Donkey Milk Bioactive Proteins&Peptides

Subjects: Food Science & Technology
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Due to its similarity with human milk and its low allergenic properties, donkey milk has long been used as an alternative for infants and patients with cow's milk protein allergy (CMPA). In addition, this milk is attracting growing interest in human nutrition because of presumed health benefits. It has antioxidant, antimicrobial, antitumoral, antiproliferative, and antidiabetic activity, stimulates the immune system, regulates the gastrointestinal flora, and prevents inflammatory diseases. Although all components of donkey milk can contribute to functional and nutritional effects, it is generally accepted that the whey protein fraction plays a major role.

Keywords: donkey milk ; mammals' milk ; whey proteins

1. Introduction

Since ancient times, donkey milk (DM) has been known for its therapeutic properties and it was used for wound healing and for treating various diseases such as bronchitis, asthma, joint pain, gastritis [1][2]. Today, it is available on the market as a commercial product to benefit newborns, people with allergies to cow's milk proteins, and older people [1][2]. Several types of milk (from goat, dromedary, donkey, and horse) are known to have lower allergenicity than cow milk, and it has been suggested that differences in nitrogen distribution and digestibility of milk proteins play an important role in determining the allergenic capacity of milk [3]. DM has become increasingly attractive due to its biological activities, such as anti-microbial, anti-viral, anti-inflammatory, antiproliferative [1][4], and antioxidant activity [5].

The characterization of milk's main constituents has fundamental importance according to the correlation between health and nutrition. In this context, proteins and peptides are considered important nutrients because some of them show bioactivity when they are native [2][5]. There is increasing evidence that many milk proteins and peptides (particularly peptides that are called 'bioactive peptides') have physiological functionality. Important effects on immune modulation, cardiovascular health, tumours, bones, and teeth have been frequently reported [5][6]. It is well known that the validity of these effects and the efficacy of functional foods based on bioactive peptides remain to be fully proven in the future, and they may lead to a new class of functional foods, for example, those based on milk proteins and their products [6][7].

According to their different solubility, the DM proteins are classified into three classes: milk fat globule membrane (MFGM) proteins, caseins, and whey proteins [4][5]. The protein content of milk may vary among species, among breeds within the same species, and even among individual animals within the same breed. Furthermore, it is well known that there is a strong qualitative resemblance between the principal classes of proteins (i.e., caseins and whey proteins) in all types of milk. These whey proteins and caseins could have biomedical applications [8][9]. Due to its high nutritional and health importance, DM is rediscovered as a functional food. Most of the studies aiming to evaluate DM qualities have been carried out in Italy, and some data are published on the DM obtained from Chinese and Balkan donkey breeds [1]. Likewise, due to the increasingly global spread of food allergies, consumers have started looking for so-called "natural milk" with good taste and useful in treating of some conditions such as cow's milk protein allergy.

2. Global Composition of Donkey Milk Compared to Other Types of Milk

Due to its chemical composition, milk is considered a complete food. It consists of water, carbohydrates, fats, proteins, and other minor components such as hormones, vitamins, minerals, cytokines [1][5]. Among the constituents of milk, proteins vary between mammalian species, ranging from 1% to 24%. These proteins exist under three categories of proteins defined by their chemical composition and their physical properties: MFGM proteins, caseins, and whey proteins. In addition, the carbohydrate (lactose) milk content varies from 0.7% to 7.0% between different species of mammals. Regarding the fat content, not only the concentration varies but also the chemical composition [4][5]. Table 1 represents the global composition of the donkey, cow, camel, goat, and human milk. The amount of DM components, such as whey protein, lactose, and caseins, are similar to that of human milk, although they differ significantly compared to cow, goat, and camel milk. The only significant difference between DM and human milk is the fat content, which is very low in DM. Nevertheless, regarding the casein-to-whey protein ratio, in DM this is intermediate between human milk and cow milk.

Table 1. Milk composition and energy value—donkey and other species [5][10][11].

