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ELECTROPHORETIC PATTERNS AND FREE AMINO ACIDS OF CAMEL'S MILK CASEINS AND WHEY AS AFFECTED BY DIFFERENT HEAT TREATMENTS

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ABSTRACT

Camel (*Camelus dromedarius*) milk caseins and whey proteins compared to bovine milk proteins and its relation to different heat treatments were detected using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Camel milk whey proteins such as serum albumin (SA) and alpha-lactalbumin (α-La) appear to possess molecular weights similar to the respective bovine whey proteins. In camel milk, no protein bands homologous to bovine neither beta-lactoglobulin (β-Lg) nor κ-casein could clearly be detected in the electrophoretic pattern and consists of large amount of serum albumin, compared to bovine whey.

Camel milk whey protein showed higher heat stability than those of cow's milk. After an initial higher denaturation of whey proteins in camel milk at 65°C/30 min, which represents the conditions of conventional pasteurization caused no visible change (denaturation) in whey proteins, whilst higher heat treatment at 80 and 100°C for 30 min which is more excessive than pasteurization, resulted visible changes of the whey proteins.

The present study demonstrates the most predominated values of free amino acids (FAA) glutamic, aspartic, isoleucine and alanine respectively in both of heated caseins and whey separated from camel's milk. The FAA concentrations of camel's milk caseins appear to be more heat stable than those of camel's milk whey, which has high loss percentages in FAA than of caseins

Keywords: Camel milk, casein, whey proteins, heat treatments, electrophoresis, free amino acids.

INTRODUCTION

There is no doubt that milk proteins provide excellent nutrition for the suckling. However, apart from that, milk proteins can also exert numerous physiological activities benefiting the suckling in a variety of ways. These activities include enhancement of immune function, defense against pathogenic bacteria, viruses, and yeasts, and development in the gut and its functions. Besides the naturally occurring, biologically active proteins present in milk, a variety of bioactive peptides are encrypted within the sequence of milk proteins that are released upon suitable hydrolysis of the precursor (Exposito and Recio 2008).

The camel (*Camelus dromedarius*) is certainly one of the most neglected species of the domestic animals. Information about camels as milk animals is very limited. Camel milk is an important component of the human diet in many parts of the world, most of it is consumed in the fresh or sour state. It contains essential nutrients as cows' milk (Knoess, 1979; Elagamy *et al.,* 1998).

Milk is the main source of nutrition for the neonate calves, and provides all the essential nutrients for growth and development, e.g. proteins, minerals, carbohydrates, fatty acids, growth factors, immune modulators, etc.

Compared to the bovine species, camel whey contains a higher content of antimicrobial factors such as lysozyme, lactoferrin and immunoglobulins (Elagamy *et al.,* 1992), whey acidic protein (Beg *et al.,* 1986) and whey basic protein (Ochirkhuyag *et al.,* 1998). To the contrary, camel whey lacks beta lactoglobulin (β-Lg), a major serum protein found in other livestock ruminant milk. Other whey proteins which have been identified in camel milk include serum albumin, lactalbumin, immunoglobulins, lactophorin and peptidoglycan recognition protein (Kappeler *et al.,* 2004).

The protein composition of camel's and cow's milks differs in some fundamental aspects. For example, β-lactoglobulin and lysozyme, which are important proteins of bovine milk, are not found in camel's milk (Beg *et al.,* 1986).

Most of camel milk is consumed in the fresh or sour state. However, the preservation of raw milk can be achieved by heat treatments such as pasteurization, boiling, ultra high temperature (UHT) or sterilization processes. Knowledge of the effect of heat treatments on individual milk proteins is of importance in understanding the changes in the biological and functional properties of milk which occur during treatment. These changes have been extensively studied for cow milk due to its wide spread industrial processing and commercialization (Jenness and Patton 1959; Lyster, 1970; Fox, 1982; deWit and Klarenbeek, 1984; Mulvihill and Donovan 1987; Pearce, 1989). In contrast, only limited studies have been carried out on camel milk casein (Farah, 1985; Mohammed and Larsson-Raznikiewicz 1991; Elagamy, 2000) or camel milk whey proteins (Farah, 1986; Elagamy, 2000; Merin *et al.,* 2001).

