Effect of heat treatment on whey proteins of camel milk

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1. Introduction

Camel milk is an important component of the human diet in many parts of the world. It contains all essential nutrients and the composition is similar to that of cow milk (l). The present knowledge about the milk production potential of camels (Camelus dromedarius) is very limited. Data available show, however, that a healthy camel on good feed can produce 2,000 J of milk per lactation period (2). Even higher milk yields have been recorded (3). Most of the camel milk is drunk fresh. It is also consumed when slightly sour. Heat processing as a means of preserving camel milk is not known. The heat treatments commonly used such as pasteurization and sterilization cause denaturation of the whey proteins. This phenomenon has been extensively studied because of its importance in understanding the changes in the properties of milk that occur with heat treatment (4, 5). However, most of these studies are limited to cow milk due to its wide-spread industrial processing and commercialization.

This investigation was undertaken to determine the degree of denaturation of the whey proteins, when camel milk is subjected to various heat treatments.

2. Materials and methods

2.1 Milk samples

Camel milk samples were taken at Ngare Ndare Camel Farm', which is situated just north of the Equator in Kenya's Laikiba District and at an altitude of between 1,730 to 1,890 m above sea level. The animals of the indigenous breed (Camelus dromedarius) were fed all year round exclusively by grazing. The milk samples were collected from 10 individual camels on 3 different occasions. For each occasion, the 10 milk samples were mixed to one batch and skimmed. Each batch was divided into 250 ml portions. One portion was kept as a control (raw milk) and the rest was individually heated at 63, 80 and 90 °c for 30 min in a water bath in 500 ml round bottom flasks equipped with a condenser and thermometer. After heating, the flasks were cooled to room temperature. For comparison, bulk cow milk was used.

2.2 Nitrogen distribution

The nitrogen distribution in the milk was determined by the procedure of ASCHAFFENBURG and DREWRY (6). The following N-fractions were determined: total protein nitrogen (TN), non casein nitrogen (NCN) and non protein nitrogen (NPN) soluble in 12 % trichloracetic acid. Denaturation of whey proteins was calculated by the difference of the whey protein nitrogen (NCN minus NPN) before and after heating of the milk.

2.3 Electrophoresis

The non casein nitrogen filtrate containing the whey proteins was dialysed against water, lyophilized and then examined by polyacrylamide gel electrophoresis (PAGE). The electrophoresis was performed in a vertical slab gel apparatus of DESAGA (Heidelberg) according to the procedure of SMITH (7). The concentration of acrylamide in the resolving gel was 10%.

Electrophoresis was run at 20 mA for 20 min and then at 1 00 mA until the marker dye (bromphenolblue) was 0.5 cm from the anodic end of the slab (about 3 h). Cooling water of 12 °c was circulated in the electrophoretic chamber. The slabs were stained for 1 h with Coomassie brilliant blue G 250 in 3.5 % perchloric acid, according to REISNER *et al.* (8) and destained with 7.5 % (v/v) acetic acid.

Polyacrylamide gel electrophoresis containing sodium dodecyl sulphate (50S-PAGE) was performed as described by LAEMMLI (9). The resolving gel contained 12.5 % acrylamide.

3. Results and discussion

The distribution of N-fractions in raw milk as well as in milk samples heated at 63, 80 and 90 °c for 30 min are presented in Table 1. The value of NCN, which consists of whey proteins and NPN expressed as percentage of the total milk nitrogen, varied in raw milk from 23 to 24 %, showing no pronounced difference between camel and cow milk. The amount of NPN in both milks ranging from 5.6 to 6.6 % was not affected by the heat treatment of milk. Denaturation susceptibility of the whey proteins was expressed as percentage denaturation relative to the control raw milk.

