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SOFT CAMEL MILK CHEESE EFFECTS OF COAGULANTS AND PROCESSING CONDITIONS

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EFFECTS OF COAGULANTS AND PROCESSING CONDITIONS

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This dissertation is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Under the Supervision of Professor Afaf Kamal-Eldin

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Declaration of Original Work

I, Mustapha Mbye the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this dissertation entitled "*Soft Camel Milk Cheese Effects of Coagulants and Processing Conditions"* hereby, solemnly declare that this dissertation is my original research work that has been done and prepared by me under the supervision of Professor. Afaf Kamal-Eldin, in the College of Agriculture and Veterinary Medicine at UAEU. This work has not previously been presented or published or formed the basis for the award of any academic degree, diploma, or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my dissertation have been properly cited and acknowledged following appropriate academic conventions. I further declare that there is no potential conflict of interest concerning the research, data collection, authorship, presentation, and/or publication of this dissertation.

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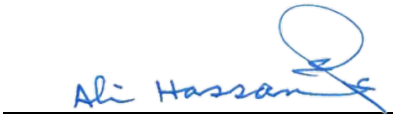
Signature  Date 27/10/2021

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Abstract

In recent years, camel milk (CM) has been acknowledged for several health benefits, including anti-diabetic, hypoallergic, and other effects. Accordingly, CM may provide a complementary or a healthier alternative to bovine milk (BM). This has led to increased interest in processing CM to products like cheese, yogurt, and powders to extend its shelf-life. However, CM is difficult to coagulate into hard gels, affecting its cheese quality and consumer preference. The current research aimed to investigate the impact of different coagulants (chymosin, *Withania coagulans*, citric and acetic acid) on CM cheese quality and sensory attributes compared to BM cheese. It also evaluated the effect of pasteurization temperatures (low-temperature long-time (LTLT) and high-temperature short-time (HTST) and high-pressure processing conditions (HPP, 350, 450, and 550 MPa) on the physical, chemical, and softness properties of CM cheese in comparison to BM cheese. Large variations were found between CM and BM milk concerning coagulation time, properties, and the microstructure of the cheeses. CM cheeses were observed to have smooth and continuous casein networks, thinner aggregate strands, and smaller pore spaces, as shown by scanning electron microscope (SEM). One important finding is that CM possesses higher proteolytic activities than BM, as demonstrated by SDS-PAGE protein/peptide analysis, which might contribute to the softness of its cheese. It was also found that HTST (75°C for the 30s) negatively affected the coagulation, especially of CM, while LTLT (65°C for 30 min) provided higher quality cheeses. HHP (450 to 550 MPa for 5 min) treatments resulted in soft cheese, while HPP (350 MPa) provided better cheese quality than HTST. Thus, HPP at low pressure may offer an alternative to conventional heat treatments in providing harder camel cheese. In conclusion, the selection of coagulants and processing conditions can be tailored to improve camel cheese quality, which opens new research avenues in this field.

Keywords: Camel Milk, Bovine Milk, Cheese, Coagulation, Chymosin, Pasteurization. High-Pressure Processing.

Title and Abstract (in Arabic)

جبن حليب الابل

النعومة وتأثيرات مواد التخثر وظروف المعالجة

الملخص

في السنوات الأخيرة، تم الاعتراف بالعديد من الفوائد الصحية لحليب الإبل مثل التأثيرات المضادة لمرض السكر، والحساسية، وتأثيرات أخرى. وفقًا لذلك، فقد يوفر حليب الإبل بديلاً مكملاً أو أكثر صحة من الحليب البقري. وقد أدى ذلك إلى زيادة الاهتمام بمعالجة حليب الإبل إلى منتجات ذات فترة صلاحية طويلة مثل الجبن والزبادي والبدرة. ولكن يصعب تخثر حليب الإبل إلى مواد هلامية صلبة مما يؤثر على قبول المستهلك.

يهدف البحث الحالي إلى دراسة تأثير مواد التخثر المختلفة على جودة الجبن المصنوع من حليب الإبل وصفاته الحسية مقارنة بالجبن المصنوع من حليب الأبقار. وقد تم تقييم تأثير درجتي حرارة للبسترة (درجة حرارة منخفضة لفترة طويلة ودرجة حرارة عالية لفترة قصيرة) وظروف معالجة عالية بالضغط (350، 450، و550 ميجا باسكال) على الخصائص الفيزيائية والكيميائية والنعومة لجبن الإبل مقارنة بجبن الأبقار. وقد وجد أن هناك اختلافات كبيرة بين حليب الإبل وحليب الأبقار فيما يتعلق بالوقت اللازم للتخثر، والخصائص، والبنية الدقيقة. وضح مسح المجهر الإلكتروني أن جبن الإبل يحتوي على شبكات كازين سلسة ومستمرة، وخيوط مجمعة أرق، ومساحات مسامية أصغر من جبن حليب الأبقار. إحدى النتائج المهمة في هذه الأطروحة هي أن حليب الإبل يمتلك أنشطة تحلل بروتينية أعلى من حليب الأبقار كما يتضح من تحليل البروتين/الببتيد بتقنية PAGE-SDS، وهذا قد يساهم في نعومة الجبن المصنوع من هذا الحليب. وجد أيضًا أن درجة حرارة البسترة العالية (75 درجة مئوية لمدة 30 ثانية) قد أثرت سلبًا على التخثر خاصةً في جبن الإبل بينما انتجت درجة الحرارة المنخفضة (65 درجة مئوية لمدة 30 دقيقة) أجبانًا عالية الجودة. أنتجت معالجات الضغط (450 إلى 550 ميجا باسكال لمدة 5 دقائق) جبن طري بينما انتجت المعالجة في ضغط (350 ميجا باسكال) أفضل جودة جبن. وعليه قد توفر معالجة الضغط المنخفض بديلاً عن المعالجات الحرارية التقليدية في توفير جبن ابل صلب. في الختام، يمكن اختيار مواد التخثر وظروف المعالجة لتحسين جودة جبن الإبل، مما يفتح آفاقًا جديدة للبحث في هذا المجال.

مفاهيم البحث الرئيسية: حليب النوق، حليب البقر، الجبن، التخثر، الكيموسين، والبسترة.

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Dedication

To my beloved family

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List of Abbreviations

BM	Bovine Milk
CM	Camel Milk
CMS	Casein Micelle Size
FTIR	Fourier Transform Infrared Spectroscopy
G’	Storage Modulus
G”	Loss Modulus
HPP	High Pressure Processing
HTST	High Temperature Short Time
IMCU	International Milk Clotting Unit
LTLT	Low Temperature Long Time
MCP	Milk Coagulation Properties
MTGASE	Microbial Transglutaminase
QDA	Quantitative Descriptive Analysis
SDS PAGE	Sodium Dodecyl Polyacrylamide
SEM	Scanning Electron Microscope
UF	Ultra-Filtration

Chapter 1: Introduction

1.1 Background

Based on the available data, camels' world population is approximately 35 million, of which around 95% are Dromedary camels, *Camelus dromedarius,* and the rest are Bactrian camels *Camelus bactrianus* (Faye, 2020). Camels produce milk for a more extended period than any other animal in the same condition of arid lands. The production of camel milk (CM) is increasing by about 2.45% yearly (FAOSTAT, 2021), which is important for at least three reasons: (i) its contribution to food security in marginal environments, (ii) new market opportunities due to its unique properties, (iii) possible health benefits, and (iv) development of camel dairy products, which could be profitable for settled producers (Faye, 2015). In recent years, the health benefits of CM and its products have attracted much attention towards the possibilities of its use as an alternative to bovine milk (BM) and other kinds of milk (Solanki & Hati, 2018; Izadi et al., 2019). Exceptional, nutritional, and therapeutic effects such as anti-diabetic (Agrawal et al., 2011; Ejtahed et al., 2015; Mohamad et al., 2009), anti-autistic (Al-Ayadhi et al., 2015), anti-carcinogenic (Magjeed, 2005), hypo-allergic (Ehlayel et al., 2011), antimicrobial (Benkerroum et al., 2004), and anti-hypertensive (Quan et al., 2008) effects were attributed to CM. Camel milk must be processed into products that can easily store for extended periods, such as cheese and yoghurt, to increase its shelf life and market opportunities. However, previous studies have reported that CM is challenging to transform into cheese (Berhe et al., 2017; Bornaz et al., 2009; Konuspayeva et al., 2009; Yagil, 1982). The longer time of coagulation compared to that of BM, 2-4 times longer, as reported by Sagar et al. (2016), and weaker curds, were attributed to the low amount of κ-casein (κ-CN) in CM compared

to BM (3.5% versus 13.6%) (El Zubeir & Jabreel, 2008; Mehaia, 2006; Ramet, 2001) and the larger casein micelles size average size in CM (260-300 nm) *versus* BM (100-140 nm) (Ibrahem & El Zubeir, 2016). Fortunately, the invention of camel chymosin has paved the way for satisfactory production of CM cheese with improved quality (Hailu et al., 2016b; Saliha et al., 2011). This opportunity would provide a future CM processing industry foundation for small and large-scale production (Ipsen, 2017). Thus, a study aiming to understand the effect of components, different coagulants, and processing conditions responsible for the peculiar quality of CM compared to BM cheese is critically needed.

1.2 Research Objective and Hypothesis

Previous literature has shown that production of conventional cheese from camel milk requires more coagulation time and leads to significantly fragile textures compared to bovine milk (Berhe et al., 2017; Bornaz et al., 2009; Konuspayeva et al., 2009; Yagil, 1982). The hypothesis of this thesis is that the peculiar behavior of camel milk during cheese manufacture is due to compositional characteristics that may be manipulated by different coagulants and processing conditions. The research presented in this thesis focused on the effects of different coagulants and specialized treatments on the quality of the CM fresh coagulum obtained in the initial stage of cheese preparation as compared with BM in terms of composition, structure, physicochemical properties, sensory attributes, and proteolytic activities. The main aim of this dissertation was to provide the camel milk dairy industries with new knowledge pertinent to CM cheese development. The specific objectives of the research were:

1. To conduct a comparative study on CM and BM cheeses coagulated with chymosin or organic acids (citric and acetic) and evaluate their physicochemical quality, microstructure, coagulation behavior, and acceptability by consumers.

2. To study the effect of camel chymosin and *Withania coagulans* aspartic proteases on the physicochemical quality and proteolysis of CM and BM cheeses.

3. To compare pasteurization and high-pressure processing effects on camel cheese's physicochemical quality, microbial load, and proteolysis activities.

1.3 Novelty of the Research

- In this study, the effect of CM microstructure related to coagulation behavior CM cheeses was reported for the first time.

- Studying the plant enzymes *Withania coagulans* in coagulating CM in cheese making was also reported for the first time.

- The effect of high-pressure processing of camel milk microbial load and cheese physicochemical quality was also reported for the first time.

- The possible contribution of the indigenous enzymes, e.g., plasmin, to camel milk proteolysis has been suggested.

1.4 Significance of the Research

Recently, the production of CM and its dairy products is expanding globally due to an increase in demand as an alternative to bovine milk (El-Agamy et al., 2009). Thus, robust scientific studies are needed to understand the properties of camel milk and the reason for its technological difficulties in product development. There is still a clear knowledge gap about the exact reason for the technological challenges encountered in

developing CM daily products compared to other dairy products. The studies included in this dissertation attempt to highlight the technical difficulties and the reason for the softness observed in camel milk cheese. The study provides essential information on camel cheese microstructure, coagulation behavior, the effect of high-pressure camel milk microbial load, and cheese physicochemical quality under the same processing conditions typically applied in the dairy industry and the best processing condition for improving cheese quality. This outcome would enable the CM dairy manufacturers to improve their processing conditions to improve the quality of the final cheese product and enhance its consumer acceptability.

1.5 Dissertation Outline

The dissertation consists of seven primary chapters. Chapter 1 includes providing the background and need for this research, states the objectives, and provides an outline for the chapters of the thesis, Chapter 2 includes a literature review of the previous work on camel milk composition, processing conditions, and physicochemical quality. It also provides a summary of the earlier studies conducted on camel milk cheese processing and its properties. Chapter 3 compares the characteristics of CM and BM cheeses coagulated with chymosin or with citric acid or acetic acid. The cheeses were evaluated for yield, moisture, microstructure, texture profile, rheology, and sensory quality. This chapter is based on a published paper (Mbye et al., 2020). Chapter 4 examines differences between a camel and bovine kinds of milk' behavior coagulated by camel chymosin and *Withania coagulans* aspartic proteases. The cheeses were evaluated in cheese yield, hardness, total solids (protein, fat, and total solids), color, titrable acidity/pH, and protein/peptide fingerprints by SDS-PAGE electrophoresis. This chapter is based on a published paper (Mbye et al., 2021a) Chapter 5 studies the

effects of pasteurization and high-pressure processing of CM and BM cheese quality and treatments on the protein profile using the SDS PAGE. The cheeses were evaluated for pH, yield, proximate composition, textural and rheological properties, microstructure, and protein profile. This chapter is based on a published paper (Mbye et al., 2021b). Chapter 6 discusses the obtained results relating them to precious obtained results in the literature. Finally, Chapter 7 presents a conclusion of the research and directions for future work.

Chapter 2: Literature Review

2.1 Camel Milk Composition

Milk is an emulsion of fat globules with suspensions of casein micelles in the serum phase, in which other components such as soluble proteins, lactose, minerals, and vitamins are also present (Figure 1). The composition of CM differs due to genetic differences, geographical origin, and other factors such as feeding conditions, seasonal, physiological variations, genetic and health status of the camel (Konuspayeva et al., 2009). Table (1) shows the average chemical composition of CM and BM.

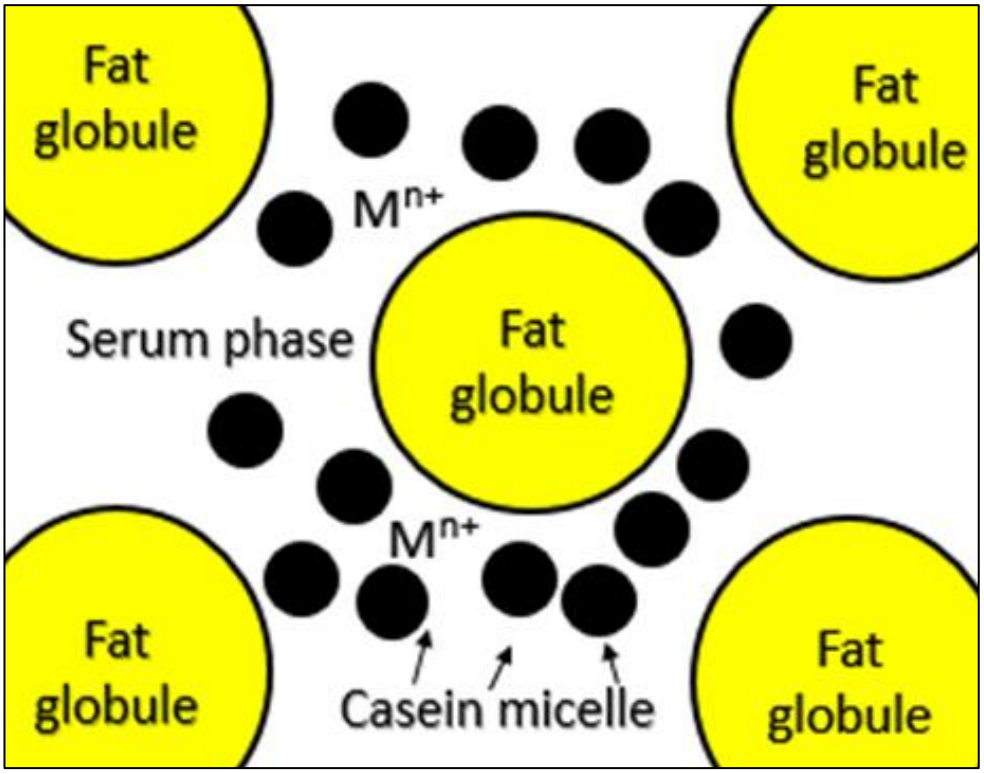

Figure 1: Milk as a colloidal suspension of fat globules, casein micelles, and minerals (Mn^{+}) in a serum phase of soluble proteins

Table 1: Shows CM and BM's average chemical compositions

Components	**Camel**	**Bovine**
Acidity (%)	0.13	0.11
pH	6.66	6.7
Fat (%)	3.5	3.7
Lactose (%)	4.4	4.8
Ash (%)	0.79	0.7
Protein (%)	3.1	3.4
Total solids (%)	12	13

Sources: Al Kanhal (2010); Mal & Pathak (2010); Mohamed et al. (2020a)

Compared with BM, CM has lower pH, higher buffering capacity, slower acidification rate, and more prolonged bacterial lag phase after inoculation (Attia et al., 2001). For example, a maximum acidification rate of 12 hours and lag phase of 5 hours was observed in CM, whereas 6 hours and 1 hour was observed in BM, respectively (Attia et al., 2001). The slow bacterial growth in CM may be related to natural protective proteins in the milk such as lysozyme, lactoferrin, and lactoperoxidase (Barbour et al., 1984; El Sayed et al., 1992).

A large part of the CM fats comprises long-chain fatty acids with little short-chain fatty acids in triacylglycerols. The concentration of saturated fatty acids is comparable in CM and BM fats; 67.7 *versus* 69.9%, respectively (Konuspayeva et al.,

2009). CM fat is homogenous and smooth and packed in smaller fat globules than BM, which is 3.2-5.6µm *versus* 4.3-8.4µm, respectively (Ibrahem & El Zubeir, 2016). The tiny fat globules in CM could contribute to its soft texture and higher digestibility than BM (Meena et al., 2014). In addition, the low quantity of the yellow pigments, carotenes, and riboflavin, could be one reason for the whitish color of CM compared with BM (Ibrahem & El Zubeir, 2016). The amount of milk proteins in milk varies with breeds, production, and environmental conditions (Khan & Iqbal, 2001). The total protein in CM ranges from 2.5-5.5 % (Al Kanhal, 2010; Zhao et al., 2015). The milk protein consists of soluble whey and insoluble caseins, as shown in Table 2 (Rafiq et al., 2016).

2.1.1 Camel Milk Caseins

The caseins are the main component of the milk proteins, representing about 52-87% of the total CM protein (Mohamed et al., 2020b). Therefore, the casein composition of CM is considerably different from that of BM. The casein content of CM is as follow: α-s1, (22.0%), α-s2 (9.5%), ß (65.0%), and κ (3.5%). While BM caseins had a high percentage of αs1-casein (38%) followed by β-casein (36-39%), followed by α-s2-casein (10%), and κ-casein (13%) (Mohamed et al., 2020b) (Table 2).

Micelles of CM casein have a larger surface area of 260-300 nm, compared with micelles of BM casein at 100-140 nm (El-Agamy et al., 2006). Therefore, the low amount of k-casein of CM couple with large micelle size, which results in a scant coverage of k-casein on casein micelles surface area (Kappeler et al., 1998), could be a significant factor in the low coagulation of CM (Berhe et al., 2017). In milk, the caseins are packed into casein micelles, complex protein assemblies linked with colloidal calcium phosphate (Fox, 2003). There are numerous studies and structural

models on the structure of BM casein micelles (De Kruif et al., 2012; Holt & Horne, 1996; Phadungath, 2005, De Kruif et al., 2012). All models agree that the casein molecules form aggregates glued together by colloidal calcium phosphate and that k-casein predominates in the outer surface of the micelle (De Kruif et al., 2012) (Figure 2).

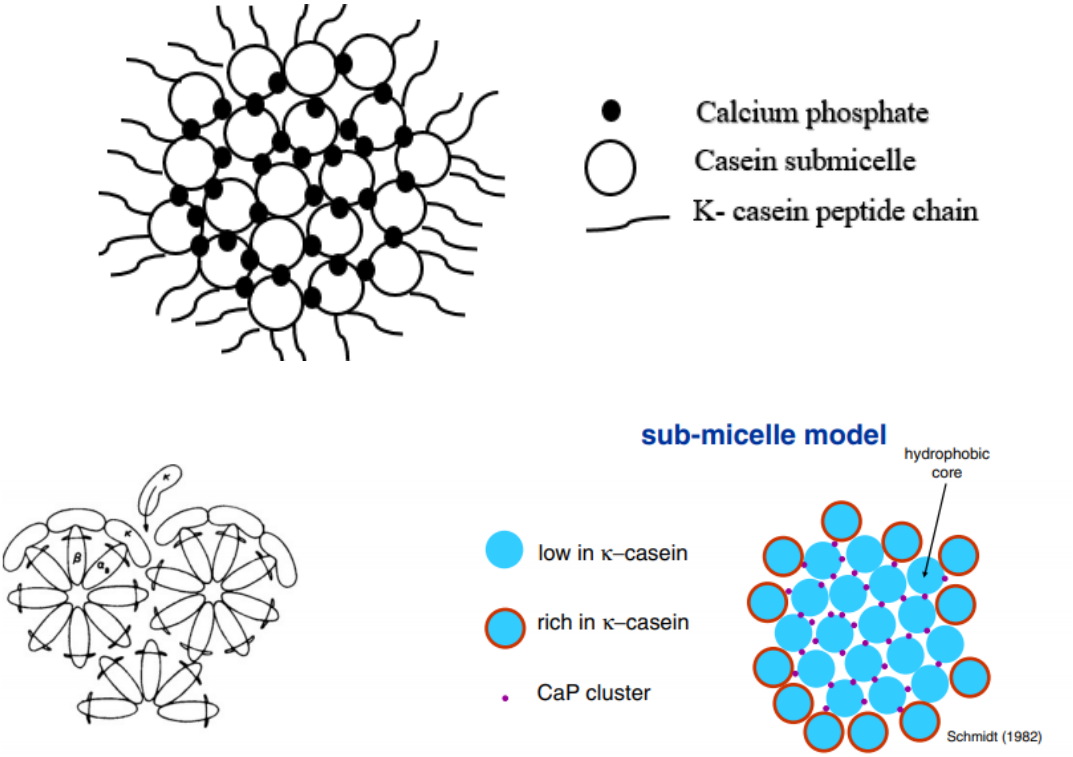

Figure 2: Diagram of casein micelle structure modified from Fox et al. (1998)

2.1.2 Camel Milk Whey Protein

The whey contains soluble proteins in the serum phase and separates from the caseins during cheese making (Saliha et al., 2013). The whey proteins of CM represent about 20-25% of the total proteins, whereas they represent around 20% in BM (Shuiep et al., 2013). CM whey proteins include *inter alias* α-lactalbumin, serum albumin, lactoferrin, acidic whey protein, glycosylation-dependent cell adhesion molecule 1, peptidoglycan recognition protein, lactoperoxidase, and immunoglobulins (Hailu et

al., 2016b; Zhao et al., 2015). The whey proteins are globular with secondary and tertiary structures that make them more sensitive to heat denaturation above 60°C (Hailu et al., 2016a). A significant difference between camel and bovine or buffalo whey proteins is that CM lacks β-lactoglobulin, which have important implications on milk functional properties (El-Agamy et al., 2009), mainly through its heat-induced association with k-casein (Oka et al., 2018). In comparison, α-lactalbumin is the major CM whey protein. In BM, α-lactalbumin constitutes just 25%, while β-lactoglobulin makes around 50% of the total whey protein (Laleye et al., 2008; El-Agamy et al., 2009). CM whey was also reported to have higher serum albumin content compared to BM (Table 2).

Table 2: Casein and whey protein concentrations (g/l) in camel milk (CM) and bovine milk (BM)

Protein	**CM**	**BM**
αs1-casein	5.3	9.5
αs2-casein	2.3	2.5
β-casein	15.6	9.8
κ-casein	0.8	3.3
β-Lactoglobulin	-	3.3
α-Lactalbumin	2.3	1.1
Serum albumin	2.2	0.35
Whey acidic protein	0.16	-
Lactoferrin	0.095	0.10
Immunoglobulins IgA, IgG, IgM	1.5	0.20

Sources: Hailu et al. (2016b)

2.2 Milk Coagulation

Milk coagulation can be described as converting the liquid milk emulsion into solid gels (Fox et al., 2017). Milk coagulation is the primary step in producing cheese

and is achieved either by enzymes or acid (Ikonen et al., 2004). The enzyme chymosin cleaves κ-casein to para-kappa-casein that remains associated with the micelle and caseinomacropeptide released into the whey fraction (Huppertz et al., 2018). On the other hand, acid coagulation directly affects casein micelles' stability by neutralizing their negative charges and dissolving the colloidal calcium phosphate. Gel formation occurs when the milk pH drops to its isoelectric point at pH 4.6 (Lucey, 2016). This reduction in pH results in a loss of electrostatic repulsion to overcome the attractive forces of interactions (Lucey, 2016) (Figure 3).

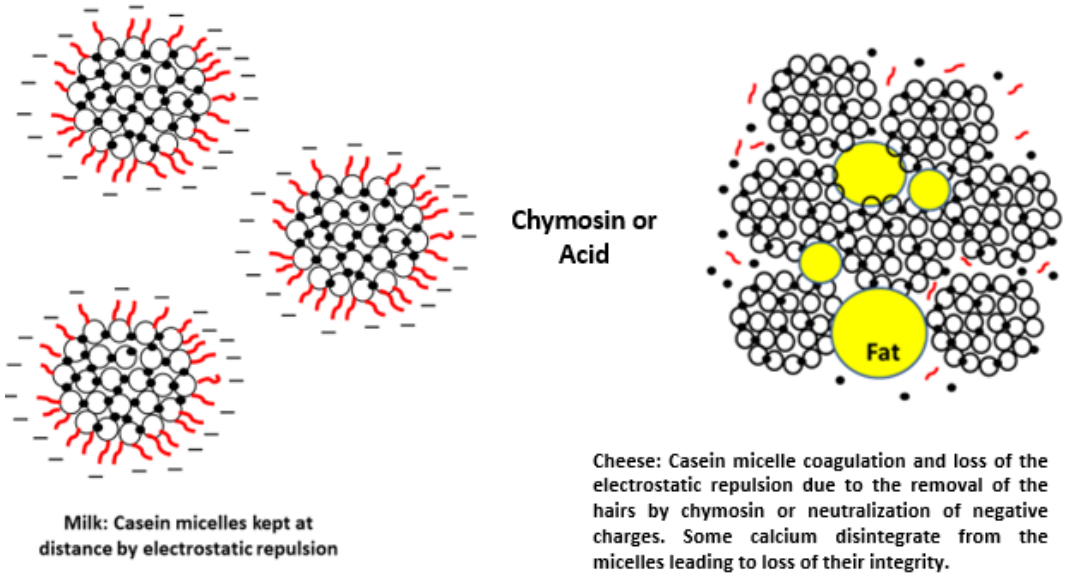

Figure 3: Diagram illustration of milk coagulation

There are three phases in rennet-mediated milk coagulation (Bathmanathan et al., 2019). The initial phase of coagulation, also called renneting, is the chymosin-catalyzed cleavage of κ-casein between phenylalanine 105 (Phe105) and methionine 106 (Met106) in BM, and phenylalanine 97 (Phe97) and isoleucine 98 (Ilu98) in CM, resulting in a hydrolytic split into an insoluble para-κ-casein (peptide 1–105) and

soluble hydrophilic κ-casein caseinomacropeptide, (peptide 106–169) (Figure 4). The para-κ-casein remains attached to the micelle while the macro-peptide diffuses into the whey, leading to micellar destabilization. The second coagulation phase is non-enzymatic and starts when approximately 85% of the κ-casein is hydrolyzed or cut, the destabilized casein micelles form a spontaneous aggregation, resulting in a gel-like network; i.e., the curd or coagulum (Ikonen et al., 2004). The third phase is syneresis, when the trapped whey is expulsed from the casein network through a contraction (Ferreira, 2011).

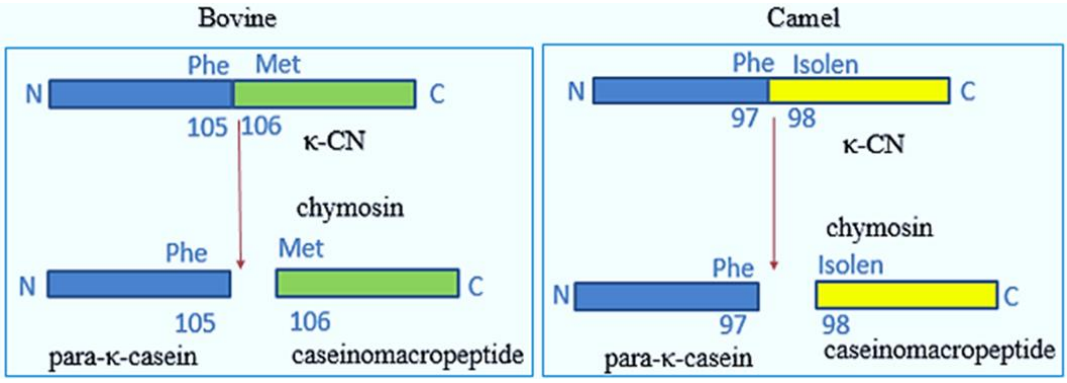

Figure 4: The action of chymosin in hydrolyzing k-casein to para-kappa casein and glycomacropeptidein bovine and camel milk

2.3 Factors that Influence Milk Coagulation

Milk coagulation properties (MCP) are critical in preparing cheese (Fox et al., 2017). Milk composition is the primary factor that affects coagulation, as shown in (Figure 5). In addition, significant variations in MCP occur in ruminant species, e.g., milk clotting time, curd firmness, and level of syneresis (Bittante et al., 2012).

