

Comparison of composition and whey protein fractions of human, camel, donkey, goat and cow milk

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Abstract

The aim of this study was to compare the physicochemical parameters of milk samples of five different species: cow, goat, donkey, camel and human. Also the analysis of whey protein profile in different milk samples was performed by anion-exchange fast protein liquid chromatography (FPLC) while polyacrylamide gel electrophoresis was used to identify a single fraction. Camel milk was the most acid (pH 6.460 ± 0.005) and the richest in total proteins (3.41 ± 0.31 %) and ash (0.750 ± 0.102 %), whereas donkey milk had a neutral pH (7.03 ± 0.02) and characterised by low proteins (1.12 ± 0.40 %) and fat (0.97 ± 0.03 %) content, being very close to human milk. Proteomic analysis of cow, goat, donkey, camel and human milk highlighted significant interspecies differences. Camel milk was similar to human milk in lacking of β -lactoglobulin and richness of α -lactalbumin. The knowledge gained from the proteomic comparison of the milk samples analysed within this study might be of relevance, both, in terms of identifying sources of hypoallergenic alternatives to bovine milk and detection of adulteration of milk samples and products.

Key words: cow, human, camel, donkey and goat milk composition, whey proteins, FPLC

Introduction

Cow milk is the most commonly consumed and processed milk. Several studies were focused on the characterization of bovine whey proteins using proteomic approaches (Manso et al., 2005; Fong et al., 2008). The major bovine whey protein fractions are β -lactoglobulin (β -LG; with molecular mass of 17 kDa) and α -lactalbumin (α -LA; 12 kDa), but contains also several predominant minor, but extensively studied, proteins, such as lactoferrin (LF; 80 kDa) and lactoperoxidase (LP; 78 kDa), (Floris et al., 2003; Sèverin and Wenshui, 2005). However, hypersensitivity to bovine milk proteins (β -LG and β -casein) is regarded as one of the a major food

allergies, which affects primarily infants who's enzyme system isn't developed yet, but may also persist throughout adulthood (Lara-Villoslada et al., 2005). For these reasons alternatives to cow's milk are being actively considered

The protein content of goat milk is quite similar to that of cow milk. The main casein fractions (CN) in goat milk are α_{s1} -CN, α_{s2} -CN, β -CN and k-CN. Whey proteins are composed of β -lactoglobulin, α -lactalbumin, immunoglobulins, glycomacropptide, bovine serum albumin and minor proteins such as LP, lysozyme and LF. Goat milk differs from cow milk in having better digestibility, alkalinity and buffering capacity (Park, 1994). Goat's milk lipids

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have higher physical characteristics than cow's milk, but there are variations between different reports (Park, 2006). In fact, goat's milk contains an appreciably higher proportion of butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0) and linoleic (C18:2) (Jenness, 1980). Fatty acid composition of goat milk fat varies depending greatly on the different regimes of feed supplementation to goats including changes of forage: concentrate ratios (Alonso et al., 1999; LeDoux et al., 2002; Sanz Sampelayo et al., 2002).

Donkey milk is gaining a growing interest in human nutrition due to its distinctive composition and physiological aspects. Considering some recent studies which focused on potential health benefits, an increase in the production of donkey milk is to be expected. Considering the contents of proteins, lactose, fat and minerals, donkey milk was found to be more similar to human milk than bovine milk (Fantuz et al., 2001). Polidori and Vincenzetti (2010) recently described that donkeys' milk digestibility was higher than that of cows' milk and similar to that of human milk. Donkey's milk lipid fraction is characterized by high levels of linoleic and linolenic acid (Salimei et al., 2004). The optimum casein/whey ratio, ranging between 0.9 and 1.1 in donkey milk (Salimei et al., 2004), was also found to be an important factor affecting the hypo-allergenicity of milk proteins (Lara-Villoslada et al., 2005). Donkey milk was characterized by low casein and high lysozyme content (1.0 mg/mL) in comparison to other kinds of milks (Vincenzetti et al., 2007).

