ADDIS ABABA UNIVERSITY COLLEGE OF NATURAL SCIENCES CENTER FOR FOOD SCIENCE AND NUTRITION

Physicochemical and casein characterization of Ethiopian camel milk (*Camelus dromedarius*)

By

Zeratsion Fesseha

A thesis presented to the College of Natural Sciences of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Food Science and Nutrition.

October, 2014

Addis Ababa

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Advisors : Dr. Abiy Zegeye

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October, 2014

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Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly and appropriately acknowledged.

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Date 16/12/2014

Place and date of submission:

Place: Center for Food Science and Nutrition, College of Natural Sciences, Addis Ababa University

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Acknowledgements

I would like to express my sincere gratitude to my advisors Dr. Abiy Zegeye and Prof. Negussie Retta for their professional guidance, encouragement and supervision.

The professional guidance, through supervision beginning from the inception of the proposal to final write up of the thesis, free provision of materials including weight markers and explored possibilities to get me laboratory space in other departments outside of Food Science and Nutrition laboratory by Dr. Abiy will always remain fresh memory in my mind.

I gratefully acknowledge W/ro Adey Feleke for her unreserved technical and material support. I gratefully thank Dr. Kassahun Tesfaye for allowing me to use cytogenetic and Isozyme research laboratory. My special thanks go to the technical supports of W/t. Woinshet Abera and Debebe Hailu rendered to me during my work in the laboratory are greatly appreciated.

My special thanks also go to center for food science and nutrition for unreserved support in materials and analytical equipments through my thesis work.

Last but not least I would like to give my special thanks to my Wife, Yeshimebet Berhe, good friends and colleagues of me for their moral support and consistent encouragement throughout the course my rigorous academic work

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Abbreviations

ANOVA	Analysis of variance
AOAC	Association Of The Analytical Chemists
α-CN	Alpha casein
β-CN	Beta casein
BSA	Bovine Serum Albumin
CN	Casein
CSA	Central Statistical Agency of Ethiopia
ESAP	Ethiopian Society of Animal Production
FAO	Food and Agricultural Organization
k-CN	Kappa casein
KDa	Kilo Dalton
LA	Lctoalbumin
LG	Lactoglobulin
NCN	Casein Nitrogen
NPN	Non-Protein Nitrogen
OD	Optical Density
PAGE	Polyacrylamide gel electrophoresis
PI	Isoelectric PH
SA	Serumalbumin
SDS	Sodium dodecyl sulphate
SPSS	Statistical Package for Social Science
TN	Total Nitrogen
UV	UltraViolet light
Vis	Visible light

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Physicochemical and casein characterization of Ethiopian camel milk (*Camelus Dromedarius)*

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Abstract

The samples were collected from East Showa zone of Oromia national regional state of Ethiopia and the analysis part conducted in Addis Ababa University college of natural Sciences Center for food Science and Nutrition laboratory with the objective of Physicochemical profile determination and characterization of Dromedary camel milk casein. Twenty milk samples were collected from secondary and tertiary partum she camels. physicochemical analysis of whole milk was done and casein samples were characterized using polyacrylamide gel electrophoresis (SDS-PAGE). Data was analyzed using computer package SPSS version 20. The following observations was made from the results of the study; the average percentage (mean ± SE) Physicochemical composition of whole camel milk for pH, titratable acidity, ash, total solids, total crude protein, fat and lactose were 6.71 ± 0.010, 0.16 ± 0.004, 0.61 ± 0.026, 11.84 ± 0.17, 2.69 ± 0.08, 3.35 ± 0.029, 5.42 ± 0.13 and 2.08 ± 0.004 respectively. Average casein yield per 100ml whole milk was 2.44% which contributes about 90% (w/w) of the total protein content (2.69%). Casein was purified by acid precipitation at its isoelectric point of pH4.6 and separated in to three major components of α-CN, β-CN and k-CN with molecular masses estimated in kDa as 28.84, 23.40 and 22.40 respectively. The molecular masses were determined using standard weight markers pageRuler^{TM} (Thermo scientific Inc.) having molecular weight range of 10,000 to 170,000Da. The relative quantities of α-, β-, and k- were also estimated using the relative quantitation method from Myimage software (Thermo scientific Inc.) and found to be in the ratio of 1.3:1.2:1. The estimated molecular sizes and ratio of casein fractions (α-CN, β-CN and k-CN) were different as compared to other research works which could be because of differences in sample breed and/or differences in analytical measurement procedure. From the physicochemical analysis result of this study, it is possible to conclude that Dromedary camel milk is a good source of protein, fat, lactose and minerals.

Key words:- Camel milk, Camel milk Protein, Camel milk fat, Camel milk Lactose, Camel milk casein and SDS-PAGE

CHAPTER ONE

1. Introduction

This chapter gives an overall introduction of the study which includes background of the study, statement of the problem, purpose of the study, research questions, general and specific objectives to be achieved.

1.1 Background of the study

The name camel is defined by the International food information service, (2005) as the common name for two species of large, herbivorous, long necked, mainly domesticated, mammals that are well adapted to living in arid conditions. Camels belong to the genus *Camelus* of the *Camelidae* family. Camels are divided into two different species belonging to the genus *Camelus*. *Dromedary* , camels (*Camelus Dromedarius*, one humped) that mainly live in the desert areas, and Bactrian camel (*Camelus bactrianus*, two-humped) which prefer living in cooler areas. The Bactrian species is domesticated in the east to the northern China and in the west to Asia minor and southern Russia, including Mongolia and Kazakhstan (Farah, 1996; Yagil, 1982). On the other hand, two-thirds of the dromedaries live in the arid area of Africa, particularly in North and East Africa (FAO, 1989). To adapt to the harsh conditions - cold or hot, arid, and poor grazing of deserts or semi-deserts, camels have developed many special abilities and attributes. They can store energy in their humps and abdomen in the form of fat, enabling them to survive long periods without any food or water. The camel's body temperature may vary from 34 to 41^0C throughout the day. Blood glucose levels in camels are twice those of other ruminants (Al-Ali *et al.*, 1988).

According to the statistics of the Food and Agriculture Organization (2008) the total population of camels in the world is estimated to be about 25 million with global market potential for camel products of USD 10 billion a year (FAO, 2012). The total population of the Dromedary species (domestic) worldwide is also estimated to be about 15 million head (FAO, 2012).

Camel produces more milk for a longer period of time than any other animal under the same condition and the milk has an important role in human nutrition in the hot regions and arid countries (Farah, Mollet *et al.*,2007: Bekele et al, 2011: Mirazeai, 2012) The general composition of camel milk varies in various part of the world with range of 3.07-5.50% fat, 3.5-

4.5% protein, 0.7-0.95% ash and 3.4-5.6 % lactose, 12.1-15% total solid based on major factors including breed, stage of lactation, feeding and management conditions of the camels (Kappeler *et al.* 1998). Camel milk contains more casein proteins and whey protein than cow milk(Al haj and Al-kanhal, 2010). The principal camel casein fractions are αs1-, αs2-, β- and K-casein in ratio 4:1:4:1 and the numbers of amino acid residues in these four casein fractions are 207, 178, 217 and 162, respectively (Al haj and Al-kanhal, 2010 : Kappeler *et al.* 1998).

The major ethnic groups owning camels in Ethiopia are the Beja, Rashaida, Afar, Somali and Borana (Workneh, 2002). According to the Ehiopian Central Statistical Agency (CSA, 2012/13) estimate, there are about 920,000 camels in the Ethiopian sedentary areas of which 237,380 (74.07 %) of them fall within the age group of 4 years and older. Only around 30.79% of the camels are used for purpose of milk production and the rest are used for other purposes like transportation. However according to (FAO 2002) report Ethiopia possesses over one million Dromedary camels which stands the country fourth in the world. Oromia national regional state, alone (CSA 2012/2013) has estimated 264, 175 heads of camels around 7000 of them are located in East Shewa zone

1.2 Statement of the problem

Camel milk is a source of food for the Ethiopian pastoralist of arid and semiarid areas, however, the milk of indigenous Ethiopian camels are not well characterized with respect to their physicochemical characteristics particularly casein composition. Camel milk has not been given as much attention in livestock research compared with bovine milk despite its significant contribution to the livelihood of the pastoralist society (Yesihak and Bekele, 2003). Most of the research conducted on camels in the past was mainly focused on their anatomical, physiological features and camel products marketing.

Recent studies have mainly concentrated on the compositional characteristics and functionality of camel milk in different countries of the world including some African countries but there is little published research works on the Ethiopian local breed camel's milk casein which constitutes 80% of milk protein (Khaskheli *et al.*, 2005). However, some researches on camel milk composition has shown that camels living in East Africa have higher fat content than the milk from camels living in other African regions and Asia. Casein, the major milk protein, is known for its high mineral content, about 2/3 of the inorganic phosphorus and 2/3 of the

magnesium are involved in the formation of the casein micelle (Farag and Kabary, 1992; Khaskheli *et al.*, 2005)

1.3 Purpose of the study

The purpose of this study is to characterize physicochemical properties of milk and elucidate the caseins of the Ethiopian local camel breeds.

1.4 Research questions

1. What are the physicochemical properties of local camel milk?
2. What are the estimated molecular weights of major casein fractions?
3. What are relative amounts of major casein fractions?

1.5 Objective of the study

1.5.1 General objectives: To determine physicochemical characteristics of camel milk and elucidate electrophoretic profile of milk casein from local indigenous camels reared in East Shewa zone of Oromia national regional state.

1.5.2 Specific objectives:

1. Determine physicochemical composition of camel milk.
2. Estimate molecular size (mass) of major casein fractions (α-, β- and k-) using SDS-PAGE
3. Estimate ratio of major casein fractions using electrophoresis gel analysis software.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Introduction

Camels are considered to be a good source of milk and meat. Camel milk has an important role in human nutrition in the hot regions and arid countries (El-Agamy *at el.*, 1998; Karue, 1998). Camel milk contains all the essential nutrients. Fresh and fermented camel milks also have been used in different regions in the world including India, Russia and Sudan as a treatment for a series of diseases such as jaundice, tuberculosis, asthma and, anti-carcinogenic anti-diabetic, and anti-hypertensive, and has been recommended to be consumed by children who are allergic to bovine and cow`s milk (Magjeed, 2005: El-Agamy *et al,*, 2009). When breast milk is not available or may not be advisable, such as when the mother is HIV positive, any substitute should have the same nutritional characteristics as breast. In addition, it should be hypoallergenic and palatable. As milk represents the main source of nutrition for infants, research to find the most valid alternative to human milk has a high priority since cows' milk allergy is the most common food allergy in childhood, with a reported prevalence of 2–7.5% (D'auria 2005: Shabo *et al.*, 2005).

2.2 Milk secretion and anatomy of the mammary gland in Camelids

Milk is made from the materials of the blood. Blood makes a complete cycle from udder to the heart and returns in 52 seconds. The chemical compounds from the blood are taken by the cells in the mammary gland or secreting tissue in the udder for the synthesis of milk. These compounds or precursors must be present in the feed if they are to get in to the blood; therefore, good nutritional feeding of the camel is important for milk production (Espe and Smith, 1952).

Only the small teats are visible as the mammary tissue in the prepuberal and nulliparous females, it does not develop until the end of the first pregnancy.

Udder of camel consists of four glandular quarters, each with its own teat . The left and right halves of the udder are separated from each other by fibro elastic tissue extending from the linea alba and prepubic tendon and a groove is generally visible between the left and right halves. The

lateral aspect of the quarters is covered by tissue from the abdominal tunic and the caudal abdominal wall. The anterior and posterior quarters are independent but there is no visible separation between them and the teats that are directed cranio-ventrally possess two openings (Skidmore, 2005; Salem, *et al.,* 2012)

Conformation of the udder can change according to breed, age and stage of lactation each quarter is composed of two distinct glands each leading to a separate streak canal within the respective teat. The udder is therefore, composed of 8 separate glands. Each mammary gland consists of parenchyma, connective stroma, ducts and alveolar systems. The gland is made of several individual lobules separated by septa of connective tissue. The glandular units of the lobule, the alveoli or acini, are separated from each other by the intralobular connective tissue which projects from the interlobular connective tissue. The duct system begins with small intralobular ducts that enlarge progressively and each duct is lined by an epithelium resting on a distinct basement membrane. The duct epithelium is low, simple and secretory in the smallest intralobular duct but becomes columnar in the larger duct. The secretor units, acini or alveoli, are small vesicles of unequal sizes that form the lobule-alveolar system. The epithelial lining of the alveoli (flattened to columnar epithelium) shows great variation. according to stage of lactation and secretory activity of the gland. In the non-lactating female, the number and size of alveoli per lobule decreases, the parenchymatous tissue regresses and the interalveolar space becomes filled with interstitial connective tissue (Skidmore, 2005; Salem, *et al.,* 2012)

2.3 Dromedary camel milk production and lactation

Camels have the capability to produce more milk than any other species and for longer periods of time, while their feed requirements are modest. Each camel (both species) produces between 1000 and 2000 L of milk per lactation period of 8 to18 months (Wilson, 1998). Their daily milk production average is estimated to be between 3 and 10 kg during a lactation period of 12 to 18 months (Wilson, 1998: Farah *et al* 2007), Milking frequency in camels varies between two to six times daily. Peak yield is normally attained during second to third month of lactation (Khan and Iqbal ,2001). The yield could increase to 20 liters per day under improved feed, husbandry practice, water availability and veterinary care (FAO, 2006). According to the latest (FAO, 2008) statistics, camel (both species) milk production in the world is reported to be about 5.3 million tons per year, only 1.3 million tons are consumed by humans whereas the remaining amount is fed to calves. Generally the world milk market is estimated to be 10 billion dollars (FAO, 2011).

Camel milk are almost exclusively produced by developing countries, particularly the Sub-Saharan Africans produced an average of around 1.3 million tones milk from 12006-2009 only. Somalia is expected to be the biggest producer of camel milk worldwide followed by Saudi Arabia (FAO,2008).

