

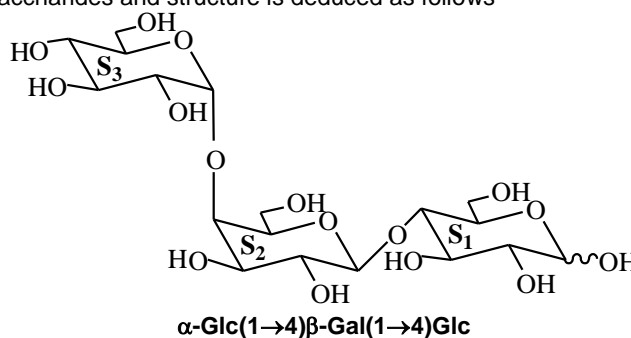
# Isolation and Structure Elucidation of Novel Oligosaccharide (Lisose) from Goat Milk

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## Abstract

Milk is an excellent source of well-balanced nutrients to exhibit a range of biological activities that influence digestion, growth and development of specific organs. Milk is a complex and dynamic biofluid comprised of many hundreds to thousands of distinct bioactive structures, among which one of the most abundant substances are non-conjugated complex carbohydrates referred to as milk oligosaccharides. Milk oligosaccharides are the third largest solid component after lactose and lipid that stimulate the growth of beneficial microorganisms in the infant colon, act as receptor analogs that inhibit the attachment of pathogenic microorganisms to colonic mucosa, participate in development of the brain, enhance intestinal epithelial barrier function and small amounts are absorbed into the circulation where they modulate immunoreactivity. Goat milk oligosaccharides have ability to stimulate specific and non-specific immunological resistance and prevention of atherosclerosis. Keeping above mentioned biological activities of Goat's milk oligosaccharides in mind we have isolated a novel trisaccharide namely **Lisose** from Goat's milk and elucidated its structure by chemical degradation and spectroscopic techniques (like  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, COSY, TOCSY, HSQC and Mass). The structure of Lisose was established by comparing the chemical shift ( $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) of anomeric signals and other important signals of isolated milk oligosaccharide with the chemical shifts of known milk oligosaccharides and structure is deduced as follows-



**Keywords:** Goat Milk, Oligosaccharides, Lisose.

## Introduction

Milk is the sole nourishment of the mammalian neonate which has a complete source of bioactive molecules that help to protect the new born against infectious disease and also promote the development of specific organs. Oligosaccharides are one of the substantial bioactive milk components which have significant influence on prebiotic activity<sup>1</sup>, anti-adhesion activity<sup>2,3</sup>, anti-inflammatory activity<sup>4</sup>, glycome modifying activity, in formation of brain and central nervous system<sup>5</sup>. Oligosaccharides play an essential role in many molecular processes impacting eukaryotic biology and diseases. The enormous biological activity of oligosaccharide such as anti-tumour<sup>6</sup>, immunostimulant<sup>7</sup>, anti-cancer<sup>8</sup>, anti-complementary, anti-inflammatory<sup>4</sup>, anti-coagulant, hypoglycaemic, antiviral, antimicrobial, antioxidant, lipid lowering<sup>9</sup>, immunological activity<sup>10</sup> and regulation of mineral absorption<sup>11</sup> reported. Goat milk oligosaccharides have ability to stimulate non-specific and specific immunological resistance and proposed to be very helpful in cure of AIDS patients and in prevention of

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atherosclerosis<sup>12</sup>. In ancient literature, Hippocrates recommended Goat milk for healing infection and wounds, liver ailments, poisoning, fever, nose bleed and for clearing of edema from the body<sup>13</sup>. Goat milk is also helpful in raising the metabolism rate of human body which helps to deplete the excess triglyceride reserve of the body<sup>13</sup>. Besides that, the presence of Omega-3 fats in its milk helps in driving away triglyceride from the body which also reduces low density lipoprotein (LDL) or in common parlance the bad cholesterol in the blood (Singh et al, 2017). Keeping in mind, biological and medicinal importance of Goat's milk oligosaccharides, Goat's milk was collected and processed by method of Kobataetal and Ginsburg<sup>14</sup> yielding oligosaccharide mixture. This oligosaccharide mixture on purification yielded a novel oligosaccharide namely **Lisose**. The structure was determined by comparing the chemical shift (<sup>1</sup>H and <sup>13</sup>C NMR) of anomeric signals and other important signals of isolated milk oligosaccharide with the chemical shifts and anomeric signals of known milk oligosaccharides. In this present study analogies between chemical shifts of certain 'structural reporter group resonances' were used to make proton resonance assignments as well as structural assignments of the oligosaccharide. All chemical shifts of anomeric proton signals of milk oligosaccharide were further confirmed by 2D (<sup>1</sup>H-<sup>1</sup>H HOMOCSY, TOCSY and HSQC) NMR experiments which were earlier assigned with the help of <sup>1</sup>H and <sup>13</sup>C NMR data. Other techniques like deacetylation, methylation, acid hydrolysis, chemical degradation and electrospray mass spectrometry were also helpful in the elucidation of the structure of oligosaccharides.

