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Immunochemical quantification of heat denaturation of camel (*Camelus dromedarius*) whey proteins

Didier Levieux[1]*, Annie Levieux[1], Halima El-Hatmi[2] and Jean-Paul Rigaudière[1]

[1] INRA, Immunochemistry Unit, Theix, 63122 Saint-Genès-Champanelle, France
[2] Arid Land Institute, Livestock and Wildlife Laboratory, 4119 Medenine, Tunisia

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The major whey proteins IgG, serum albumin and α-lactalbumin were purified from camel milk using gel permeation and ion-exchange chromatography. Specific antisera against each of them were raised and used to quantify their heat denaturation in early or mature milk over a range of 60–90 °C for 10–60 min using the single radial immunodiffusion technique. The heat denaturation midpoints for the mature milk heated 30 min were 67·2, 73·0 and 77·5 °C for IgG, albumin and α-lactalbumin respectively. The early milk was more sensitive to heat treatment with coagulation at low temperature and heat denaturation midpoints of 64·8, 71·6 and 72·6 °C respectively. This difference was related to the high IgG content of the early milk (12·6 mg/ml *v.* 0·5 mg/ml for the mature milk) and stresses the importance of monitoring the IgG level of milk to assess the absence of colostrum.

Keywords: Camel, whey, proteins, heat, denaturation.

Camel milk is one of the main components of the human diet in many parts of the world. Most of it is consumed in the fresh or sour state. However, the preservation of raw milk can be achieved by heat treatments such as pasteurization, ultra high temperature (UHT) or sterilization processes. Knowledge of the effect of heat treaments 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 whey proteins since the pioneer work of Lyster (1970). In contrast, only limited studies have been carried out on camel whey proteins, using mostly polyacrylamide gel electrophoresis (Farah, 1986; Elagamy, 2000). However, it has been demonstrated that this technique as well as size exclusion chromatography cannot be quantitative due to oligomerization of some of the whey proteins upon heat treatment (Levieux, 1980). Since heating proteins causes conformational changes in their molecular structure with concomitant loss of native epitopes, immunochemical techniques can be used for monitoring heat denaturation of proteins. Specific applications have been published on effects of heating milk, meat and egg proteins (Lyster, 1970; Levieux, 1980; Fushiki et al. 1986; Breton et al. 1988; Sajdok et al. 1989; Abe et al. 1991; Varshney et al. 1991; Ikura et al. 1992; Sanchez et al. 1992, Levieux et al. 1995).

Our objective was to measure the loss of immunoreactivity of camel whey proteins in mature and early milk upon heat treatment. To achieve this goal, the major camel whey proteins were first purified and their specific antisera were raised in rabbits to allow their quantification using the single radial immunodiffusion (SRID) technique.

*For correspondence; e-mail: didier.levieux@clermont.inra.fr

Materials and Methods

Materials and samples

Agar Noble was supplied by Difco (Detroit, MI). Bovine serum albumin, Trichlo-trifluoroethane, Freund's complete and incomplete adjuvants were purchased from Sigma Aldrich (Saint Quentin Fallavier, France).

Blood, colostrum and milk samples were obtained from multiparous lactating camels (*Camelus dromedarius*) of the Experimental Station of the Arid Land Institute of Medenine. Early milk and mature milk were obtained from animals sampled in the first week and after three months lactation respectively. Their IgG concentration were 12·6 and 0·45 mg/ml respectively, as determined by the single radial immunodiffusion assay (see later).

Serum was obtained from clotted blood by centrifugation at 2500 ***g*** and 4 °C for 20 min.

Milk was defatted by centrifugation at 2500 ***g*** for 30 min and caseins were precipitated by decreasing the pH to 4·2 with 1 M-HCl. After centrifugation at 20 000 ***g*** and 4 °C for 20 min, the supernatant was dialyzed overnight against 0·02 M-Tris-HCl buffer pH 8·4 and centrifuged at 20 000 ***g*** and 4 °C for 30 min.

Colostral whey was obtained from defatted colostrum diluted 3·5 fold (v/v) with distilled water by acid precipitation of caseins as described for milk whey.

Purified proteins

IgG were purified from colostral whey by gel permeation chromatography on a Sephadex G200 (Amersham-Biosciences, Orsay, France) column (95 × 5 cm) equilibrated in 0·02 M-Tris-HCl buffer pH 8·4. The column was previously calibrated with 15 ml horse serum defatted by treatment with trichloro-trifluoroethane (v/v) and half diluted with 0·02 M-Tris-HCl buffer pH 8·4. The colostral whey (15 ml) was passed through a 0·2 µ filter prior to injection. Elution was performed at a flow rate of 44 ml/h using a Mariott flask and monitored at 280 nm. The second peak, eluting around 160 kDa, was then passed through a Q-Sepharose Fast Flow (Amersham-Biosciences) column (19 × 2·5 cm) equilibrated in 0·02 M-Tris-HCl buffer pH 8·4. Elution was performed at a flow rate of 40 ml/h with a 0–0·75 M-NaCl gradient.

