

SHORT COMMUNICATION

Camel colostrum: Nutritional composition and improvement of the antimicrobial activity after enzymatic hydrolysis

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Abstract

Camel colostrum collected within 24 h after parturition was analyzed for physicochemical and microbiological composition. The average contents of fats, dry matter, mineral matter and proteins were 1.71 ± 0.51 , 199.55 ± 16.93 , 9.75 ± 0.5 and 143.42 ± 36.42 gL⁻¹, respectively. Microbiological analysis of colostrum samples showed richness in yeasts and Lactic acid bacteria and absence of coliforms. The good microbial quality of camel colostrum is due to a number of antimicrobial molecules such as immunoglobulins and lactoferrin. The antimicrobial activity was evaluated against the pathogens *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecium* and *Pseudomonas aeruginosa*. At concentration of 20 g L⁻¹, colostrum caused an important inhibition of growth of all tested bacteria. It therefore seemed interesting to assess whether the compounds inhibit the growth of tested strains present in camel colostrum are resisting to the action of digestive enzymes. An in vitro hydrolysis by pepsin and pancreatin was then conducted. Hydrolyzed camel colostrum was still active against all pathogenic strains with inhibition rate ranging from 15.8% to 24.18%. This finding highlights the presence of antimicrobial fragments/peptides released during proteolytic hydrolysis that may contribute to the antimicrobial activity in camel colostrum and play a significant role in the host defence system.

Key words: Camel colostrum proteins, Enzymatic digestion, Antimicrobial activity

Introduction

Colostrum, a nutrient-rich fluid produced by female mammals immediately after giving birth, is loaded with immune and growth factors. Colostrum, the early lactation, has a nutritional profile and immunological composition that substantially differs from that of mature milk. In the matter of fact, camel colostrum contains more protein, non protein nitrogen, ash, vitamins, and minerals than doe's milk. It contains significant quantities of components that act as natural antimicrobial agents to actively stimulate the maturation of a camel calf's immune system.

Camel colostrum possesses major milk proteins

like α -Lactalbumin, serum albumin, lactophorin (proteose peptone-component 3), basic whey protein, with an average concentration of 2.7, 10.8, 4.9 and 3.1 g L⁻¹, respectively (Konuspayeva et al., 2009; El Hatmi et al., 2007). A common feature of camel and human milk and colostrum is the absence of β -Lactoglobulin (β -Lg), the major whey bovine protein which causes allergy in children, and the richness in lactoferrin contents with an average of 2.3 g L⁻¹ versus 0.5 g L⁻¹ in bovine colostrum, which contribute to antimicrobial activity of camel colostrum (Benkerroum et al., 2004). Camel colostrum immunoglobulins consist of three main sub-classes, namely IgG1, IgG2, and IgG3 (Azwai et al., 1996). As reported by Hamers-Casterman et al. (1993) the two immunoglobulins sub-classes β -IgG2 and IgG3- are devoid of light chains and have a molecular mass of 42 and 45 kDa, respectively. This feature in the protein composition of camel colostrum may reflect a particular biological activity.

Only few studies have been reported that camel colostrum is rich in bioactives molecules such as antioxydant and antihypertensif peptides released

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after enzymatic hydrolysis or fermentation of milk (Jrad et al., 2014). Among the bioactive fragments, antimicrobial peptides (AMP) attract the attention of many researchers because the resistance of many pathogenic bacteria to conventional antibiotics.

Antimicrobial peptides are low molecules weight with small sequence of amino-acids (up- 50 aa) with broad-spectrum antimicrobial activity against Gram positive and negative bacteria. AMPs are often cationic peptides and extremely fast acting. Their principal mechanism is attributed to perturbation of bacteria cell membrane. Many AMPs are generated from different species milk proteins after degradation whether by enzymes produced by animals (gastro-intestinal enzymes), plants or micro-organisms or during manufacturing of dairy derived products but not from colostrum. Several antimicrobial peptides derived from milk proteins' digestion are well known now as fragments LTD1 (1-5), LDT2 (17-31/109-114), LDC (61-68/75-80); f (15-20), f (25-40), f (78-83), f (92-100); Isracidin (1-23) ; Casocidin ; Kappacin (106-169) ; f (43-97) and lactoferricin B (17-41); H (1-11/12-47, 1-16/43-48; 1-42/43-48; 1-16/17-48; 1-16/45-48; 1-11/17-47; C (14-42) from respectively digested bovine - Lactalbumin (Pellegrini et al., 1999), bovine - Lactoglobulin (Pellegrini et al., 2001), bovine S1-Casein (Recio and Visser, 1999), S2-Caseine, -Caseine, human -Caseine, bovine lactoferrin (Bellamy et al., 1992), human lactoferrin (Wakabayashi et al., 2006) and caprine lactoferrin (Recio and Visser, 2000). However, no information is available regarding peptides derived from proteins of camel milk and colostrum.