2.4 Dromedary camel milk properties and milk composition

2.4.1 Camel milk properties

Camel milk, generally opaque and white, has an acceptable taste, normally sweet and sharp taste, but sometimes can also have a salty taste due to the type of plants eaten (Yagil and Etzion, 1980 : Khaskheli *et al.,* 2005 : Magdi et al, 2010: S. Al-Hammadi *et al.*, 2010). Table 1 below is summary for physical properties of milk camel.. The changes in taste are mainly caused by the type of fodder and availability of drinking water (Farah, 1996). The viscosity of bovine milk at 20 ^{0}C is 2.04mPa s which is more viscous than camel milk. Camel milk can remain stable without souring and coagulating for a longer time at room temperature when compared with milk from other animals this may be because camel milk contains a greater content of antimicrobial components such as lysozyme, lactoferrin and immunoglobulins than do bovine or buffalo milk (Konuspayeva 2009 ; Benkerroum, 2008 ; Salmen *et al.,* 2012; Zeineb *et al.*, 2013)

Table 1: Physical properties of camel milk at 20^{0}C.

Color	White and opaque	
Taste	Sweet and sharp taste	Sometimes salty due to type of plants eaten
Average density	1.029g/cc	
Viscosity at 20^{0}C	1.72mPa s	
pH	6.5-6.7	

Modified from (Yagil and Etzion, 1980 ; Farah,1996 ; Khaskheli *et al.*, 2005).

2.4.1.1 Camel milk colostrum

Colostrum, the first milk, is white and slightly diluted as compared with the colostrums of cow (Yagil and Etzion, 1980). Camel colostrum differs in composition from regular milk in that it has a high content of whey proteins. Immunoglobulins G (IgG), providing the new-born with immunity. Camel colostral IgG consists of three main sub-classes, namely IgG1, IgG2, and IgG3

(Azwai *et al.*, 1996). Camel colostrum lacks β- lactoglobulin, whereas the average concentration of α-lactalbumin and lactoferrin were found 2.2g/L and 5.1g/L, respectively (El-Gawad *et al.*,1996). Other Studies has also confirmed that total solids level declines with time following parturition. It was found that three hours post partum total solids averaged 30.4% and found declined to 18.4% during the first two days of lactation.

2.4.2. Camel milk composition

Milk is a polyphasic secretion of mammalian glands which is considered as single food source for the neonate to meet their nutrient needs the composition of milk can vary widely among different species and may associate with the specific nutrient needs of the offspring. It is almost complete nutritional package, supplying both to the mammalian neonates and to humans.

Camel milk composition has been studied in different parts of the world by different researchers Konuspayeva 2009) have conducted meta analysis (1905-2006) and shown wide ranges of variation in camel milk composition attributed to several factors such as analytical measurement procedures, geographical locations, feeding conditions and samples being taken from different breeds in addition to other factors including stage of lactation, age, and calving number. Geographical origin and seasonal variations were found to be the most effective factors in camel milk composition (Konuspayeva *et al.* 2009: Musaad *et al.*, 2013: Aljumaah *et al.*, 2012: Desouky *et al.*, 2013).

Data of (Konuspayeva *et al.* 2009) on camel milk composition has shown effect of geographic variation for camels living in East Africa have higher fat content than the milk from camels living in other African regions and Asia. Variation in camel milk composition was also observed for camels from the same species (*Dromedary*) but domesticated in different parts of the world (Mehaia *et al.*, 1995). Seasonal variations were also found to play a role in camel milk composition even for camels from the same species *(Dromedary*) and regions (Haddadin *et al.*, 2008; Shuiep *et al.*, 2008 ; Haddadin *et al.*, 2008) has found an inverse relationship between total solids in camel milk and water intake by camels. In his study all components except lactose reached their maxima level in mid-winter and were lowest in summer may be due to availability of drinking water. Camel milk in comparison with cow, contains less fat, inorganic salts but more proteins and lactose. Indeed camel milk has low milk fat made mainly from poly-unsaturated fatty acids (Aljumaah *et al.*, 2012: Salmen *et al.*, 2012).

2.4.2.1 Proteins

Camel milk proteins contain satisfactory balance of essential amino acids. The ratio of essential to non-essential amino acids is 0.93 and 1.07 in camel and human milk proteins, respectively (Shamsia, 2009). The amino acid composition of camel milk is also similar to amino acid composition of cow and goat but camel milk is high in glutamic acid (15.16 mg/100ml) and low in alanine (0.03 mg/100ml).

Total protein content of *Dromedary* camel milk is estimated from 2.15 to 4.90%; the average is 3.1 $\pm$0.5%. Camels of same breed have similar protein content but varied for different breeds. Camel milk protein are broadly classified in to casein and whey proteins based on their solubility at pH 4.6 at 20^0C. Caseins are insoluble at their isoelectric point of pH 4.6 and constitute some 80%. Whey proteins remain soluble in milk or skim milk at the precipitation point of caseins (Cheison and Wang, 2004; Salmen *et al.*, 2012)

2.4.2.1.1 Casein proteins

Casein (CN), a phosphoprotein, which precipitates from raw skim milk upon acidification to pH 4.6 at 20 ° C, is the major protein in camel milk. *Dromedary* camel milk has about 1.63 to 2.76% casein equal to about 52 to 87% of the total milk proteins. The β-CN is the main camel milk casein followed by αs1-CN, and constitutes about 65% and 21% of total casein respectively which is similar to human milk but 36% and 38% in bovine milk, respectively (Khaskheli *et al.*, 2005).

Human and camel milk contains a high percentage of β-CN which could reflect its higher digestibility rate and lower incidence of allergy in the gut of infants, because β-CN is more sensitive to peptic hydrolysis than α-CN. Only 3.47% of the total casein corresponds to k-casein in camel milk compared with 13% in bovine milk components due to its low concentration no bands were detected for k-CN after electrophoresis in some research works (Farah and Atkins, 1992). Estimated molecular masses of β-CN and α-CN in camel milk using SDS-PAGE technique are 28.6 kDa and 35 kDa respectively which are higher than for bovine β-CN (24 kDa) and α-CN (22to25 kDa) (Attia, *et al*, 2000 ; Hinz 2012)

The numbers of amino acid residues in the three *Dromedary* caseins sequences are: α-CN, 385; β-CN, 217; k-CN, 162 similar to that of bovine milk except only glycine and cystine were found significantly lower in *Dromedary* milk casein. The casein structure of *Dromedary* camel milk is also similar to that of bovine milk with only few pronounced differences in the secondary

structure of casein. Camel milk k-CN was reported to contain an extra proline residue in its sequence (Pro95). This additional proline residue is expected to play an important role in the stability of camel milk k-CN sequence compared with bovine milk k-CN sequence (Salmen *et al.*, 2012; Salih and Hamid, 2013).

2.4.2.1.2 Whey proteins

Whey proteins are the second main component of camel milk proteins and constitute 20 to 25%(w/w) of the total proteins and 0.63 to 0.80%(w/w) of the milk (Farag and Kabary, 1992; Khaskheli *et al.*, 2005; Mehaia *et al.*, 1995).. The protein components of whey include serum albumin (SA), α-lactalbumin (α-LA), and immunoglobulins (Khaskheli *et al.*, 2005; Mehaia *et al.*, 1995). Besides, the minor proteins include lactoferrin, lactoperoxidase and lysozyme, which have important antimicrobial and carrier functions (Keri, 2004; Parodi, 2007; Buffoni et al, 2011). Lactoglobulin is the most common whey protein by a large margin. The *Dromedary* camel milk whey protein content ranges between 0.63 and 0.80% of the milk. In general, the composition of camel milk whey proteins is different to that of bovine milk whey, where camel milk is deficient in β -lactoglobulin, similar to human milk (Hinz, 2012). In bovine milk whey proteins, β-lactoglobulin is the main component (50%) and a-lactalbumin is the second (25%), whereas in camel milk whey, β-lactoglobulin is deficient and α-lactalbumin is the main component (Elagamy *et al.* 2000; Merin *et al.*, 2001).

Camel milk α-lactalbumin have a molecular mass of 14.6 kDa and comprises of 123 amino acid residues, which is similar to bovine, human and goat milk α-lactalbumin. Human whey proteins are characterized by the presence of high intensity α-lactalbumin and lactoferrin bands, whereas, α-lactalbumin and blood serum albumin (BSA) bands are dominant in camel whey proteins.

Camel milk whey contains other main components such as serum albumin, lactoferrin, immunoglobulins and peptidoglycan recognition protein (Sanchez *et al.*, 1992: El-Hatmi *et al.*, 2007). The whey released from camel milk after coagulation is known to have a white color compared with the greenish whey from bovine milk cheese manufacture. This is could be due to light scattering from the increased concentration of small particles of caseins and fat globules in camel milk whey, or may be because of the low concentration of riboflavin (Merin, 2001 ; Salmen *et al*, 2012). Camel α-lactalbumin has higher degrees of hydrolysis (digestibility) with both trypsin and chymotrypsin enzyme than bovine α-lactalbumin, but both proteins shown similar sensitivity to pepsin enzyme. (Merin, 2001 : Salmen *et al*, 2012).

2.4.2.2 Fats

The fat content of *Dromedary* camel milk is between 1.2 and 6.4% and the average is 3.5 $\pm$1.0 percent. Fat content of camel milk decreases from 4.3 to 1.1 percent in milk produced by thirsty camels. Compared with bovine milk, *Dromedary* camel milk contains smaller amounts of short chain fatty acids and a lower content of carotene (Aljumaah *et al.*, 2012). This lower carotene content could explain the whiter color of camel milk fat. Higher contents of long chain fatty acids were also reported for *Dromedary* camel milk fat compared with bovine milk fat. Similarly, the mean values of unsaturated fatty acid content (43%) were higher in Dromedary camel milk, especially the essential fatty. Human milk fat contains a higher content of unsaturated fatty acids compared with bovine and camel milk fat but that the percentage of saturated acids is higher in bovine milk fat (69.9%) than in camel milk fat (67.7%) (Aljumaah *et al.*, 2012).

The average of cholesterol content of camel milk fat (34.5 mg 100 /g) is higher (25.63 mg 100 /g) than bovine milk. The melting point and solidification temperature of camel milk fat higher in camel milk fat (41.9 $\pm$ 0.9 ^{0}C and 30.5 $\pm$2.2 ^{0}C respectively), compared with bovine milk fat (32.6 $\pm$1.5 ^{0}C and 22.8 $\pm$ 1.6 ^{0}C respectively), probably because camel milk fat contains a lower amount of short chain fatty acids (C4 -C12) and a higher amount of long chain fatty acids (C14 - C22) compared with bovine milk fat (Al-Swailem *et al.* 2010). In addition to the differences in isomeric properties of oleic acid butter is produced only at a high churning temperature of 20-25^{0}C (Ruegg and Farah, 1991). These temperatures are higher than those values reported for bovine milk butter manufacture of 8 - 12^{0}C. Some difficulties in extracting fat from camel milk was reported by Salmen *et al,* (2012) using some traditional methods such as churning sour milk, likely because fat globules are firmly bound to the proteins in camel milk.

2.4.2.3 Lactose

The lactose content of *Dromedary* camel milk varies from 2.40 to 5.80%; the average is 4.4 $\pm$ 0.7 percent. The wide variation of lactose content could be due to the type of plants eaten in the deserts. Camels usually prefer halophilic plants such as Atriplex, Salosa and Acacia to meet their physiological requirements of salts. Hence, camel milk is sometimes described as sweet, salty and at other times as bitter. Lactose content is the only milk component that almost remains

almost unchanged over with seasonal variations and under hydrated or dehydrated (Salmen *et al,* 2012)

2.4.2.4 Vitamins

Dromedary camel milk contain various vitamins, such as vitamin C, A, E, D and B group.. Camel milk is rich source of vitamin C (34.16mg/L) which is three to five times higher than in bovine milk. Hence, raw and fermented camel milk could be a good source of vitamin C for the people living in the desert area where vegetables and fruits are not available (Magdi *et al*, 2010: Salmen *et al,* 2012: Salih and Hamid, 2013) .

2.4.2.5 Mineral content

The total content of minerals is usually expressed as total ash; this amount varies from 0.60 to 0.90% in Dromedary camel milk and the average is 0.79 $\pm$ 0.07 percent. Variations in mineral content were attributed to breed differences, feeding, analytical procedures and water intake. The mean values and standard deviation of Dromedary milk minerals are as follows:
calcium, 114 $\pm$13 mg/100g; potassium, 156 $\pm$ 38 mg /100g; sodium, 59 $\pm$16 mg/ 100g; iron, 0.29 $\pm$0.09 mg /100g; magnesium, 10.5 $\pm$1.8 mg /100g; manganese, 0.05 $\pm$0.03 mg/100g and zinc, 0.53 $\pm$0.08 mg /100g (Aljumaah *et al.*, 2012: Salmen *et al,* 2012).

2.5 Dromedary camel milk functionality

It has been reported that camel milk has potential therapeutic properties, such as anti-carcinogen, anti-diabetic and antihypertensive and it has been recommended to be consumed by children who are allergic to bovine milk (Khalid *et al.*, 2011). According to the survey conducted by ESAP, (2009) pastoralists of the Somali regional state of Ethiopia also use camel milk for treatment of different diseases. The above milk therapeutic uses could be because milk has been shown to contain an array of bioactivities, which extend the range of influence of mother over young beyond nutrition. Peptides in a latent or inactive state within protein molecules, can be released during enzymatic digestion to biologically active peptides from caseins and whey proteins. Most of the bioactivities of milk proteins are latent, being absent or incomplete in the original native protein, but full activities are manifested upon proteolytic digestion to release and activate encrypted bioactive peptides from the original protein. Bioactive peptides have been identified

within the amino acid sequences of native milk proteins. They are also released from milk proteins during milk fermentation and cheese maturation, which enriches the dairy products (Park, 2009). The major biologically active milk components and functions in milk precursors and components are summarized in Table 2.

