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HEAT STABILITY OF CAMEL MILK PROTEINS AFTER STERILISATION PROCESS

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ABSTRACT

In this study, the problem associated with stability of camel milk proteins after sterilisation process were studied and discussed. Camel milk has poor heat stability at high temperature and could not be sterilised at natural pH. Various attempts were adopted to overcome this problem including the addition of κ-casein and different additives including, EDTA, phosphate and sodium hydroxide. Findings in this study showed that camel milk is likely belong to type A milk and could be converted to type B milk by adding κ-casein or EDTA at a concentration of 2 mg mL^{-1} and 6.0 mmol L^{-1}, respectively. Two contents; κ-casein and calcium were found to play major role in camel milk heat stability. The maximum and minimum heat stability of pre-adjusted pH camel milk after sterilisation process was in pH range between 7.0 - 7.2 and 6.5-6.8, respectively. Small change in pH results in great effect on heat stability.

Key words: Additives, camel milk, EDTA, heat stability, κ-casein, sterilisation

Heat stability of milk was studied intensively during the last century since the work of Sommer and Hart which was started in 1919. Heat stability was defined by Augustin (2000) as the ability of milk or concentrates to resist severe heat treatments without thickening, gelatin or coagulation. However, the actual application of a selected heat treatment process is mainly depending on the source of milk (Raynal-Ljutovac *et al*, 2007). Most of the work undertaken on heat stability was mainly focused on cow milk and other mammals including buffalo, goat and sheep milk. In general, the stability of small ruminant milk at 140°C is poor at their natural pH (Raynal-Ljutovac *et al*, 2007) but stable at sterilisation temperature of 121°C for 15 min even at their natural pH. While, camel milk at natural pH was reported not to be sterilised due to the instability of proteins (Abeiderrahmane, 2005). Among mammal's milk, this problem is expected to be unique in camel milk. However, camel milk was previously reported (Al Haj and Al Kanhal, 2010) to have some different properties from bovine milk including poor stability at high temperatures. Hence, Farah *et al* (2004) reported that the main problem of the preserved UHT camel milk is sedimentation of proteins which require selected additive to achieve physical stability. Different attempts were made to improve heat stability of camel milk. Where, neither urea nor formaldehyde addition have improved the heat stability of camel milk (Metwalli *et al*, 2000). Moreover, salt adjustment of camel milk was reported to have no effect on heat coagulation time (Al-Saleh, 1996). This could be due to the casein micelle size or deficiency of β-lactoglobulin and κ-casein in camel milk (Al-Saleh, 1996; Farah and Atkins, 1992). Nevertheless, camel milk whey proteins were found to be more heat stable than bovine or buffalo whey proteins (Al Haj and Al Kanhal, 2010; Wernery, 2006; Al-Saleh, 1996). Furthermore, camel milk is known to be effectively pasteurised by vat or HTST pasteurisation process with no evidence of precipitation in proteins. Commercially pasteurised camel milk product is now available in the markets of Gulf countries. Moreover, camel milk was shown to be stable after direct UHT process together with refrigeration to achieve 5 weeks of shelf life (Farah *et al*, 2004). However, camel milk heat stability and sterilisation has not been given such an attention in the available literature. It is believed that camel milk cannot be sterilised due to its poor stability and could coagulate in less than 3 min even after adjusting with sodium hydroxide at all pH (Al-Saleh, 1996; Farah and Atkins, 1992). Furthermore, some researchers (Abu-Taraboush *et al*, 1998) preferred pasteurised camel milk instead of sterilised one in a microbiological research. The information on camel milk heat stability is scarce. The objectives of this research were to overcome the problem associated with the stability

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of camel milk proteins after sterilisation process. Additionally, the effect of added κ-casein and other additives on camel milk heat stability after sterilisation process were also discussed.

Materials and Methods

Chemicals used

Sodium hydroxide (NaOH) and calcium chloride ($CaCl_2$) (BDH laboratory, England), κ-casein from bovine (Sigma Chemicals, USA), Sodium dihydrogen phosphate anhydrous (Fluka, Switzerland), di-sodium hydrogen orthophosphate (Fisons Analytical reagents, England) and Ethylenediaminetetraacetic acid disodium (EDTA) salt AR (WINLAB Laboratory Chemical Reagents, UK).