Camel milk is known by its therapeutic values in medicine and human nutrition. A series of metabolic and autoimmune diseases were successfully treated with camel milk (Al-Hashem, 2009). Beneficial role of raw camel milk in chronic pulmonary tuberculosis patients was observed (Mal et al., 2001). In repeated trials, it was observed that there was 30-35 % reduction in daily doses of insulin in patients suffering from type 1 diabetes after receiving raw camel milk (Agrawal et al., 2002; Sboui et al., 2010). Recent studies showed that camel milk had anti-hepatic and anti-cancer properties (El Fakharany et al., 2012; Habib et al., 2013). These medicinal properties were attributed to its unique composition. In fact, camel milk is rich in vitamin C, niacin, vitamin A and E, polyunsaturated fatty acids and minerals (sodium, potassium,

iron, copper, zinc and magnesium) and poor in cholesterol and lactose when compared to cow milk (Haddadin et al., 2008). Protein composition of camel milk differs from that of milk produced by other dairy animals. Peptidoglycan-protein (PGRP), whey acidic protein (WAP) and camel whey basic protein (CWBP) and immunoglobulins (IgGs) are specific whey proteins which were found only in camel milk (Beg et al., 1986; Kappeler, 1998; El Hatmi et al., 2007). Similar to human milk, camel milk contains high contents of α -LA and LF and lacks on β -LG (El-Hatmi et al., 2007). It has no allergenic properties and it can be consumed by lactase - and/or immune-deficient population (El-Agamy, 2009). Jrad et al. (2014) showed the existence of antioxidative peptides from camel caseins.

Human milk will remain the best nutrition for all human infants because it ensures the best healthy short- and long-term development. Human milk composition and protein fractionation are well documented and were proven to enhance immune functions, to be hypoallergenic and to promote the establishment of bifidogenic gut microbiota (Hanson et al., 1999). However, some infants may not be exclusively breast fed during the first months of life. In that case, milk substitutes play a necessary role in infant nutrition. In order to find a solution in situations when breast feeding was not possible and/or when it was not possible to use soy milk or hydrolysed infant formulas. Until the present, there have been progressive attempts to bring the composition of these formulations closer to that of human milk. This substitution results in an allergic disease known as cow milk protein allergy (CMPA) in 2-6 % of children (El-Agamy, 2007). Nowadays, most common alternatives are soy and extensively hydrolysed milk proteins formulae (El-Agamy, 2007). However, there is evidence that 10-20 % of children allergic to cow milk do not tolerate soy derivatives (Zeiger et al., 1999) and some cases of high immunological reaction to extensively hydrolysed formulae have been reported the research of another alternative of bovine milk and soy in infantile formula became a necessity.

The objectives of this study were to determine similarities of human milk with camel, cow, donkey and goat milk in terms of composition and whey protein fraction. A special focus was put on possible use of camel milk as a substitute of cow milk in order to prepare infant formulas.

Material and methods

Milk collection

Milk was collected from two breeds of camel (*Camelus dromedarius*) and one goat breed (*Capra hirta*) at the Wildlife and Livestock Laboratory, Arid lands Institute, Tunisia.

Cow (*Bos Taurus*) and donkey (*Equus asinus*) milk samples were purchased from local farms. Human milk was donated by a healthy mother and was collected over second and fourth month postpartum. The collected samples from each species were pooled separately, subdivided into 100 mL holders and stored at -20 °C until the use.

Estimation of pH, acidity, density and viscosity

The pH of different milk samples was determined using a Thermo Orion pH meter (Cumming Center Beverly, USA). The Dornic acidity and density were determined according to Afnor (1993) and the viscosity was determined by applying a shear stress of 0.1 to 100 rpm at an oscillation frequency of 1 Hz for 1 min with a Brookfield type Viscometer (model DV-E, MA, USA). The viscosity was expressed in centipoises (cP).