Table 2, major biologically active milk components and their functions

Milk Precursors	**Components**	**Bioactive Compounds and bioactivities observed**
α, β-caseins	Casomorphins	Opioid agonist (Decrease gut mobility, gastric emptying rate; increase amino acids and electrolytes uptake)
α, β-caseins	Casokinins	ACE inhibitory (Increase blood flow to intestinal epithelium)
α, β-caseins	Phosphopeptides	Mineral binding (Ca binding; increase mineral absorption, i.e., Ca, P, Zn)
α, β-caseins	Immunopeptides , Casomorphins Casokinins	Immunomodulatory (Increase immune response and phagocytic)
α, β-caseins	Isracidin	Antimicrobial
α, β-caseins	Casocidin	Antimicrobial
k-caseins	Casoxins	Opioid antagonist
k-caseins	Casoplatelins	Antithrombotic
α- lactalbumin (α - La)	Lactorphins	Opioid agonist
Serum albumin	Serorphin	Opoid agonist
α, β-serum albumin	Lactokinins	ACE inhibitory
Immunoglobulins	IgG, IgA	Immunomodulatory (Passive immunity)
Lactoferrin	Lactoferrin	Lactoferrin immunomodulatory (Increase natural killer cell activity, humoral immune response, thymocyte traffi cking immunological development, and interleukins - 6; decrease tumor necrosis factor – α. Antimicrobial (Increase bacteriostatic inhibition of Fe - dependent bacteria; decrease viral attachment to and infections of cells) Probiotic activity (Increase growth of Bifi dobacteria in GI tract)
Lactoferrin	Lactoferrin	Opioid agonist
Oligosaccharides	Oligosaccharides	Probiotic (Increase growth of bifi dobacteria in GI tract

Glycolipids	Glycolipids	Antimicrobial (Decrease bacterial and viral attachment to intestinal epithelial cells)
Prolactin	Prolactin	Immunomodulatory (Increase lymphocyte and thymocyte Trafficking and immune development

(Schanbacher *et al.*, 1998; Meisel, 1998 ; Clare and Swaisgood, 2000)

2.6 Camel milk products

2.6.1. Fermented milk

In pastoral societies, milk is traditionally consumed predominantly in the form of fermented milk. Fermentation is the only means of preserving milk under warm condition (Farah *et al.*, 1989). In addition it was found that fermented milk is more nutritious, health-promoting and digestibility of the milk proteins has been improved than fresh milk (Marshal, 1986; Deeth and Tamime, 1981). In Africa, however, where 60% of the world camel population are held, there is a long tradition in preparing fermented camel milk (Magdi, 2010). No starters are used and acidification develops after a few days (usually 24-48 hours until it becomes sour), either from natural flora of milk when it is not boiled, or from the bacteria growing on the sides of the vessel. To improve this spontaneous traditional fermentation, controlled fermentation using mesophilic lactic acid bacteria starter culture have been developed by (Farah *et al.*, 1989) and successfully introduced in camel milk processing plants in eastern African countries.

2.6.2. Cheese

In the conventional way cheese making from camel milk is difficult in getting the milk to coagulate. With the same amount of calf rennet, the coagulation time of camel milk is two to three-folds longer than in cow milk. The action of rennet on camel milk leads to coagulation in the form of flocks, with no firm coagulation. There are some reports in the literature showing that clotting enzyme from one species is more effective and specific with milk from the same species (Attia et al., 2000).

Chymosin from lamb where found to be more effective with lamb milk than with cow milk. Pig chymosin and pig pepsin have shown higher milk clotting activity against porcine milk than against bovine milk. These findings suggest an adaptation between the proteolytic specificities of the gastric proteases and the structure of the caseins. Accordingly, it can be expected that camel chymosin would be more effective in camel milk than calf chymosin. Following this, food scientists developed recombined camel chymosin, from mRNA, obtained from the stomach of a young camel and produced dried and sweetened camel cheese (Farah and Fischer, 2004)

2.6.3. Butter

Like cheese, butter is also not a traditional camel milk product as is it difficult to obtain camel milk butter following the same preparation procedures as for cow's milk. This is due to the lack of agglutinin, a protein which promotes clustering of fat globules and formation of cream layer in cold milk. Also the high melting point (41-42C°) of camel milk fat makes difficult churning camel milk cream in temperatures commonly used for churning cow milk.
A simple method for manufacturing butter from camel milk fat is by churning camel milk cream at temperatures between 20 and 25C°. This temperature is considerably higher than that of cow milk which normally varies between 8 and 12°C (Farah and Ruegg, 1991)

2.6.4. Pasteurized milk

The rate of heat denaturation of camel milk whey proteins was approximately twofold lower than cow milk whey proteins probably due to the absence of β-lactoglobulin . This indicates that camel milk can be easily pasteurised, and there are commercial small and middle scale camel milk processing plants for production of pasteurised milk in Mauritania, Kenya and Somalia.(Farah and Atkins, 1992).

2.7 Camel milk production system in Ethiopia

Camel milk is consumed as a major staple food, mainly by the desert nomad tribes because it is one of the most readily available raw food, which contains all the needful nutrients required in the dry conditions of the desert. Moreover, camel milk like any other human consumable milk consists of fat, proteins (soluble proteins and caseins) and one major carbohydrate (lactose) as major components (Farah & Fischer, 2004).

In general the average camel lactation period is 9 months with average daily camel milk production of 3.591 liters per camel and total annual milk production of over 65 million liters from over 281 thousand milking camels (CSA 2012/13). Similar studies conducted in seven camels milked twice daily in Ethiopia and has found mean daily milk yield of 6.6 liters (Knoess, 1977). According to the survey study conducted by ESAP, (2009) in Somali regional state of Ethiopia, mean milk yield per day decrease with stage of lactation from 5.01Lt to in the early stage of lactation to 3.19 Lt in the late stage of lactation.

Studies have indicated that, Ethiopia consumes approximately 17 kg/capita milk which is very low as compared to other African countries average consumption. Approximately 83% of the total milk produced is consumed at the household level and only 7% is supplied to the formal

and informal markets. The remaining balance is distributed between in-kind wages (0.43%), and used for processing local butter, yogurt, and cheese (10.06%) primarily as a means of extending the shelf life during times of surplus (Jamal *et al.*, 2010).

In the wet season, milk consumed by pastoral children can account for 67% of the mean daily energy they require and 100 % of their protein requirements. Lack of availability and access to milk in the dry season decreased daily consumption amounts by almost 25% with milk contributing only 16% and 50% of energy and protein requirements, respectively. In drought years, children's milk consumption will drop an average of 50% (Jamal *et al.*, 2010)

2.8 Protein separation and characterization

2.8.1 Protein separation

Usually, three or more separation steps are often used in sequence to prepare a pure protein for laboratory study. The biochemical properties of proteins such as protein solubility, molecular mass, isoelectricpoint, adsorption characteristics, denaturation temperature and biological affinities differences are used as bases for individual proteins separations from complex mixtures. The first separation step is often a technique that utilizes the differential solubility properties of a protein. Each succeeding step will use a different mode of separation techniques; some of the most common methods include precipitation, ion-exchange chromatography, affinity chromatography, size-exclusion chromatography and electrophoresis (Harvey, 2000: Nielsen, 2009)

2.8.1.1 Protein separation by electrophoresis

Electrophoresis is a class of separation technique in which analytes are separated based on their ability to move through a conductive medium, usually an aqueous buffer, in response to an applied electric field. (Harvey, 2000)

There are several forms of electrophoresis classified in different forms by different authors, (Nielsen, 2009) has classified in to conventional and capillary electrophoresis. Both share similar principles for the separation of protein. Proteins are separated on the basis of charge or size in an electric field. The primary difference between conventional electrophoresis and capillary electrophoresis is that capillary tubing is used in place of acrylamide gel or agarose gels cast in tubes or slabs. (Nielsen, 2009)

2.8.1.1.1 Conventional electrophoresis

In this technique, proteins can be separated on the basis of charge or size in an electric field. In this technique acrylamide gels cast in tubes or slabs are used in place of capillary tube. There are three different forms of conventional electrophoresis techniques used in protein separation (Harvey, 2000: Nielsen, 2009).

2.8.1.1.1.1 Gel electrophoresis

Proteins are separated from a complex mixture into bands by migration in aqueous buffers through a solid polymer matrix called a gel. Polyacrylamide gels are the principal medium for protein electrophoresis. Agarose is used in some applications such as for separation of proteins larger than about 500kDa and for Immunoelectrophoresis (Westermeier, 1997). Polyacrylamide gels are well suited for sieving proteins because of the following reasons;

1. The gels can be cast in range of pore sizes suitable for sieving proteins.
2. The polymerization reaction is easy and reproducible and gels can be cast in a variety of shapes.
3. Pore size is determined by the conditions of polymerization and can be easily altered by changing the monomer concentration.
4. Polyacrylamide gels are hydrophilic and electrically neutral at the time they are cast.
5. They are transparent to light at wavelengths above 250nm and do not bind protein stains. Gel electrophoresis is an important technique in biochemistry, in which it is frequently used for DNA sequencing. Although it is a powerful tool for the qualitative analysis of complex mixtures, it is less useful for quantitative work (Righetti, 1989).

Polyacrylamide gels are characterized by a pair of values, % T and % C. %T is the weight percentage of total monomers (acrylamide and plus bis) in g/100ml and % C is the proportion of bis as percentage of total monomer (Chrambach, 1985).

Separation depends on the friction of the protein within the matrix and the charge of the protein molecule as described by the following equation (Andrews, 1986).

$$\text{Mobility} = \frac{(\text{Applied voltage})(\text{Net charge on molecule})}{\text{Friction of the molecule}}$$

Proteins are amphoteric molecules, they can carry positive, negatively or zero net charge depending on the pH of local environment. For every protein there is a specific pH at which its net charge is zero. This pH is called isoelectric point or pI, of the protein. depending on solution pH and their pI. A protein is negatively charged if solution pH is above its pI, whereas a protein is positively charged if solution pH is below its pI. The magnitude of the charge and applied voltage will determine how far a protein will migrate in an electrical field. The higher the voltage and stronger the charge on the protein, the greater the migration within the electrical field. Molecular size and shape, which determine the Stokes radius of a protein, also determine migration distance within the gel matrix. Mobility of proteins decreases as molecular friction increases due to an increase in Stokes radius; thus, smaller proteins tend to migrate faster through the gel matrix. Similarly, a decrease in pore size of the gel matrix will decrease mobility (Andrews, 1986).

Gel electrophoresis could be non denaturing or native electrophoresis where proteins are separated in their native form based on charge, size, and shape of the molecule or denaturing electrophoresis where Polyacrylamide gel electrophoresis (PAGE) with an anionic detergent, sodium dodecyl sulfate (SDS), is used to separate protein subunits by size. Proteins are solubilized and dissociated into subunits in a buffer containing SDS and a reducing agent. Reducing agents, such as mercaptoethanol or dithiothreitol, are used to reduce disulfide bonds within a protein subunit or between subunits. Proteins bind SDS, become negatively charged, and are separated based on size alone (Compton and Jones,1985). Denaturing electrophoresis(SDS-PAGE) is the most commonly used technique for fractionation of proteins in to sub units and we will describe in detail because this will be our method of choice for separating our sample, camel milk casein, in to α-CN, β-CN and k-CN proteins (Nielsen, 2009).

Gel electrophoresis comprises of power supply and electrophoresis apparatus containing the polyacrylamide gel matrix and two buffer reservoirs to perform a separation (Figure 1). Power supply is used to make the electric field by providing a source of constant current, voltage, or power. The electrode buffer controls the pH to maintain the proper charge on the protein and conducts the current through the polyacrylamide gel. Commonly used buffer systems include an anionic tris-(hydroxymethyl)aminomethane buffer with a resolving gel at pH 8.8 and a cationic acetate buffer at pH 4.3 (Nielsen, 2009).

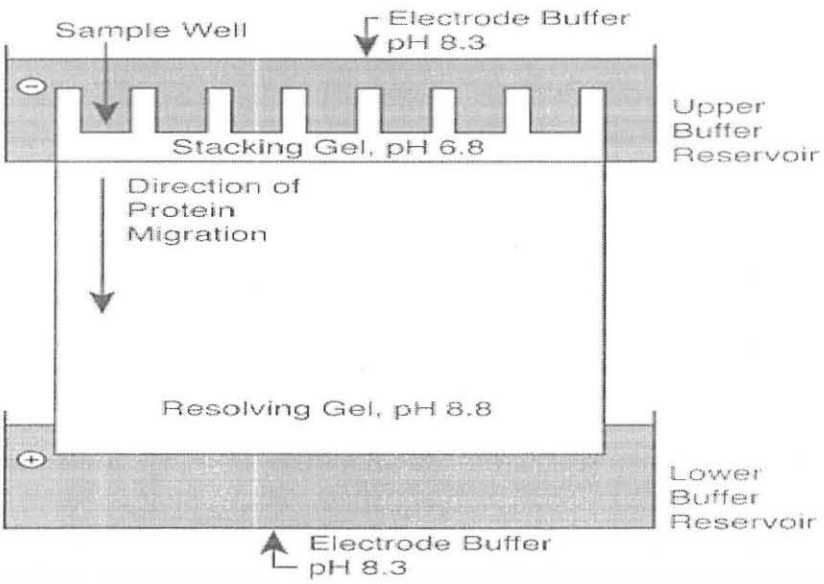

Figure 1: Schematic of a slab gel electrophoresis unit indicating the pHs of the stacking and resolving gels and the electrode buffer in an anionic discontinuous buffer system (Nielsen, 2009)

Polyacrylamide gel matrix is formed (Figure 2) by polymerizing acrylamide and a small quantity usually 5% or less) of the cross-linking reagent, N,N_- methylenebisacrylamide, in the presence of a catalyst, tetramethylethylenediamine (TEMED), and source of free radicals, ammonium persulfate (Allen and Budowle, 1994).

