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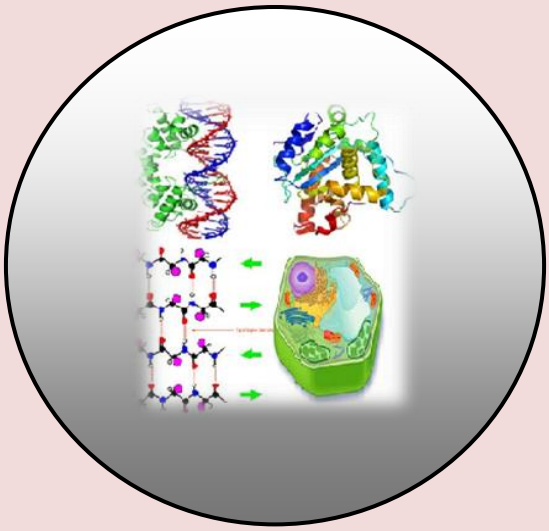

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Structure Elucidation of Novel Oligosaccharide Amelose from Camel Milk

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ABSTRACT

Camel milk is gaining increasing recognition due to its beneficial effects in the control and prevention of multiple health problems. Camel milk is different from other ruminant milk, having low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium), high vitamin C. Ayurveda has referred medicinal value of camel milk under the classification of "Dugdha Varga" (Milk Classification). Camel milk has potential therapeutic characteristics, such as anti-hypertensive, anti-diabetic and anti-carcinogenic. In continuation to our previous work on camel milk oligosaccharide, we have isolated another novel octasaccharide namely Amelose from camel milk. In this process camel milk was collected and processed by modified method of Kobata and Ginsburg followed by the HPLC and CC techniques. Structure elucidation of isolated compound (Amelose) was established by the chemical degradation, chemical transformation, NMR(^{1}H, ^{13}C NMR, 2D-NMR COSY, TOCSY, HSQC) comparison of chemical shift of anomeric signals, data of natural oligosaccharide structure reporter group theory techniques, and mass spectrometry as under:-

Gal-α(1→3)Glc-β(1→6)Gal-β(1→3)GlcNAc1-β(1→6)Gal-β(1→4)Glc
|
(1→4)Gal-β
|
(1→3)Gal-α

AMELOSE

Key words: Oligosaccharides, modified method of Kobata and Ginsburg and Amelose.

INTRODUCTION

Oligosaccharides are a heterogeneous group of carbohydrates comprising the third most abundant constituents in milk (Dai et al., 2000, Morrow and Ranges, 2004). Many of the oligosaccharides occur in free form, while others are linked to glycoproteins (e. g. Lactofferin, k-casein and slgA (Kelleher and longe dal, 2001, Newburg 2000, Shah 2000). A number of biologically active oligosaccharides have been isolated from human, buffalo, donkey, cow, mare, sheep and goat milk. Donkey milk oligosaccharides have ability to stimulate non-specific and specific immunological resistance (Ranjan A.K., et al., 2016). Mare's milk oligosaccharide fractions are having multifold properties such as antioxidant and lipid lowering activities (Srivastava, A., et al., 2012). HMOs are microbiota modulators, known to regulate immune responses directly.

Camel milk so called white gold of the desert is more similar to human milk than any other milk. Camel milk has been acknowledged for a long time to provide a potential treatment for a series of diseases such as dropsy, jaundice, anti-hypertensive, asthma, and leishmaniasis or kala-azar (Asresie et al., 2014 and Yardav et al., 2015). It has been reported that camel milk contains the low quantity of β-casein and the lack of β-lactoglobulin which cause allergic reaction in lactose intolerant person (Konuspayeva et al., 2009). Nevertheless, it contains insulin-like and protective protein used for the treatment of many ailments like diabetes, autism, and diarrhea and possesses anti-tumors properties (Gul et al., 2015). Moreover, camel milk is endowed with very strong immune system (Grader et al., 2016) and remedy for peptic ulcers and anti-malignant (Korashy et al., 2012). More recently, studies confirmed that camel's milk is unique in terms of antioxidative factors, antibacterial, antiviral, antifungal, anti-hepatitis, treatment for paratuberculosis, hypoglycaemic activity, anticancer, preventives of ageing, remedy for autoimmune diseases and have cosmetic properties ((Korashy et al., 2012, Keskes, 2015). Most of the research conducted on camels in the past was mainly focused on their anatomical and physiological features (Farah and Farah-Riesen, 1985). However, the recent studies have mainly concentrated on the compositional, characteristics and functionality of camel milk (Farah, 1993, 1996).

In the present study we have described the structure elucidation of a novel camel milk oligosaccharide isolated from camel milk by the modified method of Kobata and Ginsburg followed by chemical degradation, chemical transformation and various spectroscopic techniques like ^{1}H, ^{13}C, COSY, TOCSY, HSQC and Mass spectrometer.

