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Proteomic profiling of camel and cow milk proteins under heat treatment

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ABSTRACT

Cow and camel milk proteins before and after heat treatment at 80 °C for 60 min were identified using LC/MS and LC–MS/MS following monodimensional electrophoresis. The database used for the identification of camel and cow proteins was set from http://www.uniprot.org/. The obtained results showed that, after heating, camel milk at 80 °C for 60 min, camel α-lactalbumin (α-la) and peptidoglycan recognition protein (PGRP) were not detected while camel serum albumin (CSA) was significantly diminished. When heating cow milk at 80 °C for 60 min, α-lactalbumin (α-la) and β-lactoglobulin (β-lg) were not significantly detected. Moreover, 19 protein bands from SDS-PAGE were analyzed and a total of 45 different proteins were identified by LC–MS/MS. Casein fractions were kept intact under a heat treatment of 80 °C during 60 min of both camel and cow milks. Camel and bovine whey proteins were affected by a heat treatment of 80 °C for 60 min.

1. Introduction

Cow milk is composed of all nutrient components, mainly proteins, fat, lactose and minerals. The protein fraction of bovine milk consists essentially of whey proteins, mainly β-lactoglobulin (β-lg), α-lactalbumin (α-la), immunoglobulins (Ig) and bovine serum albumin (BSA), and caseins, assembled in micelles and accounting for about 80% of the total bovine milk protein content (Dupont, Croguennec, Brodkorb, & Kouaouci, 2013). Heat treatment is included in most dairy industries to obtain bacteriologically safe final products and to extend their shelf life. A number of structural modifications have been recognized in the milk protein component depending on time, temperature and rate of heating. Singh (1995) showed that a range of large heterogeneous protein aggregates of milk proteins occurred in heat-treated milk. The heat-induced milk protein association occurring under different heating conditions has been extensively investigated (Donato & Guyomarc'H, 2009). Previous studies have shown that both caseins and whey proteins are engaged in protein aggregates found in heat-treated milk and that the formation of intermolecular disulfide bonds is mostly responsible for heat-induced protein association in milk (Manzo, Nicolai, & Pizzano, 2015). The thermal protein denaturation has been acknowledged as the primary step of the reactions leading to the aggregation of disulfide-linked milk proteins. Thiol groups of cysteine residues, appearing in unfolded proteins, can initiate thiol-disulfide exchange reactions within hydrophobically-linked protein aggregates. Self-aggregation of heat-denatured β-lg in water (Roefs & De Kruif, 1994), heat-induced association of whey proteins and/or their aggregates with caseins (Corredig & Dalgleish, 1999) have been explained according to this mechanism.

Camel milk is an important nutrition source for inhabitants in arid and semi-arid areas (Farah, 1996). Camel milk has been shown to have nutritional and therapeutic properties which are widely exploited for human health in several countries (Mal, Sena, Jain, & Sahani, 2006). It contains higher amounts of essential fatty acids and antimicrobial agents compared to other species' milk (Shamsia, 2009). The main components of whey proteins in camel milk are similar to those in bovine, except for the lack in β-lg. Currently, most of camel milk is consumed in the fresh state. Therefore, to extend its shelf life, different heat treatments such as pasteurization may be applied to camel milk. However, heat processing as a means of preserving milk is applied to camel milk in some regions, mainly in gulf countries and in Central Asia, and up to now only a few studies have investigated the effect of heat treatment on camel whey proteins (El-Agamy, 2000; Farah, 1986). Recently, Felfoul et al. (2016) have investigated the deposit generation during camel and cow milk heating and evaluated the microstructure and the chemical composition of the obtained

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deposits. They have demonstrated that the deposit obtained after heating camel milk at 80 °C for 60 min contained 57% *w/w* proteins and 35% *w/w* minerals. Proteomic techniques are used to obtain information about the changes in the protein fraction of heat-treated milk. Although not many studies have examined non-bovine milks using the proteomic approach (Hinz, O'Connor, Huppertz, Ross, & Kelly, 2012; Pappa et al., 2008), to the best of our knowledge, a few data are available in the literature on camel milk (Zhang et al., 2016).

In the present work, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) were used to evaluate the fundamental differences in proteins aggregation after heat treatment of camel and cow milks. This study also aims to identify camel and cow proteins involved in the formation of the aggregates in both heat treated camel and cow milks.

2. Materials and methods

2.1. Milk samples

Camel milk was collected from reared camels by been directly milked into a sterile milking can. The camel milk used was pooled milk samples assembled from 20 different healthy camels (*Camelus dromedarius*) that ranged between 2 and 12 months in lactation. Cow milk was collected from 20 different Holstein cows. Both milk samples were derived from a local breeding located in the south of Tunisia. The milk samples were immediately transported to our laboratory using an ice box within 4 h. Once reaching the laboratory at 4 °C, pH (744-pH meter, Metrohm SA CH-9101, Herisau, Switzerland) was determined. Subsequently, both milks were skimmed by centrifugation at 3000*g* during 20 min at 4 °C (Gyrozen 1580MGR, Multi-purpose Centrifuge, Daejeon, Korea).

2.2. Heat treatment experiments

Heat treatment experiments of camel and bovine milks were conducted in a stainless steel recipient (316L; Total volume = 500 mL) at 80 °C for 60 min. Heat treatment consisted in heating over a hot plate without agitation (Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015). The experiments were reproduced at least 3 times.

2.3. LC/MS analysis

100 μL of fresh and heat-treated skimmed milk samples were diluted in 400 μL buffer Urea 4 M/Tris 25 mM pH8, and then placed at 37 °C for 1 h 15 min in a water bath with regular manual agitation. All solutions were diluted 2 × in 0.5% TFA solution, afterwards, filtered through Millipore® Millex®filters HVPVDF membrane, 0.45 μm Pore Size before injection.

Mass spectrometry (MS) experiments were performed using an on-line liquid chromatography mass spectrometry setup *via* an Agilent 1100 HPLC system coupled to an AB Sciex QSTAR XL Quadrupole-Time Of Flight (QTOF) mass spectrometer. The separation of proteins was performed with a separation column C4 VYDAC Grace, reference 214TP5215, 150 mm length × 2.1 mm inner diameter (i.d.) using solvent A (0.106% TFA in deionized water) and solvent B (80% (v/v) acetonitrile and 0.1% (v/v) TFA in deionized water). A linear gradient from 37 to 60% of solvent B in 40 min was applied for the elution at a flow rate of 0.25 mL/min. Protein signal was recorded by both UV detection at 214 nm wavelength and electrospray mass spectrometry. Eluted proteins were directly electrosprayed into the mass spectrometer operated in positive mode. Mass spectra were collected in the selected mass range from 800 to 3000 Thomson. The instrument was calibrated by multipoint calibration using fragment ions that resulted from the collision-induced decomposition of a peptide from β-casein, β-CN (193–209). The quantification data of the studied camel and cow milk protein fractions (expressed in μg/μL) was estimated basing on the peak areas of the chromatographic profiles as well as both milks compositional data. Measurements were performed in duplicate with independent samples.

