Investigating seasonal influences on the concentrations of bio-active milk glycans and sialic acid in dairy cows

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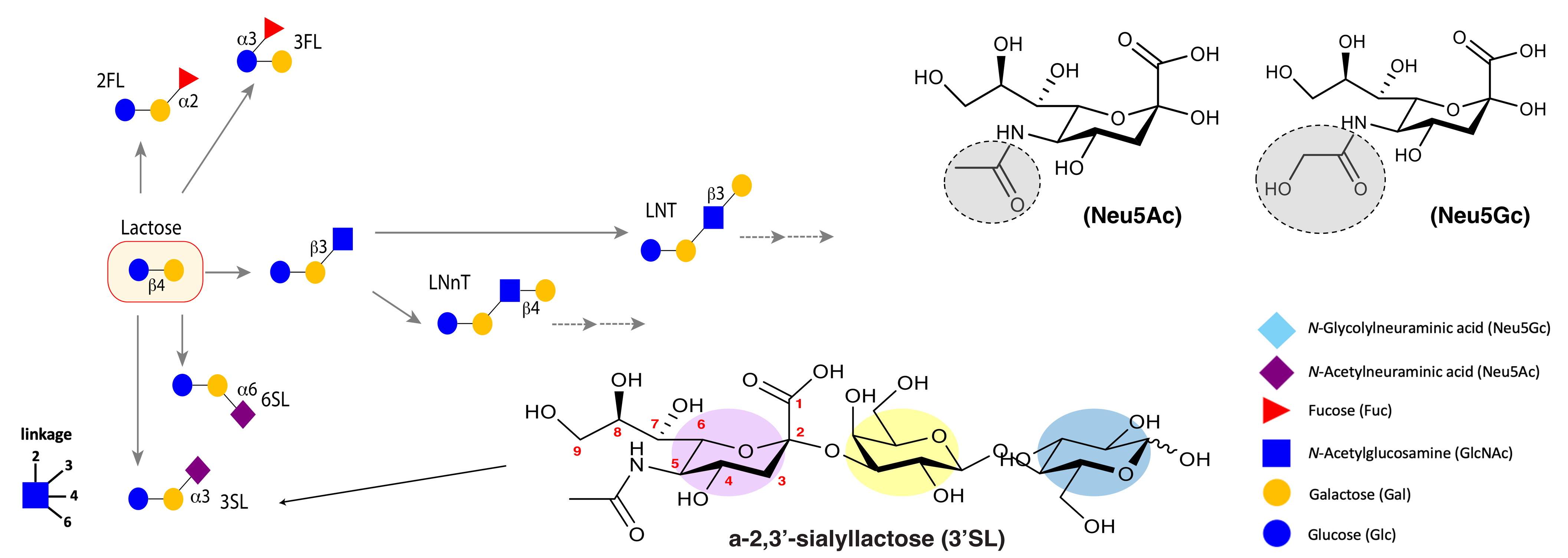
Abstract

