EFFECT OF HEAT TREATMENT ON PROPERTIES OF PROTEIN AND RENNETABILITY OF CAMEL MILK

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ALMAZ GENENE

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Almaz Genene

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APPROVAL SHEET

HARAMAYA UNIVERSITY

POST GRADUATE PROGRAM DIRECTORATE

We hereby certify that we have read and evaluated this Thesis entitled with **Effect of Heat Treatment on properties of Protein and Rennetability of Camel Milk** prepared under our guidance by Almaz Genene. we recommend that it be submitted as fulfilling the Thesis requirement.

1. Richard Ipsen (Prof.) _____ _____
   Major Advisor Signature Date

2. Mitiku Eshetu (PhD) _____ _____
   Co-Advisor Signature Date

As members of the Examining Board of the MSc Thesis Open Defense, we certify that we have read and evaluated the Thesis prepared by **Almaz Genene** and we recommend that the Thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in **Dairy Technology.**

Yosef Tadese (PhD) _____ _____
Chairman Signature Date

Yonas Hailu (PhD) _____ _____
Internal Examiner Signature Date

Mestewet Taye (PhD) _____ _____
External Examiner Signature Date

DEDICATION

This thesis is dedicated to my child Samuel who could not get full care of mine during this study and all family members for unlimited supports and strong prayer for the success of my life.

STETMENT OF THE AUTHER

First, I declare that this thesis is my glimmer work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for MSc. degree at Haramaya University and is deposited at the University Library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. brief quotations from Thesis may be made without special permission provided that accurate and complete acknowledgement of the source is made. Request for permission for extended quotation from or reproduction of this Thesis in whole or in part may be granted by the head of the School or department when in her judgment in the proposed the use of the material is in interest of scholarship. In all other instance. However, permission must be obtained from the author of the Thesis.

Name: Almaz Genene

Place: Haramaya University, Haramaya

Date ---------

signature.....

ACRONYMS AND ABBREVATIONS

AOAC	Association of Official Analytic Chemists
BME	Beta-mercaptoethanol
BSA	Bovine Serum Albumin
CMP	Caseino micro peptide
CSA	Camel Serum Albumin
ECSA	Ethiopian Central Statistical Agency
FAO	Food and Agriculture Organization of the United Nations
GMP	Glycomacropeptide
GT	Gelation Time
HCL	Hydrochloric Acid
HUDL	Haramaya University Dairy Laboratory
Ig	Immunoglobulin
PI	Point of Isoelectric
LF	Lactofferin
TCA	Trichloroacetic Acid
LTLT	Low Temperature Long Time
MOA	Ministry of Agriculture
NC	Non Coagulated
NCN	Non Casein Nitrogen
NPN	Non Protein Nitrogen
RCT	Rennet Clotting Time
SAS	Statistical Analysis System
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gelelectrophoresis
TG'MAX	Time to G'max
WPN	Whey Proteins Nitrogen
WPD	Whey Protein Denaturation
α-La	α-Lactalbumin
β-Lg	β-Lactoglobulin

BIOGRAPHICAL SKETCH

The author, Almaz Genene, was born on January 03,1984 in Chole woreda, Aris zone in Oromia region from her Father Genene Tafes and her Mother Fanaya Gizaw. She attended her primary education in Chole joiner and elementary school from 1990-1998. While secondary education from grade 9 to 10^{th} in 1999 - 2000 in Chole high school at Chole whereas grade $11^{th}$and 12^{th} from 2001-2002 at Abomsa Arbagnoch senior secondary school at Abomsa. Finally she completed her secondary education in 2002 by taking National school leaving examination. Then she joined Assella Agricultural Technical and Vocational Educational Training College (ATVETC) in 2003 for Diploma training in field of Animal Science and graduated in August 4, 2005. Then after she was employed in Chole woreda Agricultural office and served as Development Agent for Four years. She joined Ambo University in 2010 to upgrade to Bachelor Science degree in Animal Science and completed her study in 2012. She returned to her former office and assigned as supervisor of development agents. Then she join Ethiopian Meat and Dairy Industry Development Institute in 2013 and served as dairy technologist until she joined Haramaya University in September 2015 to pursue her M.Sc. programme in the field of Dairy Technology through sponsorship of Haramaya Camel Dairy Project.

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Effect of Heat Treatment on Properties of Protein and Rennetability of Camel Milk

ABSTRACT

The current study was conducted at Haramaya University Dairy laboratory with the main objective of investigating how heat treatment affects whey proteins and rennetability property of camel milk for cheese making. Completely randomized design (CRD)was used by evaluating effect temperature (heated at 40^0C,65^0C/30min,72^0C/30 sec, 75^0C/5 min, 85^0C/5 min and 90^0C/5 min).Unheated milk used for alternative reference during chemical and whey protein denaturation evaluation. Similar experimental setup was also used for cow milk for reference. Heat treatment was done in thermostatically controlled water bath. The chemical composition of milk analysed using milkoscan, whey protein denaturation analysis was done using gel electrophoresis and kjeldahal method. While for rennetabilty properties parameters Rheometery ReoRox G2-4 was used. The result showed that raw camel milk significantly ($P<0.05$) had lower percentage of protein, solid non-fat, casein and lactic acid than cow milk. Heat treatment have no significant effect ($P>0.05$) on gross chemical content of camel and cow milk. Total whey protein denaturation % increased ($P<0.05$) as heating temperature increased in both camel and cow milk. In camel milk α-lactalbumin (α-La)showed less denaturation while Camel serum albumin denatured at higher heat treatment as band become invisible. While in cow milk β-lactoglobulin and α-La denatured as temperature level increased while bovine serum albumin the denaturation percentage increased constantly as temperature increased. Gelation time, and Time to G'max were significantly increased($P<0.05$) as heat treatment increased while G'max was lowered as heating temperature increased for camel and cow milk. For camel milk short (6min) gelation time was observed for heated at 40^0C while it increased to 14 min at 65^0C. Significantly lower G'max observed at 75,85 and 90^0C/5min for camel while for cow milk at 90^0C/5min. From heat treated higher at 65^0C/30min (51pas for camel and 111 for cow) than the other temperatures. Similarly, time to G'max was also increased as heat treatment increased. Pre heat treatment of milk can determine the gelation time and curd aggregation property of cheese. Therefore, lower Pasteurization temperature that results less denaturation of whey protein was optimal for cheese milk treatment. Pasteurization temperature at 72^0C/30 sec can also be an alternative for cheese milk treatment to utilize whey proteins without affecting coagulation processes.

keywords: *Camel milk, protein, denaturation, heat treatment, rennetability, whey proteins*

1. INTRODUCTION

Camels (*Camelus dromedarius)* are the most important domesticated animals mainly in arid and semiarid areas of tropical and sub-tropical countries. From the total Camel population 27,010,350 all over the world 24,167,155 heads are estimated to be *dromedaries* Food and Agriculture organization of the United Nation (FAO, 2013). Camels can survive sever environmental conditions and produces more volume of milk than other domestic mammalians. This makes *camelus dromedarius* promising source of milk and meat for the society in arid and semi arid areas (Al haj and Al Kanhal, 2010; Hattem *et al*., 2011;Gerosa and Skoet, 2012). Ethiopian camels were Dromedar*y* type and their population was estimated to be 4.5 million Livestock Master plan (LMP, 2015). Majority of them distributed in drier areas of Eastern part and mainly kept for milk production as reviewed by Aleme and Mohammed (2014).

Camel milk contains all essential nutrient as bovine milk except some difference in its chemical composition and processing property (Farah,1993 and Marawa *et al*., 2013). Whey proteins of camel milk are relatively more heat resistant than cow's and buffalo's milk (El-Agamy, 2000). However, camel milk is poor in heat stability as, it coagulates within less than 1 min at high temperature 130^0C or 140^0C that widely used for bovine milk heat stability indication. This might expected to be due to lack of the whey protein β-lactoglobulin (β-lg) and deficiency in κ-casein in camel milk since they have greatest impact on the heat stability of bovine milk (Farah, 1993; El-Agamy, 2000; O'Connell and Fox, 2011; El haj and Freigoun, 2015; Felfuol *et al*., 2016).

In the dairy processing heat treatment is applied to ensure safety, increase shelf-life and improve desirable properties of the products (Donato and Guyomarc'h, 2009). Heat treatment can be done at least in one level that may range from mild (thermization at 65^0C for 15 second) to sever in-container sterilization at 110–115^0C for 10–15 min (O'Connell and Fox, 2011). Milk pasteurization could be batch or continuous processes (Lewis and Deeth, 2008). Knowledge of the effect of heat treatments on individual milk proteins is very important to understand changes in biological and functional properties of milk which occur during heat treatment. Heat treatments of camel milk can improve the microbial quality and also important to extended its shelf life (Mohamed and El Zubeir, 2014). However, in most areas including Ethiopia camel milk is mostly consumed in its raw form without any heat treatment and some time consumed in traditionally fermented form when

it is slightly sour or strongly soured such product traditionally known as '*dhanaan'* in Ethiopia (Eyasu, 2007) *'Garris'* in Sudan (Siddig *et al.,* 2016), *'shubat*' in kazakhstan (Ishii and Nurtazin, 2014) and '*sussa*' in Somali (Farah *et al.,* 2007) have been traditionally known by pastoralist society.

Production of cheese from camel milk is not known traditionally due to slower coagulation property (Ramet, 2001; Saliha1 *et al,* 2011; Aleme and Mohammed, 2014), lower yield and weak curd structure. This can be due to peculiar properties of camel milk with a lower κ-casein content and brooder casein micelles and lower total solid content (Farah,1993 and Khan *et al.*, 2004). However, currently different trials were done to make cheese from camel milk. Fresh soft white cheese from camel milk was manufactured using different parameter like by mixing with milk of cow, buffalo, lowering pH, adding calcium chloride (Shahein *et al*., 2014; Siddig *et al*., 2016) in Ethiopia using different coagulant (Yonas *et al*., 2014; Haileeyesus and Shimelis, 2016). Use of camel chymosin results in better camel milk coagulation for cheese making (Benkerroum *et al.*, 2011 and Yonas *et al*., 2014).

When considering cheese making, heat treatment of cheese milk is mandatory for microbial safety and quality before actual cheese processing stages since milk can be contaminated with harmful microorganisms that can lowers the quality of cheese. Heat can reduces damage to caseins by proteolytic bacteria on storage. Having such advantages heat treatment of cheese milk is an alternative method for controlling of microbiological defects in cheese manufacturing (Schreiber, 2001 and Kelly *et al*., 2008). Milk of bovine that planed for cheese making commonly pasteurized at 63-65°C, 30 minute or 72°C, 16 seconds based on this high temperature short time (HTST) pasteurization at 72°C for 15 sec is the common standard heat treatment of bovine milk for Cheese making (Hougaard *et al.,* 2010; Sbodio and Revelli, 2012).

In cheese making milk heat treatment positively affects cheese yield due to incorporation of whey proteins. This is why the dairy industry occasionally apply heat treatments more severe than pasteurisation for bovine milk in addition to inactivation of bacteria and their spores. However, higher heat treatment can negatively affects rennet coagulation process and results weak curd structures formation due to high interactions of whey protein with casein micelles interfere that affect cheese quality by reducing syneresis (Rynne *et al*., 2004; Kelly *et al*., 2008 and Hougaard *et al.,* 2010).

During heating process at a temperatures especially above 60^0C different change could occurs in milk mainly denaturation of whey proteins, interactions between denatured whey proteins and casein micelles, conversion of soluble calcium, magnesium and phosphate to the colloidal state (Singh and Waungana, 2001). Whey protein are folded stricture however during heating whey protein fraction of milk mainly of B-lactoglobulin(β-Lg) and a-lactalbumin (α-La) under goes conformational changes of the molecule which result expos of a reactive thiol (Astrid *et al*., 2003). The Heat sensitivity property of whey proteins (WPs) causes difficulty in their wider application in food products as unique functional ingredients and processing (*Dissanayake et al.,* 2013).

Even though literatures shows that camel whey protein is relatively heat resistance and also Knowing that β-Lg absent in camel milk (Farah,1993 and Hinz *et al*., 2012) knowledge of understanding enzyme coagulation properties of heat treated camel milk and denaturation of individual whey protein can provide optimization of milk heat treatment temperature during cheese making. This can have advantage to get quality product and improve cheese yield since milk microbial contamination is major problem and denatured whey proteins also affects the coagulation process (Singh and Waungana, 2001 and Rynne *et al*., 2004).

Heat denaturation of whey protein of camel milk studied by (Farah, 1998 and El-Agamy, 2000 and deposit formation (Felfuol *et al.,* 2016). While concerning factors that affecting coagulation properties of camel milk like effect of lactation stage, curd acidification, incubation temperature, calcium addition, pH and enzyme concentration on gelation property of camel milk was studied before by (Konuspayeva *et al*., 2014 and Yonas *et al.,* (2016a). However, effect of heat treatment on whey protein and effect of denatured whey protein on the coagulation property of camel milk still need to be studied in detail at different level of higher temperatures due to unique whey protein property and for utilization of whey proteins without affecting the coagulation property. Therefore this study was conducted with the general objectives of understanding the effect of heat treatment on protein and rennetabilty properties of camel milk.