2.4. SDS-PAGE

Skimmed camel and cow milks, before and after heat treatment at 80 °C for 60 min (fresh camel, heat-treated camel, fresh cow and heat-treated cow milks), were diluted with deionized water. Protein concentrations of skimmed milks were determined by dint of the Bradford method (1976) and adjusted to be the same among samples equal to 5 g/L. The proteins were then separated by polyacrylamide gel electrophoresis containing 0.1% sodium dodecyl sulphate (SDS-PAGE) in non-reducing conditions. Electrophoresis experiments were carried out using a Bio-Rad apparatus (Mini-Protean Tetra Cell, Bio-Rad Laboratories, Hercules, USA) of gels in vertical slabs. 50 μg of protein was loaded for each sample onto 12% acrylamide resolving gel. As described by Laemmli (1970), electrophoresis was run at 120 V/20 mA until the marker color (bromophenol blue, Sigma-Aldrich Chemie S.a.r.l., Saint-Quentin Fallavier, France) was 0.5 cm from the anode end of the block (approximately 3 h). The molecular weight of the different protein fractions were estimated by comparing their electrophoretic mobilities with those of marker proteins having known molecular weights. The electrophoresis experiment was repeated 3 times. All gels were photographed and the most significant one was presented.

2.5. Protein bands identification by tandem mass spectrometry

After separation by SDS-PAGE, the proteins were identified by mass spectrometry (MS) after an in-gel trypsin digestion according to Shevchenko, Wilm, Vorm, and Mann (1996). Gel pieces were excised from the gel, placed in eppendorf tubes of 0.5 mL, washed with 100 μL acetonitrile and NH_4HCO_3 solution (50%/50%) 1–3 times based on their coloration intensity. Subsequently, the supernatants were removed using a small benchtop centrifuge. Finally, the gel pieces were dried under vacuum in a SpeedVac concentrator (SVC100H-200; Savant, Thermo Fisher Scientific, Waltham, MA) for 15 min. In-gel trypsin digestion was performed overnight at 37 °C and stopped with spectrophotometric-grade trifluoroacetic acid (TFA) (Sigma-Aldrich Chemie S.a.r.l., Saint-Quentin Fallavier, France). The supernatants containing peptides were then vacuum dried in a SpeedVac concentrator for mass spectrometry analysis.

Mass spectrometry (MS) experiments were performed by means of an on-line liquid chromatography tandem mass spectrometry (MS/MS) setup using a Thermo Scientific™ Dionex™ Ultimate™ 3000 RSLC nano-liquid chromatography (nano-LC) system fitted to a Qexactive (Thermo Scientific™, San Jose, USA) equipped with a nano-electrospray ion source (ESI) (Proxeon Biosystems A/S, Odense, Denmark). The instrument was externally calibrated according to the supplier's procedure. The samples were first concentrated on a PepMap 100 reverse-phase column (C18, 5 μm, 300-μm i.d by 5-mm length) (Dionex, Amesterdam, The Netherlands). Peptides were separated on a reverse-phase PepMap column at room temperature, using solvent A (2% (v/v) acetonitrile, 0.08% (v/v) formic acid, and 0.01% (v/v) TFA in deionized water) and solvent B (95% (v/v) acetonitrile, 0.08% (v/v) formic acid, and 0.01% (v/v) TFA in deionized water). A linear gradient from 5 to 50% of solvent B in 10 min was applied for the elution at flow rate of 0.3 μL/min. Eluted peptides were directly electrosprayed into the mass spectrometer operated in positive mode. A full

continuous MS scan was carried out followed by ten data-dependent MS/MS scans. The spectra were collected in the selected mass range from 250 to 2000 *m*/*z* for MS spectra.

The peptides were identified from MS/MS spectra using the X! Tandem pipeline software (Plateforme d'Analyse Protéomique de Paris Sud-Ouest, INRA, Jouy-en-Josas, France, http://pappso.inra.fr). The peptide identification database used was a concatenation of the *Camelus* (Taxon identifier 9836) taxonomy and the *Bos Taurus* (Taxon identifier 9913) reference proteome set from http://www.uniprot.org/. Search parameters were adjusted as follows : a trypsin enzyme cleavage was used with a maximum number of missed cleavage set to 1, the mass tolerance was set to 10 ppm for MS and 0.05 Da for MS/MS spectra, and 4 variable modifications (oxidation of methionine, deamidation of asparagines and glutamine, phosphorylation of serines and lactosylation of lysine and arginine residues) were selected. For each identified protein, it was necessary to obtain a minimum of three peptides with an X! Tandem *e*-value below 0.01 for validation with a high degree of confidence.

3. Results and discussion

3.1. LC/MS of camel and cow proteins composition before and after heat treatment

The camel and cow proteins composition in fresh and heat treated cow (A) and camel (B) milks at 80 °C for 60 min are presented in Fig. 1. The different protein concentrations in fresh and heat-treated camel and cow milks at 80 °C for 60 min are pointed out in Table 1, as calculated from the integrated UV signal.

As illustrated in Fig. 1, α-la and β-lg constitute the major whey proteins in fresh cow milk (Fig. 1A). After heating cow milk at 80 °C for 60 min, several significant modifications in protein composition were observed as illustrated in Fig. 1A and Table 1. The concentrations of bovine $\alpha_{s1,2}$- and κ-caseins as well as β-casein A2 have not been significantly modified by heat treatment of cow milk ($p < 0.05$). As expected for cow whey proteins, peaks from α-la and β-lg were significantly diminished after the heat treatment of cow milk at 80 °C for 60 min (Fig. 1A) ($p < 0.05$). Bovine α-la and β-lg A concentrations were equal to 12 μg/μL in fresh cow milk (Table 1). Peak 9 in the heat-treated bovine milk is representative of the remaining denatured β-lg, either mono-meric or multimeric. 26% of β-lg (summing variant A, B and dimers) remained after heating while 100% of bovine α-la has disappeared in the heat-treated cow milk (Fig. 1A). It is well known that when exposed to heat treatment (80 °C), unfolded β-lg (monomeric form) can associate with other particles (de Jong, 1997; Petit, Six, Moreau, Ronse, & Delaplace, 2013). Besides, with heating, the amount of monomeric β-lg decreases and the aggregates of various sizes were also formed, i.e., dimers, trimers, oligomers and polymers (Kim, Cornec, & Narsimhan, 2005; Moro, Báez, Busti, Ballerini, & Delorenzi, 2011) which confirms the results found in this study where the decrease of β-lg concentrations was noted against the increase of a new peak of β-lg multimers. Moro et al. (2011) have demonstrated that under heating at 85 °C, the quantities of β-lg dimers and trimers decreased and the amount of aggregates larger than trimers increased.

