Department of Food and Nutritional Sciences

Effects of industrial processing methods on skimmed camel milk properties

Thesis submitted for the requirement for the degree of Doctor of Philosophy in Food & Nutritional Science

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Adel Omar

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Abstract

Camel milk (CM) has an integral role in the diet of the population in the arid and semi-arid regions of Africa and Asia where scarce agricultural areas, high temperatures and small amount of precipitation. Recent studies have shown that it has potential therapeutic effects, including anti-cancer, hypo-allergic and anti-diabetic properties. Nowadays, CM has become increasingly commercialised and consumed in urban areas; which has led to an increased interest in the processing of CM to improve its microbial quality and extend its shelf-life. However, there is still a scarcity of available information regarding the effects of different processing methods (e.g. thermal and high-pressure treatments) on CM properties. Therefore, the aims of the current research were to characterise and quantify CM proteins and to evaluate the effect of high-temperature short-time pasteurisation (HTST), ultra-high-temperature (UHT) and high-pressure processing (HPP) on the physical, chemical and the organoleptic properties of skimmed CM in comparison to bovine skimmed milk.

Capillary electrophoresis (CE) was successful in identifying and quantifying the major whey and casein proteins in CM (chapter 3). Major variations were found between camel and bovine milk in terms of both concentration and composition of whey and casein proteins. Unlike bovine whey, camel whey had no β-lactoglobulin (β-lg) and instead a high concentration of α-lactalbumin (α-la) followed by lactoferrin (LF) and serum albumin (SA) was observed. β-casein (β-CN) was the main camel casein followed by α-casein (α-CN) while κ-casein (κ-CN) represented only minor amount. These variations were found to have an impact on the technological properties of CM, and quality of dairy products made from CM.

In general, HTST (72^{o}C for 15s), UHT (140^{o}C for 5s) and HP (200 to 800 MPa at 20^{o}C for 30 min) treatments significantly affected components of skimmed CM and their functional

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The volatile profile of skimmed CM subjected to HTST, UHT, and HP treatments was found to differ from the volatile profile of raw CM (chapter 5). HTST pasteurisation and UHT treatment resulted in an increase of aldehydes, furans, and terpenes content in CM. Moreover, the increase of heat severity during the UHT treatment led to the formation of sulphur compounds in CM. On the other hand, HP treatments tended to enhance the formation of alcohol and ketones in CM. Both thermal and non-thermal treatments had limited effect on amino acids and lactose content of skimmed CM. The volatile profiles and sensory properties of HTST pasturised and UHT skimmed CM were different to pasteurised and UHT bovine skimmed milk. Heated CM samples were described as having attributes such as cardboard, musty, sulphur odours, as well as sour, savoury, aged, and whey taste/flavours. While, bovine milk samples were described as having cooked milk, creamy, and dairy aroma. Overall, conventional heat treatments resulted in the formation of volatile compounds which were responsible for off-flavours in processed CM.

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Chapter 1: Introduction

Milk is an important part of a balanced diet. Besides being a source of proteins and fats; it contains all micronutrients which are important for the growth of the human body. The high nutritional value and availability, as well as growth of human population are key factors which have led to the increase of demand and consumption of milk and its products.

The world milk production is expected to increase to177Mt by 2025 with a growth rate of 1.8%. About 73% of which is anticipated to come from developing countries in Africa and Asia (OECD/FAO, 2016). Although, the majority of world milk production comes from cows (83%), milk supply produced from non-cow species including: buffalo, goats, sheep and camel has grown from 15.6% in 2001 to 17% of world's milk in 2011 (Horizons, 2013). These species are increasingly being used for milk production in the developing countries representing one-third of milk produced (Minh *et al*., 2014).

Camels are the most important livestock animal in arid and semi-arid areas of Africa and Asia used for milk, meat and hides supply, as well as for transport and for field cropping. They are well adapted to harsh conditions and capable of producing more milk for longer period compared to other domestic dairy animals (Al-Owaimer *et al.*, 2014). The average length of lactation in the camel is 12–18 months, and the amount of milk produced per day varies from 3.5L under harsh condition to 40L under intensive management (Hashim *et al.*, 2009). Although camels are producing only 0.3% of total world milk production, in some countries such as Somali, Djibouti, Qatar and United Arab Emirates, camels are producing approximately 43.38, 41.21, 22.96 and 21.25% of their total milk production respectively (FAO, 2013).

Camel milk (CM) is mainly consumed in its raw state as fresh or as fermented milk with varying degrees of sourness (Kappeler *et al.*, 1998; Elagamy, 2000; Otaibi, 2013). Fermentation is the only available means of preservation of CM under such harsh warm

conditions in arid and semi arid areas. The majority of world CM production comes from East Africa region (66%) followed by West Africa (20%), Asia (9%) and North Africa (5%) (Sisay and Awoke, 2015). The interest of studying the physicochemical properties of CM and the technological aspects of its utilisation has been increased since 1980s (Farah, 1993). However, most of the research conducted on CM to date has mainly focused on its gross components, functionality, and health properties. Whereas there is still a scarcity of available information concerning technological aspects and the effects of industrial processing methods on the physical, chemical and the organoleptic properties of CM.

1.2. Research hypothesis and objectives

In the course of the current research, we have tried to develop a more detailed understanding about the technological properties of CM from different aspects. Numerous studies have extensively investigated the properties of bovine milk over the past several decades; and therefore it has been used for comparison. Distinct differences between camel and bovine milk in terms of composition and physicochemical properties have been reported (Alhaj and AlKanhal, 2010). Therefore, the research hypothesis was that the technological properties of CM differ to bovine milk when it undergoes various industrial processing methods. Thus, the objectives of this research are:

1. To develop a method to characterise and quantify CM proteins (casein and whey proteins fractions), in order to monitor their behaviour when CM is subjected to various processing methods.

2. To investigate the effect of industrial processes such as heat treatment including: pasteurisation (HTST) and ultra-high-temperature (UHT) in comparison to the non-

thermal processing (High-Pressure Processing (HPP)) on physical and chemical properties of CM.

3. To study the effects of these industrial (HTST, UHT, and HPP) processing treatments on the volatile flavour and non-volatile compounds and sensory properties of CM and comparing the results to bovine skimmed milk.

1.3. Novelty of the research

- In the current study, capillary electrophoresis technique was used to characterise and quantify CM proteins for the first time.
- In addition, CM was subjected to UHT and HPP treatments for the first time.
- The effect of HPP on whey protein denaturation, colour change, casein micelle size, and rennet coagulation time (RCT) of skimmed CM has not previously been reported.
- The effect of HTST, UHT and HP treatments on volatile flavour and non-volatile compounds and sensory properties of skimmed CM has not previously been reported.

1.4. Significance of the research

Nowadays, production of CM and its dairy products in large commercial scale is in progress in Asia, Africa and Europe due to increase in demand (Elagamy *et al.*, 2009). Thus, there is a great need for scientific studies concerning the technological challenges associated with CM which will lead to a better understanding of the quality of processed CM, and to assist in the development of such products. Moreover, there is still a clear gap of knowledge about the technological challenges of CM.

This research attempts to throw light on the technological difficulties associated with the manufacturing of CM under the same processing conditions which are typically applied in dairy industry. This research provides basic information on CM behaviour under various processing methods which will enable the dairy manufacturers to improve their processing conditions in order to improve the quality of the final product and enhance its consumer acceptability. Therefore, the current research is significant for CM manufacturers and the researchers in dairy area.

1.5. Thesis outline

The current research thesis has been written in the format of a series of published and submitted papers and it consists of 6 main chapters. The **second chapter** incorporates background information about CM and reviews the previous research conducted on CM. In the **third chapter** of the thesis, the major protein fractions of CM were successfully characterised and quantified by capillary electrophoresis (CE) and the work has been published in the International Dairy Journal:

Omar, A., Harbourne, N., & Oruna-Concha, M.J. (2016). Quantification of major camel milk proteins by capillary electrophoresis. *International Dairy Journal*, **58**, 31–35.

In **chapter four**, the effects of HTST pasteurisation, UHT and HP treatments on CM in terms of whey proteins denaturation, casein micelles size, and colour change and rennet coagulation time were studied in comparison to bovine milk. This work has been published in the International Dairy Journal:

Omar, A., Harbourne, N., & Oruna-Concha, M. J. (2018). Effects of industrial processing methods on camel skimmed milk properties. *International Dairy Journal*, **84**, 15–22.

In **chapter five**, the effects of these industrial treatments on the the profile of volatile and non-volatile compounds (amino acids and sugars) of CM, and the sensory characteristics of HTST

and UHT treated CM in comparison with the commercially available pasteurised and UHT treated bovine milk were investigated. A manuscript entitled '*Effects of industrial processing methods on the flavour and sensory properties of camel skimmed milk: a comparison with bovine skimmed milk'* in preparation for submission to Food Chemistry journal. Finally, **chapter six** presents an overall summary and conclusions of the research and directions for future work.

Chapter 2: Literature review

2.1. Dromedary camel's taxonomy and their geographical distribution

Camels belong to the camelidae family of mammals which is in the taxonomic order of Artiodactyla (even toed ungulates), suborder Tylopoda (pad-footed animals) (Al-Swailem *et al.*, 2007). The family Camelidae consist of three main genera (**Figure 2.1**): genus Camelus (the old world camels), genus Lama and Vicugna (the new world camels) (Yam and Khomeiri, 2015). The new world camel species include: *L. glama, L.guanicoe, L. pacos and V.vicugna* are characterized by their small size and living in the heights of the mountains in South America. Whereas, the two-old-world species: Dromedary camel (*C. dromedarius*) and Bactrian camel (*C. bactrianus*) are large and spread around Africa and Asia. Camels are ruminants, however, they are different to other species that belong to the suborder Ruminantia (especially bovinae family) in several aspects including foot anatomy, stomach system and the absence of horns (Faye, 2015).

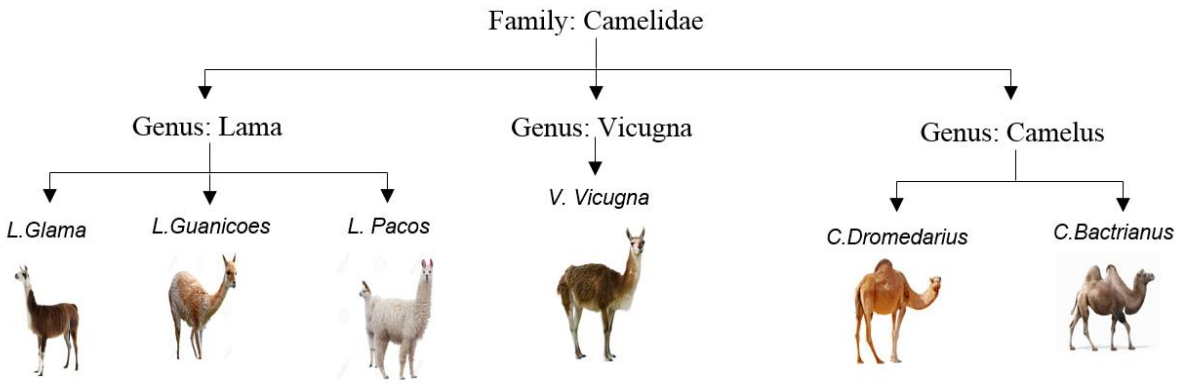

Figure 2 1. The taxonomic order of the Camelidae family.

The dromedary camels (one hump) live in the hot arid and semi-arid lands in the Northern and Eastern Africa as well as in Western Asia and Australia (**Figure 2.2**). Bactrian camel (two humps) usually inhabit cold areas in Central Asia such as the East and the Northern China, Mongolia and Southern Russia. The dromedary is slim, long-legged, short-haired whilst the

Bactrian is stockier, short-legged and has a thicker and longer coat than the dromedary (Farah, 1993). Both camels have a great ability to retain water and control their body temperature which enables them to withstand the harsh environmental conditions in such areas (Hashim *et al*., 2015).

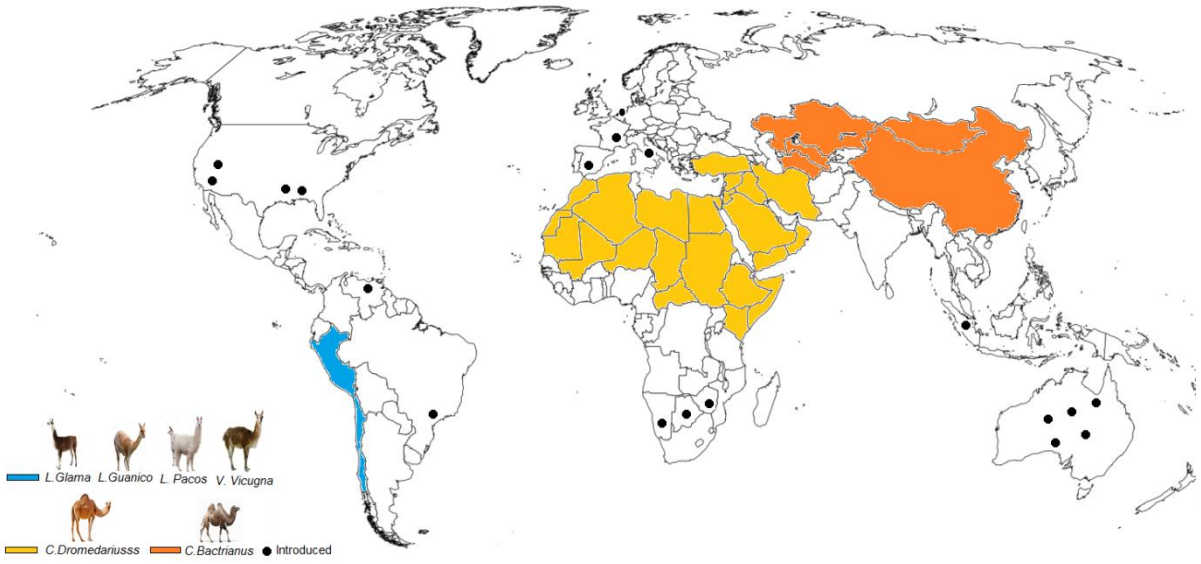

Figure 2 2. The geographic distribution of the camels.

The global population of camels is estimated to be 20 million world-wide, 15 million of which are in Africa, and 5 million in Asia. Somalia, Mali, Ethiopia, Sudan, Kenya, Niger and Saudi Arabia have the highest number of camels comparing to other countries (FAO, 2014). Approximately 94% of the estimated world's camel population were thought to be dromedary camels, whereas, the Bactrian camels comprises only 6% and is primarily in Asia (Yam and Khomeiri, 2015). The world camel population is increasing constantly since 1961 with a growth rate of 3.4% every year reaching more than double in 2014 (Faye, 2015). Thus, the population of dromedary camels has increased in several countries in Africa and Asia over the last years (**Figure 2.3**) (Yam and Khomeiri, 2015). More than 60% of the dromedary camel population is concentrated in the four North East African countries Somalia, Sudan, Kenya and Ethiopia (Farah *et al*., 2007).

The dromedary camels were domesticated in the South coast of the Arabian peninsula (Yemen and Oman) about 3000 to 4000 years ago mainly for milk, meat and hides supply, as well as for transportation (Schwartz and Dioli, 1992). They were then introduced into other regions including North and the Horn of Africa, Iran, Pakistan and India by humans as a result of the spice trade. Dromedaries were also imported to Australia in the 18th century and to the United States in the middle of the 19th century for transportation and meat production (Al-Swailem et al., 2007). The name dromedary is originally derived from the Greek word, "dromeus" which means runner or "droma"- running (Farah, 1993; Jassim and Naji, 2002). Characteristics of dromedary camels and their distribution were described by Köhler-Rollefson (1991).

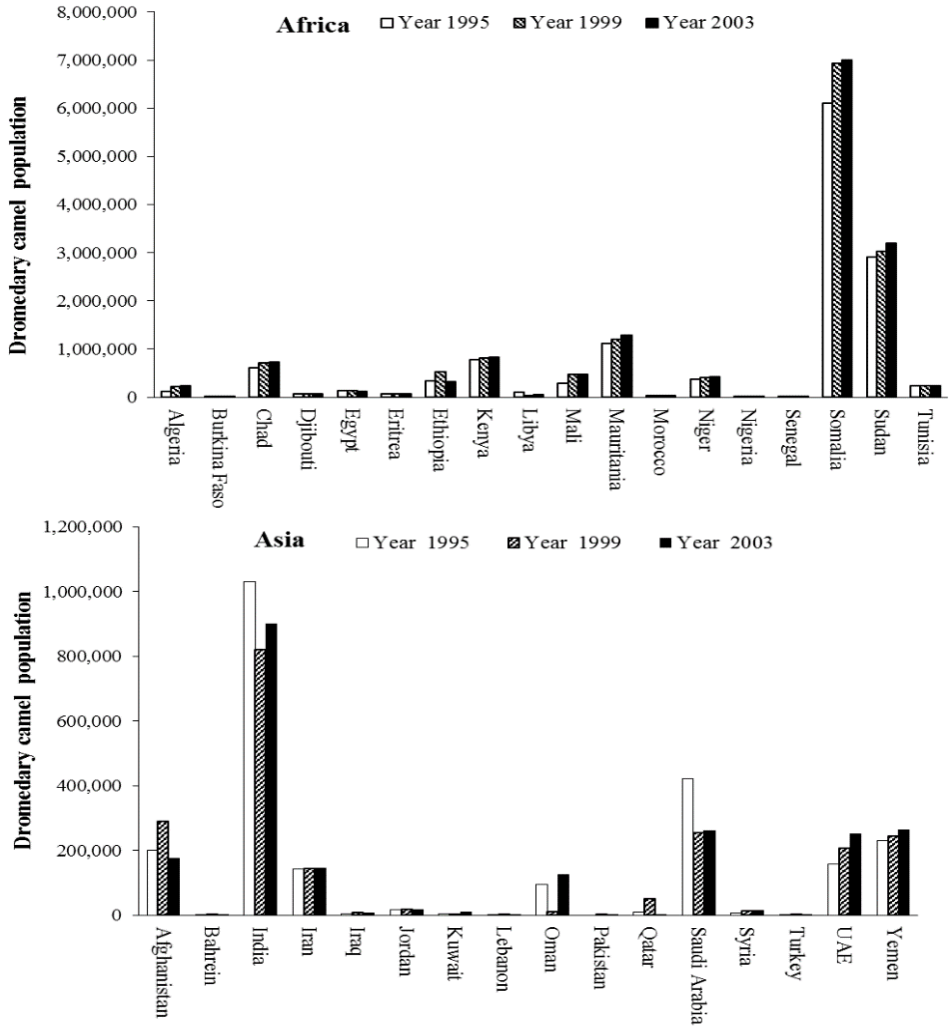

Figure 2 3. Development of the dromedary population in some countries in Africa and Asia.

2.2. Dromedary camels for milk production

Dromedary camels are important livestock animals for local population of arid and semiarid lands in Africa and Asia due to their unique anatomical, physiological and behavioural adaptive features to the harsh environmental conditions. Unlike other mammals, they can reserve up to 36 kg of fat concentrated in their humps which enables sweat to be evaporated easily over the rest of their body surface and serves as a source of water and energy when there is shortage of drinking water and scarcity of feed. In addition, in the case of hot weather and lack of water availability for long periods, camels can tolerate the loss of up to 27% of its body weight, whilst other mammals die when they lose 12–15% of body weight (Brezovečki et al., 2015).

The dromedary camels were first domesticated by the nomadic people about 3000 B.C.E. in southern Arabia as the primary source of milk and meat (Al-Swailem *et al*., 2007; Yam and Khomeiri, 2015). They are capable of producing more milk for a longer period of time than other domestic dairy animals (cattle, sheep and goats) held under these hostile conditions (Khan and Iqbal, 2001). However, the daily milk yield and the length of lactation of dromedary camels varies among geographical regions, countries in Africa and Asia. The lactation length of dromedary camels in Pakistan is between 8–9 months with a daily milk yield of 10 litres per day (Raziq *et al*., 2010). A longer period of lactation between 12–18 months and lower milk yield 7–8 L/d were reported for the camels in India (Nagpal and Patil, 2012). In Saudi Arabia, the average milk yield of different local camel breeds (Majaheem, Waddah, and Homor) was 5.4 L/d under intensive feeding management and the lactation length was 12.5 months (Musaad *et al*., 2013a). Whilst the daily milk yield of camels kept under pastoral management system in Northeast Ethiopia was ranged from 2–12 L/d over lactation period of 12 months (Simenew *et al*., 2013). Similarly, the milk yield of camels in Northeast Somalia was between 3 to 10 L/d during a lactation period of 12 to 18 months (Farah *et al*., 2007). For the Maghrebi dromedary

camel in Tunisia the length of the lactation period was 13 months with an average daily yield of 6 L/d (Jemmali *et al*., 2016). Whereas, the daily yield of Maghrebian camel raised in Egypt was within a range from 3.5–4.5 L/d throughout a lactation period of 7 months (Mostafa *et al*., 2016). This great variation in CM production and the length of lactation period might be due to several factors including high genetic variation between individuals, breed, feeding and management conditions, water availability, milking frequency, age of animal, lactation number and stage of lactation (Khan and Iqbal, 2001; Shehadeh and Abdelaziz, 2014). In general the daily milk yield of dromedary camel varies from 3.5 litres under harsh conditions to 40 litres under intensive management, and the lactation length ranges from 9 to 18 months (Khan and Iqbal, 2001).

Dromedaries have a great potential as milk livestock due to their unique ability of maintain their average daily milk yield for a long period of time (at least for one year) when there is an abundance of feed and water (Faraz *et al*., 2013). The avarage milk yield of dromedary camel kept under intensive management conditions is between 15 to 20 litres daily (Raziq *et al*., 2008). During the last decade, there has been a great progress in the intensive dairy management and machine milking of dromedary camels in several countries around the world. For example, in Saudi Arabia the camel farming moved from the pastoral system to semi-intensive and intensive feed systems, as a result of the increasing demand for CM by a growing urbanized population (Faye, 2013). Camels kept under semi-intensive feeding system were able to produce more milk of good composition for a longer period of lactation (Idrees *et al*., 2016). Similarly in Sudan, the milk yield and number of milking times per day were significantly increased after camels had been subjected to semi-intensive feeding system, compared to camels in nomadic system. (Dowelmadina *et al*., 2015).

Moreover, dromedary camels adpoted well to automatic milking equipment without significant effect on daily milk yield and the composition of CM (Ayadi *et al*., 2013).

Therefore, modern milking machines were introduced to the milking practices of dromedaries in large-scale camel dairy farms in the United Arab Emirates, Saudi Arabia, and Tunisia as well as in small-scale farms in Australia, Europe, and USA (Nagy and Juhasz, 2016).

2.3. Dromedary camel milk composition

Dromedary CM is characterised by its opaque-white colour and sweet sharp taste, however, sometimes it can be salty (Farah, 1993; Alhaj and AlKanhal, 2010). The change in its taste depends on the type of fodder and the availability of drinking water (Patel *et al*., 2016), while, its opaque white colour is becuase of the finely distribution of its fat throughout the milk (Jilo and Tegegne, 2016).

The physical properties of CM and the corresponding values from other animal species are shown in **Table 2.1** below. The average reported values of the pH, density (specific gravity) and acidity of CM (**Table 2.1**) were 6.77, 1.015, and 0.18 (Khaskheli *et al*., 2005). A slightly lower pH average value of 6.56, and higher density average of 1.029 g cm^{-3} were also reported for CM (Farah, 1993). Both were lower than in bovine milk. This low pH value of CM was found to be correlated with its high content of vitamin C, which can be masked if the animal eats salty or bitter vegetation (Al-Juboori *et al*., 2013).

Table 2 1. Physical properties of camel, bovine, buffalo, sheep, and goat milk

Types of milk	Parameters (range)		
	pH values	Acidity (%)	Density (g cm^{-3})
Camel	6.57–6.97	0.12–0.20	1.01–1.02
Bovine	6.63–6.68	0.12–0.19	1.02–1.03
Buffalo	6.60–6.90	0.11–0.18	1.02–1.02
Sheep	6.40–6.80	0.16–0.19	1.02–1.02
Goat	6.34–6.68	0.11–0.17	1.02–1.03

Adapted from:(Kanwal *et al*., 2004; Khaskheli *et al*., 2005)

The gross composition of CM and the corresponding values from other animal species are shown in **Table 2.2**. In general, CM showed great variation in its composition compared to other species, especially bovine milk (Dowelmadina *et al*., 2014). This variation was attributed to several factors including: age, stage of lactation, camel breeds, feeding conditions and geographical location. (Khan and Iqbal, 2001; Alhaj and AlKanhal, 2010; Brezovečki *et al*., 2015). Among which, geographical origin and seasonal variations were found to be the most important factors (Konuspayeva *et al*., 2009).

Table 2 2. Chemical composition of camel, bovine, buffalo, sheep, and goat milk

Types of milk	Milk components (range %)						
	Water	**Fat**	**Protein**	**lactose**	**Total solid**	**SNF**	**Ash**
Camel	86–91	1.8–4.3	2.0–3.2	3.3–5.4	7.7–12.1	5.5–8.2	0.8–1.0
Bovine	85–87	4.0–5.0	4.4–5.7	3.0–4.6	13.4–14.3	8.4–10.1	0.2–0.4
Buffalo	82–84	4.0–6.5	3.1–4.1	3.2–4.8	12.7–15.9	8.2–9.4	0.3–0.4
Sheep	79–82	8.0–9.6	5.3–7.7	3.0–4.2	17.4–19.5	9.4–10.1	0.5–0.6
Goat	87–88	3.9–5.7	1.1–3.1	4.0–5.5	12.6–15.1	8.5–9.4	0.2–0.3

Adapted from: (Kanwal *et al*., 2004; Khaskheli *et al*., 2005; Ismaili *et al*., 2016; Jilo and Tegegne, 2016)

2.3.1. *Water*

Amongst components of milk, water content was found to be the most important factor affecting the overall composition of CM. The amount of water in CM ranged from 86% in winter when there is abundance of drinking water to 91% in summer when temperature ranges between 40–45^{o}C with scarcity of water (Farah, 1993; Haddadin *et al*., 2008). During dry seasons the lactating camel loses water to milk as natural adaptation in order to provide necessary fluid to the dehydrated calf, which leads to increase of the amount of water in CM (Yadav *et al*., 2015).

2.3.2. *Fat*

Fat content of CM normally ranges between 1.8 to 4.3%, however, it was reported to decrease from 4.3 to 1.1 % in milk produced by thirsty camels (Jilo and Tegegne, 2016). Milk fat of dromedary camels differ from that of other animals in several aspects. Compared with buffalo and bovine milk fat, CM fat contains higher proportion of long chain fatty acids and lower amounts of short chain fatty acids. Furthermore, the cholesterol level of fat of CM (34.5 mg.100 g^{-1}) is higher as compared to cholesterol level (25.63 mg.100 g^{-1}) of bovine milk fat (Abbas *et al*., 2013). The fat globules in CM (2.99 µm) are smaller than those from buffalo milk (8.7 µm), but similar to that of goat milk (3.19 µm) (El-Zeini, 2006), and are characterised by a white colour due to their low content of carotene (Alhaj and AlKanhal, 2010).

2.3.3. *Protein*

The total protein content of dromedary CM varies from 2.0 to 3.2%, and is composed of two main groups, namely caseins and whey protein. Proteins of CM contain higher amount of whey proteins (0.80 %) than buffalo (0.68%), sheep (0.66%), goat (0.53%), and bovine milk (0.47%) (Rafiq *et al*., 2016). The variation in the protein content of CM was mainly attributed to the camel breeds and seasonal conditions. Milk produced by Majaheim camel showed a higher protein content (2.91%) than milk from other camel breeds such as Wadah and Hamra (2.36, 2.52% respectively) (Mehaia *et al*., 1995). Moreover, protein content of CM produced from the same camel breed was found to be maximum in February (3.32%) and minimum in October (2.76%) (Musaad *et al*., 2013b).

Casein (CN) is the main protein in CM, representing about 52–87% of total protein. It consists of β-casein (β-CN), α-casein (α-CN) and κ-casein (κ-CN) (Alhaj and AlKanhal, 2010; Abbas *et al*., 2013). The estimated molecular mass of camel β-CN, α-CN and κ-CN are 32, 35 and 22 KDa respectively, which are considerably higher than those reported for bovine β-CN (24 KDa)

and α-CN (22-27 KDa) (Farah and Farahriesen, 1985; Saliha *et al.*, 2013). The majority of camel caseins are β-CN 65% followed by 22% α_{s1}-CN, 9.5% α_{s2}-CN and 3.5% κ-CN of total casein, while, bovine caseins contains high percentage of α-CN (38%) followed by 36% β-CN and 13% κ-CN of total casein (Brezovečki *et al.*, 2015). CM has lower concentrations of κ-CN compared to bovine milk. Furthermore, camel κ-CN contains an additional proline residue in its sequence (Pro^{95}), which plays an important role in its stability, with different site for hydrolysis by chymosin (Phe^{97}-Ile^{98}) compared with bovine κ-CN (Phe^{105}-Met^{106}) (Kappeler *et al.*, 1998; Hailu *et al.*, 2016b).

The size distribution of camel casein micelles was reported to be between 260 to 300 nm, which is bigger and significantly broader than that of bovine casein 100 to 140 nm (Farah and Rüegg, 1989). Thus, the low content of κ-CN (3.47%) in camel casein compared to bovine casein (13%) could be due to its high content of large micelles, since small micelles of about 60 nm contained 12% κ-CN, large micelles of about 200 nm contained mere 2% κ-CN (Gouda *et al.*, 1984).

Whey proteins represent about 20–25% of total protein in CM and include: serum albumin (SA), α-lactalbumin (α-la), lactoferrin (LF), immunoglobulins and peptidoglycan recognition protein (Laleye *et al.*, 2008; Hinz *et al.*, 2012). Camel SA, α-la, and LF were reported to have molecular weight of 67, 15 and 79 KDa respectively (Elagamy *et al.*, 1996; Elagamy, 2009; Saliha *et al.*, 2013). Camel whey lacks β-lactoglobulin (β-lg) and contains larger amount of α-la (27%) and SA (26%) than bovine whey, whereas β-lg is the main protein in bovine whey representing 55% of total whey proteins followed by α-la (20.1%). Thus, concentration of α-la in bovine whey (1.26g/L) was found to be lower than in camel whey (3.5g/L) (Merin *et al.*, 2001; Elagamy, 2009; Hailu *et al.*, 2016a). Camel whey was also reported to have higher content of LF than bovine milk (Elagamy, 2009). In terms of amino acid composition, casein structure of dromedary CM is similar to that of bovine milk; only few differences in the primary

structure of casein were observed compared to bovine caseins. Camel casein has greater content of proline (9.2% in α_{s1}-CN, 4.5% in α_{s2}- CN, 17.1% in β-CN, and 13.6% in κ-CN) than bovine casein (8.5%, 4.8%, 16.7%, and 11.8% respectively) (Elagamy, 2009). The number of amino acid residues of camel casein fractions were estimated: α_{s1}-CN 207, α_{s2}-CN 178, β-CN 217 and κ-CN 162 (Kappeler *et al.*, 1998). The content of non-essential amino acids except arginine and the essential amino acids including: methionine, isoleucine, leucine and phenylalanine were higher in bovine α-CN than α-CN from CM. Moreover, valine, phenylalanine, histidine, glycine, and serine content were also found to be significantly higher in bovine β-CN compared to the camel β-CN. Camel κ-CN contains higher amount of arginine and lysine than bovine κ-CN (Salmen *et al.*, 2012).

The main camel whey protein α-la contains 123 residues (similar to bovine α-la) and a higher number of antioxidant amino acids residues (cysteine, tryptophan, and methionine) than bovine α-la (Salami *et al.*, 2009). However, the number of amino acids residues in camel LF is similar to bovine LF (137 and 135 respectively) (Elagamy, 2009).

