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Effect of heat treatment on camel milk proteins with respect to antimicrobial factors: a comparison with cows' and buffalo milk proteins

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Abstract

Camel, cow and buffalo skim milk samples were heated at 65, 75, 85 and 100°C for 10, 20 and 30 min. Presence and identity of whey proteins (WPs) were monitored using SDS-PAGE technique. Changes in activities of antimicrobial factors were measured. Heat-induced changes of WPs increased with increasing temperature and time of heating; they were more pronounced in buffalo and cow milk than in camel milk. Camel WPs were markedly more heat resistant than their counterparts in cow and buffalo milk. Among the WPs, the order of heat resistance found was: α-lactalbumin > β-lactoglobulin > serum albumin. Camel milk contained significantly ($P \leqslant 0.01$) higher concentrations of lysozyme (LZ), lactoferrin (LF) and immunoglobulin G (IgG) than cow and buffalo milk. Heating milk at 65°C/30 min had no significant effect on LZs and LFs, however, loss of activity of IgGs was significantly ($P \leqslant 0.01$) affected in the three kinds of milk. The whole activity of IgG in cow and buffalo milk was lost at 75°C/30 min versus 68.7% in loss activity of camel IgG. The entire activity of LFs was lost at 85°C/30 min in all kinds of milk, however, at this level of temperature, the activity losses of LZs were 56, 74 and 81.7% for camel, cow and buffalo milk, respectively. Camel milk antimicrobial factors were significantly ($P \leqslant 0.01$) more heat resistant than cow and buffalo milk proteins. Among the antimicrobial factors, the order of heat resistance found was LZ > LF > IgG.

1. Introduction

There are about 18 million camels in the world (FAO, 1996) which support the survival of millions of people in arid and semi-arid areas. Meanwhile camel milk is considered one of the main components of the human diet in many parts of the world. It contains all essential nutrients as cow milk (Elagamy, Abou-Shloue & Abdel-Kader, 1998), also it has a high biological value due to the higher contents of antimicrobial factors such as lysozyme, lactoferrin and immunoglobulins (Elagamy, Ruppanner, Ismail, Champagne & Assaf, 1992). Most camel milk is consumed in the fresh or sour state. On the other hand, the preservation of raw milk can be achieved by heat treatments such as pasteurization, boiling or sterilization processes. These treatments have direct influences on the nutritional, biological and functional properties of milk proteins. Their effects on cow milk whey proteins had been extensively studied (deWit & Klarenbeek, 1984; Lyster, 1970; Mulvihill & Donovan, 1987; Pearce, 1989). However, only limited studies were carried out on camel milk casein (Mohamed & Larsson-Raznikiewicz, 1991) or camel milk whey proteins (Farah, 1986). No data are present in the literature on the thermal effect on camel milk antimicrobial factors. The aim of this study was to determine the effect of various heat treatments on the whole camel WPs as well as the antimicrobial factors with comparison to those of cow and buffalo milk in order to have more information about the biological value of heat-treated camel milk.

2. Materials and methods

2.1. Materials

2.1.1. Milk Samples

Three bulk samples (seven individuals) of each type of milk were used in the study. Samples of camel (*Camelus dromedarius*) milk were obtained from the El-Alamin area around Alexandria. Cow and buffalo milk samples

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were collected from the herds of the Faculty of Agriculture, Alexandria University, Egypt.

2.1.2. Chemicals

Egg white lysozyme (E.C. 3.2.1.17) was purchased from Boehringer, Mannheim, Germany, *Micrococcus lysodeikticus* from Difco, Detroit, USA. Bovine lactoferrin, immunoglobulin G, α-lactalbumin and β-lactoglobulin were purchased from Sigma Chemical Co, St Louis, MO, USA.

2.2. Analytical methods

2.2.1. Heat treatment

Milk samples were defatted by centrifugation at 3000 *g* for 20 min at 4°C. Skim milk was then divided into equal portions, one portion was kept as a control (raw), the rest ones were heated at 65, 75, 85 and 100°C for 10, 20 and 30 min in a thermostatically controlled water bath in 250 ml flasks. Samples were rapidly cooled to 40°C, renneted and centrifuged at 3000 *g* for 20 min at 4°C to obtain the rennet whey.