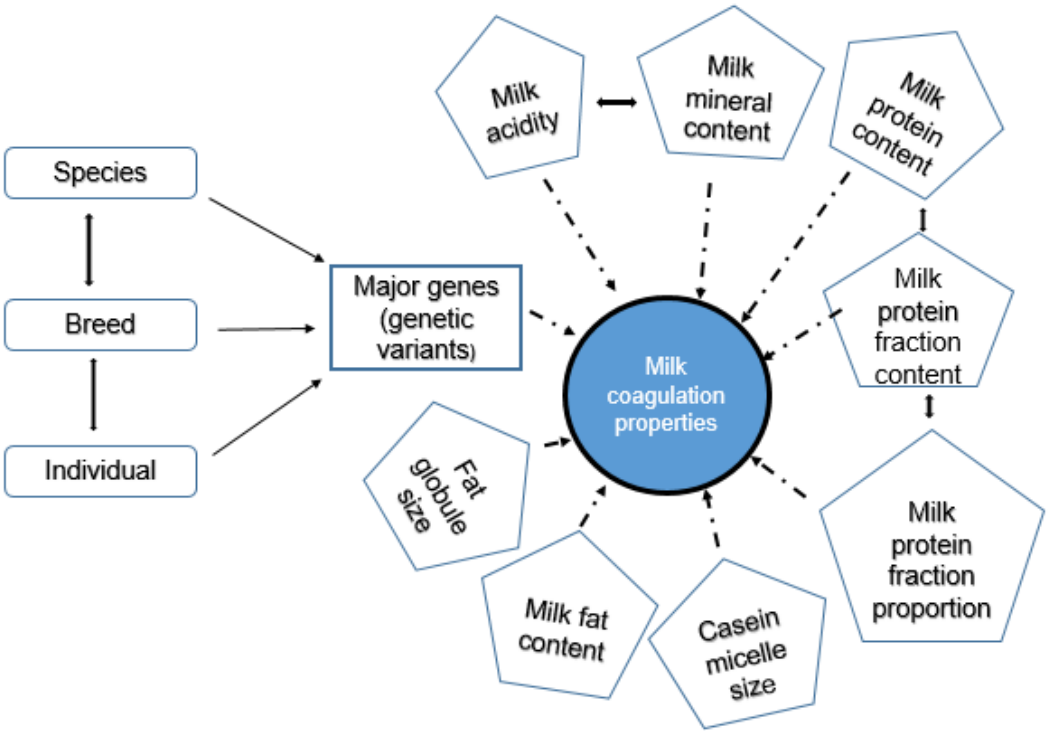

Figure 5: Factors that influenced coagulation of milk (Modified from Bittante et al., 2012)

2.3.1 Genetics

Genetics indirectly affects milk coagulation. Ruminants have different milk compositions and properties; the milk of small ruminants, such as sheep and goats Syneresis more quickly than in BM' (Park et al., 2007). The second most crucial genetic feature influencing MCP is breed within species. The most common cow breeds were the Holstein-Friesian, Ayrshire, Brown Swiss, Jersey, and Simmental. Milk from Ayrshire cows is characterized by slightly longer rennet coagulation time, longer curd firming time, and lower curd firmness than Holstein-Friesians (Bartocci & Terramoccia, 2010). Brown Swiss cows show shorter curd setting time and higher curd firmness compared with milk from Holstein-Friesian. On average, the milk of Simmental cows showed better MCP than the milk of Holstein-Friesians but not as good as those of Brown Swiss (Bartocci & Terramoccia, 2010; Glantz et al., 2009). CM was consistently shown to require longer coagulation time and provide fragile and

soft curds than BM (Mbye et al., 2020). There has yet to be a study examining how breed influences coagulation in CMs. Significant variability between individual animals and species in rennet coagulation in milk has been observed in several studies (Bartocci & Terramoccia, 2010; Bencini, 2002; Cecchinato et al., 2012; Potena et al., 2007) and its relation with several variants in milk proteins, e.g., β-casein (A1, A2, B), κ-casein (A, B), and β-lactoglobulin and (A, B) have been identified (Hallén et al., 2008). The most consistent results found were that the B variants of β-casein, κ-casein, and β-lactoglobulin are favorable for milk coagulation and cheese-making, whereas poorly coagulating milk was associated with the β-casein variant A2, κ-casein variants A and E, and β-lactoglobulin variants A and C (Hallén et al., 2008; Wedholm et al., 2006). Thus, milk with a high prevalence of A1 β-casein variant of proteins was identified to have good coagulation properties, whereas poorly coagulating milk was associated with the A2 β-casein variant.

2.3.2 Composition of Milk

The curd production phases (enzymatic coagulation, curd firmness, syneresis) are all influenced by the milk composition, particularly the concentrations and types of caseins, fat, pH, and calcium (Fox et al., 2017). Milk composition, particularly proteins and fats, has a significant effect on the cheese's yield and composition (Walstra et al., 2005). The milk's casein content also affects the coagulation and gel firming rate, increasing with the casein concentration (Lapointe-Vignola, 2002). Casein micelles' size (CMS) impacts milk coagulation with tiny micelles leading to firmer curds (Glantz et al., 2009). Tiny casein micelles have more surface area than giant casein micelles, increasing initial cheese processing by increasing rennet coagulation time and enhancing cheese curd firmness and overall cheese quality. Therefore, CMS is a

possible indicator for animal breeding exploration to improve cheese quality (Glantz et al., 2009). Higher κ-casein contents have been associated with smaller micelles, and variations in micelle size appear to influence milk curding properties and cheese yield (Dziuba & Minkiewicz, 1996). Previous research has shown that CMS and κ-casein contents positively affect milk coagulation and cheese production (Bonfatti et al., 2014; Dziuba & Minkiewicz, 1996; Freitas et al., 2019). The fat globule size exhibit different effect on the physicochemical characteristics of milk gels (Michalski et al., 2004). Native fat globules could act either as fillers or as structure breakers. Compared to BM, CM has smaller fat globules that are homogenous and smooth that may represent weaker points in the cheese matrix and enhance water-binding ability (Ibrahem & El Zubeir, 2016; Meena et al., 2014). On the other hand, larger fat globules provide a gel with a more vital linkage (Michalski et al., 2004).

2.4 Milk Coagulant Sources

2.4.1 Animal Coagulants

The rennet enzymes are aspartic peptidase, with the most known are the combinations of chymosin A, B, C, and pepsin extracted from the stomach of claves and other ruminants. By cleaving κ-casein into para-kappa-casein and caseinomacropeptide**,** renins disturb the milk emulsion and separate the caseins from the whey leading to their coagulation into cheese (Beermann & Hartung, 2012; Shieh et al., 2009). Before the early nineteenth century, cheese was made from the abomasa of young calves. However, with the establishment of dairy cooperatives in the nineteenth century, more rennet was needed to produce more volume of cheese. The need for larger rennet led to the industries' calf rennet production and commercialization as the first industrial enzyme. This shortage also prompted the

industry to look for an alternative proteolytic enzyme similar to calf rennet in cheese making (Zhang et al., 2019). This situation encouraged research on the eventual utilization of several microbial recombinant chymosin in the cheese industry to substitute rennet in cheese manufacturing (Hicks et al., 1988). One of those recombinant enzymes was recombinant camel chymosin, which proved to have 70% higher clotting activity for bovine milk. In contrast, bovine chymosin poorly coagulates camel milk (Kappeler et al., 2006). Camel chymosin clotting activity is sevenfold higher than bovine chymosin, making it attractive for commercial cheese manufacturing (Bansal et al., 2009). Camel chymosin from older camels was found to give the best milk clotting activity in both camel and bovine milk (Saliha et al., 2011).

2.4.2 Plant Coagulants

In the last few years, the challenge associated with cheese yield and quality highly contributed to exploring rennet alternatives such as plant proteolytic enzymes (Alavi & Momen, 2020; Fernández-Salguero et al., 2003). However, recombinant enzymes became unpopular in some countries due to various factors such as religious matters and diet (Bathmanathan et al., 2019). Plant proteases can coagulate milk, but their potentials as milk coagulants are less explored and understood (Shah et al., 2014). In recent years, some plant proteases have been identified as choices for milk coagulation in cheese making and have been purified, and characterized plant proteases have been divided into groups based on the hydrolytic process mechanism: aspartate, serine, and cysteine proteases (Table 3).

Table 3: Examples of the plant-based milk coagulation protease

Type Protease	Protease name	Source of protease	Reference
Aspartate	Withanine	*Withania coagulans*	(Chazarra et al., 2007; Ordiales et al., 2012; Salehi et al., 2017)
	Cardosin A and B	*Cynara cardunculus*	
	Cynara	*Cynara scolymus*	
Serine	Zingibain	*Zingiber officinale*	(Hashim et al., 2011; Mazorra-Manzano et al., 2013; Uchikoba & Kaneda, 1996)
	Cucumisin	*Cucumis melo*	
	Lettucine	*Lactuca sativa*	
Cysteine	Papain,	*Carica papaya*	(Bahmid, 2013; Devaraj et al., 2008; Monti et al., 2000)
	Ficin	*Ficus racemose*	
	Bromelain	*Ananas comosus*	

Aspartic proteases (EC 3.4.23) have two aspartic acid residues, hydrophobic residues, and beta-methylene groups in their active site (Domingos et al., 2000). Contradictory to cysteine or serine proteases, this protease does not use a covalent in cleaving. Aspartate proteases are most active in acidic media and highly specific in peptide bonds' cleavage of the substrate's hydrophobic amino acid residues (Domingos et al., 2000). The most accepted mechanism of action by aspartic proteases utilizes an acid-base, which coordinates water molecules between the conserved aspartate residues (Brik & Wong, 2003). This generates a tetrahedral oxyanion intermediary, as shown (Figure 6). Plant proteases may be less specific than animal proteases towards the cleaved bonds, which may significantly affect the softness and other characteristics of the cheese, e.g., bitterness (Jiang et al., 2012).

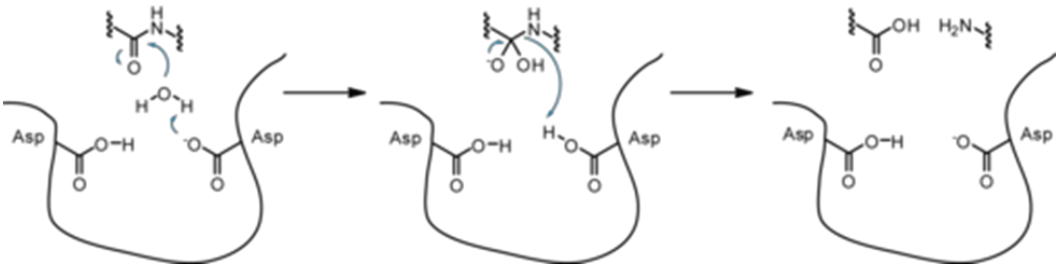

Figure 6: Suggested mechanism of peptide cleavage by aspartyl proteases (Suguna et al., 1987)

Serine proteases' molecular range in weight is from 19 to 110 kDa and are present in almost all plants, but the most significant quantity is found in the fruits. Serine enzymes are active over a wide range of pH 7-11 and temperatures 20-50°C (Feijoo-Siota & Villa, 2011). Serine protease such as *Zingiber officinale* extracts has been utilized in camel milk cheese production and the resultant cheese was acceptable (Hailu et al., 2014). Cysteine proteases include enzymes isolated from *Carica papaya, Ananas comosus, and Ficus racemosa*. Cysteine proteases have high proteolytic activity with broad specificity and action at various pH and temperature values. Papain is used to produce semi-soft Dangke cheese (Prasetyo et al., 2015), and ficin was used to make the traditional Turkish cheese Gaziantep (Piero et al., 2011).

2.4.3 Use of Organic Acids as Coagulants

The acid coagulation affects the stability of casein micelles by neutralizing their charge, dissolving some colloidal calcium phosphate crosslinks, and altering internal bonding between proteins. The development of aggregates and eventually gels occurs at the isoelectric point when electrostatic repulsion is insufficient to overcome attractive forces (Abbas et al., 2014; Lucey, 2016). The manufacturing of CM cheeses using organic acid has been documented (Mihretie et al., 2018). Mohammed et al. (2013) report on the use of acetic acid. Similarly, Mihretie et al. (2018) has made CM

cheese using citrus acids from citrus fruits, and the results show that CM can be coagulated into soft cheese.

2.5 Camel Milk Cheese Production

Cheese production from camel milk was consistently reported to meet significant challenges due to the poor rennetability of the milk as summarized in Table (4). In addition, the coagulation time of camel milk is 2-4 times longer than bovine's milk (Sagar et al., 2016), and the produced curd is weaker, which is possibly related to low κ-casein and total solid content in CM (El Zubeir & Jabreel, 2008; Mehaia, 2006; Ramet, 2001). However, the availability of camel chymosin (Chy-Max M, from Chr. Hansen A/S, Hørsholm, Denmark) enhanced the curd formation of CM and paved the way for the production of CM cheese (Kapeler et al., 2006). However, the quality of curd formation depends on the lactation stage. For example, Konuspayeva et al. (2014) reported that good curd formation occurs only 20^{th} after partum. Thus, numerous approaches to make CM cheese has been made, including the use of camel chymosin with starter cultures for acidification (Abou-Soliman et al., 2020; Al-Zoreky & Almathen, 2021; Bekele et al., 2019; Belkheir et al., 2020; Bouazizi et al., 2021; El Hatmi et al., 2020; Hailu et al., 2018). The available literature on cheese production from CM has shown the application of different processing conditions to improve the overall quality and will be discussed below. However, it is essential to note that the processing conditions for manufacturing CM cheeses significantly impact their yield and quality. For example, conditions such as cheese milk pasteurization temperature, high-pressure treatment, calcium chloride content, and pre-acidification substantially affect the final cheese product (Al-Zoreky & Almathen, 2021). In general, cheeses produced from camel milk require longer coagulation times, are significantly softer

and less affected by the addition of calcium chloride compared to corresponding bovine cheeses. Despite all these attempts (Table 4), there is still a need to understand the fundamental compositional and structural basis for these differences and to find suitable processing technologies that may overcome them.

Table 4: A summary of studies performed on CM cheese production

Reference	**Objective**	**Processing method**	**Key findings**
Khan et al. (2004)	To compare cheese prepared from CM by direct acidification and starter culture with chymosin.	Pasteurization (65°C for 30 min), cooling to 40°C, direct acidification (10% citric acid) or addition of starter culture (5%, 1 hr.), and coagulation by rennet (0.15 ml/liter, 5 hours)	Cheese prepared by starter culture and chymosin had a higher yield, total solids, protein, and fat than direct acidification.
Mehaia (2006)	The objective of this study was to determine the chemical composition, yield, and sensory characteristics of soft cheese prepared from CM by ultrafiltration.	Pasteurization (65°C for 30 min), cooling to 50°C, ultrafiltration, cooling 42°C, addition of $CaCl_2$ (0.02%) and starter culture (0.5%, 20 min), and coagulation (3 hours).	UF increased cheese yield, protein, fat and total solids recovery. In addition, CM cheese prepared by UF received a better sensory evaluation than conventional cheese.
Inayat et al. (2007)	This study aims to compare the quality of CM cheese with buffalo milk cheese to produce and improve soft, unripened CM cheese.	Pasteurization (90°C for 10 min), cooling 40°C, rennet addition, and coagulation (5 hours)	Cheese made from buffalo milk had a better yield and sensory score.
El Zubeir & Jabreel, (2008)	In this study, different salt levels (NaCl) were examined on CM cheese	Camifloc cheese was made with different salt levels (0.0%, 0.5%, 1.0%).	In sensory evaluation, CM cheese containing 1% salt performed better than cheese containing 0.5% salt.
Benkerroum et al. (2011)	To study the effect of different levels of chymosin (Chy-Max) on CM cheese yield and microbiological quality	Pasteurization (71°C, 30 sec), cooling to 37°C, addition of $CaCl_2$ (0.02%) and incubation with a starter culture (3%, 90 min), addition of chymosin (Chy-Max, 0.05-15 mL⁄L), and coagulation until a firm curd is visually observed.	Chymosin concentration of 1.7 mL⁄L obtained better yield, and 2.9 mL/L of chymosin scored the sensory properties and microbiological quality.

Table 4: A summary of studies performed on CM cheese production (continued)

Reference	Objective	Processing method	Key findings
Ibrahim & Khalifa (2013)	To examine the physicochemical and sensory properties of CM cheese in response to Microbial Transglutaminase (MTGase).	Pasteurization (72°C for 15 sec), cooling to 40°C, addition of salt (4%), starter culture (2%), $CaCl_2$ (0.03%), and rennet (1ml /L), coagulation (7 hours).	MTGase addition provided cheese having higher yield, protein, total solids, and superior sensory attributes than control.
Derar & El Zubeir (2016)	An analysis was undertaken of the processing properties and sensory evaluations of fresh soft cheeses made from sheep or camel.	$CaCl_2$, CM, Camifloc enzyme, 25%, 50%, and 75% mixed with sheep's milk.	Adding 50% sheep's milk to camel's milk reduced the coagulation time. Cheeses with the highest texture were made with 75% and 50% sheep's milk.
Habtegebriel & Emire (2016)	To evaluate the effect of total solid, fat content, and amount of coagulant.	Pasteurization (65°C, 30 min), cooling to 42°C, addition of $CaCl_2$, incubation with starter culture (60 min), rennet coagulation (8 hours).	CM cheese yield was improved to 14.57% by adjusting the CM fat content to 1.82%, the total solid level to 14%, and adding 1.5mg of rennet powder to 100ml of milk.
Siddig et al. (2016)	To investigate the effect of acid and starter culture on white cheese prepared from pure CM and a mixture of CM and BM.	Pasteurization (65°C, 30 min), cooling to 40°C, addition of either citric acid (10%) or starter culture, and rennet (0.15 ml/L), and coagulation (5 hours).	Using starter cultures, the cheeses made using starter cultures had higher protein, fat, and overall solids content than the cheeses prepared using direct acidification.
Wale et al. (2017)	To evaluate the effect of camel Chymosin levels and cooking on coagulation.	Pasteurization (65°C, 30 min), cooling to 40°C, addition of $CaCl_2$, starter culture (0.5%), coagulation with camel chymosin (40, 70, or 100 IMCU/L), and cooking or no cooking of curd.	Cooked cheese with 100 IMCU/L had the highest values for protein, total solids, and hardness. However, the best overall sensory acceptance was for the cooked cheese made using 70 IMCU/L.
Hailu et al. (2018)	Coagulant (55 and 85 IMCU L) and two NaCl levels (2% and 5%, w/w) were studied.	Pasteurization (63°C, 30 min), addition of CaCl2 (0.02%), starter culture (75 U 1000/L at 38°C), chymosin (55 or 85 IMCU/L), NaCl (2 or 5%), and coagulation 2 hours.	The softening of the cheese texture was caused by 85 IMCU/L coagulants and 5% salt.
Mihretie et al. (2018)	To evaluate the coagulating effects of different levels of lemon juice on CM cheese.	Tests were performed with different volumes of lemon juice extract (150 ml, 200 ml, 250 ml, 300 ml, 350 ml, 400 ml, 450 ml, and 500 ml) for 24 hours at ambient temperature.	Increased yield of cheese was observed in 500 ml of lemon juice added 2L of CM. The cheese is fatty, with high moisture content.

Table 4: A summary of studies performed on CM cheese production (continued)

Reference	Objective	Processing method	Key findings
Saadi et al. (2019)	An investigation of the chemical composition of cheese made from CM or a mixture of CM and sheep milk	Pasteurization (71°C, 30 sec), trypsin enzyme (0.5 g) and $CaCl_2$, (0.5 g) in 5 kg of milk treatment (1) 100% CM, (2) 75% CM, (3) 25% CM, (4) 50% CM and 50 sheep milk, and (5) 100% sheep's milk.	Solids, fat, and protein percentages gradually increase with an increase in sheep's milk percentage. In the case of only using sheep's milk, the total solids were higher.
Abou-Soliman et al. (2020)	An investigation of how the level of (MTGase) after rennet addition impacts the properties of fresh CM cheese was undertaken.	Pasteurization (65˚C, 30 min), cooling to (40˚C), addition of starter culture (0.2 g/L, 30 min), camel chymosin after 30 min), MTGase (80, 100, or 120 U/L), and coagulation (3 hours).	Soft CM cheeses with 80 U of MTGase added after 30 min of renneting has better yield, texture, and sensory properties.
Fguiri et al. (2020)	To assess the ability of enzyme extract from *Ficus carica* to coagulate CM.	Pasteurization (65°C for 30 min), cooling to 40°C and lowering pH at 5.5, addition of starter culture and enzyme (1 mL/L milk), coagulation (24 hours at 37°C)	CM cheese produced a 15% yield with increased protein compared to BM cheese.
El Hatmi et al. (2020)	A study to examine the impact of UF and the addition of Allium roseum on CM cheese	Pasteurization (90°C for 10 min.), cooling to 45°C, addition of calcium chloride (0.2 g/L), starter culture (1%), and camel chymosin, coagulation.	Cheese made using the UF process has a firmer texture, higher levels of protein, and a higher fat content.
Bouazizi et al. (2021)	Comparing the coagulation behavior of CM with that of cow's milk.	Pasteurization (36˚C), addition of camel chymosin (Chy-Max®M, 55 IMCU/L), coagulation (2 hours).	The cheese composition, color, and texture were higher for cows' milk, but CM cheeses were preferred over cow's milk.
Fguiri et al. (2021)	Compared to commercial rennet, ginger, pineapple, and kiwi extracts were evaluated for their ability to clot CM in terms of yield and texture	Pasteurization (65˚C, 30 min), cooling to (40˚C), addition of starter culture (*Lactococcus lactis*), addition of enzyme (10%) after 1 hour, coagulation (24 hours at 37°C).	Among the enzyme extracts, kiwi enzyme extract showed the highest potential for milk clotting in cheese, similar to camel chymosin.
(Al-zoreky & Almathen 2021)	To evaluate the effect of chymosin and (cultured or non-cultured) CM cheese	Pasteurization (63°C, 30 min), cooling to 35°C, addition of $CaCl_2$ (0.02%), starter culture (3%), chymosin (50 IMCU/L), coagulation (12 hours).	CM cheese made from chymosin and tarter cultures had a higher cheese yield.

2.5.1 Component Standardization

Cheese's quality and yield are significantly impacted by the protein and fat content of milk. For a particular cheese to meet its standard, it is essential to standardize these components based on the protein or fat ratio (C/F). According to Irish cheese industry specifications, the proportion of protein to fat is between 0.84 and 1.02 for Cheddar cheese, with protein ranging from 2.99 to 3.59% and fat from 3.26 to 4.2% (Guinee et al., 2007). While in Canada for instance, Canadian Mozzarella can be called Mozzarella provided it contains 20% fat and 52% moisture and has a protein/fat ratio of 1.22. Whereas cheddar is specified to contain 31% fat, 39 % moisture and 0.91 protein/fat ratio (Emmons et al., 1990). It was reported that the mean values of CM protein and fat were 3.1% and 3.5%, with protein fat ratio (P/F) of 0.88 respectively (Al Kanhal, 2010). However, some part of the fat drains into the whey during the cheese-making process due to the loose casein network of CM, which allows fat globule to pass through the curd leading to loss of fat and some total solid into whey, thus lowing the cheese total yield and quality (Al-Zoreky & Almathen, 2021). Due to these facts, standardizing the fat and total solid is crucial to guarantee good quality textural properties of CM cheese. Several strategies have been utilized to maintain the total solid of CM, as shown in (Table 4), including the addition of casein and whey power concentration (Sobti et al., 2019). Adding milk powder (Awad et al., 2015; Desouky et al., 2019; Habtegebriel & Emire, 2016). Fortifying camel milk with sweet potato powder (Elnemr et al., 2020). Ultrafiltration (UF) (El Hatmi et al., 2020; Mehaia, 2006), mixing CM with milk of other animals (Derar & El Zubeir, 2016; Inayat et al., 2007; Saadi et al., 2019) and addition of microbial transglutaminase (MTGase) (Abou-Soliman et al., 2020; Ibrahim & Khalifa, 2013). Habtegebriel & Emire (2016) studied the optimization of fat and total solid of CM and showed that the

yield increased by 14.9% by adjusting the fat level of CM to 1.82%. Desouky et al. (2019) showed that the addition of CM power to camel milk (5-15%) improved camel cheese's attributes and sensory quality. Mehaia (2006) examined the effects of camel milk ultrafiltration on yield, composition, and sensory assessment. Saadi et al. (2019) reported that mixing with sheep milk at 50% and 75% levels increased the milk's fat and protein contents to (3.6%, 4.1%) and 4 %, 4.4% respectively compared to 3% and 3.1% of CM only. CM combined with buffalo milk increased the yield of soft cheese, total solids, fat, ash, and protein contents, as well as enhanced the organoleptic properties of produced cheese (Brezovečki et al., 2015; Inayat et al., 2007; Shahein et al., 2014). The study by Shahein et al. (2014) documented that mixing CM with buffalo milk reduced rennet coagulation time and the loss of total solid into whey compared to control camel milk only due to increased curd tension in improving the curd firmness.

Mehaia (2006) showed that compared to conventional cheese processing, UF treatment of milk improved the cheese yield and protein and fat recovery rates by 45, 40, and 42%. Furthermore, cheese from UF-treated milk has a higher sensory score compared to conventionally processed cheese. Among the most recent studies looking at camel-milk soft cheese's quality and antioxidant activity, Abou-Soliman et al. (2020) studied the effect of microbial transglutaminase (MTGase). The authors found that MTGase enhanced the properties of soft cheese but negatively influenced its antioxidant activity. An excellent sensory quality score is also dependent on timing and concentration; adding 80 units of MTGase to milk after renneting produces the highest solids and protein content. The study concluded that adding MTGase to soft, fresh CM cheese at the right concentration and time improves yield, texture, and sensory properties.

2.5.2 Pasteurization

It is often preferred to use pasteurized milk for cheese making since it kills almost all pathogenic and nonpathogenic organisms (Rankin et al., 2017). Several pathogens can be eradicated by using a pasteurization temperature of 72°C for 30 seconds or 65°C for 30 minutes (Chavan et al., 2011). However, high temperatures can result in weaker gels and longer coagulation times, leading to a low curd formation (Chavan et al., 2011). In addition, a temperature above 72°C /15 leads to denaturation of serum proteins and the construction of thiol-disulfide bonds exchange reactions on the surface of casein micelles (Bulca et al., 2004). Although, in cheese making, high temperature causes the disruption of disulfide bonds between whey proteins and casein micelles, serum protein/soluble k-casein complexes hindered rennet coagulability (Kethireddipalli et al., 2010). Qadeer et al. (2015) found that CM cheese yield decreased when the temperature exceeded 65°C for 30 minutes and Farah and Fischer (2004) reported that CM does not coagulate at pasteurization temperature above 65°C for 30 minutes. They related the obtained results to CM milk's casein micelle with a loose microstructure and micelle hydration, which resulted in fragile curd. In addition, high temperatures and the denaturation of serum proteins in cheese milk may adversely affect lactic acid production by starter cultures used to inoculate cheese milk (Stulova et al., 2011). Therefore, based on the above studies, it is recommended to avoid pasteurizing cheese milk for more than 30 seconds at 72°C (Fox et al., 2017; Guinee et al., 2007).

2.5.3 High-Pressure Processing (HPP)

As discussed above, high temperature leads to undesirable sensory and nutritional changes on the product (Tiwari et al., 2009). Thus, the food industry must

develop alternative processing technologies to preserve the nutritive value, safety, freshness, and flavors (Sevenich et al., 2016). Due to its ability to inactivate microorganisms, HPP is used in the food industry as a preservation technique. In addition, HHP eradicates spoilage microorganisms at room temperature (Chawla et al., 2011). By doing so, the food is preserved while maintaining its organoleptic and nutritional quality, which cannot be achieved by traditional thermal pasteurization (Sevenich et al., 2016). Inactivation of gram-positive organisms has been demonstrated using HP treatment at 100-600 MPa at 25°C for 10 minutes and a pressure of 300-500 MPa with the same temperature and time is satisfactory in the inactivation of gram-negative organisms in milk (Chawla et al., 2011), It was also demonstrated that pressure treatments of 400-600 MPa give the same quality milk as pasteurization (Rademacher & Kessler, 1997). HPP affects the conformational structure of caseins in milk, which enhances its physiochemical properties and technological capabilities and by increasing the casein micelle size by weakening the electrostatic and hydrophobic interaction between the micelles, which leads to the aggregation of submicelles (Huppertz et al., 2006; Sivanandan et al., 2008). In addition, high pressure of 500 MPa denatures β-lactoglobulin, but the immunoglobulin and α-lactalbumin remain intact (Liu et al., 2005). HHP treatment of 500 MPa also causes a modification on the fat globule, which enhances the organoleptic properties of the milk (Chawla et al., 2011). Previous studies have shown that the coagulation properties (coagulation time, rate curd firmness, and yield) improved after HHP treatment of bovine milk (Liepa et al., 2017; Pandey et al., 2003), caprine milk (Buffa et al., 2001). The destruction of casein micelles leads to a high micellar surface area, causing the rennet to coagulate the milk faster (Huppertz et al., 2002). HPP treatment at 200 and 400 MPa enhances camel milk coagulation and consequently enhanced

coagulum strength, according to Omar et al. (2018). But HPP treatment at 600 and 800 MPa inhibits clotting.