Estimation of proximate composition

The total dry matter was determined by oven-drying at 105 °C to constant mass (Afnor, 1993). Then, the ash content was determined by combustion of the sample at 550 °C for 8 h. Crude proteins were analysed according to the Bradford method (Bradford, 1976) using a bovine serum albumin (BSA) as standard. Fat content was determined according to the methods of GERBER and VAN GULICK (Afnor, 1993).

Whey protein preparation

Milk was skimmed by centrifugation at 5000×g at 4 °C for 30 min. Casein was obtained from skimmed milk by isoelectric precipitation (pH_i, 4.6) at 20 °C using 1 M HCl. The obtained precipitate was washed twice with distilled water, solubilized at pH 7.0 by addition of 1 M NaOH, precipitated again at pH_i with 1 M HCl and washed three times with distilled water. Finally, the supernatant, containing the whey proteins and the whole caseins was solubilized at pH 7.0, dialyzed against distilled water, freeze-dried and stored at -20°C.

Fast protein liquid chromatography

Skimmed milk and whey proteins were fractionated by FPLC, Fast Protein Liquid Chromatography (AKTA purifier 10, GE healthcare, Sweden), with an anion exchange column Mono Q (50x5 mm internal diameter, particle size 10 μm). A volume of 1 mL of whey protein (10 mg/mL of Tris-HCl 20 mM, pH 8.0, containing 0.02 % (w/v) NaN₃) or skimmed milk (0.1 mL/min of Tris-HCl 20 mM, pH 7.0, 4.5 mM urea) was injected into the column, equilibrated in the elution buffer 20 mM Tris-HCl, pH 8.0 containing 0.02 % (w/v) NaN₃ for whey proteins and Tris-HCl 20 mM, pH 7.0 for skimmed milk. The flow rate used was 1 mL/min and detection is carried out at 280 nm. A linear gradient of 0 to 1 M NaCl was applied for 30 min.

Gel electrophoresis

SDS-PAGE was performed on a 5 % (w/v) polyacrylamide in 0.125 M Tris-HCl buffer, pH 6.8 stacking gel and a 15 % (w/v) polyacrylamide in 0.38 M Tris-HCl buffer, pH 8.8 containing 0.1 % (w/v) SDS separation gel (Laemmli and Favre, 1973). Samples with protein concentrations of 1 mg/mL were diluted 1:1 by SDS sample buffer and boiled for 3 min at 100 °C. A volume of 10 μL of whey samples, FPLC fractions, or skimmed milk were loaded in the gel. Proteins were stained for 120 min by 0.1 % (w/v) Coomassie blue R250 in a mixture of 50 % (v/v) ethanol and 10 % (v/v) acetic acid for 2 hours. Finally, gels are destained by several washes in a solution of ethanol (30 %) and acetic acid (10 %).

Statistical analysis

All the analyses were carried out in triplicate measurements and mean values were used for the statistical analysis. Reported values shows mean values and error bars shows standard deviations. Data were analysed by *t* test and level of significance was considered when *p* values were <0.05.

Results and discussion

Gross composition of the different milks

The physico-chemical properties of human, camel, goat, cow and donkey milk samples are presented in Table 1. Human and donkey milk had higher average pH values (7.15±0.01 and 7.03±0.02,

respectively) but a lower average of titratable acidity and viscosity than the ruminants' (camel, goat and cow) milk samples. The obtained results showed good correspondence with findings reported by Salimei et al. (2004) and may be explained by the lower casein and phosphate contents than in ruminants' milk samples. Camel milk had the highest acidity (6.46 ± 0.005), most probably due to high contents of vitamin C and certain organic acids (Farah, 1993). The observed acidity may inhibit the growth of harmful bacteria and contribute to the long-term preservation of camel milk. Regarding the fat content, donkeys' milk had noticeably lower fat (0.97 ± 0.03 %) content than ruminants' and human milk samples. The average fat content was approximately 15.8 % and correlated well with data previously reported by Rathore et al. (2011) and Salimei et al. (2004) who also found that the lipid fraction was poor in unsaturated fatty acids. Further, the observed fat content was lower in comparison to results for camel milk presented by Ereifej et al. (2011) who reported a range between 17.13 and 38.85 % of total fatty acids. Thus, the dietary fat fraction of camel milk is a useful nutritional attribute. In fact, there is a growing body of scientific evidence that increasing the supply of unsaturated fatty acids may be required to reduce the risk of disease, especially coronary heart disease (Roche, 1999).