2.2.2. Isolation of protective proteins

Camel and buffalo lactoferrins were isolated from whey as described by Law and Reiter (1977). Buffalo milk immunoglobulin G was isolated as described by Gray, Nickelson and Crim (1969). While camel milk immunoglobulin G was isolated according to the method mentioned by Elagamy, Ruppanner, Ismail, Champagne and Assaf (1996). Isolated lactoferrins and immunoglobulin G were analysed for purity using SDS-PAGE (Laemmli, 1970) and immunoelectrophoresis (Mayer & Walker, 1990).

2.2.3. Gel electrophoresis

Analytical slab gel electrophoresis of rennet-whey samples was conducted in polyacrylamide gel containing 0.1% sodium dodecyl sulphate (SDS-PAGE) according to the conventional method which involved denaturation of proteins by heating for 5 min in 1% SDS in a boiling water bath prior to applying them to the gel. After electrophoresis, proteins were localized in gels using Coomassie blue 0.1% (Laemmli, 1970). Electrophoresis was performed using PROTEAN-II Cell (Bio- Rad, Richmond, USA).

2.2.4. Protein molecular weight determination

Molecular weights (kDa) of whey protein fractions were estimated according to the method of Weber and Osborn (1969) after fractionation on SDS-PAGE and using standard protein markers.

2.2.5. Determination of lysozyme

Lysozyme concentration in whey was quantified by the modified lysoplate assay as described by Lie, Syed and Solbu (1986). The test was carried out in 1% agarose gel containing *Micrococcus lysodeikticus*.

2.2.6. Determination of lactoferrin (LF) and immunoglobulin G (IgG)

LF and IgG concentrations in whey were measured with the radial immunodiffuson assay (Carlsson, Bjorck & Persson, 1989). The test was carried out in agarose gel containing the appropriate antiserum.

2.2.7. Preparation of antisera

Antisera for camel and cow milk IgG and LF were produced according to the procedure described by Johnstone and Thorpe (1985). Antisera were elicited in rabbits by initial intramuscular injections which contained 5 mg /ml of protein in complete Freunds adjuvant (Sigma). Booster injections were given intradermally at 3 week intervals. Ten days after the last injection, blood was taken from rabbits and the antiserum titre was measured.

2.2.8. Statistical analysis

Results were analyzed using analysis of variance of the SAS package (SAS, 1985).

3. Results and discussion

3.1. Effect of heat treatment on WPs

Gel electrophoretic patterns of WPs prepared from raw and heated camel, cow and buffalo milk are shown in Fig. 1. Differences in electrophoretic patterns of raw-milk WPs of the three kinds of milk were found. It is obvious that the major WP bands in cow and buffalo milk belong to serum albumin (SA), β-lactoglobulin (β-1g) and α-lactalbumin (α-1a), whereas in camel milk WPs pattern, β-1g was a minor band. However, the bands of SA, α-la as well as another unknown fraction (F_2) were found in high intensities. When milk samples were heated at 65°C for 10, 20 or 30 min, no changes in WP electrophoretic patterns were noticed. Similar results were obtained by Farah (1986). He reported that no effect was recorded on WP prepared from cow or camel milk heated at 63°C for 30 min. Increasing the temperature to 75°C, resulted in visible changes in the electrophoretic patterns for all three kinds of milk. For example, the effect was mild on cow milk SA whilst it was drastic on buffalo milk SA but in camel milk SA was not affected. At this level of heating, no effect on α-1a fraction in all kinds of milk; however, β-1g fraction in cow and buffalo milk was affected. Its disappearance was more pronounced in buffalo milk than in cow milk. Furthermore, the disappearance increased with time. Patterns showed also that increase of β-1g disappearance in buffalo and cow WPs resulted in appearance of more