2.5.4 Effect Calcium Chloride

In addition to protein and fat, calcium plays an essential role in cheese making (Priyashantha et al., 2019). Calcium enhances the interaction between and within casein particles, stabilizing them by shrinking the porous network inside (Li & Zhao, 2019; Lucey & Horne, 2018; Huppertz et al., 2017). There are several studies on the effect of calcium salts addition in milk gel formation (Li & Zhao, 2019; Lin et al., 2018; Priyashantha et al., 2019). Priyashantha et al. (2019) investigated how calcium and citrate contents affected casein micelle size during rennet-induced coagulation in BM and found that calcium slightly decreased and citrate slightly increased casein micelle size. Gels with calcium addition were stronger and coagulated faster than those with citrate addition. When calcium salts are added to skim milk, the pH decreases (Lin et al., 2018), and this pre-acidification optimizes rennet coagulation (Li & Zhao, 2019). Calcium chloride addition to CM cheese production has not been conclusive. According to some studies, adding calcium chloride before rennet reduced clotting time and improved CM cheese yield (El Zubeir & Jabreel, 2008; Kappeler et al., 2006; Khan et al., 2004; Mehaia, 2006). Some studies have reported that modifying the pH or CaCl2 concentration did not affect camel chymosin coagulated CM (Kappeler et al., 2006, Konuspayeva et al., 2014). Hailu et al. (2016a) concluded that $CaCl_2$ effects were pH-dependent (6.6–6.0) and that 0.02% CaCl2 at pH 6.3 reduced coagulation time.

2.5.5 Pre-acidification

Pre-acidification is necessary for manufacturing many cheese types with the suitable time and dose playing essential roles in cheese ripening and quality (Ali, 2010). Milk cheese that has been acidified has enhanced nutrients, improved texture, flavor, and several other organoleptic characteristics, inhibits microbial spoilage, and enhances coagulant activity during manufacture and ripening, as well as coagulant retention in cheese curds. The abovementioned factors greatly influence cheese texture, thus increasing total solids in the cheese (Aquilanti et al., 2006; Bintsis, 2018). Milk acidification could be done either directly, e.g., by the addition (glucono-6-lactone), or indirectly via the use of lactic cultures which produce lactic acid. The acidification process improves curd firmness, suppresses undesirable bacteria, and makes flavor compounds that give fresh cheese its aroma. Starter cultures also play crucial control and cheese ripening (Rakhmanova et al., 2018). Lactic acid bacteria (LAB) constitute a significant volume of the commercial starter cultures (Liu et al., 2011; Parmjit, 2011). Control of the pH of milk is essential for milk clotting enzymes at near to 5.5 pH (Farah & Fischer, 2004). According to previous findings, the pH of raw camel milk varies between 6.55 and 6.85, depending on environmental factors (Gopal et al., 2017). Making CM cheese acidify the CM slightly before adding enzymes by Lowering the pH of CM from 6.66 to 6.40 was found to decrease the clotting time by 28%, with a further reduction of 70% caused by the addition of rennet (Ramet, 2001). Some studies reported that reducing the pH of CM to 5.6 at temperatures up to 42°C reduces the coagulation time (Mehaia et al., 1995; Siboukeur et al., 2005).

2.5.6 Proteolysis of Cheese

CM cheese's low level of k-casein has been attributed in some literature to the difficulties and peculiar differences in quality between BM cheese and CM cheese (Bornaz et al., 2009; Kappeler et al., 2003; Konuspayeva et al., 2014; Konuspayeva et al., 2009). However, the exact reason for the softness of CM cheese remains unknown. Proteolysis in cheese involves several biochemical and physical reactions, including the breakdown of residual lactose, citrate, lactate, proteolysis, lipolysis, amino acid breakdown, and fatty acid breakdown (Fox et al., 2017). In addition, the milk proteases plasmin and cathepsin decompose the caseins in cheese to produce large and intermediate peptides (Hao et al., 2021) or as a result of the action of residual rennet or other coagulants retained in the curd after milk coagulation and by enzyme action of both the starter cultures and nonstarter cultures (Lucey, 2016; Santiago López et al., 2018). Different researchers have widely investigated the effect of Starter cultures on bovine cheese ripening and proteolysis (Caldeo et al., 2016; Hao et al., 2021; Hayaloglu et al., 2005). However, no studies were previously performed on CM proteolysis activity; thus, scientific research is crucially needed to inquiry whether the soft nature of CM cheese is due to protein degradation.

2.5.7 Sensory Evaluation of Camel Milk Cheese

Processing camel milk into cheese faces sensorial limitations due to the longer coagulation and the resultant soft cheese. Few studies have performed a sensory evaluation of CM cheese using a hedonic test (Bekele et al., 2019; Bouazizi et al., 2021; El Hatmi et al., 2020). Therefore, the cheese's sensory attributes such as color, texture, aroma, taste, and overall acceptance were assessed by Bouazizi et al. (2021), who examined the physical, chemical, sensory, and coagulation properties CM and

BM cheeses. As reported by the study's authors, CM cheese had a softer texture, possibly due to smaller and fewer protein aggregates than BM cheeses (Macdougall et al., 2019). However, CM cheese was preferred by the consumers compared to BM cheese.

Chapter 3: Physicochemical Properties, Microstructure, Sensory Quality, and Coagulation Behavior of Camel versus Bovine Milk Soft Unripened Cheeses

Preface to chapter 3

Previous studies have shown apparent differences between camel milk (CM) and bovine milk (BM) regarding processing properties such as coagulation time and curd strength, which are attributed to the unique composition of CM proteins. This chapter compares the microstructure, coagulation behaviors, and sensory attributes of CM cheeses produced by acidification and chymosin coagulation to monitor the coagulation behaviors of CM and BM; three different coagulants were used, namely: camel chymosin, citric and acetic acids, and coagulation behavior and time were observed. After milk coagulation, the coagulum microstructure was observed using scanning electron microscopy, and the fresh cheese was evaluated by sensory evaluation. This chapter has been published: Mbye, M., Sobti, B., Al Nuami, M. K., Al Shamsi, Y., Al Khateri, L., Al Saedi, R., . . . Kamal-Eldin, A. (2020). Physicochemical properties, sensory quality, and coagulation behavior of camel v apparent bovine milk soft unripened cheeses. *NFS Journal, 20,* 28-36.

3.1 Abstract

This study investigated the coagulation properties of camel milk (CM) and bovine milk (BM) and the resulting soft unripened cheeses obtained by treatment with chymosin (100 IMCU/ML milk) or citric acid or acetic acid (30% acid/L milk). The cheeses were evaluated for yield, moisture, microstructure, texture profile, rheology, and sensory quality. CM cheeses were significantly lower in hardness and rheological properties than BM cheeses ($p < 0.05$). Photo images and scanning electron microscopy revealed CM cheeses' characterization by a smooth and continuous casein network, thinner aggregate strands, and smaller pore spaces, while BM cheeses showed large pore spaces with irregular aggregates. Panelists evaluated the CM and BM cheeses as comparable for some attributes and preferred the cheeses prepared using citric acid.

In conclusion, soft cheese with weak structures can be prepared from CM. The coagulation time for CM was longer than that for BM. The specific coagulation behavior of CM has been attributed to the composition of the casein fraction.

Keywords: Camel milk, bovine milk, cheese, coagulation, microstructure, sensory.

3.2 Introduction

The expansion of the dairy industry by including milk from species other than bovine is a target for the future, primarily because of the allergy associated with bovine milk (Singh et al., 2017). Dromedary camels (*Camelus dromedarius*) are raise in arid areas of the world, and camel milk (CM) is believed to have high nutritional value (Kalla et al., 2017) and slow fermentation in hot environments compared to bovine milk (BM) (Al Kanhal, 2010; Khalesi et al., 2017). CM has been acknowledged for several health benefits in recent years, including antidiabetic, antiallergic, and other effects (Chen et al., 2019; Ehlayel et al., 2011; Mihic et al., 2016; Mirmiran et al., 2017; Zibaee, 2015). Due to these therapeutic effects and enhancements to gastrointestinal functions (Al Kanhal, 2010), CM is considered a healthier alternative to bovine milk, especially for diabetics and infants suffering from bovine milk allergy. Therefore, the production and consumption of CM are expected to increase significantly (Izadi et al., 2019; Solanki & Hati, 2018; Wang et al., 2018). The global camel milk market is estimated to increase between 2018 and 2022 by 7%, mainly from fresh milk sales (Technavo, 2020). Milk's valorization increases its shelf life by increasing its shelf life through fermented contributes new flavors and tastes. However, the processing of CM into fermented dairy products (cheese and yogurt) which is vital for increasing its shelf life and commercial value faces several technological challenges compared to that of bovine milk (BM) (Berhe et al., 2017; Dantas et al., 2016).

Different types of CM cheese can be produced from raw or pasteurized milk, including soft unripened, ripened, and cooked, to name a few (Ramet, 2001; Walle et al., 2017). Previous studies have shown that CM fails to form firm curd, leading to a

fragile and soft cheese structure (Berhe et al., 2017; Hashim et al., 2009; Rahman et al., 2009). Compositional properties, related mainly to the low level of κ-casein and the large micelle sizes, are considered the main factors responsible for the differences in cheese coagulation between a camel and bovine milk (Andoyo et al., 2015; Jean et al., 2006). The destabilization of the casein micelles and milk coagulation is brought about by chymosin's renneting action, which hydrolyzes κ-casein with a release of caseinomacropeptide (Nassar et al., 2020). Direct acidification using organic acids, such as citric and acetic acid, has also been used in cheese preparation (Mihretie et al., 2018). The addition of acids lowers the milk's pH, neutralizing the negative charges in the casein micelles that are the repulsion forces keeping them apart, leading to their coagulation (Adetunji et al., 2008). In the cheese industry, milk coagulation properties are good indicators of cheese manufacturing effectiveness as it correlates with cheese yield (Frederiksen et al., 2011; Pretto et al., 2013). Milk characterized by slow coagulation produces low-quality cheese (Beux et al., 2018). Therefore, studying the microstructure of cheeses, focusing on the protein clusters, is essential in understanding their sensory properties and quality (Impoco et al., 2007; Moghanjougi et al., 2020; Ong et al., 2013; Rovira et al., 2011). Moreover, the whey drainage rate after coagulation (Macdougall et al., 2019).

Therefore, in this paper, we studied not only the physicochemical and sensory attributes but also the microstructure and coagulation behavior of CM versus BM cheeses, conducting comparative and mixing experiments to understand the differences between CM and BM cheeses and to relate these differences to the compositional differences between these two kinds of milk.

3.3 Material and Methods

3.3.1 Materials

Pooled raw camel milk was obtained from (21 lactating camels) and raw bovine milk (100 cows) from Al Ain Dairy farm. The milk was cooled to 4±1°C and delivered to United Arab Emirates University, Food Science Department Laboratory, within two hours after milking. All the samples arrived at the laboratory in refrigerated coolers (4°C). Lyophilized yogurt starter culture Yoflex Express® 1.0 (which contains *Streptococcus thermophiles and Lactobacillus bulgaricus subsp. delbrücki*) and Recombinant camel chymosin (CHY-MAX®M) with an activity of 1000 IMCU/ml (Christian Hansen A/S, Denmark). Citric acid, acetic acid, calcium chloride, and all other chemicals and reagents of analytical grade were obtained from Sigma-Aldrich (Munich, Germany).

3.3.2 Preparation of Cheeses

Based on earlier reports, raw milk samples (2L per trial) were processed into the cheese using chymosin enzyme or organic acids (Mehaia, 2006). The CM was heated to 63°C for 30 minutes, and calcium chloride 270 mmol/L was added. The milk's temperature was brought down to 43°C, and the milk was inoculated with 3% (w⁄v) of an active thermophilic yogurt starter culture Yoflex Express® 1.0 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*). The incubation continued for 60 minutes until the milk's pH was lowered to 6.2, the recombinant camel chymosin (CHY-MAX®M) was added to the milk and stirred thoroughly (Walle et al., 2017). The incubation was continued for two hours until the pH reached 4.8, and the curd was observed, and then the curd was placed in cheesecloth to drain for 8 hours (Benkerroum et al., 2011). Acid cheeses were prepared

from 2L of milk according to Mohamed et al. (2013) with some modification. The milk's pH was lowered to the two kinds of milk (4.3 for CM and 4.6 for BM) by directly adding 30% citric or acetic acid to the milk. Next, calcium chloride (270 mmol/L) was added, and the curd was transferred to cheesecloth and allowed to drain for overnight as shown in Figure 7 (Mohamed et al., 2013); photographs of the cheeses were taken.

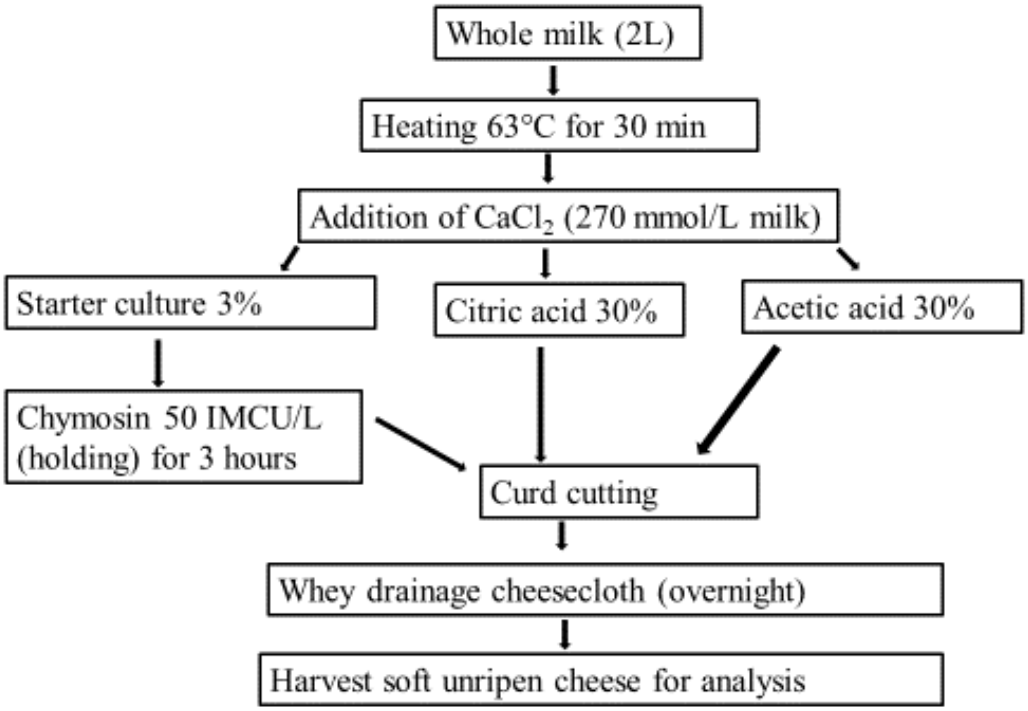

Figure 7: Cheese making process of CM and BM chymosin and acid cheeses

3.3.3 Cheese Yield, Structure, and Physicochemical Characteristics

The cheese yield was calculated as the percentage of weight recovered from the whole milk used for preparation (Akinloye & Adewumi, 2014). The pH of the samples was determined using a digital pH meter (OHAUS, Starter 3100, USA), and the titratable acidity was determined in triplicate using standard method ISO/TS 11869:2012 (IDF/RM 150:2012). The moisture content was determined by weighing five g of cheese sample on an analytical balance using a weighing boat. The samples

were placed on a glass moisture dish and dried in an oven for 5 hours at 105°C. The dried sample was cooled in a desiccator for 3 hours (Nielsen, 2010). The microstructure of different cheese samples was observed using a JEOL JSM-6010LA scanning electron microscope (SEM, Akishima, Tokyo, Japan). The lyophilized cheese samples were placed on an aluminum SEM stub with double-sided adhesive carbon tape and coated with gold. The samples were observed under a high vacuum at 20 kV voltage and recorded the samples' micrographs at a 600× magnification (Espírito-Santo et al., 2013). The hardness of the cheese samples was analyzed using a CT III texture analyzer equipped with a 4.5 kg load cell (Brook-field, Middleboro, USA). The hardness was carried out with a compression test of the cheese in a 40 ml cup using a 25-mm-diameter perplex cylindrical probe (TA11/1000) with a test speed of 1 mm/s and 3 mm of target distance (Shashikumar, Pradhan, & Mishra, 2018). The hardness pattern (force-time) was performed in triplicate. The linear viscoelastic region was determined by a stress-controlled rheometer (Discovery Hybrid Rheometer, TA Instruments, Delaware, USA) fitted with cone plate geometry (60 mm diameter and 2° of inclination angle). Samples were loaded and spread on the horizontal plate's surface, and excess samples were trimmed off. The cheese was rested for 5 minutes to allow it to attain thermal equilibrium and stress relaxation. The top plate was slowly lowered until the gap was 1 mm. Strain sweep tests were conducted from 0.01 to 100% at a frequency of 1 Hz. The linear viscoelastic region was where consecutive measurements (taken every 26.3 s) showed decreasing complex modulus (G*) in sequential measurements. The data obtained were elastic modulus (G′), viscous modulus G″), and complex viscosity (Pa. s), which gave the viscoelastic range. In all the rheological experiments, each measurement was performed in triplicate at a

controlled temperature of 25°C using a cooling water system (Thermo Cube Model 10-300-1CL, NY, USA).

3.3.4 Quantitative Descriptive Analysis (QDA)

Sensory descriptive analysis of cheese samples was conducted in the Department of Food, Nutrition & Health of UAE University, United Arab Emirates. Ten assessors (9 females, 1 male, aged 21 years and 58 years, respectively) were selected to evaluate cheeses based on specific criteria; interest, commitment, knowledge, familiarity with the product, and non-allergy to dairy products (Benkerroum et al., 2011). Panelists were trained for a total of 10 hours (2 hours/session). The sessions included a brief background about the samples, familiarization with reference materials, and sensory lexicon development. Thirty-one reference materials containing mainly dairy products were acquired from the market and provided during these sessions. The panelists developed 86 attributes and agreed on 18 features that best fit the cheeses' description. Each sensory quality was scored on an intensity scale ranging from 9 (high intensity) to 1 (low intensity). Cheeses were produced from camel milk and bovine milk with three different formulations viz. cheese from chymosin citric and acetic acid. Cheese samples were served randomly to the panelists in 3-digit labeled plastic containers. Evaluation of the final products was performed on two consecutive days. The sensory attributes appearance, odor, and texture by spoon were evaluated on the first day, while mouthfeel and flavor were assessed on the second day. Assessors used plain crackers and water to clean their palates in between the tasting of samples.

3.3.5 The Coagulation Behavior Camel and Bovine Milk

CM and BM samples' coagulation behavior treated with chymosin, citric acid, and acetic acid was observed for a length of one hour. Photos of the pieces were taken immediately after the coagulants' addition and subsequently after 20 min, 40 min, and 60 min. Two liters of each milk (camel or bovine) were acidified with 6M hydrochloric acid to pH 4 and centrifuged at 5000 × g for 20 minutes at 4°C to separate the casein from the whey proteins of each milk (Ahmad et al., 2019). Then 50 g of the casein fraction was mixed with 50 ml of the whey fraction in four combinations, namely bovine casein + bovine whey, bovine casein + camel whey, camel casein + bovine whey, and camel casein + camel whey. The casein-whey mixtures were vortexed for 2 minutes and allowed to settle for 3 hours before centrifugation and draining using cheesecloth. The obtained cheeses were subjected to texture profile and rheological analyses, as explained above.

3.3.6 Statistical analysis

Statistical analysis was performed using the commercial statistical package IBM SPSS (SPSS INC., Chicago, IL, USA). The experiments were performed in triplicate to evaluate the cheeses' physicochemical properties, and ten panelists assessed the cheeses' sensory quality. The physicochemical, rheological properties, texture, and sensory data were analyzed using a one-way analysis of variance (ANOVA) technique. The results were presented in the mean values of triplicate and their standard deviation (± SD). The means were compared using the least significant difference and considered a probability of $p \leq 0.05$ to be statistically significant.

3.4 Results and Discussion

3.4.1 Cheese Yield Physicochemical Characteristics and Microstructure

Table 5 presents data on the cheese yield, moisture content, pH, acidity, hardness, and rheology (G', G'', and complex viscosity) of six kinds of cheeses from camel milk (CM) and bovine milk (BM) using chymosin, citric acid, and acetic acid. Cheeses prepared using organic acids gave higher yields than those produced from BM due to high moisture retention than those made using chymosin ($P < 0.05$), in agreement with previous results on BM (Mihretie et al., 2018; Seth & Bajwa, 2015).

Table 5: Physico-chemical, yield, hardness and rheological properties, and moisture content of cheeses

Parameter	Camel			Bovine		
	Chymosin	Citric acid	Acetic acid	Chymosin	Citric acid	Acetic acid
Yield (%) (g cheese/100 g milk)	$11\pm.01^{e}$	$15\pm.01^{a}$	$13.5\pm.1^{c}$	12 ± 0.1^{d}	$14\pm.1^{b}$	$13.6\pm.1^{c}$
Moisture (%) cheese)	77 ± 1.0^{d}	$79\pm.8^{a}$	$79.1\pm.8^{a}$	70 ± 1.1^{d}	$74\pm.8^{c}$	73 ± 1.1^{c}
pH	$4.7\pm.01^{b}$	$4.0\pm.01^{d}$	$4.0\pm.01^{d}$	$4.9\pm.015^{a}$	$4.2\pm.01^{c}$	$4.3\pm.02^{c}$
Acidity (%)	$1.1\pm.01^{c}$	$2.7\pm.01^{a}$	$2.9\pm.01^{a}$	$0.9\pm.01^{d}$	$2.1\pm.01^{b}$	$1.9\pm.01^{b}$
Hardness (g)	681 ± 13^{d}	617 ± 5^{e}	564 ± 26^{f}	926 ± 22^{a}	881 ± 7^{b}	684 ± 2^{c}
G'(Pa)	2401 ± 1^{c}	2103 ± 2^{d}	1803 ± 3^{e}	5104 ± 3^{a}	4804 ± 4^{b}	4605 ± 4^{b}
G''(Pa)	351 ± 2^{c}	301 ± 1^{d}	308 ± 1^{d}	800 ± 1^{a}	700 ± 11^{b}	693 ± 2^{b}
Complex Viscosity (Pa)	214 ± 1^{d}	$198\pm.5^{d}$	209 ± 1^{d}	2762 ± 12^{a}	2685 ± 6^{b}	2475 ± 9^{b}

The tiny pore sizes can explain the higher moisture content in CM cheeses than in BM cheeses ($p \leq 0.05$) and higher water retention in CM compared to BM cheeses microstructure (see below). The caseins' arrangement may explain the lower cheese yields from BM into coarse matrices that contract to expel the whey (Fox et al., 2017).

Cheeses made from both kinds of milk using organic acids had significantly lower pH and higher acidity than those made using chymosin ($p \leq 0.05$). In agreement with these results, Figure 8 and Figure 9 present the visual and microstructures of cheeses prepared from CM and BM using acid coagulants and chymosin. The microstructure of CM cheeses was characterized by thin aggregate strands, homogeneous structures, and continuous networks. On the other hand, BM's cheeses had large, irregular lumps with granular forms in agreement with previous reports (Britz & Robinson, 2008).

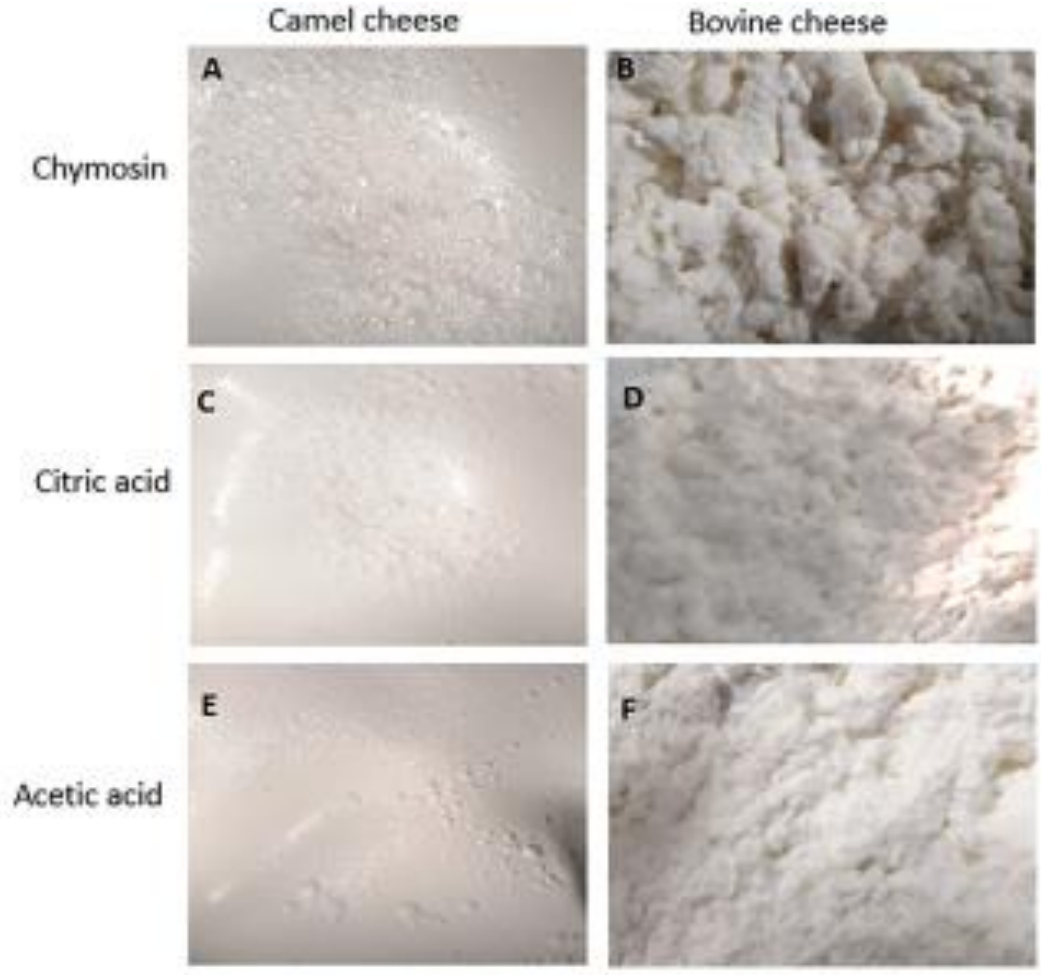

Figure 8: Photo Image of camel and bovine milk cheeses prepared using chymosin (100 IMCU/L milk), citric, and acetic acid (5 ml of 30% acid)

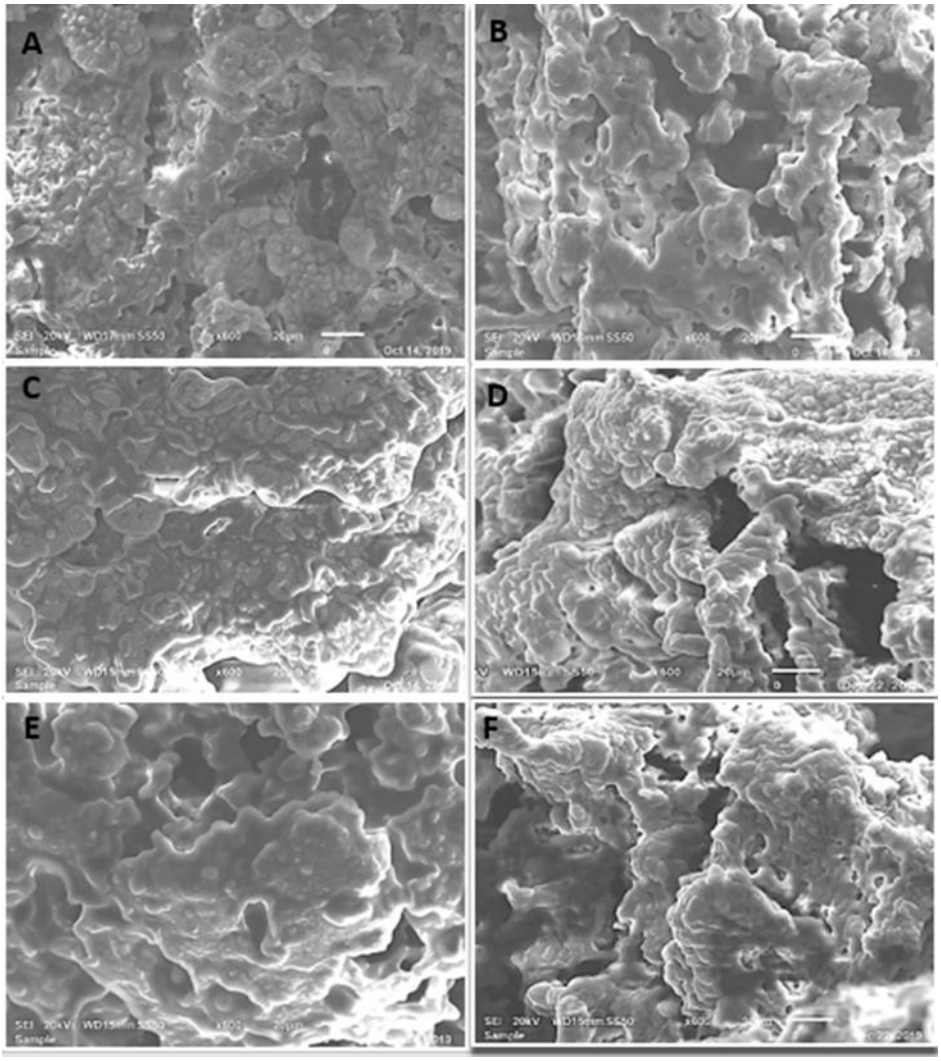

Figure 9: SEM micrographs (under 20 μM) of (a) camel acetic, (b) bovine acetic acid (c) camel citric acid, (d) bovine citric acid, (e) camel chymosin, and (f) bovine chymosin samples

Texture and rheology are essential quality parameters related to consumer acceptance of gelled foods, including cheese. In agreement with the above results and previous reports (Bekele et al., 2019; Hailu et al., 2014), BM cheeses were harder than CM cheeses. Chymosin was also observed to produce harder cheeses from both kinds of milk than those made from organic acids Table 5. The factors responsible could be associated with the faster coagulation and lower water retention of chymosin-coagulated cheeses. Chymosin reportedly triggers rapid casein cleaving and quick

rearrangement of the casein micelles, enhancing water drainage and increasing firmness (Guinee, 2003). The rheological of the cheeses are represented by elastic (G′) and viscous (loss modulus; G″) and complex viscosity as a function of amplitude sweeps (frequency at a constant value). BM cheeses had significantly ($P < 0.01$) higher G', G" and complex viscosity. The differences in the two kinds of cheese were attributed to the general stiffness of BM cheeses (Seth & Bajwa, 2015). BM cheese condensation into dense and more interwoven cheese structures (Xiong & Kinsella, 1991). The lower G', G", and complex viscosity of CM cheeses may be attributed to its soft coagulum (Macdougall et al., 2019). Similarly, higher values of complex viscosity, G', and G" were observed in yogurts made from BM than those from CM (Sobti et al., 2019). These results agree with (Bornaz et al., 2009), who reported that the low rennetability of CM resulted in weak gel strength. A greater elastic than viscous behavior ($G' > G''$) validates the dominant contribution of the storage modulus in the typical behavior of cheeses as viscoelastic solids (Salazar-Montoya et al., 2018).

