of the milks from the different breeds showed no statistical significant difference. Fat and protein content are influenced by breed type. According to Walstra, Wouters, & Geurts (2006), Jerseys have higher protein and fat content as compared to other breeds. The deviation for the lower fat content may be due to the feed provided. Apart from breed type, feed type also influences fat content of milk. The feed type determines the by-products of digestion. Feeds that are easily digestible influence the precursors of milk fat. Instead of producing more acetic acid and butyric acid, more propionic acids are rather produced. Propionic acid stimulates insulin production which reduces free fatty acid release from the adipose tissue, leading to less fat being released into the milk (Linn, 1988). This could mean that the feed provided for the Jerseys was easily digestible hence the production of less free fatty acids than their usual fat content. Even though statistically there was no difference in the fat content of the milks, it was observed that the mean fat content of the Jersey was higher than the other two breeds. This could be due to the large deviation seen on Table 4.1 for the Jersey. The deviation may have occurred perhaps due to different farming practices executed at the different farms. Even though they were both Jerseys were managed via zero grazing some practices may have differed between farms. Also, protein content of the Jersey was not higher than that of the Cross and White Fulani. This may be due to their lactation period and whether the cows were pregnant or not. After a few weeks of lactation,

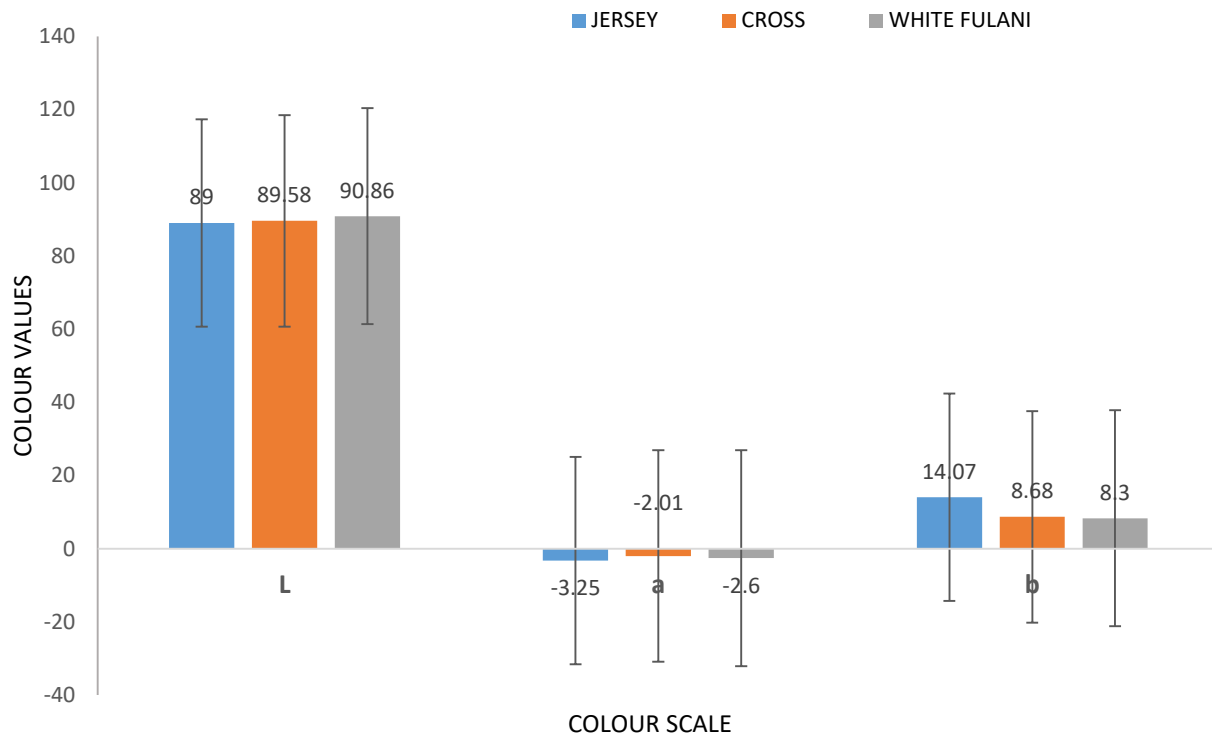
protein content in cow milk begins to increase. From the lactation history of the cows, it can be seen that the reduction in protein content may be due to the early lactation period of one Jersey (Linn, 1988).

#### **4.1.3 Total solids, Ash and Calcium content of milk samples**

For total solids, milk from the White Fulani was significantly different from the Cross and Jersey. The White Fulani had a lower total solids content. Feed affects the nutritional composition of milk. The cows that have to find their own food via grazing may not get enough as compared to those provided with feed. Those under Zero grazing (Cross and Jersey) may have the same quantity and quality of feed everyday as compared to the White Fulani cows whose eating pattern and nutritional composition of feed may not be consistent. Inconsistencies in the feeding of the White Fulani may be the cause of the lower total solid content observed. Ash content showed significant difference between the Cross and the other two breeds. Milk from the Cross had the least ash content. According to Zamberlin et al.(2012), mineral content in feed and soil influences the mineral content in cow's milk. This may mean that the feed provided for the Cross had less minerals in it as compared to that of the Jersey and the Fulani. The ash content influenced the calcium content of the milks. Calcium is a mineral hence the higher the mineral content the higher the calcium content. All the breeds showed significant difference with the Cross having the least calcium content.

#### **4.1.4 Colour of Milk Samples from the Different Breeds**

Colour of the milk samples were determined to find out if the milk from the breeds showed some differences based on the Lightness, greenness and yellowness



**Figure 4.1: Colour of Milk Samples. Where L-lightness, a- greenness and b-yellowness**

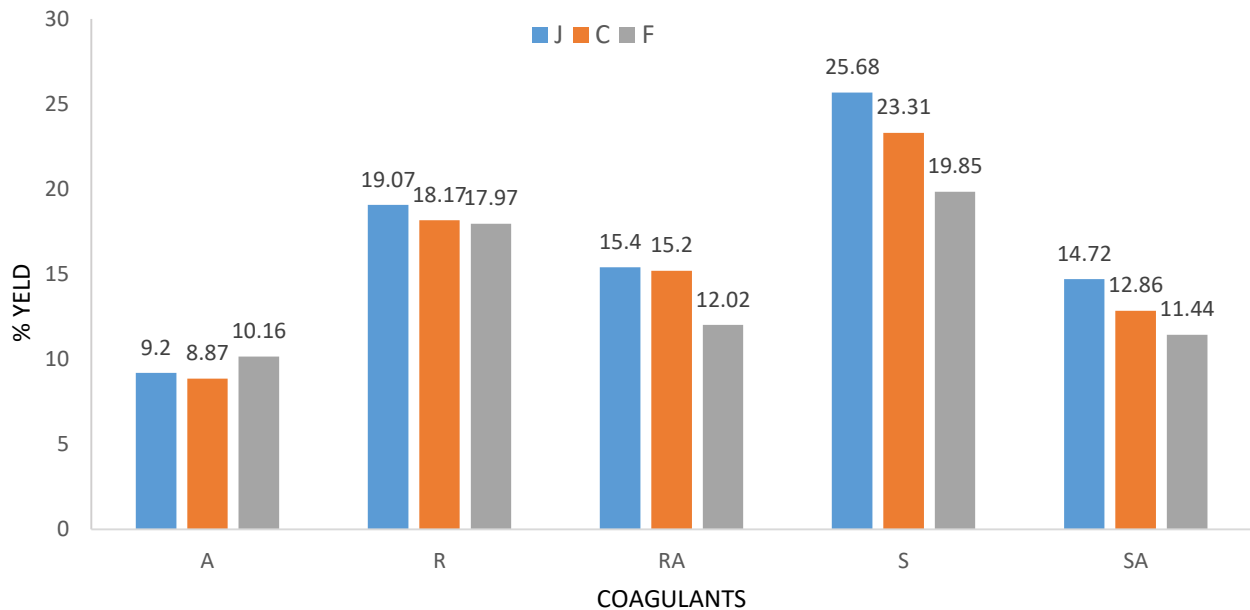
All the samples were light as seen on the lightness scale (\*L) on Figure 4.2, but statistically, milk obtained from the White Fulani was different from the other two. It was the lightest amongst the three. The \*a scale tells whether the samples are red or green. Positive values indicate redness and negative values indicate greenness. The \*a scale for the milk samples indicated, all the samples were more green than red but milk from the Jersey was seen to be the greenest as compared to the other two breeds. A positive \*b scale shows that the sample is yellow and negative means it is blue. Figure 4.1 shows that the samples are more yellow than blue. There was statistical significant difference between breeds for yellowness with the Jersey breed showing the highest values for yellow. Yellow colour in milk is normal and is as a result of beta-carotene in the fat component of milk (Walstra, Wouters, & Geurts, 2006). Ullah et al. (2017), explained that the milk fat in Jersey

cows are however more yellow coloured as they are able to breakdown the beta-carotene in their food which is then absorbed in their milk.

## 4.2 Coagulating potential of the milks from the different breeds using different coagulants

Coagulum yield was determined to find out which breed and coagulant produced the highest amount of coagulum after coagulation and dewatering. The time for coagulation was also measured to find out how fast the milks could coagulate and also know how the coagulants influenced the rate of coagulation.

### 4.2.1 Yield of Coagulum Obtained



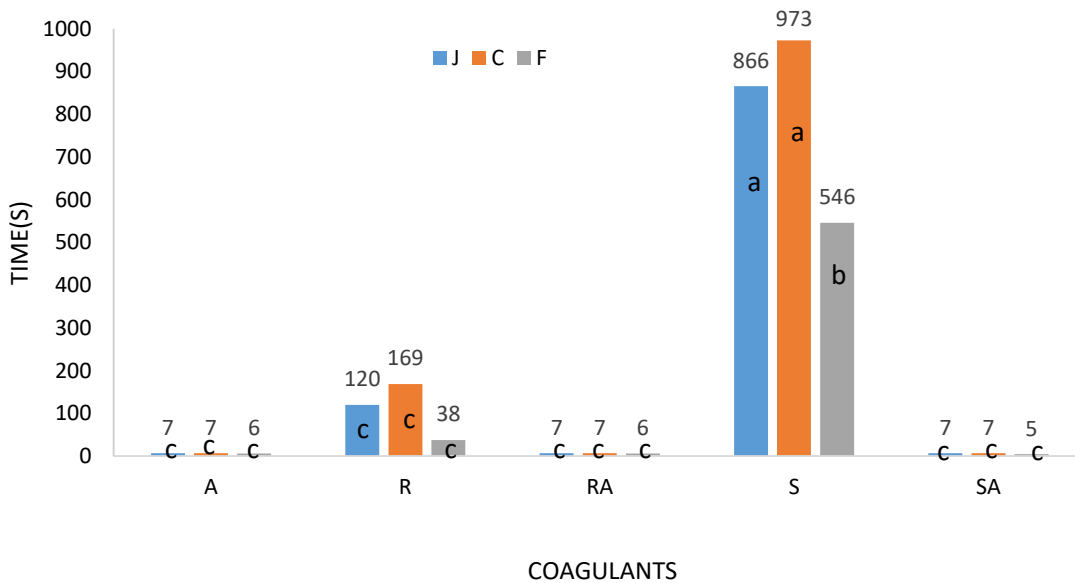
**Figure 4.2: Coagulum Yield of Milk from Different Breeds Using 5 Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**

Figure 4.2 shows that the coagulum with the least yield was processed using A and this was followed by SA and RA, then R and finally S. There was a statistical significant difference at a p value < 0.05 of the interaction between the breeds and the coagulants. Generally, the milk from the Jersey cows gave higher yield of coagulum for three out of five different coagulants used. This

may be attributed to the fact that coagulum from Jerseys are able to recover most of the nutrients in the milk. According to (Stocco et al., 2018) analysis done on milk from Jersey and five other cows showed that the recovery rate for both coagulum fat and protein content was highest. This means that, most of the proteins and fat are not lost but are retained in the coagulum as compared to other breeds.

R is known to give high coagulum yield (Ben Amira et al., 2017) but from Figure 4.2, S has the highest yield. The high yield for S maybe due to the high temperature needed for coagulation. According to El-Gawad & Ahmed (2011), high temperatures denature whey proteins which adds to the total coagulum yield. Also, longer coagulation time using enzyme produces coagulum with high yield (Johnson, Chen, & Jaeggi, 2010). Figure 4.3 showed that milks coagulated using Sodom apple had the longest coagulation time followed by rennet. Low yield in A, RA and SA may be due to loss of proteose-peptones (a peptone is a soluble protein formed in the early stage of protein breakdown and a proteose is any water-soluble compound produced during digestion by the hydrolytic breakdown of proteins) in whey due to low pH. The rate at which they are lost is more reduced in enzyme coagulated milk (Johnson, Chen, & Jaeggi, 2010).

#### 4.2.2 Time Required for First coagulum to Form



**Figure 4.3: Time Taken for First Coagulum to be Formed. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J. Values with the same alphabet are statistically the same for each physicochemical property at  $p < 0.05$**

There was a statistical significant difference at a  $p$  value  $< 0.05$  of the interaction between the breeds and the coagulants. For the breeds, milk from the White Fulani had the shortest coagulation time for coagulum processed with S as compared to the Cross and Jersey. The ability of the White Fulani milk to coagulate within a short period of time maybe due to its high calcium content (Table 4.1). Calcium content influences the rate of coagulation according to Troch et al. (2017). The higher the calcium content, the faster the rate of coagulation.