The determined protein content of the ruminant milk samples showed a remarkable similarity with camel milk which was characterised by the highest content, while in the human and donkey milk sam-

ples had considerably lower protein contents. The high proportion of protein and consequently the relatively higher amount of essential amino-acids make camel milk more favourable to human nutrition than cow's, donkey's and goat's milk. Further, human milk was poorer in mineral salts (0.11 ± 0.04 %) and dry matter (8.83 ± 0.33 %) contents when compared to donkey, camel and goat milks. Results of the present study show good correlation with findings of some earlier studies conducted by Yamawaki et al. (2005); Guo et al. (2007); Sboui et al. (2009) and Soliman et al. (2005).

Regarding the gross composition, more precisely the pH values, viscosity, dry matter and protein contents, donkey milk seemed to be the most similar to human milk.

SDS-PAGE analysis of milk samples

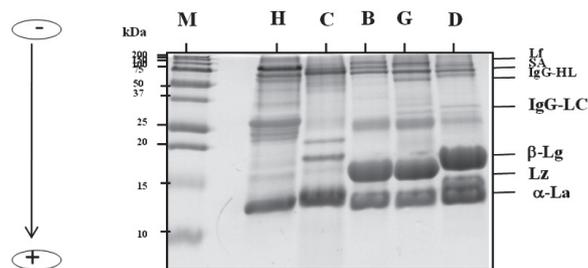
Recently there were several studies reporting that donkey, camel and goat milks might be preferable to cow milk with respect to allergenicity, particularly for infants and elder people (Vincenzetti et al., 2014; El-Agamy 2009; Reinert and Fabre, 1997; Grzesiak, 1997).

The whey protein fractions of human, camel, goat, cow and donkey were analysed by SDS-PAGE (Figure 1) and identified on the basis of their apparent molecular mass in comparison with the marker protein ladder. Different milk samples exhibited marked heterogeneity showing five different electrophoretic patterns.

Table 1. Average composition of the camel's, cows, donkey, goat's and human milk. Error bars show the standard deviation (n=2)

Parameter	Human	Camel	Cow	Goat	Donkey
pH (at 20 °C)	7.150 ± 0.007^a	$6.460 \pm 0.005^{a,b}$	$6.58 \pm 0.05^{a,c}$	$6.470 \pm 0.010^{a,d}$	$7.03 \pm 0.02^{a,e}$
Acidity (°D)	4.95 ± 0.45^a	18.30 ± 0.45^b	17.10 ± 0.45^b	16.870 ± 0.225^c	6.30 ± 0.00^a
Density	1.032 ± 0.000^a	1.0300 ± 0.0005^b	1.029 ± 0.000^c	$1.031 \pm 0.000^{a,b}$	1.025 ± 0.000^d
Viscosity (cP)	3.2 ± 0.0^a	3.6 ± 0.0^b	3.45 ± 0.07^a	$3.45 \pm 0.07^{b,c}$	3.150 ± 0.007^a
Dry matter (%)	8.83 ± 0.33^a	10.77 ± 0.03^b	8.87 ± 0.12^a	11.96 ± 0.34^c	8.53 ± 0.10^d
Ash (%)	0.11 ± 0.04^a	0.750 ± 0.102^b	$0.710 \pm 0.003^{b,c}$	$0.72 \pm 0.04^{b,c,d}$	0.41 ± 0.01^e
Fat (%)	3.5 ± 0.0^a	3.2 ± 0.0^b	2.15 ± 0.07^c	$4.45 \pm 0.49^{a,b}$	0.97 ± 0.03^d
Protein (%)	1.41 ± 0.25^a	3.41 ± 0.31^b	2.59 ± 0.2^c	2.49 ± 0.17^c	1.12 ± 0.40^d

