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The effect of frozen storage on sheep milk properties

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Scientific Interpretative Summary

New Zealand food Safety (NZFS) has a strategic priority to make food business easier while producing safe and suitable food. This report provides assistance to small cheesemakers who utilise frozen raw milk and those dairy processors producing specialty raw milk in very small quantities.

The report considers typical industry practices of freezing raw milk and determines whether various methods of freezing and thawing raw milk has an adverse effect on milk safety, composition, functional suitability and storage times post thawing. Currently utilised practices were compared with a fast-freeze technique developed for raw sheep milk under the Food Industry Enabling Technologies (FIET) research programme at Massey University.

Reported experiments were performed for milk with high fat and solids content obtained from ewes during late lactation and for low fat and solids milk from ewes at the beginning of lactation.

The research showed that both freezing and thawing methods have a significant effect on microbiological quality of thawed milk and on the milk composition. In experiments some samples of slow frozen milk showed unacceptable microbial growth for all thawing conditions, possibly indicating that microbial growth occurred during the freezing process as well as during the thawing process. Rapidly frozen milk maintained good quality provided thawing occurred at 5°C or at 55°C. Thawing at room temperature may allow unacceptable bacterial growth independently of freezing method and, consequently, room temperature should not be used to thaw frozen milk. With each freezing method, the frozen storage temperatures of -18°C and below were low enough to ensure the milk quality was maintained for at least 24 weeks.

Overall, the study showed that both early and late season milk can be frozen below -18°C for later use provided thawing is achieved rapidly and at temperatures at or below 5°C that do not encourage bacterial growth.

The reported results might be used by NZFS business units involved in developing, implementing and administrating standards for dairy products.

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Conflict of interest declaration

Massey University have applied for patent protection of a rapid freeze process for milk and could potentially benefit from the adoption of that process.
Massey is party to a contract under which we could benefit from a process that uses a rapid thawing approach developed at Massey.

Keywords

Sheep, milk, ovine, freeze, frozen, bacteriology, organoleptic, composition.

Contents

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ABSTRACT

Impacts on milk quality and composition, of different industrial methods of freezing and thawing raw sheep milk, were assessed. Early and late season sheep milk was frozen in different forms (pails, bladders, fast frozen pellets and droplets) using three different methods [slow freezing and storage at -18°C, rapid pellet freezing and storage at -25°C or freezing in liquid nitrogen (LN2) and storage below -60°C). Milk was stored frozen for 3 days, 12 weeks or 24 weeks. After frozen storage the milk was thawed in air at 5°C or 20°C. Additionally, fast frozen pellets were thawed rapidly in a liquid bath of thawed milk held at 55°C for a few minutes.

Milk from ewes during late lactation (August) has a higher level of fat and solids than milk from ewes during early lactation (May). As expected, both total bacteria-count and somatic cells count (SCC) were higher in late season milk.

Pails and bladders took about three days to thaw, allowing psychrotrophic microbial growth, especially when thawed in air at 20°C. Observed changes in pH and titratable acidity are likely due to bacterial growth during this extended thawing. Milk frozen in pellet form and thawed at 55°C took at most two minutes to thaw.

The thawing method, in combination with size and shape of the frozen mass, had a significant effect on the aerobic plate count (APC) of thawed milk and on the milk composition. Higher than average APCs were observed for milks thawed at 20°C after being stored in bladders for 3 days or 12 weeks, and after being stored in pails independently of storage times. High APCs were also noticed in several samples taken from milk stored in bladders and pails and thawed at 5°C.

Some slow frozen samples (both pail and bladder formats) showed unacceptable microbial growth for all thawing conditions, possibly indicating that microbial growth occurred during the freezing process as well as during the thawing process. Rapidly frozen milk maintained good quality provided thawing occurred at 5°C or 55°C. Thawing at 20°C, even from pellet form, may allow unacceptable bacterial growth.

With each freezing method (pail, bladder, pellets or droplets), the frozen storage temperatures (-18°C to below -60°C) were low enough to ensure the milk quality was maintained for 24 weeks (the maximum investigated), providing the freezing and thawing process was executed in a manner that minimised risk of bacterial growth.

This study showed that both early and late season milk can be frozen for later use provided thawing is achieved rapidly and at temperatures that do not encourage bacterial growth. Frozen pellets, and immersion in liquid nitrogen were the only formats that allowed this, but immersion in liquid nitrogen is unlikely to be commercially viable.

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1. Introduction

Research was commissioned, to determine whether various industrially relevant methods of freezing and thawing raw milk from sheep have an adverse effect on milk safety, composition, suitability for manufacturing and on potential storage times. Small milk producers typically freeze milk in pails or bladders (flexible bags), at various temperatures, and thaw milk in either chilled or ambient conditions.

In the current study, early and late season sheep milk was frozen in four different formats, stored frozen for three durations then thawed by three different methods. Methods and formats used included those typically used in industry now, one newly developed for the industry, plus best possible practice (cryogenic) not likely to be industrially affordable.