Milk Characteristics	Donkey (%)	Goat (%)	Cow (%)	Camel (%)	Human (%)
Proteins	**1.74**	**3.41**	**3.43**	**1.80**	**1.64**
Fat	**1.21**	**4.62**	**3.46**	**1.80**	**3.38**
Lactose	**6.23**	**4.47**	**4.71**	**2.91**	**6.69**
Dry Matter	**9.61**	**13.23**	**12.38**	**11.30**	**12.43**
Ashes	**0.43**	**0.73**	**0.78**	**0.85**	**0.22**
Water	**90.39**	**86.77**	**87.62**	**90.60**	**87.57**
Energy (KJ/Kg)	**1939.40**	**3399.50**	**2983.00**	**2745.80**	**2855.60**

3. Bioactive Proteins and Peptides in Donkey Milk Compared to Other Types of Milk

Table 2 shows the different protein fractions identified in cow, donkey, goat, camel, and human milk, and the g/L amount. Camel's milk, cow's milk, and the goat's type have high protein levels compared to human milk and DM. Cow's milk, camel's milk, and goat's milk have more caseins (80%) and fewer whey proteins (20%) [8] compared with DM, which has more whey proteins (60%) and fewer caseins (40%) [12]. Donkey's milk has a quantity of α-lactoglobulin resembling that identified in human milk and has a high level of β-lactoglobulin, which is not found in human milk. This β-lactoglobulin is the major allergen of cow's milk, besides caseins [5][8]. Another peculiarity is that lysozyme's human and donkey milk content is much higher than in cow milk:

Table 2. Main proteins of donkey milk compared to other types of milk [5][8][12].

	Cow (g/L)	Donkey (g/L)	Goat (g/L)	Camel (g/L)	Human (g/L)
Total protein content	**31–38**	**13–28**	**25–39**	**25–45**	**9–17**
Total casein	**27.2**	**6.6**	**25**	**26.4**	**5.6**
Total whey protein	**4.5**	**7.5**	**6**	**6.6**	**8**
αS1-casein	**10–15**	**0.2–1**	**0–7**	**5**	**0.3–0.8**
αS2-casein	**3–4**	**0.2**	**4.2**	**2.2**	**n.d.**
β-casein	**9–11**	**3.9**	**11–18**	**12.8**	**1.8–4**
κ-casein	**3–4**	**n.d.**	**4–4.6**	**0.8**	**0.6–1**
α-lactalbumin	**1–1.5**	**1.8–3**	**1.2**	**3.5**	**1.9–2.6**
β-lactoglobulin	**3.3–4**	**3.2–3.7**	**2.1**	**n.d.**	**n.d.**
Lysozyme	**0.00007**	**1**	**Trace**	**0.00015**	**0.04–0.2**
Lactoferrin	**0.1**	**0.08**	**0.02–0.2**	**0.22**	**1.7–2**
Immunoglobulins	**1**	**n.d.**	**1**	**1.54**	**1.1**
Albumin	**0.4**	**n.d.**	**0.5**	**0.4**	**0.4**

Several research groups have been able to characterize the protein fractions of whey in DM and have demonstrated their nutraceutical properties and their beneficial properties for human health. These proteins will be described in detail below.

3.1. Caseins

The caseins are organized into micelles (supramolecules of colloidal size) whose diameter varies from 30 to 600 nm. In particular, αS1-, αS2-, β-, κ-casein, and traces of γ-casein can be found. These micelles are made up of different proteins (94%) and colloidal calcium phosphate, made of calcium, phosphate, magnesium, and nitrate, comprises 6%. These different caseins have hydrophilic regions and other hydrophobic regions that are different from one casein to another [13]. Besides, the caseins are phosphoproteins. Therefore, they have phosphorylated regions at the level of the serine residues. The proline residues, uniformly distributed into the casein structure, prevent secondary structures such as α helices or β sheets, hence the so-called open or "random coil" conformation of casein [14]. κ-caseins have a particular role, they are first of all glycoproteins and have only one phosphoserine group, but above all, they are stable in the presence of calcium ions and thus protect all of the caseins against precipitation and stabilize the micelles [13][15].