2.3.4. *Lactose*

Lactose content in CM ranges from 3.3 to 5.4%, with an average of 4.37% (Ismaili *et al.*, 2016). It has been reported that the lactose content of CM remained almost unchanged throughout the year, from the first months up to the end of lactation (Haddadin *et al.*, 2008). The variation in the concentration of lactose in CM is associated with water intake and type of plants eaten by camels in the deserts. Camels prefer halophilic plants such as Atriplex, Salosa and Acacia to meet their physiological requirements of salts. Therefore, CM is sometimes described as salty and at other times as bitter (Alhaj and AlKanhal, 2010). In cases of dehydration the lactose content decreases in CM, thus the taste of milk is less sweet (Al-Juboori *et al.*, 2013).

2.3.5. *Total solids*

The total solids (TS) content of CM varied between 7.7 and 12.1% and the reported mean value (11.97%) was lower than that of bovine and buffalo milk, but similar to that of goat milk (Yoganandi *et al*., 2014). The TS content of CM is inversely proportional to its water content, and it is composed of milk fat, lactose, proteins, and ash (Khaskheli *et al*., 2005). Stage of lactation and season of the year were found to be the main factors affecting the TS content of CM (Brezovečki *et al*., 2015).

2.3.6. *Ash*

The amount of ash in CM varies from 0.8 to1.0%, and the lowest percentage of ash was found in the milk produced by dehydrated camel (Konuspayeva *et al*., 2009). The ash content of CM is always subject to variations depending on the breed differences, feeding, analytical procedures, and water intake (Mehaia *et al*., 1995). Dromedary CM contains relatively higher amount of ash than buffalo and bovine milk (Yoganandi *et al*., 2014). The mean values for calcium (Ca), potassium (K), sodium (Na), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) in mineral content of CM are 114, 156, 59, 0.29, 10.5, 0.05 and 0.53 mg 100 g^{-1} respectively (Alhaj and AlKanhal, 2010). The level of Na, K, Fe, copper (Cu), and Mn in CM were substantially higher than that reported for bovine milk. Furthermore, the content of Ca, phosphorus (P) and Mg of CM were comparable to bovine milk (Mehaia *et al.*, 1995; Sawaya *et al.*, 1984). CM is considered to be a rich source of chloride as result of halophilic plants consumed by camels, which usually contain a high content of salt (Alhaj and AlKanhal, 2010; Brezovečki *et al*., 2015).

It is well known that CM is a rich source of vitamin C (34.16 mg/L) and is 3–5-fold greater compared with bovine milk. Moreover, it contains more niacin (B_3), folic acid, pantothenic

acid, and vitamin B_{12}, but lower content of vitamins A, E, B, β-carotene, and riboflavin than bovine milk (Stahl *et al.*, 2006).

2.4. Therapeutic properties of dromedary camel milk

Historically, dromedary CM has been used as a remedy for several diseases including: dropsy, jaundice, tuberculosis, asthma and leishmaniasis, in different countries around the world such as India, Russia and Sudan, Iran, Somali and Libya (Alwan *et al.*, 2014; Asres and Yusuf, 2014). More recently, CM was also reported to have other potential therapeutic properties, such as anti-carcinogenic (Magjeed, 2005), anti-diabetic (Agrawal *et al.*, 2007), anti-hypertensive (Quan *et al.*, 2008), and hypoallergenic (Elagamy *et al.*, 2009) property. These potential health benefits have been attributed to the presence of several bioactive components in CM (Elagamy *et al.*, 2009).

2.4.1. *Antimicrobial and antiviral*

Dromedary CM possesses antibacterial effect against Gram-positive and Gram-negative bacteria including *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, and Salmonella typhimurium*, due to its high content of lysozyme, lactoferrin, lactoperoxidase, and immunoglobulins (Elagamy, 2000; Benkerroum *et al.*, 2004). Their amounts were found to be greater in CM than human, bovine or buffalo milk (Konuspayeva *et al.*, 2007). These protective proteins were also reported to have antiviral activities (Elagamy *et al.*, 1992). Both lactoferrin and lactoperoxidase isolated from CM exhibited higher in vitro inhibitory effects on hepatitis C virus (genotype 4a) than their counterparts in human, bovine and sheep milk. They prevented the entry and direct interaction of hepatitis C virus to Huh7.5 (hepatocyte-derived carcinoma) and HepG2 (human hepatoma) cells (EL-Fakharany *et al.*, 2013; Redwan *et al.*, 2015).

2.4.2. *Antidiabetic*

The milk of the dromedary camel has traditionally been used in the prevention and control of diabetes. Studies have suggested that drinking CM resulted in a decrease prevalence of diabetes in the Raica community in India (Agrawal *et al.*, 2007; Singh *et al.*, 2008). CM was recently recommended as safe and efficient in improving long-term glycemic control with a significant reduction in the doses of insulin in type 1 diabetic patients (24 patients) (Agrawal *et al.*, 2011; Mohamad *et al.*, 2009). The antidiabetic properties of CM were attributed to following factors: a) the high concentration of insulin and insulin-like proteins in CM; b) that fact that unlike the insulin of other animals, camel insulin is encapsulated in nanoparticles that facilitate its absorption and easy passing to the blood stream, and it does not form a coagulum in acidic conditions of human stomach; c) the effect of small size immunoglobulins of CM on β-cells (Alhaj and AlKanhal, 2010; Abdel Gader and Alhaider, 2016).

2.4.3. *Treatment of Autism*

It has been demonstrated that CM may have a therapeutic effect in the autoimmune disease such as autism. Milk protein casein may have a key role in the development of autism symptoms (Shabo and Yagil, 2005a). The incomplete metabolism of milk casein proteins (particularly β-CN and β-lg) in humans, lead to formation of β-casomorphin, which has long been considered as a risk factor for autism (Kaskous, 2016). Unlike bovine milk, CM was reported to lack these two proteins thus it may not lead to autism symptoms. Moreover, CM contains protective proteins including immunoglobulins necessary for maintaining the immune system (Yadav *et al.*, 2015). In a recent study, it was observed that the consumption of CM by children (60 males, 5 females) who were suffering from autism resulted in the disappearance of autism symptoms in some cases, or caused significant improvement in these symptoms (Adams, 2013; Al-Ayadhi *et al.*, 2015). In addition, CM was found to play an important role in decreasing oxidative stress by alteration of enzymatic and nonenzymatic antioxidant

molecules and improvement of autistic behaviour of 60 children aged 2–12 years. (AL-Ayadhi and Elamin, 2013).

2.4.4. *Treatment of Crohn's disease*

Consumption of CM was also reported to have a positive effect on the healing process from Crohn's diseases (Shabo *et al*., 2008). This disease is a bacterial infection caused by *Mycobacterium avium–subspecies paratuberculosis* (belonging to the family of tuberculosis) which could spread via bovine milk as it is unaffected by pasteurisation (Gizachew *et al*., 2014). This positive effect was attributed to the powerful bactericidal properties of CM and its high content of peptidoglycan recognition protein. In addition, camel's immunoglobulins attacked the anti-DNA and restored the immune system (Gizachew *et al*., 2014).

2.4.5. *Treatment for allergies*

Research in vitro (Elagamy *et al*., 2009) and in vivo (Shabo *et al*., 2005b; Ehlayel *et al*., 2011) showed that CM is hypoallergenic and a promising substitute for children who are allergic to bovine milk. Camel whey protein is devoid of β-lg (Omar *et al*., 2016) which might be responsible for bovine milk allergies in children. Instead, it contains great amount of α-la that has higher digestibility and more antioxidative activity than bovine α-la (Salami *et al*., 2009). Furthermore, camel casein contains higher β-CN and lower α_{s1}-CN content than bovine casein. Therefore, CM was reported to have higher digestibility rate and less allergic reactions in infants compared to bovine milk (Elagamy *et al*., 2009). In a study by Shabo *et al.* (2005b), eight children with severe food allergies were given CM for two weeks. The results showed that all children improved rapidly and recovered fully from their allergies after drinking CM. Another study by Ehlayel *et al.* (2011) suggested that consumption of CM by children (23 males and 12 females, aged 4–126 months), who suffer of cow's milk allergy, reversed allergies reactions in 28 treated children (80%). It has been reported that immunoglobulins in

CM are similar to those in mothers' milk, which could potentially reduce children's allergic reactions and strengthen their future response to foods (Al-Juboori *et al.*, 2013; Yadav *et al.*, 2015).

2.4.6. *Lactose-intolerant*

Camel's milk was also recommended as an alternative option for those individuals intolerant to lactose who show symptoms when drinking bovine milk. In a study by Cardoso *et al.* (2010), twenty-five patients (19 males and 6 females), aged 2 to 68 years, who were diagnosed with lactose intolerance were given CM for five consecutive days, on an empty stomach. 23 patients were able to accept CM without any adverse symptoms. Only two patients showed mild reactions to the maximum dosage of CM (250 mL).

Although CM has such medicinal value, its consumption is still restricted to pastoral areas. In addition, most of the reported health benefits of CM are based on small laboratory studies. Therefore, further studies in large controlled clinical trials are needed in order to fully understand the nutritional and medicinal value of CM.

2.5. The technological challenges of processing dromedary camel milk

2.5.1. *Heat treatment of camel milk*

Heat treatment of milk is an essential step to render milk safe for human consumption and extend its shelf life. Heat treatment methods include low temperature long time pasteurisation (LTLT), high temperature short time pasteurisation (HTST), sterilization and Ultra High Temperature (UHT). Amongst them, HTST pasteurisation and UHT are the most commonly used methods in the dairy industry (Benabdelkamel *et al.*, 2017). However, the actual application of a selected heat treatment process is mainly dependant on the type of milk (Alhaj *et al.*, 2011). Previous studies have shown that CM has some different properties from bovine

milk including poor stability at high temperatures (Alhaj and AlKanhal, 2010). The heat coagulation time (HCT) of CM at high temperatures of up to 140^{o}C was reported to shorter in comparison with bovine and buffalo milk (Farah and Atkins, 1992; Sagar *et al.*, 2016). This was attributed to the absence or deficiency of β-lg and κ-CN proteins in CM, as milk is more resistant to heat when it is characterized by a molar ratio of β-lg to κ-CN that equals 1 (Barłowska *et al*., 2011). Several attempts have been made to improve the heat stability of CM. As such, modification of the protein level and salt composition of CM similar to that of bovine milk did not improve its HCT (Al-Saleh, 1996). Furthermore, neither urea (10 mM) nor formaldehyde (5 mM) addition have improved the heat stability of CM (Metwalli *et al.*, 2013). In another study, Alhaj *et al*. (2011) demonstrated that the heat stability of CM at 121°C could be improved by increasing the milk pH to 7.0–7.2 and addition of κ-CN, EDTA or sodium phosphate.

Whey proteins in CM were found to be significantly affected by heat treatment at 98°C for 60 min, while they remained slightly stable under heat treatment at 63°C for 60 min. Their denaturation increased significantly as the temperature increased from 63 to 98 °C. The fold change in the abundance of proteins identified between untreated CM and heated milk at 63◦C ranged from 15%–61% and for untreated CM and at 98°C from 79%–98% (Benabdelkamel *et al*., 2017). Felfoul *et al.* (2017) reported that heating CM at 80 °C for 60 min induced a complete disappearance of α-la and peptidoglycan recognition protein and a decrease of 42% of SA concentration. Similarly, bovine α-la was not detected and only 26% of β-lg remained in bovine milk after heating at 80 °C for 60 min. However, contradictory results were reported regarding the heat stability of whey proteins in CM when they are isolated from the milk and studied in model systems. CM whey proteins were found to be more resistant to heat denaturation than those in bovine and buffalo milk (Elagamy, 2000). Morevoer, camel α-la had greater stability (in both holo and apo states) and its secondary structure was better preserved

than that of bovine α-la during heat denaturation. This was mainly due to difference in the quantity of hydrophobic interactions involved in their folding (Atri *et al.*, 2010). Laleye *et al.* (2008) showed that there was no significant difference in heat stability between bovine and camel whey proteins in liquid form. However, heat induced aggregation of camel whey proteins was found to increase at pH lower than 5 because of its high content of α-la, leading to the conclusion that camel whey protein is more sensitive to acidity than bovine whey protein (Laleye *et al.*, 2008).

Heat preservation of CM was reported to be successfully done by LTLT and HTST pasteurisation process (Tay and Chua, 2015). Mohamed and El Zubeir (2014) reported that LTLT (63^{o}C for 30 min) and HTST (72^{o}C for 15s) pasteurisation of CM improved its microbial quality and extended its shelf life up to 20 days under refrigeration temperature compared to raw CM (7 days at refrigeration temperature). However, heat treatments were reported to have a significant effect on the composition and properties of CM. Elhasan *et al.* (2017) found that LTLT, HTST, and sterilization treatments caused a decrease of pH, protein and lactose content of full-fat CM with an increase of its acidity, while solid not fat (SNF), fat and density of milk remained stable. Hattem *et al*. (2011) indicated that thermal treatments (LTLT, HTST, 80 and 90°C for 30 min) had a significant impact on protein, total solids, ash content, and distribution of nitrogen in CM. In addtion, the rennet clotting time (RCT) of CM in the presence of different concentrations of calcium chloride (0–20 mg /100 ml) was also found to increase with rise in temperature. In another study, Kamal *et al.* (2017) observed that preheating of CM at 50°C negatively affected its gelation properties, while the preheating at 70°C prevented the formation of rennet-induced gelation of CM. In contrast, no effect was observed on the gelation properties of bovine milk.

2.5.2. *Fermented camel milk*

In pastoral societies, dromedary CM is consumed mostly as fresh or in the form of fermented milk as the only means of preserving CM under warm conditions (Farah *et al*., 2007). Traditionally, CM is allowed to ferment naturally at ambient temperature (26–29^{o}C) without prior heat treatment and without addition of starter cultures for one or two days (Lore *et al*., 2005). The resulting fermented CM has various names in different countries of the world. For instance, in Eastern Africa, Kenya and Somalia it is known as Suusac and characterized with its low viscosity, smoky aroma and an astringent taste (Lore *et al*., 2005; Mwangi *et al*., 2016). Whereas, in Sudan, and Ethiopia is known as Gariss or Dhanaan which is made by a semi-continuous fermentation process where the fermentation is carried out in two leather bags of tanned goat skin embedded in green or wet grass carried on the bag of camels and subjected to continuous shaking by the jerky walk inherent to camels (Abdelgadir *et al*., 2008; Biratu and Seifu, 2016). In the north- and central-Asia particularly, China, Iran, Turkey, Kazakhstan and Turkmenistan it is known as chal or Shubat which is a sparkling white fermented milk product with extremely sour taste (Lü *et al*., 2014; Yam *et al*., 2014). It is prepared by adding water (1:1 ratio) and previously fermented milk (1:3 or 1:5 ratio) to raw CM where spontaneous fermentation takes place in a skin bag or ceramic vessels at ambient temperature (25–30^{o}C) for 8 hours (Brezovečki *et al*., 2015).

However, several drawbacks have been reported to be associated with the production process of fermented CM under pastoral conditions including unpredictable production environment, unknown microbiology in processing, lack of process control and unknown toxicological status (Mwangi *et al*., 2016). Moreover, fermented CM manufactured by the traditional methods often shows a great variation in taste and flavour and is usually of poor hygienic quality that do not meet the acceptable quality requirements (Farah *et al*., 1990; Elmoslih *et al*., 2016). It has also been reported that growth of lactic acid bacteria and yeast species as well as the chemical

compositions, microbial counts and pH of fermented camel milk differ according to preparation methods (Shori, 2012a).

In order to improve the spontaneous traditional fermentation, mesophilic lactic acid bacteria have been suggested to be used as starter culture in production of fermented CM Gariss (Farah et al., 1990). The resulting fermented CM product has a uniform taste and a longer shelf life compared to that produced by traditional spontaneous fermentation. Pasteurisation of CM at 63^{o}C for 30 min prior to fermentation process was reported to enhance the microbiological quality of the final Gariss (Hassan *et al*., 2006). Similarly, the microbial quality of fermented CM Dhanaan was also improved by following hygienic processing conditions during its preparation, handling and production in the laboratory compared to the traditionally made Dhanaan samples (Biratu and Seifu, 2016).

2.5.3. *Yoghurt manufacturing of camel milk*

Manufacturing of yoghurt from dromedary CM is reported to be difficult. According to Attia *et al.* (2001), the starter culture in CM showed a longer lag phase and an earlier decline phase than in bovine milk, resulting in a fragile and heterogeneous coagulum that consists of dispersed flakes. This was attributed to the natural presence of antibacterial factors such as lysozymes, lactoferrin immunoglobulin in CM that retard microbial starter activities, thus hindering acidification and curd formation (Attia *et al*., 2001). The concentrations of these antibacterial proteins in CM are significantly higher and they are more heat stable compared with their counterparts in bovine and buffalo milk. Moreover, CM viscosity is reported to remain unchanged during the gelation process of yogurt compared to bovine, ovine, and caprine milk mainly due to differences in chemical composition of milks, particularly total solids and protein content (Jumah *et al*., 2001). Dromedary CM has lower total solid content than bovine milk (Yoganandi *et al*., 2014). In addition, camel proteins lack β-lg (Omar *et al*., 2016).

Nevertheless, it has been demonstrated that acidification of CM by using commercial starter cultures is possible although their growth rate is limited by the rate of proteolysis. The acidification rate in CM, however, was found to be lower than in bovine milk (Berhe *et al.*, 2018). Additionally, yoghurt produced solely from CM with no additives was reported to have a thin, flowable and very soft texture (Alhaj and AlKanhal, 2010). Therefore, attempts have been made to increase firmness and prevent syneresis of CM yoghurt through the addition of both sodium alginate (0.75%) and calcium chloride (0.075%) (Hashim *et al*., 2009), corn starch (2%) (Muliro *et al*., 2013), and colloids such as κ-carrageenan (3%), and xanthan gum (3%) (Kavas, 2016). Moreover, thermal treatment of CM at 95°C for 30 min was also reported to have a good significant impact on the physicochemical, texture and sensory properties of resultant strained yogurt (Labneh) (Desouky *et al.*, 2013). Other types of yoghurt have also been manufactured from CM including probiotic yoghurt (Attia *et al.*, 2001), banana frozen yoghurt (Ahmed *et al*., 2010), yoghurt made of mixing CM with bovine milk (50%: 50% v /v) (Ahmadoon, 2012), and yoghurt supplemented with different spices (Shori *et al*., 2013) in order to improve its sensory properties and consumer acceptability.

2.5.4. *Cheese processing of camel milk*

Processing of CM into cheese under traditional conditions used for milk of other livestock is difficult and has even been considered as impossible (Brezovečki *et al.*, 2015; Berhe *et al.*, 2017). Difficulties including long coagulation time, weak curd, and low cheese yield were reported to be associated with the production of cheese from CM (Farah and Bachmann, 1987; Bornaz *et al.*, 2009). The large casein micelle size, low casein content and small amount of κ-CN in CM are found to be responsible for these problems (Barłowska *et al*., 2011).

The rennet clotting time of milk varies based on the micelle size and reaches an optimum in the medium and small size micelles (Bornaz *et al*., 2009). In addition, small micelles contain

higher concentrations of κ-CN (Gouda *et al*., 1984) and form stronger coagulum than the big casein micelles (Glantz *et al*., 2010). Dromedary CM casein contains a great number of large micelles with average diameter of 380 nm, whereas the smallest micelles are found in bovine (150 nm), sheep (180 nm), and goat milk (260 nm) (Barłowska *et al*., 2011). Therefore, CM exhibited longer rennet coagulation time (2 to 3-fold) and less firm coagulum than bovine milk during cheese processing (Farah and Bachmann, 1987). Furthermore, CM showed the lowest cheese yield compared to bovines', goats', and ewes' milk due to its lower dry matter and casein content (Bornaz *et al*., 2009). Attia *et al.* (2000) showed a reverse correlation between the casein concentration and micelle size in CM. In addition, the suitability of CM for cheese making decreases significantly during hot dry seasons as a results of the high reduction in its total solids under water restrict conditions (Khan *et al*., 2004). Lactation stage was also found to have an impact as CM was only able to coagulate and form curd suitable for cheese making after 20 days day post-partum (Konuspayeva *et al*., 2014).

Despite of the previously mentioned difficulties, efforts have been made for cheese production from CM and improve its quality. The coagulation of CM has been improved after using the dromedary camel gastric enzyme extracts as a substitute for the commercial chymosin for cheese making using CM (Haroun *et al*., 2012). CM hydrolysis κ-CN takes place on peptide connection Phe^{97}-Ile^{98} by chymosin action, while in the bovine milk it takes place on Phe^{105}-Met^{106} (Hailu *et al*., 2016b). Camel chymosin has been shown to have a 70% higher clotting activity towards milk and more selectively to cleave κ-CN compared to bovine chymosin (Langholm Jensen *et al*., 2013). The addition of starter culture of lactic acid bacteria and calcium chloride to CM was also reported to reduce clotting time and increase cheese yield (Zubeir and Jabreel, 2008; Khan *et al*., 2004; Ahmed and Zubeir, 2011). Higher yield of cheese from CM with better microbiological quality and organoleptic properties was obtained when mixing CM with bovine milk (1:1) (Siddig *et al*., 2016), buffalo milk (70:30) (Shahein *et al*.,

2014), and sheep milk (50: 50 and 25:75) (Derar and El Zubeir, 2016). Mehaia (2006) reported that production of soft white cheese from CM by using ultrafiltration process resulted in higher cheese yield with better sensory quality compared to conventional processes.

Different varieties of cheeses have been reported to be manufactured from camel milk including semi and hard cheese (Mohamed *et al*., 1990), soft white cheese (Mehaia, 1993; Ama and Iem, 2014), soft unripened cheese (Hailu *et al*., 2014).

2.5.5. *Butter manufacturing of camel milk*

The production of butter from CM by using the conventional churning methods is not as easy as from milk of other animals owing to its unique milk-fat properties (Asresie and Adugna, 2014). The fat in CM is distributed as small globules that are firmly bound to the protein and contain a higher proportion of long chain fatty acids (Abbas *et al*., 2013; El-Zeini, 2006). Butter can be only made from CM by churning fresh or soured CM at 24 to 25°C, which is higher than the churning temperature for bovine milk (8–12°C), due to the high melting point of its fat (41–42°C) (Brezovečki *et al*., 2015). Dromedary CM butter is prominently white with a more buttery and viscous consistency than bovine milk butter. Whereas, its taste and the aroma of are neutral (Berhe *et al*., 2017; Brezovečki *et al*., 2015). Pastoralists in Sahara region, Ethiopia, and north-eastern Kenya produce small amounts of butter from CM by different traditional methods and they usually use it for medicinal purposes or cooking (Mourad and Nour-Eddine, 2006; Bereda *et al*., 2014). More efficient methods of obtaining butter from CM alone or mixed with other animals milk have also been described by Farah *et al*. (1989), Berhe *et al*. (2013), and Asresie et al. (2013).

2.5.6. *Sensory and flavour characteristics of camel milk and its dairy products*

The acceptance and preference of milk and dairy products by consumers is determined by their sensory characteristics (Nursten, 1997). Therefore, sensory evaluation is regarded as a test that

help providing the processors and producers with a guide to consumer acceptance for the products (ul Haq *et al*., 2014). Sensory characteristics of milk from ruminants differ from one species to another (Wolf *et al*., 2013). The sensory attributes of bovine milk and its dairy products have been extensively studied but information on the sensory properties of CM and its products are very limited. In addition, most of the available studies have focused mainly on the sensory attributes and acceptability of dairy products made from CM, while, research on the organoleptic properties of CM subjected to various technological treatments (thermal and non-thermal) is scarce.

Fresh CM was described as having very good colour and slight good odour with fair taste, flavour and overall acceptability (Ahmed *et al*., 2014). Addition of 10% orange syrup and 15% cherry syrups to HTST pasteurised CM was reported to improve its flavour, texture, appearance and overall acceptability (Toloun *et al*., 2013). Rahman *et al.* (2009) studied the sensory properties of fermented CM inoculated with five selected pure starter cultures including *Streptococcus thermophilus 37*, *Lactobacillus delbrueckii sp. bulgricus CH2*, *Lactococcus lactis*, *Lactobacillus acidophilus* and mixed yogurt culture (*S. thermophilus* and *L. bulgaricu* 1:1) at 43ºC for 6 h, using a hedonic scoring scales (9 = excellent; 1 = extremely poor). The results showed that the consistency of all fermented CM samples was watery and showed a fragile, poor structure (poor scores), however, CM fermented by mixed yogurt culture was the most accepted.

CM set yogurt containing 0.75% alginate and 0.075% calcium showed similar hedonic ratings for sensory attributes and acceptability to that of bovine milk yogurt (Hashim *et al*., 2009). Similarly, no differences were observed in sourness, bitterness, and overall preference scores between plain yogurts made from camel and bovine milk. Moreover, the addition of *Allium sativum and Cinnamomum verum* did not affect the organoleptic properties of both yogurts (Shori and Baba, 2012b). Desouky *et al.* (2013) found that strained yogurt (Labneh)

manufactured from CM thermally treated at 95°C for 30 min had higher overall acceptability scores (for flavour, consistency and appearance) than yogurt from CM treated at 63°, and 85°C for 30 min or pasteurised at 72°C for 15 sec.

Fresh CM cheese produced using the Camifloc enzymes as a clotting agent was reported to have a higher mean scores for colour, flavour, taste, body texture, saltiness, and overall acceptability than cheese produced using the Camifloc enzymes and calcium chloride (Zubeir and Jabreel, 2008). In another study, soft unripened cheese made from CM by using camel chymosin was found to have significantly higher sensory score values for colour, appearance, taste, texture and overall acceptability than cheese made using ginger crude extract as a coagulant (Hailu *et al*., 2014). White cheese produced form CM and mixture of camel and bovine milk (1:1) using 5% starter culture had more acceptability than cheese prepared by direct acidification process (addition of 10% citric acid) (Siddig *et al*., 2016).

Recently, ice cream made from CM showed to have acceptable sensory properties either with or without addition of different kinds of dates (Salem *et al*., 2017). Morevoer, Ahmed and El Zubeir (2015) indicated that the addition of vanilla and coconut flavours have increased the overall acceptability for the taste of CM ice cream.

2.5.7. *Characterisation methods of CM proteins*

Proteins are one of the primary components of milk which have major impact on its nutritional value and technological suitability (Gizachew *et al*., 2014). Based on previous discussion, the technological processing applied to CM including pasteurisation, sterilisation, fermentation, as well as, yogurt and cheese making were found to be largely influenced by the properties and composition of its proteins. Additionally, CM proteins were reported to be responsible for several health properties of the milk such as anticancer, hypoallergenic and anti-diabetic

properties (Hailu *et al*., 2016b). Therefore, the interest in characterisation and quantification of CM proteins has increased during the last two decades.

The separation and quantification of CM proteins has been traditionally performed by using polyacrylamide gel electrophoresis (Native, SDS, and Urea–PAGE) and chromatographic techniques, which supply information on molecular mass and purity. Ereifej *et al.* (2011) described protein composition for raw CM collected from eight Jordanian locations using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). Camel whey proteins LF, SA and α-la were found to have molecular masses of 80, 66, and 10.9 kDa, respectively. While, casein fractions with molecular masses of 23.2, 24.5 and 19.9 kDa were corresponding to β-CN, $\alpha_{s1,2}$-CN and κ-CN, respectively. However, several bands in the polyacrylamide gel of CM from different locations were not identified. Elhaj and Freigoun (2015) reported that the fractionation of CM proteins during the first week of lactation by using the SDS–PAGE electrophoresis was very difficult. However, during the period from over one week up to 48 weeks of lactation, α-la was the main whey protein and β-lg was not detected in camel whey. β-CN and α-CN were the major components of camel casein, while κ-CN was detected in very low concentration or absent. Recently, Yelubaeva *et al.* (2017) identified casein proteins of CM by suing the SDS–PAGE electrophoresis in the presence of 0.1% SDS-Na. The proportion of individual fractions of casein proteins in CM was α_s-CN 31.5%, β-CN 64.5%, κ-CN 4%, with molecular weights of 25, 23.8 and 22.4 kDa, respectively. Whereas, it was 40.12%, 24.28%, and 27.93%, with molecular weights of 25.3, 22 and 20.5 kDa in bovine milk caseins in the same order. Two-dimensional electrophoresis (2-DE) followed by MALDI-TOF mass spectrometry (MALDI–TOF MS) was also used to identify principal proteins in CM in comparison with milk from other species. CM was found to be devoid of β-lg, whereas it was the major whey protein in bovine, buffalo, caprine, and equine milk. Moreover, the

migration of α_s-, β-, and κ-CN in the casein of CM was the slowest comparing with casein of other milks (Hinz *et al.*, 2012).

In another study by Saliha *et al.* (2013), major casein and whey proteins in raw Algerian dromedary's milk were separated using DEAE-cellulose ion exchange chromatography and Sephacryl S200 permeation gel chromatography respectively, and then identified by different polyacrylamide gel electrophoresis (Native, SDS, and Urea–PAGE). The results showed that β-CN and $\alpha_{s1,2}$-CN were the only identified proteins in camel casein. In addition, whey proteins were separated into two fractions, namely SA and α-la with molecular masses of 66.0 kDa and 14.0 kDa, respectively (Saliha *et al.*, 2013). Whey proteins from camel colostrum and milk were separated by cation-exchange fast protein liquid chromatography (FPLC) and identified by the SDS–PAGE electrophoresis. The main proteins found in colostrum were IgG1 and enzyme inhibitory antibodies IgG2 and IgG3, while SA was the major whey protein present in CM. β-lg was not detected neither in the colostrum nor in the CM (El-Hatmi *et al.*, 2007; Merin *et al.*, 2001). Niaz *et al.* (2017) used the same techniques (FPLC and SDS–PAGE) for isolation and characterisation of LF from raw CM. The results revealed that the maximum amount of the LF recovered from the CM was 2.3 mg/mL, with a molecular weight of 76 kDa. The migration of CM LF on the SDS–PAGE was slower than the bovine and buffalo milk Lf.

Heat treatment is essential for hygienic safety and for extension of shelf-life of milk and its products.Thus stability of CM proteins, either in milk or in separated purified form, against heat denaturation was studied using several methods including SDS–PAGE electrophoresis and differential scanning calorimetry (DSC) (Elagamy, 2000; Laleye *et al.*, 2008; Felfoul *et al.*, 2015), fluorescence spectroscopy and circular dichroism (CD) (Atri *et al.*, 2010), 2-DE and MALDI–TOF MS (Benabdelkamel *et al.*, 2017), and liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) (Felfoul *et al.*, 2017). Although these techniques are considered as the most commonly used for the separation of CM proteins, however, they are

time consuming, costly, consuming large amount of solvents, require sophisticated analytical equipment, and skilled operators (Hristov and Radoslavov, 2015). Therefore, there is still a need for a rapid method for routine assessment of technological process effects on CM, which enables simultaneous identification and quantification of its major proteins in their native and denatured status.

In conclusion, CM and its products have an important role in the diet of the population in the arid and semi-arid areas of Africa, Asia and the Middle East, which have scarce agricultural areas, high temperatures and small amount of rain fall. The major differences in composition between camel and bovine milk could lead to the milk behaving differently during processing and thus, could affect the final quality of camel's milk dairy products. However, most of the available studies in the literature have mainly focused on the compositional, characteristics and medicinal properties of CM. Hence, there is limited information concerning technological properties of CM. Therefore, the aim of the current research was to study the effect of varous industrial treatments including: HTST, UHT and HP treatments on the physical, chemical and the organoleptic properties of CM in comparison to bovine milk.

Chapter 3: Quantification of major camel milk proteins by capillary electrophoresis

Preface to chapter 3

From the literature review, it is clear that the main reason for the differences observed between camel and bovine milk regarding technological properties such as thermal stability, coagulation time, curd strength and cheese yield, is due to the unique composition of CM proteins. In addition, CM proteins are of interest for applications in infant foods, for food preservation and in functional foods. In this chapter, a simple method for rapid analysis of the major whey and casein proteins in CM was developed using capillary electrophoresis for the first time, in order to monitor their behaviour when CM is subjected to various processing methods.

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Abstract

Proteins from dromedary camel milk (CM), in Europe were separated and quantified by capillary electrophoresis (CE). CE analysis showed that CM lacks β-lactoglobulin and consists of high concentration of α-lactalbumin (2.01 ± 0.02 mg mL^{-1}), lactoferrin (1.74 ± 0.06 mg mL^{-1}) and serum albumin (0.46 ± 0.01 mg mL^{-1}). Among caseins, the concentration of β-casein (12.78 ± 0.92 mg mL^{-1}) was found the highest followed by α-casein (2.89 ± 0.29 mg mL^{-1}) while κ-casein represented only minor amount (1.67 ± 0.01 mg mL^{-1}). These results were in agreement with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) patterns.