3.4.2 Sensory Evaluation

The cheeses' physicochemical characteristics determine their sensory quality, essential in characterizing and defining their primary attributes (Ciappini et al., 2013). Therefore, quantitative descriptive analysis (QDA) was used to define and quantify the most important sensory attributes of the CM and BM cheeses prepared in this study. QDA was based on panelists' training and the development of a lexicon, or protocol, for detailed profile analysis of the experimental cheeses, which we then used as a standard to quantify the level of each attribute using a hedonic scale (Ciappini et al., 2013; Micşunica, 2016; Stone et al., 2012). The results of the QDA of the six different kinds of cheese prepared from CM and BM are shown in Table 6. The panelists did

not detect any significant effect of treatment, namely, chymosin, citric acid, and acetic acid, on any of the sensory attributes describing the appearance of the unripe cheeses of CM versus BM except for the evenness of color, which was the lowest for CM cheese prepared with citric acid. In terms of odor attributes, while they did not detect an effect of chymosin or citric acid on the odor strength, creamy odor, and pungent odor of CM and BM cheeses, the result of acetic acid on these odor attributes was more prominent in the case of CM cheese ($P \leq 0.05$).

Table 6: Assessment of the six different kinds of cheese by trained panelists

Sample	Camel			Bovine		
	Chymosin	**Citric acid**	**Acetic acid**	**Chymosin**	**Citric acid**	**Acetic acid**
Appearance						
Color	2±1.3^{ab}	3.1±1.6^{ab}	1.6±0.7^{a}	1.7±0.6^{a}	3.6±1.9^{b}	2±1.3^{ab}
Evenness of Color	8.5±0.8^{b}	6.1±1.1^{a}	8.3±0.8^{b}	8.2±0.8^{b}	7.6±1.8^{b}	8.5±0.8^{b}
Smoothness	7.6±2.4^{b}	6.1±0.9^{ab}	8.4±1.1^{b}	8.2±0.9^{b}	3.8±2.1^{a}	7.6±2.4^{b}
Presence of Lumps	2.2±2.3^{a}	5.7±1.3^{b}	1.5±1^{a}	1.9±1.1^{a}	6.5±2.7^{b}	2.2±2.3^{a}
Odor						
Odor Strength	3.3±1.3^{a}	6.4±1.4^{b}	5.8±2.3^{b}	4.6±1.8 ^{ab}	5.2±2.3^{ab}	3.3±1.3^{a}
Creamy Odor	4.2±1.9^{a}	6.3±2.0^{a}	4.9±2^{a}	4.6±1.1^{a}	6.1±2^{a}	4.2±1.9^{a}
Pungent odor	2.0±1.1^{a}	3.7±2^{ab}	5.2±2.7^{b}	2.7±1.4^{a}	2.4±1.8^{a}	2.0±1.1^{a}
Texture by Spoon						
Hardness	3.6±2.4^{a}	4.4±1.6^{ab}	3.0±2.3^{a}	4.8±1.82^{ab}	6.4±1.4^{b}	3.6±2.4^{a}
Spreadability	7.4±1.2^{c}	5±1.2^{b}	7.9±1.1^{c}	8.5±0.7^{c}	2.2±0.9^{a}	7.4±1.2^{c}
Crumbliness	7.4±2.5^{b}	5±2.2^{ab}	6.5±3.2^{b}	6.6±3.4^{b}	2.3±2.1^{a}	7.4±2.5^{b}
Mouthfeel						
Chewiness	2.1±2.1^{a}	5.4±1.3^{b}	2.9±2.8^{ab}	1.2±0.4^{a}	5.2±2.3^{b}	2.1±2.1^{a}
Firmness	1.8±1.5^{a}	4.7±1.1^{b}	1.9±1.4^{a}	1.5±0.5^{a}	6.0±1.1^{b}	1.8±1.5^{a}
Mouth-coating	3.9±1.9^{a}	5.8±1.5^{a}	4.4±2^{a}	4.6±2^{a}	6.0±1.8^{a}	3.9±1.9^{a}
Lumpiness	1.6±1.3^{a}	5.6±1.1^{b}	1.7±1.3^{a}	1.1±0.2^{a}	6.0±2.1^{b}	1.6±1.3^{a}
Flavor						
Saltiness	1.5±0.8^{a}	2.8±1.4^{a}	2.7±2.6^{a}	2.4±2.2^{a}	1.8±1.9^{a}	1.5±0.8^{a}
Sourness	2.2±1.9^{a}	1.5±0.8^{a}	5.8±2.1^{b}	5.3±2.6^{b}	1.3±0.5^{a}	2.2±1.9^{a}
Creaminess	6.0±1.5^{a}	4.6±1.4^{a}	6.1±1.2^{a}	5.7±1.5^{a}	6.2±1.9^{a}	6.0±1.5^{a}
Pungent	3.4±2.3^{ab}	4.0±1.3^{ab}	4.9±2.3^{b}	5.1±2.3^{b}	1.8±1.2^{a}	3.4±2.3^{ab}

On assessing the texture of cheeses with a spoon, the panelists observed the BM cheeses to be generally more rigid than the CM cheeses, although their responses were not sensitive enough to allow for statistical differences between the six kinds of cheese. CM cheese prepared from citric acid was more spreadable and less crumbly than its counterparts ($P \leq 0.05$) were. There was no significant treatment effect other than sourness for both CM and BM cheeses regarding mouthfeel and flavor. CM cheese prepared using acetic acid was experienced as more sour than the equivalent BM cheese. Thus, we suggest that CM is more suitable for manufacturing soft and spreadable cheese types and that cheeses made with citric acid may be preferable.

3.4.3 The Role of CM and BM Casein and Whey Proteins in Cheese Properties

The milk coagulation properties are crucial in cheese making (Cassandro et al., 2008). These properties are associated with several traits, such as the proportion of casein content and the titratable milk (Penasa et al., 2010). Results in Figure 10 show that BM has a substantially shorter coagulation time (about 20 minutes in agreement with (Cassandro et al., 2008), who reported 16.9 min, faster than CM. The results also revealed that coagulation times had a strong negative correlation with curd firmness. In agreement with (Ikonen et al., 2004). In this study, Table 5 and Figure 9, the chymosin coagulation time and the curd hardness were 60-minute and 422 g for CM cheese in contrast to 20 minutes and 4612 g for BM cheese, respectively. Bovine milk has about tenfold curd hardness value compared to CM. The factors responsible for the slow coagulation properties of CM could be low casein to whey ratio (Berhe et al., 2017), low κ-casein to total casein (3.5% of the whole casein) as compared to about 13.6% in BM (Mohamed et al., 2020b), and low calcium content (Beux et al., 2018).

The amount of casein in CM accounts for just 60% of the total protein (Farah, 1993) compared to 80% in BM (Rodriquez et al., 1985).

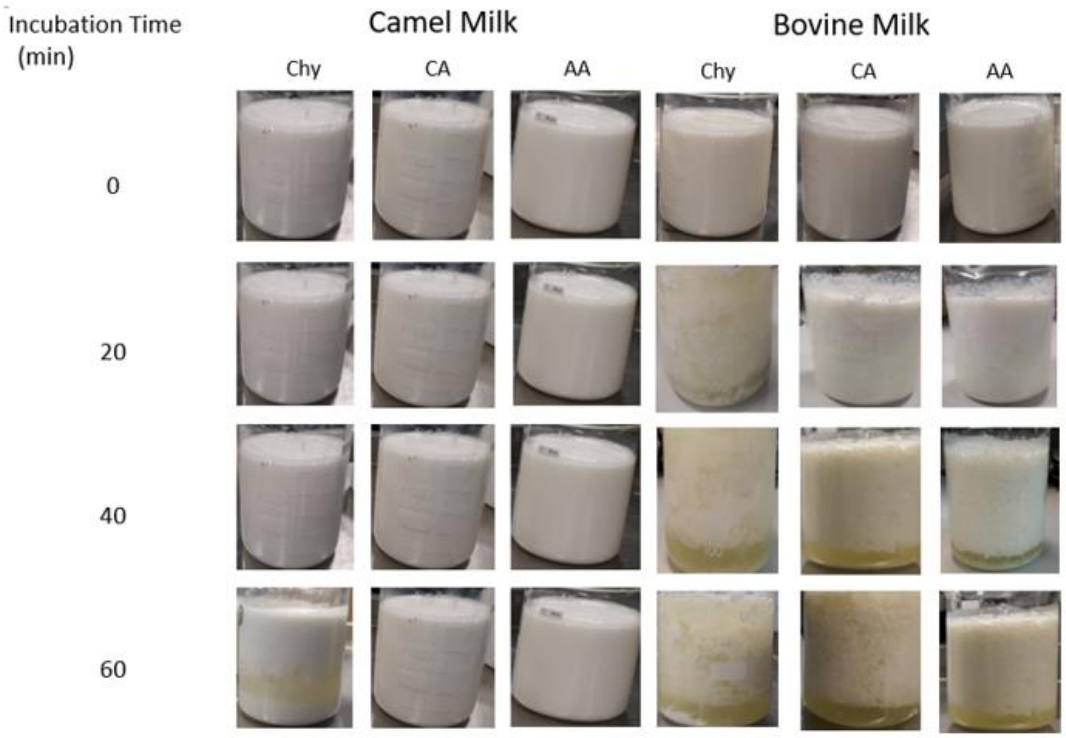

Figure 10: Photo Image of coagulation behaviors with time (0 – 60 min). chy (chymosin), CA (citric acid), AA (acetic acid)

Figure 11 presents photographs, microstructures, and hardness and rheological properties of CM and BM cheeses made by the four possible casein whey combinations, namely, (i) bovine casein + bovine whey, (ii) bovine casein + camel whey, (iii) camel casein + bovine whey, and (iv) camel casein + camel whey. Cheeses produced by these combinations present a clear and substantial difference between CM and BM proteins' effects on their structure, hardness, and rheological properties. The results show that CM cheeses were smooth and less granulated than the BM cheeses and that this effect is mainly due to the casein fractions. The κ-casein concentration and its relative proportions to αS- and β-caseins were reported to play an essential role in BM coagulation (Wedholm et al., 2006).

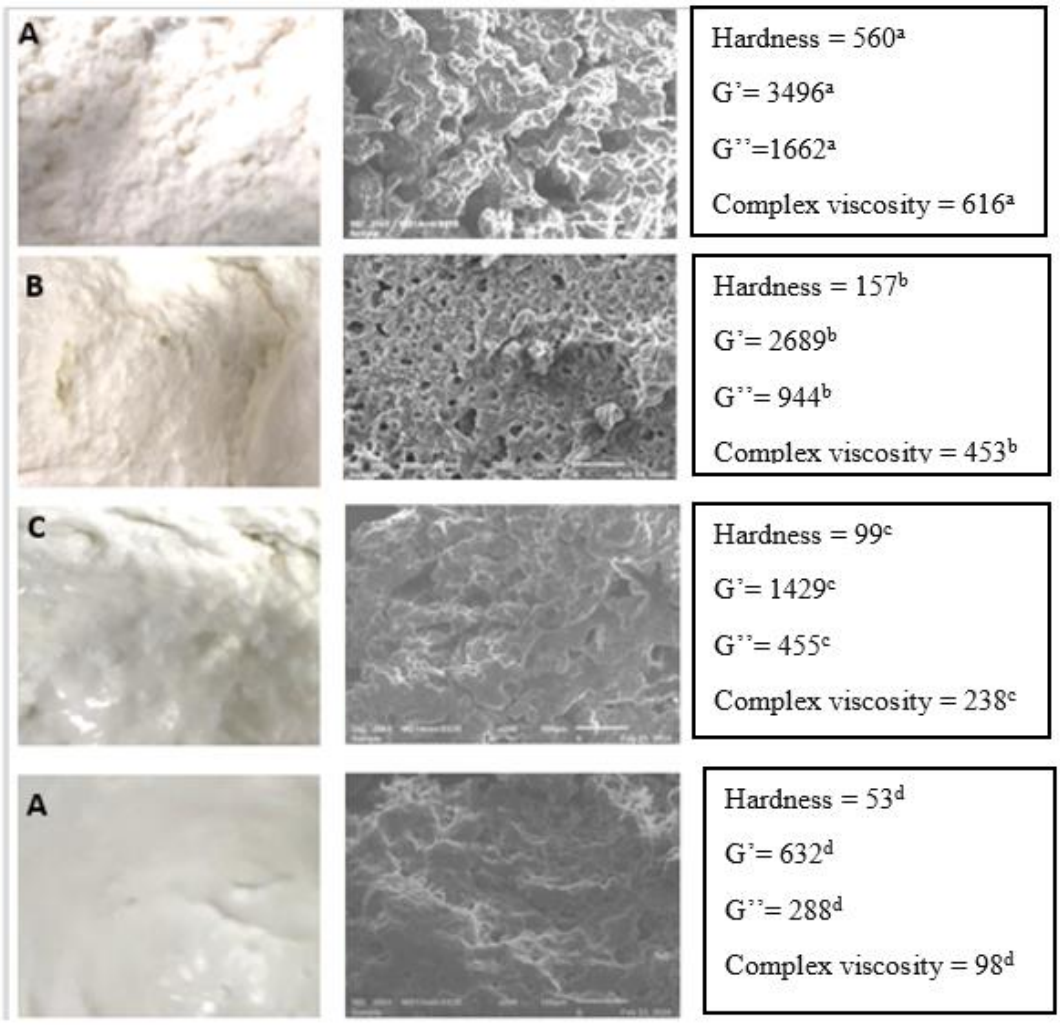

Figure 11: Photo Image, SEM, hardness, and rheological properties of cheeses casein x whey combination (A) bovine casein x bovine whey, (B) bovine casein x camel whey, (C) camel casein bovine whey, and (D) camel casein x came whey

Similarly, it was reported that higher levels of κ-casein in milk contribute to curd firmness and reflect the importance of the hydrolysis of κ-casein to para- κ-casein supports the aggregation and formation of the building blocks in the chymosin cheese matrix (Freitas et al., 2019). Thus, milk with higher κ-casein content and smaller casein micellar size enhance cheese gel strength (Creamer et al., 1998). The relative concentrations of αs1 support our CM cheese results -, αs2-, β- and κ- caseins in CM reported being approximately 22: 9.5: 65: 3.5 total casein (Mohamed et al., 2020b) in contrast to 40: 10: 40: 10 in BM total casein (Chandan, 2008). The poor coagulation

of CM was mainly attributed to the low concentration of κ-casein (Berhe et al., 2017; Kamal et al., 2017; Wedholm et al., 2006), but the high prevalence of β-caseins in CM may play a role in the observed differences in the cheese quality since β-casein has more chaperone-like activities causing suppression of milk protein aggregation (Zhang et al., 2005). Increased levels of β-casein in BM were reported to affect its coagulation properties negatively (Zhang et al., 2018). β-casein possesses a higher hydrophobicity than the other milk proteins (Zhang et al., 2005), which supports extended structures with small pore sizes, while the low level of κ-casein mainly contributes to suppressed protein aggregation (Swaisgood, 1993; Zhang et al., 2018). The small pore size in CM cheeses explains the water retention and the soft nature of CM cheeses shown in Table 5. The findings that cheese prepared from CM or BM using organic acids gave higher yields than those produced using chymosin suggest that the hydrolysis of κ-casein by the enzyme modifies the structure making more porous aggregations and lower water retention.

3.5 Conclusions

In this study, photo images and scanning electron microscopy revealed CM cheeses' characterization by smooth and continuous casein network, thinner aggregate strands, and smaller pore spaces compared to BM cheeses with large pore spaces and large irregular aggregates. Furthermore, mixing experiments between CM and BM caseins and whey proteins suggested that CM caseins were the main contributors to these structural features. Thus, the low level of κ-casein and the very high level of β-casein in CM compared to BM leads to the smooth structure and the tiny pore sizes in the CM cheese microstructure. This effect causes retention of moisture in the CM cheeses, thus, explaining their soft systems.

Chapter 4: The Effects of Camel Chymosin and *Withania coagulans* Extract on Camel and Bovine Milk Cheeses

Preface to chapter 4

Milk coagulation by chymosin is a primary step in commercial cheese manufacturing. Over the past decades, efforts have been made to find appropriate coagulant substitutes suitable for the coagulation of camel milk (CM). *Withania coagulans,* a plant base protease, has been in traditional cheese preparation from bovine, sheep, and goat in Pakistan, Afghanistan, and India. In this chapter, we compare the coagulation behaviors of CM and BM induced by this plant enzyme with chymosin. This chapter has been published: Mbye, M, Huda Mohamed, H, Raziq, A & Kamal-Eldin, (2021a). The effects of camel chymosin and *Withania coagulans* extract on a camel and bovine milk cheeses. *Scientific Reports*, *11*(1), 1-14.

4.1 Abstract

Withania coagulans (W. coagulans) extract and camel chymosin have aspartic protease capable of coagulating milk for cheese production. This study investigated the quality of camel and bovine milk cheeses set using *Withania* extracts, came chymosin, and their mixture in two experiments. In Experiment (1), a factorial design with four factors (*W. coagulans*, camel chymosin, incubation time, and incubation temperature) was performed. The effect of these factors on cheese's yield and hardness were assessed. An enzyme concentration corresponding to a 36 μg/L of milk of *W. coagulans*, 50 IMCU/L of camel chymosin, holding time of 4 hours, and incubation temperature of 60°C provided the optimal textural hardness for both camel and bovine milk cheeses. In Experiment (2), seven treatments were analyzed for physicochemical properties, yield, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results showed that pure Withania extract exhibited the lower coagulating effect resulting in cheeses with a soft product, hardness, fat, protein, and total solids. In addition, the SDS-PAGE electropherograms of camel cheese showed several low molecular weight bands compared to bovine cheese. This phenomenon is due to excessive proteolysis in camel cheese, which we believed is caused by endogenous enzymes.

Keywords: Camel milk, bovine milk, cheese, chymosin, *Withania coagulans.*

4.2 Introduction

The production and consumption of camel milk (CM) have increased over the years. The global production of camel milk and its products is projected to increase to 7% between 2018 and 2027 (Faostat, 2017; Technavo, 2020). CM is acknowledged for its nutritional and therapeutic qualities (Solanki & Hati, 2018). However, despite the exceptionality of CM, it's technically more difficult to process into cheese (Berhe et al., 2017; Bornaz et al., 2009; Konuspayeva et al., 2009; Yagil, 1982). The difficulty in producing high-quality cheeses from CM is attributed to larger casein micelles size (Ibrahem & El Zubeir, 2016; Kamal et al., 2017), long coagulation time (Mbye et al., 2020), low amount of κ casein (El Zubeir & Jabreel, 2008; Mehaia, 2006; Ramet, 2001), the small size of fat globules (Meena et al., 2014) compared to bovine milk.

Production of cheese from CM has been challenging due to the lack of coagulants that can specifically cleave CM κ casein. Thus, numerous approaches to make cheese from CM have been studied. Some of these studies include the use of camel chymosin with started cultures for acidification and better curd quality (Abou-Soliman et al., 2020; Al-Zoreky & Almathen, 2021; Bekele et al., 2019; Belkheir et al., 2020; Bouazizi et al., 2021; El Hatmi et al., 2020; Hailu et al., 2018; Mbye et al., 2020). In addition to chymosin, there has been a steady growth in plant enzymes in the cheese industry because they are readily accessible and have simple extraction and refining processes (Grozdanovic et al., 2013). Furthermore, animal enzymes are becoming unpopular in some countries due to diet and religious matters (Bathmanathan et al., 2019). Plant proteases can be obtained from extracts of *Cynara cardunculus* (Abd El-Salam et al., 2017), Algerian spontaneous *Cynara cardunculus* (Zikiou & Zidoune, 2019*)*, artichoke *(Cynara scolymus*, L.) (Chazarra et al., 2007), crude extracts of ginger rhizome (*Zingiber officinale*) (Hailu et al. 2014), and *Withania*

coagulans (W. coagulans) (Ebrahimnejad et al., 2019; Kazemipour et al., 2017; Salehi et al., 2017). *W. coagulans* belongs to the family Solanaceae and grows in Afghanistan, Pakistan, India, and Iran. Its extract has been traditionally used as a substitute for rennet in the preparation of cottage cheeses from bovine, goat, and sheep milk, especially in Baluchistan, Pakistan (Qazalbash et al., 2018; Ben et al., 2017). The active proteolytic enzyme in *Withania coagulans* was estimated to have a molecular weight of 66 KDa optimum activity at 70 °C and pH 4 (Beigomi et al., 2014; Kazemipour et al., 2017). The high proteolytic nature of most plant proteases may result in bitter flavors and low cheese yields; thus, their use is limited in cheese production (Egito et al., 2007; Shah et al., 2014).

Thus, this work aimed to conduct a comparative study of camel and bovine milk cheese coagulated with *W. coagulans* extract, camel chymosin, and mixtures of the two enzymes to explore the differences between the two milk sources. Two experiments were performed. In the first experiment, the effect of four factors, i.e. (*Withania coagulans* concentration, camel chymosin concentration, incubation time, and incubation temperature) cheese's yield and hardness, was assessed. In the second experiment, the effect of the two individual enzymes and five mixtures on yield, physicochemical parameters, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of the proteins of cheese and whey were analyzed.

4.3 Material and Methods

4.3.1 Materials

The milk used in cheese preparation pooled raw CM from 300 camels and BM from 800 bovines and was obtained from Al Ain Dairy farm, Al Ain City, Emirates of Abu Dhabi, UAE. The milk samples were delivered to the Food Science Department at United Arab Emirates University in refrigerated coolers (4°C). Milk composition was as follows: camel milk (pH, 6.5; acidity, 0.16%; total solids, 12.4%; protein, 2.8%, and fat, 3.3%) and bovine milk (pH, 6.69; acidity, 0.16%; total solids, 12.5%; protein, 3%, and fat, 3.4%). The lyophilized yogurt starter culture Yoflex Express® 1.0 (1:1) mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* subsp. *delbrückii* was used. Recombinant camel chymosin (CHY-MAX®M), with an activity of 1000 IMCU/mL, was kindly provided by Chr. Hansen Denmark. Fresh extracts from *W. coagulans* seeds, obtained from Loralai, Balochistan, Pakistan, were used. Urea Bio Ultra (for molecular biology, >99%), N, N, N′, N′-Tetramethylethylenediamine (TEMED), calcium chloride, and all other chemicals and reagents, all of the analytical grade, were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Precision Plus Protein–unstained standard (molecular weight marker), 4× Laemmli sample buffer (62.5 mM Tris HCl, pH 6.8, 10% glycerol, 1% lithium dodecyl sulfate, 0.005% bromophenol blue), resolving gel buffer (1.5 M Tris HCl, pH 8.8), stacking gel buffer (0.5 M Tris HCl, pH 6), SDS solution (10%), dithiothreitol (DTT), ammonium persulphate (APS), 10× TGS buffer (0.25 M Tris, 1.92 M glycine, and 1% sodium dodecyl sulfate), QC Colloidal Coomassie stain, and 30% acrylamide/Bis solution (29:1, v/v) were purchased from Bio-Rad Laboratories Inc. (Hercules, California, USA).

4.3.2 Experimental Design

The first experiment was performed using a central composite rotatable design, with varying combinations of *W. coagulans* concentration (7, 21, 36, 50, and 65 µg/1000 mL milk), camel chymosin concentration (10, 30, 50, 70, and 90 IMCU/1000 mL milk), incubation time, (1, 2, 4, 6 and 8 hour), and incubation temperature (40, 50, 60, 70 and 80°C), which were independent variables; then, the response variables (cheese yield and hardness) were measured (Table 7). In the second experiment, which was performed in triplicate, three sets of cheeses were made from each treatment using *Withania* extract, camel chymosin, or their mixture, as shown in (Table 9). In this experiment, several other traits were measured in addition to yield and hardness, including cheese color, titratable acidity/pH, protein, fat content, and SDS-PAGE electrophoretic profiles of both camel and bovine cheese, whey, and milk.

4.3.3 Enzyme Extraction

Enzymes were extracted from *W. coagulans* following a previously described method (Beigomi et al., 2014). *W. coagulans* berries were washed and dried in a cool place and then ground. The powder (10 g) was mixed with 100 mL of 1% saline solution for 24 h at 4°C with agitation. The mixture was centrifuged at 9,000 ×g at 4°C for 30 min. The supernatant was filtered through Whatman paper No. 1 (Kazemipour et al., 2017). The protein content in *W coagulans* crude extract was determined following the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as a standard. The absorbance of the supernatant was measured at 595 using a UV/visible spectrophotometer (Pharmacia Biothch ultrospect 3000, Cambridge, England). A freshly crude extract was used in making the cheeses.

4.3.4 Cheese Preparation

One litter of raw camel or bovine milk was processed into cheeses, in each of three repetitions per treatment, using camel chymosin or *W. coagulans*, or the mixture of the two coagulans. The milk was heated to 63°C for 30 minutes, and calcium chloride (270 mmol/L) was added based on earlier reports (Mehaia, 2006). The milk's temperature was brought down to 43°C, and it was inoculated with 3% (w⁄v) of an active thermophilic yogurt starter culture Yoflex Express® 1.0 (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*). The incubation continued for 60 minutes until the milk's pH was lowered to 6.2, the camel chymosin (CHY-MAX®M), *W. coagulans* extract, or the mixture was added to the milk and stirred thoroughly (Walle et al., 2017). The enzyme concentrations, incubation time, and incubation temperature in Experiment (1) are given in Table 7. While in Experiment (2) after adding (3%) starter culture and 50 IMCU/ L of milk camel chymosin or 36 µg /L of milk or the mixture Figure 12. The milk was incubated at room temperature (25°C) for four hours until the pH reached 4.8, and the firm curd was observed, and then the curd was placed in cheesecloth to drain for overnight Figure 12 (Al-Zoreky & Almathen, 2021; Benkerroum et al., 2011; Mbye et al., 2020).

