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Effect of Heat Treatment on Camel Milk Proteins

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EFFECT OF HEAT TREATMENT ON CAMEL MILK PROTEINS

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Food Science

Under the Supervision of Professor Afaf Kamal Eldin

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Declaration of Original Work

I, Crystal Dcosta Clement Dcosta, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Effect of Heat Treatment on Camel Milk Proteins*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Professor Afaf Kamal Eldin, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Camel milk is important in the dry and arid lands because of its cultural, nutritional, and therapeutic properties. Milk proteins are known to be affected by various treatments including heating. The structure of proteins are known to change upon exposure to temperatures due to unfolding/folding and intra- and inter-molecular interactions. The aim of this thesis was to study the effect of various heating temperatures (60-130 °C) and times (0, 1, 10, and 30 min) on camel milk proteins. Electrophoresis (SDS-PAGE), free thiol groups, and hydroxymethylfurfural were used to observe the changes in proteins after the heat treatments. It was found that considerable changes in the proteins happen already during the first minute of heating. Camel whey proteins were more sensitive to heat than the caseins. As camel milk is devoid of β-Lactoglobulin, the major whey protein is α-Lactalbumin. α-Lactalbumin showed an increase in intensity with heating, which was not reported before. This might be due to complexation with fatty acids and formation of Alpha-lactalbumin Made Lethal to Tumor cells (AMLETs). The free thiol content decreased while hydroxymethylfurfural increased with heating time and temperature.

Keywords: Camel milk proteins, a-lactalbumin, heat treatment, SDS-PAGE, hydroxymethylfurfural.

Title and Abstract (in Arabic)

تاثير المعالجات الحرارية على بروتينات حليب الابل

الملخص

يعد حليب الابل مصدر غذائى هام فى الاماكن الجافة لاسباب تتعلق بالارث الثقافى والقيمة الغذائية والعلاجية. تتاثر بروتينات الحليب بالحرارة فتتغير تركيبتها وتتفاعل مع المركبات الاخرى الموجودة فى الحليب. الهدف من هذه االأطروحة هو دراسة أثر درجات الحرارة المختلفة (60-130 درجة مئوية) ومدة التسخين (0، 1 ،10 و 30 دقيقة) على بروتينات حليب الابل. تمت متابعة التغيرات بواسطة الفصل الكهربائى الهلامى ومجموعات الثيول الحر وهايدروكسيد ميثيل الفارفارال. وفد لوحظ فى هذه الدراسة ان التغيرات فى البروتينات تبدأ خلال الدقيقة الأولى من التسخينوأن البروتينات فى مصل الحليب أكثر تأثرا بالحرارة من بروتينات التجبن (الكاسينات). ونسبة لخلو حليب الابل من البتا لاكتوقلوبيولين فان البرتين الرئيسى فىمصل الحليب هو الألفا لاكتالبيمين الذى يتواجد مع اللاكنوفيرين والبيومين المصل. بينما تقل كميات كل البروتينات بتاثير الحارة، فقد لوحظ ان كثافة الألفا لاكتالبيومين تزيد مما يمكن تفسيره بتكوين مركبات معقدة مع الأحماض الدهني'. كما لوحظ ان كميات الثيوت الحر تنقص بينما تزيد كميات وهايدروكسيد ميثيل الفارفارال وهو أمر متوقع.

مفاهيم البحث الرئيسية: حليب الابل، بروتينات الحليب، التاثير الحرارى، الفصل الكهربائى الهلامى، مجموعات الثيول الحر، هايدروكسيد ميثيل الفارفارال.

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I thank my God for giving me my wonderful parents and family who have encouraged me, prayed for me and helped me along the way. Though this was long and tiresome, their love and support has strengthened me throughout my journey.

Dedication

To my beloved parents and family

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List of Abbreviations

α-CN	Alpha-Casein
α-LA	Alpha-Lactalbumin
β-CN	Beta-Casein
β-LG	Beta-Lactoglobulin
BSA	Bovine Serum Albumin
CE	Capillary Electrophoresis
CIEF	Capillary Isoelectric Focusing
CSA	Camel Serum Albumin
CZE	Capillary Zone Electrophoresis
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
DTT	Dithiothreitol
ESI	Electrospray Ionization source
FAO	Food and Agriculture Organization
HMF	Hydroxy Methyl Furfural
HTST	High Temperature Short Time
IEF	Isoelectric Focusing
κ-CN	Kappa-Casein
LC	Liquid Chromatography
LF	Lactoferrin
LTLT	Low Temperature Long Time
m/z	Mass/Charge Ratio
MALDI-TOF	Matrics-Assisted Laser Desorption Ionization Time of Flight
MEKC	Micellar Electro Kinetic Chromatography

MS	Mass Spectrometry
pI	Isoelectric Point
RP-HPLC	Reversed Phased High Performance Liquid Chromatography
Rf	Relative Front
RSD	Relative Standard Deviation
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
TBA	2-Thiobarbituric Acid
TCA	Trichloroacetic Acid
UHT	Ultra-High Treatment
UPLC	Ultra-High Performance Liquid Chromatography

Chapter 1: Introduction

1.1 Overview

Heat treatment is a crucial step in milk processing performed to reduce bacterial load and enzyme activity in order to extend the shelf-life of the final product (Sakkas *et al*., 2014; Felfoul *et al*., 2017). In addition, heat treatment modifies protein secondary and tertiary structures and enables certain processes such as fermentation of yoghurt and cheese. The common heat treatments applied to milk are pasteurization and sterilization aiming to reduce the number of pathogens to an extent that there is no risk of a health hazard (Sakkas *et al*., 2014; Patel and Patel, 2015). High temperature short time (HTST) is a common pasteurization technique used, where the milk is heated at 72-80 °C for 15-30 s, but may vary depending on different countries (Lewis and Deeth, 2008). A less commonly used method is low temperature long time (LTLT), in which milk is heated at 63 °C for 30 min. LTLT is not used as much as HTST because the longer processing time leads to more chemical changes in protein and non-protein components (Lewis and Deeth, 2008). Another method is ultra-high temperature (UHT) treatment where the milk is heated at temperatures between 135-150 °C for 1-10 s. This sterilization process is able to destroy all microorganisms and spores present in milk. The efficiency of the heat treatment and its effect are related to the time and temperature combinations, method used for heating along with pH and any pre-treatment conditions (Fox and Kelly, 2006; Lewis and Deeth, 2008).

As milk is heated at temperatures above 60 °C, different reactions may take place. Heat treatments lead to varied degrees of denaturation, aggregation, and interactions of proteins (Sakkas *et al*., 2014; Patel and Patel, 2015). The heat treatment causes denaturation of whey proteins, which can be reversible or irreversible

depending on temperature and other conditions such as pH (Sakkas *et al*., 2014). In addition, non-enzymatic browning occurs because of heating and can cause off-flavours, changes in colour due to substances formed by Maillard reactions, as well as loss of nutritive value (Morales *et al.,* 1996; Van Boekal, 1998; Sakkas *et al.*, 2014).

In UHT treatment of milk, different degrees of interactions among whey proteins and between whey proteins and caseins occur to form protein aggregates (Anema, 2008; Wang *et al*., 2012; Wijayanti *et al.,* 2014). When bovine milk is heated at high temperatures, aggregates *via* disulphide bonding and hydrophobic interactions between β-lactoglobulin (β-LG) and κ-casein (κ-CN) are formed (Nolet and Toldra, 2010; Wijayanti *et al.,* 2014). The hydrophobic interactions are predominant at temperatures below 70 °C whereas disulphide bond interactions take place at higher temperatures (O'Connell and Fox, 2011). When bovine milk is heated with LTLT protocols, its major whey protein β-LG, will have enough time to unfold and associate with the casein micelles. On the other hand, with HTST treatment, β-LG will not unfold completely, which allows it to refold into a non-native structure and form aggregates with other monomers instead of κ-CN (Oldfield *et al*., 1998). The other complexes formed during heat treatment are those between α-lactalbumin (α-LA) and β-LG and between α-LA and κ-CN protein. β-LG can form a heat induced complex with α-La through thiol-disulphide interchange (Oldfield *et al*., 1998; Tolkach and Kulozik, 2007; Donato and Guyomarc'h, 2009; Sakkas *et al*., 2014). The interactions between β-LG, α-La, and κ-CN depend *inter alias* on their total and relative concentrations, the heating time and temperature combination, pH, and ionic strength (Oldfield *et al*., 1998; Donato and Guyomarc'h, 2009).

Up to now, it is not possible to attain UHT milk from camel milk (*Camelus dromedaries*) and the reason for this is not completely understood. While camel whey

proteins were mentioned not to be affected by heating at temperatures below 70 ℃, noticeable changes are observed at higher temperatures (Farah, 1986; Elagamy, 2000). Although camel milk lacks β-LG, this milk is much unstable during UHT treatment compared to bovine (*Bos taurus*) milk suggesting that the chemistry of gelation of milk proteins is not well understood. This gives researchers even more reason to investigate the chemistry of camel milk proteins and how they are affected by heat treatment.

1.2 Statement of the Problem

Camel milk can be used as an alternative to bovine's milk and its products (Mayer and Fiechter, 2012). Compositional and conformational changes in milk proteins are known to affect the functional properties of dairy ingredients, such as solubility, gelation, heat stability and emulsification, which ultimately affect their performance in the dairy products. Understanding the effect of temperature on camel (*Camelus dromedaries*) milk proteins is very important since these proteins have unique nutritional and technological properties. Therefore, the aim of this thesis was to study the effect of various heating processes on the denaturation of camel milk caseins and whey proteins.

1.3 Relevant Literature

Milk and milk products are consumed by more than 6 billion people all around the world. Milk is a complex and dynamic nutritional system with multiple nutritional, functional, and therapeutic benefits (FAO, 2012). Milk has become an important source of dietary energy, fats, and proteins thus making it wholesome especially for children and older people. Milk is known to provide several essential nutrients including calcium, magnesium, selenium, riboflavin, vitamin B12, and pantothenic acid (Tache Kula and Tegegne, 2016; Mati *et al.*, 2017). Milk is produced by different mammalian species with the milk quality being affected by the animal species, its breed, age, diet, stage of lactation, geographical location, farming system, and season. The season has an influence on the colour, flavour and composition of milk and allows the production of a variety of milk products (FAO, 2012).

1.3.1 Camel Milk

Camel (*Camelus dromedaries*) milk is considered an important source of nutrition in different parts of the world. They are part of the staple diet in most arid lands especially in the Middle East. A very small percentage of raw milk is still drunk fresh as consumers are aware of the hazards and risks associated with its consumption. Some of the products made from milk are butter cheese and khoya. Fermented camel milk is available in different forms, e.g. Shubat (chal), Suusac (susa) and gariss (Brezovečki *et al.*, 2015). Both camel and goat can survive arid land climates with meagre fodder and harsh environmental conditions (Muehlhoff *et al.*, 2013). Camel milk is known to have many nutritional and therapeutic properties (Table 1) like hypo-allergic (Shabo *et al.*, 2005) anti-carcinogenic, anti-hepatic (EL-Fakharany *et al.*, 2012; Habib *et al.*, 2013) and anti-diabetic properties (Agarwal *et al.*, 2007) which

makes it suitable to be consumed by children allergic to bovine milk. It has also been studied previously that goat milk is easily digested due to the smaller fat globule size as compared to bovine milk. This suggests that goat milk has lower allergenicity than bovine milk though it has similar proteins (β-lactoglobulin) as that of bovine milk.

Table 1: Nutritional and therapeutic properties of camel milk

Nutritional and therapeutic properties	**References**
Anti-carcinogenic	Agarwal *et al.*, 2007; EL-Fakharany *et al.*, 2012; Habib *et al.*, 2013
Anti-diabetic properties	Shabo *et al.*, 2005; Agarwal *et al.*, 2007; Khalesi *et al.*, 2017
Hypo-allergenic	Shabo *et al.*, 2005; Al haj and Al Kanhal, 2010
Anti-hypertensive	Shabo *et al.*, 2005; Khalesi *et al.*, 2017
Immuno-modulatory, anti-inflammatory	Khalesi *et al.*, 2017
Therapeutic properties for autism	Gizachew *et al.*, 2014; Kaskous, 2016
Antigenotoxic, anticytotoxic	Khalesi *et al.*, 2017

Camel milk is usually drunk fresh which may have a sharp and salty taste. The milk is opaque white having a pH ranging from 6.2-6.5 (Farah, 1996). Goat (*Capra hircus*) milk on the other hand is known to have a characteristic "goaty and muttony flavour" (Muehlhoff *et al.*, 2013). It has been studied that camel milk contains a higher concentration of vitamin C, vitamin A and E, niacin, minerals and poly-unsaturated fats. The vitamin C content was reported to be three to five times higher as compared to bovine milk (Haddadin *et al.*, 2008).

1.3.2 Comparison of Camel Milk Composition with other Milks

Milk is one of the most widely produced agricultural commodities worldwide. The main components of milk are lactose, proteins, fat, and minerals (FAO, 2012). Milk is a good source of high quality and multifunctional proteins but these proteins are susceptible to the processing conditions used by the food industry. The fat present in milk is mainly composed of triacylglycerol in the form of an emulsion of fat globules. Minerals associate with proteins as salts or bound ions. Lactose, a soluble carbohydrate molecule is a disaccharide of glucose and galactose (Patel and Patel, 2015). Table (2) draws a comparison of basic nutrients in different milk.