MATERIAL AND METHODS

GENERAL PROCEDURE

General procedure was same as described in our previous communication (Gangwar et al., 2017).

Isolation of camel milk oligosaccharide by the modified method of Kobata and Ginsberg

Isolation of camel milk oligosaccharides was done by the modified method of Kobata and Ginsburg method (Kuldeep, K., et al., 2016), which was described in our previous communication (Gangwar et al., 2017, Kuldeep et al., 2016) except the isolation, was done from 10 litre of camel milk and the yield of oligosaccharide mixture was 315 gm.

Acetylation of oligosaccharide mixture

For acetylation 12 g of pooled fractions obtained after Sephadex chromatography which gave positive phenol-sulphuric acid test (Dubois et al., 1956) were acetylated by standard method of acetylation which yielded 13.5 gm acetylated mixture of oligosaccharides. TLC of this oligosaccharide mixture showed eight spots namely a, b, c, d, e, f, g and h.

Deacetylation of compound

Compound e (68.6 mg) was obtained from column chromatography 4 of acetylated oligosaccharide mixture by column chromatography. 35 mg of compound e was dissolved in acetone (2 ml) and 3 ml of NH_4OH was added in it and was left overnight in a stoppered hydrolysis flask. After 24 hrs ammonia was removed under reduced pressure and the compound was washed thrice with $CHCl_3$ (5 ml) (to remove acetamide) and water layer was finally freeze dried giving the natural oligosaccharide E (28.5 mg).

Methyl glycosidation/Acid hydrolysis of compound E

Compound E (8 mg) was ref1uxed with absolute MeOH (2 ml) at 70°C for 18 h in the presence of cation exchange IR-l20 (H) resin. The reaction mixture was filtered while hot and filtrate was concentrated. In the reaction mixture, 1,4-dioxane (1 ml), and 0.1N H_2S0_4 (1 ml) was added and the solution was warmed for 30 minutes at 50°C. The hydrolysis was complete after 24 h. The hydrolysate was neutralized with freshly prepared $BaCO_3$, filtered and concentrated under reduced pressure to afford α-and β-methylglucosides along with the Glc, Gal and GlcNAc. Their identification was confirmed by comparison with authentic samples (TLC, PC).

Killiani hydrolysis of compound

Compound E (5 mg) was dissolved in 2 ml Kiliani mixture (AcOH-H_20-HCI, 7: 11:2) and heated at l00°C for 1 h followed by evaporation under reduced pressure. It was dissolved in 2 ml. of H_20 and extracted twice with 3 ml $CHCl_3$.

The aqueous residual solution was made neutral by addition of 1-2 drops of 2N NaOH to it and was evaporated under reduced pressure which afforded glucose, Gal and GlcNAc which were identified by comparison with authentic sample of glucose, galactose and GlcNAc.

Description of isolated compound

Substance e (66 mg) obtained from fraction 21-26 of column chromatography 4. On deacetylation of 35 mg of acetylated compound with NH_3/ acetone it afforded substance E (28.5 mg) as a viscous mass, $[\alpha]_D$ +94.12^0 (c, 4, H_2O).

For experimental analysis, this compound was dried over P_2O_5 at 100^0 C and 0.1 mm pressure for 8 hr.

$C_{50}H_{85}NO_{41}$	%C	%H	%N
Calad.	44.28	6.25	1.03
Found	44.24	6.25	1.02

It gave positive Phenol-sulphuric acid test, Feigl test and Morgon-Elson test.

δ in D_2O: ^{1}H NMR

2.05 [s, 3H, $NHCOCH_3$, β-GlcNAc (S-3)], 3.56[t, 1H, β-Glc(S-1), H-2], 3.93[β-Gal (S-2), H-4], 4.06[t, 1H, β-GlcNAc(S-3), H-3], 4.47[d, 2H, J=7.8 Hz, β-Gal (S-2 and S-4), H-1], 4.53[d, 3H, J=7.8Hz, β-Glc(S-1 & S-5), β-Gal(S8),H-1], 4.65[d, 1H, J=8.4 Hz, β-GlcNAc (S-3), H-1], 5.227[d, 2H, J=3.6 Hz, α-Gal (S-6 & S-7), H-1], 5.28 [d, 1H, J=3.8 Hz, α-Glc (S-1), H-1].

δ in D_2O: ^{13}C NMR

89.50[α-Gal (S-7),C-1], 90.10 [α-Gal (S-6),C-1], 91.80 [α-Glc (S-1),C-1], 94.50[β-Glc [(S-1), C-1], 96.50[β-Glc (S-5), C-1], 102.30 [β-Gal (S-2 & S-4), C-1], 102.80 [β-GlcNAc [(S-3)], C-1], 103.73 [β-Gal (S-8), C-1].