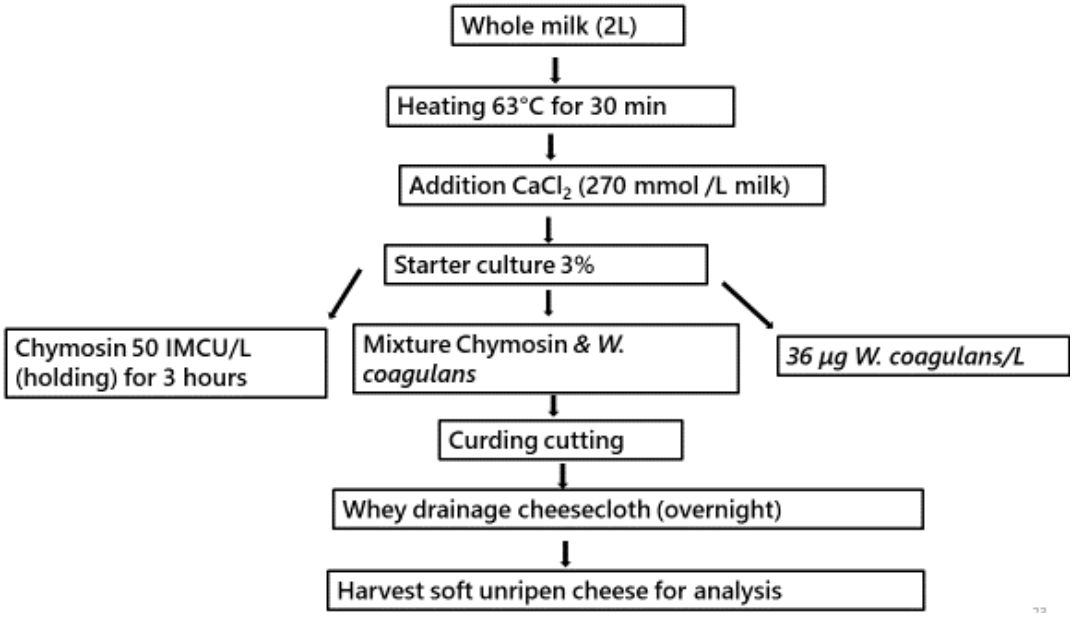

Figure 12: Cheese making process of CM and BM coagulated with camel chymosin or *W. coagulans*

4.3.5 Cheese Yield and Physicochemical Properties

Fresh cheese prepared from 1 liter of milk per trial was weighed, after 8 hours of draining, using Metis digital weighing scale (Dubai, UAE). The cheese yield was calculated as the percentage of the weight of the fresh cheese as follows (Yield = kg of fresh cheese*100/mL of processed milk) (Akinloye & Adewumi, 2014). The pH of the samples from both experiments was determined using a digital pH meter (Starter3100; Ohaus, New Jersey, USA), and the titratable acidity was determined in triplicate using the standard method ISO/TS 11869:2012 (IDF/RM 150:2012) (Mbye et al., 2020). The seven treatments of camel and bovine cheeses and wheys in the experiment (2) were evaluated for their contents of fat, protein, and total solid using a Near-Infrared Multipurpose Analyzer (Bruker Optik GmbH, Ettlingen, Germany). All the samples were tested on the same day, with each sample analyzed in triplicate. The texture profile analysis (TPA) of the camel and bovine cheeses from both experiments was analyzed for textural hardness using a CT III texture analyzer equipped with a 4.5-

kg load cell (Brook-field, Middleboro, Massachusetts, USA). TPA was performed with a compression test of the cheese in a 40-mL cup using a 25-mm-diameter perplex cylindrical probe (TA11/1000) with a test speed of 2 mm/s and a target distance of 5 mm (Mbye et al., 2020). The hardness pattern (force-time) was analyzed in triplicate. The color characteristics of camel and bovine cheeses in the experiment (2) were measured using a HunterLab color analyzer (Mini Scan XE Plus, Model 45/0-S, Hunter Lab Inc., Reston, Virginia, USA). Color values L*, a*, and b* were recorded, with each value being the average of four measurements. This color system comprises a lightness component (L*), a* component for green (−a) to red (+a), and a b* component from blue (−b) to yellow (+b).

4.3.6 SDS-PAGE of Cheeses and Whey Proteins

Proteolytic activity of the seven treatments of camel and bovine cheese, whey, and milk samples from camel and bovine in Experiment (2) were analyzed using SDS-PAGE. Cheese samples were prepared using previously reported methods (Hailu et al., 2018). The fresh cheese samples (0.6 g) were dissolved in 25 mL of 8 M urea, and whey samples (0.6 g) were dissolved in 8.3 mL of 8 M urea. The samples were homogenized for 2 min using T 25 digital Ultra-Turrax (IKA-Werke GmbH and Co. KG, Staufen, Germany). For the complete solubilization of caseins, the sample and urea mixtures were incubated in a temperature-controlled water bath at 37°C for 2 hours. The cheese and urea mixture was defatted by centrifugation at 9150 ×g at 4°C for 35 min. The solution was filtered through Whatman no. 1 filter paper (pore size, 11 µm). 10 µl of the filtered sample was added to 30 µL of 4× Lamelli buffer solution containing 50 mM Dithiothreitol (added freshly). The sample and sample buffer mixture were heated in a temperature-controlled water bath for 5 min at 90°C. From

this mix, 6 µL was loaded on the hand-casted polyacrylamide gels. Electrophoresis was performed at 200 V using a power supply from PowerPacTM Basic Power supply (Bio-Rad Laboratories Inc., Hercules, California, USA). The running buffer (pH=8.3) used was a 10 x Tris/Glycine/Sodium dodecyl sulphate Buffer (25mM Tris, 192 mM glycine, and 0.1% (w/v) sodium dodecyl sulphate. Gels with 1 mm thickness were prepared using the gel hand casting accessories provided with the Bio-Rad Mini-PROTEAN Tetra cell (Bio-Rad Laboratories Inc., Hercules, California, USA). A 12% resolving gel and 4% stacking gel were prepared. To prepare a quantity of 15 mL of 12% resolving gel solution the following were added: 6 mL 30% acrylamide / Bis Solution 29:1, 3.75 mL 1.5M Tris HCl (pH 8.8), 150 µl of SDS solution 10% (w/v), 5.03 mL deionized water, 75 µL of 10% APS (ammonium persulphate), 7.5 µl N, N, N′, N′-Tetramethylethylenediamine. To prepare a quantity of 15 mL of 4 % stacking gel solution the following were added: 1.98 mL 30% acrylamide / Bis Solution (29:1, v/v), 3.78 mL 0.5 M Tris HCl (pH 6.8), 150 µL SDS solution 10% (w/v), 9 mL deionized water, 75 uL 10% APS, 15 µL TEMED. The gels were kept for one hour in a solution of 40% ethanol and 10% acetic acid to fix the protein bands. Gels were stained for 20 hours using the QC colloidal Coomassie stain. The gels were de-stained for three hours by changing the distilled water three times. Gel DocTM XR+ and ChemidocTM XRS+ Imaging Systems (Bio-Rad Laboratories Inc., Hercules, California, USA). For imaging the gels, a UV/White light conversion screen was used. The instrument was operated by the Image lab software (Bio-Rad Laboratories Inc., Hercules, California, USA). The software was used to determine the protein bands' molecular weights, integrate the peaks, and determine their relative densities.

4.3.7 Statistical Analysis

A full factorial central composite design was used in the experiment (1). The values of four independent factors (*W. coagulans* extract concentration, camel chymosin concentration, incubation time, and incubation temperature) and their response variable is shown in (Table 7). The design consisted of 31 experiments, including seven central point repetitions that would account for the error in the model. This experiment was designed using Minitab®19 (Connecticut, USA). The model design was fitted to each response using the following equation:

$$y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j$$

Where y is the response, β_0 is a constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient. X_i and X_j are two independent variables. In this experiment, raw data from freshly extracted cheese was analyzed.

In the second experiment, the physicochemical and yield from freshly extracted cheese were analyzed in triplicate, and the mean values were used in the calculations. The statistical analysis was performed using IBM SPSS (SPSS Inc., Chicago, Illinois, USA). Data were analyzed using a one-way analysis of variance. The results were presented as the mean values of triplicate and their standard deviations. Mean values were compared using the least significant difference test, and $p \leq 0.05$ was considered to represent statistical significance.

4.4 Results and Discussion

4.4.1 Effects of Camel Chymosin and *Withania coagulans* on the Yield and Hardness of Camel and Bovine Cheeses

Table (7) presents the experimental design for the independent variables (*W. coagulans* concentration, camel chymosin concentration, incubation time, and incubation temperature) and the results of two associated response variables (cheese yield and hardness) in the experiment (1). Among the different combinations, crude extracts of *W. coagulans* (36 µg/L milk), camel chymosin (50 IMUC/L milk), coagulation time (4 hours), and incubation temperature (60°C) were the optimum conditions providing the lower fresh yield (Figure 13) and the highest hardness (Figure 14) for both camel and bovine milk cheeses. The negative correlation between the new outcome and the hardness of both camel and bovine cheeses ($p < 0.001$), as shown in Figure (15), is consistent with our previous observations and is explained by the increased retention of moisture in soft cheeses (Mbye et al., 2020). As the concentrations of added enzyme and the incubation temperature and time exceeded the optimum concentrations, the cheese became softer in agreement with the literature (Chazarra et al., 2007). This decrease in hardness may be explained by increased proteolysis of the casein proteins (Guo et al., 2004; Mbye et al., 2020; Seth & Bajwa, 2015).

Table (8) presents mathematical models that indicate the significance of the independent variables and their interactions in affecting the yield and hardness of camel and bovine cheeses in the experiment (1). All models significantly ($p<0.05$) suggest that the independent variables (concentration of *W. coagulans* and camel chymosin, and incubation time and temperature) collectively have similar effects on

the yield and hardness of both camel and bovine cheese. However, the magnitude of the impact is different. However, higher product and lower hardness are noticed more in camel than bovine cheeses (Konuspayeva et al., 2016). The results also show that chymosin interactions are more critical than Withania's in affecting cheese yield and hardness. The essential terms of the models were the constants related to the kinds of milk and the quadratic terms C*C, TP*TP, and TM*TM, all contributing negatively to the hardness of camel and bovine cheeses. These models suggest that the independent variables affected the camel and bovine milk cheeses fresh yield and hardness in similar ways, but the effects differed due to differences between the two kinds of milk.

Table 7: Experimental design of the independent variables (enzyme concentration, incubation time, and temperature) and results of the associated response variables (cheese yield and hardness)

Run order	**INDEPENDENT VARIABLES**				**RESPONSE VARIABLES**			
	W. coagulans extract (µg protein/1000 mL milk)	Chymosin (IMCU/1000 mL milk)	Incubation time (h)	Incubation temperature (°C)	**Yield (%)**		**Hardness (g)**	
					Camel cheese	**Bovine cheese**	**Camel cheese**	**Bovine cheese**
1	65	50	4	60	14.66	11.88	445	1302
2	36	50	4	60	12.98	8.44	609	1769
3	36	50	4	40	14.87	10.23	431	1396
4	50	70	6	70	16.76	14.02	260	799
5	21	30	6	70	15.08	12.75	398	1245
6	21	30	6	50	15.23	12.59	392	1168
7	7	50	4	60	14.51	11.01	491	1424
8	50	30	2	70	15.55	12.88	378	1009
9	21	70	6	70	16.71	13.89	258	844
10	21	30	2	50	13.13	8.78	580	1752
11	36	50	4	60	14.36	10.95	498	1490
12	36	50	4	60	13.44	9.54	561	1645
13	36	10	4	60	16.47	14.23	259	781
14	50	30	2	50	16.33	13.98	261	860
15	21	70	2	70	16.02	13.72	299	909
16	36	50	0	60	16.78	14.45	254	770
17	21	70	2	50	15.52	12.91	382	1009
18	36	50	4	60	13.89	9.88	511	1567

Table 7: Experimental design of the independent variables (enzyme concentration, incubation time, and temperature) and results of the associated response variables cheese yield and hardness (continued)

Run order	INDEPENDENT VARIABLES				RESPONSE VARIABLES			
	W. coagulans extract (µg protein/1000 mL milk)	Chymosin (IMCU/1000 mL milk)	Incubation time (h)	Incubation temperature (°C)	**Yield (%)**		**Hardness (g)**	
					Camel cheese	**Bovine cheese**	**Camel cheese**	**Bovine cheese**
19	36	50	4	60	14.02	10.8	501	1555
20	21	30	2	70	15.4	12.87	391	1156
21	36	50	4	80	15.06	12.98	354	988
22	50	30	6	50	15.03	12.56	400	1250
23	36	50	4	60	12.89	7.03	671	2297
24	50	30	6	70	15.71	13.01	345	940
25	36	50	8	60	14.96	11.44	411	1344
26	36	50	4	60	14.91	11.61	426	1336
27	36	90	4	60	16.66	13.87	276	876
28	21	70	6	50	15.92	13.62	309	918
29	50	70	2	50	15.87	13.57	311	925
30	50	70	2	70	15.81	13.34	322	931
31	50	70	6	50	16.22	13.88	287	895

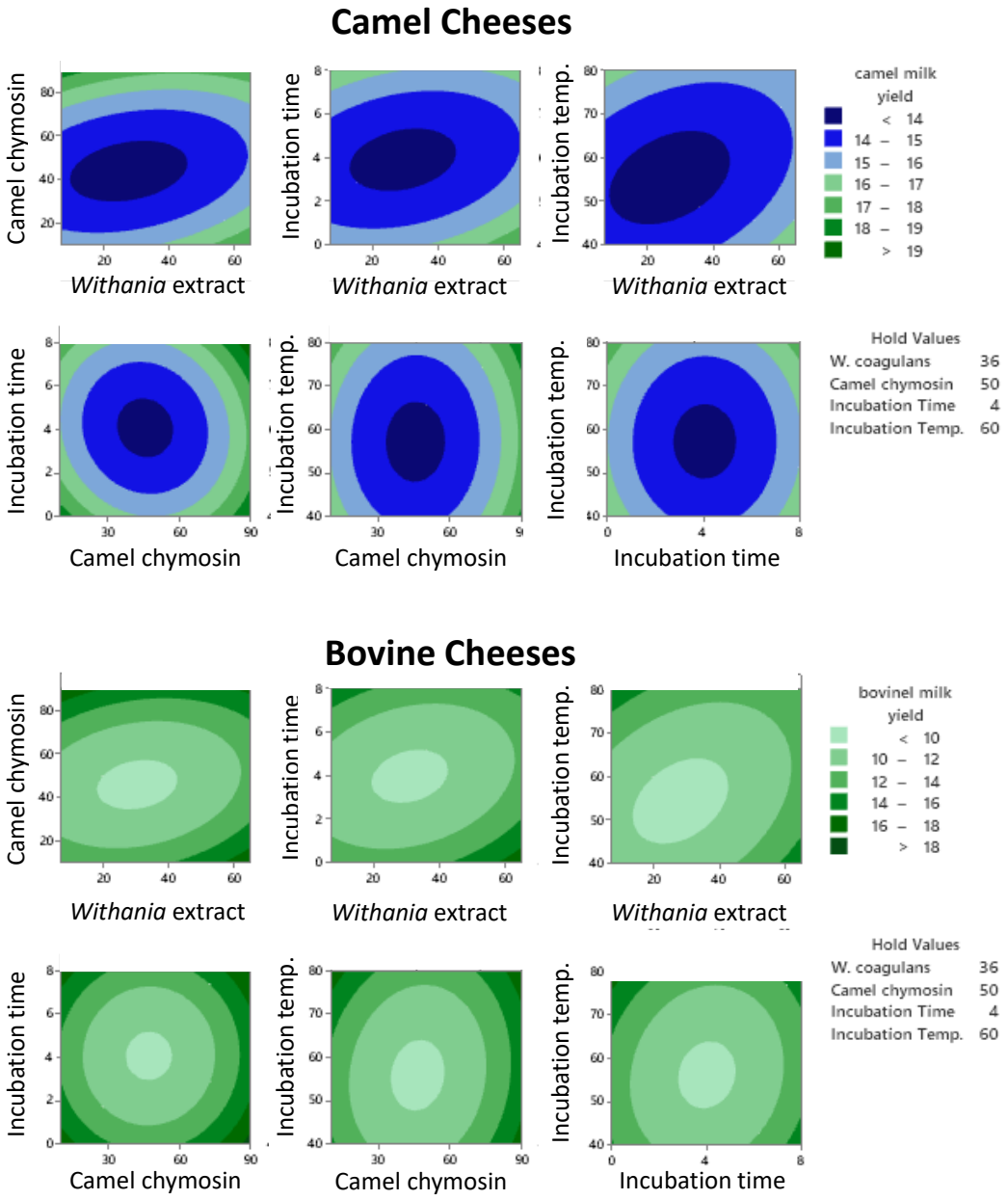

Figure 13: Interaction effects of four independent variables on the yield of camel and bovine milk cheeses

Camel Chymosin (IMCU/1000 mL milk), Withania extract (µg protein/1000 mL milk), Incubation time (hours), and Incubation temperature (°C) (Experiment 1).

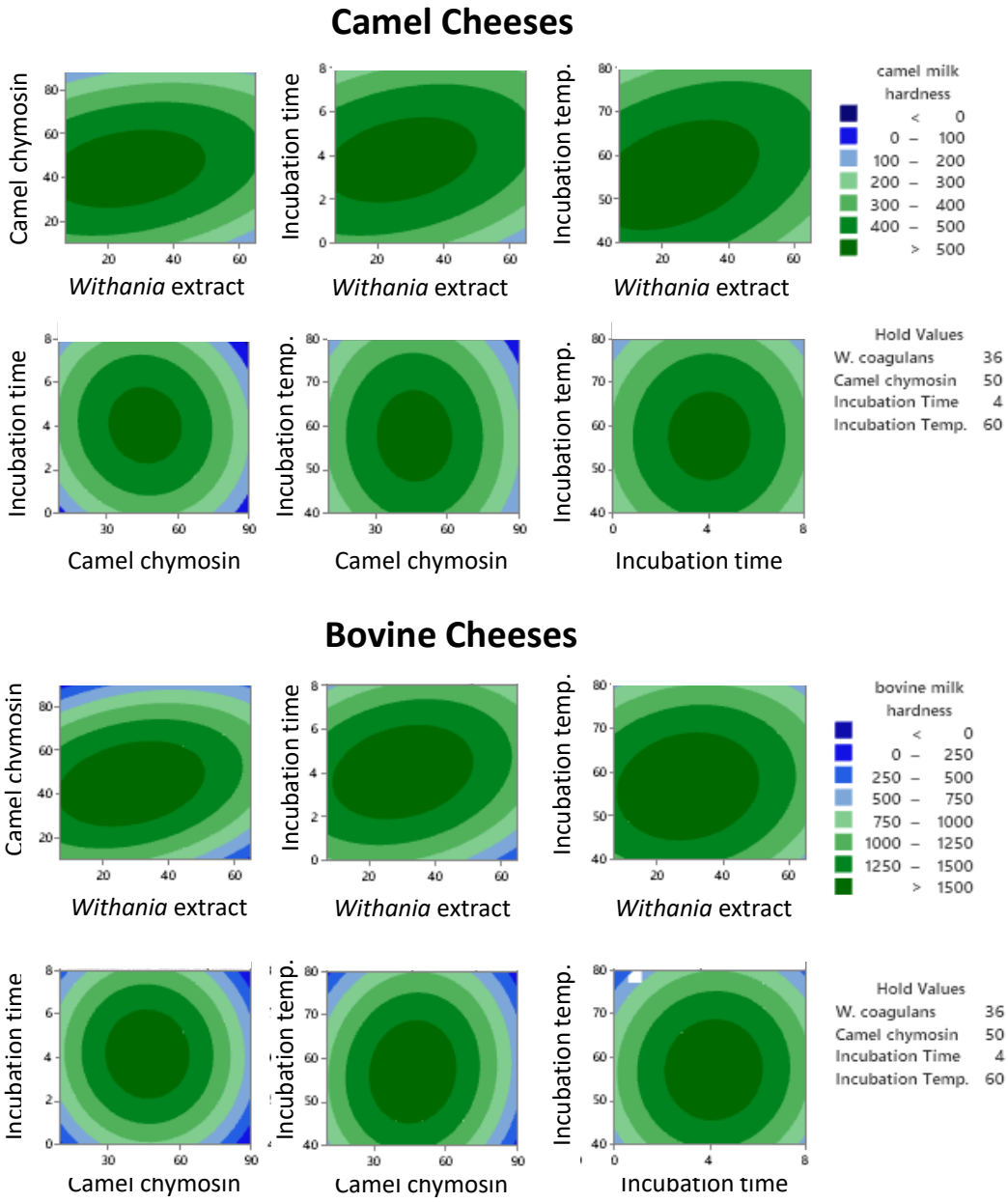

Figure 14: Interaction effects of four independent variables on the hardness of camel and bovine milk cheeses

Camel Chymosin (IMCU/1000 mL milk), Withania extract (µg protein/1000 mL milk), Incubation time (hours), and Incubation temperature (°C) (Experiment 1).

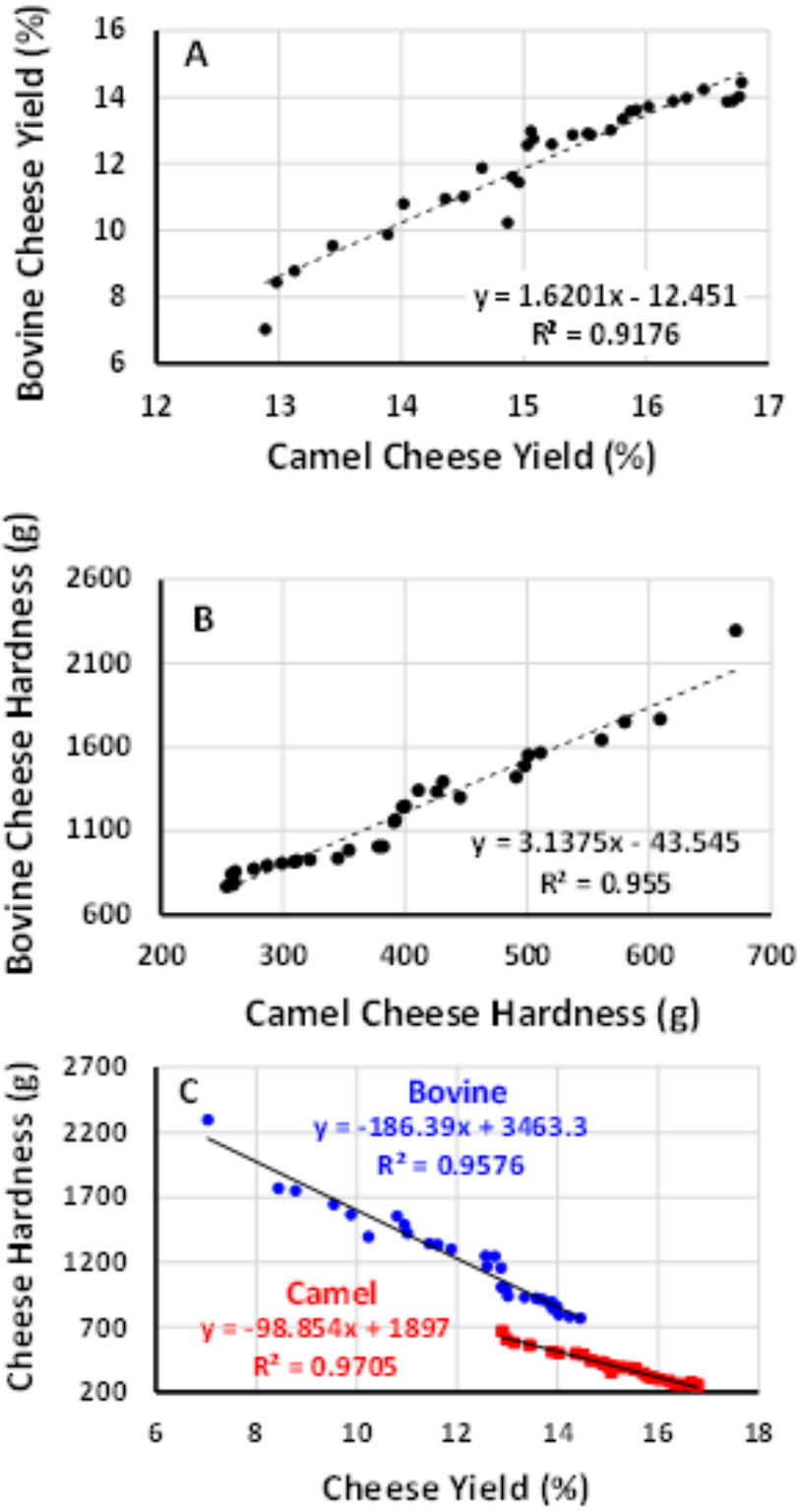

Figure 15: Correlations between (A) camel and bovine cheese yield (%), (B) camel and bovine cheese hardness (g), and (C) cheese yield and cheese hardness for camel (red) and bovine (blue) cheeses

Table 8: Model for the relations between dependent and independent cheese variables and estimated regression coefficients and their significance

Models constants & coefficients	Yield (%)		Hardness (g)	
	Camel milk cheese	Bovine milk cheese	Camel milk cheese	Bovine milk cheese
Constant	+ 26.39***	+ 25.80***	- 939***	- 3298***
C1(W)	+ 5.28	+ 7.1	+ 713	+ 369
C2(C)	- 0.014	- 0.018	+ 1.519	+ 3.55
C3(TM)	- 1.016	- 0.90	+ 91.9	+ 264
C4(TP)	- 0.314	- 0.449	+ 40.3	+ 134
C5(W*W)	+ 5.62	+ 12.73	- 517	- 2191
C6(C*C)	+ 0.006***	+ 0.000***	- 0.001***	- 0.005***
C7(TP*TP)	+ 0.13**	+ 0.221**	- 13.64**	- 41***
C8(TM*TM)	+ 0.003*	+ 0.005*	- 0.396**	- 1.304**
C9(W*C)	- 0.005	- 0.007	+ 0.483	+ 1.77
C10(W*TP)	- 0.423	- 0.761	+ 49.2	+ 127
C11(W*TM)	- 0.094	- 0.190	+ 11.34	+ 13.8
C12(C*TP)	+ 0.000	- 0.000	- 0.019	- 0.023
C13(C*TM)	-0.000	- 0.008	- 0.001	+ 0.013
C14(TP*TM)	+-0.002	- 0.008	+ 0.053	+ 0.43
Model p-value	0.01**	0.02*	0.009**	0.02*

*Abbreviations: C (chymosin), W (W coagulant), TM (Time) and TP (Temperature), Significance of model parameters: *($p < 0.05$, ** ($p < 0.01$, and *** ($p < 0.005$)

The second experiment, based on seven treatments Table (9), was performed by combining different concentrations of chymosin and *W. coagulans* extracts at fixed incubation temperature (60°C) and incubation time (4 h) that were chosen based on the results of Experiment 1.

Table 9: Withania and chymosin enzyme mixing protocols for detailed studies on cheese characteristics*

Treatment	**Abbreviation**	***Withania*** **(µg/1000 mL of milk)**	**Chymosin (IMCU/1000 mL of milk)**
Withania	W	65	0
Chymosin	C	0	70
Low *Withania*–Low Chymosin	LWLC	7	10
Low *Withania*–High Chymosin	LWHC	7	70
High *Withania*–Low Chymosin	HWLC	65	10
Medium *Withania*–Medium Chymosin	MWMC	36	40
High *Withania*–High Chymosin	HWHC	65	70

Table (10) presents results of the yield, hardness, and color of camel and bovine milk cheeses prepared using *W. coagulans* extracts, pure chymosin, and their mixtures. Cheese made from pure *W. coagulans* alone has the lowest cheese yield and hardness and is more yellow than the other cheeses. This yellow color of the *Withania*-treated cheeses may result from the presence of some water-soluble compounds in the berries (Salehi et al., 2017). The low yield and hardness of cheeses coagulated with the *W. coagulans* extract could be associated with poor coagulating properties of its protease compared with chymosin. This agrees with the results of experiment 1; the camel chymosin was more crucial for the cheese yield and hardness than the Withania extract. However, as the concentration of either enzyme increased, the cheeses became soft and fragile with high moisture content due to excessive hydrolysis of caseins (Guo et al., 2004; Mbye et al., 2020; Seth & Bajwa, 2015).

Thus, the results from the two experiments showed that unripened cheeses produced from camel milk have higher moisture contents and lower hardness than bovine cheeses, in agreement with our previous findings (Mbye et al., 2020). This difference was suggested to result from low κ-casein content in camel milk than bovine milk (Bornaz et al., 2009; Kappeler et al., 1999). However, we have suggested that the high percentage of β-casein might also contribute to camel cheeses' soft and smooth nature (Park & Jin, 1998). Moreover, the SDS-PAGE electropherograms (see below) suggest that some endogenous enzymes in camel milk might contribute to the softness of camel cheeses.