Table 2: Average composition of basic nutrients in camel, goat, bovine and human milks

Composition	**Camel (*Camelus dromedarius*)**	**Goat (*Capra hircus*)**	**Bovine (*Bos taurus*)**	**Human (*Homo sapiens*)**
Fat (%)	3.5	3.8	3.6	4.0
Solids-non-fat (%)	7.4	8.9	9.0	8.9
Protein (%)	3.4	3.4	3.2	1.2
Lactose (%)	4.4	4.4	4.7	6.9
Casein (%)	2.1	2.4	2.6	0.4
Ash (%)	0.7	0.8	0.7	0.3
Casein/whey ratio	1.68:1	3.5:1	4.7:1	0.4:1

Sources: Farah, 1986; Elagamy, 2000; Park *et al*., 2007; Konuspayeva *et al*., 2009; Al haj and Al Kanhal, 2010; Muehlhoff *et al*., 2013; El-Hatmi *et al*., 2015

Camel milk protein content ranges between 2.1-4.9%, fat content 1.2-6.4%, and lactose 2.4-5.0% (Konuspayeva *et al*., 2009) while goat milk protein content ranges between 2.9-3.8%, fat content 3.3-4.5% and lactose 4.2-4.5% (Muehlhoff *et al.*, 2013). About 52-87% of camel milk is comprised of caseins (Khaskheli *et al*., 2005). Raynal-Ljutovac *et al*. (2008) stated that the protein content in goat milk depends on the genetic polymorphism of α_{S1}-casein. It has also been observed that goat milk contains less α_{S1}-casein than other milk and that the total protein is indirectly dependent on the goat breed depending on the allele frequency of α_{S1}-casein (Raynal-Ljutovac *et al*., 2008). Compared to other bovine species, camel whey contains a higher content of lysozyme, lactoferrin and immunoglobulins and whey acidic and whey basic proteins (El-Hatmi *et al*., 2007). It has also been reported that lactoferrin from goat milk mimics the functionality of lactoferrin from human milk, thus making it a better source for infant formulae (Le Parc *et al*., 2014). The composition of camel milk has been studied in different parts of the world, especially in the Middle East. The mean values of the composition of camel milk and goat milk varies with time due to factors like camel diet, climate, availability of water and several other factors (Raynal-Ljutovac *et al*., 2008; Al haj and Al Kanhal, 2010). However, there have been variations in camel milk composition as mentioned earlier. In comparison with bovine milk, camel milk fat contains a higher concentration of unsaturated fatty acids (Haddadin *et al*., 2008; Konuspayeva *et al*., 2008) which contributes to the milk's overall dietary quality.

There isn't much difference between the amino acid composition of camel milk and bovine milk. The concentration of essential and non-essential amino acids is higher in bovine milk than camel milk, except for arginine. Essential amino acids in bovine β-casein is higher than the concentration of it in the β-casein of camel breeds.

The protein contents of dairy animals differ between breeds and could also differ due to regions and their lactation stage (Raynal-Ljutovac *et al.*, 2008; Al haj and Al Kanhal, 2010). Table (3) depicts a comparison between the protein fractions of different types of milk. Thus, the differences between camel milk and bovine milk with respect to protein composition shows that camel milk, unlike bovine milk does not contain β-LG. However, camel milk may contain a higher amount of α-LA than bovine milk. Another notable difference is the lesser amount of κ-CN in camel milk.

Table 3: Comparison of proteins (in g/L) in camel, goat, bovine and human milks

Protein fraction	**Camel Milk**	**Goat Milk**	**Bovine milk**	**Human Milk**
Total Casein	22.1-26	23.3-46.3	24.6-28	2.4-4.2
α casein	2.89	5.9	12.79	0.77
β-casein	12.78	0-29.6	11.66	3.87
κ-casein	1.67	2.8-13.4	4.39	0.14
Micelle size (nm)	380	260	150-182	64-80
Total whey protein	5.9-8.1	3.7-7.0	5.5-7.0	6.2-8.3
α-lactalbumin	2.01	0.7-2.3	1.08	1.9-3.4
β-lactoglobulin	-	1.5-5.0	5.97	-
Immunoglobulin	1.5	-	0.5-1.0	0.96-1.3
Serum Albumin	0.46	1.6-5.5	0.36	0.4-0.5
Lactoferrin	1.74	0.02-0.2	-	1.5-2.0
Lysozyme	(60-1350) x 10^{-6}	250 x 10^{-6}	(70-600) x 10^{-6}	0.1-0.89

Source: Elagamy, 2000; Park *et al.*, 2007; Claeys *et al.*, 2014; El- Hatmi *et al.*, 2014; Ali Al-Alawi and Laleye, 2015; Brezovečki *et al.*, 2015; Hailu *et al.*, 2016; Omar *et al.*, 2016

Camel milk, as compared to other milk has a lower fat content. As compared to bovine milk, it is also known that camel milk has a lower percentage of short chain fatty acids and higher percentage of long chain fatty acids like stearic and palmitic acids (Khalesi *et al.*, 2017). From Figure (1) it can be noted that camel milk has the smallest fat globules as compared to buffalo, cow and goat milks. This is another reason why camel milk has a higher digestibility than other milks (Khalesi *et al.*, 2017).

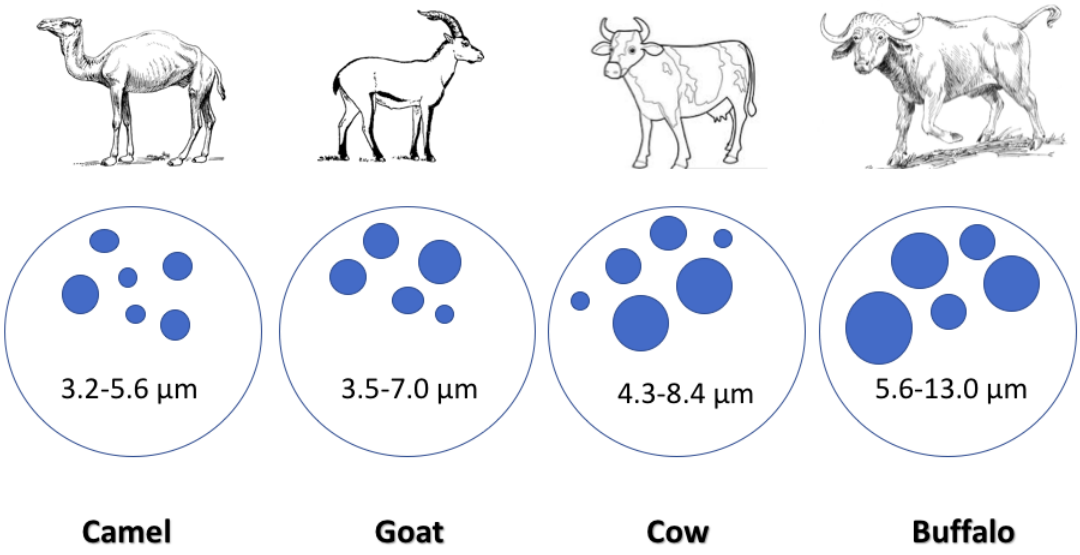


Figure 1: Size distribution of fat particles in different milks

Modified from Khalesi *et al.* (2017)

1.3.3 Caseins

The main types of casein fractions in milk are α_{s1}-casein, α_{s2}-casein, β-casein and κ-casein (Figure 2) (Ghnimi and Kamal-Eldin, 2015). Camel milk has lower concentrations of α- and κ-caseins and a higher concentration of β-casein compared to bovine milk (Brezovečki *et al.*, 2015; Ghnimi and Kamal-Eldin, 2015) while goat milk is composed of α_{s2}-casein, β-casein and κ-casein (Hinz *et al.*, 2012). The content of β-

casein, which comprises 65% of the total caseins in camel milk is higher than in bovine milk (36%) leading to improved digestibility and lower incidences of allergy in infants (El-Agamy *et al*., 2009; Brezovečki *et al*., 2015). Among the caseins in goat milk, β-casein is the major casein fraction which is like human milk and different from bovine milk. Caprine milk also lacks α_{s1}-casein in some types, which could explain the fact that caprine milk is less allergenic than bovine milk (Hinz *et al*., 2012; Muehlhoff *et al*., 2013). Nonessential amino acids in κ-casein in bovine milk is higher in comparison with camel milk, except arginine which is found in greater amounts in camel milk κ-casein. Bovine milk κ-casein contains a higher concentration of essential amino acids in comparison with camel milk, except for lysine whose concentration is higher in the camel κ-casein (Brezovečki *et al*., 2015).

Farah (1996) reported that camel milk caseins have higher molecular weights as compared to bovine milk. According to the author, the molecular weights of β-CN and α-CN in camel milk were 28.6 kDa and 35 kDa, respectively. In bovine milk these β-CN and α-CN had molecular weights of 24 kDa and 22-25 kDa, respectively (Farah, 1996). Camel milk contains ~3.5% κ-CN whereas 13% of the same is found in bovine milk (Al haj and Al Kanhal, 2010). The amount of κ-CN present in camel milk is much lower as compared to bovine milk (Figure 2) (Farah & Atkins, 1992; Kappeler *et al*., 2003). κ-CN is known to be an important factor in stabilizing the casein micelle in milk. At a higher pH, the negative charges on κ-CN micelle increases thus increasing the stability of the milk. As the amount of κ-CN is lower in camel milk, the casein micelles in this milk become more susceptible to Ca^{2+} induced precipitation which decreases their stability (Al haj, Metwalli and Ismail, 2011). κ-CN also contains an additional proline residue (Kappeler *et al*., 1998), which could be responsible for stability of camel milk (Kappeler *et al*., 1998). α-, β-, and κ-caseins group together

with calcium in stable casein micelles, in which κ-CN resides in the exterior with hydrophilic hairs extending in the water phase or the whey (Figure 3).

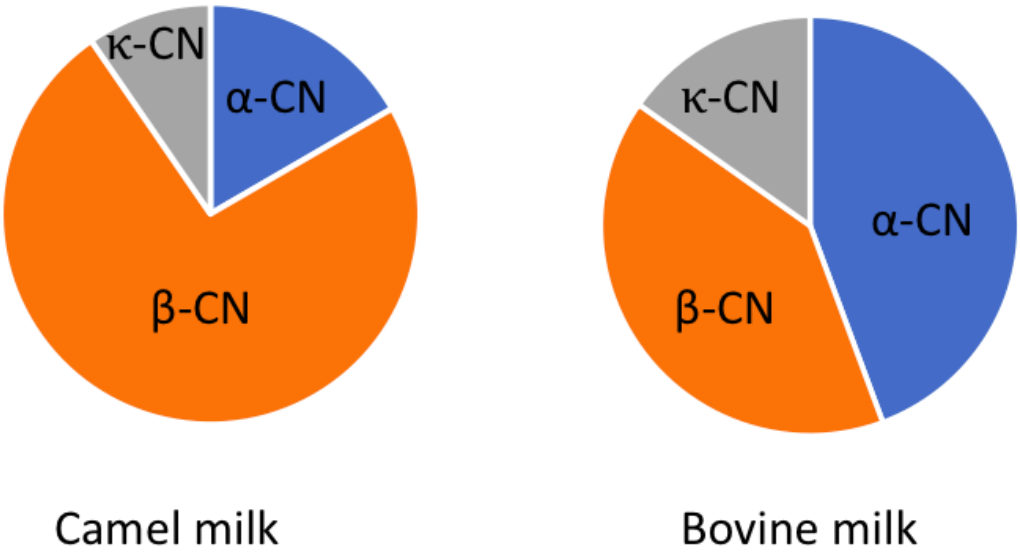


Figure 2: Distribution of the main caseins in camel and bovine milk

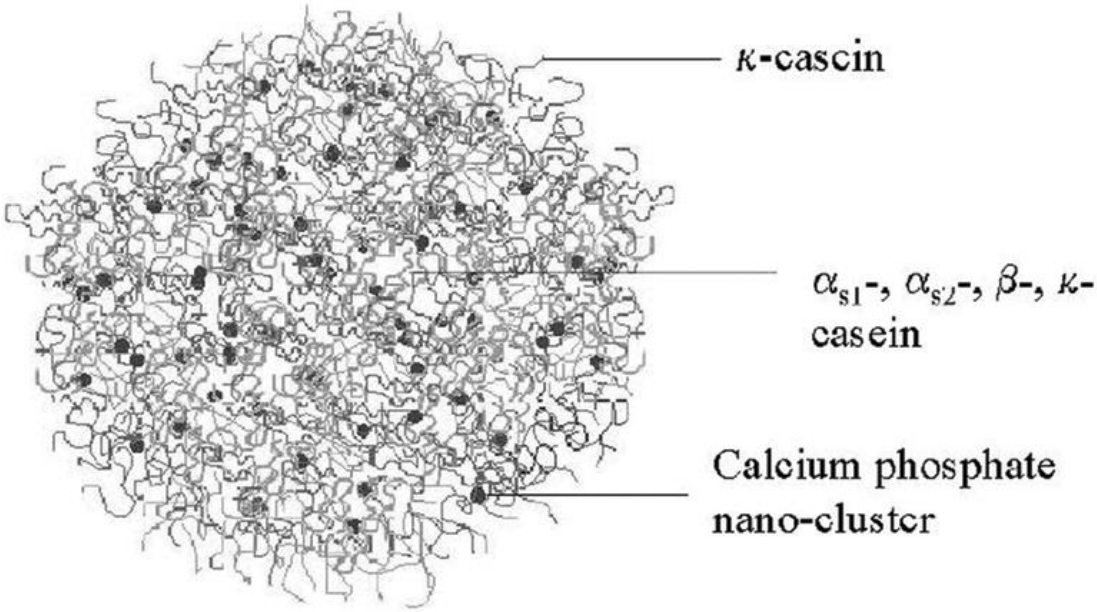


Figure 3: Model of a casein micelle structure

Reproduced from Hristov *et al*. (2016**)** with permission from InTech

1.3.4 Whey Proteins

The whey proteins in camel milk make up 20-25% of the total caseins (Al haj and Al Kanhal, 2010; Brezovečki *et al.*, 2015; Elhaj and Freigoun, 2015). The crucial whey proteins found in camel milk are α-lactalbumin, lactoferrin, serum albumin while lysozyme, lactoperoxidase and immunoglobulins are also among the other whey proteins found in camel milk (Khaskheli *et al.*, 2005; Brezovečki *et al.*, 2015). The main components of camel whey proteins are α-lactalbumin and camel serum albumin along with lactoferrin (Figure 4) (Zhao *et al.*, 2015). Camel milk does not contain β-lactoglobulin, which is present in higher concentration in bovine whey (El-Agamy *et al.*, 2009). Bovine whey is yellow-green whereas camel whey is white in color, possibly due to increased concentrations of caseins and fat globules and/or lower concentrations of riboflavin in camel milk (Elagamy, 2000; Haj & Kanhal, 2010). The amino acid sequence of camel milk proteins compared to bovine milk proteins is shown in Figure (5). α-lactalbumin in camel milk has a molecular mass of 14.6 kDa containing 123 residues. This is also similar to bovine, goat and human milk α-lactalbumin (Beg *et al.*, 1984; Beg *et al.*, 1986).