#### Aim of the Study

Milk oligosaccharides exhibit several biological activities such as immunostimulant, hypoglycemic, anti-tumour, antiviral, anti-cancer, anticoagulant, anti-complementary, immunological and anti-inflammatory activities. Therefore, we have isolated a novel trisaccharide namely **Lisose** from Goat's milk and elucidated its structure by chemical degradation and spectroscopic techniques (like <sup>1</sup>H NMR, <sup>13</sup>C NMR, COSY, TOCSY, HSQC and Mass).

#### Experimental

##### General Procedures

For evaporation of alcohol from crude extract of milk oligosaccharides, Buchi Rotary evaporator was used. Freeze drying of the compounds was done with the help of CT 60e (HETO) lyophilizer and centrifuged by a cooling centrifuge Remi instruments C-23 JJRCI 763. Optical rotations were measured with a Buchi automatic Polarimeter in 1.2 cm tube. The C, H and N analyses were recorded on CARLO-ELBA 1108 elemental analyzer. All melting points were recorded on BOETIUS micro-melting point apparatus and are uncorrected. <sup>1</sup>H and <sup>13</sup>C NMR and 2D experiments were recorded in solvent CDCl<sub>3</sub> and D<sub>2</sub>O at 25° on a Bruker AM 300 MHz FT NMR spectrometer. The Electrospray mass spectra were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer. The milk oligosaccharide sample (dissolved in water as solvent) was introduced into the ESI source through a syringe pump at the rate of 5ul per min. The ESI capillary was set at 3.5 kV and the cone voltage was 40 V. The spectra were collected in 6 s scans and the print outs are averaged spectra of 6-8 scans. Spectrum recorded in higher mass scale is computerized deconvoluted. Authentic samples of glucosamine, galactosamine, galactose, glucose, fucose and sialic acid were purchased from Aldrich Chemicals.

##### Isolation of Goat Milk Oligosaccharides

Goat's milk (10 L) was collected from a domestic Goat and was stored at -20°C. The milk was processed by the method of Kobataet al<sup>14</sup>. It was centrifuged for 15 min at 5000 rpm at -4°C. The solidified lipid layer was removed by filtration through glass wool column in cold. Ethanol was added to the clear filtrate to a final concentration of 68% and

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the resulting solution was left overnight at 0°C. The white precipitate formed, mainly of lactose and protein was removed by centrifugation and washed twice with 68% ethanol at 0°C. The supernatant and washings were combined and filtered through a micro filter (0.24 mm) (to remove remaining lactose) and lyophilized affording crude oligosaccharide mixture (110 g). This lyophilized material (mixture of oligosaccharide) was further purified by fractionating it on Sephadex G-25 chromatography using glass triple distilled water as eluent at a flow rate of 5 ml/m. Each fraction was analyzed by phenol-sulphuric acid reagent (Dubois et al, 1956) for the presence of neutral sugar.

##### Goat Milk Oligosaccharide Mixture Chromatographed Over Sephadex G-25 (1.6 x 40 cm) Column

The repeated gel filtration was performed by Sephadex G-25 chromatography of crude Goat milk oligosaccharide mixture. The oligosaccharide mixture (22.5 g) was packed in a column (1.6 x 40 cm) (void volume = 25 mL) equilibrated with glass triple distilled water (TDW) and left for 10-12 h to settle down (18.6 g). The material was applied onto a Sephadex G-25 column and was eluted for separation of protein and glycoprotein from oligosaccharide mixture (low molecular weight component). Presence of neutral sugars was monitored in all eluted fractions by phenol-sulphuric acid test<sup>15</sup>. The sephadex G-25 chromatography of Goat milk oligosaccharide mixture which was monitored by UV spectrophotometry showed five peaks i.e. I, II, III, IV and V. A substantial amount of proteins, glycoproteins and serum albumin were eluted in the void volume which was confirmed by positive colouration with p-dimethylaminobenzaldehyde reagent<sup>16</sup> and phenol-sulphuric acid reagent. Fractions under peaks II, III and IV gave a positive phenol-sulphuric acid test which showed the presence of oligosaccharide mixture in Goat milk. They were pooled together and lyophilized.