2. Methodology

2.1 Material collection

Early-season (May 2019) and late-season (August 2019) chilled milk was collected from a producer in the Wairarapa in sterile 10 litre bladders. The early-season (May) collection was from two consecutive milkings (the day of collection and the previous day). The ewes had lambed between 3 to 6 weeks prior so the milk should have been free of colostrum. The late-season (August) collection was from three consecutive milkings, collected and processed over two days due to a lower than expected milk yield. The first collection was from the milking of the collection day and the previous day; the second collection was from the collection day's milking. Chilled milk was collected from the farm vat,the contents of which were maintained stirred at below 6°C between milkings.

Milk was transported packed in ice directly to the FoodPilot food processing facility at Massey University's Palmerston North campus. To establish a baseline, the milk was sampled immediately on arrival and tested in triplicate. These samples were termed "fresh".

2.2 Freezing

To replicate current and recent industry practice, milk was frozen in 10 litre pails and in 10 litre bladders (pillow-shaped bags) using conventional walk-in freezers at a temperature of -18°C. This is believed to represent typical industry practice; the FoodPilot freezers are reasonably typical of industrial freezer practice. These storage freezers were not designed as high velocity blast freezers, but each increment of milk added was < 2% of the freezer room capacity.

Milk was also frozen using a fast-freeze technique developed for raw sheep milk under the Food Industry Enabling Technologies (FIET) research programme at Massey University. This method forms the milk into frozen pellets, cooling the bulk of the milk from its initial temperature to near its final storage temperatures within approximately three minutes. The maximum residence time within this freezer was 184 seconds. As the method is currently undergoing patent proceedings and commercialisation, further details cannot yet be disclosed. Once frozen, the milk was stored at -25°C. These samples are referred to as fast-frozen or "FF" samples.

As a control, milk was frozen using liquid nitrogen in what were considered to be optimum conditions. Droplets were dropped into stirred liquid nitrogen to freeze them while preventing agglomeration. Solidification of droplets was observed to be almost instantaneous. Liquid nitrogen boiled off allowing the frozen milk droplets to be recovered. This cryogenically frozen milk was stored in sterilised Ziplock™ bags in a freezer. The temperature within this freezer was measured periodically and found to be reliably between -65°C and -76°C. In this report, this temperature is referred to as "below -60°C". This control was used both to confirm that the analysis methods worked and to provide what is considered the benchmark for stored frozen milk. Selective sampling only, and not the full range of tests, was applied for milk treated by this method.

Milk was frozen for six combinations of season/duration, with each collection of milk (May and August) being stored for 3 days and 12 weeks and 24 weeks. It was considered that samples held frozen for only three days represented freshly frozen milk with little or no influence from storage. After three days it was assumed that the samples would have reached a steady state at storage temperature with a stable frozen fraction in the water and solid fraction in the fat. For the storage durations, 12 weeks and 24 weeks were selected as the short and long durations. However, it is accepted there is no "typical" industry storage duration and 24 weeks is still short when a year or two of storage could be desirable.

2.3 Thawing

At the end of each freezing period milk was thawed at both chilled (5°C) and ambient (20°C) temperatures to replicate typical methods in industry. The fast-frozen milk (in free-flow pellet form) was thawed at 5°C, 20°C, and also at 55°C as a fast thawing temperature.

Thawing was carried out in the Massey FoodPilot, which has a walk-in chiller at 5°C, and a process area maintained at 20°C. Thawing at 55°C was carried out by placing frozen milk inside a sterilised stainless-steel bowl placed in a jacketed vessel with water held at 55°C (bain-marie). A sterilised spatula was used to manually agitate the milk until it thawed, observed to take less than two minutes.

2.4 Testing

Thawed milk was agitated with a sterilised hand-held stick-blender for 30 seconds and then transferred to sterile bottles for laboratory testing. The surface of the blender was sterilised with ethanol prior to mixing.

- Ash was determined by ashing at 550°C according to AOAC 942.05,
- Fat was determined by the Mojonnier method according to AOAC 989.05.
- Total solids were determined by vacuum drying according to AOAC 990.19 and 990.21.
- The Crude Protein was determined by multiplying the Total Nitrogen value by 6.38.
- Total Nitrogen was determined according to the Dumas method following AOAC 968.06.
- Lactose was determined by an enzymatic method following AOAC 984.15.
- Osmolality was determined from the freezing point of the milk using a Digimatic Osmometer Model 3D2, Advanced Instruments Inc, Massachusetts, USA.

