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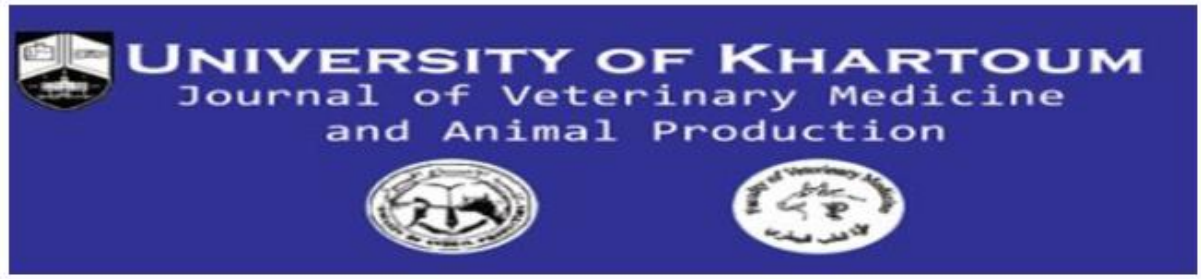

Isolation and Characterization of Three Camel (*Camelus dromedarius*) Milk Casein fractions

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Abstract

This study was designed to isolate and characterize camel (*C. dromedarius*) milk caseins (CN) using Ion Exchange Chromatography (IEC), Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Isoelectric Focusing (IEF) techniques. Whole camel casein was precipitated by means of acidification (pH 4.3), freeze-dried and then fractionated using IEC. Dissolved casein revealed three (I, II and III) well resolved peaks. Elutes representing different peaks were loaded together with whole camel milk on the same IEF gel to identify bands corresponding to each fraction based on their isoelectric points (p*I*). Elutes under peak I and II revealed a single band each corresponding to κ-CN and β-CN, respectively, while elute under peak III showed two bands corresponding to α_{s1}-CN and β-CN. Band corresponding to α_{s1}-CN fraction was focused on the most acidic side of the IEF gel followed by β-CN on the middle, while κ-CN was the less acidic fraction. Furthermore, casein fractions were subjected to SDS-PAGE, and their molecular masses were estimated at 36.325, 31.732 and 25.044 kDa, respectively. However α_{s2}-CN was not detected and κ-CN was only observed in IEC product (due to low concentration in milk), β-CN fraction revealed the most intensive band on SDS-PAGE and IEF gel indicating its relatively higher content in camel milk.

Key words: Camel milk, casein fractions, IEC, IEF, SDS-PAGE

المستخلص

صُممتْ هذه الدراسة لعزل وتوصيف كازينات لبن الإبل وحيدة السنام باستخدام التبادل الأيونى الطيفى (IEC) و هلام كبريتات دوديكل الصوديوم متعدد الأكريلامايد (SDS-PAGE) وهلام التركيز عند التعادل الكهربى (IEF). رُسب الكازين الكلى للبن الإبل بواسطة الحموضة (أُس هيدروجينى 4.3) وجُففَ بالتبريد ومن ثم تمت تجزئته بواسطة التبادل الأيونى الطيفى. أظهر الكازين المذاب ثلاثة قممٍ (I و II و III) جليةٍ. حُملت نواتج التبادل الأيونى الطيفى المقابلة للقمم المختلفة مع لبن الإبل الكامل على ذات هلام التعادل الكهربى بغرض تحديد الحزم الممثلةِ لكل شقٍ اعتماداً على تقنية نقطة التعادل الكهربى (*p*I) لها. أظهر ناتجا التبادل الأيونى الطيفى تحت القمتين I و II حزمةً وحيدةً لكلٍ، ممثلتين للشقين كابا كازين وبيتا كازين على التوالي ، بينما أظهر الناتج تحت القمة الثالثة (III) حزمتين ممثلتين للشقين الفا س$_1$ كازين وبيتا كازين. رُكزت الحزمة الممثلة للشق الفا س $_1$ كازين على الجانب الحمضى من هلام التعادل الكهربى، تلاها بيتا كازين فى المنتصف، بينما كان الشق كابا كازين هو الأقل حمضيةً. بالإضافةِ إلى ذلك عُرضت شقوق الكازين لهلام كبريتات دوديكل الصوديوم متعدد الأكريلامايد حيث قدرت أحجامها الجزيئية ب 36,325 و 31,732 و 25,044 كيلو دالتون، على التوالي . بينما لم يُتحصل على الشق الفا س $_2$ كازين، وشوهد الشق كابا كازين فقط فى ناتج التبادل الأيونى الطيفى (بسبب انخفاض التركيز فى اللبن) ، أظهر الشق بيتا كازين أشد الحزم كثافةً على هلام كبريتات دوديكل الصوديوم متعدد الأكريلامايد وهلام التركيز عند التعادل الكهربى مما يُشير إلى محتواه العالى نسبياً فى لبن الابل.