##### Acetylation of Oligosaccharide Mixture

The pooled fraction (II, III and IV) (10.35 g) which gave positive phenol-sulphuric acid test was acetylated with pyridine (10 mL) and acetic anhydride (10 mL) at 60°C and the solution was stirred overnight. The mixture was evaporated under reduced pressure and the viscous residue was taken in CHCl<sub>3</sub> (250 mL) and washed in sequence with 2 N HCl (1 x 25 mL), ice cold 2 N NaHCO<sub>3</sub> (2 x 25 mL) and finally with H<sub>2</sub>O (2 x 25 mL). The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to dryness yielding the acetylated mixture (12.51 g). The acetylation converted the oligosaccharides into their non-polar acetyl derivatives which were resolved very nicely on TLC.

##### Purification of Acetylated Milk Oligosaccharide Mixture on Silica Gel Column

Separation of the acetylated products (12.51 g) was carried over silica gel using varying proportions of C<sub>6</sub>H<sub>12</sub>:CHCl<sub>3</sub>, CHCl<sub>3</sub> and CHCl<sub>3</sub>:CH<sub>3</sub>OH mixture which was resolved into eleven fractions. Repeated column chromatography of fraction IV led to the isolation of one chromatographically pure compound Lisose (173 mg).

##### Deacetylation of Compound Lisose

The compound Lisose (30 mg) obtained from column chromatography of acetylated oligosaccharide mixture was dissolved in acetone (2 mL) and NH<sub>3</sub> (2 mL) was added and left overnight in a stoppered hydrolysis flask. Ammonia was removed under reduced pressure and the product was washed with CHCl<sub>3</sub> (3 x 3 mL) (to remove acetamide) and was finally freeze dried giving the deacetylated oligosaccharide (23.2 mg).

##### Methylglycosidation/Acid Hydrolysis of Compound Lisose

The compound Lisose (10 mg) was refluxed with absolute MeOH (2 ml) at 70°C for 20 h in the presence of cation exchange IR-140 (H<sup>+</sup>) resin. The reaction mixture was filtered while hot and filtrate was concentrated. To a solution of methylglycoside of Lisose in 1, 4-dioxane (1 ml), 0.1 N

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H<sub>2</sub>SO<sub>4</sub> (1 ml) was added and the solution was warmed for 45 minutes at 50°C. The hydrolysis was complete after 26 h. The hydrolysate was neutralized with freshly prepared BaCO<sub>3</sub> filtered and concentrated under reduced pressure to afford  $\alpha$ - and  $\beta$ -methylglucosides along with the Glc and Gal. Their identification was confirmed by comparison with authentic samples (TLC, PC).

#### Kiliani Hydrolysis of Compound Lisose

The compound Lisose (5 mg) was dissolved in 2 ml Kiliani mixture (AcOH-H<sub>2</sub>O-HCl, 9:13:3) and heated at 100°C for 1 h followed by evaporation under reduced pressure. It was dissolved in 2 ml of H<sub>2</sub>O and extracted twice with 3 ml CHCl<sub>3</sub>. The aqueous residual solution was made neutral by addition of 1-2 drops of 2N NaOH to it and was evaporated under reduced pressure to afford glucose and galactose on comparison with authentic samples of glucose and galactose on PC.

#### Description of Isolated Compound Lisose

##### Elemental Analysis

For experimental analysis, this compound was dried over P<sub>2</sub>O<sub>5</sub> at 100°C and 0.1 mm pressure for 8 hr. The molecular formula of compound Lisose was C<sub>18</sub>H<sub>32</sub>O<sub>16</sub>.

##### Elemental Analysis

Calculated	%C 42.86,	%H 6.35
Found	%C 42.85,	%H 6.34

#### <sup>1</sup>H NMR of Acetylated Lisose in CDCl<sub>3</sub>

$\delta$  3.59 (t, 1H, Glc(S<sub>1</sub>), H-4), 4.015 (t, 1H,  $\beta$ -Glc(S<sub>1</sub>), H-4), 5.74 (d, 1H, J = 3.3 Hz,  $\alpha$ -Glc (S<sub>1</sub>), H-1), 5.63(d, 1H, J = 7.8 Hz,  $\beta$ -Glc(S<sub>1</sub>), H-1), 5.40(d, 1H, J = 3.0 Hz,  $\alpha$ -Glc(S<sub>3</sub>), H-1), 4.57 (d, 1H, J = 8.1 Hz,  $\beta$ -Gal (S<sub>2</sub>), H-1)