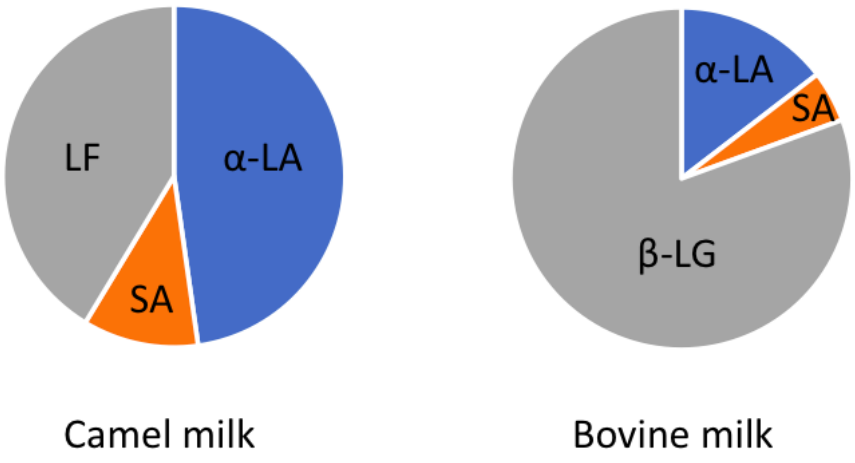


Figure 4: Distribution of the main whey proteins in camel and bovine milk

Omar *et al.* (2016)

Table 4: Physio-chemical properties of camel milk proteins, mean concentration (in g/L) as compared to bovine and caprine proteins

Camel protein	**Molecular mass (mature chain; Da)**	**Goat Protein**	**Molecular Mass (Goat protein; Da)**	**Homologous bovine protein**	**Molecular Mass (Bovine protein; Da)**
α_{S1}-casein B	25,307.33			α_{S1}-casein B	22,974
α_{S1}-casein A	24,289.24	α_{S1}-casein	24,290		
β-casein	24,650.76	β-casein	24,865	β-casein A2	23,583.2
α_{S2}-casein	21,265.90	α_{S2}-casein	26,389	α_{S2}-casein A	26,019
κ-Casein	18,209.79	κ-Casein	21,441	κ-Casein A	18,974.4
Lactoferrin	75,250.83	Lactoferrin	77,358	Lactoferrin/Lactotransferrin	76,143.9
Serum albumin	67,092.60	Serum albumin	66,313	Serum albumin	69,293
α-Lactalbumin	14,430.36	α-Lactalbumin	16,255	α-Lactalbumin B	14,186

Modified from Mati *et al.* (2017); Swiss Prot.

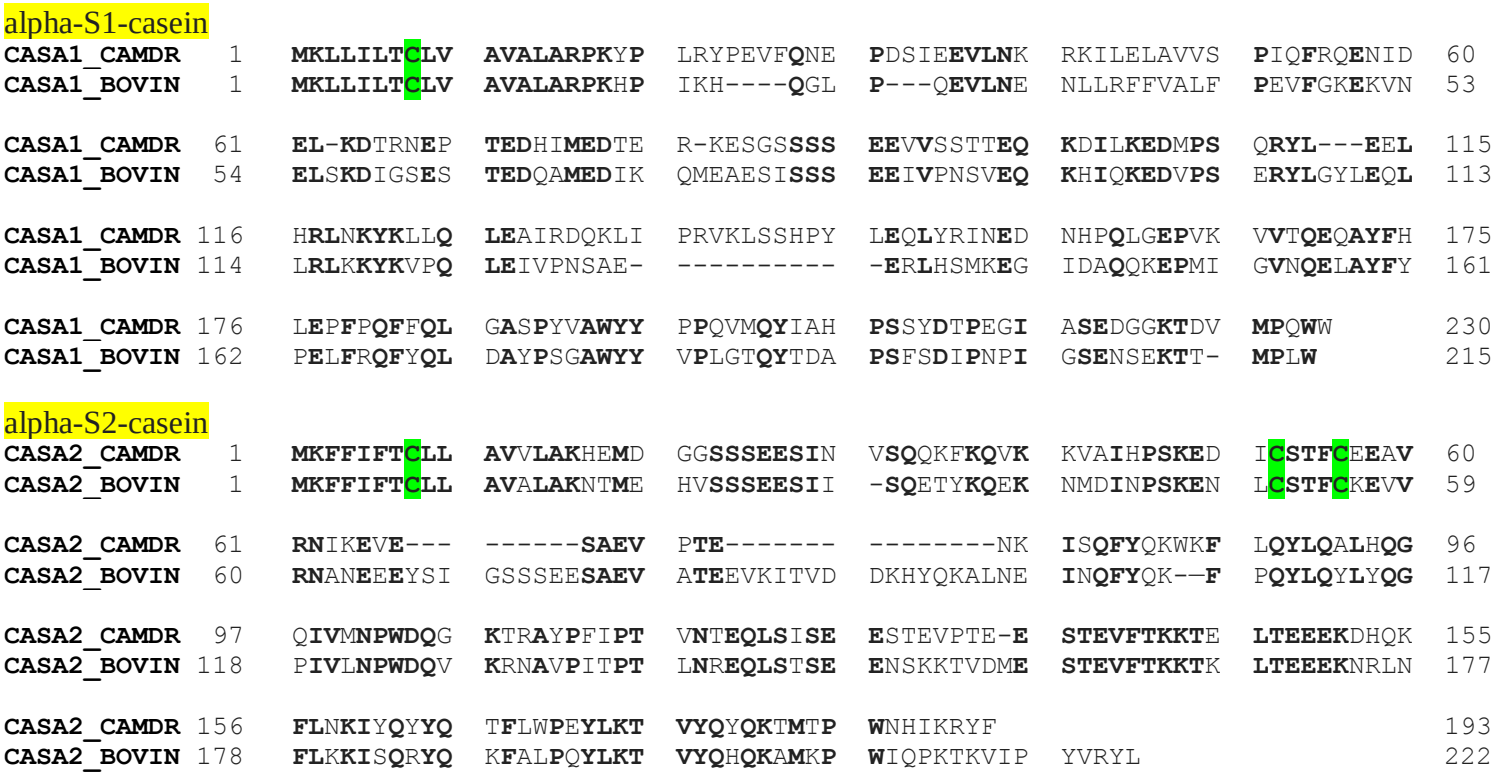


Figure 5: The amino acid sequence of major camel milk caseins and whey proteins aligned with bovine milk proteins

Corresponding amino acid residues are bold and cysteine residues (C) are highlighted green. The amino acid sequences are retrieved from UniProt/NCBI database and comparison of protein sequences was performed using BLAST (www.uniprot.org). Amino acid residues: Alanine (A), Cysteine (C), aspartic AciD (D), glutamic Acid (E), phenylalanine (F), Glycine (G), Histidine (H), Isoleucine (I), lysine (K), Leucine (L), Methionine (M), asparagiNe (N), Proline (P), glutamine (Q), aRginine (R), Serine (S), Threonine (T), Valine (V), tryptophan (W), and tYrosine (Y).

beta-casein

```
CASB_CAMDR    1   MKVLILACRV  ALALAREKEE  FKTAGEALES  ISSSEESITH  INKQKIEKFK  IEEQQQTEDE  60
CASB_BOVIN    1   MKVLILACLV  ALALARELEE  LNVPGEIVES  LSSSEESITR  INK-KIEKFQ  SEEQQQTEDE  59

CASB_CAMDR   61   QQDKIYTFPQ  PQSLVYSHTE  PIPYPI---L  PQNFLPPLQ-  -PAVMVPFLQ  PKVMDVPKTK  115
CASB_BOVIN   60   LQDKIHPFAQ  TQSLVY----  PFPGPIHNSL  PQN-IPPLTQ  TPVVVPPFLQ  PEVMGVSKVK  114

CASB_CAMDR  116   ETIIPKRKEM  PLLQSPVVPF  TESQSLTLTD  LENLHLPLPL  LQSLMYQIPQ  PVPQTPMIPP  175
CASB_BOVIN  115   EAMAPKHKEM  PFPKYPVEPF  TESQSLTLTD  VENLHLPLPL  LQSWMHQPHQ  PLPPTVMFPP  174

CASB_CAMDR  176   QSLLSLSQFK  VLPVPQQMVP  YPQRAMPVQA  VLPFQEPVPD  PVRGLHPVPQ  PLVPVIA     232
CASB_BOVIN  175   QSVLSLSQSK  VLPVPQKAVP  YPQRDMPIQA  FLLYQEPVLG  PVRGPFPIIV              224
```

kappa-casein

```
CASK_CAMDR    1   -MKSFFLVVT  ILALTLPFLG  AEVQNQEQPT  CFEKVERLLN  EKTVKYFPIQ  FVQSRYPSYGI  59
CASB_BOVIN    1   MMKSFFLVVT  ILALTLPFLG  AQEQNQEQPI  RCEKDERFFS  DKIAKYIPIQ  YVLSRYPSYGL  60

CASK_CAMDR   60   INYYQHRLAV  PINNQFIPYP  NYAKPVAIRL  HAQIPQCQAL  PNI-------  -DPPTVERRP  111
CASB_BOVIN   61   LNYYQQKPVA  LINNQFLPYP  YYAKPAAVRS  PAQILQWQVL  SNTVPAKSCQ  AQPTTMARHP  121

CASK_CAMDR  112   RPRPSFIAIP  PKKTQDKTVN  PAINTVATVE  PPVIPTAEPA  VNTVVIAEAS  SEFITTSTPE  172
CASB_BOVIN  122   HPHLSFMAIP  PKKNQDKTEI  PTINTIASGE  PTSTPTIEAV  ESTVATLEAS  PEVI-ESPPE  181

CASK_CAMDR  173   TTTVQITSTE  I  182
CASB_BOVIN  182   INTVQVTSTA  V  191
```

Figure 5: The amino acid sequence of major camel milk caseins and whey proteins aligned with bovine milk proteins (continued)

Serum albumin

```
ALBU_CAMDR    1   MKWVTFISLL  FLFSSVYSRG  VFRRDTHKSE  IAHRFKDLGE  DDFKGLVLIA  FSQYLQQCPF  60
ALBU_BOVIN    1   MKWVTFISLL  LLFSSAYSRG  VFRRDTHKSE  IAHRFKDLGE  EHFKGLVLIA  FSQYLQQCPF  60

ALBU_CAMDR   61   DDHVKLVNEV  TEFAKTCVAD  ESAADCDKSL  HTLFGDKLCT  VASLRETYGE  MADCCEKQEP  120
ALBU_BOVIN   61   DEHVKLVNEL  TEFAKTCVAD  ESHAGCEKSL  HTLFGDELCK  VASLRETYGD  MADCCEKQEP  120

ALBU_CAMDR  121   ERNECFLQHK  SDNPDLPKLK  PEPEALCTAF  QENEKRFGGK  YLYEIARRHP  YFYAPELLYY  180
ALBU_BOVIN  121   ERNECFLSHK  DDSPDLPKLK  PDPNTLCDEF  KADEKKFWGK  YLYEIARRHP  YFYAPELLYY  180

ALBU_CAMDR  181   AHQYKHVFEE  CCKDADKAAC  LLPKLDALKE  RILASSARQR  LRCTSIQKFG  DRALKAWSVG  240
ALBU_BOVIN  181   ANKYNGVFQE  CCQAEDKGAC  LLPKIETMRE  KVLTSSARQR  LRCASIQKFG  ERALKAWSVA  240

ALBU_CAMDR  241   HLSQKFPKAD  FAEISKIVTD  LTKIHKECCQ  GDLLECADDR  ADLAKYFCDN  QETISSKLKE  300
ALBU_BOVIN  241   RLSQKFPKAE  FVEVTKLVTD  LTKVHKECCH  GDLLECADDR  ADLAKYICDN  QDTISSKLKE  300

ALBU_CAMDR  301   CCEKPLLEKS  HCIHEAERDE  MPENLPAITE  QFAEDKDVCK  HYTEEKDVFL  GMFLHEYARR  360
ALBU_BOVIN  301   CCDKPLLEKS  HCIAEVEKDA  IPENLPPLTA  DFAEDKDVCK  NYQEAKDAFL  GSFLYEYSRR  360

ALBU_CAMDR  361   HPEYAVSLLL  RIAKEYEATL  EDCCAKDDPH  ACYATVFDKL  QHLADEPQNL  VKQNCELFEK  420
ALBU_BOVIN  361   HPEYAVSVLL  RLAKEYEATL  EECCAKDDPH  ACYSTVFDKL  KHLVDEPQNL  IKQNCDQFEK  420

ALBU_CAMDR  421   LGEYGFQNDI  LVRYTKRLPQ  VSTPTLVEVA  RGLGRVGTKC  CTLPESNRMS  CAEDYLSLIL  480
ALBU_BOVIN  421   LGEYGFQNAL  IVRYTRKVPQ  VSTPTLVEVS  RSLGKVGTRC  CTKPESERMP  CTEDYLSLIL  480

ALBU_CAMDR  481   NRLCVLHEKT  PVSPRVTKCC  TESLVNRRPC  FSSLTADETY  EPKEFDEKTF  TFHADLCSVS  540
ALBU_BOVIN  481   NRLCVLHEKT  PVSEKVTKCC  TESLVNRRPC  FSALTPDETY  VPKAFDEKLF  TFHADICTLP  540

ALBU_CAMDR  541   EPEKQIKKQT  ALAELLKHKP  KATDEQLKTV  MEKFVAFVDK  CCAAVDKEAC  FTVEGPLLVA  600
ALBU_BOVIN  541   DTEKQIKKQT  ALVELLKHKP  KATEEQLKTV  MENFVAFVDK  CCAADDKEAC  FAVEGPKLVV  600

ALBU_CAMDR  601   ATRTALA      607
ALBU_BOVIN  601   STQTALA      607
```

Figure 5: The amino acid sequence of major camel milk caseins and whey proteins aligned with bovine milk proteins (continued)