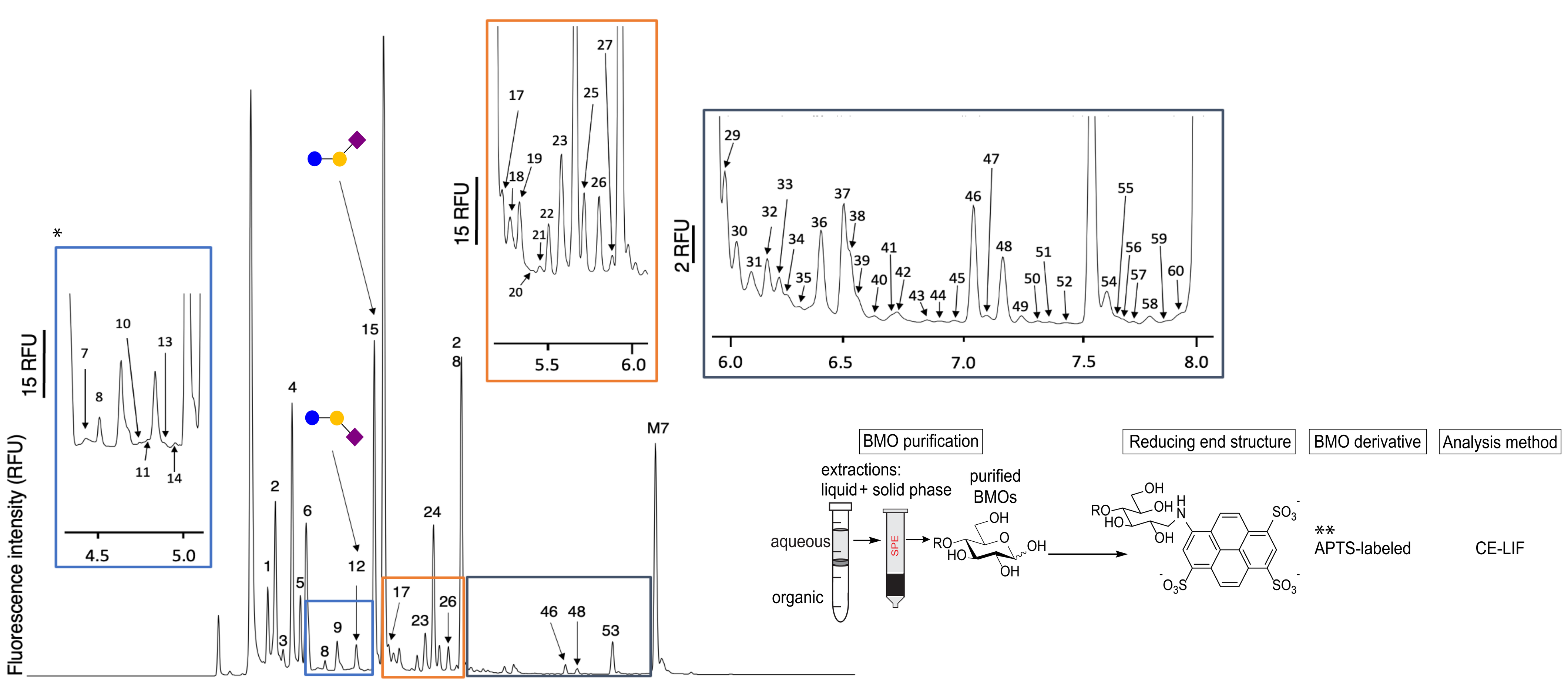
Milk is the first food consumed by all neonatal mammals and, accordingly, it fills many bio-active roles beyond basic nutrition[1]. To date, the factors that influence milk oligosaccharides (MOs) concentrations in dairy cow milk have not been extensively studied, albeit seasonal variation has been noted and it is known that different breeds have different relative MO concentrations. ***It is my hypothesis*** that a) relative levels (%) of bovine MOs (BMOs) and sialic acids will vary across seasons, and b) there is no significant difference between the two different BC milk producers. In order to deduce seasonal influences, off-the-shelf milk samples were collected weekly from the two producers over two years. High performance liquid chromatography-mass spectrometry (HPLC-MS) was used to quantitate the sialic acids present in all samples. In addition, capillary electrophoresis with fluorescence detection (CE-FLD) was used to determine the concentrations of up to 63 unique MOs. Our data suggested differences in relative levels (%) of BMO's and sialic acids across seasons and year as well as differences between the two BC milk producers. These analyses identified a strong negative correlation between sulfated and sialyllated BMOs. Additionally, we also identified a negative correlation between levels of sialyllated BMOs and increasing temperature as well as in Neu5Ac but not Neu5Gc.

Introduction

It is established that majority of BMOs consist of the well-known sugar lactose. Moreover, BMOs comprises a large number of complex oligosaccharide structures, consisting of three or more monosaccharides. Such a diversity is further amplified by combining these monosaccharides to sialic acids primarily the non-human, mammal-specific N-Glycolylneuraminic acid (Neu5Gc); N-Acetylneuraminic acid (Neu5Ac) is only found in humans.