- Non-Protein-Nitrogen (NPN) was determined by precipitating and filtering protein and determining the nitrogen in filtrate according to the Kjeldahl method, following AOAC 991.21.
- Non-Casein-Nitrogen (NCN) was determined by acid precipitation and filtration of casein followed by Kjeldahl analysis of nitrogen in the filtrate, following AOAC 998.05.
- Titratable Acidity (TA) was determined by AOAC 947.05.
- Free Fatty Acids (FFA) was determined according to the method presented by Perrin and Perrin (1958).
- Vitamin B2 was determined by a fluorometric method (Dunbar & Stevenson, 1979).
- SCC was determined by a Delaval cell counter (DCC), (Delaval, Tumba, Sweden.) (DeLaval Inc., 2018).
- The amount of insoluble matter in a sample was determined by collecting sediment after centrifuging 15mL aliquot at 3,000g for 10 minutes followed by forced air drying at 108°C. Values are presented as g insoluble matter per 250mL of liquid sample.
- The particle size of the milk was measured by dynamic light scattering using a Malvern Mastersizer 3000, (Malvern Instruments, Malvern, UK) (Malvern Panalytical Ltd., 2020). The particles were treated as spherical with a refractive index of 1.46 and an absorption index of 0.001 (Michalski, Briard, & Michel, 2001).
- Microbial measurements were carried out in duplicate. Aerobic Plate Counts (APC) were determined by the incubation of sample on Standard Plate Count Agar for 48 hours at 30°C according to the method presented in the Compendium of Methods for the Microbiological Examination of Foods(Downes, Ito, & American Public Health Association, 2001).
- Coliforms were determined by a Most Probable Number (MPN) method involving incubation in Lauryl Sulphate broth with MUG for 24-48 hours at 35°C (Downes et al., 2001).
- The assessment of the quality of the milk before and after freezing and thawing was based on the NZFSA DPC2: Approved Criteria for Farm Dairies (New Zealand Food Safety Authority, 2008), which sets an Action Limit for Ovine and Caprine milk of 100,000 CFU/mL. Milk samples with microbial counts above this limit result in a range of corrective actions including penalisation of producers, repeat sampling, investigations and inspections, and refusal of producers to accept milk. NZFSA DPC2 also requires that further screening be conducted if coliform counts over 500 CFU/mL are recorded

Tests were undertaken in the Massey Microbiology and Nutrition laboratories on campus and by an off-site laboratory (accredited for the tests specified).

3. Results

3.1 Basis for quality evaluation

Evaluation of milk quality before and after freezing and thawing is based on Fonterra's current standard for raw milk, itself based on the NZFSA DPC2[1] standard, as summarised in Table 1, below. This standard is for milk from cows but is also used for sheep and goat milk.

Microbiological contamination.	Three tests per month per farm. See Note 1.	Bulk milk tank of each farm dairy at the time of collection.	Aerobic plate count (APC) at 30 °C or Bactoscan®.	100, 000 cfu/ml.

***Table 1**: Standard criteria for milk quality. DPC2*

The following tables show the microbiology and nutrition laboratory test results obtained from triplicate analyses for the fresh early-season (May) and late-season (August) milk prior to freezing.

3.2 Fresh milk

The properties of the fresh milk are listed in Table 2 below.

		Early -season	Late-season
Aerobic PC	cfu/mL	$2.4*10^4$ - $4.4*10^4$	$1.1*10^4$ - $9.6*10^4$
Coliforms Total	MPN/10mL	>2400	>2400
Total solids	%	15.03 - 15.10	18.58 - 19.50
Ash	%	0.91 - 0.93	0.99 - 1.03
Nitrogen	%	0.85 - 0.86	1.11 - 1.17
Fat	%	3.65 - 3.67	6.11 - 7.24
Lactose	g/100ml	3.68 - 3.87	3.44 - 4.03
Osmolality	mOsm/kg	300	332 - 356
pH		6.8	6.54 - 6.7
NPN	%	0.05 - 0.06	0.05
Casein Protein	%	4.262 - 4.326	5.583 - 5.614
Free FA	% m/v	0.008	0.042 - 0.064
TA % m/v	% m/v	268	0.22 - 0.32
Vit B2	µg/100ml	268	378 - 429
Protein	%	5.42 - 5.49	7.08 - 7.46

***Table 2**: Fresh milk properties showing the range measured across triplicate samples*

[1] NZFSA DPC2: Approved Criteria for Farm Dairies (New Zealand Food Safety Authority, 2008)

It was observed that, for fresh late season milk, all higher fat contents, Nitrogen, ash, TS, pH and B12 and protein value were for milk collected on the second collection day. All lower Lactose, Osmolality, FFA, and TA results were for milk collected on the second collection day.

3.3 Frozen milk

For each measured value a general linear model was fitted in 'Minitab 17'[2] using the freezing method, thawing temperature, month of milk collection and storage time as independent variables. The factors that were statistically significant predictors are reported where relevant.