Albumin (CSA) was purified from camel serum defatted by treatment with trichlo-rotrifluoroethane (v/v) and half diluted with 0·02 M-Tris-HCl buffer pH 8·4. Ten ml were passed through a 0·2 µ filter and injected on the Sephadex G200 column. Elution was performed as described for IgG purification. The second half of the third peak, eluting around 70 kDa, was then passed through the Q-Sepharose Fast Flow as described for IgG.

α-Lactalbumin (α-la) was purified from camel whey by gel permeation chromatography on a Sephadex G100 column (90 × 5 cm) equilibrated in 0·02 M-Tris-HCl buffer pH 8·4. The column was previously calibrated with bovine whey supplemented with 3 mg/ml of bovine serum albumin. Samples (20 ml) were 0·2 µ filtered before injection. Elution was performed at 58 ml/h flow rate using a Mariott flask. The last peak, eluting around 14 kDa, was passed through the Q-Sepharose Fast Flow equilibrated in the same buffer. Elution was performed at a flow rate of 40 ml/h with a 0–0·5 M-NaCl gradient. The major peak was finally passed through a Mono-Q HR 10/10 column (Amersham-Biosciences) equilibrated in 0·02 M-Tris-HCl buffer pH 8·6. Elution was performed at 2 ml/min with a 0–1 M-NaCl gradient using HPLC equipment (Pump 420, detector 430; Kontron Instrument, St-Quentin-en-Yvelines, France).

Purity was checked by polyacrylamide gel electrophoresis (12·5% acrylamide) with or without denaturating agents (SDS and mercapto-ethanol with heating for 5 min in a boiling water bath).

Polyclonal antibodies

Rabbits were immunized at monthly intervals by multiple intradermal injections of antigen-adjuvant mixture (Vaitukaitis et al. 1971) prepared by emulsifying one volume saline containing 0·5–1 mg purified protein/ml and one volume complete (first injection) or incomplete (booster injections) Freund's adjuvant. Each rabbit received 2 ml of the emulsion. Animals were bled 7 d after each booster injection and the sera were analysed for antibody activity and specificity by immunoelectrophoresis (Scheidegger, 1955) and single radial immunodiffusion (Mancini *et al*. 1965). Immunogens used were purified IgG, CSA, and α-la.

Heat treatment

Aliquots (1·5 ml) of crude camel milk in stoppered (5 ml) glass tubes were heated in triplicate in a thermostatically controlled water bath (Polystat 44, Bioblock Scientific, Illkirch, France) maintained ±0·05 °C of the required temperature. Heat treatment was halted immediately by immersion in ice water. An unheated aliquot was used as a control. All samples were centrifuged at 16 000 ***g*** for 5 min and the immunochemical analyses were performed on the supernatants.

Single radial immunodiffusion assay of proteins

Residual native proteins were determined by single radial immunodiffusion using 1·9 mm-thick agar plates containing 1·2% agar Noble in 0·005 M-barbital buffer pH 7·3 and suitable quantities of each specific antiserum. Circular wells (1·5 mm diameter) were punched out in the gel and filled with 3 µl aliquots of adequately diluted heated milk or 3 µl of purified proteins of known concentrations as standards. The purified proteins were diluted in the barbital buffer containing 1 mg/ml human serum albumin and 1 mg/ml sodium azide. Plates were incubated in a moist box at 37 °C for 15–20 h and the diameter of the ring-shaped precipitates was measured using a magnifying video camera system (Levieux, 1991). Standard curves were constructed by plotting the diameter of the precipitating ring *v*. the square root of the protein concentration. With the diffusion time used, a linear regression was always obtained. Samples and standards were plated in duplicate. The CVs of the assays were 3–5%. Results were expressed as residual native protein (%):

$$\text{Residual native protein (\%)} = c/c_0 \times 100$$

where c_0 is the protein concentration before heating and c the concentration of undenatured protein after heating.