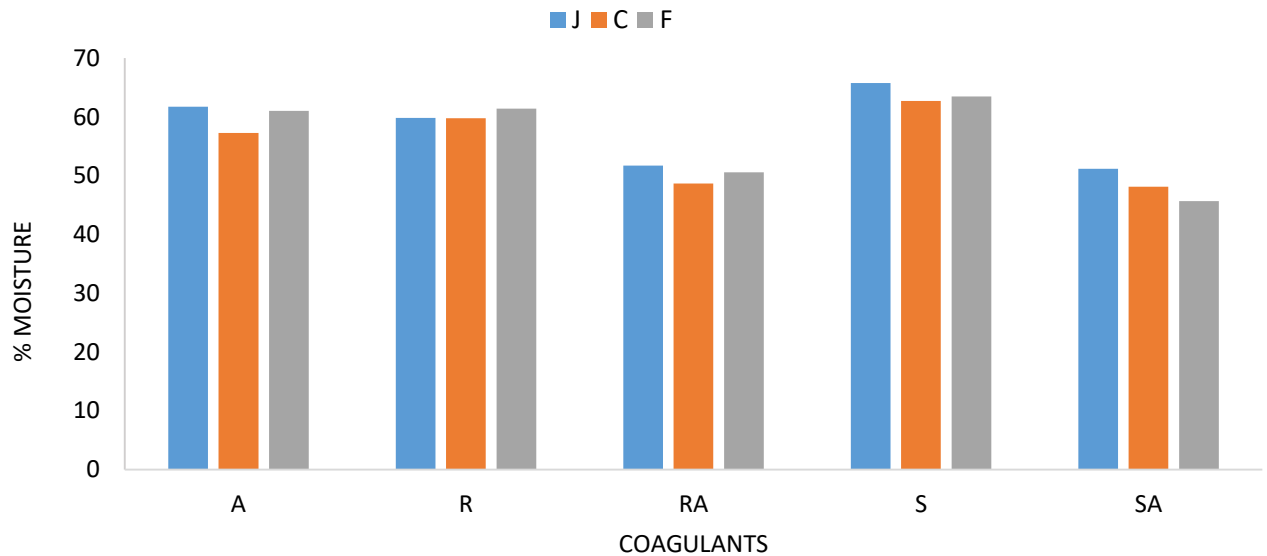
All milk samples that had an initial addition of acid had the first coagulum formed in less than 10 seconds. It took a longer period for coagulum processed with sample R and sample S to coagulate, and between the two, sample R had a shorter coagulation time. The differences in time may be due

to the process by which the different coagulants react. The change in milk pH by adding acid immediately neutralizes the casein micelle. When this happens, the caseins start to coagulate. This takes a very short time to occur as compared to the enzyme, hence the quick rate of coagulation for coagulum processed with A, SA and RA (Troch et al., 2017). Sodom apple and Rennet are enzymes and the process of coagulation is a proteolytic reaction. Coagulum processed with S took a longer time to coagulate and this may be because proteolysis is not as specific as that of R (Rayanatou et al., 2017). It means a longer time would be needed to cleave the right bonds for coagulation to occur.

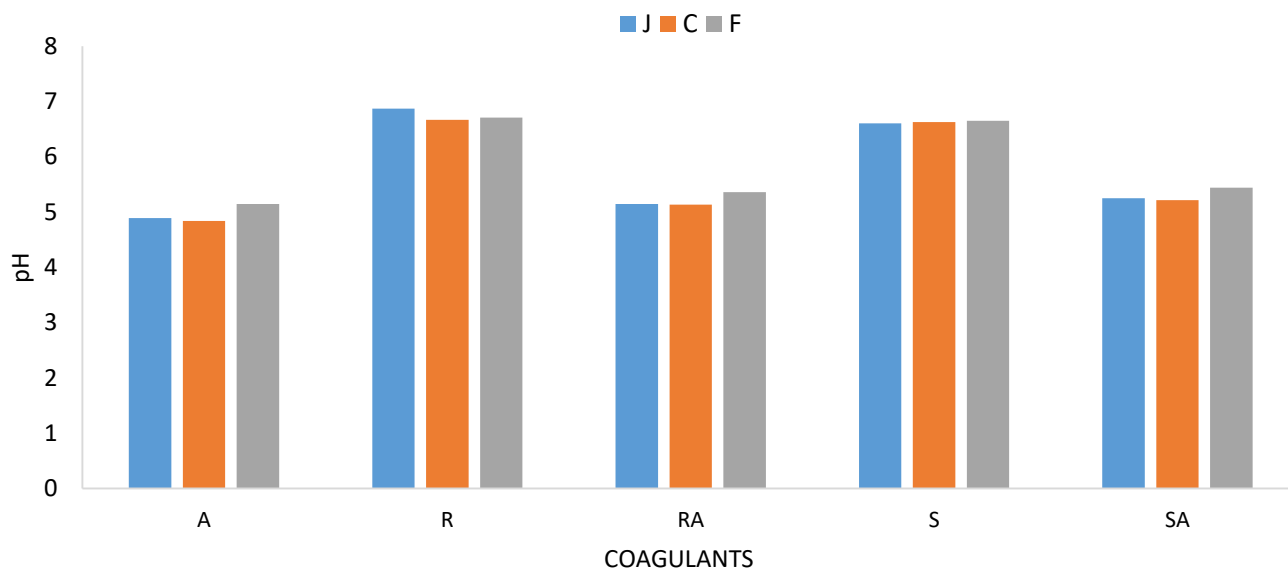
### 4.3 Physicochemical properties of dewatered coagulum obtained

Coagulum moisture, pH, fat, protein and colour were determined. This was done to find out if the milk from the different breeds and different coagulants influenced the final physicochemical properties of the coagulum.

#### 4.3.1 Moisture Content and pH of Coagulum



**Figure 4.4: Moisture Content of Coagulum from Different Breeds Using Different Coagulant Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**



**Figure 4.5: pH of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**

#### 4.3.1.1 Moisture content

There was a statistical significant difference at a p value < 0.05 of the interaction between the breeds and the coagulants

Coagulum from the Jersey showed the highest moisture content for four coagulums. A study conducted by Stocco *et al.* (2018) also showed that the moisture retained in coagulum processed from Jersey milk retained more water as compared to five other cattle breeds.

Coagulum processed with S had the highest moisture content while coagulum processed from RA and SA were the driest. This may be due to continuous syneresis that occurs after cutting or stirring coagulum made from enzymes. Syneresis is the oozing out of moisture in the form of whey when the coagulum is disturbed (Lucey, 2002). In the case of sample S, it means even after dewatering more whey was being let out hence the high moisture content. Longer coagulation time also has an influence on the final moisture content of the product. According to Johnson, Chen, & Jaeggi,

(2010), the longer the coagulation time for enzyme coagulation the higher the moisture content of the final coagulum.

Coagulum processed from A also had a high moisture content. This could be attributed to low calcium -casein interaction leading to more hydrated proteins during direct acidulation (Ismail & Hamad, 2015). The drier state of coagulum from RA and SA may also be due to the low pH of the coagulum. According to Ismail & Hamad, (2015) there is a decrease in hydration of protein due to hydrophobic interactions when the pH of acid-rennet coagulum is closer to the isoelectric point <5.4.

#### **4.3.1.2 pH of coagulum**

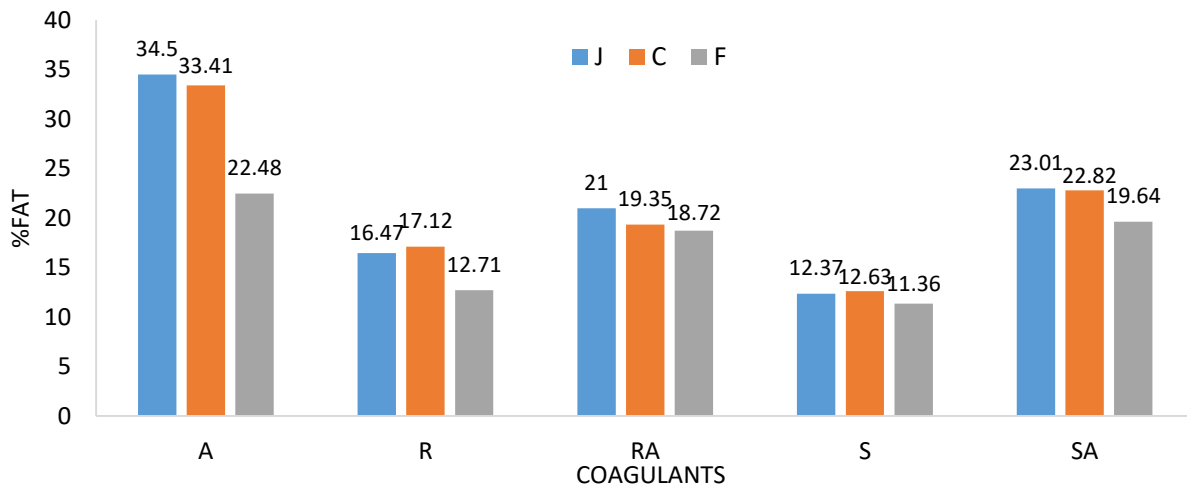
There was a statistical significant difference at a p value < 0.05 of the interaction between the breeds and the coagulants.

pH values of coagulum obtained in Figure 4.5 varied for all the coagulants. The breeds showed almost the same pH pattern for all the coagulum obtained. The White Fulani showed the highest pH values for coagulum that had acid. Apart from the Jersey which showed high pH for coagulum processed with R the other breeds showed almost the same pH for coagulum processed with R. All three breeds showed similar pH values for coagulum processed with S.

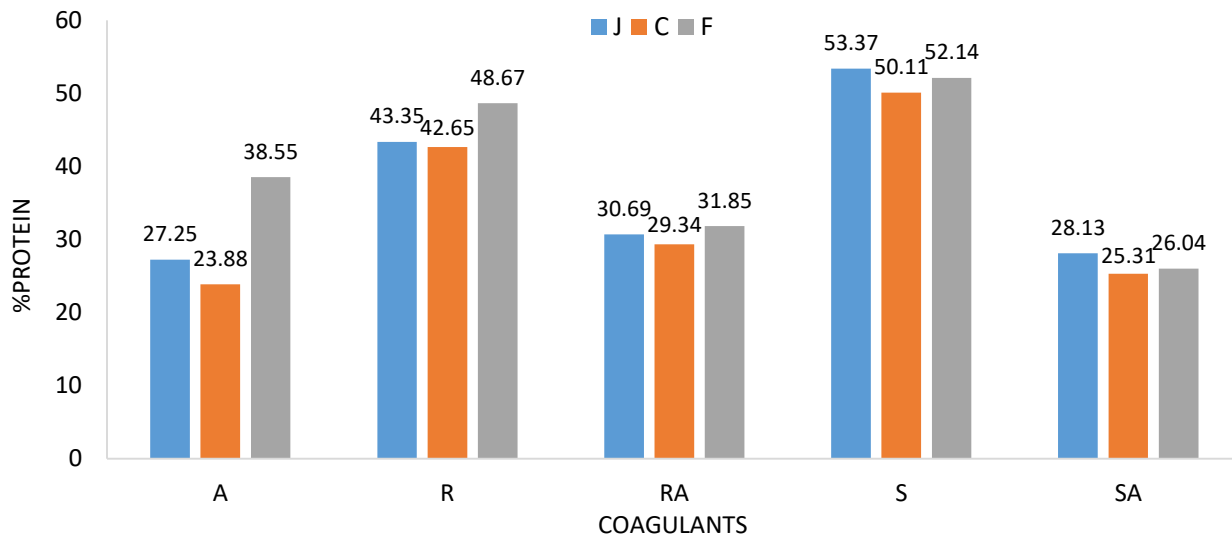
From the graph, the pH of the coagulum processed with S reduced slightly as compared to the pH of the milks. This may be due to the slightly acidic nature of Sodom apple which is 6.36. Coagulum processed with R had its pH values closer to that of the milk used in coagulating. The very close values of coagulum processed with R and S to the original pH value of the milk samples is because enzyme coagulation does not change the pH but rather cleaves  $\kappa$  caseins (Ricardo et al. 2017). The rest of the samples which had acid being added had a more acidic pH. Coagulum processed with A was the most acidic and this was followed by sample RA and finally sample SA. When pH of

milk is lowered, casein micelles reduce in size. The reduced size influences texture of coagulum obtained. The coagulum is much firmer as compared to large sized micelles (Sinaga, Bansal, & Bhandari, 2017). Also, the smaller the micelle size the less lighter the colour of the acidified coagulum. This was seen in Figure 4.11. Samples A, RA and SA were not as light as sample R and S which had no acid added to them.

### 4.3.2 Fat and Protein Content of Coagulum



**Figure 4.6: % Fat Content of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**



**Figure 4.7: % Protein Content of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**

#### 4.3.1.3 Fat content

There was a statistical significant difference at a p value < 0.05 of the interaction between the breeds and the coagulants. Coagulum obtained from the Jersey showed high fat content for most of the samples (Figure 4.6). This was followed closely by the Cross with White Fulani having the least fat content. This may be because coagulum processed from Jerseys usually retain more fat than other cattle breeds (Stocco et al., 2018). Also, the fat content for the cow milks (Table 4.1) showed a similar pattern. Milk from Jersey cows had the highest fat content followed by the Cross and White Fulani. Milk composition has an influence in the final coagulum composition (El-Gawad & Ahmed, 2011). It is therefore not surprising to see that coagulum from the Jersey has higher fat content than the other cows.