3.3.1 Frozen early-season milk results

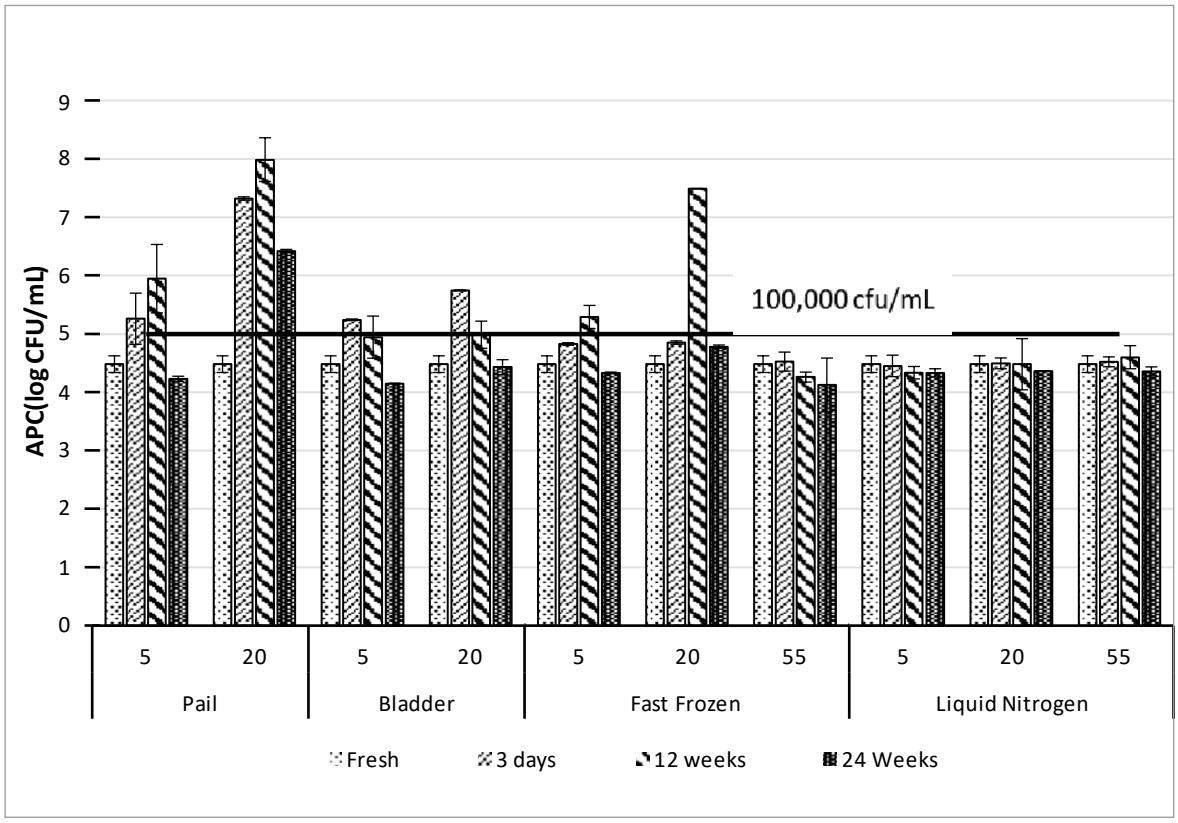

***Figure 1**: APC counts of early-season milk, stored frozen for up to 24 weeks. Error bars represent the standard deviation for triplicate subsamples.*

Based on APC results in Figure 1, the poorest quality milk was predominantly from frozen pails and bladders, reflecting the slow freeze/thaw conditions. The greatest increase in APC was seen in milk stored in pails and thawed at 20°C, which showed 1.9 to 3.5 log

[2] Minitab statistical analysis software. Minitab Coventry CV3 2TE UNITED KINGDOM

increases in APC relative to fresh. The best quality milk had been fast frozen (pelletized), or frozen with LN2 (with the exception of one sample of pelletized milk thawed at 20°C).