D and Z values

D-values, time required for 90% denaturation were calculated by regression analysis, as the reciprocal of the

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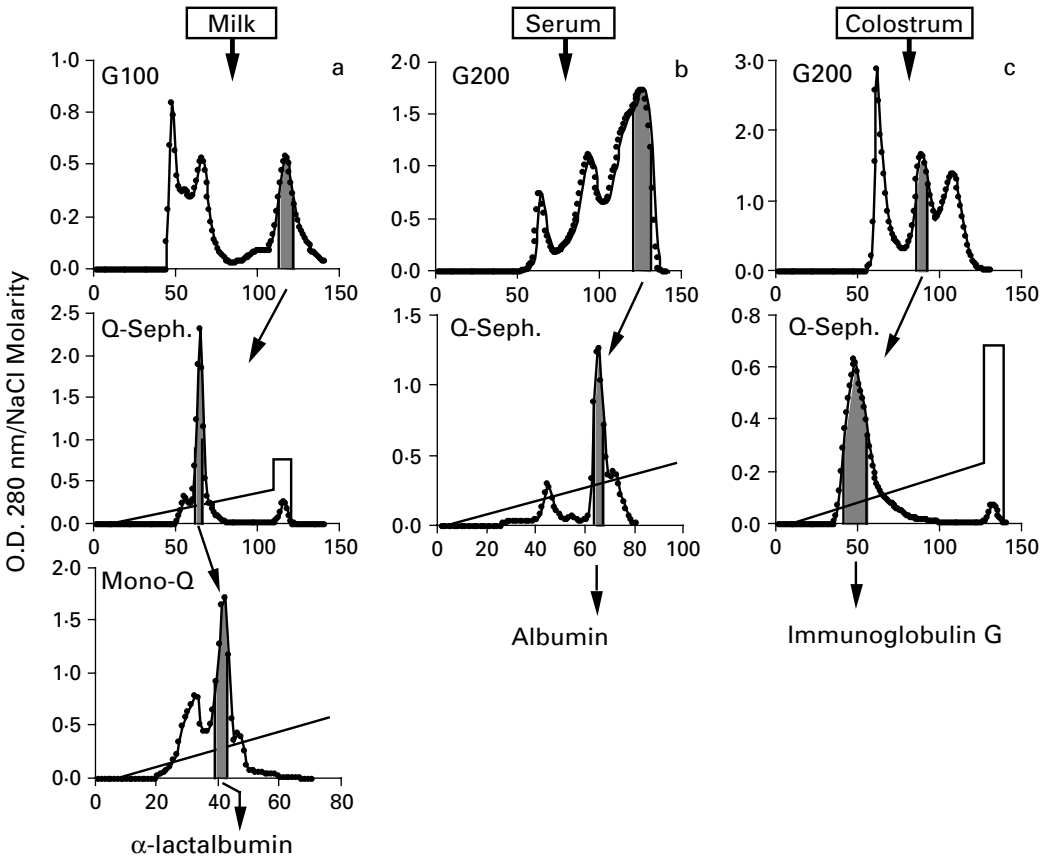

Fig. 1. Flow chart of the procedure used for the purification of the major camel whey proteins. Q-Seph: Q-Sepharose Fast Flow.

slope of lines obtained for each temperature by plotting the logarithm of residual native protein (%) as a function of holding time. For second order reaction, *D*-values were calculated from the general equation:

$$c^{1-n} = c_0^{1-n} + (n-1)kt$$

For a decimal value of c_0:

$$(c_0/10)^{1-n} = c_0^{1-n} + (n-1)k\ D_\theta$$

For a second-order reaction

$$D_\theta = 9/(c_0 k_2)$$

Z-values (degrees needed for a 10 fold decrease in *D*) were calculated, by regression analysis, as the reciprocal of the slope of the line obtained by plotting the logarithm of *D*-values as a function of temperature, in a range which showed a linear relationship.

Results

Protein purification and production of antisera

Results of a typical purification procedure are presented in Fig. 1. The third major peak obtained by gel permeation chromatography of camel whey on Sephadex G100 (Fig. 1a) was recovered for α-la purification. This peak contained minor contaminants that were removed by ion exchange chomatography on Q-Sepharose Fast Flow. On Mono-Q, with increased resolution, two major fractions were obtained.

Three peaks were obtained by gel permeation chromatography of camel serum on Sephadex G200 (Fig. 1b), the second one containing IgG of 160 kDa. The atypical shoulder of the ascending part of the third peak contained 80 kDa IgG which contaminated the CSA. Thus, in order to obtain pure CSA, fractions of the second half of the third peak were pooled and loaded on the Q-Sepharose Fast Flow. IgG and CSA were respectively eluted around 0·18 and 0·3 M-NaCl.

Three peaks were obtained by gel permeation chromatography of colostral whey on Sephadex G200 (Fig. 1c). The second peak contained essentially IgG of 160 kDa and the ascending part of the third peak contained IgG of 80 kDa. Fractions of IgG 160 kDa were pooled and loaded on the Q-Sepharose column. IgG were eluted between 0·05 and 0·1 M-NaCl and a minor contaminant was eluted with 0·75 M-NaCl.