Overall, CE offers a quick and reliable method for the determination of major CM proteins, which may be responsible for the many nutritional and health properties of CM.

3.1. Introduction

Camel milk (CM) is becoming more popular in many countries in Asia, Africa and Europe due to its claimed therapeutic properties such as anti-cancer, hypo-allergic and anti-diabetic (El-Agamy, Nawar, Shamsia, Awad, & Haenlein, 2009). As the result, there is an increase in the production of CM on large commercial scale from modern camel farms (Alhaj *et al*., 2013).

The interest in characterization of CM proteins and studying their composition has increased during the last decades (Kappeler, Farah, & Puhan, 1998). Therefore, several analytical techniques have been used for the separation and quantification of individual CM proteins. Major whey and casein protein fractions in CM were successfully separated by different electrophoretic techniques such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE), native–PAGE and urea–PAGE (Ereifej, Alu'datt, AlKhalidy, Alli, & Rababah, 2011; Saliha, Dalila, Chahra, Saliha, & Abderrahmane, 2013). Two-dimensional gel electrophoresis technique was also used to characterise CM proteins (Hinz, O'Connor, Huppertz, Ross, & Kelly, 2012). Chromatographic techniques in their different modes such as a reversed phase-high performance liquid chromatography (RP–HPLC), ion-exchange and gel chromatography have also been applied for the separation and quantification of CM proteins (Conesa et al., 2008; Kappeler, Farah, & Puhan, 1998). More recently, RP-HPLC was used to identify and follow the enzymatic degradation of camel whey proteins (Salami *et al*., 2010).

Capillary electrophoresis technique (CE) has well-known advantages (speed, excellent resolution, simplicity and low operation costs) in milk proteins analysis compared to the traditional electrophoretic and chromatographic methods (Gutierrez, & Jakobovits, 2003; Kinghorn, Norris, Paterson, & Otter, 1995). The advent of CE has resulted in the development of simple, rapid and automated technique with excellent separation of individual milk proteins

based on their charge to mass ratio, allowing their identification and quantification (Recio, Amigo, & López-Fandiño, 1997). The major whey and casein proteins in human, bovine, goat and ewe milk were successfully characterized and quantified by the CE technique (Cattaneo, Nigro, Toppino, & Denti, 1996; De Jong, Visser, & Olieman, 1993; Manso, Miguel, & López-Fandiño, 2007). In addition, CE methods have been used for several commercial applications in the control of the quality of dairy products including: assessment of technological process effects on milk proteins, detection of adulteration of dairy products, proteolysis in milk and cheese, and the analysis of the genetic polymorphism of milk from different species (Frazier, 2001).

Therefore, the aim of the current study was to develop a rapid and simple capillary electrophoresis method, enabling the simultaneous separation and quantification of CM proteins. The CE patterns were compared with traditional polyacrylamide gel electrophoresis and the suitability and reliability of using CE technique for the determination of major CM proteins was discussed.

3.2. Material and methods

3.2.1. Materials

3.2.1.1. Chemicals & reagents

Protein standards (from bovine milk) β-lactoglobulin (β-lg) (purity ≥90%), serum albumin (SA) (≥ 98%), α-lactalbumin (α-la) (≥85%), lactoferrin (LF) (≥85%), β-casein (β-CN) (≥98%), α-casein (α-CN) (≥ 70) and κ-casein (κ-CN) (≥70%) were obtained from Sigma-Aldrich (Poole, Dorset, UK). Dialysis sucks were also obtained from Sigma-Aldrich. Sodium dihydrogen orthophosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) was obtained from BDH Laboratory supplies (Poole, Dorset, UK). All chemicals were HPLC grade and used without any further purification.

3.2.1.2. Milk samples

In this study 20 litres (40 bottles of 500 mL size) of dromedary camel raw milk produced by Kamelenmelkerij Smits (Cromvoirt, The Netherlands), were supplied by UK Camel Milk Ltd (Bolton, Lancashire, UK). Samples were kept at -18^{o}C for subsequent analysis. For each experiment 6 bottles were analysed in triplicate and the results were expressed as mean values ± standard deviation. For comparison, raw bovine milk obtained from a local retailer was used.

3.2.2. Methods

3.2.2.1. Chemical composition analysis of raw whole camel milk

Chemical composition of raw whole CM including percentage of fat, total protein and lactose was determined by LactoScope Filter Auto (QuadraChem Laboratories Ltd, Forest Row, UK). The device was calibrated for whole milk analysis and samples (100 mL) were homogenised before being introduced to the machine for analysis. In addition, the content of ash, fat and proteins in CM were estimated by traditional methods (ash content , Gerber and Kjeldahl, respectively) according to the British Standard Institute (BSI, 1970, 2008, 2014). All analyses were done in triplicate and results of milk components were expressed as g 100 mL^{-1}.

3.2.2.2. Preparation of whey and casein proteins

Following the procedure of Saliha, Dalila, Chahra, Saliha, and Abderrahmane (2013), samples of whole CM were defatted by centrifugation (SIGMA, Laborzentrifugen, 3K10, Newtown Shropshire, UK) at 4000 x *g*, 4°C for 15 min. Casein was obtained from the skimmed CM by precipitation with 1M HCl to pH 4.3 at 22°C.The samples were then centrifuged at 4000 x *g*, 4°C for 15 min. The precipitated casein was washed twice with distilled water and solubilized at pH 7.0 by addition of 1M NaOH. The casein was then freeze–dried (VirTis Bench top 2, 4, 6 K; Leybold Vacuum UK Ltd, Plough Lane, UK) and stored at -18°C until analysis. The

remaining supernatant, containing whey proteins was dialyzed against distilled water, freeze dried and kept at -18°C until analysis. Bovine milk was subjected to the same treatment as CM.

3.2.2.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

Both camel and bovine milk proteins (casein and whey) were separated in terms of their molecular weights using an XCell SurelockTM unit (Invitrogen Ltd, Renfrew, UK) according to the protocol provided by the supplier. Specifically, protein samples were reduced by treatment with NuPAGE LDS buffer and reducing agent (dithiothreitol) at 70°C for 10 min. Electrophoresis was performed on a 1.0 mm 4–12% Bis-Tris pre-casted gel (NuPAGE Novex, Paisley, UK) with NuPAGE MES SDS as running buffer, at constant voltage (200V) for 35 min. 10 µL of the sample was loaded in the gel. Gels were washed three times with purified water, stained with SimplyBlue SafeStain buffer (Life Technologies, Paisley, UK) for 1 h at room temperature, followed by an overnight destain with distilled water to obtain a clear background. Molecular weights of the bands were estimated using Novex Sharp pre-stained protein standard (Invitrogen Ltd, Renfrew, UK).

3.2.2.4. Capillary electrophoresis (CE) analysis of camel caseins and whey proteins

CE analysis was carried out by using HP3D CE with diode array detection and a HP3D Chemstation for instrument control (Agilent, Palo Alto, CA, United States). The separation of camel and bovine milk proteins was performed by using an extended light path capillary of 48.5 cm total length (40 cm to detector) x 50 µm I.D and BF (bubble factor) 3. All samples were filtered through 0.22 µm filter (Merck Millipore Ltd, Tullagreen Carrigtwohill, Co Cork, Ireland) and the sample introduction was achieved by constant pressure (50 mbar) with a 5s injection. During sample analysis, a constant voltage was applied (15 kV) and the temperature was kept at 25°C. For all experiments, 100 mM phosphate ($NaH_2PO_4 \cdot H_2O$) buffer was freshly

prepared, the pH was adjusted to 2.5 with 1M HCl and filtered (0.22 µm) before use for separation. The capillary was rinsed sequentially between electrophoretic runs, with 0.1 M sodium hydroxide for 3 min followed by 3 min with the running buffer. Detection was carried out at 200 nm. The external standard method was used for the quantification of proteins. Standard curves were made with different concentrations (0.01–2.5 mg mL^{-1}) of purified bovine milk proteins (SA, β-lg, α-la, α-CN, β-CN and κ-CN) and analysed in triplicate.

3.3. Results and discussions

3.3.1. Composition of camel milk

The analysis of the composition of raw CM (g 100 mL^{-1}) indicated that the total solid, lactose and ash content in CM samples were 11.10 ± 0.02, 4.06 ± 0.05 and 0.65 ± 0.07 g 100 mL^{-1} respectively. These figures are in accordance with those reported by Mehaia (1996) and Abbas, Ashraf, Nazir, and Sarfraz (2013). The average fat content was 3.39 ± 0.01 g 100 mL^{-1}, similar to the values reported by Guliye, Yagil, and Hovell (2000) and Abdoun, Amin, and Abdelatif (2007). Whereas, higher values of fat matter (5.22 and 4.14 g 100 mL^{-1}) were reported by Konuspayeva, Faye, and Loiseau (2009). The mean value for protein content of CM was 2.65 ± 0.05 g 100 mL^{-1}. Similar mean figures were reported by Abu-Lehia (1987) and Mehaia (1993) however, it was slightly lower than the values (3.11 and 3.07 g 100 mL^{-1}) recorded by Abbas *et al*., (2013). These differences in the mean values of CM composition to some of the previously reported results could be attributed to several factors including geographical locations, feeding conditions, water availability, different breeds, stage of lactation and age (Alhaj & Kanhal, 2010). Moreover, it could be due to differences in measurement methods and analysis procedures.

3.3.2. SDS–PAGE of camel milk whey and casein proteins

CM casein and whey protein fractions were characterized by SDS–PAGE (**Figure 3.1**). The molecular masses of CM proteins were estimated by comparing them with the standard marker proteins with molecular weights between 160 to 3.5 kDa. The electrophoretic pattern of camel casein showed three fractions corresponding to α-CN, β-CN and ϰ-CN similar to bovine caseins and no difference in the mobility was observed. Similar casein fractions were reported by Hinz, O'Connor, Huppertz, Ross, and Kelly (2012). The isolated camel caseins α-CN and β-CN were found to have molecular masses of 30 and 35 kDa, respectively, which is in agreement with the reported observations by Saliha, Dalila, Chahra, Saliha, and Abderrahmane (2013). However, differences in composition of whey proteins between camel and bovine milk were observed. According to the electrophoretic pattern in **Figure 3.1**, camel whey did not contain a band at the expected position of β-lg, whereas bovine whey showed clear band corresponding to β-lg (18 kDa) which is the dominant protein in bovine whey (El-Agamy, Nawar, Shamsia, Awad, & Haenlein, 2009). Other camel whey proteins such as SA and α-la showed identical bands with bovine whey at molecular weight of 66 kDa and 14 kDa, respectively (Salami, *et al*., 2008). LF was only identified in camel whey with a protein band apparent at molecular weight 80 kDa (Ereifej, Alu'datt, AlKhalidy, Alli, & Rababah, 2011). An unidentified band in camel whey was observed at molecular weight around 20 to 23 kDa. This protein band could be attributed to camel light chains immunoglobulins proteins (IgG) (Farah, 1993).

3.3.3. Capillary electrophoresis (CE) analysis of camel caseins and whey proteins

The identification of isolated proteins from skimmed CM sample was carried out based on the observation of migration time of bovine milk protein standards. Camel whey showed different separation patterns to bovine whey (**Figure 3.2 A and B**).

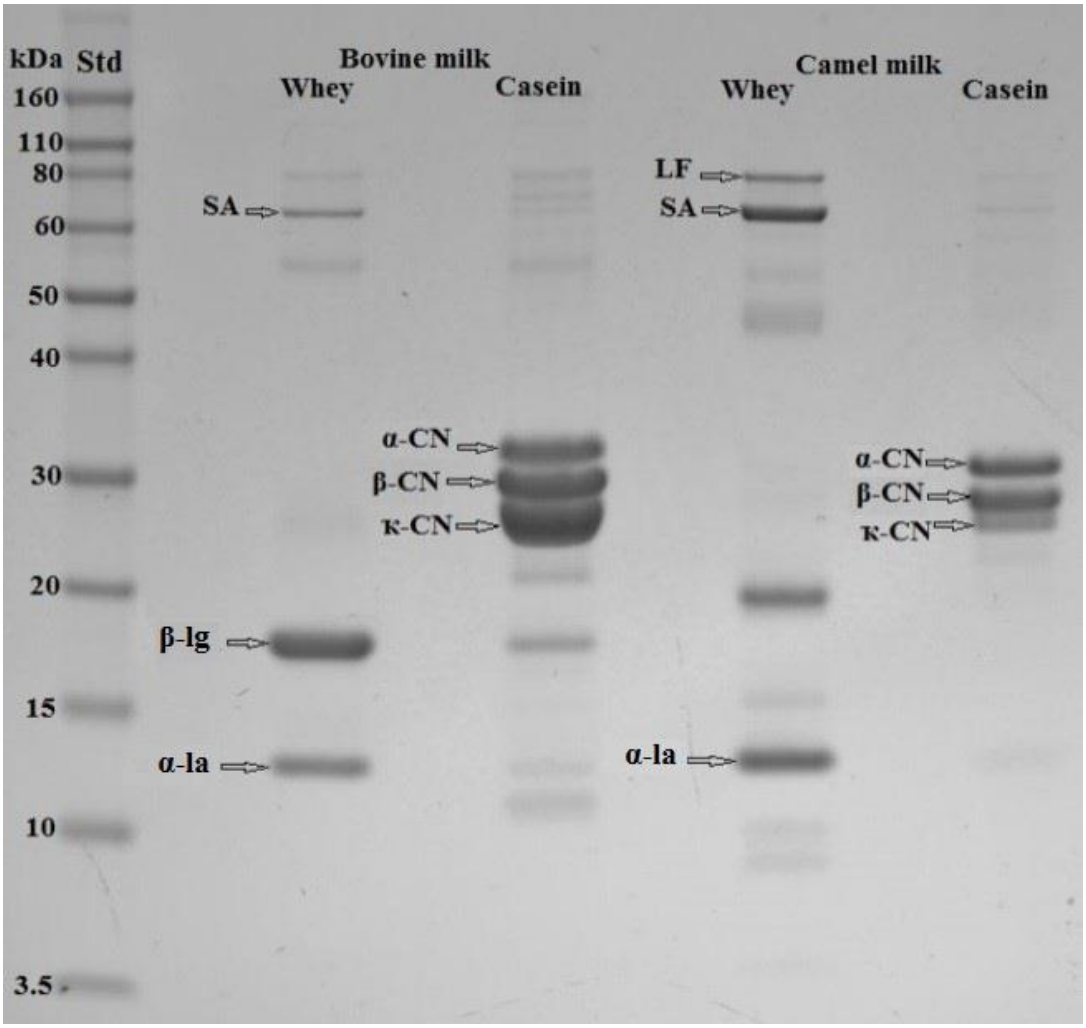

Figure 3 1. SDS–PAGE electrophoretogram of bovine and camel milk casein and whey proteins. Std: Standard Protein Marker. SA: serum albumin, α-la: α-lactalbumin, β-lg: β-lactoglobulin, LF: lactoferrin, β-CN: β-casein, α-CN: α-casein and κ-CN: κ-casein.

Three peaks corresponding to α-la, LF and SA were identified in camel whey while as expected, no peak corresponding to β-lg was detected. In contrast, the elution profile of bovine whey showed that β-lg was the main whey protein followed by α-la and BSA. CE electropherograms of camel and bovine casein proteins are shown in **Figure3.3 A** and **B**. Camel caseins showed similar protein composition to the bovine milk with major peaks identified as α-CN, κ-CN and β-CN. The characterized proteins in camel milk samples by CE were comparable to the results of the traditional SDS–PAGE technique.

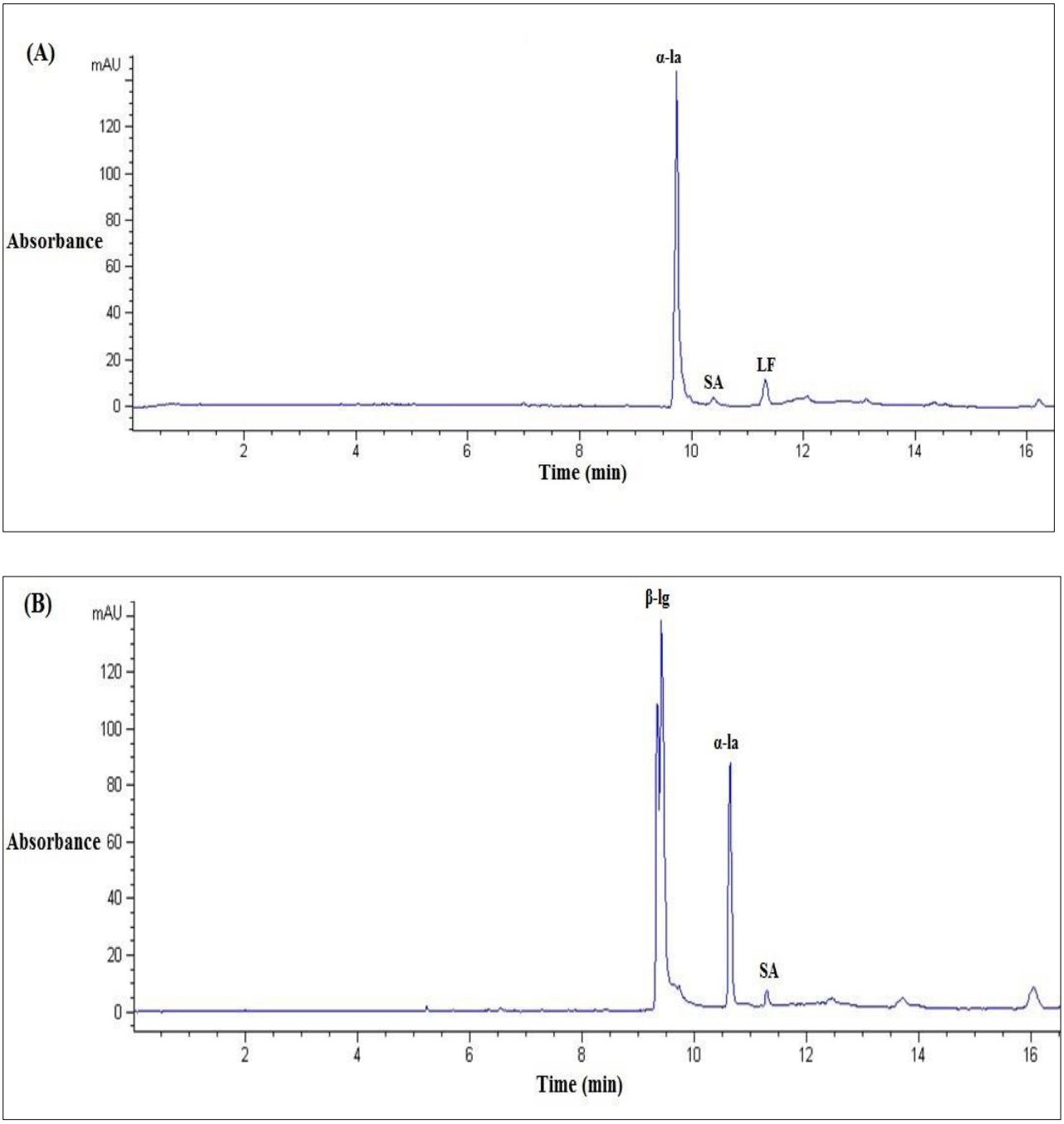

Figure 3 2. Electropherograms of camel (A) and bovine (B) whey proteins. SA: serum albumin, α-la: α-lactalbumin, β-lg: β-lactoglobulin and LF: lactoferrin.

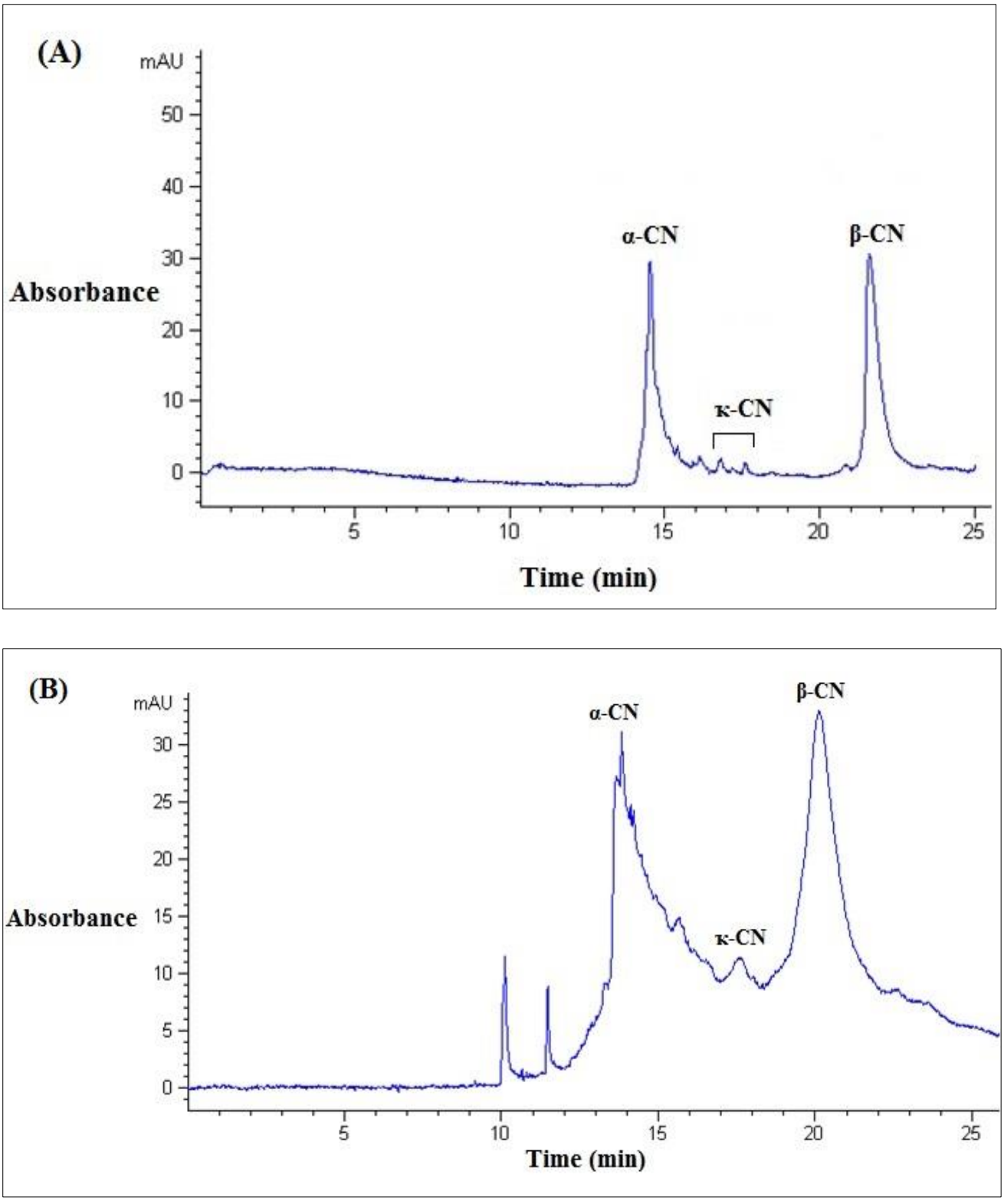

Figure 3 3. Electropherograms of camel (A) and bovine (B) caseins. β-CN: β-casein, α-CN: α-casein and κ-CN: κ-casein.

3.3.4. Quantification of major camel milk proteins by CE

The major casein and whey proteins in bovine and CM were quantified by CE (**Table 3.1**). Standard curves at various concentrations were made by using purified commercial bovine milk protein standards. The obtained peak areas from the CE electropherograms *versus* different protein concentrations showed linear correlation between protein concentration and resulting peak area. The estimated correlation coefficient (R^2) for protein standard curves was 0.999. CE results showed that α-la was the main whey protein in CM and represents 2.01 ± 0.02 mg mL^{-1} which was higher than the concentration of α-la in bovine milk (1.08 ± 0.04 mg mL^{-1}), followed by LF 1.74 ± 0.06 mg mL^{-1}. Whereas, in bovine milk β-lg was the main whey protein with concentration of 5.97 ± 0.14 mg mL^{-1}.

Table 3 1. Mean values and standard deviations of major proteins (mg mL^{-1}) in camel and bovine milk

Whey proteins	**Camel**	**Bovine**
β-lg	ND	5.97 ± 0.14
α-la	2.01 ± 0.02	1.08 ± 0.04
SA	0.40 ± 0.01	0.36 ± 0.04
LF	1.74 ± 0.06	ND
Caseins	**Camel**	**Bovine**
α-CN	2.89 ± 0.29	12.79 ± 2.31
β-CN	12.78 ± 0.92	11.66 ± 0.87
κ-CN	1.67 ± 0.01	4.39 ± 0.31

ND = not detected

Data are the means (samples n = 6) and standard deviation (SD) of three independent experiments.

The content of SA in CM was 0.40 ± 0.01 mg mL^{-1} of total whey proteins, which was higher than that of SA in bovine milk (0.36 ± 0.04 mg mL^{-1}). On the other hand, the main camel casein protein was β-CN with a concentration of 12.78 ± 0.92 mg mL^{-1} followed by α-CN 2.89 ± 0.29 mg mL^{-1} while κ-CN represented only 1.67 ± 0.01 mg mL^{-1}. Bovine milk had a higher amount of α-CN (12.79 ± 2.31 mg mL^{-1}) and κ-CN (4.39 ± 0.31 mg mL^{-1}), and lower content of β-CN (11.66 ± 0.87 mg mL^{-1}) than CM. These results were in agreement with previously reported data in the literature (Alhaj & Kanhal, 2010; Ereifej, Alu'datt, AlKhalidy, Alli, & Rababah, 2011).

3.4. Conclusion

CE was successful in identifying and quantifying the major whey and casein proteins in CM. The CE method has several advantages compared to the traditional SDS–PAGE method including the simplicity, excellent resolution, and low operative costs. It offers a quick and reliable method for routine determination of CM protein composition and it can be also used for the evaluation of the quality of dairy products made from CM.

According to the obtained CE results in the current study, it can be concluded that the protein composition of camel and bovine milk differs with regards to both, casein and whey proteins. Major variations in quantitative and structural aspects of whey and casein proteins were found between the two species. The protein profile of CM showed that CM contains higher amount of β-CN and lower amount of α-CN and κ-CN than bovine milk. Moreover, CM is devoid of β-lg which is the main whey protein in bovine milk and instead it contains high amount of α-la and LF. Therefore, CM proteins might behave differently to bovine milk during various manufacturing processes due to these variations.

Chapter 4: Effects of industrial processing methods on camel skimmed milk properties

Preface to chapter 4

Since the early 1980s, interest in research concerning both the physicochemical and technological characteristics of CM has grown. However, such studies are still scarce and isolated with little impact on improving of CM processing. This chapter covers the study on effect of HTST pasteurisation (72.5 ºC for 15s), UHT treatment (144 ºC for 5s) and high-pressure processing (200, 400, 600 and 800 MPa) at 20 °C for 30 min on some selected processing related parameters of skimmed CM including: colour, casein micelle sizes, whey proteins denaturation and rennet coagulation time (RCT) of milk.

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Omar, A., Harbourne, N., & Oruna-Concha, M. J. (2018). Effects of industrial processing methods on camel skimmed milk properties. *International Dairy Journal*, **84**, 15–22.

Abstract

The aim of this research was to investigate the effect of pasteurisation (high-temperature-short-time; HTST), ultra-high-temperature (UHT), and high-pressure (HP) treatments on some of the physical and chemical properties of camel milk (CM), including whey protein denaturation, colour change, casein micelle size, and rennet coagulation time (RCT). UHT treatment caused the biggest colour change and highest whey proteins denaturation in the CM. In contrast, HP treatments considerably reduced the denaturation of whey proteins and colour change in CM compared to UHT process. Casein micelle size decreased after all treatments. The RCT of CM significantly delayed and coagulum strength (G') decreased after HTST. HP treatment at 200 and 400 MPa increased the RCT of CM and G' value was the highest after treatment at 200 MPa. Processing at 600 and 800 MPa inhibited coagulation of CM. Both thermal and non-thermal treatments affected many constituents and properties of CM differently from bovine milk.

4.1. Introduction

Camels (*Camelus dromedarius*) have traditionally been the primary source of milk in many countries in Africa and Asia particularly during dry seasons because camels are well adapted to harsh conditions in arid and semi-arid regions. Camel milk (CM) is mainly consumed in its raw state or as a fermented milk with varying degrees of sourness (Alhaj and AlKanhal, 2010).

Heat treatments, such as high-temperature, short-time pasteurisation (HTST) and ultra-high-temperature (UHT) are typically applied to milk to ensure better microbiological quality and increase its shelf life for human consumption. It is well known that these treatments influence the physical and chemical properties of bovine milk (McSweeney and Fox, 2013). Recently, there has been an increase in consumer demand for low-fat dairy products, including skimmed milk. In addition, CM is becoming more popular due to its potential beneficial effects on human health such as anti-cancer, hypo-allergenic and anti-diabetic effects (Kaskous, 2016). It is also has lower cholesterol, lower sugar, higher minerals (sodium, potassium, iron, copper, zinc and magnesium) and vitamin C than bovine milk (Jilo & Tegegne, 2016). Therefore, investigating the effects of heat treatments on skimmed CM is of a great technological importance, as thermal treatment is an important step involved in the processing of milk and milk products. Nevertheless, very few studies have focused on the influence of heat treatments on CM and the results from published studies are contradictory and mostly in relation to whey proteins. Farah and Atkins, (1992) and Sagar, Mehta, Wadhwani, Darji, & Aparnathi (2016) reported that skimmed and whole CM had poor heat stability at high temperatures (100–140^{o}C) compared to bovine and buffalo milk. Similarly, Alhaj, Metwalli, & Ismail (2011) showed that heat treatment (121°C for 15 min) of whole CM at its natural pH resulted in partial or complete protein precipitation indicating poor heat-stability, however they demonstrated that the heat stability could be improved by increasing the milk pH to 7.0–7.2 and addition of κ-casein, EDTA or sodium phosphate. Furthermore, CM whey proteins were also reported to be more

sensitive to heat treatments with denaturation rates faster than those of bovine milk (Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015a). Benabdelkamel *et al*. (2017) indicated that heat treatment of CM whey at 98ºC for 60 min caused a significant denaturation of camel α-lactalbumin (α-la), lactoferrin (LF), and serum albumin (SA). Similarly, Felfoul, Jardin, Gaucheron, Attia, & Ayadi (2017) found that whey proteins in skimmed camel and bovine milk were significantly affected by heat treatment at 80°C for 60 min, whereas, casein fractions were kept intact under the same heat conditions for both types of milk. In contrast, several studies reported that CM whey proteins were more heat stable than bovine whey proteins. Elagamy (2000) reported that camel whey proteins were considerably more heat resistant than their counterparts in bovine milk after pasteurisation at 65, 75, 85, and 100°C for 10, 20, and 30 min. Furthermore, camel α-la was found to be more heat stable than bovine α-la during pasteurisation due to the secondary structure of camel α-la being conserved better than that of bovine α-la during heat denaturation (Atri et al., 2010). Laleye, Jobe, &Wasesa (2008) reported that there was no significant difference in the heat stability of liquid whey separated from camel or bovine milk during pasteurisation at 60, 70, 80, 90, and 100°C. Preliminary work on dried whey in the same study suggests that camel whey proteins were slightly more sensitive to heat denaturation than bovine whey. Factors including, stage of lactation, camel breeds, feeding conditions and geographical location might be responsible for the conflict of the reported results regarding the heat stability of whey proteins in CM (Alhaj and AlKanhal, 2010). Levieux, Levieux, El-Hatmi, and Rigaudie (2006) found that whey proteins in early CM (the first week lactation) were more sensitive to heat treatment than those in CM after three months. This difference in the heat denaturation was attributed to the high content of IgG 12.6 mg mL^{-1} in early CM compared to 0.5 mg mL^{-1} in milk from camels during the later stages of lactation.