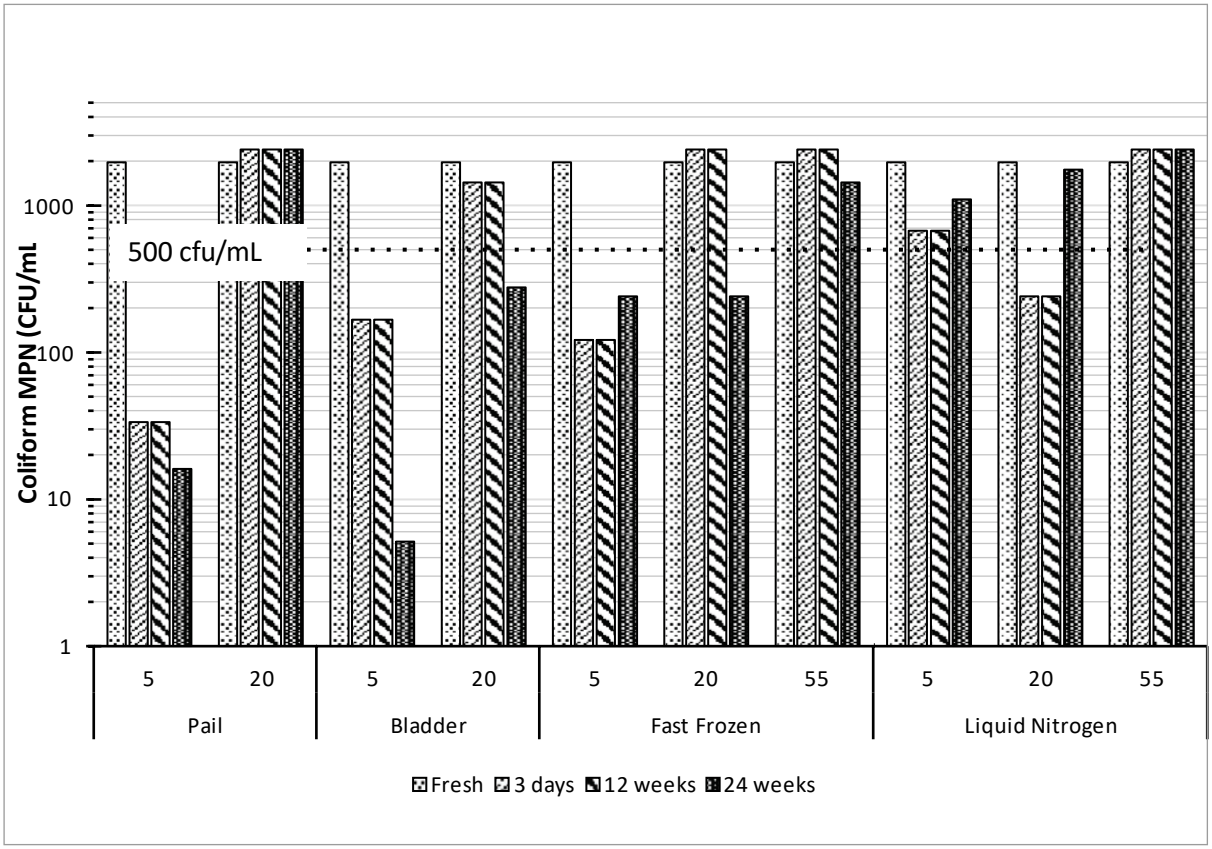

***Figure 2**: Coliform MPN-counts (CFU/mL) of early season milk, stored frozen for up to 24 weeks.*

As seen in Figure 2, the fresh milk collected in early-season (May) had a coliform count above the DPC2 action limit, as did a number of the samples after frozen storage and thawing. Since the fresh milk coliform levels reached the highest that could be registered by MPN at the dilution used, it was impossible to register any microbial growth occurring during the freezing and thawing process for any format.

Note that these results represent the quality of the raw milk collected for further processing and do not measure the safety of the milk.