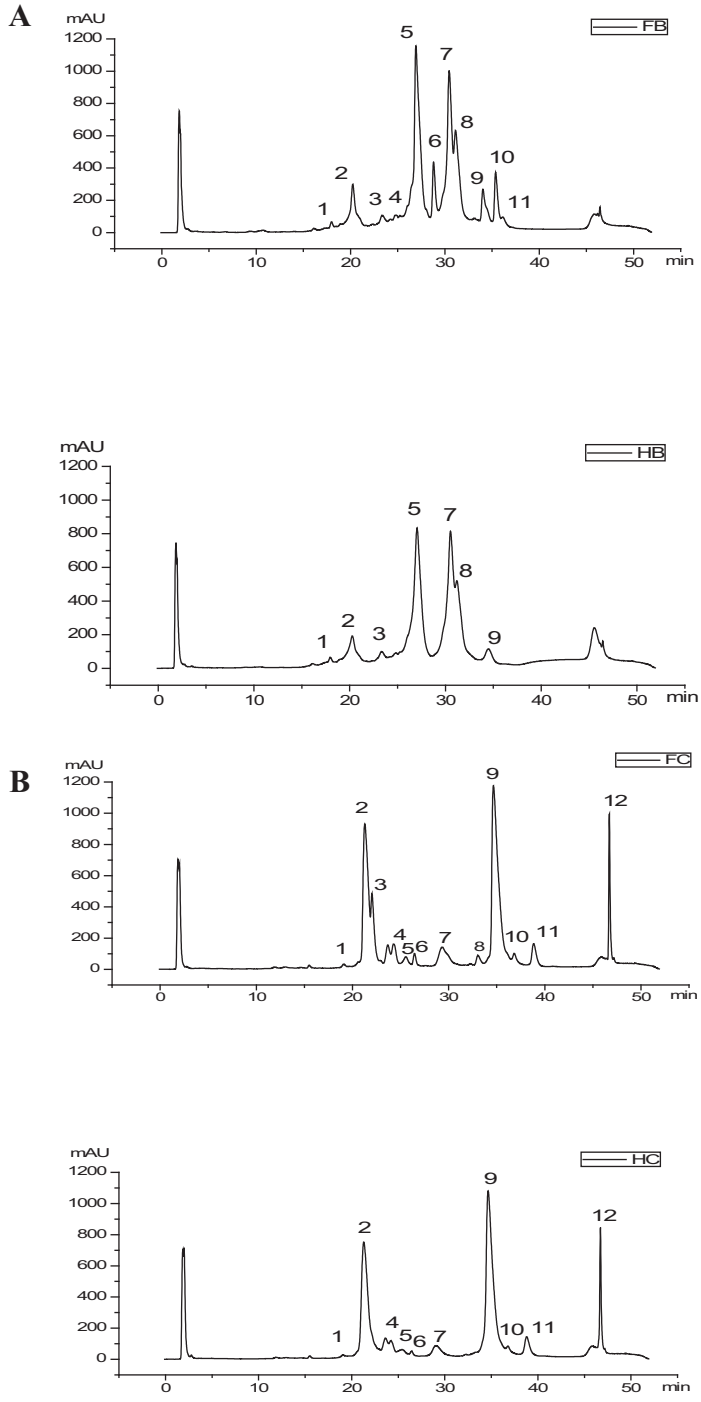
Otherwise, fresh camel milk contains α-la, peptidoglycan recognition protein (PGRP), serum albumin (CSA) and casein proteins as major peaks. In the same way as cow milk, the heat treatment of camel milk at 80 °C for 60 min (Fig. 1B) also caused various significant modifications in protein composition. Table 1 indicates that camel α_{s2}-, β- and γ-caseins concentrations have not been significantly modified by heat treatment ($p < 0.05$). The major camel whey proteins (α-la, CSA and PGRP) were significantly affected by heat treatment ($p < 0.05$) which is in accordance with recently reported results (Zhang et al., 2016). The corresponding peak of CSA decreased significantly after heating at 80 °C for 60 min while camel α-la and PGRP disappeared from the chromatograms (peaks 3 and 6) ($p < 0.05$). Indeed, the initial concentration of CSA in fresh camel milk was 13 μg/μL. From UV integration, a 42% decrease of this protein was observed after heating camel milk at 80 °C for 60 min (Table 1). Furthermore, α-la and PGRP contents were 17 and 3 μg/μL in fresh camel milk, respectively. After heating camel milk at 80 °C for 60 min, 100% of α-la disappeared from the chromatogram while PGRP concentration drastically decreased by 68%. As illustrated in Fig. 1B and Table 1, the most heat-sensitive whey protein in camel milk obviously corresponds to α-la which confirms the results found in our recent study (Felfoul et al., 2015). These results showed that the order of heat sensitivity for camel milk proteins is as follows: α-la < PGRP < CSA. These results were not consistent with those reported by Levieux, Levieux, El-Hatmi, and Rigaudière (2006). A similar secondary structure for camel and bovine α-la was reported from circular dichroism experiments in unheated milk (Atri et al., 2010), which may explain their comparable decrease after heating at 80 °C for 60 min, in this study. However, El-Agamy (2000) proved that CSA was not affected after heating camel milk at 75 °C for 30 min. Levieux et al. (2006) have demonstrated that after heating camel milk at 90 °C for 10 min, 55% of α-la and only 9% of CSA remain undenatured by the single radial immunodiffusion assay. On the other hand, these authors have reported that upon heating camel milk at 80 °C and 75 °C for 10 min, only 37% of residual native CSA is quantified by the single radial immunodiffusion assay. They have also confirmed that purified CSA and α-la are denatured at 73 and 77.5 °C, respectively.

3.2. LC–MS/MS of camel and cow milk proteins bands

Milk sample proteins were separated onto electrophoresis gel. Afterwards, in-gel tryptic digestion was applied and the protein bands were identified by LC–MS/MS analysis. As a result, differences in milk protein composition, after heating camel and cow milks at 80 °C for 60 min, were observed. There were clear differences between the electrophoretograms of fresh bovine milk and fresh camel milk (Fig. 2). In fact, the most abundant proteins of bovine milk were the caseins, α_{s1}-, α_{s2}-, β- and κ-caseins and the whey proteins β-lg, α-la and BSA. Otherwise, the major proteins of camel milk were α_{s1}-, α_{s2}-, β- and κ-caseins and for camel whey proteins, CSA, PGRP and α-la. β-lg was not detected in camel milk (Fig. 2), which is in agreement with previous reports (Felfoul et al., 2015; Kappeler, Farah, & Puhan, 2003).

From Fig. 2, a notable difference can be observed between fresh and heat-treated camel and cow milks samples. Actually, the visual observation of the SDS-PAGE gel patterns shows that the bands corresponding to whey proteins decreased or disappeared after heating. However, those of caseins were kept intact which confirms the results obtained in Section 3.1. This tendency is noted for both camel and cow milks samples. Besides, as illustrated in Fig. 2, new bands of high molecular weight were observed on the gel after heating camel and cow milks. These results suggest that heating both milks at 80 °C for 60 min induced significant physicochemical and structural changes in milks proteins.