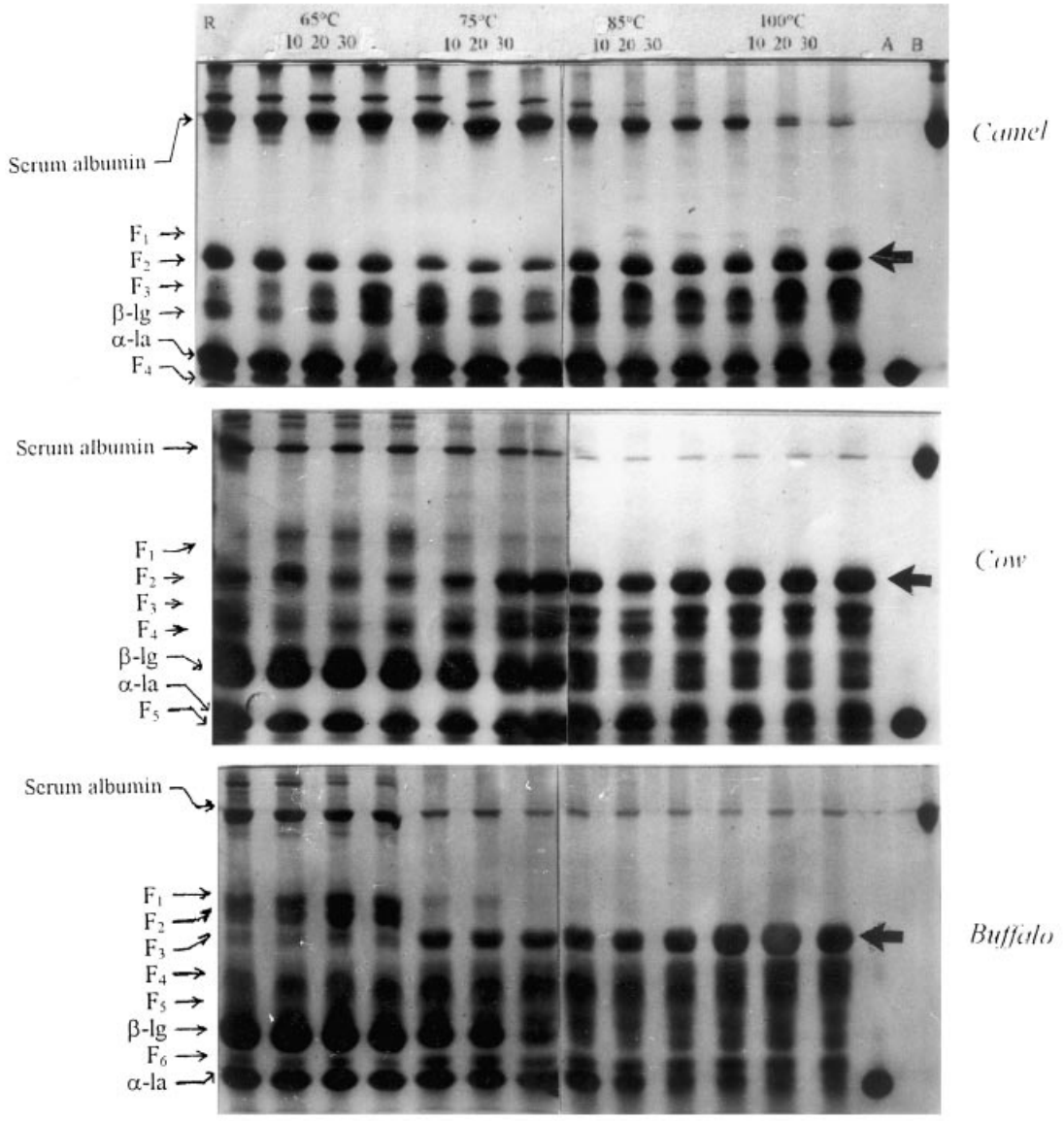

Fig. 1. SDS-PAGE (12.5% T) of whey proteins prepared from camel, cow and buffalo milk heated at 65, 75, 85 and 100°C for 10, 20 and 30 min. R: raw; A & B: standard bovine α-lactalbumin and serum albumin, respectively.