Hence, this study was aimed to characterize the microbiological and physicochemical quality of camel colostrum. In order to evaluate the effect of enzymatic digestion on antimicrobial proteins present in camel colostrum, the growth of four pathogenic bacteria in presence of camel colostrum proteins before and after gastro-intestinal digestion in vitro was monitored.

Material and Methods

Samples collection

Camel colostrum and milk were handily collected from eleven healthy camel (*Camelus dromedarius*) reared in Livestock and Wildlife Laboratory of Arid Land Institute in south of Tunisia. Samples were stored at -20°C until use.

Gross composition analysis

pH and acidity

The pH of sample was determined using a Thermo Orion pH meter (Cumming Center

Beverly, USA) and the Dornic acidity was measured by titration of 10 ml of milk or colostrum by the sodium hydroxide N / 9 in the presence of phenolphthalein (AFNOR, 1993).

Fat analysis

It was determined by the method of acid-butyrometric Gerber using butyrometer graduates (AFNOR, 1993). This method consisted of an attack of milk with sulfuric acid and separation of the fat released by centrifugation in the presence of iso-amyl alcohol.

Proteins analysis by Kjeldhal method:

The levels of crude protein (CP) of milk were determined by the Kjeldahl method ($\text{N} \times 6.38$) (AFNOR, 1993) after distillation unit NITRO PRO-I and titration with 0.1 N hydrochloric acid.

Viscosity

The viscosity was expressed in centipoises (cP) and 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).

Dry matter and ash content

Dry matter expressed in grams per liter milk was calculated after weighing the sample at 105°C for 24 hours of its dry residue. Ash content, expressed in g L^{-1} of milk, was determined after dry mineralization at 505°C (AFNOR, 1993).

Bacteriological analysis of samples

The techniques used were conventional methods and reflected the recommendations of French law or official French method (AFNOR, 1996) as follows:

Milk samples (1 ml) were diluted in buffered peptone saline (10^{-1} to 10^{-3}) and mixed in stomacher bag. In order to quantify the various microbial groups, appropriate dilutions were used as follows:

Aerobic total plate counts (ATPC) (Sharlau Chemie S.A) were carried out in depth on plate count agar (PCA) and incubated at 32°C for 72h (El-Ziney and Al-Turki, 2007).

Yeast and moulds were surface plated on Sabouraud Chloramphenicol (Pronadisa Micro & Molecular Biology) and incubated at 25°C for 3 to 5 days.

Total coliform were grown in Violet Red Bile Agar (VRBA) (AppliChem.Biochemica.Chemica services) in double layer. After solidifying of the agar, the plates were incubated at 30°C for 22 h (Federal Register, 1990).

Lactic acid bacteria (LAB) were shown on the surface on MRS agar (de Man, Rogosa and Sharpe)

(De Man et al., 1960) and then incubated 30°C for 48 h.

Colostrum protein preparation

Samples of camel colostrum were defatted by centrifugation (5000g; 30 min; 4°C). Then caseins were precipitated by HCl (1 M) at pH 4.2 and centrifuged again (1500g; 20 min, 20°C). The supernatant contained whey proteins was neutralized at pH 7 by addition of NaOH (1M) and dialyzed at 4°C for 72 h. Whey proteins were then freeze-dried and kept at - 20°C until further analysis.

Enzymatic hydrolysis *in vitro*

Samples were digested by pepsin and pancreatin at 37°C in shaking water bath to mimic gastro-intestinal conditions according to protocol adopted by Parrot et al. (2003). Briefly, camel colostrum proteins were incubated with pepsin at acidic condition (pH=2) for 30 min and after with pancreatin for 4h at pH 7.5. The reaction was stopped by heating the mixture at 85°C for 5 min to inactivate digestive enzymes. The E/S ratio was 1/200 and 1/400 for pepsin and pancreatin, respectively. Digested samples were stored at - 20°C until analysis.

Antibacterial activity assay

The antibacterial activity was determined using a semi-automatic spectrophotometer Bioscreen (Thermofisher, IllKirch, France) in liquid medium at wavelength 600 nm. For this reason, 30 µL of tested bacteria (10^6 CFU ml⁻¹) were inoculated with 270 µL of Brain Heart Infusion (BHI) supplemented with undigested and digested camel colostrum proteins at different concentrations and stirred under medium agitation at 30°C for 24 h after sterilization by filtration onto 0.2-µm pore size membranes (Millipore Corporation, Billerica, MA, USA).

Results and Discussion

Gross composition of camel milk and colostrum

The average contents of fat, dry matter, mineral matter and proteins were 1.71±0.51, 199.55±16.93,

9.75±0.5 and 143.42±36.42 gL⁻¹, respectively (table 1). The pH value of camel colostrum was lower than that of milk due to the richness of colostrum in proteins (143.42±36.42 g L⁻¹) especially immunoglobulins G (Ig G1, Ig G2 and Ig G3) and camel serum albumin (CSA) (El Hatmi et al., 2007).

The highest content of dry matter was observed in camel colostrum due mainly to the high content of proteins. The Ca and K contents which could be necessary to bone growth of the newborn, were higher in camel colostrum than milk. The content of fat in camel colostrum was very low compared to that of bovine colostrum. A similar trend was noted for dromedary and Alxa Bactrian camel as is reported by El Hatmi et al. (2006) and Zhang et al. (2005).