The goal of this work was to characterize caseins and whey protein fractions from dromedary's milk using electrophoresis when camel milk is subjected to various heat treatments. Also, free amino acids pattern will be quantified using HPLC.

MATERIALS AND METHODS

Five individual dromedary milk samples from a herd of lactating dairy camels (*Camelus dromedarius*) at random in Berkash Village Market, Giza governorate and cow's milk was obtained from private farm in West Omrania, Giza, Cairo, Egypt, during the period January and March 2008. Milk samples were collected in cleaned sample bottles and brought to the laboratory of Dairy Department, National research Centre for analysis and stored at -18°C.

For the preparation of caseins and whey proteins , the milk samples were defatted by centrifugation at 4000 rpm for 30 min. and casein was precipitated at pH 4.6 by adding 1 ml of 10% (v/v) acetic acid and 1 ml of 1M sodium acetate to 10 ml of milk. After centrifugation at 10000 rpm for 20 min., the precipitation product was washed three times with distilled water, while the supernatant was filtered through membrane filter.

Bulk normal milk samples were collected from 5 individual camels (*Camelus dromedarius*) and defatted by centrifugation. Aliquots (5 ml) of camel milk in stoppered (10 ml) glass tubes were heated at 65, 80 and 100°C for 30 min in a thermostatically controlled water bath, the tubes were cooled

immediately by immersion in ice water. An unheated aliquot was used as a control. All samples were centrifuged at 10000 rpm for 10 min and the gel electrophoresis analysis was performed on the supernatants. For comparison, bulk cow milk was used.

Both casein and whey proteins profiles were carried out according to Laemmli, (1970), using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Five gram of sample completed to 25 ml in volumetric flask with sulphosalicicilic acid 5 %. About 1 ml of the solution was filtered through 0.45 μm Millipore sample filter. The analysis of free amino acids was performed by analytical High Performance Liquid Chromatography (HPLC) method according to Millipore co-operative (1987). The apparatus used is Waters 600E Multi solvent Delivery System, Pico Tag analysis column, Waters 484 Detector and Workstation with Millennium Chromatography Manager Software. The analysis was carried out using a gradient of Pico-Tag solvent A and B at 40 °C and flow rate 1 ml/min. The separated PTC-amino acids were detected at 254 nm wavelength. Before injecting the sample, the instrument was calibrated by two injections of the standards.

RESULTS AND DISCUSSION

Proteins represent one of the greatest contributions of milk to human nutrition. They perform a variety of functions in living organisms ranging from providing structure to reproduction. The main components of milk proteins are casein and whey.

Milk proteins were separated from camel milk using SDS-PAGE electrophoresis technique. Samples of milk casein and whey proteins from five individual dromedary milk as well as their pooled milk were examined to determine whether they have similar composition in comparison to cow milk.

From Fig. (1), no difference could be observed and the electrophoretic patterns show the same main bands of equal intensity and mobility for both pooled and individual milk caseins. Compared with cow milk, casein of camel milk has considerably lower electrophoretic mobility.

Fraction of β-casein is clearly separated and consists of a single strong band, while fraction of α-casein shows one strong band and some diffuse slow moving bands, it occurs as a mixture of many sub fractions such as α_{s1} and α_{s2}-casein. The strong band (α_{s2}) to be homologous to bovine α-casein and the diffuse band moving behind to be α_{s1}-casein. This result agreed with those mentioned by Mohamed *et al.,* (1989); Abd El-Salam *et al.,* (1992), who observed that β- and α_{s2}-casein present as a major component and its mobility was much less than that of cow milk. No protein bands homologous to bovine κ-casein could clearly be detected in the electrophoretic pattern. These data are typically corresponding to that those of (Pant and Chandra 1980; Hassan *et al.,* 1987; Restani *et al.,* 1999). Also, Pant and Chandra 1980 found that camel milk caseins and its fractions were minor when compared with cow milk.

Dromedary's caseins showed electrophoretic characteristics and protein amounts different from other ruminants (Kappeler *et al.,* 1998), featuring high amounts of β-casein and low amounts of κ-casein in contrast to the milk from ruminants traditionally used in technological processing. The low content of κ-casein and the relatively large size of casein micelles may act as an obstacle during the coagulation process (Farah, 1993; Attia *et al.,* 2000).