Tab 1: Effect on heat treatment on the distribution of N-fractions in camel and cow milk

Batch No.	Temperature for 30 min	TN mg/100g Cow	TN mg/100g Camel	NCN mg/100g Cow	NCN mg/100g Camel	NCN % of TN Cow	NCN % of TN Camel	NPN mg/100g Cow	NPN mg/100g Camel	NPN % of TN Cow	NPN % of TN Camel	Percentage denatured WPN mg/100g Cow	Percentage denatured WPN mg/100g Camel	Percentage denatured WPN mg/100g Cow	Percentage denatured WPN mg/100g Camel
1	raw	522	433	122	106	23	24	34	29	6.5	6.6	88	77		
	63°C			115	93	22	22	33	28	6.3	6.5	82	65	7	16
	80°C			55	79	11	18	33	29	6.3	6.6	22	SO	75	3S
	90°C			51	70	10	16	34	29	6.5	6.6	17	41	81	47
2	raw	530	543	129	124	24	23	32	31	6.0	S.7	97	93		
	63°C			122	113	23	21	32	32	6.0	5.8	90	81	7	13
	80°C			57	95	11	17	31	32	5.8	S.8	26	63	73	32
	90°C			SO	81	9	15	32	32	6.0	5.8	18	49	81	53
3	raw	550	548	124	130	23	24	33	30	6.0	S.6	91	100		
	63°C			113	115	21	21	32	30	5.8	5.6	81	85	7	15
	80°C			58	97	11	18	32	30	5.8	5.6	26	67	70	33
	90°C			55	79	10	14	32	30	5.8	5.6	23	49	74	51

The amount of denaturated whey proteins in the cow milk is in agreement with the values reported in the literature (10, 11). The lowest time-temperature combination (63 °C/30 min), which represents the conditions of conventional pasteurization caused little whey protein denaturation, while higher heat treatment at 80 and 90°C for 30 min which is more excessive than pasteurization, resulted in a 70 to 81 % denaturation of the whey proteins .

The camel milk whey protein showed generally a higher heat stability than cow milk. After an initial higher denaturation of whey proteins in camel milk at 63°C, the heal stability of the camel milk whey proteins increased in comparison to cow whey proteins markedly with temperature during heat treatment. The degree of denaturation of the whey proteins varied in camel milk from 32 to 35%at80°C and 47 to 51 % at 90°C heating temperature. This means that the susceptibility of camel whey protein to heat denaturation under the applied conditions is nearly twofold lower than that of the cow whey proteins, as shown in Table 1.

By means of polyacrylamide electrophoresis it has been possible to give visual evidence of heat effect on the whey proteins, thus confirming the results of the distribution of the N-fractions.

Fig. 1 gives the whey protein gel patterns of the raw and heated cow and camel milk. The obtained electrophoretic patterns of the individual whey proteins in cow milk agree with the results of previous investigations (10, 11, 12). Pasteurization temperature (63°C) caused no visible change in the whey protein gel pattern. At 80°C (slot C) immune globulins and serum albumin disappeared from the electrophoresis pattern. Portions of β-lactoglobulins (A and B) and α-lactalbumin remain undenaturated at 80°C, but disappear after heat treatment of 90°C (slot D).

Following earlier investigations of electrophoretic separation of camel milk (13), camel milk whey proteins showed lower mobility than cow milk whey protein (Fig. 1). The main whey protein bands of camel milk are designated by the numbers 1, 2, 3, and 4. The electrophoretic patterns in slots E F and G show 1 broader band in the upper part of the gel (component 1) followed by 2 sharp bands and I faint band in the lower part of the gel (components 2, 3 and 4). The gel patterns indicate that a pronounced heat effect can be observed in the sample of 90°C (slot H) where band intensities decrease and component 2 disappears.