Purity of the purified proteins was checked on native and SDS PAGE electrophoresis (Fig. 2). IgG and CSA

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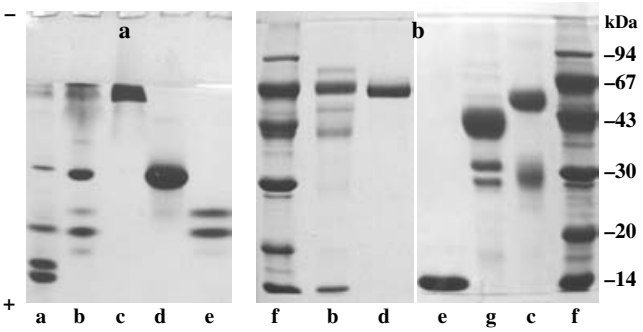

Fig. 2. Electrophoretic analysis of the purified camel whey protein using native PAGE (*a*) or SDS-PAGE (*b*). a: bovine whey; b: camel whey; c: purified IgG; d: purified albumin; e: purified α-lactalbumin; f: molecular weight markers (phosphorylase b 94 kDa, bovine albumin 67 kDa, ovalbumin 43 kDa, carbonic anhydrase 30 kDa, trypsin inhibitor g 20·1 kDa, α-lactalbumin 14·4 kDa); g: IgG 80 kDa (ascending part of the third peak obtained by chromatography of camel serum on Sephadex G200).

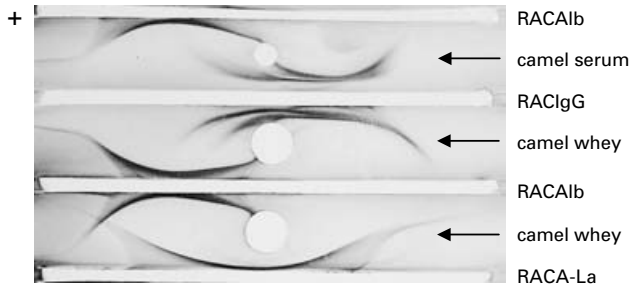

Fig. 3. Immunoelectrophoretic analysis of the antisera raised against the purified proteins of camel whey. RACAlb: rabbit antisera against camel albumin; RACIgG: rabbit antisera against camel IgG; RACA-La: rabbit antisera against camel α-lactalbumin.

fractions were more than 95% pure. The two fractions obtained for α-la by Mono-Q chromatography were quite similar in composition; two bands were observed in native PAGE, one of them being the major component of the second fraction and migrating as bovine α-la. In SDS PAGE, only one band was observed at a MW of 14 kDa.

High titer antisera were obtained in rabbits and their specificity was checked by immunoelectrophoretic analysis (Fig. 3). One strong arc was observed for the anti-CSA and anti-α-la. Three typical confluent arcs, corresponding to three IgG subclasses, were observed for the anti-IgG rabbit serum tested against camel serum or whey.

Proteins concentration in the mature and early milk

Concentrations of IgG, α-la, and CSA in the mature milk were found at 0·45, 1·62 and 0·30 mg/ml respectively. In the early milk these concentrations were 28 fold higher for IgG (12·6 mg/ml), 3 fold higher for α-la (4·3 mg/ml) and 11 fold higher for CSA (3·4 mg/ml). Thus there is a total protein excess of 18 mg/ml for these three major whey proteins.

Heat denaturation

Coagulation upon heating was visually detected for the early milk when heat treatments were equal or higher than 20 min incubation at 70 °C. No coagulation was observed in the mature milks.

The denaturation of the various proteins during heat treatment of early and mature milk was compared. In both milks the denaturation was clearly a function of time as well as temperature (Fig. 4).

α-La was the most resistant whey protein in both milks (Fig. 4a). The slope obtained was particularly low for the mature milk with 55% of the protein remaining undenatured after heating at 90 °C for 10 min. In the early milk the heat sensitivity was dramatically higher with only 28% of the protein remaining undenatured for the same heat treatment.

The slope obtained for the CSA was more pronounced (Fig. 4b) with 9% and 2% of the protein remaining undenatured upon heating the mature and early milk at 90 °C for 10 min.

IgG was the most sensitive protein to heat treatment with only 2–3% residual native protein at 80 °C and 75 °C (10 min holding time) for the mature and early milk (Fig. 4c).

In Table 1 are reported the temperatures of half denaturation of the proteins upon heating for 30 min and the percentage of undenatured protein after a pasteurization-like treatment. Compared with mature milk, the heat denaturation mid-points (temperature for 50% protein denaturation) in the early milk were significantly decreased by 2·4 °C ($P<0·01$), and 4·9 °C for IgG and α-la respectively. Upon a pasteurization-like treatment (65 °C for 30 min) IgG was 48% and 66% denatured in the mature and early milk respectively ($P<0·001$).