^{a, b, c, d, e} means with the different letter are statistically different in the same line $P < 0.05$



M: molecular mass standards; H: human; C: camel; B: cow; G: goat; D: donkey. Lf: lactoferrin; SA: serum albumin; IgG-HL: high chains of IgG; IgG-LC: light chains of IgG; β-Lg: β-lactoglobulin; LZ: lysozyme; α-La: α-lactalbumin

Figure 1. SDS-PAGE profiles of whey proteins from camel, human, donkey, goat and cow milk

Most of the milk samples presented a common band with different intensity of 12 kDa molecular weight. This band corresponds to α-LA which is more intense in human and camel milk with 72 % of homology (Lisak et al., 2013). Human α-LA had about the same migration position as their counterpart in cow, goat and donkey milks, whereas the migration of camel α-LA was slightly slower. The content of α-LA is the highest in human milk (2.45 g/L) and camel milk (2.2 g/L) than cow (0.5 g/L), goat (2.0 g/L) and donkey (1.43 g/L) milk (El-Hatmi et al., 2007; Salimei et al., 2004). Both, camel and human α-LA showed attractive nutritional and biological values due to high contents of essential amino acids, antioxidant and anti-tumor activities (Salami et al., 2009; El Hatmi et al., 2014). The attention was focused on producing α-La-enriched formulae with high nutritional value. The α-LA in camel milk presented great homology with its cow counterpart and with high content. Due to the high contents and the high nutritional value of α-LA, it might be feasible to utilize camel milk as a supplement for food products, especially infant formula, in order to substitute bovine α-LA.

Human and camel milk samples were also free of β-LG, while it was found to be the major whey protein fraction of cow's, goat's and donkey's milks (band of 17 kDa molecular mass), which was in agreement with previous reports (Kappler et al., 2003; El Hatmi et al., 2007; El-Agamy et al., 2009). In addition, no fraction corresponded to camel β-LG included in the Swiss Prot and UniProt database, it was not possible to confirm the presence of this protein in milk and thus allow its isolation and primary sequence determination. As β-LG is responsible for the onset of allergic forms to milk proteins that affect a significant percentage of

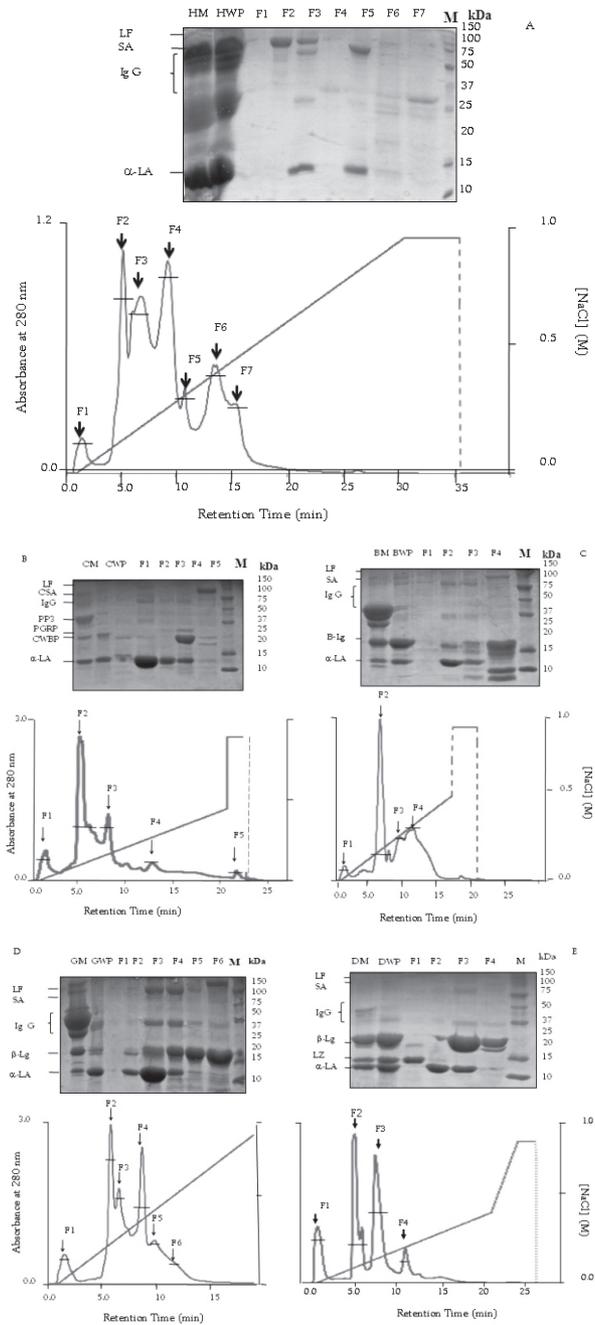
infants nourished with maternal milk replacement based on cow milk, camel milk was suggested to be a good substitute for people suffering from cow milk allergy.