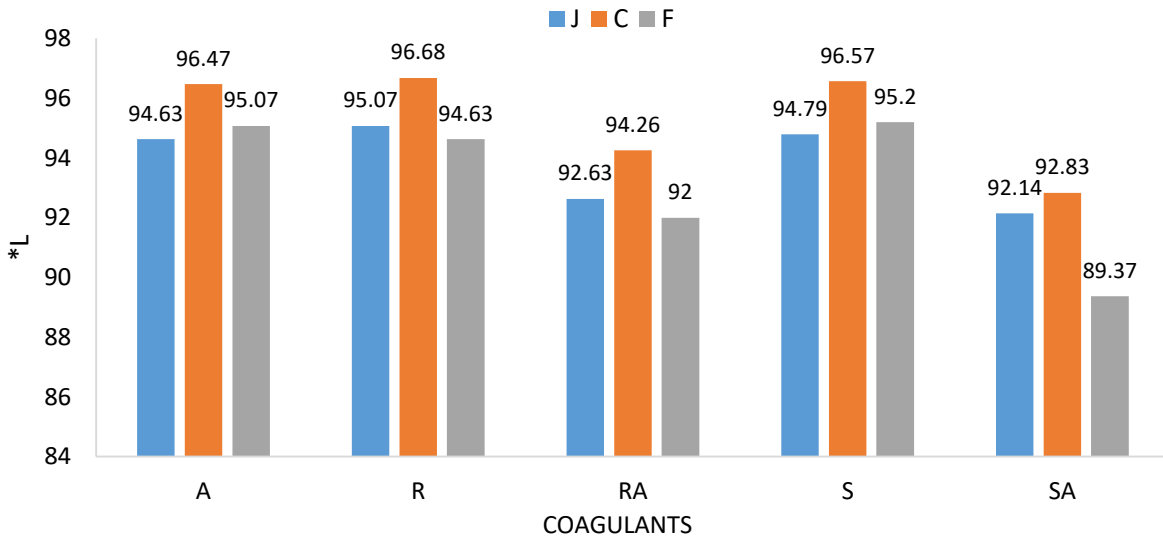
Coagulum processed with A had the highest fat content as compared to the other samples. This was not observed by Ismail & Hamad (2015). According to them milk cheese produced via direct acidulation using glucono-  $\delta$ -lactone had lower fat content as compared to cheese coagulated using

microorganisms. The difference may be due to the type of acid used. Coagulum processed with R and S had the least fat content. This could be accounted for by the firmness of the curd. Firmer curds lose more fat than soft curds. The process of breaking curd before dewatering may have also influenced the fat content too (El-Gawad & Ahmed, 2011). Longer coagulation time also influences the fat recovery of coagulum. S was seen to take a very long time to coagulate as compared to the other coagulants. This could have accounted for the low fat content in S (Johnson, Chen, & Jaeggi, 2010)

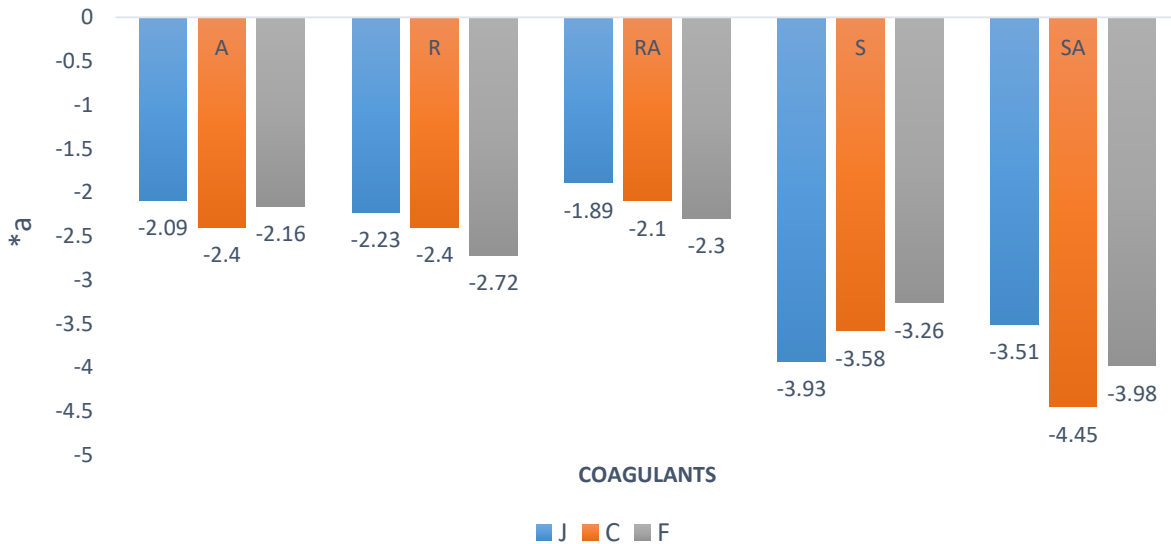
#### **4.3.1.4 Protein content**

From Figure 4.7, protein content was lowest for coagulum processed with A and SA. The highest was that of S. The protein content of the various coagulum follows the same trend as that of the yield. Samples with acid had lower protein content as compared to those that had enzyme only. This may be due to the fact that proteins coagulate at different pH's (Phadungath, 2005a). That is, all the proteins in the milk were not able to coagulate hence the low protein content. Coagulum from S had the highest protein content because some of the whey proteins in the milk got denatured, therefore remained in the coagulum increasing the protein content (El-Gawad & Ahmed, 2011). For breeds, the Cross showed the least protein content for all coagulum. This could be due to the inability of proteins to be retained in the coagulum (Stocco et al., 2018). Unlike milk from the Jerseys that are able to retain proteins in coagulum, the Crosses was not able to do so. This means that most of the proteins were lost since there was no statistical difference in the protein content of the milks from the different breeds (Table 4.1).

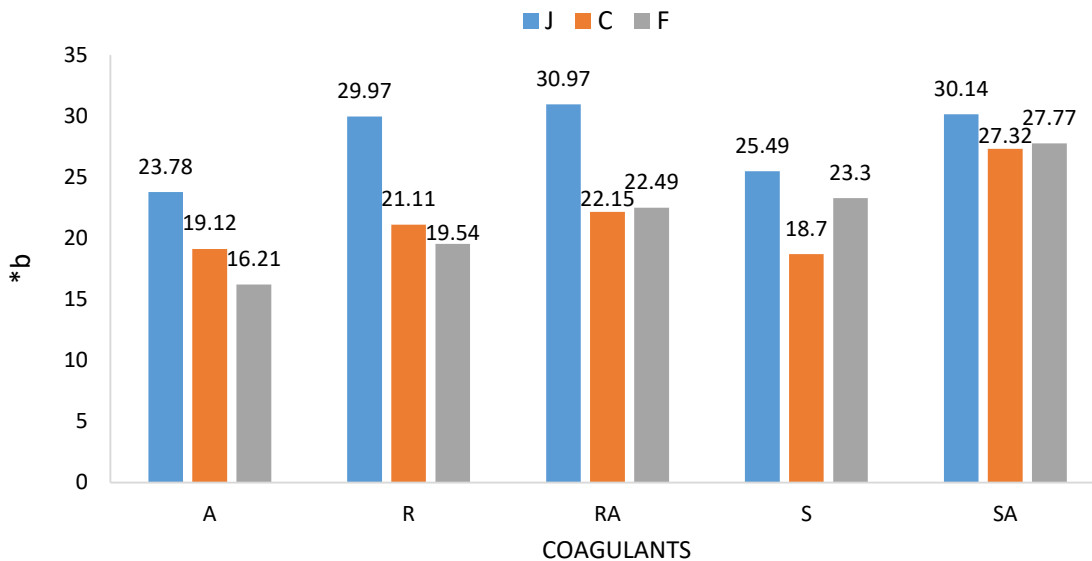
### 4.3.3 Colour of Coagulum - \*L \*a and \*b.



**Figure 4.8: \*L of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**



**Figure 4.9: \*a of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**



**Figure 4.10: \*B of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**

#### 4.3.1.5 Colour of coagulum

There was a statistical significant difference at a p value < 0.05 of the interaction between the breeds and the coagulants for all the colour indices. For the lightness scale (\*L), in Figure 4.8 coagulum samples processed with coagulants A, R and S were observed to be lighter than RA and SA. For the breeds, coagulum from the Cross were lighter than that of the Jersey and the White Fulani

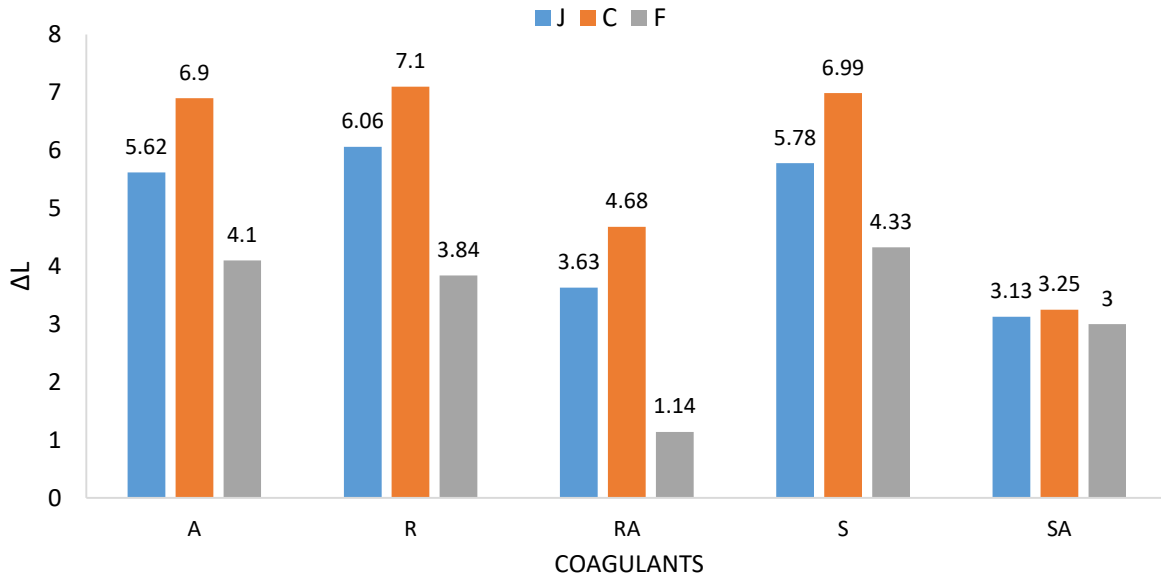
The negative values of the \*a scale Figure 4.9 are an indication that the samples are greener than red. Coagulum made with coagulants S and SA were seen to be greener as compared to the other coagulum. This may be due to the influence of the colour of the S as stated by Akinloye &

Adewumi, (2014). Coagulum processed from SA using milk from the Cross was the greenest amongst all the samples with sample RA processed from the Jersey having the least greenness. Generally, the greenest coagulum was observed to be from the White Fulani and the least from the Jersey.

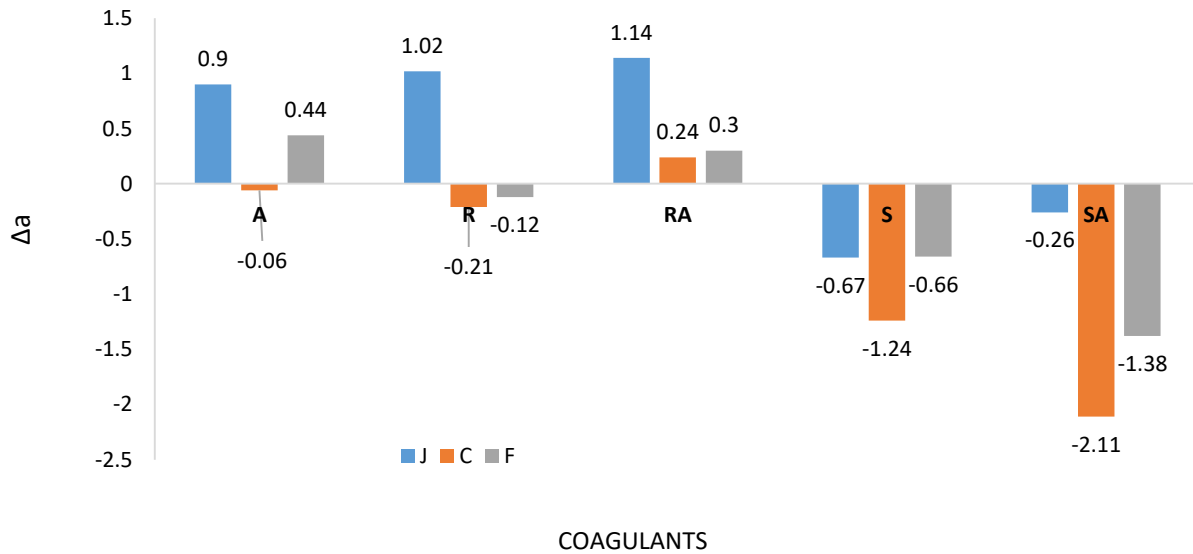
The \*b scale Figure 4.10 indicated that the samples were more yellow than blue. All coagulum processed using milk from the Jersey had high yellowness as compared to the other breeds. This may be due to the yellowness of the Jersey milk as observed in Figure 2. For the Jersey breed, coagulum with the highest yellowness were those processed with R, RA and SA and the least was the sample from A. For the Cross breed, coagulum processed from SA was the yellowest and the least yellow was the coagulum with S. Finally, for the White Fulani breed, coagulum with SA was the most yellow and coagulum with A being the least. For all the coagulants used coagulum obtained using A, was generally the lowest for yellowness and the highest was generally coagulum with SA.