#### <sup>1</sup>H NMR of Lisose in D<sub>2</sub>O

$\delta$  3.296 (t, 1H, J = 8.4 Hz,  $\beta$ -Glc(S<sub>1</sub>), H-2), 3.599 (t, 1H, J = 9.0 Hz, Glc(S<sub>1</sub>), H-4), 4.065 (t, 1H, J = 5.4 Hz,  $\beta$ -Glc(S<sub>1</sub>), H-4), 4.46(d, 1H, J = 7.8 Hz,  $\beta$ -Gal (S<sub>2</sub>), H-1), 4.67(d, 1H, J = 8.1 Hz,  $\beta$ -Glc(S<sub>1</sub>), H-1), 5.23(d, 1H, J = 3.6 Hz,  $\alpha$ -Glc(S<sub>1</sub>), H-1), 5.26(d, 1H, J = 3.0 Hz,  $\alpha$ -Glc(S<sub>3</sub>), H-1)

#### <sup>13</sup>C NMR of Lisose in D<sub>2</sub>O

$\delta$  61.73, 62.04, 68.68, 69.58, 70.46, 70.74, 71.36, 71.48, 76.36, 76.99, 77.63, 82.07, 89.11, 101.86

#### Mass Spectral Fragments of Compound Lisose

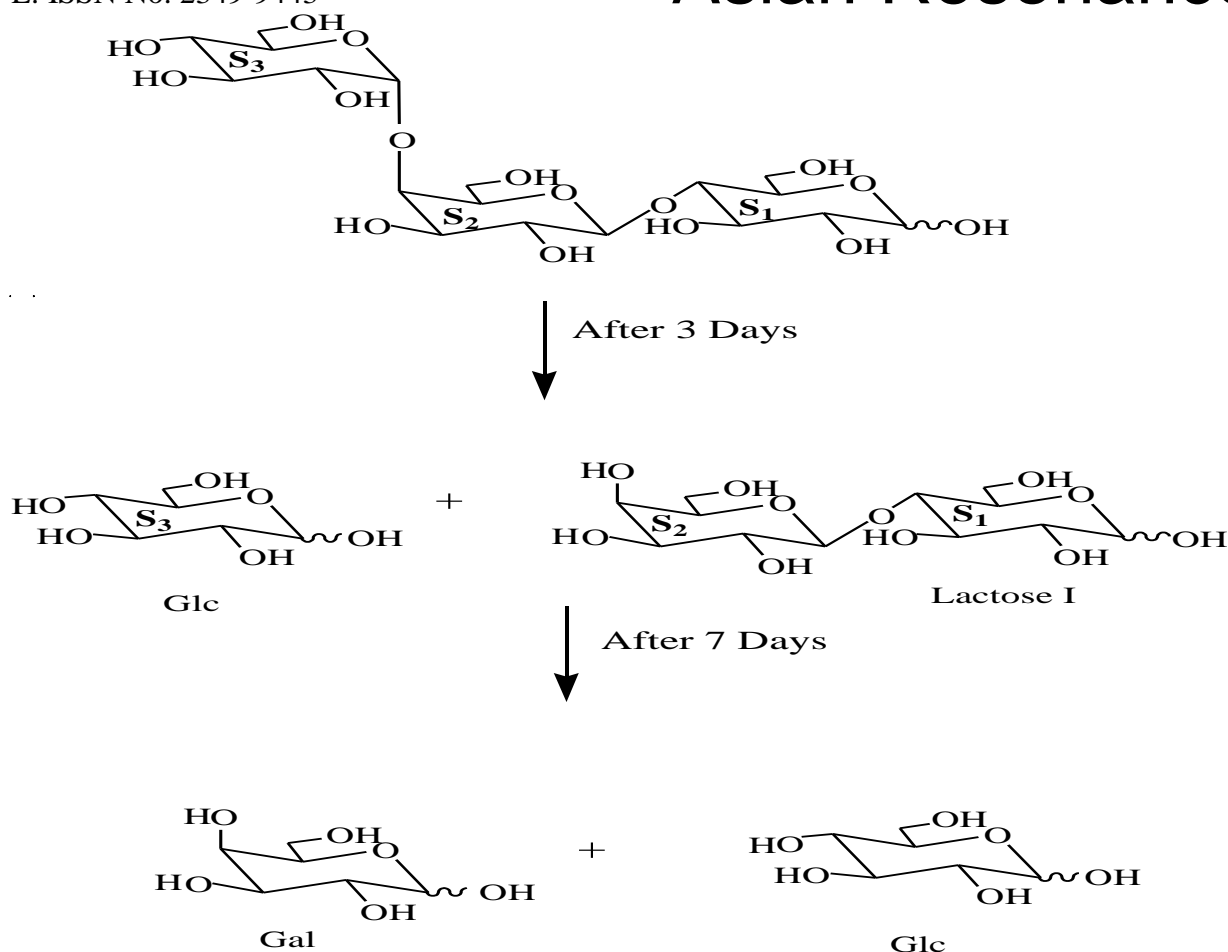
ES-MS m/z: 566, 543, 486, 462, 444, 384, 349, 342, 325, 311, 307, 289, 269, 251, 180.