Camel and human milks revealed common band with high molecular weight close to lactoferrin (80 kDa and 76 kDa for human and camel milk respectively). Difference in band intensity reflected difference in LF concentration. The contents of LF (an antimicrobial factor) determined in camel and human milk (0.34 g/L and 1.95 g/L, respectively) were higher than in cow (0.006 g/L), goat and donkey milk (Konuspayeva et al., 2007; Shamsia, 2009). SDS-PAGE pattern of donkey's milk highlighted band above the α-LA which may correspond to lysozyme (LZ; approx. M_r 17 kDa). A similar trend was reported by Fantuz et al. (2001). Solaroli et al. (1993) who found that antimicrobial defence in donkey's milk most probably originated from the presence of LZ and, to a lesser degree, from LF.

Donkeys' milk might be regarded as a very rich source of lysozyme (1 g/L) (Vincenzetti et al., 2008) and was suggested to have significantly higher contents than human (0.4 g/L) or cow milk (Solaroli et al., 1993), while the level of lysozyme in donkey milk was found to be quite similar to that of mare's milk (0.4-1 g/L) (Floris et al., 2003). At the contrary, in the present study LZ was only found in donkey's milk. Serum albumin was observed in all samples. The molecular weight of camel serum albumin estimated in this study was similar to data previously reported by Ereifej et al. (2011), who suggested it to be 66 kDa. However, there seems to be a close taxonomic proximity between camel serum albumin and bovine serum albumin.

Whey proteins fractionation by Fast protein liquid chromatography

The chromatographic protein profile of FPLC followed by 15 % SDS-PAGE performed on whey proteins of human, camel, cow, goat and donkey milks are presented in Figure 2. Separation of total whey proteins of different species by anion exchange column (Mono Q) showed considerably different elution profiles. Such findings might be correlated to differences in composition and structure between the proteins of five species. Different FPLC fractions were collected: F2-F7 for human milk, F1-F5 for camel, F2 to F4 for bovine milk, F2-F6 for goat milk and F1-F4 for donkey milk.



A.U.: absorbance unit; M: molecular mass standards; LF: lactoferrin; CSA: camel serum albumin; IgG: immunoglobulins G; CWBP: camel whey basic protein; α -LA: α -lactalbumin, SA: serum albumin; β -Lg: β -lactoglobulin; Lz: Lysozyme; PGRP: Peptidoglycan recognition protein; HWP: human whey protein; CWP: camel whey protein; BWP: bovine whey protein; GWP: goat whey protein and DWP: donkey whey protein

Figure 2. Cation-exchange fast protein liquid chromatography of whey proteins (WP) of human (A) camel (B), cow (C), goat (D) and donkey milk (E) onto Mono Q columns connected to an ÄKTA-FPLC system and SDS-PAGE analysis of the fractions collected