Therefore, changes in camel milk composition occurred along of lactation stage, because towards the end of the lactation, the fat, protein, solids and mineral contents increase, while the lactose content decreases (Benkerroum et al., 2003; Konuspaveva et al., 2007; Musaad et al., 2013).

Enumeration of microorganisms

The bacteriological results found in camel colostrums and milk (table 3) did not meet the cow milk standard ($< 5 \times 10^4$ CFU ml⁻¹). This result is due to the good health status of milking dromedaries (with no mastitis) and to precautions taken to avoid any milk contamination.

Table 1. Gross composition of camel milk and colostrum ± SEM.

Gross composition	colostrum	milk
pH	6.28±0.04	6.45 ±0.025
Dornic acidity (°D)	31±0.55	17± 0.46
Viscosity (cP)	372±5.30	192±3.05
Fats (g L ⁻¹)	1.71±0.22	22± 6.36
Porteins (g L ⁻¹)	143.42±5.91	31.5 ±0.79
Dry matter (g L ⁻¹)	199.55±5.9	106 ± 5.08
Ash (g L ⁻¹)	9.75±0.59	7.5±0.102

Table 2. Mineral content of camel colostrum and milk.

Sample	Mineral content (g L ⁻¹)			
	Ca	K	Mg	Na
Colostrum	2.03 ± 0.31	1.26 ± 0.34	0.08 ± 0.07	0.75 ± 0.08
Milk	1.47 ± 0.38	0.98 ± 0.24	0.07 ± 0.01	0.65 ± 0.11

Table 3. Microbiological examination of raw camel and colostrums samples (cfu ml⁻¹).

Micro-organisms (CFU ml ⁻¹)	ATPC	Yeasts	LAB
Colostrum	3.10 ²	2.10 ^{2a}	10 ²
Milk	7.10 ³	50 ^b	17.10 ¹
Effect	N.S	**	N.S

Table 4. Rate of inhibition of bacterial growth after 15 h of incubation in presence and absence of digested and undigested camel colostrum at concentration of 10 and 20 g L⁻¹, respectively.

Sample	Inhibition (%)			
	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>E. faecium</i>	<i>S. aureus</i>
Colostrum (20 g L ⁻¹)	28.97±0.018	21.82±0.028	16.59±0.019	21.45±0.014
Colostrum hydrolysates (10 g L ⁻¹)	16.47±0.013	15.80±0.012	24.18±0.147	17.52±0.022

The level of ATPC, LAB and coliform were different in camel colostrum and milk but those differences were not significant. Camel colostrum possessed a significantly higher content of yeasts than camel milk, due to the ability of yeasts to grow in substrates with high salt concentration and low pH. Generally, yeasts were regarded as normal flora of camel milk (Nikkhah, 2011) but their presence in a large number is a consequence of proteolytic and lipolytic activity, as well as their ability to ferment and/or assimilate lactose and to utilize lactic, citric and succinic acids (Corbaci et al., 2012). Yeasts may play a therapeutic role in the dairy production and be responsible for antimicrobial property (Lopandic et al., 2006). Three predominant yeasts from twelve species identified from fermented camel milk were *Kazakhstania unispora*, *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* (Akhmetsadykova et al., 2014).

Antimicrobial activity

The inhibitory activity of camel colostrum before and after hydrolysis was tested against different pathogenic microorganisms by calculating the rate of growth inhibition (Table 4). On the whole, the camel colostrum displayed a different inhibitory capability ranging from 16.59% to 28.97%. Literature reports that camel colostrum and milk have antimicrobial activity against different microorganisms (El-Agamy et al., 1992; Benkerroum et al., 2004).

The antimicrobial activity in camel colostrum might be partially due to high level of antimicrobial proteins namely lactoferrin (Conesa et al., 2008). A large number of studies have demonstrated bactericidal and bacteriostatic effect of lactoferrin from other species. The camel colostrum proteins still exhibits a bacterial modulating effect following digestion even at lower concentration (10 g L⁻¹). Interestingly, the inhibitory effect against *E. faecium* was markedly higher for the digested

samples, thus the antimicrobial activity might result from a synergistic effect of substances, possibly peptides released by gastrointestinal enzymes.

Conclusion

These preliminary results suggest that most compounds in camel colostrum inhibiting the growth of diverse bacteria are hydrolysed to more active compounds by successive actions of pepsin and pancreatin. At this moment, it is not possible to decide if the inhibition is caused by a single peptide or protein, or by a mixture and synergic effect of more than one compound. To assess this further fractionation of hydrolysates, it is necessary to identify the proteins (or peptides) at the origin of the observed effects. It is now necessary to study the antibacterial activity of various purified proteins from the camel colostrum and their hydrolysates.

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Author contributions

J. Z. designed the study, did the analysis, O. N. and A. I. designed the study, E. H. wrote the article and D. P. and K. T. wrote and corrected the article.

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