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Effects of industrial processing methods on camel skimmed milk properties

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ACCEPTED MANUSCRIPT

Effects of industrial processing methods on camel skimmed milk properties

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ABSTRACT

Effects of pasteurisation (high-temperature-short-time; HTST), ultra-high-temperature (UHT), and high-pressure (HP) treatments on some physical and chemical properties of camel milk (CM), including whey protein denaturation, colour change, casein micelle size, and rennet coagulation time (RCT), was investigated. UHT treatment caused the biggest colour change and highest whey protein denaturation in CM; in contrast, the effects of HP treatments on these properties were considerably less. Casein micelle size decreased after all treatments. The RCT of CM was significantly delayed and coagulum strength (*G'*) decreased after HTST. HP treatment at 200 and 400 MPa increased the RCT of CM and the *G'* value was the highest after treatment at 200 MPa. Processing at 600 and 800 MPa inhibited coagulation of CM. The effects of both thermal and non-thermal treatments on many constituents and properties of CM were different from those on constituents and properties of bovine milk.

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1. Introduction

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

Heat treatments, such as high-temperature, short-time pasteurisation (HTST) and ultra-high-temperature (UHT) are typically applied to milk to ensure better microbiological quality and increase its shelf life for human consumption. It is well known that these treatments influence the physical and chemical properties of bovine milk (McSweeney & Fox, 2013).

Recently, there has been an increase in consumer demand for low-fat dairy products, including skimmed milk. In addition, CM is becoming more popular due to its potential beneficial effects on human health such as anti-cancer, hypo-allergenic and anti-diabetic effects (Kaskous, 2016). It also has lower cholesterol, lower sugar, higher minerals (sodium, potassium, iron, copper, zinc and magnesium) and vitamin C than bovine milk (Jilo & Tegegne, 2016). Therefore, investigating the effects of heat treatments on skimmed CM is of great technological importance, as thermal treatment is an important step involved in the processing of milk and milk products. Nevertheless, very few studies have focused on the influence of heat treatments on CM and the results from published studies are contradictory and mostly in relation to whey proteins.

Farah and Atkins, (1992) and Sagar, Mehta, Wadhwani, Darji, & Aparnathi (2016) reported that skimmed and whole CM had poor heat stability at high temperatures (100–140 °C) compared with bovine and buffalo milk. Similarly, Alhaj, Metwalli, and Ismail (2011)

showed that heat treatment (121 °C for 15 min) of whole CM at its natural pH resulted in partial or complete protein precipitation indicating poor heat-stability; however, they demonstrated that the heat stability could be improved by increasing the milk pH to 7.0–7.2 and addition of κ-casein, EDTA or sodium phosphate. Furthermore, CM whey proteins were also reported to be more sensitive to heat treatments, with denaturation rates faster than those of bovine milk (Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015a). Benabdelkamel et al. (2017) indicated that heat treatment of CM whey at 98 °C for 60 min caused a significant denaturation of camel α-lactalbumin (α-la), lactoferrin (LF), and serum albumin (SA). Similarly, Felfoul, Jardin, Gaucheron, Attia, and Ayadi (2017) found that whey proteins in skimmed camel and bovine milk were significantly affected by heat treatment at 80 °C for 60 min, whereas casein fractions remained intact under the same heat conditions for both types of milk.

In contrast, several studies reported that CM whey proteins were more heat stable than bovine whey proteins. Elagamy (2000) reported that camel whey proteins were considerably more heat resistant than their counterparts in bovine milk after pasteurisation at 65, 75, 85, and 100 °C for 10, 20, and 30 min. Furthermore, camel α-la was found to be more heat stable than bovine α-la during pasteurisation due to the secondary structure of camel α-la being conserved better than that of bovine α-la during heat denaturation (Atri et al., 2010). Laleye, Jobe, and Wasesa (2008) reported that there was no significant difference in the heat stability of liquid whey separated from camel or bovine milk during pasteurisation at 60, 70, 80, 90, and 100 °C.

Preliminary work on dried whey in the same study suggests that camel whey proteins were slightly more sensitive to heat denaturation than bovine whey. Factors including stage of lactation, camel breeds, feeding conditions and geographical location might be responsible for the conflicting results reported regarding the heat stability of whey proteins in CM (Alhaj

& AlKanhal, 2010). Levieux, Levieux, El-Hatmi, and Rigaudie (2006) found that whey proteins in early CM (the first week lactation) were more sensitive to heat treatment than those in CM after three months. This difference in the heat denaturation was attributed to the high content of IgG, 12.6 mg mL^{-1} in early CM compared with 0.5 mg mL^{-1} in milk from camels during the later stages of lactation.