Milk samples

Whole camel milk samples were collected from dromedary camels (Majaheim) from the region around Riyadh city (central region of Saudi Arabia) and refrigerated immediately and transferred to the laboratory. Upon arrival, camel milk samples were homogenised at 15 MPa and then used according to the designed experiment.

Sterilisation process

All camel milk samples were sterilised using the standard laboratory method of sterilisation at 121°C for 15 min in autoclave. Camel milk was also sterilised using Arnold steam steriliser method (1897). The microbial test of camel milk after using both sterilisation methods was negative.

Determination of heat stability of camel milk proteins after sterilisation process

The pH of camel milk samples at 4°C were adjusted to various pH values by adding 1 M- NaOH usually in a range 6.6-7.4, unless, otherwise stated depending on the experiment design. All samples were equilibrated overnight in the refrigerator before determining heat stability and were run in triplicate. All samples in this research were removed from the autoclave directly after sterilisation treatment. The determination analysis of heat coagulation time of camel milk using oil bath was not carried out at this stage of study because it does not meet our aim. Partial or complete protein precipitation was the only indication of unsuccessful heat stability and sterilisation of camel milk.

Effect of κ-casein addition on heat stability

The κ-casein from bovine was added to camel milk samples (pH 6.5) at the concentration of 1 mg mL^{-1} and 2 mg mL^{-1} and dissolved using magnetic stirrer prior to pH adjustment. Samples were then adjusted to pH range of 6.6-7.0 (κ-casein dissociates at higher pH) by adding 1 M NaOH and equilibrated overnight in the refrigerator prior to sterilisation process.

Effect of phosphate, EDTA and calcium addition on heat stability

In order to determine the interrelation effect between pH and phosphate, EDTA or calcium addition, the following concentrations were dissolved in camel milk (pH 6.5) prior to pH adjustment; sodium phosphate (1.0 and 2.0 mmol L^{-1} of mixed mono- and di-phosphate, pH 6.7); EDTA (2.0, 4.0 and 6.0 mmol L^{-1}) and calcium chloride (1.0 and 2.0 mmol L^{-1}). Samples were then adjusted to pH range of 6.6-7.4 by adding 1 M- NaOH and equilibrated overnight in the refrigerator prior to sterilisation process.

Results and Discussion

In the current research, 3 main methods were practiced to overcome the problem associated with camel milk sterilisation with emphasis on protein stability.

The effect of pH/NaOH adjustment on camel milk proteins stability after sterilisation process

The natural pH and acidity of camel milk were 6.54 and 0.14%, respectively. Camel milk was noticed to separate into 2 parts after normal laboratory sterilisation process (121°C for 15 min), where all casein and whey proteins were precipitated. This occurred when camel milk was heated to 110°C for 15 min NaOH. was added to whole camel milk to increase the pH from 6.6 to up to 8.0, thus improved heat stability at certain pH (Fig 1). Results in this work showed that whole camel milk proteins at natural pH of 6.5 and pre-adjusted samples at pH 6.6, 6.7, 6.8, 7.7, 7.8, 7.9 and 8.0 were unstable directly after sterilisation process (121°C for 15 min), where all casein and whey proteins precipitated and found to be irreversible (Fig 1). These results (pH 6.5 - 6.8) are in agreement with that reported by Farah and Atkins (1992) and Al-Saleh (1996). The low pH decrease is usually associated with the induced increase in Ca^{2+} activity or content, which leads to shield the negative charge of the micelles and consequently micelle aggregation (Raynal-Ljutovac *et al*, 2007). Result of this study showed that camel milk probably belongs to the predominant type A milk and has 2 stages of coagulation in proteins similar to cow milk. This phenomenon was suggested by Sweetsur and White (1974) and cited by O'Connell and Fox (2000) as "*the premature coagulation of large casein micelles caused by adsorption of precipitated calcium phosphate, followed by a second coagulation involving smaller casein*

micelles, which also adsorb precipitated calcium phosphate but are inherently more stable than the larger micelles". Two coagulation stages in camel milk proteins was noticed at pH below 6.9 and above 7.6 as shown in Fig 1. Camel milk casein was reported (Al Haj and Al Kanhal, 2010; Farah and Rüegg, 1989) to contain a higher number of large micelles (200-500 nm) than cow milk (220-300 nm). This could theoretically explain the poor heat stability of camel milk in this study, where as large casein micelles of cow milk was reported to coagulate rapidly in 4.4 min, while smaller micelles increase in size with heating (O'Connell and Fox, 2000).