Table:10 Yield, hardness, and color of camel and bovine milk cheeses*

Treatment*	Yield (%)	Hardness (g)	Color		
			L*	a*	b*
	Camel Milk Cheese				
W	10.0 ± 0.75^{f}	181 ± 6.66^{e}	83.2 ± 0.2^{e}	-1.5 ± 0.09^{b}	14.7 ± 0.31^{a}
C	13.9 ± 0.17^{b}	279 ± 5.29^{c}	92.3 ± 0.3^{a}	-1.7 ± 0.10^{d}	6.7 ± 0.06^{f}
LWLC	11.0 ± 0.16^{e}	552 ± 19.5^{a}	89.6 ± 0.3^{c}	-1.7 ± 0.01^{e}	8.3 ± 0.19^{e}
LWHC	12.3 ± 0.31^{d}	424 ± 9.85^{b}	91.3 ± 0.3^{b}	-0.9 ± 0.01^{a}	5.8 ± 0.05^{g}
HWLC	13.4 ± 0.34^{c}	413 ± 8.33^{b}	83.6 ± 0.4^{e}	-1.6 ± 0.02^{c}	13.9 ± 0.09^{b}
MWMC	14.2 ± 0.22^{b}	254 ± 8.14^{c}	89.4 ± 0.1^{c}	-1.3 ± 0.03^{b}	9.9 ± 0.05^{c}
HWHC	15.1 ± 0.36^{a}	215 ± 17.3^{d}	88.6 ± 0.2^{d}	-1.7 ± 0.03^{d}	9.1 ± 0.11^{d}
	Bovine Milk Cheese				
W	9.4 ± 0.98^{e}	681 ± 55^{f}	79.9 ± 0.03^{g}	0.5 ± 0.03^{b}	22 ± 0.06^{a}
C	12.9 ± 0.09^{b}	1022 ± 35^{d}	90.7 ± 0.07^{a}	-1.2 ± 0.03^{f}	8.9 ± 0.11^{e}
LWLC	10.5 ± 0.2^{d}	1628 ± 18^{a}	88.7 ± 0.04^{c}	0.8 ± 0.03^{a}	11.7 ± 0.08^{c}
LWHC	11.5 ± 0.47^{c}	1240 ± 27^{b}	89.7 ± 0.03^{b}	0.8 ± 0.02^{a}	9 ± 0.11^{e}
HWLC	11.9 ± 0.24^{c}	1139 ± 35^{c}	85.9 ± 0.05^{f}	-0.1 ± 0.03^{c}	12.3 ± 0.14^{b}
MWMC	13.3 ± 0.3^{b}	824 ± 24^{e}	88.5 ± 0.04^{d}	-0.9 ± 0.03^{e}	10.3 ± 0.23^{d}
HWHC	14.0 ± 0.3^{a}	735 ± 36^{ef}	86.5 ± 0.03^{e}	-0.8 ± 0.03^{d}	11.7 ± 0.13^{c}

*Comparison was made between the different treatments for each cheese. Values within each column and cheese category (camel milk cheese or bovine milk cheese) carrying different superscripts are statistically different ($p<0.05$, n=3 per treatment)
**Abbreviations are shown in Table 9.

4.4.2 Effects of camel chymosin and *Withania coagulans* on the physicochemical properties of camel and bovine cheeses

Table 11 presents the pH/acidity, fat, protein, and total solids in camel and bovine cheeses and whey. The camel milk cheeses were generally more acidic than bovine milk cheese. *Withania*-treated cheeses possessed higher acidity and lower pH in both camel and bovine cheeses and whey products. The high pH in the chymosin-treated cheeses may be associated with rapid coagulation of milk after the addition of chymosin, which triggers rapid casein cleaving and quick rearrangement of the caseins (Fox et al., 2017). This results in larger pore spaces in the casein microstructure (Mbye et al., 2020) that enhance water drainage leading to increased pH due to lower activities of lactic acid bacteria in the dry gels (Guinee, 2003). In this study, we added calcium chloride (270 momol/L) to both kinds of milk as usually done during the preparation of bovine cheeses, but it was reported that there is no observed improvement by adding calcium chloride with camel chymosin (Konuspayeva et al., 2014). Table (11) shows significant differences between the different treatments to the pH and acidity of the other cheeses. The importance of electrolyte balance for enzyme activities and casein coagulation during cheese-making is not well understood and deserves further investigation. The equilibria involving minerals (mainly calcium and magnesium but also sodium) and anions (such as phosphate, citrate, and acetate) are essential determinants of casein micelle stability, pH, and enzyme activity (Kazemipour et al., 2017). Moreover, the camel's high acidity and low pH compared with the bovine cheeses may be explained by an increased degree of proteolysis in camel milk cheeses because proteolytic activities may produce peptides with acidifying effects (see below).

Cheeses made from a mixture of chymosin and *W. coagulans* had the highest total solids, protein, and fat. Furthermore, the results also show that fat, protein, and total solid contents were significantly higher in the bovine milk cheeses than in the camel milk cheeses, which agrees with previous findings (Hailu et al., 2018; Yirda et al., 2020). The lower protein, fat, and total solid contents of camel cheeses and the higher total solid in whey shown in Table (11) may be associated with the softer nature of these cheeses. Another factor could be a lower concentration of κ-casein in camel milk than bovine milk (3.3% vs. 13%). The proportions of αs1-: αs2-: β-: κ- caseins in camel milk were 2.6:0.4:6.7:0.3, compared with 4:1:4:1 in bovine milk (Mohamed et al., 2020b). κ-casein is known to enhance the coagulation properties of milk, leading to a denser casein matrix, which reduces the loss of fat and protein to the whey (Lomholt & Qvist, 1997; Dai et al., 2019; Ong et al., 2012). The κ-casein concentration and its relative proportion to αS1- and β-casein concentrations are usually low in poorly and non-coagulating bovine milk (Wedholm et al., 2006). Determination of the exact contribution of the different caseins in camel milk to the texture of camel milk cheese remains a challenge.

Table 11: Chemical composition of camel and bovine milk cheeses and whey

Treatment	pH	Acidity (%)	Total solids (%)	Fat (%)	Protein (%)	pH	Acidity (%)	Total solids (%)	Fat (%)	Protein (%)
	Camel Milk Cheese					Camel Milk Whey				
W	4.3±0.03^{d}	2.6±0.04^{a}	37.2±0.16^{f}	19.7±0.06^{e}	12.8±0.06^{de}	4.0±0.01^{e}	5.2±0.04^{a}	7.9±0.06^{a}	2.0±0.01^{a}	1.6±0.04^{c}
C	4.9±0.02^{a}	0.8±0.03^{f}	45.5±0.18^{c}	28.4±0.25^{b}	12.6±0.26^{e}	4.5±0.01^{a}	3.6±0.03^{g}	6.9±0.051^{c}	1.2±0.00^{c}	1.7±0.02^{bc}
LWLC	4.6±0.02^{b}	1.7±0.025^{d}	53.5±0.16^{a}	32.2±0.13^{a}	17.2±0.23^{a}	4.3±0.02^{c}	4.3±0.04^{d}	5.5±0.087^{d}	1.2±0.01^{d}	1.5±0.02^{d}
LWHC	4.7±0.04^{b}	1.7±0.02^{de}	49.5±0.38^{b}	28.6±0.42^{b}	15.6±0.28^{b}	4.4±0.01^{bc}	4.1±0.011^{e}	5.6±0.07^{d}	1.1±0.00^{de}	1.5±0.02^{d}
HWLC	4.4±0.04^{c}	2.4±0.02^{b}	45.8±0.16^{c}	23.5±0.34^{d}	14.6±0.28^{c}	4.2±0.02^{d}	4.6±0.04^{b}	5.7±0.09^{d}	1.1±0.007^{e}	1.5±0.03^{d}
MWMC	4.5±0.03^{c}	2.0±0.04^{c}	42.4±0.39^{d}	24.4±0.26^{c}	13.4±0.24^{d}	4.3±0.01^{d}	4.4±0.04^{c}	7.0±0.10^{c}	1.2±0.01^{c}	1.8±0.05^{a}
HWHC	4.7±0.03^{b}	1.6±0.07^{e}	40.5±0.47^{e}	17.9±0.09^{f}	12.7±0.09^{e}	4.4±0.04^{ab}	3.8±0.04^{f}	7.5±0.10^{b}	1.5±0.02^{b}	1.8±0.01^{ab}
	Bovine Milk Cheese					Bovine Milk Whey				
W	4.6±0.03^{d}	1.2±0.03^{a}	51.6±0.29^{e}	29.4±0.025^{f}	18.3±0.11^{e}	4.3±0.02^{c}	3.5±0.01^{a}	6.9±0.08^{a}	1.2±0.01^{c}	1.3±0.01^{abc}
C	5.3±0.05^{a}	0.2±0.04^{e}	68.9±0.33^{c}	41.4±0.08^{c}	19.5±0.31^{d}	4.6±0.02^{a}	2.0±0.05^{g}	6.3±0.03^{b}	1.4±0.01^{a}	1.4±0.01^{a}
LWLC	4.9±0.03^{b}	0.5±0.03^{d}	77.2±0.23^{a}	40.1±0.18^{d}	22.3±0.20^{a}	4.4±0.03^{b}	2.5±0.03^{e}	6.0±0.17^{b}	1.3±0.02^{b}	1.3±0.03^{bc}
LWHC	4.8±0.02^{bc}	0.6±0.04^{d}	76.7±0.22^{a}	44.3±0.08^{a}	21.4±0.23^{b}	4.4±0.02^{c}	2.9±0.02^{d}	6.1±0.16^{b}	1.3±0.02^{b}	1.3±0.02^{c}
HWLC	4.8±0.02^{c}	1.0±0.05^{b}	74.8±0.13^{b}	42.7±0.12^{b}	21.0±0.13^{b}	4.3±0.02^{c}	3.3±0.03^{b}	6.1±0.13^{b}	1.3±0.03^{b}	1.3±0.02^{bc}
MWMC	4.8±0.03^{c}	0.9±0.035^{c}	68.2±0.25^{c}	41.4±0.22^{c}	20.1±0.11^{c}	4.4±0.01^{c}	3.1±0.03^{c}	6.3±0.02^{b}	1.3±0.02^{b}	1.3±0.02^{ab}
HWHC	5.2±0.03^{a}	0.3±0.042^{e}	59.2±0.26^{d}	33.2±0.09^{e}	20.5±0.11^{c}	4.6±0.03^{a}	2.2±0.03^{f}	6.8±0.11^{a}	1.2±0.00^{c}	1.3±0.02^{abc}

* Comparison was made between the different treatments for each cheese. Values within each column and each of the four categories (camel cheese, bovine cheese, camel whey, or bovine whey) carrying different superscript are statistically different ($p<0.05$, n=3 per treatment)

4.4.3 SDS-PAGE Results on the Proteolysis of Camel and Bovine Milk Cheeses

The SDS-PAGE electropherograms showing differences in the protein and peptide profiles of camel and bovine cheeses and whey are presented in Figure (16). It can be observed that camel cheeses show several low molecular weight bands compared to bovine cheeses suggesting that excessive proteolysis of caseins has occurred presumably catalyzed by endogenous enzymes such as plasmin in camel milk (Baer et al., 1994; Rauh et al., 2014). The proteolysis of β-CN by the natural milk proteases (plasmin) was successfully found in milk samples analysis before (Ryskaliyeva et al., 2018). Thus, the high proportion of β-casein and possibly more active proteolytic activity in camel milk may increase the level of proteolytic products. It was reported that high levels of β-casein affect milk coagulation causing softness of cheeses (Zhang et al., 2018). We have observed similar behavior in camel milk fermented by the lactic acid bacteria used to make yogurt (results not shown). Some of the low molecular weight peptides from camel milk cheese seem to migrate into the whey fraction, explaining the low total solid content in camel milk cheeses and casein bands seen in the SDS-PAGE whey results.

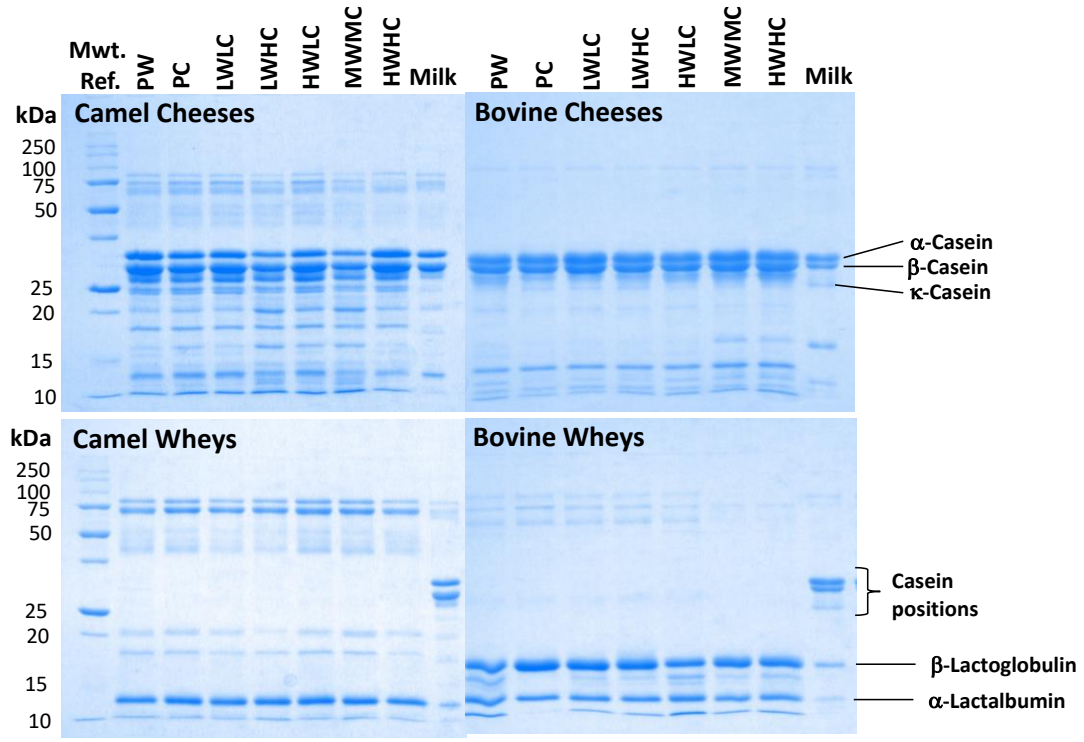

Figure 16: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of camel and bovine cheeses, wheys, and milk

4.5 Conclusions

This study investigated the effect of different combinations of *W. coagulans* extract and camel chymosin on the yield, hardness, and total solids (protein, fat, and other solids) of cheeses prepared from cheeses camel and bovine kinds of milk. The results revealed that too high concentrations of the enzymes resulted in the production of soft cheeses. *W. coagulans* extract protease alone is not sufficient to produce good quality cheese, especially camel milk cheese but a mixture of *W. coagulans* and camel chymosin produced better quality camel and bovine milk cheeses than chymosin alone. SDS-PAGE showed camel cheeses to more hydrolysis products than bovine cheeses suggesting possible participation of endogenous enzymes in camel milk. Further

studies are needed to identify the enzyme(s) responsible for proteolytic activity in camel milk and their contribution to milk coagulation and cheese softness.

Chapter 5: Effects of Pasteurization and High-Pressure Processing of Camel and Bovine Milk on Cheese Quality and Proteolysis Contribution to Camel Cheese Softness

Preface to chapter 5

Over the years, research regarding the effect of processing technologies on the physicochemical characteristics of camel milk cheese has grown. This chapter describes a study on the impact of two pasteurization treatments: heat treatment (65°C for 30 min and 75°C for 30 seconds) and high-pressure processing treatments (350, 450, and 550 MPa at 4°C for 5 min) on a camel and bovine cheese quality parameter including microbial load, yield, and proteolytic activities in cheeses, whey, and milk.

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5.1 Abstract

The effects of high-pressure processing (HPP) compared to thermal treatments on the quality of camel versus bovine cheeses were studied. The study showed that camel milk has a lower microbial load than bovine milk, which was maintained during the processed milk's seven days. The effect of three HPP treatments (350, 450, and 550 MPa for 5 minutes at 4°C) and two pasteurization treatments (65°C for 30 min and 75°C for 30 seconds) on the quality of soft unripened camel and bovine milk cheeses were accessed. SDS-PAGE electrophoresis evaluated the cheeses for pH, yield, proximate composition, textural and rheological properties, microstructure, and protein profile. The effects of the treatments on cheese's hardness were different between the camel and bovine cheeses; while heat treatment at 65°C for 30 min gave the hardest bovine milk cheese (1253±20), HPP treatment at 350 MPa for 5 min gave the highest value for camel milk cheese (519±5) ($p<0.05$). The hardness of the cheeses was associated with low yield and moisture content. SDS-PAGE electrophoresis revealed that extensive proteolysis might have contributed to the softness of camel cheeses compared to bovine and suggested the involvement of some residual enzyme activities.

Keywords: Camel milk, bovine milk, cheese quality, pasteurization, high-pressure processing.

5.2 Introduction

Nowadays, great attention is given to camel milk (CM) production and consumption because of its high nutritional value and digestibility (Izadi et al., 2019). Bacterial fermentation and cheese manufacture are typical of perverse dairy products (Muehlhoff et al., 2013). Still, up to now, their application to CM is limited due to the extreme softness of the produced coagulum (Mbye et al., 2020; Sobti et al., 2019; Sobti et al., 2020). The most crucial step in cheese making is chymosin-induced milk coagulation (Liepa et al., 2017). The coagulation rate and the outcome of the cheese are significantly influenced by different factors, including the animal species and breed, the composition of the milk, and pretreatment of the milk such as pasteurization homogenization, and pressure treatment (Huppertz et al., 2005). Milk pasteurization is an essential step in cheese making to ensure the safety of the cheese (Rankin et al., 2017). However, higher temperatures may lead to adverse effects on curd formation due to longer coagulation times and weaker gels (Chavan et al., 2011; Chawla et al., 2011) and are less suitable for cheese production (Huppertz et al., 2005). Thus, non-thermal technologies, such as high-pressure processing (HPP), have emerged as alternatives to traditional heat treatment in milk and dairy products (Muñoz-Cuevas et al., 2013).

HPP provides a valuable food preservation method that eliminates food bacteria by disrupting their cell membranes and the intermediate layer between the cell wall and the cytoplasmic membrane, deactivating membrane ATPase, and destroying the nucleic acids and ribosomes involved in protein synthesis (Datta & Deeth, 1999). Unlike heat treatments, HHP also maintains the quality of fresh foods with little effect on flavor and nutritional factors such as vitamins and other bioactive compounds (Norton & Sun, 2008). HHP of milk induces electrostatic interactions between proteins

leading to their disruption, solubilization of colloidal calcium phosphate, reduction in the size of casein micelles, and the whey protein's denaturation (Lopez-Fandino et al., 1996; Nassar et al., 2020). This modification was reported to improve the milk coagulation time and gel firmness of bovine milk cheese (Nassar et al., 2020; Pandey et al., 2003). To the best of our knowledge, no data is available to describe the effect of HPP on camel milk's microbial load and milks utilization in cheese manufacture. To explore a wider range of pressure-time combinations at a fixed temperature of 4°C, two experiments were performed. In the first experiment, the effect of pressure (350, 450, and 550 MPa) and time (3, 6, and 9 min) on microbial count load, cheese yield, hardness, and viscosity were assessed. In the second experiment, the effects of the two pasteurization temperatures (65°C for 30 min, 75°C for 30 seconds) and three high-pressure treatments (350, 450, and 550 MPa for 5 minutes at 4°C) on the textural and physicochemical properties of cheeses made from camel and bovine milk were studied. Analysis of the cheeses by SDS-PAGE electrophoresis showed that proteolytic activities generate many peptides in the camel but not bovine cheeses, which might be responsible for the softness of the camel milk cheeses.

5.3 Material and Methods

5.3.1 Materials

The milk used in cheese preparation pooled raw camel milk from 220 camels and bovine milk from 600 bovines and was obtained from Al Ain Dairy farm, Al Ain City, Emirates of Abu Dhabi, UAE. The milk was delivered to the Food Science Department at United Arab Emirates University in refrigerated coolers (4°C). The lyophilized yogurt starter culture used was Yoflex Express® 1.0, a 1:1 mixture of *Streptococcus thermophiles* and *Lactobacillus bulgaricus* subsp. *delbrückii*.

Recombinant camel chymosin (CHY-MAX®M, activity of 1000 IMCU/mL) was from Chr. Hansen (Hoersholm, Denmark). TEMED Ultra for molecular biology (N, N, N′, N′-Tetramethylethylenediamine, >99%), calcium chloride, and all other chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Unless otherwise stated, all the media and supplements used throughout microbial analysis are purchased from Oxoid (Oxoid, Basingstoke, Hampshire, England). Precision Plus Protein–unstained standard (molecular weight marker), 4x Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 1% lithium dodecyl sulphate, 0.005% bromophenol blue), resolving gel buffer (1.5M Tris HCL, pH 8.8), stacking gel buffer (0.5 M Tris HCL, pH 6), sodium dodecyl sulphate (SDS) solution (10%), dithiothreitol (DTT), ammonium persulphate (APS), 10 x TGS buffer (0.25M Tris, 1.92M glycine and 1% sodium dodecyl sulphate), QC colloidal Coomassie stain and 30% acrylamide/Bis solution 29:1 (v/v) were purchased from Bio-Rad Laboratories Inc. (Hercules, CA, USA).

5.3.2 Heat Treatments and High-Pressure Processing of Milk

The first experiment was performed using a central composite rotatable design with varying combinations of the independent variable pressure (308, 350, 450, 550 and 590 MPa at 4°C) and time (1, 3, 6, 9 and 10 Mins) (Raghubeer et al., 2020) and the response variables (cheese yield. hardness and viscosity) were measured Table (12). Similarly, microbiological analyzes were carried out on the milk samples after various pressure-time combinations or after the pasteurization treatments Table (14). The milk samples were subjected to two pasteurization regimes and three high-pressure treatment levels in the second experiment. In this experiment, several other parameters were measured in addition to yield, hardness, and viscosity.

Heat treatments of milk samples were performed by low-temperature long-time (LTLT, 65°C for 30 min) or high-temperature short-time (HTST, 75°C for 30 seconds) pasteurization. For the high-pressure processing (HPP), the two kinds of milk (camel and bovine) were filled in plastic bottles (330 mL) without any headspace and subsequently *vacuum* seal packed in polyethylene bags using a vacuum packaging machine Multivac Sepp C350 (Haggenmuller SE & CO. KG, Düsseldorf, Germany) before pressurizations. HPP treatments were performed using an Iso-Lab high-pressure pilot food processor S-FL-100-250-09-W (Stansted fluid power LTD Essex, UK). The HP unit consisted of a system that generates a maximum pressure of 700 MPa, an inlet and outlet temperature of 2-4°C, a pressure rate of 5 MPa/sec, and a heating rate of (0.5°C/100 MPa). This study performed HPP at three pressures (350, 450, and 550 MPa) at 4°C for different times, as explained in experiments 1 and 2. The system was equipped with a water jacket that allows temperature control in the pressure chamber by circulating cold water. The pressure chamber was filled with distilled water as the transmitting fluid. The plastic bottles containing the milk samples were submerged in the pressure chamber and then subjected to varying combinations of pressure and time, as described in Tables 12 and 14.

5.3.3 Microbiological and Raw Milk Composition Analysis

Milk samples (25 ml) were diluted in buffered peptone saline (225 ml, 0.5% w/v; peptone; 0.85% w/v; NaCl), mixed in stomacher bag (Seward 400, England) for 2 minutes. To quantify the various microbial groups, Increased sensitivity to <1 CFU (colony-forming unit) per mL was achieved by spread plating 1 mL of the undiluted sample onto the agar media as well as the 1:10 dilutions to eliminate any inhibitory effect that may be present in the undiluted sample. Total plate count (TPC) was carried

out on plate count agar (PCA), incubated at 32°C for 72 h (Marshall, 1992). According to the US standard method, the coliforms were determined by the most probable number (MPN) method (Register, 1990). *Staphylococcus aureus* was enumerated on Baird Parker agar supplemented with egg yolk, according to (Haaber et al., 2016). *Listeria monocytogenes* were detected according to (Hitchins et al., 2004), while the *E. coli* was examined with MacConkey agar followed by 24 hours' incubation at 37°C according to (Lupindu, 2017).

Lactose, protein, fats, and total solids contents (%) were evaluated using Near Infra-Red Multipurpose Analyzer (MPA), Bruker Optik Gmbh, (Ettlingen, Germany) (Mohamed et al., 2020a). The pH of the samples was determined using a digital pH meter (Starter3100; Ohaus, New Jersey, USA), and the titratable acidity was determined in triplicate using the standard method ISO/TS 11869:2012 (IDF/RM 150:2012) (Mbye et al., 2020).

5.3.4 Preparation of the Cheeses

Two liters of treated camel or bovine milk was processed into cheeses, three repetitions per treatment, supplemented with calcium chloride (3%) and incubated with 3% (w⁄v) of an active thermophilic yogurt starter culture at 43°C for 60 min to allow the pH to fall to 6.2 (Mbye et al., 2020). After that, recombinant camel chymosin (CHY-MAX®M, 50 IMCU) was added to the milk (Al-Zoreky & Almathen, 2021), and the incubation was continued for 3 hours until the pH reached 4.8, and firm curd was observed. Then, the curd was placed in cheesecloth to drain overnight Figure 17 (Benkerroum et al., 2011).

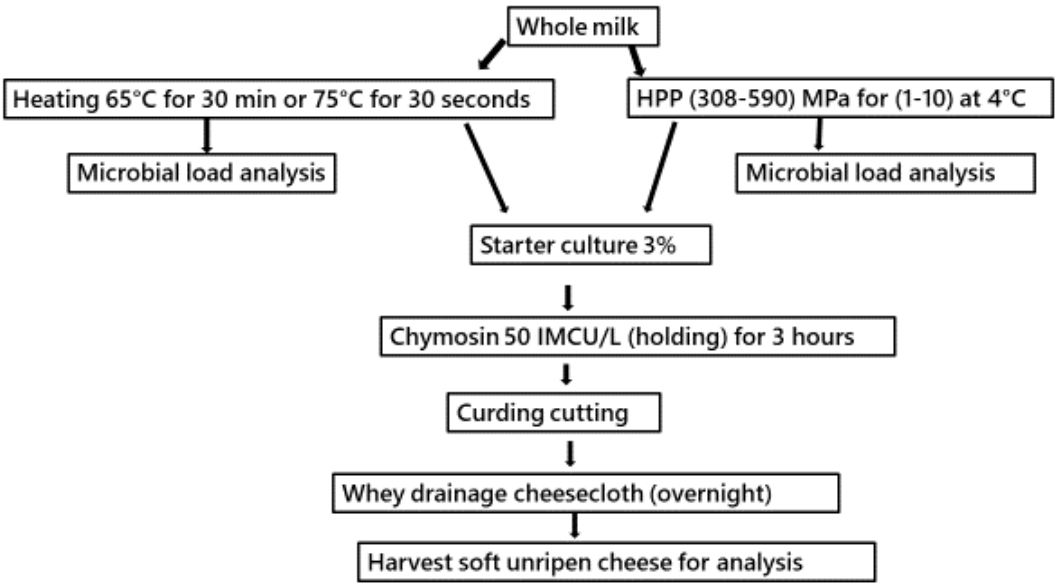

Figure 17: Cheese making process of CM and BM from pasteurized and pressured milk

5.3.5 Cheese Yield and Physicochemical Properties

The cheese yield was calculated as the percentage of weight recovered from the whole milk used for preparation (Yield = kg of fresh cheese*100/mL of processed milk) (Akinloye & Adewumi, 2014). The pH of the samples was determined using a digital pH meter (OHAUS, Starter 3100, New Jersey, USA), and the titratable acidity was determined in triplicate using the standard method ISO/TS 11869:2012 (IDF/RM 150:2012 (Mbye et al., 2020). The cheese samples' texture profile analysis (TPA) was analyzed using a CT III texture analyzer equipped with a 4.5 kg load cell (Brookfield, Middleborough, Massachusetts, USA). TPA was carried out with a compression test of the cheese in a 40 mL cup using a 25-mm-diameter perplex cylindrical probe (TA11/1000) with a test speed of 1 mm/s and 3 mm of target distance (Mbye et al., 2020). The hardness, cohesiveness, gumminess, and chewiness were performed on cheese samples at room temperature (Ong et al., 2012).

Linear Viscoelastic Region was determined in a stress-controlled rheometer (Discovery Hybrid Rheometer, TA Instruments, Delaware, USA) fitted with cone plate geometry (30 mm diameter and 2° of inclination angle). Samples were loaded and spread on the horizontal plate's surface, and leftover pieces were trimmed off. The cheese was rested for 5 min to allow it to attain thermal equilibrium and stress relaxation. The top plate was slowly lowered until the gap was 1 mm. Strain sweep tests were conducted from 0.01 to 100% at a frequency of 1 Hz (Mbye et al., 2020). The linear viscoelastic region was defined as the point where consecutive measurements (taken every 26.3 s) showed decreasing complex modulus (G*) in sequential measurements. The data obtained were elastic modulus (G′), viscous modulus (G″), and complex viscosity (Pa), which gave the viscoelastic range. Each measurement was performed in triplicate at a controlled temperature of 25°C using a water-cooling system (Thermo Cube Model 10–300-1CL, New York USA).