A discontinuous gel matrix is usually used to improve resolution of proteins within a complex mixture. The discontinuous matrix consists of a stacking gel with a large pore size (usually 3–4% acrylamide) and a resolving gel of a smaller pore size. The stacking gel, as its name implies, is used to stack or concentrate the proteins into very narrow bands prior to their entry into the resolving gel. At pH 6.8, a voltage gradient is formed between the chloride (high negative charge) and glycine ions (low negative charge) in the electrode buffer, which serves to stack the proteins into narrow bands between the ions. Migration into the resolving gel of a different pH disrupts this voltage gradient and allows separation of the proteins into discrete bands (Allen and Budowle, 1994).The pore size of the resolving gel is selected based on the molecular mass of the proteins of interest and is varied by altering the concentration of acrylamide in solution. Proteins are usually separated on resolving gels that contain 4–15% acrylamide. Acrylamide concentrations of 15% may be used to separate proteins with molecular mass below 50,000. Proteins greater than 500,000 Da are often separated on gels with acrylamide concentrations

below 7% at the end of an electrophoresis run, the bands on the gels are generally visualized using a protein stain such as Coomassie Brilliant Blue or silver stain. Specific enzyme stains or antibodies can be used to detect a protein (Dunn, 1993).

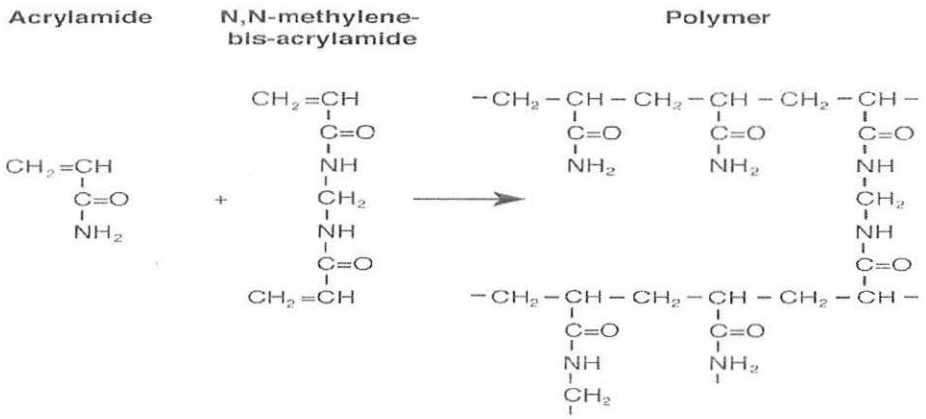

Figure 2: Free radical polymerization reaction of polyacrylamide (Dunn, 1993)

2.8.1.1.1.2 Isoelectric Focusing

In Isoelectric focusing proteins are separated by charge in an electric field on a gel matrix in which a pH gradient has been generated using ampholytes. Proteins are focused or migrate to the location in the gradient at which pH equals the pI of the protein. Resolution is among the highest of any protein separation technique and can be used to separate proteins with pIs that vary less than 0.02 of a pH unit (Harvey, 2000: Nielsen, 2009).

2.8.1.1.1.3 Two-dimensional electrophoresis

Two-dimensional electrophoresis is produced by combining isoelectric focusing and SDS-PAGE which is extremely useful for separating very complex mixtures of proteins. Proteins are first separated in tube gels by isoelectric focusing. The tube gel containing the separated proteins is then placed on top of an SDS-PAGE slab gel, and proteins are separated. Thus, proteins are separated first on the basis of charge and then according to size and shape. Pretreatment of samples for isoelectric focusing (IEF) involves solubilisation, denaturation and reduction to completely break the interactions between the proteins and to remove nonprotein sample components such as nucleic acids (Rabilloud, 1997)

2.8.1.2 Capillary electrophoresis

Capillary electrophoresis system comprises a capillary column, power supply, detector, and two buffer reservoirs (Figure 3). The sample is introduced into the inlet side of the capillary tube by simply replacing the inlet buffer reservoir with the sample solution and applying low pressure or voltage across the capillary until the desired volume of sample has been loaded onto the column. As the sample migrates through the capillary, its components separate and elute from the column at different times. The resulting electropherogram looks similar to the chromatograms obtained in gas chromatography (GC) or High performance liquid chromatography (HPLC) and provides both qualitative and quantitative information (Nielsen, 2009).

Capillaries are composed of fused silica with internal diameters that commonly range from 25 to 100 μm. Column length varies from a few centimeters to 100 cm. High electric fields (100–500 V/cm) can be used as the narrow columns dissipate heat very effectively, allowing for short run times of 10–30 min. (Harvey, 2000: Nielsen, 2009)

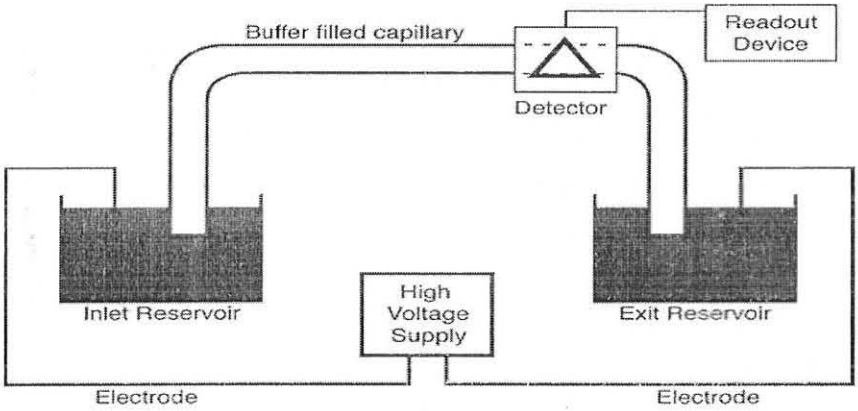

Figure 3: Schematic of a capillary electrophoresis system (Nielsen, 2009)

At the end of a run protein bands are detected on the column as they migrate past a detector. The detectors are Ultraviolet (UV)–visible detectors, fluorescence and conductivity detectors similar to those used in HPLC (Ljungberg et al, 1998; Lee, 1989).

Capillary zone electrophoresis, SDS capillary gel electrophoresis and capillary isoelectric focusing electrophoresis are three types of capillary electrophoresis commonly used for protein separations (Nielsen, 2009).

In capillary zone electrophoresis(CZE) or free solution electrophoresis proteins are separated in free solution inside capillary tubes filled with buffer of the desired pH. Diffusion is prevented within the narrow diameter of capillaries but electroosmotic flow influences the separation of

proteins within capillary tubes. The mechanism of action is such that negatively charged fused silica capillary wall [containing silanol groups (SiO−)], silanol groups ionize to form negatively charged silanate ions at pH levels greater than approximately 2 or 3, then attracts positively charged ions (cations) from the buffer to form a double ion layer at the interface between the capillary column wall and the buffer. When the electric field is applied, the cations forming the double layer are attracted toward the cathode and "pull" other molecules (independent of charge) in the same direction producing the electroosmotic flow. Thus, in CZE cations, anions, and uncharged molecules can be separated in a single run (Harvey, 2000: Nielsen, 2009).

SDS capillary gel electrophoresis techniques can be used to separate proteins by size and to determine molecular mass. In this technique proteins are denatured and dissociated in the presence of SDS and a reducing agent, then fractionation occurs in polyacrylamide gel-filled capillaries of specific pore sizes. Alternatively, linear polymers, such as methyl cellulose, dextrans, or polyethylene glycol, are added to the buffer within the capillary in a technique called dynamic sieving capillary electrophoresis. These entangled polymers act like the pores of the polyacrylamide gel to slow migration of the larger proteins and allow separation by size. advantages of this method over the classical SDS-PAGE include: on-column detection, automated operation, great resolving power, and capability of accurate protein quantification and molecular weight determination (Guttman, 1994).

In capillary isoelectric focusing, ampholytes, which are small polymers (molecular mass of about 5000 Da) containing both positively and negatively charged groups are used to form a pH gradient within the capillary tube. Proteins migrate to the location in the gradient at which pH equals the pI of the protein. In this technique, electroosmotic flow is minimized by coating the capillary walls with buffer additives to prevent undesirable effects caused by surface charge. (Guttman, 1994).

2.9 Protein detection and analysis

Once the gel is stained, it can be imaged and analyzed using imaging instruments and accompanying software (Miller *et al.* 2006):

2.9.1 Protein stains:

The choice of staining technique depends on the availability of imaging equipment. However, a protein staining technique should offer the following features (Miller *et al.*, 2006):

- High sensitivity and reproducibility

- Wide linear dynamic range
- Compatibility with downstream technologies such as protein extraction and assay, blotting or mass spectrometry
- Fast, and uncomplicated protocol

Staining protocols unusually involve protein fixation, exposure to dye solution and washing to remove excess dye (detain). Stains are grouped in to total stains and specific stains. Total stains allow non selective visualization of protein separation pattern which includes coomassie, fluorescent and silver stains. Whereas, specific protein stains are used to visualize specific protein classes such as glycoprotein, and phosphor proteins examples include Pro-Q Diamond and Pro-Q Emerald (Hart *et al.* 2003; Steinberg *et al.* 2003)

2.9.2 Gel Imaging

Electrophoresis patterns (called electropherograms) are digitized by dedicated image acquisition devices and data are analyzed with sophisticated software. Once the gels are digitized, the raw data can be stored for further reference.

Selecting image acquisition devices for the digitization of electrophoresis gels depends on the staining technique used. Some examples of imaging system used in electrophoresis include: densitometers, laser based scanners, CCD (charge-coupled device) camera. Densitometer utilize visible light for analysis of electrophoresis gels (transmission mode) stained with visible dyes. calibrated densitometer has a linear response up to 3.0 optical units in gray levels (Steinberg *et al.* 2003).

2.9.3 Gel imaging analysis software

Software package is required for image acquisition to analyze data and draw conclusions from PAGE applications. Gel analysis software provides a variety of tools that enhance the user's ability to evaluate the acquired data. The software adjusts contrast and brightness, magnifies, rotates, resizes, and annotates gel images, which can then be printed using standard and thermal printers. The software can measure total and average quantities and determine relative and actual amounts of protein. Gel imaging software is also capable of determining the presence/absence and up/down regulation of proteins, their molecular weight, pI, and other values (Hames, 1998; Steinberg *et al.* 2003)

2.9.3.1 Molecular Weight (Size) estimation

SDS-PAGE is a reliable method for estimating the molecular weight (MW) of an unknown protein, since the migration rate of a protein coated with SDS is inversely proportional to the logarithm of its MW. The key to accurate MW determination is selecting separation conditions that produce a linear relationship between log MW and migration within the likely MW range of the unknown protein. The electrophoretic or relative mobility (Rm) of each protein band is calculated as (Nielsen, 2009),

$$Rm = \frac{\text{Distance protein migrated from start of resolving gel}}{\text{Distance between start of running gel and tracking dye}}$$

SDS-PAGE is used in characterization protocols to determine subunit composition of a protein and to estimate subunit molecular mass. Molecular mass can usually be estimated within an error of ±5%. Molecular mass is determined by comparing Rm of the protein subunit with Rm of protein standards of known molecular mass. Commercially prepared protein standards are available in several molecular mass ranges. To prepare a standard curve, logarithms of protein standard molecular mass are plotted against their corresponding Rm values. The molecular mass of the unknown protein is determined from its Rm value using the standard curve (Harvey, 2000; Nielsen, 2009; Steinberg *et al.* 2003)

The accuracy of MW estimation by SDS-PAGE is in the range of 5–10% for glycoproteins and lipoproteins, usually not fully coated with SDS and will not behave as expected in SDS-PAGE, sometimes leading to false estimations (Hames, 1998)

2.9.3.2. Protein quantification by electrophoresis

Two types of quantification are possible: relative quantification (quantification of one protein species relative to the quantity of another) and absolute quantification (quantification of a protein by using a calibration curve generated by a range of known concentrations of that protein). Since proteins interact differently with protein stains, the staining intensity of different proteins at identical protein loads can be very different. Thus, only relative quantitative values can be determined in most cases (Fenselau, 2007: Hames, 1998).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Sample collection and sampling units

The samples were collected from East Shewa zone of the Oromia national regional state of Ethiopia and the analysis part conducted in Addis Ababa University College of Natural Sciences Center for Food Science and Nutrition. East Shewa is located in the middle of Oromia, connecting the western regions to the eastern ones. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA, 2007), this Zone has a total population of 1,356,342 of whom 664 (or 0.05%) them are pastoralists further this zone a population density of 162.03 per square kilometer. In Oromia regional state, CSA has estimated the total number of camels to be 264, 175 heads and around 7000 of them are located in East Shewa zone

The basic sampling units were second and third partum lactating she camels within age range of six to ten years having no history of mastitis. Lactating mothers ranged from 3 to 10 months of lactation. Sample size of twenty lactating she camels were purposive selected because strictly stratified simple random sampling procedure were not possible due to mobile, scattered and less accessible nature of pastoral communities.

3.2 Sample preparation

3.2.1 Washing and sterilization

The sampling glass bottles and lid was soaked in 20% nitric acid to avoid stained dirty and other contaminants then washed with warm detergent water three times, rinsed with tap water and distilled water. Finally sampling bottles were sterilized by autoclave at 121^0C for 15 minutes.

3.2.2 Sample collection and handling

Individual milk samples of approximately 450ml were collected by a single visit through direct milking in to sterile bottles from unwashed udder of left and right teats after discharging the first squirt. Collected milk samples were transported to food science and nutrition center laboratory within 24 hours using cold box filled with ice bags to maintain 4^0c. Collected samples were stored at -20^0C without any preservatives until analysis as recommended by Saliha *et al.*, (2012).

3.3 Physicochemical analysis of camel milk

Milk samples were tested for pH, acidity, ash, total solids, fat, crude protein and lactose in duplicates.