Kinetic constants

Experimental points were plotted according to the following equation:

$$\ln c_t/c_o \times 100 = -kt \quad (1)$$

which was obtained from the integrated form of the equation for first-order reaction kinetics:

$$-dc/dt = kc$$

where c_o is the initial concentration, c_t the concentration of native protein at time t, and k the rate constant. The straight lines from linear regression showed a low coefficient of correlation (not shown). Best fits were

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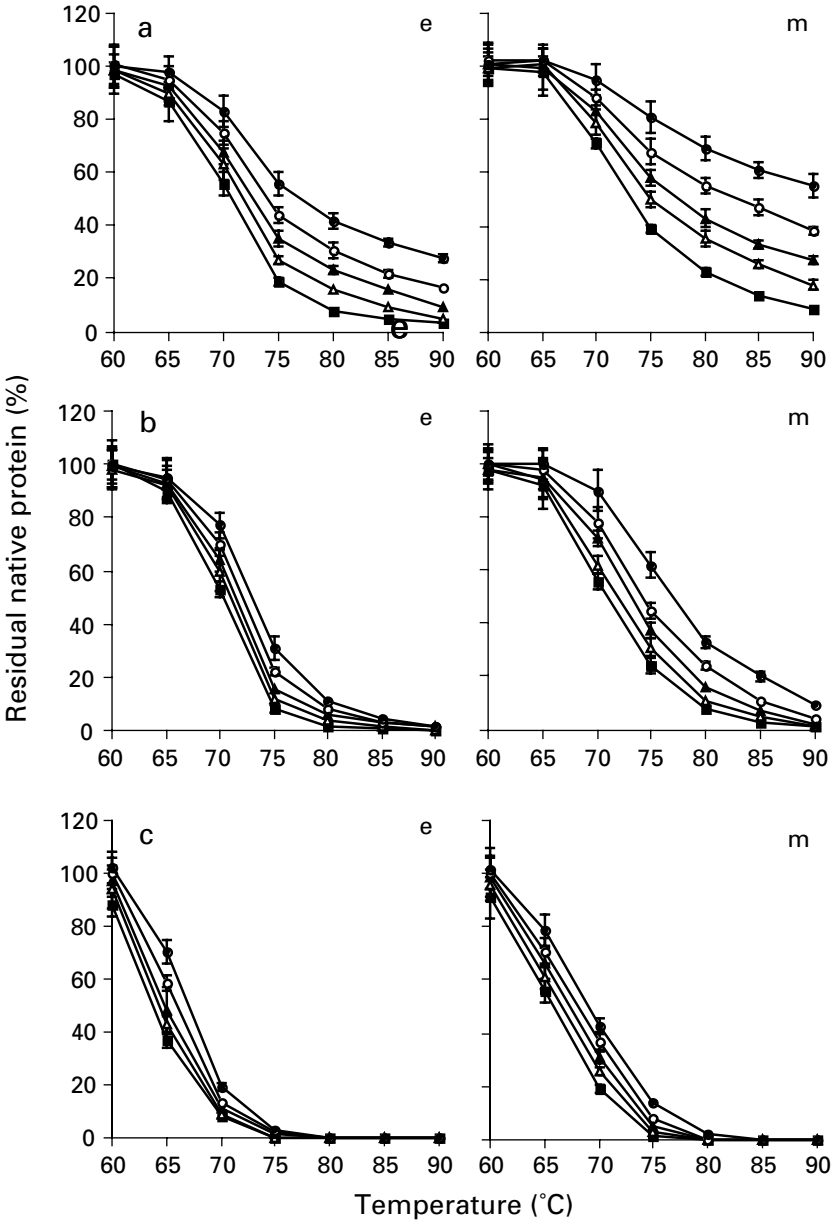

Fig. 4. Thermal denaturation of camel α-la (a), albumin (b) and IgG (c) in the range 60–90 °C after heating early (e) or mature (m) milk for 10 min (closed circles), 20 min (open circles), 30 min (closed triangles), 40 min (open triangles), or 60 min (closed squares). Residual native protein was determined by the single radial immunodiffusion assay and expressed as mean and standard deviation ($n=3$) of percent of initial concentration.

obtained using the equation for second-order kinetics:

$$1/c - 1/c_0 = k_2 t \quad (2)$$

obtained, using $n=2$, from the integrated form of the equation for n-order reaction kinetics:

$$dc/c^n = -kdt$$

Temperature dependence and thermodynamic parameters

The rate constant k_2, calculated from the slope of equation (2), was related to the temperature of treatment according to the Arrhenius equation:

$$k_2 = A \exp(-Ea/RT) \quad (3)$$

where A is a constant, Ea the apparent activation energy, R the universal gas constant and T the absolute temperature. From equation (3):

$$\ln k_2 = \ln A - Ea/RT \quad (4)$$

was obtained. When the logarithm of the rate constant was plotted *v.* the reciprocal of the absolute temperature