#### **4.3.4 Change in Coagulum Colours $\Delta L$ , $\Delta a$ , and $\Delta b$**

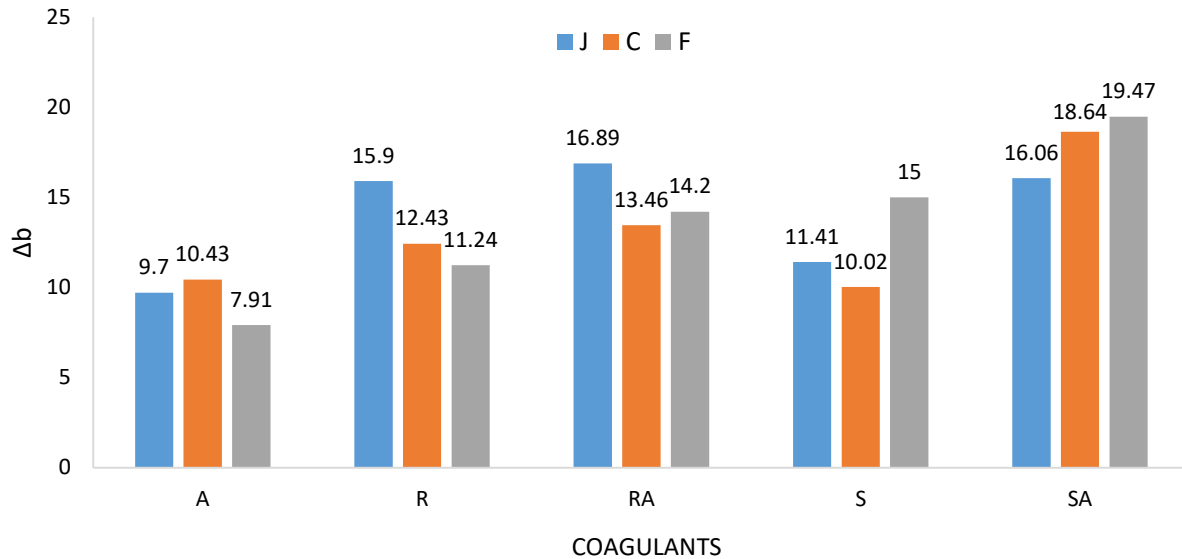
Change in coagulum colour was done to see if there was a change in the final coagulum when compared to the milk that was used in processing it.



**Figure 4.11:  $\Delta L$  of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only - R, Rennet +Acid - RA, Sodom Apple Only - S, Sodom Apple + Acid - SA, White Fulani - F, Cross - C, Jersey - J**



**Figure 4.12:  $\Delta a$  of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only - R, Rennet +Acid - RA, Sodom Apple Only - S, Sodom Apple + Acid - SA, White Fulani - F, Cross - C, Jersey - J**



**Figure 4.13:  $\Delta B$  of Coagulum from Different Breeds Using Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA, White Fulani – F, Cross – C, Jersey – J**

There was a statistical significant difference at a p value  $< 0.05$  of the interaction between the breeds and the coagulants for the change in L, a and b. For  $\Delta L$  Figure 4.11, coagulum obtained from the White Fulani changed the least, followed by the Jersey and finally the Cross breed. The positive values give an indication that the samples are lighter than the milk samples used. Amongst all the samples, coagulum processed with A, R and S were the lightest when compared to the milk and those processed with RA and SA were the least light.

From Figure 4.12, all the three breeds had a greener colour for coagulum obtained from S and SA when compared to the milk samples. Coagulum from coagulant RA was red across all breeds, meaning that it was redder than the milk. Coagulum obtained from the Cross showed the highest

level of greenness when compared to the other breeds and coagulum from the Jersey showed 3 samples being redder than the milk samples. Apart from coagulum processed from RA which was redder than the milks for all three breeds, the other coagulum showed both some redness and greenness depending on the breed.

From the graph all the coagulums were more yellow than the milk. Generally, the sample that was most yellow for all three breeds was coagulum processed with SA. And the least was coagulum processed with A. Coagulum from the Jersey breed generally showed high level of yellowness as compared to the other breeds.

## 4.4 Sensory and physicochemical properties of pooled milk coagulum

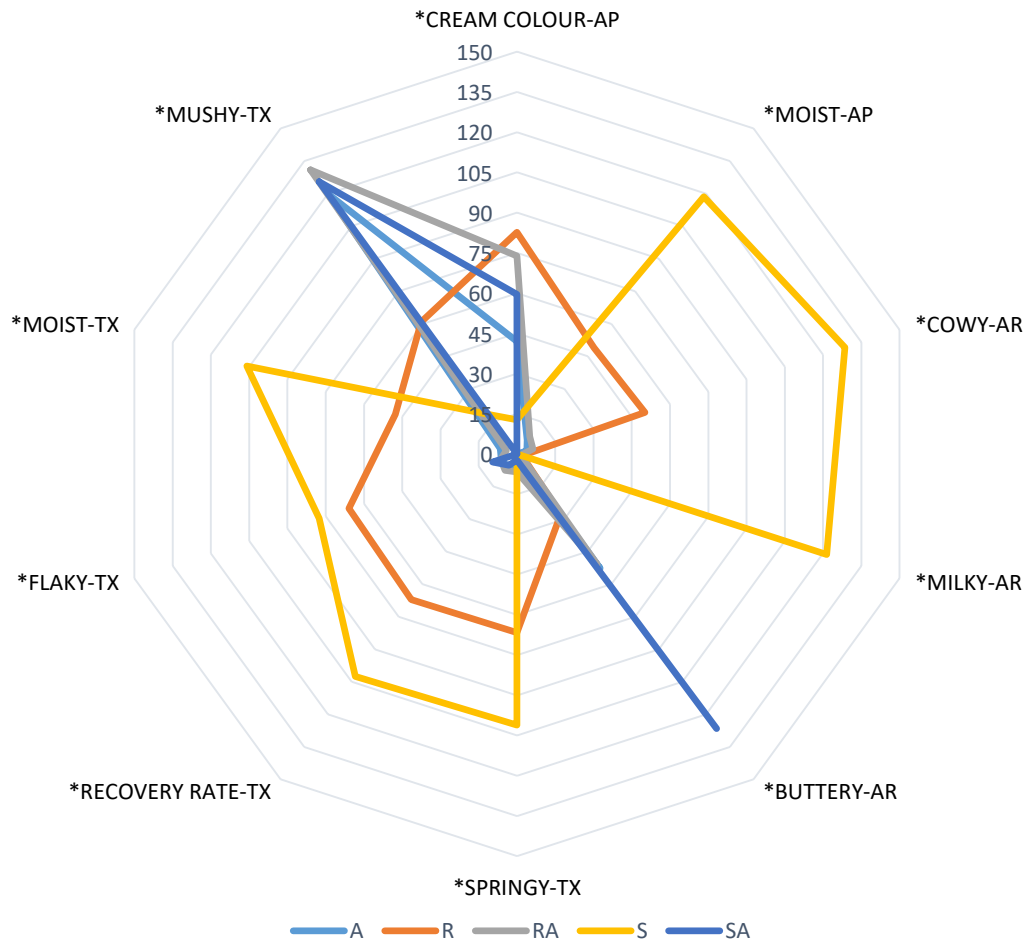
### 4.4.1 Sensory properties of pooled milk Coagulum

Three modalities of the coagulum samples: appearance, aroma and texture in hand were analysed using QDA. A total of 10 attributes were obtained. These consisted of 2 appearance attributes, 3 aroma attributes and 5 texture in hand attributes. Some of the attributes were similar to that obtained by (Aday et al., 2010).

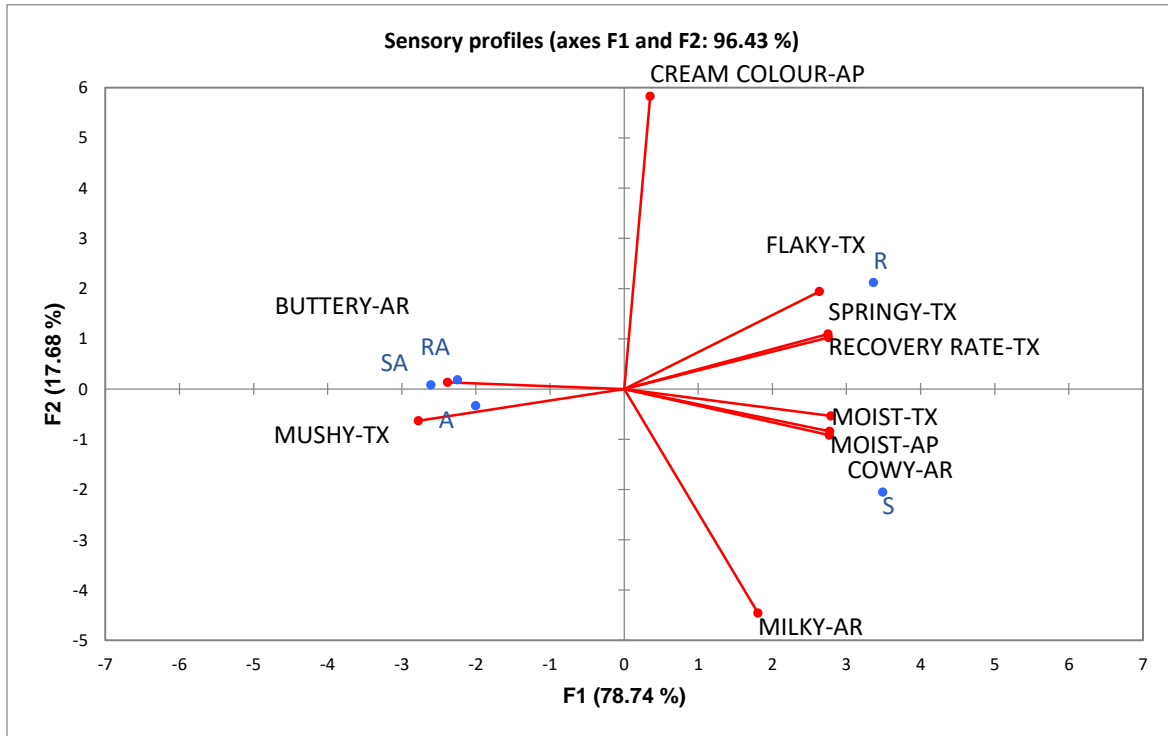
**Table 4.2: Sensory Attributes for the Coagulum Samples**

<b>Modality</b>	<b>Descriptor</b>	<b>Definition</b>	<b>Reference</b>	<b>Anchor</b>	<b>Protocol</b>
<b>Appearance</b>	Cream colour			Light- Dark	<b>Open disposable cup Observe sample from top</b>
	Moist	Having a wet appearance		Not- Very	
<b>Aroma</b>	Cowy	Characteristic aroma of raw cow meat	Raw cow meat	Not- Very	<b>Shake disposable cup Bring close to nose, open to perceive aroma note</b>
	Milky	Characteristic aroma of hot powdered milk drink	Full cream Cowbell powdered milk mixed with hot water	Not- Very	
	Buttery	Characteristic aroma of unsalted butter	Lucke unsalted butter	Not- Very	
<b>Texture in hand</b>	Mushy	Becoming squashy when rubbed between fingers		Not- Very	<b>Cut a quarter of sample Gently press sample between fingers Flaky &amp; Mushy: Take a quarter of sample Rub sample with thumb and middle finger in a forward</b>
	Springy	“Returning” to original state after being compressed		Not- Very	

Recovery rate	Time required for sample to return to original shape	High - Low	and motion	backward
Flaky	Breaking into small fragments	Not- Very		
Moist	Having a wet feel	Not- Very		



**Figure 4.14: Sensory Profile of the Dewatered Coagulum. \*Attributes are Statistically Significant. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA**



**Figure 4.15: PCA of Dewatered coagulum Samples. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA**

All 10 attributes statistically significantly discriminated ( $\alpha=0.05$ ) amongst the samples, showing how the samples differed from each other. Figure 4.14 shows the full appearance, aroma and texture in hand profile of the samples. All the samples were cream in colour with some having a lighter intensity than others. Sample R and S were observed to be the moistest and this was replicated in the attribute moist for the modality texture in hand. This may be due to continuous syneresis that occurs after cutting or stirring coagulum made from enzymes. Syneresis is the oozing out of moisture in the form of whey when the coagulum is disturbed (Lucey, 2002)

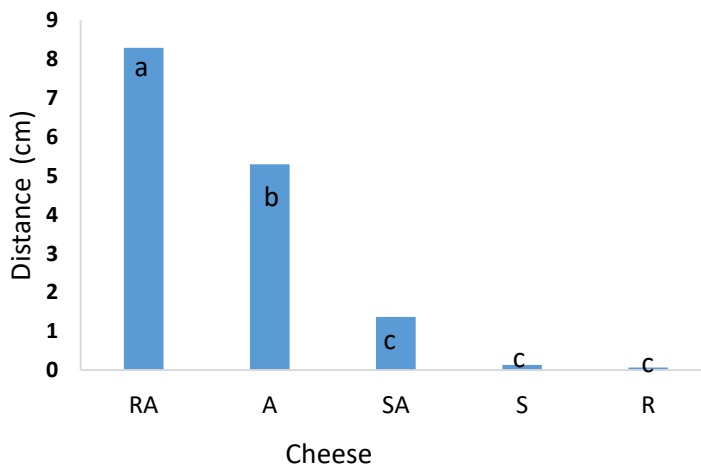
Sample S was also seen to have the highest intensity for cowy and milky aromas. For texture in hand, sample S was the springiest, had the highest recovery rate and was the flakiest. This was closely followed by sample R.

Sample SA had the highest buttery aroma as compared to the other samples and sample R, RA and SA were observed to be mushier as compared to samples S and R for texture in hand.

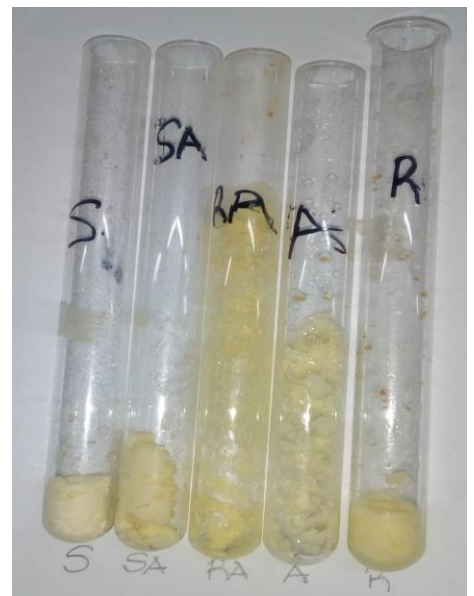
From the PCR in Figure 4.15, it was observed that samples SA, RA and A had a mushy texture when in hand and they were generally buttery. The samples on the positive side: S and R had a more cowy aroma. In terms of texture in hand, S and R were generally springy, moist and flaky.