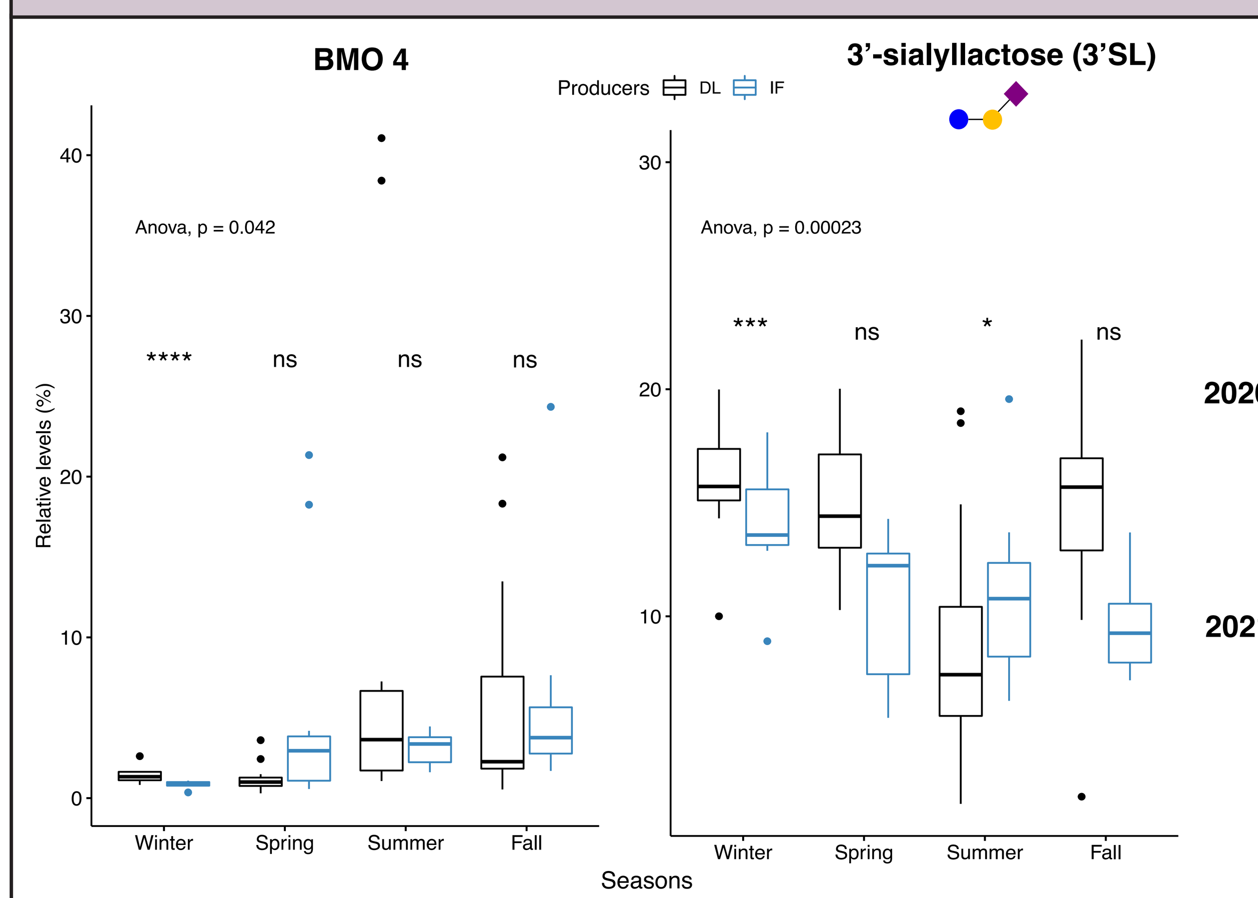


Identification and characterization of BMOs by CE-FLD.



* Region denotes BMO (1-10) species that are highly charged or smaller than 3'-sialyllactose (3'SL).
** 8-aminopyrene-1,3,6-trisulfonic acid (APTS)

Significant differences in relative levels (%) of BMOs across seasons and between BC milk producers.



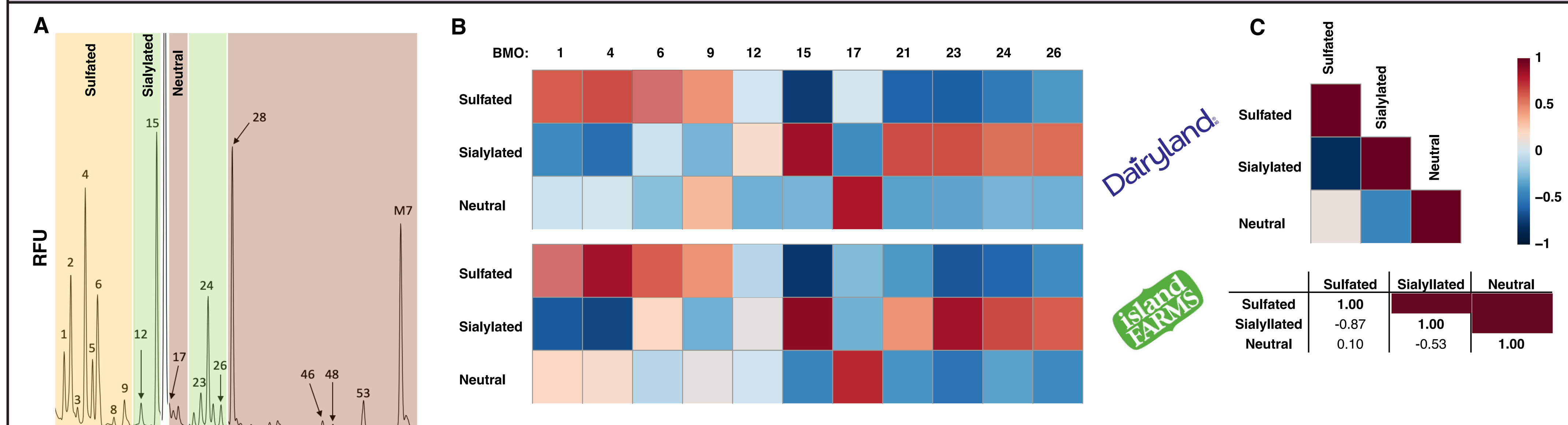
Winter: December - February **Spring:** March - May
Summer: June - August **Fall:** September - November