δ in $CDCl_3$: ^{1}H NMR (Acetylated)-

3.60 [α-Glc (S-1), H-4], 3.65[β-Glc(S-1), H-4], 3.74[β-GlcNAc (S-3),H-3 & β-Glc (S-5),H-3], 3.77 [β-Gal (S-2 and S-4), H-6] , 3.80[β-Glc(S-1), H-3], 3.88[α-Glc (S-1), H-3] , 4.49[d, 2H, J=7.8 Hz, β-Gal (S-2 and S-4), H-1], 4.51[d, 2H, J=7.8 Hz, β-GlcNAc (S-3 & S-8), H-1], 4.76[d, 1H, J=9.1Hz, β-Glc(S-5),H-1], 5.36 [d, 1H, J=7.8Hz, β-Glc(S-1), H-1], 5.36 [d, 2H, J=3.6 Hz, α-Gal (S-6 & S-7), H-1], 6.25[d, 1H, J=3.8 Hz, α-Glc (S-1), H-1].

δ in $CDCl_3$: ^{13}C NMR (Acetylated)-

72.33[α-Glc (S-1), C-3], 72.34[β-Glc (S-1),C-3], 72.75[α-Glc (S-1) & β-GlcNAc (S-3),C-4], 73.60[β-Glc (S-1),C-3], 76.47[β-Gal (S-2 & S-4), H-6 & β-GlcNAc (S-3), H-3], 90.3[α-Gal(S-6 & S-7)], 91.40[β-Glc (S-1),C-1], 91.7[α-Glc (S-1),C-1], 95.4[], 101.10[β -Gal (S-2 & S-4,C-1}], 101.20 [β-GlcNAc S-3), C-1], 101.30 [β-Gal (S-8),C-1].

ES mass

1394$[M+K]^+$, 1378$[M+Na]^+$, 1355$[M]^+$, 1319, 1297, 1288, 1271, 1263, 1232, 1193, 1175, 1158, 1131, 1124, 1104, 1089, 1071, 1031, 1000, 995, 983, 973, 964, 953, 942, 908, 869, 838, 811, 807, 796, 789, 772, 747, 746, 730, 712, 712, 707, 645, 642, 628, 586, 584, 566, 545, 504, 486, 455, 393, 375, 357, 342, 325, 289, 180.

RESULT AND DISCUSSION

Compound E, Amelose, $C_{50}H_{85}NO_{41}$ $[\alpha]_D$ + 94.12^o gave positive Phenol- sulphuric acid test (Dubois et al., 1956) Feigl test (Fiegl, F., 1975) Morgon-Elson test (Partrige S.M. et al., 1948) showing the presence of normal and amino sugar(s) in the compound. The ^{1}H NMR spectrum of compound E at 300 MHz exhibited five doublets for nine anomeric proton signals at δ 5.23 (1H), 5.22 (2H), 4.65 (1H), 4.53 (3H), and 4.47 (2H) indicating that the compound E may be a octasaccharide in its reducing form giving signals for α and β anomers of glucose at its reducing end. Methylglycosidation of amelose by MeOH/H^+ followed by its acid hydrolysis led to isolation of α and β- methyl glucoside, which suggested the presence of glucose at the reducing end of the oligosaccharide. It was also confirmed by the presence of two anomeric proton signals at δ 5.23 and δ 4.53 for α- and β- Glc. The octasaccharide nature of compound E was further confirmed by the presence of nine-anomeric carbon at δ 90.30 (2C), δ 91.70 (1C), δ 91.40 (1C), δ 95.40(1C), δ 101.10 (2C), δ101.20 (1C) and δ101.30 (1C) in ^{13}C NMR of amelose acetate. The eight monosaccharides present in compound E have been designated as S_1, S_2, S_3, S_4, S_5, S_6, S_7 and S_8 for convenience starting from reducing end.

