COMPARATIVE ASSESSMENT OF CHEESE PRODUCED USING PLANT RENNETS STORED IN WHEY AND BAOBAB PULP EXTRACT WITH MOZZARELLA CHEESE

BY

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B. Agriculture (University of Ilorin) 2015
P16AGAN8011

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ANIMAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE,
FACULTY OF AGRICULTURE,
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ZARIA, NIGERIA

DECEMBER, 2019

DECLARATION

I hereby declare that the work in this dessertation entitled “COMPARATIVE ASSESSMENT OF CHEESE PRODUCED USING PLANT RENNETS STORED IN WHEY AND BAOBAB PULP EXTRACT WITH MOZZARELLA CHEESE’’ was carried out by me at the Animal Product Laboratory, Ahmadu Bello University, Zaria under the supervision of Prof. Duru and Dr Oyadeyi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

_____ _____

BADMUS, Abass Olayemi Date

CERTIFICATION

This dessertation entitled COMPARATIVE ASSESSMENT OF CHEESE PRODUCED USING PLANT RENNETS STORED IN WHEY AND BAOBAB PULP EXTRACT WITH MOZZARELLA CHEESE by BADMUS, Abass Olayemi meets the regulation governing the award of the degree of Masters of Science (Animal Science) of the Ahmadu Bello University, Zaria. It is approved for its literary presentation and contribution to scientific knowledge.

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DEDICATION

This project work is dedicated to the Almighty God and my dearly parents, Late Alh. S. A. Badmus and Alh. T. M. Badmus, for their parental care and support.

ACKNOWLEDGEMENTS

My sincere and ultimate gratitude goes to the Almighty Allah, who in His infinite mercies saw me through in the course of this project work and the stay in the University. All glory, honour, power and adoration belong to Him, for He is worthy of my praise.

My appreciation goes to my Supervisors;Late Prof S. Duru and Dr. O.S. Oyadeyi for their support. It is impossible to quantitate all their effort towards the improvement of this work. May the Almighty continue to bless them.

My immense gratitude goes to all staff members of the Faculty of Agriculture, the Head of Animal Science Department. Prof.M. Kabir and to the teaching and non-teaching staff of the department whom through their personality imparted incomparable virtues in my life. And to all the lecturers from,Prof S.B. Abdu, Dr. M.R. Hassan, Prof. J.J. Omage, Dr (Mrs) Adedibu, Prof. D. Zahraddeen,Late Dr Alli-Balogun, Prof. P.A. Onimisi, Prof. T.S. Olugbemi, Dr. Musa Abubakar, Dr. M. AbdulRasheed and Mal. Ibrahim Taofik of Department of Animal Science and Prof. Ehoche, Prof. S.M Otaru, Prof. P.P. Barje, Prof. Sekoni, Prof. Abekeof NAPRI and all the other lecturers that I could not mention, I am grateful for your tutelage.

Also, I appreciate the efforts of my entire family members, my parents; Late Alh. S.A. Badmus, Alh. T.M. Badmus. My sisters; Mrs. A.A Olasupo, Mrs. Z.O Dikko and Mrs L.A Agbaje. My brothers; Mr A.A Badmus and Mr K.A Badmus. My nieces and nephews; Kaosara, Sukurat, Abdul-Lateef, Ayesha, Warriz, Awwal, Kafayat, Idris, Farid and Khalid. I also appreciate the effort of my uncles; Dr. Adeleke and Dr. Monsur and to my entire family members. I remain indebted to you forever.

To the love of my life; my wife Fatai Ruqqoyah Omolara, you are a blessing to me. To all my friends and coursemates,few include; Adebayo Micheal, Madaki Jubril, Oyewo Teslim, Moses

Eunice, Danladi Yussuf, Abubakar Sodiq, Yussuf Ahmad, Talabi Adeola, Bello Muizzat, Millah, Duke, Kayode, Hamman and all 2016 classmates. I deeply apologize for not mentioning all the names.I appreciate you all.

To all my colleagues at Rico Gado Nutrition, thank you all for the support.

ABSTRACT

Cheese was produced using coagulants from plant sources (Sodom Apple, Moringa and Tamarind) and stored for 7 days in whey and Baobab pulp extract. Lime was combined with each of the plant rennet using *pasta filata* (stretched curd) method and the resulting cheese compared with commercial Mozzarella. The milk utilized in this study was purchased from the open market in Zaria and the cheese stored under room condition (32^{o}C) in Zaria, Nigeria. There was significant ($P<0.05$)difference in all chemical composition parameters measured. Dry matter ranged from 39.67-42.65% across the plant rennet. Crude Protein percent recorded ranged from 21.39 to 24.77 while the ether extract ranged from 6.52 to 10.68. Total Yeast count (TYC) ranged from $0.5 \times 10^{7}$to $19.3 \times 10^{7}$with storage day 4 recording the highest. Taste and colour ranged between 4.83 to 5.98 and 6.34 to 6.75 respectively. Texture and salt of 6.75, 6.34, 6.67 and 6.03, 5.56, 5.33 were recorded for storage days 1, 4 and 7 respectively. Acceptability figure of 6.50, 5.84 and 6.04 was recorded for storage days 1, 4 and 7 respectively. Taste and colour along the storage duration were 5.98, 4.83, 5.12 and 6.75, 6.34, 6.67 respectively for day 1, 4 and 7 of storage.Moringa coagulated cheese has a higher Crude Protein compared to Mozzarella cheese. Crude protein increases as the storage days increases owing to the deposition of microbial protein during ripening.Cheese stored in whey was more acceptable to the panelist compared to cheese stored in baobab pulp extract. Profitability ratio ranged from 0.17 to 0.20 and effiency ratio of 1.17 to 1.20 due to low cost of production. It was concluded that production of cheese with Moringa seed extract is acceptable and profitable. Also, plant rennet coagulated cheese can be stored for a longer period as bitter tasting and green colouration were not observed in this study following the use of *pasta filata* to inactivate the proteases in the plant rennet.

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CHAPTER ONE

1.0 INTRODUCTION

Diversification of the economy with focus on Agriculture in Nigeria has made all branches of Agriculture to look inward to solve our challenging problems. Post-harvest losses remain one of the foremost problems of Agriculture, the dairy industry inclusive (Kurwijila and Boki, 2003). Dairy production is an important part of the livestock production systems in Nigeria where cattle, sheep and goats are the main livestock species that produce this milk, cows contributing the highest percentage (Obadina, 2014). It is well known that milk is highly perishable, therefore milk losses encountered due to improper handling and lack of storage is usually high (Turkoglu *et al*., 2003; Hill, 2011; Ayeni *et al*., 2014). Thus, processing and value addition becomes necessary. Cheese production is one of the classical examples of food preservation, and common to households in almost every country in the world with the existence of more than 2,000 varieties increasing the shelf-life of milk (O'Connor, 1993; Turkoglu*et al*., 2003). Preservation of the most important constituents of milk (*i.e.* fat and protein) as cheese exploits two of the classical principles of food preservation, *i.e.* lactic acid fermentation and reduction of water activity through removal of water and addition of salt (NaCl) (Bereda *et al*., 2014).Cheese is a solid milk product from the milk of animals and is an excellent source of protein, fat, minerals, vitamins, andessential amino acids (Buckman, 2003). The ingredients used for cheese processing are milk, starter, and coagulants (Beresford *et al.*, 2001). The general steps ofcheese manufacturing involve heat treatment of milk, addition of starter or coagulant, and removal of whey with changes depending on the variety of the cheese (Adegoke *et al*., 1992; O'Connor *et al.*, 1993).

Mozzarella cheese is a southern Italian cheese traditionally made from Italian buffalo milk by the *pasta filata* method (Stretched-curd) which originated from Italy (Citro, 1981; Jana, 2001). The name *pasta filata* refers to a unique plasticizing and texturing treatments ofthe fresh curd in hot water that imparts to the finished cheese its characteristic fibrousstructure and melting properties.Mozzarella is one of the most important cheese in the world as it is considered an industrial cheese (Kosikowski, 1982; Jana, 2001). It utilizes two different coagulants; out of which one should be acidic, so as to bind calcium while the other is useful during aging or ripening (Coker *et al.*, 2014).Kosikowski (1982) reported that the addition of salt and additional heat treatment used in stretching mozzarella may be responsible for the long shelf-life.Mozzarella Cheese account for 33.33% of USA's 11.8 billion pounds of annual cheese production with about 20% export earnings (Geisler, 2012). Central Bank of Nigeria (2012) reported an increase in importation of milk products including cheese, World Bank (2016) data showed a sharp increase in cheese consumption in urban areas in Nigeria, while Konwea (2012) observed an increase in pizza consumption in Nigeria. However, despite the huge value of cheese, exploiting the economic potentials of locally made cheese in Nigeria is still low.

Rennet is described as variety of enzymes of young animals (especially calves), plant or microbial origins used to coagulate milk during cheese preparation (Craw, 1983; O 'Connor, 1993). The shortage in the world supply and high prices of calf rennet (Rogelj *et al*., 2001) and refusal to accept animal and porcine rennet by vegetarians and muslims respectively (Roseiro *et al*., 2003), necessitated the need to substitute animal rennet with easily available, cheap, and acceptable source of rennet for cheese preparation. The proteases present in the plant extracts and those naturally present in milk are believed to play a role in the impartation of aroma and texture to cheese (Visser, 1977; O 'Connor, 1993). Examples of plants with such properties are

pawpaw(papain), pineapple (bromelain), castor seed oil, sodom apple (*Calotropis procera)* plant, tamarind pulp, *Moringa oleifera* seed extract and citric acid (lime or lemon*)* are also used as coagulants (O'Connor, 1993; Chazarra *et al*., 2007; Maijalo *et al*., 2016). Other alternative includes treatment of milk with the common inorganic coagulants which hassome disadvantages (Johnson*et al*., 2001). Cheese making with natural coagulants has reduced the level of milk wastage and associated diseases which result from inorganic coagulants (Hill, 2011).

Mozzarella and local cheese can either be stored in whey or brine; however, storing in brine may reduce palatability of deep salted cheese (Zeak, 2013; Orhevba *et al*., 2016). Aqueous extract of baobab has been reported to have flavonoids, terpenoids, saponins, tannins, alkaloids, anthraquinones, steroids, resins and phenols. It also contains ten times the vitamin C content of oranges, with antimicrobial, antioxidant and anti-inflammatory properties which makes it effective against pathogenic microbes (Vertuani *et al.*, 2002; Kamatou *et al.*, 2011; Abiona *et al.*, 2015).

1.1 JUSTIFICATION

Cheese is nutritionally important to the body (Arnarson, 2014). It also helps in reducing the quantity of raw milk wastage, because the milk which would have been lost as a result of the spoilage is converted to cheese. However, cheese consumption in Nigeria is very low which may be due to the quick spoilage of the locally produced soft cheese as a result of low post-harvest processing and refrigeration. Spoilage in locally produced cheese can be traced to microbial and enzymatic sources, although researches have been done to improve the storage medium, it has resulted in little or no improvement on the cheese shelf-life. Hence, the need to improve the cheese shelf-life is inevitable through alternative technique. The excessive proteolytic nature of plant coagulants has limited their use in cheese manufacturing due to lower yields of cheese, bitter flavors and texture defects especially during ripening (Shah *et al.,* 2013), curd stretching in Mozzarella has been reported to deactivate proteolytic enzymes due to the high temperature of stretching (Kosikowski, 1982; Jana, 2001; Ahmed *et al*., 2008). Controlling the activities of proteolytic enzymes and microbial load is very important in the cheese industry.Mozzarella cheese (also refers to as the pizza cheese) production is similar to the locally produced cheese although, it employs post-production processing with emphasis on pH and salting. Production of cheese from plant rennet will not only increase the acceptability but also add to the nutrients available to consumer. Sodom Apple, Tamarind and Moringa are common plant in the tropics which can be used as coagulants while Baobab has the quality to be a cheap alternative storage medium. Cheese can be a source of export earning as demonstrated by the USA with Mozzarella at the fore-front. Over 60% of the Mozzarella produced in the USA is for commercial purposes (export, baking and pizza industries) which are as a result of its ability to give high returns and

long shelf-life. In view of the inconsistencies on the efficacy of plant rennet on the quality attributes and shelf-life of cheese despite the economic potential of cheese, there is a need for additional studies with emphasis on control of the proteolytic enzymes to allow tailored decisions and informed choices on the use of plant rennet in cheese production.

1.2 Objectives

The broad objective of the study was the comparative assessment of cheese produced using plant rennets stored in whey and baobab pulp extract with mozzarella cheese

The specific objectives of the study were to determine the;

- suitability of plant rennet in making Mozzarella cheese
- organoleptic properties and shelf-stability of Mozzarella cheese in Zaria
- proximate properties and microbial analysis of Mozzarella cheese
- economic and marketing potential of Mozzarella cheese

1.3 HYPOTHESES

1.3.1 Experiment 1

Null Hypothesis (H_0) = Plant rennet and storage medium have similar effect on the mozzarella cheese production

Alternative Hypothesis (H_1) = Plant rennet and storage medium have different effect on mozzarella cheese production

Null Hypothesis (H_0) = Plant rennet and storage medium have similar effect on the proximate properties and microbial analysis of mozzarella cheese

Alternative Hypothesis (H_1) = Plant rennet and storage medium have different effect on proximate properties and microbial analysis of mozzarella cheese

1.3.2 Experiment 2

Null Hypothesis (H_0) = Plant rennet and storage medium have the same effect on organoleptic properties and shelf-stability of mozzarella cheese storage

Alternative Hypothesis (H_1) = Plant rennet and storage medium have different effect on organoleptic properties and shelf-stability of mozzarella cheese storage

Null Hypothesis (H_0) = Plant rennet and storage medium have the same effect on the economic and marketing potential of mozzarella cheese

Alternative Hypothesis (H_1) = Plant rennet and storage medium have different effect on the economic and marketing potential of mozzarella cheese

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Cheese

Cheese refers to the solid mash obtained after the separation of whey following curdling. Fox and McSweeney (2004) reported that coagulation of milk by the in-situ production of lactic acid was probably accidental, as lactic acid bacteria have the ability to grow in milk and produce enough acid to lower the pH of milk, causing the milk proteins to coagulate. Similarly, the use of rennets to coagulate milk may also have initially been accidental; milk was commonly stored in bags made from the stomachs of slaughtered animals (Fox and McSweeney, 2004). The earliest indication of cheesemaking descends from cave paintings around 5000 BC (Harboe *et al*., 2010). Enzymes (chymosin and some pepsin) in the stomach tissue would have caused the milk to coagulate on storage. People have come to realize that the shelf-life of the curds could be extended by dehydration or by adding salts. Given the large variety of cheeses – over 1400 varieties (Fox and McSweeney, 2004) – and considerable body of literature available, cheese production is therefore an important part of dairy industry.

2.2*Wara*

In Nigeria, milk production is mainly done by the nomadic Fulani (Aduku and Olukosi, 2012). Fulani women process the surplus fresh milk into a soft; unripen cheese called "*warankasi*" or "*wara*" to avoid wastage (Ayeni *et al*., 2014). The *wara* is widely consumed at home and sold in the markets especially in South-western Nigeria (Aduku and Olukosi, 2012; Ayeni *et al*., 2014).

The cheese is usually stored in a mixture of whey at room temperature (28ºC). Under these storage conditions, '*wara*' is highly perishable and has a shelf life of 2-3 days (Ayeni *et al*., 2014). The coagulants used for '*wara*' processing include the leaves and soft stem extracts of Sodom apple (*Calotoropis procera*), Lime juice and *Moringa olifera* seed. The *Moringa olifera* seed extract processed cheese has a sweeter flavour compared to the cheese processed with pawpaw leaf extracts (O'Connor *et al*., 1993). Mahami *et al*. (2012) demonstrated that *Moringa oleifera* seed extract enrichment resulted in significant increase in the yield, protein and mineral content of cottage cheese. Maijalo *et al*. (2016) demonstrated that both Tamarind pulp (*Tamarindus indica*) and lime are suitable as coagulants in making soybean cheese, however, lime juice has higher yield. In addition, plant rennet not only coagulates the milk but also improves the mineral content of the resultant cheese (Chikpah *et al*., 2014).

The extracts from the *Calotropis* plant has been used traditionally as the sole coagulant of milk in a number of African countries for the production of a soft cheese called '*wagashie*' (Ibiama and Griffiths, 1987; O'Connor, 1993; Belewu and Aina 2000; Ashaye *et al*., 2006; Chikpah *et al*., 2014). However, a green colouration and bitter nature of the cheese produced are some problems that are associated with the use of plant extract as coagulant of cheese (Omueti and Jaiyeola, 2006; Chikpah *et al*., 2014). Alalade and Adeneye (2010) reported that there is no significant change in the cheese microbial load stored for 87 h in whey.Cheese in Nigeria is still a niche category. Although Nigerians do consume of unpackaged local cheese, packaged cheese is not popular among Nigerians(Euromonitor, 2016). Growth is, however, being driven by an increased presence in retail outlets, such as supermarkets and independent small grocers, which increasingly stock western-style products. Growth is also in line with the fast rate of urbanisation growth.Cheese is expected to grow at 1% increase at constant 2016 prices (Euromonitor, 2016).

Continuing urbanisation, westernisation, growth in the expatriate population, growth in incomes and the rapid development of modern retail channels will help to fuel the increase in sales. The expected strong growth of modern retail channels such as hypermarkets and supermarkets will be the most significant factor spurring growth, as these channels make such products more visible to the middle classes, which are demanding more western-style products; they are also more capable of the better storage and preservation of these products (Euromonitor, 2016). Economic analysis of product is based on either cost effectiveness (reduction) or cost-benefit ratio (Dixon and Pagiola, 1998). Cost-benefit analysis is desirable when the product will incur additional cost of production. The benefit (revenue) of the project can be measured from price and quantity. The price can be calculated from consumer economic valuation (measures of tangible and intangible benefits) less the switch cost (MIT OpenCourseWare, 2010). The tangible benefit can be measured using a fair price. A fair price is defined as how much a customer thinks a service (or good) should cost, and so to assess the fairness of a price, customers may rely on the reference price which includes the last price paid, the price most frequently paid, market prices, posted prices and price of the closest alternative (Kahneman *et al*., 1986;Seperich *et al*., 2002). However, the intangible benefit and switch cost can be neglected because of the difficulty and low monetary value (MIT OpenCourseWare, 2010). Marketing is said to be profitable if the profitability ratio is greater than 0, while market is operationally efficient if the efficiency ratio is greater than 1 and the higher the benefit-cost ratio (profitability index rate), the better the investment (Mafimisebi *et al*., 2013). It is a common assertion that a product is valued for its quality and utility generating attributes which buyers evaluate during purchase (Seperich *et al*., 2002). The observed market price for a product is therefore the sum of the implicit prices paid for each quality attributes. However, in most studies observed that prices may reflect not only

consumer preferences but also attributes of buyers and sellers and the political organization of the market setting (Seperich *et al*., 2002; Mafimisebi *et al*., 2013). Hence the importance of a price strategy formation index to identify the critical areas of interest by the consumer which can be used to maximize the fair price by improving the best ranked index during *wara* production.