	BMO	Dairyland Winter	Dairyland Spring	Dairyland Summer	Dairyland Fall	Island Farms Winter	Island Farms Spring	Island Farms Summer	Island Farms Fall
2020	4	ab	a	ab	ab	ab	ab	b	ab
	13 (3'SL)	a	a	a	a	a	a	a	a
	Sulfated**	abc	a	abc	abc	bc	bc	c	ab
	Sialyllated	a	a	a	a	a	a	a	a
	Neutral	a	ab	ab	ab	ab	b	ab	ab
2021	4	a	a	a	a	a	a	a	a
	13 (3'SL)***	d	cd	a	cd	bcd	ab	abc	ab
	Sulfated**	a	a	c	abc	a	ab	ab	bc
	Sialyllated***	c	c	a	bc	bc	ab	abc	ab
	Neutral*	a	a	a	a	ab	b	ab	a

Highlighted regions denote a statistically significant interaction effect between producers Dairyland and Island farms across seasons.

Signif. code: 0, '***' 0.001, '**' 0.01, '*' 0.05, ' . ' 0.1, ' ' 1

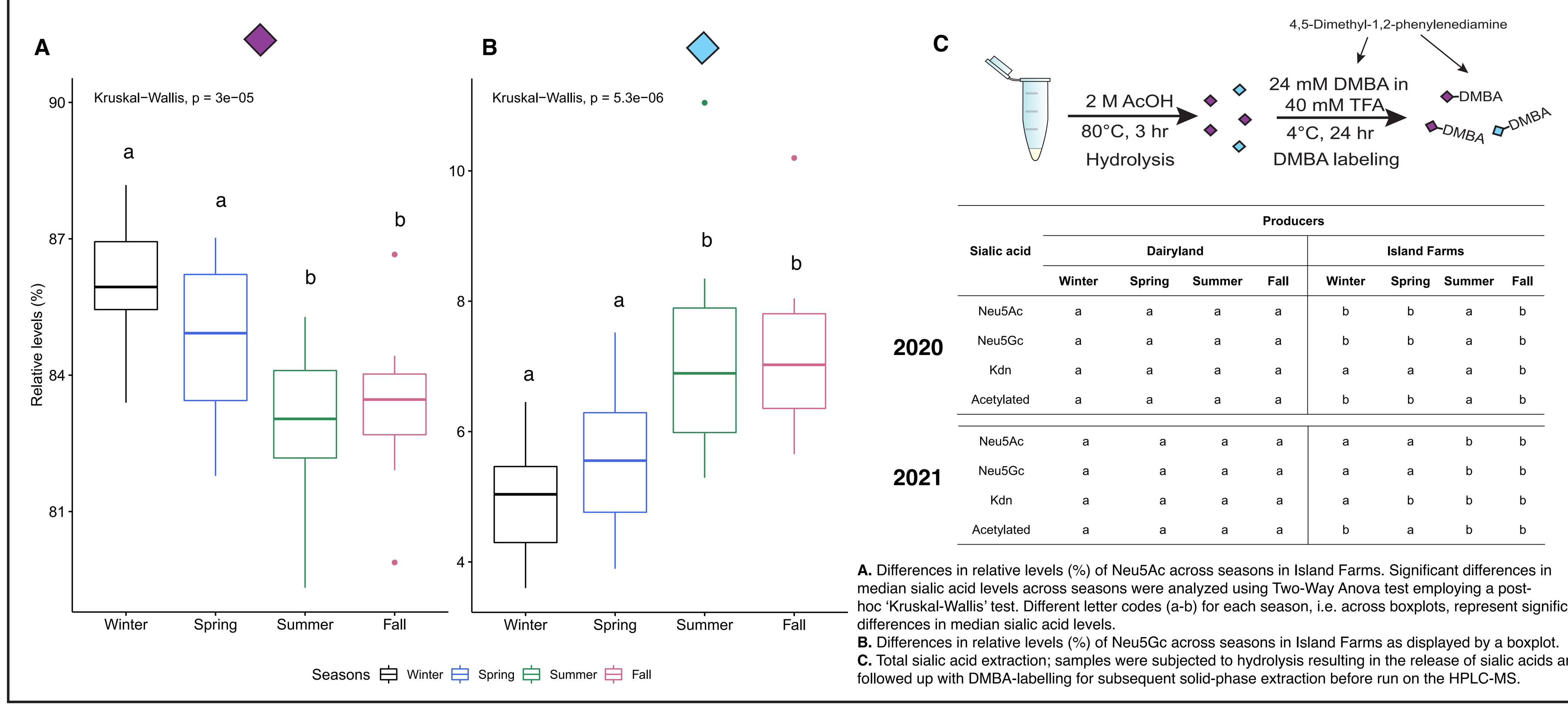
Negative correlation identified between sulfated and sialyllated BMOs.