To confirm the monosaccharide constituents in compound E, it was hydrolysed under strong acidic conditions. In Killiani hydrolysis under strong acid condition, it gave three monosaccharides i.e. glucose, galactose and N-acetyl-glucosamine, confirming that the octasaccharide is consist of three types of monosaccharide units i.e. glucose, galactose and N-acetyl-glucosamine. Since the glucose was present in its reducing form which was supported by ^{1}H NMR of amelose which contains two anomeric proton signals for α- and β-Glc at δ 5.23 (1H) and at δ 4.53 (J=7.8) (Mani A. et al., 2016). Further the presence of another anomeric proton doublet signal at 4.48 (J=7.8) was due to presence of β-Gal moiety in the amelose. β-Gal moiety present in amelose suggested the presence of a lactosyl moiety i.e Gal-β-(1→4) Glc. Since it showed H-2 signal of β-Glc (S_1) as a triplet at δ 3.56 in the downfield region, which indicated that both the equatorially oriented hydroxyl groups at C-3 and C-4 of the reducing β-Glc (S_1) were substituted and involved in glycosidation (Singh, A. K., et al. 2015). It was also supported by the presence of β-Glc H-3 proton resonance at δ 3.80 and β-Glc H-4 proton resonance at δ 3.55 in acetylated derivative of amelose. Another anomeric signal, which appeared at δ5.22, was due to presence of α-Gal (S_7) moiety. In amelose acetate the up field shifted values of H-3 of S-1 at δ 3.80 along with H-2 triplet of S-1 which is structure reporter group suggested that the Gal S-7 was linked to H-3 of Glc (S_1). The Gal-α-(1→3) Glc linkage was confirmed by reporter group value of α-Gal at H-1 at δ5.22 and H-5 at δ4.13 (Urashima et al., 2000, 1999). Further the presence of another anomeric proton doublet at δ 4.65 (J=8.4Hz) along with signal of amide methyl group at δ 2.05, was due to the presence of β-GlcNAc moiety (Urashima et al., 1994). The H-4 proton resonance (Shahi S. et al., 2017, V. K Dua et al., 1983) of β-Gal (S_2), appeared at δ 3.93 in amelose and the presence of H-6 and C-6 resonance of β-GlcNAc (S_3) of amelose acetate at δ 3.77 and δ 76.47 confirmed that β-GlcNAc (S_3) may be 1→6 linked to β-Gal (S_2). This was confirmed on the basis of assignments made by 2D NMR spectra of acetylated compound E. The 2D COSY, HSQC and TOCSY NMR spectra of amelose acetate assignments ascertained the positions of H-3 and H-4 protons of β-GlcNAc (S_3) at δ 3.74 and δ 3.62 respectively which imply that H-3 and H-4 of β-GlcNAc (S_3) were involved in the glycosidation. Further another anomeric signals appeared at δ 4.47 (J=7.8 Hz) was due to presence of a β-Gal moiety (S_4) which was linked to H-3 of β-GlcNAc (S_3). This linkage was confirmed by the chemical shift value of this β-Gal moiety (S_4), that is identical with the chemical shift value of β-Gal (S_2) of lactosyl moiety, which is structure reporter group for β-Gal (S_4) and β-GlcNAc (S_3) linkage (Chaturvedi P. et al., 1983). On the basis of chemical shift analogies (Chaturvedi P. et al., 1988), the compound amelose appeared to have another β-Gal (S_8) moiety linked to H-4 of β-GlcNAc (S_3), which was identified by its anomeric proton signal at δ 4.53 (J=7.8 Hz). This downfield shifting of β-Gal may be due to crowding and steric hindrance at the non reducing end. The next anomeric proton signal appeared as doublet at δ 4.53 (J=7.8Hz) was due to the presence of another β-Glc (S_5) moiety in amelose. The presence of H-6 and C-6 resonance of β-Gal (S_4) of amelose acetate at δ 3.77 and δ 76.47 confirmed that β-Glc (S_5) is linked to H-6 of β-Gal (S_4) which was further supported by structure reporter group value of H-4 of β-Gal (S_4) at δ 3.93.

Table 1. ^{1}H NMR values in D_2O.