Mass spectrometry was performed on the tryptic digests of protein bands excised from this gel to identify the 40 relevant bands highlighted in Fig. 2 (results included in the Supplementary data). Based on the results found in Section 3.1 and because of the large number of the obtained data, we will be limited to follow how BSA, β-lg and α-la for cow milk become and in the same way for CSA, PGRP and α-la regarding camel milk (19 bands). A total of 45 different proteins were successfully identified by mass spectrometry



Peak number	Mass measured (Da)	Hypothesized protein identification
1	19036	κ-CN
2	25227	α_{s2}-CN
3	-	N.D
4	11169	-
5	23616	α_{s1}-CN
6	14179	α-la
7	24024	β-CN A_1
8	23983	β-CN A_2
9	18278	β-lg B
10	18364	β-lg A
11	36642	Dimers of β-lg

Peak number	Mass measured (Da)	Hypothesized protein identification[a]
1	5436.7	Monoisotopic
2	24770	α_{s1}-CN
3	14423	α-la
4	22065, 22145	α_{s2}-CN + 10P, α_{s2}-CN +11P
5	-	N.D
6	19138	PGRP
7	66600	CSA
8	-	N.D
9	24972	β-CN +4P
10	17825	-
11	25699	-
12	13902	γ-CN

[a] P = Phosphorylation

Fig. 1. HPLC-UV chromatograms recorded at 214 nm for A) fresh and heated (80 °C/60 min) bovine (FB, HB) milk and B) fresh and heated (80 °C/60 min) camel (FC, HC) milk. Mass spectrometry measurement of labeled peaks are indicated. Peaks with no mass measured have not been identified by LC/MS.

after trypsin hydrolysis. These proteins were sorted by the *e*-value to highlight the most confidently identified proteins in each of the 19 relevant bands (Table 2).

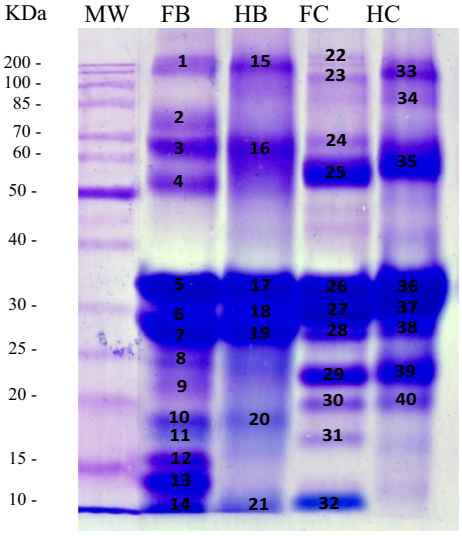
It is clear from Table 2 that band 4 contains 13 proteins among which BSA, lipoprotein lipase, κ- and $\alpha_{s1,2}$-caseins, with BSA on the top. After heat treatment, no band corresponding to BSA was observed. This protein has completely disappeared in the heat-treated cow milk at 80 °C for 60 min. Indeed, as shown in Table 2, bands 15 and 16 were both identified as mixtures of 4 casein proteins and β-lg. In this respect, Rüegg, Morr, and Blanc (1977) have studied the denaturation mechanism of whey proteins in simulated cow milk ultrafiltrate and demonstrated that BSA is the most heat sensitive bovine whey protein.

Bands 12 and 13 corresponded to β-lg based on the theoretical molecular weight value as indicated in Fig. 2. The identification by LC–MS/MS analysis showed that these two bands contained mixtures of proteins, including β-lg as the major protein with very low *e*-value, high sequence coverage and high number of identified peptides. After heating cow milk at 80 °C for 60 min, no band was observed on the gel patterns corresponding to β-lg. However, β-lg existed in bands 15, 16, 17, 18, 19 and 21 for heat-treated cow milk with high sequence coverage, 53, 36, 40, 19, 30 and 40%,

Table 1
Protein concentrations as measured from HPLC-UV (214 nm) peak integration for camel and cow milks before and after heat treatment at 80 °C for 60 min.

	Peaks	Proteins	Before heating			After heating		
			Area (AU*s)	Proportion (%)	Concentration (μg/μL)	Area (AU*s)	Proportion (%)	Concentration (μg/μL)
Cow milk	1	κ-CN	0.3 ± 0.03	0.2 ± 0.03	1 ± 0.03[a]	0.9 ± 0.02	0.7 ± 0.02	2 ± 0.02[a]
	2	α_{S2}-CN	10.9 ± 0.01	7.8 ± 0.01	17 ± 0.01[a]	8.9 ± 0.01	6.9 ± 0.01	15 ± 0.01[a]
	5	α_{S1}-CN	47.9 ± 0.04	34.4 ± 0.04	74 ± 0.04[a]	44.4 ± 0.02	34.5 ± 0.02	76 ± 0.02[a]
	6	α-la	7.5 ± 0.19	5.4 ± 0.19	12 ± 0.19[b]	0 ± 0.00	0 ± 0.00	0 ± 0.00[a]
	7	β-CN A_1	35.8 ± 0.01	25.7 ± 0.01	56 ± 0.01[a]	37.6 ± 0.01	29.3 ± 0.01	64 ± 0.01[b]
	8	β-CN A_2	21.2 ± 0.00	15.2 ± 0.00	33 ± 0.00[a]	21.8 ± 0.01	17.0 ± 0.01	37 ± 0.01[a]
	9	β-lg B	6.4 ± 0.10	4.6 ± 0.10	10 ± 0.10			
	10	β-lg A	7.8 ± 0.10	5.6 ± 0.10	12 ± 0.10 8 ± 0.10[a]	15.0 ± 0.00	8.5 ± 0.00	19 ± 0.00[b]
	11	Dimers of β-lg	1.5 ± 0.10	1.1 ± 0.10	2 ± 0.10			
Camel milk	2	α_{S1}-CN	34.8 ± 0.01	28.0 ± 0.01	57 ± 0.01[a]	37.9 ± 0.02	33.7 ± 0.02	67 ± 0.02[b]
	3	α-la	10.3 ± 0.12	8.3 ± 0.12	17 ± 0.12[b]	0 ± 0.00	0 ± 0.00	0 ± 0.00[a]
	4	α_{S2}-CN	3.9 ± 0.01	3.1 ± 0.01	6 ± 0.01[a]	2.1 ± 0.01	1.9 ± 0.01	4 ± 0.01[a]
	6	PGRP	1.2 ± 0.10	1.3 ± 0.10	3 ± 0.10[b]	0.5 ± 0.23	0.4 ± 0.23	1 ± 0.23[a]
	7	CSA	8.2 ± 0.15	6.6 ± 0.15	13 ± 0.15[b]	4.7 ± 0.19	4.2 ± 0.19	8 ± 0.19[a]
	9	β-CN	55.6 ± 0.01	44.8 ± 0.01	91 ± 0.01[a]	57.1 ± 0.01	50.8 ± 0.01	102 ± 0.01[a]
	12	γ-CN	1.0 ± 0.00	8.0 ± 0.00	16 ± 0.00[a]	10.1 ± 0.00	9.0 ± 0.00	18 ± 0.00[a]

Averages ± Standard deviation (SD) of three replicates. [a–b] Values within the same row with different superscripts differed significantly by Duncan's multiple-range test ($p < 0.05$).