3.3.1 pH measurement

The pH of milk was measured through electronic digital pH meter (PH-016 PH Meter). Buffer solution of pH 4 and 7 was used to calibrate the pH meter. Ten ml of milk sample of each was placed in a beaker; electrode of pH meter was immersed in the sample to determine pH. The measurement was done in duplicate for each milk sample

3.3.2 Titratable acidity determination

Acidity in milk samples was determined by the method (No. 947.05) given in (AOAC, 2000). Nine ml of milk sample was taken in a titration flask and 3 drops of phenolphthalein was added to it. The sample containing indicator was titrated slowly while stirred continuously against 0.1N NaOH until light pink end point appeared persistently. Volume of 0.1 N NaOH used was recorded from the burette to determine acidity of milk in terms of lactic acid by using following expression:

$$\% \text{ acidity (Lactic acid)} = \frac{\text{ml of 0.1N NaOH X0.009 X100}}{\text{9ml sample}}$$

The measurement was done in duplicate for each milk sample.

3.3.3 Determination of Ash (%)

Ash concentration in milk was estimated by gravimetric methods using the method No 945.46 as given in AOAC, (1990). Five ml milk sample was taken in a crucible and moisture was evaporated to dryness of sample on steam bath. Then crucible was placed in muffle furnace at 550^0C until carbon free ash was obtained. The crucible containing ash was placed in desiccator for 30 minutes and weighed. The ash content was calculated as given below.

$$\text{Ash (\%)} = \frac{\text{Wt of ash}}{\text{Wt of sample,}} \text{X100}$$

The measurement was done in duplicate for each milk sample

3.3.4 Total solids (%) determination

Total solids of milk were determined according to the AOAC, (1990) method described in No.925.23. Five gram sample was taken in clean dried and tarred aluminum dish. Heated for 15

minutes in water bath and kept in hot air oven for 3 hours at 100°C and then cooled in a dessicator and weighed quickly. The total solids were calculated as under

$$\text{Total solids (\%)} = \frac{\text{residue after drying (g)}}{\text{Weight of sample (g)}} \times 100$$

The measurement was done in duplicate for each milk sample

3.3.5 Fat determination

Determination of fat in milk was done using the (AOAC, 2000) method 905.02. The milk sample was treated with ammonium and ethyl alcohol; the former to dissolve the protein and the later to help precipitate the protein. Fat was extracted with diethyl ether and petroleum ether. Mixed ethers were evaporated and the residue weighed.

Two gram raw milk sample was weighed and transferred in to an empty volumetric flask. Ammonia of 0.25ml was added, mixed and shaken thoroughly. Two ml ethyl alcohol was added and mixed again. Five ml of diethyl ether was added, stopper and shaken vigorously for 1minute. Then 5ml petroleum ether was added and shaken again vigorously for 30 minutes. The flask Stood until the upper ethereal layer had separated completely and became clear up on addition of 2ml of ethanol. Clear ethereal layer decanted off in to pre weighed aluminum dish. The volumetric flask was washed with 5ml ether and added the washings in to the aluminum dish. The liquid remained in the volumetric flask was extracted two times using 3ml each solvent every time. Ethereal extract was added to the same container and evaporated off completely. Aluminum dishes dried in air oven at 102 $\pm$ 2°C for 3 hours. Cooled in desicator and weighed where two successive weights did not exceed 1mg. The total fat content was determined using the formula

$$\text{.Fat \% (w/w)} = \frac{\text{Weight of extracted fat}}{\text{weight test portion}} \times 100$$

3.3.6 Total nitrogen and crude protein

The nitrogen content in milk sample was estimated based on Kjeldahl's method (991.20) of (AOAC, 2000). One ml of milk sample was digested in digestion tubes containing two digestion catalysts namely cupper sulphate and potassium sulphate. . Twenty ml of sulphuric acid and potassium permanganate was added to the tubes and digested by applying heat at 370^{0}c for 4 hours in laminar air flow hood until clear solution is formed. Digested sample was diluted with 25ml water and neutralized with 25ml NaOH. The ammonium formed was distilled in to 25ml

of boric acid solution containing methyl blue and titrated against 0.1N HCl. The protein content in milk was estimated by multiplying the percent nitrogen with 6.38

$$\text{Nitrogen}(\%) = \frac{\text{Vol (sample-blank)HCL X 0.1NHCL X 0.014 X100}}{\text{Weight of sample}}$$

Protein % (w/w) = N(%) X6.38

3.3.7 Lactose determination

Lactose (%) = Total solids (%) – (Total protein (%) + Fat (%) + ash (%)), according to (AOAC, 2003)

3.4 Absorbance assay of casein

Casein concentration was measured using spectrophotometer ultraviolet light with absorbance maxima at 280nm. (Layne. E. 1957: Whitaker, 1993) using the following procedure; Casein fractions was taken out of the -20°C freezer to thaw. UV lamp was warmed for 15 minutes and wavelength adjusted to 280nm. Each casein sample of 20mg was suspended in 25ml distilled water by adjusting pH to 7.0 with 1M NaOH. Finally each sample was transferred to cuvette for absorbance reading. The UV spectrophotometer was auto zero with quartz cuvette filled with distilled water of PH 7. Concentration of casein was calculated using the formula, concentration

$$\text{(mg/ml)} = \frac{\text{Absorbance at 280nm}}{\text{path length (cm)}}$$

3.5 Electrophoretic analysis of Casein using Polyacrylamide gel electrophoresis (SDS–PAGE)

3.5.1 Preparation of milk casein

Frozen milk samples were thawed at room temperature for 3 hours. About 100ml raw milk samples were defatted by centrifugation at 3000rpm at room temperature for 15 minutes followed by separation of the protein fraction into casein and whey proteins. Whole casein was obtained from skimmed milk by isoelectric precipitation (pH 4.6) using 1N HCl. The precipitate was washed twice with distilled water at pH 4.6, suspended at pH 7 by addition of 1M NaOH, precipitated again at pH 4.6 with 1N HCl and washed three times with distilled water. Finally, the whole casein was suspended at pH 7, freeze dried (using MINI LYODEL freeze drier), yield in gram per 100ml was recorded and stored at - 20°C until used for SDS-PAGE separation. The supernatant containing whey proteins was discarded (Shammet *et al.* 1992).

3.5.2 SDS-PAGE

SDS PAGE was carried out using the methods described by (Laemmli 1970 ; Liljeruhm et al, 2014) and α-, β- and k- caseins were separated by the methods of Hipp *et al.* (1952); Girdhar and Hansen, (1978).

3.5.2.1 SDS-PAGE procedure

a) Apparatus cleaning (cleaver VS30D, 20X20cm, 1.5mm spacer thickness)

The units of the elctrophoretic device were cleaned with warm distilled water (40-50°C) and soap two times. Set of glass plates (one notched glass plate and one plain glass plate with bonded spacers) was cleaned with distilled water and then with 70% ethanol. Finally air dried before use

b) Vertical gel casting

The bonded spacer plain glass plate with the notched glass plate facing inter most to form a gel cassette was placed together. The cassettes were placed on to the casting base. Tightened the screws to secure the gel cassette in position and repeated all the above steps other side with the remaining gel cassettes.

c) Gel preparation

Resolving gel, stacking gel, sample buffer and running buffer solutions were prepared above according to the given protocol. Total volume of gel solutions were calculated and prepared for two 20X20cm, 1.5mm spacers. A resolving gel concentration of 12.5% which has resolving capacity of 17-66kDa and stacking gel solution of 4.5% was selected for this experiment because casein fractions` molecular sizes are expected to be within 17-66kDa.

d) Gel pouring

First a comb was inserted in to the glass plates and marked a point on the glass plate 1 cm below where the comb teeth finish, which indicated where to add the resolving gel to. 215µl of TEMED was added to the resolving gel solution and mixed while avoiding generation of air bubbles. Each glass plate was filled quickly with the 50ml gel avoiding generation of any air bubbles. Filling was done quickly before the TEMED caused the gel too viscous. The gel was over laid extremely carefully with 1ml of distilled water. A sharp line between water layer and gel was an

indication of completion of polymerization. The resolving gel was polymerized in around 20 minutes. Over laid liquid was poured off and rinsed the gel with distilled water. 4.5% of stacking gel was carefully mixed and up on addition of 78µl TEMED the glass pates was filled with the help of Pasteur pipette up to the top (15ml for each plate). Comb was carefully inserted immediately following the gel.

e) Preparation of protein samples for loading

Casein samples of 5mg was weighed and dissolved in 1.25ml loading buffer at a ratio of 1:4. Denatured for 5 minutes at 90^0C in water bath. The diluted samples were transferred to 1ml micro centrifuge tube and then mixed in a micro centrifuge for 1 minute at 12000rpm. Finally twenty samples each 25µg size (table 3) was loaded on 20 wells. Weight marker (PageRuler) was directly loaded on the gel.

Table 3: Volume of samples in µL loaded in each well to attain 25µgof casein.

Sample No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Loading Vol(µl) /25µg	7.9	8.	7.9	7.9	8.0	8.	7.9	7.8	8	8.0	7.8	8.	7.8	7.9	7.6	7.8	7.9	7.7	8.	7.9

f) Loading of samples and weight markers

Once the gel has set, the combs were removed carefully from the glass plates without damaging the wells. A total of 25µg were loaded in to the wells with micro pipette and 15µL weight markers to either ends. Pre-frozen cooling packs were inserted to the outside part of the glass plates inside the running buffer. Any unused wells were filled with sample loading buffer. The orientation and order the samples loaded was noted. The inner gel module containing cast gels was transferred in to the main tank in the correct orientation as indicated + ve on the module aligned with +ve on the tank, -ve on the module aligned with –ve on the tank.

g) Gel running

The upper tank lid was fitted and connected to the power supply. Running conditions were set as follows; Voltage: 225V, current: 110mA, power: 20W and running time 6-7 hours. Power supply was turned off when the loaded dye and the blue 10kDa marker reached the bottom of the gel. The inner buffer was emptied in to the main tank. The glass plate was unscrewed and gently

separated apart. The gel was removed from the plate by first wetting running buffer and gently lifted with a spatula in to the staining chamber.

h) Staining and destaining of gel

Gel was stained by immersing gel in staining solution (see solution h) for 3 hours and destained in destaining solution (see solution i) over night until background was clear and bands appear distinct (Merin 2001; Feligini et al, 2005; Salmen , 2012).

i) Electrophoregram analysis

The migration distance from the top of the resolving gel was measured using ruler to each size marker band and to the dye front. The Rf value of each band in the standard was measured using the formula

$$Rf = \frac{\text{migration distance of the size markers' band}}{\text{Migration distance of the dye front}}$$

Rf values for the bands of the casein samples was also measured using the above formula. The logMW of the size markers as a function of Rf was plotted. Finally the equation ($y = -1.32x + 5.14$) was generated and the value of y was solved to estimate the molecular weight of the casein sample or lanes .of unknown proteins (Hames , 1998: Higgins and Hames, 1999)

3.6 Casein fractions ratio estimation

Relative quantities of the principal fractions of camel milk casein, α-, β- and k- are estimated from the gel using the Myimage analysis software (Thermo Fisher scientific Inc. 2012). Relative quantitation methods were selected to estimate the casein fraction ratios.

3.7 Statistical analysis

Physicochemical analysis of whole milk was performed in duplicates, absorbance assay using spectrophotometer and electrophoretic profile with SDS-PAGE. Values of these different tests were expressed as the mean ± standard error ($x\square \pm SE$). SPSS packet program for Windows (SPSS, version 20, USA) was used for the statistical analysis.

CHAPTER FOUR

4. Result and discussion

4.1 Results

4.1.1 Physicochemical analysis

Physicochemical analysis was conducted on 20 *Dromedary* camel milk samples (pH, acidity, ash value, total solids, total N and crude protein, fat and lactose) and analysis results are summarized in table 4.

Table 4: Physicochemical (%) characterization of camel milk (mean ± SE)

Components (%)	N	Minimum	Maximum	Mean ± SEM
pH value	20	6.58	6.80	6.71± 0.010
Acidity (%)	20	0.11	0.19	0.16± 0.004
Ash value (%)	20	0.46	0.80	0.618± 0.018
Total solids (%)	20	9.99	13.79	11.84± 0.17
Total protein (%)	20	2.23	3.70	2.69± 0.08
Fat (%)	20	3.01	3.67	3.35 ± 0.029
Lactose	20	4.00	6.38	5.19±0.13

4.1.2 Absorbance assay of casein

Below (table 5 and table 6) shows yield and absorbance assay of casein in camel milk.