Lactoferrin

```
LF_CAMDR    1    MKLFFPALLS  LGALGLCLAA  SKKSVRWCTT  SPAESSKCAQ  WQRRMKKVRG  PSVTCVKKTS  60
LF_BOVIN    1    MKLFVPALLS  LGALGLCLAA  PRKNVRWCTI  SQPEWFKCRR  WQWRMKKLGA  PSITCVRRAF  60

LF_CAMDR   61    RFECIQAIST  EKADAVTLDG  GLVYDAGLDP  YKLRPIAAEV  YGTENNPQTH  YYAVAIAKKG  120
LF_BOVIN   61    ALECIRAIAE  KKADAVTLDG  GMVFEAGRDP  YKLRPVAAEI  YGTKESPQTH  YYAVAVVKKG  120

LF_CAMDR  121    TNFQLNQLQG  LKSCHTGLGR  SAGWNIPMGL  LRPFLDWTGP  PEPLQKAVAK  FFSASCVPCV  180
LF_BOVIN  121    SNFQLDQLQG  RKSCHTGLGR  SAGWIIPMGI  LRPYLSWTES  LEPLQGAVAK  FFSASCVPCI  180

LF_CAMDR  181    DGKEYPNLCQ  LCAGTGENKC  ACSSQEPYFG  YSGAFKCLQD  GAGDVAFVKD  STVFESLPAK  240
LF_BOVIN  181    DRQAYPNLCQ  LCKGEGENQC  ACSSREPYFG  YSGAFKCLQD  GAGDVAFVKE  TTVFENLPEK  240

LF_CAMDR  241    ADRDQYELLC  PNNTRKPVDA  FQECHLARVP  SHAVVARSVN  GKEDLIWKLL  VKAQEKFGRG  300
LF_BOVIN  241    ADRDQYELLC  LNNSRAPVDA  FKECHLAQVP  SHAVVARSVD  GKEDLIWKLL  SKAQEKFGKN  300

LF_CAMDR  301    KPSGFQLFGS  PAGQKDLLFK  DSALGLLRIS  SKIDSGLYLG  SNYITAIRGL  RETAAEVELR  360
LF_BOVIN  301    KSRSFQLFGS  PPGQRDLLFK  DSALGFLRIP  SKVDSALYLG  SRYLTTLKNL  RETAEEVKAR  360

LF_CAMDR  361    RAQVVWCAVG  SDEQLKCQEW  SRQSNQSVVC  ATASTTEDCI  ALVLKGEADA  LSLDGGYIYI  420
LF_BOVIN  361    YTRVVWCAVG  PEEQKKCQQW  SQQSGQNVTC  ATASTTDDCI  VLVLKGEADA  LNLDGGYIYT  420

LF_CAMDR  421    AGKCGLVPVL  AESQQSPESS  GLDCVHRPVK  GYLAVAVVRK  ANDKITWNSL  RGKKSCHTAV  480
LF_BOVIN  421    AGKCGLVPVL  AENRKSSKHS  SLDCVLRPTE  GYLAVAVVKK  ANEGLTWNSL  KDKKSCHTAV  480

LF_CAMDR  481    DRTAGWNIPM  GLLSKNTDSC  RFDEFLSQSC  APGSDPRSKL  CALCAGNEEG  QNKCVPNSSE  540
LF_BOVIN  481    DRTAGWNIPM  GLIVNQTGSC  AFDEFFSQSC  APGADPKSRL  CALCAGDDQG  LDKCVPNSKE  540

LF_CAMDR  541    RYYGYTGAFR  CLAENVGDVA  FVKDVTVLDN  TDGKNTEQWA  KDLKLGDFEL  LCLNGTRKPV  600
LF_BOVIN  541    KYYGYTGAFR  CLAEDVGDVA  FVKNDTVWEN  TNGESTADWA  KNLNREDFRL  LCLDGTRKPV  600

LF_CAMDR  601    TEAESCHLAV  APNHAVVSRI  DKVAHLEQVL  LRQQAHFGRN  GRDCPGKFCL  FQSKTKNLLF  660
LF_BOVIN  601    TEAQSCHLAV  APNHAVVSRS  DRAAHVKQVL  LRQQALFGKN  GKNCPDKFCL  FKSETKNLLF  660

LF_CAMDR  661    NDNTECLAKL  QGKTTYEEYL  GPQYVTAIAK  LRRCSTSPLL  EACAFLMR    708
LF_BOVIN  661    NDNTECLAKL  GGRPTYEEYL  GTEYVTAIAN  LKKCSTSPLL  EACAFLTR    708
```

Figure 5: The amino acid sequence of major camel milk caseins and whey proteins aligned with bovine milk proteins (continued)

alpha-lactalbumin

```
LALBA_CAMDR   1    ----------  ---------K  QFTKCKLSDE  LKDMNGHGGI  TLAEWICIIF  HMSGYDTETV  41
LALBA_BOVIN   1    MMSFVSLLLV  GILFHATQAE  QLTKCEVFRE  LKDLKGYGGV  SLPEWVCTAF  HTSGYDTQAI  60

LALBA_CAMDR  42    VSNNGNREYG  LFQINNKIWC  RDNENLQSRN  ICDISCDKFL  DDDLTDDKMC  AKKILDKEGI  102
LALBA_BOVIN  61    VQNNDSTEYG  LFQINNKIWC  KDDQNPHSSN  ICNISCDKFL  DDDLTDDIMC  VKKILDKVGI  121

LALBA_CAMDR 103    DYWLAHKPLC  SEKLEQWQCE  KW  125
LALBA_BOVIN 122    NYWLAHKALC  SEKLDQWLCE  KL  144
```

Figure 5: The amino acid sequence of major camel milk caseins and whey proteins aligned with bovine milk proteins (continued)

1.3.5 Heat Treatment of Milk

During pasteurization and sterilization, milk is heated at different temperatures as shown in Table (5). Heat treatment (pasteurization and sterilization) is one of the important steps in milk processing performed to reduce bacterial load and to extend the shelf-life of products while maintaining its nutritional properties. These heat treatments variably lead to protein denaturation, interactions, and aggregations, non-enzymatic Maillard reaction browning, and loss of nutritive value (Sakkas *et al*., 2014).

Table 5: Common heat treatments used for processing milk and milk products

Heat treatment	**Conditions**
Thermisation	65 ℃/30 s
High temperature short time (HTST)	72-80 ℃/15-30 s
Low temperature long time	63 ℃/30 min
Sterilization	110-120 ℃/5-20 min
Ultrahigh temperature (UHT)	135-150 ℃/1-10 s

Source: Fox and Kelly, 2006; Lewis and Deeth, 2008.

The Maillard reaction is a reaction between amino groups and reducing sugars. This reaction is significant in the food industry as the quality of the food product is strongly affected with the formation of brown pigments with strong flavour. The reaction includes condensation of the sugar with an amino group to form a Schiff's base and the Amadori product as seen in Figure (6). The reaction is commonly known

as non-enzymatic browning reaction and is responsible for the desirable and undesirable products formed (Van Boekel, 1998).

Camel milk whey proteins show a higher heat stability when compared to bovine milk due to the absence of β-lactoglobulin but the heat sensitivity of whey proteins in camel milk is considerably low (Farah, 1986; Elagamy, 2000). The stability of camel whey has also found to be better than bovine or buffalo whey. Recent studies showed that at higher temperature, camel whey proteins, namely α-lactalbumin and camel serum albumin were greatly affected while the caseins were not affected much (Felfoul *et al*., 2017). Some of the studies on heat treatment of camel milk are depicted in Table (6).

Table 6: Previous studies on heat treatment of camel milk

Studies	Findings	Reference
70 and 90 °C for 30 min	α-La was found in highest concentration among camel whey Denaturation of α-La was 73.5 °C for sweet camel whey and 60.5 °C for acid whey Heat improved foaming properties of acid whey and increased foam stability	Lajnaf *et al*., 2018
80 °C for 60 min	α-La was not detected CSA was significantly diminished	Felfoul *et al*., 2017
60-90 °C for 60 min and 120 min SDS-PAGE, Thiol and DSC	Denaturation temperature is 77.8 °C. Deposit formation at 70 °C At 90 °C α-La, CSA, and κ-casein bands decreased	Felfoul *et al*., 2015
60 and120 min; temperatures: 60, 70, 80 and 90 °C Free thiol, SDS-PAGE, DSC	Denaturation temperatures were 73.8 °C for camel rennet whey and 60.5 °C for camel acid whey No deposit formation at 60 °C but severe fouling above 70 °C At 90 °C, CSA disappeared for both rennet and acid whey α-La concentration decreased with increase in time	Felfoul *et al*., 2015
63 °C for 30 min and 72 °C for 15 s	Increased quality and extended shelf life	Mohamed and El Zubeir, 2014
Lactoferrin heated at 65 °C, 85 °C and 100 °C for 15 and 30 min RP-HPLC, SDS-PAGE	Strong anti-bacterial activity at 65 and 85 °C, anti-bacterial zone reduced at 100 °C Lactoferrin slightly disappeared at 100 °C/30 min	Abdel-Salam *et al*., 2014
65, 75, 85 and 100 °C for 10, 20 and 30 min SDS-PAGE	Whey proteins were more heat resistant compared to cow and buffalo milk SA not affected at 75 °C Unknown band intensified at 85 and 100 °C	Elagamy, 2000
63, 80 and 90 °C for 30 min Nitrogen distribution, PAGE	70-81% denaturation at 80 and 90 °C Higher heat stability SA disappeared at 80 °C	Farah, 1986

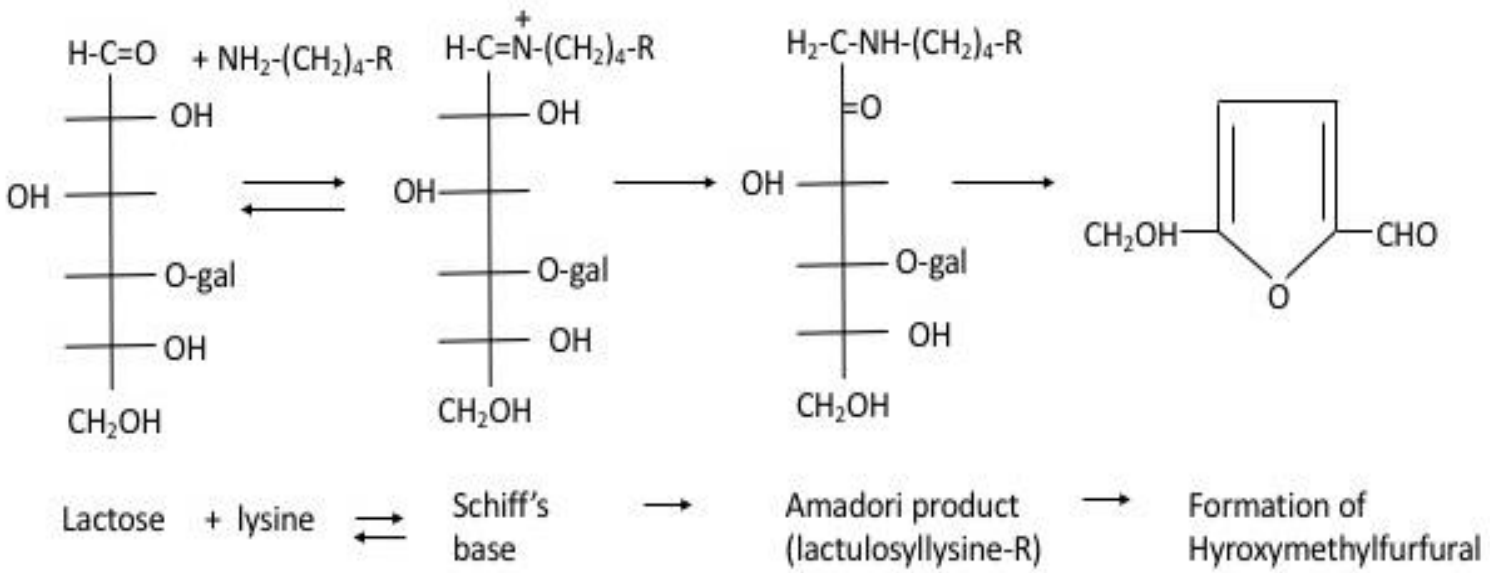


Figure 6: Schematic overview of Maillard reaction in milk leading to the formation of hydroxymethylfurfural (HMF)

(Modified from Van Boekel, 1998)

Heat treatment can also enhance organoleptic properties of the milk and its products by increasing the functionality of the proteins (Hristov *et al*., 2010). Caseins are generally heat resistant due to their structure and it has been noted that they are able to withstand higher temperatures. Heating casein micelles below 140 °C has little effect on their stability and structure. It has been reported that above 100 °C, κ-casein dissociates from the surface of the micelle. This causes the micelle to decrease in size (Singh and Waungana, 2001).

The globular structure of native whey proteins is maintained by hydrogen bonding, van der Waal forces, hydrophobic and electrostatic forces. It is known that at temperatures above 60 °C, proteins denature. This causes unfolding of globular whey proteins which exposes its hydrophobic residues and disulphide bonds. An unfolded protein can refold into its native form when the temperature is reduced. However, at higher temperatures, the unfolded proteins interact to form new hydrophobic interactions which can be irreversible (Al haj and Al Kanhal, 2010; Al haj *et al*., 2011).

The heat stability of bovine and buffalo whey proteins was reported to be lower than camel milk whey proteins (Al haj and Al Kanhal, 2010; Al haj *et al*., 2011). Goat's milk heat stability is lower than bovine's milk as reported by Montilla and Calvo (1997). They observed that change in pH at different temperatures affected the denaturation of proteins. Significant changes in whey proteins were observed at temperatures higher than 130 °C with β-LG being affected more than α-LA (Montilla and Calvo, 1997). At 80 °C/30 minutes, the denaturation of camel milk whey proteins is lower (32-35%) in comparison with bovine milk whey (70-75%) (Brezovečki *et al*., 2015). An unfolded protein can aggregate with other proteins. The process of aggregation occurs mainly through the formation of disulphide linkages by sulphydryl-disulphide interchanges. This denaturation process can occur in two steps. In the first

step the native globular protein will unfold, thus exposing its hydrophobic residues and disulphide bonds. At lower temperatures, these unfolded proteins can refold to their native forms. However, as temperatures increase, new interactions can give rise to random structures which lead to the second step of denaturation. Moreover, once the whey proteins unfold, they are free to form aggregates with other molecules through disulphide and covalent bonds as shown in Figure (7) (Wijayanti *et al.*, 2014). Immunoglobulins and BSA are found to be less stable, whereas β-LG can be intermediate (Nollet and Toldrá, 2010; Sakkas *et al*., 2014; Felfoul *et al*., 2015). α-LA is found sensitive to heat as it denatures at around 62 ℃. At this temperature however, its unfolding can be reversible. It does not form aggregates at heating temperatures below 80 ℃ (Donato and Guyomarc'h, 2009). At these temperatures, α-LA is deformed to a molten state (Chang and Li, 2000).