	Sulfated	Sialyllated	Neutral
Sulfated	1.00		
Sialyllated	-0.87	1.00	
Neutral	0.10	-0.53	1.00

A. CE-LIF electropherogram of assigned BMOs into different classes; yellow = **sulfated**, green = **sialyllated**, brown = **neutral**; excluding lactose.
B. Spearman's rank correlation analysis performed on top 11 unique BMOs and correlated to sum of BMOs within each class (sulfated, sialyllated, and neutral) across 2020 and 2021. All unique BMOs displayed statistically significant correlation, * (P < 0.05).
C. Sum of BMOs within each class were correlated. Dairyland and Island farms displayed equal correlations, and statiscally significant correlation; * (P < 0.05).

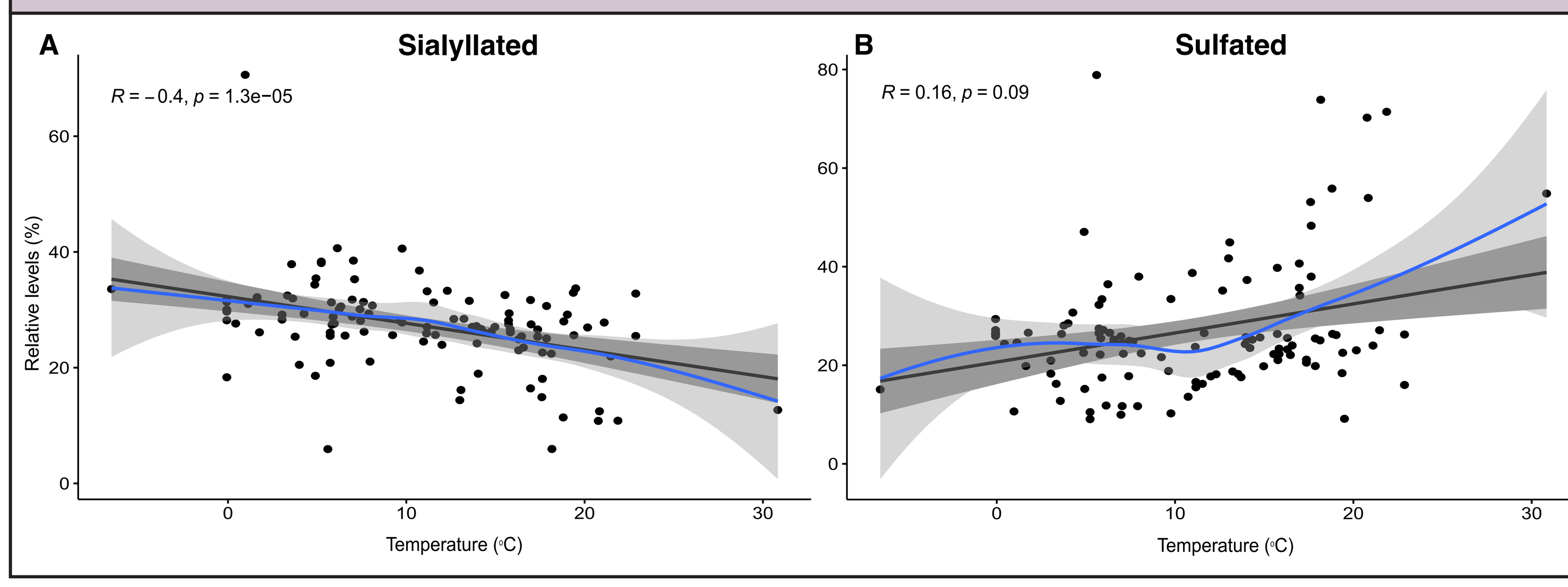
Few significant differences in relative levels (%) of Neu5Ac and Neu5Gc across seasons.