Our results are in close agreement with those of Larsson-Raznikiewicz and Mohammed, (1986); Ochirkhuyag *et al.,* (1997), who obtained three casein fractions with molecular weights of (25, 27 and 31); (26.3, 27.5 and 35.3) respectively, identified as being homologous with the α_{s1}-, α_{s2}- and β-casein of bovine milk, and partial with those of Farah and Farah-Riesen (1985) isolated only two bands 32 and 35 kDa, as well as with those of Kherouatou *et al.,* (2003) who separated four casein components.

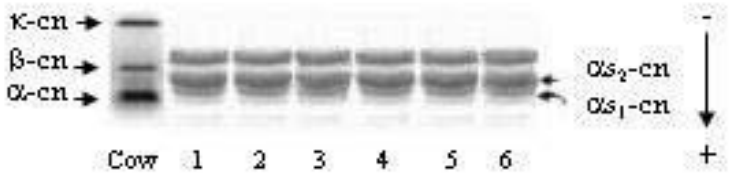

Fig. (1): Sodium dodecyl sulfate polyacrylamide gel electrophoresis of camel casein compared with cow; 1, 2, 3, 4, 5: casein from five individual camels; 6: pooled camel milk.

The same bands of equal intensity and mobility for both pooled and individual camel whey were clearly detected in the electrophoretic patterns (Fig. 2). Most of the whey proteins in camel milk resemble bovine whey proteins, except the lack of β-Lg, which was in agreement with data previously reported (Ochirkhuyag *et al.,* 1998; Merin *et al.,* 2001). Also, the lack of β-Lg has been reported for human colostrum and milk (Jenness, 1985). Thus, the major whey proteins to be considered were alpha-lactalbumin (α-La), serum albumin (SA) and another unknown fractions. High SA concentration has been observed in camel colostrum (Merin *et al.,* 2001). α-La was separated from camel milk whey, which was only observed in one band in all whey samples, but identified of two variants in sample 4. This observation is corresponding with those of (Beg *et al.,* 1985; Conti *et al.,* 1985; Farah, 1986; Ochirkhuyag *et al.,* 1998).

Moreover, β-Lg, the predominant bovine whey protein has not been detected in camel milk. Conversely, β-Lg was not detected neither in the colostrum nor in the camel milk. This is in agreement with the previously reported findings (Ochirkhuyag *et al.,* 1998; Restani *et al.,* 1999). The lack of β-Lg in colostrum and milk has also been reported in other species, including rodent and human (Hambling *et al.,* 1992). In this regard, camel milk could

be more suitable for the production of "humanised" milk, to be used as a substitute of human milk in infant diet and for alleviating some allergic reactions, especially in children.

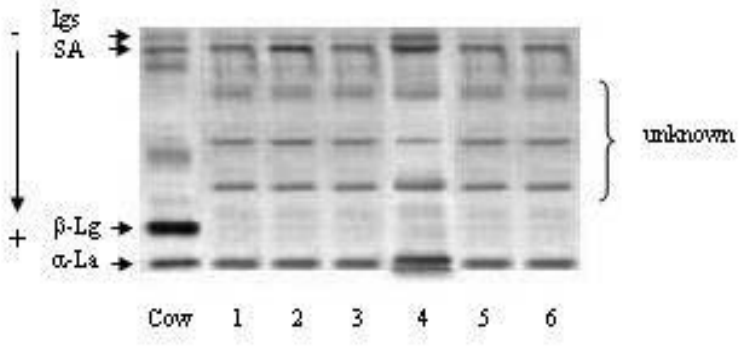

Fig. (2): Sodium dodecyl sulfate polyacrylamide gel electrophoresis of camel whey proteins compared with cow; 1, 2, 3, 4, 5: whey from five individual camels; 6: pooled camel milk.

Fig. (3) shows the whey protein gel patterns of the raw and heated cow and camel milk. Little changes in gel electrophoretic patterns of whey proteins in raw and heated camel milk were observed when compared with cow one. Since heating proteins causes conformational changes in their molecular structure with concomitant loss of native epitopes, specific applications have been published on effects of heating milk (Lyster, 1970; Levieux, 1980; Farah, 1986; Elagamy, 2000).