Heat treatment during milk processing was reported to cause significant changes above 60 ℃, which along with denaturation, include interaction of the denatured whey protein and casein micelles as well as conversion of soluble calcium, magnesium and phosphate to the colloidal state (Jovanovic *et al*., 2007). The main aggregates formed due to the applied heat treatment are complexes between β-LG and κ-CN in bovine milk (Nollet and Toldrá, 2010; O'Connell and Fox, 2011; Wijayanti *et al.*, 2014). This occurs *via* disulphide bonds and hydrophobic interactions. At lower temperatures (below 70 ℃) the association of whey protein and casein micelles occur *via* hydrophobic interactions while at higher temperatures these interactions are caused by disulphide bonds (Wijayanti *et al.*, 2014). Studies have shown that the association is stronger when the complex is formed on the surface of casein micelles. The formation of these complexes may depend on rate of heating. When the milk is heated at a slower rate or heated for a long time at lower temperatures, β-LG is given a longer

time to unfold and associate with the micelle. On the other hand, when rapid heating occurs, β-LG is not allowed to unfold completely, which allows it to refold into a non-native structure and form aggregates with other monomers instead of κ-casein. Also, when the proportion of β-LG increases, it causes more β-LG to interact thus increasing the ratio of β-LG/κ-CN complexes. Thus, the association of β-LG with casein micelles is dependent on the heating conditions, protein concentration and even salt concentration. The other complexes formed during heat treatment are between α-LA and β-LG as well as α-LA and κ-CN. β-LG can form a heat induced complex with α-LA through thiol-disulphide interchange between β-LG and α-LA. By itself, α-LA does not associate with casein micelles (Sakkas *et al*., 2014). It must form complexes with β-LG which can then associate with the casein micelle. This occurs during prolonged heating as at lower temperatures the unfolding of α-LA can be reversible (Oldfield *et al*., 1998; Tolkach and Kulozik, 2007; Donato and Guyomarc'h, 2009; Sakkas *et al*., 2014). In bovine milk, these reactions are caused primarily by the single S-H group (Cys 121) on β-LG (Table 7).

Table 7: Free sulfhydryl group (SH) and disulfide (S-S) bonds in milk whey proteins

Protein	**-SH groups**	**S-S bonds**
α-LA	-	4 (Cys6-Cys120, Cys28-Cys111, Cys61-Cys77, and Cys73-Cys91) (Brew, 2003)
β-LG	1 (Cys121)	2 (Cys66-Cys160 and Cys106-Cys119) (Hambling *et al*., 1992)
BSA	1 (Cys34)	17 (Wijayanti *et al.*, 2014)

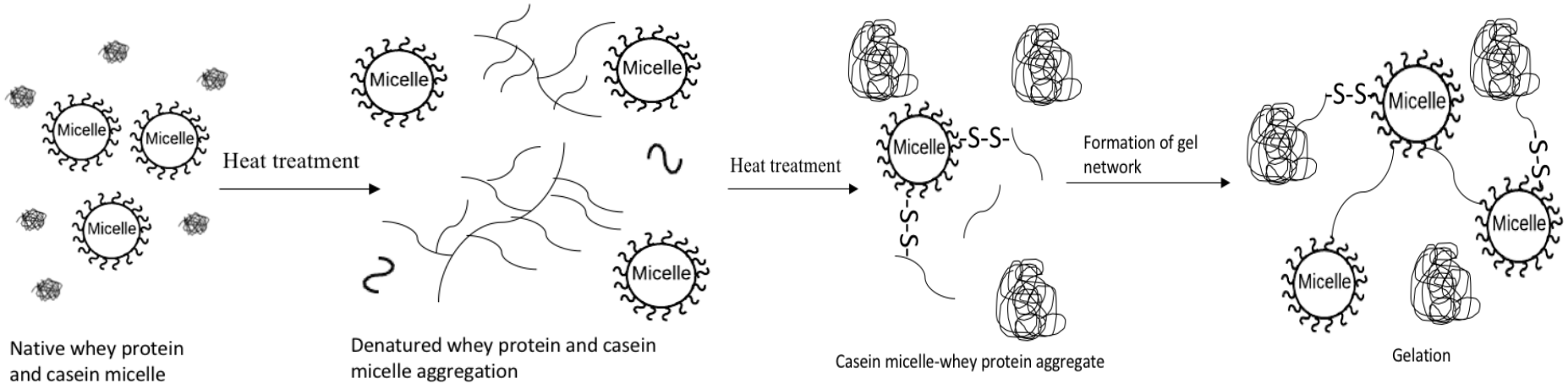


Figure 7: Schematic diagram showing possible interactions in the heat-treated milk system

(Modified from Patel and Patel, 2015)

1.3.6 Methods Used for Analysis of Proteins

Different methods are available to measure protein denaturation and each method relies on measuring a different aspect of physiochemical changes that the protein undergoes. Proteins can be characterized based on their size or any chemical modification it undergoes during processing (Felfoul *et al.*, 2017). Depending on the type of protein separation required, different types of electrophoresis like SDS-PAGE, isoelectric focusing (IEF) or native or urea PAGE can be employed (Nollet and Toldrá, 2010).

The most common electrophoretic method used for milk protein analysis is the SDS-PAGE described by Laemmli (1970). This method has been used in several proteomics studies to determine the molecular of proteins and its subunits as well as the size of the protein subunits (Considine *et al.*, 2007). In this method, separation occurs in the presence of the detergent SDS, which denatures the tertiary and secondary protein structure and conceals the protein in negative charges (Nollet and Toldrá, 2010). In order to separate the protein aggregates in a mixture, the disulphide bridges can be reduced. Due to the different charges, proteins migrate differently. This migration is proportional to the molecular mass of the protein (Nollet and Toldrá, 2010). The method also allows a high resolution and wide rages of molecular sizes which makes its efficient and reliable (Considine *et al.*, 2007). This method can be followed by Scanning densitometry to quantify individual protein bands on a gel (Jovanovic *et al.*, 2007).

It is also possible to separate proteins based on their isoelectric point (pI), a pH at which the protein is having a zero charge, which is used in a technique known as isoelectric focusing (IEF) that works around an electric filed. (Jensen *et al.*, 2012a).

The proteins migrate towards their isoelectric point in the electric field. The migration then stops when the pI is reached and they have no net charge. This technique along with mass spectrometry is often used in proteomics to identify individual spots on the gel (Jensen *et al.*, 2012a).

To increase quantification of a proteins, capillary electrophoresis can be used as an alternative to PAGE (Strickland *et al*., 2001). It allows the separation of smaller to larger sized proteins. The major advantage of CE when compared with HPLC is the use of very low amount of chemicals. The CE includes different methods of separation like capillary zone electrophoresis (CZE) which is based on the differences in the electrophoretic mobility (depending on charge and size of the compounds) and electroosmotic flow; micellar electro kinetic chromatography (MEKC) based on hydrophobic interactions, ion interactions, electrophoretic mobility, and electroosmotic flow; and gel electrophoresis (SDS-CE) in the presence of SDS based on molecular sieving or capillary isoelectric focusing (CIEF) based on the differences in pI (Nollet and Toldrá, 2010).

Liquid chromatography (LC) is also used for the separation and analysis of milk proteins based on polarity, size, hydrophobicity, or certain biological function e.g., Affinity Chromatography, whereby the protein of interest is purified by virtue of its specific binding properties to an immobilized ligand. A fast and accurate method that is being used for quantification of milk proteins is reversed phased high performance liquid chromatography (RP-HPLC) and ultra-high performance liquid chromatography (UPLC) (Nollet and Toldrá, 2010). Due to high resolution, the RP-HPLC allows the separation of the genetic variants of milk proteins (both caseins and whey proteins) as well as their quantitative analysis. For better separation, it is important to know the sample preparation protocol where caseins would have to be

reduced prior to separation while whey proteins like α-LA and β-LG are better separated without reduction. When whey proteins denature irreversibly, their globular structure is refolded into a non-native structure (Wijayanti *et al.*, 2014). When the pH is adjusted to pH 4.6, the denatured whey proteins forms a sediment due to aggregation. Due to this sedimentation, they disappear when the soluble phase is analyzed. The amount of protein present in the native form can be determined through RP-HPLC. Protein that is denatured can be calculated by comparing the amount of native proteins in treated and untreated samples (Nollet and Toldrá, 2010).

Different spectroscopic methods like fluorescence spectroscopy, mass spectrometry have been used to analyze proteins. Protein separation by HPLC can be coupled with mass spectrometry to identify the separated fractions. This is useful to identify unknown peaks and to analyze the purity of peaks, the combined technique is called liquid chromatography mass spectrometry (LC-MS). Mass spectrometry separated protein fractions according to their masses based on their mass to charge ratios (m/z). The m/z of proteins will depend on their molecular weight. An example of LC-MS is the proteomic profiling of bovine and camel milk proteins conducted by Felfoul *et al*. (2017). MS is important for analyzing proteins and peptides as it is high in sensitivity and speed. This technique also requires a small sample size as compared to conventional methods that require larger sample sizes. Other mass analyzers are used for analysis and quantification of milk proteins like HPLC-electrospray-ionization mass spectrometer. These methods are preferred because of their accuracy. The other highly specific methods include liquid chromatography tandem mass spectrometry (LC-MS/MS) having an electrospray ionization source (ESI) or Matrics-assisted laser desorption ionization time of flight mass spectrometer (MALDI-TOF-MS) (Nolet and Toldra, 2010). Another type of spectrophotometer is the quadrupole

mass spectrometer that is used because of its specificity. This type of spectrophotometer will only transmit ions of a definite mass/charge ratio (m/z) (Nolet and Toldra, 2010).

Chapter 2: Materials and Methods

2.1 Chemicals and Reagents

Acrylamide, tris HCl, Sodium Dodecyl Sulphate (SDS) solution, temed, ammonium persulphate, dithiothreitol (DTT), 4X laemmli buffer solution, coomassie blue staining solution was obtained from Biorad (California, United States). Bovine serum albumin (BSA), lactoferrin, β-lactoglobulin (β-LG), α-lactalbumin (α-LA), β-casein (β-CN), α-casein (α-CN), κ-casein (κ-CN), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), and thiobarbituric acid (TBA) were obtained from Sigma-Aldrich (St Louis, MO, USA). A protein mixture/solution was made from the above seven proteins, having 95% purity, was used as a reference for SDS-PAGE electrophoresis. All other chemicals and reagents were of analytical grade and were also obtained from Sigma Chemical Company.

2.2 Milk Samples

The milk used in the experiments was raw camel milk obtained from Al Ain dairy in Abu Dhabi, United Arab Emirates. The raw milk obtained was immediately poured into glass bottles and subjected to varied time and temperature combinations. Raw goat milk was obtained from a local farm in Al Ain, Abu Dhabi.

2.3 Heat Treatment

The milk samples were heat treated from 70 °C to 130 °C for 1, 10, and 30 minutes in an autoclave. The heated samples were then immediately chilled in an ice bath and stored for further testing at -20 °C.

2.4 Determination of Free Thiol Content

Free thiol content is determined using the method described by Muangthai and Surapat (2003). Milk samples (25 ml) were mixed with 0.77 ml of 33.3% v/v acetic acid and incubated at 45 °C for 15 minutes. This was then mixed with 0.77 ml of 27.35% w/v sodium acetate solution. After thorough mixing, the solution was filtered using Whatman No. 40 filter paper. A 600 µl portion of the filtrate was mixed with 600 µl of 10 mM DTNB in ethanol, 1200 µl of phosphate buffer (pH 8.0) and 600 µl of sodium dodecyl sulphate (SDS, 1 g/L) and diluted to 6 ml with deionized water. The absorbance of this solution was measured against a blank at 412 nm using Varian Cary 50 UV-Visible Spectrophotometer, Varian Inc. Free thiol content was then calculated using a molar extinction coefficient 13,600 $Lmole^{-1}cm^{-1}$.

2.5 Electrophoresis

A 1:10 dilution was made for all heat-treated milk samples as well as the pure protein mixture. Diluted camel milk samples (3 µl) and protein standard marker (20 µl) were mixed with 10 µl dithiothreitol (DTT) and 25 µl of 4X Laemmli buffer solution, heated for 5 minutes under boiling conditions and then loaded onto 12% acrylamide gel (Resolving gel: 3 ml 30% acrylamide, 1.875 ml 5M Tris HCL, 75 µl 10% SDS solution, 2.515 ml deionized water, 3.75 µl Temed, 37.5 µl of 10% ammonium persulphate solution; Stacking gel: 0.99 ml 30% acrylamide, 1.89 ml 0.5 M Tris HCL, 75 µl 10% SDS solution, 4.5 ml deionized water, 7.5 µl Temed, 37.5 µl 10% ammonium persulphate solution). This was then run at 200 V under non-reducing conditions until the bands reached the end of the gel. The gel was then transferred to a fixing solution (40 ml methanol + 10 ml acetic acid + 60 ml deionized water) for an

hour and then transferred to the Coomassie blue staining solution and left overnight on a shaker. The gels were then scanned and read using ChemiDoc MP Imaging System (Biorad, USA) and the intensity of each band was estimated through the obtained electropherograms.

2.6 Determination of Hydroxymethylfurfural (HMF)

Determination of HMF was carried out as described by Morales *et al.* (1996) at absorbance 433 nm. 10 ml milk samples were digested with 0.3 N oxalic acid (5 ml) solution for 1 hour at 100 °C. This was then rapidly cooled in ice and slowly deproteinized with 40% w/v trichloroacetic acid (5 ml TCA) solution and filtered through Whatman filter paper No. 42. Of this filtrate, 4 ml was then mixed with 1 ml of 0.05 M thiobarbituric acid (TBA) solution and absorbance at 433 nm was read using Varian Cary 50 UV-Visible Spectrophotometer, Varian Inc, after incubation at 40 °C/30 min.

2.7 Statistical Analysis

The precision of quantification of protein band intensities was calculated from three separate runs and results were presented as mean ± SD. Heating experiments were repeated twice and in the quantitative estimation, peak areas (representing band intensities) of treated relative to untreated milk are presented.

Chapter 3: Results

3.1 Electrophoresis of Raw Milk Proteins

Figure (8) shows a typical SDS-PAGE gel pattern of raw camel milk compared to raw goat milk. The absence of β-LG in camel milk is noted while its presence in goat milk is well appreciated. Lactoferrin in goat milk appears to be faint as compared to camel milk. These results show that camel milk is richer in lactoferrin as compared to goat milk which is in agreement with literature (Table 3). The caseins (α-CN, β-CN and κ-CN) appear more pronounced in goat milk than camel milk. α-LA being the smallest protein (about 10 KD) appears at the bottom of the gel while lactoferrin, being the heaviest appears at the top (about 100 KD).