The microstructures of different cheese samples were observed using a JEOL JSM-6010LA scanning electron microscope (SEM, Akishima, and Tokyo, Japan). The lyophilized cheese samples were placed on an aluminum SEM stub with double-sided adhesive carbon tape and coated with gold. The samples were observed under a high vacuum and a voltage of 20 kV and recorded the micrographs of the pieces were at a 400× magnification (Mbye et al., 2020).

5.3.6 The Chemical Composition of the Cheeses and whey

The total solid, fat, and protein in camel and bovine milk cheese and whey samples were determined by near infra-red multipurpose analyzer using the equipment calibration model (MPA, Bruker Optik Gmbh, Ettlingen, Germany). All the pieces were analyzed on the same day in triplicate. Protein analysis of camel and bovine

cheese samples was performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). Cheese samples were prepared using the method described before (Park & Jin, 1998). Cheese samples (0.6 g) were dissolved in 25 ml. of 8M urea. The cheese samples were homogenized for 2 min using T 25 digital Ultra-Turrax (IKA-Werke GmbH and Co. KG, Staufen, Germany). To dissociate caseins, the urea-cheese dispersion was incubated in a temperature-controlled water bath at 37°C for 2 hours and then defatted by centrifugation at 9150 g at 4°C for 35 minutes and filtered through Whatman no. 1 filter paper (pore size, 11 µm). Of the filtered sample, a 10 µl portion was added to 30 µl of 4X Lamelli buffer solution containing 50 mM Dithiothreitol (added freshly). The sample and sample buffer mix were heated in a temperature-controlled water bath for 5 minutes at 90°C. From this mix, 6 µl was loaded on the hand-cast polyacrylamide gels.

Gels with 1 mm thickness were prepared using the gel hand casting accessories provided with the Bio-Rad Mini- PROTEAN Tetra cell (Bio-Rad Laboratories Inc., Hercules, California, USA). A 12% resolving gel and 4% stacking gel were prepared. To prepare a quantity of 15 ml of 12% resolving gel solution the following were added: 6 ml 30% acrylamide / Bis Solution 29:1, 3.75 ml 1.5M Tris HCL (pH 8.8), 150 µl 10% SDS solution, 5.03 ml deionized water, 75 µl of 10% APS (ammonium persulphate), 7.5 µl TEMED. To prepare a quantity of 15 ml of 4% stacking gel solution the following were added: 1.98 ml 30% acrylamide / Bis Solution 29:1, 3.78 ml 0.5 M Tris HCL (pH 6.8), 150 µl 10% SDS solution, 9 ml deionized water, 75 ul 10% APS, 15 µl TEMED. Electrophoresis was executed at 200 V using a power supply from Bio-Rad power basic. The gels were kept for one hour in a solution of 40% ethanol and 10% acetic acid to fix the protein bands. Gels were stained for 20 hours using the QC colloidal Coomassie stain. The gels were destained for three hours by

changing the distilled water three times. Gel DocTM XR+ and ChemidocTM XRS+ Imaging Systems (Bio-Rad Laboratories Inc., Hercules, California, USA) performed gel image acquisition and densitometry. The Image lab software (version 6) operated the instrument. The software was used to determine the protein bands' molecular weights, integrate the peaks, and determine their relative densities.

5.3.7 Statistical Analysis

A central composite rotatable design **(**Table 12) and the dependent and independent variables' model relationships (Table 13) were designed using Minitab®19 (USA). The physicochemical, textural, rheological, and proximate composition data were analyzed using a one-way analysis of variance (ANOVA) technique. The statistical data were analyzed using the commercial statistical package IBM SPSS (SPSS INC., Chicago, IL, USA). Cheese preparation and analytical measurements were executed in triplicate, and mean values and standard deviations were used in the calculations. Means were related using the least significant difference, and a probability of $p \leq 0.05$ was considered statistically significant.

5.4 Results and Discussion

5.4.1 Milk composition

The gross composition of camel milk (pH, 6.61; acidity, 0.15%; Lactose, 4.43%; total solids, 12.4%; protein, 2.7%; and fat, 3.1%) was slightly different from that of bovine milk (pH, 6.68; acidity, 0.15%; lactose, 5.08%; total solids, 12.6%; protein, 2.98%; and fat, 3.4%) in agreement with other researchers (Al Kanhal, 2010; Konuspayeva et al., 2009; Mohamed et al., 2020a; Nagy et al., 2019).

5.4.2 The Effects of (HPP) on the Yield, Hardness, and Complex Viscosity of CM and BM Cheese

Table (12) presents the experimental design for the first study of the effect of high pressure and time (independent variables) on cheese yield, hardness, and complex viscosity (associated response variables). Plots showing the interaction effects of the independent variables are shown in Table (13). The results clearly show that the increase in pressure results in higher yield but lower hardness and viscosity in both camel and bovine cheeses (Figure 18), which agrees with others (Huppertz et al., 2004; Nassar et al., 2020). The negative correlation between cheese yield and hardness is consistent with our previous observations that increased moisture content in the soft cheeses (Mbye et al., 2020). Certain HPP and pressurization conditions may promote extensive whey protein denaturation and interaction with the κ-casein on the surface of the casein micelle (Fox et al., 1998; Lopez-Fandino et al., 1996). Denatured whey proteins were suggested to protect the casein micelles from dissociation and serve as barriers against their aggregation, resulting in cheeses with a relatively open structure and high moisture retention (Gazi & Huppertz, 2015). In the case of cheeses made from LTLT and HPP at 350 MPa, this effect might have been minimal, explaining the more rigid texture and lower moisture content.

Table 12: Experimental design of the independent variables (pressure and time at 4°C) and results of associated response variables (cheese yield hardness and complex viscosity

Run Order	Independent Variables		Response Variables					
	Pressure (MPa)	Time (min)	Yield (g/100 g milk)		Hardness (g)		Complex Viscosity (Pa.s)	
			Camel Cheese	Bovine Cheese	Camel cheese	Bovine Cheese	Camel Cheese	Bovine Cheese
1	550	3	15.8	19.2	271	684	7990	10358
2	590	6	16.3	20.6	212	583	7819	9499
3	450	6	13.4	16.0	334	810	8778	13488
4	450	1	12.9	15.8	362	843	8821	13188
5	450	6	13.6	16.3	341	812	8766	13452
6	450	6	13.4	16.8	343	819	8758	13423
7	450	10	14.0	18.8	311	783	8655	13288
8	350	9	12.0	15.2	519	927	9431	16699
9	350	3	12.4	15.5	498	900	9212	16241
10	450	6	13.5	17.1	337	814	8722	13417
11	308	6	12.2	15.4	507	913	9330	16441
12	550	9	16.0	19.8	243	652	7919	10058
13	450	6	13.7	16.6	335	822	8768	13488

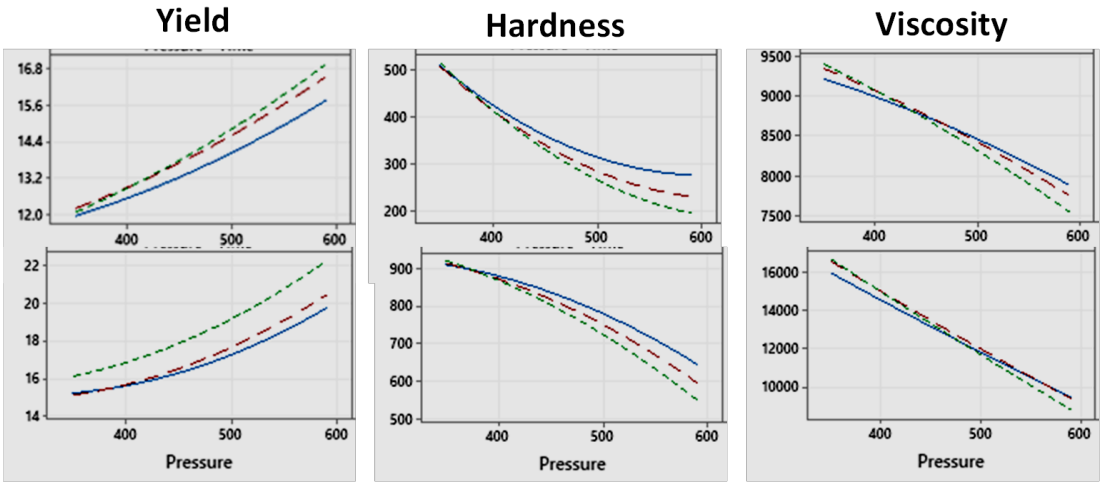

Figure 18: Effect of HPP pressure and time at 4°C on the yield, hardness, and complex viscosity of camel (Upper panel) and bovine (lower panel) cheeses.

Table (13) presents the mathematical models that show the significance of the independent variables and their interactions in affecting the camel and bovine cheeses' yield, hardness, and complex viscosity. The most important terms for both camel and bovine cheeses include the constant, which signifies the inherent differences between

the camel and bovine milk, the pressure, and the time and pressure square interactions. The effect of the independent variables on hardness and viscosity were qualitatively similar between the camel and bovine cheeses despite the notable quantitative differences where camel cheeses are significantly ($p < 0.001$) softer than the corresponding bovine cheeses. These observed difference between bovine and camel cheeses is reported to be mainly due to difference in caseins composition. There is a major difference between camel milk and bovine milk caseins. Camel milk caseins consist of α-s1, (22.0%), α-s2, (9.5%), β, (65.0%), and κ (3.5%) whereas bovine milk caseins consist of a high percentage of α-casein (38%) followed by 36-39% β-casein and 13% κ-casein (Mohamed et al., 2020b). Milk κ-casein is the major player in cheese quality because coagulation is initiated when the enzyme chymosin cleavages κ-casein to para-kappa-casein and caseinomacropeptide. CM coagulation takes longer than bovine, resulting in soft cheese texture with high moisture content (El Zubeir & Jabreel, 2008; Mehaia, 2006; Ramet, 2001).

Table 13: The model equation of independent and dependent variables and its estimated cheeses' estimated constant values. response variable value = constant + C1*pressure + C2*time + C3*pressure2 + C4*time2 + C5*pressure*time + residuals

Models constants & coefficients	Yield (%)		Hardness (g)		Viscosity (Pa.s)	
	Camel Cheese	Bovine Cheese	Camel Cheese	Bovine Cheese	Camel Cheese	Bovine Cheese
Constant	11***	11***	729***	1586***	9851***	26374***
C1	-0.00 5***	- 0.037***	+1.44***	-4.36***	+0.01***	-32.93***
C2	- 0.06	- 0.47*	+17.5 **	+ 12.2*	+123	+433
C3	+0.0002	- 0.0006	- 0.003**	+0.0037***	-0.006	+0.007
C4	- 0.009	+0.027	+0.082	+0.26	-1.69	-11.98*
C5	- 0.0006	+0.0007	- 0.049	- 0.0408	-0.243	-0.63*

5.4.3 Effect of HPP and Thermal Pasteurization on the Microbial Loads in Camel and Bovine Kinds of Milk

Table (14) revealed that all the pressure-time combinations used in this study were enough to maintain the total plate count and other bacteria below the acceptable limit in camel but not in bovine milk (Council, 2003). Studies have shown that HPP treatments at 350 and 450 MPa at room temperature and times less than 15-20 minutes are not adequate to reduce the microbial population (pathogenic and deteriorating) in bovine milk (Rendueles et al., 2011). Camel's antimicrobial effects against different pathogens such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Mycobacterium tuberculosis*, and *Salmonella typhimurium* have been reported (Almehdar et al., 2019; Mahmoudi et al., 2016; Sharma & Singh, 2014). Presence of antimicrobial agents in camel milk has been suggested as the reason for the lower bacterial growth compared to bovine milk (El Khasmi & Faye, 2019). These agents include peptidoglycan recognition protein (PGRP) enzyme, immunoglobulins (Igs), N-acetyl-β-glucosaminidase (NAGase), lactoferrin (LF) lactoperoxidase (LP), and lysozyme (LZ) (Mal & Pathak, 2010). The levels of LF, NAGase, and LZ are higher in camel milk compared to bovine milk (Berhe et al., 2018; Ho et al., 2019) while no PGRP is found in cow milk (Felfoul et al., 2017). However, Berhe et al. (2018) followed the growth of different bacterial cultures in camel milk and showed that they were not inhibited by the antimicrobial agents in the milk but by limited rates of proteolysis and availability of nutrients.

Table 14: Experimental design of the independent variables (pressure, time and at 4°C) on the associated total plate count (TPC) and *Staphylococcus aureus* (S) of camel and bovine HPP and pasteurization milk

Sample Code	Pressure (MPa) or Temperature (C)	Time (min)	Camel milk microbial load (log10 CFU/ml)				Bovine milk microbial load (lo10 CFU/ml)			
			Day 0		Day 7		Day 0		Day 7	
			TPC	S	TPC	S	TPC	S	TPC	S
1	550	3	5.1	4.6	4.7	5.0	7.1	4.0	7.8	5.4
2	590	6	5.0	4.8	4.3	5.1	7.0	4.0	7.7	5.4
3	450	6	5.2	5.0	4.4	5.2	7.2	4.3	7.8	5.4
4	450	1	5.5	5.0	4.8	5.2	7.3	4.7	7.8	5.4
5	450	6	5.3	5.0	4.7	5.2	7.3	4.6	7.8	5.4
6	450	6	5.3	5.0	4.4	5.0	7.3	4.8	7.8	5.6
7	450	10	5.1	5.0	4.3	5.1	7.3	4.7	7.8	5.5
8	350	9	5.3	5.2	5.0	5.2	7.3	4.3	7.8	5.4
9	350	3	5.2	5.1	5.0	5.0	7.4	4.8	7.8	5.5
10	450	6	5.3	5.2	4.6	5.2	7.3	4.8	7.8	5.5
11	308	6	5.5	5.2	4.8	5.2	7.5	4.3	7.8	5.5
12	550	9	5.0	5.0	4.6	5.0	7.0	4.4	7.7	5.4
13	450	6	5.4	5.0	4.7	5.1	7.3	4.4	7.8	5.4
HTST	75	30 sec	4.0	0.0	4.4	4.6	5.5	4.3	7.7	5.4
LTLT	65	30 min	4.7	4.0	4.6	4.6	5.6	4.7	7.7	5.4

5.4.4 Comparing the Effects of Pasteurization and HPP on Cheese Yield and Acidity

The second experiment compared the effect of five treatments: heat pasteurization (LTLT, 65°C, 30 min, and HTST, 75°C, 30 seconds) and HPP (350, 450, and 550 MPa, 5 min at 4°C each) on the cheese yield, physicochemical, rheological and microstructural properties of camel and bovine milk cheeses. The information presented in Table (15) shows that curd yield HTST treatments resulted was significantly (21±0.2 and (17±0.3) percent higher in both bovine and camel kinds of cheese than all other treatments, i.e., LTLT, and HHP (350, 450, and 550 MPa for 5 min at 4°C) followed by the HHP treatment at 550 MPa ($p < 0.05$). The high yield from HTST- treated milk samples can be due to whey protein denaturation and its interactions with the κ-casein on the surface of the casein micelles (Lucey et al., 1997; Zobrist et al., 2005). Furthermore, high pressurization promotes whey protein denaturation, especially β-lactoglobulin, which interacts with casein micelle (Fox et al., 1998; Lopez-Fandino et al., 1996). Thus, the denatured whey proteins would serve as barriers against the re-formation of casein aggregates during curd formation, resulting in cheeses with open structure and high moisture content, consequently higher yield. (Fox et al., 1998). Thus, the slight increase in cheese yield is due to the HPP-induced whey proteins' denaturation, causing an increase in moisture and fat retention (Huppertz et al., 2004; Pandey et al., 2003; San Martin-González et al., 2009). According to our results, HTST-treated camel milk has the lowest suitability for cheese production due to its soft, weak curd firmness.

The HPP treatment decreased the titrable acidity and increased the pH of the camel and bovine cheeses significantly ($p<0.05$). This has been explained by the disaggregation of the colloidal casein micelles and the increased dissolution of ionic

calcium phosphate in response to the pressure effect on BM (Chopde et al., 2014; Huppertz & de Kruif, 2007; Nassar et al., 2020; Orlien et al., 2010). Cheese produced from the HTST-pasteurized CM samples had the lowest pH and the highest acidity compared to bovine milk samples ($p<0.05$), which can be explained by enhanced hydrophobic contacts within the casein micelles conferring stability against dissociation with increased temperature (Gebhardt et al., 2005).

Table 15: Chemical composition of camel and bovine milk cheeses prepared from pasteurized and HPP-treated milk*

Quality Parameter	Camel Milk Cheeses					Bovine Milk Cheeses				
	LTLT, 65°C (30 min)	**HTST, 75°C (30 seconds)**	**350 MPa (5 min at 4°C)**	**450 MPa (5 min at 4°C)**	**550 MPa (5 min at 4°C)**	**LTLT, 65°C (30 min)**	**HTST, 75°C (30 seconds)**	**350 MPa (5 min at 4°C)**	**450 MPa (5 min at 4°C)**	**550 MPa (5 time at 4°C)**
Yield (%)	12 ± 0.02^{g}	17 ± 0.3^{c}	11.5 ± 0.2^{h}	13.5 ± 0.2^{f}	14.7 ± 0.3^{e}	14 ± 0.3^{e}	21 ± 0.2^{a}	15 ± 0.15^{d}	17 ± 0.4^{c}	19 ± 0.31^{b}
pH	5.3 ± 0.01^{f}	5.2 ± 0.03^{f}	5.5 ± 0.02^{e}	5.6 ± 0.01^{d}	5.8 ± 0.03^{cd}	5.6 ± 0.04^{d}	5.4 ± 0.02^{e}	5.8 ± 0.02^{bc}	5.9 ± 0.05^{b}	6.4 ± 0.15^{a}
Acidity (%)	2.7 ± 0.04^{b}	2.9 ± 0.05^{a}	2.5 ± 0.05^{c}	1.9 ± 0.02^{d}	1.7 ± 0.04^{e}	1.2 ± 0.03^{f}	1.3 ± 0.02^{f}	1.1 ± 0.05^{g}	0.8 ± 0.04^{h}	0.7 ± 0.05^{i}
Total solids (%)	40.9 ± 0.2^{f}	37.4 ± 0.16^{h}	40.6 ± 0.26^{f}	39.7 ± 0.22^{g}	39.4 ± 0.13^{g}	53 ± 0.13^{a}	41 ± 0.13^{e}	51 ± 0.11^{b}	49 ± 0.21^{c}	47 ± 0.12^{d}
Fat (%)	20.5 ± 0.1^{g}	17.6 ± 0.37^{i}	21.2 ± 0.1^{f}	21.8 ± 0.13^{e}	22.2 ± 0.12^{e}	29 ± 0.09^{a}	20 ± 0.08^{h}	28 ± 0.05^{b}	25 ± 0.1	23 ± 0.05^{d}
Protein (%)	15.14 ± 0.1^{d}	17.8 ± 0.03^{c}	14.3 ± 0.3^{e}	13.2 ± 0.41^{f}	13.3 ± 0.21^{f}	19.2 ± 0.3^{a}	15.5 ± 0.31^{d}	18.6 ± 0.24^{abc}	18 ± 0.74^{bc}	18 ± 0.45^{ab}
Hardness (g)	367 ± 6^{g}	228 ± 7^{i}	519 ± 5^{d}	341 ± 5^{g}	276 ± 7^{h}	1253 ± 20^{a}	438 ± 14^{e}	913 ± 9^{b}	810 ± 12^{c}	645 ± 7^{d}
Cohesiveness	0.62 ± 0.02^{c}	0.82 ± 0.02^{a}	0.53 ± 0.01^{d}	0.65 ± 0.01^{bc}	0.68 ± 0.01^{b}	0.37 ± 0.01^{g}	0.52 ± 0.02^{d}	0.41 ± 0.02^{f}	0.45 ± 0.01^{ef}	0.47 ± 0.02^{e}
Gumminess (g)	228 ± 8^{d}	185 ± 8^{e}	281 ± 5^{c}	224 ± 5^{d}	187 ± 7^{e}	463 ± 17^{a}	227 ± 5^{d}	374 ± 7^{b}	356 ± 7^{b}	305 ± 12^{c}
Chewiness (MJ)	$8.9.\pm0.36^{e}$	$3.7.8\pm0.13^{f}$	12 ± 0.2^{e}	7.7 ± 0.31^{ef}	7.4 ± 0.21^{ef}	99 ± 2.5^{a}	51 ± 1.5^{d}	93 ± 1.5^{a}	79 ± 6.7^{b}	59 ± 1^{c}
Complex Viscosity (Pa)	9717 ± 15^{e}	3448 ± 235^{i}	9804 ± 81^{e}	8746 ± 22^{f}	7945 ± 38^{g}	17419 ± 119^{a}	6859 ± 43^{h}	16429 ± 240^{b}	13539 ± 320^{c}	12376 ± 327^{d}
G-prime (Pa)	58240 ± 150^{e}	22657 ± 213^{i}	58671 ± 252^{e}	53429 ± 129^{f}	46334 ± 350^{g}	99360 ± 233^{a}	38499 ± 186^{h}	97753 ± 336^{b}	81586 ± 351^{c}	80201 ± 156^{d}
G-double prime(Pa)	22172 ± 129^{e}	11336 ± 267^{h}	23289 ± 139^{d}	21424 ± 260^{f}	20621 ± 308^{g}	37694 ± 204^{a}	11786 ± 153^{h}	32173 ± 141^{b}	24520 ± 164^{c}	23353 ± 228^{d}

*Values within a raw having different superscripts are significantly different ($p<0.05$, n=3 per treatment)

5.4.5 Comparing the Effects of Pasteurization and HPP Treatments on Cheese Hardness, Rheology, and Microstructure

Bovine milk cheeses had significantly higher textural properties than camel milk cheeses except for cohesiveness (Table 15), which can be explained by the higher content of β-casein with a sticky hydrophobic C-terminal in camel milk (Mohamed et al., 2020b). The LTLT-treated bovine milk cheese showed improved hardness, gumminess, and chewiness. This can be associated with the milk's faster coagulation, enhancing water drainage, and increasing curd firmness (Guinee, 2003). On the other hand, HPP treatment at 350 MPa produced the hardest camel milk, possibly due to the "optimal" disruption of the casein micelles. It was reported that a mild HPP treatment would not cause complete disruption of the casein micelles but rather dissociate parts of their surfaces (Sandra & Dalgleish, 2005). The micelle fragments would surround fat globules rather than intact casein micelles and make them behave as casein micelles rather than embedded fat globules observed on average in higher pressures (Hayes et al., 2005). Such structures could enhance gel firmness and aggregation by increasing particle associations the significantly lower textural profile of the bovine cheeses made from HPP-treated milk at 450 and 550 MPa ($p < 0.05$) compared to HPP 350 MPa was reported (Messens et al., 2000). The reduction in firmness upon high-pressure treatments was attributed to increased water retention due to the protein network's hydration. Water in the protein matrix plays a plasticizer role decreasing its elasticity and making it prone to fracture during compression.

Rheology describes the gel system's stress-strain characteristic parameters. G', the "storage modulus" describes the protein network's elastic (solid) component predicting gel strength (Zhang et al., 2018). The rheological properties (G', G" and complex viscosity) of bovine milk cheese samples were significantly ($p<0.05$) higher

than that of camel milk cheese samples (Table 15). This could be due to the rapid coagulation of bovine caseins into dense and more interwoven structures (Mbye et al., 2020; Xiong & Kinsella, 1991) compared to soft gel texture in camel caseins (Macdougall et al., 2019) as well as yogurts (Sobti et al., 2019; Sobti et al., 2020). The study also observed a decrease in the gel strength and associated rheological properties of cheeses on HPP-treated milk from 350 to 550 MPa (Tables 12 and 15, Figure 15). The LTLT samples of bovine milk cheese showed the highest G', significantly different from the other treatments ($P<0.05$). In contrast, the complex viscosity and G' of HTST-treated milk samples were considerably lower than all the other treatments showing positive relationships with hardness and hostile relations with moisture content and yield.

Figure 19 presents the microstructures of the two pasteurization levels (LTLT and HTST) and two HHP levels of milk treatment (350 and 550 MPa). Large, irregular lumps characterized the camel and bovine cheeses' microstructure resulting from the LTLT and HHP 350 MPa-treated milk with granular structures, which permit faster drainage of the whey to enhance cheese hardness (Britz & Robinson, 2008; Mbye et al., 2020). On the other hand, cheeses produced from HTST and HHP 500 MPa-treated milk showed tight aggregate strands, homogeneous structures, and continuous networks as observed before (Mimouni et al., 2010; Zhang et al., 2018). The water-holding capacity of curds is directly linked to the gels' porosity (Lucey et al., 2001). Thus, microstructures with smoother protein networks have fewer pore spaces and retain moisture explaining the increased yield and softness (Green et al., 1983).

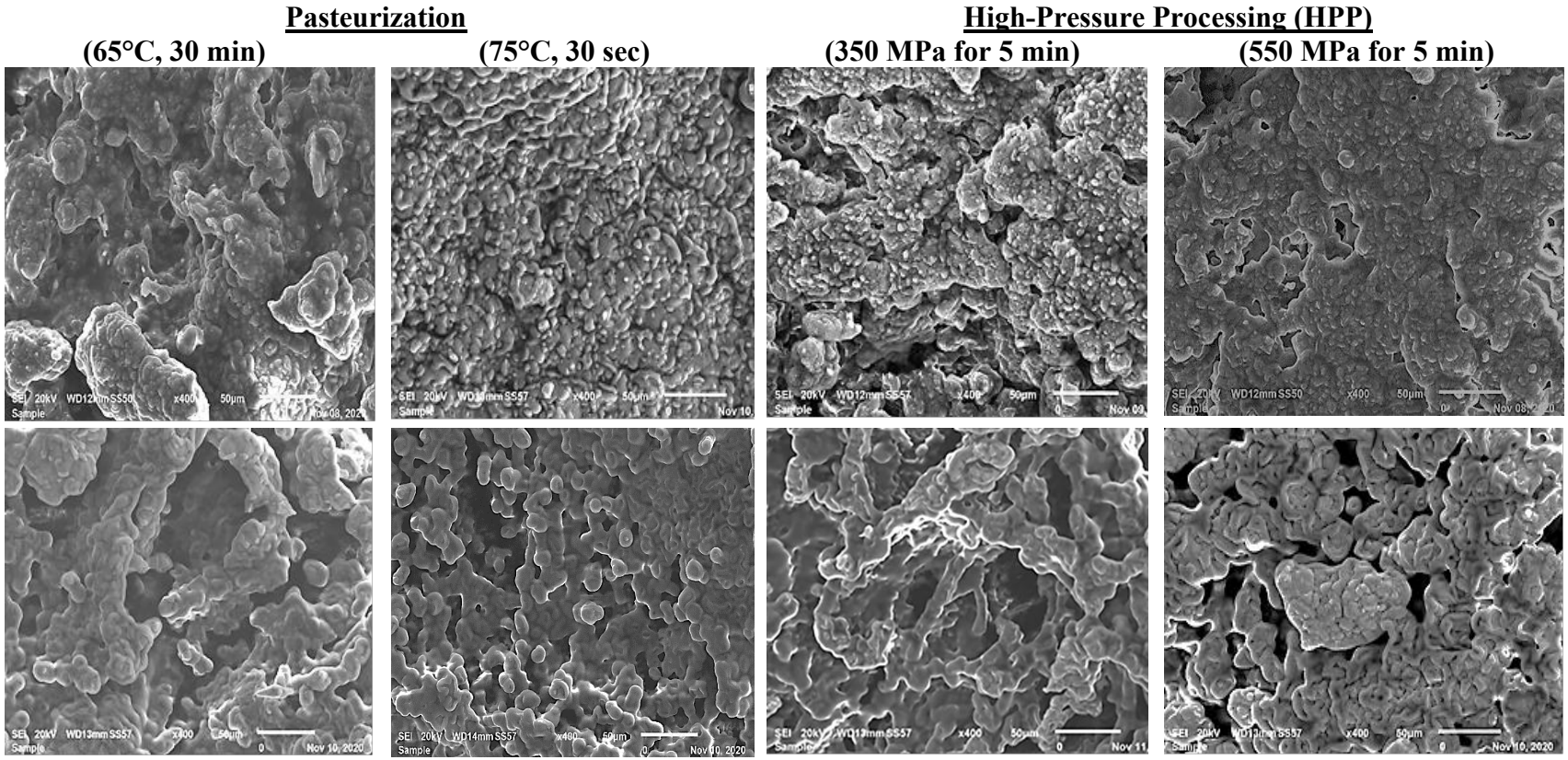

Figure 19: Scanning electron micrograph of camel cheeses (Upper panel) and bovine cheeses (Lower panel) with two pasteurization temperatures and two high-pressure treatments applied to the milk (Magnification: ×, 400)

5.4.6 Proteolytic Activities May Be Involved in the Softness of CM Cheese

Figure (20) shows that except for the HTST cheese, the fat, protein, and total solids contents were significantly higher in bovine milk cheeses than camel milk cheeses ($p<0.001$), which agrees with previous findings (Hailu et al., 2018; Yirda et al., 2020). This can be related, at least partly, to the higher level of κ-casein in bovine milk (Mohamed et al., 2020b). κ-Casein is known to enhance the coagulation properties by forming a denser casein matrix, which reduces the loss of fat and protein to the whey (Dai et al., 2019; Ong et al., 2012).