#### 4.4.2 Meltability of Samples

Meltability of the samples was determined in order to find out which of them had the capability of melting when heat was applied.



**Figure 4.16: Meltability of Cheese Samples. Samples with Different Alphabets are Statistically different at  $P < 0.05$ . Where: Acid Only - A, Rennet Only - R, Rennet + Acid - RA, Sodom Apple Only - S, Sodom Apple + Acid - SA**



**Figure 4.17: Picture Showing the Meltability of the Cheese Samples.**

Figure 4.16 showed that sample RA melted more than the other samples and this was followed by sample A. Sample SA showed some level of melting, but there was no statistical significance difference amongst it, sample S and R. Melting in cheese actually occurs due to fat melting (Lucey, Johnson, & Horne, 2003). According to Cais-Sokolińska & Pikul (2009), the higher the fat content the higher the meltability. This is evident in Figure 4.7, where sample RA, SA and A were seen to generally have a higher fat content as compared to the other samples. SA deviated from this fact and this could be due to the optimum temperature at which coagulation occurs for S. According to Lucey, Johnson, & Horne (2003), cheese processed from milks at very high temperatures are not able to melt. The high rate of melting seen in sample RA and not in sample R may be due to the influence of the acid present. The acid may have reduced the protein-protein interaction that takes place during enzyme coagulation thereby reducing the association of calcium with the casein molecules. Strong protein-protein interactions prevents cheese from melting (Lucey, Johnson, & Horne, 2003).

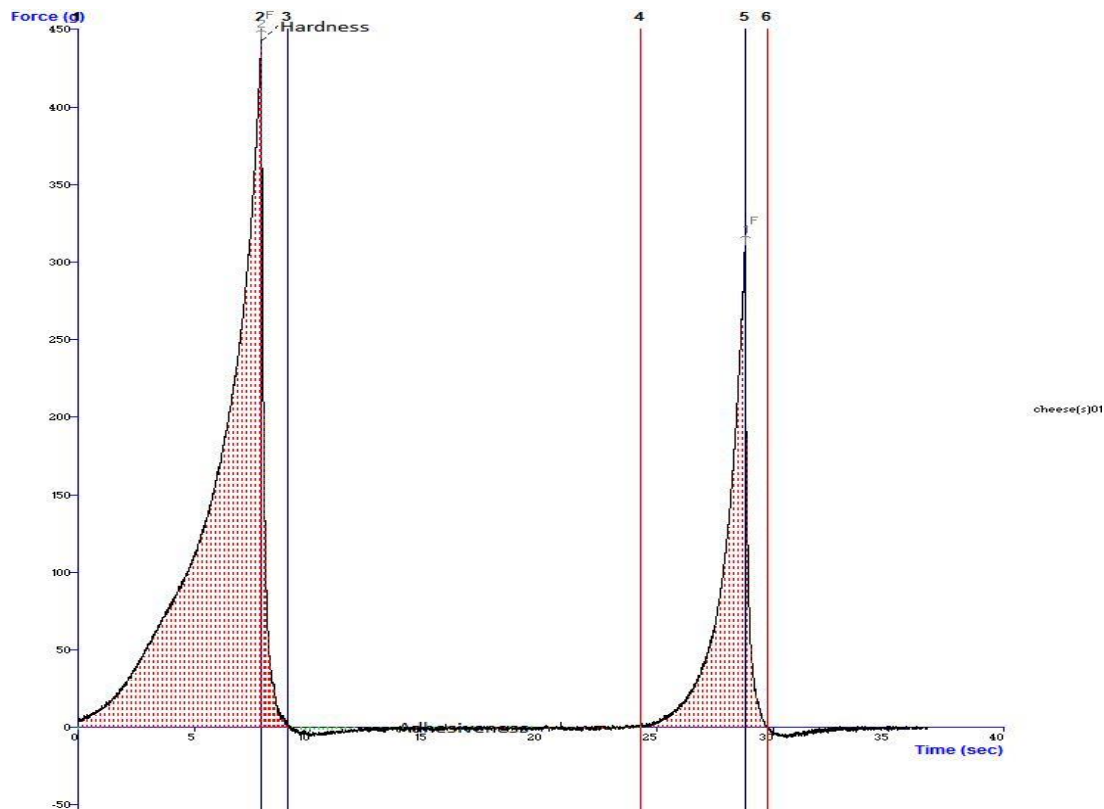
#### 4.4.3 Texture Profile of Dewatered Coagulum Samples

Texture of the samples were determined to know the textural properties of the individual coagulum's processed from different coagulants.

**Table 4.3: Texture Profile of the Cheese Samples**

<b>Sample</b>	<b>Hardness (g)</b>	<b>Adhesiveness (gs)</b>	<b>Springiness (s)</b>	<b>Cohesiveness</b>	<b>Chewiness (gs)</b>
<b>A</b>	962.73±7.25 <sup>b</sup>	-35.61±10.29 <sup>c</sup>	0.45±0.036 <sup>c</sup>	0.24 ±0.008 <sup>c</sup>	103.13±5.98 <sup>b</sup>
<b>R</b>	939.00±19.9 <sup>b</sup>	-10.76±1.29 <sup>a</sup>	0.71±0.031 <sup>a</sup>	0.36±0.010 <sup>a</sup>	235.36±4.68 <sup>a</sup>
<b>RA</b>	1066.80±17.5 <sup>a</sup>	-74.71±0.67 <sup>d</sup>	0.22±0.02 <sup>d</sup>	0.20±0.004 <sup>b</sup>	45.86±4.74 <sup>c</sup>
<b>S</b>	530.60±31.7 <sup>c</sup>	-20.80±0.58 <sup>ab</sup>	0.62±0.047 <sup>b</sup>	0.33±0.026 <sup>a</sup>	107.40 <sup>b</sup> ±17.30 <sup>b</sup>
<b>SA</b>	1029.78±7.15 <sup>a</sup>	-34.92±5.65 <sup>bc</sup>	0.38±0.014 <sup>c</sup>	0.26±0.015 <sup>b</sup>	101.22±9.68 <sup>b</sup>

Values with the different alphabets are statistically different for each textural property at p<0.05. Values are means ± standard deviations. Where: Acid only - A, Rennet only – R, Rennet +Acid – RA, Sodom apple only - S, Sodom apple + Acid – SA



**Figure 4.18: A Typical Texture Profile Graph of Dewatered Coagulum**

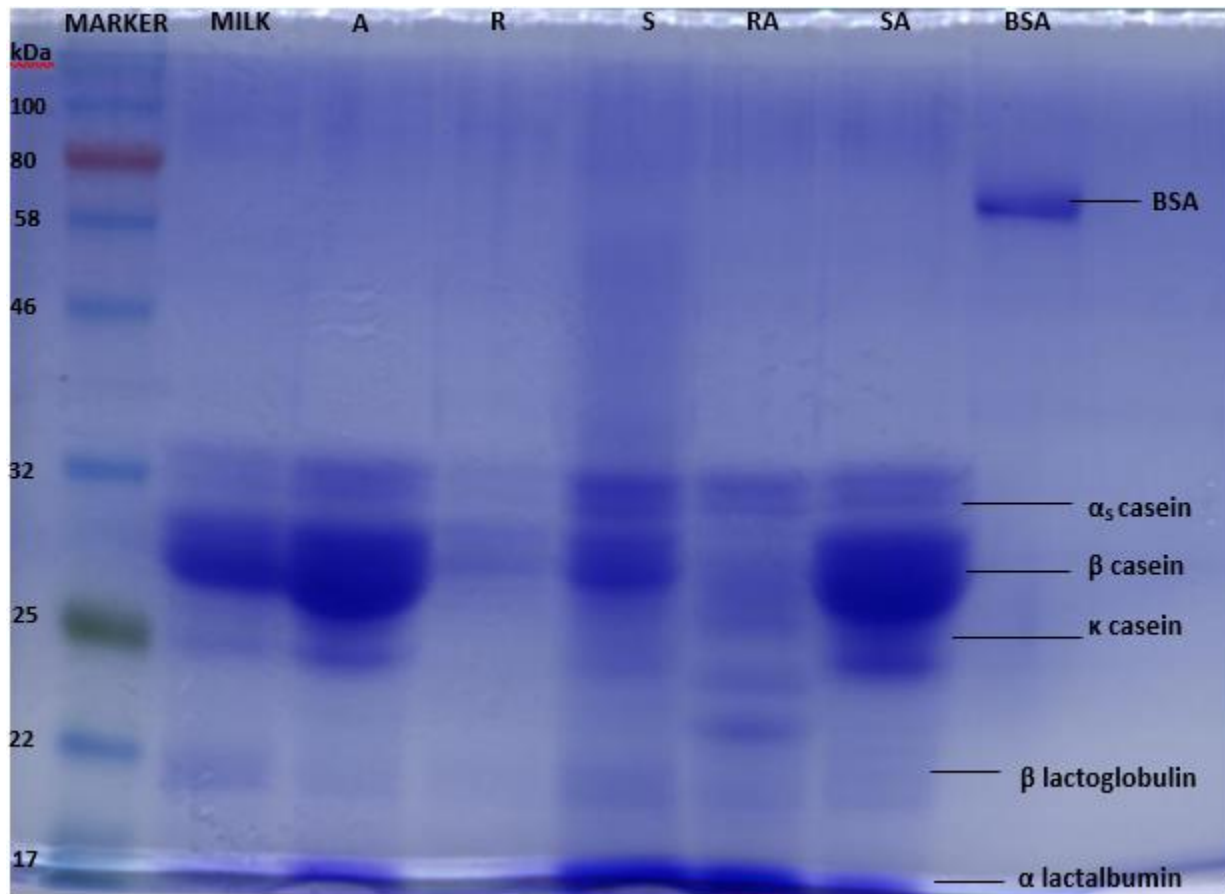
From Table 4.3, samples RA and SA were the hardest amongst all the samples, while sample S was the softest. Comparing sample R to S, sample R was seen to have a harder texture. This correlates with Ben Amira et al. (2017), when they make mention of the fact that rennet or chymosin produces firmer gels as compared to plant enzymes.

Sample R was observed to be the most adhesive. This means that for sample R, more force was required in pulling the compression force off. The least adhesive was sample RA. This trend can be accounted for on the basis that the harder the sample, the less adhesive it becomes (Saunders, Hamann, & Lineback, 1992). This can be observed in Table 4.3 where RA was the hardest among all the samples.

The springiest samples were R and S. This indicates that they recovered more of their original height as compared to the other samples. Similar results were seen for texture in hand in the sensory analysis. More energy (chewiness) was needed to break down or masticate sample R and S as compared to the other samples. The least chewy sample was RA. According to Foegeding & Drake (2007), low fat content of coagulum produces springy and chewy samples. This could be the reason why sample R and S were the springiest and chewiest. They had the least fat content in (Figure 4.6). Samples R and S were also the most cohesive. Cohesiveness is the degree to which the sample deforms before shearing.

#### 4.4.4 Protein Profile of Dewatered Coagulum and Milk Samples

Proteins in the milk undergo various processes during coagulation. This test was done to find out which proteins were lost and which ones were retained in the various coagulums.



**Figure 4.19: SDS-PAGE of Dewatered Coagulum Made from Different Coagulants. Where: Acid Only - A, Rennet Only – R, Rennet +Acid – RA, Sodom Apple Only - S, Sodom Apple + Acid – SA**

From Figure 4.19 visible bands indicate presence of a protein. The deeper the band the more the protein present. The milk sample had all three caseins in it with  $\beta$  casein being the most. Generally, sample R had had the faintest bands for all proteins. For all the samples there was a band that

showed presence of  $\alpha_s$  casein. The bands for A and S were the deepest and that of R was the faintest. Amongst all the caseins,  $\beta$  casein was observed to be the most abundant. It was lowest in R and RA and highest in A and SA. The same was observed for  $\kappa$  casein; deeper bands were observed for A and SA. The faint bands seen in sample R could be because more  $\kappa$  caseins were hydrolyzed allowing other caseins to bind to calcium to form paracaseinate (Lucey, 2002) . The specificity of the rennet enzyme could also account for this (Ben Amira et al., 2017). Acids do not have any influence on the caseins as compared to enzymes (Troch et al., 2017), hence the high concentration of the caseins. SA had similar bands as that of A. This may be due to denaturation of the Sodom apple enzyme due to the presence of the acid.

#### 4.5 Cost of setting up, running and producing cheese in a cheese factory

The assumptions made in estimating the economic potential of using local raw materials to make cheese from any of the coagulum developed in the current experiment are outlined below:

Working period: 144 days per year continuously (three times a week)

- Production rate for cheese: 80kg/day (800L of milk/day) assuming that 1L of milk gives 100g of cheese.
- Plant life: 20 years.
- Exchange rate: \$1 = GH¢ 4.41 (Bank of Ghana rate: 28/05/2018).
- Interest rate (i) = 17% (Bank of Ghana rate: 28/05/2018).
- Tax rate- Corporate tax (t): 25% (Ghana revenue authority: 28/05/2018)

#### 4.5.1 Total Capital Investment

##### 4.5.1.1 Equipment Cost

One of the major costs involved in a plant is the cost of equipment. This involves all the major and minor equipment and the vehicles needed. The spare parts, contingency, handling and transportation of the equipment were also calculated using factors proposed by (Peters & Timmerhaus, 1991).