In order to estimate the molecular weight of the individual whey proteins of camel milk, the proteins were further examined by PAGE in the presence of sodium dodecyl sulphate

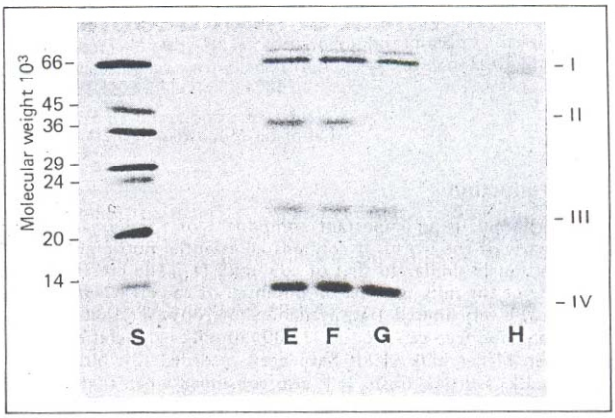

Fig. 2: 50S-PAGE patterns of whey protein filtrates prepared from camel milk heated at various temperatures for 30 min.
E, raw; F, 63°C; G, 80°C, H, 90°C. 5, standard marker proteins from top to bottom: bovine albumin (mol. wt. 66,000); egg albumin (mol. wt. 45,000); glyceraldehyde phosphate dehydrogenase (mol. wt. 36,000); carbonic anhydrase (mol. wt. 29,000); trypsinogen (mol. wt. 24,000); trypsin inhibitor (mol. wt. 20,000); α-lactalbumin (mol. wt. 14,000).

(SDS, Fig. 2). Comparing with the standard mixture of proteins in slot 5, band I and band IV are identical with standard bovine serum albumin (molecular weight 66,000) and α-lactalbumin (molecular weight 14,000). The estimated molecular weights of bands II and III were 43,000 and 23,000 respectively. These 2 protein bands are presumably β-lactoglobulins. It must be noted, however, that the sequence of the bands in Fig. 2 (I-IV) is not identical with the sequence of bands in Fig. 1 (1-4).

The electrophoresis indicates that the degree of susceptibility to heat denaturation of the individual camel whey proteins is not as pronounced as it is in cow whey proteins. It seems that the differences in heat stability among camel milk whey proteins are smaller than among the cow milk whey proteins. Thus, a more intensive heat treatment of camel milk is necessary to obtain the same degree of denaturation as in cow milk.

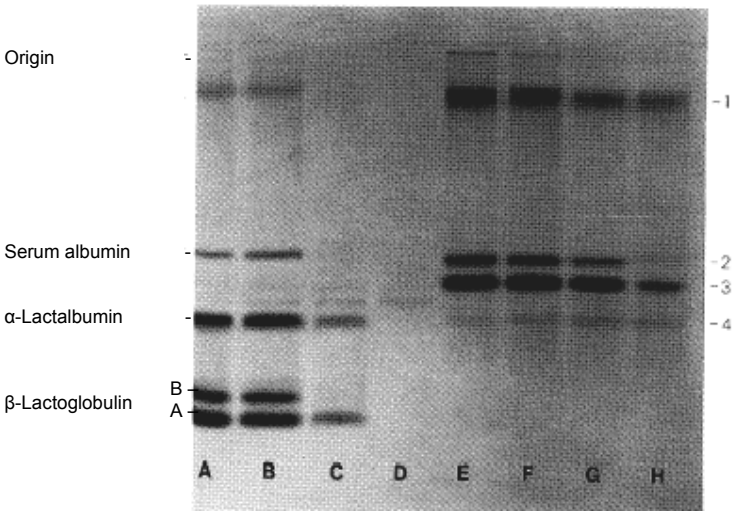

Fig. 1: Polyacrylamide gel patterns of whey protein filtrates prepared from camel and cow milk heated for 30 min at various temperatures. Cow milk: A, raw; B, 63 °C; C, 80 "'C; D, 90 °C Camel milk: E, raw; F, 63°C; G, 80°C; H, 90°C.

From the results of this investigation it can be concluded that the heat sensitivity of whey proteins in camel milk is considerably lower than in cow milk. At the time being no adequate explanation can be offered for this relative heat stability. Further studies are needed to elucidate the mechanism involved in the heat denaturation of camel milk whey proteins.

Acknowledgement

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5. Summary

FARAH, Z.: Effect of heat treatment on whey proteins of camel milk. Milchwissenschaft 41 (12) 763-765 (1986).
24 Camel milk (heat denaturation)

Heat denaturation of the whey proteins of camel and cow milk was compared. The milk was heated to 63, 80 and 90 °C for 30 min and nitrogen distribution was determined in raw and heated milk. The whey proteins were also examined by polyacrylamide gel electrophoresis.