Results also showed that the pH of these samples (pH 6.5 - 6.8) were decreased to 6.4. Such a decrease in milk pH after heating could be mainly due to the acid production from lactose especially formic acid. Precipitation of soluble calcium phosphate occurs with the concomitant release of H^+, and finally de-phosphorylation of casein with subsequent precipitation as $Ca_3(Po_4)_2$ with release of H^+ (Fox and McSweeney, 1998).

Singh and Fox (1985) reported that negative charge on κ-casein micelles increase at high pH values, consequently increase milk heat stability. The percentage of κ-casein (3.47% of the total casein) in camel milk (Al Haj and Al Kanhal, 2010; Kappeler *et al,* 2003) is lower than that reported (13%) for cow milk (Davies and Law, 1980). Hence, casein micelles in camel milk especially large micelles become more susceptible to Ca^{2+} induced precipitation, hence, decreases heat stability (O'Connell and Fox, 2000).

Little sedimentation in protein was noticed in the top of camel milk samples with pH value of 6.9, 7.4, 7.5 and 7.6 after 4 days of storage at room temperature (Fig 1) after being stable directly following sterilisation process as shown in fig 2. The protein sedimentation of these samples was found to be reversible. Contrarily, no sedimentation was noticed directly after sterilisation process or storage of camel milk samples having pH values of 7.0, 7.1, 7.2 and 7.3 (Fig 2). Results in this work disagree with that reported by Farah and Atkins (1992) where camel milk proteins were unstable after adding NaOH at all pH values (range 6.3-7.1) and heated at 120°C or 130°C and were coagulated in less than 3 min.

The results showed that the maximum heat stability of camel milk proteins after sterilisation process was in pH range between 7.0 - 7.2 (Fig 2). These results are in agreement with that reported for caprine milk where the maximum heat stability of unstable milk samples were at pH values of 7.0-7.1 (Raynal-Ljutovac *et al*, 2007; Morgan *et al,* 2000). Minimum heat stability was noticed in samples having pH range between 6.5-6.8 (Fig 1). Similarly, minimum heat stability in cow milk was at pH value of 6.8 (Farah and Atkins, 1992). Maximum heat stability term is used in the current work to indicate the protein heat stability of camel milk after sterilisation process with no sedimentation or reversible protein sedimentation, while, minimum heat stability term is used to indicate the protein heat stability of camel milk after sterilisation process with irreversible protein sedimentation.

Browning Maillard reaction was noticed in this type of sterilisation (Fig 1), similar to cow milk due to the formation of some complex compounds between casein and lactose (Raynal-Ljutovac *et al*, 2007). Browning colour was noticed to increase especially, samples with high NaOH addition or high pH values. Maillard reaction was expected to increase heat stability of cow milk due to the formation of low molecular weight carbonyl (Fox and McSweeney, 1998), but in camel milk it appears to decrease heat stability (Fig 1).

The effect of κ-casein addition and pre-adjusted pH on camel milk proteins stability (type A) after sterilisation process

The κ-casein is known to be the main factor stabilising casein micelle in milk. Its content in camel milk is lower than that in cow milk (Al Haj and Al Kanhal, 2010; Kappeler *et al,* 2003) which makes it less stable. However, different concentration of κ-casein was added to camel milk to examine its effect on protein stability at different pH after sterilisation process. The addition of κ-casein was reported (Horne and Muir, 1990; Tessier and Rose, 1964) to increase the stability of cow milk type A at the pH range (6.7-6.8) of the minimum heat stability. Similarly, the addition of κ-casein at a concentration of 1 mg mL^{-1} has shown to increase the heat stability of pre-adjusted camel milk protein type A at the pH range of the minimum heat stability of pH 6.8-6.9. The stabilising effect of κ-casein on heating at high temperature for milk proteins was reported (Van Boekel *et al,* 1989) to protrude the hairy layers of c-terminal of κ-casein providing steric repulsion for casein which leads to increase heat stability. However, no effect on camel milk protein heat stability was noticed below this pH range. Further increase of κ-casein concentration to 2 mg mL^{-1} in camel milk type A has shown to increase the heat stability and eliminate the minimum heat stability at pH range 6.7-6.9, as compared to normal camel milk type A containing no added κ-casein. In the mean time, the addition of κ-casein to camel

Fig 1. Sterilised whole camel milk after 4 days of storage at natural pH 6.5 or after adding NaOH to adjust the pH up to 8.0. Figure shows stability and instability of camel milk at different pH values.