Specific objectives

- Investigate the effect of different level of heat treatment on whey proteins or denaturation of whey proteins of camel milk
- Investigate effect of heat treatment on Gelation time and coagulum strength or gel firmness of camel milk at different level of heat treatment

2. LITERATURE REVIEW

2.1. Camel Milk Production

Camels are a multipurpose animal that can offer milk, meat, wool, used for transport, tourism and agricultural work from all purpose of keeping camel milk production is principal especially dromedaries camels are good source for the desert society including in prolonged drought period (Saliha *et al*., 2013 and Faye, 2015). The global share of camel milk was 0.3% which is comparatively less than that of 83% of cow, 13 % buffalo, 2.4% goat and 1.4% sheep (Gerosa and Skoet, 2012). Gestation length of camel is 13 months and can give birth once per two year (Ishii and Nurtazin, 2014). Camel have natural adaptation mechanism to provide calf with enough water during dry season by increasing moisture contain of their milk (Farah, 1993;Yadav *et al*., 2015). Camel can produces 1000 to 2000 litre of milk per lactation period of 8 to 18 months (FAO, 2006). From the two camel species *Camelus* b*actrianus* know to be lower in its milk production relatively than *Camelus dromedarius* (Faye, 2015). Dromedary camel can produce up to 5.5 litres of milk per day (Ishii and Nurtazin, 2014). In Ethiopian camel dromedary can give up to 9 litre per day if managed in better condition according to the report of Eyasu (2007). Lactating camels that supplemented with concentrate feed showed a considerable increase in milk yield and quality with is increases milk yield from 5.5 to 11.3 litre when feed supplementary feed (Moges *et al*., 2016). Camel have a potential to produce milk for a longer period even in dry seasons than other mammals.

2.2. Physical Property of Camel Milk

The awareness of physical and chemical properties of milk is crucial and basic in any dairy industry, laboratory and dairy input production industry. Physically camel milk is opaque white due to the fat as finely homogenized throughout the milk. Camel milk has sweet and sharp taste while sometimes salty. The taste can be affected by type of fodder and the availability of drinking water which also leads to variation in total solid content (Farah, 1993;Yadav *et al*., 2015). Camel milk have 6.6 pH value and specific gravity of 1.029 from the investigation done in Sudan Khartoum (Sabahelkhier *et al*., 2012) on the other hand 1.033 ±0.015 reported by Shamsia (2009). According to Yonas *et al.,* (2014) pH of camel milk was 6.64 ± 0.02 and 0.15 ± 0.01% titirable acidity. Camel milk acidity varied from 0.13 to 0.17%, with grand mean value of 0.15% which is higher than 0.12% of cow milk

(Sabahelkhier *et al*., 2012; Abbas *et al.,* 2013). The titratable acidity of milk is the measure of lactic acid formed in camel milk. The result of titratable acidity is dependent on the hygienic condition of milk collection and handling (Kouniba *et al.,* 2005).

2.3. Chemical Property of Camel Milk

2.3.1. Total solids

Milk is a highly diverse fluid consisting a number of components like water, lactose, fat, protein, organic acids, and minerals (Hallén, 2008). Total solids content of camel milk is reported to be 9.9±1.189 % Hattem *et al*., (2011) while Shahein *et al.* (2014) report indicates 11.07% as compared to 17.60% buffalos milk and 13% of bovine milk which were higher than camel milk. Whereas total solids content of camel milk from Errer valley of Ethiopia found to be 11.6 ± 0.27% (Yonas *et al*., 2014).

2.3.2. Fat

Milk fat naturally present as small globules which are surrounded by membrane protein called milk fat globule membrane with size dependent on individual cow milk. For camel milk the average fat globule size 2.9 micrometres which is smaller than 3.78 mm that of bovine milk fat globules size. Camel milk is poor in creaming properties which results from a deficiency in agglutinin a protein that cover the layer of fat globules and responsible for cluster formation. Camel milk fat consist of polyunsaturated fatty acid, completely homogenized and gives the milk a smooth white appearance (*El-Agamy*, 2009; Al Haj and Kanhal, 2010; Claeys *et al*., 2014; Yadav *et al.,* 2015). Camel milk fat is characterized by very low content of short chain fatty acids and higher contents of long chain fatty acids (Shamsia, 2009). The fat % of camel milk including other animal were indicated in table1. Fat composition can be affected by feed type ,breed (Meiloud *et al*., 2011) and species of the animals. In cheese production fat plays in development of flavour, smell and body or texture as the fat globules are trapped in the protein network created in gel formation (Kelly *et al*., 2008).

Table 1.Gross chemical composition (%) of milk from different animal species

Species	Protein	Lactose	Fat	Total solid	Ash
Human	1.94	6.45	2.1	10.71	0.22
Goat	3.30	4.40	3.90	12.0	0.70
Camel	2.95	4.30	3.60	11.7	0.75
Cow	3.40	4.80	3.75	12.8	0.71
Sheep	6.35	5.00	6.90	19.3	0.85

Source (Shamsia, 2009 and Sabahelkhier *et al*., 2012)

2.3.3. Proteins

Proteins represent one of the greatest contributions of milk to the human nutrition and milk and can be grouped in to two major part. The precipitate formed when adjusting milk to pH 4.6 is casein, whereas the protein remaining in solution is whey protein, or serum protein. Bovine milk generally contains about 3.5% protein from this around 80% are caseins and 20% are whey proteins (Hallén, 2008). Camel milk has protein content of 2.86% at pastoral and 3.30% at farm level (Osman, 2016). Almost similar result of protein 2.95% reported by Sabahelkhier *et al*., 2012 in table 1. The protein content of milk vary based on species and management condition (Osman, 2016). Camel milk protein has health benefits as hypocholesterolaemic, hypoglycaemic, antimicrobial and alternative protein source for consumer with hypersensitive to bovine milk protein or allergic (Hinz *et al*., 2012 and Al Haj and Al Kanhal, 2010). In cheese production, the composition of protein is crucial since the major part of cheese is produced from the casein which is major part of milk protein (Konuspayeva *et al*., 2014).

2.3.3.1. Casein

Casein is a major and important protein fraction of milk in cheese production (Kouniba *et al*., 2005). Casein fraction of bovine milk exists as polydisperse, large, roughly spherical colloidal particles in average ~150 nm diameter called “casein micelles”. The size, form and structure of the casein micelle have great importance for the milk industry especially

for cheese making. The average casein micelle size varies widely between milk of individual cows. Casein micelle size is also variable and can range between 154 and 230 nm in bovine milk (Hristov *et al*., 2016). While in camel milk casein micelles size range from 260-300 nm which is relatively broad than bovine milk (Farah, 1993).

The casein micelle in milk consists of four caseins: alpas$_1$, alphs$_2$, βeta- casein and kappa -casein figure1 (Fox and Brodkorb, 2008 and Dalgleish and Corredig, 2012). From the caseins κ -casein is important for the stability and properties of the casein micelle in milk due to this casein micelles have hydrophilic property. The glycomacropeptide hairy like and negative charge part of k-casein on the micelles helps to for the stability of milk. The hairy layer can be collapses by acidification, rennet enzyme and heat and then losses its stabilizing role The stability of casein micelles is critical for the technology of most dairy products. It can be affected by rennet, heat treatment, acidification (Dalgleish, 2007; Dalgaleish; Corredig, 2012 and Hristov *et al*., 2016). These properties of casein micelles in different condition that is in native, during the effect of rennet, heat treatment and acidification, are indicated in figure 1.

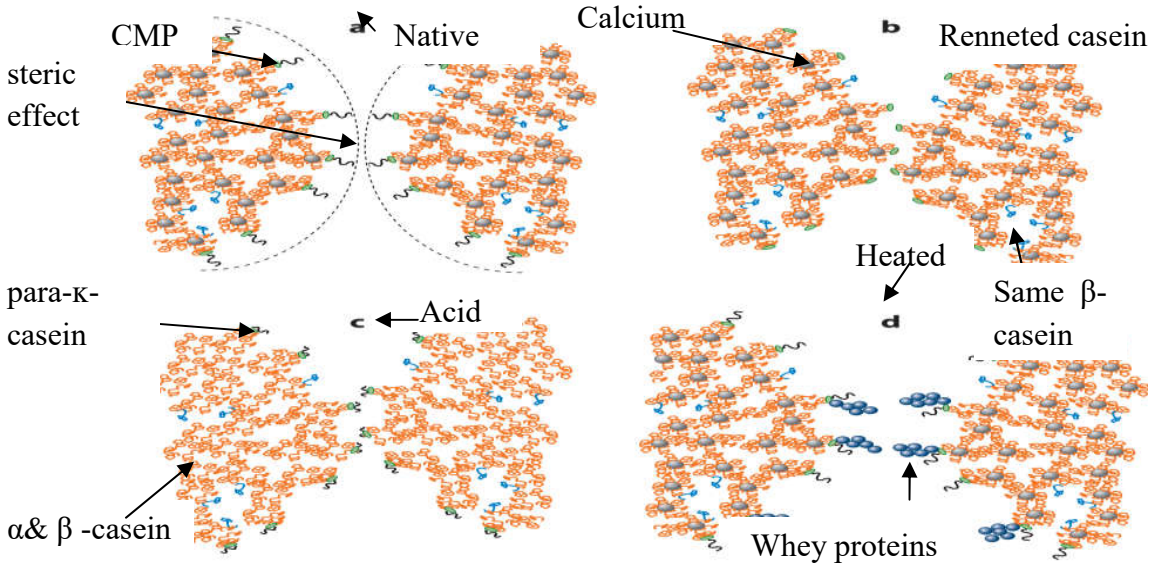

Figure 1. The structure of a native and modified casein micelle while effected by enzyme, acid and heat.

Source: (Dalgleish and corredig, 2012).

NB. The colours stands for Para-κ-casein is green, the caseinomacropeptide chains is black, αs- and β-caseins is orange, and calcium phosphate nanoclusters are represented by

grey spheres, denatured whey proteins are represented by dark blue spheres and some β-casein by blue.

The casein content of dromedary camel ranges 1.63 to 2.76 percent casein protein which caver 52 to 87 percent of total milk protein (Khaskheli *et al.,* 2005). The molecular weights and amount of each casein fractions are indicated in table 2 below. Camel milk lower in its κ- casein (Elhaj and Freigoun, 2015), higher in its β-casein content. According to Wangoh *et al.,* (1998) camel milk casein and whey proteins separation is possible at pH 4.3 and temperature of 20℃ which was lower than the Point of Isoelectric (PI) of bovine milk at which casein precipitation at pH 4.6 during acid precipitation if the precipitation of camel casein is performed at pH 4.6 small fraction of casein may remain in the whey and lead the overestimation of non-casein nitrogen (NCN). In the production of ether traditional or varies new dairy products casein micelles are the base as their aggregation property was very important. However, it was needed or avoided based on product type. For instance aggregation is needed in cheese and yogurt production but did not needed in liquid milk production.

2.3.3.2. Whey proteins

Whey protein are the second protein components next to casein. Whey proteins exist as soluble globular proteins and are characterised by a relatively high level of intra-molecular disulphide bonding. Can be grouped in to four major fractions β-Lactoglobulin (β-Lg), α-Lactalbumin (α-La), Bovine Serum Albumin (BSA) and Immunoglobulins (Ig's).and other smaller fraction including lactoperoxidase, serum transferrin (Fox and Kelly, 2006; Jovanovic *et al*., 2007; Donato and Guyomarcho, 2009; El-Hatmi *et al*., 2015). Camel milk whey protein cover 20 to 25% total protein (Abbas *et al.*, 2013). The major camel milk whey portion is α-la followed by Camel serum albumin (CSA) whereas in bovines β-Lg cover 50% of the total whey protein, α-La cover 25 % (Kappeler *et al*., 2003) table 2. Camel milk have no β-lg (Elhaj and Freigoun, 2015; Felfoul *et al.,* 2015; Omar, 2016). Camel milk whey proteins has high biological value such as lysozyme which is 4.9 times higher than cow's milk and 11 time that of buffalo milk. Camel milk is also higher in immunoglobulin 1.64 mg/ml verse 0.67mg/ml of cows (Farah, 1986; El-Agamy, 2000; Shamsia, 2009; Marawa *et al*., 2013).

Camel milk is also know to have unique whey protein components like whey acidic protein and higher amount of GLYCAM-1(Glycosylation-dependent cell adhesion molecule 1). Camel milk contains protease peptone component (PP3) fraction called lactophorin, belongs to the glycosylation-dependent cell adhesion molecule (GLYCAM-1) family. GLYCAM 1 have an immunological function for camel or its suckling young since have an antibacterial activity against pathogenic agents as reviewed by El-Salam and El-Shibiny,(2013). The molecular weight and volume of whey proteins in camel and bovine milk is indicted in table 2 .

Table 2. Whey and casein protein component of camel and bovine milk.

Whey protein	**Molecular weight (KDa)**	**Camel (g /L)**	**Bovine (g /L)**
β-lactoglobulin	18.4	-	3.1** (3.2$^{\#\#}$)
α- lactalbumin	14.2-14.4	2.3 **	1.1** (1.2##)
Serum albumin	66-69	2.2**	0.35a
Lactoferrin	75-76	1.74±0.06 mg/l* (0.22mg/mL)+	0.10-0.50mg/mL+
Immunoglobulin IgMg heavy and light chain	60,29 &55.26 respectively for camel and bovine+	1.5**	0.20**
GLYCAM-1	21$^{\#}$	0.95**	0.30**
Whey acidic protein	12.5	0.16**	-
Caseins			
β-Casein	24.9++	15.6**	9.8(9.3##)
αs1-Casein	24.7++	5.3**	9.5(10##)
αs2-Casein	21.9++	2.3**	2.5(2.6##)
k-Casein	22.29-22.98++	0.8**	3.3++

Source:-(Kappeler *et al.*, 1998++;Korhonen, 2009; El Agmay, 2009+, Raikos, 2010## ; Omar *et al*., 2016*, Yonas *et al*., 2016b **, unpblishd datof Sonia Su, 2016$^{\#}$).

2.3.4. Lactose

Lactose is the major carbohydrates of milk and dromedary camel milk contains lactose 4.91 ± 0.70% (Meiloud *et al*., 2011). The work done in Sudan which compares the composition of cow and camel milk resulted that the lactose content was 4.85% in camel milk while 4.97% for cow milk which is slightly higher than that of camel (Siddig *et al*., 2016).