Warankasi is a soft unripened cheese made from cow's milk by coagulating the proteins with Sodom apple juice to obtain the curd, in Benin Republic, it is called *Woagachi*, Its Western substitute; Cottage Cheese, its Latin American substitute; Queso Blanco, Zaire; *Mashanza* and its Indian substitute is Paneer. The local production of *warankasi* dates back for a long time and used as a mode of preservation of milk by the nomadic Fulani women, although it is believed to originate from Wara town in Kwara State (Ogundiwin and Oke, 1993). The Sodom-apple juice contains a rennet-like enzyme which was isolated and identified as calotropain (Ibiama and Griffiths, 1997). The enzyme produces activities which do not stop at milk clotting but leads to the breakdown of the primary covalent bonds in milk proteins. It is also well known that milk proteins gelation is generally considered irreversible, resulting from enzyme reaction of rennet and other proteases (Ganguli and Bhalerac, 1995). It could also be initiated by heat or cation reactions, whereby formation of paracaseinates occurs (Gangali and Bhalerac, 1995). During hydrolysis peptides of varying sizes are formed but it is well known that some peptides, especially those rich in hydrophobic amino acids taste bitter. Bitterness is influenced by the cheese making procedure and by factors such as strain of starter culture, rennet concentration, heat treatment of the milk, acidity or pH of cheese and salt concentration (Harboe *et al*. 2010; Jacob *et al*. 2011). Lemieux and Simard (1992) observed that Valine, Leucine, Proline and Phenylalanine are major free amino acids (FAAs) which forming bitterness. Kabelova *et al*. (2009) reported that individual amino acids, seven amino acids were more concentrated in all

cheese samples, and came from three distinctive taste groups: bitter tasting amino acids (Leucine, Lysine, and Phenylalanine), bitter sweet amino acids (Proline and Valine), and salty-umami amino acids (Glutamic acid and Aspatic acid). The amino acids Phenylalanine, Leucine - Isoleucine, Glycine, Valine, Proline and Alanine were found most abundantly in the fractions of FAAs of ripened White cheeses. The ratio of basic bitter taste free amino acidswas not changed depending on rennet type. The coagulated curds tend to lose water or synerese on standing (Schmidt and Morris,1994). Warankasi is a fermented milk product that is consumed by just a few majorities of Nigerians due to lack of knowledge of its nutritional status. Hence, the need to create awareness about its nutritional value becomes necessary.

2.3Mozzarella

Mozzarella cheese is a soft, unripened cheese variety of the *Pasta-filata* family which had its origin in the Battipaglia region of Italy (Citro, 1981; Jana, 2001). Conventionally, Mozzarella cheese was made from buffalo milk. However, these days it is being manufactured in Italy, in other European countries and USA from cow milk which needs appropriate modifications (Ghosh *et al.,* 1990). The cheese is white, soft with a glossy surface and is valued for its stretch property. This makes it suitable for the preparation of *lasagna*, veal *cutlet alla Parmigiana* and as a topping on pizza (Jana, 2001). Mozzarella cheese is used as a topping on pizza pie owing to its inherent stretching qualities. The popularity of pizza parlour, especially amongst youngsters has boosted the production of Mozzarella cheese. In Italy Mozzarella and pizza cheeses accounts for 78% of the total Italian cheese product (Merrill *et al.,* 1994)

In '*Pasta filata*' principle of cheese making, the smooth texture and grain of the cheese is achieved through a skillful stretching of curd in hot water. The desirable characteristics of Mozzarella cheese is brought about by the action of lactic acid on dicalcium paracaseinate, viz.,

the rennet cheese curd. At a pH of 5.2 to 5.4, much of this compound gets converted to mono-calcium paracaseinate which provides the strings and sheen to the cheese (Kosikowski, 1958). Mozzarella and Pizza cheeses requirements are depicted in Table 2.1 (Jana, 2001).

Buffalo milk is reported to be more suitable than cow milk for Mozzarella cheese due to high yield, its characteristic aroma and physical attributes (Bonassi *et al.,* 1982; Scott, 1986; El-Koussy *et al.,* 1995). Bonassi *et al.* (1982) did not find any difference in taste, aroma or texture of Mozzarella cheese made from cow milk, buffalo milk or their combination (1:1). The cheese yield and fat content were significantly higher for buffalo milk product but the difference in pH, Titratable Acidity (TA), moisture content, protein, soluble nitrogen and ash content were not significant. Singh and Ladkani (1984) standardized the method for Mozzarella cheese from cow and buffalo milk, with and without cheddaring. The stretch ability was relatively inferior in buffalo cheese. Patel *et al.* (1986) standardized the Starter Culture (SC) and Direct Acidification (DA) method for the manufacture of Mozzarella cheese from buffalo milk. The type of milk (viz., cow, buffalo and their admixture) used for Mozzarella had a profound influence on the cheese composition.

The cheese obtained from a blend of cow and buffalo milk had superior organoleptic quality as well as meltability compared to those made from individual milks; buffalo cheese had superior nutritional value (Sameen *et al.,* 2008).

Traditionally, Mozzarella cheese was manufactured from raw milk. However, pasteurization of milk is recommended for Mozzarella cheese that is to be consumed fresh, because the plasticizing process does not always destroy pathogens (Caserio *et al.*, 1977; Jana and Manthal, 2011). Heat treatment of milk (72°C, no hold) meant for Mozzarella cheese making improved protein and Total Solids (TS) recovery but decreased the fat recovery, gave soft-bodied cheese,

improved flavor score and keeping quality and ensured public health safety (Patel *et al.*, 1986). The starter used for fresh Italian Mozzarella is only *Streptococcus thermophilus*. To achieve consistent quality in the

Table 2.1: Classification of Mozzarella cheese (%)

Type of cheese	Moisture	Fat-on-dry matter
Mozzarella (Regular)	**52.0-60.0**	**Min. 45.0**
Mozzarella (Part skim)	**52.0-60.0**	**30.0-45.0**
Mozzarella (low moisture)	**45.0-52.0**	**Min. 45.0**
Mozzarella (low moisture, part skim)	**45.0-52.0**	**30.0-45.0**
Mozzarella (Homemade)	**Max. 60.0**	**Min. 35.0**
Pizza-grade Mozzarella	**Max. 54.0**	**Min. 35.0**

Source: Jana and Mandal (2011).

end product, the parameters to be controlled include avoiding batch-to-batch variation in the starter strain ratio and constant acidification (Sigsgaard, 1994).

Manufacture of high-moisture Mozzarella entails use of starters like *S. lactis, S. durans* or *S. faecalis*, whereas for low-moisture Mozzarella the starters recommended are *S. thermophilus, L. bulgaricus* and/or *L. helveticus* (Christensen, 1966; Jana and Manthal, 2011). According to Quarne *et al.*(1968) cheese made with porcine pepsin or fungal rennet gave an organoleptically superior product than veal rennet. Micketts and Olson (1974) found that cheese made with porcine pepsin was slightly softer than the one made using calf rennet. Christensen (1972) found identical result using Marzyme and calf rennet in cheese making.

Rossi (1972), Matteo *et al.* (1982), Patel *et al.* (1986), Mikacic (1986) andPizaia *et al.*(2003) studied the changes during storage of Mozzarella cheese. Mozzarella cheese kept in brine with 1.0% NaCl had the highest sensory score and had excellent quality, even after 12 days, however, higher percentage of salt gave low sensory score. Storage of Mozzarella cheese at 4°C over 4 weeks decreased the apparent viscosity; the rough fibrous texture changed to a smooth one and changes also occurred in cheese melting and stretching characteristics (Kindstedt and Guo, 1997). Patel *et al.* (1986) reported that cheeses made by direct acidification (DA) method had lower shelf life (1 week) as compared to those prepared by starter method (>2 weeks), when stored at 8°C. The unsalted cheese packaged in polyethylene pouch had a shelf life of 3 months

at 8-10°C (Ghosh and Kulkarni, 1996). Soluble Nitrogen (SN) and soluble tyrosine and tryptophan contents elevated with increasing storage period. Advancement of cheese storage led to an increase in the melt ability of cheese (Ahmed *et al.,* 2011)

Several researchers studied the microbiological aspects of Mozzarella cheese during manufacture, as well as of market samples (Sforzolini *et al.,* 1956; Kosikowski, 1960; Catellani and Giordano, 1962; Nilson and La Clair, 1976; Caserio *et al.,* 1977; Nieradka *et al.,* 1979; Ottogalli *et al.,* 1979; Asperger, 1982; Asperger and Brandi, 1982). A decrease of about 2 log of *Listeria monocytogenes* was observed after stretching of the curd in hot water (95°C). *Listeria* could not survive in cheese after 24 h when stored in the conditioning liquid (skim water from stretch, pH ~4.0) (Villani *et al.,* 1998). Stretching of Mozzarella cheese curd at 66°C for 5 min or 77°C for 1 min can effectively control *L. monocytogenes* during the production of Mozzarella cheese; brining too had a lethal effect but less effective than stretching treatment (Kim *et al.,* 1998). The US Food and Drug Administration (FDA) permits addition of mould inhibiting ingredients, upon declaration on the label, during kneading and stretching process or applying it to the surface of the cheese (FDA, 1977). Few reports dwell on the effects of storage on the microbiological quality of the product (Rossi, 1972; Costamagna, 1976; Asperger, 1982; Asperger and Brandi, 1982; Mikacic, 1986).

During refrigerated (4°C) storage of Mozzarella cheese for 50 days, the textural characteristics viz., hardness, cohesiveness and springiness of unmelted cheese decreased, meltability increased, apparent viscosity of melted cheese decreased and free oil formation from melted cheese increased (Yun *et al.,* 1993). During storage there was an increase in the water holding capacity of the cheeses (Yazici and Akbulut, 2007). With advancement in ripening time of Low Moisture Mozzarella (LMM), there was a significant decrease in the concentration of intact casein,

firmness, melt time and apparent viscosity. Nevertheless, the flow ability and stretchability of the molten cheeses were found to increase significantly during storage (Guinee *et al.*, 2001). There was a slight continual decrease in moisture of cheese during refrigerated storage; the pH decreased from initial 5.36 to 5.16 in 49 days, while titrable acidity(TA) showed a linear increase (Ghosh and Singh, 1992). Refrigerated storage of Mozzarella cheese led to significant decrease in the moisture content culminating in increased fat, cholesterol and protein content; total volatile fatty acids (TVFA) also showed an increasing trend. The pH of cheese decreased with increase in storage period of Mozzarella cheese (Ali and Abdel-Razig, 2011).

The soluble nitrogen of LMM increased significantly during 7 weeks of ripening but at different rates for different coagulants (*Endothia parasitica, Mucor miehei* or chymosin). Substantial breakdown of caseins was evident during ripening. Texture characteristics, meltability, apparent viscosity and free oil formation changed significantly during ripening; the rate of change differed among coagulants and corresponded with proteolysis rates (Kindstedt, 1993). The melting and fat leakage characteristics increased while stretch tended to decrease with advancement in storage period of Mozzarella cheese (Ghosh and Singh, 1992).

2.4Rennet

Rennet is used to describe the wide range of coagulants that can be used for the coagulation of milk into a gel structure. Calf rennet, which consists primarily of chymosin, has traditionally been used in cheese making, but a growing cheese industry, combined with a limited supply of calf rennet, has led to the search for calf rennet substitutes (Sousa *et al*., 2001). Rennet can originate from other animal sources; porcine pepsin, bovine pepsin and chicken pepsin, microbial sources; *Rhizomucor miehei* (Mucor), *Rhizomucor pusillus* and *Cryphonectria parasitica* (Endothia), or plant sources; *Cynara cardunculus* (Sousa *et al*., 2001). Inserting the coding

sequence for bovine chymosin into microorganisms has made fermentation-produced chymosin (FPC) widely available (Kumar *et al*., 2010). More recently, recombinant camel chymosin, also known as fermentation-produced camel chymosin (FCC) (Kappeler *et al*., 2006), has also become commercially available. The type of rennet can influence the primary proteolysis. Sheehan *et al*. (2004) showed that reduced-fat Mozzarella cheese made with *R. pusillus* proteinase resulted in higher levels of pH 4.6 soluble nitrogen during ripening, when compared to cheeses made with either FPC or *R. miehei*proteinase. A study compared the effects of calf rennet with bovine pepsin and reported that a more compact structure was usually observed in Cheddar cheese made with the calf rennet (Stanley and Emmons, 1977). The rennet concentration applied may also affect the coagulation process and the quality of cheese. Increasing the rennet concentration increases the rate of enzymatic hydrolysis, with a resulting decrease in the time required for rennet-induced coagulation (Spangler *et al*., 1991). Creamer *et al*. (1987) found that rennet concentration did not majorly affect Cheddar cheese making, besides producing a slightly soft gel when very low concentrations of rennet with a similar fat in dry matter. A reduction of up to 40% of rennet concentration was found to only minimally affect the composition of Mozzarella cheese, resulting in cheeses with similar protein, moisture, salt and calcium concentration and pH, although the fat content did differ (Kindstedt *et al*., 1995).

Most enzyme preparations used for cheese have been extracts from the stomachs of ruminants, but coagulants from microbes and plants were also used at very early dates (Harboe *et al*., 2010; Jacob *et al*., 2011). The cheese production increased by a factor of approximately 3.5 since 1961 but the rennet supply decreased due to the limited availability of ruminant stomachs (Jacob *et al*., 2011). Various factors such as high price of rennet, religious concerns (e.g., Islam and Judaism), diet (vegetarianism) or ban on recombinant calf rennet (in France, Germany and The

Netherlands) have encouraged the search for alternative milk-clotting sources (Roseiro *et al*., 2003). The research has been directed towards discovering milk-clotting enzymes which would satisfactorily replace calf rennet in cheesemaking, including microbial, recombinant, and plant-based enzymes (Jacob *et al*., 2011). The most important substitutes which fulfill the requirements of cheese manufacture include microbial, recombinant, and plant-based enzymes which have been isolated and studied.

2.5Plant Rennet

Plant extracts as a coagulant for cheesemaking has been largely used for the production of hard and semi-hard cheese products from ovine and caprine milk. Cheeses prepared with plants coagulants have a characteristic soft texture and a slightly bitter flavor (Roseiro*et al*. 2003). Their application to bovine milk has sometimes resulted in poor quality cheeses, especially in terms of texture and flavor. The plant coagulants such as cardoon extract can be used for production of bovine cheeses if the cow's milk could be modified to resemble to those of sheep milk (Agboola *et al*., 2009). Low *et al*. (2006) showed that the undesirable proteolysis inherent in the application of plant coagulants to regular cow's milk can be significantly reduced when concentrated UV milk was employed as the milk source. Proteolytic, textural, and sensory studies showed that while UV may have reduced the extent of milk and cheese proteolysis in the presence of cardoon extract, it did not result in improved sensory qualities unless a significant amount of regular milk was present (Agboola *et al*. 2009).

Tamer and Mavituna (1997) observed enzymes are present in almost all kinds of plant tissues and all proteolytic enzymes have the ability to clot milk under appropriate conditions. Almost all

the enzymes used as milk coagulants belong to aspartic proteases, but enzymes from other groups such as cysteine and serine proteases have also been used. Plant extracts have been used as milk coagulants in cheesemaking since ancient times. Cheeses made with vegetable coagulant can be found mainly in Mediterranean, West African, and southern European countries. Spain and Portugal have the largest variety and production of cheeses using *Cynara* sp. as the vegetable coagulant (Roseiro *et al*., 2003). The extracts of *Cynara* spp. have been used in the making of Portuguese *Serra* and *Serpa* cheeses (Macedo *et al*., 1993) and Spanish *Los Pedroches*, *La Serena* (Roa *et al*., 1999) and *Torta del Casar* cheeses (from ewes' milk) as well as *Los Ibores* cheese (from goats' milk) and *Flor de Guía* cheese (from a mixture of ewes' and cows' milk) (Fernández-Salguero *et al*., 1991; Fernández-Salguero, 1999; Sanjuán *et al*., 2002).

In West African countries like Nigeria and the republic of Benin, extracts from *Calotropis procera* (Sodom apple) have been used in traditional cheesemaking (Roseiro *et al*., 2003). However, the excessive proteolytic nature of most vegetable coagulants has limited their use in cheese manufacturing due to lower cheese yield and defects in flavor and texture (Lo Piero *et al*., 2002). Therefore, the search for new potential milk-clotting enzymes from plants is in continuous process, so as to make them industrially useful and go with the increasing global demand for diversified and good quality cheese production (Hashim *et al*., 2011). Sousa and Malcata (2002) reviewed the role of plant coagulant (*Cynara cardunculus*) *in vitro* and during ripening of cheeses from several milk species, while Roseiro *et al*. (2003) reviewed the use of plant extracts with special reference to *Cynara* species. Jacob *et al*. (2011) reviewed the important types of milk-clotting enzymes including animal rennet, microbial coagulants, recombinant coagulants, and plant-derived clotting enzymes. Yegin and Dekker (2013) have recently reviewed the progress in the field of aspartic proteinases from animal, plant and

microbial origin with a special emphasis on structures, functions, catalytic mechanism, inhibition and engineering.

Proteases are required by plants in all aspects of their life cycle. They are involved from the mobilization of storage proteins during seed germination to the initiation of cell death and senescence program (Schaller, 2004). Proteases have been divided into groups based on the catalytic mechanism used during the hydrolytic process. The main catalytic types are aspartate, serine, cysteine, and metalloproteases (Bah *et al*., 2006), but the plant proteases used as milk coagulants have been reported only from first three types and none from metalloproteases. Serine and cysteine proteases are catalytically very different from aspartic and metalloproteases in that the nucleophile of the catalytic site is part of an amino acid, whereas it is an activated water molecule in the other two groups (Bruno *et al*., 2006).

Aspartic proteases have two aspartic residues at their catalytic site. They are most active at acidic pH and show preferential specificity for cleavage at peptide bonds between hydrophobic amino acid residues responsible for the catalytic activity (Domingos *et al*., 2000). Aspartic proteases with milk-clotting activity have been reported in *Onopordum turcicum* (Tamer, 1993);artichoke (*Cynara scolymus* L.) (Llorente *et al*., 1997); rice kernels (Asakura *et al*., 1997); *Centaurea calcitrapa* (Domingos *et al*., 2000); milk thistle (*Silybum marianum* L. Gaertn.) (Vairo-Cavalli *et al*., 2005). Cardoon (*C. cardunculus*) flowers are traditionally used in the Mediterranean region for cheesemaking (Barros *et al*., 2003). It produces cardosins and cyprosins, aspartic proteases that have been found to accumulate in mature flowers but not in leaves or seeds (Cordeiro *et al*., 1998). Cardosin A is an abundant aspartic protease from pistils of *C. cardunculus*. Also, three cyprosins with milk-clotting activity, from dried flowers of *C. cardunculus*, were isolated, purified, and characterized by Heimgartner *et al*. (1990). These flowers contain aspartic

proteases, previously described, which have been shown to share specificity and kinetic parameters with chymosin and pepsin (Verissimo *et al*., 1995; 1996).

Cysteine proteases, also known as thiol proteases, and the catalytic mechanism of these enzymes involve a cysteine group in the active site. Cysteine proteases have great potential in the food, biotechnology, and pharmaceutical industries owing to their property of being active over a wide range of temperature and pH. Plants offer an attractive alternative for the production of CPs as they occur naturally in different tissues, in some cases in excessive amount (Gonzalez-Rabade *et al*., 2011). Ficin isolated from the latex of different *Ficus* species possess certain characteristic properties. A ficin isolated from the latex of *Ficus racemosa*, showed an ability to digest casein, suggestive of a milk-clotting property (Devaraj *et al*., 2008). Protein extracts from sunflower and albizia seeds were prepared to determine the milk-clotting activity and the action of these milk-clotting plant extracts on bovine whole casein (Egito *et al*., 2007). Actinidin was isolated from ripe fresh kiwifruits (*Actinidia chinensis*) (Katsaros *et al*., 2010). Ginger protease was isolated from ginger rhizomes (*Zingiber officinale*) having a molecular mass of 36 kDa (Hashim *et al*., 2011).