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Table 1. Heat sensitivity of the major whey proteins in camel milk as quantified by radial immunodiffusion assay using specific antisera. Results are expressed as means ± SD. Means with the same letter differ significantly at $P<0{\cdot}01$ (lower-case letter) or $P<0{\cdot}001$ (upper case letter)

	α-lactalbumin	Albumin	IgG
Temperature for 50% denaturation upon heating 30 min (°C)			
Mature milk	$77{\cdot}5 \pm 0{\cdot}8^{a}$	$73{\cdot}0 \pm 1{\cdot}0$	$67{\cdot}2 \pm 0{\cdot}6^{b}$
Early milk	$72{\cdot}6 \pm 1{\cdot}1^{a}$	$71{\cdot}6 \pm 0{\cdot}8$	$64{\cdot}8 \pm 0{\cdot}7^{b}$
% residual native protein upon heating at 65 °C for 30 min			
Mature milk	$99{\cdot}0 \pm 3{\cdot}5$	$95{\cdot}0 \pm 3{\cdot}7$	$66{\cdot}0 \pm 2{\cdot}8^{C}$
Early milk	$93{\cdot}0 \pm 3{\cdot}0$	$93{\cdot}2 \pm 3{\cdot}2$	$48{\cdot}2 \pm 2{\cdot}3^{C}$

Table 2. Energy of activation (Ea) and changes in enthalpy of activation (ΔH), free energy of activation (ΔG) and entropy of activation (ΔS) for denaturation of camel whey protein

Protein	Temp (°C)	Ea ($kJ.mol^{-1}$)	ΔH ($kJ.mol^{-1}$)	ΔG ($kJ.mol^{-1}$)	ΔS ($kJ.mol^{-1}K^{-1}$)
	70		126·6	77·7	0·143
	75		126·6	76·9	0·143
α-lactalbumin	80	129	126·5	76·2	0·143
	85		126·5	75·4	0·143
	90		126·5	74·7	0·142
	70		254·8	72·8	0·530
	75		254·7	70·1	0·530
Albumin	80	258	254·7	67·4	0·530
	85		254·6	64·7	0·530
	90		254·6	62·1	0·530
	60		368·1	77·4	0·872
	65	371	368·0	73·0	0·872
IgG	70		368·0	68·7	0·872
	75		367·9	64·3	0·872

(Arrhenius plots) according to equation (4), straight lines were obtained by linear regression from which slopes (Fig. 5), and thus activation energy values (Table 2), were calculated. The coefficient of correlation of the Arrhenius plots were r = 0·997, 0·999 and 0·998 for respectively α-la, CSA and IgG.

The activation energy value enabled determination of enthalpy (ΔH), entropy (ΔS), and free energy of activation (ΔG), according to the following expressions:

$$\Delta H = Ea - RT$$

$$\Delta S = R(\ln A - \ln Kb/hp - \ln T)$$

$$\Delta G = \Delta H - T\Delta S$$

where lnA is the ordinate intersection of the straight line obtained by linear regression for Ea calculation (equation 4), Kb is the Boltzmann constant ($1{\cdot}38066 \times 10^{-23}$ J/K), hp the Planck constant ($6{\cdot}62618 \times 10^{-34}$ J s), R the gas constant and T the absolute temperature.

D and *Z*-values for the three camel whey proteins were calculated for second order reactions and the results are presented in Table 3.

Discussion

Most of the whey proteins in camel milk resemble bovine whey proteins, except the lack of β-lactoglobulin (β-lg; Ochirkhuyag et al. 1998; Merin et al. 2001a). Thus, the major whey proteins to be considered were IgG, α-la and CSA.

In the camelids, three IgG subclasses have been described, two of them being devoid of light chains (Hamerscasterman et al. 1993). Since information about the selective concentration of these subclasses is lacking, colostral whey was chosen as the starting material to obtain representative IgG subclass(es) suitable for antibody production and as standard for SRID analysis.

While high CSA concentration has been observed in camel colostrum (Merin et al. 2001a), camel serum was used for its purification due to much higher concentration.

α-La was purified from milk whey. The observation of two bands in native PAGE and only one band in SDS PAGE, corresponding to an expected MW of 14 kDa is in accordance with the identification of two α-la variants in camel milk having pI of 5·1 and 5·3 (Beg et al. 1985; Conti et al. 1985; Farah, 1986; Ochirkhuyag et al. 1998).