Band	Identified protein*	Band	Identified protein*
1	Complement C3	21	α_{S1}-CN
2	LTF	22	XDO
3	PIgR	23	Uncharacterized protein
4	BSA	24	LF
5	α_{S1}-CN	25	CSA
6	κ-CN	26	α_{S1}-CN
7	κ-CN	27	β-CN
8	α_{S1}-CN	28	α_{S2}-CN
9	Uncharacterized protein	29	GLYCAM1
10	α_{S2}-CN	30	GLYCAM1
11	α_{S1}-CN	31	PGRP
12	β-lg	32	α-la
13	β-lg	33	α_{S1}-CN
14	α-la	34	α_{S2}-CN
15	α_{S1}-CN	35	α_{S1}-CN
16	α_{S2}-CN	36	α_{S1}-CN
17	α_{S1}-CN	37	β-CN
18	α_{S1}-CN	38	α_{S2}-CN
19	κ-CN	39	GDCAM
20	α_{S2}-CN	40	α_{S1}-CN

*Identified protein from the band based with the best *e*-value.
LTF : Lactotransferrin ; PIgR : Polymeric immunoglobulin receptor ; BSA : Bovine serum albumin ; XDO : Xanthine dehydrogenase/oxidase ; LF : Lactoferrin ; CSA : Camel serum albumin ; GLYCAM1 : Glycosylation-dependent cell adhesion molecule 1 ; PGRP :Peptidoglycan recognition protein 1.

Fig. 2. SDS-PAGE separation of proteins from fresh and heated (80 °C/60 min) bovine (FB, HB) and camel (FC, HC) milks. Labeled bands were further in-gel digested with trypsin before identification of peptides by LC/MS/MS.

respectively. Bands 15, 16, 17, 18 and 19 contained mixtures of $\alpha_{s1,2}$-, β- and κ-caseins and β-lg (Table 2). In addition to the previously mentioned proteins, band 21 contained α-la. This result validates those reported earlier by several researchers. In a previous investigation (Donato & Guyomarc'H, 2009), it has been indicated that once unfolded due to heat treatment, β-lg, irreversibly denature.

Bovine α-la existed in band 14 for fresh cow milk. The quantity of band 14 exhibited a clear decline with heating (band 21, Fig. 2). Indeed, α-la is present only in band 21 for heat-treated cow milk, with a sequence coverage of 52% and 9 identified peptides, however, it was not the protein with the best *e*-value for this band. This could justify the decreasing of the α-la band after heat treatment of cow milk. Jovanović, Barać, and Maćej (2005) have demonstrated that at high temperature, β-lg denatures first then interacts with κ-casein at the micelle surface. The newly-formed micelle surface is ragged with numerous filaments originated from β-lg. At a higher temperature of 90 °C for 10 min, α-la denatures and binds to the filaments of β-lg, thus filling "gaps" on the micelle surface, which is converted from ragged into regular spherical form.

Regarding camel milk, Table 2 shows that band 25 consists of 18 proteins with CSA having the best *e*-value with 44 identified peptides. After heating, this band disappeared from the gel patterns (Fig. 2). In fact, band 35 contains $\alpha_{s1,2}$-, β- and κ-caseins, PGRP, lactadherin-like protein, L-amino-acidoxidase and glycosylation-dependant cell adhesion molecule 1 as detailed in Table 2. This band does not contain CSA, which endorses the decrease in the concentration of this protein when heating camel milk at 80 °C for 60 min (Fig. 1B). Furthermore, CSA existed only in band 34 in heat-treated camel milk with low *e*-value, sequence coverage and number of identified peptides. Table 2 revealed that after heating cow milk at 80 °C for 60 min, BSA has completely disappeared in the heat-treated bovine milk while CSA still existed in the heat-treated milk after heating camel milk at 80 °C for 60 min.

Table 2
Proteins identified by proteomic analysis following by an in-gel tryptic digestion before and after heating camel and cow milks at 80 °C for 60 min. Band number refers to the gel image visible on Fig. 2.

Band	Accession	Protein name	Log (*e*-value)	Coverage (%)	MW (kDa)	No. of identified peptides	PAI
4	P02769	Serum albumin	−387.40	75	69.1	56	6.8
	P02668	Kappa-casein	−137.25	84	21.2	24	16.2
	P11151	Lipoprotein lipase	−69.55	29	53.2	10	1.4
	P02663	Alpha-S2-casein	−56.79	45	25.9	13	8.1
	P02662	Alpha-S1-casein	−55.01	38	24.4	8	1.4
	G5E5H7	Uncharacterized protein	−45.98	68	19.8	7	1.6
	P24627	Lactotransferrin	−26.54	11	77.9	6	0.2
	P34955	Alpha-1-antiproteinase	−26.35	17	46.0	6	0.5
	G3N0V0	Uncharacterized protein (Fragment)	−25.33	19	35.8	7	1.6
	P02666	Beta-casein	−22.44	19	25.0	3	3.0
	A2I7N3	Serpin A3-7	−18.80	11	46.8	4	0.3
	P08037	Beta-1,4-galactosyltransferase 1	−19.71	12	44.7	3	0.4
	Q2UVX4	Complement C3	−16.50	5	187.0	3	0.1
12	P02754	Beta-lactoglobulin	−100.90	79	19.8	19	6.2
	P02663	Alpha-S2-casein	−31.14	42	25.9	9	2.1
	P02662	Alpha-S1-casein	−24.20	24	24.4	4	0.9
	P02668	Kappa-casein	−24.15	36	21.2	6	2.0
	Q8SPP7	Peptidoglycan recognition protein 1	−23.91	36	21.0	5	1.0
	P00711	Alpha-lactalbumin	−17.05	31	16.2	4	1.3
	Q58DP6	Ribonuclease 4	−15.63	41	16.9	4	1.3
	P10152	Angiogenin-1	−13.39	30	16.9	4	1.0
	P80195	Glycosylation-dependent cell adhesion molecule 1	−12.52	27	17.1	3	0.6
13	P02754	Beta-lactoglobulin	−217.52	87	19.8	35	18.2
	P02663	Alpha-S2-casein	−40.32	38	25.9	10	2.2
	P02662	Alpha-S1-casein	−35.46	26	24.4	6	0.9
	P02668	Kappa-casein	−33.72	39	21.2	8	2.2
	P02666	Beta-casein	−24.30	18	25.0	3	4.3
	G3MZ19	HRPE773-like	−17.47	34	17.5	4	0.4
	P00711	Alpha-lactalbumin	−15.90	23	16.2	3	0.8
	P10790	Fatty acid-binding protein, heart	−15.35	26	14.7	3	0.3
	P10152	Angiogenin-1	−14.08	38	16.9	4	0.7
	F1N1Z8	Uncharacterized protein (Fragment)	−13.33	20	22.0	4	0.4
	P62803	Histone H4	−11.62	42	11.3	4	1.0
14	P00711	Alpha-lactalbumin	−72.71	64	16.2	12	7.8
	P02754	Beta-lactoglobulin	−62.31	68	19.8	9	3.6
	P02666	Beta-casein	−37.05	34	25.0	7	10.5
	P10790	Fatty acid-binding protein, heart	−28.91	51	14.7	5	0.8
	P02663	Alpha-S2-casein	−26.78	30	25.9	8	1.6
	P61626	LYSC_HUMAN	−21.14	34	16.5	3	0.4
	P02662	Alpha-S1-casein	−19.78	21	24.4	5	0.6
	P01888	Beta-2-microglobulin	−12.81	18	13.6	3	1.3
	P02668	Kappa-casein	−12.45	20	21.2	3	0.8
	P62803	Histone H4	−9.08	30	11.3	3	0.8
15	P02662	Alpha-S1-casein	−131.29	73	24.4	24	8.5
	P02668	Kappa-casein	−112.08	64	21.2	19	10.8
	P02663	Alpha-S2-casein	−98.57	65	25.9	20	9.2
	P02666	Beta-casein	−46.05	48	25.0	8	12.0
	P02754	Beta-lactoglobulin	−45.23	53	19.8	9	1.9
16	P02663	Alpha-S2-casein	−113.69	68	25.9	25	11.6
	P02662	Alpha-S1-casein	−98.96	66	24.4	18	8.2
	P02668	Kappa-casein	−67.04	59	21.2	13	5.8
	P02666	Beta-casein	−48.50	52	25.0	7	12.3
	P02754	Beta-lactoglobulin	−33.14	36	19.8	6	0.9
17	P02662	Alpha-S1-casein	−263.27	80	24.4	41	25.7
	P02663	Alpha-S2-casein	−135.28	65	25.9	26	13.7
	P02668	Kappa-casein	−55.50	51	21.2	9	4.0
	O97943	Alpha-S1-casein	−38.20	48	26.8	8	0.9
	P02666	Beta-casein	−36.89	40	25.0	6	7.5
	P02754	Beta-lactoglobulin	−29.45	40	19.8	6	0.8
	Q9TVD0	Beta-casein	−22.97	30	26.1	4	1.8
18	P02662	Alpha-S1-casein	−103.87	65	24.4	16	8.5
	P02666	Beta-casein	−103.25	67	25.0	18	25.5
	P02668	Kappa-casein	−87.13	62	21.2	17	6.3
	P02663	Alpha-S2-casein	−83.71	60	25.9	16	7.7
	P80195	Glycosylation-dependent cell adhesion molecule 1	−20.49	27	17.1	4	1.0
	P02754	Beta-lactoglobulin	−17.33	19	19.8	3	0.4
19	P02668	Kappa-casein	−160.34	77	21.2	27	19.7
	P02666	Beta-casein	−111.80	78	25.0	19	27.3
	P02662	Alpha-S1-casein	−71.36	47	24.4	10	4.2
	P02663	Alpha-S2-casein	−44.80	43	25.9	11	2.8
	P80195	Glycosylation-dependent cell adhesion molecule 1	−24.34	38	17.1	4	1.7
	P02754	Beta-lactoglobulin	−18.23	30	19.8	3	0.7