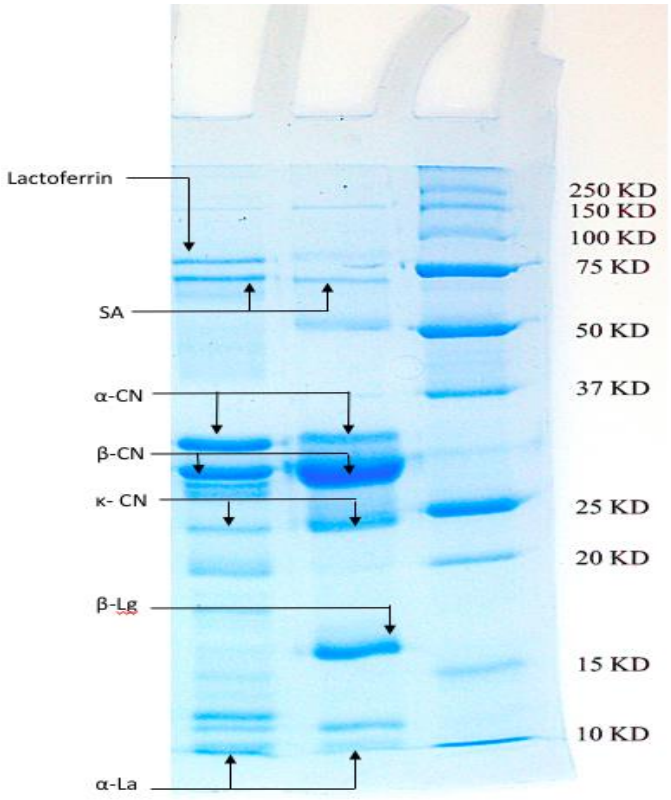


Figure 8: Electrophoretic gel pattern of raw camel milk (RC) and raw goat milk (RG)

All gels were scanned using the ChemiDoc MP Imaging System (Biorad, USA) providing electropherograms similar to the one presented as an example in Figure (9). It can be seen that peak overlap occurs in some cases, therefore results presented in this thesis should be regarded as estimated values. Repeatability of the semi-quantitative SDS-PAGE electrophoresis/ChemiDoc technique was tested for triplicate analysis of the reference proteins and the results are presented in Table (8). The analytical variabilities for the different proteins, expressed as relative standard deviation (RSD), were: lactoferrin (2%), bovine serum albumin (42%), α-casein (21%), β-casein (21%), κ-casein (31%), β-lactoglobulin (21%), and α-lactalbumin (15%). Therefore, quantitative data presented in this thesis should be considered as indicative rather than strictly quantitative.

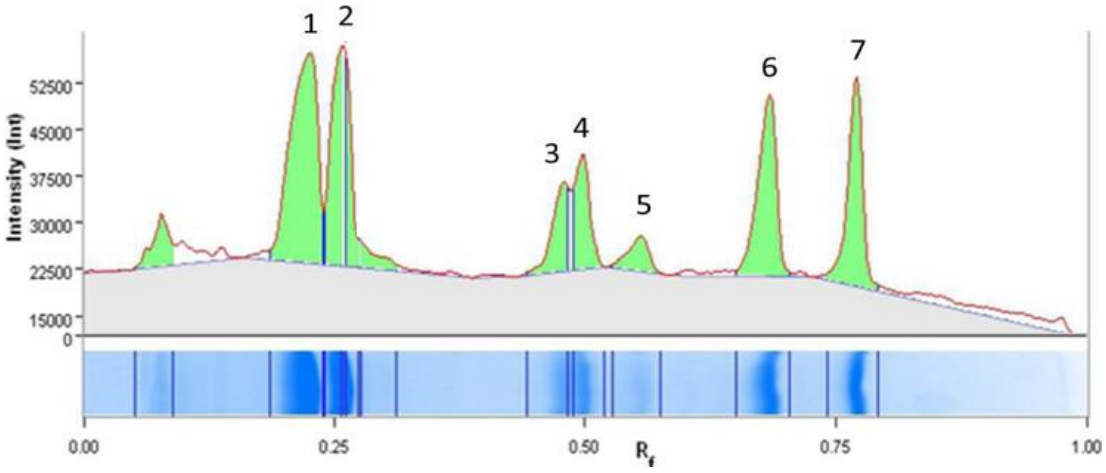


Figure 9: Conversion of band intensities of different proteins in electrophoretic gels to electropherogram using BioRad ChemiDoc

Peaks: (1) lactoferrin, (2) bovine serum albumin, (3) α-casein, (4) β-casein, (5) κ-casein, (6) β-lactoglobulin, and (7) α-lactalbumin.

Table 8: Mean values of electropherograms of the reference bovine proteins (n+3)

Proteins	**Rf**	**Mean Peak Area ± SD**	**Peak Area RSD (%)**
Lactoferrin	0.22	24,356,255±420,747	2
Serum albumin	0.25	13,035,672±5,440,447	42
α-Casein	0.47	4,676,743±1,002,982	21
β-Casein	0.49	5,845,297±1,210,985	21
κ-Casein	0.55	4,040,536±1,243,474	31
β-Lactoglobulin	0.68	14,910,961±3,180,710	21
α-Lactalbumin	0.77	12,663,226±1,897,890	15

Figure (10) shows changes in the proteins of camel and goat milk occurring at 60 ℃, 95 ℃ and 120 ℃. Differences in the gel patterns are observed as the milks were heated for 1, 10, 20 and 30 minutes. While comparing the electrophoretic pattern of camel and goat milk, the most notable difference is the absence of β-LG in camel milk while a clear band is observed in goat milk for all temperatures which agrees with literature (Farah and Atkins, 1992; Farah, 1996; Elagamy, 2000; Kappeler *et al*., 2003; Konuspayeva *et al*., 2009; EL-Fakharany *et al*., 2012; Habib *et al*., 2013). Lactoferrin is more pronounced in camel milk as compared to goat milk. β-CN appears as a thicker band in goat milk as compared to camel milk. While other unknown bands appear in camel milk below the caseins, the same is not observed in goat milk. It is observed that lactoferrin depletes with time for goat milk after 95 °C/20 minutes itself while the band fades at 120 °C for camel milk. In general, whey proteins depleted faster that caseins in both camel and goat milks.

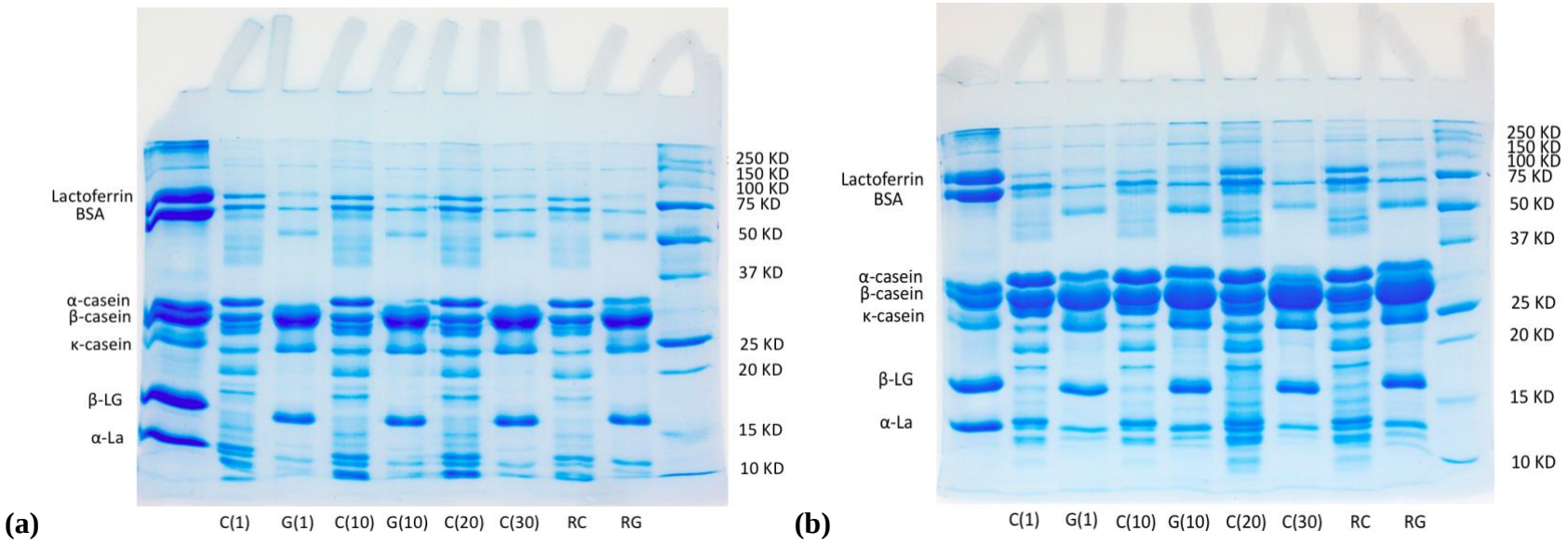


Figure 10: SDS-PAGE pattern of camel and goat milks as affected by heating at 60, 95, and 120 °C for 1, 10, 20, and 30 min

Reference proteins are shown on both ends of the gel with their names on the left and molecular masses on the right.

(a) 60 °C for 1, 10, 20 min, (b) 95 °C for 1, 10, 20 min, (c) 120 °C for 1, 10 and 20 min, and (d) 60 °C, 95 °C and 120 °C for 30 min.

Panels (a, b, c): C(1)- Camel milk heated for 1 minute; G (1)- Goat milk heated for 1 minute. C (10) Camel milk heated for 10 minutes; G(10)- Goat milk heated for 10 minutes. C(20) Camel milk heated for 20 minutes; G(20) Goat milk heated for 20 minutes. ; RC Raw camel milk; RG Raw goat milk

Panel (d): C(60) Camel milk heated at 60 °C; G(60) Goat milk heated at 60 °C; C(95) Camel milk heated at 95 °C; G(95) Goat milk heated at 95 °C; C(120) Camel milk heated at 120 °C; G(120) Goat milk heated at 120 °C; RC Raw camel milk; RG Raw goat milk

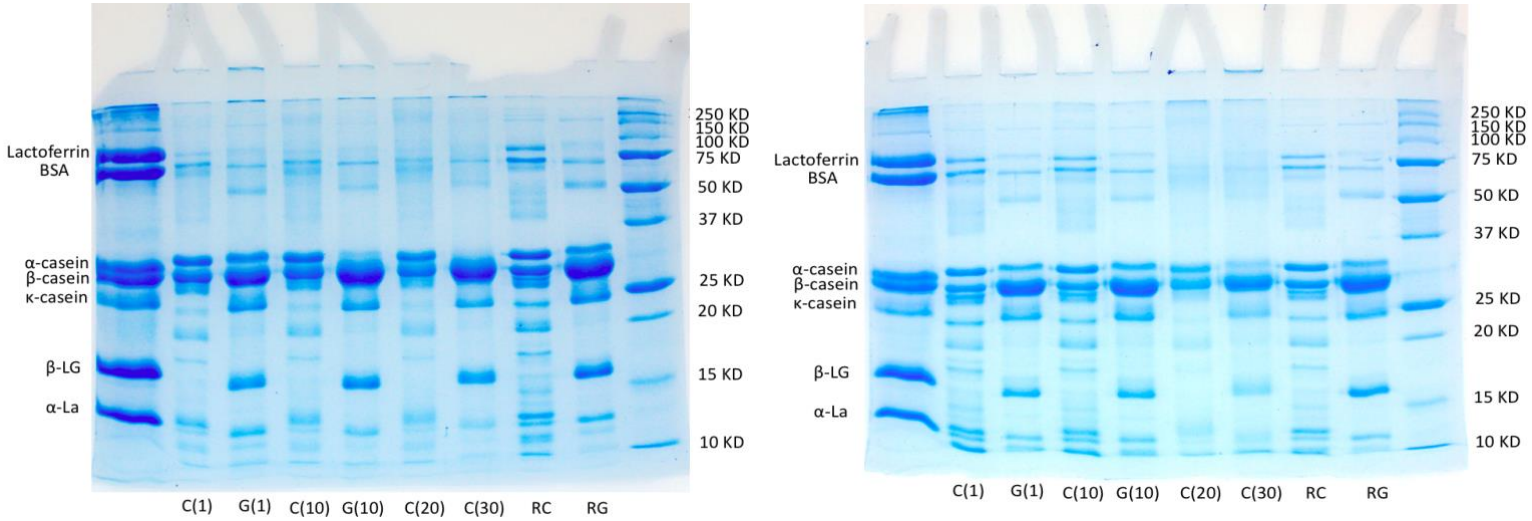
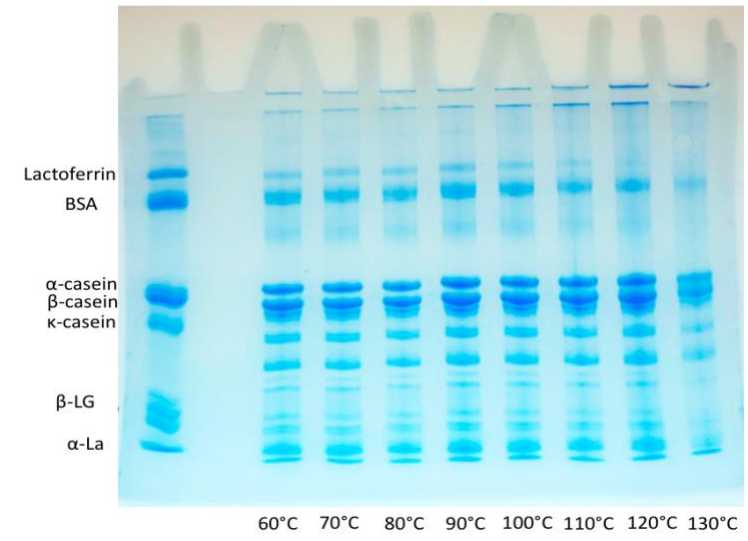


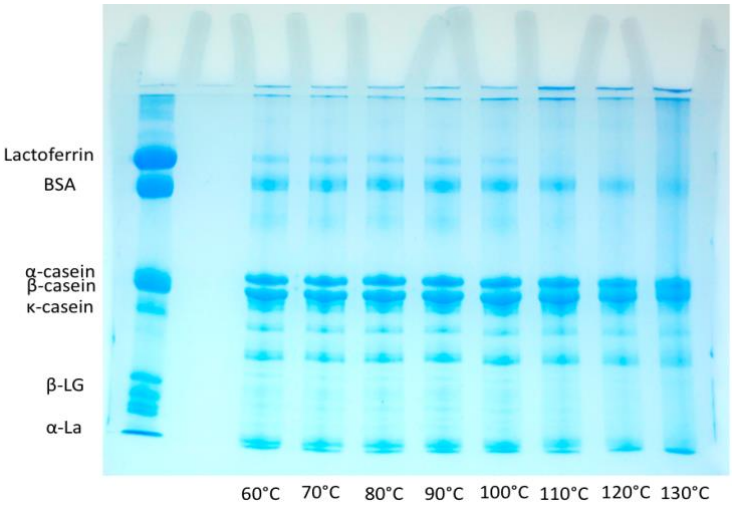
Figure 10: SDS-PAGE pattern of camel and goat milks as affected by heating at 60, 95, and 120 °C for 1, 10, 20, and 30 min (continued)

Figure (11) shows gel patterns of camel milk heated from 60-130 ℃ for 1, 10 and 30 minutes. The whey proteins assessed in this experiment are α-LA, SA and lactoferrin. Lactoferrin (Mwt 75 KDa) and SA (Mwt 67 KDa) being heavier than the other proteins (Table 4) appeared at the top of the gel. As mentioned previously, camel milk contains a higher amount of lactoferrin and SA as compared to other milks, which is also confirmed in Table (3). The lower molecular weight protein, α-LA (~14 KD), moved towards the bottom of the gel, while caseins positioned themselves somewhere in between. From this figure, it is clearly observed that heating time and temperature showed a greater impact on the stability of the whey proteins as compared to the caseins. The electrophoretic gel patterns showed that among the whey proteins, lactoferrin and SA we easily affected by heat. Lactoferrin disappeared after 100 °C/30 min while SA disappeared after 110 ℃/30 min. The general trend in lactoferrin and SA showed bands fading and disappearing in the gels which was confirmed in Figures 10 and 11. After 1 minute of heating, α-LA appeared as two bands. These two bands came closer after 10 minutes and merged into one intense band after 30 minutes.