High-pressure (HP) processing is an alternative preservation method to traditional heat treatments. Previous research has shown that HP processing can cause changes in milk

including upsetting the mineral balance of the milk, denaturing whey proteins, inducing aggregation or disruption of casein micelles, changing the activity of native milk enzymes, changing the colour of the milk and altering the rennet coagulation properties (Huppertz, Smiddy, Upadhyay, & Kelly, 2006; López-Fandiño, 2006; Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002). The majority of studies focusing on the effect of HP on milk have been conducted using bovine, buffalo, ewe, or ovine milk (Gervilla, Ferragut, & Guamis, 2001; Huppertz *et al*., 2005, Moatsou *et al*., 2008a, Moatsou *et al*., 2008b). However, the effects of HP on the physicochemical and functional properties of CM have not been studied to date. Therefore, the aim of this study was to investigate the effects of commonly used food-processing methods (HTST, UHT, and HP) on some components and properties of skimmed CM, including whey protein denaturation, casein micelle size, appearance, and rennet coagulation properties. In addition, the obtained results were compared to bovine milk.

4.2. Materials and Methods

4.2.1. Chemicals and reagents

Pure camel chymosin (FAR-M®) available in powder form (CAS: 9001-98-3) suitable for both camel and bovine milk was obtained from Chr. Hansen Laboratories A/S (Copenhagen, Denmark). Sodium dihydrogen orthophosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) was obtained from BDH Laboratory supplies (Poole, Dorset, UK). Protein standards (from bovine milk) β-lactoglobulin (β-lg) (purity ≥90%), serum albumin (BSA) (≥98%), α-la (≥85%), and lactoferrin (LF) (≥85%) were obtained from Sigma-Aldrich (Poole, Dorset, UK). Propanediol oil was obtained from Sigma-Aldrich. All chemicals were HPLC grade (Sigma-Aldrich) and used without any further purification.

4.2.2. Milk samples

Forty litres (80 bottles, 500 mL in size) of commercially available raw camel (*Dromedary camel*) milk produced by Kamelenmelkerij Smits (Cromvoirt, The Netherlands) were purchased from the UK Camel Milk Ltd (Bolton, Lancashire, UK) in January (winter season). The CM was frozen and directly transported using ice boxes. For comparison, raw bovine milk of Holstein Friesian dairy cows was obtained from the University of Reading's farm. Upon arrival, the frozen milk samples were kept at -18°C until further treatment. Prior to processing, milk samples were defrosted at 4°C overnight (13h) and then kept at room temperature (23°C) for 30 min and gently mixed. The milk samples were then skimmed and directly subjected to industrial treatments. Each treatment was conducted once, and large batch of processed milk was obtained. The processed milk samples were taken for analysis directly after each treatment. All the analyses were conducted in triplicate, and the results were expressed as mean values ± standard deviation.

4.2.3. High-Temperature, Short-Time Pasteurisation

Both camel and bovine milk were pasteurised using an APV HXP pasteuriser (APV UK Limited, Crawley, West Sussex, UK). The holding section of the pasteuriser consisted of a plate-and-frame heat exchanger system. The pasteuriser unit was sterilised by circulating water at 85°C through the entire system prior to the treatment. The milk was then pasteurised at 72.5°C and held for 15s in a holding section. The pasteurised milk was cooled to 4°C and collected in 500 mL sterile bottles (Ascott Ltd. Newton Abbot, UK).

4.2.4. Ultra-High-Temperature Processing

A tubular UHT plant (U.H.T.A.C, Fareins, France) was used for the indirect UHT treatment of camel and bovine milk. Heating was obtained in two stages using two hot oil baths. The unit was sterilised by circulating pressurized hot water prior to the treatment. The temperature of

the milk samples was raised from 4 to 90°C in a preheating unit (oil bath 1). The temperature was raised from 90 to 144°C (oil bath 2), and the milk was held for 5s at this temperature. The processed milk was cooled to 4°C and collected in 500 mL sterile bottles.

4.2.5. High-Pressure Treatment

High-pressure treatment of camel and bovine milk was performed as described by Huppertz, Fox, and Kelly (2004a) Camel and bovine milk samples (50 mL) were vacuum-packed in polyethylene bags and HP-treated using a Stansted Iso-Lab 900 High Pressure Food Processor (Stansted Fluid Power, Stansted, Essex, UK), at pressures of 200, 400, 600, and 800 MPa for 30 min. The temperature of the HP unit vessel was maintained at 20°C. A mixture of water and 1, 2-Propanediol oil (70: 30) was used as the pressurizing fluid.

4.2.6. Proximate composition analysis

The chemical composition of raw skimmed and processed skimmed camel and bovine milk including the percentage of fat, total protein and lactose was determined using a LactoScope Filter Auto (QuadraChem Laboratories Ltd, Forest Row, UK). The machine was calibrated for skimmed milk analysis, and the samples (100 mL) were homogenised prior to analysis. The analyses were conducted in triplicate, and the results are expressed as g 100 mL^{-1}.

4.2.7. Determination of whey proteins denaturation

Denaturation of whey proteins in camel and bovine milk samples was estimated by determining the level of residual native whey protein fractions: SA, α-la, β-lg, and LF in milk by capillary electrophoresis (CE) (Agilent, Palo Alto, CA, United States) following the method described by Omar, Harbourne & Oruna-Concha (2016). Briefly, the pH of the milk samples was adjusted to pH 4.3 by adding 1M HCl. Then the samples were centrifuged at 4000 x *g*, 4°C for 15 min to separate the whey proteins from the precipitated casein. The supernatant, containing whey

proteins was dialyzed (Dialysis sacks Avg. flat width 25 mm (1.0 in.), MWCO 12,000 Da, Sigma-Aldrich) against distilled water and kept at -18°C until analysis. Purified bovine milk proteins (BSA, β-lg, α-la, LF) at concentrations between 0.01-2.5mg mL^{-1} were used to identify and quantify the proteins present in the milk samples. The degree of protein denaturation was expressed as the percentage of protein not detected compared to the untreated milk sample, which is stated to have a native protein percentage of 100% and thereby no denaturation.

4.2.8. Determination of average casein micelle Size

The casein micelle sizes in milk were determined using a Malvern Zetasizer Nano ZS (Malvern instruments Ltd., Malvern, Worcestershire, UK), as described by Chen, Grandison, & Lewis (2011).

4.2.9. Determination of colour parameters

Colour attributes were measured using the Hunter Lab Colour Quest (Hunter Associates Laboratory, Inc. Reston, VA, United States) according to Chugh *et al*. (2014). The colour values were expressed as L^* (lightness), a^* (redness to greenness), and b^* (yellowness to blueness). In order to compare the total colour difference (ΔE) between the colour properties of untreated milk samples and those obtained after subjecting raw skimmed milk to different treatments (HTST, UHT, and HP), we used the following equation:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

Where $\Delta L^* = L_{\text{raw milk}} - L_{\text{treated milk}}$, $\Delta a^* = a_{\text{raw milk}} - a_{\text{treated milk}}$,

and $\Delta b^* = b_{\text{raw milk}} - b_{\text{treated milk}}$.

The whiteness (WI) of the milk samples was determined by converting Hunter Lab to CIE 1931 XYZ colour space values:

$$Y = (L^*/10)^2 \quad (2)$$

$$X = [Y + (L^*/10 \times a^*/17.5)]/1.02 \quad (3)$$

$$Z = [Y - (b^* /7) \times (L^* /10)]/0.847 \quad (4)$$

$$WI = (3.388 \times Z) - (3 \times Y) \quad (5)$$

Colour measurements were conducted in triplicate for each milk sample.

4.2.10. Determination of rennet coagulation time and rheological properties of milk

The rheological assessment of the rennet-induced coagulation of milk was performed with a Bohlin Gemini HRnano rheometer (Malvern Instruments Ltd, Worcestershire, UK) using a cylinder cup (27mm in diameter) and bob (25mm outer diameter) system (Bohlin C-25, Malvern Instruments Ltd). The milk sample (13 mL) was pre-warmed in a water bath at 30°C for 20 min. Then, 0.013 mL of a 0.4% (v/v) liquid solution of rennet enzyme (Chr. Hansen, Copenhagen, Denmark) was added and the mixture was stirred for 1 min before being poured into the cup. The storage modulus, *G'*, was measured at constant temperature of 30°C for 60 min at an applied strain of 1% and a frequency of 0.1 Hz. The time point at which the storage modulus *G'* was ≥1Pa was defined as the gelation time as described by Moynihan *et al*. (2014).

4.2.11. Statistical analysis

Analyses were performed in triplicate and the results were presented as the mean ± standard deviation. The analysis of variance (ANOVA) was used to compare the effects of the different treatments and the Tukey test to determine the differences between them at a 95% confidence level (XLSTAT Version 2015.6.01.24797, Kovach Computing Services, Wales, UK). Principal component analysis (PCA, Pearson n-1; XL Stat) was performed to differentiate between milk samples subjected to different processing methods.

4.3. Results and Discussion

4.3.1. Composition of thermally and high-pressure treated camel and bovine milk

The mean values of protein, lactose, and total solids in the raw skimmed bovine milk were 3.17±0.01, 4.50±0.01, and 7.68±0.01 g100mL^{-1} respectively. These values were in agreement with literature (McSweeney and Fox, 2013). In CM, the protein, lactose, and total solids were lower (2.10±0.01, 3.59±0.01, and 5.86±0.03 g100mL^{-1} respectively) than bovine milk. These compositional variations were consistent with previously reported interspecies differences between the milks of camels and cows (Alhaj and AlKanhal, 2010).

The compositional analysis of the processed CM revealed that the protein, lactose, and solids content after HTST treatment were similar to those of raw milk (2.09±0.01, 3.57±0.01, and 5.86±0.01 g 100 mL^{-1}, respectively). However, a slight variation in the protein content (1.90±0.12 g 100 mL-1) of CM was observed after UHT treatment, which might be due a decrease in soluble proteins. Whereas the lactose and total solids were not affected (3.56±0.01, 5.67±0.04 g 100 mL^{-1}, respectively). High-pressure treatments at 200–800 MPa did not alter the composition of CM.

4.3.2. Whey proteins denaturation of thermally and high-pressure treated camel and bovine milk

The levels of individual whey proteins in processed camel and bovine milk are presented in **Table 4.1**. The major identified whey protein in CM was α-la, followed by LF and SA. The highest level of denaturation in camel whey proteins occurred in the UHT-treated CM sample, which was consistent with the results of its compositional analysis. Among the camel whey proteins, α-la underwent the highest level of denaturation (65.55±0.29%), followed by SA (12.58±0.88%) and LF (3.65±0.54%). In pasteurised CM, the amount of denatured α-la was about 27.13±3.23%, considerably lower than that of UHT-treated CM, and only small denaturation in SA and LF was observed (2.97±0.65 and 1.13±0.51%, respectively). The

results showed that α-la was the most sensitive whey protein to HTST pasteurisation and UHT treatments. Similar findings were reported by Felfoul *et al*. (2017) who found that α-la was the most heat-sensitive whey protein in CM heated at 80°C for 60 min. High-pressure treatment of CM at 200 MPa caused a lower level of denaturation of camel whey proteins compared with UHT treatment. However, increasing the pressure from 400 to 800 MPa resulted in a significant increase in denatured α-la up to 32.50±2.05%, however it was still significantly lower than in the UHT treated samples. Whilst, SA and LF were more resistant to pressure with lower denaturation levels of 3.94±0.07% and 2.93±0.38%, respectively.

In contrast, β-lg was the primary whey protein in bovine milk, followed by α-la and BSA. The levels of heat-induced denaturation of α-la and BSA in bovine milk after HTST (4.23±1.37, and 2.70±0.31% respectively) and UHT (51.06±2.11 and 5.37±1.46% respectively) treatments were considerably lower than those of their counterparts in heat-treated CM. This finding is consistent with that observed by Felfoul *et al*. (2015a) and Sagar *et al*. (2016) who reported that camel α-la and SA were less heat stable and their temperatures of denaturation were lower than their bovine counterparts. Some studies have attributed the high heat sensitivity of camel whey proteins to the absence or deficiency of β-lg and κ-CN proteins in CM (Alhaj *et al*., 2011; Farah & Atkins, 1992; Sagar *et al*., 2016). However, the variation in the thermal stability between the major whey protein in CM α-la and its bovine milk counterpart could be also due to differences in their conformational stabilities and structural features. The primary structure of the intact camel α-la, as bovine α-la, consists of 123 amino acids, but with 39 positional differences compared to bovine α-la (Beg, Bahr-Lindström, Zaidi, & Jörnvall, 1985). Camel α-la contains, 8 cysteine, 5 tryptophan, 4 phenylalanine, 3 methionine and 3 tyrosine, while its bovine counterpart contains 8 cysteine, 4 tryptophan, 4 phenylalanine, 1 methionine, and 4 tyrosine residues (Atri et al., 2010; Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015b). Atri *et al*., (2010) found that the conformation of both camel and bovine α-la was sensitive to calcium

removal. However, camel α-la showed greater change in exposure of buried hydrophobic areas upon calcium depletion than its bovine equivalent. Redington, Breydo, Almehdar, Redwan, and Uversky (2016) reported that purified camel α-la was more stable towards thermal denaturation than its bovine counterpart. However, it was less conformationally stable, aggregated faster and was more disordered than bovine α-la.

Other factors such as pH and calcium concentration could also influence the stability of CM proteins (Levieux, *et al*.,2006). Alhaj *et al*., (2011) reported that heat treatment of CM at high temperature (121ºC) induced precipitation of calcium phosphate, which led to casein micelle dissociation, and increased the calcium ion content with a decrease of milk pH, which lowered the stability of milk proteins. Increasing level of soluble Ca^{2+} may neutralise the net negative charge on unfolded whey proteins which increases their thermal denaturation (Huppertz, Fox, & Kelly, 2004b). Therefore, the higher level of thermal denaturation of whey proteins in CM, compared with bovine milk, might be also due to an increase Ca^{2+} level as result of heat-induced disintegration of camel casein micelles (**Table 4.2**).

Unlike heat treatments, the stability of camel whey proteins was higher than that of their counterparts in bovine milk during HP processing. The level of denatured α-la (32.50±2.05%) after treatment at 800 MPa, was considerably lower than that of bovine milk (55.23±1.66%), buffalo milk (91.8±2.2%) (Huppertz *et al*., 2005) and ovine milk (79.3±3.1%) (Moatsou *et al*., 2008b). HP treatments induced the disintegration of casein micelle in bovine milk through disruption of hydrophobic, electrostatic interactions and solubilization of colloidal calcium phosphate, resulting in an increased level of soluble calcium which may enhances denaturation of whey proteins (Huppertz *et al*., 2004b). Therefore, the lower extent of HP-induced denaturation of α-la in CM compared with bovine milk might be explained by limited effect of HP treatments on casein micelle of CM (**section 4.3.3**). Furthermore, camel SA was more stable (3.94±0.07%) at 800 MPa than BSA (16.17±1.85%), and LF was the most stable among camel

Table 4 1 . Major whey proteins: β-lactoglobulin (β-lg), α-lactalbumin (α-la), serum albumin (SA), and lactoferrin (LF) identified in raw and processed skimmed camel and bovine milk (n = 3) at 72ºC, 15sec (HTST), 140ºC, 5sec (UHT), and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20ºC.

Treatment	Whey proteins content (mg mL^{-1})							
	β-lg		**α-la**		**SA**		**LF**	
	Camel	Bovine	Camel	Bovine	Camel	Bovine	Camel	Bovine
Raw skim milk	-	5.59 ± 0.26^{a}	1.96 ± 0.07^{a}	1.08 ± 0.01^{a}	0.40 ± 0.01^{a}	0.43 ± 0.07^{a}	1.74 ± 0.05^{a}	-
HTST	-	4.18 ± 0.16^{a}	1.43 ± 0.12^{b}	1.04 ± 0.01^{ab}	0.39 ± 0.00^{b}	0.41 ± 0.01^{ab}	1.72 ± 0.00^{ab}	-
UHT	-	0.80 ± 0.01^{c}	0.68 ± 0.03^{c}	0.53 ± 0.02^{d}	0.35 ± 0.01^{c}	0.40 ± 0.01^{b}	1.68 ± 0.00^{d}	-
HP200	-	3.88 ± 0.63^{ab}	1.58 ± 0.14^{b}	1.02 ± 0.00^{b}	0.40 ± 0.00^{ab}	0.36 ± 0.00^{cd}	1.74 ± 0.00^{a}	-
HP400	-	1.98 ± 0.18^{bc}	1.39 ± 0.04^{b}	0.81 ± 0.01^{c}	0.40 ± 0.00^{ab}	0.37 ± 0.00^{c}	1.69 ± 0.01^{c}	-
HP600	-	1.14 ± 0.06^{c}	1.35 ± 0.03^{b}	0.53 ± 0.01^{d}	0.39 ± 0.00^{b}	0.36 ± 0.00^{cd}	1.69 ± 0.00^{c}	-
HP800	-	1.05 ± 0.07^{c}	1.32 ± 0.01^{b}	0.48 ± 0.01^{d}	0.39 ± 0.00^{b}	0.35 ± 0.01^{d}	1.69 ± 0.00^{cd}	-

[a-d] Means within a column with different superscripts were significantly different ($p < 0.05$).
(-) not detected

camel whey proteins over both thermal and pressure treatments with treatments with only a small reduction in its concentration.

4.3.3. Casein micelle size distribution in thermally and high-pressure treated camel and bovine milk

The size distribution of casein micelles in raw skimmed camel and bovine milk was measured and the results indicated that the distribution of casein micelles in raw CM was broader and contained a higher proportion of large particles than in bovine milk. The average diameter of casein micelles in CM was 171.23±4.18 nm; the corresponding value in bovine milk was 143.45±2.96 nm. These results are consistent with data reported by (Farah and Rüegg, 1989). The effects of thermal and pressure treatments on casein micelle size in processed camel and bovine milk are listed in **Table 4.2**. The results revealed that HTST and UHT treatments caused a significant decrease in casein micelles size in CM by 16.39 and 19.55%, respectively, compared with untreated milk.

Micelle size in bovine milk was not significantly affected after HTST, and it increased by 14.00% after UHT treatment. Similar observations with bovine casein micelles have been reported by Freeman and Mangino (1981). This increase in micelle size in bovine milk was mainly due to the heat-induced association of denatured whey proteins, particularly β-lg and α-la with the micelles. Heat treatment of bovine milk at temperature above 80^{o}C induces formation of β-lg/α-la complex through sulfhydryl-disulphide interchange reactions which then associates with the micelle. The ratio of β-lg and α-la associated with the micelle increases with increasing temperature (Oldfield, Singh, Taylor, and Pearce 2000). However, structural differences and variation in proportions of individual caseins between bovine and dromedary milk have been reported (Kappeler, Farah, & Puhan, 1998). It has been established that a high content of β-CN and a low content of κ-CN adversely affect some of the processing characteristics of casein micelles such as stability towards ethanol and heat (Schmidt, 2009).

In CM, β-CN is predominant while κ-CN is present in very small amount compared to bovine milk (Omar *et al*., 2016). Therefore, the significant decrease in the micelle size of heat-treated CM could possibly be due to the dissociation of κ-CN from micelles or the result of precipitation of calcium phosphate out of the casein micelles, which caused them to decrease in size (Anema and Li, 2003).

After HP treatment, casein micelles in CM behaved differently than casein micelles in bovine milk. Treatment of CM at 200 MPa caused a significant ($p < 0.05$) decrease in the size of casein micelles by 21% compared with untreated milk. After increasing the pressure from 400 to 800 MPa, a decrease of 25% in the size of micelle in CM was observed. Treatment of bovine milk at 200 MPa caused a small reduction ($p < 0.05$) in micelle size by 6% compared with untreated milk. However, casein micelles in bovine milk were more susceptible to disintegrate due to increasing pressure (400–800 MPa) during HP treatment than were casein micelles in CM.

Table 4 2. The average diameter of casein micelle size (n = 3) in raw and processed skimmed camel and bovine milk at 72°C, 15sec (HTST), 140°C, 5sec (UHT), and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20°C.

Treatment	Casein micelle size (nm)	
	Camel milk	Bovine milk
Raw skimmed milk	171.23±4.18[a]	143.45±2.96[b]
HTST	143.18±2.34[b]	140.05±2.29[b]
UHT	137.77±1.52[c]	163.60±3.70[a]
HP200	135.22±2.68[c]	134.90±1.52[c]
HP400	128.28±2.75[d]	73.13±0.54[d]
HP600	127.57±1.76[d]	70.96±0.59[d]
HP800	129.40±0.78[d]	71.52±0.81[d]

[a-d] Means within a column with different superscripts were significantly different ($p < 0.05$).

Treatment at pressure 400 to 800 MPa considerably reduced micelle size in bovine milk by 50% compared with controls. Similar observations of bovine casein micelles were reported by Huppertz *et al*. (2004a) and Needs *et al*. (2000). Studies on goat milk by Law *et al*. (1998) found that treatments at 200 MPa and temperatures between 20 and 45°C had little effect on

casein micelle size. Treatment at 300 MPa caused an increase in micelle size due to the formation of insoluble aggregates of denatured β-lg with κ-CN. However, higher pressures (>350 MPa) at 45°C caused a reduction in the size of casein micelles in goat milk. Different observations of buffalo milk have been reported by Huppertz *et al*. (2005) who found that treatment of buffalo milk at 250 MPa for 30min at 20°C reduced micelle size slightly and that treatment at ≥400 up to 800 MPa increased it by 35%. The reduction in casein micelle size in bovine milk is likely to be due to the HP-induced disintegration of casein micelles into smaller particles via the disruption of the intra-micellar van der Waals, hydrophobic and electrostatic interactions and changes in the solubilisation of micellar calcium phosphate (Needs *et al*., 2000, Huppertz *et al*., 2006).

In contrast, the decrease in the size of casein micelles in CM was considerably smaller than in bovine milk after HP treatments, which might be due to the differences in the primary structure of micelles between the two kinds of milk. The CM micelles have spherical shape, as bovine milk micelles, with relatively larger diameters and higher mineral content compared to bovine milk micelles (Hailu *et al*., 2016). Moreover, minerals such as magnesium, inorganic phosphorus and citrate are involved to a more important extent in the formation of the CM micelles, about 2/3, 2/3 and 1/3 respectively, than in the bovine milk micelles (2/5, 3/5 and 1/10 respectively) (Attia, Kherouatou, Nasri, & Khorchani, 2000). Thus, they are more mineralised and contain more saline bridges binding submicelles than bovine milk (Kherouatou, Nasri, & Attia, 2003). Nevertheless, further investigation is necessary in order to explain the reasons behind this phenomenon.

4.3.4. Changes in the colour values of thermally and high-pressure treated camel and bovine milk

The values of the Hunter colour attributes L^*, a^*, b^*, total colour difference ΔE, and WI of processed camel and bovine milk samples compared with untreated skimmed milk are listed in

Table 4.3. The HTST process caused a decrease in L^* ($p < 0.05$) and an increase in a^* (greenness) and b^* (yellowness) ($p < 0.05$) in both camel and bovine milk. The lowest L^* value ($p < 0.05$) was observed in UHT-treated CM, which indicates increased darkness for the highest positive yellowness value (b^*). This reduction in the lightness of CM during the UHT treatment may be the result of disintegration of casein micelles into smaller particles (**Table 4.2**). On the other hand, L^* and b^* were the highest ($p < 0.05$) in UHT-treated bovine milk, which indicates an increase in the lightness and yellowness of the milk. Similar results for bovine milk have been reported by Rufian-Henares, Guerra-Hernandez, and Garcıa-Villanova (2006). These authors found that after UHT treatment bovine milk had higher a^* and b^* values and that there was an increase in the lightness of milk by 11 units compared with the untreated samples. This increase in the lightness of UHT-treated bovine milk may be due to denaturation and association of whey proteins with casein micelles, in particular β-lg (Burton and Rowland, 1955). The values of WI and ΔE in CM were markedly higher than those in bovine milk after UHT treatment.

High-pressure treatment of bovine milk at 200 MPa caused a significant ($p < 0.05$) decrease in L^* with an accompanying increase in ΔE and WI. Increasing the pressure up to 800 MPa resulted in a further decrease in L^* and an increase in ΔE and WI. Devi, Buckow, Singh, Hemar, and Kasapis (2015) reported similar findings on the behaviour of bovine milk colour under HP treatments. This significant reduction in L^* of bovine milk is mainly attributed to the destruction of casein micelles by pressure into smaller particles, which increases the translucence of the milk. In contrast, a small reduction in L^* in HP-treated CM by up to 1.88 units was observed after treatment at 200 MPa. Treatment at higher pressures (≥400 up to 800 MPa) resulted in a further decrease ($p < 0.05$) in L^* in CM by up to 3.35 units. This slight reduction in L^* in CM compared with bovine milk during HP treatments is possibly the result of the limited HP-induced disruption of its casein micelles (**Table 4.2**).

Table 4 3. Changes of colour parameters (n = 3), *L** (lightness), *a** (redness to greenness), *b** (yellowness to blueness), total colour difference (Δ*E*), and whiteness (WI) measured in raw and processed skimmed camel and bovine milk at 72°C, 15sec (HTST), 140°C, 5sec (UHT), and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20°C.

Camel milk					
Treatment	*L**	*a**	*b**	Δ*E*	WI
Raw skim milk	67.77±0.29^{a}	-1.99±0.37^{d}	-0.23±0.19^{b}	0±0.0^{e}	14.47±0.81^{b}
HTST	66.34±0.12^{b}	-1.97±0.11^{d}	-0.22±0.38^{b}	1.48±0.10^{d}	14.13±1.44^{b}
UHT	61.83±0.33^{d}	-1.19±0.12^{a}	0.45±0.43^{a}	6.05±0.34^{a}	10.78±1.57^{c}
HP200	65.89±0.55^{b}	-2.46±0.16^{e}	-1.23±0.44^{c}	2.26±0.35^{c}	17.85±1.75^{a}
HP400	64.01±0.32^{c}	-1.44±0.12^{ab}	-1.13±0.10^{c}	3.91±0.32^{b}	16.93±0.31^{a}
HP600	64.43±0.46^{c}	-1.91±0.04^{cd}	-1.03±0.10^{c}	3.44±0.45^{b}	16.69±0.45^{a}
HP800	64.42±0.14^{c}	-1.63±1.33^{bc}	-1.07±0.14^{c}	3.57±0.13^{b}	16.84±0.56^{a}
Bovine milk					
Treatment	*L**	*a**	*b**	Δ*E*	WI
Raw skim milk	66.81±0.05^{b}	-3.53±0.1^{b}	-0.25±0.08^{b}	0±0.0^{e}	14.33±0.33^{c}
HTST	66.77±0.17^{b}	-3.11±0.17^{ab}	-0.21±0.31^{b}	0.61±0.15^{e}	14.15±1.30^{c}
UHT	68.94±0.22^{a}	-2.98±0.22^{ab}	1.51±0.79^{a}	2.93±0.14^{d}	7.83±3.16^{d}
HP200	59.99±0.46^{c}	-2.53±0.13^{a}	-1.27±1.45^{b}	7.08±0.61^{c}	16.34±4.90^{c}
HP400	49.51±0.42^{d}	-2. 30±0.71^{a}	-9.61±0.46^{c}	19.72±0.43^{b}	37.11±1.39^{a}
HP600	47.13±0.74^{e}	-3.01±0.95^{ab}	-9.85±0.29^{c}	21.92±0.70^{a}	35.97±0.96^{ab}
HP800	45.78±0.87^{f}	-2.67±0.63^{ab}	-8.95±0.29^{c}	22.79±0.73^{a}	32.60±1.31^{b}

$^{a-e}$ Means within a column with different superscripts were significantly different ($p < 0.05$).

Similar observations of buffalo milk have been reported by Huppertz *et al*. (2005) who found that treatments at 250 or 400 MPa reduced L^* of buffalo milk slightly and that treatment at 600 or 800 MPa reduced L^* significantly, by up to 17 units after treatment at 800 MPa. The results revealed that ΔE and WI of CM significantly increased ($p < 0.05$) with increasing pressure (200, 400, 600, and 800 MPa). These parameters attained maximum values of 3.57 and 16.83, respectively, after treatment at 800 MPa. The degree of change of these values in CM was considerably less than that of bovine milk. Furthermore, the HP-treated bovine milk had more yellow and green characteristics than CM after treatments at 400, 600, and 800 MPa. Gervilla *et al*. (2001) reported a decrease in L^* and an increase in greenness and yellowness in ewe's milk when the pressure was incrementally increased to 200, 300, 400, and 500 MPa during HP treatment. These changes were due to HP-induced disruption of casein micelles in ewe's milk.

4.3.5. Rennet coagulation properties of thermally and high-pressure treated camel and bovine milk

The development of rennet-induced coagulum in camel and bovine milk was monitored using dynamic oscillatory rheology. The effect of thermal and HP treatments on the storage modulus G' of camel and bovine milk after renneting for 60 min at 30°C is shown in **Figure 4.1**. The initial pH of the CM samples prior to the addition of rennet (**Table 4.4**) varied between 6.71 and 6.51, consistent with the reported pH values of CM in the literature (Farah, 1993). The final pH values of the gel formed after 60 min incubation were ranged from 6.32–6.64, which was lower than those measured for the curd formed by the bovine milk.

The RCT of the raw CM was much shorter than that of bovine milk. Camel chymosin initiates coagulation of milk by hydrolysing bovine κ-CN at the Phe^{105}–Met^{106} scissile bond and disconnecting the C-terminal part of 106–169 amino acids (Langholm Jensen *et al*., 2013). Whereas, the chymosin cleavage site of camel κ-CN is at the Phe^{97}–Ile^{98} bond and the enzyme cuts off a C-terminal glycol-macropeptide of 65 amino acids residues (Hailu *et al*., 2016b). The

coagulation of CM occurs only after hydrolysis of 95% of camel κ-CN by camel chymosin, while, the gelation of bovine milk starts at level of 60–70% of bovine κ-CN hydrolysis (Hailu *et al*., 2016a). The HTST treatment of CM at 72.5°C for 15s significantly ($p < 0.05$) delayed the RCT of the milk by 70.99% compared with untreated milk (**Table 4.4**) and the final G' value was considerably lower than that of the control (**Figure 4.1A**). The RCT of bovine milk was also significantly delayed after HTST by14.36% and the G' value was decreased but not statically significant compared with untreated milk. Similar results about the effects of heat treatment on the RCT of bovine (Singh and Waungana, 2001), ewe, and goat milk (Calvo and Balcones, 1998) clotted using bovine chymosin have been reported. Kethireddipalli and Hill (2015) noted that heat treatment at temperatures above 75°C led to an increase in the RCT of milk and the formation of weak curds. Vasbinder, Rollema and Kruif (2003) and Blecker et al. (2012) reported that the decrease in the rate of gel development and final G' value in pasteurised milk could be the result of the association of denatured whey protein aggregates with casein micelle surfaces. The formation of whey protein–κ-casein complex affects the reactive sites on the micelles that are formed by the action of rennet, which leads to fewer and weaker bonds and therefore a weaker coagulum (Singh and Waungana, 2001).

As expected, UHT-treated camel and bovine milk failed to coagulate. Similar observations have been found using bovine chymosin in bovine milk by Ham *et al*. (2008). These authors found that UHT treatment hindered the coagulation of milk compared with HTST treatment. This heat-induced inhibition of rennet coagulation is mainly attributed to effects arising during the secondary stage of rennet coagulation or micelle aggregation, the decreased concentration of ionic calcium, and the association of denatured whey proteins with the casein micelles in heat-treated milk (Vasbinder *et al*., 2003).

Table 4 4. Rennet coagulation time (RCT), the final storage modulus *G'* after 60 min at 30ºC, and pH of raw and processed skimmed camel and bovine milk (n = 3) at 72ºC, 15sec (HTST), 140ºC, 5sec (UHT), and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20ºC.