High-pressure (HP) processing is an alternative preservation method to traditional heat treatments. Previous research has shown that HP processing can cause changes in milk including upsetting the mineral balance of the milk, denaturing whey proteins, inducing aggregation or disruption of casein micelles, changing the activity of native milk enzymes, changing the colour of the milk and altering the rennet coagulation properties (Huppertz, Smiddy, Upadhyay, & Kelly, 2006; López-Fandiño, 2006; Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002).

The majority of studies focusing on the effect of HP on milk have been conducted using bovine, buffalo, ewe, or ovine milk (Gervilla, Ferragut, & Guamis, 2001; Huppertz et al., 2005; Moatsou et al., 2008a,b). However, the effects of HP on the physicochemical and functional properties of CM have not been studied to date. Therefore, the aim of this study was to investigate the effects of commonly used food-processing methods (HTST, UHT, and HP) on some components and properties of skimmed CM, including whey protein denaturation, casein micelle size, appearance, and rennet coagulation properties. In addition, the results obtained were compared with those from bovine milk.

2. Materials and methods

2.1. Chemicals and reagents

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

2.2. Milk samples

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

2.3. High-temperature, short-time pasteurisation

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

2.4. Ultra-high-temperature processing

A tubular UHT plant (UHTAC, Fareins, France) was used for the indirect UHT treatment of camel and bovine milk. Heating was obtained in two stages using two hot oil baths. The unit was sterilised by circulating pressurised hot water prior to the treatment. The temperature of the milk samples was raised from 4 to 90 °C in a preheating unit (oil bath 1). The temperature was raised from 90 to 144 °C (oil bath 2), and the milk was held for 5s at this temperature. The processed milk was cooled to 4 °C and collected in 500 mL sterile bottles.

2.5. High-pressure treatment

High-pressure treatment of camel and bovine milk was performed as described by Huppertz, Fox, and Kelly (2004a) Camel and bovine milk samples (50 mL) were vacuum-packed in polyethylene bags and HP-treated using a Stansted Iso-Lab 900 High Pressure Food Processor (Stansted Fluid Power, Stansted, Essex, UK), at pressures of 200, 400, 600,

and 800 MPa for 30 min. The temperature of the HP unit vessel was maintained at 20 °C. A mixture of water and 1,2-propanediol oil (70:30) was used as the pressurising fluid.

2.6. Proximate composition analysis

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

2.7. Determination of whey proteins denaturation

Denaturation of whey proteins in camel and bovine milk samples was estimated by determining the level of residual native whey protein fractions: SA, α-la, β-lg, and LF in milk by capillary electrophoresis (CE) (Agilent, Palo Alto, CA, United States) following the method described by Omar, Harbourne and Oruna-Concha (2016). Briefly, the pH of the milk samples was adjusted to pH 4.3 by adding 1 M HCl. Then the samples were centrifuged at 4000 × *g* at 4 °C for 15 min to separate the whey proteins from the precipitated casein. The supernatant, containing whey proteins was dialysed (dialysis sacks average flat width of 25 mm, cut off 12,000 Da; Sigma-Aldrich) against distilled water and kept at –18 °C until analysis. Purified bovine milk proteins (BSA, β-lg, α-la, LF) at concentrations between 0.01 and 2.5 mg mL^{-1} were used to identify and quantify the proteins present in the milk samples. The degree of protein denaturation was expressed as the percentage of protein not detected

compared with the untreated milk sample, which is stated to have a native protein percentage of 100% and thereby no denaturation.

2.8. Determination of average casein micelle Size

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

2.9. Determination of colour parameters

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

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

where $\Delta L^* = L_{\text{raw milk}} - L_{\text{treated milk}}$, $\Delta a^* = a_{\text{raw milk}} - a_{\text{treated milk}}$, and $\Delta b^* = b_{\text{raw milk}} - b_{\text{treated milk}}$.

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

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

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

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

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

Colour measurements were conducted in triplicate for each milk sample.