Fig 2. Heat stability of sterilised pre-adjusted (NaOH) whole camel milk proteins. No sedimentation and separation in proteins was noticed in camel milk directly after sterilisation process.

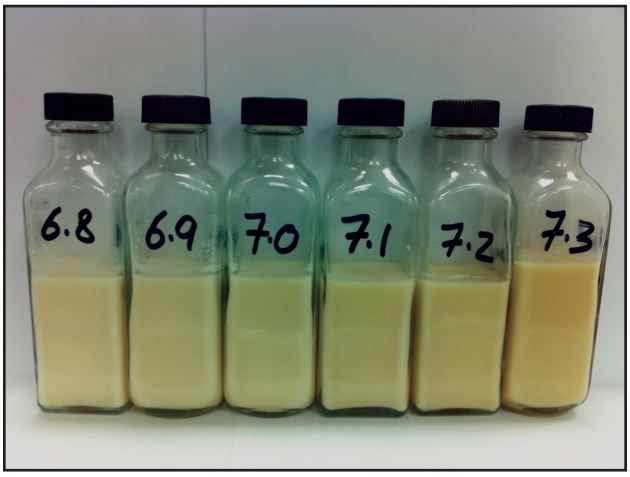

Fig 3. Heat stability of sterilised whole camel milk proteins containing sodium phosphate at a concentration of 1 mmol L-1 after adding NaOH to adjust the pH up to 7.3. No sedimentation and separation in proteins was noticed in camel milk directly after sterilisation process.

Fig 4. Stability of sterilised whole camel milk using autoclave apparatus on each 3 successive days.

milk type A was shown to convert it to type B due to reduction or complete elimination of the minimum heat stability in camel milk type A. The mechanism by which κ-casein eliminate minimum heat stability was discussed by Fox and McSweeney (1998) as κ-casein dissociate from micelles on heating; however, the presence of β-lactoglobulin in milk could reduce this dissociation at pH below 6.7, but at pH above 6.7, it can accentuate dissociation. However, camel milk is less stable than cow milk because it is deficient in β-lactoglobulin and k-casein, which leads to decreased colloidal stability in camel milk.

The effect of EDTA addition and pre-adjusted pH on camel milk proteins stability (type A) after sterilisation process

It has been reported that content of calcium in dromedary camel milk were close to that reported in bovine milk (Al Haj and Al Kanhal, 2010; Sawaya *et al,* 1984). In this experiment, EDTA was added to camel milk as a chelating agent to disrupt casein micelles (Lucey and Horne, 2009) by reducing calcium ion contents, as well as colloidal calcium phosphate (Udabage *et al,* 2000) which led to casein micelle dissociation (Gaucheron, 2005) and consequently increased heat stability of camel milk. Different concentration of EDTA (2.0, 4.0 and 6.0 mmol L^{-1}) was added to determine its effect on camel milk protein heat stability after sterilisation process. The addition of 2.0 mmol L^{-1} was found to have no effect on camel milk heat stability at the studied range of 6.5-7.4. Further increase in EDTA concentration to 4.0 or 6.0 mmol L^{-1} was shown to extend the heat stability range towards the acidic side from 7.0-7.3 to 6.6-7.4 for both concentrations, compared to normal camel

milk containing no added EDTA. But the maximum heat stability was at the pH range 6.7-7.2 and 6.7-7.3, respectively; where proteins sedimentation of samples above or below these ranges were reversible except the natural pH of camel milk of 6.5 where protein sedimentation was irreversible. The addition of EDTA to camel milk type A at a concentration of 4.0 or 6.0 mmol L^{-1} was noticed to convert it to type B and eliminate its minimum heat stability due to calcium binding to EDTA.