Serine proteases possess a serine residue in their active site and have been found and extracted from latex, seeds, flowers, stems, leaves and roots. Serine proteases from cucurbits, cereals, and trees are usually classified together (Rawlings and Barrett, 2004). Neriifolin, a chymotrypsin-like serine protease, has been purified from the latex of *Euphorbia neriifolia* (Yadav *et al*., 2011). Neriifolin S, a dimeric serine protease of molecular mass 94 kDa with milk-clotting activity has been purified from the latex of *E. neriifolia* (Yadav *et al*., 2012). Religiosin (43.3 kDa), Religiosin B (63 kDa) and Religiosin C (80 kDa) were isolated from *Ficus religiosa* latex by Kumari *et al*. (2010), Kumari *et al*. (2012) and Sharma *et al*. (2012) respectively. Streblin, a

thermostable enzyme having a molecular mass of 63 kDa was purified from *Streblus asper* (Tripathi *et al*., 2011). Dubiumin was purified from the seeds of *Solanum dubium*, having a molecular mass of 66 kDa (Ahmed *et al*., 2009). Cucumisin from *Cucumis melo* (Uchikoba and Kaneda, 1996) and lettucine from *Lactuca sativa* (Lo Piero *et al*., 2002) were isolated and used as milk coagulants.

2.5.1Lime

2.5.1.1 *Lime Tree*

Citrus aurantifolia (family: *Rutaceae*) is mainly used in daily consumption, in many cultural cuisines, and in juice production. It is widely used because of its antibacterial, anticancer, antidiabetic, antifungal, anti-hypertensive, anti-inflammation, anti-lipidemia, and antioxidant properties (Narang and Jiraungkoorsku, 2016). Moreover, it can protect heart, liver, bone, and prevent urinary diseases. Its secondary metabolites are alkaloids, carotenoids, coumarins, essential oils, flavonoids, phenolic acids, and triterpenoids. The other important constituents are apigenin, hesperetin, kaempferol, limonoids, quercetin, naringenin, nobiletin, and rutin, all of these contribute to its remedial properties (Narang and Jiraungkoorsku, 2016).

C. aurantifolia is a perennial evergreen tree that can grow to a height of 3–5 m. Stem is irregularly slender branched and possesses short and stiff sharp spines or thorns 1 cm or less. Leaves are alternate, elliptical to oval, 4.5–6.5 cm long, and 2.5–4.5 cm wide with small rounded teeth around the edge. Petioles are 1–2 cm long and narrowly winged. Flowers are short and axillary racemes, bearing few flowers which are white and fragrant. Petals are 5, oblong, and 10–12 mm long. Fruits are green, round, 3–5 cm in diameter, it is yellow when rip (Manner *et al*., 2006; Enejoh *et al*., 2015).

2.5.1.2 *Lime Fruit*

All citrus fruits present the same anatomical structures: The flavedo is the external part of the fruit and has a lot of flavonoids as its name. The outer cell wall is composed of wax and cutin for prevention of water loss from the fruit; The albedo is the white spongy portion, below the flavedo layer; The carpal membranes or septum presenting around 8–11 glandular segments, usually aligned and situated around the soft central core; The juice sacs are yellow-green pulp vesicles; and the seeds are small, plump, ovoid, pale, and smooth with white embryo (Rivera-Cabrera *et al*., 2010).

C. aurantifolia contains active phytochemical substances as follows: flavonoids including apigenin, hesperetin, kaempferol, nobiletin, quercetin, and rutin, (Berhow *et al*., 1994; Kawaii *et al*., 1999; Caristi *et al*., 2003; Aranganathan *et al*. 2008; Lee *et al*., 2011; Loizzo *et al*., 2012), flavones (Piccinelli *et al*., 2008), flavanones (Peterson *et al*., 2006) and naringenin, (Arul *et al*., 2013) triterpenoid (Jayaprakasha *et al*., 2008), and limonoids (Poulose *et al*., 2005). In addition, Lota *et al*. (2002) reported at least 62 volatile compounds in the fruit peel oils and 59 in the leaf oils of several lime species. In the fruit peel oils, limonene was the major volatile component, followed by terpinene, pinene, and sabinene. For leaf oils, limonene, pinene, and sabinene were the major components, followed by citronellal, geranial, linalool, and neral. The bioactive compounds from citrus in many countries were reported, for example, Spadaro *et al*. (2012) reported the fruit essential oils of *C. aurantifolia* as limonene (59%), β-pinene (16%), γ-terpinene (9%), and citral (5%), Costa *et al*. (2014) reported the fruit essential oils of *C. aurantifolia* as limonene (54%), γ-terpinene (17%), β-pinene (13%), terpinolene (1%), α-terpineol (0.5%), and citral (3%) in Italy. Okwu and Emenike (2006) determined the phytochemical and vitamin contents of five varieties of citrus species; *C. sinensis, C. reticulata, C. limonum, C. aurantifolia, and C. grandis* in Nigeria. The presence of bioactive compounds in 100 g of citrus comprise

alkaloids (0.4 mg), flavonoids (0.6 mg), phenols (0.4 mg), tannins (0.04 mg), ascorbic acids (62 mg), riboflavin (0.1 mg), thiamin (0.2 mg), and niacin (0.5 mg). They also reported that these citrus fruits contain crude protein (18%), crude fiber (8%), carbohydrate (78%), moisture (6%), crude lipid (1%), ash (8%), and food energy content was (363 g/cal) of fresh fruits. The most important minerals detected in the fruit include calcium (3%), phosphorus (0.4%), potassium (1%), magnesium (0.6%), and sodium (0.4%). Lawal *et al*. (2014) reported that the leave essential oil of *C. aurantifolia* contains limonene (45%) and geranial (38%). Wang *et al*. (2007) reported that the chemical substances from citrus fruit contain hesperidin; the major flavanone (6 mg/g), naringin (2 mg/g), diosmin; the major flavone (0.7 mg/g), kaempferol; the major flavanol (1 mg/g), chlorogenic acid; the major phenolic acid (0.1 mg/g), β-cryptoxanthin; the major carotenoid (7 μg/g), and β-carotene (4 μg/g), followed by total pectin (87 mg/g) in Taiwan. Sandoval-Montemayor *et al*. (2012) reported that *C. aurantifolia* fruit peels consist of 44 volatile compounds, for example, dimethoxycoumarin (16%), cyclopentanedione (9%), methoxycyclohexane (8%), corylone (7%), palmitic acid (7%), dimethoxypsoralen (6%), α-terpineol (6%), and umbelliferone (5%) in Mexico.

The traditional uses or phytochemical properties of *C. aurantifolia* from literatures are described as antibacterial (Aibinu *et al*., 2006; Pathan *et al*., 2012; Nwankwo *et al*., 2015), antidiabetic (Daniels, 2006; Karimi *et al*., 2014), antifungal (Dongmo *et al*., 2009; Tagoe *et al*., 2009; Balamurugan *et al*., 2014) antihypertensive (Souza *et al*., 2011), anti-inflammation (Dongmo *et al*., 2013), anti-lipidemia (Yaghmaie *et al*., 2011; Ezekwesili-Ofili *et al*., 2015), antioxidant (Reddy *et al*., 2012; Kumari *et al*., 2013), anti-parasitic (Abdelqader *et al*., 2012; Enejoh *et al*., 2014; Enejoh *et al*., 2015; Domínguez-Vigil *et al*., 2015), and antiplatelet (Piccinelli *et al*., 2008) activities. It is used for the treatment of cardiovascular (Yamada *et al*., 2011), hepatic

(Gokulakrishnan *et al*., 2010), osteoporosis (Shalaby *et al*., 2011), and urolithiasis (Tosukhowong *et al*., 2008; Anggraini, 2015) diseases and acts as a fertility promoter (Okon and Etim, 2014). Moreover, it can be used for insecticide activity (Effiom *et al*., 2012; Soonwera, 2015).

Patil *et al*. (2009) reported that *C. aurantifolia* fruit from Texas, USA, consists of at least 22 volatile compounds, and its major compounds were limonene (30%) and dihydrocarvone (31%). About 100 µg/ml of *C. aurantifolia* extract can inhibit the growth of colon cancer cell after 48 h of exposure. Patil *et al*. (2013) also found out three new coumarins from *C. aurantifolia* peel from Texas as 5-geranyloxy-7-methoxycoumarin, limettin, and isopimpinellin and that about 25 µM of *C. aurantifolia* extract can inhibit the growth of colon cancer cell in after 72 h of exposure. Gharagozlo *et al*. (2002) reported that the 125–500 µg/ml of *C. aurantifolia* fruit juice extract from Iran inhibits the growth of breast cancer cell after 24 h of exposure. Adina *et al*. (2014) reported that the 6 and 15 µg/ml of *C. aurantifolia* peel extract from Indonesia inhibits the growth of breast cancer cell at G1 and G2/M phase, respectively, after 48 h of exposure.

Moreover, the citrus secondary metabolites were studied for anticancer activity, for example, flavonoids on skin cancer (Pan *et al*., 2012), hesperetin on colon cancer (Aranganathan *et al*., 2013), limonoids on colon cancer (Chidambara Murthy *et al*., 2013), nobiletin on gastric cancer (Yoshimizu *et al*., 2004), naringenin on prostate cancer (Gao *et al*., 2006), gastric cancer (Ekambaram *et al*., 2008), and hepatocarcinoma (Arul and Subramanian, 2013). Li *et al*. (2010) reported the relationship between the citrus consumption and the reduction of cancer incidence and revealed a positive relationship that citrus consumption could prevent the occurrence of cancer. Wang *et al*. (2014) reviewed the protective effects of polymethoxyflavones from citrus

and proposed that it inhibits carcinogenesis by several pathways in the metastasis, cell mobility, proapoptosis, and angiogenesis.

2.5.2Sodom apple

2.5.2.1*Sodom apple Tree*

Calotropis procera of family *Asclepiadaceae* is a tropical plant growing wild in warm climate up to an altitude of about 1050 meters. It is a native plant of North Africa. It also found in Pakistan, Africa, Mexico, Australia, Egypt, Central and South America and Caribbean islands (Belewu *et al*., 2005;Pusapati *et al.,* 2012). From pre-historic times to the modern era in many parts of the world and India, plants, animals and other natural objects have profound influence on culture and civilization of man. Since the beginning civilization, human beings have worshiped plants and such plants are conserved as a genetic resource and used as food, fodder, fiber, fertilizer, fuel, and febrifuge in every other way, *Calotropis procera* is one such plant (Kumar *et al.,* 2013). In ancient Ayurvedic medicine the plant *Calotropis procera* is popularly known as *Raktha Arka* and *Calotropis gigantea* as *Sweta Arka*. Both of them are often similar in their botanical aspects and also have similar pharmacological effects (Gamble, 1935; Kumar *et al.,* 2013). Common names for the plant include apple of Sodom, Sodom apple, Stabragh, Kapok tree, King's crown, Rubber bush or Rubber tree with local names like *Bomubomu*(Yoruba) and *Timpapia* (Hausa). A number of ethanomedicinal uses of the drug are reported. Whole plant was used either alone or with other herbs for the treatment of common diseases such as fever, rheumatism, indigestion, cold, eczema and diarrohea (Kew, 1985), paste of root bark was locally applied in the treatment of elephantiasis (Chopra *et al.,* 1956)

The plants contain the cardenolide, proceragenin, while the root bark contains benzoylinesolone and benzoylisolinelone. The leaves and stalk contain calotropin and calotropagenin while the

flower contains calotropenyl acetate and multiflavenol and the latex contains uzarigenin, and terpenol ester (Quazi *et al.,* 2013). Traditionally it was used to treat cholera, extracting guinea worms, diarrhea, dysentery and indigestion (Jain *et al*., 1985).

Chemical analysis showed the presence of triterpenoids, calotropursenyl acetate and calopfriedelenyl, a norditerpenyl ester, calotropternyl ester oleanene triterpenes like calotropoleanyl ester, procerleanol A and B (Ansari and Ali, 2001) and cardiac glycosides calotropogenin, calotropin, uscharin, calotoxin and calactin (Ahmed *et al.,* 2005). The plant also has been investigated for the presence of cardenolides (Seiber *et al.,* 1982) and anthocyanins. Phytochemical investigation of the roots of *Calotropis procera* yields two new phytoconstituents, procerursenyl acetate and proceranol, together with the known compounds N-dotriacont-6-ene, glyceryl mono-oleolyl-2-phosphate, methyl myrisate, methyl behenate and glyceryl-1, 2-dicapriate-3- phosphate. In the leaves, mudarine is the principal active constituent as well as a bitter yellow acid, resin and 3 toxic glycosides calotropin, uscharin and calotoxin. The structures of the new compounds have been identified as urs-18 alpha-II-12, 20 (30)-diene-3 beta-yl acetate and n-triacontan-10 beta-ol on the basis of spectral data analysis and chemical reactions (Quazi *et al.,* 2013). The root bark has also been found to possess α-amyrin, β-amyrin, lupeol, β-sitosterol (Quazi *et al.,* 2013) and flavanols like quercetin-3-rutinoside.In the leaves, mudarine is the principal active constituent as well as a bitter yellow acid, resin and 3 toxic glycosides calotropin, uscharin and calotoxin.

2.5.2.2*Sodom apple Latex*

The latex contains a powerful bacteriolytic enzyme, a very toxic glycoside calactin (the concentration of which is increased following insect or grasshopper attack as a defense mechanism), calotropin D I, calotropin D II, calotropin-F I, calotropin F II and a non-toxic

proteolytic enzyme calotropin (2-3 %). This calotropin is more proteolytic than papain, and bromelain; coagulates milk, digests meat, gelatin and casein. The whole plant of *Calotropis gigantea* contains a- and b- amyrin, teraxasterol, gigantin, giganteol, isogiganteol, beta-sitosterol and a wax (Perwez and Mohammad, 2009) which are poisonous.

Calotropis procera plant latex contains the cardenolide, proceraenin, while the root bark contains benzoylinesolone and benzoylisolinelone. The leaves and stalk contain calotropin, and calotropagenin while the flower contains calotropenyl acetate, and multiflavenol and the latex contains uzarigenin, and terpenol ester (Yoganarasimhan, 2000).

Calotropin a compound in the latex, is more toxic than strychnine and similar in structure to two cardiac glycosides which are responsible for the cytotoxicity of Apocynum. Extracts from the flowers of Bomubomu (*C. procera*) have shown strong cytotoxic activity in the patients of colorectal cancer. Quercetin-3-rutinoside is identified in the roots, stem, leaves, flowers and latex. Voruscharin is isolated from African plant. Cardenolides contents in leaf (2.04 mg/gm) and in latex (162.0 mg/g), mostly calotropagenin – derived cardenolides present from *Calotropis gigantea*, two triterpene esters – 3 methylbutanoates of amyrin and taraxasterol are isolated from latex of *C. gigantea*. calotropins D1 and D2 had been isolated from *C. gigantea* (Pal and Sinha, 1980). The new oxiopregnane- oligoglycosides named Calotropis A and B have been isolated from the root of *C. gigantea* and their chemical structure have been elucidated by chemical and spectroscopy methods (Kitagawa *et al.,* 1992). The cytotoxic principles of 'Akond mul' (Root of *C. gigantea*) cardenoloids glycosides, calotropin frugoside and 4-O-Beta-D-glucopyranosyl frugoside were obtained as the cytotoxic principles (Kiuchi *et al.,* 1998). Gigantin is a chemical extracted only from *C. gigantea*.

2.5.3Moringa

2.5.3.1*Moringa Tree*

Moringa oleifera Lam. tree grows in many tropical and subtropical countries. It is grown for commercial purposes in places like South and Central America, Africa, India, Hawaii, Mexico and Asia. It was named mothers best friend, 'horseradish tree' on the basis of ground root taste preparations, also named "drumstick tree" on the basis of its immature seed pods appearance, the "ben oil" tree on the basis of seed-derived oils and locally called *Zogale* in Hausa, Nigeria. In some places, the immature seed pods are eaten, while the fresh leaves are commonly used as a basic food due to their high nutritional composition (Thurber and Fahey, 2009; Mbikay, 2012; Razis *et al.,* 2014). *M. oleifera* tree belongs to the family of *Moringaceae*. It was well known for its multipurpose attributes, wide adoptability, and ease of establishment. According to Dalla (1993) its leaves, pods, and flowers are packed with nutrients important to both human and animals. *M. oleifera* is not a nitrogen fixing tree, but its fruits, flowers and leaves all contain 5to 10% protein on average. All of these parts are eaten widely as vegetables; provide excellent food, for both humans and eaten like green beans. These roots taste similar to horse radish and is popular food (leaves) in the East Africa. Moringa flowers also produce a good honey. The seeds are usually used as anticoagulant. Harvested dry seed are shelled crushed in to powdered form and made in to paste with the water before mixing with more water. Muyibi, and Evison (1995) stated that, the mixed water is then allowed to settle and sieved its turbidity is extensively reduced including hardness reduction. *Moringa oleifera* is the most nutrient-rich plant yet discovered. This humble plant has been making strides in less-developed societies for thousands of years, and significant nutritional research has been conducted since the 1970s. Moringa provides a rich and rare combination of nutrients, amino acids, antioxidants, antiaging and anti-inflammatory properties used for nutrition and healing. Since 1998, the World Health

Organization has promoted Moringa as an alternative to imported food supplies to treat malnutrition (Waterman *et al.,* 2014).

A large number of reports on the nutritional qualities of Moringa now exist in both the scientific and the popular literature. Moringa has been in use since centuries for nutritional as well medicinal purposes. These include vitamin C, for colds and flu; vitamin A, against eye disease, skin disease, heart ailments, diarrhea, and many other diseases; Calcium, for strong bones and teeth and helps prevent osteoporosis; Potassium, for the functioning of the brain and nerves, and Proteins, the basic building blocks of all our body cells (Waterman *et al.*, 2014).

2.5.3.2*Moringa Leaves*

Moringa leaves contain all of the essential amino acids, which are the building blocks of proteins. It is very rare for a vegetable to contain all of these amino acids. And Moringa contains these amino acids in a good proportion, so they may serve as alternative to meat. Moringa even contains arginine and histidine two amino acids especially important for infants. Moringa could be an extremely valuable food source (William *et al.,* 1994; Makker and Becker, 1997; Foidi *et al.*, 2001; Bashir *et al.*, 2014; Razis *et al.,* 2014).

Given its nutritional value, it can be utilized in fortifying sauces, juices, spices, milk, bread, and most importantly, instant noodles. A comparative study of Moringa fresh leaves gram for gram with other foodstuffs puts Moringa on top (Table 2.2). It contains (seven times the vitamin C of oranges); (four times the vitamin A of carrots), (four times the calcium of milk), (three times the potassium of banana) and (two times the protein of yogurt). But the micro-nutrient content is even more in dried leaves; (ten times the vitamin A of carrots), (17 times the calcium of milk), (15 times the potassium of bananas), (25 times the iron of spinach) and (nine times the protein of yogurt). However, Vitamin C drops to half that of oranges Moringa contains a range of fairly

unique phytochemicals. containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates. Six such phytochemicals have been reported to have hypotensive, anticancer, and antibacterial activity include benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-{a-L-rhamnopyranosyloxy} benzyl glucosinolate (Dalla, 1993; William *et al.,* 1994; Price, 2000; Foidi *et al.,* 2001; Bashir *et al.,* 2014; Arnarsan, 2015).

Numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with Moringa or with phytochemicals isolated. Moringa have shown antiulcer effect, effect on immune response, spasmolytic activities, hypocholesterolemic effects, antibacterial activity, sympatholytic activity and antiviral activity against herpes simplex virus type-1. (Guevara *et al.,* 1999; Nambiar *et al.,* 2010; Gupta *et al.,* 2013; Sadek, 2013)

Table 2.2: Nutritional value of Moringa compared with other food

Content	***Moringa***	***other food***
Vitamin A	6,780 mg	caroot: 1,890 mg
Vitamin C	220 mg	Orange: 30 mg
Calcium	440 mg	cow milk: 120 mg
Potassium	259 mg	Banana: 88 mg
Protein	6. 6 g	Cow milk: 3.2 g

Source; Shankar *et al.* (2007)

2.5.3.3*Moringa Seed*

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. Different report suggests that the extracts of *Moringa oleifera* seed, both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage (Saleem, 1995; Bennett *et al.,* 2003).

