Chemical characterisation of oligosaccharides in commercially pasteurised dromedary camel (Camelus dromedarius) milk

O.A. Alhaj a, E. Taufik b,1, Y. Handa b, K. Fukuda b, T. Saito c, T. Urashima b,*

a Department of Food Science and Nutrition, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia
b Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan
c Graduate School of Agriculture, Tohoku University, Tsutsumidori-Amamiya machi 1-1, Aoba-Ku, Sendai, Miyagi 981-8555, Japan

A R T I C L E  I N F O

Article history:
Received 12 April 2012
Received in revised form 23 August 2012
Accepted 27 August 2012

A B S T R A C T


1. Introduction

There are two species of camels: the dromedary or Arabian camel (Camelus dromedarius, one hump) and the bactrian camel (Camelus bactrianus, two humps) (Al haj & Al Kanhal, 2010). In arid and semi-arid areas where heat and lack of water and feed severely affect dairy cows, cameldids play an important role in providing milk for the population (Yagil, 1982). As the result, establishment of modern camel farms for large scale milk production is currently underway. It is expected that in the future, modern large-scale camel farms can also become sources for the production of bio-functional milk components.

The lactose and protein contents of dromedary and bactrian camel milks are similar but their fat contents differ (Medhammar et al., 2012). The composition of bactrian camel colostrum obtained 2 h post-partum has been estimated to be 14.23% protein, 2.91% fat, 3.52% carbohydrate, and 0.04% minerals (Zhang et al., 2005). Aljumaah et al. (2011) reported that the mean (±standard error) levels of milk constituents from healthy lactating dromedary camel were 2.91 ± 0.04% fat, 3.52 ± 0.02% protein, 5.13 ± 0.03% lactose and 9.40 ± 0.05% solids-non-fat (SNF).

Al Haj and Al Kanhal (2010) reported that dromedary camel milk has been acknowledged to have many functional properties due to its bioactive components. However, most of the reports on these components and their functionality were concerned with protein-related components. Reports on functionality related to oligosaccharides are very limited.

2. Materials and methods

2.1. Milk samples

Three litres of commercially available pasteurised (at 75 °C for 15 s) bulk camel milk sample was purchased from the local market of Riyadh city (central region of Saudi Arabia), in February (winter season). This milk is a pasteurised product processed by Alwatania Co. Ltd. (Riyadh, Saudi Arabia), one of the food companies in Saudi Arabia that is ISO 9001 certified and supplies HACCPC in their production. Compositional analysis results of the sample showed that fat and solids-non-fat levels were 3% and 8.5%, respectively, and pH and acidity were 6.54 and 0.15%, respectively. The sample was then freeze-dried using a Virtis freeze dryer, model Unipot 600SL (The Virtis Co., Gardiner, NY, USA), stored in a sealed nylon pack in –20 °C and then shipped to Obihiro University of Agriculture and Veterinary Medicine, Japan, for further analysis.

2.2. Materials

Lacto-N-neohexaose [LNnH; Gal(β-1-4)GlcNAc(β-1-3)]Gal(β-1-4)GlcNAc[β-1-6]Gal(β-1-4)Glc] was purchased from Seikagaku Co. (Tokyo, Japan), and 3′-sialylactose [3′-NAC-SL; Neu5Ac(α2-3)Gal(β-1-4)Glc] and 6′-sialyllactose [6′-NAC-SL; Neu5Ac(α2-6)Gal(β-1-4)Glc] were obtained from Sigma Co. (St. Louis, MO, USA). 3′-galactosylactose [3′-GL; Gal(β1-3)Gal(β-1-4)Glc] was isolated from caprine colostrum (Urashima, Bubb, Messer, Tsuji, & Taneda, 1994), while sialyl 3′-galactosylactose [sialyl 3′-GL; Neu5Ac(α2-3)Gal(β-1-3)Gal(β-1-4)Glc], sialyllacto-N-novopentaose a [sialyl LNP a; and Wang et al. (2007), to possess anti-adhesive effects against certain pathogens, as well as important nutrients for brain development.

According to the Agriculture Statistical Year Book (2009) of Saudi Arabia, as cited by Aljumaah et al. (2011), the population of dromedary camels in Saudi Arabia consists of more than 800,000 animals. Of an estimated 18 million camels in the world, only 2 million are bactrian camels (Allhadrami, 2003). Thus, dromedary species supply more milk for human consumption than bactrian.