#### Result and Discussion

Compound Lisose, [ $\alpha$ ]<sub>D</sub> + 85° (c, 0.23, H<sub>2</sub>O), C<sub>18</sub>H<sub>32</sub>O<sub>16</sub> gave positive Phenol-sulphuric acid test<sup>15</sup> and Feigl test<sup>17</sup> indicating the presence of normal sugar(s) in the moiety. The <sup>1</sup>H NMR spectrum of Lisose at 300 MHz exhibited four signals in the anomeric proton region as doublets at  $\delta$  5.26 (1H), 5.23 (1H), 4.67 (1H), and 4.46 (1H) for four protons indicating that it is a trisaccharide in its reducing form. It was further supported by the appearance of two signals for four anomeric carbons at  $\delta$  89.112 (2C), and 101.861 (2C) in the <sup>13</sup>C NMR spectrum of acetylated product of compound Lisose. The ES mass spectrum of Lisose showed the highest mass ion peak at m/z 566 [M+Na+K]<sup>+</sup> and at m/z 543 [M+K]<sup>+</sup>, which was in agreement of derived molecular formula C<sub>18</sub>H<sub>32</sub>O<sub>16</sub> with the molecular ion expected at m/z 504 for the trisaccharide. The reducing nature of Lisose was confirmed by methylglycosidation of MeOH/H<sup>+</sup> followed by its acid hydrolysis led to the isolation of  $\alpha$  and  $\beta$ -methyl glucoside confirming the presence of glucose at the reducing end in the oligosaccharide. The three monosaccharide units present in Lisose have been designated as S<sub>1</sub>, S<sub>2</sub>, and S<sub>3</sub> for convenience starting from