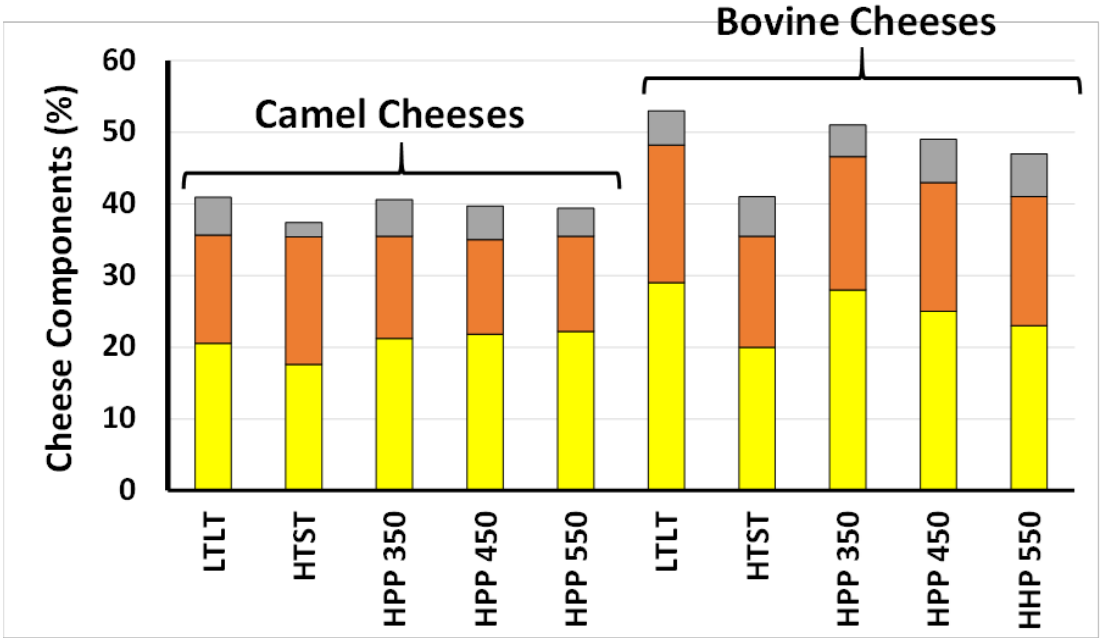

Figure 20: The percentages of fat (yellow), protein (orange), and other solids

Figure (21) presents sodium dodecyl polyacrylamide gel (SDS-PAGE) electropherograms of camel and bovine cheeses and wheys. It is observed that the camel milk cheeses show more bands below and above the caseins suggesting extensive proteolysis compared with bovine cheeses. The observed proteolysis may

result from the action of two proteolytic enzymes; the residual chymosin used in milk coagulation or the indigenous milk proteinase, plasmin (EC 3.4.21.7) (Bastian et al., 1997; Bastian et al., 1993; Fox, 1992; Mulvihill & McCarthy, 1994; Song et al., 1993). The recombinant camel chymosin used in this study is known to hydrolyze bovine and camel κ-caseins at different positions, Phe105-Met106, and Phe97-Ile98, respectively, leading to the release of other macro peptides (Kappeler et al., 1998) and possibly fewer hydrolysis products in camel milk because κ-casein is present at a low concentration (3.5%) compared to bovine milk (about 13%) (Mohamed et al., 2020b). It was reported that cheese coagulation by chymosin is slower and weaker in camels than in bovine milk (Mbye et al., 2020), but the mechanisms behind these differences are still unknown. Chymosin hydrolysis of κ-casein is the most essential proteolytic reaction in bovine milk during cheese making (Beermann & Hartung, 2012). Of the rennet used in bovine milk coagulation, about 6-10% is retained in the bovine cheese curd (Fox, 1989), but the residues chymosin contained in camel cheese curd to further hydrolyze other caseins requires further investigation. Notably, the raw and processed camel kinds of milk showed similar proteolytic bands, of less prevalence than those observed in cheese, absent in bovine milk types **(**Figure 18), suggesting that some endogenous proteolytic enzymes are endogenous in the camel milk may have been activated during cheese processing. Thus, the hydrolysis of camel's milk caseins, predominantly β- and αs1-casein, by the enzymatic plasminogen/plasmin system may contribute to the observed extensive proteolytic activity in camel milk cheeses (Garía-Risco et al., 2000). The numerous peptide bands observed in camel milk cheeses may be explained by plasmin (EC 3.4.21.7) degradation of β-casein, which accounts for 65% of camel milk *versus* 40% of bovine milk caseins, and the lack of β-lactoglobulin in this milk (Mohamed et al., 2020b). γ2-Casein, a C-terminal peptide originating from

highly specific proteolysis of β-casein by plasmin, was found in raw camel milk (Baer et al., 1994). Plasmin activity in milk is affected by the level of its precursor, plasminogen, and some activators/deactivators (Bastian et al., 1993). For example, β-lactoglobulin, the major whey protein in bovine milk that is lacking in camel milk (Berhe et al., 2018; Berhe et al., 2017), was reported to act as an inhibitor through thiol-disulfide exchange with plasmin, causing reduced plasmin activity (Mazri et al., 2012). HPP treatment of bovine milk at pressures higher than 100 MPa was reported to induce β-lactoglobulin denaturation (Lopez-Fandino et al., 1996).

There are conflicting reports on the effect of HPP on plasmin/plasminogen activity in bovine milk. For example, one study reported that HPP treatment enhances this activity (Garcia-Risco et al., 2003), while other studies said that plasmin activity was not affected by HPP up to 400 MPa for 30 min (Lopez-Fandino et al., 1996) or 600 MPa for 20 min (Scollard et al., 2000a). Thus, the plasmin activity may explain the softness of camel cheeses, but it does not explain the hardening effect of HPP on camel milk cheeses. Future studies should investigate the differences between the plasmin/plasminogen systems in camel and their cheese-making results. As already discussed, the significant difference between camel and bovine milk relates to the composition, i.e., the relative percentages of the four caseins and the nature of the casein micelles in the two kinds of milk. Furthermore, the hydrophilicity/hydrophobicity of the micelle and the access of the hydrolytic enzymes to the reactive sites on the caseins affects the proteolytic activities. In addition, the higher hydration level and concentrations of minerals, mainly calcium, magnesium, phosphate, and citrate, in the casein micelles of camel compared to bovine milk (Attia et al., 2000) may also play an essential role in the micelle structure and its vulnerability to proteolytic attacks (Bhat et al., 2016).

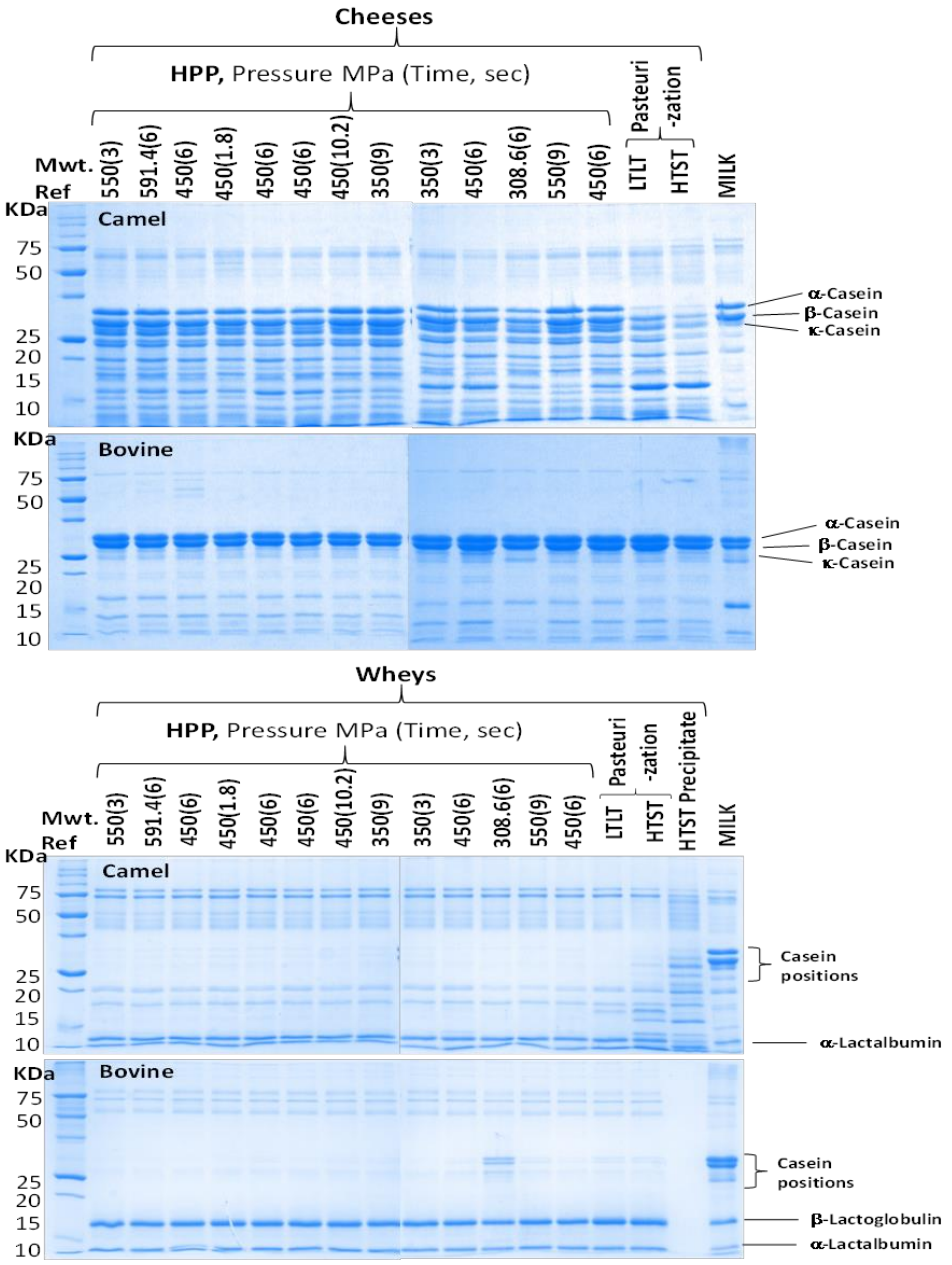

Figure 21: SDS-PAGE electropherograms of the camel and bovine cheeses and whey. For processing conditions, see Table 12.

The loose casein micelle structure in camel cheeses may be responsible for water retention and associated with higher yield and lower hardness, viscosity, and rheology, thus resulting in loss of total solid protein and fat into the whey. Our study has revealed camel milk whey had significant ($p < 0.05$) higher solid protein and fat.

LTLT treatment whey had a total solid of (6.9 ± 0.051), fat (1.2 ± 0.00), and protein (1.6 ± 0.04). The results obtained from this study are less than what was reported by (Al-Zoreky & Almathen, 2021). At the same time, HTST treatment whey had significantly ($p < 0.05$) the highest protein and fat (Table 16), this is due to the infiltration of fat and protein into the whey fraction due to hydrolysis of micro casein during coagulation.

Table 16: Chemical of camel and bovine milk whey proteins (n=3)

Treatment	pH	Total solids (%)	Fat (%)	Protein (%)
	Camel Milk Whey			
LTLT, 65°C (30 min	4.5 ± 0.01^{a}	6.9 ± 0.051^{c}	1.2 ± 0.00^{d}	1.6 ± 0.04^{b}
HTST, 75°C (30 seconds)	4.0 ± 0.01^{d}	7.9 ± 0.06^{a}	2.0 ± 0.01^{a}	1.7 ± 0.02^{a}
350 MPa (5 min) at 4°C	4.3 ± 0.02^{c}	7.5 ± 0.087^{b}	1.3 ± 0.01^{d}	1.5 ± 0.02^{c}
450 MPa (5 min) at 4°C	4.4 ± 0.01^{b}	7.6 ± 0.07^{b}	1.4 ± 0.00^{b}	1.5 ± 0.02^{c}
550 MPa (5 min) at 4°C	4.4 ± 0.02^{b}	7.7 ± 0.09^{b}	1.4 ± 0.007^{b}	1.5 ± 0.03^{c}
	Bovine Milk Whey			
LTLT, 65°C (30 min)	4.6 ± 0.02^{a}	6.0 ± 0.17^{d}	1.3 ± 0.02^{b}	1.3 ± 0.03^{d}
HTST, 75°C (30 seconds)	4.4 ± 0.03^{b}	6.3 ± 0.03^{e}	1.4 ± 0.01^{b}	1.4 ± 0.01^{c}
350 MPa (5 min) at 4°C	4.4 ± 0.02^{b}	6.1 ± 0.16^{f}	1.3 ± 0.02^{c}	1.3 ± 0.02^{d}
450 MPa (5 min at 4°C	4.3 ± 0.02^{c}	6.1 ± 0.13^{f}	1.3 ± 0.03^{c}	1.3 ± 0.02^{d}
550 MPa (5 min) at 4°C	4.4 ± 0.01^{b}	6.1 ± 0.02^{f}	1.3 ± 0.02^{c}	1.3 ± 0.02^{d}

A comparison was made between the different treatments for each whey.

The whey fractions from camel milk had tiny casein particles, especially from the HTST-treated dairy, where a higher amount of total solid can be observed (Figure 22). The increase in cheese hardness by HPP may also be affected by factors other than plasmin activity, e.g., disruption and destabilization of the camel milk micelles and

enhancement of coagulation. It was reported that when milk is pressurized at room temperature, micelle disruption might enhance the susceptibility of casein to proteolysis by increasing the protein surface area available to the plasmin enzymes and the exposure of new substrate sites (Garía-Risco et al., 2000).

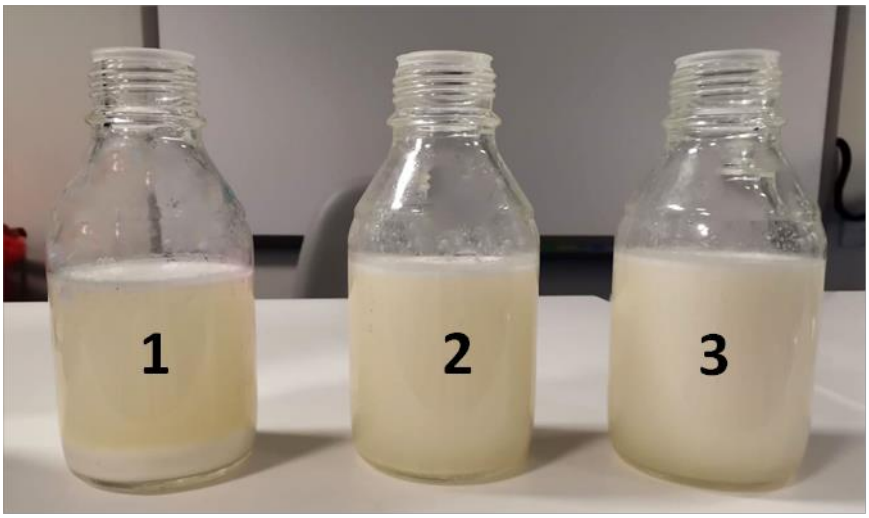

Figure 22: The whey fractions from cheeses produced from camel milk (1) pasteurized (75°C, 30 seconds), (2) HPP (350 MPa, 5 min at 4°C), and (3) HPP (550 MPa, 5 min at 4°C)

5.5 Conclusions

This study investigated the effects of high-pressure milk processing and pasteurization on the yield and physicochemical properties of soft unripe cheeses produced from camel milk compared with bovine milk. It was found that camel milk cheeses were affected differently from bovine milk cheeses by the different treatments. Camel milk cheeses were relatively softer than bovine cheeses, possibly due to an active endogenous protease proposed to be the plasmin/plasminogen system. The results revealed that mild processing conditions (e.g., LTLT pasteurization (65°C, 30 minutes) and HPP-treatment (350 MPa, 5 min at 4°C) were effective in productizing semi-hard cheeses from camel milk. It was also shown that HPP treatment could

replace pasteurization HTST (75°C 30 seconds) in camel milk microbial preservation before cheese production. Further studies are needed to investigate the possibility of camel cheese production further using HPP processing and evaluate ripened cheeses' safety and sensory quality. Other studies are also required to identify the proteolytic products in camel milk cheeses, their protein source(s), and their role in camel milk coagulation and cheese quality.

Chapter 6: General Discussion

The overall global consumption of cheese has increased steadily and is expected to increase to ˜13.5% between 2016 and 2025 (Cosme, 2017). Furthermore, an increase in camel dairy products is expected to be about 7% between 2018 and 2022 (Technavo, 2020). At the same time, consumers are very particular about enhancing the Physico-chemical properties, sensory attributes, and nutritional quality of dairy products. Due to these demands, dairy science researchers and cheese producers focus on the improvement in the quality of existing products or the design of new innovative products.

The general objective of this dissertation was to explore the effects of various coagulants (organic acids, chymosin, and a plant enzyme) and processing conditions (pasteurization temperature and high-pressure processing) on the physicochemical properties and quality of camel milk cheese compared to bovine milk cheese. The results of these assessed traits are discussed below in the given headings.

6.1 Chemical Analysis of Camel and Bovine Milk

Fresh camel milk (CM) has average milk composition (pH, 6.61; acidity, 0.15%; Lactose, 4.43%; total solids, 12.4%; protein, 2.7%; and fat, 3.1%) compared with bovine milk (BM) (pH, 6.68; acidity, 0.15%; lactose, 5.08%; total solids, 12.6%; protein, 2.98%; and fat, 3.4%), which is in agreement with previous findings (Al Kanhal, 2010; Konuspayeva et al., 2009; Mohamed et al., 2020a; Nagy et al., 2019). Some variation in the composition of raw milk could be a result of various factors such as the breed of animals, feeding behavior, seasonal variations, and water intake. Hot seasons can reduce CM fat from 4.2 to 1.2% due to dehydration (Konuspayeva et al., 2009). Genotypes of the animal and changes in season can also result in variation in

the overall variation in milk composition. Al Kanhal (2010) found that the protein content to be low in summer (2.48%) and high in winter (2.9%). However, CM is generally known to resist fermentation and enzymatic coagulation regardless of such variability in raw milk composition (Ipsen, 2017).

6.2 Exploration of Various Milk Coagulants (Camel Chymosin, Citric Acid, Acetic Acid, and *Withania coagulans*) for Camel Milk Cheese Production Compared to Bovine Milk

In the manufacturing of cheese, the coagulant properties, and microstructure significantly affect the properties and the overall quality and consumer acceptability of CM cheese (Lamichhane et al., 2018). For instance, microstructure plays an essential role in determining the rheological, texture perception, flavor release, digestion, and the absorption of nutrients (Guinee, 2016; Rogers et al., 2009; Singh et al., 2015; Taylor, 2002). Studying and getting a better understanding of the complex interrelationship between the coagulation properties, microstructure, and sensory quality is necessary to design cheese types. However, the full extent of the relationships between the structure and functionality of CM cheese is not fully understood. Thus, one of the aims of this study is to provide knowledge of how cheese structure may influence the properties and quality of cheese. In the first study, we compared the coagulation behaviors and the systems of CM and BM cheeses by scanning electron microscopy (SEM) and how it relates to coagulation, whey off, cheese quality, and sensory attributes Significant differences were found in coagulation time, texture, yield, and microstructure among the various coagulants and between the CM and BM cheeses ($p<0.05$). Our study revealed three times longer coagulation time of CM compared to BM coagulated with chymosin, while no coagulation occurred in CM coagulated with citric and acetic acid after 60 minutes.

While W. coagulans extract protease alone can set BM cheese but not sufficient to produce strong coagulum to make CM cheese. However, a mixture of W. coagulans and chymosin was acceptable to produced CM and BM cheeses. This study also showed that CM cheeses had a softer texture with higher moisture content than BM cheeses. The poor coagulation, soft texture, and high moisture content of CM cheese is responsible for the microstructure of CM cheeses. The CM cheese microstructure is characterized by a smooth and continuous casein network, thinner aggregate strands, and smaller pore spaces, while BM cheeses showed large pore spaces with irregular aggregates. Thus, the microstructure of CM cheese has a direct relationship to slow coagulation, soft textural profile, and higher moisture content compared to BM cheeses. The tiny pore sizes can explain the higher retention of moisture in CM cheese than the more significant and coarser pore space in BM cheese that has enhanced faster whey drainage resulting in a more rigid cheese texture with low moisture content (Fox et al., 2017).

6.3 The Effects of Pasteurization and High-Pressure Processing (HPP) on the Quality Camel Milk Cheeses

Milk pasteurization is vital to assure the cheese's safety (Rankin et al., 2017). However, high pasteurization temperature could affect the quality of cheese, such as yield and texture. Therefore, the cheese product obtained from cheese milk is a vital criterion regarding the profitability of the cheese industry. At the same time, the texture is an important attribute that influences the cheese's appearance, texture, and preference or acceptability. The results of the third study revealed that (65°C for 30 min), compared to high pasteurization temperature (72°C, 30 seconds), prolonged the CM rennet coagulation time and resulted in cheeses of high moisture content and soft texture. This finding is in line with previous studies showing that the low heat stability

of CM could be due to its larger micelles size compared with BM (Al Kanhal, 2010; O'Connell & Fox, 2000; Omer & Eltinay, 2009). Similarly, Qadeer et al. (2015) reported that the yield of CM cheese is lower when the temperature exceeded 65°C /30 minutes and (Farah & Fischer, 2004) said that camel milk does not coagulate at pasteurization temperature above 65°C. They related the obtained results to CM milk's casein micelle with a loose microstructure and micelle hydration, which resulted in fragile curd. Furthermore, Guinee et al. (1997) stated that the set time at 20 Pascal for cheese milk heat-treated at 88°C for 15 seconds was about (70 min) compared (33.3 min) faster coagulation time to that raw cheese milk. According to these results, it is recommended that milk for cheese making should not undergo high heat treatment (i.e.,>72°C 15 s) due to high levels of serum protein denaturation (Fox et al., 2017; Guinee et al., 1997). The results from the above studies agreed with the current study's findings that HTST-treated milk is the least favorable treatment for cheese production due to the resultant longer coagulation time soft curd firmness.

HPP treatment of milk induces micelle disintegration, whey protein denaturation, and interactions of whey and caseins (Lopez-Fandino et al., 1996). It is hypothesized that denatured whey proteins protect the casein micelles from excessive dissociation and serve as barriers against the re-formation of casein aggregates during curd formation, resulting in cheeses with a relatively open structure with high concentration moisture retention consequently greater yield (Huppertz et al., 2006). Omar et al. (2018) reported that HPP treatment of 200 and 400 MPa increases camel milk coagulation and, consequence, enhanced coagulum strength, but HPP treatment of 600 and 800 MPa inhibits clotting. Those findings agreed with our study in the case of cheeses made from HPP at 350 MPa, where a little whey protein denaturation is

achieved, and the dissociated micelles re-form into dense aggregates resulting in cheeses with more rigid texture with lower moisture content.

6.4 The Effects of Proteolytic Activities on Camel Milk and Cheeses

SDS-PAGE of CM cheeses have shown proteolytic bands below and combination bands above the casein's bands suggesting extensive proteolysis compared with BM cheeses. The observed proteolysis may result from the action of the residual chymosin used in milk coagulation, the indigenous milk proteinase, e.g., plasmin (EC 3.4.21.7) (Bastian et al., 1997; Bastian et al., 1993; Fox, 1992; Mulvihill & McCarthy, 1994; Song et al., 1993), and enzymes belonging to the starter culture used before milk coagulation (Pereira et al., 2008). The recombinant camel chymosin used in this study is known to hydrolyze BM and CM κ-caseins at different Phe105-Met106 and Phe97-Ile98, respectively leading to the release of other macro peptides (Kappeler et al., 1998). In BM, chymosin hydrolysis of κ-casein is the most crucial proteolytic reaction during cheese making (Beermann & Hartung, 2012). Of the rennet used in bovine milk coagulation, about 6-10% is retained in the bovine cheese curd (Fox, 1989), but the residues chymosin contained in camel cheese curd to further hydrolyze other caseins requires further investigation. The activities of residual enzymes of the starter culture(s) in the degradation during cheese ripening were reported (Fox, 1989; Pereira et al., 2008). However, the cheeses analyzed in the current study are fresh. Thus, the effect of residual chymosin and starter culture to cause proteolysis is expected to be negligible. It is, therefore, a hypothesis that some endogenous proteolytic enzymes in the CM may have been active during cheese processing. Thus, the hydrolysis of CM caseins, predominantly β- and αs1-casein, by the enzymatic plasminogen/plasmin system may contribute to camel milk's observed

extensive proteolytic activity cheeses (Garía-Risco et al., 2000). The numerous peptide bands observed in camel milk cheeses may be explained by plasmin (EC 3.4.21.7) degradation of β-casein, which accounts for 65% of camel milk *versus* 40% of bovine milk caseins, and the lack of β-lactoglobulin in this milk (Mohamed et al., 2020b). γ2-Casein, a C-terminal peptide originating from highly specific proteolysis of β-casein by plasmin, was found in raw camel milk (Baer et al., 1994). β-lactoglobulin, the major whey protein in BM lacking in CM (Berhe et al., 2018; Berhe et al., 2017), was reported to act as an inhibitor through thiol-disulfide exchange with plasmin, causing reduced plasmin activity (Mazri et al., 2012). There are conflicting reports on the effect of HPP on plasmin/plasminogen activity in BM. For example, one study reported that HPP treatment enhances this activity (Garcia-Risco et al., 2003), while other studies said that plasmin activity was not affected by HPP up to 400 MPa for 30 min (Lopez-Fandino et al., 1996) or 600 MPa for 20 min (Scollard et al., 2000b). Thus, the plasmin activity may explain the softness of camel cheeses, but it does not explain the hardening effect of HPP on camel milk cheeses. Future studies should investigate the endogenous enzymes in CM and their impact on cheese making. As already discussed, the significant difference between CM and BM relates to the composition, i.e., the relative percentages of the four caseins and the nature of the casein micelles in the two kinds of milk, the hydrophilicity/hydrophobicity of the micelle, and the hydrolytic enzymes and their access to the reactive sites on the caseins affect the proteolytic activities. In addition, the higher hydration level and concentrations of minerals, mainly calcium, magnesium, phosphate, and citrate, in the casein micelles of camel compared to bovine milk (Attia et al., 2000) may also play an essential role in the micelle structure and its vulnerability to proteolytic attacks (Bhat et al., 2016).

Chapter 7: Conclusions and Future work

7.1 Conclusion

In this thesis, the effects of different coagulants (camel chymosin, citric, acetic acid, and *Withania coagulans*) and processing conditions: namely low-temperature long-time (LTLT), high-temperature short-time (HTST), and high-pressure processing (HPP) on the quality of cheeses produced from camel milk (CM) and bovine milk (BM) was studied. The study revealed that camel milk processing presents several challenges, including milk component behavior and technology adaptations. Despite these constraints, camel milk cheese production is significantly changing due to structural innovations. This study suggested that CM's milk component and microstructure cause the peculiar differences in quality between CM and BM cheeses. It concludes the following points:

- Camel chymosin is the most suitable coagulation in the production of CM cheeses.
- CM cheese has fewer aggregated protein clusters compared to BM cheeses. Thus, CM is more suitable for the production of soft cheese types.
- High-pressure processing (HPP) treatments have tremendous processing potential in producing semi-hard CM cheeses than heat treatments.

  CM cheeses were more prone to proteolysis than BM due to plasmin activity, higher β-casein, increased hydration, and mineralization.

7.2 Future Research Needs

In this study, some exciting phenomena were detected about the effect of coagulant, heat, and HPP treatments on CM properties. But, not all were explored fully due to the time limitation of the study. The following can be explored further to fill in

the gap of information and provide exciting results about the processing features of CM:

- Studies are further required to understand the chemical basis for the different behavior of camel milk during cheese making, particularly the enzymes responsible for extensive proteolytic activity in CM and their impact on camel dairy products,
- It would be interesting to perform further studies on HPP treatments using less than 350 MPa and to investigate the addition of microbial transglutaminase (MTGase) after HHP treatment of milk before cheese making,
- Studies on the combined ultrafiltration and HHP treatment on the quality and sensory properties of CM cheese can be compared with conventional heat treatments,
- Further studies on the impact of different treatments on camel cheese ripening are also required
- Optimization of the sensory characteristics of camel milk cheeses to achieve consumer acceptance/appreciation are highly warranted.

7.3 Implications for the Food Industry

The results obtained in this thesis will be useful to the camel milk industry, which is currently expanding towards the utilization of camel milk in dairy products with longer shelf-life such as cheese. The obtained knowledge is also important for understanding the relation between milk protein composition and characteristics and their effects on product quality and consumer acceptability. Further optimization of milk pasteurization, selection of additives and other treatments prior to coagulation, as well as selection of coagulation enzymes are believed to lead to improved camel milk

cheese that will meet consumers acceptance. The camel milk cheese is not necessarily having the same sensory characteristics of bovine milk cheeses.

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