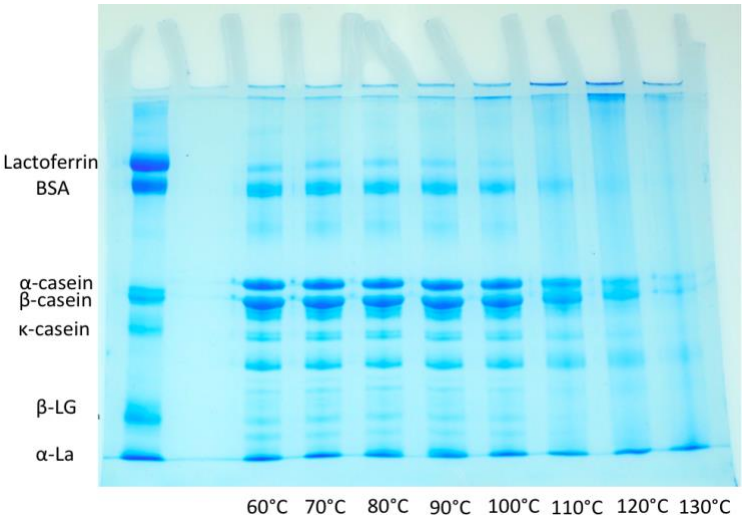
Results from the semi-quantitative SDS-PAGE electrophoresis/ChemiDoc analysis of the intensities of heated milk electrophoresis bands are presented in Figures 12 and 13. From these results, we can observe all camel milk proteins decreased in response to heating times (1, 10, and 30 min) and temperatures (60-130 ℃) except for α-LA. Camel milk κ-CN and LF was found to be highly susceptible to heat as it was depleted at temperatures above 100 ℃. CSA depleted after 110 ℃ while α-LA increased with time. Camel milk α-CN and β-CN were affected by heating temperature and time but they were more stable than LF, CSA, and κ-CN.



1 minute

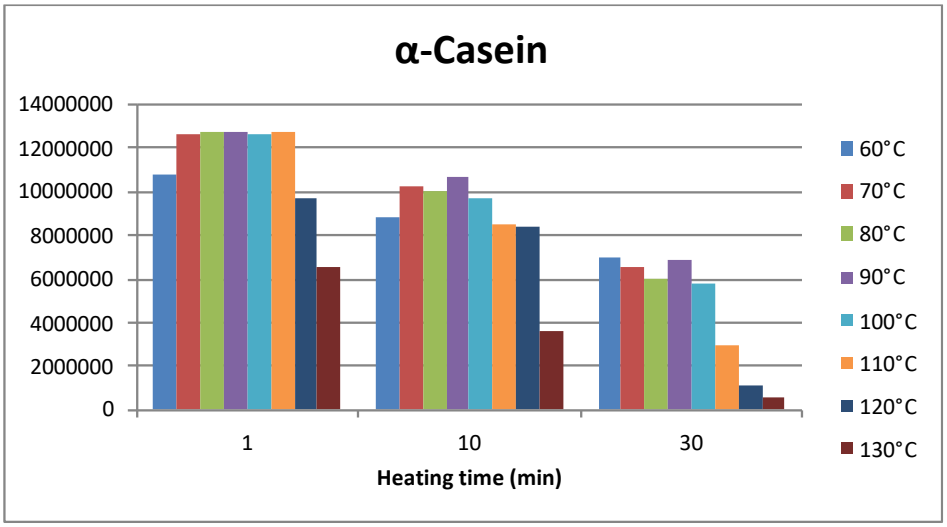


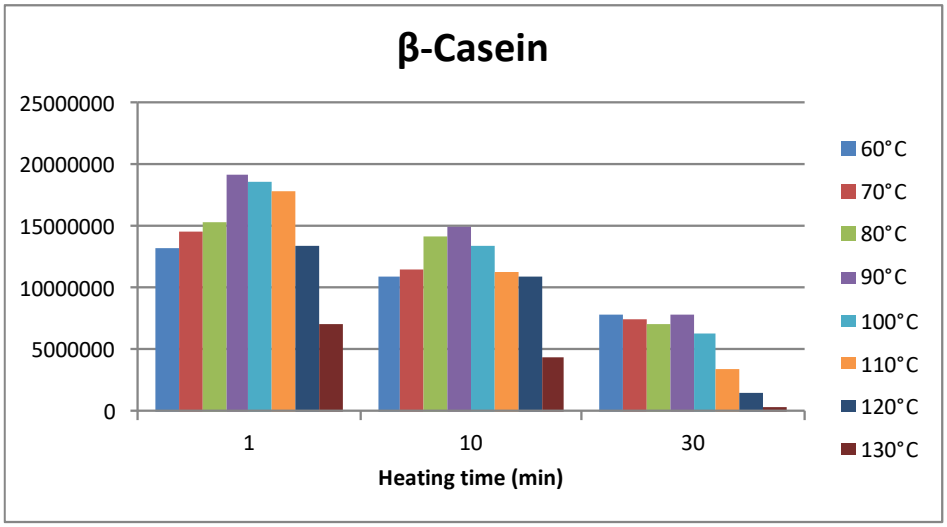
10 minutes



30 minutes

Figure 11: SDS-PAGE patterns of camel milk heated from 60-130 °C for 1, 10 and 30 minutes





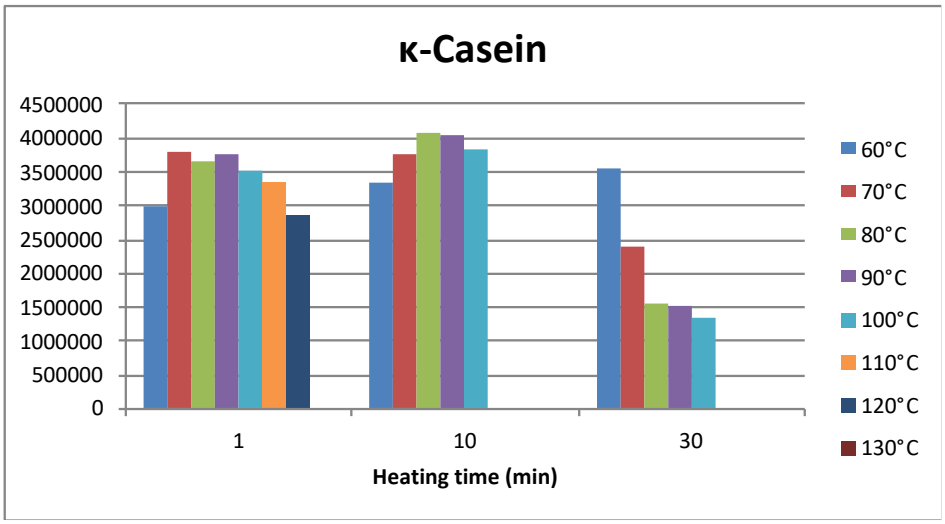
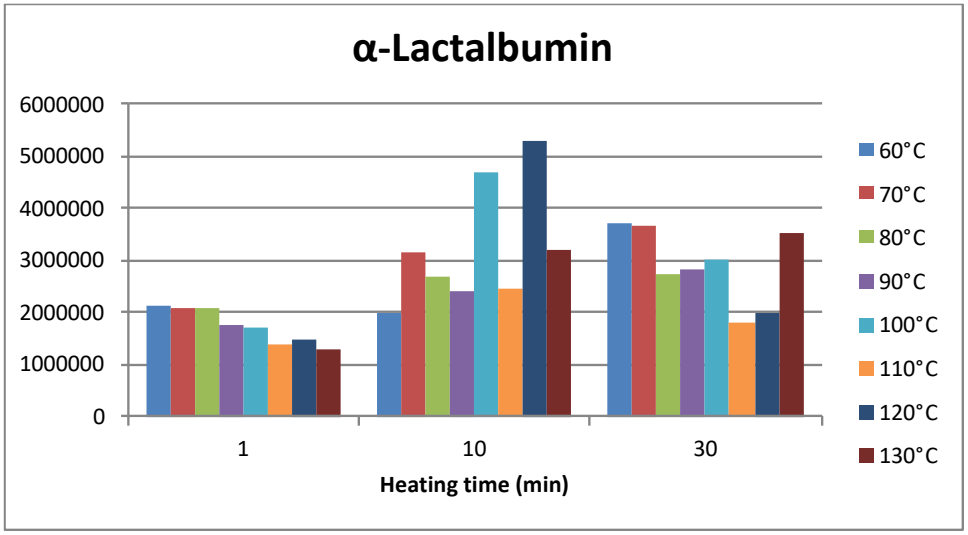
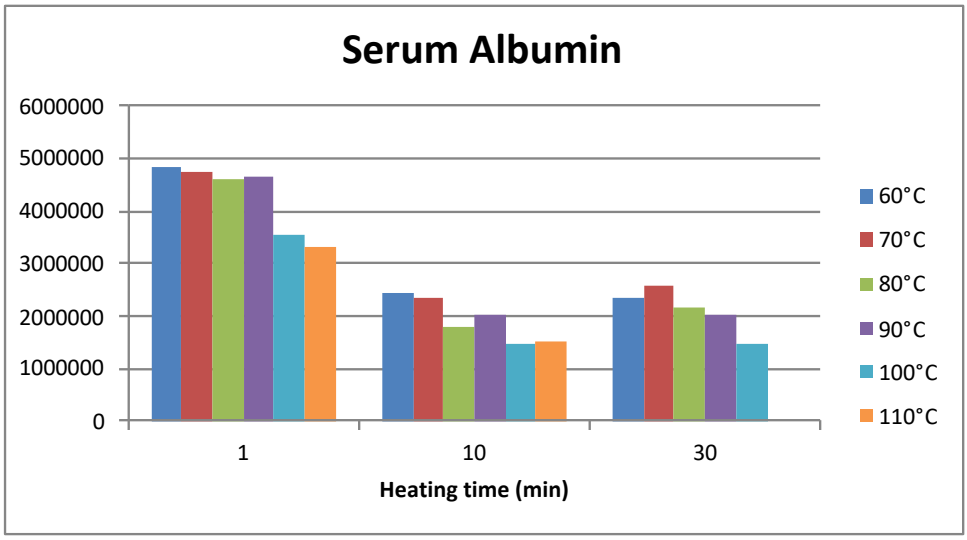


Figure 12: Effect of heat treatment on the relative peak intensity of camel milk caseins





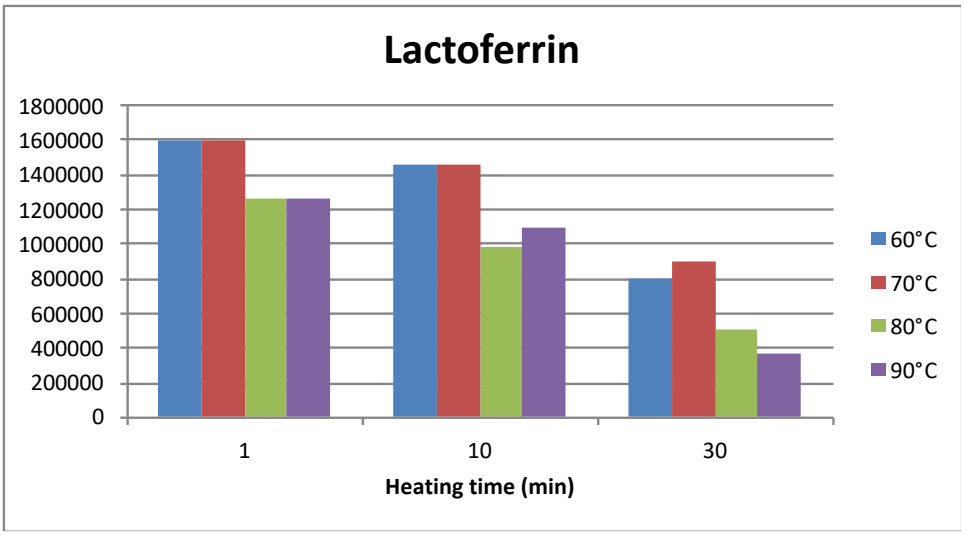


Figure 13: Effect of heat treatment on the relative peak intensity of camel milk whey proteins

Interestingly, α-La showed an increase in intensity with increase in heating time as compared to the other milk proteins. The variability of the band intensities of α-LA in response to temperature, may be due to the involvement of a complex phenomenon in this effect, which needs to be studied further. While comparing the gel patterns, the band of α-LA appeared as 2 bands (Figure 11). It has been reported that heating of α-LA makes it to release Ca^{2+} and changes its conformation from holo to apo α-LA, which starts protein unfolding. An equilibrium intermediate state between the native state (NS) and the fully unfolded state (US) is called the molten globule (MG) state (Arai and Kuwajima, 1996: Kuwajima, 1996).

The unfolding of α-LA was found to expose inner hydrophobic amino acids (Lala and Kaul, 1992), which can then form complexes with lipids (Atri *et al*., 2011). Cytotoxic **A**lpha-lactalbumin **M**ade **LE**thal to **T**umor cells (AMLET) complexes have been shown to form by the combination of oleic acid and α-LA from human bovine and camel milk (Atri *et al*., 2011).

3.2 Changes in Thiol Content

Figure 14 presents changes in thiol content in camel milk with heating time. After 1 minute of heating, the free -SH content at different temperatures was 60 °C ($1.149*10^{-3}$ M), 70 °C ($1.235*10^{-3}$ M), 80 °C ($1.280*10^{-3}$ M), 90 °C ($1.074*10^{-3}$ M), 100 °C ($0.787*10^{-3}$ M), 110 °C ($0.509*10^{-3}$ M), 120 °C ($0.441*10^{-3}$ M) and 130 °C ($0.401*10^{-3}$ M). At 60 and 70 °C, the free thiol content continued to increase slowly up to 10 minutes of heating and then decreased. At 80, 90 and 100 °C, the free thiol content decreased sharply up to 30 minutes. For 110, 120 and 130 °C, the free thiol

content remained constant between 1-30 minutes. Overall the graph shows a decreasing trend in residual thiol with increasing time and temperature.