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the reducing end. To confirm the monosaccharide constituents and their sequence in compound Lisose, it was hydrolyzed under strong acidic i.e., Kiliani hydrolysis<sup>18</sup> and mild acidic conditions i.e., Mannich-Siewert method<sup>19</sup> respectively. In Kiliani hydrolysis the reducing trisaccharide gave only two spots i.e. Glc and Gal, which were identified by comparison with authentic samples of Glc and Gal (PC) suggesting that the trisaccharide is comprised of two monosaccharide units i.e. Glc and Gal. To confirm the sequences of monosaccharides in Lisose, it was hydrolyzed under mild acidic conditions. After three days the reaction mixture gave two spots, one of which was identical in mobility to Glc while the other may be disaccharide (I) which had a comparable mobility with lactose (PC) showing that the Glc (S<sub>3</sub>) was the terminal monosaccharide in the trisaccharide. Further after seven days the reaction mixture showed two spots which were identified as Gal and the Glc (PC, [ $\alpha$ ]<sub>D</sub>). The results obtained from acid hydrolysis confirmed that the trisaccharide was made up of Gal and Glc in the sequence Glc-Gal-Glc. The chemical shifts of anomeric carbons observed in <sup>13</sup>C NMR spectrum and of anomeric protons observed in <sup>1</sup>H NMR spectrum of compound Lisose were also in agreement with the reported values of <sup>1</sup>H and <sup>13</sup>C anomeric chemical shifts of Glc and Gal. The presence of glucose at the reducing end was further supported by presence of two anomeric proton signals as doublets at  $\delta$  5.233 (1H) and  $\delta$  4.675 (1H) and their coupling constants as 3.6 Hz and 8.1 Hz for  $\alpha$  and  $\beta$ Glc respectively. One of the anomeric proton appearing as a doublet at  $\delta$  4.463 (1H) with a coupling constant 7.8 Hz was assigned for  $\beta$ -Gal (S<sub>2</sub>). Its linkage to S<sub>1</sub> i.e.  $\beta$ -Glc by a (1 $\rightarrow$ 4) linkage which was confirmed by the presence of an H-4 methine proton triplet of  $\beta$ -Glc (S<sub>1</sub>) at  $\delta$  3.599 (J = 9.0 Hz) in <sup>1</sup>H NMR spectrum of acetate of compound Lisose. The presence of these two monosaccharide units i.e. Gal and Glc in the sequence Gal 1 $\rightarrow$ 4 Glc confirm the presence of lactosyl moiety in the compound Lisose. The presence of lactosyl moiety in Lisose was further confirmed by the structural reporter group (SRG) appeared as a triplet of H-2 of  $\beta$ -glc at  $\delta$  3.296 in its <sup>1</sup>H NMR spectrum. Isolation of lactose during the hydrolysis of Lisose supported the presence of these two monosaccharide units i.e. Gal and Glc in the sequence Gal 1 $\rightarrow$ 4 Glc (S<sub>2</sub> $\rightarrow$ S<sub>1</sub>) in Lisose. The third sugar in the trisaccharide was identified as  $\alpha$ -Glc which gave an anomeric proton doublet at  $\delta$  5.269 (1H, J=3 Hz). Its linkage to S<sub>2</sub> i.e.  $\beta$ -Gal by a (1 $\rightarrow$ 4) linkage was confirmed by the presence of a H-4 methine proton triplet of  $\beta$ -Gal (S<sub>2</sub>) at  $\delta$  4.065 in <sup>1</sup>H NMR spectrum of compound Lisose. The 1 $\rightarrow$ 4 linkage between S<sub>3</sub>-S<sub>2</sub> was confirmed by the COSY spectrum of acetylated Lisose, in which the anomeric proton doublet at  $\delta$  4.57 (J= 8.1 Hz, Gal, S<sub>2</sub>) shows connectivity with H-2 at  $\delta$  5.265, H-2 showed connectivity with H-3 at  $\delta$  5.002 and H-3 showed connectivity with H-4 at  $\delta$  4.015 respectively. The upfield shifted signal of H-4 of Gal (S<sub>2</sub>) indicated that Gal (S<sub>2</sub>) was substituted at position 4 by Glc (S<sub>3</sub>). Simultaneously the downfield shifted chemical shifts of H-2, H-3, and H-4 methine protons also confirm that the  $\alpha$ -Glc was the terminal sugar in the trisaccharide. All the <sup>1</sup>H NMR assignments for ring protons of monosaccharide units of Lisose were confirmed by HOMOCOSY and TOCSY experiments. The trisaccharide nature of Lisose was further confirmed by the spectral studies of acetylated of Lisose, which contained signals of methyl protons of acetyl groups in its <sup>1</sup>H NMR spectrum at  $\delta$  2.00 to 2.12 ppm besides the signals of ring protons and anomeric protons present in acetate of Lisose.



#### Mannich Hydrolysis of Compound Lisose under Mild Acidic Conditions

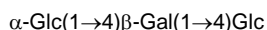
The  $^{13}\text{C}$  NMR data of acetylated compound Lisose was also in conformity with the derived structure. The chemical shifts of the anomeric carbons of compound Lisose at  $\delta$  89.112 (2C,  $\alpha$ -Glc), 101.861 (1C,  $\beta$ -Glc), and 101.861

(1C,  $\beta$ -Gal) present in the  $^{13}\text{C}$  NMR spectrum were in accordance with the anomeric carbon values of Glc and Gal. The values of chemical shifts of ring carbons of trisaccharide also supported the derived structure.