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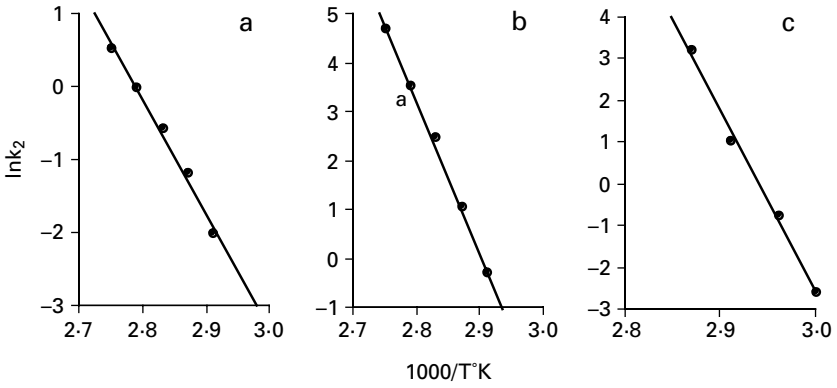

Fig. 5. Arrhenius plots of kinetic constants for α-la (a), albumin (b) and IgG (c) of camel mature milk. Each point represents an average of three determinations.

The purified proteins allowed the production of high titre specific antisera on rabbits which were used to monitor the heat sensitivity of the proteins by SRID analysis. This technique has proven useful for the study of thermal denaturation of milk proteins (Lyster, 1970; Levieux, 1980; Fushiki et al. 1986; Sanchez et al. 1992) and has been already used for IgG quantification in llama (Hutchinson et al. 1995) or camelids (Elagamy, 2000).

Heat denaturation of camel milk protein occurred in the following order: IgG >CSA >α-la. Quantifification of IgG may be thus appropriate for the control of heating temperature between 60 and 70 °C while CSA and α-la are more suitable for the ranges 65–75 °C and 70–90 °C respectively.

A study of the kinetics of thermal denaturation of the individual whey proteins should lead to a better understanding of the relationship between heat treatment and its effect on the functional properties of milk products. Kinetic parameters were calculated for the camel mature milk which is representative of normal milk. In the first week of lactation the protein composition of milk varies largely and the IgG level decreases abruptly. Our early milk is only representative of a sample obtained around the third day of lactation and thus kinetics parameters were not calculated.

We found that heat denaturation of the three major camel whey proteins in mature milk can be described by second order kinetics. While the half-time (when $c=1/2c_o$) for a first-order reaction is independent of starting concentration it can be seen from equation (2) that for a second order reaction it should vary inversely with the initial concentration. This can be related to an increased probability of intermolecular interactions induced by the thermal processing such as disulphide interchanges leading to oligomers, polymers and then precipitating aggregates when the protein concentration increases.

Values found for free energy of activation are characteristic of protein denaturation (Daemen, 1981). The high values of enthalpy of activation and the positive values of the entropy of activation indicate that, during denaturation, the proteins experienced a large change in conformation (Sanchez et al. 1992). These values were consistent with those reported for thermodynamic studies of proteins in complex media such as β-lg A and B and α-la in bovine milk: 260–310 kJ/mol in the range 70–80 °C (Lyster, 1970; Dannenberg & Kessler, 1988; Gotham et al. 1992), rennin in whey: 376 kJ/mol (Daemen, 1981), LDH M4 in bovine plasma: 400 kJ/mol (Matzinos & Hall, 1993), LDH M4 in meat soluble protein extract: 343 kJ/mol (Levieux et al. 1995).

D and *Z*-values were calculated for second order reactions. *Z*-values were found between 6 and 18·5. These values are consistent with published values for proteins or enzymes heated in complex media such as bovine milk or blood serum (Daemen, 1981; Sanchez et al. 1992; Matzinos & Hall, 1993; Levieux et al. 1995).

Studies have generally reported a first-order reaction for the denaturation of α-la in bovine milk (Lyster, 1970; Manji & Kakuda, 1986; Dannenberg & Kessler, 1988; Anema & McKenna, 1996). However, according to Hillier & Lyster (1979), kinetic behaviour displayed by α-la is probably pseudo first-order since the irreversible denaturation of bovine α-la probably involves disulphide interchange, that is, the second order reaction between a disulphide bond and a –SH group to form a new disulphide link.