In mature cows' milk, caseins make up 80% (*w*/*w*) of all proteins, whereas, in humans [16] and equines [17], they represent only 35% and 50% of the total protein content, respectively. The DM essentially comprises αS1-, and β-casein while αS2- and κ-casein are minor components. β-casein can represent up to 80% of the total casein in human milk [18] and is also the predominant protein in the casein fraction of DM [16]. Several studies revealed that caseins and β-lactobglobulin are the main allergens in cow milk [19], and the low allergenicity of DM is explained by low casein content [5].

3.2. β-Lactoglobulin

β-lactoglobulin, a globular protein containing 162 amino acids that belong to the family of lipocalin proteins, has a molecular mass of 18.36 kDa. Lipocalin molecules have pockets capable of hosting iron complexes. Iron binds to protein through iron chelators called "siderophores" [5]. β-lactoglobulin is known for its richness in lysine, leucine, glutamic acid, and aspartic acid. Its secondary structure is mainly composed of β sheets (≈50%), but there are also α helices (10%), β elbows (8%), and a high proportion of disordered structures (35%) [20]. Its structure is also reinforced by two disulfide bridges and a tertiary structure mainly composed of antiparallel β sheets. Studies showed that two different isoforms of β-lactoglobulin could be found in DM: the major isoform is β-lactoglobulin I (80%), while β-lactoglobulin II is encountered in lower quantities.

In DM, the β-lactoglobulin content of 3.75 g/L resembles that found in cow's milk [7], and is lower than that found in goat's milk, while it is absent in camel's [21] and human's milk [3]. In DM, β-lactoglobulins correspond to one genetic variant of β-LGI (β-LGIB), two genetic variants of β-LGII (β-LGIIB, and β-LGIIC), and a third minor β-LGII variant (β-LGIID) [5].

β-lactoglobulin is known to have several functions, both nutritional and functional. One of the most studied functions is the protein's ability to bind specific nutritional interest molecules and serve as a protective matrix during digestion. β-lactoglobulin was shown to bind specific vitamins (D2, D3), cholesterol, particular catechins, and even mercury [20][22]. These interactions occur mainly in the protein's central area, denominated as the calyx (also known as β-barrel), and formed of β sheets. This hydrophobic cavity, which makes it possible to fix a large variety of ligands, is regulated by an EF loop, which works as a gate to the site of binding. At low pH, this loop is in the "closed" position, and interactions are impossible. When the pH increases, the loop opens, allowing the ligands to insert into the hydrophobic cavity [20]. This change in the Tanford transition structure generally occurs between pH 6.5 and 7.5 [23].

3.3. α-Lactalbumin

The α-lactalbumin, a protein composed of 123 amino acid residues, with a molecular weight of 14.2 kDa, has in its tertiary structure four disulfide bridges. Native α-lactalbumin is made up of two distinct domains, and a large section is produced of α helices and a small β sheet domain. A calcium fixation loop bonds the two sections. This protein is found in DM as two isoforms with different isoelectric points (pI) values: 4.76 and 5.26. α-lactalbumin content in DM is 1.8 g/L, a value very close to that found in cow and human milk [4][5].

α-lactalbumin is a protein recognized for its nutritional qualities, mainly for infants' nutrition. First, α-lactalbumin plays an essential role in milk production in mammals because it binds to the enzyme β-1,4-galactosyltransferase and creates the lactose synthase essential for lactose formation. Another important nutritional element of this protein is its high tryptophan content since it is an essential amino acid. This amino acid has demonstrated positive effects on the development of newborns' brains and nervous systems and contributes to these systems' functioning as a direct precursor of serotonin or niacin (also known as vitamin B3). Studies have also shown that regular intake of α-lactalbumin in adult subjects makes it possible to increase the plasma quantities of tryptophan, thus improving certain neurological functions (such as attention, cognitive performance, and morning alertness) [24][25]. This protein also has good digestibility and a low allergenic capacity [26][27].

3.4. Lysozyme

Lysozyme, or muramidase, is a globular enzyme consisting of 129 amino acids and a class of hydrolases [28]. The latter consists of two domains: a domain composed essentially of α helices and a β anti-parallel sheet and two α helices. Three disulfide bridges provide the three-dimensional configuration of the molecule: two are found in the α-helix domains, while one is located in the β sheet. Lysozyme can catalyze the hydrolysis of the glycoside 1 → 4 bond of peptidoglycans in the bacterial wall and chitin present in fungi walls [1][5].