**Table 4.4: Cost of Major Equipment**

Equipment	Material of construction	Quantity
Batch pasteurizer and fermentation tank	Stainless steel	1 SET
Automatic control panel	-	1 SET
Milk pump	Stainless steel	1 UNIT
Cream separator	Stainless steel	1 UNIT
Cream collection trolley	Plastic	1 UNIT
Homogenizer	Stainless steel	1 UNIT

Fermentation tank	Stainless steel	1 UNIT
Cheese press	Stainless steel	1 UNIT
Cheese molds	Plastic	1 SET
Cheese working table	Stainless steel	1 SET
Hot water boiler	-	1 UNIT
<b>TOTAL</b>	<b>\$95,000</b>	
<b>DELIVERY COST- Shipping and packaging</b>	<b>\$2,500</b>	
<b>TOTAL COST IN \$</b>	<b>\$97,500</b>	
<b>TOTAL COST IN GH¢</b>	<b>429,975.00</b>	

**Table 4.5: Cost of Minor Equipment**

<b>Equipment</b>	<b>Material of construction</b>	<b>Quantity</b>	<b>Unit cost (GH¢)</b>	<b>Total cost (GH¢)</b>
Cheese cloth	Cotton	50 yards	7.00	350.00
Knives	Stainless steel	5	5.00	25.00
Basin	Stainless steel	5	35.00	175.00
Thermometer		1	550.00	550.00
Hand held pH meter		1	50.00	50.00
Aerometer		1	250.00	250.00
Electronic scale		1	155.00	155.00
Measuring cup		5	6.00	30.00
Spatula	Stainless steel	5	35.00	175.00

*Industrial grinder		1	7,497.00	7,497.00
Glass door Refrigerator		1	1,800.00	1,800.00
<b>*TOTAL</b>				<b>11,057.00</b>
<b>TOTAL</b>				<b>3,560.00</b>

\*Required only for producing sample Sodom apple and Sodom apple + acid

$$\begin{aligned}
 \text{*Total cost of equipment} &= \text{Cost of major equipment} + \text{Cost of minor equipment} \\
 &= 429,975.00 + 11,057.00 \\
 &= \text{GH}\text{\textasciitilde} 441,032.00
 \end{aligned}$$

$$\begin{aligned}
 \text{*Estimated: Spare parts} &= 10\% \\
 &= 1.10 \times 441,032.00 = \text{GH}\text{\textasciitilde} 485,135.20 \\
 \text{Contingency} &= 2\% \\
 &= 1.02 \times 485,135.20 = \text{GH}\text{\textasciitilde} 494,837.90 \\
 \text{Handling and Transportation} &= 0.5\% \\
 &= 1.005 \times 494,837.90 = \text{GH}\text{\textasciitilde} 497,312.09
 \end{aligned}$$

$$\begin{aligned}
 \text{Total cost of equipment} &= \text{Cost of major equipment} + \text{Cost of minor equipment} \\
 &= 429,975.00 + 3560.00 \\
 &= \text{GH}\text{\textasciitilde} 433,535.00
 \end{aligned}$$

$$\begin{aligned}
 \text{Estimated: Spare parts} &= 10\% \\
 &= 1.10 \times 433,535.00 = \text{GH}\text{\textasciitilde} 476,888.50 \\
 \text{Contingency} &= 2\%
 \end{aligned}$$

$$= 1.02 \times 476,888.50 = \text{GH¢ } 486,426.27$$

Handling and Transportation = 0.5%

$$= 1.005 \times 486,426.27 = \text{GH¢ } 488,858.40$$

**Table 4.6: Cost of Vehicle for transporting milk and cheese**

Cost of vehicle	Quantity	Unit cost (GH¢)	Total cost (GH¢)
Refrigerated truck	1	138,000.00	138,000.00
<b>TOTAL</b>			<b>138,000.00</b>

**\*Total purchased equipment cost (PEC)** = total cost of equipment + cost of vehicle

$$\begin{aligned} \text{PEC} &= \text{GH¢ } 497,312.09 + \text{GH¢ } 138,000 \\ &= \text{GH¢ } 635,312.09 \end{aligned}$$

**Total purchased equipment cost (PEC)** = total cost of equipment + cost of vehicle

$$\begin{aligned} \text{PEC} &= \text{GH¢ } 488,858.40 + \text{GH¢ } 138,000 \\ &= \text{GH¢ } 626,858.40 \end{aligned}$$

#### 4.5.1.2 Estimation of Fixed Capital Investment (CF)

Costs associated with Fixed Capital investment include expenditures for raw materials, direct operating labor; supervisory; plant maintenance and repairs; operating supplies; power; utilities etc. (Peters & Timmerhaus, 1991).

**Table 4.7: Fixed Capital Investment**

Item	Cost factor of PEC	Estimated cost (GH¢)	*Estimated cost (GH¢)
Purchased equipment cost (PEC)	1.00	626,858.4	635,312.09
Equipment installation	0.30	188,057.52	190,593.6

pipng	0.15	94,028.76	95,296.81
Electrical installation	0.10	62,685.84	63,531.21
Building/ auxiliary	0.20	125,371.68	127,062.4
Service and land improvement	0.40	250,743.36	254,124.8
Instrumentation control	0.20	125371.68	127062.4
Engineering and supervision	0.05	31342.92	31765.6
Construction expense and contractor	0.12	75223.008	76237.45
Contingency	0.01	6268.584	6353.121
<b>C<sub>F</sub> TOTAL</b>		<b>1,585,951.75</b>	<b>1,607,339.59</b>

Calculations are based on the PEC

#### 4.5.1.2.1 Working Capital

According to Peters and Timmerhaus (1991), Working Capital (C<sub>w</sub>) is 15% of the Total Capital investment (C<sub>T</sub>).

$$C_T = C_F + C_W$$

$$C_W = 0.15 C_T$$

$$C_T = C_F + 0.15C_T$$

$$C_F = C_T - 0.15C_T$$

$$C_F = 0.85 C_T$$

#### 4.5.1.2.2 Total Capital Investment

$$*C_T = \frac{C_F}{0.85} = \frac{1,607,339.59}{0.85}$$

$$*C_T = 1890987.75$$

$$*C_W = 0.15C_T$$

$$= 0.15 \times 1890987.75$$

$$= \text{GH}\text{¢} 283648.163$$

$$C_T = \frac{C_F}{0.85} = \frac{1,585,951.75}{0.85}$$

$$C_T = \text{GH}\text{C}1865825.59$$

$$C_W = 0.15C_T$$

$$= 0.15 \times 1865825.59$$

$$= \text{GH}\text{C} 279873.839$$

**Table 4.8: Summary of Results**

<b>SAMPLES</b>	<b>Total Capital Investment (C<sub>T</sub>) (GH¢)</b>	<b>Working Capital (C<sub>W</sub>) (GH¢)</b>	<b>Fixed Capital Investment (C<sub>F</sub>) (GH¢)</b>
Sodom apple and Sodom apple +acid	1890987.75	283648.163	1,607,339.59
Rennet only, Acid only and Rennet + Acid	1865825.59	279873.839	1,585,951.75

#### 4.5.2 Estimation of Total Production Cost

Total production is expressed as:

**Total production cost (TPC)** = manufacturing cost (C<sub>M</sub>) + general expenses

**Manufacturing cost (C<sub>M</sub>)** = Direct production cost + fixed charges + plant overhead cost

##### 4.5.2.1 Direct Production Cost

Costs associated with this section include expenditures for raw materials direct operating labor; supervisory; plant maintenance and repairs; operating supplies; power; utilities etc. (Peters and Timmerhaus, 1991).

#### 4.5.2.2 Fixed Charges

These are expenses which remain quite constant from year to year and do not vary substantially with changes in production rate. These may include depreciation among others (Peters & Timmerhaus, 1991).

#### 4.5.2.3 Plant-overhead

This may include safety services and general plant maintenance and overheads (Peters & Timmerhaus, 1991).

#### 4.5.2.4 Estimation of Total Product Cost

**Table 4.9: Production Rate for Cheese**

	<b>Rennet only</b>	<b>Acid only</b>	<b>Sodom apple only</b>	<b>Sodom apple and acid</b>	<b>Rennet and acid</b>
Cost of milk/L (GH¢)	4.00	4.00	4.00	4.00	4.00
Production rate for cheese/day (g)	147000	72000	184000	104000	114000
Amount of milk needed per day(L)	800	800	800	800	800
Cost of milk per day (GH¢)	3,200	3,200	3,200	3,200	3,200
Annual cost of milk (GH¢)	460,800	460,800	460,800	460,800	460,800
Amount of coagulum needed per day (kg)	0.4	2.56	28.24	28.24+2.56	0.4+2.56
Cost of Coagulant per day (GH¢)	2,389.86	2,905.36	50.00	2,955.36	5,295.22
Annual cost of coagulant (GH¢)	344,140.88	418,372.49	7,200.00	425,571.84	762,511.68
Cost of packaging (vacuum package)	7,200.00	7,200.00	7,200.00	7,200.00	7,200.00
Estimated Miscellaneous	1,000.00	1,000.00	1,000.00	1,000.00	1,000.00
<b>Total cost of raw materials GH¢</b>	<b>813,140.88</b>	<b>887,372.49</b>	<b>476,200.00</b>	<b>894,571.84</b>	<b>1,231,511.68</b>

Amount of coagulum needed per day (kg) was estimated from the quantity used in coagulating 100ml of milk.

#### 4.5.2.5 Depreciation Cost (d)

This is the assumed decrease in value of a material that can occur throughout its lifespan.

Several methods are available for determining the depreciation cost. The straight-line method was used for this project

$$d = \frac{V - V_s}{n}$$

Where:

d = annual depreciation (GHC/yr)

V = the Initial Fixed Capital Investment

V<sub>s</sub> = Salvage value = 10% C<sub>F</sub>

N = Service life or useful life of plant (yrs) = 20yrs

For rennet only

$$d = \frac{1,585,951.75 - 158,595.18}{20} = \text{GH}\text{¢} 64,606.6053/\text{yr}$$

**Table 4.10: Depreciation (d)**

Depreciation	Rennet only	Acid only	Sodom apple only	Sodom apple and acid	Rennet and acid
C <sub>F</sub>	1,585,951.75	1,585,951.75	1,607,339.59	1,607,339.59	1,585,951.75
V <sub>s</sub>	158,595.18	158,595.18	160,734.96	160,734.96	1,585,95.18
V	1,585,951.75	1,585,951.75	1,607,339.59	1,607,339.59	1,585,951.75
n	20	20	20	20	20
<b>d (GHC/yr)</b>	<b>71,367.83</b>	<b>71,367.83</b>	<b>72,330.28</b>	<b>72,330.28</b>	<b>71,367.83</b>

#### 4.5.2.6 Total Operating Labour

This involves the wages for all the workers employed in the factory.

**Table 4.11: Total Operating Labour**

<b>Personnel</b>	<b>Number</b>	<b>Monthly salary (GH¢)</b>	<b>Total annual salary (GH¢)</b>
Managing director	1	2,000.00	288,000.00
Product manager	1	1,500.00	216,000.00
Quality assurance manager	1	1,500.00	216,000.00
Accountant	1	1,000.00	144,000.00
Casual workers	4	500.00	288,000.00
Driver	1	400.00	57,600.00
Canteen attendants	2	350.00	100,800.00
Nurse	1	500.00	72,000.00
Security	2	300.00	86,400.00
Cleaners	2	300.00	86,400.00
Receptionist	1	500.00	72,000.00
<b>TOTAL</b>			1,627,200.00
Social security		18.5%	301,032.00

<b>TOTAL OPERATING LABOUR COST (GH¢)</b>	<b>1627200+301032</b>	<b>1,928,232.00</b>
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#### 4.5.2.7 Total Production Cost

**Table 4.12: Total Production Cost**

<b>Direct production cost</b>						
	<b>FACTOR</b>	<b>TOTAL COST (GH¢)</b>				
		<b>Rennet only</b>	<b>Acid only</b>	<b>Sodom apple</b>	<b>Sodom apple +Acid</b>	<b>Rennet +acid</b>
Raw material	10% TPC	0.1TPC	0.1TPC	0.1TPC	0.1TPC	0.1TPC
Utilities	15% TPC	0.15 TPC	0.15 TPC	0.15 TPC	0.15 TPC	0.15 TPC
Maintenance	5% equipment cost	31342.92	31342.92	31765.60	31765.60	31342.92
Supervision	15% operating labour	289,234.80	289,234.80	289,234.80	289,234.80	289,234.80
Operating labour	15% TPC	0.15 TPC	0.15 TPC	0.15 TPC	0.15 TPC	0.15 TPC
<b>Total</b>		<b>320,577.72 +0.4TPC</b>	<b>320,577.72 +0.4 TPC</b>	<b>321,000.40 +0.4 TPC</b>	<b>321,000.40 +0.4 TPC</b>	<b>320,577.72 +0.4 TPC</b>
<b>FIXED CHARGES</b>						
Plant overheads	10% TPC	0.10TPC	0.10TPC	0.10TPC	0.10TPC	0.10TPC
Depreciation		71,367.83	71,367.83	72,330.28	72,330.28	71,367.83

Insurance	1% C <sub>F</sub>	15,859.52	15,859.52	16,073.4	16,073.4	15,859.52
Rates	5% C <sub>F</sub>	79,297.59	79,297.59	80,366.98	80,366.98	79,297.59
<b>Total</b>		166,524.93 <b>+0.1TPC</b>	166,524.93 <b>+0.1TPC</b>	168,770.66 <b>+0.1TPC</b>	168,770.66 <b>+0.1TPC</b>	166,524.93 <b>+0.1TPC</b>
<b>GENERAL EXPENSES</b>						
Sales expenses	2% TPC	0.02TPC	0.02TPC	0.02TPC	0.02TPC	0.02TPC
Research and development	2% TPC	0.02TPC	0.02TPC	0.02TPC	0.02TPC	0.02TPC
General overhead	2% TPC	0.02TPC	0.02TPC	0.02TPC	0.02TPC	0.02TPC
<b>Total</b>		<b>0.06TPC</b>	<b>0.06TPC</b>	<b>0.06TPC</b>	<b>0.06TPC</b>	<b>0.06TPC</b>