Table 2 (*continued*)

Band	Accession	Protein name	Log (*e*-value)	Coverage (%)	MW (kDa)	No. of identified peptides	PAI
21	P02662	Alpha-S1-casein	−48.01	44	24.4	8	1.8
	P00711	Alpha-lactalbumin	−45.58	52	16.2	9	3.8
	P02666	Beta-casein	−43.10	33	25.0	8	10.8
	P02754	Beta-lactoglobulin	−32.09	40	19.8	7	1.2
	P18892	Butyrophilin subfamily 1 member A1	−24.93	15	59.1	6	0.4
	P02663	Alpha-S2-casein	−22.15	27	25.9	6	1.0
	P61626	LYSC_HUMAN	−19.58	34	16.5	3	0.6
	P02668	Kappa-casein	−14.07	41	21.2	4	2.8
25	S9WI87	Serum albumin	−316.68	27	125.0	44	2.8
	S9Z0L8	L-Amino-acidoxidase	−196.37	67	55.3	26	2.7
	O97944	Alpha-S2-casein	−107.37	49	22.9	15	9.6
	S9X1L5	Lipoprotein lipase isoform 3 (Fragment)	−76.73	37	46.5	11	1.4
	S9X3X3	Butyrophilin subfamily 1 member A1	−65.18	37	51.1	11	0.8
	L0P3Z7	Kappa casein	−61.95	49	20.3	11	6.6
	W6GH05	Lactoferrin	−58.88	24	77.1	12	0.3
	S9WF76	Lactadherin-like protein	−46.99	21	56.2	8	0.5
	P02662	Alpha-S1-casein	−30.58	40	24.4	4	0.4
	P02754	Beta-lactoglobulin	−30.25	39	19.8	5	1.0
	S9W7K0	Uncharacterized protein	−29.77	30	28.3	6	0.7
	Q9TVD0	Beta-casein	−29.19	27	26.1	4	1.4
	O97943	Alpha-S1-casein	−27.89	29	26.8	6	0.7
	S9WCV2	Sulfhydryloxidase	−26.45	11	72.2	5	0.2
	S9YC53	Alpha-1-antitrypsin-like protein	−23.15	12	51.9	4	0.3
	S9XT33	Lipopolysaccharide-binding protein	−12.69	7	47.5	3	0.3
	S9X358	Tissue alpha-L-Fucosidase	−8.90	8	42.7	3	0.2
	S9WZP7	Serpin A3-8	−7.99	5	74.9	3	0.1
31	Q9GK12	Peptidoglycan recognition protein 1	−224.97	72	21.3	41	26.6
	P02662	Alpha-S1-casein	−36.39	28	24.4	6	1.0
	P02754	Beta-lactoglobulin	−34.73	47	19.8	7	1.6
	P02668	Kappa-casein	−26.74	40	21.2	5	1.2
	P84080	ADP-ribosylation factor 1	−26.07	43	20.6	5	0.8
	P02666	Beta-casein	−21.42	18	25.0	3	2.0
	O97944	Alpha-S2-casein	−20.91	25	22.9	5	0.7
	Q9TVD0	Beta-casein	−20.85	24	26.1	4	1.6
	P00710	Alpha-lactalbumin	−18.94	42	14.3	6	1.2
	P15522	Glycosylation-dependent cell adhesion molecule 1	−15.31	35	17.2	4	1.0
	S9X4G0	Neutrophilgelatinase-associated lipocalin-like protein	−13.66	16	28.3	3	0.2
	S9WVH2	Beta-2-microglobulin isoform 2-like protein	−13.37	42	13.6	3	1.2
	O97943	Alpha-S1-casein	−9.21	32	26.8	3	0.5
32	P00710	Alpha-lactalbumin	−186.39	83	14.3	27	18.0
	Q9GK12	Peptidoglycan recognition protein 1	−41.74	40	21.3	3	1.2
	S9XVK5	Transthyretin	−34.49	56	16.0	4	0.5
	P15522	Glycosylation-dependent cell adhesion molecule 1	−31.15	35	17.2	6	1.1
	P61626	LYSC_HUMAN	−25.01	34	16.5	3	0.4
	O97943	Alpha-S1-casein	−23.50	26	26.8	6	0.6
	O97944	Alpha-S2-casein	−22.27	19	22.9	4	0.7
	S9XI30	Uncharacterized protein	−20.10	28	22.0	4	0.4
	P79139	Kappa-casein	−18.45	35	20.3	4	0.8
	P02754	Beta-lactoglobulin	−14.09	31	19.8	4	0.7
	P02668	Kappa-casein	−13.24	30	21.2	4	0.8
	S9XL94	C-C motif chemokine 23-like protein	−13.23	23	15.0	3	0.8
	S9WVH2	Beta-2-microglobulin isoform 2-like protein	−9.57	18	13.6	3	0.6
	P02663	Alpha-S2-casein	−7.76	11	25.9	3	0.4
33	O97943	Alpha-S1-casein	−105.09	69	26.8	19	5.8
	O97944	Alpha-S2-casein	−96.16	55	22.9	14	6.6
	Q9TVD0	Beta-casein	−42.91	48	26.1	8	9.0
	Q9GK12	Peptidoglycan recognition protein 1	−41.73	48	21.3	5	1.6
	L0P3Z7	Kappa casein	−41.19	49	20.3	9	4.2
	P02662	Alpha-S1-casein	−36.87	28	24.4	6	1.2
	F5BZ34	Milk fat globule EGF factor 8 (Fragment)	−22.39	30	32.7	6	0.5
	P02663	Alpha-S2-casein	−20.68	37	25.9	7	1.0
	P02666	Beta-casein	−20.55	34	25.0	4	2.0
	S9W7K0	Uncharacterized protein	−15.91	25	28.3	4	0.5
	P00710	Alpha-lactalbumin	−15.83	43	14.3	4	0.7
	S9Z0L8	L-Amino-acidoxidase	−13.36	19	55.3	4	0.3
	P15522	Glycosylation-dependent cell adhesion molecule 1	−11.21	28	17.2	3	0.4
34	O97944	Alpha-S2-casein	−103.95	49	22.9	14	6.7
	O97943	Alpha-S1-casein	−89.71	67	26.8	16	5.2
	Q9TVD0	Beta-casein	−55.17	51	26.1	10	8.2
	L0P3Z7	Kappa casein	−53.28	49	20.3	10	3.6
	Q9GK12	Peptidoglycan recognition protein 1	−35.73	48	21.3	6	1.4
	S9W7K0	Uncharacterized protein	−26.12	27	28.3	4	0.5
	F5BZ34	Milk fat globule EGF factor 8 (Fragment)	−25.06	25	32.7	6	0.5
	S9Z0L8	L-Amino-acidoxidase	−20.85	25	55.3	6	0.4
	P15522	Glycosylation-dependent cell adhesion molecule 1	−20.26	35	17.2	4	0.9