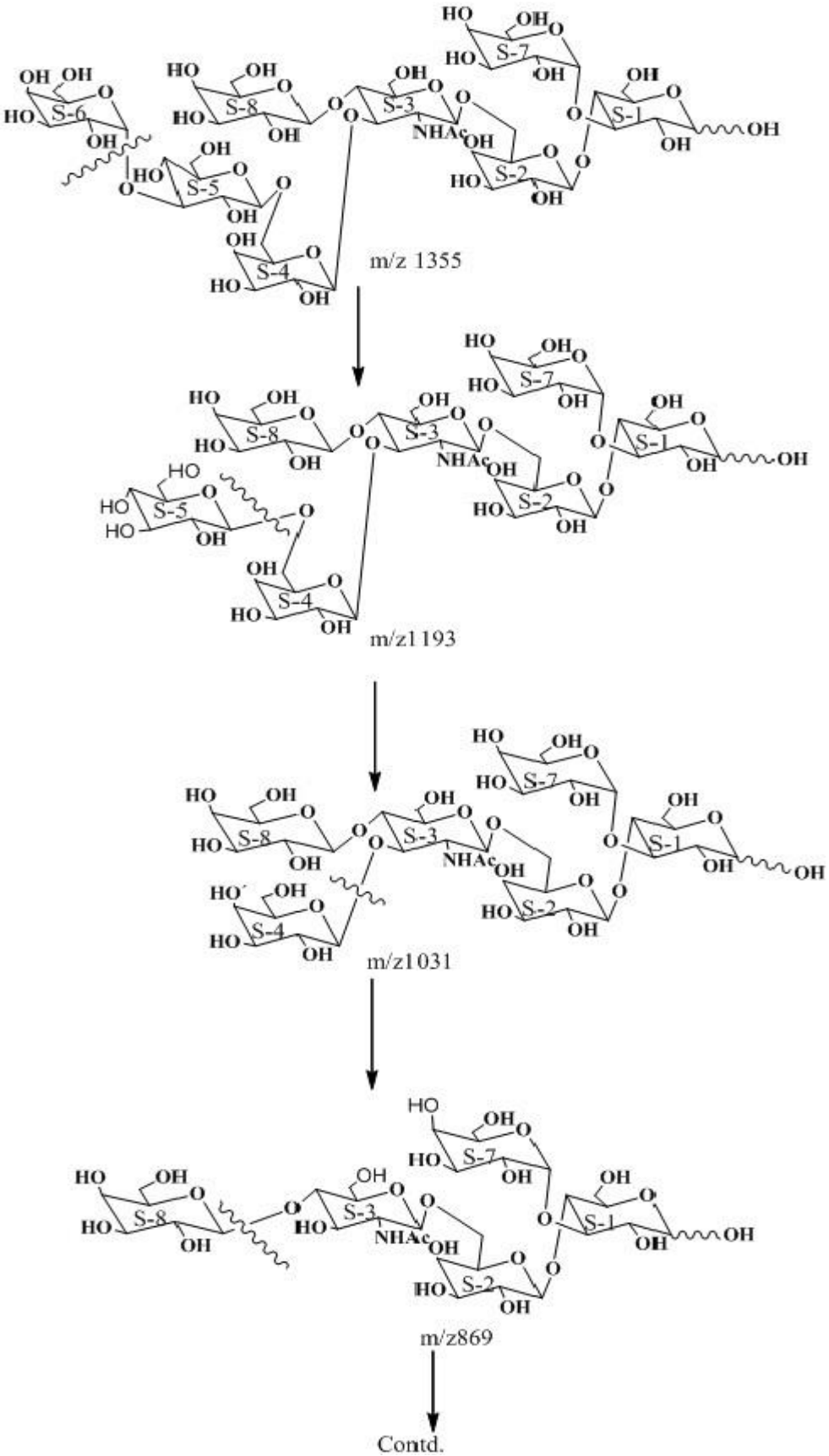
Moieties	^{1}H NMR	Coupling Constant (J in Hz)
α-Glc	5.23	3.8
β-Glc	4.53	7.8
β-Gal	4.47	7.8
β-GlcNAc	4.65	8.4
β-Gal	4.47	7.8
β-Glc	4.53	7.8
α-Gal	5.22	3.6
α-Gal	5.24	3.6
β-Gal	4.53	7.8

Table 2. ^{13}C NMR values of Amelose acetate.

	C-1	C-2	C-3	C-4	C-5	C-6
α-Glc	91.7	71.50	72.33	72.75	69.1	60.8
β-Glc	91.4	71.22	73.60	72.34	69.5	61.9
β-Gal	101.1	70.38	71.50	61.01	73.4	76.47
β-GlcNAc	101.2	61.01	76.47	72.75	70.38	60.8
β-Gal	101.1	70.38	71.50	67.5	73.47	76.47
β-Glc	95.4	73.6	76.4	72.75	72.5	68.27
α-Gal	90.3	71.50	68.38	63.0	66.83	62.07
α-Gal	90.3	71.50	68.38	63.0	66.83	62.07
β-Gal	101.3	69.31	71.5	68.0	73.60	61.01

The next anomeric signal appeared at δ 5.22 were recognized for the presence of another α-Gal (S_6) moiety. The presence of α-Gal, H-1 at δ5.22 and H-5 at δ4.13 suggested that α-Gal (S_6) moiety was linked by (1→3) linkage to β-Glc (S_5) moiety (Urashima et al., 2000, 1999). Since α-Gal (S_6) was present at non-reducing end, H-1 α-Gal was downfield shifted. The chemical shift value of anomeric carbons at δ 90.30(2C), δ 91.70(1C), δ 91.4 (1C), δ 95.40(1C), δ 101.10 (2C), δ101.20(1C) and δ101.30 (1C) present in the ^{13}C NMR spectrum of amelose acetate are in accordance with the anomeric carbon values of S_1, S_2, S_3, S_4, S_5, S_6, S_7 and S_8 respectively. The ^{13}C NMR values of anomeric carbons and ring carbons of amelose are given in table 2. The various values of ring carbons are in accordance with ^{13}C value of their respective monosaccharides, which also supports the derived structure. The octasaccharide nature of compound E was further confirmed by the spectral studies of acetylated derivative of compound E. These studies were made on the basis of COSY, TOCSY and HSQC connectivities. The heteronuclear single quantum coherence (HSQC) spectrum of acetylated compound confirmed anomeric assignments in ^1H and ^{13}C NMR spectra by showing cross peaks of an α-Glc (S_1) H-1 & C-1 at (δ 6.25 x 91.7) and β-Glc (S_1) H-1 & C-1 at (δ 5.23 x 91.4).