Pasteurization temperature, 65°C/30 min (lane 2) caused no visible change in the whey proteins gel pattern. This result is in agreement with the results of Farah, (1986); Elagamy, (2000), who mentioned that no effect was noticed in whey proteins of both cow and camel milk heated at 63 °C/30 min. At 80°C/30 min (lane 3), Igs and SA disappeared from the electrophoresis pattern, while portions of α-La, and β-Lg not completly denaturated, but disappeared after heat treatment of 100°C/30 min (lane 4). Furthermore, these results are in accordance with those of Wangoh, *et al.,* (1998), who heated skimmed camel milk at 90°C for 20 min and found 46% undenatured soluble proteins.

Gel electrophoretic pattern of heated camel milk whey protein shows several components in high intensities, two sharp (α-La, SA) and one faint (Igs) as well as another unknown fractions, but β-Lg component was not recorded. The electrophoretic patterns in lanes 5, 6, 7 and 8 show two components in the upper part of the gel (Igs and SA) followed by three bands unknown in the middle of the gel, followed by one sharp band in the lower part of the gel (α-La). The gel patterns indicate that α-La was of no visible affects, SA became very lighter intensity at 100°C/30 min (lane 8), where a visible decrease in intensity of Igs fraction was noticed by heat temperature increasing (Farah, 1986; Elagamy, 2000).

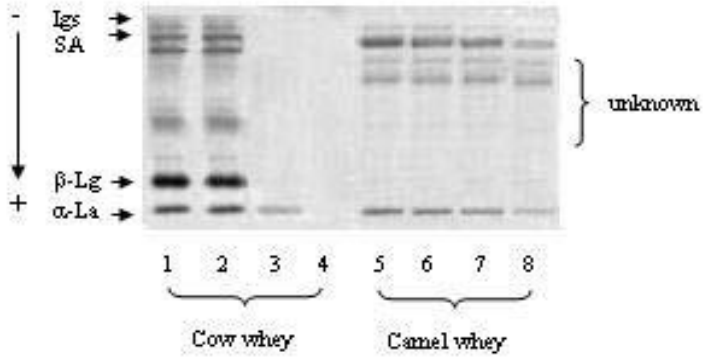

Fig. (3): Sodium dodecyl sulfate polyacrylamide gel electrophoresis of whey proteins filtrates prepared from camel and cow milks heated to 30 min at various temperatures. Cow milk: 1, raw; 2, 65°C; 3, 80°C; 4, 100°C and camel milk: 5, raw; 6, 65°C; 7, 80°C; 8, 100°C.

From these results, it could be concluded that there are high heating sensitivity of whey proteins in camel milk. This finding is well in conformity with recently demonstrated by Levieux *et al.,* (2006). Generally, the camel milk whey proteins were considered of higher heat stability than cow milk. After an initial higher denaturation of whey proteins in camel milk at 65°C, the heat stability of the camel milk whey proteins markedly increased in comparison to cow whey proteins with the increase of temperature during heat treatment. These results are in accordance with the heat sensitivity of α-La the predominant whey protein.

Restani *et al.,* (1999) mentioned that the camel milk is considered a useful component of the diet for individuals that show allergic reactions to the protein fraction of cow, ewe or goat milk, as camel milk does not contain β-Lg and the content of α-casein is much lower than in milk of the other herbivores.

The effect of heat treatments on the free amino acids concentration of camel's milk caseins and whey are seldom discussed in view of the importance of these nutrients for dairy foods. In this study we investigated the free amino acids concentration of camel's milk caseins and whey at different heat treatments (65, 80 and 100 °C) for 30 min.

Camel's milk was rich in free glutamic, aspartic, isoleucine and alanine relatively to other free amino acids. Sulaimanova *et al.,* (1998) reported that the camel milk is rich in free amino acids.

From Table (1), it could be seen that the concentrations of total free amino acids (FAA) of camel's milk whey decreased after heat treatments (65, 80 and 100 °C) from 11.1 % for control unheated to 9.51, 6.42 and 5.71 % respectively.

The mean loss concentrations (%) of individual amino acids were increased after heating from 14.36 % at 65 °C to 39.96 and 48.28 % at 80 and 100°C respectively. Also, the amino acid cystine is more heat sensitive than other amino acids, as its concentration decreased from 0.18 for control to 0.15, 0.10 and 0.06 % after heated to 65, 80 and 100 °C in the same order. High heat (like the sustained high temperatures above 72 °C associated with the pasteurization process) denatures whey proteins, destroying some bioactive compounds, such as the amino acid cystine.