Camel milk				
Treatment	RCT (min)	Final *G'* value (Pa)	Initial pH	Final pH
Raw skim milk	16.16±1.86^{c}	8.85±1.17 ^{b}	6.68±0.03	6.47±0.19
HTST	27.64±1.46^{b}	4.04±0.70^{c}	6.64±0.01	6.64±0.01
UHT	-	-	6.65±0.04	6.63±0.02
HP200	25.20±1.41^{b}	17.86±1.53^{a}	6.53±0.13	6.35±0.01
HP400	33.84±0.41^{a}	3.68±6.37^{c}	6.51±0.00	6.32±0.01
HP600	-	-	6.56±0.01	6.32±0.02
HP800	-	-	6.71±0.00	6.58±0.04
Bovine milk				
Treatment	RCT (min)	Final *G'* value (Pa)	Initial pH	Final pH
Raw skim milk	31.18±1.07^{b}	13.74±2.82 ^{ab}	6.73±0.14	6.64±0.15
HTST	35.66±0.46^{a}	7.44±2.24^{b}	6.90±0.03	6.71±0.03
UHT	-	-	6.70±0.01	6.69±0.01
HP200	22.79±0.98^{c}	22.72±6.92^{a}	6.71±0.03	6.53±0.17
HP400	30.92±1.86^{b}	13.41±4.04^{ab}	6.87±0.00	6.82±0.04
HP600	30.92±1.86^{b}	15.41±2.19^{ab}	6.42±0.05	6.42±0.05
HP800	32.09±2.15^{b}	19.49±2.63^{a}	6.83±0.01	6.66±0.13

$^{a-d}$ Means within a column with different superscripts were significantly different ($p < 0.05$).

(-) milk failed to coagulate

In HP treatment, the RCT of bovine milk treated at 200 MPa was shortened significantly ($p < 0.05$) by 26.91% (**Table 4.4**) and the *G′* value was the highest (**Figure 4.1B**) compared with the control milk. However, HP treatments at higher pressures (400, 600, and 800 MPa) resulted in an increase of the RCT and the final *G′* value was similar to untreated milk. These results are consistent with previously reported observations for bovine milk coagulated with recombinant bovine chymosin by Needs *et al*. (2000) and Zobrist *et al*. (2005). The reduction in the RCT of bovine milk after HP treatment at 200 MPa is believed to be the result of the dissociation of micellar κ-casein and the disruption of casein micelles, which led to an increase in the surface area for intermicellar interactions with less κ-casein available to provide steric stabilisation (Huppertz *et al*., 2006; Needs *et al*., 2000).

In contrast, the rheological properties of CM differed from that of bovine milk for HP treatments. The HP treatment at 200 and 400 MPa significantly ($p < 0.05$) delayed the RCT of CM by 55.90 and 109.39%, respectively, compared with untreated milk. The rate of gel formation in HP-treated CM at 200 MPa (**Figure 4.1A**) was lower than that of untreated milk during the first 30 min, following the addition of rennet enzymes. However, after 40 min the rate of increase of *G′* was higher than that of control milk and the final value of *G′* after 60 min of incubation was the highest. This increase in the RCT and strength of the rennet-induced coagulum from CM treated at 200 MPa might be due to restricted effect of HP on disruption of casein micelles and whey protein denaturation in CM.

In HP-treated CM at 400 MPa, the rate of coagulum formation was considerably slower with a significantly lower final G' value than that of untreated milk. Meanwhile, no progress in the rennet-induced coagulum was observed in CM treated at 600 and 800 MPa. Similar observations regarding effect of HP treatments on the RCT of buffalo milk coagulated with bovine chymosin (Maxiren 180) have been reported by Huppertz *et al*. (2005). These authors found that HP treatment at 100 MPa had no effect on the RCT of buffalo milk.

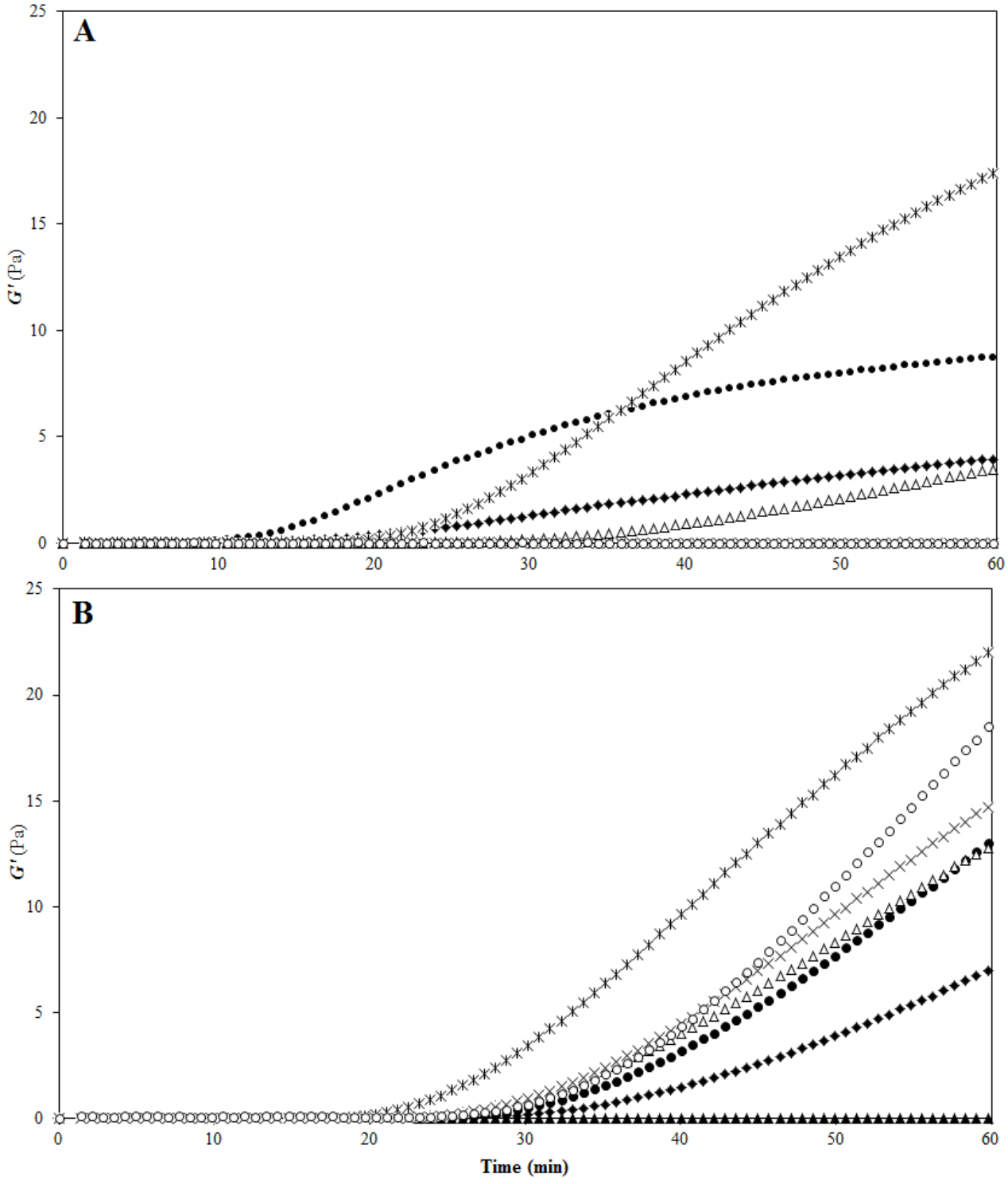

Figure 4 1. Influence of incubation time at 30ºC following addition of rennet on the storage modulus, *G'* of processed camel (A) and bovine (B) milk, raw skimmed untreated milk (●), HTST (72ºC, 15sec) milk (♦), UHT (140ºC, 5sec) milk (▲), High-pressure at 200 (ж), 400 (Δ), 600 (×), 800 (○) MPa for 30 min at 20ºC. Values are means of data from experiments on three individual milk samples.

On the other hand, the RCT of buffalo milk increased significantly by 50% after treatment at 200 MPa and continued to increase with pressure to a maximum of 100% after treatment at 800 MPa. In another study by López-Fandiño and Olano (1998), the RCT of ovine milk, clotted using standard bovine chymosin, increased significantly after treatment at 200 and 300 MPa, but treatment at 400 MPa decreased the RCT. These authors also found that HP treatment of caprine milk at 200 MPa did not affect the RCT, and treatment at 300 and 400 MPa increased the RCT.

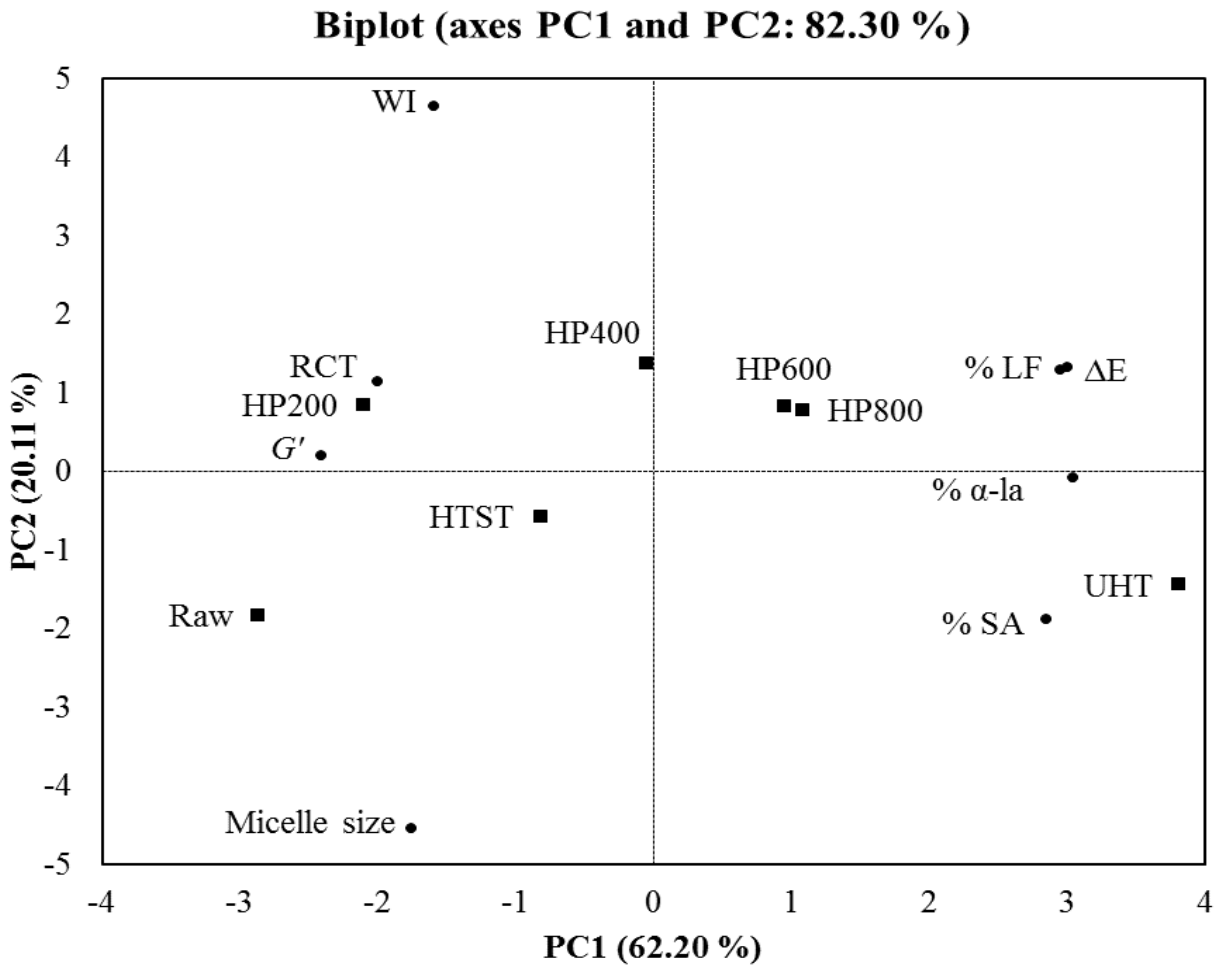

Figure 4 2. Principal component analysis of skimmed CM samples subjected to HTST (72ºC, 15sec) and UHT (140ºC, 5sec) and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20ºC, and variables: final storage modulus (*G'*), whiteness (WI), rennet coagulation time (RCT), total colour difference (Δ*E*), and denaturation of whey protein (%): serum albumin (SA), lactoferrin (LF), and α-lactalbumin (α-la).

In order to visualise the effects of the different treatments on CM properties, principal component analysis (PCA) was used (**Figure 4.2**). The two principal components accounted for 82% of the variation in the data. Processed CM samples were separated according to intensity of heat and high-pressure along PC1, which explains 62.20% of the total variance in the data. UHT CM was clearly separated from the raw and the pasteurised milk, while HP-treated CM samples at 600 and 800 MPa were clustered in the upper right tope of the PCA. These CM samples were correlated with the highest levels of denaturation of whey proteins and maximum colour difference (ΔE). On the other hand, the PCA revealed a distinct separation between HP-treated CM samples and HTST and UHT milk samples along the second PC, which explains 20.11% of the variability. CM samples treated at 200 and 400 MPa were associated with the RCT, G' and WI. While, raw and pasteurised CM were correlated with the size of casein micelle.

4.4. Conclusions

Heat and pressure treatments considerably affected many constituents and properties of CM. In UHT CM the colour change, and level of whey proteins denaturation were markedly greater than those observed in pasteurised and HP-treated CM. While, casein micelles size was significantly decreased in both heated and HP-treated CM. The RCT of CM was significantly delayed and coagulum strength (G') decreased after HTST pasteurisation. HP treatment at 200 MPa increased the RCT and enhanced the G' value of CM. However, treatment at pressures higher than 400 MPa impaired the rennet coagulation properties of CM. These findings will be beneficial to the dairy processors in terms of design, evaluation and optimization conditions of industrial operations such as pasteurisation and UHT for camel milk processing. They also can be helpful for evaluating the potential commercial use of HP treatment for CM preservation as an alternative to thermal methods, and in developing and manufacturing of various dairy products from CM.

Chapter 5: Effects of industrial processing methods on the flavour and sensory properties of camel skimmed milk: a comparison with bovine skimmed milk

Preface to chapter 5

Based on the literature, there is very limited information on the organoleptic properties of fluid CM under thermal and non-thermal processing. This chapter address the effects of heat (HTST and UHT) and high-pressure treatments on the sensory characteristics of CM.

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Omar, A., Harbourne, N., & Oruna-Concha, M.J. (2016). Effect of industrial processing on the camel skimmed milk flavour characteristics. The First Food Chemistry Conference in Amsterdam, the Netherlands, 30 October –1 November 2016, poster presentation.

Omar, A., Harbourne, N., & Oruna-Concha, M.J. (2016). Influence of heat treatments on the flavour and sensory properties of camel skimmed milk: a comparison with bovine skimmed milk. The 5th Nursten Postgraduate Flavour Symposium in Belfast, Jun 29–30, 2017, oral presentation.

Omar, A., Harbourne, N., & Oruna-Concha, M.J. (2018). Effects of industrial processing methods on the flavour and sensory properties of camel skimmed milk: a comparison with bovine skimmed milk. Manuscript in preparation for submission to Food Chemistry journal.

Abstract

The effect of high-temperature short-time pasteurisation (HTST), ultrahigh-temperature (UHT) and high-pressure (HP) treatments on the volatile and non-volatile compounds of camel milk (CM) was studied. Aroma volatile compounds were extracted and analysed by headspace solid-phase micro extraction and gas chromatography/mass spectrometry (HS–SPME–GC/MS). Non-volatile compounds including amino acids and sugars were analysed by GC–MS and HPLC respectively. Quantitative descriptive analysis (QDA) was used to describe the key sensory attributes of HTST and UHT CM in comparison with bovine milk, and the sensory data were correlated with the chemical analysis.

Volatile profiles showed that HTST and UHT treatments resulted in an increase of aldehydes, furans, and terpenes content in CM. Moreover, the increase of heat severity in UHT treatment led to the formation of sulphur compounds in CM. In contrast, HP treatments tended to enhance the formation of alcohol and ketones in CM. Both heat and HP treatments had limited effect on amino acids and lactose content of CM. The overall sensory properties of pasteurised and UHT CM were different to bovine milk, and described as having flavours such as cardboard, musty, sulphur, bitter, and sour, which are not associated normally with fresh milk and may be undesirable to consumers. This difference in sensory properties was supported by their profiles of volatile compounds.

5.1. Introduction

Camel milk (CM) popularity and demand continue to rise in many countries due to growing population, which has led to the development of camel dairy farms, especially around urban areas (Faye, Madani, & El-Rouili, 2014). However, in order for CM to be commercially successful it needs to be preserved to extend its shelf-life. Heat treatments such as high-temperature short-time pasteurisation (HTST) at 72°C for 15s and ultra-high-temperature (UHT) at 135–150°C for 5s are usually applied to raw milk in order to achieve microbial safety by destroying pathogenic microorganisms, and prolong shelf-life of milk by inactivating enzymes and killing spoilage microorganisms (Cadwallader & Singh, 2009). However, heat treatments can affect the composition of volatile and non-volatile compounds in milk and cause significant changes in the sensory properties of milk. For instance, it has been seen that UHT treatment induced the development of thermally derived off-flavours in bovine milk, due to formation of sulphur compounds and change in concentration of particular volatile compounds such aldehydes and methyl ketones, thus limiting its acceptance (Contarini, Povolo, Leardi, & Toppino, 1997). The intensity of these changes depends on the duration and temperature of the heat treatment (Zahir Al-Attabi, D'Arcy, & Deeth, 2014).

In recent years, there is an increasing demand for fresh milk which is minimally processed, thereby preserving its composition and sensory properties (Chawla, Patil, & Singh, 2011). As a result, several alternative non-thermal technologies, such as microfiltration, high-intensity ultrasound (US), ultra-high-pressure homogenization (UHPH), pulsed electric field (PEF), and high-pressure (HP) treatments have being studied in order to achieve this goal (Pereda et al., 2008). Among them, HP treatment offers unique advantages over traditional thermal treatments; it destroys pathogenic microorganisms and extends the shelf life of milk without compromising its sensory and nutritional quality (McSweeney & Fox, 2013). The effects of HP processing on microorganism destruction in bovine milk and on its various properties have

been extensively studied, and well documented in the literature (Huppertz, Smiddy, Upadhyay, & Kelly, 2006; López-Fandiño, 2006; Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002). The effect of HP treatments on the aroma profile and sensory properties of bovine milk has also been studied. It has been reported that HP treatment at pressure in the range 480-620 MPa at low temperature (25°C) causes a minimum change of volatile compounds of bovine milk, however, HP treatment of milk at temperature higher than 60°C favours the formation of aldehydes which is thought to be the main cause for the stale off-flavour in milk (Vazquez-Landaverde, Torres, & Qian, 2006a). Information on flavour generation in non-cow milk under heat and HP treatments is still very limited. Therefore, the objectives of the current study were 1) to investigate the effect of thermal (HTST and UHT) and HP treatments on the volatile and non-volatile compounds in CM; 2) to study the sensory characteristics of CM as a result of HTST and UHT treatments; and 3**)** to investigate the correlation between aroma and sensory properties of the heat processed CM and comparing the results to bovine skimmed milk.

5.2. Material and methods

5.2.1. Chemicals and reagents

Internal standard (IS) 1, 2-dichlorobenzene (130.6 µg/mL) in methanol and the alkane standards C_6–C_{25} (100 µg/mL) in diethyl ether were purchased from Sigma-Aldrich (Poole, Dorset, UK). The EZ-Faast amino acid analysis kit was purchase from Phenomenex (Torrence, CA, USA). Lactose (≥98%) was supplied by Sigma-Aldrich (Poole, Dorset, UK). Propanediol oil was obtained from Sigma-Aldrich. All other chemicals were HPLC grade (Sigma-Aldrich) and used without any further purification.

5.2.2. Milk samples

Forty litres (80 bottles, 500 mL in size) of commercially available raw camel (*Dromedary camel*) milk produced by Kamelenmelkerij Smits (Cromvoirt, The Netherlands) were supplied

by UK Camel Milk Ltd (Bolton, Lancashire, UK). The milk samples were skimmed and directly subjected to industrial treatments. Milk samples were taken for analysis directly after each treatment. All of the analyses were conducted in triplicate, and the results were expressed as mean values ± standard deviation. For sensory analysis commercially available pasteurised and UHT skimmed bovine milk from a local market were used for comparison.

5.2.3. High-Temperature, Short-Time Pasteurisation

Camel milk was pasteurised using an APV HXP pasteuriser (APV UK Limited, Crawley, West Sussex, UK). The holding section of the pasteuriser consisted of a plate-and-frame heat exchanger system. The pasteuriser unit was sterilised by circulating water at 85°C through the entire system prior to the treatment. The milk was then pasteurised at 72.5°C and held for 15s in a holding section. The pasteurised milk was cooled to 4°C and collected in 1L sterile bottles (Ascott Ltd. Newton Abbot, UK).

5.2.4. Ultra-High-Temperature

A tubular UHT plant (U.H.T.A.C, Fareins, France) was used for the indirect UHT treatment of CM. Heating was obtained in two stages using two hot oil baths. The unit was sterilised by circulating pressurized hot water prior to the treatment. The temperature of the milk samples was raised from 4 to 90°C in a preheating unit (oil bath 1). The temperature was raised from 90 to 144°C (oil bath 2), and the milk was held for 5s at this temperature. The processed milk was cooled to 4°C and collected in 1L sterile bottles.

5.2.5. High-Pressure Treatment

High-pressure treatment of camel and bovine milk was performed as described by Huppertz, Fox, and Kelly (2004). Camel milk samples (50 mL) were vacuum-packed in polyethylene bags and HP-treated using a Stansted Iso-Lab 900 High Pressure Food Processor (Stansted

Fluid Power, Stansted, Essex, UK), at pressures of 200, 400, 600, and 800 MPa for 30 min. The temperature of the HP unit vessel was maintained at 20°C. A mixture of water and 1, 2-Propanediol oil (70: 30) was used as the pressurizing fluid.

5.2.6. Analysis of volatile compounds

5.2.6.1. Headspace solid-phase microextraction (HS-SPME)

The extraction of volatile compounds in processed CM was performed using a headspace solid-phase microextraction system (HS–SPME). A SPME fibre (50/30µm DVB/CAR/PDMS) was used (Supelco, Bellefonte, Pennsylvania, USA). 5mL of the raw and processed skimmed milk samples were weighed into 15mL size SMPE vials and were tightly capped with a polytetrafluoroethylene septum. The fibre was exposed in the head space placed at 5mm above the liquid surface of the samples for 30 min, after incubation at 40^{o}C for 10 min. The sample was stirred magnetically for 20 min at 40^{o}C before the fibre was inserted into the GC–MS injector port for desorption at 230^{o}C for 3 min. The extraction by SPME fibre for each sample was performed in duplicate for GC–MS analysis.

5.2.6.2. GC-MS analysis of HS-SPME extracts

After extraction, samples passed through a DB-5 (30m × 250µm × 0.25µm) capillary column (Agilent Technologies, Inc., Palo Alto, CA, USA). Helium at 20 psi was used as the carrier gas with a constant flow of 2.1 mL /min at 40°C. Volatile compounds in CM sample extracts were identified on a mass spectrometer Agilent Technologies 5975C coupled to an Agilent 7890A gas chromatography system (both Agilent Technologies, Inc., Palo Alto, CA, USA) as described by Morales-Soto et al. (2015). Volatiles were determined by comparison of each mass spectrum with spectra from authentic compounds analysed in our laboratory, or from the National Institute of Standards and Technology (NIST) mass spectral database (NIST/EPA/NIH Mass Spectral database, 2008), or spectra published elsewhere. In order to

confirm the identification, a series of n-alkanes (C_6–C_{33}) were run under the same chromatographic conditions in order to calculate the linear retention index (LRI) of detected compounds to be compared with the LRI which was provided by the NIST database in the same capillary column. In addition, the identified volatiles in CM samples were quantified by comparison of their peak areas with that of the (IS) 1, 2-dichlorobenzene. The pasteurised and UHT skimmed bovine milk samples were treated the same and used for comparison.

5.2.7. Analysis of non-volatile compounds

5.2.7.1. Determination of free amino acids by GC-MS

Skimmed CM samples (15 mL) were centrifuged at 4000×*g* at 4°C for 20 min then filtered through a 0.2µm syringe filter (Merck Millipore Ltd, Tullagreen Carrigtwohill, Co Cork, Ireland). An aliquot of the supernatant (100µL) was derivatised using the EZ-Faast amino acid derivatization technique (Phenomenex, Torrance, CA, USA). GC-MS analysis of the derivatised samples was carried out using an Agilent GC–MS 6890/5975 instrument (Agilent, Santa Clara, CA, USA) as described by Elmore, Koutsidis, Dodson, Mottram, and Wedzicha (2005). Sample analyses were done in triplicate and results were expressed as µg/mL of milk. The pasteurised and UHT skimmed bovine milk samples were treated the same and used for comparison.

5.2.7.2. Sugar analysis

Skimmed CM samples were diluted 20-fold in HPLC grade-water and filtered through a 0.2 µm syringe filter (Merck Millipore Ltd, Tullagreen Carrigtwohill, Co Cork, Ireland). Sugar analysis were carried out using a Dionex ion chromatography system with a 250 × 4mm Carbopac PA1 column (Dionex Corp., Sunnyvale, CA, USA) as described by Muttucumaru, Powers, Elmore, Mottram, and Halford (2015). Sugar standard (lactose) was run under same conditions and used to prepare standard curves, which were then used to determine sugar

concentration in milk samples. Each sample was analysed in triplicate and results were expressed as g /100 mL of milk. The pasteurised and UHT skimmed bovine milk samples were treated the same and used for comparison.

5.2.8. Sensory analysis

Quantitative Descriptive Analysis (QDA) of milk samples was performed using the profile method (ISO 22935–1; ISO 22935–2:2009). Samples were assessed by a panel of 10 assessors, 1 male and 9 females. All panellists were members of an established sensory panel at the University of Reading. The HTST pasteurised and UHT CM samples were collected in 1 L bottles (Ascott Ltd. Newton Abbot, UK) and immediately, cooled and stored at -18°C until sensory analysis. After 7 days of storage samples were defrosted overnight in a fridge at 4°C and were presented to panellists at 22°C (ISO 22935–2:2009).

5.2.8.1. Training session

Training sessions (4 sessions) of 30 min duration were conducted on consecutive days during the first week prior to the evaluation of milk samples in order for the assessors to be familiarised with the descriptors and intensity of scales. Samples were presented in a random, coded style during these sessions. Assessors evaluated the milk samples for the appearance, odour, mouthfeel, taste, flavour and aftereffects. Different samples as examples and specific references were used where appropriate to ensure consensus about the descriptive terms obtained as shown in **Table 5.1**.

5.2.8.2. Sensory assessment

The descriptive sensory assessment was conducted in isolated sensory booths in an air-conditioned room (~ 22°C) and normal fluorescent lighting in the Sensory Science Centre at the Department of Food & Nutritional Sciences, University of Reading, UK. Samples were

coded with 3-digit random numbers using balanced-block design and evaluated in duplicate by each of the ten panellists in two different days. The obtained sensory descriptors: 8 appearance, 12 odour, 6 mouthfeel, 6 taste, 5 flavour, and 7 aftereffects were scored on anchored unstructured line scales (15cm, scaled 0-100) by using Compusense software (version 5.2; Guelph, ON, Canada). Panellists were provided with water (at room temperature) and crackers (United Biscuits Ltd. Carlisle, Cumbria.UK) were served to cleanse palate between samples and remove an aftertaste. There was a 60 seconds time delay between the finishing of one sample and the presenting of the next.

5.2.9. Statistical analysis

The quantitative results of the identified volatiles compounds, amino acids and sugars by GC–MS and DIONEX–HPLC were analysed by one-way ANOVA using XLSTAT version 2015.6.01.24797 (Kovach Computing Services, Wales, UK). Statistical significance of differences between mean values was analysed by using the multiple comparison Tukey's HSD test. The quantitative descriptive data of the sensory assessments were separately analysed by ANOVA using Senpaq software version 4.2 (Qi Statistics Ltd., Reading, UK). The significance of differences between the samples was calculated using Tukey's test for the multiple comparison of means ($p < 0.05$). Principal component analysis (PCA, Pearson n-1; XL Stat) was conducted to simplify interpretation of differences between the processed milk samples. Multiple factor analysis (MFA) was used to evaluate the relationships between volatile and non-volatile compounds and sensory properties of milk by using the means of the sensory data (Pagès, 2004).

Table 5 1. Definitions of attributes used to describe sensory properties of HTST (72°C, 15sec) and UHT (140°C, 5sec) camel and bovine skimmed milk

Attributes	Definition
Appearance:	
Yellow colour	Degree of intensity of the colour yellow
Creamy colour	The extent to which the sample resembles cream
Body	The extent to which the sample is thick and rich
Opaque	Opaque colour of milk
White	Degree of intensity of the colour white
Separation	Separation of why proteins
Watery	The extent to which the sample resembles diluted milk with water
Powdery	Presence of small particles like flour or powder
Odour:	
Intensity	The overall intensity of the aroma of milk sample
Butyric	Aroma associated with butyric acid and cheesy
Powdery	Aroma associated with milk powder (SMA)
Dairy	Aroma associated with products made from cow`s milk.
Savoury	Aroma associated with slightly salty or spicy food
Cooked milk	Aroma associated with heat treated milk
Sour	Aroma associated with wet fermented milk
Cardboard	Aroma associated with wet paper/cardboard
Goat	Aroma associated with goat hair/skin
Dry	Aroma associated with dryness
Musty	Aroma associated with a cellar/old cupboard/cabinet
Sulphur	Aroma associated with eggs
Taste/ Flavour:	
Sweet	Fundamental taste sensation associated with sucrose in water

Bitter	Basic taste typical of caffeine in water
Salt	Basic taste typically associated with sodium chloride as diluted in water
Sour	Acidic taste associated with lactic or citric acid
Metallic	Taste associated with metal
Savoury	Taste associated with slightly salty or spicy food
Aged	The extent to which the sample resembles aged mutton meat
Cheesy	The extent to which the sample resembles blue cheese (Butyric acid)
Creamy	The extent to which the sample resembles cream- milky products
Whey	The extent to which the sample resembles whey proteins.
Dairy	The extent to which the sample resembles cow's milk or its products
Mouthfeel:	
Watery	The sensation of water in the mouth (no body)
Mouth coating	The extent to which the sample sticks to the mouth.
Drying	The extent to which the sample produces a drying effect in the mouth
Powdery	The sensation of small particles like flour or powder
Body	The extent to which the sample is thick and rich in the mouth
Tooth coating	A sticky sensation on palate and between the teeth
Aftereffects:	
Dairy	Persistence of flavour associated with cow's milk or its products.
Sweet	Persistence of the sweet taste in the mouth after the milk is swallowed.
Savoury	Persistence of the salty or spicy taste in the mouth after the milk is swallowed.
Salt	Persistence of the salty taste stays in the mouth after the milk is swallowed
Sour	Persistence of the acidic taste in the mouth after the milk is swallowed
Animal	Persistence of flavours resembles goat hair/skin after the milk is swallowed
Lingering	The extent to which taste, and flavour lingers in the mouth after the milk is swallowed

5.3. Results and Discussion

5.3.1. Effect of heat and high-pressure processing on the non-volatile compounds in camel milk

5.3.1.1. Amino acids

The amino acids content of raw and processed CM is shown in **Table 5.2**. Glutamic acid and glycine were the predominant amino acids in the raw CM as previously reported by Sabahelkheir, Fat en, and Hassan (2012) and Shamsia (2009).

In general, there were no significant differences between the levels of most amino acids in pasteurised and UHT CM and their initial concentrations in the raw milk, with the exception of glutamic acid which levels significantly ($p < 0.05$) increased after UHT treatment. This amino acid has been reported to be responsible of the sour and umami taste in heated milk (Newton, Fairbanks, Golding, Andrewes, & Gerrard, 2012). Other amino acids including alanine, glycine, serine (sweet taste properties), and aspartic acid (sour and umami taste) also exhibited a slight increase in their levels after heat treatments although this change was not significant. Furthermore, some small reductions ($p > 0.05$) in the amount of some amino acids such as phenylalanine, leucine, and lysine, which have a bitter taste, occurred in both HTST and UHT CM. Similarly, glutamic acid and glycine were the prevalent amino acids in the pasteurised and UHT bovine skimmed milk (Appendix 1). However, HTST and UHT CM had higher amount of glycine and lower level of glutamic acid than heated bovine milk. No significant difference in the levels of amino acids was observed between the HTST and UHT bovine milk sample. Payne-Botha and Bigwood (1959) reported that sterilisation processes (122–124^{o}C for 20 min) had negligible effect on the amino-acid content of skimmed bovine milk compared to the raw milk.