It also contains cross peak of 2 α-Gal (S_6 & S_7) H-1 & C-1 at (δ 5.36 x 90.30), β-Glc (S_5) H-1 & C-1at (δ 4.76 x 95.40), β-GlcNAc (S_3) H-1 & C-1 moiety at (δ 4.51 x 101.20), β-Gal (S_8) H-1 & C-1 at (δ 4.51 x 101.30) and a cross peak of 2 β-Gal (S_2 & S_4) H-1 & C-1 at (δ 4.49 x 101.10). The glycosidic linkages were assigned by the cross peaks for glycosidically linked carbons with their protons in HSQC spectrum of amelose acetate. The values of these cross peaks were as α -Glc (S_1) H-3 and C-3 at δ 3.88 x 72.33 showed (1→3) linkage of S_7 and S_1 and also its H-4 and C-4 at δ 3.60 x 72.75 shows (1→4) linkage of S_2 and S_1, β-Glc (S_1) H-3 and C-3 at δ 3.80 x 73.6 showed (1→3) linkage of S_7 and S_1 and also H-4 and C-4 at δ 3.65 x 73.6 shows (1→4) linkage of S_2 and S_1 i.e. its 3-position and 4-position of Glc (S_1) were involved in linkage, β-Gal (S_2 & S_4) H-6 and C-6 at δ 3.77 x 76.47 shows (1→6) linkage of $S_3 \rightarrow S_2$ and $S_5 \rightarrow S_4$, β-GlcNAc(S_3) H-3 and C-3 at δ 3.74 x 76.47 shows (1→3) linkage of S_4 and S_3, β-GlcNAc (S_3) H-4 and C-4 at δ 3.62 x 72.75 shows (1→4) linkage of S_8 and S_3, and β-Glc (S_5) H-3 and C-3 at δ 3.74 x 76.47 shows (1→3) linkage of S_6 and S_5. COSY spectrum gave assignments of ring hydrogens involved in linkage at δ 3.80 (3-position), δ 3.65 (4-position) for β-Glc, δ 3.77 (6-position) for β-Gal (S_2), 3.74 (3-position), δ 