dense bands, F_2 (33.9 kDa) in cow and F_3 (32.7 kDa) in buffalo milk (indicated by big arrows, Fig. 1). Each band of those had a molecular weight higher than that of β-lg (18.5 kDa), therefore ,it is suggested that each band is may be (1) an aggregation of β-lg. Hill (1988) and Lametti, Cairoli and Bonomi (1997) found that the aggregates of β-lg with itself during heating of cow milk are formed (2) a complex resulted from the interaction of α-la and β-lg or casein and β-lg. The interaction of α-la and β-lg (Elfagm & Wheelock, 1977, 1978; Gezimati, Creamer & Singh, 1997) or β-lg and K-casein (Parnell-Clunies, Kakuda, Irvine & Mullen, 1988) in cow milk during heating has been established and confirmed.

Regarding camel milk, neither α-la nor β-lg was affected; however, the band intensity of the unknown fraction, F_2 (34.6 kDa) became lighter and further increase of the intensity of this band by the increase of heating up to 85°C then to 100°C was noticed.

Moreover, at this level of heating, buffalo WPs pattern showed the disappearance of two distinguishing bands (F_1 & F_2, Fig. 1) from the gel after 30 min. By contrast, in cow WPs pattern, two distinguishing bands, F_3 (30.4 kDa) and F_4 (28.6 kDa) appeared. It is obvious that their intensities were increased with the decline of α-la and disappearance of β-lg from the gel.

At 85°C, there was a pronounced decrease of SA band intensity in cow and buffalo WPs; however, a smaller decrease in camel milk SA was noticed. It reached the same rate of disappearance as cow and buffalo SA after heating up to 100°C for 20 min. At this level of heating,

β-lg fraction had disappeared from the pattern of cow and buffalo WPs; however, camel β-lg was not affected. Buffalo α-la was highly affected and its disappearance increased with time compared to cow and camel milk α-la.

After heating up to 100°C the effects were less on α-la and β-lg of camel milk compared to those of cow and buffalo. Both cow and buffalo β-lg were extremely affected but cow α-la was little affected. High disappearance of cow and buffalo β-1g resulted in: (1) increase of the bands' intensities of the unknown fraction (F_2 in cow and F_3 in buffalo WPs). (2) increase of the bands, intensities of F_3 (30.4 kDa) and F_4 (28.6 kDa) in cow and F_4 (29.4 kDa) and F_5 (27.5 kDa) in buffalo WPs. All of these fractions are lower in molecular weights than F_2 (33.9 kDa) in cow and F_3 (32.7 kDa) in buffalo WPs. In camel WPs, a similar behavior was noticed.

3.2. Effect of heat treatment on immunity factors

The concentrations of lysozyme (LZ) in raw milk significantly ($P \leqslant 0.01$) differed among the three kinds of milk. Camel milk contained 4.9 and 11 times as much LZ as cow and buffalo milk, respectively (Table 1).

Fig. 2A shows the effect of various heat treatment on LZ activity in camel, cow and buffalo milk. Heating milk at 65 or 75°C for 30 min had no significant effect on LZ activity in the three kinds of milk (Table 1). However, highly significant differences between the effect of 75 and 85°C were observed especially in camel and cow milk but not in buffalo milk. Increasing the temperature to 85°C for 30 min resulted in a significantly greater loss of activity for all LZs.Buffalo milk LZ was more heat labile than camel and cow milk LZ. Although at 100°C/30 min, the entire activity of buffalo and cow milk LZ was lost versus 94% of activity loss of camel milk LZ, there was no significant differences among them. Results revealed also that there was no significant difference between the effect of 85 and 100°C on both cow and buffalo LZ but it was highly significant on camel LZ.