Table 5: Mean percentage yield (mean% ± SE) of casein in Dromedary camel milk

ample No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	mean ± SE
Weight of freeze dried (gm)/100ml whole milk	2.4	2.5	2.4	2.5	2.3	2.5	2.5	2.4	2.3	2.5	2.4	2.4	2.5	2.3	2.5	2.4	2.5	2.5	2.4	2.5	2.44± 0.074
% casein in milk	2.4	2.5	2.4	2.5	2.3	2.5	2.5	2.4	2.3	2.5	2.4	2.4	2.5	2.3	2.5	2.4	2.5	2.5	2.4	2.5	2.44± 0.074

4.1.3 Electrophoretic profile of caseins

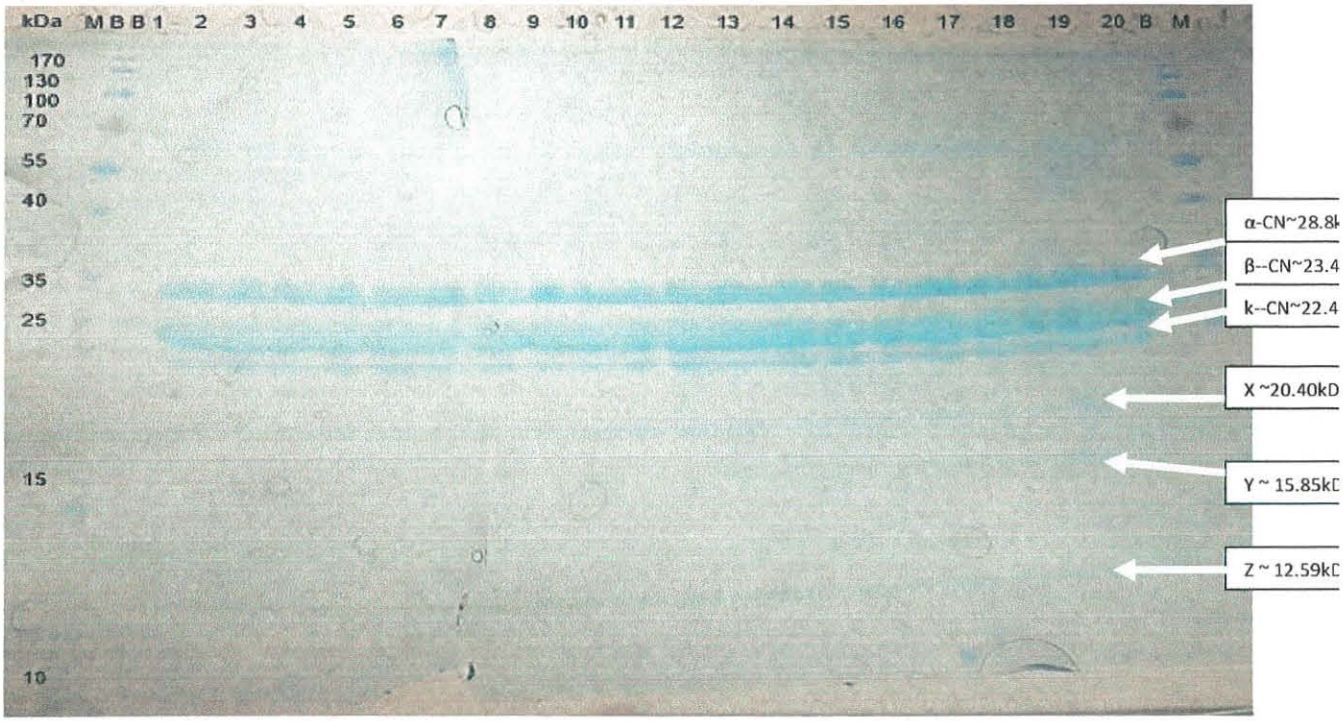

Figure 4: SDS-PAGE profile of camel milk caseins, lane (1 to 20) are casein samples (M) is the molecular weight of marker bands (10-170kDa) and (B) are the blank lanes

When the relative electrophoretic mobilities (Rf) are plotted (figure 5) against the logarithm of the weight markers` molecular weights (Log_{MW}), a linear relation was observed.

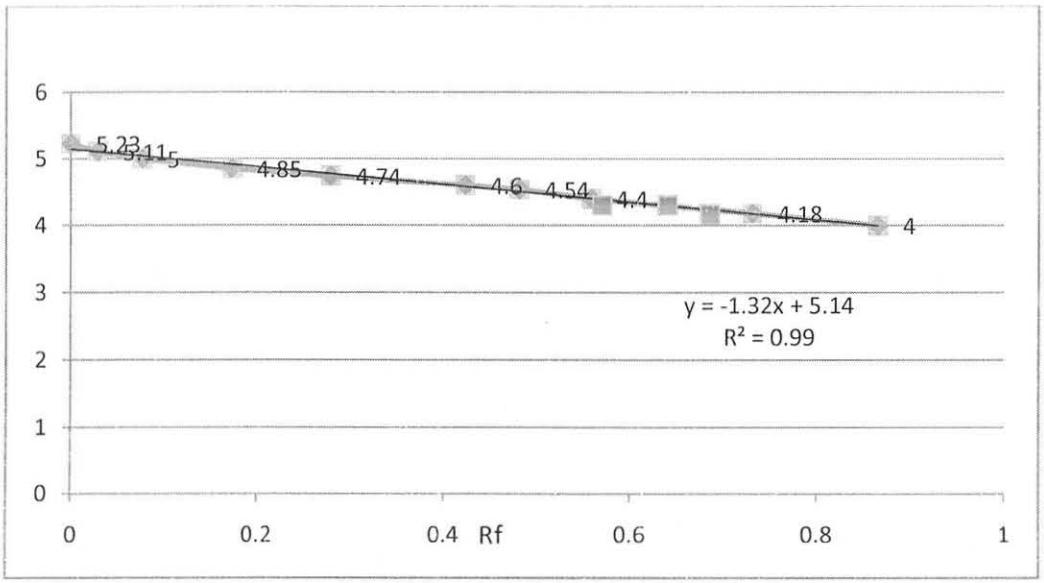

Figure 5: Calibration curve obtained when the logarithm (LogMW) of the molecular weight markers and samples (■) are plotted against their relative nobilities (Rf) in 12.5% SDS-PAGE.

Table 6: Relative mobility, LogMW and molecular weight (MW) of the weight marker (10-170kDa) and samples

Marker bands				Sample bands					Tracking dye
Molecular weight (kDa)	LogMW	Mobility (cm)	Relative mobility (Rf)	Molecular weight (kDa)	LogMW	Mobility (cm)	Relative mobility (Rf)	Band name	Mobility (cm)
170	5.23	0.00	0.00						
130	5.11	0.30	0.029						
100	5.0	0.80	0.077						
70	4.85	1.80	0.173						
55	4.74	2.9	0.279						
40	4.60	4.4	0.423						
35	4.54	5.0	0.481						
-				28.84	4.46	5.2	0.50	α-	
25	4.40	5.8	0.558						
-				23.40	4.37	6.0	0.58	β-	
-				22.39	4.35	6.2	0.60	k-	
-				20.40	4.31	6.6	0.65	x-	
-				15.85	4.20	7.4	0.71	y-	
15	4.18	7.6	0.731						
-				12.59	4.10	8.4	0.81	z-	
10	4.0	9.0	0.865						
-									10.40

4.1.4 Casein fractions ratio

Table 7: Ratio of casein fractions (α-, β- and k-casein)

Casein fraction	Sum of relative amount	ratio
α-CN	49.85	1.3
β-CN	44.82	1.2
k-CN	38.39-	1

From the above table (table 7) the principal camel casein fractions, α-, β- and K- are in ratio of 1.3:1.2:1or in quantity percentage of 37.5, 33.6 and 28.9, respectively.

4.2 Discussions

4.2.1 Physicochemical analysis of camel milk

pH, the hydrogen ion concentration of milk, indicates its quality. Jenssen (1995) indicated that pH values higher above 7.5 are indicative of mastitis.

Results presented in table 4 show pH values of 20 camel milk samples. The results varied from 6.58 to 6.80 with average value of 6.71 $\pm$ 0.010. The result is slightly higher than the Egyptian camel milk as reported by Shamsia (2009) who found pH value of 6.64$\pm$ 0.05 and than those of Sawaya *et al.* (1984) and Ahmed (1990) who reported 6.49 and 6.53, respectively, while in agreement with the result 6.70 reported by others Khaskheli *et al.*(2005) and Samet-Bali and Attia (2012). The mean result is also similar to 6.77 as reported by (FAO, 1982). The average pH result indicates that camel milk samples have the expected acceptable quality with no indication of contamination during collection and infection during delivery.

Milk acidity indicates the level of lactic acid which is produced by the action of bacteria during fermentation by converting lactose. It has been reported by Shori (2012) fermented milk for being more nutritious and health-promoting than fresh milk Acidity in milk mainly reflects temperature of milk after collection, husbandry techniques and marketing practices (Iqbal, 1999). Camel milk was reported to remain stable for a longer time at room temperature when compared with milk from other animals. Whereas bovine milk took 3 h to turn sour (to reach a pH of 5.7 or less) at 30^0 C, camel milk took a longer time of 8 h to reach a pH of 5.8 at the same temperature (Lakosa and Shokin, 1964). This may be because camel milk contains a greater content of antimicrobial components such as lysozyme, lactoferrin and immunoglobulins than do bovine or buffalo milk (Benkerroum, 2008; El-Agamy, 2000). The average percentage acidity (lactic acid)

values of twenty camel milk samples in terms of lactic acid (table 1) varied from 0.11 to 0.19 with an average of 0.16% ± 0.004. The experiment result was in line with those reported by Shamsia (2009) and Samet-Bali and Attia (2012) as 0.162% ± 0.007 and 0.16%, respectively; slightly higher than the result of 0.13% as reported by Ahmed (1990), while lower than 0.18% as reported by Khaskheli *et al.*(2005). Acidity test of this study was conducted within 24 hours after samples being collected which is confirmed by test result where the pH value of all samples are higher than 6.0 (fresh). This is also supported by the observations made by Hafiz and Hamzawi, (1991), when camel milk was left to stand the lactic acid content did not show any noticeable increase until approximately 8-10 h.

The total content of minerals is usually expressed as total ash. Average percentage ash values of this study varied from 0.46 to 0.80 with an average of 0.61% ± 0.026. The result is similar to the Egyptian camels ash value of 0.68% ±0.096 as reported by Hattem (2011) and lower than the result 0.81% ± 0.045 as reported by Desouky *et al.* (2013). The ash value is also lower than those reported by Ahmed (1990:) who found between 0.75 to 0.83gm /100gm with average 0.89 ± 0.03/100gm and Khaskheli *et al.* (2005) who found between 0.85 to 1.00 gm/100gm with average 0.94 ± 0.02gm /100gm. The ash value of this study is at a lower limit range as compared to most research results which indicate that the total mineral content of raw milk are also lower. Generally variations in mineral content are attributed to breed differences, feeding condition, analytical procedures Mehaia *et al.*(1995) and water intake (Haddadin *et al.*, 2008).

Results presented in table 4 show wide variations in average percentage total solid content of camel milk. The values varied from 9.99 to 13.69 % with average of 11.84 ± 0.17%. The average result is in agreement with the results of 11.7% as reported by Sawaya *et al.* (1984), however, the results of this study are lower than the results of Desouky *et al.* (2013) and Atigui *et al.* (2013) who reported 12.05 ± 0.868% and 12.1 ± 2.6, respectively. But average result of this study is higher than the results reported by Hattem *et al.*,(2011) and Khaskhell., *et al.*,(2005) who found 9.9 %±1.189 and 9.74% ± 0.49, respectively. Wide variations in average percentage total solids of the samples are because of wide variation in average percentage of total protein, fat and lactose content with in the samples

The average protein content of camel milk varied from 2.23 to 3.70% with average content of 2.69% ± 0.08. This result is similar to the result of Abu-Lehia (1990) and Iqbal (1999) who

found 2.78% ±0.12 and 2.7%, respectively. However, mean percentage result of this study is found little higher than 2.54 ±0.19 as reported by Khaskhell., *et al.* (2005). The protein result is lower as compared to the results of 3.1% ± 0.157 and 3.44 ±0.113% as obtained by Hattem *et al.* (2011) and Desouky *et al.* (2013), respectively. Elamin and Wilcox, (1992); Sawaya *et al.*, (1984) had studied variations in camel milk protein composition and reported as protein content (casein and whey proteins) was found to be similar for camel milk of the same breed but varied for other breeds when comparing the result of this study and the above researchers. We can infer that the camels from which we obtained the samples may be from different breed.

Variation in fat content was observed to be directly or indirectly related to the total solids content of camel milk, as the total solids increased, the fat content also increased and vice versa (FAO, 1982). This observation is supported by the result of this study in camel No. 3(refer annex page 54 and 57) where, the lowest amount of total solids and fat content are recorded. Fat content of camel milk (table 4) was observed to vary from 3.01 to 3.67 with an average of 3.35 ± 0.029 g per 100g of milk. The mean result is higher than those reported by Khaskhell (2005) and Konuspayeva *et al.* (2013) who found to be 2.6± 0.40 g per 100g and 2.9g per 100ml, respectively. However, the mean result is lower as compared to the result of 4.4% as reported by Ibrahim (1990) and to 3.5 % ±1.0 as reported by Konuspayeva *et al.* (2009). Compared with bovine milk, Dromedary camel milk fat contains higher values of unsaturated fatty acid content Haddadin *et al.* (2008) however; Bracco, (1972) has reported that human milk fat contains a higher content of unsaturated fatty acids compared with bovine and camel milk fat. Palmquist *et al.* (1993) has studied that the sum of short chain fatty acids(C4 to C8) which are promoters of atherosclerosis, were only 0.52% in camel milk, and 8.99% in the milk of cows fed with a nutritionally balanced diet. Whereas the long chain fatty acids (C15 to C22) which protect atherosclerosis were much higher (81.8%) in camel milk than in cow's milk (66.1%).

Results presented in table 4 showed wide variations in lactose content of camel milk ranged from 4.00 to 5.98 with average content of 5.42 ± 0.126 g per 100g of milk sample. The wide variation of lactose content could be due to the type of plants eaten in the deserts (Khaskheli *et al.*, 2005). Camels usually prefer halophilic plants such as Atriplex, Salosa and Acacia to meet their physiological requirements of salts (Yagil and Etzion, 1980). Hence, camel milk is sometimes described as sweet, salty and at other times as bitter. However, lactose is the only component that

almost remains almost unchanged over a seasonal variation (Haddadin *et al.*, 2008) and under hydrated or dehydrated conditions (Yagil and Etzion, 1980). This study result does not agree with the results reported by Konuspayeva *et al.* (2009) who found it to be in the range of 2.40 to 5.80%, with average of 4.4 $\pm$ 0.7 %. The results are also higher than the average content of 3.65 $\pm$0.16% as reported by Khaskheli *et al.* (2005). Results of this study are also similar to the results of Khan *et al.* (2001) who found it to be 5.5% from milk of Somalia and Kenyan camels.

Generally, we can make explanations based on the physicochemical results seen in our samples. We have observed wide variations in camel milk composition as compared to certain other research works. The observed variations in camel milk composition could be attributed to several factors such as difference in analytical measurement procedures, feeding conditions and/or the samples are not from same breed, in addition to other factors including stage of lactation, age, and calving number (Khaskheli *et al.*, 2005). Geographical origin and seasonal variations were found to be the most important factors in camel milk composition (Konuspayeva *et al.* (2009).