3.62 (4-position) for β-GlcNAc (S_3), δ 3.77 (6-position) for β-Gal (S_4), and δ 3.74 (3-position) for β-Glc, and it was further confirmed by the presence of same peaks in TOCSY spectrum. Based on the pattern of chemical shifts of ^1H, ^{13}C, COSY, TOCSY and HSQC NMR experiments it was interpreted that the compound was octasaccharide. The result obtained from the ES mass spectrum further substantiated the structure of compound E which was derived by its ^1H and ^{13}C NMR, 2D NMR Spectra. The highest mass ion peak were recorded at m/z 1394 which were due to $[M+K]^+$, further mass ion peaks at m/z 1378 and 1355 for $[M+Na]^+$ and $[M]^+$ confirmed the molecular weight of compound was 1355. Further the mass fragments were formed by repeated H transfer in the oligosaccharide and was accompanied by the elimination of terminal sugar less water. The fragmentation pathway confirmed the sequence of monosaccharides in the oligosaccharide (scheme 8). The octasaccharide m/z 1355 on fragmentation gave heptasaccharide at m/z 1193(I), which was due to loss of S-6 sugar unit i.e. Gal (S-6) sugar unit linked to the S-5 of octasaccharide. It was supported by its respective fragment at m/z 180 that confirmed the presence of Gal (S-6) at non-reducing end.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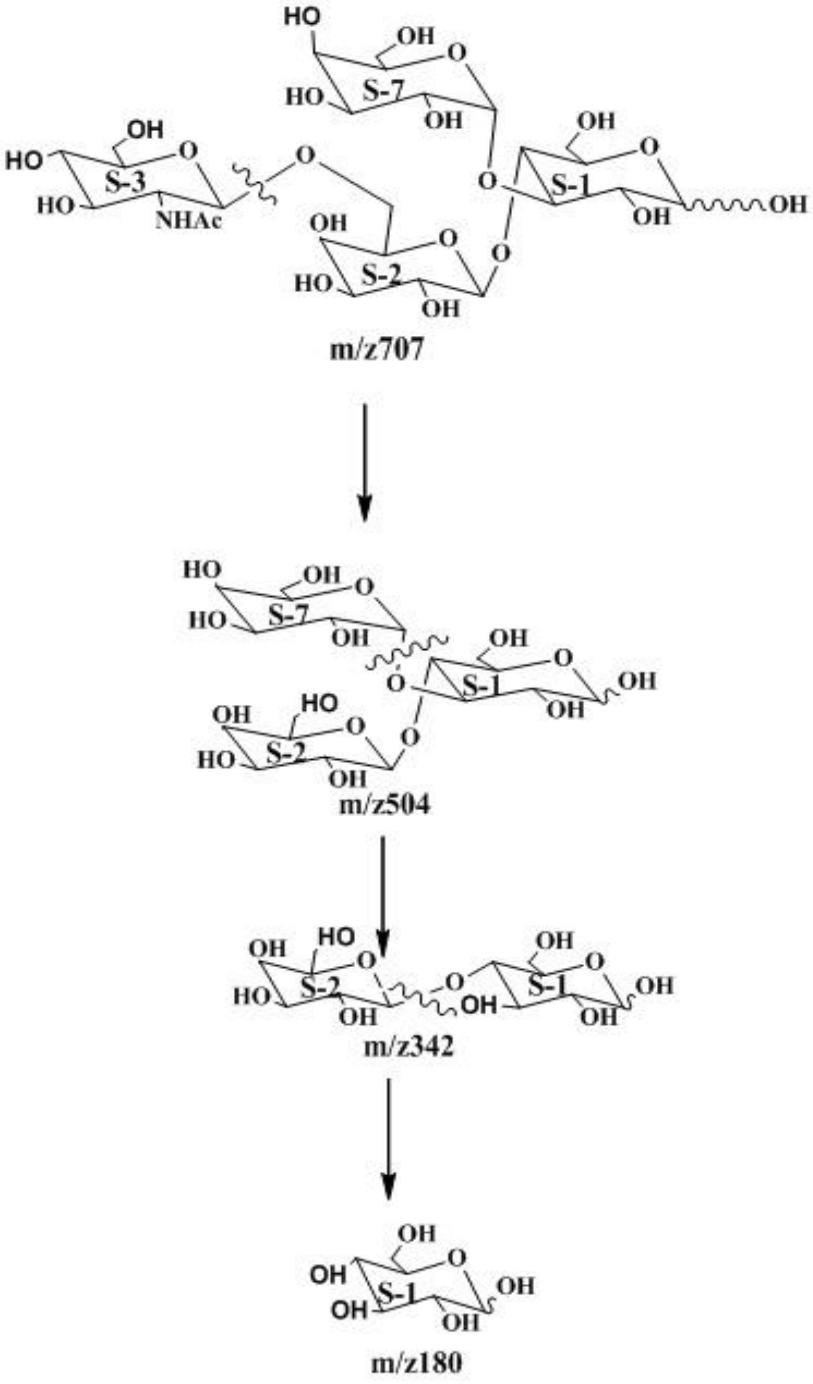
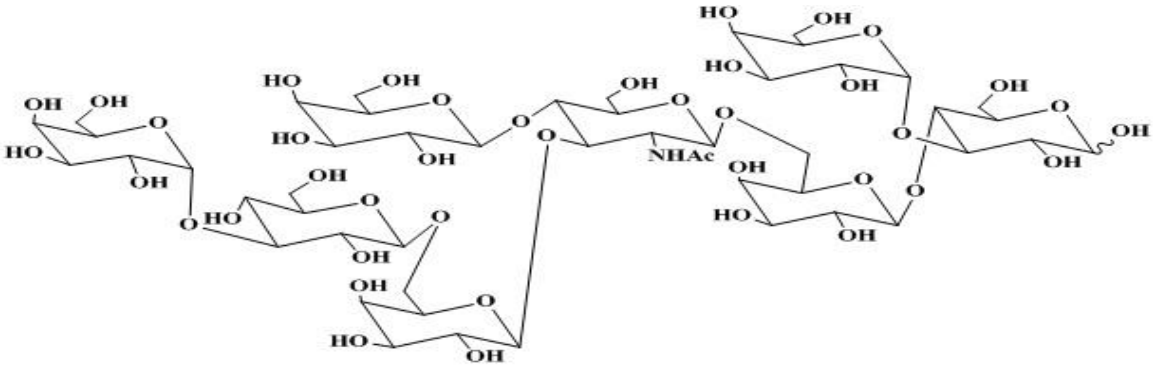