The viability and functionality of a cell partly depends on a favorable redox state (ROS), i.e., on its ability to prevent excessive oxidation of its macromolecules, including DNA, proteins, and lipids (Ryter *et al.*,2007; Limon-Pacheco and Gonsebatt, 2009). ROS and free radicals are the

major mediators of the oxidative process. Cellular inability to reduce ROS leads to oxidative stress.

All cells are variably capable of endogenous self-protection against this stress through the actions of enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, as well as through reducing molecules such as glutathione. Nutritional antioxidants such as vitamins A, C, and E provide additional protection from the stress (Limon-Pacheco and Gonsebatt,2009). Oxidative stress is widely accepted as a major contributing factor in the pathogenesis of CVD and diabetes (Dhalla *et al.,* 2000; Kaneto *et al.,* 2007; Rodrigo *et al.,* 2011). The therapeutic actions of *M. oleifera* medication has been linked to the relatively high antioxidant activity of its leaves, flowers, and seeds (Chumark *et al.,* 2008; Sreelatha and Padma, 2009; Verma *et al.,* 2009; Atawodi *et al.,* 2010). Among the major classes of phytochemicals found in the plant, flavonoids appear to carry most of this activity. Anti-inflammatory properties inflammation with its wide array of cytokines secreted by immune cells is an integral part of the pathophysiology of obesity, hypertension, atherosclerosis, and diabetes (Rana *et al.*,2007). Extracts from *M. oleifera* leaves have been shown to modulate humoral and cellular immunity in rats and mice (Gupta *et al.,* 2010; Sudha *et al.*,2010). They have exhibited strong anti-inflammatory properties in rodent models of chemically induced inflammation of the paw (Sulaiman *et al.,* 2008; Mahajan and Mehta, 2009). These properties have been more extensively studied with fruit and seed extracts (Cheenpracha *et al.,* 2010; Mahajan and Mehta, 2010; Muangnoi *et al.,* 2011). They may also contribute to the observed anti-atherogenic and anti-diabetic effects of *M. oleifera* therapy.

Medicinal interest in *M. oleifera* are three structural classes of phytochemicals: glucosinolates, flavonoids, and phenolic acids (Saleem, 1995; Bennett *et al.,* 2003; Lako *et al.,* 2007; Manguro and Lemmen, 2007; Coppin, 2008; Amaglo *et al.,* 2010; Kasolo *et al.,* 2010). Their content in *M.*

oleifera leaves varies somewhat with the geographic and climatic conditions under which the plant was grown, as well as with the processing methods for the collected leaves (Bennett *et al.,* 2003; Coppin, 2008; Mukunzi *et al.,* 2011). Glucosinolates are characterized by β-thioglucoside N-hydroxy sulfate motif. In *M. oleifera* leaves, most phytochemicals of this class carry a benzyl-glycoside group linked to the single carbon of the motif. The most abundant of the mis 4-O-(α-l-rhamno pyranosyl-oxy)-benzyl glucosinolate, otherwise known as glucomoringin (Amaglo *et al.,* 2010). Enzymatic hydrolysis of the glucosinolate motif of members of this class leads to the formation of corresponding isothiocyanates, thiocyanates, or nitriles. Several of these by-products have been shown to possess antihypertensive properties (Faizi *et al.*,1992,1994,1998). Flavonoids and phenolic acids are collectively referred to phenolic compounds. The structural skeleton of flavonoids is made of two aromatic rings joined by a three-carbon link; that of the sub-class of flavonols is 3-hydroxy-2-phenylchromen-4-one. Quercetin and kaempferol, in their as 3-O-glycoside forms, are the predominant flavonols in *M. oleifera* leaves. The sugar moieties include, among others, rhamnogly- cosyl (rutinosides), glucosyl (glucosides),6_ malonyglucosyl, and 2-galloylrutinoside groups (Bennett *et al.*,2003; Manguro and Lemmen, 2007; Amaglo *et al.,* 2010). Biologically, flavonoids are best known for their antioxidant properties, but their metabolic pathways of activity remain to be fully elucidated (Rice-Evans, 2001). Phenolic acids have benzoic acid and cinnamic acid as backbones, with one or several hydroxyl groups. Chlorogenic acid, which is a nester of dihydrocinnamic acid (caffeic acid) and quinic acid, is a major phenolic acid in *M. oleifera* leaves (Bennett *et al.,* 2003; Amaglo *et al.,* 2010).

2.5.4Tamarind

2.5.4.1*Tamarind Tree*

Tamarind (*Tamarindus indica*) is a leguminous tree in the family *Fabaceae* indigenous to tropical Africa. The genus *Tamarindus* is a monotypic taxon, having only a single species (Diallo*et al.,* 2007). The tamarind tree produces pod-like fruit, which contain an edible pulp that is used in cuisines around the world. Other uses of the pulp include traditional medicine and metal polish. *Tamarindus indica* is probably indigenous to tropical Africa, (Diallo*et al.,* 2007) but has been cultivated for so long on the Indian subcontinent that it is sometimes reported to be indigenous there (Abukakar*et al.,* 2008), where it is known as *imli* in Hindi-Urdu (Raghavan, 2006) and *Samia* in Hausa. It grows wild in Africa in locales as diverse as Sudan, Cameroon, Nigeria, and Tanzania. It reached South Asia likely through human transportation and cultivation several thousand years BC (Popenoe, 1974; Morton, 1987). The fruit pulp is edible. The hard, green pulp of a young fruit is considered by many to be too sour, but is often used as a component of savory dishes, as a pickling agent or as a means of making certain poisonous yams in Ghana safe for human consumption (Portman, 2013)

2.5.4.2*Tamarind Fruit*

The ripened fruit is considered the more palatable, as it becomes sweeter and less sour (acidic) as it matures. It is used in desserts, as a jam, blended into juices, or sweetened drinks (Portman, 2013) sorbets, ice creams and other snacks. In Western cuisine, it is found in Worcestershire Sauce (Portman, 2013). In most parts of India, tamarind extract is used to flavor foods, in curries and traditional dishes (Doughari, 2006), and tamarind sweet chutney is popular in India and Pakistan (Doughari, 2006) as a dressing for many snacks. Tamarind pulp is a key ingredient in flavoring curries and rice in south Indian cuisine, as well as in the Chigali lollipop. Across the

Middle East, tamarind is used in savory dishes, notably meat-based stews, and often combined with dried fruits to achieve a sweet-sour tang (Nathan, 2004). Throughout Southeast Asia, the fruit of the tamarind is used as a poultice applied to foreheads of fever sufferers (Doughari, 2006). The fruit exhibits laxative effects due to its high quantities of malic acid, tartaric acid, and potassium bitartrate. Its use for the relief of constipation has been documented throughout the world (Panthong*et al.,* 2008;Havinga*et al.,* 2010). Seeds can be scarified or briefly boiled to enhance germination. They retain their germination capability for several months if kept dry (Havinga*et al.,* 2010).

In hens, tamarind has been found to lower cholesterol in their serum, and in the yolks of the eggs they laid and has been recommended for the treatment of hypercholesterolemia or diabetes (Chowdhury*et al.,* 2005; Salma*et al.,*2007). Tamarind fruit pulp is found in the pea-like pods (up to 15cm long by 2.5cm across) surrounding one to twelve seeds. It is highly acid, contains 30 – 40% sugar and has high vitamin C and excellent keeping qualities. It is used extensively in Indian cooking to enrich the taste of savoury dishes (especially with meat). It is a popular ingredient in curries, chutneys, preserves and refreshing drinks. The acidic pulp is also used to clean silverware.

Tamarind fruit pulp has a sweet acidic taste due to a combination of high contents of tartaric acid and reducing sugars. The pulp is used for seasoning, in prepared foods, to flavour confections, curries and sauces, and as a major ingredient in juices and other beverages. Vitamin B content is quite high; carotene and vitamin C contents are low. Presence of tannins and other dyeing matters in the seed testa make the whole seed unsuitable for consumption, but they become edible after soaking and boiling in water (Salma*et al.,* 2007). Tamarind kernel powder is an important sizing material in textile, paper and jute industries. Seeds are gaining importance as an

alternative source of proteins, and are besides rich in some essential minerals. Seed pectin can form gels over a wide pH range. Leaves and flowers can be eaten as vegetables, and are prepared in a variety of dishes. They are used to make curries, salads, stews and soups. Tamarind leaves are a fair source of vitamin C and β-carotene; mineral content is high, particularly P, K, Ca and Mg. Anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal activity has been documented from several plant parts. Tamarind is also extensively used in traditional medicine (Salma*et al.*, 2007).

2.6Whey

Whey proteins are unique as they contain all the essential amino acids of good quality protein. The nutrient composition of whey is shown in Table 2.3. Milk whey and whey proteins have different biological and functional properties. Bioactive peptides from dairy sources are majorly classified on the basis of their biological roles as anti-hypertensive, anti-oxidative, immmuno-modulant, anti-mutagenic, anti-microbial, opioid, anti-thrombotic, anti-obesity and mineral-binding agent (Yalcin, 2006). Furthermore, it is also associated with lower risks of hypertension, coagulopathy, stroke and cancer insurgences (Sultan *et al*., 2014). The major components of milk whey of nutraceutical potential include beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin (BSA), lactoferrin (Lf), immunoglobulins (Igs), lactoperoxidase (Lp) enzymes, glycomacropeptides (GMP), lactose and minerals. The composition of the whey depends upon the source of milk; whey derived from buttermilk contains more lipid sphingomyelin as compared to those derived from cheese. Whey is a popular dietary protein supplement that is well known to possess antimicrobial activity, immune modulation, improved muscle strength and body composition. It can prevent cardiovascular disease and osteoporosis. The important whey components that provide antimicrobial action in the intestinal tract are the immunoglobulins like

IgG, IgM and IgA. IgG binds to toxin produced by *Clostridium difficile*, thereby reducing diarrhoea, dehydration and muscle aches. Lactoferrin, an iron binding protein present in milk whey, possesses antibacterial properties. It sequesters iron from bacteria (Troost *et al*., 2001). Since pathogens in particular have high iron requirements for metabolism and growth, this property of lactoferrin makes it broadly antimicrobial in nature. Lactobacilli can utilize lactoferrin-bound iron, thus allowing lactoferrin to both inhibit pathogenic bacteria and support the growth of lactobacilli. Moreover, lactoferrin possess important antiviral activity asit inhibits virus replication and their ability to attach to colonic epithelial cells. Another whey-derived important protein component is lactoperoxidase (Lp) with potent antimicrobial properties. It catalyzes the oxidation of thiocyanate into hypothiocynate ion, which is a strong oxidizing agent. Hypothiocynate ions disrupts bacterial cell membranes. Whey-derived phospholipids such as sphingolipids are metabolized in the gastrointestinal (GI) tract and produce sphingosine and lysosphingomyelin. These compounds possess powerful bactericidal agents *in vitro*.

The lactoferrin and lactoferricin produced from whey exhibit inhibitory activity against a broad range of microorganisms including Gram-negative and, Gram-positive bacteria, yeast, fungi and parasitic protozoa (Takakura *et al*., 2003). They also inhibit the growth of food-borne pathogens such as *E. coli* and *Listeria monocytogenes* (Floris *et al*., 2003). They exhibit antiviral property

Table 2.3: Nutrient content and characteristics of cheese whey types (dry matter basis).

Characteristics	Sweet Whey	Whey Permeate
Total nitrogen (TN)	1.30	0.26
Non-protein nitrogen (NPN)	0.34	0.24
Calcium	0.058	0.055
Phosphorus	0.052	0.045

Net energy lactation (Mcal/lb)	0.90	0.85
Non-protein nitrogen (NPN)	0.34	0.24
Total digestible energy (Mcal/lb)	1.86	1.7
Specific gravity (kg/L)	1.025	1.030
pH	6.40	6.55
Titrable acidity	0.05	0.089
Water (%)	91.95	94.45
Dry matter (DM%)	8.05	5.55
- Solid Not fat (SNF%)	7.55	5.55
- Fat (%)	0.50	0.00
Crude protein (CP%)	1.10	0.25
Soluble carbohydrates (%)	5.20	4.90
Total ash (%)	0.52	0.50

Source: Gupta and Prakash (2017).

against HIV, Human Cytomegalovirus (HCMV), herpes viruses, Human Papilloma Virus (HPV), alpha-virus and hepatitis C, B and G viruses. Overall, whey proteins activate immune cell and/or prevent infection (Wolber *et al*., 2005). Whey protein concentrate supplementation can thus decrease the occurrence of xerostimia (dry mouth) and associated co-infections (Solak and Akin, 2012). Several scientific studies on animal experiments have shown the therapeutic effect of bovine lactoferrin (BLF) in treating distinct types of cancer (Gill and Cross, 2000), including

colon cancer due to its iron-binding capacity (Masuda *et al*., 2000). Whey protein increases the level of glutathione in the body and helps to maintain a healthy immune system (Gupta *et al*., 2012). Numerous studies have proved that whey proteins help in weight management and obesity control through the influence on the hormones that controls appetite and hunger (Hall *et al*., 2003), lactalbumin enhances the lipid oxidation and rapidly delivers amino acids for use during exercise, thereby decreasing adiposity (Bouthegourd *et al*., 2002). Whey proteins are a rich source of essential amino acids that contributes to its anabolic properties. There is an increase in availability of postprandial plasma amino acids that stimulates synthesis of muscle proteins (Pennings *et al*., 2011). Whey proteins comprises of good quality proteins and are therefore often recommended by physicians after any surgery or burn therapy (Gupta *et al*., 2012).

Beta-lactoglobulin comprises approximately half of the total protein content in bovine whey, while it is absent in human milk. It is a source of both essential and branched chain amino acids. A retinol binding protein (a carrier of small hydrophobic molecules including retinoic acid) is present within the beta-lactoglobulin structure. This protein, has an ability to modulate lymphatic responses (Yolken *et al*., 1985; Gupta *et al*., 2012). It also has the property to bind to hydrophobic ligands like fatty acids. Le Maux *et al*. (2012) demonstrated that beta-lactoglobulin acts as a carrier molecule that alters the bio-accessibility of linoleate and linoleic acid. It also provides resistance against gastric and simulated duodenal digestions. It also serves as a potential carrier for delivering gastric labile hydrophobic drugs. Alpha-lactalbumin is reported to be the second most important protein in whey quantitatively and it represents approximately 20% (w/w) of the total whey protein. It is completely synthesized in the mammary gland.

Bovine serum albumin (BSA) is not produced in the mammary gland, but is secreted in milk after its passive leakage from the blood stream. The most important property of bovine serum

albumin is its ability to bind to various ligands reversibly. It is the principal carrier of fatty acids and can bind to free fatty acids and other lipids as well as flavoring compounds (Huang *et al*., 2004). In addition, BSA possess antioxidant activities (Tong *et al*., 2000). BSA is a source of all major essential amino acids. Lactoferrin is an iron-binding glycoprotein that is present in whey fraction of milk and colostrum. It is a non-enzymatic antioxidant and consists of approximately 689 amino acid residues (Pierce *et al*., 1991). The concentration of lactoferrin in human milk and colostrum is reported to be about 2 mg/ml and 7 mg/ml, respectively, whereas in bovine milk and colostrum it is around 0.2 mg/ml and 1.5 mg/ml, respectively (Levay *et al*., 1995). Lactoferrin is a pre-dominant component of whey protein in human breast milk. Lactoferrin is also known to be an important host defense molecule and performs a range of physiological functions such as antimicrobial, antiviral, immuno-modulatory and antioxidant activity, anti-infective, anti-cancer and anti-inflammatory effects. Thus, lactoferrin possess the potential to be used as a food additive (El-Loly and Mahfouz, 2011).

Immunoglobulins (Ig) are antibodies and chemically gamma-globulins. Milk whey contains many types of enzymes, such as lactoperoxidase, hydrolases, transferases, lyases, proteases and lipases. Lactoperoxidase (LPO) accounts for 0.25–0.5 percent of total protein found in whey. Lactoperoxidase is the most abundant enzyme in whey. It has the property to catalyze certain molecules, and reduction of hydrogen peroxide. This enzyme system catalyzes peroxidation of thiocyanate and some halides (such as iodine and bromium), which ultimately generates products that inhibit and/or kill a range of bacterial species. It is heat tolerant and therefore lactoperoxidase is not inactivated during the pasteurization process, thereby suggesting its stability as a preservative. The biological significance of this enzyme is that it has a natural protection system against the invasion of microorganisms. Besides its antiviral effect, it protects

animal cells against various damages and peroxidative effects (de Wit and Van Hooydonk, 1996). The LPO enzyme functions as a non-immune biological defense system of mammals and catalyzes the oxidation of the thiocyanate ion into the antibacterial hypothiocyanate (Reiter and Perraudin, 1991).

Glycomacropeptides are also known as Casein Macro-Peptide (CMP) and constitutes about 10–15% of protein in whey. It is produced in whey due to the breakdown of casein by chymosin enzyme during cheese production. It contains large numbers of branched chain amino acids; but lacks the aromatic amino acids such as phenylalanine, tryptophan and tyrosine. The other advantage is that it can be safely administered to patients suffering with phenylketonuria (PKU) as it lacks phenylalanine (Marshall, 2004). Lysozyme is a hydrolytic enzyme that is widely distributed in nature and occurs in many body fluids and tissues of living organisms (Fox and Kelly, 2006). Although the highest concentration of the enzyme is found in tears and egg white protein, it is also present in milk whey. It exhibits antibacterial activity only against gram-positive bacteria. It is also used in the treatment of bacterial and viral infections, skin and eye diseases, periodontitis, leukemia and cancer (Lesnierowsk, 2009; Benkerroum, 2008).

2.7Baobab

2.7.1*Baobab Tree*

Baobab (*Adansonia digitata*) occurs naturally in most countries of Sahara as a scattered tree in the savannah, and is also present in human habitation. The various parts of the plant (leaves, bark and seeds) are used as a panacea, that is, to treat almost any disease and specific documented

uses include the treatment of malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery, toothache, etc. (Table 2.4) (Watt and Breyer-Brandwijk, 1962; Brendler *et al.,* 2003). The leaves and fruit pulp are used as febrifuge as well as an immune stimulant (Nguta *et al.,* 2010). In India, it is reported that baobab pulp is used externally with buttermilk for the relief of diarrhea and dysentery, while the young leaves are crushed and used to treat painful swellings (Sidibé *et al.,* 2002).Baobab plant is traditionally used to treat conditions like insect bites, Guinea worm and internal pains, dysentery, diseases of the urinary tract, opthalmia and otitis in some parts of Africa (Sidibé and Williams, 2002; Abiona *et al.*, 2015). It is a rich source of amino acids and minerals especially iron and calcium (Lockett and Grivetti,2000; Boukari *et al.*, 2001; Sidibé and Williams, 2002; Abiona *et al.*, 2015).

2.7.2*Baobab Fruit*

In some countries in West Africa, the leaves, fruit pulp and seeds are the main ingredients in sauces, porridges and beverages (El-Rawy *et al.*, 1997; Chadare *et al.*, 2009; Nguta *et al.*, 2010). Recently, baobab has been referred to as a "super fruit" based on its nutritional profile (e.g., vitamin, fatty acid, mineral) (Yusha'u *et al.,* 2010). The energy value of pulp is similar to that of baobab leaves (Becker, 1983; FAO, 1988; Yusha'u *et al.*, 2010). The major interest in baobab products is as a result of its ascorbic acid and dietary fiber content. The level of vitamin C contained in fruit pulp is high and can range from 2.8 to 3 g/kg (Gruenwald, 2009). It was noted

Table 2.4: Analysis of baobab fruit pulp (mg/100 g).