α-La in camel mature milk appears more resistant to heat denaturation than α-la in bovine milk. Lyster (1970) and Levieux (1980) found 50% residual native protein after heating bovine milk at 80 °C for 10 min. In the same heating conditions we found 69% residual native protein for the camel milk. Due to the difference in the slope of the denaturation kinetics, the difference between the two species is more striking at 90 °C with 8 and 55% residual native protein for the bovine and camel milk respectively. Using native-PAGE Farah (1986) analysed camel milk and bovine milk heated at 63, 80 and 90 °C for 30 min. Although the results were not quantified, the denaturation obtained at 80 °C for the bovine α-la was roughly similar

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Table 3. *D* and *Z*-values for camel whey proteins

	D values (sec × 10^{-3})							
	60 °C	65 °C	70 °C	75 °C	80 °C	85 °C	90 °C	*Z* values (°C)
α-lactalbumin	1106·3	358·0	**41·9**	**17·9**	**9·9**	**5·7**	**3·3**	18·5 (0·995)
Albumin	1826·4	332·5	**39·5**	**10·1**	**2·5**	**0·9**	**0·3**	9·3 (0·998)
IgG	**264·4**	**41·6**	**7·1**	**0·8**	0·1			6·0 (0·999)

Values in bold show temperature range used for *Z* determination
Correlation coefficient in parentheses

to that obtained for the camel α-la. For Elagamy (2000), the camel α-la was not denatured after heating milk at 100 °C for 10 to 30 min, while the bovine α-la was slightly affected. However this author used SDS-PAGE electrophoresis to monitor the protein heat denaturation. In this technique results are overestimated since denatured but not precipitated proteins are partly solubilized by the boiling-SDS treatment.

Hillier & Lyster (1979) have observed that the denaturation of albumin in bovine skim-milk cannot adequately be described by simple first-order kinetic and they have suggested that this complexity may result from the formation of heat stable intermediates. According to Elagamy (2000) CSA was not affected by heating milk at 75 °C for 30 min. In this work we found only 37% residual native CSA in the mature milk and 16% in the early milk for the same heat treatment. In bovine milk we found a similar heat sensitivity with 45% residual native bovine albumin for the same heat treatment (unpublished data). These discrepancies clearly illustrate the strong overestimation obtained using SDS-PAGE.

The concentration effect was particularly marked for IgG since their concentration in the early milk we studied was 28 fold higher than in the mature milk. Thus we found 35% *v.* 11% residual native protein for respectively mature and early milk upon heating at 70 °C for 30 min. The heat sensitivity found for IgG in camel mature milk is slightly higher than that reported by Elagamy (2000) using the same SRID technique: 50% residual native IgG for the same heating and is similar to that obtained for IgG in bovine milk (Hubert, 1977).

The heat sensitivity of whey proteins in camel milk has been generally considered lower than in cow milk. Wangoh (1997) heated skimmed camel milk at 90 °C for 20 min and found 46% undenatured soluble proteins. This result is in accordance with the heat sensitivity of α-la the predominant whey protein, since we found 40% residual native protein for the same heat treatment. However the heat sensitivity we found for camel milk IgG and albumin is similar to the heat sensitivity of their bovine counterparts.

Opposite results have been recorded for the ability of camel milk to withstand high processing temperatures. The time required to induce coagulation at a given temperature (heat coagulation time, HCT) has been compared for the bovine, ovine, caprine and camelid milk by Farah & Atkins (1992). Within the four species camel milk has the greatest heat sensitivity. However, numerous inter-related factors influence the heat stability of milk such as pH, concentration of salts (calcium and phosphate) and concentration of proteins (reviewed by Singh & Creamer, 1992). In bovine milk, the interaction between heat denatured β-lg and κ-casein via disulphide bonds is responsible for the influence of pH on the sensitivity of milk to heat treatment. This sensitivity is controlled by the proportion of κ-casein and β-lg. In camel milk, the high heat sensitivity can be related to its low κ-casein concentration. Moreover, β-lg, the predominant bovine whey protein has not been described in camel milk.

Our results clearly show that the denaturation of the camel soluble proteins in heated milk is markedly influenced by the IgG concentration. In early milks, the higher heat sensitivity of α-la could be related to its co-precipitation with aggregates of denatured IgG or by an increased interaction with the denatured IgG via intermolecular disulphide bonds. High IgG levels have been observed in bovine milk adulterated with colostrum or early milk and create several problems such as reduced heat stability, low yield of cheese production, weak curd formation and poor curd characteristics (Feagan, 1979; Zawitowski & Mackinnon, 1993). These problems have also been observed with goat and sheep milk and the measurement of IgG concentration is officially done in France for the payment of goat milk for its protein quality. In the camelids, the quantity of colostral milk is usually very small and exclusively suckled by newborn camel calves. However, very high IgG levels have been observed in camel colostrum (Bravo et al. 1997; Merin et al. 2001b) and throughout the first week of lactation (unpublished data) and the possibility of abnormal IgG concentrations in consumed milk cannot be totally excluded.

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