2.3.5. Mineral

Milk is a good source of most the essential minerals that is required for human health. The mineral content of milk also termed as total ash content of milk. In the milk of dromedary camel the total ash content ranges between 0.60 to 0.90% with the average of 0.79± 0.07% (Al Haj and Kanhal, 2010 and Abbas *et al.*, 2013). On the other hand mineral content is reported to be as 1.30 ±0.09 % of Mauritanian dromedary camel which looks higher the above literatures (Meiloud *et al*., 2011).

2.3.6. Vitamins

In dromedary camel milk vitamins like D, E, A, C and vitamins of B group are vitamins know to be found form which the content of vitamin C has been estimated to be two to three folds higher than that of the cow's milk this make camel milk a good alternative source of vitamin C to society in harsh environment where fruits and vegetables are lacking. The content of vitamin A, E and B1 as 20.1±10.0 µg, 32.7±12.8 µg and 19.6±6.4 mg in camel milk and 60.9±25.6 µg, 171.0±114.4 µg and 34.7±8.1 mg in cow milk respectively (Shamsia, 2009 , Alwan and Igwegbe, 2014 and Abbas *et al*., 2013).

2.4. Application of Heat Treatment in Dairy Processing

High water and nutrition content make milk easily damaged by microbial contamination and leads to health risk for consumer (Winarso *et al*., 2011). The processing of milk in to different product can extend and improve product quality. As date shows pasteurization was applied in dairy industry in 1885 by N. Fjord to improve the microbiological quality of cream for butter making after Louis Pasteur applied pasteurization in about 1860 to preserve wine and beer (O'Connell and Fox, 2011).

The most common pasteurization type in dairy batch pasteurization 63^0C,30 min which is longer time lower temperature (LTLT), High temperature short time (HTST pasteurization 72^0C for 15 sec, higher heat shorter time 88.3 1 se , ultra pasteurization 137.8 for 2 sec and UHT for 137 - 150 for 4-15 sec (Walstral *et al*., 2006). The processing in almost all milk and dairy products involves heat treatment. The adjustment of pasteurisation depend on product type . For instance low pasteurisation can be used for treatment of milk that use for direct consumption and cheese while high pasteurisation is used for yoghurt, butter and

kefir production (Ebing and Rutgers, 2006). Heat treatment of milk can also improve the texture of dairy products (Donato and Guyomarc'h, 2009). Heat treatment that leads to denaturation of whey proteins and then association of casein micelles and denatured whey proteins can form additional cross-linkages within the yoghurt gel that can improve the texture (Krzeminski *et al.*, 2011). High thermally treatment resulted in an increase in gel strength of Labneh samples that made from camel milk (Marwa *et al*., 2013).

2.4.1. Improve cheese yield

Heat treatment of the milk to denature the whey proteins and aggregate them with the casein fraction is one of the known method for whey protein utilization during cheese making (Guyomarch, 2006). There were a need occasionally to apply heat treatments more severe than pasteurisation that will results denaturation of whey proteins and their incorporation into cheese curd to improve cheese yield and composition (Kelly *et al*., 2008). This incorporation of whey protein into cheese increased cheese yield especially in the case of low fat cheese (Hinirichs, 2001). situation shown as that report about Domaiti cheese which was made from buffalo's milk in comparison with raw and heat treated milk at 65^0C/15 sec and 72^0C/15 sec shows that highest cheese yield was obtained in pasteurized milk cheese either in fresh or during storage period. This can be because of effect of heat treatment and pasteurization (Salwa and Galal, 2002). Heat treatment can cause important difference on cheese yield. For instance according to the review report of Abd El-Gawad and Ahmed (2011) theoretical cheese yield increment obtained about 0.01 to 0.04 kg for milk with Cheddar cheese yield of 10 kg/100 kg of milk due to heat denaturation of whey protein caused by Higher Temperature Short Time (HTST) of milk prior to cheese making. Approximately 5% of the whey proteins originally present in the milk, especially ß-lactoglobulin can be associated with casein micelles after milk pasteurization. More whey protein denaturetion occur at high heat treatment temperature or above 90°C of cheese milk which can lead a greater increase in cottage cheese yield compared to minimum legal pasteurization (72°C/15 sec) (Vakaleris 1962 as cited by (Abd El-Gawad and Ahmed, 2011).

2.4.2. Improve milk quality

Milk is sterile product in the udder but its quality can be affected after production due to contamination with pathogenic microorganism from animal itself, environment, person during milking, handling and transportation. The initial flora of raw milk can determinant and influence final microbiological quality of milk and milk products (Ritcher and Vadamuthu, 2001). Heat treatment of milk can increase the shelf life of milk. The report from Sudan indicates that, heated camel milk can stay up to 20 days while raw camel milk up to 7 days under refrigerator due to reduction of total bacteria, coliforms, total yeast and mould, psychrotrophic bacteria. Low thermoduric bacterial count in the heat-treated milk which was 6.2×10^5 to 2.03×10^6 at Lower temperature long time (LTLT) and 6.1×10^5 to 2.03×10^6 cfu/ml at HTST than raw milk (Mohamed and El Zubeir, 2014). The authors also conclude that camel milk whey protein were heat resistance and can be pasteurized in order to reduce pathogenic microbial hazards.

2.5. Effect of Heat Treatment on Milk Protein Properties

2.5.1. Effect of heat treatment on gross chemical composition of camel milk

Heat treatment of milk applied to increase milk shelf life can lead to a number of other changes such as decrease in pH, precipitation of calcium phosphate, denaturation of whey proteins and interaction with casein, Miallard browning, modification of casein, hydrolysis of κ-casein can occur (O'Connell and Fox, 2011). The ability of milk product to withstand a particular heating temperature without start of visible aggregation when subjected at a particular temperature is termed as milk 'heat stability'(Kouniba *et al.,* 2005;O'Connell; Fox, 2011).

Pasteurization of camel milk at 72°C/15 second or at 63°C for 30 min have no effect on fat however it changes only if temperature goes more than 85°C and also no effect on protein but at 95^0C (Marwa *et al*., 2013). This authors conclude that no change is seen on gross chemical composition at normal pasteurization. Thermal treatment at 63°C,80°C and 90°C for 30 min and 72°C for 15 sec also have no effect on fat content of camel milk (Hattem *et al*., 2011). Heating have very little effect on mineral content with exception of Cu and Zn.

On the other hand pasteurization does not destroy zinc, copper, iron and calcium in camel milk (Suliman *et al*., 2013).

2.5.2. Effect of heat treatment on whey proteins properties

During heat treatment of milk especially at temperature above 60^0C results significant changes such as the denaturation of whey proteins, then leads to interactions between the denatured whey proteins and the casein micelles and the conversion of soluble calcium to the colloidal state (Singh and Waungana, 2001). Denaturation is unfolding and an exposure of hydrophobic group whey proteins (side chain groups formerly buried in the native structure, especially the reactive thiol groups) or it was simply a structural change of whey proteins which intern depending on the heating conditions that applied on milk (Rynne *et al*., 2004;. Raikos *et al*., 2010). This change affects many properties of the micelles heat stability and rennet coagulation properties (Fox and Brodkorb, 2008).

Most heat-induced changes of whey proteins of bovine milk was on *β*-Lg while *a*-La does not polymerize by itself during heating as it need β-Lg (Hessey, 2011). Therefore it has been indicted that β-Lg was the main responsible for interaction (Donato and Guyomarcho, 2009). During heat treatment *β*-lactoglobulin loss native structure occurs via both disulfide-linked aggregate formation and noncovalently linked aggregates. There are at least three possible interaction of denatured β-Lg; unfolded monomeric β-Lg, self-aggregated β-Lg and β-Lg/α-La aggregates that can association with the casein micelles (Oldfield *et al*. 1998b) as cited by (Singh and Waungana, 2001). The denatured whey proteins can interact via thiol–disulphide bonds with other whey proteins or with κ-casein (Guinee and O'Brien, 2010).

Interaction of β-lg with κ-casein occur on the exterior of the casein micelle and leads to coating of the casein micelles with β-lg , mixture of native whey proteins and denatured whey proteins present as whey protein aggregates, casein-whey protein aggregates and whey protein coated casein micelles (Donato and Guyomarcho, 2009; Raikos, 2010). At room temperature and physiological pH β-lg exists mainly as a dimer, it also dissociates into monomers at higher temperatures. β-lg monomer contains two disulfide bridges and one free cysteine (Cys121). α-L in milk is a compact, low molecular mass have four disulfide bridges. differences between the whey proteins upon heating are caused by the

fact that α-L contains only disulfide bridges, while β-lg has a free thiol group in addition to two disulfide bridges. Therefore α-lac, which can renature completely when heated alone, is also irreversibly denatured in the presence of β -lg due to thiol group-disulfide bond exchange reactions (Astrid *et al*., 2003; Guyomarcho, 2006 and Walstral *et al.*, 2006).

According to Hattem *et al*., (2011) the denaturation of camel whey proteins was highest at thermal treatment 90°C/30 min and lowest at 63°C /30 min heat treatment temperature. Thermal denaturation was higher for bovine whey proteins than for camel whey proteins (60 and 69 %) for camel and cow whey proteins respectively (Felfoual *et al.,* 2016). Increasing pasteurization temperature from 63°C/30 min to 75° C causes visible change in electrophoresis patterns for cow, camel and buffalo milk. As the effect on bovine serum albumin(BSA) was mild while it was severe on buffalo milk but no effect on camel milk while effect on β-Lg fraction in cow and buffalo milk were observed but no effect on α-La fraction of all kinds of milk. Further increment of temperature to 85°C, decrease of SA band intensity in buffalo and cow milk whey proteins while smaller decrease of CSA (El Agamy, 2000). The report conclude that heat-induced changes of whey proteins increased with increasing temperature and time of heating and effect were different with in milk of different species for instance it was more pronounced in buffalo and cow milk than camel milk.

The study conducted (Felfoul *et al*., 2015) to know the fouling properties of camel milk at different heating temperature which include 70^0C, 80^0C, and 90°C for 30min to 120 min indicates that pasteurization temperature at 70^0C had no visible changes in camel milk protein gel patterns. However, increases in temperature to 80°C camel serum albumin (CSA) band become less intense than at 70°C while starts to disappear after heating camel milk for 60 min at 80°C. On the other hand camel milk α-La's band remained constant after heating camel milk at 70 and 80°C. However, at 90 °C α-La and CSA bands as well as κ-casein decreased or hydrolysed. Whereas in cow milk bovine serum albumin disappearance at 70°C while β-Lg and α-La bands remained constant but disappeared after heat treatment of 90°C. From the result of the experiment the authors confirmed that β-Lg plays main role in deposit formation during heating of bovine milk while for camel milk CSA and α-la were responsible alone or by interaction with other proteins and minerals since both are affected by heat treatment but CSA was the most affected protein then α-La . The report of these authors also indicates the absent of β-Lg in camel milk.

2.5.3. Effect of heat treatment on casein protein

Casein present in micelles rather than in solution and important for the properties of milk. Casein is more heat resistant Raikos (2010) as compared to whey protein due to lack of secondary and tertiary structure (Hallén, 2008). However, it undergoes changes, mostly hydrolytic when subjected to severe heat treatment. The effect of higher heat treatment regarding on casein content it can cause modification of the casein micelle surface by partial hydrolysis of the κ-casein hair of the micelle surface that leads to aggregation. In addition result formation of casein micelles with a "new" surface, that coated by denatured whey proteins which can reduces their susceptibility to rennet and making them difficult to coagulate (Dalgleish, 2007).

2.5.4. Effect of heat treatment on milk nitrogen fractions

Protein is an important constituent of milk which contains about 95% of the total nitrogen present (Khan *et al*., 2016). In camel the fraction of nitrogen is similar to that of cow milk even if the NPN content of camel milk relatively higher than cow milk (Farah, 1993). NPN components are primarily urea, creatine, creatinine, amino acids, and other minor nitrogen containing compounds (Felfoul *et al*., 2015). While the NCN of milk is a fraction obtained by casein precipitation of using acetic acid and sodium acetate at the point of isoelectric (wangoh *et al*., 1998).Total nitrogen remain constant at all temperatures such as 63^0C,80^0C,90^0C for 30 min and 72^0C for 15 sec .On the other hand the amount of Non protein Nitrogen (NPN) of raw milk was is largest than all heat treated sample on hand it is reported that NPN was not affected by the heat treatment of milk and in both camel and cow milk the mount milks ranging from 5.6 to 6.6 % of total nitrogen (Farah,1998). Whey Protein Nitrogen (WPN) is significantly decreased due to the effect of different thermal treatments in comparison to the raw milk sample (Hattem *et al.,* 2011). Heating at 85 for 5 min decreased WPN and NCN in camel milk as compared to raw milk (Hannsey, 2009).

2.6. Rennetability Properties of Heat Treated Milk

2.6.1. Milk clotting enzymes

In the production of cheese the most important inputs next to milk were milk coagulant. Milk clotting enzyme can found from different source such as from animal, plant microbial

or produce by fermentation processes (Vallejo *et al*., 2012). Rennet is a mixture of chymosin and pepsin, which is mainly obtained from animal sources. Due to shortage of animal rennet and increase cheese production alternative source like fermentation produced chymosin identical to animal origin but produced by gene expression in selected microorganisms as reviewed by Harboe *et al*., (2010). For instance, in recent time camel chymosin produced by fermentation from gene of camel (*Camelus dromedarius*) by expressing in *Aspergillusniger* (Kappeler *et al*., 2006) and now it is available as Chymax M® from Chr.HansenA/S. Camel chymosin shows higher specificity and there by reduced proteolysis activity which is beneficial for the cheese production and the quality of the final cheese. It has higher thermal stability and the clotting to proteolysis ratio is increased seven fold compared to that of bovine chymosin. This enzyme is also better for camel milk coagulation than other coagulate like ginger curd extract (Benkerroum *et al.*, 2011 Yonas *et al.,* 2014).