Fraction F1 contained proteins not retained on the Mono Q column essentially basic proteins: LF for human's milk, CWBP (Camel Whey Basic Protein with theoretical pHi is of 9.30) according to Ochirkhuyag et al. (1998) and LZ for donkey's milk (accession number P11375). The characterization of those proteins by SDS-PAGE displayed apparent molecular masses of 80, 20 and 15.5 kDa for LF, CWBP and LZ respectively. The fraction F2 of donkey milk contained almost pure α -LA which was together with β -LG fully recovered in fraction F3 of donkey whey protein. This finding is consistent with that observed by Girardet et al. (2004) who mentioned that α -LA of equine's milk showed two isoforms separated in fraction F2 and F3 eluted by anion exchange chromatography and displayed the same apparent molecular masses (14.4 kDa). Acidic proteins were highly retained onto the anion-exchange column as shown by electrophoresis of the five investigated milk samples, but the nature of these proteins differed among the species. The β -LG was the principal protein found in the last FPLC fraction of cow, goat and donkey milk samples. Since α -LA and SA were ultimately eluted in samples of human and camel milk samples, this might be regarded as the other common point between the two types of milk besides the absence of β -LG protein. With respect to that, camels' milk was favoured as a potential new paediatric diet and a good alternative for infant nutrition.

Conclusion

In this study the gross composition of the milk of five dairying species (human, camel, cow, goat and donkey) was characterized. Donkeys' milk appeared to be the most similar to human milk considering the physicochemical properties such as pH value (7.15 for human milk and 7.03 for donkey milk), viscosity (3.2 ± 0.0 for human milk 3.150 ± 0.007 cP for donkey milk) and protein content (1.41 ± 0.25 % and 1.12 ± 0.40 % for human and donkey milk respectively). The results observed by the whey protein fractionation provided further evidence that the whey protein profile of donkey milk was considerably different from human milk and, as expected, the qualitative whey protein profile of camel milk was very similar to that of human milk. Human and camel milk were found to have high contents of α -LA, LF and β -CN and to be devoid of β -LG, whereas it was the major whey protein fraction detected in samples

of cow, goat and donkey milk. Further, thus camel milk was suggested to be the most suitable substitute for cow milk when considering preparation of infant formulas. Hence, future studies should focus on purification of single camel whey protein fractions in order to evaluate their immunoreactivity.

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Usporedba sastava i profila proteina sirutke majčinog, devinog, magarećeg, kozjeg i kravljeg mlijeka

Sažetak

Cilj ovog istraživanja bio je usporediti fizikalno-kemijska svojstva 5 različitih vrsta mlijeka - kravljeg, kozjeg, magarećeg, devinog i majčinog. Primjenom ionske izmjene i brzo-proteinske tekućinske kromatografije (FPLC) provedena je i analiza profila proteina sirutke, dok je pomoću poliakrilamid gel elektroforeze identificirana svaka pojedina frakcija. Devino mlijeko imalo je najveću kiselost ($\text{pH } 6,460 \pm 0,005$) te najviše udjele proteina ($3,41 \pm 0,31 \%$) i mineralnih tvari ($0,750 \pm 0,102 \%$). S druge strane, magareće mlijeko imalo je pH vrijednost u neutralnom području ($7,03 \pm 0,02$) te niske udjele proteina ($1,12 \pm 0,40 \%$) i mliječne masti ($0,97 \pm 0,03 \%$) uslijed čega je bilo najsličnije majčinom mlijeku. Proteomička analiza uzoraka kravljeg, magarećeg, devinog i majčinog mlijeka rezultirala je značajnim razlikama ovisno o vrsti mlijeka. Devino mlijeko pokazalo se vrlo slično majčinom zbog odsutnosti frakcije β -laktoglobulina te visokog udjela α -laktalbumina. Rezultati dobiveni proteomičkom analizom različitih vrsta mlijeka mogu poslužiti za pronalaženje hipoalergenih alternativa kravljem mlijeku, ali i kao pomoć pri određivanju patvorenja mlijeka i mliječnih proizvoda.

Ključne riječi: sastav mlijeka, kravlje, majčino, devino, magareće, kozje, proteini sirutke, FPLC

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