(*continued on next page*)

Table 2 (*continued*)

Band	Accession	Protein name	Log (*e*-value)	Coverage (%)	MW (kDa)	No. of identified peptides	PAI
	P00710	Alpha-lactalbumin	−12.26	37	14.3	3	0.7
	S9WI87	Serum albumin	−11.86	3	125.0	4	0.1
35	O97943	Alpha-S1-casein	−143.95	69	26.8	23	7.7
	O97944	Alpha-S2-casein	−115.75	54	22.9	16	10.8
	Q9TVD0	Beta-casein	−85.98	64	26.1	13	15.4
	L0P3Z7	Kappa casein	−63.25	50	20.3	13	6.2
	P02754	Beta-lactoglobulin	−37.60	59	19.8	7	1.1
	Q9GK12	Peptidoglycan recognition protein 1	−30.06	35	21.3	4	1.0
	S9WF76	Lactadherin-like protein	−27.82	18	56.2	6	0.3
	S9Z0L8	L-Amino-acidoxidase	−26.75	17	55.3	5	0.2
	P02662	Alpha-S1-casein	−22.85	20	24.4	4	0.5
	P02666	Beta-casein	−20.04	15	25.0	4	1.3
	P02663	Alpha-S2-casein	−16.48	14	25.9	4	0.9
	P15522	Glycosylation-dependent cell adhesion molecule 1	−12.53	24	17.2	3	0.3
37	Q9TVD0	Beta-casein	−158.95	70	26.1	29	28.4
	O97943	Alpha-S1-casein	−120.62	67	26.8	19	6.3
	O97944	Alpha-S2-casein	−65.41	48	22.9	10	3.3
	L0P3Z7	Kappa casein	−60.48	50	20.3	12	6.2
	Q9GK12	Peptidoglycan recognition protein 1	−34.58	42	21.3	4	0.8
	P02754	Beta-lactoglobulin	−19.99	33	19.8	3	0.6
	P80195	Glycosylation-dependent cell adhesion molecule 1	−13.12	23	17.1	3	0.4
	P15522	Glycosylation-dependent cell adhesion molecule 1	−9.76	28	17.2	3	0.6
38	O97944	Alpha-S2-casein	−115.25	50	22.9	14	9.4
	O97943	Alpha-S1-casein	−104.04	64	26.8	21	4.5
	P02662	Alpha-S1-casein	−42.87	24	24.4	7	1.1
	L0P3Z7	Kappa casein	−38.62	48	20.3	8	2.8
	Q9TVD0	Beta-casein	−37.61	51	26.1	8	7.8
	P02754	Beta-lactoglobulin	−30.14	40	19.8	7	0.8
	P15522	Glycosylation-dependent cell adhesion molecule 1	−23.18	35	17.2	4	0.9
	Q9GK12	Peptidoglycan recognition protein 1	−22.89	47	21.3	5	1.2
	P02666	Beta-casein	−18.30	15	25.0	3	1.5
	P02668	Kappa-casein	−14.47	29	21.2	3	0.7
	P02663	Alpha-S2-casein	−10.69	21	25.9	3	0.6
40	O97943	Alpha-S1-casein	−76.18	67	26.8	15	3.5
	P15522	Glycosylation-dependent cell adhesion molecule 1	−71.21	47	17.2	13	5.1
	O97944	Alpha-S2-casein	−55.20	47	22.9	10	1.6
	Q9GK12	Peptidoglycan recognition protein 1	−38.43	32	21.3	5	1.4
	S9X4G0	Neutrophilgelatinase-associated lipocalin-like protein	−36.03	32	28.3	6	0.7
	P02754	Beta-lactoglobulin	−32.17	46	19.8	7	0.9
	Q9TVD0	Beta-casein	−31.58	33	26.1	5	3.6
	W0K8B9	Kappa-casein (Fragment)	−19.39	42	17.1	5	1.0

PAI = Protein Abundance Index Shevchenko et al. (1996); MW (kDa) = Molecular Weight calculated from the database used in this study (http://www.uniprot.org/).

This observation is in accordance with those found by Farivar, Moosavi-Movahedi, Salami, Bohlooli, and Niasari-Naslaji (2013) who have reported that the thermal stability of CSA was higher than that of BSA.

Band 31 includes 13 proteins with PGRP having the highest PAI, a sequence coverage of 72% and 41 identified peptides. This band has disappeared from the gel patterns after heating camel milk at 80 °C for 60 min (Fig. 2). However, PGRP existed in heat-treated milk in bands 33, 34, 35, 37, 38 and 40 as shown in Table 2 with high sequence coverage, 48, 48, 35, 42, 47 and 32%, respectively. In addition to PGRP, bands 33, 34, 35, 37, 38 and 40 consisted mainly of mixtures of $\alpha_{s1,2}$-, β-, κ-caseins and PGRP. Besides, these proteins, bands 33 and 34 contained α-la.