The present study focused on the structural characterisation of commercially available pasteurised dromedary camel milk marketed in Riyadh city, Saudi Arabia. Thus, the chemical structures of some neutral and acidic oligosaccharides from pasteurised dromedary camel milk were characterised using 1H nuclear magnetic resonance spectroscopy (1H NMR).

2.3. Isolation of milk oligosaccharides and lactose

Milk powder (2 g) was dissolved in 18 mL of water and the solution was centrifuged at 4000 × g. The supernatant was extracted with 4 volumes of chloroform/methanol (2:1, v/v). The emulsion was centrifuged at 4000 × g for 30 min at 4 °C, and the lower chloroform layer and the denatured protein were discarded. The methanol was removed from the upper layer by rotary evaporation, and the residue was called the carbohydrate fraction.

The carbohydrate fraction was separated into four fractions (DC-1 to DC-4, see Fig. 1) by gel filtration with a BioGel P-2 column as previously described (Fukuda et al., 2010). The above procedures were repeated eight times. The saccharides in the fractions constituting peaks DC-2, DC-3 and DC-4 (Fig. 1) were separately pooled, freeze-dried and subjected to 1H NMR spectroscopy to characterise their chemical structures. The components in peak DC-
Camel milk.

The peak fractions of oligosaccharides were subjected to 1H NMR liquid chromatography (HPLC) on a TSK gel Amide-80 column as components in DC-1 were further subjected to high performance anion-exchange chromatography. The unadsorbed fraction, redesignated as DC-1 (Fig. 2), was pooled and passed through a BioGel P-2 column. The components contained in this fraction were then separated by HPLC (Fig. 3).

Table 2

<table>
<thead>
<tr>
<th>Reporter group</th>
<th>Residue</th>
<th>Chemical shifts, δ (coupling constants, Hz)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DC-1-3</td>
</tr>
<tr>
<td>H-1</td>
<td>Glc z</td>
<td>5.222 (3.4)</td>
</tr>
<tr>
<td></td>
<td>Glc β</td>
<td>4.663 (8.0)</td>
</tr>
<tr>
<td></td>
<td>Gal[β-1-4]</td>
<td>4.427 (8.0)</td>
</tr>
<tr>
<td></td>
<td>Gal[β-1-3]</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>GlcNAc[β-1-3]</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>GlcNAc[β-1-6]</td>
<td>4.638 (8.5)</td>
</tr>
<tr>
<td>H-4</td>
<td>Gal[β-1-4]</td>
<td>4.145 (3.4)</td>
</tr>
<tr>
<td></td>
<td>GlcNAc[β-1-3]</td>
<td>2.030</td>
</tr>
<tr>
<td></td>
<td>GlcNAc[β-1-6]</td>
<td>2.060</td>
</tr>
</tbody>
</table>

a The numbers in the brackets are coupling constants (J). Coupling constant is a measure of the interaction between neighbouring resonances or a pair of protons.

b J_1,4.

c J_3ax.
d J_3ax,4.
e J_3eq.
f J_3eq,6.

The carbohydrate fraction from pasteurised dromedary camel milk was subjected to gel chromatography as shown in Fig. 1. The peak that reacted positively for sialic acid (DC-1) was subjected to anion-exchange chromatography. The unadsorbed fraction, redesignated as DC-1 (Fig. 2), was pooled and passed through a BioGel P-2 column. The components contained in this fraction were then separated by HPLC (Fig. 3).

2.4. 1H NMR spectroscopy

Nuclear magnetic resonance spectra were recorded in D_2O (100.00 atom D%, Sigma–Aldrich, Milwaukee, WI, USA) at 500 or 600 MHz for 1H NMR with a JEOL ECP-500 Fourier transform-NMR spectrometer (JEOL, Tokyo, Japan) or a Varian INOVA 600 spectrometer (Varian Inc., Palo Alto, CA, USA), respectively, operated at 293.1 K. Chemical shifts are expressed as change relative to internal 3-(trimethylsilyl)-1-propane sulphuric acid, sodium salt, but measured by reference to internal acetone (δ = 2.225).

3. Results

3.1. Separation of oligosaccharides from pasteurised dromedary camel milk


3.3. Structural characterisation of acidic oligosaccharides

As the 1H NMR spectra of DC-1-3 and DC-1-4 (chemical shifts in Table 2) were identical to those of authentic 3'-NAc-SC and 6'-NAc-SC, respectively, the oligosaccharides in these peaks were characterised as Neu5Ac(s2-3)Gal[β-1-4]Glc and Neu5Ac(s2-6)Gal[β-1-4]Glc. The 1H NMR spectra of DC-1-5, DC-1-8 and DC-1-9 (chemical shifts in Table 2, Fig. 4) showed that their characteristic resonances...