Table:  $^{13}\text{C}$  NMR Values of Compound Lisose

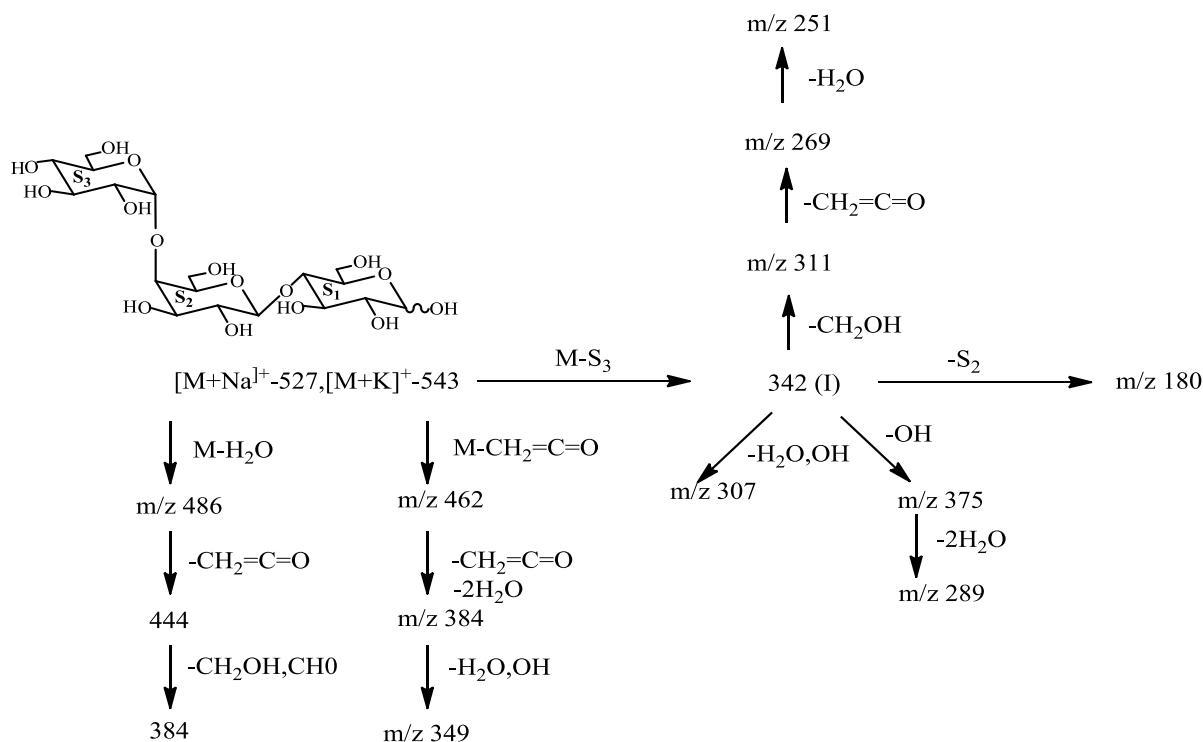
Sugar	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>
$\alpha$ Glc	89.112	70.74	71.48	82.07	71.36	61.73
$\beta$ Glc	101.86	76.99	77.63	77.63	76.99	62.04
$\beta$ Gal	101.86	68.68	70.74	69.58	76.36	62.04
$\alpha$ Glc	89.112	70.46	71.48	62.04	71.36	61.73

Based on the pattern of chemical shift of  $^1\text{H}$ ,  $^{13}\text{C}$ , HOMOCOSY, and TOCSY NMR experiments, it was interpreted that the compound Lisose was a trisaccharide having two Glc and one Gal moieties. Thus, based on the pattern of chemical shifts of  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, HOMOCOSY, TOCSY and HSQC NMR experiments it was interpreted that the compound was a trisaccharide having structure as-



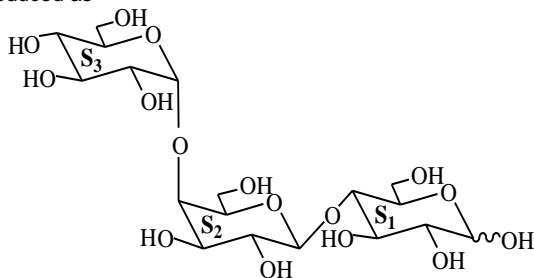
The derived structure of Lisose as Glc- $\alpha$ -(1 $\rightarrow$ 4) Gal- $\beta$ -(1 $\rightarrow$ 4) Glc was further supported by its ES Mass spectrum. The highest mass ion peaks were recorded at  $m/z$  566 and 543 which were due to  $[\text{M}+\text{Na}+\text{K}]^+$  and  $[\text{M}+\text{Na}]^+$  confirming the molecular weight of compound Lisose as 504 with the derived composition  $\text{C}_{18}\text{H}_{32}\text{O}_{16}$ . The molecular ion at  $m/z$  504 further fragmented to give mass ion fragment at  $m/z$  342 (I) corresponding to lactosyl moiety comprising of Glc