Non casein nitrogen and non protein nitrogen in raw camel milk expressed as percentage of the total milk nitrogen varied from 23 to 24 % and 5.6 to 6.6 %, respectively. Application of electrophoresis in sodium dodecyl sulphate polyacrylamide gel(SDS-PAGE) to identify camel whey proteins revealed beside α-lactalbumin and bovine serum albumin, the presence of two whey proteins of different molecular weights, which can be regarded as possibly homologous to bovine β-lactoglobulins. Compared with cow milk, camel whey proteins exhibited markedly lower sensitivity to heat denaturation. Under the selected experimental conditions the rate of heat denaturation of camel milk whey proteins was approximately twofold lower than cow milk whey proteins.

FARAH, Z.: Einfluss der Hitzebehandlung auf die Molkenproteine der Kamelmilch. Milchwissenschaft 41 (12) 763-765 (1986).
24 Kamelmilch (Hitzedenaturierung)

Die Hitzedenaturierung der Molkenproteine von Kamel- und Kuhmilch wurde verglichen. Die Milch wurde bei; 63,80 und 90 °C während 30 min erhitzt und die Stickstoffverteilung in der rohen und in der erhitzten Milch bestimmt. Die Molkenproteine wurden auch mit Hilfe der Polyacrylamidgel-Elektrophorese untersucht. Der Anteil des Nicht-Casein· und des Nicht-Protein-Stickstoffes in der rohen Kamelmilch am Gesamtstickstoff variierte von 23 bis 24 %, bzw. *von* 5,6 bis 6,6 %. Die Anwendung der Elektrophorese in Natrium-dodecylsulfat-Polyacrylamidgel (SDS-PAGE) zur Identifizierung von Kamelmolkenproteinen zeigte neben α-Laktalbumin und Serumalbumin die Anwesenheit von zwei Molkenproteinen unterschiedlichen Molekulargewichtes. Diese können als Homologe der β-Laktoglobuline der Kuhmilch betrachtet werden. Die Molkenproteine der Kamelmilch sind bedeutend weniger empfindlich für Hitzedenaturierung als diejenigen der Kuhmilch. Unter den gewählten experimentellen Bedingungen war der Grad der Hitzedenaturierung der Molkenproteine bei der Kamelmilch annähernd zweimal geringer als bei Kuhmilch.

FARAH, Z.: Influence du traitement thermique sur les protéines du sérum du lait de chamelle. Milchwissenschaft 41 (12) 763-765 (1986).
24 Lait de chamelle (denaturation thermique)

FARAH, Z.: Influjo del tratamiento térmico en las proteínas de suero de leche de camella. Milchwissenschaft 41 (12) 763-765 (1986).
24 Leche de camella (denaturaci6n térmica)

Whey proteins present in camel milk were less affected by heating at 63 Â°C than at 98 Â°C. This experimental study showed that denaturation increased significantly as the temperature increased from 63 to 98 Â°C.Â Proteomic Profiling Comparing the Effects of Different Heat Treatments on Camel (Camelus dromedarius) Milk Whey Proteins. by Hicham Benabdelkamel 1,*, Afshan Masood 1 , Ibrahim O. Alanazi 2, Dunia A. Alzahrani 1,2, Deema K. Alrabiah 1,2, Sami A. AlYahya 3 and Assim A. Alfadda 1,4. 1. Camel milk is consumed in the Middle East because of its high nutritional value. Traditional heating methods and the duration of heating affect the protein content and nutritional quality of the milk. We examined the denaturation of whey proteins in camel milk by assessing the effects of temperature on the whey protein profile at room temperature (RT), moderate heating at 63 Â°C, and at 98 Â°C, for 1 h. The qualitative and quantitative variations in the whey proteins before and after heat treatments were determined using quantitative 2D-difference in.