4.2.2 Absorbance assay and yield of casein

Volume of samples equivalent to 25µg loaded in each well was roughly estimated based on the respective absorbance assay result of each sample (table 6). The amount 25µg was determined though optimization works producing sharp and distinct bands. Absorbance ranging 0.3150 to 0.3286 equivalents to 0.3150 to 0.3286 mg/ml was recorded at 280nm wavelength. Volume of samples loaded in each well was computed from the absorbance assay and found to be in the range of 7.6 to 8µl

Casein is the major camel milk protein that contains most of the essential amino acids in high ratios (Salmen, 2005). Casein yield or content in raw milk was varied between 2.3% to 2.5% with average content of 2.44 $\pm$ 0.074 % (table 5). This result is in agreement with 1.63- 2.76% as reported by Khaskheli *et al.* (2005). The average casein content 2.44$\pm$ 0.074 % is slightly lower than 2.65% but higher than 2.22% reported by Shamsia, (2009) and Khaskheli *et al.*, (2005) respectively.

The average casein content of this study is about 90%(w/w) of the total protein content (2.69%) which is higher than the result 80 - 87% as reported by El-Agamy *et al.*, (2009) this could be due to difference in analytical measurements or difference in our samples breed type.

4.2.3 Electrophoretic analysis of camel milk casein

In order to identify the different casein fractions from camel milk, SDS–PAGE electrophoresis of twenty casein samples were compared against the weight marker (molecular weight from 10kDa to 170kDa, purchased from Thermo scientific, Lot number 00138717). SDS-PAGE patterns of camel caseins are presented in Figure 4. The marker proteins as well as the samples are properly separated in the selected acrylamide gel concentration (12.5%).

From this figure it is possible to observe six bands (three major expected bands and three very faint smaller bands). The observed three major bands are in between the weight markers of 15kDa and 35kDa in their mobility and the three faint bands (x, y and z) are in between 10kDa and 25kDa. The three major bands correspond to the expected sizes α- , β- and k-caseins. From calibration curve of the weight markers (figure 5), estimated molecular weights of α-, β- and k-caseins are 28.84kDa, 23.4kDa and 22.4kDa, respectively. These observed results are similar to the results reported by Salmen *et al.,(*2012) who found that the molecular weights of α- , β-and k-caseins to be 27.6kDa, 23.8kDa and 22.4kDa, respectively . The estimated results of α-and k-caseins are also similar to the results of 28 and 22.9 as reported by (El-Agamy *et al.*, 2009: Kappeler,1998) and less than 35.5kDa and 32kDa for α- and β-caseins as reported by Saliha *et al.,(* 2012); Frah and Riesen, (1985). The α- and k- casein results of this study are also lower than the results of Ochirkhuyag *et al.*, (1997) who obtained 35.3kDa and 27.5kDa, respectively.

From the results of this study, we have observed that molecular weights of casein fractions are different from other similar studies done in other countries. This molecular weight difference may be due to differences in amount of amino acid residues per molecule of casein. therefore, we may infer that the Ethiopian local bread camels' casein fractions have different amino acid residue. This differences in molecular weight attribute to differences in breeds and the camel samples may be from different breed. From figure 4, we have observed that the bands size and intensity for the three casein fractions are different. α - has larger size and intensity followed by β- and k- bands which indicate differences in relative amount in casein. The smaller three faint bands (figure 4) may be degraded products of the major bands. The lowest two bands (y and z) with expected molecular mass of 12.59kDa and 15.85kDa as calculated from the calibration curve of the weight markers may be the result of degraded products of α-casein. Sum of the two bands weight mass is similar with α-casein molecular mass (28.8kDa). But it is possible to conjecture for the third faint band (x) with expected molecular mass of 20.40kDa may be

degraded product of β- or k- where other smaller size degraded products are away from the gel after running.

4.2.4. Casein fractions ratio

The principal camel casein fractions are α-, β- and K-casein in ratio of 1.3:1.2:1 or in percentage of 37.5, 33.6 and 28.9, respectively. This ratio result is different as compared to other research works. Kappeler *et al.*, (2004) research work showed β-CN as the main camel milk casein fraction followed by α-CN and k-CN in the percentage of 65, 31.53 and 3.47%, respectively. (Schmidt, D.G, 1982) has also reported ratio of 3.8;3;1 which is different from this study ratio result. High percentage of β-CN could reflect its higher digestibility rate and lower incidence of allergy in the gut of infants since it is more sensitive to peptic hydrolysis than α- and k-CN (Abou- Soliman, 2005 ; El-Agamy, et al, 2009). In this study all fractions have similar quantities and it is likely the sample camels to have higher incidence of allergy in the gut of infants. Difference in ratio is mainly attributed to difference in breed and type of image analysis software.

CHAPTER FIVE

5.1 . Conclusion

In view of the observed results of the physiochemical and electrophoretic properties of the *Dromedary* camel milk, camel milk contains good amounts of protein, fat, lactose and minerals besides casein has three major fractions. The physicochemical values revealed that Dromedary camel produces nutritious milk for its calves and for human consumption. Camel milk is a good source of protein, fat, lactose and minerals. As observed from the SDS-PAGE profile and analysis software, the major casein fractions are α-, β- and k-caseins with relative quantities of 1.3:1.2:1. The major protein, casein contributes about 90% of the total protein content of the milk. The three major casein fractions (α-, β- and k-casein) has an estimated molecular weight of 28.84kDa, 23.4kDa and 22.40kDa, respectively as determined from the standard weight markers. Wide variations in camel milk composition have been observed compared to other research works. The observed variations could be attributed to several factors such as difference in analytical measurement procedures, feeding conditions and/or the samples are from different breed, in addition to other factors including stage of lactation, age, and calving number.

Ratio and molecular weights of these fractions are also different from other research works which indicated that our casein samples have different amino acid residue and the camels could be from different breed and /or may be due difference in analytical measurement procedures

5.2 Recommendations

Based on the findings of this study, Dromedary camel milk is a good source fat, protein, lactose and minerals for the calves and the people living in the arid and urban areas, however, more extensive research is needed to confirm on the characteristics of casein and nutritional composition of Ethiopian local breed camel milk. It is also recommended to study amino acid sequence analysis of casein.

Limitations of the study

This work was done in a situation where the electrophoresis devices were incomplete and without any additional laboratory supported training on protein separation and analysis of electrophoretic bands. Besides it was very difficult to get electrophoretic grade chemicals particularly TEMED(N,N,N\N'-tetramethylethylenediamine), N, N'-methylene bisacrylamide and sodium dodecyl sulfate (SDS) from the local market with in the course of study. The above listed conditions has affected the quality my work and made the work very time taking and financially expensive.

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Appendix

1. SPSS Result
2. Ratio analysis report for casein fractions using my image analysis soft ware
3. Preparation of stock solutions and buffers (Laemmli 1970 ; Liljeruhm et al, 2014)

1. SPSS Result

Descriptive Statistics

Total solids

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
1	2	12.2535	1.04157	.73650	2.8954	21.6116	11.52
2	2	12.7305	.44053	.31150	8.7725	16.6885	12.42
3	2	9.9980	.53740	.38000	5.1696	14.8264	9.62
4	2	13.6910	1.78332	1.26100	-2.3315	29.7135	12.43
5	2	12.4550	.88106	.62300	4.5390	20.3710	11.83
6	2	11.0685	1.17168	.82850	.5414	21.5956	10.24
7	2	12.6370	.42851	.30300	8.7870	16.4870	12.33
8	2	11.6685	.49427	.34950	7.2277	16.1093	11.32
9	2	11.1470	.44406	.31400	7.1573	15.1367	10.83
10	2	12.0350	.25173	.17800	9.7733	14.2967	11.86
11	2	12.0770	.02546	.01800	11.8483	12.3057	12.06
12	2	10.1505	.06576	.04650	9.5597	10.7413	10.10
13	2	11.9085	2.30588	1.63050	-8.8090	32.6260	10.28
14	2	11.1945	.69650	.49250	4.9367	17.4523	10.70
15	2	12.1860	1.00975	.71400	3.1138	21.2582	11.47
16	2	12.1810	.80186	.56700	4.9766	19.3854	11.61

17	2	11.5540	.31537	.22300	8.7205	14.3875	11.33
18	2	12.9455	1.36118	.96250	.7158	25.1752	11.98
19	2	10.8745	.52255	.36950	6.1796	15.5694	10.51
20	2	12.1200	.54730	.38700	7.2027	17.0373	11.73
Total	40	11.8438	1.12345	.17763	11.4845	12.2031	9.62

Descriptive statistics

Total protein

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
1	2	2.3223	.00000	.00000	2.3223	2.3223	2.32
2	2	2.5456	.31579	.22330	-.2917	5.3829	2.32
3	2	2.4612	.19636	.13885	.6969	4.2254	2.32
4	2	3.7078	.69615	.49225	-2.5469	9.9624	3.22
5	2	2.8311	.18950	.13400	1.1285	4.5337	2.70
6	2	2.4515	.10571	.07475	1.5017	3.4012	2.38
7	2	2.5544	.32817	.23205	-.3941	5.5028	2.32
8	2	2.5010	.63159	.44660	-3.1736	8.1756	2.05
9	2	2.3708	.05770	.04080	1.8524	2.8892	2.33
10	2	2.6530	.41663	.29460	-1.0902	6.3962	2.36
11	2	2.5189	.07623	.05390	1.8340	3.2038	2.47
12	2	2.2330	.12629	.08930	1.0983	3.3677	2.14
13	2	3.5248	1.19529	.84520	-7.2145	14.2641	2.68
14	2	2.5623	.22627	.16000	.5293	4.5953	2.40

15	2	2.5923	.50254	.35535	-1.9229	7.1074	2.24
16	2	2.7602	.26502	.18740	.3791	5.1413	2.57
17	2	2.4384	.14899	.10535	1.0998	3.7769	2.33
18	2	3.5369	1.31395	.92910	-8.2684	15.3422	2.61
19	2	2.5208	.15443	.10920	1.1333	3.9083	2.41
20	2	2.6796	.37901	.26800	-.7257	6.0849	2.41
Total	40	2.6883	.54540	.08624	2.5139	2.8627	2.05

Descriptive statistics

Fat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
1	2	3.2050	.06364	.04500	2.6332	3.7768	3.16
2	2	3.1850	.09192	.06500	2.3591	4.0109	3.12
3	2	3.0100	.01414	.01000	2.8829	3.1371	3.00
4	2	3.2850	.09192	.06500	2.4591	4.1109	3.22
5	2	3.2350	.02121	.01500	3.0444	3.4256	3.22
6	2	3.2750	.14849	.10500	1.9408	4.6092	3.17
7	2	3.6700	.07071	.05000	3.0347	4.3053	3.62
8	2	3.5600	.07071	.05000	2.9247	4.1953	3.51
9	2	3.2250	.07778	.05500	2.5262	3.9238	3.17
10	2	3.5550	.02121	.01500	3.3644	3.7456	3.54
11	2	3.4050	.17678	.12500	1.8167	4.9933	3.28
12	2	3.2000	.04243	.03000	2.8188	3.5812	3.17

13	2	3.3200	.16971	.12000	1.7953	4.8447	3.20
14	2	3.3600	.12728	.09000	2.2164	4.5036	3.27
15	2	3.4000	.01414	.01000	3.2729	3.5271	3.39
16	2	3.3550	.12021	.08500	2.2750	4.4350	3.27
17	2	3.6400	.18385	.13000	1.9882	5.2918	3.51
18	2	3.3400	.11314	.08000	2.3235	4.3565	3.26
19	2	3.2200	.00000	.00000	3.2200	3.2200	3.22
20	2	3.5650	.09192	.06500	2.7391	4.3909	3.50
Total	40	3.3505	.18555	.02934	3.2912	3.4098	3.00

Descriptive statistics

Lactose

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
1	2	5.9827	1.03379	.73100	-3.3055	15.2709	5.25
2	2	6.3864	.41818	.29570	2.6292	10.1436	6.09
3	2	4.0014	.17204	.12165	2.4556	5.5471	3.88
4	2	6.1923	.87929	.62175	-1.7078	14.0923	5.57
5	2	5.7474	.69084	.48850	-.4596	11.9544	5.26
6	2	4.8786	.95919	.67825	-3.7394	13.4965	4.20
7	2	6.0383	.31770	.22465	3.1839	8.8928	5.81
8	2	4.9060	.11116	.07860	3.9073	5.9047	4.83
9	2	4.8142	.50091	.35420	.3137	9.3147	4.46
10	2	5.2585	.21793	.15410	3.3005	7.2165	5.10

11	2	5.5576	.11681	.08260	4.5081	6.6071	5.48
12	2	4.0610	.07679	.05430	3.3711	4.7509	4.01
13	2	4.5137	.75561	.53430	-2.2752	11.3026	3.98
14	2	4.6892	.36557	.25850	1.4046	7.9738	4.43
15	2	5.6768	.52984	.37465	.9164	10.4371	5.30
16	2	5.4273	1.07183	.75790	-4.2027	15.0573	4.67
17	2	4.8112	.34245	.24215	1.7343	7.8880	4.57
18	2	5.2661	.04511	.03190	4.8608	5.6714	5.23
19	2	4.6342	.43671	.30880	.7105	8.5579	4.33
20	2	5.1119	.81671	.57750	-2.2259	12.4497	4.53
Total	40	5.1977	.79093	.12506	4.9448	5.4507	3.88

Descriptive statistics

Ash

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
1	2	.7435	.05586	.03950	.2416	1.2454	.70
2	2	.6135	.20153	.14250	-1.1971	2.4241	.47
3	2	.5255	.15486	.10950	-.8658	1.9168	.42
4	2	.5060	.11597	.08200	-.5359	1.5479	.42
5	2	.6415	.02192	.01550	.4446	.8384	.63
6	2	.4635	.04172	.02950	.0887	.8383	.43
7	2	.6010	.03253	.02300	.3088	.8932	.58