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

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

2.11. Statistical analysis

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

3. Results and discussion

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

The mean values of protein, lactose, and total solids in the raw skimmed bovine milk were 3.17±0.01, 4.50±0.01, and 7.68±0.01 g 100 mL^{-1}, respectively. These values were in agreement with those in the literature (McSweeney & Fox, 2013). In CM, the protein, lactose, and total solids were lower (2.10±0.01, 3.59±0.01, and 5.86±0.03 g 100 mL^{-1}, respectively) than in bovine milk. These compositional variations were consistent with previously reported interspecies differences between camels' milk and cows' milk (Alhaj & AlKanhal, 2010).

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

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

The levels of individual whey proteins in processed camel and bovine milk are presented in Table 1. The major identified whey protein in CM was α-la, followed by LF and SA. The highest level of denaturation in camel whey proteins occurred in the UHT-treated CM sample, which was consistent with the results of its compositional analysis. Among the camel whey proteins, α-la underwent the highest level of denaturation (65.55±0.29%),

followed by SA (12.58±0.88%) and LF (3.65±0.54%). In pasteurised CM, the amount of denatured α-la was 27.13±3.23%, considerably lower than that of UHT-treated CM, and only small denaturation in SA and LF was observed (2.97±0.65 and 1.13±0.51%, respectively).

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

In contrast, β-lg was the primary whey protein in bovine milk, followed by α-la and BSA. The levels of heat-induced denaturation of α-la and BSA in bovine milk after HTST (4.23±1.37, and 2.70±0.31%, respectively) and UHT (51.06±2.11 and 5.37±1.46% respectively) treatments were considerably lower than those of their counterparts in heat-treated CM. This finding is consistent with that observed by Felfoul et al. (2015a) and Sagar et al. (2016) who reported that camel α-la and SA were less heat stable and their temperatures of denaturation were lower than their bovine counterparts.

Some studies have attributed the high heat sensitivity of camel whey proteins to the absence or deficiency of β-lg and κ-CN proteins in CM (Alhaj et al., 2011; Farah & Atkins, 1992; Sagar et al., 2016). However, the variation in the thermal stability between the major whey protein in CM α-la and its bovine milk counterpart could be also due to differences in their conformational stabilities and structural features. The primary structure of the intact

camel α-la, as bovine α-la, consists of 123 amino acids, but with 39 positional differences compared with bovine α-la (Beg, Bahr-Lindström, Zaidi, & Jörnvall, 1985).

Camel α-la contains, 8 cysteine, 5 tryptophan, 4 phenylalanine, 3 methionine and 3 tyrosine residues, while its bovine counterpart contains 8 cysteine, 4 tryptophan, 4 phenylalanine, 1 methionine, and 4 tyrosine residues (Atri et al., 2010; Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015b). Atri et al., (2010) found that the conformation of both camel and bovine α-la was sensitive to calcium removal. However, camel α-la showed greater change in exposure of buried hydrophobic areas upon calcium depletion than its bovine equivalent. Redington, Breydo, Almehdar, Redwan, and Uversky (2016) reported that purified camel α-la was more stable towards thermal denaturation than its bovine counterpart. However, it was less conformationally stable, aggregated faster and was more disordered than bovine α-la.

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

Unlike heat treatments, the stability of camel whey proteins was higher than that of their counterparts in bovine milk during HP processing. The level of denatured α-la (32.50±2.05%) after treatment at 800 MPa, was considerably lower than that of bovine milk (55.23±1.66%), buffalo milk (91.8±2.2%) (Huppertz et al., 2005) and ovine milk

(79.3±3.1%) (Moatsou et al., 2008b). HP treatments induced the disintegration of casein micelle in bovine milk through disruption of hydrophobic, electrostatic interactions and solubilisation of colloidal calcium phosphate, resulting in an increased level of soluble calcium which may enhances denaturation of whey proteins (Huppertz et al., 2004b). Therefore, the lower extent of HP-induced denaturation of α-la in CM compared with bovine milk might be explained by limited effect of HP treatments on casein micelle of CM (section *3.3*). Furthermore, camel SA was more stable (3.94±0.07%) at 800 MPa than BSA (16.17±1.85%), and LF was the most stable amongst camel whey proteins over both thermal and pressure treatments with only a small reduction in its concentration.