3.3.2 Frozen late-season milk results

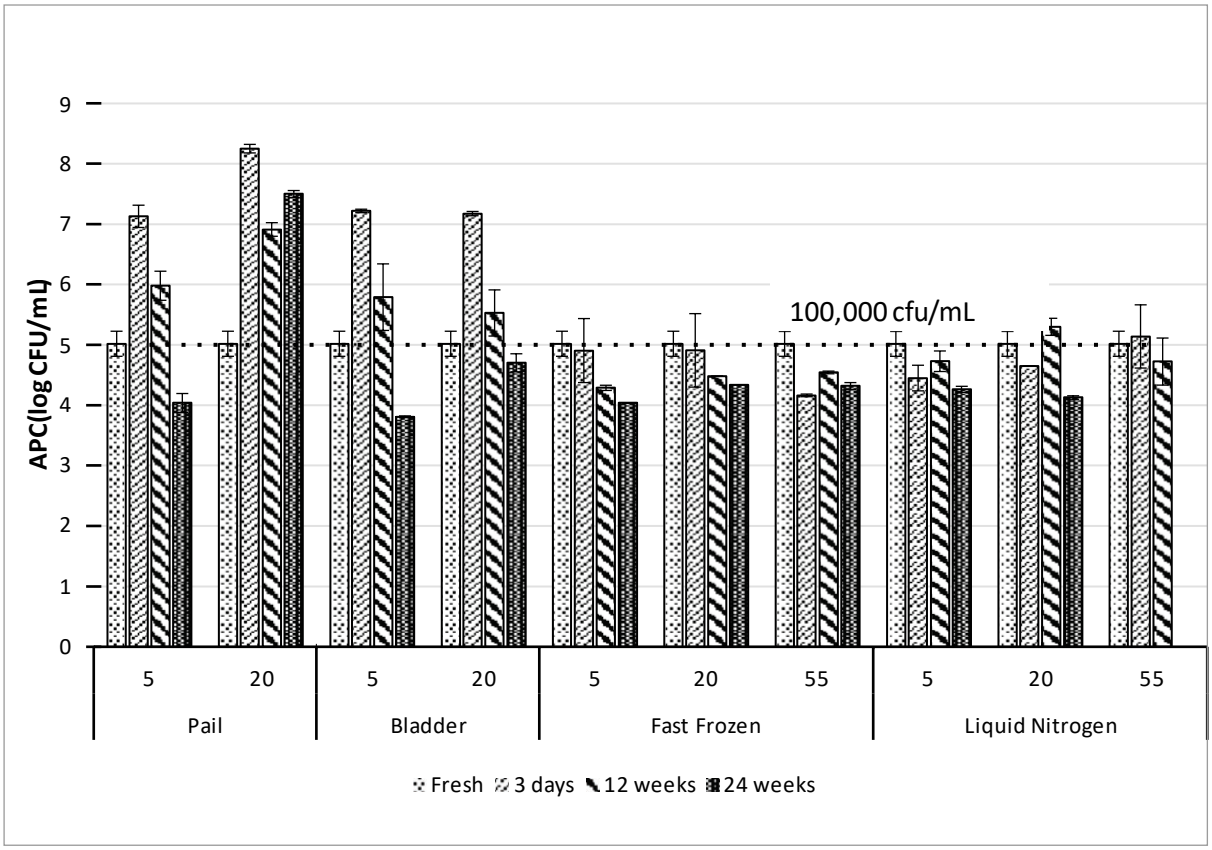

***Figure 3**: APC counts of late-season milk and stored frozen for up to 24 weeks. Error bars represent the standard deviation for triplicate subsamples.*

The APC counts of milk collected in late-season, frozen and thawed at different temperatures, is shown in Figure 3. APC counts of the fresh milk collected in late-season were higher than for milk collected in early-season (May) (5.01 log CFU/mL vs. 4.48 log CFU/mL).

For the milk collected in late-season, the largest increases in APC counts were seen in milk stored in pails and thawed at 20°C (increases of 1.90 to 3.24 log CFU/mL) or thawed at 5°C (increases of 0.99 to 2.11 log CFU/mL). Samples frozen in bladders showed increases of 0.52 to 2.21 log CFU/mL.

After 24 weeks of storage most samples showed a decrease in microbial count of 0.30 to 1.20 log CFU/mL, however the milk frozen in pails and thawed at 20°C showed an increase of 1.49 log CFU/mL.

In the late-season milk fast frozen in pellet form, the outgrowth during thawing seen with early-season milk did not occur.