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Introduction

Milk is the main source of nutrition for the neonate mammal; as it provides all essential nutrients such as proteins, minerals, carbohydrates, fatty acids, growth factors and immune modulators (El Agamy, 2009). In human nutrition, milk occupies an important position and has a significant role as a source for growth and development elements (Caroli *et al.*, 2009). Among other chemical constituents in milk, protein is a very important one. Due to their importance, milk proteins have earlier been subjected to intensive and deep research work (Aschaffenburg and Drewry, 1957). The outcome of these studies was identification and characterization of different casein fractions in mammals which was reviewed by Rijnkels (2002).

The one humped camel (*Camelus dromedarius*) has a major role among traditional rural communities where it is mainly reared. It has important social and economical roles (Shuiep *et al.*, 2014a). Camel milk, in particular, has special importance as a unique and sometimes a single nutrient source for a wide sector of people (Shuiep *et al.*, 2014b). Due to its importance, the compositional quality of camel milk has been subjected to intensive studies in different regions (Al Haj and Al Kanhal, 2010).

Reviewing the literature of research in milk and milk constituents indicated that many biochemical methods were used to separate and characterize milk protein in bovine milk as well as other mammals. Among these methods, Ion exchange chromatography (IEC) is the most frequently used for purification of milk proteins. The principle of the operation involves the binding of the proteins to the fixed charges, and elution of the proteins (Rossomando, 1990). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is also widely used for proteins isolation with reference to their relative molecular weights (Shapiro *et al.*, 1967 and Laemmli, 1970). In addition to that, separation of milk proteins based on their relevant isoelectric points (p*I*) which is the principle of the Isoelecric Focusing (IEF) technique is also applicable (Shuiep *et al.*, 2013 and Wangoh *et al.*, 1998).

According to their behavior by acidification, camel milk proteins are basically divided into water soluble whey proteins and precipitable caseins (Kappeler *et al.*, 2003). Four casein fractions (α_{s1}-, α_{s2}-, β- and κ-CN) have been recognized and characterized in camel milk (Larsson-Raznikiewicz and Mohamed, 1986 and Alim *et al.*, 2005). Bovine rennet was successfully used for precipitation of camel casein (Wangoh *et al.*, 1993), as well as for camel cheese processing (El Zubeir and Jabreel, 2008). Nevertheless, longer coagulation time was reported (Benkerroum *et al.*, 2011). Moreover, individual camel caseins were characterized by lower electrophoretic mobility, smaller casein micelles and higher molecular masses compared to the respective counter fractions in cows (Farah and Farah-Riesen, 1985; Farah and Rüegg, 1989 and Farah, 1996). Moreover, total camel casein content has been reported to be lower, compared to bovine; consequently, the concentrations of individual camel caseins were also markedly lower (Kappeler *et al.*, 2003).