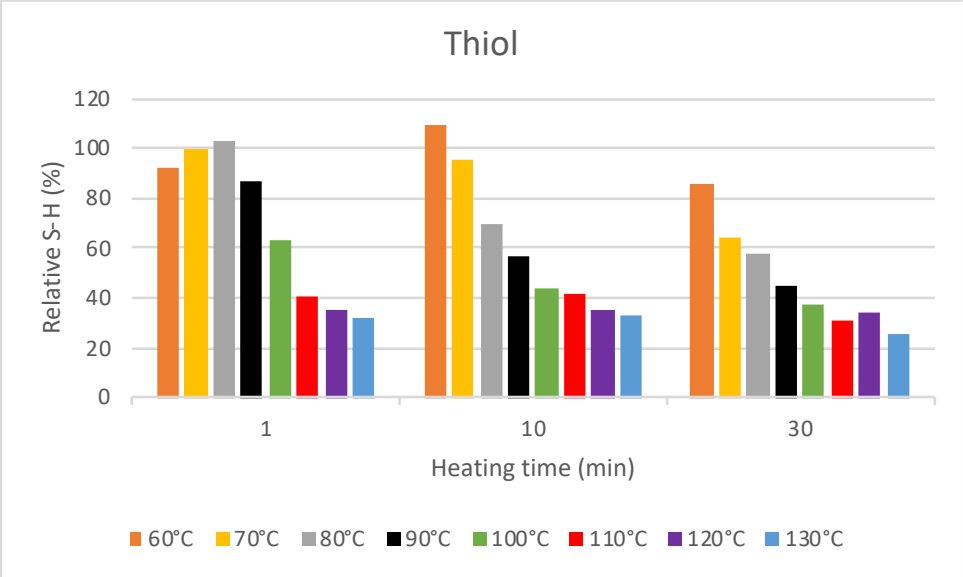


Figure 14: Relative S-H in heated camel milk (Reference raw milk)

3.3 Contribution of Maillard Reaction

Besides protein interaction and conformational changes, milk proteins may be consumed by Maillard reactions with reducing sugars (Morales *et al*., 1996; Van Boekel, 1998). Figure 15 shows that temperatures from 60-100 °C showed slight increase in HMF content up to 10 minutes and a higher increase after 10 minutes. At 110 and 120 °C, HMF increased more than at the lower temperatures after 10 minutes of heating. On the other hand, heating at 130 °C demonstrates a sharp increase in HMF content after 10 minutes of heating right up to 30 minutes. The general trend showed an increase in HMF content at all temperatures agrees with other literature (Morales *et al*., 1996; Muangthai and Surapat, 2003).

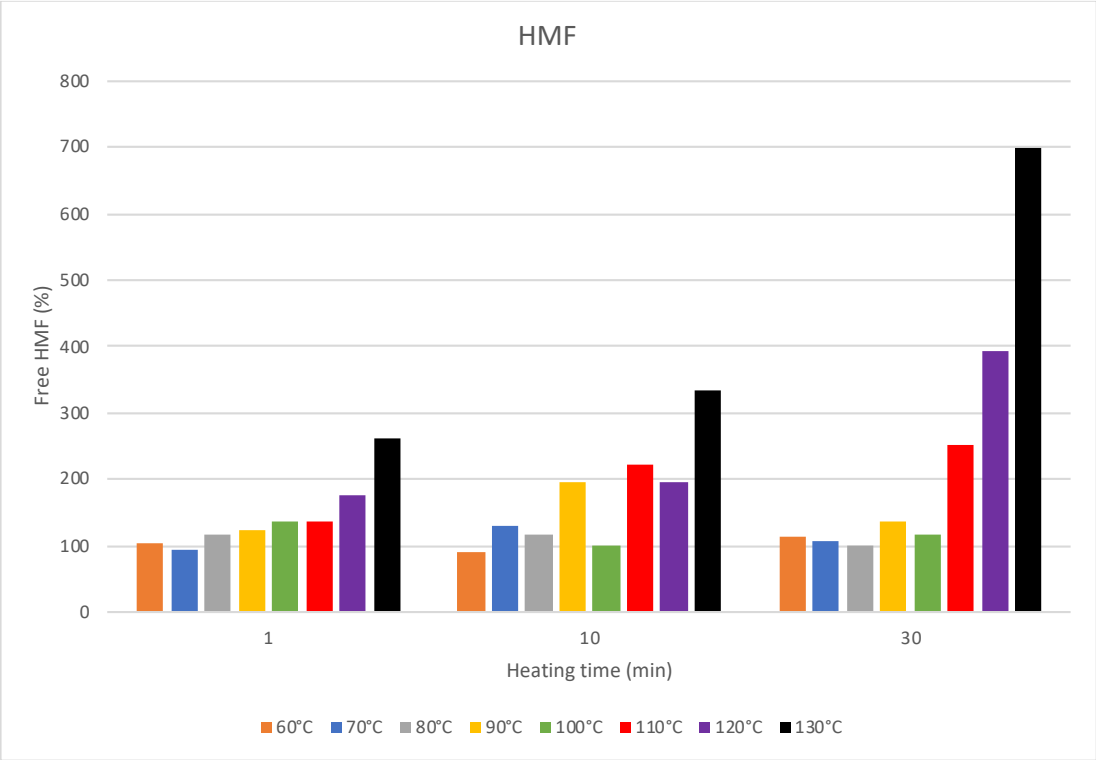


Figure 15: Hydroxymethylfurfural (HMF) content in heated camel milk

Chapter 4: Discussion

4.1 Electrophoresis

The dairy industry is growing rapidly across the globe and new technologies and techniques are being employed to study the milk proteins in order to produce novel foods from different kinds of milk. In this study, the effect of temperature and time combinations on camel milk proteins were studied. In agreement with literature, we find that caseins are found to be more stable as compared to the whey proteins. This is also observed in the data obtained through this research. New bands below the caseins were noted along with the known protein bands. These could probably be variants of caseins or the polymerization reactions occurring at high temperatures. As the temperature and time treatment increased, the casein bands slowly started fading. The electrophoretic gel pattern of 30 minutes' (Figure 11) treatment clearly shows the effect of heat on the caseins. Bands fade after 100 ℃ which is also confirmed through the graphs (Figure 10, 11, 12, 13). The graphs of the caseins show a decreasing trend which is also in agreement with literature. New bands observed below caseins could be variants of caseins or the result of polymerization or interaction with other unknown proteins.

It was observed that the whey proteins, lactoferrin (LF) and camel serum albumin (CSA), were easily denatured during thermal processing starting at 60 ℃ in agreement with literature (Abdel-Salam *et al*., 2014; Elagamy 2000). LF, CSA, and κ-CN depleted with increasing temperature from 100 °C/1 min, 110 ℃/30 min, and 110 °C/10 min degrees, respectively. If the concentration of CSA is high, it can form a gel above 70 ℃ as it is available to intermolecular interactions by forming aggregates (Considine *et al*., 2007). This means that the native globular structures of these whey proteins undergo conformational changes while unfolding. Since no free thiol is

available, α-LA will have little effect on aggregation and disulfide interchange (Considine *et al*., 2007). In this study, we have observed that α-LA showed increase in intensity with heating time, which is possibly due to a combination of its molten globule with fatty acids and formation of CAMLET, Camel Alpha-lactalbumin Made Lethal to Tumor Cells (Atri *et al*., 2011). Another possible explanation for this increase could be the phenomenon of lactosylation of α-LA, which is a reaction between the milk proteins and lactose (Abd El-Salam, 2014). Proteomics has been used to study lactosylation especially at UHT treatments as lactosylation of α-LA is enhanced in whole milk as compared to aqueous model systems (Czerwenka *et al*., 2006).

4.2 Changes in Thiol Content

In the native structure of bovine whey proteins, the free thiol is buried within the globular protein. When this protein undergoes heat treatment, the free thiol changes because of the interactions between the reactive thiol groups. This leads to formation of new polymers. Therefore, free thiol content is an indicator of heat treatment of milk (Muangthai and Surapat, 2003). The decrease in thiol content indicates lower levels of surface –SH groups due to the formation of S-S bonds. It has been studied previously through literature that denaturation of whey proteins at a higher temperature causes irreversible unfolding of the whey proteins. This action exposes the surface –SH groups which can lead to protein aggregates *via* –SH/S-S interactions (Havea *et al*., 2000; Wijayanti *et al*., 2014). Camel whey proteins were reported to be void of free –SH groups. α-LA however, contains 8 cysteine and 3 methionine residues, having 4 S-S groups while CSA has 14 cysteine and 3 methionine residues and has seven S-S groups (Felfoul *et al*., 2015).

The present study suggests that the unfolding of proteins and the conversion of S-S groups to -SH occurs already during the first minute of heating for all temperatures and that this transformation continued up to 10 minutes a 60 and 70 °C. From 80-100 °C no further increase was observed but a sharp decrease in free –SH was noted. DSC studies (Felfoul *et al*., 2015) revealed that the denaturation temperature for camel milk is 77.8 °C which may explain the difference in behavior between 70 and 80 °C. At temperatures 110,120 and 130 °C, the decrease in –SH with increasing temperatures could suggest oxidation of –SH groups as a major reaction (Wijayanti *et al*., 2014).

The decrease in free –SH after protein denaturation was explained by further polymerization of different whey proteins. The denaturation process in heated milk can either be reversible or irreversible. When milk is heated at lower temperatures, the native globular structure of whey proteins unfolds. During this time, if the temperature is not increased, the unfolded protein can re-fold to its original form. However, when the temperature is increased, the unfolded protein structure can undergo aggregation involving sulfhydryl (-SH)/disulfide (S-S) interchange reactions, hydrophobic or electrostatic interactions. For bovine milk, abundant in β-lactoglobulin, the major interactions at high temperatures are between β-LG and caseins. Since camel milk is supposed to be devoid of β-LG, the possible interactions would involve α-LA as it is one of the major whey proteins of camel milk (Lajnaf *et al*., 2018). Wijayanti, Bansal and Deeth (2014) wrote about the inability of α-LA to form aggregates without the presence of β-LG. This means that α-LA would need a donor –SH group to form aggregates with other proteins. Alone, α-LA was found to be heat stable even at 130 °C when heated for 30 minutes. This could be due to its secondary structure which does not have a free –SH group (Wijayanti *et al*., 2014). When heated to temperatures above 90 °C, irreversible denaturation of α-la takes place where S-S linked polymers

are formed. At another stage, it is noted that the free –SH on nonnative monomeric species can take part in (-SH)/disulfide (S-S) interchange reactions forming oligomers. Even then, α-LA is unable to form a gel. BSA on the other hand is an important gelling protein. This protein also undergoes unfolding at 62 °C and aggregation at a faster rate as compared to β-LG (Wijayanti *et al*., 2014). Conformational changes were mentioned to occur in the native structure of BSA causing its –SH group to move to the outer surface (Havea *et al*., 2000). This allows for the protein to interact with another BSA molecule to form dimers and trimers via (-SH)/disulfide (S-S) interchange reactions. The molten globule state (MG state) of α-LA and BSA can associate irreversibly *via* hydrogen bonding. It has also been noted by the authors that the presence of BSA in solutions containing BSA and α-LA accelerates the formation of α-LA dimers, trimers and polymers. This could be due to the number of S-S bonds present in BSA and β-LG.

4.3 Contribution of Maillard Reaction

Morales, Romero and Jimenez-Perez (1996) observed higher HMF values using the TBA method and they attributed this to the interference from highly reactive aldehydic compounds. They also deduced that about 72% of total HMF could be obtained because of interferences from yellow complexes at 433 nm. This phenomenon can be observed through the figure above especially at 130 °C/20 minutes which involves drastic heating conditions. In this research, total HMF determination of a yellow complex formed with 2-thiobarbituric acid (TBA) was carried out by a colorimetric method at 433 nm. This method alone, however, lacks specificity due to general reactivity of TBA towards aldehydic group. Therefore, other methods using HPLC would be beneficial to corroborate the results obtained by colorimetric methods.

Nevertheless, the results obtained here are clearly indicating an increase in HMF with increased heating time and temperature.

Chapter 5: Conclusions and Recommendations

Camel milk is important in the dry and arid lands. Nowadays, many publications are highlighting the nutritional and medicinal value of camel milk as an alternative to bovine milk. Special interest is on camel milk anti-diabetic, anti-allergic, anti-autistic and other health related properties (Shabo *et al*., 2005; Gizachew *et al*., 2014; Khalesi *et al*., 2017). Camel milk is very different from bovine milk with regards to its proteins, both caseins and whey proteins (Elagamy, 2000; Park *et al*., 2007; Claeys *et al*., 2014; El- Hatmi *et al*., 2014; Al-Alawi and Laleye, 2015; Brezovečki *et al*., 2015; Hailu *et al*., 2016).

Over the years, heat treatment has become a crucial step in the dairy industry. Different heating methods are applied to produce the best possible results of dairy products while maintaining its nutritional properties and shelf life. In fact, the dairy industry is working towards increasing the shelf life of milk and milk products. Therefore, this thesis has focussed on the effect of different heat treatments on the stability of camel casein and whey proteins. In this thesis, camel milk was subjected to temperatures of 60-130 ℃ for 1, 10 and 30 minutes. LF, CSA, and κ-CN depleted with increasing temperature from 100 ℃/1 min, 110 ℃/30 min, and 110 ℃/10 min degrees, respectively, in agreement with literature (e.g. Farah, 1986; Elagamy 2000; Sakkas *et al*., 2014; and Felfoul *et al*., 2015) while α-LA increased with increase in time. The instability of camel and other milks proteins will have implications on the characteristics and nutritional value of dairy products produced from these milks. The results obtained here need to be verified with more robust analytical methods like HPLC or capillary electrophoresis. These methods along with detailed mass spectroscopic analysis will enable researchers to get a better understanding of the chemistry of camel milk proteins. Understanding the chemistry of unfolding and

gelation during the thermal behaviour of camel milk proteins, especially α-LA, κ-CN and SA at ultra-high temperatures is very important as this will help future researchers solve the issue of developing stable products from camel milk. This research has thrown light on the possible reasons for the different behaviors of camel milk proteins at higher temperatures and highlighted a different behavior of α-LA compared to the other proteins. Further research in this area would be beneficial to future developments in the field.

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