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

The size distribution of casein micelles in raw skimmed camel and bovine milk was measured and the results indicated that the distribution of casein micelles in raw CM was broader and contained a higher proportion of large particles than in bovine milk. The average diameter of casein micelles in CM was 171.23±4.18 nm; the corresponding value in bovine milk was 143.45±2.96 nm. These results are consistent with data reported by Farah and Rüegg (1989). The effects of thermal and pressure treatments on casein micelle size in processed camel and bovine milk are listed in Table 2. The results revealed that HTST and UHT treatments caused a significant decrease in casein micelles size in CM by 16.4 and 19.5%, respectively, compared with untreated milk. Micelle size in bovine milk was not significantly affected after HTST, and it increased by 14% after UHT treatment. Similar observations with bovine casein micelles have been reported by Freeman and Mangino (1981). Heat treatment of bovine milk at temperature above 80 °C induces formation of β-

lg/α-la complexes through sulphydryl-disulphide interchange reactions, which then associate with the micelles and lead to an increase in their size (Oldfield, Singh, Taylor, & Pearce, 2000). However, structural differences and variation in proportions of individual caseins between bovine and dromedary milk have been reported (Kappeler, Farah, & Puhan, 1998). It has been established that a high content of β-CN and a low content of κ-CN adversely affect some of the processing characteristics of casein micelles such as stability towards ethanol and heat (Schmidt, 2009). In CM, β-CN is predominant while κ-CN is present in very small amount compared with the levels in bovine milk (Omar et al., 2016). Therefore, the significant decrease in the micelle size of heat-treated CM could possibly be due to the dissociation of κ-CN from micelles or the result of precipitation of calcium phosphate out of the casein micelles, which caused them to decrease in size (Anema & Li, 2003).

After HP treatment, casein micelles in CM behaved differently than casein micelles in bovine milk. Treatment of CM at 200 MPa caused a significant ($p < 0.05$) decrease in the size of casein micelles by 21% compared with untreated milk. After increasing the pressure from 400 to 800 MPa, a decrease of 25% in the size of micelle in CM was observed. Treatment of bovine milk at 200 MPa caused a small reduction ($p < 0.05$) in micelle size by 6% compared with untreated milk. However, casein micelles in bovine milk were more susceptible to disintegrate due to increasing pressure (400–800 MPa) during HP treatment than were casein micelles in CM. Treatment at pressure 400 to 800 MPa considerably reduced micelle size in bovine milk by 50% compared with controls. Similar observations of bovine casein micelles were reported by Huppertz et al. (2004a) and Needs et al. (2000).

Studies on goat milk by Law et al. (1998) found that treatments at 200 MPa and temperatures between 20 and 45 °C had little effect on casein micelle size. Treatment at 300 MPa caused an increase in micelle size due to the formation of insoluble aggregates of denatured β-lg with κ-CN. However, higher pressures (>350 MPa) at 45 °C caused a

reduction in the size of casein micelles in goat milk. Different observations of buffalo milk have been reported by Huppertz et al. (2005) who found that treatment of buffalo milk at 250 MPa for 30min at 20 °C reduced micelle size slightly and that treatment at ≥400 up to 800 MPa increased it by 35%. The reduction in casein micelle size in bovine milk is likely to be due to the HP-induced disintegration of casein micelles into smaller particles via the disruption of the intra-micellar van der Waals, hydrophobic and electrostatic interactions and changes in the solubilisation of micellar calcium phosphate (Huppertz et al., 2006; Needs et al., 2000).

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

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

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

listed in Table 3. The HTST process caused a decrease in L^* ($p < 0.05$) and an increase in a^* (greenness) and b^* (yellowness) ($p < 0.05$) in both camel and bovine milk. The lowest L^* value ($p < 0.05$) was observed in UHT-treated CM, which indicates increased darkness for the highest positive yellowness value (b^*). This reduction in the lightness of CM during the UHT treatment may be the result of disintegration of casein micelles into smaller particles (Table 2). On the other hand, L^* and b^* were the highest ($p < 0.05$) in UHT-treated bovine milk, which indicates an increase in the lightness and yellowness of the milk. Similar results for bovine milk have been reported by Rufian-Henares, Guerra-Hernandez, and Garcıa-Villanova (2006). These authors found that after UHT treatment bovine milk had higher a^* and b^* values and that there was an increase in the lightness of milk by 11 units compared with the untreated samples. This increase in the lightness of UHT-treated bovine milk may be due to denaturation and association of whey proteins with casein micelles, in particular β-lg (Burton & Rowland, 1955). The values of WI and ΔE in CM were markedly higher than those in bovine milk after UHT treatment.