Constituent	**Content (mg/100g)**
Ascorbic acid (vitamin C)	280.00-300.00**
Calcium	293.00**

Phosphorus	96.00-118.00
Carbohydrates	75.60
Soluble and insoluble dietary fibers	52.00
Potassium	2.31
Protein	2.30
Lipids	0.27

**: Ascorbic acid compared with oranges of 51 mg/100 g and calcium compared with milk of 125 mg/100 g.
Source: Manfredini *et al.* (2002)

that baobab fruit pulp has very high vitamin C content (280–300 mg/100 g), which is seven to ten times more than oranges (51 mg/100 g) (Manfredini *et al.*, 2002; Vertuani *et al.*, 2002). One study demonstrated that the consumption of 40 g of baobab pulp provided 100% of the recommended daily intake of vitamin C in pregnant women (19–30 years) (El-Rawy *et al.*, 1997). The ascorbic acid content was evaluated in the fruit of *A. digitata* (Täufel *et al.*, 1993);

and it was found tocontain 337 mg/100 g of ascorbic acid (Diop *et al.,* 1988; Eromosele *et al.,* 1991). Sidibé and Williams recommended that baobab leaves should be stored as whole leaves rather than ground leaf powder in order to preserve the high vitamin content (Sidibé *et al.,* 2002).

Although *A. digitata* is mostly regarded as a fruit-bearing forest tree, it is a multipurpose and widely used species with medicinal properties, numerous food uses of various plant parts, and bark fibers that are used for a variety of purposes.

Centuries ago, the products were traded: it was well known in Cairo markets in the sixteenth century (Sidibé *et al.,* 2002). There is no doubt that baobab pulp is a valuable source of vitamin C. Bioavailability studies are necessary for a better appreciation of the contribution to human health since the dietary intake can never be fully utilized by the human body (Gebauer *et al.,* 2002). The fruit pulp has very high vitamin C content, almost ten times that of oranges (Table 4). It contains sugars but no starch and is rich in pectin. It can bedissolved in water or milk. The liquid is then used as a drink and sauce for food, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking.

Baobab plant parts have been used for centuries to treat microbial infections both in humans and animals and many scientific studies have been carried out in order to validate its traditional antimicrobial uses. Kubmarawa *et al.* (2007) found that *A. digitata* extract was not active against *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. This was in contrast to a study carried out by Yagoub (2008) who investigated the antibacterial activity of a solvent extract of *A. digitata* against *E. coli* isolated from urine and water. The results clearly indicated that the solvent extract inhibited bacterial growth with the inhibition zone ranging from 20 to 30 mm depending on the concentration at which the sample was tested. In a recent study, Masola *et al.* (2009) investigated the antimicrobial activity of

baobab plant parts (stem and root barks) against Gram-positive bacteria, Gram-negative bacteria and yeast. The results indicated that the aqueous and ethanolic root and stem bark extracts inhibited the growth of various micro-organisms with the Microbial Inhibition Count values ranging from 1.5 to 6 mg/ml. The antibacterial activity of the plant could be attributed to the presence of tannins, phlobatannins, terpenoids and saponins in the stem bark (Masola *et al.*, 2009).

The anti-oxidant activity of fresh ripe fruit of *A. digitata* was 1000 mg AEAC/100 g (ascorbic acid equivalent anti-oxidant content) (Lamien-Meda *et al.*, 2008). In a separate investigation, Nhukarume *et al.* (2008) showed that phenolic content of baobab fruit pulp (≈ 52 mg/100 ml Gallic Acid Equivalent) was not statistically different from that of *Citrus sinensis* Pers, suggesting the use of baobab fruit pulp as an anti-oxidant botanical dietary supplement. Brady (2011) found that the anti-oxidant capacity of the hydrophilic extract of fruit pulp was higher (7.65 mg ascorbic acid equivalent/g) compared to the lipophilic extract of fruit pulp (3.32 mg ascorbic acid equivalent/g). Carlsen *et al.* (2010) summarised the anti-oxidant activity of various fruits and vegetables. The anti-oxidant activity of the baobab varies according to the plant part used. The anti-oxidant content of dried leaves was 48.1 mmol/100 g which was almost five times higher than that of fruit pulp. The anti-oxidant content of fruit or dried leaves was higher than that of orange (0.9 mmol/100 g); mango (1.7 mmol/100 g); apple (3.8 mmol/100 g) and papaya (0.6 mmol/100 g). Nhukarume *et al.* (2008) investigated the ability of solvent extracts of various fruits including baobab to inhibit the peroxidation of lipids. The results showed a significant difference in the activity of the beverage extracts with *A. digitata* having the greatest activity. The order of activity was *A. digitata*> ascorbic acid >*C. sinensis* Pers. (orange) >*Strychnos spinosa* Lam. >*Parinari curatellifolia* Planch. ex Benth. > baobab nectar. Besco *et al.* (2007)

evaluated the anti-oxidant activity of baobab red fibre. The lipid soluble anti-oxidant capacity, the water-soluble anti-oxidant content and the ascorbic acid anti-oxidant capacity were determined. The results obtained demonstrated the lipid soluble anti-oxidant capacity of baobab red fibre was high (508.0 ± 0.008 μmol/g, Trolox® equivalent). Similarly, the water-soluble anti-oxidant capacity, corresponding to the activity expressed as μmol/g equivalents of ascorbic acid for each gram showed the same pattern with the ascorbic acid equivalents of baobab red fibre particularly high (386.0 μmol/g). The same study also showed that the integral anti-oxidant capacity (sum of the anti-oxidant capacity of hydrophilic and lipophilic anti-oxidants) of baobab red fibre (1617.3 μmol/g) was 66 times higher than that of orange pulp (24.3 μmol/g) (Besco *et al.,* 2007).

Several *in vitro* and *in vivo* studies have been carried out to determine the antiviral activity of various baobab plant parts. Ananil *et al.* (2000) investigated the antiviral activity of several plants against the herpes simplex, sindbis and polio viruses. The baobab extract from leaves was found to have the most potent effect. All the extracts from any part of Baobab contained antiviral photosensitisers (Hudson *et al.,* 2000; Vimalanathan and Hudson, 2009).

Table 2.5: Traditional medicinal uses of *A. digitata* in different types of aliments.

Aliments	Parts used	Preparation	References
Diarrhoea, fever, inflammation, kidney and bladder diseases,	Leaves	Decoction, infusion	(Brendler *et al.,* 2003)

blood clearing, asthma			
Malaria, fever	Leaves	Mixed with water	(Watt, MG Breyer-Brandwijk, 1962)
Toothache, gingivitis	Leaves	Decoction	(Tapsoba *et al.*, 2006)
Diaphoretic, fever remedy	Leaves	Decoction	(Odetokun, 1996)
Diaphoretic, kidney and bladder diseases, asthma, insect bites	Leaves	Decoction	(Perrier de la Bâthie, 1952)
Fever, diarrhea	Seeds	Mixed with water	(Watt, MG Breyer-Brandwijk, 1962)
Microbial diseases	Fruits	Decoction	(Abbiw, 1990)
Anaemia	Bark	Aqueous extract	(Wickens, 1982)
Coughs	Powdered seeds	Decoction	(Watt, MG Breyer-Brandwijk, 1962)
Wound healing	Stem bark	Decoction	(Perrier de la Bâthie, 1952)

Source: Rahul*et al.* (2015)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Site of Experiment

This study was conducted at the Animal Product laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State. Zaria is located at Latitude $11^{0}12^{'}$N, Longitude $7^{0}33^{'}$ E and Altitude of 610m above sea level (Metrological Unit, Institute of Agricultural Research, Ahmadu Bello University, 2016) within the Northern Guinea Savanna, the experiment was conducted in the month of May with the following weather condition;Temperature was 32^{o}C, humidity was 56%, Barometer was 1005mbar and wind was 13km/h in SSE direction. The microbial analysis was conducted at the microbiology laboratory, Faculty of Veterinary Medicine while the proximate composition and other analyses was conducted at the Department of Animal Science of the same institution.

3.2 Experimental Design

3.2.1*Experimental I*

Experiment I was a 3 x 3 factorial arrangement with control (with 3 replicates) in a completely randomized design. Each treatment was prepared with 3 unique plant rennet. A total of two litres of milk was used for each replicate. Each Treatment was stored for a duration of 7 days with 3 distinct storage period.

3.2.2*Experimental II*

Experiment II was a 3 x 2 factorial arrangement (with 3 replicates) in a completely randomized design. Each treatment was stored in the whey and Baobab solution giving a total of three replicates in each storage medium. Commercial Mozzarella Cheese was purchased to serve as the control.

3.3Treatments and Coagulants

Yoghurt of 50ml + 30ml lime juice added to each replicate

Treatment one (control) was the commercial Mozzarella

Treatment two contained 120ml Sodom Apple solution per replicates

Treatment three contained 120ml Tamarind solution per replicates

Treatment four contained 120ml Moringa seed solution per replicates.

3.4SAMPLE COLLECTION

3.4.1 *Milk Procurement*

Total of 36 litres of milk was purchased from open market in Samaru, Zaria very early in the morning. The milk was transported to the Department of Animal Science which is the site of the experiment. The milk was divided into three treatments with different coagulants, stored in two storage media (whey and baobab pulp extract) with three replicates.

3.4.2 *Lime Juice Procurement* (*Citrus aurantiifolia*)

Lime fruit was purchased from the open market in Samaru, Zaria a day to the experiment. Twenty lime fruits were rinsed with distilled water, squeezed and sieved with cheese cloth to obtain 800ml of lime juice.

3.4.3 *Sodom apple Leaves and Stem Procurement (Calotropis procera)*

The succulent leaves and the stems of *Calotropis procera* plant were harvested around Samaru, Zaria. After harvesting, the leaves and stems were washed with distilled water, weighed and sliced into smaller sizes. The mixture was then ground to homogenized mixture. 100 g of the mixture was soaked in 1L of water for 1hr. The homogenized plant materials werefiltered and the filtrate used as Sodom apple extract(Chikpah *et al*., 2014).

3.4.4 *Moringa Seeds Procurement* (*Moringa oleifera*)

Moringa seed was purchased from the open market in Samaru, Zaria a day to the experiment. 150 g of dry *Moringa oleifera* seeds was ground and soaked in 1 L of distilled water for 2 h as described by Orhevba and Taiwo (2016). The resulting solution was allowed to settle for an hour which was then filtered, and the filtrate was taken as the Moringa seed coagulant.

3.4.5 *Tamarind pulp procurement* (*Tamarindus indica*)

Tamarind fruit was harvested from the main campus, Ahmadu Bello University Zaria a day to the experiment. 5 tamarind fruit was rinsed with distilled water and then soaked in 1 L of distilled water and was left for 5 h. The solution was then filtered, and the filtrate taken as Tamarind pulp coagulant.

3.4.6 *Baobab pulp procurement* (*Adansonia digitata*)

Baobab fruit was harvested from the main campus, Ahmadu Bello University Zaria a day to the experiment. 10 baobab fruit was rinsed with distilled water and then soaked in 1 L of distilled water and was left for 5 h. The solution was then filtered, and the filtrate taken as Baobab pulp extract.

3.5 Cheese Production

The recipe wass adapted from McDaniel and Kendall (2004). The cheese was produced and stored for duration of 7 days and analyses carried out at a 3-day interval.

3.5.1 *Ingredients*

Two (2) L of milk,NAPRI yoghurt; Rennet mixture (Lime + Moringa seed, Lime + Tamarind pulp or Lime + Sodom apple); Table Salt.

3.5.2 *Procedure*

The yoghurt was added to the milk and heated to 90°F (32°C). The mixture was stirred slowly for 15 min while maintaining the constant temperature. Rennet mixture was added and stirred for 5 min at 1-2% of the milk volume (Omotosho *et al*., 2011). The pot was covered so as to maintain a temperature at 90°F (32°C) and allowed standing until coagulated, for about 30 min. The curd was sliced cut into cubes. The temperature was slowly increased to 118°F (48°C) over a period of 45 min and maintained for another 15 minutes (Total time is 1 h). The curd was allowed to settle in whey. The curd was removed and then put in hot water 180°F (82°C). Using wooden spoons, the cheese cut was tumbled and stretched under hot water for 15 min. The curd was removed from hot water and shaped by hand; salt (10g) was added to develop the taste. The cheese was then put in storage medium.

3.6 Cheese Storage

The cheese was stored in well labeled plastic containers which was bought from the market; the storage media (whey or baobab solution) was also added. The containers were covered and kept in a cool dry place at the room temperature (32^0C). The cheese taken from the containers for each analysis with one sample per replicate.

3.7 Parameters Measured

3.7.1 *Proximate analysis*

Total Solids Content (Dry Matter), Protein Content, Ash Content, Fat Content and Moisture Contentwas determined according to the method of AOAC (2005).

3.7.2 *Microbiological Analyses*:

3.7.2.1 Microbiological Count

The cheese slices were subjected to counts of yeasts, molds and bacteria duringthe storage time. The count was done using the standard plate count procedure using a set of serial dilution by plating the sample in agar media on a petri dish. The agar solidifies and the colonies grown within the agar before the count was done. 36 plates were bought for the serial dilution; 0.5ml of the dissolved cheese added to a 4.5ml saline solution in a test tube followed by 1.0ml of the diluted solution in a 9.0ml saline solution. 0.1ml of the second solution which was later plated in a culture media of nutrient agar. The cultures were left for 12 h after which the total microbial count was done. Total viable count was done using Bacteria Nutrient Agar (EDQM, 2007) while total fungi count was done using Potato Dextrose Agar (AOAC, 2005)

3.7.2.2 Microbial Characterization

The following methods were employed for the microbial characterization in sequencial order; Morphological Characterization or Gram staining reaction (Larry and Kandel, 1996), Durham Tube Sugar Fermentations: Dextrose, Lactose, Sucrose (Prescott *et al.* 2002), Indole Test: Tryptophan Hydrolysis, Methyl Red Testand Voges Proskauer Test (Cheesbrough, 2000), Simmon's Citrate test (Prescott *et al*. 2002) Catalase Test (Cheesbrough, 2000) Oxidase Test (Prescott *et al.* 2002) Urease Test (Cheesbrough, 2000), followed by Triple Sugar Iron Agar test and Coagulase test(Stuart, 2005).

3.7.3 *Organoleptic Assessment*

The products were assessed against commercial Mozzarella for flavour and aroma, taste, texture, saltiness, colour and overall acceptability with questionnaires on a 9-point Hedonic scale (9, like extremely; 8, like very much; 7, like moderately; 6, like slightly; 5, neither like nor dislike; 4,

dislike slightly; 3, dislike moderately; 2, dislike very much; 1, dislike extremely) which involved a total of 30 trained respondents(Omotosho *et al*., 2011). Clean water, tooth-picks, detergent and serviette were provided for the entire panelist before the evaluation. A sachet of water for rinsing the mouth was also provided for each sample was given a code known only to the researcher to avoid bias.

3.7.4 *Economic and Marketing Analysis*:

3.7.4.1 Revenue to Cost Analysis

Cost, revenue and benefit- cost ratio was calculated for each treatment of fresh cheese. The benefit (revenue) of the project was measured from price and quantity. The benefit was based on the mean fair price suggested by the consumer while providing them with the reference price (Commercial Mozzarella), only the variable cost was considered as the cost of production. Budgeting technique adopted from Mafimisebi *et al.* (2013) was used to determine the gross margin. Profit (gross margin) is the excess of the expected income (benefit) over expenditure (cost), Profitability and efficiency ratios was calculated as folllows;

Profit =Total Benefit - Total Cost

Profitability ratio = Profit/Total Cost

Efficiency ratio = Total Benefit/Total Cost

The milk used was purchased at the rate of ₦400/litre, lime for ₦30/20 fruits, Sodom apple for ₦15/kg, Moringa for ₦115/kg, Tamarind for ₦65/kg and Mozzarella cheese for₦900/kg.

3.7.4.2 Price Formation Strategy

The prominent factors considered in discovering the prices of cheese by consumers was identified using a Price Formation Strategy Index (PFSI). The prominent factors suggested by

Mafimisebi *et al.* (2013) for dairy industry was utilized by administering questionnaire to 30 respondents. PFSI was conducted for only the freshly produced cheese. The degree of importance of each factor in the PFSI was expressed using a four-point scale with the scoring order 4, 3, 2, 1 for very important, important, slightly important and not important, respectively. The formula used to obtain the PFSI score was adopted from Mafimisebi *et al.* (2013). The PFSI used in rank order to reflect the relative position of each of the PFSI in terms of their importance

PFSI = N1X4 + N2X3 + N3X2 + N4X1

Where PFSI = price formation strategy index

N1 - number of respondents who ranked factor as very important (X4)

N2 - number of respondents who ranked factor as important (X3)

N3 - number of respondents who ranked factor as slightly important (X2)

N4 - number of respondents who ranked factor as not important (X1)

3.8 Statistical Analysis

All data generated were subjected to two-way ANOVA using the General Linear Model (GLM) procedure of SAS (2004) while the mean separation was done with Dunnett (Upton and Cook, 2006).

3.9 Experimental Model

3.9.1 *Experiment IModel*

$Y_{ijk} = a + T_i + Sj + (TxS)_{ij} + e_{ijk}$

Y_{ijk} = Response parameters of the Cheese,

a= population mean,

T_i = effect of the rennet (Control, Sodom apple, Moringa and Tamarind coagulated cheese)

S_j = effect of storage period (day 1, 4 and 7)

$(TxS)_{ij}$ = effect of interaction between plant rennet and storage period,

e_{ijk} = error term

3.9.2 *Experiment II Model*

$Y_{jkl} = a + S_j + M_k + (SxM)_{jk} + e_{jkl}$

Y_{ijk} = Response parameters of the Cheese,

S_j = effect of storage period (day 1, 4 and 7)

a= population mean,

M_k = effect of storage medium (Whey and Baobab pulp extract)

$(MxS)_{jk}$ = effect of interaction between plant rennet and storage medium,

e_{jkl} = error term

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Plant Rennet, Storage Period (Day) and their Interactions on Proximate Composition of Mozzarella Cheese Produced

Table 4.1 shows the Effect of Plant Rennet, Storage Day and their Interactions on Proximate Composition of Cheese Produced. There was significant difference ($P<0.05$) in all proximate composition parameters measured except for NFE of plant rennet. Dry matterof plant rennet coagulated cheese ranged from 38.43-46.99. Crude protein content of 20.94, 22.08,23.37 and 23.16% was reported for Control, Sodom Apple, Moringa and Tamarind coagulated cheese respectively. NFE ranged from 5.71 to 6.31 with Control having the highest.

There was significance difference ($P<0.05$) in all proximate parameters measured. Dry matter of storage day ranged from 39.67-42.65% across treatment. Crude protein content of 21.39, 21.63 and 24.77% was reported for storage day 1,2,3 respectively. NFE ranged from 7.58 to 4.69% with day 1 recording the highest. There was no significant($P>0.05$) in the interaction between plant rennet and day.