The effect of phosphate or calcium addition and pre-adjusted pH on camel milk proteins stability (type A) after sterilisation process

Disodium phosphate is usually used during UHT treatment to prevent destabilisation of goat milk (Raynal-Ljutovac *et al,* 2007). In the current work, the addition of sodium phosphate at a concentration of 1 mmol L^{-1} to whole camel milk type A has broadened the stability range from 7.0-7.3 to 6.8-7.3 as shown in fig 3. Moreover, added phosphate has no effect on camel milk stability below or above this range (pH 6.8-7.3). However, the maximum heat stability of camel milk containing phosphate at a concentration of 1 mmol L^{-1} was found to be slightly shifted to the acidic side at the pH range 6.8-7.1. Moreover, increasing sodium phosphate concentration to 2 mmol L^{-1} has further broadened the maximum heat stability range into 6.8-7.2. The presence of added phosphate in camel milk helps to decrease the effect of high temperature on pH decrease, in addition to increase the heat stability of camel milk at pH points (6.8 and 6.9), which was considered as minimum in normal milk containing no added phosphate. It is believed that added phosphate could increase the heat stability by binding soluble Ca^{2+} hence induced calcium phosphate precipitation on the micelle or lower calcium activity (Raynal-Ljutovac *et al,* 2007). In another study, the calcium ion activity of concentrated milk was reported to decrease from 0.60 mmol L^{-1} to 0.27 mmol L^{-1} at 120°C, pH 6.5 when phosphate was added, while decreased from 0.83 mmol L^{-1} to 0.71 mmol L^{-1} when phosphate was not added (Nieuwenhuijs *et al,* 1988).

On the other hand, the addition of calcium chloride at a concentration of 1 mmol L^{-1} has decreased the heat stability of camel milk proteins at the range studied and coagulated all samples except the one at pH 7.2. As expected, further increase in the concentration of calcium chloride of 2 mmol L^{-1} has sedimented all sample proteins within the range studied. It has been reported that the addition of calcium decreased heat stability of cow milk through displacement of coagulation into less stable region of $CaCl_2$ (Miller and Sommer, 1940). This was explained that added calcium has decreased the pH in milk more rapidly, and decreased the stability factors of casein micelles (Van Boekel *et al,* 1989); while the presence of added phosphate has decreased the pH less rapidly than in the normal milk containing no added calcium and phosphate.

Camel milk proteins stability after sterilisation process using Arnold steam method

In this method, camel milk was sterilised by exposing milk to the steam in an open valve autoclave apparatus on each 3 successive days. This method is similar to that used by Arnold steam steriliser (1897). The pH value of camel milk (no additive) was found to decrease from 6.5 to 6.3 after sterilisation process; this could be attributed to the production of acids in milk that caused by lactose degradation (Raynal-Ljutovac *et al,* 2007; Jenness and Patton, 1976). Additives such as phosphate and citrate were reported to limit pH decrease during heat treatment process (Raynal-Ljutovac *et al,* 2007). Minor but reversible protein sedimentation could be noticed after this type of sterilisation (Fig 4). No further sedimentation was noticed in this type of sterilisation even after 6 month of storage at room temperature or cold room. However, no microbial growth was noticed after using this type of sterilisation in camel milk.

Camel milk proteins stability after sterilisation process using partial amount of cow milk

In this method, whole camel milk at a percentage of 69% (natural pH and acidity) was mixed with whole cow milk at a percentage of 31% (no additives). This milk was found to remain stable after sterilisation process (121°C for 15 min), No sedimentation was noticed in this type of sterilisation. The addition of 31% of cow milk has shown to increase the stability of camel milk. This percentage is expected to be high for such a research. In addition, the mechanism behind this effect is still unclear pending further investigation. However, increasing the percentage of camel milk to more than 69% was found to coagulate the whole milk.

Conclusion

Findings in this research showed that κ-casein and calcium content are expected to be the main factors affecting the low heat stability of camel milk protein. Furthermore, camel milk appears to belong to type A milk and has 2 stage of coagulation in proteins. Results also exhibited that camel milk could

be converted from type A to type B and eliminate its minimum heat stability by adding κ-casein or EDTA. Results also showed that pH increase was beneficial for camel milk heat stability; however, very small change in pH could result in large effect on protein heat stability. Camel milk was shown to be sterilised if the pH increased to 7.0 - 7.2 or certain additives added. Some additives such as phosphate could be used in commercial skill to improve heat stability of whole camel milk.

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