2.6.2. Mechanism of rennet coagulation

Gel formation of milk proteins is the basis for the manufacture of cheese and fermented milk products. In cheese making coagulation of milk is major steps in which milk under goes gelation process that is formed due to aggregation of casein protein. It is deep physical and rheological change (Sundaram, 2003 and Walstr *et al.,* 2006. Various approaches can be used to destabilize the milk proteins, including heating which focuses on whey proteins, use of rennet enzyme (caseins) and acidification (caseins and denatured whey proteins).

Coagulation of milk by rennet probably know accidentally from use of animal skin. Milk coagulation/gel formation start when the hydrophilic and predominantly negatively charged C-terminus that make outer layer of the casein micelles called κ-casein removed by rennet enzyme. The enzyme cleaves the C-terminal part of the k-casein into para κ-casein and casein-macropeptide (CMP). In bovine milk chymosin cleavage site of k-casein is specifically at Phe105-Met 106. However, in camel milk the cleavage site at Phe 97-Ile 98 that leave macro peptide of 6.774 kDa, and 65 amino acids while in bovine 6.707 kDa and 64 amino acids (Kappeler *et al*., 1998; Fox, 2007; Lucey, 2014).

2.6.3. Effect of temperature on milk coagulation

Milk coagulation by rennet can be influenced by processing applied to the milk. Temperature affect on gel formation of milk that have been heated at a temperature greater than pasteurization results poor gel formation characteristics (Hennessy, 2011). Denatured whey proteins that interacted with the casein micelles and those present at the surface of fat globules may reduce casein to casein interactions that affect gel structure due to less complete fusion gel system (Rynne *et al.,* 2004). The denaturation level can determine gelation time, low denaturation degree results in slight increase coagulation time while linear increase could be due to 20-90 % whey protein denaturetion degree (Schreiber and Hinrichs, 2000).

2.6.4. Other factors that affect milk coagulation

In addition to the effect of heat treatment the property of milk coagulation can be affected by other factors. The Rennet Clotting Time (RCT) varies between a breeds that leads variation in milk composition. As literature shows milk from Jersey cows was to found have significantly have higher in coagulation property than for milk of Danish Holstein-Friesian cows and breeds due to higher protein content (Frederiksen *et al*., 2011). The type of enzyme used for coagulation of milk also determinate factor that cause variation in coagulation property of milk during cheese making (Yonas *et al*., 2014). Normally gel development of camel milk during cheese making can be improved by increasing temperature at which the enzyme camel chymosin is added and using higher concentration of chymosin. Increasing incubation temperature may have role in aggregation of casein fractions from camel milk while higher camel chymosin concentration reduce gelation time (Yonas *et al*., 2016a). This report shows increasing gel temperature from 30 to 40^0C result higher clotting activity and better accessibility of κ - casein t for the action of camel chymosin.

2.6.5 . The coagulation property of heated camel and cow milk

Effect of milk heat treatment on camel cheese making property that was studied by Hattem *et al*., (2011) using calf rennet and visual method of observation the result shows that RCT of un treated camel milk had the lowest RCT than thermally treated milk and can also be

improved by addition of calcium chloride. Raw camel milk RCT 17±1.186 min while it was 20±1.275, 26±1.256 min, 28±1.248 min and 23±1.169 min for milk heated at 65, 80°C, 90°C for 30 min. and 72°C/ 15 sec, respectively.

From study done the on soft white cheese production from camel milk by acid and heating at different temperature including 60^0C,65^0C,70^0C and 75 ^{0}C for constant time of 30 min. The optimum temperature for curdling of milk was reported to be 66.24°C while pH was at 4.3 according to the report of Ahmed *et al*., (2013*).* Lower cheese yield due to increases in temperature above 65^0C while decreased in pH decreases coagulation time and cheese yield Qadeer *et al*., (2015) authors found a shortest coagulation time of 37.67 min at pH 5.5 comparing with pH value at 6.5,6.3,6.0 5.7. RCT of cow milk increased as temperature increases as compared to goat milk according to Alloggio *et al*., (2000). It can range from 18.8 min at 70°C /1 min to 60.7 min at 95°C /10 min by observing until first sign of sudden breakdown of the film on the test tube wall. On the other hand experiment that was done on goat milk after treating cheese milk at 65^0C and 72^0C for 30 min and then incubated at 37^0C which results higher stress value cheese at 65^0C than sample treated at 75^0C (Frau *et al.,* 2014).

2.7. Cheese Production from Camel Milk

Different researchers tried to find solution to improve cheese making from camel milk by lowering the pH of milk and addition of calcium chloride before rennet addition in order to make κ-casein available for action of rennet enzyme. Mixing of buffalo milk with camel milk resulted in decreasing the RCT. Increasing the CT due to increment of the casein content of mixtures which improved the rennet ability and cured properties. In Egypt the yield of soft cheese can be increased by mixing camel and buffalo milk at a ratio of 70% and 30% respectively (Shahein *et al*., 2014). Similarly good fresh soft white cheese "Jibna-beida" can be produced by mixing camel and cow milk, lowing the pH of milk and addition of calcium chloride prior to rennet addition (Siddig *et al*., 2016). The other interesting trial to make cheese from camel milk was observed from literature report that done in Ethiopia which showed that soft unripe cheese can be made from camel milk by using plant origin milk coagulant called ginger crude extract. The author indicted that most of the quality factors such yield, curd and texture.etc of cheese made using ginger curd extract were lower than cheese made using the commercial camel chymosin even if using locally available material is economically advantages (Yonas *et al*., 2014).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

Erer valley was located in of Babile district in Eastern part of Ethiopia in Oromia regional state. The area is far from main city Addis Ababa by 550 km and 25 km from Harar city at 9° 14'N latitude and 42° 14'E longitude at an altitude of 1300–1600 metre above sea level. The areas has semi-arid climate condition with average annual rainfalls and temperature of 400-500 mm and 17°C-31°C, respectively. It is also characterized by long rainy (July–September), short rainy (March–April), long dry (October–February) and short dry (May–June) Seasons of the year and sandy-dry-loam with some alluvial nature in some places were the soil type in the area. Dwarf shrubs such as *Indigofera species*, large shrubs and trees like *Acacia and Boscia* were the main vegetation in the area (Merga *et al.*, 2014). Camel production dominates in the area. The collection of milk and experiment was done from March to May 2017.

3.2 Materials

Fresh Camel milk sample that bought from the pastoralist in Erer valley while cow milk from Haramaya University Dairy farm was milk used for the experiment. Recombinant camel chymosin (CHY-MAX®M) was enzyme used. MinProtean TGXstain-free precasted gels, Tris/glycine/SDS running buffer (TG10x), laemmli sample buffer, Beta-mercaptoethanol (BME) and broad range precision plus Protein Standard (Protein™ Unstained) from BIO-RAD (USA) were chemicals used for SDS-PAGE analysis. Double distilled was also used for dilution chymosin while de-ionized water SDS -PAGE analysis. Sulpheric acid, kjeltabs, sodium hydroxide, boric acid and Hydrochloric acid were chemicals used for kjeldhal analysis.

3.3. Milk Sample Collection

Milk samples were purchased from pastoralist who have lactating camels that have willingness to supply wholesome camel milk. The lactation stage of the camels were more than two month and were parity two and above. Whereas cow milk was collected from Harmaya University Dairy farm. For both milk types and for all experiments morning milk was collected after being pooled together in clean stainless steel containers. The seven litre

of milk was collated in two times for camel milk and the same volume also collected for cow milk. Then the milk samples were transported to Haramaya University Dairy Laboratory (HUDL) and immediately refrigerated at 4^0C until subdivide for different heat treatments.

3.3. Experimental Procedure

3.3.1. Heat treatment of milk

After raw milk samples were analysed for chemical composition and pH using milkoscan FT1 (Foss Electric, Hillerød, Denmark) and digital pH meter, then 250 ml milk sample was filled in to 6 bottle and randomly assigned for heat treatment at different temperature of 40°C, 65°C for 30min,72°C for 30 sec, 75°C for 5 min, 85°Cfor 5 min and 90°C for 5 min . heated milk sample at 40°Cwas used as reference. In similar experimental setup bovine milk was also heat treated for reference. Therefore, a total of 12 treatments (6 for camel milk and 6 for cow milk) were prepared in duplication of the same experiment. Then the samples were refrigerated until heat treated using thermostatically controlled water bath (model memmert) according to El-Agamy (2000) by adjusting temperature level for each sample. Milk samples temperature was monitored using thermometer. After the desired temperature combination was attained heating was stopped. Then portion of milk samples were taken for rennetebility test and chemical composition analysis while the rest milk samples stored at 4^0C for whey protein analysis.

3.3.2. Physicochemical analysis

The gross chemical composition of milk such percentage of Fat, protein, lactose, total solid, solids-not-fat, casein number and lactic acid and density were measured using automatic milk analyser milkoscan FT1(Model MilkoScan™ FT1- FOSS, Hillerød, Denmark) and for this two solution were used for automatic cleaning of the instrument as indicated in the guideline of the instrument. While pH of the samples was measured using digital pH meter after calibration at 4 and 7 pH solution. The measurements for both fresh and heat treated milk samples were done by taking 80 ml milk samples from each treatments.

3.3.3. Total whey protein denaturation analysis

To know total whey protein denaturation of whey proteins the value of non-casein nitrogen (NCN), and non protein nitrogen (NPN) were analysed by Kjeldhal method as per (AOAC, 1995) procedure. The activity including sample preparation, digestion, distillation, titration and calculation of values which were done to get total whey protein denaturation for each treatment.

Sample Preparation

For NCN 10 ml milk sample and 75 ml distilled water were taken at 37 ^{0}C then 1ml 10% v/v acetic acid was added and hold for five minutes. Then pH was adjusted at 4.3 for camel milk as recommended by Wangoh *et al.,* (1998) and 4.6 for cow milk using 1ml sodium acetate. After filtration 50 ml of clear filter was poured into kjeldhal tubes with kjeltabs as catalyst and concentrated sulphuric acid for digestion, and 1 ml acetic acid and 0.5 ml sodium acetate solution poured into tube for blank sample. While for NPN 10 ml of milk was mixed with 40 ml of 15% Trichloroacetic acid (TCA) solution and then after five minutes filtered with whatman paper. Finally 20 ml clear filter was poured to a kjeldhal tubes with kjeltabs as catalyst and concentrated sulphuric acid while 16 ml TCA solution used for blank test.

Digestion, Distillation and Titration steps

Chemicals used for analysis per sample were 20 ml concentrated sulphuric acid, 2 digestion tablet (kjeltabs that contain 5gm potassium sulphate, 0.15g copper sulphate, 0.15g Titanium Dioxide) and 6 boiling chips for digestion, 75 ml of 40% sodium hydroxide solution, 40 ml of 4% boric acid solution, three drop of bromocresol green/methyl red indicator during distillation whereas 0.1 and 0.01 N Hydrochloric acid were used for titration of NCN and NPN, respectively. Whatman filtration papers no 42 was used for filtration. While kjeldhal tubes, Erlenmeyer flasks, beakers, pipits and pH meter were the major materials used. The solutions were prepared as per the directions indicated by manufactures and based on the standard methods.

The digestion was done for three hours at temperature of 120- 410^{0}C in kjeldhal digester (Heizblock 8, Germany). The samples were cooled down and then distillation was done

using BUCHI Distillation Unit (K-350 Switzerland) with 40% sodium hydroxide solution, 4% boric acid solution and three drops of indicator until blue colour persist. Finally the titration was done in a titration burette using 0.1 N HCl for NCN and 0.01N HCl solution was used for titration of NPN until pink colour seen. Then the following formula was applied based on AOCA.

Non casein nitrogen (NCN) =

$$\frac{1.4007 * (\text{volume sample} - \text{volume blank}) * 0.1 * 2 * 0.994}{(\text{Weigh})\text{of sample}}$$

Non protein nitrogen =

$$\text{NPN\%} = \frac{1.4007 * (\text{Volume sample} - \text{volume of blank}) * \text{N}}{\frac{\text{Wfx Wm}}{(\text{Wt} - (\text{Wmx}0.065)}}$$

N=Normality of HCL solution

Wf= Weight g of 20 ml filtrate

Wm= Weight, g, of milk

Wt= Weight, g, of milk and 40lm 15% of T CA solution

Whey protein nitrogen=

Whey protein nitrogen (WPN) = NCN - NPN

Total whey protein Denaturation % =

$$\text{WPD\%} = \frac{\text{WPN of raw milk} - \text{WPN of heat milk}}{\text{WPN of raw milk}} \text{x } 100$$

3.3.4. Gel -Electrophoresis

The denaturation level of individual whey proteins (β-Lg, α-La, serum albumin, lactoferrin) were determined using Sodium Dodecylsulphate-polyacrylamid Gelelectrophoresis (SDS-PAGE) methods of Laemmli (1970) using a vertical gel electrophoresis BIO-RAD. Milk samples were taken from heat treated and raw milk for preparation of whey protein samples by precipitation of casein at its PI at pH 4.3 for camel milk and at pH 4.6 for cow milk using1N HCl. Centrifugation was done at 4000 gravitational force (g) for 15 min using centrifuge (Model Sigma, Germany) to remove the fat and re centrifuged to get clear acid-whey sample that was free from casein as per the method described by Omar (2016).

Finally a clear supernatant whey protein was extracted using disposable syringe in endorphins tubes.

MinProtean TGXstain-free precast gel having 15 µl well size, Tris/glycine/SDS running buffer (TG 10x), laemmli sample buffer, beta-mercaptoethanol (BME) and broad range precision plus Protein Standard (Protein™ Unstained) were chemicals used. All instrument and chemical were from BIO-RAD (USA). The dilution of chemicals and preparation of solution were done according to the instruction from the manufactures while de-ionized water prepared at Haramaya University.