Table 4.1: Effects of Plant Rennet, Storage Period (Day) and their Interactions on Proximate Composition of Mozzarella Cheese Produced

Treatment	DM	CP	EE	Ash	NFE
Control	46.99[a]	20.94	14.62[a]	5.19[a]	6.31
SAC	41.08[b]	22.08	7.87[b]	5.73[a]	5.30
MC	39.53[bc]	23.37	6.54[c]	3.11[b]	6.41
TC	38.43[c]	23.16	6.61[c]	3.38[b]	5.71
SEM	0.79	0.83	0.44	0.41	0.71
LOS	*	*	*	*	NS
Day					
1	42.65[a]	21.39[b]	10.68[a]	2.89[c]	7.58[a]
4	39.84[b]	21.63[b]	7.00[b]	5.83[a]	5.36[b]
7	39.67[b]	24.77[a]	6.59[b]	3.98[b]	4.69[b]
SEM	1.06	1.10	0.58	0.54	0.95
LOS	*	*	*	*	*
Interaction					
P x D	0.054	0.337	0.001	0.001	0.004

[abc]means with different superscript along column differ significantly, DM = dry matter, CP = crude protein, EE = ether extract, NFE = nitrogen free extract, SEM = standard error of mean, LOS = level of significance, P = plant rennet, D = day, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese

4.2: Interaction Effect of Plant Rennet and Storage Period (Day) on Ether Extract,Ash and Nitrogen Free ExtractContent of Mozzarella Cheese Produced in Zaria

Table 4.2 shows the interaction Effect of Plant Rennet and Storage Days onEther Extract, Ash and Nitrogen Free Extractcontent of cheese produced. Significant ($P<0.05$) differences were observed in all the parameters recorded. Value range of 13.51 to 15.17% and 5.34 to 12.81% were observed in the interaction effect between control, Sodom apple and day. Moringa and Tamarind interaction with days ranged between 5.72 to 8.30% and 5.26 to 8.69%. Ash content of 4.85, 5.52 and 5.19% were recorded for interaction between control and day 1, 4 and 7. Value range of 3.66 to 9.78% and 1.84 to 3.80% were observed in the interaction effect between, Sodom, Moringa and day.Tamarind coagulated cheese recorded 2.09, 4.17 and 3.88 for days 1, 4 and 7 respectively.

Nitrogen Free Extract of 13.51 to 15.17% and 5.34 to 12.81% were observed in the interaction effect between control, Sodom apple and day. Moringa and Tamarind interaction with days ranged between 5.72 to 8.30% and 5.26 to 8.69%.Sodom apple coagulated cheese had the highest Ether extract content (12.81) among the plant rennet coagulated cheese while Moringa coagulated cheese had the highest Nitrogen Free extract content (11.76) among all the cheese on the day of production.

Table 4.2: Interaction Effect of Plant Rennet and Storage Period (Day) on Ether Extract, Ash and Nitrogen Free Extract Content of Mozzarella Cheese Produced

		Storage Period (Day)			
Parameter		1	4	7	SEM
Ether Extract	Control	15.17[a]	15.17[a]	13.51[a]	
	SAC	12.81[a]	5.34[c]	5.45[c]	
	MC	8.30[b]	5.72[c]	5.59[c]	0.44
	TC	8.69[b]	5.86[c]	5.26[c]	
Ash	Control	4.85[b]	5.52[b]	5.19[b]	
	SAC	3.76[bc]	9.78[a]	3.66[bc]	0.41
	MC	1.84[d]	3.69[b]	3.80[bc]	
	TC	2.09[c]	4.17[b]	3.88[bc]	
NFE	Control	6.64[b]	5.97[b]	6.31[b]	
	SAC	5.72[b]	4.80b[c]	5.37[b]	0.71
	MC	11.76[a]	4.42b[c]	3.04[c]	
	TC	5.74[b]	6.55[b]	*4.86[bc]*	

[abc]means with different superscript across parameters differ significantly, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese, NFE = Nitrogen Free Extract

4.3Effect of Storage Media, Storage Period (Day) and their Interactions on Proximate Composition of Mozzarella Cheese Produced

Effect of Storage, Day and their Interactions on Proximate Composition of Cheese Produced is presented in Table 4.3. There was no significant difference (P<0.05) in Storage Dry matter, ether extract, crude protein, Ash and NFE. DM and CP of 40.16 and 22.81 for whey and 39.20 and 22.94% for Baobab. EE of 7.49 and 6.52% were recorded for whey and Baobab. There were significant differences (P<0.05) in CP,EE, Ash and NFE for day of storage. Dry matter of storage day ranged from 39.67-42.65% across treatment. Crude protein content of 21.39, 21.63 and 24.77% were reported for storage day 1,4,7 respectively. NFE ranged from 7.58 to 4.69% with day 1 recording the highest. There was no significant(P>0.05) difference between the interaction between storage medium and day.

4.4: Interaction Effect of Storage Media and Storage Period (Day) on Crude Protein and Ether ExtractContent of Mozzarella Cheese Produced in Zaria

Table 4.4 shows the interaction effect of storage and day on crude protein and ether extract content of cheese produced. Significant (P<0.05) differences were observed in all the CP interaction parameters recorded. Crude protein value recorded for the interaction between Whey and day were 19.59, 23.40 and 25.43%. Conversely, CP contents of 23.41, 20.27 and 25.12% were observed for Baobab and day interaction. Day 7 had the highest crude protein for both whey and baobab pulp extract. Significant (P<0.05) differences were also observed in all the EE interaction parameters recorded. Ether extract value recorded for the interaction between Whey and day were 11.49, 5.84 and 5.13%. Conversely, Ether extract contents of 8.38, 5.45 and 5.73%

were observed for Baobab and day interaction. Day 7 had the highest CP and day 1 had the highest EE for both cheese storage media.

Table 4.3: Effect of StorageMedia, Storage Period (Day) and their Interactions on Proximate Composition of Mozzarella Cheese Produced in Zaria

Treatment	DM	CP	EE	Ash	NFE
Storage					
CW	40.16	22.81	7.49	4.32	5.44
CB	39.20	22.94	6.52	3.83	6.18
SEM	1.11	1.04	0.61	0.75	1.09
LOS	NS	NS	NS	NS	NS
Storage Period (Day)					
1	42.65[a]	21.39[b]	10.68[a]	2.89[b]	7.58
4	39.84[b]	21.63[b]	7.00[b]	5.83[a]	5.36
7	39.67[b]	24.77[a]	6.59[b]	3.98[b]	4.69
SEM	1.11	1.04	0.61	0.75	1.09
LOS	*	*	*	*	*
Interaction					
S x D	0.078	0.023	0.004	0.733	0.830

[abc]means with different superscript along column differ significantly, DM = dry matter, CP = crude protein, EE = ether extract, NFE = nitrogen free extract, SEM = standard error of mean, LOS = level of significance, S = storage, D = day, CB = Cheese stored in Baobab Pulp Extract, CW =

Cheese stored in Whey

Table 4.4: Interaction Effect of Storage Media and Storage Period (Day) on Crude Protein and Ether Extract Content of Mozzarella Cheese Produced in Zaria

		Storage Period (Day)			
	Storage	1	4	7	SEM
Crude Protein	CW	19.59^{bc}	23.40^{ab}	25.43^{a}	
	CB	23.41^{ab}	20.27^{bc}	25.12^{a}	1.04
Ether Extract	CW	11.49^{b}	5.84^{d}	5.13^{d}	
	CB	8.38^{c}	5.45^{d}	5.73^{d}	0.61

^{abc}means with different superscript across parameters differ significantly, CB = Cheese stored in Baobab Pulp Extract, CW = Cheese stored in Whey

4.5: Interaction effect of plant rennet and Storage Media on at different storage period onProximate Analysis (%) of MozzarellaCheese produced in Zaria

Table 4.5 shows the interaction effect of storage medium and plant rennet at different storage period. There was no significant interaction between the plant rennet, storage media and storage period.

4.6: Effect of Plant Rennet, Storage Period (Day) and their Interactions on Microbial Count of MozzarellaCheese Produced in Zaria

Effect of Plant Rennet, Day and their Interactions on Microbial Count of Cheese Produced is presented in Table 4.6. There was significant (P>0.05) difference on microbial count done on the cheese with the type of rennet used. Total viable bacteria (TVC) count was higher in Moringa (8.41 x 10^7cfu/g), follow by Tamarind (2.91 x 10^7 cfu/g) and lowest value observed in the control (0.00012 x 10^7cfu/g). Highest Total coliform count (TCC) was observed in Moringa (0.039 x 10^7) while control had the lowest value of 0.00032 x 10^7 cfu/g. Total Yeast count (TYC) ranged from 1.29 x 10^7 to 2.17 x 10^7 with Moringa recording the highest and control being the lowest. Value range of 00.6 x $10^7$to 67.6 x $10^7$was recorded for Total mould count.

Significant difference was not observed on microbial count done on storage period of the cheese. Total viable bacteria (TVC) count was higher in Day 7 (9.61 x 10^7cfu/g), follow by Day 1(0.2 x 10^7cfu/g) and lowest value observed on Day 4. Highest Total coliform count (TCC) was observed on day 7 (0.042 x 10^7) while Day 1 had the lowest value of 0.00032 x 10^7cfu/g. Total Yeast count (TYC) ranged from 0.50 x 10^7 to 19.3 x 10^7 with day 4 recording the highest and Day 1 the lowest. Value range of 0.000078 x $10^7$to 68.9 x 10^7 was recorded for Total mould count

(TMC). There was no significant (P>0.05) difference on the interaction between plant rennet and day on microbial count done on the cheese.

Table 4.5: Interaction effects of Plant Rennet and Storage mediumat different storage period on Proximate Composition of Mozzarella Cheese Produced

Treatment	DM	CP	EE	Ash	NFE
Rennet					
Control	46.99[a]	20.94	14.62[a]	5.19[a]	6.31
SAC	41.08[b]	22.08	7.87[b]	5.73[a]	5.30
MC	39.53[bc]	23.37	6.54[c]	3.11[b]	6.41
TC	38.43[c]	23.16	6.61[c]	3.38[b]	5.71
SEM	0.79	0.83	0.44	0.41	0.71
LOS	*	*	*	*	NS
Storage Period					
1	42.65[a]	21.39[b]	10.68[a]	2.89[c]	7.58[a]
4	39.84[b]	21.63[b]	7.00[b]	5.83[a]	5.36[b]
7	39.67[b]	24.77[a]	6.59[b]	3.98[b]	4.69[b]
SEM	1.06	1.10	0.58	0.54	0.95
LOS	*	*	*	*	*
Storage Medium					
CW	40.16	22.81	7.49	4.32	5.44
CB	39.20	22.94	6.52	3.83	6.18
SEM	1.11	1.04	0.61	0.75	1.09
LOS	NS	NS	NS	NS	NS
Interaction					
P x D	0.054	0.337	0.001	0.001	0.004
S x D	0.078	0.023	0.004	0.733	0.830
P x S	0.00	0.00	0.00	0.00	0.00
P x S x D	0.00	0.00	0.00	0.00	0.00

[abc]means with different superscript along column differ significantly, DM = dry matter, CP = crude protein, EE = ether extract, NFE = nitrogen free extract, SEM = standard error of mean, LOS = level of significance, CB = Cheese stored in Baobab Pulp Extract, CW = Cheese stored in Whey, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese, P = Plant rennet, D = storage period, S = storage mediu

Table 4.6: Effect of Plant Rennet, Storage Period (Day) and their Interactions on Microbial Count of Mozzarella Cheese Produced in Zaria (x 10^7)

Treatment	TVC	TYC	TMC
Control	0.00012	1.29	0.60
SAC	0.19	1.39	12.40
MC	8.41	2.17	1.90
TC	2.91	21.6	67.60
SEM	3.72	9.50	29.70
LOS	*	*	*
Storage Period (Day)			
1	0.20	0.50	0.000078
4	0.06	19.30	9.31
7	9.61	2.27	68.90
SEM	4.97	12.60	39.7
LOS	NS	NS	NS
Interaction			
P x D	0.000000066	0.000000066	0.000000058

[abc]means with different superscript along column differ significantly, SEM = standard error of mean, LOS = level of significance, P = plant rennet, D = day, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese, TVC = Total Viable Count, TMC = Total Mold Count, TCC = Total Coliform Count, TYC = Total Yeast Count

4.7: Effect of Storage Media, Storage Period (Day) and their Interactions on Total Microbial Count of MozzarellaCheese Produced in Zaria

Effect of storage, Day and their Interactions on Microbial Count of Cheese Produced is presented in Table 4.7. Storage medium and days of storage was not significant (P>0.05) on microbial count done on the cheese.Total viable bacteria (TVC) count of 5.23 x 10^7 and 2.44 x 10^7 were observed for whey and Baobab. Total Yeast count (TYC) ranged from 2.4 x $10^7$to 14.3 x $10^7$with Whey recording the highest. Total mould count values of 52.6 x $10^7$and 8.09 x 10^7 was recorded for Whey and Baobab respectively. Significant difference was not observed on microbial count done on stored cheese.Total viable bacteria (TVC) count was higher in Day 7 (9.61 x 10^7cfu/g), follow by Day 1(0.2 x 10^7cfu/g) and lowest value observed on Day 4. Total Yeast count (TYC) ranged from 0.50 x 10^7 to 19.30 x $10^7$with day 4 recording the highest and Day 1 being the lowest. Value range of 0.00078 x 10^7 to 68.90 x 10^7 was recorded for Total mould count(TMC). No Significance difference (P>0.05) was recorded for interaction between storage medium and days of storage.

4.8: Interaction effect of Plant rennet and Storage media on Microbial Count ofMozzarella Cheese produced in Zaria

Table 4.8 shows the interaction effect of plant rennet and storage medium on Microbial count. There is no significant difference in the Total Yeast count. Control, Sodom Apple Coagulated Cheese stored in Baobab Pulp Extract and Tamarind Coagulated Cheese stored in Whey had the lowest Total Viable Count. TVC ranged from 1.23 x 10^3 to 6.48 x 10^7 cfu/g. There was no mold present for control while Moringa coagulated cheese in whey had the highest mold count.

Table 4.7: Effect of Storage Media, Storage Period (Day) and their Interactions on Total Microbial Count ofMozzarella Cheese Produced in Zaria (x 10^7)

Treatment	TVC	TYC	TMC
CW	5.23	14.30	8.10
CB	2.44	2.40	52.60
SEM	5.06	12.70	39.20
LOS	NS	NS	NS
Storage Period (Day)			
1	0020	0.50	0.00078
4	0.06	19.30	9.31
7	9.61	2.27	68.90
SEM	5.06	12.70	39.20
LOS	NS	NS	NS
Interaction			
S x D	0.000000085	0.00000007	0.000000053

[abc]means with different superscript along column differ significantly, SEM = standard error of mean, LOS = level of significance, S = storage, D = day, CB = Cheese stored in Baobab Pulp Extract, CW = Cheese stored in Whey, TVC = Total Viable Count, TMC = Total Mold Count, TCC = Total Coliform Count, TYC = Total Yeast Count

4.9: Interaction effect of Plant rennet, Storage media and Storage Period (Day) on Microbial characterization

There was no significant difference in microbial characterization accros the treatments. There was absence of *Salmonella* sp. on day 1 in control however there was presence of the same pathogen in subsequent days.

4.10: Effect of Plant Rennet, Storage Period (Day) and their Interactions on Sensory Characteristics of Mozzarella Cheese Produced in Zaria

Table 4.10 shows the result of effect of plant rennet, day and their interactions on sensory analysis of cheese produced. Sensory properties differ significantly amidst plant rennet. Aroma of control (7.14) was most preffered by the panelist, followed by Sodom apple (6.05) which was at par with Moringa and Tamarind. Similarly, Taste (6.37) and colour (8.22) of control were more preferred followed by Sodom apple (5.20 and 6.45) which was at par with Moringa and Tamarind. Texture, salt and Acceptability follow similar trend with Control recording highest value of 7.70, 6.41 and 7.29 respectively.The taste of Lime was reported to be prominent in all the cheese on the first day.

There was no significant ($P>0.05$) difference among day of storage. Aroma of day 1 (6.56) was most preffered by the panelist, followed by Day 4 (6.10) and day 7 (5.75). Taste and colour range between 4.83 to 5.98 and 6.34 to 6.75 with Day 1 recording the highest preference by the panelist. Texture and salt of 6.75,6.34, 6.67 and 6.03,5.56, 5.33 were recorded for Day 1, 4 and 7 of storage. Acceptability figure of 6.50, 5.84 and 6.04 was recorded for Day 1, 4 and 7 respectively. There was no significant ($P>0.05$) difference in the interaction between Plant rennet and day of storage. Ferment and salty taste increased as the storage days increased while the Moringa coagulated cheese became sweeter as fermentation progressed.

Table 4.8: Interaction effect of Plant rennet and Storage media on Microbial Count of Mozzarella Cheese produced in Zaria (x 10^7)

	CONTROL	SAC		MC		TC			
		CW	CB	CW	CB	CW	CB	SEM	LOS
TVC	0.00066^{c}	0.37^{b}	0.00012^{c}	15.30^{a}	1.49^{b}	0.005.54^{c}	5.82^{b}	6.48	*
TYC	0.0004	0.012	0.00011	0.0037	0.074	0.00005	0.029	0.031	NS
TMC	0	1.44^{b}	23.53^{b}	21.30^{b}	0.63^{b}	1.55^{b}	133.67^{a}	49.60	*

^{abc}means with different superscript along row differ significantly, SEM = standard error of mean, LOS = level of significance, S = storage, D = day, CB = Cheese stored in Baobab Pulp Extract, CW = Cheese stored in Whey, TVC = Total Viable Count, TMC = Total Mold Count, TCC = Total Coliform Count, TYC = Total Yeast Count, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese

Table 4.9: Interaction effect of Plant rennet, Storage media and Storage Period (Day) on Microbial characterization

MICROBES	CHEESE		DAY 1	DAY 4	DAY 7
Salmonella sp.	Control		-	+	+
	SAC	CW	+	+	+
		CB	+	+	+
	MC	CW	+	+	+
		CB	+	+	+
	TC	CW	+	+	+
		CB	+	+	+
Shigella spp.	Control		+	+	+
	SAC	CW	+	+	+
		CB	+	+	+
	MC	CW	-	+	+
		CB	-	+	+
	TC	CW	-	+	+
		CB	+	+	+
E. coli	Control		+	+	+
	SAC	CW	+	+	+
		CB	+	+	+
	MC	CW	+	+	+
		CB	+	+	+
	TC	CW	+	+	+
		CB	+	+	+
Staphylococus	Control		+	-	+
aureus	SAC	CW	-	+	+
		CB	-	+	+
	MC	CW	+	+	+
		CB	+	+	+
	TC	CW	+	+	+
		CB	-	+	+

+ = present and - = absent, CB = Cheese stored in Baobab Pulp Extract, CW = Cheese stored in Whey, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese

4.11: Effect of Storage Media, Storage Period (Day) and their Interactions onSensory Characteristics of MozzarellaCheese Produced in Zaria

Table 4.11 shows the result of Effect of Storage, Day and their Interactions on Sensory characteristics of Cheese Produced. Sensory properties differ significantly amidst storage medium except for salt. Aroma of Whey (6.38) was most preffered by the panelist followed by Baobab (5.56). Similarly, Taste (5.43) and colour (6.84) of Whey were more preferred than Baobab (4.83and 5.79). Whey recorded a texture of 6.30 while Baobab was 5.58. Whey was more acceptable (6.22) than Baobab who recorded a figure of 5.64. There was no significant ($P>0.05$) difference among day of storage. Aroma of day 1 (6.56) was most preffered by the panelist, followed by Day 4 (6.10) and day 7 (5.75). Taste and colour range between 4.83 to 5.98 and 6.34 to 6.75 with Day 1 recording the highest preference by the panelist. Texture and salt of 6.75,6.34, 6.67 and 6.03, 5.56, 5.33 were recorded for Day 1, 4 and 7 of storage. Acceptability figure of 6.50, 5.84 and 6.04 was recorded for Day 1, 4 and 7 respectively. There was no significant ($P>0.05$) difference in the interaction between Storage medium and day of storage

4.12: Interaction effect of plant rennet and storage media on Organoleptic analysis of the Mozzarella Cheese Produced

Table 4.12 showed the interaction between plant rennet and storage medium and the effect on the Organoleptic attributes of the cheese. Aroma value recorded ranged from 7.53 to 5.30 with commercial Mozzarella having the best aroma. Taste ranged from 6.44 to 4.77 in Sodom Apple coagulated cheese stored in Baobab. Acceptability ranged from 7.09 to 5.59 with Sodom apple coagulated cheese in whey having the best acceptability among plant coagulated cheese. Texture value of 6.84 was recorded for Tamarind coagulated cheese in Baobab pulp extract compared to 7.59 recorded for control.