Table 5 2. Free amino acids and sugars in raw and processed skimmed camel milk at 72°C, 15sec (HTST), 140°C, 5sec (UHT), and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20°C.

Code	Amino acids *(µg/ mL)*	Raw	HTST	UHT	HP200	HP400	HP600	HP800	*LSD*[c]	*P*[d]
Ala	Alanine	0.94^{b}	1.16^{ab}	1.18^{ab}	1.34^{ab}	1.44^{a}	1.41^{a}	1.42^{a}	0.25	**
Gly	Glycine	7.83^{a}	8.29^{a}	8.00^{a}	8.59^{a}	8.72^{a}	8.74^{a}	8.65^{a}	0.94	ns
Val	Valine	2.67^{a}	2.63^{a}	2.66^{a}	3.00^{a}	2.82^{a}	2.44^{a}	2.45^{a}	0.89	ns
Leu	Leucine	0.56^{ab}	0.39^{b}	0.41^{b}	0.65^{a}	0.65^{a}	0.61^{a}	0.54^{ab}	0.12	**
Ile	Isoleucine	0.89^{a}	0.93^{a}	0.90^{a}	1.12^{a}	0.87^{a}	0.87^{a}	0.83^{a}	0.41	ns
Thr	Threonine	0.53^{a}	0.50^{a}	0.50^{a}	0.58^{a}	0.54^{a}	0.53^{a}	0.51^{a}	0.12	ns
Ser	Serine	0.37^{a}	0.54^{a}	0.47^{a}	0.54^{a}	0.46^{a}	0.38^{a}	0.45^{a}	0.28	ns
Pro	Proline	0.13^{a}	0.10^{a}	0.25^{a}	0.30^{a}	0.23^{a}	0.23^{a}	0.25^{a}	0.21	ns
Asn	Asparagine	0.53^{a}	0.41^{a}	0.25^{a}	0.34^{a}	0.30^{a}	0.28^{a}	0.34^{a}	0.18	ns
Asp	Aspartic acid	1.16^{a}	2.18^{a}	2.21^{a}	1.69^{a}	1.13^{a}	1.14^{a}	1.22^{a}	0.80	ns
Met	Methionine	0.04^{a}	0.10^{a}	0.05^{a}	0.06^{a}	0.10^{a}	0.09^{a}	0.07^{a}	0.05	ns
Glu	Glutamic acid	8.82^{b}	9.50^{b}	11.38^{a}	10.11^{ab}	9.65^{b}	9.64^{b}	10.17^{ab}	0.94	**
Phe	Phenylalanine	0.17^{abc}	0.15^{bc}	0.14^{c}	0.20^{abc}	0.25^{a}	0.26^{a}	0.24^{ab}	0.06	**
Gln	Glutamine	0.17^{a}	0.22^{a}	0.19^{a}	0.21^{a}	0.25^{a}	0.23^{a}	0.13^{a}	0.13	ns
Orn	Ornithine	0.40^{a}	0.25^{a}	0.39^{a}	0.39^{a}	0.59^{a}	0.57^{a}	0.58^{a}	0.31	ns
Lys	Lysine	1.94^{a}	1.23^{a}	1.69^{a}	1.65^{a}	2.29^{a}	1.90^{a}	1.96^{a}	0.70	ns
His	Histidine	0.12^{a}	0.11^{a}	0.12^{a}	0.13^{a}	0.09^{a}	0.07^{a}	0.13^{a}	0.06	ns
Tyr	Tyrosine	0.05^{a}	0.06^{a}	0.07^{a}	0.07^{a}	0.08^{a}	0.06^{a}	0.09^{a}	0.04	ns
Trp	Tryptophan	0.03^{a}	0.02^{a}	0.04^{a}	0.03^{a}	0.04^{a}	0.04^{a}	0.03^{a}	0.01	ns
Sugar (g/100mL)										
Lct	Lactose	3.02^{a}	2.70^{a}	2.91^{a}	2.74^{a}	3.04^{a}	2.97^{a}	2.99^{a}	0.41	ns

[c] Least significant difference at $p = 0.05$. [d] Means in the same row followed by different letters differ significantly at the 5% level. ns, no significant difference between means ($p > 0.05$); *P<0.05; ** P< 0.01. Values are means of triplicate analysis from three independent experiments. (-) Not detected.

In terms of HP processing, the majority of amino acids present in CM showed no significant difference ($p > 0.05$) in their concentrations after all HP treatments in comparison to raw milk. Only alanine (sweet taste) content was significantly increased after HP treatment of CM at 400, 600 and 800 MPa. In addition, the content of phenylalanine and leucine in the HP-treated CM were higher ($p < 0.05$) than in the heated milk samples.

5.3.1.2. Sugars

Lactose was the only sugar identified in raw and processed CM (**Table 5.2**). Its concentration in the raw CM was 3.02 g/100 mL, which was within the range of reported values in the literature by Khan and Iqbal (2001), Omar, Harbourne, and Oruna-Concha (2016) and Jilo and Tegegne (2016). Lactose content of CM was not significantly affected ($p > 0.05$) by pasteurisation and UHT treatments. The amount of lactose in HTST and UHT CM was slightly lower ($p > 0.05$) than that of pasteurised and UHT bovine skimmed milk (3.26, and 3.25 g/100mL respectively). Similar observations were reported by Pestana, Gennari, Monteiro, Lehn, and Souza (2015) who found that pasteurisation (75°C for 15s) and UHT (140°C for 3s) treatments of whole bovine milk had no significant effect on the concentration of lactose in pasteurised and UHT treated milk compared to raw milk. On the other hand, no significant differences were found between the lactose levels in all HP-treated CM samples and the raw milk. Similar findings regarding the effect of HP treatment on lactose level in whole bovine milk were reported by Lopez-Fandino, Carrascosa, and Olano (1996) and Dhineshkumar, Ramasamy, and Siddharth (2016).

5.3.2. Effect of heat and high-pressure processing on the volatile compounds of camel milk

A complete list of volatile compounds identified in raw and processed skimmed CM, grouped in classes, is shown in **Table 5.3**. Overall, the profile of volatiles in the raw CM was

significantly different ($p < 0.05$) after the application of HTST, UHT and HP treatments, indicating that these processing methods affected the volatile profile of CM.

5.3.2.1. Aldehydes

The total aldehyde levels in CM significantly increased ($p < 0.05$) following heat treatments (**Figure 5.1A**). Amongst these aldehydes, hexanal, heptanal, octanal, nonanal, and (E)-2-nonenal were the main constituents that contributed to this increase (**Table 5.3**) and levels were increased with the increasing intensity of heat, from HTST pasteurisation to UHT treatment. Heptanal and (E,E)-2,6-nonadienal were only detected in the heat-treated CM. Hexanal was the main aldehyde in pasteurised and UHT bovine skimmed milk and its concentration was significantly higher in the UHT milk (Appendix 2). In addition, some aldehydes such as (E)-2-hexenal, heptanal, (E, E)-2, 6-nonadienal, and [E, E]-2, 4-nonadienal were only detected in the heat-treated CM. These three compounds impart the fresh fatty green herbal note (Lloyd, M.A.Drake, & P.D.Gerard, 2009).

Aldehydes can be found in the raw milk as a consequence of light-induced lipid oxidation (Calvo & Hoz, 1992) or transferred to milk from animal feed (Scanlan, Lindsay, Libbey, & Day, 1968). UHT treated CM had higher concentrations of total aldehydes than raw and pasteurised milk samples. Similar observations were reported by Vazquez-Landaverde, Velazquez, Torres, and Qian (2005) who investigated the thermally derived volatile compounds in low, full-fat and skimmed bovine milk. The authors found that the total aldehydes concentration in low-fat UHT milk was greater than in the raw and low-fat and skimmed pasteurised milk. The Strecker degradation of amino acids during Maillard reactions and the spontaneous decomposition of hydroperoxides in milk induced by heat, can also promote the formation of aldehydes (Vazquez-Landaverde et al., 2006a). The study concluded that these aldehydes could be major contributors to the off-flavour of the heated milk. They

were also reported to be responsible for the "stale" flavour of UHT-treated milk (Perkins, D'Arcy, Lisle, & Deeth, 2005).

In contrast, no statistical difference was found in the total concentration of total aldehydes between the raw and all HP-treated (200, 400, 600, and 800 MPa) CM samples at 20°C for 30min. Furthermore, the total aldehyde content in the HP- treated CM was significantly lower than in the pasteurised and UHT milk. Some aldehydes such as octanal, nonanal, decanal, (E)-2-nonenal, (E)-2-heptenal, and (E)-2-undecenal showed decrease concentrations after HP treatments compared to their initial value in the raw CM, but they were not significant ($p > 0.05$). Only (E)-2-decenal was significantly decreased after HP treatments at 800 MPa. Vazquez-Landaverde, Torres, and Qian (2006) reported that the levels of aldehydes in whole bovine milk under HP treatments (482, 586, and 620 MPa), at 25°C for 1, 3, and 5 min holding time were lower than those in the raw milk, except, heptanal and 2-methylpropanal.

5.3.2.2. Alcohols

Several alcohols were detected in the raw CM, of which 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol were the most abundant compounds (**Table 5.3**). These primary alcohols can be naturally present in the raw milk mainly due to reduction of the respective aldehydes and it is improbable that they contribute to the odour of fresh milk (Moio, Dekimpe, Etievant, & Addeo, 1993; Toso, Procida, & Stefanon, 2002). The total content of alcohols in the UHT CM was significantly lower ($p < 0.05$) than in pasteurized and raw milk (**Figure 5.1B**). In general, there was a decrease in these compounds after UHT treatment with the exception of the level of 1-octen-3-ol which was almost double in concentration ($p < 0.05$) after the UHT treatments. This increase in its concentration might be the result of heat-induced autoxidation (Calvo & Hoz, 1992). This compound is characterised as having earthy, green, and mushroom odour (Cornua et al., 2009). The alcohols (E)-2-Nonen-1-ol and 2-Decen-1-ol were not detected in milk after

both heat treatments. As in heat-treated CM samples, alcohols 1-hexanol, 1-heptanol, 1-octen-3-ol, 2-ethyl-1-hexanol, and 1-octanol were detected also in headspace of the pasteurised and UHT bovine skimmed milk samples. The concentrations of these compounds in CM were significantly higher than in the heated bovine milk samples, whereas 1-pentanol and 1-nonanol (floral odour) were only detected in the HTST and UHT-treated CM. Although no statistical differences were found in alcohols content between the pasteurised and UHT bovine milk, their levels were lower in the UHT bovine milk. Vazquez-Landaverde et al. (2005) found that the concentration of 3-Methylbutanol (which was the only identified alcohol in their study) in low-fat and whole raw bovine milk was significantly higher than in UHT. However, it was not important for the aroma of raw and heated milks. Alcohols were also reported to be significantly decreased or not detected in skimmed bovine milk subjected to pasteurisation at 80, 100, and 120^{o}C (Hougaard, Vestergaard, Varming, Bredie, & Ipsen, 2011).

Unlike heat treatments, the alcohols 1-heptanol, 1-octanol, 1-nonanol, and 2-decen-1- showed increased levels in CM after all HP treatments. This increase could be as a result of converting the straight-chain aldehydes (heptanal, octanal, nonanal, decanal, and (E)-2-decenal) to the corresponding alcohols under reducing conditions, which might be in line with the decrease in their levels in milk after HP treatments (Nursten, 1997). Overall, the total amount of alcohols in the HP-treated CM was significantly higher ($p < 0.05$) than in the UHT milk (**Figure 5.1B**). There is very limited information in the literature with regards to the mechanism of volatile formation in bovine milk under the HP processing (Cadwallader & Singh, 2009). However, Contador, Delgado, García-Parra, Garrido, and Ramírez (2015) reported that HP treatment of human milk (full-fat) at 600 MPa for 6 min caused an increase in the alcohol content due to lipid oxidation.

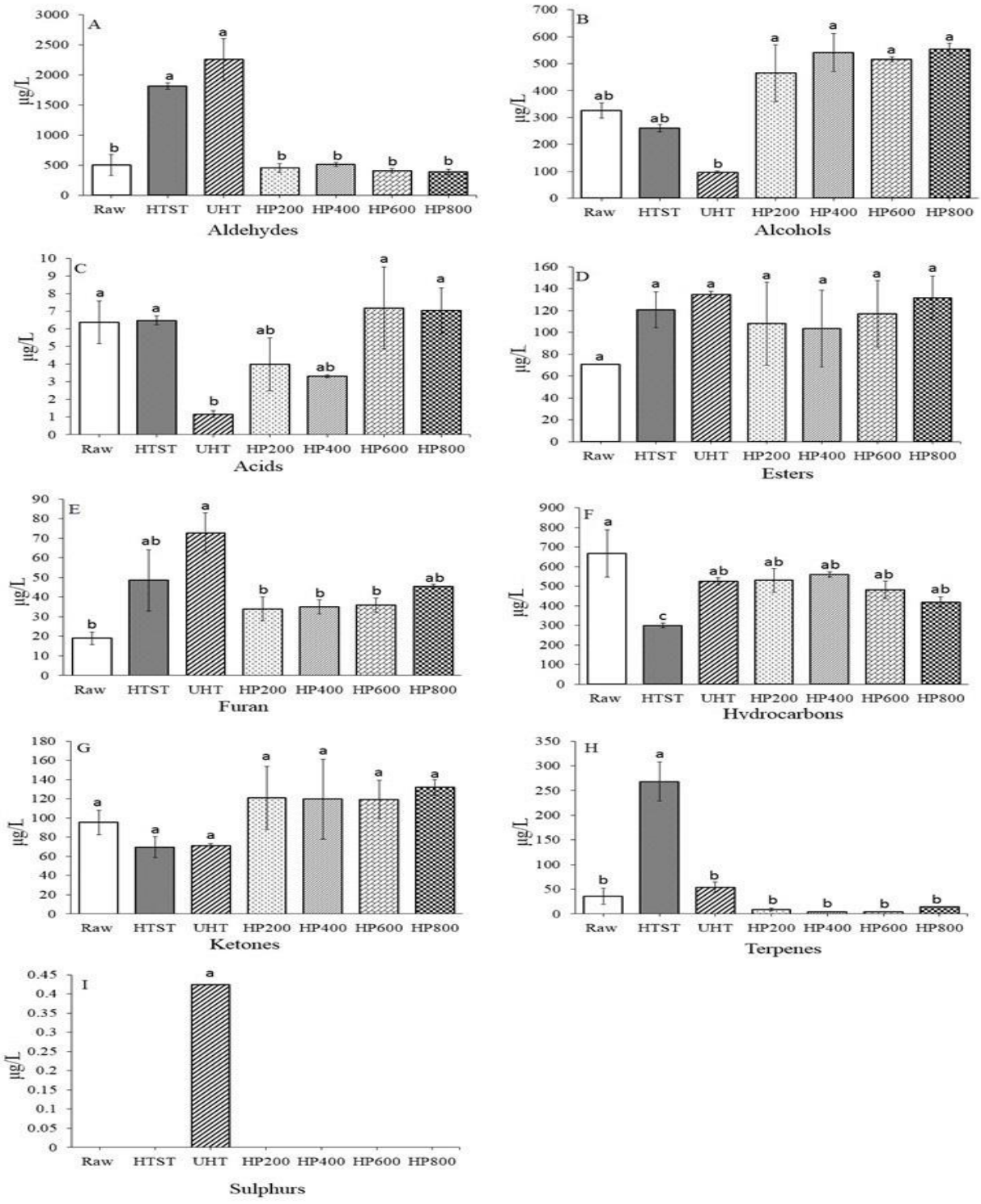

Figure 5 1. Sum of volatile compounds (µg/mL) in each chemical group: aldehydes (A), alcohols (B), acids (C), esters (D), furans (E), hydrocarbons (F), ketones (G), terpenes(H), and sulphur compounds (I) isolated from raw and processed skimmed camel milk at 72ºC, 15sec (HTST), 140ºC, 5sec (UHT), and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20ºC.

5.3.2.3. Acids

Acids were the least abundant group detected in the headspace of the raw skimmed CM. Acetic acid was the main organic acid detected in the raw milk and it can be produced by bacteria or yeast. Short chain free fatty acids originate in milk mainly from degradation of milk triglycerides due to their catalysis by lipase and microbial action (Tunick, Iandola, & Hekken, 2013). They were reported to be responsible of the development of rancid flavour in milk (Zhang et al., 2011). Consequently, they were considered to have a key role in the flavour of skimmed milk (Shiratsuchi, Shimoda, Imayoshi, Noda, & Osajima, 1994). The total acids concentration in UHT milk samples (**Figure 5.1C**) was significantly lower ($p < 0.05$) than in the raw and pasteurised milk. A significant reduction ($p < 0.05$) in the level of acetic acid which contributes to the pungent sour odour (Attaie, 2009) and disappearing of decanoic acid in CM samples was observed after heat treatments. 2-oxooctanoic acid which imparts sweet brown caramel note (Smit, Smit, & Engels, 2005) was only detected in the pasteurised and UHT milk samples. On the other hand, n-decanoic acid which contributes to the fatty note (Zhang et al., 2011), was the only acid detected in the pasteurised skimmed bovine milk. In whole bovine milk, hexanoic, octanoic, and dodecanoic acids were reported to be the main fatty acids (Tunick et al., 2013). No significant differences were found in their concentrations between the raw and HTST pasteurised full-fat bovine milk (Zhang et al., 2011). However, pasteurisation of reduced-fat milk at 79, 82, and 85^{o}C was reported to cause an increase in their levels (Gandy et al., 2008).

On the other hand, the total concentration of acids in HP-treated CM samples at 600 and 800 MPa was significantly ($p < 0.05$) higher than in the UHT CM. Acetic acid which imparts acidic note was decreased after HP treatments at 200, 400, 600, and 800 MPa, while an increase in the level of decanoic acid which is responsible for the unpleasant rancid fatty odour was observed. Dodecanoic acid, which has mild fatty odour was only formed in the HP- treated CM

at pressure 400, 600, and 800 MPa, which might be the result of the degradation of the milk triglycerides due to the high pressure (Contador et al., 2015).

5.3.2.4. Esters

Esters represented the fifth most abundant group of the volatile compounds in CM (**Table 5.3**). The main identified components were short-chain fatty acid ethyl esters and long-chain fatty acid methyl esters. Amongest them, ethyl 2-methylbutanoate, 2-heptanol acetate, and methyl acetate were predominant constituents, and described as having fruity, sweet, citrus, and ethereal odour (Delgado, González-Crespo, Cava, & Ramírez, 2011). Esters are formed in the milk via esterification of short and medium-chain fatty acids with aliphatic or aromatic alcohols by enzymatic reactions (Wang, Zheng, Liu, Hu, & Deng, 2014). They can also be the result of bacterial actions in milk, particulary lactic bacteria (Moio et al., 1993). Esters were reported to be accountable for a fruity aroma in milk and dairy products (Cadwallader & Singh, 2009).

The levels of the main esters (methyl acetate, hexyl acetate, 2-heptanol, acetate and butyl butanoate) in CM exhibited an increase after heat treatments. However, there was no signfifcant difference in total content of esters in the pasteurised and UHT CM compared to the raw milk (**Figure 5.1D**). Esters such as butyl acetate (ethereal, and furity note), methyl octanoate (waxy, green, and sweet note), and 4-nitrophenyl nonanoate were not detected in CM after the HTST and UHT treatments. Whereas, (Z)-3-hexenyl butanoate, 2-ethylhexyl formate, and pentanoic acid,2,2,4-trimethyl-3-carboxyisopropyl isobutyl ester were present only in the heated CM, and characterised as having fruity, sweet and metallic odour. Heat treatments catalyse the esterification of alcohols with fatty acids in milk (Hougaard et al., 2011), which may explain the increase of some esters sucha as methyl acetate, hexyl acetate, 2-heptanol, acetate and butyl butanoate and the significant reduction in the concentration of alcohols and fatty acids in the UHT CM. Similarly, the level of methyl acetate in the UHT bovine skimmed milk was

significantly higher than in the pasteurised milk; however, it was significantly lower than in the skimmed UHT CM (Appendix 2). Both methyl hexanoate, (Z)-3-hexenyl butanoate were detected in the pasteurised camel and bovine milk. However, other compounds such as hexyl acetate, ethyl 2-methylbutanoate, 2-heptanol acetate, 2-ethylhexyl formate, butyl butanoate, octyl thiocyanate, pentanoic acid, 2, 2, 4-trimethyl-3-carboxyisopropyl-isobutyl ester, were only identified in the heated CM samples. While methyl octanoate, methyl butyrate and methyl decanoate were only found in heated bovine milk samples. Concentrations of ester compounds in bovine milk were reported to be increased after both HTST and UHT treatments. For instance, the content of ethyl acetate in the low and full-fat UHT bovine milk was 10 times higher than in the raw samples (Vazquez-Landaverde et al., 2005).

Under HP treatments, the formation of methyl hexanoate, ethyl 2 methylbutanoate, methyl octanoate, 2-heptanol acetate, and 4-nitrophenyl nonanoate in CM was increased with increasing the pressure. Only methyl acetate seemed to be inhibited under the HP treatments. Methyl esters of octanoic, decanoic, thiocyanic, and dodecanoic acid were only detected in the HP-treated CM. There is no available explanation in the literature for the mechanisms of esters formation under the HP treatments. Vazquez-Landaverde et al. (2006) found that increasing both pressutre and temperature caused an increase in the levels of esters in whole bovine milk.

5.3.2.5. Furans

2-pentylfuran was the only furan compound detected in raw CM. It is formed in milk as a result of lipid oxidation (Perez Locas & Yaylayan, 2004). The level of 2-pentylfuran in UHT-treated CM was significantly higher ($p < 0.05$) than in HTST and raw milk (**Figure 5.1E**). Moreover, its concentration in both HTST and UHT CM was significantly higher than in the pasteurised and UHT skimmed bovine milk. This compound has been reported to have a fruity, green, beany, metallic, and musty odours (Yuan & Chang, 2007). 2-ethylfuran (chemical-like note)

and 2-n-octylfuran were only detected in the UHT-treated CM. The increased formation of furan compounds in the UHT milk was highly associated with the Maillard reaction (Cadwallader & Singh, 2009; Calvo & Hoz, 1992). Similar observations regarding to the effects of heat treatments on furan derivatives in whole bovine milk were reported by Jansson et al. (2014) and Tunick et al. (2013).

Similar to heat treatments, the formation of 2-pentylfuran in CM was increased after HP processing at all pressures (200, 400, 600, and 800 MPa). Contador et al. (2015) reported that HP treament of human milk at 400 and 600 MPa for 6 min caused a significant increase in furans concentrations. The author suggested that this increase might be the result of induced Maillard reaction and carbohydrate degradation by pressure leading to formation of furan derivatives.

5.3.2.6. Hydrocarbons

In raw CM, most of the identified hydrocarbons belong to aliphatic hydrocarbons. However, toluene was the most abundant component and the only aromatic hydrocarbon identified in the raw milk. Although the origin of these hydrocarbons is not well understood (Toso et al., 2002), they can be formed in milk either by lipid autoxidation processes, or by the decomposition of carotenoids (Wang et al., 2014). Generally, hydrocarbons have no role in the aroma of fresh milk due to their low concentrations and weak odour (Moio et al., 1993), however they contribute indirectly to the flavour of skimmed milk especially the aromatic hydrocarbons (Cadwallader & Singh, 2009; Shiratsuchi et al., 1994). In general, concentrations of aliphatic hydrocarbons in CM were reduced after the application of HTST pasteurisation (**Figure 5.1F**). The concentration of the acyclic hydrocarbon, nonane (gasoline-like odour) was the highest among the identified aliphatic hydrocarbon in the raw CM. However, after applying the heat treatments this compound was not detected in the CM, suggesting that it might be transformed into other complex volatile compounds during the thermal treatments. Aromatic hydrocarbons

including styrene, and 1,3-dimethylbenzene were only formed in the UHT CM. These compounds have low odour threshold, therefore they may contribute to the off-flavour (strong plastic odour) of milk (Wang et al., 2014).

Both aromatic and aliphatic hydrocarbons have been detected in whole raw (Toso et al., 2002), pasteurised at 72°C for 15s (Wang et al., 2014) and UHT skimmed bovine milk (Valero, Villamiel, Miralles, Sanz, & MartõÂnez-Castro, 2001); their levels were increased in the processed bovine milk. Unlike CM, nonane was present at higher concentration in the UHT bovine milk compared to pasteurised milk. Moreover, other compounds such as 1-heptene, heptane, 1-octene, 2,2,4,6,6-pentamethyl-3-heptene, tridecane, hexadecane, and pentadecane were not detected in bovine milk samples. The contents of toluene, which has a sweet, pungent, benzene-like odour (Cornua et al., 2009) and undecane (gasoline-like to odourless) in the UHT CM were significantly higher than in the UHT bovine milk. The aromatic hydrocarbon 1,3-dimethylbenzene was detected in both UHT camel and bovine milk at similar levels, whilst styrene, which has a strong plastic note (Wang et al., 2014), was only detected in the UHT CM. The concentrations of most hydrocarbons in CM remained constant after the application of HP treatments, with two exceptions. The level of undecane significantly decreased after all HP treatments, while nonane level was only significantly decreased after HP treatment at 800 MPa. These two hydrocarbons are characterised by having gasoline-like odours or being odourless.

5.3.2.7. Ketones

Ketones are compounds present naturally in milk as consequence of oxidation of fatty acids (mainly from $C_{6:0}$ to $C_{12:0}$) to β-keto acids, which are then decarboxylated to the corresponding methyl ketones with one carbon atom less (Cadwallader & Singh, 2009). Due to their low perception threshold these compounds have been reported to have a key role in the aroma of milk and dairy products such as blue cheese (Moio et al., 1993). Six ketones were detected in

the volatile profile of CM, of which, 2-nonanone was the most abundant one. In general, ketone levels in UHT CM were lower than in untreated milk (**Table 5.3**), except 2-heptanone that was slightly higher after UHT treatment. Whereas, the concentrations of 6-methyl-2-heptanone, 2,3-butanedione and 2-undecanone were slightly increased in CM after the HTST pasteurisation. Floral, fruity and musty notes are usually related to the ketones content in particular 2-nonanone, 2-decanone, and 2-undecanone, whereas blue cheese note is attributed to 2-heptanone (Curioni & Bosset, 2002). Compounds such as 2,3-pentanedione and 2-decanone were only detected in pasteurised and UHT CM, respectively. Heat treatments induce the formation of ketones in milk either by β-oxidation of saturated fatty acids or by decarboxylation of β-keto acids present in milk fat (Calvo & Hoz, 1992; Pereda et al., 2008). These compounds were reported to be responsible for the heated- milk flavour in the processed milk (Contarini et al., 1997). The obtained results regarding the effect of heat treatments on the ketones in CM were different to those observed for bovine milk (Appendix 2). Ketones levels in the UHT bovine milk were higher than in the pasteurised milk, except 6-Methyl-2-heptanone. Moreover, acetone was only isolated in bovine milk and it was the most abundant compound in UHT milk. The content of 2,3-octanedione in the HTST CM was significantly higher than in the pasteurised bovine milk. In addition, ketones including 2,3-pentanedione, 3-octanone, and 2-decanone were only detected in the heat-treated CM samples. Similarly, Hougaard et al., (2011); Li et al., (2013) and Wang et al., (2014) found that ketones concentration in skimmed bovine milk were significantly increased after pasteurisation. Further increase in their concentration when heat severity increased during the UHT processing was also observed (Contarini et al., 1997; Vazquez-Landaverde et al., 2005).

HP treatment had no significant effect on the total ketone content of skimmed CM. Their levels in all HP-treated CM samples at various pressures were close to those of the raw milk. 2-decanone was detected in HP-treated CM samples and its concentration showed an increase

with increasing pressure from 200 to 800 MPa. Similar observations for bovine milk were reported by Vazquez-Landaverde et al. (2006a) who found that HP treatment at 25°C had no major effect on the formation of methyl ketones in whole bovine milk.

5.3.2.8. Sulphur compounds

Dimethyl disulphide and dimethyl trisulphide were only detected in the UHT CM at low levels (**Table 5.3**). However, their concentrations in the UHT CM were more than double of their contents in the UHT skimmed bovine milk (Appendix 2). These two sulphur compounds can be formed in heated milk as result of the decomposition of milk proteins containing sulphur-containing amino acids and Maillard reactions (Al-Attabi, D'arcy, & Deeth, 2009). They have strong and unpleasant cabbage, sulphur-like aroma with very low sensory threshold values (0.16 and 0.008µg L in water, respectively), thus any small change in their concentration could affect milk aroma (Calvo & Hoz, 1992). They were also detected in the raw, pasteurised and UHT skimmed bovine milk and their concentrations were associated with the intensity of heat treatment. (Zahir Al-Attabi et al., 2014; Contarini et al., 1997; Vazquez-Landaverde, Torres, & Qian, 2006b). Dimethyl trisulphide was strongly linked to the sulphurous flavour of the UHT bovine milk and considered to be a major contributor to milk flavour (Vazquez-Landaverde et al., 2006b). In general, sulphur compounds were reported to be responsible for the "cooked" off-flavour defect of heated bovine milk and (Valero et al., 2001).

5.3.2.9. Terpenes

D-limonene (citrus, fresh, and sweet odour) was the only terpene detected in the raw CM. The origin of this compound in milk was linked to the plants eaten by the animals, particularly from dicotyledonous mixtures in highland pasture (Toso et al., 2002; Villeneuve et al., 2013). Its concentration in CM was significantly increased after HTST pasteurisation (**Figure 5.1H**).

Table 5 3. Approximate quantities of volatile compounds identified in raw and processed skimmed camel milk at 72°C, 15sec (HTST), 140°C, 5sec (UHT), and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20°C.