**TPC for rennet only**

= Direct production cost + Fixed Charges + General Expenses

= 320,577.72+0.4TPC+ 166,524.93+0.1TPC + 0.06TPC

TPC = 487,102.65+0.56TPC

TPC (1- 0.56) = 487,102.65

0.44 TPC = 487,102.65

$$\text{TPC} = \frac{487,102.65}{0.44}$$

= GH¢ 1,107,051.49

**Table 4.13: Summary of Total Production Cost**

<b>TOTAL PRODUCTION COST (TPC) (GH¢)</b>					
	<b>Rennet only</b>	<b>Acid only</b>	<b>Sodom apple</b>	<b>Sodom apple +Acid</b>	<b>Rennet +acid</b>

Direct production cost	320,577.72 <b>+0.4TPC</b>	320,577.72 <b>+0.4 TPC</b>	321,000.40 <b>+0.4 TPC</b>	321,000.40 <b>+0.4 TPC</b>	320,577.72 <b>+0.4 TPC</b>
Fixed Charges	166,524.93 <b>+0.1TPC</b>	166,524.93 <b>+0.1TPC</b>	168,770.66 <b>+0.1TPC</b>	168,770.66 <b>+0.1TPC</b>	166,524.93 <b>+0.1TPC</b>
General Expenses	0.06TPC	0.06TPC	0.06TPC	0.06TPC	0.06TPC
Total production cost	487,102.65 <b>+0.56TPC</b>	487,102.65 <b>+0.561TPC</b>	489,771.06 <b>+0.56TPC</b>	489,771.06 <b>+0.56TPC</b>	487,102.65 <b>+0.56TPC</b>
<b>TOTAL(GH¢)</b>	<b>1107051.49</b>	<b>1107051.49</b>	<b>1113116.05</b>	<b>1113116.05</b>	<b>1107051.49</b>

#### 4.5.2.8 Manufacturing Cost (C<sub>M</sub>)

For rennet only

$$\text{General expenses} = 0.06 \text{ TPC}$$

$$= 0.06 \times 1107051.49$$

$$= \text{GH¢ } 66423.09$$

$$\text{TPC} = \text{Manufacturing Cost (C}_M\text{)} + \text{General Expenses}$$

$$\text{C}_M = \text{TPC} - \text{general expenses}$$

$$= 1107051.49 - 66423.09$$

$$= \text{GH¢ } 1173474.57$$

**Table 4.14: Manufacturing Cost (C<sub>M</sub>)**

	<b>Rennet only</b>	<b>Acid only</b>	<b>Sodom apple</b>	<b>Sodom apple +Acid</b>	<b>Rennet +acid</b>
Total production cost (TPC)	1,107,051.49	1,107,051.49	1,113,116.05	1,113,116.05	1,107,051.49
General expenses	66,423.09	66,423.09	66,786.96	66,786.96	66.423.09

<b>(C<sub>M</sub>) (GH¢)</b>	<b>1,040,628.40</b>	<b>1,040,628.40</b>	<b>1,046,329.09</b>	<b>1,046,329.09</b>	<b>1,040,628.40</b>
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### 4.5.3 Profitability Analysis

This is to find out if there would be some profit realized from running the project. Profitability evaluation can be categorized under the following headings:

Rate of return on investment

Discounted cash flow based on full-life performance

Net present worth

Capitalized costs

(Peters and Timmerhaus, 1991)

**Table 4.15: Summary**

<b>SUMMARY</b>					
	<b>Rennet only</b>	<b>Acid only</b>	<b>Sodom apple</b>	<b>Sodom apple +Acid</b>	<b>Rennet +acid</b>
<b>Cost of cheese/100g (GH¢)</b>	<b>8.00</b>	<b>16.00</b>	<b>6.00</b>	<b>12.00</b>	<b>10.00</b>
Total production/day of cheese (g)	147000	72000	184000	104000	114000
Total Capital Investment (C <sub>T</sub> ) (GH¢)	1865825.59	1865825.59	1890987.75	1890987.75	1865825.59
Manufacturing cost (C <sub>M</sub> ) (GH¢)	1040628.40	1040628.40	1046329.09	1046329.09	1040628.40

d (GH¢/yr)	71,367.83	71,367.83	72,330.28	72,330.28	71,367.83
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#### 4.5.3.1 Sales(S)

$$S = \frac{\text{total amount of cheese (g)}}{100g} \times \text{number of working days} \times \text{amount /100g}$$

For rennet only:

$$\text{Annual sales} = 147000g/100g \times 144\text{days} \times 10GH\text{¢}$$

$$= GH\text{¢}1693440/\text{year}$$

#### 4.5.3.2 Profit

This is the gross profit or profit before tax

For rennet only

$$\text{Profit} = S - C_M$$

$$S - C_M = 1693440 - 1040628.4$$

$$= 652811.6GH\text{¢}/\text{year}$$

#### 4.5.3.3 Net Profit After Tax (P)

This is the profit obtained after the tax has been deducted

$$P = (S - C_M - d)(1 - t)$$

$$\text{Tax (t)} = 0.20 \text{ (20\%)}$$

$$\text{Net profit for rennet only} = (1693440 - 1040628.4 - 71367.8288)(1 - 0.20)$$

$$= GH\text{¢} 595717.337/\text{year}$$

**Table 4.16: Sales, Profit and Net Profit**

	<b>Rennet only</b>	<b>Acid only</b>	<b>Sodom apple</b>	<b>Sodom apple +Acid</b>	<b>Rennet +acid</b>
<b>Cost of cheese/100g (GH¢)</b>	<b>8.00</b>	<b>16.00</b>	<b>6.00</b>	<b>12.00</b>	<b>10.00</b>
Total production/day of cheese (g)	147000	72000	184000	104000	114000
Total production/day of cheese/(100g)	1470	720	1840	1040	1140
<b>Total annual sales (S) (GH¢)</b>	1,693,440	1,658,880	1,589,760	1,497,600	1,641,600
<b>Gross profit S-C<sub>M</sub></b>	652,811.60	618,251.60	543,430.91	451,270.91	600,971.60
<b>Net profit after tax (P)</b>	595,717.34	561,157.34	485,566.68	393,406.69	543,877.34

**4.5.3.4 Annual Cashflow (ACF)**

This is the amount of money that would be available yearly

For rennet only

$$\begin{aligned}
 \text{ACF} &= P - d \\
 &= 595717.34 - 71367.83 \\
 &= \text{GH¢ } 524,349.51
 \end{aligned}$$

**4.5.3.5 Cumulative Cash Flow (CCF)**

This helps to determine the long-term strength of a company

For rennet only

$$\begin{aligned}
 \text{CCF} &= -C_T + (n \times \text{ACF}) \\
 \text{CCF} &= -1865825.59 + (20 \times 524349.51)
 \end{aligned}$$

$$CCF = \text{GH}\text{¢ } 8,621,164.58$$

#### 4.5.3.6 Payback Period (PBP)

This is the period or time required for a project to recover the money invested in it. It is usually expressed in years.

For rennet only

$$PBP = \frac{CT}{P}$$

$$PBP = \frac{1865825.59}{595717.34}$$

$$PBP = 3.1 \text{ years}$$

Therefore, the Payback Period is approximately 36 months.

#### 4.5.3.7 Return on Investment (ROI)

This deals with the money you invest in the company and the return you realize on that money based on the net profit of the business.

This is expressed on annual percent basis:

$$\text{Return on investment} = \frac{\text{yearly profit}}{\text{Total initial investment } C_T} \times 100$$

For rennet only

$$\begin{aligned} \text{Return on investment} &= \frac{595717.34}{1865825.59} \times 100 \\ &= 31.93\% \end{aligned}$$

#### 4.5.3.8 Net Present Value (NPV)

A positive NPV generally means the investment will be profitable and a negative NPV will result in a net loss.

$$\text{Capital Recovery Factor, } e = \frac{i(1+i)^n}{(1+i)^n - 1}$$

Where  $i$  is interest rate

$$= \frac{0.17(1 + 0.17)^{20}}{(1 + 0.17)^{20} - 1}$$

$$= 0.178$$

$$\text{NPV} = -C_T + \frac{P}{e}$$

For rennet only

$$\text{NPV} = \left[ -1865825.59 + \frac{595717.34}{0.178} \right]$$

$$\text{NPV} = \text{GH}\text{¢ } 3,346,726.61$$

#### 4.5.3.9 Profitability Index (PI)

It is a ratio that shows how much profit results from a project. If a project has a profitability index greater than 1, it should be accepted; if lower than 1, it should be rejected.

$$\text{PI} = \frac{\text{NPV}}{C_T}$$

For rennet only

$$= \frac{1,480,901.02}{1865825.59}$$

$$\text{PI} = 1.79$$

**Table 4.17: Summary of Profitability Analysis**

	<b>Rennet only</b>	<b>Acid only</b>	<b>Sodom apple</b>	<b>Sodom apple +Acid</b>	<b>Rennet +acid</b>
Annual cashflow ( <b>ACF</b> ) ( <b>GH¢</b> )	524,349.51	489,789.51	413,236.40	321,076.40	472,509.51
Cumulative Cash Flow ( <b>CCF</b> ) ( <b>GH¢</b> )	8,621,164.58	7,929,964.58	6,373,740.30	4,530,540.31	7,584,364.58
Payback Period in years ( <b>PBP</b> )	3.1	3.3	3.9	4.8	3.4
Return on Investment ( <b>ROI</b> )%	31.93	30.08	25.68	20.80	29.15
Net Present Value ( <b>NPV</b> ) ( <b>GH¢</b> )	1,480,901.02	1,286,743.72	836,914.97	319,162.164	1,189,665.067
Profitability Index ( <b>PI</b> )	1.79	1.69	1.44	1.17	1.64

It is therefore viable to run a cheese factory or plant in Ghana, using the different coagulants. The estimated cost of the cheese samples varies. They are as follows.

Rennet only - **GH¢ 8.00**

Acid only - **GH¢ 16.00**

Sodom apple only (wagashie) - **GH¢ 6.00**

Sodom apple +acid - **GH¢ 12.00**

Rennet +acid - **GH¢10.00**

Based on the survey done in some shops in Accra, and on the informal market, price of 100g of cheese ranged from GH¢ 14.00 to GH¢17.00 but some of the cheeses cost as low as GH¢ 6. For wagashie, on the informal market, 100g was sold averagely for GH¢3.00.

In terms of sales, cheese processed from acid only was the most expensive with rennet only being the least expensive. Sodom apple only may not be lucrative if sold on the informal market because the price has doubled but can be sold on the formal market where the middle-income earners and high-income earners consumers can have access to it.

## CHAPTER FIVE

### 5.0 CONCLUSION AND RECOMMENDATION

#### 5.1 Conclusions

There was a significant difference amongst the three milk samples for all the physicochemical properties except for protein and fat content. Milk from the Jersey and the White Fulani were similar for, ash and pH, and milk from the White Fulani showed highest calcium content.

Coagulum obtained showed that there was an interaction between the breeds and the coagulants used in coagulation. Sodom apple gave the highest yield and the addition of Acid first to the milk shortened the time for coagulation. Coagulum from the Jersey gave the highest yield and coagulation time was the shortest with White Fulani.

Physicochemical properties of coagulum obtained showed that there was an interaction between the breeds and the coagulants used in coagulation. Acid influences the moisture content, fat content and pH of coagulum when combined with an enzyme. Coagulum processed from Jersey produces coagulum with high fat content.

Dewatered coagulum samples showed significant difference for the coagulants used in processing them. Rennet + acid and acid only can be used for cheeses that need to be melted. Hard cheeses could be obtained from Rennet + acid and Sodom apple coagulum retained more of the milk properties in terms of their sensory properties. The combination of Sodom apple with acid does not allow the full potential of the Sodom apple to hydrolyse  $\kappa$  caseins.

It is economically viable to establish a cheese factory in Ghana using Sodom apple, acid or rennet as coagulant.

## **5.2 Recommendations**

It would be very helpful if the enzymes in Sodom apple can be extracted and purified. This would reduce the time needed for processing. It takes about an hour for the coagulation process and extra time would be needed to crush and obtain the crude extract for coagulation.

Even though Sodom apple grows easily, it would be beneficial if farmers start cultivating it to make it more available.

Instead of processing the cheese with fresh coagulants all the time, they can be kept and used as starter culture to reduce cost.

Sensory acceptance test can be done to determine which cheese would be liked most by consumers.

If the coagulants can be out sourced locally instead of importing, the rate of production can be minimized to an extent.

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## APPENDIX- ANOVA TABLES AND TUKEY'S PAIRWISE COMPARISONS

### One-way ANOVA: total solid versus BREED

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	49.95	24.977	8.46	0.001
Error	48	141.73	2.953		
Total	50	191.68			

### One-way ANOVA: FAT versus BREED

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	9.597	4.799	2.92	0.064
Error	45	74.004	1.645		
Total	47	83.601			

### One-way ANOVA: pH versus BREED

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	0.08911	0.044555	7.92	0.001
Error	48	0.26999	0.005625		
Total	50	0.35910			

a letter are significantly different.

### One-way ANOVA: PROTEIN versus BREED

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	2.029	1.015	0.42	0.660
Error	48	116.235	2.422		
Total	50	118.264			

### One-way ANOVA: ASH versus BREED

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	0.2069	0.103432	11.89	0.000

Error	48	0.4177	0.008703
Total	50	0.6246	

**One-way ANOVA: CALCUIM versus BREED**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	141638	70819	70.29	0.000
Error	48	48365	1008		
Total	50	190002			

**One-way ANOVA: \*L versus BREED**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	32.45	16.2230	22.03	0.000
Error	48	35.35	0.7364		
Total	50	67.79			

**Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

BREED	N	Mean	Grouping
F	18	90.864	A
C	15	89.580	B
J	18	89.004	B

Means that do not share a letter are significantly different.