Compositional quality, technological properties and nutritional value of milk proteins from different farm animals including cattle, sheep and goat have been well studied (Caroli *et al.*, 2009, Giambra *et al.*, 2014 and Salem *et al.*, 2009). However, information about camel milk caseins in particular is relatively fewer and only scattered studies are available (Alim *et al.*, 2005 and El Agamy, 2006). Studying camel milk caseins and characterization of different fractions provide more information about this species. Hence, the aim of the current study was to precipitate, isolate and characterize camel milk casein fractions using different biochemical methods.

Materials and Methods

Collection and preparation of milk samples

Camel milk samples (10 ml ×2) were collected from individual she camels (n=5) in clean bottles. Milk samples were collected from Kamelhof Rotfelden (Rotfelden-Ebhausen, Germany) and transported to the laboratory under cooling (-4º C). Whole milk samples were left standing at 4º C overnight to allow the separation of fat. Whole casein was then harvested from the skimmed milk by precipitation with 50% acetic acid (v/v) at pH 4.3; and centrifuged at 10000 rpm for 10 minutes. The precipitated curd was dissolved in water, and the pH was set to the initial milk pH (6.4- 6.6) by sodium hydroxide (25% w/v). Casein was then precipitated again by acidification. The samples were washed twice, and the whole casein was dissolved at pH 6.5 after which caseins were freeze-dried and stored at -20º C.

Isolation and characterization of casein by IEC, IEF and SDS-PAGE techniques

Total casein was subjected to ion exchange chromatography (IEC) to obtain pure fractions. The middle size column (2.5 cm diameter and 46.5 cm long) was used. Thirty grams of DEAE cellulose were dissolved in 55 mL IEC buffer, and transferred carefully into the column, with restriction to avoid formation of air bubbles. A layer of sea sand (2- 3 mm) was carefully added onto the column. The column was equilibrated in starting buffer containing 8 M urea, 10 mM 2-Mercaptoethanol in 10 mM imidazol. When needed, the pH of the buffer was corrected to 7 by HCl. The lyophilized casein (0.6- 1.0 g) was dissolved in IEC buffer and applied directly to the column. Ismatec ip-4 pump to control the flow rate and UV cord Lampe type 1 to record the absorbance were used.

A step gradient (0.075, 0.130 and 0.170M NaCl) was used to elute casein fractions from the column. Absorbance was recorded at 280 nm and the flow rate was adjusted at 12 drop/min. Elutes were collected on multi rack. The column was regenerated by flushing with buffer containing 1.50M NaCl followed by equilibration with starting buffer before next separation.

Elutes related to different peaks were subjected to IEF gel to determine their purity and at the same time to define different casein fractions based on their isoelectric point according to Seibert et al. (1985). Elutes of different peaks were pooled, dialyzed against distilled water and lyophilized. Afterwards, molecular masses of different fractions were estimated using SDS-PAGE according to the procedure described by Laemmli (1970).

Results and discussion

In this study the most used biochemical methods for fractionation and separation of proteins such as IEC and SDS-PAGE, were used to identify and characterize casein fractions in camel (*C. dromedarius*) milk. Fractionation of camel milk casein by ion exchange chromatography using stepwise elution revealed well resolved three peaks. As the IEF is widely used as a routine screening method for typing most protein variants in bovine as well as in small ruminants, the present study tried to apply the same procedure for camel. Whole camel milk and elutes under the three peaks obtained from IEC were subjected to IEF on the same gel. Elutes related to peak I and II revealed a single band each, corresponding to κ- and β-CN, respectively, while elute III showed two bands corresponding to α_{s1}- and β-CN. Bands corresponding to α_{s1}- and β-CN were clearly recognized. However, bands corresponding to κ-CN were only detected IEC product but not in milk samples (Fig. 1). Missing of bands corresponding to κ-CN could be due to the low concentration of this fraction in camel milk (Kappeler *et al.*, 2003 and El Agamy, 2006) or lack of κ-CN in camel milk (Alim *et al.*, 2005). Moreover, κ-CN might be obscured by other caseins. Another reason for missing κ-CN in IEF gel could the resolution of the gel

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which affected by the concentration of the carrier ampholytes. From a technological view, κ-CN fraction is a limiting factor as it has a special importance regarding milk properties for cheese processing. It has a dominant role as it influences the formation and stabilization of casein micelles (Farah and Rüegg, 1989).