Code	Volatile compounds (µg /L)	Treatments										
		LRI[a]	ID[b]	Raw	HTST	UHT	HP200	HP400	HP600	HP800	*LSD*[c]	*P*[d]
	Aldehydes											
a01	Pentanal	**697**	RI, MS	1.97^{c}	7.30^{ab}	8.06^{a}	2.06^{c}	3.11^{c}	4.58^{abc}	3.77^{bc}	2.10	**
a02	Hexanal	**800**	RI, MS	126.72^{c}	448.37^{b}	724.49^{a}	155.03^{c}	199.47^{c}	149.44^{c}	169.92^{c}	66.79	**
a03	(E)-2-Hexenal	**853**	RI, MS	2.66^{ab}	5.78^{a}	2.56^{ab}	1.06^{b}	2.72^{ab}	2.72^{ab}	1.10^{b}	2.12	*
a04	Heptanal	**901**	RI, MS	-	446.34^{a}	417.73^{a}	-	-	-	-	54.23	**
a05	(E)-2-Heptenal	**957**	RI, MS	8.78^{b}	21.18^{a}	7.37^{b}	4.65^{b}	9.53^{b}	4.98^{b}	5.78^{b}	3.88	**
a06	Octanal	**1003**	RI, MS	58.07^{b}	222.85^{a}	248.93^{a}	33.48^{b}	33.18^{b}	29.36^{b}	30.91^{b}	33.21	**
a07	(E)-2-Octenal	**1059**	RI, MS	30.59^{b}	64.50^{a}	21.47^{b}	28.07^{b}	33.15^{b}	23.78^{b}	20.35^{b}	11.37	**
a08	Nonanal	**1103**	RI, MS	173.32^{c}	422.30^{b}	766.01^{a}	154.25^{c}	148.79^{c}	127.48^{c}	124.53^{c}	105.86	**
a09	(E,E)-2,6-Nonadienal	**1154**	RI, MS	-	2.82^{a}	-	-	-	-	-		
a10	(E)-2-Nonenal	**1160**	RI, MS	50.24^{bc}	96.17^{b}	275.61^{a}	37.16^{c}	39.34^{c}	31.29^{c}	14.42^{c}	33.65	**
a11	Decanal	**1205**	RI, MS	4.78^{ab}	8.10^{a}	3.28^{b}	2.75^{b}	3.79^{ab}	2.67^{b}	2.58^{b}	2.70	*
a12	[E,E]-2,4-Nonadienal	**1216**	RI, MS	3.31^{ab}	5.51^{a}	-	2.59^{ab}	1.54^{ab}	1.58^{ab}	1.05^{ab}	3.12	ns
a13	(E)-2-Decenal	**1262**	RI, MS	28.54^{ab}	46.28^{a}	12.47^{bc}	20.89^{bc}	27.99^{ab}	20.02^{bc}	5.11^{c}	12.21	**
a14	(E)-2-Undecenal	**1363**	RI, MS	13.52^{ab}	19.42^{a}	6.18^{b}	11.65^{ab}	10.96^{ab}	10.98^{ab}	9.55^{b}	5.87	*
	Alcohols											
b01	1-Pentanol	**763**	RI, MS	6.95^{a}	5.55^{a}	4.19^{a}	7.64^{a}	7.41^{a}	6.66^{a}	6.93^{a}	5.04	ns
b02	1-Hexanol	**866**	RI, MS	111.34^{ab}	179.23^{a}	9.38^{b}	123.57^{a}	124.48^{a}	109.76^{ab}	141.61^{a}	60.92	**
b03	1-Heptanol	**967**	RI, MS	77.51^{bc}	38.47^{c}	42.37^{c}	111.64^{ab}	137.80^{a}	122.03^{ab}	122.65^{ab}	27.14	**
b04	1-Octen-3-ol	**978**	RI, MS	12.02^{b}	13.83^{b}	20.38^{a}	10.85^{b}	10.19^{b}	11.64^{b}	10.26^{b}	2.62	**
b05	2-ethyl-1-Hexanol	**1027**	RI, MS	4.67^{a}	2.83^{a}	3.75^{a}	4.84^{a}	3.31^{a}	1.88^{a}	1.84^{a}	2.62	ns
b06	1-Octanol	**1068**	RI, MS	72.06^{b}	59.74^{b}	11.34^{c}	103.65^{a}	113.37^{a}	107.74^{a}	116.93^{a}	15.48	**
b07	(E)-2-Nonen-1-ol	**1167**	MS	7.08^{bc}	-	-	22.27^{ab}	30.88^{a}	34.50^{a}	31.60^{a}	11.38	**
b08	1-Nonanol	**1169**	RI, MS	33.83^{bc}	5.63^{c}	5.14^{c}	70.07^{abc}	113.64^{a}	108.92^{a}	101.79^{ab}	41.89	**
b09	2-Decen-1-ol	**1267**	MS	0.30^{c}	-	-	14.34^{b}	-	17.85^{ab}	20.35^{a}	2.14	**

	Acids											
c01	Acetic acid	**621**	MS	6.19^{a}	3.40^{ab}	1.03^{b}	3.77^{ab}	2.90^{b}	2.07^{b}	1.16^{b}	1.78	**
c02	2-Oxooctanoic acid	**1080**	MS	-	3.08^{a}	0.14^{b}	-	-	-	-	0.20	**
c03	Decanoic acid	**1353**	MS	0.18^{c}	-	-	0.22^{c}	0.28^{bc}	3.01^{a}	2.34^{ab}	1.48	**
c04	Dodecanoic acid	**1550**	MS	-	-	-	-	0.14^{b}	2.10^{ab}	3.18^{a}	1.70	*
	Esters											
d01	Methyl acetate	**524**	MS	11.10^{c}	22.13^{b}	39.42^{a}	2.90^{d}	1.73^{d}	4.90^{cd}	2.74^{d}	3.88	**
d02	Butyl acetate	**813**	MS	3.75^{a}	-	-	2.09^{a}	1.22^{a}	1.51^{a}	2.43^{a}	4.59	ns
d03	Methyl hexanoate	**923**	MS	4.04^{b}	1.70^{b}	-	23.62^{a}	23.81^{a}	23.26^{a}	25.11^{a}	3.63	**
d04	Hexyl acetate	**1010**	MS	2.61^{b}	28.07^{a}	-	2.39^{b}	1.65^{b}	1.60^{b}	3.33^{b}	5.67	**
d05	Ethyl 2-methylbutanoate	**1084**	MS	23.42^{a}	32.11^{a}	31.29^{a}	25.43^{a}	24.27^{a}	25.20^{a}	26.37^{a}	12.22	ns
d06	Methyl octanoate	**1122**	MS	4.18^{ab}	-	-	18.21^{ab}	18.27^{ab}	19.22 ^{ab}	24.07^{a}	13.96	*
d07	(Z)-3-hexenyl butanoate	**1184**	MS	-	1.79	-	-	-	-	-		
d08	2-Heptanol, acetate	**1187**	MS	16.64^{b}	21.27^{b}	38.91^{a}	20.26^{b}	19.35^{b}	21.22^{b}	23.43^{ab}	10.32	*
d09	Methyl 4-methyloctanoate	**1221**	MS	-	-	-	-	-	1.30^{a}	0.94^{a}	1.01	ns
d10	2-Ethylhexyl formate	**1236**	MS	-	-	1.40	-	-	-	-		
d11	4-Nitrophenyl nonanoate	**1278**	MS	2.56^{a}	-	-	3.56^{a}	2.67^{a}	3.10^{a}	2.46^{a}	0.83	ns
d12	Methyl decanoate	**1319**	MS	-	-	-	-	7.61^{a}	6.36^{a}	7.09^{a}	0.95	**
d13	Butyl butanoate	**1379**	MS	3.30^{b}	2.63^{b}	20.61^{a}	2.06^{b}	1.86^{b}	2.01^{b}	1.55^{b}	1.30	**
d14	Octyl thiocyanate	**1393**	MS	-	9.57^{a}	-	9.47^{a}	-	10.17^{a}	11.97^{a}	3.27	ns
d15	Methyl dodecanoate	**1518**	MS	-	-	-	2.62^{a}	3.29^{a}	3.29^{a}	2.27^{a}	3.55	ns
d16	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	**1601**	MS	-	1.48^{a}	2.94^{a}	-	-	-	-	2.55	ns
	Furans											
e01	2-ethyl-Furan	**702**	RI, MS	-	-	2.11	-	-	-	-		
e02	2-pentyl-Furan	**993**	RI, MS	19.08^{b}	48.54^{ab}	68.38^{a}	33.94^{b}	35.08^{b}	35.93^{b}	45.55^{ab}	18.57	**
e03	2-n-Octylfuran	**1294**	MS	-	-	2.17	-	-	-	-		
	Hydrocarbons											
f01	n-Hexane	**599**	MS	7.36^{a}	4.44^{a}	5.76^{a}	5.54^{a}	7.78^{a}	5.55^{a}	5.23^{a}	2.35	ns
f02	1-Heptene	**689**	RI, MS	6.51^{a}	1.44^{b}	1.00^{b}	6.23^{a}	6.09^{a}	5.31^{a}	7.72^{a}	1.92	**
f03	Heptane	**700**	MS	7.22^{a}	4.51^{ab}	1.50^{b}	5.08^{ab}	8.95^{a}	8.16^{a}	4.54^{ab}	1.682	**

f04	Toluene	**769**	RI, MS	356.84^{ab}	265.63^{b}	404.69^{a}	308.56^{ab}	287.24^{b}	250.96^{b}	278.63^{b}	75.07	*
f05	1-Octene	**791**	RI, MS	7.61^{abc}	5.95^{bc}	3.61^{c}	9.60^{ab}	11.07^{a}	10.08^{ab}	10.08^{ab}	2.66	**
f06	1,3-Dimethylbenzene	**873**	RI, MS	-	-	2.71^{a}	-	-	-	-		
f07	Styrene	**895**	RI, MS	-	-	43.79^{a}	-	-	-	-		
f08	Nonane	**900**	MS	257.30^{a}	-	-	182.07^{ab}	223.91^{a}	187.76^{ab}	103.77^{bc}	73.07	**
f09	Decane	**1000**	MS	11.37^{a}	7.71^{a}	9.84^{a}	5.95^{a}	7.21^{a}	6.07^{a}	5.46^{a}	5.13	ns
f10	2,2,4,6,6-Pentamethyl-3-heptene	**1010**	RI, MS	-	-	31.18	-	-	-	-		
f11	Undecane	**1098**	MS	12.04^{a}	5.89^{b}	14.67^{a}	4.68^{b}	5.64^{b}	5.13^{b}	5.20^{b}	1.58	**
f12	3-Dodecyne	**1130**	MS	-	2.67	-	-	-	-	-		
f13	Tridecane	**1393**	MS	-	-	3.32	-	-	-	-		
f14	Hexadecane	**1457**	MS	-	-	1.23	-	-	-	-		
f15	Pentadecane	**1494**	MS	1.70^{a}	1.96^{a}	1.76^{a}	1.92^{a}	1.72^{ab}	1.84^{a}	1.82^{a}	1.13	ns
	Ketones											
g01	2,3-Pentanedione	**692**	RI, MS	-	1.70^{a}	-	-	-	-	-		
g02	2-Heptanone	**890**	RI, MS	10.85^{ab}	5.0^{b}	16.54^{a}	11.82^{ab}	11.14^{ab}	12.12^{ab}	14.46^{ab}	5.77	*
g03	6-Methyl-2-heptanone	**955**	RI, MS	6.14^{ab}	8.59^{a}	5.31^{b}	5.22^{b}	5.36^{b}	5.07^{b}	5.31^{b}	1.62	*
g04	2,3-Octanedione	**982**	RI, MS	22.93^{a}	27.94^{a}	25.81^{a}	25.88^{a}	27.28^{a}	18.69^{a}	23.95^{a}	8.79	ns
g05	3-Octanone	**986**	RI, MS	17.40^{a}	-	2.98^{bc}	11.98^{a}	11.11^{ab}	12.70^{a}	12.04^{a}	5.18	**
g06	2-Nonanone	**1090**	RI, MS	24.31^{ab}	5.92^{b}	13.95^{ab}	29.76^{a}	24.38^{a}	29.21^{a}	26.33^{a}	11.60	*
g07	2-Decanone	**1191**	MS	-	-	2.95^{d}	7.96^{c}	14.75^{b}	17.62^{b}	25.11^{a}	2.95	**
g08	2-Undecanone	**1290**	RI, MS	13.73^{ab}	19.43^{ab}	3.68^{b}	22.92^{ab}	20.58^{ab}	23.67^{ab}	24.69^{a}	11.99	*
	Sulphurs											
h01	Dimethyl disulphide	**746**	RI, MS	-	-	0.19	-	-	-	-		
h02	Dimethyl trisulphide	**978**	RI, MS	-	-	0.23	-	-	-	-		
	Terpenes											
i01	α-Pinene	**940**	RI, MS	-	8.32^{a}	1.17^{c}	-	-	-	3.31^{b}	0.81	**
i02	D-Limonene	**1035**	RI, MS	36.26^{b}	260.02^{a}	53.28^{b}	9.32^{b}	3.49^{b}	4.37^{b}	11.49^{b}	38.86	**

[a] Linear retention index (LRI) of identified compounds on DB-5 capillary column calculated against the GC/MS retention time of n-alkanes (C6–C33). [b] LRI matching with retention index of authentic compounds; MS, compared with Nist11.L Mass Spectral Database. [c] Least significant difference at $p = 0.05$. d Means in the same row followed by different letters differ significantly at the 5% level. ns, no significant difference between means ($p > 0.05$); * $P<0.05$; ** $P< 0.01$. Values are means of duplicate analysis from two independent experiments. (-) Not detected. Estimated quantities in the headspace from 5 mL of milk, calculated by comparison with 130.6 µg/mL of 1,2-dichlorobenzene used as internal standard.

α-Pinene, which imparts herbal aroma was only detected in the pasteurised, UHT, and HP treated CM at 800 MPa. These two terpenes (D-limonene and α-pinene) have been detected in raw whole bovine milk, and D-limonene was the predominant one (Toso et al., 2002). The level of D-limonene in reduced-fat and skimmed bovine milk was found to be significantly increased after pasteurisation (Gandy et al., 2008; Hougaard et al., 2011; Li, Zhang, & Wang, 2013) and UHT treatments (Contarini et al., 1997). However, the concentrations of D-limonene and α-pinene in pasteurised and UHT skimmed bovine milk were lower than in CM samples (Appendix 2). Moreover, β-pinene (herbal notes) was only identified in the UHT bovine milk. Ultra-high-pressure homogenisation treatment of whole bovine milk at pressures 200 and 300 MPa and temperature 30–40^{o}C were also found to cause and increase in the content of D-limonene in milk (Pereda et al., 2008). However, none of the previous studies in the literature provided an explanation about the mechanism of terpenes formation under those industrial treatments or their contribution to milk aroma.

Principal component analysis (PCA) was performed on all the six processed CM samples (HTST, UHT, HP treatments at 200, 400, 600, and 800MPa) (**Figure 5.2**). The first two principal components accounted for 84.99 % of the variation in the data. Principal component 1 (PC1) displayed 52.18% of the variation and principal component 2 (PC2) displayed 32.81%. PC1 separated the pasteurised and UHT milk samples from the raw and HP-treated CM, while PC2 separated the raw milk from the HP treated CM. No obvious separation between the HP-treated CM samples at different pressures was observed on both PC1 and PC2, whereas, the pasteurised CM sample was clearly separated from the UHT milk sample on the PC2.

The distribution of the variables showed that the composition of volatile compounds in processed CM was different from the composition of volatile compounds in the untreated CM as it shown in **Figure 5.2B**. Most of aldehydes (a01, a02-a13), esters (d01, d04, d07, d08, d10, d13), all furans (e01, e02, e03), hydrocarbons (f04, f06, f07, f10, f11, f12, f13, f14), all sulphur

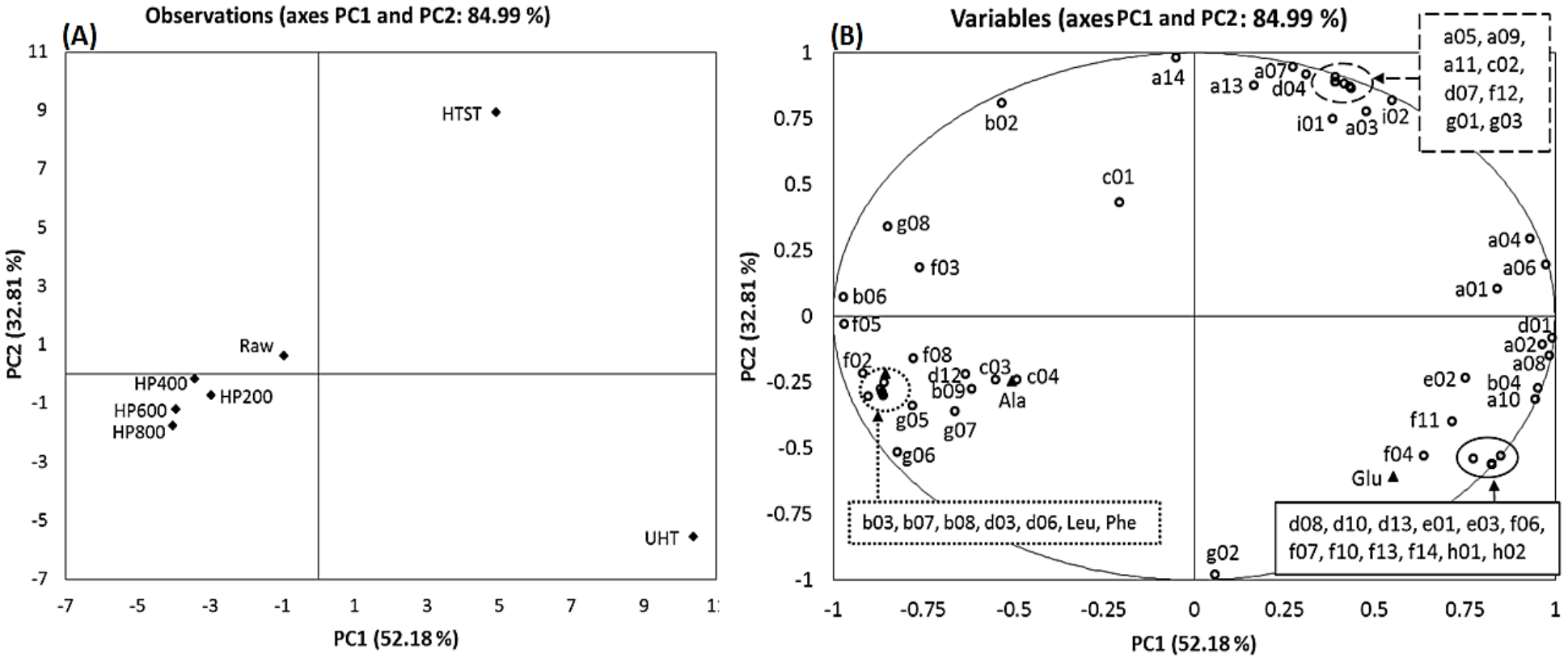

Figure 5 2. Principal component analysis of processed camel milk showing correlation between volatile (○) and non-volatile compounds (▲). (A) Projection of camel milk samples subjected to HTST (72ºC, 15sec), UHT (140ºC, 5sec) and High-Pressure (HP) at 200, 400, 600, 800 MPa for 30 min at 20ºC. (B) Distribution of variables (codes on plot refer to compound codes in Table 5.2 and 5.3).

compounds (h01, h02), and terpenes (i01, i02) were positively correlated with the PC1. Whereas, (E)-2-undecenal (a14), 1-hexanol (b02), acetic acid (c01), 2-undecanone (g08), heptane (f03), and 1-octanol (b06) were positively correlated with the PC2.

Both heat treatments were positively correlated with the first axis. UHT CM was characterised by the high level of glutamic acid (Glu) and some aldehydes (hexanal, nonanal, (E)-2-nonenal) with great number of esters, hydrocarbons, furans, and sulphur compounds which were only present in UHT milk. Pasteurised milk was distinguished by a greater number of aldehydes, some esters, and all terpene compounds. In contrast, all HP-treated CM samples were negatively correlated with both PC1 and PC2. Thus, the levels of aldehydes, esters, and furans in HP-treated milk samples were lower than in the HTST and UHT milk. Moreover, sulphur compounds were not detected in any of the HP treated milk samples. However, they were characterised by having more alcohols, acids, and ketones as well as amino acids including alanine (Ala), leucine (Leu), and phenylalanine (Phe). In addition, all HP-treated CM samples were close to the raw milk sample, which indicates that HP treatments had minimal effect on the composition of volatile compounds in CM comparing to the heat treatments.

5.3.3. Sensory properties of heat treated camel milk in comparison with bovine milk

Compositional and quantitative differences were observed between the pasteurised and UHT-treated milk samples from both camel and bovine milk in terms of volatile and non-volatile compounds. The PCA of the four heat treated milk samples (HTST, UHT camel milk, pasteurised and UHT bovine milk) and the 74 variables (58 volatile compounds and 16 non-volatile compounds) showed that principal components 1 and 2 accounted for 54.54 and 36.26% of the variability, respectively (**Figure 5.3**). The PCA reveals four distinct processed milk samples, separated according to milk type (CM vs bovine milk), and applied heat processing method (HTST pasteurisation and UHT treatment) as it shown in **Figure 5.3A**.

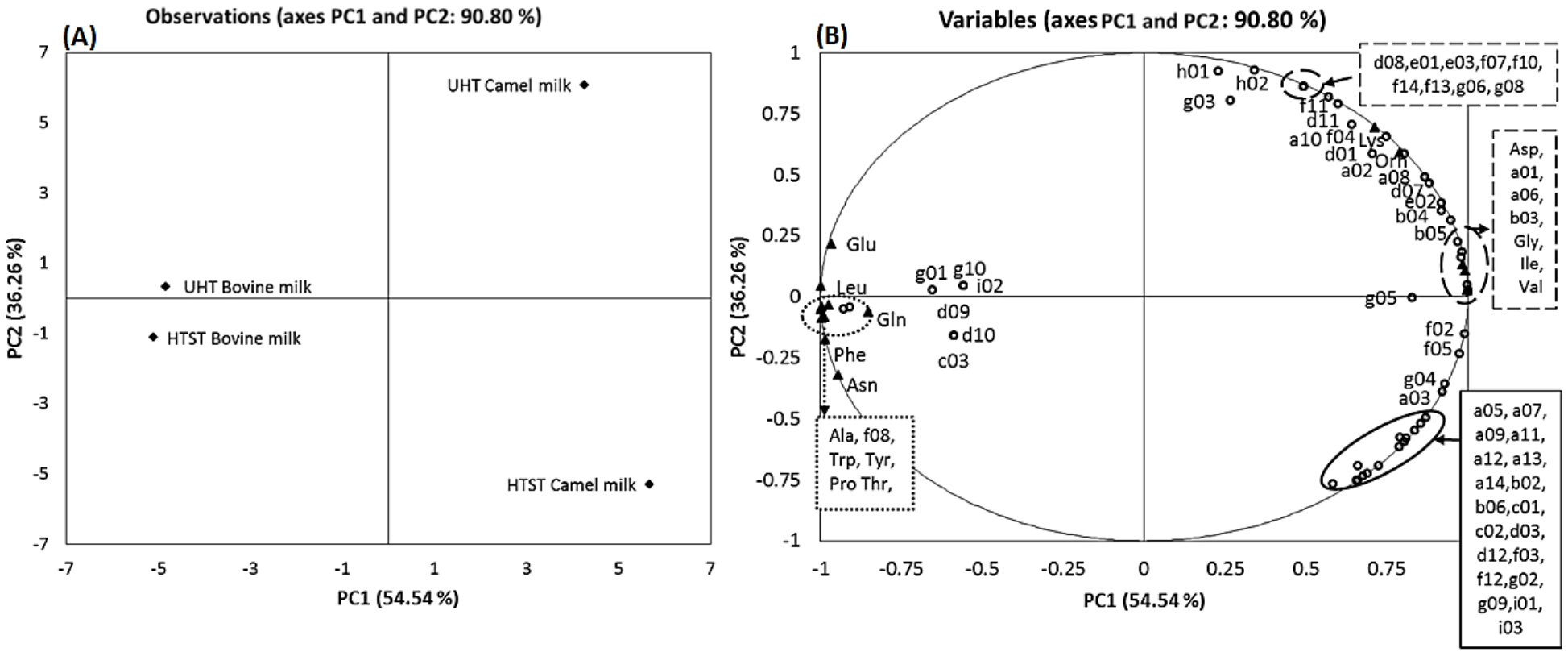

Figure 5 3. Principal component analysis of heat treated camel and bovine milk showing correlation with volatile (○) and non-volatile compounds (▲). (A) Projection of camel and bovine milk samples subjected to HTST (72ºC, 15sec) and UHT (140ºC, 5sec) treatments. (B) Distribution of variables: c03, n-Decanoic acid; d09, Methyl butyrate; d10, Methyl decanoate; g01, Acetone; rest of codes on plot refer to compound codes in Table 5.2 and 5.3.

It can be seen that HTST and UHT CM samples were clearly separated from the bovine milk along the PC1 mainly due to the contribution of the variables, most of them were positively associated with the PC1 (**Figure 5.3B**). Furthermore, the HTST pasteurised milk samples (camel and bovine) were discriminated from the UHT treated milk samples on PC2. Similar observations were reported by Contarini and Povolo (2002) on bovine milk subjected to different heat treatments (pasteurisation , UHT, and "in-bottle" sterilization). The authors found that the concentration of volatile compounds particularly methyl ketones, 2-heptanone played an important role in the separation between the UHT and pasteurised milk.

HTST pasteurised CM was positively correlated with the PC1 and distinguished by having high amounts of aldehydes (a03, a05, a07, a09, a11, a12, a13, a14), alcohols (b02, b06), acids (c01, c02), esters (d03, d12), hydrocarbons (f02, f03, f05, f012), ketones (g02, g04, g09), and terpenes (i01, i03). Whereas, pasteurised bovine milk sample was negatively associated with the PC1. Therefore, it had lower amounts of these compounds and higher concentrations of methyl octnouate (d05), methyl butyrate (d09), methyl decanoate (d10), nonane (f08), n-decanoic acid (c03), and amino acids including alanine (Ala), proline (Pro), asparagine (Asn), phenylalanine (Phe), glutamine (Gln), tyrosine (Tyr), and tryptophan (Trp). UHT camel and bovine milk samples were positively associated with both PC1 and PC2 respectively. However, the distance between the UHT and pasteurised bovine milk was very short compared to the distance between the UHT and pasteurised CM, which may suggest that the effects of these heat treatments on the composition of volatile compounds were less pronounced in bovine milk than in CM. UHT CM was characterised by greater levels of aldehydes (a01, a02, a06, a08, a10), alcohols (b03, b04, b05), esters (d01, d07, d08, d11), hydrocarbon (f04, f07, f10, f11, f13, f14), furans (e01, e02, e03), sulphur compounds (h01, h02), and non-volatile compounds such as lysine (Lys), ornithine (Orn), glycine (Gly), isoleucine (Ile), and valine (Val). On the contrary, two ketones acetone (g01), 2-tridecanone (g10), one terpene β-pinene (i02), glutamic

acid (Glu), leucine (Leu) were positively associated with the UHT bovine milk. These results explained that the effect of HTST pasteurisation and UHT treatment on volatiles generation in CM was greater than in bovine milk.

The mean panel scores for the descriptive sensory attributes for the HTST pasteurised and UHT CM samples compared with bovine milk are shown in **Table 5.4**. The results showed that 40 out of 44 attributes were found to be significantly different ($p < 0.05$) between the four milk samples as determined by ANOVA. Both, pasteurised and UHT CM were distinguished by white colour, and higher scores for separation, watery, and powdery attributes especially for the UHT CM. Whereas, pasteurised and UHT bovine milk samples were characterised by yellow and creamy colour, as well as, higher rating for body and opaque than CM.

In relation to odour attributes, the intensity of odour of CM samples was significantly higher ($p < 0.05$) than the bovine milk, hence confirming the GC–MS results, where the concentrations of most volatile compounds were significantly higher in CM. Moreover, other attributes including butyric, powdery, savoury, sour, cardboard, goat, dry, musty, sulphur received the highest scores in the pasteurised and UHT CM. Only dairy and cooked odours were found to be perceived higher for the pasteurised and UHT bovine milk compared to CM. These observations confirmed the quantitative results of the GC–MS, in which the levels of acetic acid, which imparts the sour odour, and 2-pentyl-furan which imparts the musty flavour were significantly higher in both heat-treated CM samples.

In addition, the GC–MS data showed that the concentrations of aldehydes including pentanal, hexanal, heptanal, and nonanal, sulphur compounds were significantly greater in CM than in bovine milk. These compounds were reported to be responsible for the cardboard flavour especially in the presence of dimethyl trisulphide (Whitson, Miracle, & Drake, 2010).

Table 5 4. Mean values of panel scores for 44 sensory descriptors for HTST (72°C, 15sec) and UHT (140°C, 5sec) camel and bovine skimmed milk.

Cod	Sensory descriptor	Treatments				*LSD*[c]	*P*[d]
		Camel milk		Bovine milk			
		HTST	UHT	HTST	UHT		
Appearance:							
A01	Yellow colour	0.03[c]	0.99[bc]	8.29[ab]	14.24[a]	8.23	**
A02	Creamy colour	0.38[b]	3.63[b]	28.06[a]	30.83[a]	20.28	**
A03	Body	28.83[bc]	19.33[c]	36.03[ab]	42.66[a]	10.43	***
A04	Opaque	77.12[b]	56.53[c]	84.13[a]	87.90[a]	6.91	***
A05	White	76.62[a]	43.53[b]	16.84[c]	11.61[c]	26.60	***
A06	Separation	18.67[b]	64.50[a]	5.90[bc]	4.24[c]	13.07	***
A07	Watery	34.57[b]	62.00[a]	17.64[c]	12.41[c]	15.39	***
A08	Powdery	18.14[ab]	33.32[a]	11.19[b]	4.88[b]	15.31	**
Odour:							
O01	Intensity	53.93[a]	63.31[a]	13.64[b]	19.34[b]	13.63	***
O02	Butyric	17.26[a]	15.94[a]	0.68[b]	2.27[b]	13.08	*
O03	Powdery	22.02[a]	18.43[a]	4.83[b]	3.92[b]	13.59	*
O04	Dairy	9.00[bc]	7.70[c]	22.46[ab]	31.14[a]	13.50	**
O05	Savoury	27.02[a]	33.17[a]	3.30[b]	4.81[b]	12.49	***
O06	Cooked milk	7.11[b]	8.94[b]	13.39[ab]	24.12[a]	15.18	*
O07	Sour	11.99[ab]	16.10[a]	2.90[c]	4.69[bc]	8.56	**
O08	Cardboard	10.12[a]	6.90[ab]	0.27[b]	0.04[b]	8.98	*
O09	Goat	28.30[a]	32.85[a]	0.03[b]	0.02[b]	17.09	***
O10	Dry	13.60[a]	11.74[ab]	2.51[bc]	0.93[c]	9.64	**
O11	Musty	8.37[a]	10.38[a]	0.62[b]	0.43[b]	5.90	***
O12	Sulphur	5.25[a]	16.66[a]	0.01[b]	0.04[b]	15.26	*
Taste/ Flavour:							
T01	Sweet	24.01[a]	23.54[a]	29.68[a]	29.06[a]	12.28	ns
T02	Bitter	8.54[a]	9.66[a]	1.49[b]	0.97[b]	6.17	*
T03	Salt	13.29[a]	12.90[a]	2.86[b]	2.88[b]	7.24	**
T04	Sour	14.93[a]	14.33[a]	7.05[b]	7.35[b]	6.97	*
T05	Metallic	5.55[a]	6.57[a]	2.47[a]	3.84[a]	4.50	ns

T06	Savoury	38.80[a]	35.82[a]	5.49[b]	4.78[b]	17.24	***
T07	Aged	29.71[a]	39.68[a]	0.02[b]	0.01[b]	17.64	***
T08	Cheesy	12.73[a]	11.35[a]	1.31[b]	2.23[b]	8.31	*
T09	Creamy	4.41[b]	6.89[b]	20.54[ab]	36.96 [a]	16.82	***
T10	Whey	22.32[a]	23.65[a]	4.50[b]	2.72[b]	13.34	**
T11	Dairy	5.75[b]	7.70[b]	31.72[a]	35.58[a]	16.11	***
Mouthfeel:							
MF01	Watery	42.63[a]	40.53[a]	24.88[ab]	20.86[b]	18.03	*
MF02	Mouth coating	43.45[a]	51.10[a]	24.32[b]	27.76[b]	11.99	***
MF03	Drying	42.98[a]	44.84[a]	21.18[b]	21.22[b]	11.75	***
MF04	Powdery	30.72[a]	40.32[a]	9.30[b]	9.06[b]	11.45	***
MF05	Body	36.64[a]	40.39[a]	28.19[a]	35.66[a]	12.74	ns
MF06	Tooth coating	14.40[ab]	27.03[a]	4.89[b]	4.69[b]	16.13	*
Aftereffects:							
AF01	Dairy	7.95[b]	9.58[b]	33.12[a]	38.58[a]	13.54	***
AF02	Sweet	17.96[a]	15.35[a]	19.81[a]	19.84[a]	7.16	ns
AF03	Savoury	30.00[a]	28.50[a]	4.43[b]	5.60[b]	15.35	***
AF04	Salt	10.91[a]	11.45[a]	2.85[b]	2.94[b]	5.84	**
AF05	Sour	11.46[a]	10.45[ab]	5.51[b]	7.11[ab]	5.12	*
AF06	Animal	21.07[a]	27.08[a]	0.01[b]	0.05[b]	15.61	***
AF07	Lingering	42.62[a]	45.73[a]	22.21[b]	25.44[b]	10.23	***

[c] Least significant difference at $p = 0.05$

[d] Means in the same row followed by different letters differ significantly at the 5% level. ns, no significant difference between means ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

There was no significant difference ($p > 0.05$) among all heat processed milk samples (camel and bovine milk) in terms of sweet and metallic taste. However, other taste/flavour descriptors including bitter, salt, sour, savoury, aged, cheesy, and whey were scored significantly higher in the pasteurised and UHT CM samples than in bovine milk. Whilst, pasteurised and UHT bovine milk samples were characterized by higher scores for creamy and dairy flavour. Significant differences regarding mouthfeel descriptors were also found between the camel and bovine milk samples. Pasteurised and UHT CM samples received higher mean scores for

watery, mouth, coating, drying, powdery, tooth coating than bovine milk. After swallowing, pasteurised and UHT CM samples were described as having significantly less intensities of dairy, but more persistence of savoury, salt, sour, animal, and lingering in the aftereffects than bovine milk.