High-pressure treatment of bovine milk at 200 MPa caused a significant ($p < 0.05$) decrease in L^* with an accompanying increase in ΔE and WI. Increasing the pressure up to 800 MPa resulted in a further decrease in L^* and an increase in ΔE and WI. Devi, Buckow, Singh, Hemar, and Kasapis (2015) reported similar findings on the behaviour of bovine milk colour under HP treatments. This significant reduction in L^* of bovine milk is mainly attributed to the destruction of casein micelles by pressure into smaller particles, which increases the translucence of the milk. In contrast, a small reduction in L^* in HP-treated CM by up to 1.88 units was observed after treatment at 200 MPa. Treatment at higher pressures (≥400 up to 800 MPa) resulted in a further decrease ($p < 0.05$) in L^* in CM by up to 3.35 units. This slight reduction in L^* in CM compared with bovine milk during HP treatments is possibly the result of the limited HP-induced disruption of its casein micelles (Table 2).

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

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

The development of rennet-induced coagulum in camel and bovine milk was monitored using dynamic oscillatory rheology. The effect of thermal and HP treatments on the storage modulus G' of camel and bovine milk after renneting for 60 min at 30 °C is shown in Fig. 1. The initial pH of the CM samples prior to the addition of rennet (Table 4) varied between 6.71 and 6.51, consistent with the reported pH values of CM in the literature (Farah, 1993). The final pH values of the gel formed after 60 min incubation were ranged from 6.32–6.64, which was lower than those measured for the curd formed by the bovine milk.

The RCT of the raw CM was much shorter than that of bovine milk. Camel chymosin initiates coagulation of milk by hydrolysing bovine κ-CN at the Phe^{105}–Met^{106} scissile bond

and disconnecting the C-terminal part of 106–169 amino acids (Langholm Jensen et al., 2013). Whereas, the chymosin cleavage site of camel κ-CN is at the Phe^{97}–Ile^{98} bond and the enzyme cuts off a C-terminal glycomacropeptide of 65 amino acids residues (Hailu et al., 2016). The coagulation of CM occurs only after hydrolysis of 95% of camel κ-CN by camel chymosin, while, the gelation of bovine milk starts at level of 60–70% of bovine κ-CN hydrolysis (Hailu et al., 2016). The HTST treatment of CM at 72.5 °C for 15s significantly ($p < 0.05$) delayed the RCT of the milk by 70.99% compared with untreated milk (Table 4) and the final *G′* value was considerably lower than that of the control (Fig. 1A). The RCT of bovine milk was also significantly delayed after HTST by14.36% and the *G′* value was decreased, but not statistically significant, compared with untreated milk. Similar results about the effects of heat treatment on the RCT of bovine (Singh & Waungana, 2001), ewe, and goat milk (Calvo & Balcones, 1998) clotted using bovine chymosin have been reported. Kethireddipalli and Hill (2015) noted that heat treatment at temperatures above 75 °C led to an increase in the RCT of milk and the formation of weak curds. Vasbinder, Rollema, and Kruif (2003) and Blecker, Habib-Jiwan, and Karoui (2012) reported that the decrease in the rate of gel development and final *G′* value in pasteurised milk could be the result of the association of denatured whey protein aggregates with casein micelle surfaces. The formation of whey protein-κ-casein complex affects the reactive sites on the micelles that are formed by the action of rennet, which leads to fewer and weaker bonds and therefore a weaker coagulum (Singh & Waungana, 2001).

As expected, UHT-treated camel and bovine milk failed to coagulate. Similar observations have been found using bovine chymosin in bovine milk by Ham et al. (2013). These authors found that UHT treatment hindered the coagulation of milk compared with HTST treatment. This heat-induced inhibition of rennet coagulation is mainly attributed to effects arising during the secondary stage of rennet coagulation or micelle aggregation, the

decreased concentration of ionic calcium, and the association of denatured whey proteins with the casein micelles in heat-treated milk (Vasbinder et al., 2003).