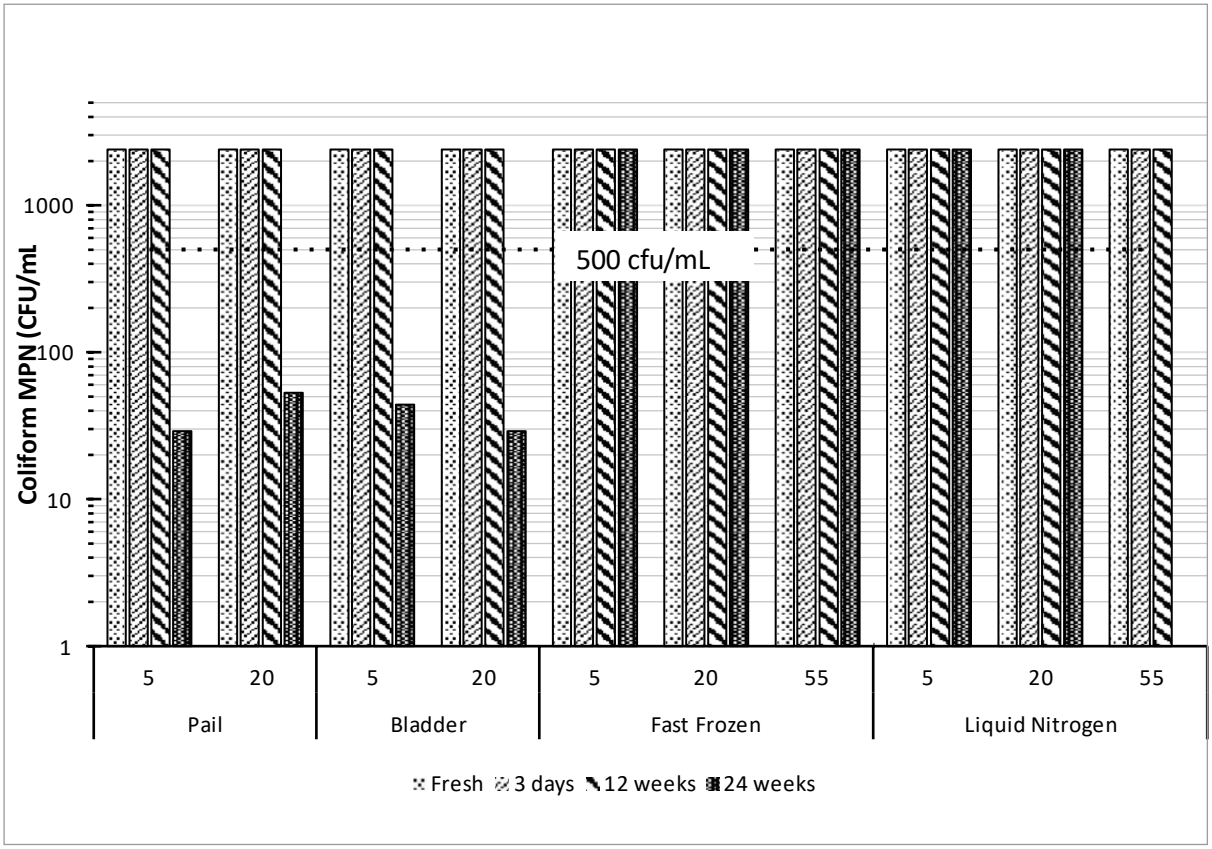

***Figure 4**: Coliform MPN counts (CFU/mL) of late-season milk, stored frozen for up to 24 weeks.*

Coliform counts were typically higher in the milk freshly collected in late-season than in early-season. Most samples, before or after frozen storage, showed coliform counts above the DPC action limit of 500 CFU/mL, as seen in Figure 4. The month of milk collection ($p<0.01$), thawing temperature ($p<0.01$) and storage method ($p=0.048$) all had significant effects on the measured coliform count.

Coliform counts generally decreased with increased storage time. These decreases may be a result of freezing stresses leading to a reduction in viable microbes. Decreases in APC are also seen in 75% of bladder and pail samples after 24 weeks of frozen storage. Beyond this, there was no apparent relationship between APC and coliform count, which seems to respond differently to freezing, holding and thawing treatments.

As the coliform count is an MPN measure, ">2400 CFU/mL" is the maximum estimate recorded and the actual counts are for those samples with a reading of >2400 CFU/mL are not known.

3.4 Effect of Freezing Method, Thawing Temp, and Storage Time

3.4.1 Effect on Microbial Quality.

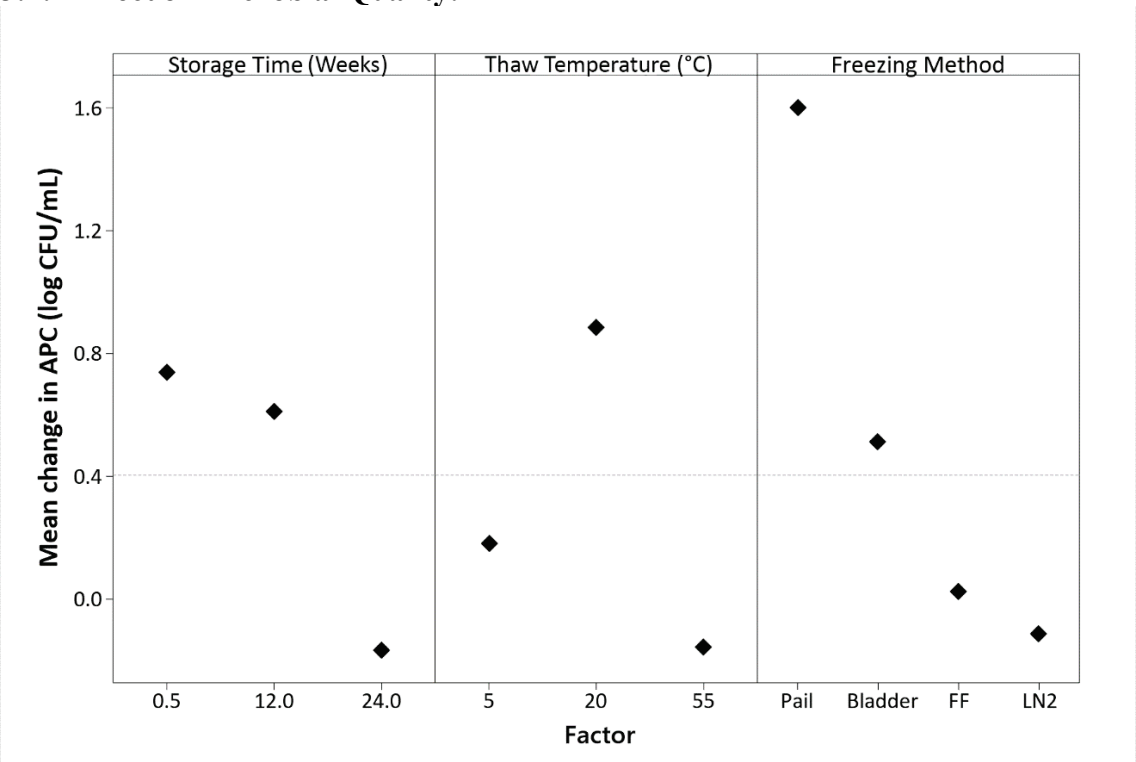

***Figure 5**: Main effect plots for the change in log APC from fresh to frozen and thawed milk.*

Regression analysis showed a significant effect of freezing method ($p<0.01$), thawing temperature ($p<0.01$) and storage time ($p<0.01$), on the change in APC in the thawed milk comparing with fresh. The month of collection was not shown to have a significant effect on the change in APC level ($p=0.066$). Figure 5 shows the effect on APC, as expressed in log CFU/mL, caused by freezing method, thawing temperature and storage time: the mean for each level of a factor is averaged across all other factors. APC counts increased after 3 days storage and 12 weeks storage and decreased by 24 weeks. The APC counts were low in response to thawing at 55°C and are higher when thawing at 5°C and 20°C. Freezing in pellets and LN2 led to minimal change in microbial count, whereas frozen storage in bladders led to an average increase of 0.45 log CFU/mL and frozen storage in pails led to an average increase of 1.6 log CFU/mL.