8	2	.7015	.09687	.06850	-.1689	1.5719	.63
9	2	.7370	.03677	.02600	.4066	1.0674	.71
10	2	.5685	.03182	.02250	.2826	.8544	.55
11	2	.5955	.00919	.00650	.5129	.6781	.59
12	2	.6565	.05869	.04150	.1292	1.1838	.62
13	2	.5500	.18526	.13100	-1.1145	2.2145	.42
14	2	.5830	.02263	.01600	.3797	.7863	.57
15	2	.5170	.03677	.02600	.1866	.8474	.49
16	2	.6385	.12516	.08850	-.4860	1.7630	.55
17	2	.6645	.00778	.00550	.5946	.7344	.66
18	2	.8025	.11526	.08150	-.2331	1.8381	.72
19	2	.4995	.06859	.04850	-.1168	1.1158	.45
20	2	.7635	.01768	.01250	.6047	.9223	.75
Total	40	.6186	.11547	.01826	.5817	.6555	.42

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
camelmilk	20	1	20	10.50	5.916
casein	20	2.30	2.50	2.4350	.07452
Valid N (listwise)	20				

2. Ratio analysis report for casein fractions using my image analysis soft ware

Image Report: 20140805_142830.jpg

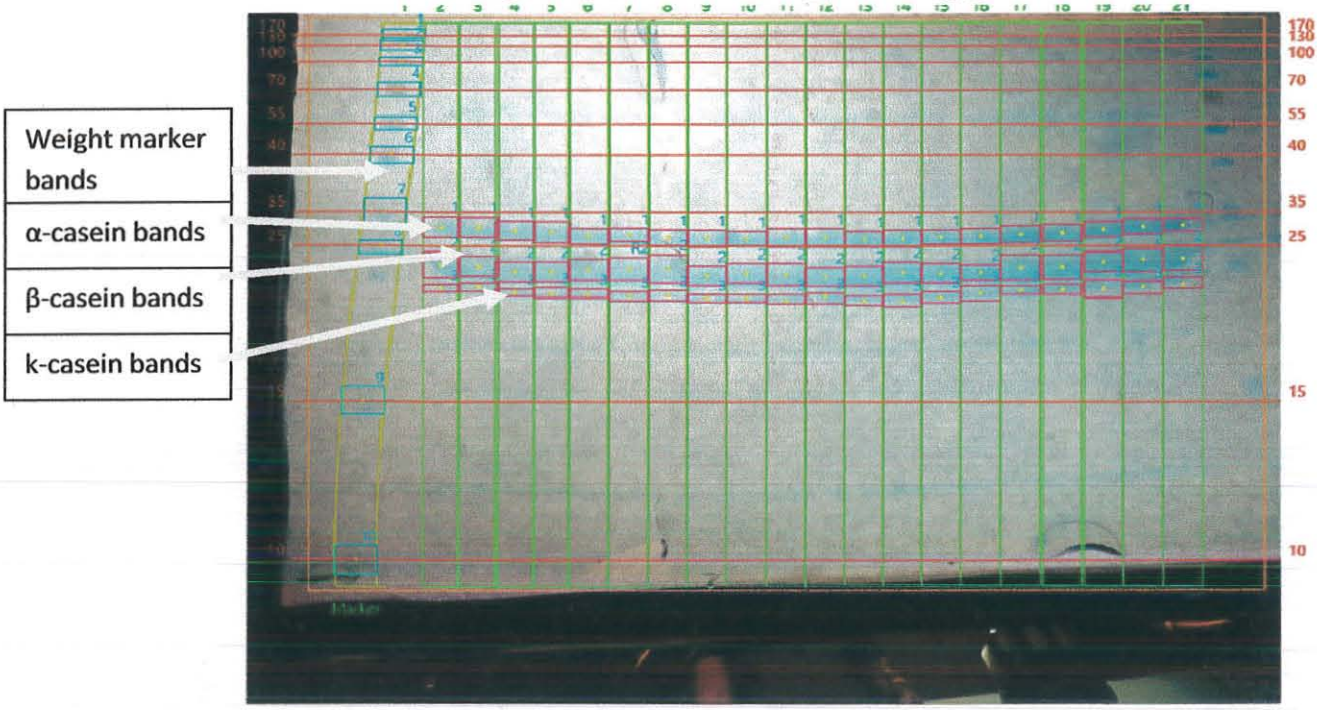

Image Information

Creation Date	8/5/2014 4:04:04 PM
User Name	
Image Area (mm)	X: 225.778 Y: 169.333
Image Pixels	X: 640 Y: 480
Pixel Size (um)	X: 352.778 Y: 352.778

Molecular Weight Analysis Details

Standard	PageRuler Prestained Protein Ladder
Standard Lanes	1
Regression Method	Linear (semi-log)

Lane and Band Analysis

Lane 1(weight marker)

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	MW (kDa)	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	3424268	158	21672	24929	245092.00	1551.22	0.020	170	170.00	0.55	1.78	1.05
2	5514963	185	29810	25443	1230060.00	6648.97	0.040	130	130.00	2.76	8.96	1.69
3	4864239	159	30592	25957	1331005.00	8371.10	0.069	100	100.00	2.99	9.69	1.49
4	7737756	266	29089	26085	1699860.00	6390.45	0.117	70	70.00	3.82	12.38	2.38
5	6528571	239	27316	24672	1472747.00	6162.12	0.177	55	55.00	3.31	10.72	2.00
6	8231710	292	28190	25700	1565506.00	5361.32	0.234	40	40.00	3.52	11.40	2.53
7	13211085	452	29228	27242	1592985.00	3524.30	0.333	35	35.00	3.58	11.60	4.06
8	7059533	237	29787	29041	645529.00	2723.75	0.394	25	25.00	1.45	4.70	2.17
9	18011074	505	35665	33667	1954401.00	3870.10	0.670	15	15.00	4.39	14.23	5.53
10	24382618	532	45831	42919	2488557.00	4677.74	0.952	10	10.00	5.59	18.12	7.49

Lane 2

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	6281594	324	19387	25186	1538902.00	4749.70	0.362	38.82	3.46	66.19	3.00
2	9315993	408	22833	26728	1142364.00	2799.91	0.429	32.02	2.56	49.13	4.45
3	3009984	112	26874	26471	356281.00	3181.08	0.471	28.36	0.80	15.32	1.44

Lane 3

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	8843370	346	25558	25443	725960.00	2098.15	0.362	38.78	1.63	70.31	3.31
2	9105253	346	26315	25957	474072.00	1370.15	0.431	31.78	1.06	45.92	3.41
3	3265185	146	22364	26214	167582.00	1147.82	0.469	28.47	0.38	16.23	1.22

Lane 4

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	7990901	326	24511	24672	337265.00	1034.56	0.369	38.11	0.76	25.16	3.11
2	8217061	347	23680	25186	45182.00	130.21	0.441	30.87	0.10	-3.37	3.20
3	3849089	123	31293	25700	1048502.00	8524.41	0.478	27.74	2.35	78.21	1.50

Lane 5

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	8171315	341	23962	23901	577622.00	1693.91	0.371	37.79	1.30	17.47	3.60
2	9048713	318	28455	24672	1744642.00	5486.30	0.441	30.86	3.92	52.76	3.98
3	4448413	157	28333	24415	984779.00	6272.48	0.481	27.54	2.21	29.78	1.96

Lane 6

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	7033062	271	25952	23644	1157095.00	4269.72	0.379	36.98	2.60	69.26	2.95
2	8632887	372	23206	24543	102379.00	275.21	0.438	31.21	0.23	6.13	3.62
3	3598514	147	24479	23901	411157.00	2796.99	0.482	27.43	0.92	24.61	1.51

Lane 7

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	4524228	227	19930	22616	236224.00	1040.63	0.376	37.27	0.53	14.35	1.95
2	9046657	395	22902	22616	445374.00	1127.53	0.436	31.32	1.00	27.06	3.90
3	4679970	152	30789	23644	1436655.00	9451.68	0.485	27.21	3.23	87.29	2.02

Lane 8

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity /area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	6428084	271	23719	22616	698909.00	2579.00	0.381	36.72	1.57	24.10	2.90
2	11861835	487	24356	23130	1441537.00	2960.04	0.436	31.33	3.24	49.71	5.35
3	4307577	170	25338	22616	759476.00	4467.51	0.484	27.33	1.71	26.19	1.94

Lane 9

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	7070841	292	24215	22359	935209.00	3202.77	0.383	36.57	2.10	56.24	3.22
2	7179809	339	21179	23130	135585.00	399.96	0.449	30.21	0.30	8.15	3.27
3	3320183	121	27439	23130	863184.00	7133.75	0.489	26.93	1.94	51.91	1.51

Lane 10

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	6213232	243	25568	23901	832639.00	3426.50	0.383	36.57	1.87	30.49	2.78
2	8283881	340	24364	22873	1021557.00	3004.58	0.444	30.63	2.29	37.41	3.70
3	3392143	121	28034	23901	876822.00	7246.46	0.489	26.93	1.97	32.11	1.52

Lane 11

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	7846467	316	24830	23644	884634.00	2799.48	0.381	36.71	1.99	49.34	3.42
2	9191348	387	23750	23901	550083.00	1421.40	0.446	30.43	1.24	30.68	4.00
3	3447912	145	23778	24286	358064.00	2469.41	0.492	26.64	0.80	19.97	1.50

Lane 12

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	8168488	316	25849	24672	1046517.00	3311.76	0.381	36.71	2.35	56.07	3.44
2	9003995	317	28403	24929	1519625.00	4793.77	0.450	30.08	3.41	81.42	3.79
3	3036455	169	17967	25186	699751.00	4140.54	0.486	27.13	1.57	37.49	1.28

Lane 13

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	7726962	267	28939	26985	1345982.00	5041.13	0.384	36.44	3.02	39.81	3.13
2	9706376	340	28548	26985	1114652.00	3278.39	0.449	30.19	2.50	32.97	3.93
3	4200151	145	28966	25443	920208.00	6346.26	0.492	26.64	2.07	27.22	1.70

Lane 14

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	8685058	292	29743	28013	1310945.00	4489.54	0.383	36.57	2.94	33.77	3.38
2	10939976	365	29972	28527	1418913.00	3887.43	0.443	30.75	3.19	36.55	4.26
3	5695891	194	29360	26214	1152540.00	5940.93	0.492	26.64	2.59	29.69	2.22

Lane 15

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	7713341	243	31742	29298	1395480.00	5742.72	0.380	36.84	3.13	55.05	2.88
2	10447564	340	30728	29041	1056481.00	3107.30	0.444	30.64	2.37	41.68	3.90
3	4638850	182	25488	27499	83041.00	456.27	0.487	27.08	0.19	3.28	1.73

Lane 16

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	8808675	267	32991	30840	1399443.00	5241.36	0.379	36.98	3.14	35.61	3.14
2	8904022	267	33348	30840	1383825.00	5182.87	0.443	30.75	3.11	35.21	3.18
3	7461995	243	30707	28270	1146755.00	4719.16	0.480	27.64	2.57	29.18	2.66

Lane 17

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	10437541	292	35745	33924	1741909.00	5965.44	0.375	37.39	3.91	54.39	3.57
2	14599399	438	33331	32125	1775954.00	4054.69	0.434	31.56	3.99	55.45	4.99
3	3605196	145	24863	30069	315010.00	-2172.48	0.475	28.05	0.71	9.84	1.23

Lane 18

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	10553962	292	36143	34181	1479058.00	5065.27	0.373	37.67	3.32	32.64	3.45
2	14759510	419	35225	32639	1963948.00	4687.23	0.433	31.64	4.41	43.35	4.83
3	5915369	171	34592	31097	1087821.00	6361.53	0.473	28.15	2.44	24.01	1.93

Lane 19

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	10305700	267	38598	35980	1568819.00	5875.73	0.366	38.37	3.52	59.23	3.20
2	10765216	340	31662	34695	77516.00	227.99	0.424	32.51	0.17	2.93	3.35
3	9822797	292	33639	31611	1157241.00	3963.15	0.472	28.25	2.60	43.69	3.05

Lane 20

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	10670640	267	39964	37008	1457467.00	5458.68	0.361	38.94	3.27	1975.91	3.21
2	12836636	386	33255	35466	233478.00	604.87	0.419	32.94	0.52	316.53	3.87
3	5494146	217	25318	32382	1150227.00	5300.59	0.468	28.59	2.58	1559.38	1.66

Lane 21

Analysis results table

Band Id	Volume (Intensity)	Area (Pixels)	Density (Intensity/area)	Median (Intensity)	Local Bkg. Corr. Volume (Intensity)	Local Bkg. Corr. Density (Intensity/Area)	Rf	Calc. MW (kDa)	Relative Quantity	% Purity	% Purity (Lane)
1	9264336	219	42302	39064	1532019.00	6995.52	0.358	39.22	3.44	25.64	2.68
2	16578556	419	39566	36751	2321782.00	5541.25	0.417	33.08	5.21	38.86	4.79
3	8226570	193	42624	34695	2120516.00	10987.13	0.464	28.89	4.76	35.49	2.38

3 Preparation of stock solutions and buffers (Laemmli 1970 ; Liljeruhm et al, 2014)

a) 30% acrylamide solution

Acrylamide (29.2g) and bisacrylamide (0.8 g) were taken and volume was made up to 100ml with distilled water and it was filtered and stored at 4°C.

b) 1.5M Tris –HCl (PH8.8)

Tris (18.24g) was taken and dissolved in 60ml distilled water and pH was adjusted to 8.8 with 1N HCl and volume was made up to 100ml in volumetric flask with distilled water and stored at 4°C.

C) 0.5M Tis –Hcl (PH6.8)

Tris (6.08g) was taken and dissolved in 60ml distilled water and pH was adjusted to 6.8 with 1N HCl and volume was made up to 100ml in volumetric flask with distilled water and stored at 4°C.

d). 10% SDS (Sodium Dodecyl sulphate)

SDS (10gm) was taken and dissolved in 80ml distilled water and volume was made up to 100ml in volumetric flask and stored at room temperature.

e) 10% Ammonium persulphate (APS)

A fresh solution of ammonium persulphate was prepared for each gel by dissolving 1g ammonium persulphate in distilled water and made up to 10ml in volumetric flask with distilled water.