Both essential and non essential free amino acids were similarly affected by different heat treatments (2.76 and 6.75 %) at 65 °C; (1.90 and 4.52 %) at 80°C and decreased to (1.16 and 4.55 %) at 100 °C respectively. This data is comparable with published by Yeung *et al.,* (2006).

Table (2) shows the FAA concentrations of heat treated caseins separated from camel's milk. It could be revealed that the FAA concentrations of camel's milk caseins are more heat stable than the FAA of camel's milk whey. However, there is little decrease in the pattern of FAA between control and treated samples. The heat treatments caused more loss percentages in FAA in camel milk whey than in the caseins (at 65, 80 and 100 °C) in comparing to control samples, being, (14.36 and 1.34 %), (39.96 and 3.30 %) as well as (48.28 and 5.03 %) in order. It is due to action of denaturation on whey proteins.

Table (1): Free amino acids composition (%) of camel's milk whey as affected by different heat treatments.

Amino acids	Control	65 °C/ 30 min.	Loss (%)	80 °C/ 30 min.	Loss (%)	100 °C/ 30 min.	Loss (%)
Essential							
Arginine	0.42	0.36	14.29	0.24	42.86	0.22	47.62
Histidine	0.07	0.06	14.29	0.05	28.57	0.04	42.86
Iso Leucine	0.77	0.66	14.29	0.44	42.86	0.42	45.46
Leucine	0.28	0.25	10.71	0.18	35.71	0.15	46.43
Lysine	0.40	0.35	12.50	0.24	40.00	0.20	50.00
Methionine	0.14	0.12	14.29	0.11	21.43	0.08	42.86
Phenylalanine	0.42	0.36	14.29	0.24	42.86	0.22	47.62
Theronine	0.39	0.33	15.38	0.22	43.59	0.19	51.28
Valine	0.32	0.27	15.63	0.18	43.75	0.16	50.00
Non-essential							
Alanine	0.67	0.57	14.93	0.38	43.28	0.36	46.27
Aspartic	2.52	2.16	14.29	1.44	42.86	1.34	46.83
Cystine	0.18	0.15	16.67	0.10	44.44	0.06	66.67
Glycine	0.39	0.33	15.39	0.24	38.46	0.22	43.59
Glutamic	3.36	2.88	14.29	1.92	42.86	1.64	51.19
Proline	0.42	0.36	14.29	0.24	42.86	0.23	45.24
Serine	0.35	0.30	14.29	0.20	42.86	0.18	48.57
Tyrosine	-	-	-	-	-	-	-
TFAA [1]	11.10	9.51	-	6.42	-	5.71	-
EFAA [2]	2.23	2.76	-	1.9	-	1.16	-
Non-EFAA [3]	8.87	2.76	-	4.52	-	4.55	-
Loss mean (%)	-	-	14.36	-	39.96	-	48.28

***: The average of duplicate individual samples.**

1: Total free amino acids, 2: Essential free amino acids, 3: Non-essential free amino acids.

From these data, it could be concluded that the heat treatments clearly resultede in decrease in the concentrations of FAA in camel's milk caseins and whey. Furthermore, glutamic, aspartic, isoleucine and alanine values were the most abundant free amino acids in camel's milk proteins relatively to other free amino acids.

These values were 8.64, 6.48, 1.96 and 1.71 % for caseins, while values for whey were 3.36, 2.52, 0.77 and 0.67 % in the same order, these amino acids decreased as temperature increased.

Table (2): Free amino acids composition (%) of camel's milk caseins as affected by different heat treatments.