Figure. ES-MASS fragmentation of compound Amelose.

The heptasaccharide m/z 1193 further fragmented to side mass ion peak at m/z 1031(II), which aroused due to loss of Glc (S-5) sugar unit i.e. Glc (S-5) its corresponding hexasaccharide(II) moiety of heptasaccharide. The hexasaccharide m/z 1031 on fragmentation gave pentasaccharide at m/z 869(III), which was due to loss of Glc (S-4) sugar unit i.e. S-4 sugar unit is linked to the S-3 of hexasaccharide. The pentasaccharide on fragmentation gave a mass ion peak at m/z707 (IV), corresponding to tetrasaccharide unit, which was due to loss of S-8 sugar unit i.e. Gal (S-8) sugar unit linked to the S-3 of pentasaccharide. The tetrasaccharide m/z 707 on fragmentation gave trisaccharide at m/z 504(V), which was due to loss S-3 sugar unit i.e.

GlcNAc (S-3) sugar unit is linked to the S-2 of tetrasaccharide unit. The trisaccharide m/z 504 on fragmentation gave mass ion peak at m/z 342(VI), which was due to loss of S-7 sugar unit i.e. Gal (S-7) sugar unit linked to the S-1 of trisaccharide unit. This disaccharide m/z 342 on further fragmentation gave a mass ion peak at m/z 180(VII), which was due to loss of S-2 sugar unit i.e. Gal (S-2) sugar unit linked to the S-1 of disaccharide. The other important signal obtained at m/z 1093 [M-S_7-S_8] or [M-S_6-S_5], 869[M-S_7-S_8 –S_6] or [M-S_6-S_5 –S_4], 707 [M-S_6-S_5–S_7 –S_1] or [M-S_6-S_5–S_4 –S_3] or [M-S_6-S_5–S_4 –S_8], and 545 [M-S_6-S_5–S_4 –S_7-S_8] suggested the anchoring nature of sugar S_3 (GlcNAc). The ES-mass of compound E also showed other mass ion peaks at m/z 1297[M-$NHCOCH_3$], 1319 [M-$2H_2O$], 1263 [1297-OH-OH], 1232[1263-CH_2OH], 1288 [1319-CH_2OH] and 1271 [1288-OH]. The mass ion peak at m/z 1193 further fragmented to give important mass ion fragment at 1145 [1193-CH_2OH-OH], 1114[1145-CH_2OH], 1175 [1193-H_2O], 1158 [1175-OH], 1124[1158-2 OH], 1131 [1193-2 CH_2OH], 1089 [1131-CH_2CO], 1071 [1089-H_2O] and 1104 [1193-$NHCOCH_3$]. The mass ion peak at m/z 1193 further fragmented to give important mass ion fragment at m/z 973[1031-$NHCOCH_3$], 942[973-CH_2OH], 908 [942-2 OH], 964 [1031-CH_2OH- 2 H_2O], 1000 [1031-CH_2OH], 983 [1000-OH], 995 [1031- 2 H_2O] and 953 [995- CH_2CO]. The mass ion fragment m/z 1031 fragmented by the loss of other terminal sugar (S_6) gave the corresponding pentasaccharide mass ion fragment at m/z 869 which confirmed that three Gal moieties were present at three non reducing ends of the octasaccharide moiety. Other mass ion fragments corresponded to the moiety m/z 869 appeared at m/z 807 [869-2CH_2OH], 789[807-H_2O], 772 [789-OH], 712[772-CH_2OHCHO], 838 [869-CH_2OH], 796 [838-CH_2CO], 747 [796-CH_2OH-H_2O], 730 [747-OH], 712 [730-H_2O], 811 [869-$NHCOCH_3$] and 746[811-CH_2OH-2OH]. The mass ion peak at m/z 869 further fragmented to give mass ion fragment for tetrasaccharide moiety at m/z 707 which was aroused by the loss of sugar (S_5) other mass ion fragments corresponds to the moiety m/z 707 appeared at m/z 645 [707-2CH_2OH], 628[645-OH], 586[628-CH_2CO], 545 [586-CH_2CO], 642 [707-CH_2OH-2OH], 584 [642-$NHCOCH_3$] and 566 [584-H_2O]. The tetrasaccharide mass ion fragment on further fragmentation gave a important trisaccharide segment at m/z 504. The trisaccharide segment at m/z 504 on further fragmentation gave a disaccharide segment at m/z 342 and other mass ion fragment at m/z 455 [504-CH_2OH-H_2O], 393 [455-2CH_2OH], 375[393-H_2O] and 357[375- H_2O]. The disaccharide segment at m/z 342 on fragmentation gave mass ion fragment at m/z 325 [342-OH] and 289 [325-2H_2O]. The disaccharide segment at m/z 342 on further fragmentation gave monosaccharide unit at m/z 180. The mass ion fragment at m/z 869 was due to fragment A (S_6, S_5, S_4, S_3 and S_8) and fragment B (S_1, S_2, S_3, S_8 and S_7) along with other fragment at m/z 707 due to fragment C (S_1, S_2, S_3, and S_7), fragment D (S_8, S_2, S_3, and S_4) , fragment E (S_1, S_2, S_3, and S_7) and at m/z 545 due to fragment F (S_2, S_3, and S_4), fragment G (S_3, S_4, and S_8) confirmed the anchoring nature of sugar S_3 in compound E.
Based on the results obtained from chemical degradation and chemical transformation, mass spectrometry and ^1H, ^{13}C, HOMOCOSY, TOCSY, HSQC NMR, the structure of the isolated octasaccharide is deduced as:



Gal-α(1→3)Glc-β(1→6)Gal-β(1→3)GlcNAc1-β(1→6)Gal-β(1→4)Glc
|
(1→4)Gal-β
|
(1→3)Gal-α

AMELOSE

CONCLUSION

In summary, the novel milk oligosaccharide E, an octasaccharide namely (Amelose) has been isolated from Camel milk and elucidated with the help of 1H, 13C, 2D NMR spectroscopy and mass spectrometry.

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