Two isoforms of lysozyme, which differ by an oxidized methionine at position 79, were described in DM: lysozyme A with a molecular weight of 14.631 kDa and lysozyme B with a molecular weight of 14.646 kDa [29][30].

Compared to human milk, donkey milk has a higher content of lysozyme (1 g/L), while in goat and cow milk, lysozyme is missing [3][5]. Due to the high amount of lysozyme [3][5] and its thermostability [29][31], the DM is resistant to alteration.

3.5. Lactoferrin

Lactoferrin is a glycoprotein that belongs to the transferrin family and has a molecular weight of 80 kDa. Its structure is built by two homologous domains, which bind ferric and carbonate ions. The anti-microbial activity of lactoferrin applies to a wide range of Gram-positive and Gram-negative bacteria. On the one hand, it is partly dependent on its capacity to bind iron, resulting in an environment scarce in iron, which limits the bacterial growth; on the other hand, it depends on its capacity to bind to the lipopolysaccharides of bacterial cell walls via its N-terminus, resulting in the permeabilization of the bacterial cells [5][32]. Likewise, once digested in the stomach, lactoferrin is fragmented into small peptides: lactoferrampin and lactoferricin; the second one has an important action against several bacteria, viruses, fungal pathogens, and protozoa. Moreover, this peptide has other activities, such as inhibition of tumor metastasis in mice [33] and induction of apoptosis in THP-1 human monocytic leukemic cells [34].

3.6. Lactoperoxidase

Lactoperoxidase (LPO) is an oxidoreductase enzyme and has a protective function against infections by microorganisms. It is found in low concentrations in fresh DM, as well as in human milk (about 100 times lower than in bovine milk) [1]. LPO can catalyze the oxidation of diverse substrates by using hydrogen peroxide. The oxidation products possess bactericidal activity against bacteria (Mycoplasmas), and bacteriostatic effects against Listeria monocytogenes [4][5]. It has been shown that immunoglobulins found in the milk exert a synergistic effect on the activity of these non-specific anti-microbial factors with LPO, whose anti-microbial activity against Streptococcus mutants increases considerably if the system is incubated with secretory IgA. The anti-microbial activity enhancement seems to be due to binding between LPO and immunoglobulins (IgA) [5][7]. The result of this interaction is a stabilization of the enzymatic activity of lactoperoxidase [5].

References

1. Martini, M.; Altomonte, I.; Licitra, R.; Salari, F. Nutritional and Nutraceutical Quality of Donkey Milk. J. Equine Vet. Sci. 2018, 65, 33–37, doi:10.1016/j.jevs.2017.10.020.

2. Karami, Z.; Akbari-adergani, B. Bioactive food derived peptides: A review on correlation between structure of bioactive peptides and their functional properties. J. Food Sci. Technol. 2019, 56, 535–547, doi:10.1007/s13197-018-3549-4.

3. Fantuz, F.; Salimei, E.; Papademas, P. Macro- and micronutrients in non-cow milk and products and their impact on human health. In Non-Bovine Milk and Milk Products, 1st ed.; Tsakalidou, E., Papadimitriou, K., Eds.; Elsevier Academic Press: London, UK, 2016; pp. 209–261, doi:10.1016/B978-0-12-803361-6.00009-0.

4. Aspri, M.; Economou, N.; Papademas, P. Donkey milk: An overview on functionality, technology, and future prospects. Food Rev. Int. 2017, 33, 316–333, doi:10.1080/87559129.2016.1175014.

5. Vincenzetti, S.; Pucciarelli, S.; Polzonetti, V.; Polidori, P. Role of proteins and of some bioactive peptides on the nutritional quality of donkey milk and their impact on human health. Beverages 2017, 3, doi:10.3390/beverages3030034.

6. Boland, M.; Harjinder, S. Milk Proteins: From Expression to Food, 3rd ed.; Elsevier Academic Press: London, UK, 2020; pp. 715–730, doi:10.1016/C2017-0-03077-X.