Fraction DC-1-1 contained lactose, which had contaminated DC-1. The oligosaccharides in the other peaks were not characterised, because it was not possible to assign their 1H NMR signals.

4. Discussion

Commercial processed milk is normally produced from bulk milk that is collected from many animals at different lactation stages, and the milk quality is guaranteed by food quality and safety systems during processing and along the supply chain. During the pasteurisation step, a small portion of sialic acid might have been removed from the sialyl oligosaccharides. However, as this study focused on characterisation of each oligosaccharide, such small release of sialic acid has negligible effect on the characterisation. Moreover, Bertino et al. (2008) reported that pasteurisation does not affect the concentration or pattern of analysed oligosaccharides in human milk.

The structures of the oligosaccharides of dromedary milk characterised in this study are shown in Table 3, which also provides a comparison of the neutral and acidic oligosaccharides of dromedary milk and of bactrian camel colostrum and milk. The following oligosaccharides, 3'-GL and 3'-SL, which were found in this study, also exist in bactrian camel milk (Fukuda et al., 2010). LNnH was detected only in this study, while Gal[1-3][Gal[1-4]GlcNAc[1-6]Gal[1-4]Glc (lacto-N-novopentaose I) was found only in bactrian camel milk (Fukuda et al., 2010). However, as sialyllacto-N-novopentaose a was found in dromedary camel milk, it is possible that lacto-N-novopentaose I existed at a low concentration in this milk, too. In addition, 3'-GL, 3'-SL, 6'-SL, sialyl-3'-galactosylactosylactose, sialyllacto-N-novopentaose a and MSLNnH, which were found in this study, were also detected in bactrian camel colostrum. Although the oligosaccharides in dromedary colostrum were not characterised in this study, the colostral oligosaccharide profile is probably similar to that found in dromedary milk; i.e., the oligosaccharides, which were found in dromedary milk in this study, might be found in dromedary colostrum, too. The oligosaccharide 3'-GL is a prebiotic molecule, which may suggest that dromedary camel milk oligosaccharides are prebiotic and could be used as a food additive with this function (Urashima et al., 2009). On the other hand, the biological function of another neutral oligosaccharide, LNnH, is still unclear.

Sialyl -3'-galactosylactosylactose and sialyllacto-N-novopentaose a have not been found in human milk/colostrum. However, other oligosaccharides, present in dromedary milk, including 3'-GL, LNnH, 3'-SL, 6'-SL and MSLNnH, are also present in human milk. Sialyllacto-N-novopentaose a, LNnH and MFLNnH contain Gal[1-4]GlcNAc (N-acetyllactosamine); these oligosaccharides are categorised as type II oligosaccharides. In camels, only type II oligosaccharides were found in this study and our previous study. The type II oligosaccharides, LNnH, MFLNnH, as well as Neu5Ac[2-6]Gal[1-4]GlcNAc[1-3]Gal[1-4]Glc (LST c), which was found in bactrian colostrum in our previous study (Fukuda et al., 2010), have

![Graph showing NMR spectrum](image)


| Type of oligosaccharide | Milk oligosaccharide | Dromedary camel mature milk (fraction) | Bactrian camelb |
|-------------------------|----------------------|--------------------------------------|-----------------|--------|
|                         | Codea                | Structure                            | Colosum         | Mature milk |

a For the neutral oligosaccharides, the codes refer to: 1, 3'-galactosylactose (3'-GL); 2, 6'-galactosylactose (6'-GL); 3, 3-fucosyllactose (3-FL); 4, lacto-N-novopentaose 1 (Novol NP 1); 5, Lacto-N-neohexose [LNnH]. For acidic oligosaccharides, the codes refer to: 1, 3'-sialyllactose (3'-N-acetylactosamine); 2, 6'-sialyllactose (6'-N-acetylactosamine); 3, sialyl-3'-galactosylactosylactose (5-3'-GL); 4, sialyllacto-N-neotetraose c (LST c); 5, sialyllacto-N-novopentaose a (Novol NP a); 6, sialyllacto-N-novopentaose b (Novol NP b); 7, monosialyllacto-N-neohexose (MSLNnH). b The tick (✓) indicates the oligosaccharide was isolated, the dash (–) indicates was not isolated.
been reported in human milk, too (Urashima et al., 2009). However, in human milk or colostrum, type I oligosaccharides, which contain Gal[b1–3]GlcNac (lacto-N-biose I), predominate over the type II.