Table 4.10: Effect of Plant Rennet, Storage Period (Day) and their Interactions on Sensory Characteristics of Mozzarella Cheese Produced in Zaria

	Aroma	Taste	Color	Texture	Salt	Acceptability
Control	7.14[a]	6.37[a]	8.22[a]	7.70[a]	6.41[a]	7.29[a]
SAC	6.05[b]	5.20[b]	6.45[b]	5.96[b]	5.79[a]	5.97[b]
MC	5.88[b]	5.14[b]	5.96[b]	5.65[b]	5.46[a]	5.71[b]
TC	5.98[b]	5.06[b]	6.53[b]	6.21[b]	5.28[b]	6.11[b]
SEM	0.44	0.45	0.43	0.44	0.45	0.41
LOS	*	*	*	*	*	*
Storage Period (Day)						
1	6.56	5.98	6.75	6.34	6.03	6.50
4	6.10	4.83	6.34	5.99	5.56	5.84
7	5.75	5.12	6.67	6.25	5.33	6.04
SEM	0.59	0.60	0.58	0.58	0.60	0.54
LOS	NS	NS	NS	NS	NS	NS
Interaction						
P x D	0.24	0.061	0.057	0.065	0.062	0.072

[abc]means with different superscript along column differ significantly, SEM = standard error of mean, LOS = level of significance, P = plant rennet, D = day, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese

Table 4.11: Effect of Storage Media, Storage Period (Day) and their Interactions on SensoryCharacteristics ofMozzarella Cheese Produced in Zaria

	Aroma	Taste	Color	Texture	Salt	Acceptability
CW	6.38[ab]	5.43[ab]	6.84[b]	6.30[b]	5.72	6.22[a]
CB	5.56[b]	4.83[b]	5.79[b]	5.58[b]	5.30	5.64[b]
SEM	0.58	0.60	0.57	0.59	0.60	0.54
LOS	*	*	*	*	NS	*
Storage Period (Day)						
1	6.56	5.98	6.75	6.34	6.03	6.50
4	6.10	4.83	6.34	5.99	5.56	5.84
7	5.75	5.12	6.67	6.25	5.33	6.04
SEM	0.59	0.60	0.58	0.58	0.60	0.54
LOS	NS	NS	NS	NS	NS	NS
Interaction						
S x D	0.37	0.066	0.056	0.065	0.065	0.075

[abc]means with different superscript along column differ significantly, SEM = standard error of mean, LOS = level of significance, S = storage, D = day, CW = Cheese stored in Whey, CB = Cheese stored in Baobab pulp extract

Table 4.12: Interaction effect of plant rennet and storage media on Organoleptic analysis of the Mozzarella cheese produced

Parameter	CONTROL	SAC		MC		TC			
		CW	CB	CW	CB	CW	CB	SEM	LOS
Aroma	7.53[a]	6.81[ab]	5.30[c]	6.00[b]	5.76[b]	6.35[ab]	5.63[b]	0.18	*
Taste	6.44[a]	5.65[ab]	4.77[b]	5.30[ab]	4.98[b]	5.34[ab]	4.78[b]	0.18	*
Colour	7.94[a]	7.20[ab]	5.72[c]	6.26[b]	5.68[c]	7.073[ab]	5.99[b]	0.18	*
Texture	7.59[a]	6.28[b]	5.65[c]	5.78[c]	5.53[c]	6.84[ab]	5.59[c]	0.18	*
Saltiness	5.69[ab]	6.28[a]	5.31[b]	5.37[b]	5.56[ab]	5.50[ab]	5.06[b]	0.18	*
Overall acceptability	7.09[a]	6.42[ab]	5.53[b]	5.84[b]	5.59[b]	6.42[ab]	5.82[b]	0.17	*

[abc]means with different superscript along row differ significantly, SEM = standard error of mean, LOS = level of significance, CW = Cheese stored in Whey, CB = Cheese stored in Baobab pulp extract, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese

4.13: Interaction Effect of Plant rennet and Storage media on Organoleptic Analysis across the storage period

Aroma ranged from 5.72 to 7.53 on Day 1, 4.91 to 7.38 on Day 4 and 4.63 to 6.75 on Day 7. Colour ranged from 4.31 in Sodom Apple coagulated cheese in Baobab to 8.44 in Commercial Mozzarella across the storage period. Texture ranged from 8.03 to 4.84 on Day 7. Saltiness ranged from 6.66 to 4 on Day 7.

4.14: Comparative Cost-Benefit Analysis of Mozzarella Cheese Produced in Zaria

Moringa coagulated cheese had the highest estimated benefit and therefore profit. Tamarind coagulated cheese had the worst economic parameters. Profit ranged from ₦356.88 to ₦425.64. Efficiency ratio ranged from 1.17 to 1.20 while Profitability ratio ranged from 0.17 to 0.20.

4.15: Ranking of Factors Considered Important in Price Formation

Source of milk used (121) is the most paramount criteria considered by the customers, followed by the appearance of the seller. Surprisingly, categories of buyers were the least considered factor (97).

Table 4.13: Interaction Effect of Plant rennet and Storage media on Organoleptic Analysis across the storage period

Parameter	CONTROL	SAC		MC		TC			
		CW	CB	CW	CB	CW	CB	SEM	LOS
Storage Period (Day 1)									
Aroma	7.53^{a}	6.63^{b}	5.72^{d}	6.53^{c}	6.19^{c}	6.97^{ab}	6.41^{c}	0.32	*
Taste	6.44^{a}	6.44^{a}	6.03^{b}	5.53^{c}	5.72^{c}	6.094^{b}	5.63^{c}	0.32	*
Color	7.94^{a}	6.78^{ab}	6.66^{b}	7.03^{ab}	6.53^{b}	6.47^{b}	5.84^{c}	0.31	*
Texture	7.59^{a}	6.09^{b}	6.00^{b}	6.41^{ab}	6.38^{ab}	6.031^{b}	5.94^{b}	0.31	*
Saltiness	5.69^{b}	6.41^{a}	5.53^{b}	6.31^{a}	6.19^{a}	5.88^{b}	6.22^{a}	0.32	*
Overall acceptability	7.09^{a}	6.59^{b}	6.25^{b}	6.22^{b}	6.53^{b}	6.44^{b}	6.41^{b}	0.29	*
Storage Period (Day 4)									
Aroma	7.38^{a}	7.06^{a}	4.91^{c}	5.91^{b}	5.84^{b}	5.78^{b}	5.84^{b}	0.32	*
Taste	6.03^{a}	4.84^{c}	3.88^{d}	5.09^{b}	4.063^{c}	5.19^{b}	4.75^{c}	0.32	*
Colour	8.44^{a}	6.91^{ab}	4.31^{d}	5.84^{bc}	5.00^{c}	7.31^{ab}	6.63^{b}	0.31	*
Texture	8.03^{a}	5.72^{b}	4.84^{c}	5.25^{bc}	4.94^{bc}	7.13^{ab}	6.03^{b}	0.31	*
Saltiness	6.91^{a}	6.19^{b}	5.34^{b}	5.13^{c}	5.094^{c}	5.34^{b}	4.97^{c}	0.32	*
Overall acceptability	7.69^{a}	6.03^{b}	4.41^{d}	5.84^{c}	4.88^{d}	6.38^{b}	5.69^{c}	0.29	*
Storage Period (Day 7)									
Aroma	6.53^{a}	6.75^{a}	5.28^{bc}	5.56^{b}	5.25^{bc}	6.31^{ab}	4.63^{c}	0.31	*
Taste	6.66^{a}	5.66^{ab}	4.41^{c}	5.28^{b}	5.16^{b}	4.75^{b}	3.97^{c}	0.32	*
Colour	8.31^{a}	7.91^{ab}	6.19^{bc}	5.91^{bc}	5.50^{c}	7.44^{b}	5.5^{c}	0.30	*
Texture	7.50^{a}	7.03^{ab}	6.13^{b}	5.69^{bc}	5.28^{bc}	7.38^{a}	4.81^{c}	0.31	*
Saltiness	6.66^{a}	6.25^{ab}	5.06^{c}	4.66^{d}	5.41^{b}	5.28^{b}	4.00^{e}	0.32	*
Overall acceptability	7.09^{a}	6.63^{b}	5.94^{c}	5.47^{d}	5.38^{d}	6.44^{b}	5.38^{d}	0.29	*

^{abc}means with different superscript along row differ significantly, SEM = standard error of mean, LOS = level of significance, CW = Cheese stored in Whey, CB = Cheese stored in Baobab pulp extract, SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese

Table 4.14: Comparative Cost-Benefit Analysis of Mozzarella Cheese Produced in Zaria

	SAC	MC	TC
Total Costof Production (₦)	2045	2145	2095
Estimated Benefit (₦)	2449.36	2570.64	2451.88
Cost-benefit ratio (₦)	0.83	0.83	0.85
Profit margin (₦)	404.36	425.64	356.88
Profitability Ratio	0.20	0.20	0.17
Efficiency Ratio	1.20	1.20	1.17

SAC = Sodom Apple Coagulated Cheese, MC = Moringa Coagulated Cheese, TC = Tamarind Coagulated Cheese

Table 4.15: Ranking of Factors Considered Important in Price Formation

Identified factors	Very Important (4)	Important (3)	Slightly Important	Not Important (1)	PFSI	RANK
Source of Milk	28	3	1	0	121	1
Type of Rennet	10	17	5	0	101	11
Appearance of Sellers	23	9	0	0	119	2
Category of Buyers	9	15	8	0	97	12
Location of Market	13	17	2	0	107	9
Appearance of Cheese	20	9	3	0	113	6
Storage Medium	19	12	1	0	114	5
Packaging	19	11	1	1	112	7
Flavour (Aroma)	17	11	4	0	109	8
Date of Production	22	10	0	0	118	3
Type of Milk	22	9	1	0	117	4
Variety of cheese	11	16	5	0	102	10

PFSI = Price Formation Strategy Index

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Plant Rennet, Storage Period (Day) and their Interactions on Proximate Composition of MozzarellaCheese Produced in Zaria

There was significance ($P<0.05$) difference across the rennet in almost all chemical composition parameters measured except for crude protein and NFE. Moisture content range reported in this study was in line with Adetunji and Salawu, (2008) who used *Calotropis procera* and *Carica papaya* as coagulant and that of Anka Popović *et al.* (2016) for organic hard cheese, Nevertheless, it was higher than values reported by James *et al*. (2016). High moisture content observed in all coagulant could make it susceptible to the growth and proliferation of microorganism thereby shortening the shelf life (James *et al*., 2016). Crude protein observed in this study was in line with Augustine *et al*. (2014) but lower than James *et al*. (2016). Ash content was significantly high for Control and Sodom Apple than Moringa and Tamarind. The ash content is a measure of mineral elements in a food.

Fat content observed decreased across storage day, the significantly high fat of the control agrees with Ojedapo *et al*. (2014) who reported value of 12.68% for unsalted cheese. Albeit higher values were reported by James *et al*. (2016). High fat content of control could be attributed to higher fat content in type of milk used in the production of the commercial Mozzarella cheese. Significant ($P<0.05$) differences were observed in all the Ether extract parameters recorded for storage period and Coagulant interaction. Highest values were observed in control. There was numerical decrement on Ether extract value with increase in storage period and plant rennet interaction. The progressive increase in the crude protein agrees with that of Alalade and Adeneye (2007) although they recorded the fat content to be 38.26% compared to 10.68%

obtained however, this is dependent on the proximate analysis of the milk used. Moringa coagulated cheese has a higher crude protein as Moringa has more protein. The crude protein is lower than that reported by Salawu and Adeniyi, (2008) but similar to that of Ayeni *et al.* (2006). Both the fat and Crude Protein progressively decrease as the cheese ripen. However, both fat and crude protein is lower than that reported by Jana and Manthal, (2011) even though the moisture content is similar to that of Mozzarella Cheese. There is notable increase in the Nitrogen Free Extract due to lactic acid production. Moringa coagulated cheese had the highest Crude Protein (23.37%) probably because moringa seed is proteinous in nature while the ash content of Sodom apple coagulated cheese has the highest ash content due to the minerals present in the Sodom apple latex used.

The Crude Protein reported by Jana and Manthal, (2011) increased by 4.5% during ripening compare to 3% increase reported in this work, however, this may be because of the longer ripening duration for commercial Mozzarella. Generally, the proximate analysis recorded is consistent with that of Alalade and Adeneye, (2010).

5.2 Interaction Effect of Plant Rennet and Storage Period (Day) on Ether Extract, Ashand Nitrogen Free Extract Content of MozzarellaCheese Produced in Zaria

Commercial Mozzarella Cheese are deeply salted and preserved in the freezer in a condition not favourable for microbial growth (Henny, 2010). Moringa and Tamarind showed little of antimicrobial properties even though lipid is highly degradable by microbes. In all, there is a decrease in the value for Ether extract as storage days' increase due to microbial degradability except for Sodom apple coagulated cheese which recorded slight increase on Day 7 of storage.

Alalade and Adeneye (2010) reported a decrease of about 4% from the initial 39% reported on the first dayof wara production, this is comparable to the 4.2% decrease reported in this work.

Total ash increases as the storage days increased. Ash is chemically the most undegradable of all nutrients. The value for total ash increases as microbial degradation increases due to ripening except for Sodom apple and Tamarind coagulated cheese on Day 7. As observed in several works, ash content increases as the microbial load increases (Belewu *et al*., 2005; Alalade and Adeneye, 2010; Oladipo and Jadesinmi, 2013). Alalade and Adeneye reported an increment of 0.5% during storage compared to the 2% reported in this work. Ash content of extract are mosly low (Kadam, 2018) due to ash being poorly absorbed by water, this may be responsible for the notable low percentage of ash in Moringa and Tamarind coagulated cheese.

The percent Soluble carbohydrate was highest in Moringa coagulated cheese, the value decreased as storage days increased except for Sodom apple coagulated cheese on Day 7 and Tamarind coagulated cheese on Day 4, the decrement may be due to the fact that Nitrogen Free extract are easily utilized by Microbes (Belewu *et al*., 2005) while lactic acid is later depostited from galactose catabolism. On average, the value agrees with that of Oladipo and Jadesinmi (2010) which displayed similar attributes when Cheese was stored for 7 days.

5.3 Effect of Storage Media, Storage Period (Day) and their Interactions on Proximate Composition of MozzarellaCheese Produced in Zaria

There was no significant difference across the storage media. This shows that storing in whey is as efficient as storing in Baobab pulp extract. It is worthy of note that both media progressively undergo fermentation similar to what was reported by Belewu *et al.* (2005) where they used ginger extract as the preservative. Crude protein and the Ash content increased as the storage days increased, Microbial protein and the Protein in the storage media might have found a way

into the cheese as the storage days increased (Alalade and Adeneye, 2010), while increase in the ash content might be because of decrease in soluble carbohydrates. However, the fat content decreases across the storage days.

5.4: Interaction Effect of Storage Media and Storage Period (Day) on Crude Protein and Ether Extract Content of MozzarellaCheese Produced in Zaria

Crude Protein increased as the storage period increased except for Cheese stored in Baobab pulp extract for 4 days. Alalade and Adeneye (2010) reported high CP utilization by microbes during ripening which may be responsible for the initial decrease in CP.Alhough, the general increase in CP recorded agree with what was obtained by Oladipo and Jadesinmi (2010). The result on day 7 for both storage media indicated that there is likelihood of further increase in CP as ripening progresses.

Ether extract decreased as the storage period increased except for cheese stored in Baobab pulp extract for 7 days. Utilization of fat by microbes is not retarded by the storage media type. Lee *et al*. (2010) indicated that fermented extract is weak as an antimicrobe which may be responsible for the decrease in EE.

5.5: Interaction effect of plant rennet and storage medium at different storage period on Chemical Analysis (%) of MozzarellaCheese produced in Zaria

There was no significance ($p<0.05$) difference in the interaction between plant rennet and storage medium across the storage period.

5.6: Effect of Plant Rennet, Storage Period (Day) and their Interactions on Microbial Count of MozzarellaCheese Produced in Zaria

There was no significance difference in the total Microbial count of all the plant rennet across the storage days. Lime, Sodom apple, Moringa and Tamarind have all been reported by different authors (Bennett *et al.,* 2003; Salma *et al.,* 2007; Quazi *et al.,* 2013; Nwankwo *et al*., 2015) to have antimicrobial properties although no report about their compared action against microbes. The reported levels for each vary with the inclusion as plant rennet however it might have little effect on the antimicrobial properties. The total Microbial load reported in this work is higher than that reported by National Agency for Food and Drug Administration and Control standard recommendation of 5.0×10^5 and 1.0×10^6 cfu/g. Total Viable count was higher than the satisfactory levels ($< 10^4$) in all the treatment (Taiwo *et al*., 2017). This shows that all the rennets used did not impact any antimicrobial property on cheese at the levels used. As noted by Taiwo *et al*. (2017) the microbial count of most agricultural produce in Nigeria are usually higher than the standard due to the high humidity of the tropics.

5.7: Effect of Storage Media, Storage Period (Day) and their Interactions on Microbial Count of MozzarellaCheese Produced in Zaria

There was no significance difference between the storage media (whey and Baobab) and their efficiency on microbes. Whey contains several bacteriostatic (Takakura *et al*., 2003) compounds while Baobab pulp is essentially rich in Vitamin C which is efficient against microorganisms (Yagoub, 2008). All the microbial counts recorded are higher than the acceptable limit set by NAFDAC (Taiwo *et al*., 2017) even though both whey and baobab are reported to have high antimicrobial properties.

5.8: Interaction effect of Plant rennet and Storage media on Microbial Count of Mozzarella Cheese produced in Zaria

The Total Viable Count recorded in this work is higher than the normal safe recommendation by NAFDAC (Taiwo *et al*., 2017) except for that of Control and Sodom apple coagulated cheese stored in Baobab pulp extract. Therefore, only Sodom apple coagulated cheese stored in Baobab is safe for consumption following NAFDAC regulation.

5.9: Interaction Effect of Plant rennet, Storage media and Storage Period (Day) on Microbial characterization

There was presence of pathogenic microbes in all the treatments as against the regulations of NAFDAC. This result shows that there is likely contamination of either the raw materials used or the cheese produced during ripening. Also, lack of hazard anaylysis and critical control point (HACCP) implementation during milk collection and transport. The microbial characteristic of the milk determines that of the cheese.

5.10: Effect of Plant Rennet, Storage Period (Day) and their Interactions on Sensory Characteristics of MozzarellaCheese Produced in Zaria

The research showed that the Commercial Mozzarella cheese has the highest acceptable sensory attributes, Sodom apple and Moringa coagulated cheese followed closely then followed by that of Tamarind.Lime was described by James *et al.* (2016) has having a strong taste which may be why the panelist reported that the taste of lime in all the cheese. The improvement in the texture recorded in this study is similar to that of Jana and Manthal, (2011) during the stage of ripening, however the cheese did not melt as described. Melting of the cheese depends on how effective the *pasta filata* (stretched curd) was, and this is greatly affected by the coagulating enzyme action. Belewu *et al*. (2005) Alalade and Adeneye (2010) reported that locally produced *wara* become bitter after storing for more than 72hrs, however the result of this experiment disagreed

with them. Only on storage day 4 did the result indicated slightly bitter while it was neutral to sweet throughout the experiment.