7. Suharoschi, R.; Bartalici, C.I.; Petrut, R.; Semeniuc, C.A.; Rotar, A.M. Two-Dimensional gel electrophoresis of peptides as lap in bovine milk. J Agroaliment Processes Technol, 2010, 16, 169-176.

8. Mati, A.; Senoussi-Ghezali, C.; Si Ahmed Zennia, S.; Almi-Sebbane, D.; El-Hatmi, H.; Girardet, J.-M. Dromedary camel milk proteins, a source of peptides having biological activities—A review. Int. Dairy J. 2017, 73, 25–37,

doi:10.1016/j.idairyj.2016.12.001.

9. Nagarajan, S.; Radhakrishnan, S.; Kalkura, S.N.; Balme, S.; Miele, P.; Bechelany, M. Overview of Protein-Based Biopolymers for Biomedical Application. Macromol. Chem. Phys. 2019, 220, 1900126, doi:10.1002/macp.201900126.
10. Shori, A.B. Comparative study of chemical composition, isolation and identification of micro-flora in traditional fermented camel milk products: Gariss, Suusac, and Shubat. J. Saudi Soc. Agric. Sci. 2012, 11, 79–88, doi:10.1016/j.jssas.2011.12.001.
11. Ellouze, S.; Kamoun, M. Evolution of Dromedary's Milk Composition According to Lactation Stage. In Rabat (Morocco); 25–27 October 1988; CIHEAM: Montpelier, France, 1989; pp. 307–311.
12. Hazebrouck, S. Laits de chèvre, d'ânesse et de chamelle: Une alternative en cas d'allergie au lait de vache? Innov. Agron. 2016, 52, 73–84.
13. Kessler, A.; Menendez-Aguirre, O.; Hinrichs, J.; Stubenrauch, C.; Weiss, J. Alphas-casein-PE6400 mixtures: A fluorescence study. Faraday Discuss 2013, 166, 399–416, doi:10.1039/c3fd00102d.
14. Swaisgood, H.E. Review and update of casein chemistry. J. Dairy Sci 1993, 76, 3054–3061, doi:10.3168/jds.S0022-0302(93)77645-6.
15. Swaisgood, H.E. Chemistry of the Caseins. In Advanced Dairy Chemistry—1 Proteins; Springer: Boston, MA, USA, 2003; pp. 139–201, doi:10.1007/978-1-4419-8602-3.
16. Lonnerdal, B. Nutritional and physiologic significance of human milk proteins. Am. J. Clin. Nutr. 2003, 77, 1537s–1543s, doi:10.1093/ajcn/77.6.1537S.
17. Malacarne, M.; Martuzzi, F.; Summer, A.; Mariani, P. Protein and fat composition of mare's milk: Some nutritional remarks with reference to human and cow's milk. Int. Dairy J. 2002, 12, 869–877, doi:10.1016/S0958-6946(02)00120-6.
18. Kroening, T.A.; Mukerji, P.; Hards, R.G. Analysis of beta-casein and its phosphoforms in human milk. Nutr. Res. 1998, 18, 1175–1186, doi:10.1016/S0271-5317(98)00098-0.
19. Tafaro, A.; Magrone, T.; Jirillo, F.; Martemucci, G.; D'alessandro, A.; Amati, L.; Jirillo, E. Immunological properties of donkey's milk: Its potential use in the prevention of atherosclerosis. Curr. Pharm. Des. 2007, 13, 3711–3717, doi:10.2174/138161207783018590.
20. Kontopidis, G.; Holt, C.; Sawyer, L. Invited Review: β-Lactoglobulin: Binding Properties, Structure, and Function. J. Dairy Sci. 2004, 87, 785–796, doi:10.3168/jds.S0022-0302(04)73222-1.
21. Hinz, K.; O'Connor, P.M.; Huppertz, T.; Ross, R.P.; Kelly, A.L. Comparison of the principal proteins in bovine, caprine, buffalo, equine and camel milk. J. Dairy Res. 2012, 79, 185–191, doi:10.1017/S0022029912000015.
22. Diarrassouba, F.; Remondetto, G.; Liang, L.; Garrait, G.; Beyssac, E.; Subirade, M. Effects of gastrointestinal pH conditions on the stability of the β-lactoglobulin/vitamin D3 complex and on the solubility of vitamin D3. Food Res. Int. 2013, 52, 515–521, doi:10.1016/j.foodres.2013.02.026.
23. Taulier, N.; Chalikian, T.V. Characterization of pH-induced transitions of β-lactoglobulin: Ultrasonic, densimetric, and spectroscopic studies. Edited by C. R. Matthews. J. Mol. Biol. 2001, 314, 873–889, doi:10.1006/jmbi.2001.5188.
24. Markus, C.R.; Jonkman, L.M.; Lammers, J.H.; Deutz, N.E.; Messer, M.H.; Rigtering, N. Evening intake of α-lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention. Am. J. Clin. Nutr. 2005, 81, 1026–1033, doi:10.1093/ajcn/81.5.1026.
25. Markus, C.R.; Olivier, B.; de Haan, E.H. Whey protein rich in α-lactalbumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. Am. J. Clin. Nutr. 2002, 75, 1051–1056, doi:10.1093/ajcn/75.6.1051.
26. Gurgel, P.V.; Carbonell, R.G.; Swaisgood, H.E. Studies of the Binding of α-Lactalbumin to Immobilized Peptide Ligands. J. Agric. Food Chem. 2001, 49, 5765–5770, doi:10.1021/jf010462b.
27. Velusamy, V.; Palaniappan, L. Compositional analysis α-lactalbumin. Am. J. Biochem. Mol. Biol 2011, 1, 106–120, doi:10.3923/ajbmb.2011.106.120.
28. Carminati, D.; Tidona, F. Chapter 31—Nutritional Value and Potential Health Benefits of Donkey Milk. In Nutrients in Dairy and Their Implications on Health and Disease; Watson, R.R., Collier, R.J., Preedy, V.R., Eds.; Academic Press: London, UK, 2017; pp. 407–414, doi:10.1016/B978-0-12-809762-5.00031-0.
29. Vincenzetti, S.; Polidori, P.; Mariani, P.; Cammertoni, N.; Fantuz, F.; Vita, A. Donkey milk protein fractions characterization. Food Chem. 2008, 10, 640–649, doi:10.1016/j.foodchem.2007.06.026.
30. Vincenzetti, S.; Amici, A.; Pucciarelli, S.; Vita, A.; Micozzi, D.; Carpi, F.M.; Polzonetti, V.; Natalini, P.; Polidori, P. A Proteomic Study on Donkey Milk. Biochem. Anal. Biochem. 2012, 1, 109, doi:10.4172/2161-1009.1000109.