(S<sub>1</sub>) and Gal (S<sub>2</sub>) after the elimination of terminal Glc (S<sub>3</sub>) unit, indicating the presence of Glc (S<sub>3</sub>) at the nonreducing end. The molecular ion peak at  $m/z$  504 was further supported by some other mass ion peak at  $m/z$  486 (M-H<sub>2</sub>O), 462 (M-CH<sub>2</sub>=C=O), 444 (486-CH<sub>2</sub>=C=O), 384 (444-CH<sub>2</sub>OH, CHO or 462-CH<sub>2</sub>=C=O, 2H<sub>2</sub>O) and 349 (384-H<sub>2</sub>O, OH). The mass ion peak at  $m/z$  342 which were due to a lactosyl moiety (Gal  $\beta$  1-4 Glc) was further supported by some other mass ion peaks present at  $m/z$  325 (342-OH), 311 (342-CH<sub>2</sub>OH), 307 (342-H<sub>2</sub>O, OH), 289 (325-2H<sub>2</sub>O), 269 (311-CH<sub>2</sub>=C=O), and 251 (269-H<sub>2</sub>O). The mass ion peak at  $m/z$  342 (I) further fragmented to give another mass ion peak at  $m/z$  180 corresponding to the glucose moiety (S<sub>1</sub>), which was present at the reducing end of the reducing trisaccharide. These mass ion fragments not only confirmed the derived structure but also fixed the sequence of the sugars Glc—Gal—Glc in the trisaccharide.



SCHEME : MASS FRAGMENTATION OF COMPOUND (LISOSE)

Based on the results obtained from chemical degradation/acid hydrolysis, chemical transformation, Electro spray mass spectrometry and 1D-NMR viz.  $^1H$  NMR,  $^{13}C$  NMR and 2D-NMR viz. COSY, TOCSY and HSQC NMR spectra of Lisose acetate and Lisose, the structure and sequence of isolated novel trisaccharide Lisose was deduced as-



### Conclusion

From the above informations, we conclude the structure of isolated Goat milk oligosaccharide, Lisose. This oligosaccharide was reported for the first time from any natural source or any milk and its structure was elucidated with the help of spectroscopic techniques like  $^1H$ ,  $^{13}C$ , 2D-NMR (COSY, TOCSY and HSQC) spectroscopy and mass spectrometry.

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### Endnotes

- Coppa G V, Zampini L, Galeazzi T, and Gabrielli O, *Digest. Liver Dis.*, 2006, 38, S291-S294.
- Hakkarainen J, Toaiven M, Leinonen A, Frangsmyr L, Stromberg N, Lapinjoki S, Nassif X, and Tikkanen-Kaukanen, *CJ.Nutr.*, 2005, 135, 2445-2448.
- Zivkovic A M and Barile D, *Adv. Nutr.*, 2011, 2, 284-288.

- Maslowski K M, Vieira A T, Ng A, Kranich J, Sierro F, Yu D, Schilter H C, Rolph M S, Mackay F, Artis D, Xavier R J, Teixeira M M, and Mackay C R, *Nature.*, 2009, 461, 1282-1286.
- Wang B and Brand-Miller J, *Eur. J. Clin. Nutr.*, 2003, 57, 1351-1369.
- Schwonzen M, Schmits R, Baldus S E, Vierbuchen M, Hanish F G, *Br. J. Cancer*, 1992, 65, 559-565.
- Abe K, Mckibbin J M J, Hakomori S, *Eur. J. Biochem. Biol. Chem.*, 1983, 258, 11793-11797.
- Fang J N, Proksch H, Wagner H, *Phytochemistry*, 1985, 24, 2619-2622.
- Srivastava A, Tripathi R, Bhatia G, Khanna A K and Deepak D, *Asian Pacific Journal of Tropical Biomedicine*, 2012, 1-6.
- Srivastava R, Kulshretha D K, *Phytochemistry*, 1989, 28, 2877-2883.
- Boehm G, Stahl B and MattilaSandholm T, Woodhead publishers, Cambridge, UK, 2003, pp. 203-243.
- Ranjan A K, Rathore R S, Deepak D, Khare A, Sahai R and Srivastava V M L, *Asian Journal of Organic & Medicinal Chemistry*, 2016, 1(2), 55-60.
- Singh P, Maurya R K, Rizvi A H and Deepak D, *J. Biol. Chem. Research*, 2017, Famiose: A novel hexasaccharide from Goat's milk, 34(2), 548-556.
- Kobata A and Ginsburg V, *J.Biol.Chem*, 1970, 245, 1484.
- Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smith F, *Anal. Chem*, 1956, 28(3), 350-356.
- Freeden O, Goldschmidt L, *MikrochimActa*, 1937, 1, 351.
- Feigl F, *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 1975, edn7, p. 337.
- Kiliani H, *ÜberDigitalinumverum. Ber. Deutsch. Chem. Ges*, 1930, 63, 2899
- Mannich C, Siewert G, *Über g-strophanthin (ouabain) und g-strophanthidin. Ber. Deutsch. Chem. Ges*, 1942, 75, 737.