**One-way ANOVA: \*a versus BREED**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	12.78	6.3876	10.56	0.000
Error	48	29.04	0.6049		
Total	50	41.81			

**Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

BREED	N	Mean	Grouping
C	15	-2.007	A
F	18	-2.6017	A
J	18	-3.253	B

Means that do not share a letter are significantly different.

**One-way ANOVA: \*b versus BREED**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	366.8	183.409	51.89	0.000
Error	48	169.6	3.534		
Total	50	536.5			

**Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

BREED	N	Mean	Grouping
J	18	14.074	A
C	15	8.683	B
F	18	8.296	B

Means that do not share a letter are significantly different.

**General Linear Model: YIELD versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	288.2	144.12	9.42	0.000
COAGULANT	4	5508.8	1377.20	89.98	0.000
BREED*COAGULANT	8	272.1	34.01	2.22	0.027
Error	240	3673.5	15.31		
Total	254	9757.8			

**Tukey Pairwise Comparisons: Response = YIELD, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
J S	18	25.6827	A
C S	15	23.3097	A B
F S	18	19.8486	B C
J R	18	19.0647	B C D
C R	15	18.1657	C D
F R	18	17.9706	C D
J RA	18	15.4044	C D E
C RA	15	15.1987	C D E
J SA	18	14.7176	D E
C SA	15	12.8596	E F
F RA	18	12.0177	E F
F SA	18	11.4425	E F
F A	18	10.1552	F
J A	18	9.1997	F
C A	15	8.8719	F

Means that do not share a letter are significantly different.

**General Linear Model: TIME versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	539967	269983	3.58	0.029
COAGULANT	4	23937081	5984270	79.32	0.000
BREED*COAGULANT	8	1251290	156411	2.07	0.039
Error	240	18107240	75447		
Total	254	43390527			

**Tukey Pairwise Comparisons: Response = TIME, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
C S	15	973.333	A
J S	18	865.833	A
F S	18	546.333	B
C R	15	169.400	C
J R	18	120.389	C
F R	18	51.611	C
J A	18	7.556	C
J RA	18	7.222	C
C SA	15	7.000	C
J SA	18	6.944	C
C A	15	6.867	C
C RA	15	6.800	C
F A	18	6.278	C
F RA	18	5.833	C
F SA	18	5.056	C

Means that do not share a letter are significantly different.

**General Linear Model: MOISTURE versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	307.8	153.91	7.56	0.001
COAGULANT	4	9531.1	2382.77	117.02	0.000
BREED*COAGULANT	8	331.9	41.49	2.04	0.043
Error	240	4886.9	20.36		
Total	254	15182.3			

**Tukey Pairwise Comparisons: Response = MOISTURE, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
J S	18	65.7354	A
F S	18	63.4934	A B

C S	15	62.7415	A	B	C
J A	18	61.7559	A	B	C
F R	18	61.3857	A	B	C
F A	18	61.0202	A	B	C
J R	18	59.8167		B	C
C R	15	59.7676		B	C
C A	15	57.2927			C
J RA	18	51.6921			D
J SA	18	51.1530			D
F RA	18	50.5672			D E
C RA	15	48.6904			D E
C SA	15	48.1264			D E
F SA	18	45.6743			E

Means that do not share a letter are significantly different.

**General Linear Model: Ph versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	1.140	0.5702	18.74	0.000
COAGULANT	4	145.694	36.4235	1196.91	0.000
BREED*COAGULANT	8	1.230	0.1538	5.05	0.000
Error	240	7.303	0.0304		
Total	254	155.829			

**Tukey Pairwise Comparisons: Response = Ph, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping		
J R	18	6.87167	A		
F R	18	6.70778	A	B	
C R	15	6.67067	A	B	
F S	18	6.65333		B	
C S	15	6.63133		B	
J S	18	6.60389		B	
F SA	18	5.44056			C
F RA	18	5.36167			C D
J SA	18	5.25444			C D E
C SA	15	5.21733			D E
J RA	18	5.14722			E
F A	18	5.14667			E
C RA	15	5.13800			E
J A	18	4.89333			F
C A	15	4.84333			F

Means that do not share a letter are significantly different.

**General Linear Model: FAT versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	1086.6	543.31	14.38	0.000
COAGULANT	4	9562.6	2390.65	63.26	0.000
BREED*COAGULANT	8	857.8	107.22	2.84	0.005
Error	240	9070.3	37.79		
Total	254	20503.0			

**Tukey Pairwise Comparisons: Response = FAT, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
J A	18	34.5041	A
C A	15	33.4083	A
J SA	18	23.0131	B
C SA	15	22.8169	B
F A	18	22.4745	B
J RA	18	21.0012	B
F SA	18	19.6390	B C
C RA	15	19.3469	B C D
F RA	18	18.7171	B C D
C R	15	17.1181	B C D E
J R	18	16.4686	B C D E
F R	18	12.7122	C D E
C S	15	12.6268	C D E
J S	18	12.3677	D E
F S	18	11.3552	E

Means that do not share a letter are significantly different.

**General Linear Model: Protein versus BREED, COAGULANT**

Factor Information

Factor	Type	Levels	Values
BREED	Fixed	3	C, F, J
COAGULANT	Fixed	5	A, R, RA, S, SA

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	1118	558.79	8.12	0.000
COAGULANT	4	24535	6133.67	89.15	0.000
BREED*COAGULANT	8	1478	184.71	2.68	0.008
Error	240	16512	68.80		
Total	254	43728			

**Tukey Pairwise Comparisons: Response = Protein, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
J S	18	53.3677	A
F S	18	52.1381	A B
C S	15	50.1148	A B
F R	18	48.6735	A B
J R	18	43.3481	B C
C R	15	42.6495	B C
F A	18	38.5458	C D
F RA	18	31.8501	D E
J RA	18	30.6908	D E
C RA	15	29.3435	D E
J SA	18	28.1399	E
J A	18	27.2518	E
F SA	18	26.0353	E
C SA	15	25.3095	E
C A	15	23.8845	E

Means that do not share a letter are significantly different.

**General Linear Model: \*L versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	190.89	95.446	37.90	0.000
COAGULANT	4	699.77	174.944	69.47	0.000
BREED*COAGULANT	8	61.44	7.680	3.05	0.003
Error	240	604.35	2.518		
Total	254	1567.14			

**Tukey Pairwise Comparisons: Response = \*L, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
C R	15	96.6840	A
C S	15	96.5733	A B
C A	15	96.4693	A B C
F S	18	95.1967	A B C D
J R	18	95.0683	A B C D
F A	18	94.9594	A B C D
J S	18	94.7889	A B C D
F R	18	94.7033	B C D E
J A	18	94.6267	C D E
C RA	15	94.2580	D E F
C SA	15	92.8313	E F G
J RA	18	92.6300	F G
J SA	18	92.1378	G
F RA	18	91.9983	G
F SA	18	89.3706	H

Means that do not share a letter are significantly different.

**General Linear Model: \*b versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	2306.0	1153.01	27.84	0.000
COAGULANT	4	2114.0	528.51	12.76	0.000
BREED*COAGULANT	8	672.6	84.08	2.03	0.044
Error	240	9938.1	41.41		
Total	254	15051.1			

**Tukey Pairwise Comparisons: Response = \*b, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
J RA	18	30.9656	A
J SA	18	30.1378	A B
J R	18	29.9739	A B
F SA	18	27.7683	A B C
C SA	15	27.3207	A B C
J S	18	25.4883	A B C D
J A	18	23.7778	A B C D
F S	18	23.2961	B C D E
F RA	18	22.4928	C D E
C RA	15	22.1453	C D E
C R	15	21.1120	C D E
F R	18	19.5350	D E
C A	15	19.1173	D E
C S	15	18.7027	D E
F A	18	16.2056	E

Means that do not share a letter are significantly different.

**General Linear Model: \*a versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	3.292	1.6461	4.61	0.011
COAGULANT	4	147.138	36.7846	102.98	0.000
BREED*COAGULANT	8	12.365	1.5457	4.33	0.000
Error	240	85.728	0.3572		
Total	254	247.423			

**Tukey Pairwise Comparisons: Response = \*a, Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
J RA	18	-1.88944	A
J A	18	-2.09444	A B
C RA	15	-2.10067	A B
F A	18	-2.16333	A B
J R	18	-2.23389	A B
F RA	18	-2.30333	A B

C A	15	-2.39600	A B			
C R	15	-2.54400	A B C			
F R	18	-2.72167	B C			
F S	18	-3.25944	C D			
J SA	18	-3.51222	D E			
C S	15	-3.57867	D E			
J S	18	-3.92500	D E F			
F SA	18	-3.98444	E F			
C SA	15	-4.44467	F			

Means that do not share a letter are significantly different.

**General Linear Model:  $\Delta L$  versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	40.7	20.36	0.61	0.546
COAGULANT	4	2114.0	528.51	15.76	0.000
BREED*COAGULANT	8	672.6	84.08	2.51	0.012
Error	240	8049.5	33.54		
Total	254	10897.3			

**Tukey Pairwise Comparisons: Response =  $\Delta L$ , Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
C R	15	7.10420	A
C S	15	6.99353	A
C A	15	6.88953	A
J R	18	6.06383	A B
J S	18	5.78439	A B C
J A	18	5.62217	A B C D
C RA	15	4.67820	B C D E
F S	18	4.33317	C D E
F A	18	4.09594	D E
F R	18	3.83983	E
J RA	18	3.62550	E
C SA	15	3.25153	E
J SA	18	3.13328	E
F RA	18	1.13483	F
F SA	18	-1.49294	G

Means that do not share a letter are significantly different.

**General Linear Model:  $\Delta a$  versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	52.11	26.0528	71.56	0.000
COAGULANT	4	135.84	33.9604	93.28	0.000
BREED*COAGULANT	8	12.84	1.6051	4.41	0.000

Error	240	87.37	0.3641
Total	254	286.37	

**Tukey Pairwise Comparisons: Response =  $\Delta a$ , Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
J RA	18	1.13528	A
J R	18	1.01861	A B
J A	18	0.90139	A B C
F A	18	0.43817	B C D
F RA	18	0.29817	C D E
C RA	15	0.23613	C D E
C A	15	-0.05920	D E F
F R	18	-0.12017	D E F
C R	15	-0.20720	D E F
J SA	18	-0.25972	E F
F S	18	-0.65794	F G
J S	18	-0.67250	F G
C S	15	-1.24187	G H
F SA	18	-1.38294	H
C SA	15	-2.10787	I

Means that do not share a letter are significantly different.

**General Linear Model:  $\Delta b$  versus BREED, COAGULANT**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
BREED	2	522.29	261.146	121.02	0.000
COAGULANT	4	699.77	174.944	81.07	0.000
BREED*COAGULANT	8	61.44	7.680	3.56	0.001
Error	240	517.88	2.158		
Total	254	1812.07			

**Tukey Pairwise Comparisons: Response =  $\Delta b$ , Term = BREED\*COAGULANT**

Grouping Information Using the Tukey Method and 95% Confidence

BREED*COAGULANT	N	Mean	Grouping
F SA	18	19.4723	A
C SA	15	18.6379	A B
J RA	18	16.8911	A B C
J SA	18	16.0633	A B C D
J R	18	15.8994	A B C D
F S	18	15.0001	A B C D
F RA	18	14.1968	A B C D E
C RA	15	13.4625	A B C D E
C R	15	12.4292	B C D E
J S	18	11.4138	C D E
F R	18	11.2390	C D E

C A	15	10.4345	C D E
C S	15	10.0199	C D E
J A	18	9.7033	D E
F A	18	7.9096	E

Means that do not share a letter are significantly different.

**One-way ANOVA: Hardness versus Sample**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	559332	139833	386.07	0.000
Error	10	3622	362		
Total	14	562954			

**One-way ANOVA: Adhesiveness versus Sample**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	7096.6	1774.16	63.26	0.000
Error	10	280.4	28.04		
Total	14	7377.1			

**One-way ANOVA: Springiness versus Sample**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.44751	0.111878	108.97	0.000
Error	10	0.01027	0.001027		
Total	14	0.45778			

**One-way ANOVA: Cohesiveness versus Sample**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	0.050986	0.012746	59.87	0.000
Error	10	0.002129	0.000213		
Total	14	0.053115			

**One-way ANOVA: Chewiness versus Sample**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	58772.7	14693.2	154.91	0.000
Error	10	948.5	94.8		
Total	14	59721.2			

**One-way ANOVA: Meltability(cm) versus Sample**

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	4	158.593	39.6483	75.66	0.000
Error	10	5.240	0.5240		
Total	14	163.833			

**Two- way Anova- sensory profile**

	CREAM COLOUR-AP	MOIST- AP	COWY-AR	MILKY-AR	BUTTERY-AR
R <sup>2</sup>	0.879	0.895	0.938	0.978	0.934
F	14.811	17.474	30.886	89.562	28.707
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PANELIST	6.878	1.410	2.403	5.929	2.714
	< 0.0001	0.194	0.016	< 0.0001	0.007
PRODUCT	143.057	189.916	349.570	948.037	324.978
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PANELIST*PRODUCT	2.545	2.329	2.598	15.084	2.286
	0.000	0.001	0.000	< 0.0001	0.001

	SPRINGY-TX	RECOVERY RATE- TX	FLAKY- TX	MOIST- TX	MUSHY- TX
R <sup>2</sup>	0.957	0.962	0.880	0.923	0.846
F	45.687	51.627	14.902	24.455	11.221
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PANELIST	3.378	6.717	1.533	6.729	6.110
	0.001	< 0.0001	0.147	< 0.0001	< 0.0001
PRODUCT	529.058	584.697	162.216	251.351	106.875
	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PANELIST*PRODUCT	2.557	3.625	1.876	3.676	1.870
	0.000	< 0.0001	0.008	< 0.0001	0.008