5.11: Effect of Storage Media, Storage Period (Day) and their Interactions on Sensory of MozzarellaCheese Produced in Zaria

The research showed that cheese stored in Whey was more acceptable compared to those stored in Baobab pulp extract, however there was no significant difference between consumer perception of the cheese as the storage days increased. Cheese undergoes different processes during ripening, these processes improves the quality of the resulting cheese. Cheese stored in baobab pulp extract however recorded a decrease in quality.

5.12: Interaction effect of plant rennet and storage media on Organoleptic analysis of the Mozzarellacheese produced

Sodom apple coagulated cheese in whey had the best aroma among the plant coagulated cheese second only to control. Aroma of cheese stored in whey were judged to be better than that of cheese stored in Baobab pulp extract probably owing to the excessive fermentation in Baobab (Abiona *et al.*, 2015). The Texture of Tamarind coagulated cheese in whey was very close to that of control followed by Sodom apple coagulated cheese in whey. It is possible that the storage medium has a far more influence on the organoleptic attributes of cheese than what has been reported in literatures. Only Sodom apple coagulated cheese in whey was judged as being extremely salty while all the other cheese slices were slightly salty, this is in contrast with what was reported by Jana (2001) who mentioned that commercial Mozzarella are deeply salted to improve the shelf life. Commercial Mozzarella was more acceptable than all the plant coagulated cheese with cheese stored in Baobab pulp extract having the lowest figure.

5.13: Interaction Effect of Plant rennet and Storage media on Organoleptic Analysis across the storage period

During ripening, quality attributes like aroma, taste and texture of Mozzarella develops (Jana, 2001), however this was not observed in this study. The value obtained for texture decreased from Day 1 to day 4 except for Tamarind coagulated cheese in whey. Acceptability was highest on the day 1 for all the cheese. The Taste and Colour of Sodom Apple coagulated Cheese is similar to what was reported by Orhevba and Taiwo (2016) but better than what was reported by Belewu *et al.* (2005). The result showed that there is an improvement on the sensory attribute compared to local cheese, however the improvement does not exceed that of commercial Mozzarella cheese. This may be as a result of a short ripening period utilized in this study. In summary, Commercial Mozzarella cheese had the best sensory attributes. Alalade and Adeneye (2007) reported a decrease in sensory attributes during storage which is similar what was observed in this work for plant coagulated cheese.

5.14: Comparative Cost-Benefit analysis of the MozzarellaCheese Produced in Zaria

As shown in Table 4.13, the profitability ratio of 0.18 to 0.21 shows that the business is profitable. This shows that no matter the initial investment, there will always be a profit. Moringa and Sodom coagulated cheese had the best economic parameters even though Moringa coagulated cheese (₦425.64) had more profit compare to the Sodom Apple coagulated cheese (₦404.36), this shows that profit on its on is not a good measure of economic potential of any commodity. Reducing the cost of production is paramount in agricultural businesses (Mafimisebi

et al., 2013), however, it should not be done at the detriment of the quality as was observed in Tamarind coagulated cheese (₦2095).

5.15: Ranking of Factors Considered Important in Price Formation

Source of milk was identified as being important as hygiene of the cheese starts from the cow itself. Factors that are closely related to food safety (Appearance of Seller, Date of Production etc) appears to be of importance to the majority of the consumers and are more liable to pay extra if safety is guaranteed. Organoleptic attribute and aesthetics factors are however of little importance in price determination that is, consumers are less likely to pay extra for packaging and Aroma. This shows that organoleptic attributes of cheese are secondary to the safety of the cheese.

CHAPTER SIX

6.1 SUMMARY

A 7-day experiment was conducted whereby three unique plant rennets were utilized in producting cheese using *pasta filata* method. NAPRI yoghurt (50ml) was utilized as the starter culture added to 2 litre of milk purchased from Zaria open market while Lime (30ml) was also added to aid the acidity of the medium. Moringa seed, Tamarind pulp and Sodom apple extracts at 120ml each were used as the rennet for the cheese produced. The cheese was stretched under hot water after formation for effective syneresis. The produced cheese was later stored in whey or baobab pulp extract for 7 days. Proximate analysis, Microbial analysis and Sensory evaluation were all conducted at a 3-day interval while Economic analysis was only done on the day of production. Commercial Mozzarella cheese had the lowest Crude Potein (20.94 %) while the Moringa coagulated cheese had the highest crude protein (23.37 %). However, Commercial Mozzarella had the best Dry matter and Ether extract content of 46.99 and 14.62 % respectively compared with Tamarind coagulated cheese with Dry Matter and Ether Extract of 38.43 and 6.61 % respectively. There was no significance ($p<0.05$) difference in the interaction between storage medium and plant rennet.

Microbial analysis conducted showed the total viable count of all the cheese is higher than the x 10^4limit set by NAFDAC, high Total Viable Count recorded can be either as a result of contamination of the cheese during storage or that of the milk before production. Also, the prescence of at least one pathegonic microbes shows that the cheese analysed is not

recommended for consumption. Economic analysis indicated that Moringa coagulated had the same profitability ratio (0.2) with Sodom apple coagulated cheese even though producing cheese with Sodm apple is the cheapest of all the plant rennet tested.

Cheese stored in whey had the best aroma (6.56), taste (5.43), texture (6.30) compared with that of baobab pulp extract. The result obtained for taste and colour along the storage duration was 5.98, 4.83, 5.12 and 6.75, 6.34, 6.67 respectively for day 1, 4 and 7 of storage. This is positive as previous literatures particularly indicated that those two parameters yield very low score during storage.The result indicated that there is improvement in cheese shelf life if stretch curd method was utilized during production. However, adequate precautions should be taken so that the cheese does not get contaminated during the ripening process.

6.2 CONCLUSION

It can therefore be concluded that;

1. Crude Protein of Moringa coagulated cheese (23.37%) was higher than that of commercial Mozzarella cheese (20.94%).
2. Respondents ranked the commercial Mozzarella cheese (7.14) highest followed by Sodom apple coagulated cheese (6.05), tamarind coagulated cheese (5.98) and Moringa coagulated cheese (5.88) in terms of aroma.
3. Best profitability ratio (0.20) was obtained for Sodom apple coagulated cheese and Moringa coagulated cheesecompared with Tamarind coagulated cheese at 0.17
4. Respondents ranked the source of milk (121) higher than type of rennet (101) in price formulation strategy index

6.3RECOMMENDATION

The following are the recommendations;

1. Cheese production using moringa seed as coagulant should be encouraged.
2. Further research with adjusted stretching time should be conducted to determine the utilization of plant rennt in Mozzarella making.

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Appendix

The GLM Procedure

Dependent Variable: TVC TVC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2.2495791E17	2.0450719E16	0.92	0.5353
Error	30	6.6764835E17	2.2254945E16		
Corrected Total	41	8.9260626E17			

R-Square	Coeff Var	Root MSE	TVC Mean
0.252024	453.1188	149180914	32923131

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DAY	2	5.8777811E16	2.9388905E16	1.32	0.2821

TRT 3 4.9645861E16 1.654862E16 0.74 0.5345
DAY*TRT 6 9.1378492E16 1.5229749E16 0.68 0.6636
1 11:42 Thursday, March 23, 2019 3

The GLM Procedure

Dependent Variable: TCC TCC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	4.6620477E12	423822513962	0.82	0.6169
Error	30	1.5411805E13	513726830741		
Corrected Total	41	2.0073853E13			

R-Square	Coeff Var	Root MSE	TCC Mean
0.232245	422.6981	716747.4	169564.8

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DAY	2	932145917542	466072958771	0.91	0.4144
TRT	3	885920737208	295306912403	0.57	0.6360
DAY*TRT	6	2.4453796E12	407563267525	0.79	0.5824

1 11:42 Thursday, March 23, 2019 4

The GLM Procedure

Dependent Variable: TYC TYC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1.2453026E18	1.1320933E17	0.78	0.6541
Error	30	4.3361298E18	1.4453766E17		
Corrected Total	41	5.5814324E18			

R-Square	Coeff Var	Root MSE	TYC Mean
0.223115	514.8948	380181089	73836652

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DAY	2	2.226254E17	1.113127E17	0.77	0.4719
TRT	3	3.4090139E17	1.136338E17	0.79	0.5111

DAY*TRT 6 6.0019678E17 1.000328E17 0.69 0.6577

The GLM Procedure

Class Level Information

Class	Levels	Values
DAY	3	1 4 7
TRT	7	1 2 3 4 5 6 7
STORAGE	3	0 1 2

Number of Observations Read 672
Number of Observations Used 672

The GLM Procedure

Dependent Variable: Aroma Aroma

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	20	391.410714	19.570536	6.56	<.0001
Error	651	1940.875000	2.981375		
Corrected Total	671	2332.285714			

R-Square	Coeff Var	Root MSE	Aroma Mean
0.167823	28.10851	1.726666	6.142857

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DAY	2	72.96387131	36.48193565	12.24	<.0001
STORAGE	0	0.00000000	.	.	.
TRT	4	42.50694444	10.62673611	3.56	0.0069
DAY*STORAGE	0	0.00000000	.	.	.
DAY*TRT	8	51.32638889	6.41579861	2.15	0.0294
TRT*STORAGE	0	0.00000000	.	.	.
DAY*TRT*STORAGE	0	0.00000000	.	.	.

The GLM Procedure

Dependent Variable: TVC TVC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1.2946016E17	1.618252E16	0.70	0.6893
Error	33	7.6314609E17	2.3125639E16		
Corrected Total	41	8.9260626E17			

R-Square	Coeff Var	Root MSE	TVC Mean
0.145036	461.8976	152071165	32923131

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DAY	2	3.9201429E16	1.9600715E16	0.85	0.4376
STORAGE	2	1.4552301E16	7.2761504E15	0.31	0.7322
DAY*STORAGE	4	3.0974308E16	7.743577E15	0.33	0.8525

Response Taste
Whole Model
Actual by Predicted Plot

Residual by Predicted Plot
Effect Tests

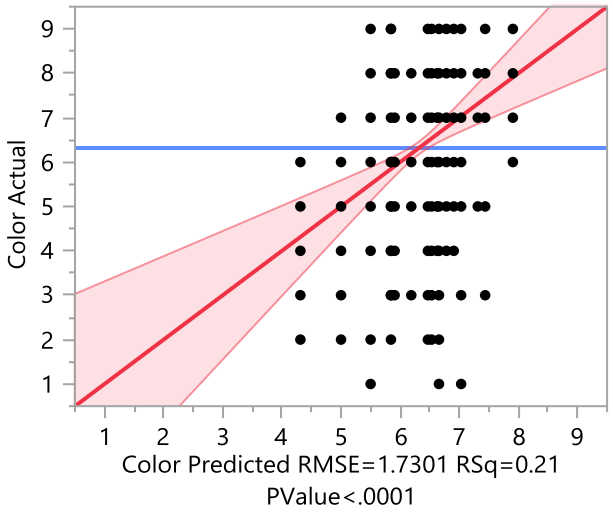
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Trt	5	5	58.98785	3.5948	0.0033*
DAY	2	2	175.62847	26.7574	<.0001*
Trt*DAY	10	10	68.26736	2.0801	0.0243*

Trt

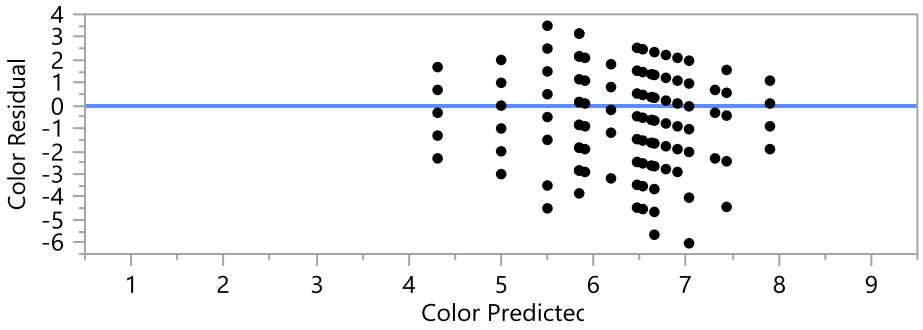
Response Color

Whole Model

Actual by Predicted Plot



Residual by Predicted Plot



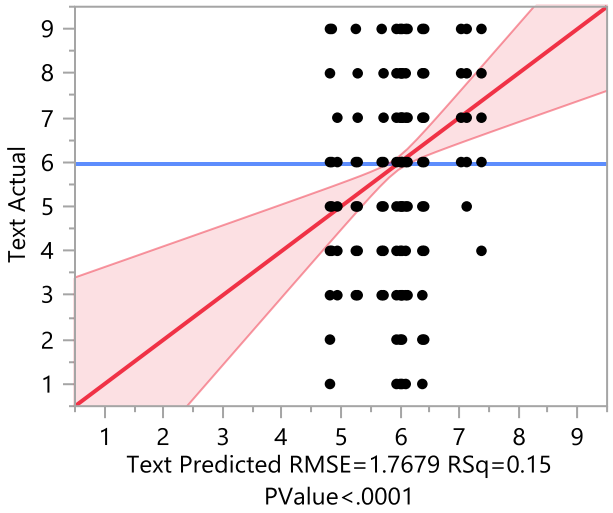
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Trt	5	5	213.61806	14.2737	<.0001*
DAY	2	2	31.43056	5.2504	0.0055*
Trt*DAY	10	10	197.98611	6.6146	<.0001*

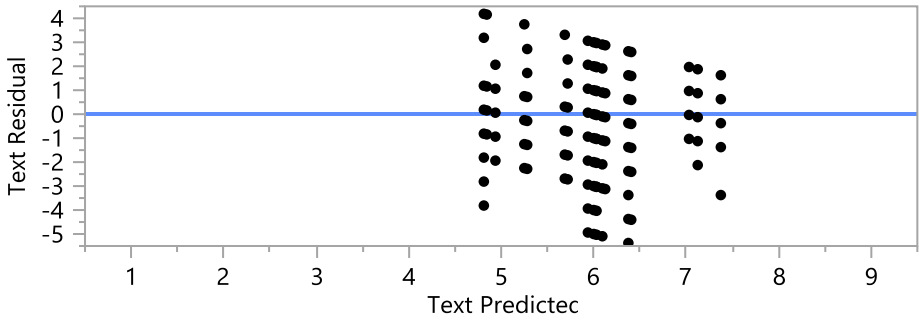
Response Texture

Whole Model

Actual by Predicted Plot



Residual by Predicted Plot



Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Trt	5	5	127.25000	8.1428	<.0001*
DAY	2	2	26.13542	4.1811	0.0158*
Trt*DAY	10	10	155.05208	4.9610	<.0001*

Isolate code	Gram R	Shape	Cat	M R	V P	Lac	Glu	Suc	Oxi	Cit	Mot	Ind	Ure	H_2S	Slope	Gas	Co	Inference species
CHD01A1	-	B	-	+	-	+	+	+	-	-	+	+	-	-	Y	+	-	*Escherichia coli*
CHD01A2	+	C	-	N	N	N	N	N	N	N	N	N	N	N	N	N	+	*S aureus*
CHD01A3	-	B	-	+	-	-	-	-	-	-	-	+	-	-	R	-	-	*Shigella spp.*

Key: Gram R= Gram Reaction, **B**= Bacilli, **C**= Cocci, **MR=** Methyl red, **VP** = Voges-Proskauer, **Lact** = Lactose, **Glu** = Glucose, **Suc** = Sucrose, **Oxi** = Oxidase test, **Cit** = Citrate test, **Mot** = Motility, **Ind** = Indole test, **Ure** = Urease, **H2S** = Hydrogen sulphide (blackening), **R** = Red-pink (alkaline reaction), **Y** = Yellow (acid reaction), **Co**= Coagulase, **Cat**= Catalase, **N**= Not performed

BIOCHEMICAL TEST FOR THE IDENTIFICATION AND CHARACTERIZATION OF BACTERIA

Isolate code	Gram R	Shape	Cat	M R	V P	Lac	Glu	Suc	Oxi	Cit	Mot	Ind	Ure	H_2S	Slope	Gas	Co	Inference species
CHD01B1	-	B	-	+	-	+	+	+	-	-	+	+	-	-	Y	+	-	*Escherichia coli*
CHD01B2	-	B	-	+	-	-	-	-	-	-	-	+	-	-	R	-	-	*Shigella spp.*
CHD01B3	-	B	-	-	-	-	+	-	-	-	+	-	-	+	R	-	-	*Salmonella typhi*

Key: Gram R= Gram Reaction, **B**= Bacilli, **C**= Cocci, **MR=** Methyl red, **VP** = Voges-Proskauer, **Lact** = Lactose, **Glu** = Glucose, **Suc** = Sucrose, **Oxi** = Oxidase test, **Cit** = Citrate test, **Mot** = Motility, **Ind** = Indole test, **Ure** = Urease, **H2S** = Hydrogen sulphide (blackening), **R** = Red-pink (alkaline reaction), **Y** = Yellow (acid reaction), **Co**= Coagulase, **Cat**= Catalase, **N**= Not performed



Lime Juice (*Citrus aurantiifolia*)



Sodom Apple *(Calotropis procera)*





Baobab pulp (*Adansonia digitata*)



Moringa Seeds (*Moringa oleifera*)



Tamarind pulp (*Tamarindus indica*)



Wara



Mozzarella Cheese

AHMADU BELLO UNIVERSITY

FACULTY OF AGRICULTURE

DEPARTMENT OF ANIMAL SCIENCE

Dear Respondent,

This interview is designed to obtain data on a research titled ‘’**QUALITY ASSESSMENT OF MOZZARELLA CHEESE PRODUCED WITH PLANT RENNET STORED IN WHEY AND BAOBAB PULP EXTRACT**’’. The information solicited will be used for research purpose only and your response will be treated with outmost confidentiality.

Thank you for your cooperation.

Please tick (√) the appropriate boxes and fill where necessary

SECTION A: SOCIOECONOMIC CHARACTERISTICS OF RESPONDENTS

1. Gender: Male ( ), Female ( )
2. Age (in years): _____
3. Marital Status: Single ( ), Married ( ), Divorced ( ), Widowed ( ), Separated ( )
4. Household size: _____ (in persons)
5. Religion: Christianity ( ), Islam ( ), Traditional ( ), Others (specify)_____
6. Education: Non formal Education ( ), Adult literacy class ( ), Primary School ( ), Secondary Education ( ), Tertiary Education ( )
7. Primary Occupation: _____
8. Secondary Occupation (if any):_____
9. Annual Income _____naira
10. Do you have allergies to milk and milk products? Yes ( ), No ( ), if yes, state_____
11. Do you have access to dairy products? Daily ( ), Weekly ( ), Monthly ( ), Yearly ( )
12. How often do you consume dairy products? Daily ( ), Weekly ( ), Monthly ( ), Yearly ( )
13. Do you have access to cheese? Yes ( ), No ( )
14. How often do you consume cheese or cheese products? Daily ( ), Weekly ( ), Monthly ( ), Yearly ( )
15. Will you be more interested in cheese if supply improves? Yes ( ), No ( )

SECTION B: ORGANOLEPTIC PROPERTIES

Cheese Sample	Aroma	Taste	Colour	Texture	Saltiness	Overall Acceptability
A						
B						
C						
D						
E						
F						
G						
H						
I						
J						
K						
L						
M						
N						
O						
P						
Q						
R						
S						

COMMENT:

9, like extremely; 8, like very much; 7, like moderately; 6, like slightly; 5, neither like nor dislike; 4, dislike slightly; 3, dislike moderately; 2, dislike very much; 1, dislike extremely.

SECTION C: FAIR PRICE SUGGESTION (PFSI)

If Local Cheese is ₦650/KG and foreign cheese is 900/KG

CHEESE	SUGGESTED PRICE
A	
B	
C	
D	
E	
F	
G	
H	
I	
J	
K	
L	
M	
N	
O	
P	
Q	
R	
S	