Amino acids	Control	65 °C/ 30 min.	Loss (%)	80 °C/ 30 min.	*Loss (%)*	100 °C/ 30 min.	*Loss (%)*
Essential							
Arginine	1.06	1.05	0.94	1.03	*2.83*	1.01	*4.72*
Histidine	0.18	0.18	-	0.17	*5.56*	0.17	*5.56*
Iso Leucine	1.96	1.94	1.02	1.91	*2.55*	1.90	*3.06*
Leucine	0.72	0.71	1.39	0.69	*4.16*	0.68	*5.56*
Lysine	1.07	1.05	1.87	1.03	*3.74*	1.01	*5.61*
Methionine	0.36	0.36	-	0.35	*2.78*	0.34	*5.56*
Phenylalanine	1.08	1.06	1.85	1.04	*3.70*	1.02	*5.56*
Theronine	0.99	0.98	1.01	0.96	*3.03*	0.93	*6.06*
Valine	0.81	0.80	1.24	0.79	*2.47*	0.77	*4.94*
Non-essential							
Alanine	1.71	1.69	1.17	1.67	*2.34*	1.64	*4.09*
Aspartic	6.48	6.38	1.54	6.35	*2.00*	6.30	*2.78*
Cystine	0.64	0.61	4.69	.060	*6.25*	0.59	*7.81*
Glycine	0.99	0.98	1.01	0.95	*4.04*	0.93	*6.06*
Glutamic	8.64	8.50	1.62	8.44	*2.31*	8.40	*2.78*
Proline	1.06	1.05	0.94	1.03	*2.83*	1.01	*4.72*
Serine	0.90	0.89	1.11	0.88	*2.22*	0.85	*5.56*
Tyrosine	-	-	-	-	-	-	-
TFAA [1]	*28.65*	*28.23*	-	*27.89*	-	*27.53*	-
EFAA [2]	*8.23*	*8.13*	-	*6.97*	-	*7.83*	-
Non-EFAA [3]	*20.42*	*20.10*	-	*19.92*	-	*19.72*	-
Loss mean (%)	-	-	*1.34*	-	*3.30*	-	*5.03*

***: The average of duplicate individual samples.**
1: Total free amino acids, 2: Essential free amino acids, 3: Non-essential free amino acids.

Conclusions:

It was shown in camel milk that most whey proteins are similar in molecular weights to bovine whey proteins. The main differences are the lack of β-Lg and the high amount of camel serum albumin and the different intensity of the various proteins.

The analytical results suggested the absence of κ-casein in samples, the compositional characteristics of its casein make this milk close to the human one and could therefore be useful to promote this product as a substitute of bovine milk for allergic people.

Camel milk is considered a useful component of the diet for individuals that show allergic reactions to the protein fraction of cow, ewe or goat milk, as camel milk does not contain β-Lg and the content of α-casein is much lower than in milk of the other herbivores.

The camel milk whey protein showed generally higher heat stability and it had ability to withstand higher processing temperatures than that from cows' milk.

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فصل بروتينات لبن الجمال وتقدير محتواها من الأحماض الأمينية الحرة وعلاقتها بالمعاملات الحرارية المختلفة

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تم تجميع عينات ألبان النوق (الإبل) والأبقار من المزارع الخاصة الموجودة فى سوق برقاش ، العمرانية الغربية على الترتيب - الجيزة ، حيث تم دراسة خصائص مكونات كلاً من الكيزين وبروتينات الشرش فى لبن الجمال ، وكذلك تأثير بعض المعاملات الحرارية على ما يحتويه هذا اللبن من بروتينات الشرش ومقارنتها مع لبن الأبقار وذلك عن طريق الهجرة الكهربية Electrophoresis.

أوضحت النتائج أن درجة السريان الكهربى لكيزين لبن الإبل أقل عنه فى لبن الأبقار ، ويتميز لبن الإبل بإرتفاع محتـواه مـن مشـتق السـيرم ألبيـومين (SA) ، وعـدم إحتـواءه علـى كـلٍ مـن مشـتقى الكابـا كيـزين (κ-cn) و البيتـا لاكتوجلوبيولين (β-Lg) عند مقارنته بلبن الأبقار مما يجعله أكثر إستخداماً كبديل للبن الأمهات فى غذاء الأطفال نظراً لعدم ظهور الحساسية أو تقليلها.

أما بالنسبة لدراسة تأثير المعاملات الحرارية على ما يحتويه لبن الجمال من الأحماض الأمينية الحرة ، أظهرت النتائج إنخفاض محتواها تدريجياً بزيادة درجة الحرارة فى كلٍ من الكيزين والشرش ، وعلى العكس من ذلك حيث كانت هناك علاقة طردية فى معدل الإنخفاض (الدنترة) بالنسبة لزيادة درجات الحرارة حيث كان أقل فى الكيزين عنه فى الشرش مما يدل على أن مكونات الكيزين أكثر ثباتاً للحرارة. بينما كان حامض السيستين أكثر حساسيةً للحرارة.