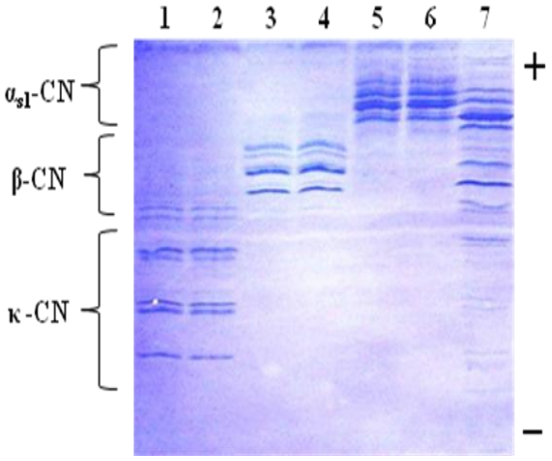

Fig. 1: IEF gel for whole camel milk and pure casein fractions after IEC. IEC products lanes 1 and 2: peak 1 (κ-CN), lane 3 and 4: peak II (β-CN), lane 5 and 6: peak III (αs1-CN) and lane 7: whole camel milk

The three casein fractions defined after IEF were further subjected to SDS-PAGE (Fig. 2). Again elute I and II revealed single band each, while the third one showed two bands. Similar pattern using the same technique was reported by Ochirkhuyag *et al.* (1997). They concluded that camel α_{s1}- and β-CN fractions eluted together in ion exchange chromatography. The molecular masses of the three proteins were estimated as 25.044, 31.732 and 36.325 kDa, corresponding to κ-, β- and α_{s1}-casein, respectively. This result is within the range reported by El Agamy (2006) and Farah and Farah-Riesen (1985). However, the molecular weight of β-CN in the present study is higher than that reported by Ochirkhuyag *et al.* (1997). They reported a molecular mass of 27.500 kDa for the same fraction. This difference might be due to the different genetic pools as the samples in the two studies were from different population. This would further suggest genetic variation in gene responsible for this fraction.

The separation pattern obtained in this study is different compared to that of cattle, as five peaks were reported after fractionation of casein using the same technique (Ng-Kwai-Hang and Pelissier, 1989). They reported two peaks for α_{s1}-CN in addition to the α_{s2}-CN peak, which was not detected in this study. When the gene coding for a certain fraction in milk protein is represented by two different alleles (heterozygous), two peaks for the same fraction will be obtained; each peak being an expression of a single allele, while a single band is obtained when the locus is homozygous or the two proteins expressed by the two alleles were not differently charged.

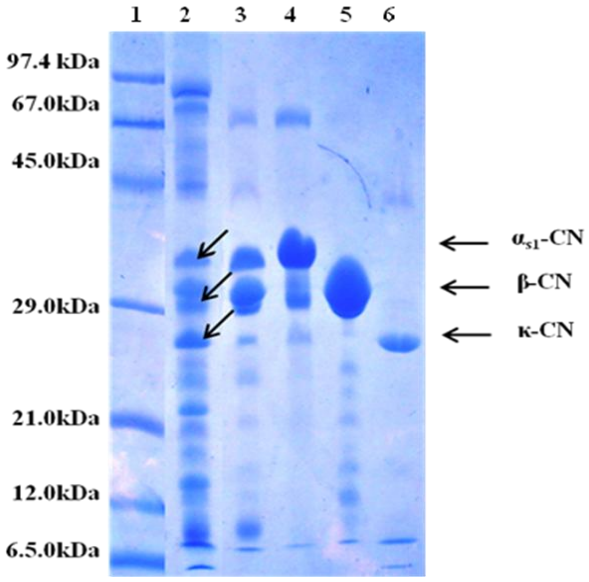

Fig. 2: Separation patterns of camel milk proteins on SDS-PAGE (T=15%)