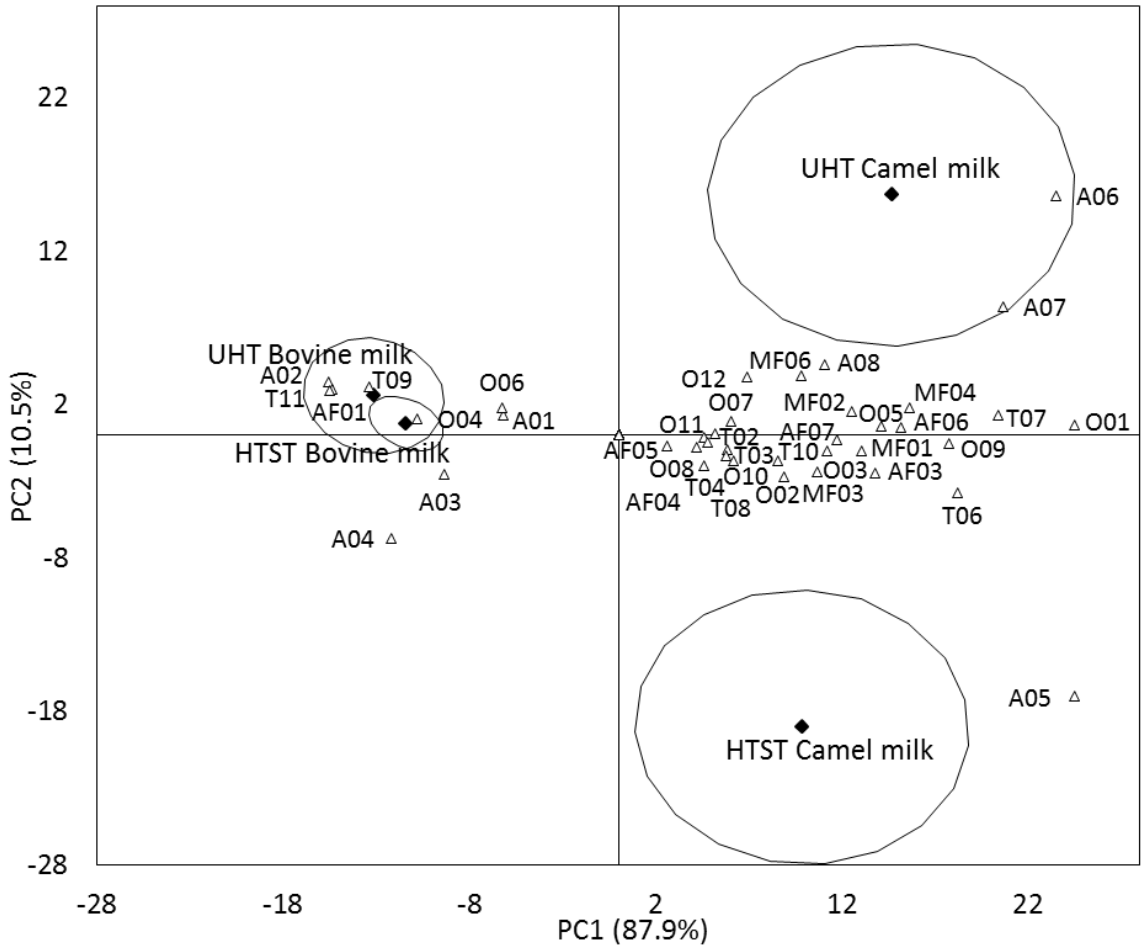

Figure 5 4. Principal component biplot of sensory data from evaluation of HTST (72ºC, 15sec) and UHT (140ºC, 5sec) treated camel and bovine milk samples (codes on plot refer to compound codes in Table 5.4).

The sensory attributes of all milk samples analysed by PCA (**Figure 5.4**) revealed that the first and second principal components described 98.4 % of the total variance in the data. The separation between the camel and bovine milk samples along the first axis (87.9% variance) was primarily related to the type of milk. On the second axis (explaining 10.5% of total

variance) pasteurised CM was clearly distinguished from the UHT milk, whereas pasteurised and UHT bovine milk samples were very close and positively associated with PC2, indicating that their sensory properties were less affected by heat treatments compared with CM samples. These observations confirm the quantitative results of the GC–MS. The PCA demonstrated that the effect of HTST and UHT treatments on the sensory properties of CM was greater than of bovine milk.

5.3.4. Correlation of volatile of non-volatile compounds with sensory properties

Multiple factor analysis (MFA) was performed to determine the correlation between volatile, non-volatile compounds and sensory descriptors. In this analysis, all variables were simultaneously analysed as active variables, rather than analysing only one set of variables and considering the rest as supplementary variables (Pagès, 2004). The first two dimensions of the MFA accounted for 94.17% of the total variance of data as shown in **Figure 5.5A** and **B**. Both HTST pasteurised and UHT CM samples were positively correlated with most of the variables (volatile and non-volatile) on the first dimension of the MFA. These variables were then highly related with sensory descriptors such as sour, salt, savoury, aged, whey taste/ flavour, as well as, musty odours. UHT CM sample was clearly separated from the pasteurised milk along the second dimension, and correlated with sulphur compounds (h01, h02) which were strongly associated with sulphur odour in processed milk.

On the other side of the MFA map, pasteurised and UHT bovine milk samples were located near to each other in the negative side of the first dimension. They were negatively correlated with most of the variables (volatiles) and differentiated from CM samples in terms of sensory attributes by having a strong cooked and dairy taste/flavour. Based on the MFA map it can be said that the quantitative results of the GC–MS were correlated well with the sensory data. In addition, most of these variables and sensory attributes were highly associated with pasteurised

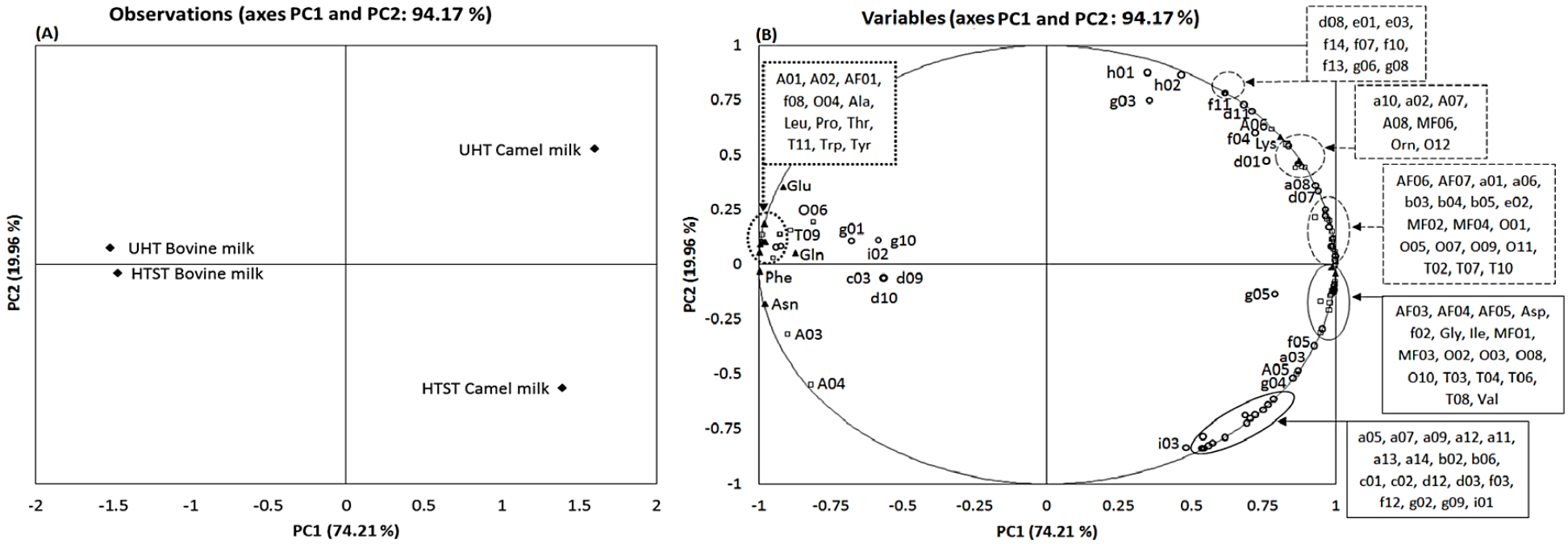

Figure 5 5. Multiple factor analysis: (A) Representation of camel and bovine milk samples subjected to HTST (72ºC, 15sec) and UHT (140ºC, 5sec) treatments. (B) Distribution of variables: volatile (○), non-volatile compounds (▲), and sensory attributes (□). Codes on plot refer to c03, n-Decanoic acid; d09, Methyl butyrate; d10, Methyl decanoate; g01, Acetone; rest of codes on plot refer to compound codes in Tables 5.2, 5.3 and 5.4.

and UHT-treated CM, indicating that these heat treatments had significant effects on the properties of CM.

5.4. Conclusion

Both thermal (HTST and UHT) treatments resulted in an increase of aldehydes, furans, and terpenes in CM which contributed to the off-flavour in milk. The UHT processing had the biggest effect on the aroma profile of CM, and led to the formation of sulphur compounds (sulphur-like aroma) in processed CM. In contrast, HP treatments (200 to 800 MPa, at 20^{o}C, for 30min) increased content of alcohols and ketones in CM.

Descriptive analysis was used to reveal the sensory profiles of the HTST and UHT processed CM and were compared to the sensory profiles of commercially available pasteurised and UHT bovine milk. Pasterised and UHT CM exhibited higher levels of volatile compounds particularly aldehydes, hydrocarbons, and sulphur compounds than bovine milk, and were described by the assessors as having attributes such as cardboard, musty, sulphur odours, as well as sour, savoury, aged, and whey taste/flavours. While, bovine milk samples were described as having cooked milk, creamy, and dairy aroma. Overall, the effects of HTST pasterisation and UHT treatment on the aroma and sensory properties of CM were markedly greater than in bovine milk, and they resulted in the formation of volatile compounds which were responsible for off-flavours in processed CM.

Chapter 6: Concluding remarks

In this research, quantitative and compositional aspects of dromedary CM proteins were studied. In addition, the impact of HTST, UHT and HP treatments, which are typically used in dairy industry, on different properties of skimmed CM was investigated in comparison with bovine milk.

Major variations were found, when identified caseins and whey proteins in CM were compared with their counterparts in bovine milk, in terms of both concentration and composition. These variations were found to have an impact on the processing characteristics of CM, and quality of dairy products made from CM.

The degree of denaturation of whey proteins and colour change in skimmed CM following HTST pasteurisation (72^{o}C for 15s) and UHT treatment (140^{o}C for 5s) was greater compared to bovine milk. Moreover, both heat treatments resulted in a significantly decrease in the size of casein micelles of CM, whereas, an increase in the micelles size of bovine milk was observed after UHT processing. The rennet coagulation properties of skimmed camel and bovine milk were significantly affected by the thermal treatments. HTST pasteurisation led to an increase in the RCT of camel and bovine milk, while UHT process impeded the coagulation of the milk from both species.

HP treatments (200, 400, 600, and 800 MPa, at 20^{o}C for 30 min) caused considerably less colour change, lower denaturation of whey proteins, and limited disruption in micelles size of skimmed CM compared with bovine milk. HP treatment at 200 and 400 MPa increased the RCT of CM, while treatment at pressures higher than 400 MPa impaired the rennet coagulation properties of skimmed CM. The highest G' value of CM coagulum was observed after HP treatment at 200 MPa. In contrary, the RCT of bovine skimmed milk was significantly shortened and the G' value was the highest after HP treatment at 200 MPa. HP treatments at

higher pressures (400, 600, and 800 MPa) resulted in an increase of the RCT and the final *G'* value was similar to untreated milk.

The quantitative and qualitative analysis of aroma volatile compounds by HS–SPME/ GC–MS showed that there were differences between the volatile profile of raw skimmed CM and the pasteurised, UHT, and HP-treated milk. UHT processing had the most severe impact on the aroma of CM and resulted in formation of sulphur compounds which are mainly responsible for the development of the "cooked" off-flavour defect in heated milk. The total concentration of aldehydes was the highest after thermal treatments. They could contribute much to the aroma of heated milk (stale flavour) because of their low sensory thresholds. Unlike thermal treatments, HP treatments favoured the formation of alcohols and ketones in CM. The concentrations of amino acids and lactose content remained unaffected under heat and pressure treatments.

The influence of heat treatments on the aroma and sensory characteristics of skimmed CM was more pronounced when compared with skimmed bovine milk. The concentration of most of the volatile compounds was higher in the HTST pasteurised and UHT CM samples than that in bovine milk samples. Panelists were able to clearly discriminate between CM versus bovine milk, as well as pasteurised vs UHT milk for both species. In the unstructured line evaluation, pasteurised and UHT CM samples obtained the highest scores for the odour and flavour attributes such as cardboard, musty, and sulphur odour, which seemed to have a strong influence on the milk's acceptability.

A correlation was also attempted to be established between analytical and sensory results. Based on the multiple factor analysis (MFA), The MFA analysis suggested that some compounds including pentanal, hexanal, octanal, nonanal, and (E)-2-nonena, 2-pentyl-furan, toluene, dimethyl disulphide, and dimethyl trisulphide were present in significantly higher

amounts in the pasteurised and UHT CM compared to bovine milk, and they were responsible for the sulphurous flavour of heated CM.

Overall, both thermal and non-thermal treatments affected constituents and functional properties of skimmed CM differently compared to bovine skimmed milk. Thus, the results of the current research support the hypothesis that the processing characteristics of CM differ from bovine milk under various industrial processing methods. Nevertheless, further studies are needed to assess these differences in greater detail.

Based on the results of this research, thermal treatments considerably affected many constituents and properties of CM and resulted in great change in its organoleptic qualities. In contrast, HP treatments had less effect on the components and aroma profile of CM even under severe conditions of pressure (600 and 800 MPa). Thus, it may offer an alternative to conventional heat treatments for the preservation of CM with minimum impact on its flavour.

6.1. Contribution to Knowledge

While there is abundance literature regarding the impact of heat and HP treatments on bovine milk, this is the first complete study concerning the effect of these processing methods on CM. The results of this research provide useful information for the food scientists and dairy processing industries which can be used to design and develop production of fresh CM and its dairy products such as cheese and yogurt and extend their shelf life. The contributions to scientific knowledge are summarized as follows:

- A reliable and rapid method for simultaneous separation and quantification of proteins in raw and processed CM using capillary electrophoresis technique was successfully developed.

- The impact of HTST pasteurisation and UHT processing on the colour change, whey proteins denaturation and casein micelles size of CM was investigated in comparison with HP treatments.
- The rennet coagulation properties of the HTST pasteurised, UHT and HP-processed CM was evaluated and compared with those from bovine milk.
- The effect of HTST pasteurised, UHT, and HP treatments on the aroma profile of CM was studied and compared with those from raw milk.

6.2. Limitations of the research

There are some limitations that need to be considered in the present research:

- Due to the long distance between production areas (arid and semi-arid regions of Africa and Asia) of CM and the place where this study took place (The UK), it was extremely difficult to obtain raw CM from those areas. Therefore, the current research was conducted on commercially available frozen raw CM in Europe, which was produced in camel dairy farm called Kamelenmelkerij Smits, a camel dairy farm in the Netherlands. As a result, information on the origin of the CM such as camel breed, stage of lactation, feeding conditions was not available in the current research. It is well known that the composition of CM is greatly varied according to age, stage of lactation, camel breeds, feeding conditions and geographical location. Therefore, further investigation is needed to fully understand the role of various factors influencing CM composition and its processing characteristics.
- The gross composition of raw CM was reported to be not changed or affected by freezing (Smits *et al.*, 2011). In this research the frozen raw CM samples were slowly thawed (at 4^{o}C overnight, 13h) and processed within a week of arrival to avoid the influence of frozen storage for long period. Nevertheless, the process of freezing and

thawing can lead to the alteration of milk constituents such as milk fat, solids-not-fat, total solids, protein stability, casein micelles, calcium caseinate phosphate (Weese et al., 1969). Thus, further research regarding unfrozen fresh CM is required.

- In this research, the impact of HP treatments on the physico-chemical properties and volatile and non-volatile compounds of skimmed CM were studied and compared to thermal treatments. However, it was not possible to evaluate the sensory quality attributes of the HP- treated CM samples due to limited ability of producing safe milk for human consumption by using the high-pressure processing unit in the pilot plant. Thus, it was not possible to investigate the correlation between the aroma and sensory properties of the HP treated CM.

6.3. Future studies

During this research, several interesting phenomena were observed regarding the impact of heat and HP treatments on CM properties. However, not all were explored fully due to limitations with respect to the time frame and scope of the study. The following can be explored further to fill in the gap of knowledge and provide interesting results regarding the processing characteristics of CM:

- It would be of interest to investigate the effect of heat and HP treatments on CM taking into consideration the impact of different factors such as stage of lactation, camel breeds and, feeding conditions and the milk composition.
- It would be worthwhile to study the effect of heat and HP treatments on casein micelles of CM, with the consideration of the effects of the level of soluble calcium and phosphate, as well as the concentration of colloidal calcium phosphate and the nature of its binding to casein.

- To establish the full commercial potential use of HP treatment for CM preservation, further research is required regarding the influence of HP treatments on the microbiological quality and sensory properties of CM, in comparison with conventional heat treatments.
- Further detailed study on the effect of HP treatment on the functional properties of CM proteins to be commercially used to improve the properties of its dairy products such cheese and yogurt.
- Further consumer research studies would be of great importance in order to truly understand consumer perceptions and evaluate the consumers acceptability of CM in different countries around the world.

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Appendix 1 Free amino acids and sugars in HTST (72ºC, 15sec) and UHT (140ºC, 5sec) camel and bovine skimmed milk

Cod	*Amino acids (µg /mL)*	Treatments				*LSD*[b]	*P*[c]
		Camel milk		Bovine milk			
		HTST	**UHT**	**HTST**	**UHT**		
Ala	Alanine	1.16[b]	1.18[b]	2.39[a]	2.35[a]	0.22	**
Gly	Glycine	8.29[a]	8.00[a]	5.23[b]	5.24[b]	0.43	**
Val	Valine	2.63[a]	2.66[a]	1.10[b]	1.03[b]	0.11	**
Leu	Leucine	0.39[b]	0.41[ab]	0.50[a]	0.50[a]	0.07	*
Ile	Isoleucine	0.94[a]	0.90[a]	0.51[b]	0.48[b]	0.17	**
Thr	Threonine	0.50[b]	0.50[b]	0.64[ab]	0.66[a]	0.10	*
Ser	Serine	0.54[a]	0.47[a]	0.58[a]	0.62[a]	0.12	ns
Pro	Proline	0.10[b]	0.25[b]	2.38[a]	2.31[a]	0.17	**
Asn	Asparagine	0.41[b]	0.25[b]	0.87[a]	0.80[a]	0.20	**
Asp	Aspartic acid	2.18[a]	2.21[a]	0.95[b]	1.18[b]	0.18	**
Met	Methionine	0.10[a]	0.05[a]	0.10[a]	0.11[a]	0.07	ns
Glu	Glutamic acid	9.50[c]	11.39[b]	14.64[a]	13.89[a]	0.87	**
Phe	Phenylalanine	0.15[b]	0.14[b]	0.25[a]	0.24[a]	0.03	**
Gln	Glutamine	0.22[b]	0.19[b]	0.48[b]	0.85[a]	0.21	**
Orn	Ornithine	0.25[b]	0.39[a]	0.17[bc]	0.15[c]	0.06	**
Lys	Lysine	1.23[ab]	1.69[a]	1.06[ab]	1.05[b]	0.46	*
His	Histidine	0.11[a]	0.12[a]	0.13[a]	0.13[a]	0.07	ns
Tyr	Tyrosine	0.06[c]	0.07[c]	0.22[b]	0.27[a]	0.02	**
Trp	Tryptophan	0.02[c]	0.04[c]	0.25[a]	0.22[b]	0.02	**
	Sugar (g/100mL)						
Lact	Lactose	2.75[ab]	2.91[ab]	3.26[a]	3.25[a]	0.22	ns

[b] Least significant difference at $p = 0.05$.

[c] Means in the same row followed by different letters differ significantly at the 5% level. ns, no significant difference between means ($p > 0.05$); * $P<0.05$; ** $P< 0.0001$.

Values are means of duplicate analysis from three independent experiments.

Appendix 2 Volatile compounds detected in HTST (72ºC, 15sec) and UHT (140ºC, 5sec) camel and bovine skimmed milk.

Cod	*Volatile compounds (µg/ L)*	LRI[a]	Treatments				*LSD*[b]	*P*[c]
			Camel milk		Bovine milk			
			HTST	UHT	HTST	UHT		
	Aldehydes							
a01	Pentanal	**697**	7.30[a]	8.06[a]	2.76[b]	3.63[b]	2.17	**
a02	Hexanal	**800**	448.37[b]	724.49[a]	189.17[d]	298.20[c]	43.68	**
a03	(E)-2-Hexenal	**853**	5.78[a]	2.56[b]	-	-	0.93	**
a04	Heptanal	**901**	446.34[a]	417.73[a]	-	-	84.24	ns
a05	(E)-2-Heptenal	**957**	21.18[a]	7.37[b]	3.04[c]	0.93[d]	0.61	**
a06	Octanal	**1003**	222.85[a]	248.93[a]	22.52[b]	26.32[b]	19.96	**
a07	(E)-2-Octenal	**1059**	64.50[a]	21.47[b]	5.46[c]	15.22[b]	6.52	**
a08	Nonanal	**1103**	422.30[b]	766.01[a]	46.03[c]	55.14[c]	140.75	**
a09	(E,E)-2,6-Nonadienal	**1154**	2.82 [a]	-	-	-		
a10	(E)-2-Nonenal	**1160**	96.17[b]	275.61[a]	9.62[c]	11.01[c]	23.08	**
a11	Decanal	**1205**	8.10[a]	3.28[b]	1.88[b]	2.03[b]	1.99	*
a12	[E,E]-2,4-Nonadienal	**1216**	5.51	-	-	-		
a13	(E)-2-Decenal	**1262**	46.28[a]	12.47[b]	6.14[bc]	1.82[c]	4.40	**
a14	2-Undecenal	**1363**	19.42[a]	6.18[b]	3.48[bc]	-	2.67	**
	Alcohols							
b01	1-Pentanol	**763**	5.55[a]	4.19[a]	-	-	7.18	ns
b02	1-Hexanol	**866**	179.23[a]	9.38[b]	6.70[b]	2.08[b]	35.06	**
b03	1-Heptanol	**967**	38.47[a]	42.37[a]	6.70[b]	2.80[b]	3.78	**
b04	1-Octen-3-ol	**978**	13.83[b]	20.38[a]	2.76[c]	0.05[c]	2.19	**
b05	2-ethyl-1-Hexanol	**1027**	2.83[a]	3.75[a]	-	0.53[b]	1.34	*
b06	1-Octanol	**1068**	59.74[a]	11.34[b]	9.01[b]	2.55[b]	12.01	*
b07	1-Nonanol	**1169**	5.65[a]	5.14[a]	-	-	3.35	ns
	Acids							
c01	Acetic acid	**621**	3.40[a]	1.03[b]	-	-	0.16	**
c02	2-Oxooctanoic acid	**1080**	3.08[a]	0.14[b]	-	-	0.31	**
c03	n-Decanoic acid	**1354**	-	-	1.41	-		
	Esters							
d01	Methyl acetate	**524**	22.13[b]	39.42[a]	1.99[c]	20.07[b]	4.20	**
d02	Methyl hexanoate	**923**	1.70[a]	-	3.31[a]	0.89[a]	2.88	ns
d03	Hexyl acetate	**1010**	28.07[a]	-	-	-		
d04	Ethyl 2-methylbutanoate	**1084**	32.11[a]	31.29[a]	-	-	12.01	ns
d05	Methyl octanoate	**1122**	-	-	10.14[a]	17.13[a]	9.78	ns
d06	(Z)-3-hexenyl butanoate	**1184**	1.79[a]	-	1.95[a]	-	0.27	ns
d07	2-Heptanol, acetate	**1187**	21.27[b]	38.91[a]	-	-	7.40	**
d08	2-Ethylhexyl formate	**1236**	-	1.40	-	-		
d09	Methyl butyrate	**1312**	-	-	1.12	-		
d10	Methyl decanoate	**1320**	-	-	10.02[a]	-		
d11	Butyl butanoate	**1379**	2.63[b]	20.61[a]	-	-	1.77	**
d12	Octyl thiocyanate	**1393**	9.57	-	-	-		
d13	Pentanoic acid, 2,2,4-trimethyl-3-	**1601**	1.48[a]	3.03[a]	-	-	3.05	ns

	carboxyisopropyl, isobutyl ester							
	Furan							
e01	2-ethyl-Furan	**702**	-	2.11	-	-		
e02	2-pentyl-Furan	**993**	48.54[ab]	68.38[a]	15.15[b]	13.10[b]	26.25	*
e03	2-n-Octylfuran	**1294**	-	2.17[a]	-	-		
	Hydrocarbons							
f01	n-Hexane	**599**	4.44[a]	5.76[a]	4.11[a]	3.01[a]	3.26	ns
f02	1-Heptene	**689**	1.44[a]	1.00[b]	-	-	0.13	**
f03	Heptane	**700**	4.51[a]	1.50[ab]	-	-	2.62	*
f04	Toluene	**769**	265.63[b]	404.69[a]	175.39[b]	266.38[b]	81.24	**
f05	1-Octene	**791**	5.95[a]	3.61[b]	-	-	1.05	**
f06	1,3-Dimethylbenzene	**873**	-	2.71[a]	-	2.84[a]	0.70	ns
f07	Styrene	**895**	-	43.79	-	-		
f08	Nonane	**900**	-	-	75.64[ab]	138.91[a]	75.68	*
f09	Decane	**1000**	7.71[a]	9.84[a]	4.73[a]	5.19[a]	5.59	ns
f10	2,2,4,6,6-Pentamethyl-3-heptene	**1010**	-	31.18	-	-		
f11	Undecane	**1098**	5.89[b]	14.67[a]	4.29[b]	5.62[b]	1.31	**
f12	3-Dodecyne	**1130**	2.67	-	-	-		
f13	Tridecane	**1393**	-	3.32	-	-		
f14	Hexadecane	**1457**	-	1.23	-	-		
f15	Pentadecane	**1494**	1.96[a]	1.76[a]	-	-	0.88	ns
	Ketones							
g01	Acetone	**500**	-	-	4.78[b]	38.26[a]	3.36	**
g02	2,3-Pentanedione	**692**	1.70	-	-	-		
g03	2-Heptanone	**890**	5.00[c]	16.54[a]	1.79[c]	12.19[b]	2.58	*
g04	6-Methyl-2-heptanone	**955**	8.59[a]	5.31[b]	2.63[c]	1.67[d]	0.53	**
g05	2,3-Octanedione	**982**	27.94[a]	25.81[ab]	15.03[b]	22.95[ab]	7.53	*
g06	3-Octanone	**986**	-	2.98	-	-		
g07	2-Nonanone	**1090**	5.92[a]	13.95[a]	2.15[a]	13.78[a]	8.61	ns
g08	2-Decanone	**1191**	-	2.95	-	-		
g09	2-Undecanone	**1290**	19.43[a]	3.68[b]	1.11[c]	5.48[b]	1.35	**
g10	2-Tridecanone	**1491**	-	-	-	0.98		
	Sulphurs							
h01	Dimethyl disulfide	**746**	-	0.19[a]	-	0.09[b]	0.03	**
h02	Dimethyl trisulfide	**978**	-	0.23[a]	-	0.07[b]	0.07	*
	Terpenes							
i01	α-Pinene	**940**	8.32[a]	1.17[b]	1.41[b]	0.79[b]	1.20	**
i02	β-Pinene	**985**	-	-	-	1.21		
i03	D-Limonene	**1035**	260.02[a]	53.28[bc]	107.68[b]	12.16[c]	56.41	*

[a] Linear retention index (LRI) of identified compounds on DB-5 capillary column calculated against the GC/MS retention time of n-alkanes (C6–C33).

[b] Least significant difference at $p = 0.05$.

[c] Means in the same row followed by different letters differ significantly at the 5% level. ns, no significant difference between means ($p > 0.05$); * $P<0.05$; ** $P< 0.0001$.

Values are means of duplicate analysis from two independent experiments.

(-) Not detected

Estimated quantities in the headspace from 5 mL of milk, calculated by comparison with 130.6 µg/mL of 1,2-dichlorobenzene used as internal standard.

Appendix 3 Microbiology analysis of pasteurised and UHT processed camel milk for the sensory analysis

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UKAS TESTING
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CERTIFICATE OF ANALYSIS

FAO: Dr Sameer Khalil Ghawi
Company: University of Reading
Address: Department of Food and Nutritional Sciences
University of Reading
Whiteknights

Order Number: 3205000
Date Received: 16/09/2016
Analysis Started 17/09/2016
Report Date: 22/09/2016

Reference:

Report Number: 16-99061

Lab No	Client Reference	Sample Description	Specification
906726	15/09/2016	Pasteurised Camel Milk 1	
906727	15/09/2016	Pasteurised Camel Milk 2	
906728	15/09/2016	UHT Camel Milk 3	
906729	15/09/2016	UHT Camel Milk 4	

Lab No.	Test Ref	Analysis	Result
906726	MIC1004	201 Aerobic Colony Count 72h at 30°C	< 1.00x10^4 cfu/ml
906726	MIC1018	203 Enterobacteriaceae (presumptive)	< 1 cfu/ml
906726	MIC1021	205 Coagulase Positive Staphylococci	< 20 cfu/ml
906726	MIC1019	215 Listeria spp. (detection)	Not Detected in 25ml
906726	MIC1023	217 Salmonella spp. (detection)	Not Detected in 25ml
906727	MIC1004	201 Aerobic Colony Count 72h at 30°C	< 1.00x10^4 cfu/ml
906727	MIC1018	203 Enterobacteriaceae (presumptive)	< 1 cfu/ml
906727	MIC1021	205 Coagulase Positive Staphylococci	< 20 cfu/ml
906727	MIC1019	215 Listeria spp. (detection)	Not Detected in 25ml
906727	MIC1023	217 Salmonella spp. (detection)	Not Detected in 25ml
906728	MIC1004	201 Aerobic Colony Count 72h at 30°C	< 1 cfu/ml
906728	MIC1018	203 Enterobacteriaceae (presumptive)	< 1 cfu/ml
906728	MIC1021	205 Coagulase Positive Staphylococci	< 20 cfu/ml
906728	MIC1019	215 Listeria spp. (detection)	Not Detected in 25ml
906728	MIC1023	217 Salmonella spp. (detection)	Not Detected in 25ml

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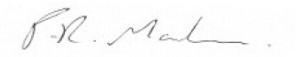
Results marked with an "E" are estimated counts.

906729	MIC1004	201 Aerobic Colony Count 72h at 30°C	1 cfu/ml
906729	MIC1018	203 Enterobacteriaceae (presumptive)	< 1 cfu/ml
906729	MIC1021	205 Coagulase Positive Staphylococci	< 20 cfu/ml
906729	MIC1019	215 Listeria spp. (detection)	Not Detected in 25ml
906729	MIC1023	217 Salmonella spp. (detection)	Not Detected in 25ml
			cfu = colony forming units

END

Signed for and on behalf of Geneius Laboratories Ltd



Phil Marsden, Data Systems Manager

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