Based on its theoretical molecular weight value, band 32 corresponds to camel α-la (Fig. 2). LC–MS/MS analysis proved that α-la was identified in band 32 along with other 13 proteins in fresh camel milk (Table 2). Camel α-la had the best *e*-value and PAI in band 32 with 83% sequence coverage and 27 identified peptides (Table 2). After heating camel milk at 80 °C for 60 min, this band disappeared completely from the gel patterns (Fig. 2). However, after heat treatment, camel α-la existed in bands 33 and 34. On the other hand, Table 2 displayed the existence of bovine α-la in heat-treated cow milk in band 21 only. This indicates that bovine α-la is more heat-resistant than camel α-la, which disagrees with previously reported data (El-Agamy, 2000; Farah, 1986).

4. Conclusion

This study aims at identifying the modifications in protein composition during the heat treatment of camel and cow milks at 80 °C for 60 min. A total of 19 protein bands were separated using SDS–PAGE and identified by LC–MS/MS. The obtained results showed that camel α-la and PGRP were significantly affected by heat treatment. These proteins co-existed in several bands with other protein components in the heat-treated camel milk. For cow milk, β-lg and α-la disappeared completely from the gel patterns with heating, however, they are present with other milk proteins. Casein fractions were kept intact under heat treatment at 80 °C during 60 min for both camel and cow milks. Based on this experimental study, future research works will be dedicated to identify the proteins involved in the deposits formation on the hot surfaces during the heat treatment of camel milk.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.08.007.

References

Atri, M. S., Saboury, A. A., Yousefi, R., Dalgalarrondo, M., Chobert, J. M., Haertle, T., et al. (2010). Comparative study on heat stability of camel and bovine apo and holo α-lactalbumin. *Journal of Dairy Research, 77*, 43–49.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Corredig, M., & Dalgleish, D. G. (1999). Effect of temperature and pH on the interactions of whey proteins with casein micelles in skim milk. *Food Research International, 29*, 49–55.

de Jong, P. (1997). Impact and control of fouling in milk processing. *Trends in Food Science & Technology, 8*, 401–405.

Donato, L., & Guyomarc'H, F. (2009). Formation and properties of the whey protein/kappa-casein complexes in heated skim milk – A review. *Lait, 89*, 3–29.

Dupont, D., Croguennec, T., Brodkorb, A., & Kouaouci, R. (2013). Quantitation of proteins in milk and milk products. In P. L. H. McSweeney & P. F. Fox (Eds.), *Advanced dairy chemistry: Volume 1A: Proteins: Basic aspects* (4th ed., pp. 87–134). New York: Springer.

El-Agamy, E. I. (2000). Effect of heat treatment on camel milk proteins with respect to antimicrobiol factors: A comparison with cow's and buffalo milk proteins. *Food Chemistry, 68*, 227–232.

Farah, M. (1996). Is face recognition ''special''? Evidence from neuropsychology. *Behavioural Brain Research, 76*, 181–189.

Farah, Z. (1986). Effect of heat treatment on whey proteins of camel milk. *Milchwissenschaft, 41*, 763–765.

Farivar, F., Moosavi-Movahedi, A. A., Salami, M., Bohlooli, M., & Niasari-Naslaji, A. (2013). Heat-induced conformational unfolding of fatty acid-free and fatted camel serum albumin: A comparative study. *Cell Biochemistry and Biophysics, 67*, 727–734.

Felfoul, I., Beaucher, E., Cauty, C., Attia, H., Gaucheron, F., & Ayadi, M. A. (2016). Deposit generation during camel and cow milk heating: Microstructure and chemical composition. *Food and Bioprocess Technology*. http://dx.doi.org/10.1007/s11947-016-1714-1.

Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., & Ayadi, M. A. (2015). Fouling behavior of camel and cow milks under different heat treatment. *Food and Bioprocess Technology, 8*, 1771–1778.

Hinz, K., O'Connor, P., Huppertz, T., Ross, R. P., & Kelly, A. L. (2012). Comparison of the principal proteins in bovine, caprine, buffalo, equine and camel milk. *Journal of Dairy Research, 79*, 185–191.

Jovanović, S., Barać, M., & Maćej, O. (2005). Whey proteins – Properties and possibility of application. *Mljekarstvo, 55*, 215–233.

Kappeler, S. R., Farah, Z., & 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.

Kim, D. A., Cornec, M., & Narsimhan, G. (2005). Effect of thermal treatment on interfacial properties of β-lactoglobulin. *Journal of Colloid and Interface Science, 285*, 100–109.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head bacteriophage T4. *Nature, 277*, 680–685.

Levieux, D., Levieux, A., El-Hatmi, H., & Rigaudière, J. P. (2006). Immunochemical quantification of heat denaturation of camel (*Camelus dromedarius*) whey proteins. *Journal of Dairy Research, 73*, 1–9.

Mal, G., Sena, D. S., Jain, V. K., & Sahani, M. S. (2006). Therapeutic value of camel milk as a nutritional supplement for multiple drug resistant (MDR) tuberculosis patients. *Israel Journal of Veterinary Medicine, 61*, 88–91.

Manzo, C., Nicolai, M. A., & Pizzano, R. (2015). Thermal markers arising from changes in the protein component of milk. *Food Control, 51*, 251–255.

Moro, A., Báez, G. D., Busti, P. A., Ballerini, G. A., & Delorenzi, N. J. (2011). Effects of heat-treated β-lactoglobulin and its aggregates on foaming properties. *Food Hydrocolloids, 25*, 1009–1015.

Pappa, E. C., Robertsona, J. A., Rigbya, N. M., Mellona, F., Kandarakisb, I., & Mills, E. N. C. (2008). Application of proteomic techniques to protein and peptide profiling of Teléme cheese made from different types of milk. *International Dairy Journal, 18*, 605–614.

Petit, J., Six, T., Moreau, A., Ronse, G., & Delaplace, G. (2013). β-Lactoglobulin denaturation, aggregation and fouling in a plate heat exchanger: Pilot-scale experiments and dimensional analysis. *Chemical Engineering Science, 101*, 432–450.

Roefs, S. P., & De Kruif, K. G. (1994). A model for the denaturation and aggregation of β-lactoglobulin. *European Journal of Biochemistry, 226*, 883–889.

Rüegg, M., Morr, U., & Blanc, B. (1977). A calorimetric study of the thermal denaturation of whey proteins in simulated milk ultrafiltrate. *Journal of Dairy Research, 44*, 509–520.

Shamsia, S. M. (2009). Nutritional and therapeutic properties of camel and human milk. *International Journal of Genetics and Molecular Biology, 1*, 52–58.

Shevchenko, A., Wilm, M., Vorm, O., & Mann, M. (1996). Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Analytical Chemistry, 68*, 850–858.

Singh, H. (1995). Heat-induced changes in casein, including interactions with whey proteins. In P. F. Fox (Ed.), *Heat induced changes in milk* (2nd ed., pp. 86–104). Brussels: International Dairy Federation.

Zhang, L., Boeren, S., Smits, M., van Hooijdonk, T., Vervoort, J., & Hettinga, K. (2016). Proteomic study on the stability of proteins in bovine, camel, and caprine milk sera after processing. *Food Research International, 82*, 104–111.