Lane **1**: molecular weight marker, lane 2 and 3: whole camel milk, lane 4: IEC product under peak III (αs1- and β-CN), lane 5: IEC product under peak II (β-CN), lane 6: IEC product under peak I (κ-CN)

In this study, camel milk α_{s1}- and κ-CN showed lower mobility on SDS-PAGE than that reported for bovine counterpart (Ng-Kwai-Hang and Pelissier, 1989). The lower mobility could be due to their degree of phosphorylation (Farah and Farah-Riesen, 1985). However, β-CN showed similar mobility to that of the counter fraction in bovine milk. Similarly, Ochirkhuyag *et al.* (1997) reported the same behavior for this fraction in camel milk.

The results revealed no elutes corresponding to α_{s2}-CN after IEC, therefore bands representing this fraction were not defined on the IEF gel. Farah and Farah-Riesen (1985), Ng-Kwai-Hang and Pelissier (1989) and Ochirkhuyag *et al.* (1997) were also not able to detect α_{s2}-CN fraction in camel milk, which might be due to the low concentration of this fraction in camel milk. Another reason for missing α_{s2}-CN fraction could be the step gradient used in IEC. Nevertheless, missing casein fraction in milk is well documented such as missing α_{s2}-CN fraction in human milk (Crittenden and Bennett, 2005). However, Larsson-Raznikiewicz and Mohamed (1986) reported detection of α_{s2}-CN but as diffuse bands in camel milk.

Casein fractions obtained from camel skim milk showed diffused wavy bands on IEF gel. The same behavior was also observed in cow's (Seibert *et al.*, 1985). This phenomenon could be used to define and recognize casein fraction when IEF technique is used. Important observation on IEF gel is that β-CN was focused in the middle of the gel. It revealed a similar p*I* to that of bovine counterpart. Moreover, this fraction showed the most intense band on the gel. Similarly, Larsson-Raznikiewicz and Mohamed (1986) reported that α_{s1}- and β-CN are the dominant fractions in camel milk. Kappeler *et al.* (2003) concluded that β-CN represents 65% of total camel casein. Therefore, the high intensity of this fraction was expected.

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References

Al haj, O. A. and Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects of dromedary camel milk. International Dairy Journal, 20: 811- 821.

Alim, N.; Fondrini, F.; Ionizzi, I.; Feligini, M. and Enne, G. (2005). Characterization of casein fractions from Algerian dromedary (*Camelus dromedarius*) milk. Pakistan Journal of Nutrition, 4: 112- 116.

Aschaffenburg, R. and Drewry, J. (1957). Genetics of the β-lactoglobulins of cows' milk. Nature, 180: 376- 378.

Benkerroum, N.; Dehhaoui, M.; El fayq, A. and Tlaiha, R. (2011). The effect of concentration of chymosin on the yield and sensory properties of camel cheese and on its microbiological quality. International Journal of Dairy Technology, 64: 232- 239.

Caroli, A. M.; Chessa, S. and Erhardt, G. (2009). Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. Journal of Dairy Science, 92: 5335- 52.

Crittenden, R. G. and Bennett, L. E. (2005). Cow's milk allergy: A complex disorder. Journal of the American College of Nutrition, 6: 582- 591.

El Agamy, E. I. (2006). Camel milk. In: Park, Y. W. and Haenlein, F. W. (^{eds}) Handbook of non-Bovine Mammals. Blackwell Publisher Professional, Iowa, USA.

El Agamy, E. I. (2009). Bioactive component in camel milk. In: Park, Y. W. (^{ed}). Bioactive

Components of Milk and Dairy Products. Wiley-Blackwell, Iowa, USA.