The concentration of IgG in raw camel milk was significantly ($P \leqslant 0.01$) higher than the corresponding values in cow and buffalo milk (Table 2).

Fig. 2B shows the effect of various heat treatments on camel, cow and buffalo milk IgG. At 65°C for 30 min significant differences in the extents of loss of activity for IgG were found among the three kinds of milk.

Early study on total cow milk immunoglobulins and their heat treatments was done by Larson and Rolleri (1955) who found that heating skim milk at 70°C for 30 min resulted in 89% loss in immunoglobulins activity. Li-Chan, Kummer, Losso, Kitts and Nakai (1995) found that no change in bovine IgG after heating of cow milk at 62.7°C for 30 min but Dhar, Fichtali, Skura, Nakai and Davidson (1996) reported that pasteurization of cow milk at 71°C for 9 s resulted in retention of 75% of IgG. In this study, increasing the temperature to 75°C for 30 min had a significant effect on IgG activity in the three kinds of milk. At 85°C for 30 min, camel-IgG

Table 1
Effect of heat treatment on camel, cow and buffalo milk Iysozyme[a]

Temperature (°C)	Milk (concentration[b], μg/ml)		
	Camel	Cow	Buffalo
0	1.32 ± 0.088a	0.273 ± 0.061c	0.120 ± 0.005cdef
65	1.32 ± 0.089a	0.264 ± 0.06cd	0.115 ± 0.005cdef
75	1.14 ± 0.059a	0.213 ± 0.049cde	0.094 ± 0.005cdef
85	0.582 ± 0.085b	0.071 ± 0.015ef	0.022 ± 0.002ef
100	0.0767 ± 0.012def	0.00 ± 0.00f	0.00 ± 0.00f

[a] $LSD_{0.01}$, for milk type at the same level of temperature = 0.0858; $LSD_{0.01}$, for termperature at the same level of milk = 0.1108; $LSD_{0.01}$, for milk × temperature level interaction = 0.1918.

[b] Means of duplicate analyses on each of three bulk samples. Means having different letters are significantly different ($P \leqslant 0.01$).

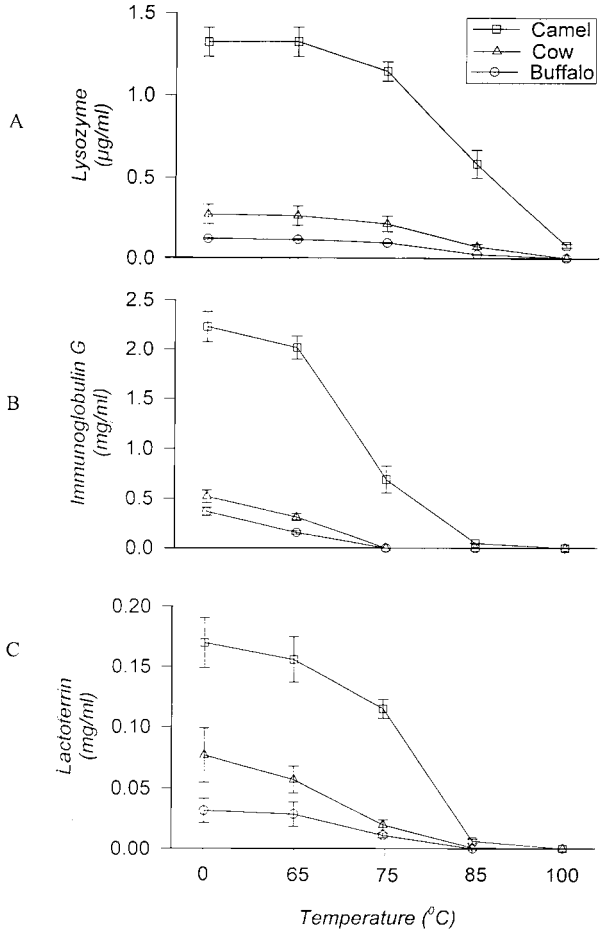

Fig. 2. Effect of heat treatment on camel, cow and buffalo milk lysozyme, immunoglobulin G and lactoferrin.