One to one ratio of whey protein precipitated sample and sample and sample buffer with 5% BME were mixed (20:20 µL). And denatured at 90^0C for 5min as according to (El agamy, 2000). Then by assembling MinProteanTGXstain-free precast gels on the electrophoresis running buffer was filed in buffer dam up to the level then 15µl mixed sample were loaded using sample loading micropipette per lane and electrophoresis was run at 200 voltage for 30 min until the dye rich the end of gel. Finally, the gel was visualised using gel Doc™ EZ imager BIO-RED (gel imager). This analysis was done in Animal Genetics and Breading Laboratory of Haramaya University. The data was extracted in figure from the soft ware output. The location of each whey protein were compared with the bands of standard and previous literatures for accuracy.

3.3.5. Rennatablity properties of camel milk

Gelation time (GT), maxima elasticity(G' max) and time to G'max (time to maxima elasticity) were measured using free oscillating rheometry (ReoRox G2-4 MRX 505-2, Nykoping Sweden) according to Frederiksen *et al*. (2011).

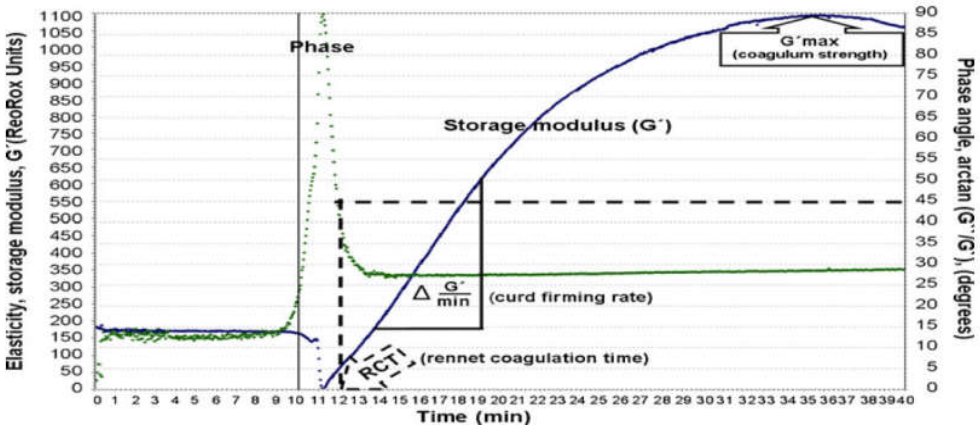

Figure 2. Example of Rennatablity parameters

For this experiment, 50 ml of milk sample (Hougaard *et al*., 2010) was taken after the heat treatment and cooled down to 40^0C for addition of camel chymosin (CHY-MAX) (Chr. Hansen, Denmark). The enzyme was added at pH 6.3 by adjusted the pH using 1NHCl.The concentration of Camel chymosin was 20-50 IMCU/1000 ml according to the manufacturer guideline. After dilution of 1ml stock solution to ten times in 9 ml distilled water then 70 µl (7 IMCU/50ml) chymosin were taken and used for the final work after pre-trial at 35 µl, 50µl and 70µl.

Before the commencement of the measurement the instrument was calibrated using 1 ml of visco-diluents calibrator (MediRox AB Studsvik 61182, Nykoping, Sweden) calibration solution and distilled water following the instruction in manufacturers manual for calibration of this instrument. After camel chymosin addition, immediately 1ml milk sample was loaded in to the bob cup where sample introduced using 1ml volume disposable syringe. Measurement was done for one hour continually at oscillation frequency of 10 Hz.

- Coagulation or Gelation time= point time where the increase in elesticity was recorded
- G'max =maximum coagulum strength.
- Time to maximum elasticity at which G'max rich maximum point within one hour measurement of ReoRox was also studied.

3.4. Experimental Design

The experiment was done by complete randomize designed (CRD) with factorial arrangement in which 6 level of temperatures (40°C ,65^0C ,72^0C, 75^0C, 85^0C and 95^0C) were assigned as 6 treatment. Heated milk at 40°C used as reference. In similar experimental set up cow milk also studied for reference. The experiment was done in duplicate following the same steps for both milk sources

With a model of

$y_{ij} = \mu + Tj + \varepsilon_{ij}$

where:

Y_{ij}= observation milk sample in treatment i (Temperatures)

μ = overall mean

Tj = effect of heat treatment (the reference, 65^0C /30 min, 72^0C/30 sec, 75^0C/ 5min, 85^0C 5 min, 90^0C 5 min)

ε_{ij} = experimental error a = the number of treatments; $_j$ = the number of experimental unit

3.5. Statistical Analysis

The research designed in CRD with 2 x 6 factorial arrangement in which 2 milk source and six heat treatment levels. The experimental data was analysed using Analysis of Variance (ANOVA) with Statistical Analysis System (SAS) 2009 version 9.2 (SAS Institute Inc., Cary, NC USA).The data were expressed as the means ± standard deviation of values obtained from duplicates of the experiments. Statistically significant differences at (P<0.05) between mean of different treatment levels were determined by least significant difference (LSD).

4. RESULT AND DISCUSSION

4.1. Physicochemical Properties of Fresh Milk

The physic-chemical properties of raw dromedary camel and cow milk samples were indicated (Table 3). The physical property like the pH value in the current study was significantly(P<0.05) higher in camel milk than in cow's milk. The pH value of camel milk closer to pH 6.6 result from the investigation done in comparison with Goat, Sheep, Cow, Camel and Human Milk in Sudan Khartoum (Sabahelkhier *et al.*, 2012). It also agree with pervious study of Yonas *et al.* (2014) that reported pH value of 6.64 ± 0.02 for camel milk in the same study area. On the other hand the lactic acid percentage slightly higher in cow milk at (P<0.05).These might be due to more content of antimicrobial components such as lysozyme, lactoferrin and immunoglobulin in camel milk than bovine milk that results relatively slower conversion of lactose in to lactic acid (El-Agamy, 2000).

While the difference in total solid (TS) content was not significantly different between the two milk sources (P>0.05) even if numerical higher value of TS was observed in cow milk. Even though value TS of the current result slightly lower than 11.6 ± 0.27% of previous works of Yonas *et al.* (2014), it was higher than 9.9±1.189% for Egyptian camel that reported by Hattem *et al*., (2011). Whereas the TS of cow milk closes to 13% for bovine milk which is similar to the work of Shahein *et al*. (2014). The variation in TS might be due to the changes in fat, lactose, minerals and protein content of camel milk (Abbas *et al.,* 2013).

Table 3. Physicochemical property of raw camel and cow milk

Milk component	**Milk source (Mean +SD)**		**P value**
	Camel	Cow	
Fat %	3.39±1.32	3.82±0.63	ns
Protein%	2.47±0.04[b]	3.37±0.12[a]	*
SNF%	7.70±0.05[b]	8.87±0.05[a]	**
TS %	11.25±1.48	12.7±0.74	ns
Lactose %	4.80±0.08	4.82±0.11	ns
Casein %	1.75±0.05[b]	2.44±0.04[a]	**
Lactic acid%	0.11±0.01[b]	0.15±0.00[a]	*
pH	6.66±0.01[a]	6.47±0.03[b]	*

Mean value with Same superscripts letter in the same row are not significantly different at $P<0.05$. TS: Total Solid, SNF: Solid Non-Fat.

The difference fat% and lactose content were in significantly different between the two milk samples ($P>0.05$). However, their values were within the range of report of Siddig *et al*. (2016) for both camel and cow milk. On the other hand the difference in protein%, casein% and SNF % content were significantly different ($P>0.05$) for camel and cow milk in current study in which lower value was seen for camel milk than that of cow milk. The protein content of camel milk lower than 2.95 % and 2.86% reports of Sabahelkhier *et al*., (2012) and Osman, (2016) respectively. However, the value was with the range of 2.15 to 4.90 % as reported by Abbas *et al*. (2013).Whereas the value for cow milk agree with 3.40 % that reported by Sabahelkhier *et al*, (2012).The percentage of camel milk casein was within the range of 1.63 to 2.76 percent of camel milk that reported by Khaskheli *et al.,* (2005).

Variation in milk protein might be observed due to influencing factors such as breed, stage of lactation and season play a role in camel milk protein content (Al haj and Al Kanhal, 2010; Siddig *et al*., 2016). Therefore, the variation in protein content between camel and cow milk may be related to the species effect and the management condition. Milk composition varies depends on genetic, feeding and management conditions, season, milking frequency, age, stage and number of lactation, methods used to determine

composition (Khan and Iqbal, 2001, Walstra *et al.,* 2006, Abba *et al* ., 2013;Khan *et al* , 2016).

4.2. Effect of Heat Treatment on Camel Milk Composition

The effect of heat treatment on the major chemical composition of milk that treated at indicated temperatures was analysed and summarized (Table 4) based on this the % of fat, protein, lactose, and total solid of camel milk were not significantly affected ($P>0.05$). However, a slight significant effect was seen on casein% in which their value increase as temperature of treatment increases when seen the variation between samples effect was seen at higher at 90 ^{0}C/5min at ($P<0.05$) than all even if the variations it have with 75^{0}C/5 min and 85^{0}C/5min was insignificant. The current result for gross chemical composition of camel milk like fat protein and lactose disagree with report of Hattem *et al*., (2011) who observe significant change at higher temperature after heating camel milk at 63°C, 80°C and 90°C for 30 min and 72°C for 15 sec. Insignificant effect observed in this study than the report of Hattem *et al*., (2011) might be due difference in temperature level, duration of holding time and method or temperature control.

Similarly the effect of heat treatment on cow milk indicated in table 4 and the result showed that% of fat, protein , lactose and total solid content of cow milk were not significantly affected ($P>0.05$) as heat treatment increases. Pasteurisation temperature had no significant effect on nutritional value except minimal whey protein denaturation and little change on colour, flavour and appearance of the milk (Lewis and Deeth, 2008 and Marwa *et al*., 2013). While significantly higher casein number was recorded at 85^{0}C/5 min and 90^{0}C/5 min than all. The casein is heat resistant at pasteurization due to lack of secondary and tertiary structure unlike whey protein (Hallen, 2008; Raikos, 2010). However whey proteins denatured and interact via thiol–disulphide bonds with other proteins or with κ-casein and results in co-aggregate (Guinee and O'Brien, 2010; Hessey, 2011). Therefore the increment in casein content might be due to indirect effect of denatured whey protein that attached on the surface casein micelles that was with κ-casein.

Table 4. Effect of heat treatment on gross chemical components of camel and cow milk

Milk source	Parameters	Temperature/treatments						P value
		Heated(40^0C)	65^0C/30min	72^0C/30sec	75^0C/5min	85^0C/5min	90^0C/5min	
Camel								
	Fat %	3.39±1.32	3.39±1.32	3.37±1.32	3.32±1.20	3.26±1.29	3.33±1.31	ns
	Protein%	2.47±0.04	2.46±0.49	2.46± 0.49	2.46±0.03	2.47±0.02	2.48±0.01	ns
	Lactose %	4.80±0.08	4.85±0.09	4.87±0.09	4.86±0.08	4.86±0.07	4.91±0.04	ns
	TS	11.25±1.48	11.35±1.48	11.35±1.58	11.27±1.37	11.35±1.35	12.00±0.70	ns
	Casein%	1.75 ± 0.05^{d}	$1.82\pm0.03b^{cd}$	1.81 ± 0.04^{cd}	1.87 ± 0.04^{abc}	1.92 ± 0.02^{ab}	1.96 ± 0.01^{a}	*
Cow								
	Fat %	3.82±0.63	3.71±0.68	3.64±0.48	3.77±0.80	3.52±0.50	3.38±0.31	ns
	Protein%	3.37±0.12	3.38±0.13	3.40±0.14	3.38±0.12	3.40±0.15	3.48±0.16	ns
	Lactose %	4.82±0.11	4.85±0.10	4.86±0.12	4.86±0.12	4.89±0.14	4.89±0.12	ns
	TS	12.70±0.74	12.71±0.63	12.72±0.31	12.76±0.16	12.84±0.08	12.92±0.03	ns
	Casein %	2.44 ± 0.04^{c}	2.51 ± 0.05^{bc}	2.55 ± 0.07^{bc}	2.59 ± 0.13^{bc}	2.66 ± 0.02^{ab}	2.84 ± 0.04^{a}	*

Mean + SD Value with same superscripts letter in the same row were not significantly different at ($P<0.05$)

4.2.2. Effect of heat treatment on total whey protein of camel milk

The analysis for NCN, NPN and WPN is indicted table 5. Non-casein nitrogen of camel milk was significantly affected by heat treatment ($P< 0.05$) as it decrease as temperature increase even though no significant difference was observed between the untreated milk and at lower heat treatment or pasteurization temperature of 65^0C/30 min. The clear variation was observed above 72^0C/30 sec as the highest effect was observed at 90^0C/5 min. Similarly the NCN value of cow milk decreases as level of heat treatment increases ($P<0.05$). This could be due to interaction of heat induced denaturation of whey proteins with other protein and fat globs causes a decrease in the NCN contents (Felfoul *et al*., 2016).

The value of NPN in camel milk was not affected significantly ($P>0.05$) by heat treatment as it show slightly increase than untreated milk even though the variation between with 65^0C, 72^0C and 75^0C and between 85^0C and 90^0C, respectively was insignificant. Whereas NPN content in cow milk was significantly affected by heat treatment ($P<0.05$). The value increase at temperature increases especial at 90^0C. Regarding NPN content, lower value for bovine than that of camel milk due to higher enzymes and free amino acids concentrations in camel milk than in cow milk, and during heat treatment it might increases due to presence of heat-induced protein degradation products (Kappeler 1998; El-Agamy *et al*. 2009; Felfoul *et al*., 2016).