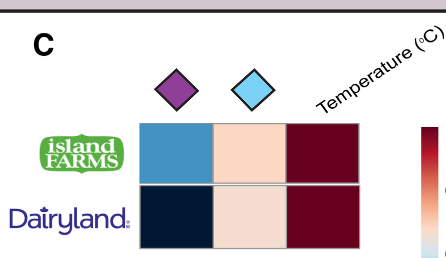


	Sialic acid	Dairyland Winter	Dairyland Spring	Dairyland Summer	Dairyland Fall	Island Farms Winter	Island Farms Spring	Island Farms Summer	Island Farms Fall
2020	Neu5Ac	a	a	a	a	b	b	a	b
	Neu5Gc	a	a	a	a	b	b	a	b
	Kdn	a	a	a	a	a	a	a	b
	Acetylated	a	a	a	a	b	b	a	b
2021	Neu5Ac	a	a	a	a	a	a	b	b
	Neu5Gc	a	a	a	a	a	a	b	b
	Kdn	a	a	a	a	a	b	b	b
	Acetylated	a	a	a	a	b	a	b	b

A. Differences in relative levels (%) of Neu5Ac across seasons in Island Farms. Significant differences in median sialic acid levels across seasons were analyzed using Two-Way Anova test employing a post-hoc 'Kruskal-Wallis' test. Different letter codes (a-b) for each season, i.e. across boxplots, represent significant differences in median sialic acid levels.
B. Differences in relative levels (%) of Neu5Gc across seasons in Island Farms as displayed by a boxplot.
C. Total sialic acid extraction; samples were subjected to hydrolysis resulting in the release of sialic acids and followed up with DMBA-labelling for subsequent solid-phase extraction before run on the HPLC-MS.

Negative correlation between sialyllated BMOs and temperature as well as in Neu5Ac but not Neu5Gc.





Producers	Neu5Ac	Neu5Gc	Temperature (°C)
Island Farms	-0.32	0.27	1.00
Dairyland	-0.96	0.18	1.00

A. Spearman rank correlation between sum of sialyllated BMOs and milking data temperature. Correlation is definde by Rho (p): -0.4
B. Spearman rank correlation between sum of sulfated BMOs and milking data temperature. Correlation is definde by Rho (p): 0.16
C. Neu5Ac and Neu5Gc were correlated to milking date temperature.

Conclusions

Through analysis by CE-FLD, there were seasonal influences on the relative levels (%) of BMOs with significant differences in sulfated and sialyllated BMOs. Additionally, there was an observable difference between the two different BC milk producers. A statistically significant difference was observed in Neu5Ac and Neu5Gc between the producers and year, notably with a seasonal shift from Winter-Spring to Summer-Fall. Neu5Ac levels decreased with season recording highest and lowest levels in Winter and Summer, respectively. The opposite was observed in Neu5Gc. A negative correlation was identified between the sulfated and sialyllated BMOs in all the individual BMOs and sum of BMOs within each class[2]. Despite differences in producers, similar correlations were observed between the BMOs. The data presented was analyzed against milking date temperature and revealed strong negative correlation between the sialyllated BMOs and increasing temperature. There was a strong negative correlation to Neu5Ac and temperature whereas Neu5Gc displayed a slight postive correlation. A weak (positive) correlation was identified in sulfated BMOs with greater variation.

References

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[2] Gray, T. E.; Narayana, K.; Garner, A. M.; Bakker, S. A.; Yoo, R. K. H.; Fischer-Tlustos, A. J.; Steele, M. A.; Zandberg, W. F. *Food Chem.* **2021**, 361, 130-143

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