El Zubeir, I. E. M. and Jabreel, S. (2008). Fresh cheese from camel milk coagulated with Camifloc. International Journal of Dairy Technology, 61: 90- 95.

Farah, Z. (1996). Camel milk: Properties and products. SKAT Swiss Centre for Development Cooperation in Technology and Management. Gallen, Switzerland.

Farah, Z. and Farah-Riesen, M. (1985). Separation and characterization of major components of camel milk casein. Milchwissenschaft, 40: 669- 671.

Farah, Z. and Rüegg, M. W. (1989). The size distribution of casein micelles in camel milk. Food Microstructure, 8: 211- 216.

Giambra, I. J.; Brandt, H. and Erhardt, G. (2014). Milk protein variants are highly associated with milk performance traits in East Friesian Dairy and Lacaune sheep. Small Ruminant Research, 121: 382- 394.

Kappeler, S., Farah, Z. and Puhan, Z. (2003). 5´-Flanking regions of camel milk genes are highly similar to homologue regions of other species and can be divided into two distinct groups. Journal of Dairy Science, 86: 498- 508.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 27: 680- 685.

Larsson-Raznikiewicz, M. and Mohamed, M. A. (1986). Analysis of casein content of camel (*Camelus dromedarius*) milk. Swedish Journal of Agricultural Research, 16: 13- 18.

Ng-Kwai-Hang, K. F. and Pelissier, J. P. (1989). Rapid separation of bovine caseins by mass ion exchange chromatography. Journal of Dairy Research, 56: 391- 397.

Ochirkhuyag, B.; Chobert, J.; Dalgalarrondo, M.; Choiset, Y. and Haertlte, T. (1997). Characterization of caseins from Mongolian Yak, Khainak and Bactrian camel. Lait, 77: 601- 613.

Rijnkels, M. (2002). Multispecies comparison of the casein gene loci and evolution of casein gene family. Journal of Mammary Gland Biology and Neoplasia, 7: 327- 345.

Rossomando, E. F. (1990). Ion exchange chromatography. Methods in Enzymology, 182: 309- 317.

Salem, S. A.; El-Agamy, E. I.; Salama, F. A. and Abo-Soliman, N. H. (2009). Isolation, molecular and biochemical characterization of goat milk casein and its fractions. Tropical and Subtropical Agroecosystems, 11: 29- 35.

Seibert, B.; Erhardt, G. and Senft, B. (1985). Procedure for simultaneous phenotyping of genetic variants in cow's milk by isoelectric focusing. Animal Blood Groups and Biochemical Genetics, 16: 183- 191.

Shapiro, A. L.; Vinuela, E. and Maizel, J. V. (1967). Molecular weight estimation of poly peptide chain by electrophoresis in SDS polyacrylamide gel. Journal of Biochemical and Biophysical Research Communications, 28: 815- 820.

Shuiep, E. S.; Giambra, I. J.; El Zubeir, I. E. M. and Erhardt, G. (2013). Biochemical and molecular characterization of polymorphisms of α_{s1}-casein in Sudanese camel (*Camelus dromedarius*) milk. International Dairy Journal, 28: 88- 93.

Shuiep, E. S.; El Zubeir, I. E. M. and Yousif, I. A. (2014a). Compositional quality of camel milk and some husbandry practices associated with camel milk production in two production systems in Sudan. Journal of Agricultural and Veterinary Sciences, 15: 10- 18.

Shuiep, E. S.; El Zubeir, I. E. M. and Yousif, I. A. (2014b). Socioeconomic aspects of rearing camels under two production systems in Sudan. Livestock research for Rural Development, 26 (11).

Wangoh, J.; Farah, Z. and Puhan, Z. (1993). Extraction of camel rennet and its comparison with calf rennet extract. Milchwissenschaft, 48: 322-325.

Wangoh, J.; Farah, Z. and Puhan, Z. (1998). Isoelectric focusing of camel milk protein. International Dairy Journal, 8: 617- 621.