Table 2
Effect of heat treatment on camel, cow and buffalo milk immunoglobulin G (IgG)[a]

Temperature (°C)	Milk (concentration[b], mg/ml)		
	Camel	Cow	Buffalo
0	2.227 ± 0.153a	0.520 ± 0.064bc	0.367 ± 0.041cd
65	2.017 ± 0.119a	0.312 ± 0.036cd	0.155 ± 0.019de
75	0.696 ± 0.134b	0.00 ± 0.00e	0.00 ± 0.00e
85	0.0479 ± 0.003e	0.00 ± 0.00e	0.00 ± 0.00e
100	0.00 ± 0.00e	0.00 ± 0.00e	0.00 ± 0.00e

[a] $LSD_{0.01}$, for milk type at the same level of temperature = 0.1127; $LSD_{0.01}$, for termperature at the same level of milk = 0.1455; $LSD_{0.01}$, for milk × temperature level interaction = 0.2520.
[b] Means of duplicate analyses on each of three bulk samples. Means having different letters are significantly different ($P \leqslant 0.01$).

activity loss was significantly increased. No significant difference between the effect of 85 and 100°C on camel-IgG activity loss. This result reveals that the heat stability of camel milk IgG is significantly greater than that of either cow or buffalo milk IgG.

Raw camel milk contained a significantly ($P \leqslant 0.01$) higher level of LF compared to cow and buffalo milk. Camel milk contained 2 and 6 times more LF than that of cow and buffalo milk, respectively (Table 3). The effect of heat treatments on LF of camel, cow and buffalo milk is shown (Fig. 2C). Heating milk at 65°C for 30 min had no significant effect on LF activity in the milk of all three species. However, increasing the temperature to 75°C for 30 min resulted in significantly greater loss of activity. Paulsson, Svensson, Kishore and Naidu (1994) found that cow milk LF was unaffected by pasteurization but completely denatured by UHT treatment. In the same respect, Luf and Rosner (1997) found that HTST treatment of cow milk has no significant effect on LF denaturation, whereas, heat treatment at 63°C for 30 min reduced the native LF content by 40%.

Table 3
Effect of heat treatment on camel, cow and buffalo milk lactoferrin[a]

Temperature (°C)	Milk (concentration[b], mg/ml)		
	Camel	Cow	Buffalo
0	0.170 ± 0.021a	0.0767 ± 0.022cd	0.0317 ± 0.01ef
65	0.156 ± 0.019ab	0.057 ± 0.011de	0.0287 ± 0.01ef
75	0.115 ± 0.008bc	0.020 ± 0.004ef	0.0113 ± 0.002f
85	0.063 ± 0.002f	0.0009 ± 0.00f	0.00 ± 0.00f
100	0.00 ± 0.00f	0.00 ± 0.00f	0.00 ± 0.00f

[a] $LSD_{0.01}$, for milk type at the same level of temperature = 0.01832; $LSD_{0.01}$, for termperature at the same level of milk = 0.02366; $LSD_{0.01}$, for milk × temperature level interaction = 0.04097.
[b] Means of duplicate analyses on each of three bulk samples. Means having different letters are significantly different ($P \leqslant 0.01$).

In this study although, heating of milk at 85°C for 30 min resulted in complete loss of LFs activity in cow and buffalo milk versus 96.5% of denaturation of camel LF, which became complete at 100°C for 30 min, no significant differences among the three kinds of milk were found. Generally, on the basis of these findings, it can be concluded that camel milk LF is more resistant than that of cow and buffalo milk.

4. Conclusions

From the results obtained it can be concluded that (a) Camel milk WPs are more heat stable than cow and buffalo milk ones. (b) Antimicrobial factors are significantly present in higher concentration in camel milk than in cow or buffalo and they are more heat resistant than their counterparts in cow and buffalo milk. This means that the biological activity of protective proteins in heat-treated camel milk at 75°C/30 min is higher than that of cow and buffalo milk proteins.

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