31. Ozturkoglu-Budak, S.; Effect of different treatments on the stability of lysozyme, lactoferrin and b-lactoglobulin in donkey’s milk. Int. J. Dairy Technol. 2016, 71, 36–45, doi:10.1111/1471-0307.12380.
32. Brumini, D.; Criscione, A.; Bordonaro, S.; Vegarud, G.E.; Marletta, D. Whey proteins and their antimicrobial properties in donkey milk: A brief review. Dairy Sci. Technol. 2016, 96, 1–14, doi:10.1007/s13594-015-0246-1.
33. Yoo, Y.C.; Watanabe, S.; Watanabe, R.; Hata, K.; Shimazaki, K.I.; Azuma, I. Bovine lactoferrin and lactoferricin, a peptide derived from bovine lactoferrin, inhibit tumor metastasis in mice. Jpn. J. Cancer Res. 1997, 88, 184–190, doi:10.1007/978-1-4757-9068-9_35.
34. Yoo, Y.-C.; Watanabe, R.; Koike, Y.; Mitobe, M.; Shimazaki, K.-I.; Watanabe, S.; Azuma, I. Apoptosis in human leukemic cells induced by lactoferricin, a bovine milk protein-derived peptide: involvement of reactive oxygen species. Biochem. Biophys. Res. Commun. 1997, 237, 624–628, doi:10.1006/bbrc.1997.7199.