In HP treatment, the RCT of bovine milk treated at 200 MPa was shortened significantly ($p < 0.05$) by 26.91% (Table 3) and the *G′* value was the highest (Fig. 1B) compared with the control milk. However, HP treatments at higher pressures (400, 600, and 800 MPa) resulted in an increase of the RCT and the final *G′* value was similar to untreated milk. These results are consistent with previously reported observations for bovine milk coagulated with recombinant bovine chymosin by Needs et al. (2000) and Zobrist, Huppertz, Uniacke, Fox, and Kelly (2005). The reduction in the RCT of bovine milk after HP treatment at 200 MPa is believed to be the result of the dissociation of micellar κ-casein and the disruption of casein micelles, which led to an increase in the surface area for intermicellar interactions with less κ-casein available to provide steric stabilisation (Huppertz et al., 2006; Needs et al., 2000).

In contrast, the rheological properties of CM differed from that of bovine milk for HP treatments. The HP treatment at 200 and 400 MPa significantly ($p < 0.05$) delayed the RCT of CM by 55.90 and 109.39%, respectively, compared with untreated milk. The rate of gel formation in HP-treated CM at 200 MPa (Fig. 1A) was lower than that of untreated milk during the first 30 min, following the addition of rennet enzymes. However, after 40 min the rate of increase of *G′* was higher than that of control milk and the final value of *G′* after 60 min of incubation was the highest. This increase in the RCT and strength of the rennet-induced coagulum from CM treated at 200 MPa might be due to restricted effect of HP on disruption of casein micelles and whey protein denaturation in CM. In HP-treated CM at 400 MPa, the rate of coagulum formation was considerably slower with a significantly lower final *G′* value than that of untreated milk. Meanwhile, no progress in the rennet-induced coagulum was observed in CM treated at 600 and 800 MPa. Similar observations regarding effect of HP

treatments on the RCT of buffalo milk coagulated with bovine chymosin (Maxiren 180) have been reported by Huppertz et al. (2005). These authors found that HP treatment at 100 MPa had no effect on the RCT of buffalo milk. On the other hand, the RCT of buffalo milk increased significantly by 50% after treatment at 200 MPa and continued to increase with pressure to a maximum of 100% after treatment at 800 MPa. In another study by López-Fandiño and Olano (1998), the RCT of ovine milk, clotted using standard bovine chymosin, increased significantly after treatment at 200 and 300 MPa, but treatment at 400 MPa decreased the RCT. These authors also found that HP treatment of caprine milk at 200 MPa did not affect the RCT, and treatment at 300 and 400 MPa increased the RCT.

To visualise the effects of the different treatments on CM properties, principal component analysis (PCA) was used (Fig. 2). The two principal components accounted for 82% of the variation in the data. Processed CM samples were separated according to intensity of heat and high-pressure along PC1, which explains 62.20% of the total variance in the data. UHT CM was clearly separated from the raw and the pasteurised milk, while HP-treated CM samples at 600 and 800 MPa were clustered in the upper right tope of the PCA. These CM samples were correlated with the highest levels of denaturation of whey proteins and maximum colour difference (ΔE). On the other hand, the PCA revealed a distinct separation between HP-treated CM samples and HTST and UHT milk samples along the second PC, which explains 20.11% of the variability. CM samples treated at 200 and 400 MPa were associated with the RCT, G' and WI. While, raw and pasteurised CM were correlated with the size of casein micelle.

4. Conclusions

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

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Figure legends

Fig. 1. Influence of incubation time at 30 °C following addition of rennet on the storage modulus, *G'*, of processed camel (A) and bovine (B) milk, raw skimmed untreated milk (●), HTST (72 °C, 15 s) milk (◆), UHT (140 °C, 5 s) milk (▲), high-pressure at 200 (∗), 400 (△), 600 (×), 800 (○) MPa for 30 min at 20 °C. Values are means of data from experiments on three individual milk samples.

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

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Table 1

Major whey proteins β-lactoglobulin (β-lg), α-lactalbumin (α-la), serum albumin (SA), and lactoferrin (LF) identified in raw and processed skimmed camel and bovine milk.[a]

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

[a] Processing parameters were: HTST 72 °C, 15sec; UHT, 140 °C, 5sec; high-pressure (HP), 200, 400, 600, 800 MPa for 30 min at 20 °C. Values are means ± standard deviation (n = 3; -, not detected); means within a column with different superscript letters are significantly different ($p < 0.05$).

Table 2

The average diameter of casein micelle size in raw and processed skimmed camel and bovine milk. [a]

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

[a] Processing parameters were: HTST 72 °C, 15sec; UHT, 140 °C, 5sec; high-pressure (HP), 200, 400, 600, 800 MPa for 30 min at 20 °C. Values are means ± standard deviation (n = 3); means within a column with different superscript letters are significantly different ($p < 0.05$).