Whey protein nitrogen value in camel milk was significantly affected($P<0.05$) at higher heat treatment of 90^0C/ 5 min which results higher effect than all. While the variation in between 65^0C and 72^0C, 75^0C and 85^0C respectively is insignificant. Total whey protein denaturetion percentage of camel milk increases at ($P< 0.05$) as heat treatment temperature increases. For instance it was higher at 75 and above than that of 65^0Cand 72^0C heating temperature. Similarly WPN content of cow milk significantly affected at 75^0C/5 min and above with a more effect at 90^0C/5min. Total whey protein denaturetion increases as temperature level increase at ($P<0.05$). Related report of Rynne *et al*., (2004) indicates that the levels of total whey protein denaturation increased from 2.8%, to 34% due to increasing pasteurization temperature from 72^0C to 87^0C for 26 second.

The denaturation percentage of whey protein in camel milk looks lower than for cow milk in the current study. This could be due to relatively heat resistance of camel whey protein than that of bovine whey protein (El-Agamy *et al*., 2000). Higher thermal denaturation for bovine whey proteins 69 % than 60% whey protein denaturation of camel milk as reported by Felfoul *et al*., (2016). Lower effect at lowest time-temperature combination or conventional pasteurization while at higher heat treatment had higher effect (Farah,1993).

Table 5. Effect of heat treatment on total whey protein of camel milk and cow milk

Milk source	Parameters	Temperature/treatments (Mean +SD)						P
	mg/100g	Heated(40^0C)	65^0C/30min	72^0C/30sec	75^0C/5min	85^0C/5min	90^0C/5min	value
Camel								
	NCN	110.75 ± 1.76^{a}	108 ± 1.41^{a}	105.2 ± 1.13^{a}	98.8 ± 1.97^{b}	87.25 ± 3.88^{c}	74.00 ± 2.82^{d}	***
	NPN	29.75 ± 0.70^{c}	30.55 ± 0.77^{bc}	31.60 ± 2.26^{abc}	32.55 ± 1.34^{abc}	33.25 ± 0.35^{ab}	33.90 ± 0.14^{a}	ns
	WPN	81.00 ± 1.41^{a}	77.5 ± 2.12^{ab}	73.70 ± 0.98^{b}	66.50 ± 3.53^{c}	54.00 ± 4.24^{d}	40.10 ± 2.68^{e}	***
	WPD%	0	4.30 ± 0.98^{ed}	9.00 ± 2.82^{d}	$18.00\pm0.2.82^{c}$	33.37 ± 4.06^{b}	50.50 ± 2.51^{a}	***
Cow								
	NCN	131.00 ± 1.41^{a}	126.00 ± 1.41^{ab}	122.50 ± 0.70^{b}	115.00 ± 4.24^{c}	87.55 ± 3.60^{d}	76.00 ± 2.82^{e}	***
	NPN	25.50 ± 0.70^{c}	26.00 ± 1.41^{c}	27.65 ± 0.49^{bc}	29.50 ± 0.70^{abc}	31.65 ± 1.9^{ab}	33.40 ± 3.67^{a}	*
	WPN	105.50 ± 2.12^{a}	100.50 ± 3.53^{ab}	95.00 ± 1.41^{b}	85.50 ± 7.77^{c}	55.90 ± 4.87^{d}	42.60 ± 0.84^{e}	***
	WPD%	0	$5.00\pm1.41d^{ed}$	10.07 ± 2.92^{d}	22.00 ± 5.65^{c}	47.00 ± 0.56^{b}	60.75 ± 0.07^{a}	***

Mean value with same superscripts letter in the same row were not significantly different at ($p < 0.05$)

NCN =Non casein nitrogen, NPN= Non protein nitrogen, WPN = whey protein nitrogen, WPD= whey protein denaturation

4.2.3. Effects of heat treatment on individual whey proteins of camel and cow milk

The effect of heat treatment on individual whey proteins was identified using SDS-PAGE. This was done through visual observation at the band of each fraction of whey protein based on their migration which in turn depends on their molecular weight. Figure 3 shows the denaturation level of individual whey protein at different heat treatment temperature level for both camel and bovine milk including the unheated milk that used as a reference.

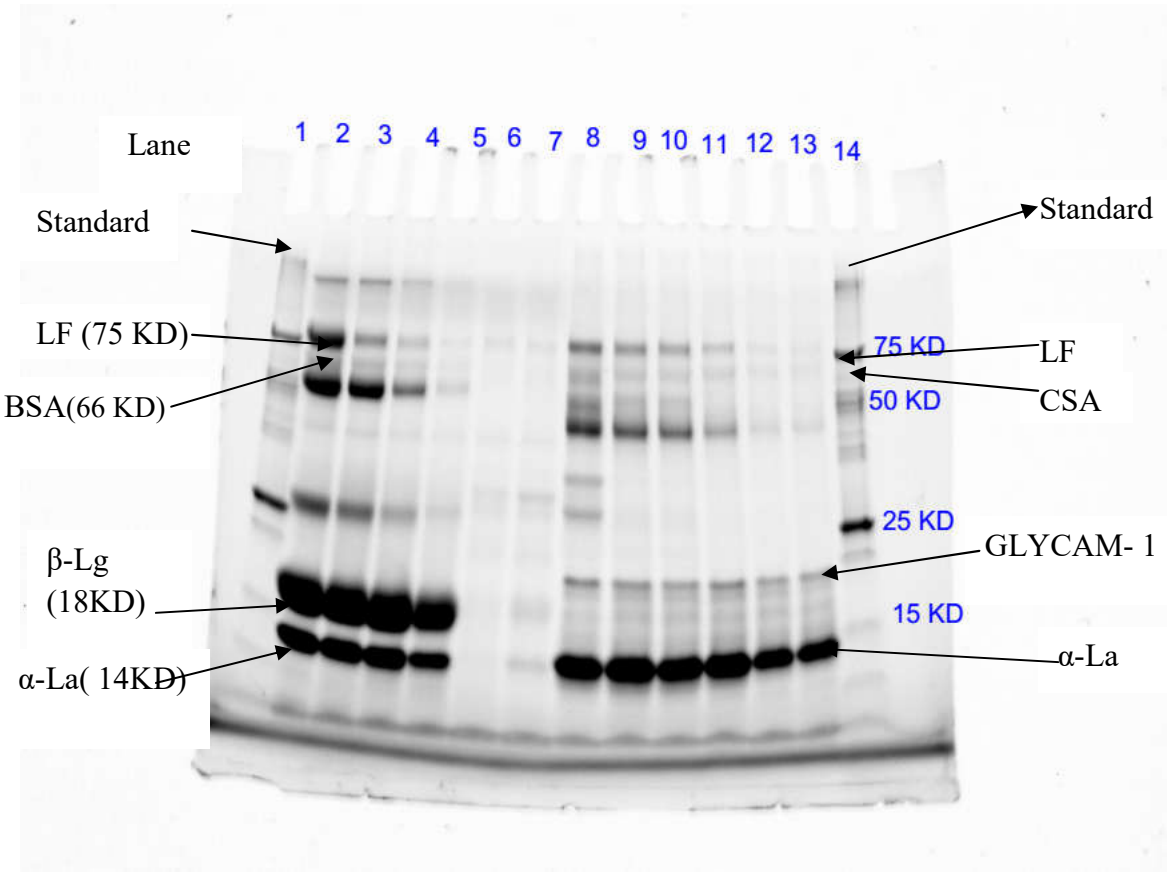

Figure 3. SDS-PAGE of whey proteins of heated camel and cow milk at different time temperature combination.

NB: Whey proteins of cow milk at lane 2, 3,4,5,6,7 stands for heated at 40^0C, 65^0C/30min, 72^0C /30sec, 75^0C /5min, 85^0C /5min and 90^0C /5min, respectively while for camel milk lane number 8, 9,10,11,12 and 13 stands for the temperature level indicted for cow milk respectively. At Lane number 1and 14 are precision plus protein Standard with molecular weight of 10-250 KDa . KDa= kilo Dalton.

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The position of whey protein on the band was indicated by comparing the molecular weight of whey proteins with the protein standard and with previous literature (Farah, 1986; Hinz *et al*., 2012 and omar *et al*., 2016).

Whey protein move through the gel electrophoresis according to their molecular weight (Salmen *et al.,* 2011). In bovine milk β-Lg the dominate whey protein (Farah, 1993; Hinz *et al*., 2012;Omar *et al*., 2016) indicted from lane number 2 to 7 in second lower band. The intensity of β-Lg band in cow milk seen less change at standard pasteurization however, increases at temperature to 75^0C the band intensity decreases even also invisible at lane 6 and 7 at higher heat treatments 85^0C and 90^0C. As compared to the band intensity percentage of β-Lg in milk that only heated at 40^0C with heat treated the band intensity level decreased. For instance, at 75^0C it was 64% with 36% denaturation while at 90^0C band intensity decreased or denaturation by 96% figure 3A. However, from the current electrophoresis analysis any band that related to β-Lg was not seen clearly for camel milk neither in raw nor for heat treated samples in lane 8 to13. This agree with different literatures that report no β-Lg in camel milk (Kappeler *et al*., 2003; Hinz *et al*., 2012; Saliha *et al*., 2013; El haj and Freigoun, 2015; Felfuol *et al*., 2016; Omar *et al*., 2016).

In cow milk α-La band intensity also reduced as heat treatment level increases and even become invisible at lane 6 and 7. Band percentage of α-La of cow milk (Figure 4B) 94%, 82%, 45%,1% and 4% at 65^0C 72^0C,75^0C,85^0C and 90^0C, respectively as compared to control milk percentages with a reduction of 6%, 18%, 55%, 99 % and 96% respectively. This might be due to availability of β-Lg in bovine milk that helps interaction of α-La with casein micelles or other whey protein (Hessey, 2011). While in camel milk (Figure 3) α-La band seen less visible change as heat treatment level increases. Even though there was some variation, the band percentage of α-la was 67% at 90^0C with less reductions of 33 % (figure 3A) as compared to controlled milk band percentage. This might be due to lack of β-Lg in camel milk that leads less interaction with other protein.

Decreased in band intensity of β-Lg and α-La attribute of denaturation and interaction with casein micelles or with other whey proteins due to heat-treatment of milk at temperatures above 60°C that leads to denaturation(unfolding) of the whey proteins mainly β-lactoglobulin, followed by aggregation through hydrophobic interaction and disulphide-thiol interchanges to form heat-induced aggregates, either on the surface of the casein micelles (micelle bound aggregates), or in the serum phase of the milk (serum aggregates)

depend on heating temperature and time (Singh and Waungana, 2001; Rynne *et al*., 2004; Guyomarch, 2006; Li and Wang, 2015).

The mechanism of association denatured whey protein with casein micelles in camel milk is not known clearly because of lack of β-Lg. However; according to Felfoul *et al.*, (2015) denatured α-La and Camel serum albumin (CSA) is able to adhere on the hot surfaces alone or interacting with the casein micelles mainly κ-casein as β-Lg was responsible for cow milk. From this point of view in the current result of electrophoresis CSA looks responsible for interaction with the κ- casein in camel milk during heat treatment than α-la due to less denaturetion of α-la as temperature increases.

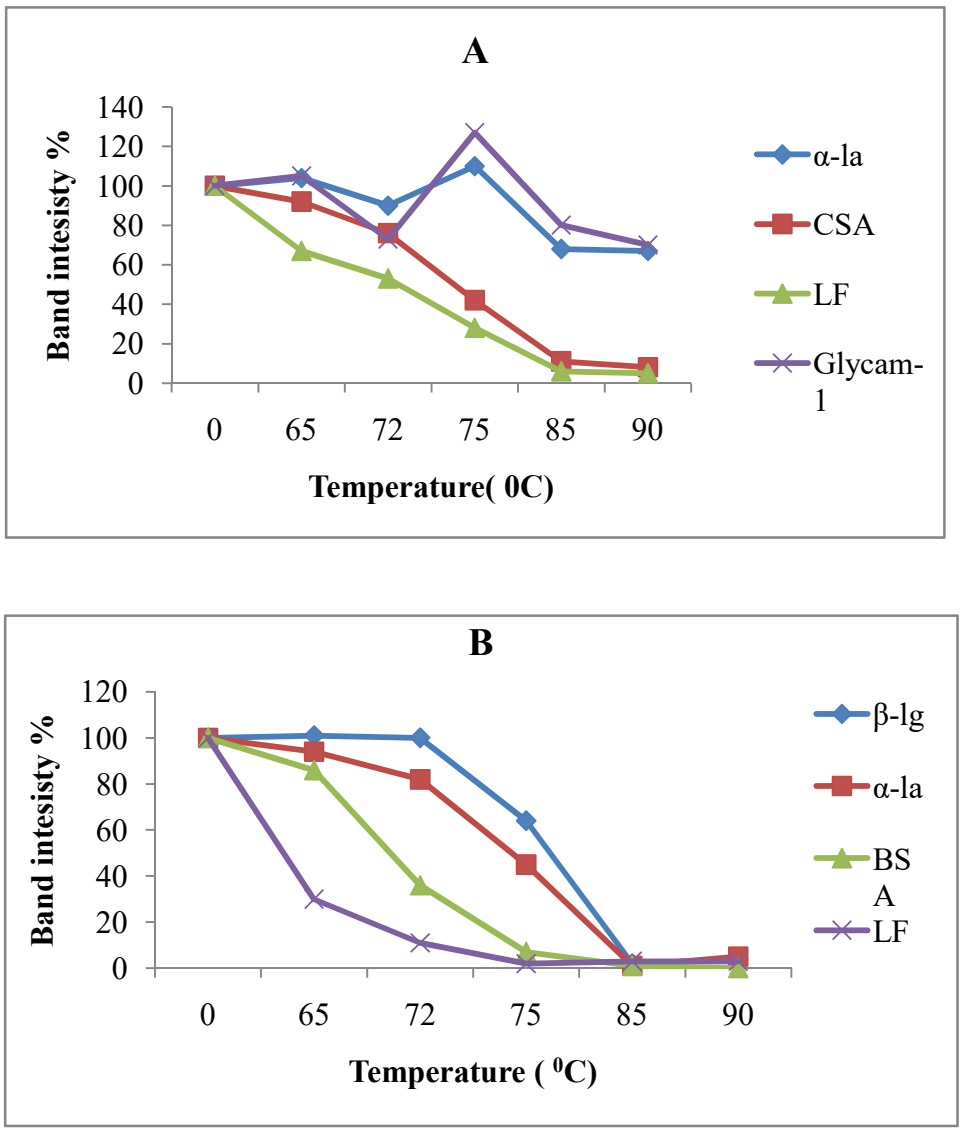

Figure 4. Band intensity% of β-lg, α-al, CSA/BSA, Lacoferin and Glycam-1 in heat treated Camel (A) and Cow milk (B) as compared to controlled milk.