As mentioned previously, milk oligosaccharides are thought to have biological significances. It can therefore be expected that oligosaccharides from camel milk may in future be incorporated into human infant formulae. Mature bovine milk contains only low concentrations of milk oligosaccharides (Gopal & Gill, 2000; Martin-Sosa, Martin, Garcia-Pardo, & Hueso, 2003; McJarrow & Amelsfort-Schoonbeek, 2004; Nakamura et al., 2003). The addition of bovine milk oligosaccharides to infant formulae has therefore been limited. Although fifteen neutral and twenty four sialyl oligosaccharides have been characterised in bovine colostrum (Marino et al., 2011; Urashima et al., 2009), the bulk composed of only four oligosaccharides, i.e., 3′-SL, 6′-SL, Neu5Ac(2′-6′)Gal[β1–4]GlcNAc (6′-sialyl-N-acetyllactosamine) and Neu5Ac(2′-8′)Neu5Ac(2′-3′)Gal[β1–4]Glc (disialylactose). The others, including neutral and sialyl oligosaccharides, are present in only trace amounts (Fong, Ma, & McJarrow, 2011; Tao et al., 2008; Tao, DePeters, German, Grimm, & Lebrilla, 2009). However, the concentrations of sialyl oligosaccharides, including 3′-SL, 6′-SL and 6′-sialyl-N-acetyllactosamine, dramatically decrease after 48 h post-partum (Nakamura et al., 2003). Furthermore, the total concentration of oligosaccharides in bovine mature milk is very low (Fong et al., 2011), although processed bovine milk and ‘waste streams’ from such processing (e.g., cheese whey) should be easy sources from which to separate sialyl as well as neutral oligosaccharides. In a previous study, the profiles of sialyl oligosaccharides from colostrum and mature bovine milk were obtained (Fukuda et al., 2010), and these profiles could be compared with the oligosaccharides profile of dromedary camel milk (Fig. 3) in this study. The ratios of other sialyl oligosaccharides to 3′-SL and 6′-SL were lower than that in dromedary milk, suggesting that dromedary milk may be a better commercial source for the isolation or enrichment of these types of sialyl oligosaccharides than bovine milk and colostrum.

Camel milk oligosaccharides, especially sialyl oligosaccharides, could be isolated and added to infant formulae, as it has been reported that sialic acid may exhibit a number of health benefits for human infants, including the promotion of infant brain development (Nakamura & Urashima, 2004). There are technologies for the production of oligosaccharides, such as Neu5Ac(2′-3′)Gal[β1–4]Glc (3′-SL, 3′-Nac-SL) by bacterial coupling (Endo, Koizumi, Tabata, & Ozaki, 2000) or GlcNAc[β1–3]Gal[β1–4]Glc (lacto-N-triose 2, LNT 2), Gal[β1–4]GlcNAc[β1–3]Gal[β1–4]Glc (lacto-N-neotetraose, LNT4), and 3′-Nac-SL by metabolically engineered bacteria (Priem, Gilbert, Wakarchuk, Heyraud, & Samain, 2002). However, in the food industry, the use of such technologies for production of oligosaccharides has some constraints, such as very high costs and the un-“natural” origin factor. The production of oligosaccharides that originate from natural sources, such as colostrum and milk/milk components of dairy animals, supported by relatively low cost and simple technology such as the use of ultrafiltration membranes (Oliveira, Wilby, Grandison, Duarte, & Roseiro, 2012), might be preferred by the food industry.

5. Conclusion

Two neutral oligosaccharides and five acidic oligosaccharides were characterised from commercially pasteurised dromedary camel milk. It is concluded that dromedary camel milk is likely to be a good source of oligosaccharides that could be utilised on industrial scale for the manufacture of functional foods, including human infant formulae. Apart from supplying milk for consumption, in the future, camel dairy farming can be developed to utilise the biofunctional properties of camel milk as an industrial resource.

Acknowledgements

This work was supported by funding from the Global COE Program “Frontier Program for Animal Global Health and Hygiene”, Ministry of Education, Culture, Sports, Science, and Technology, Japan, and Yotsuba Milk Products Co.

References