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Table 3

Changes of colour parameters, *L** (lightness), *a** (redness to greenness), *b** (yellowness to blueness), total colour difference (Δ*E*), and whiteness (WI) measured in raw and processed skimmed camel and bovine. [a]

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

[a] Processing parameters were: HTST 72 °C, 15sec; UHT, 140 °C, 5sec; high-pressure (HP), 200, 400, 600, 800 MPa for 30 min at 20 °C. Values are means ± standard deviation (n = 3); means within a column with different superscript letters are significantly different ($p < 0.05$).

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Table 4

Rennet coagulation time (RCT), the final storage modulus *G′* after 60 min at 30 °C, and pH of raw and processed skimmed camel and bovine milk. [a]

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

[a] Processing parameters were: HTST 72 °C, 15sec; UHT, 140 °C, 5sec; high-pressure (HP), 200, 400, 600, 800 MPa for 30 min at 20 °C. Values are means ± standard deviation (n = 3; -, milk failed to coagulate); means within a column with different superscript letters are significantly different ($p < 0.05$).

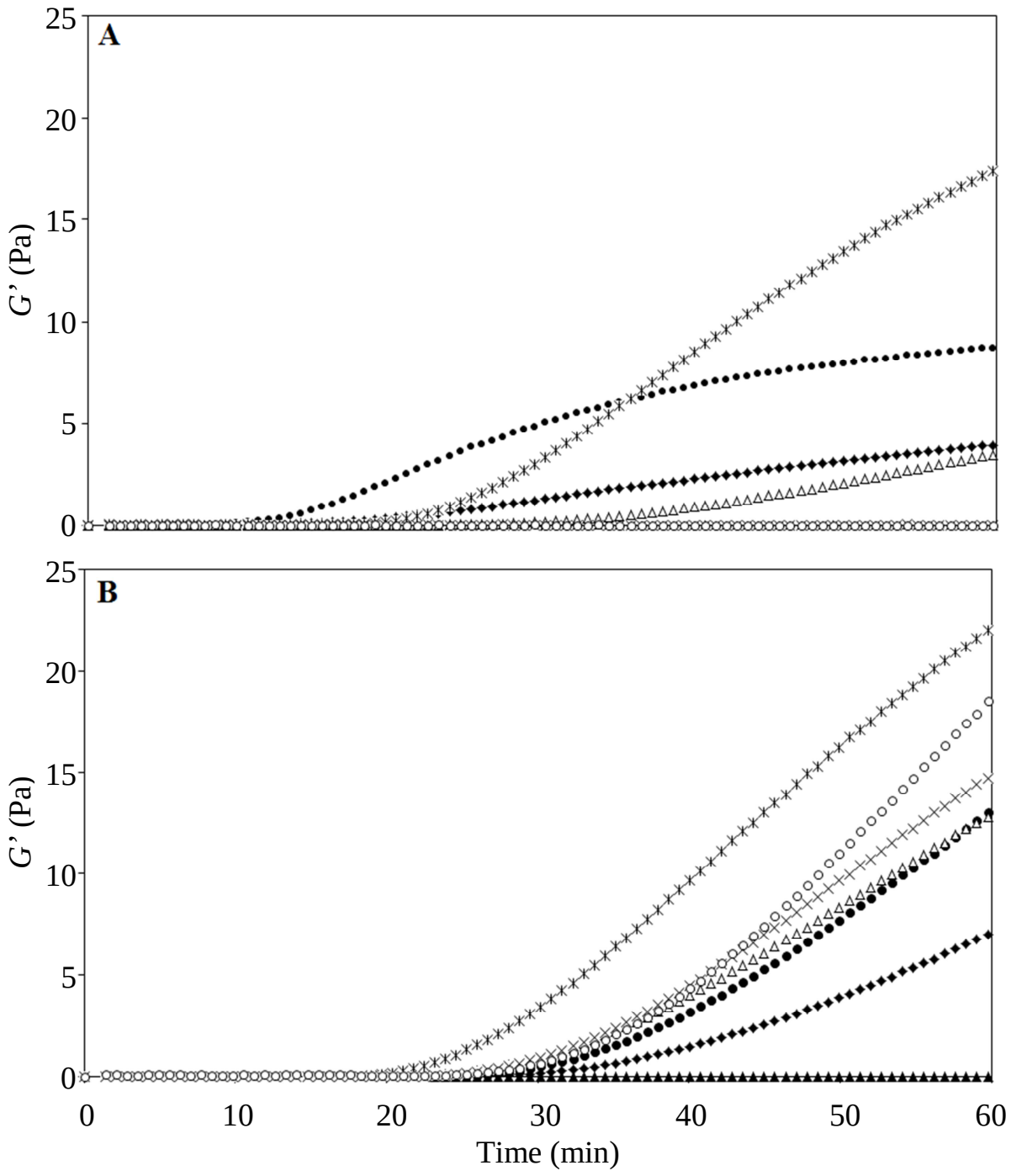

Figure 1.

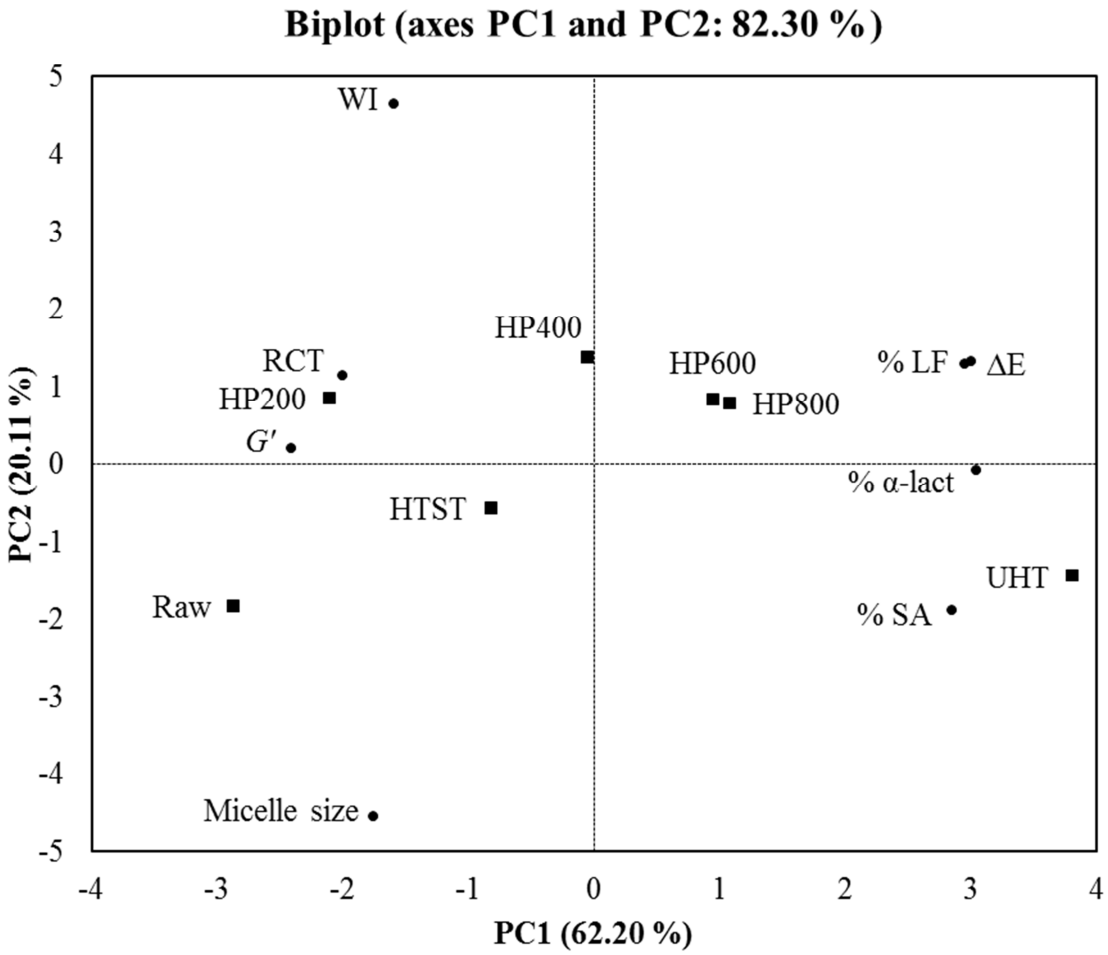

Figure 2.

ACCEPTED