Camel serum albumin band intensity continually decreased as heat treatment increased and even invisible after 85^0C(figure 4A). The band intensity reduction of CSA as compared to the references sample and the reduction was 8, 24, 58, 89 and 92% at 65 ,72 ,75,85 and 90^0C, respectively. Similarly in cow milk BSA band show continual decrease as heat treatment level increased and disappeared after 75^0C with reduction percentage 14 %, 64%, 93%, 99% 100% at 65^0C,72^0C,75^0C, 85^0Cand 90^0C, respectively as compared to % of reference milk reduction. The reduction in band intensity both in CSA and BSA supported by the literature that shows serum albumin was faster to denature (Donato and Guyomarc'h, 2009).

For camel milk the band of lactoferrin band was visible for raw milk however the intensity of the band deceases as heat treatment increases (figure 3). The band intensity percentage of camel milk LF was 67%, 53%, 28% ,6% and 5% at 65,72, 75 ,85 and 90^0C respectively compared to reference milk sample % with a reduced of 33%, 47%, 72%, 94% as increasing order of heat treatment. The band of LF in cow's milk also decreased as level of heat treatment increases. However, looks more than what is seen in camel milk since pronounced after 75^0C whereas for cow milk it looks after 72^0C.

It have been reported that BSA and LF form complex to a minor extent with αs_2-casein through thiol/disulphide exchanges while Immunoglobulin partially associate through hydrophobic interactions only (Donato and Guyomarc'h, 2009). Therefore denatured bovine serum albumin and LF might be attached to micelles or other whey protein and that could be why their band become less visible as heating treatment increased. Denaturation rate of individual whey protein agree with the literature that indicates the thermal stability order serum albumin, β-Lg and α-La, respectively (Singh and Waungana, 2001, Donato and Guyomarc'h, 2009). The trend of β-Lg and α-La in bovine milk agree with previous work of (Farah., 1986). As indicated on report of El-Algamy (2000), increasing temperature to 75°C resulted visible changes on whey protein of bovine, buffalos and camel milk even though observe less denaturation of α-La in camel milk this finding supports the results from the current study.

On the other hand the current SDS-PAGE results at mild lower band of camel milk visible band was seen around 22-23kda. This might be GLYCAM-1 (Glycosylation-Dependent Cell Adhesion Molecule 1) whey protein. According to the unpublished information Sonia (2016) with SDS-PAGE and Liquid chromatography–mass spectrometry identification

methods GLYCAM-1 was seen in camel milk at 21 KDa. On the other hand Farah (1986) found a new band that had estimated molecular weight of 23KDa nearly in similar band position of the current study. According to El-Salam and El-Shibiny (2013) Glycam-1 had antibacterial activity against pathogenic and found in camel milk in higher concentration. However, further study about its property during heat treatment is important due to limited references regarding this whey proteins.

4. 3. Effect of Heat Treatment on Rennatablity Property of Camel Milk

4.3.1. Gelation time of camel milk

Gelation time (GT) is a time taken for the milk to become viewed as a gel or coagulum (Sundaram, 2003).GT of camel milk (Table 6) was significantly increased by heat treatment milk (P<0.05). As compared to milk sample that heat treated at 40^0C only camel milk coagulation time increased as the level of heat treatment increased. Significantly lower time was recorded for heated milk at 40^0C that was up to incubation temperature than other heat treatment levels. The samples with longer time were considered as non coagulated sample that failed to form gel with in 60 min of ReoRox measurement time. GT of camel milk was longer as heat treatment increase and non coagulated at a temperature 75^0C/5 min , 85^0C/5 min and 90^0C/5 min.

Table 6. Effect of heat treatment on gelation time of camel and cow milk samples.

	Temperatures						*P*.V
	Heated ($40\ ^0$C)	65^0C/30 min	72^0C/30 sec	75^0C/5 min	85^0C/5 min	90^0C/5 min	***
Camel	6.79 ± 0.57^{c}	14.85 ± 1.62^{b}	19.55 ± 6.4^{ab}	NC	NC	NC	***
Cow	4.20 ± 0.28^{e}	6.00 ± 1.46^{de}	8.50 ± 3.53^{cd}	10.61 ± 0.54^{bc}	12.76 ± 1.74^{b}	NC	***

Mean values with same superscripts letter in the same row were not significantly different at P <0.05. GT= Gelation time, NC= Non coagulated samples.

Similarly gelation time of cow milk was significantly increased as heat treatment increases at (*P<0.05*). From the result (table 6) GT of milk heated at 40 ^{0}C have lower time value than at all heat treatment while the longer time at 90^0C/5 min than all which show more

pronounced heat effect treatment and considered as non coagulated. On supportive and related works indicated that coagulation time of bovine milk that was pasteurized at 72^0C for 20 sec show 168 second which was 32 second longer than raw milk during using CHY-MAX as coagulant and using Nephelo-turbidimetry methods (Pytel *et al*., 2016). Alloggio *et al*.,(2000) report shows longer coagulation time for cow milk due to temperature effect. This authors found RCT of cow milk is 17.4 min for raw milk and 21.1 min at temperature 70^0C for 10 min and longer time 60.7 min at 95^0C heating with equal amount of rennet.

Gel formation start when the hydrophilic and predominantly negatively charged C-terminus that make outer layer of the casein micelles part is κ-casein removed by coagulating enzyme specifically at Phe105-Met106 for bovine milk (Fox, 2007). While camel milk cleavage site is at Phe97-Ile98 (Al-Haji and Al-Kanhal 2010). Gel formation for bovine milk can occur when at list 85% of the κ-CN has been cleaved (Dalgleish and Corredig., 2012) while for camel milk initiated only after more than 95% of camel milk κ-casein was hydrolysed by camel chymosin (Yonas *et al*., 2016). In current study it was observed that GT increased as heat treatment increased. This might be due to retardation of the hydrolysis process due to less exposure of κ casein for enzyme action or cleavage that related with effect whey protein denaturetion that interact with casein micelles or simply aggregate themselves (Singh and Waungana, 2001; Rynne, 2004 and Sbodio and Revelli, 2012). For the reduction of κ-casein as substrate for the enzyme up to 7% could be due to interactions between κ -casein and whey protein fraction or between a specific fraction of κ-casein and whey proteins (Leaver *et al.*, 1995).

The GT recorded for all camel milk samples looks longer than that of cow milk. The variation might be attributed to casein micelles content and size. As camel milk have broader casein micelles that range 350- 500 nm than that of bovine (154-230–160 nm) which difficult for the availability κ- casein for reaction of coagulating enzyme and this difference makes coagulation time twice or three times longer than cow's milk with the equal amount of rennet (Farah, 1993 , and Hristov *et al*., 2016).

4.3.2. Gel development (G'max) of heated milk

The second rennetabilty properties of cheese milk is G'max in which gel development condition expressed by maximum elasticity or the G'max that indicate gel firmness. As indicated Figure 5, the G'max of camel milk significantly ($P < 0.05$) reduced with

increasing of heating temperature that was applied to treat the milk before addition of enzyme. Significantly firmer gel development was observed for heated milk at 40^0C tha was only up to incubation temperature and followed by 65^0C (51 pascal (pas)).and 72^0C (24 pas) . However, the other samples that were treated at 75^0C, 85^0C and 90^0C resulted in weaker gel that give 0 G'max value during measurement with in 60 min.

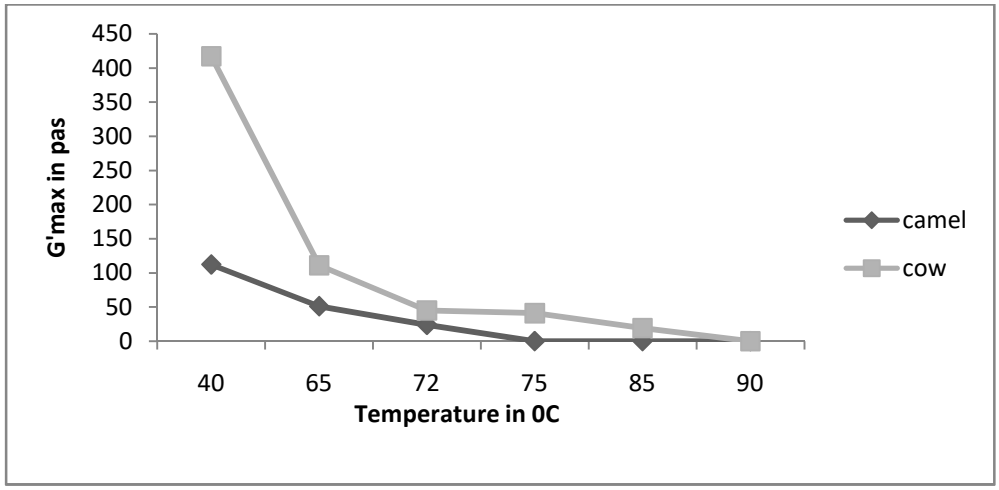

Figure 5. Effect of heat treatment on G'max of camel and cow milk G'max = maximum elasticity; pas= Pascal

Significantly weaker curd was formed in cow milk samples as heat treatment increases (P <0.05). Raw milk samples incubated at 40 ^{0}C showed higher G'max than all the others and at 65^0C heat treated milk sample gave better gel firmness than the other heat treated milk samples. Cheese milk can be pasteurized at a temperature of 63^0C to 65^0C/ 30 min and 72^0C for 15 sec due to risk of microbial contamination of raw milk (Sbodio and Revelli, 2012). However, higher temperature leads to a longer coagulation times and weaker or finer gel matrix structure (Singh and Waungana, 2001). Even though rennet enzymes does not directly affected by heat treatment, the denatured whey protein that coated to casein micelles can affect the primary phase of coagulation through stearic hindrance and changes in electro negativity. While the second phase due to lowering the potential for interaction or aggregation and fusion of the Para-casein micelles or lowering surface hydrophobicity (Schreiber and Hinrichs, 2000; Vasbinder *et al*., 2003 Rynne *et al*., 2004; Guyumich, 2006; Dalgaleish and Corredig, 2012).

If the amount of β-lg fused with micelles increases from 0% to 50%, results almost linear decrease in G' max while the association β-Lg with casein micelles of κ-casein is greater than 50% very low G' values could occur (Singh and Waungana, 2001). From gel electrophoresis analysis β-Lg is responsible for poor gel formation of bovine milk and α-La to less extent while for camel milk CSA might be responsible whereas α-la to less extent.

As literatures shows camel milk lack whey protein β-lg and deficient in κ-casein, poor in rennet coagulation property and poor heat stability (Farah,1993). In addition to effect of heat treatment lower G'max value obtained in current study as heat treatment increase and even lower than cow milk might be aggravated due to native milk properties such as lower protein content (Li and Wang, 2015), broader casein micelles that could results in flocks or weaker coagulum (Farah,1993) due to lower surface area for reaction (Maciel *et al*., 2015). Similarly lower κ- casein to casein ration (law and Tamime, 2010) that can also determine degree of casein aggregation and arrangement of the *para*-casein micelles during gel formation.

The effect of initial protein content on curd strength also reported by Frederiksen *et al*., (2011) who observe higher G'max (705.71±28.22) for Danish Jersey breed with higher protein content relatively to that of Danish red and Danish Holstein Friesian after evaluating milk at incubation temperature of 37^0C due to higher protein content of the milk that can improve the coagulation properties of milk in addition to increasing cheese yield.

4.3.3. Effect of heat treatment on time to G'max (G') of camel

The time in which the maximum gel development observed was also evaluated for both camel and cow milk after addition of the clotting enzyme (Figure 6). Therefore, for camel milk the time to rich G'max was longer than the time to obtain maximum G'max development as temperature increases (P<0.05). heated milk at 40^0C shows faster to attain its maximum elasticity than other treatments. However heat treatment at75^0C/5min.85 ^{0}C/5min and 90^0C/5min showed higher G'max constantly 0 until end of 60 min since these treatment resulted no curd or non coagulated.

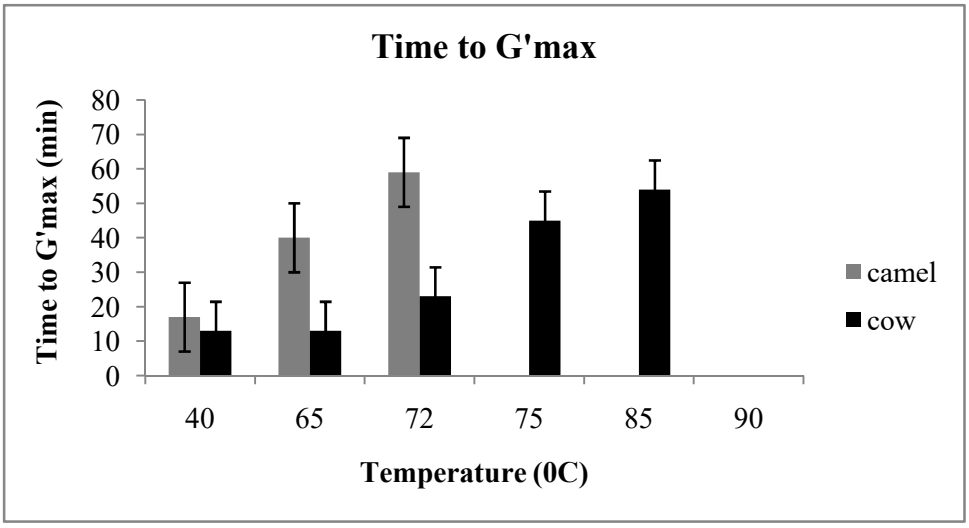

Figure 6. Effect of heat treatment on Time to G'max of camel and cow milk.

The time to G'max for cow milk was significantly (P<0.05) increased as heat treatment increases (Figure 6). When compares the mean value between treatment short time to rich higher G'max development was observed for heat treated milk at 40^0C while longer time was at higher heat treatment 85^0C. While samples that treated at 90^0C have 0 G'max until 60 min measurement time. The longer time to rich maximum gel development might be due to longer onset time of coagulation and slower curd aggregation that hindered of chymosin activity by structural change of whey protein due to heat treatment (Rynne *et al.,* 2004).

5. SUMMARY, CONCLUSION and RECOMANDETION

5.1. Summary

The present study was under taken to investigate the effect of different level of pasteurization temperatures on whey protein denaturation, rennetability property of camel milk to determine optimal temperature for treatment of cheese milk. The experiment was done in a completely randomized design (CRD) experimental design, by evaluating effect of temperature level at 40°C 65^0C/30 min, 72^0C/30sec, 75^0C/ 5 min,85^0C /5 min and 90^0C/ 5 min. Untreated milk samples were used as reference for chemical compositions and whey protein denaturation analysis instead of heated milk at 40^0C. The effect heat treatment on major chemical components such as fat, protein, lactose was evaluated at different temperatures. The effect of heat treatment on the total whey proteins denaturation and individual whey proteins denaturation such as β-lg α-la, bovine serum albumin, camel serum albumin and lactoferrin were also evaluated. While the rannetability parameter such as gelation time, G'max and time to G'max. were also determined. Heat treatment was done using water-bath while milkoscan was used for milk composition analysis and SDS-PAGE and kjeldhal method were used for individual and total whey protein denaturetion analysis whereas rennetabilty property analysed using free oscillation rheometry ReoRox G2.

The result of this study indicated that Fat%, lactose%, and total solid %, were not significantly different between camel and cow milk. Whereas the percentage of protein, casein, soiled not fat and Lactic acid were significantly higher in cow milk than that of camel milk at (P<0.05). Heat treatment of milk at 65^0C/30 min, 72^0C/30sec,75^0C/5 min ,85^0C/5min and 90^0C5min had no significant effect on the gross chemical composition of fat, protein and lactose of both camel and cow milk (P>0.05). A significant effect was observed on casein% in both camel and cow milk at higher temperature. Heat treatment significantly (P< 0.05)decreases the value of Non-casein nitrogen of camel and cow milk as temperature increase while the effect on Non-protein nitrogen was insignificant. Total whey protein denaturetion percentage increase as level of heat treatment increases for both camel and cow milk (P<0.05). Significantly higher WPD % was seen at 90^0C/5 min heat treatment than all other heat treatment temperatures. On the other hand at 65^0C/ 30 min total WPD% was significantly lower than the other heat treatment level. From whey

proteins camel milk α-La intensity shows less denaturation while CSA and LF band intensity decreases constantly that was due to denaturation as heat treatment level increases. β-Lg was absent in camel milk while in cow milk band intensity of β-Lg and α-La was decreased that due to increase of denatured level as heat treatment increases. Whereas BSA and lactoferrin show a steady decrease as heat treatment level increases. the reduction in band intensity was due to denaturation of whey protein and their association with casein micelles of κ- casein or other proteins.

The second experiment was done on rennetability property heated cheese showed that gelation time of heat treated camel and cow milk increased as the level of heat treatment increases (P<0.05). For camel milk coagulation time for heat treated at 40^0C milk was 6 min while it was 14 min and 19 min at 65^0C and 72^0C respectively. Further increase of heat treatment to 75 and above resulted None coagulated milk. In cow milk it took longer time to form gel and even become none non coagulated at 90^0C/5min. G'max of camel milk significantly affected by heat treatment (P<0.05). Significantly firmer gel development was observed for heat treated milk at 40^0C for both milk. For heat tread samples at 65^0C/30 min results (51 pas) while at 72^0C/30sec 24 pas. Whereas for cow milk non coagulated at 90^0C/5min. For both camel and cow milk the time to rich G'max was longer as temperature increases (P< 0.05).

5.2 Conclusion

Generally heat treatment of milk resulted the denaturation of whey proteins and this denaturation rate depends on the level of heat treatment temperature for both camel and cow milk and also it depends on the individual whey protein. Non-casein nitrogen of camel and cow milk decreased as heat treatment temperature increase while the effect on Non-protein nitrogen was insignificant. following NCN and NPN value whey protein nitrogen of also decreased as heat treatment increased. Finally total whey protein denaturetion % increased as heat treatment significantly increased. From the individual whey proteins camel milk α-La intensity shows less denaturation while CSA and LF band intensity decreases constantly that due to denaturation as heat treatment level increases. β-Lg was absent in camel milk while in cow milk band intensity of β-Lg and α-La was decreased as it show denaturation as heat treatment increased. Even if the standard temperature for

treatment of camel milk for cheese making and the interaction formed during heat treatment is not well know it can be concluded that the level of cheese milk pre-treatment temperature can determinant the rennetabilty property that related with coagulation and curd aggregation in both camel and cow milk. The short gel formation time and stronger gel resulted at reference milk that heat treated at 40^0C comparatively to higher temperatures that resulted in longer time and lower G'max. However, due to poor raw milk quality as the cases of most areas including Ethiopia and for the quality of final product a low pasteurization at min that showed better rennetebility in terms gelation time, G'max value and minimum whey protein denaturation than higher heat treated milk can be optimum for cheese milk pasteurization for camel milk.

5.3 Recommendations

- Lower temperature long time at 65^0C/30 should be used for camel milk cheese milk treatment. However Higher temperature short time heat treatment at 72^0C/30sec could be other alternative if a need to incorporate whey proteins to improve yield .
- Camel milk heat treatment is possible without any significant effects on the gross chemical composition. This could be un advantages to reduce risk related to raw milk consumption through awareness creation by the concerned organization on importance and possibility of camel milk heat treatment to pastoralists society.
- Further research should be done on property of denatured whey proteins of camel milk to known in detail the complex formation during heat treatment.

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7. APPENDIX

Appendix 1. Analysis of variance(ANOVA) table fresh camel and cow milk analysis

Dependent variable Fat					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model	1	0.18490000	0.18490000	0.17	0.7199
Error	2	2.17220000	1.08610000		
Corrected total	3	2.35710000			
Dependent variable Protein					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	0.81000000	0.81000000	95.86	0.0103
Error	2	0.01690000	0.01690000		
Corrected total	3	0.82690000			
Dependent variable NSF					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	1.36890000	1.36890000	427.78	0.0023
Error	2	0.00640000	0.00320000		
Corrected total	3	1.37530000			
Dependent variable Total solid					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	2.11702500	2.11702500	1.54	0.3409
Error	2	2.75625000	1.37812500		
Corrected total	3	4.87327500			
Dependent variable Lactose					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	0.00040000	0.00040000	0.04	0.8600
Error	2	0.02000000	0.01000000		
Corrected total	3	0.02040000			
Dependent variable Casein					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	0.48302500	0.48302500	170.98	0.0058

Error	2	0.00565000	0.00565000		
Corrected total	3	0.48867500			

Dependent variable lactic acid					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	0.00230400	0.00230400	67.76	0.0144
Error	2	0.00006800	0.00003400		
Corrected total	3	0.00237200			

Dependent variable Density					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	0.72250000	0.72250000	1.99	0.2935
Error	2	0.72500000	0.36250000		
Corrected total	3	1.44750000			

Dependent variable pH					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ species	1	0.03610000	0.03610000	55.54	0.0175
Error	2	0.00130000	0.00065000		
Corrected total	3	0.03740000			

Appendix 2. Analysis of variance(ANOVA) table effect of heat treatment on gross chemical composition of camel

Dependent variable Fat					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/temperature	5	0.02276667	0.00455333	0.00	1.0000
Error	6	10.13210000	1.68868333		
Corrected total	11	10.15486667			

Dependent variable Protein					
Source	*DF*	Sum of squares	Mean square	F Value	Pr>f
Model/ temperature	5	0.00040000	0.00008000	0.05	0.9976
Error	6	0.00960000	0.00160000		
Corrected total	11	0.01000000			

Dependent variable Lactose					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	0.01250000	0.00250000	0.37	0.8524
Error	6	0.04050000	0.00250000		
Corrected total	11	0.05300000			
Dependent variable Casein					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	0.05884167	0.01176833	7.80	0.0133
Error	6	0.00905000	0.00150833		
Corrected total	11	0.06789167			
Dependent variable Total solid					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	0.79794167	0.15958833	0.09	0.9912
Error	6	10.82615000	1.80435833		
Corrected total	11	11.62409167			

Appendix 3. Analysis of variance(ANOVA) table effect of heat treatment on gross chemical composition of cow milk

Dependent variable Fat					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/temperature	5	0.28226667	0.05645333	0.17	0.9647
Error	6	1.99860000	0.33310000		
Corrected total	11	2.28086667			
Dependent variable Protein					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	0.00096667	0.00019333	0.01	1.0000
Error	6	0.11760000	0.01960000		
Corrected total	11	0.11856667			
Dependent variable Lactose					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	0.00784167	0.00156833	0.11	0.9871

Error	6	0.08945000	0.01490833		
Corrected total	11	0.09729167			

Dependent variable Casein

Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	0.19170000	0.03834000	6.27	0.0225
Error	6	0.03670000	0.00611667		
Corrected Total	11	0.22840000			

Dependent variable Total solid

Source	*DF*	Sum of squares	Mean square	F Value	Pr>f
Model/ temperature	5	0.07804167	0.01560833	0.09	0.9918
Error	6	1.09125000	0.18187500		
Corrected total	11	1.16929167			

Appendix 4. Analysis of variance(ANOVA) table effect of heat treatment on total whey protein denaturetion of camel milk

Dependent variable NCN

Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/temperature	5	2007.876667	401.575333	72.03	<.0001
Error	6	7.80000000	5.575000		
Corrected total	11	2041.326667			

Dependent variable NPN

Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	25.54666667	5.10933333	3.93	0.0630
Error	6	7.80000000	1.3800000		
Corrected total	11	33.34666667			

Dependent variable WPN

Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	2455.564167	491.112833	66.90	<0.0001
Error	6	44.045000	7.340833		
Corrected total	11	2499.609167			

Dependent variable WPD%					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	3743.758667	748.751733	113.42	<0.0001
Error	6	39.611100	6.601850		
Corrected total	11	3783.369767			

Appendix 5. Analysis of variance(ANOVA) table effect of heat treatment on total whey protein denaturetion of cow milk

Dependent variable NCN					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/temperature	5	5075.237500	1015.047500	139.99	<0.0001
Error	6	43.505000	7.250833		
Corrected total	11	5118.742500			
Dependent variable NPN					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	95.8866667	19.1773333	5.10	0.0360
Error	6	22.5700000	3.7616667		
Corrected total	11	118.4566667			
Dependent variable WPN					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	6563.390000	1312.678000	257.39	<0.0001
Error	6	30.600000	5.100000		
Corrected total	11	6593.990000			
Dependent variable WPD%					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	6028.990667	1205.798133	168.66	<0.0001
Error	6	42.894800	7.149133		
Corrected total	11	6071.885467			

Appendix 6. Analysis of variance(ANOVA) table for of effect of heat treatment Rennetebility of camel milk

Dependent variable GT					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/temperature	5	6589.319500	1317.863900	178.15	<0.0001
Error	6	44.386200	7.397700		
Corrected total	11	6633.705700			
Dependent variable G' max					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	19781.17750	3956.23550	10.54	0.0062
Error	6	2251.84500	375.30750		
Corrected total	11	22033.02250			
Dependent variable T to G'max					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	3092.751042	618.550208	4.22	0.0544
Error	6	880.301250	146.716875		
Corrected total	11	3973.052292			

Appendix 7. Summary of Analysis of variance(ANOVA) table for effect of heat treatment on Rennatability of cow milk.

Dependent variable GT					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/temperature	5	4529.584767	905.916953	303.20	<0.0001
Error	6	17.926900	2.987817		
Corrected total	11	4547.511667			
Dependent variable G' max					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f
Model/ temperature	5	247394.6642	49478.9328	424.76	0.0001
Error	6	698.9250	116.4875		
Corrected Total	11	248093.5892			
Dependent variable T to G'max					
Source	*DF*	Sum of squares	Mean square	F-Value	Pr>f

Model/ temperature	5	4361.646067	872.329213	21.76	0.0009
Error	6	240.530500	40.088417		
Corrected total	11	4602.176567			

Appendix table 8. Band % of individual Whey protein

Temperature	Band % of whey protein compared to the % of raw milk band intensity				
Camel milk	β-lg	α-la%	CSA%	Lf%	GLYCAM-1%
40^0C	-	100	100	100	100
65^0C/30min	-	104	91.59	66.57	105
72^0C/30sec	-	89.9	75.9	52.7	73
75^0C/5min	-	110	42.3	27.8	127
85^0C/5 min	-	68.2	10.8	6.46	80.3
90^0C/5min	-	66.5	7.8	5.07	70.2
Temperature					
Cow milk	β-lg	α-la	BSA	LF	
40^0C	100	100	100	100	
65^0C/30min	101	94.3	85.5	30	
72^0C/30sec	100.2	82.3	35.6	10.9	
75^0C/5min	64.4	45.37	7.2	1.7	
85^0C/5 min	1.9	0.49	0.56	3.28	
90^0C/5min	4.17	4.6	0.0	3.28	