The change in APC in response to the interaction between thawing temperature and freezing method is shown in Figure 6. Divergence between the 5°C and 20°C line for the pail samples indicates that there is an interaction between the freezing format and thawing temperature, leading to greater increases in APC at a given thawing temperature for

samples frozen in pails.

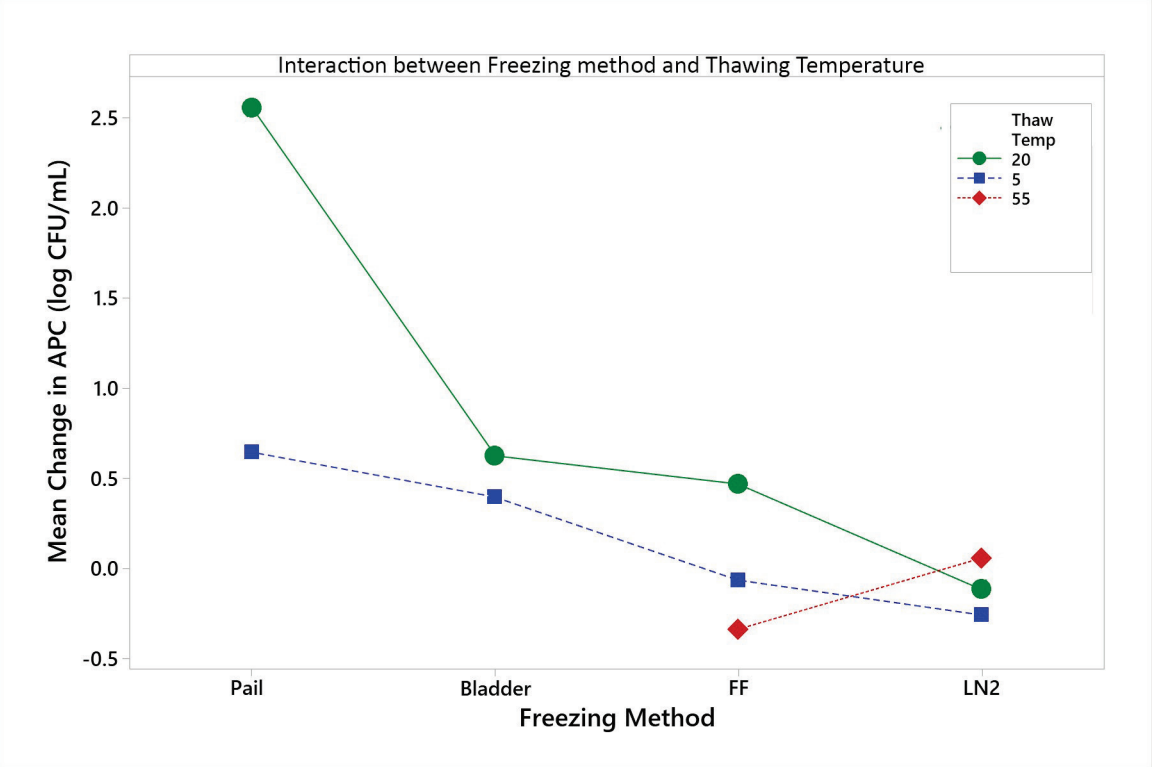

***Figure 6**: Response of APC to the interaction between freezing method and thawing temperature.*

3.4.2 Effects on Chemical composition of thawed milk.

The distributions of measured values of osmolality, protein, lactose and fat content, and pH are shown in Tables 3 & 4 and Figure 7. In Figures 6 and 7, freezing methods are presented generally in order of decreasing freezing and thawing time. Additional detail is provided in appendices to this report. The chemical composition of the milk collected in early-season (May) differs from that of milk collected in late-season (August). Due to this difference, statistical tests were carried out on the change relative to fresh, separately for different collection dates.

Analyses showed no significant effect of storage time, freezing method, or thawing temperature on the change in protein levels, pH, or TA between fresh milk and milk after frozen storage. The freezing method ($p=0.01$ for lactose, $p<0.01$ for osmolality) and month of milk collection ($p<0.01$ for lactose, $p<0.01$ for osmolality) both had significant effects on changes in lactose levels and osmolality of the thawed samples. A significant effect on the change in fat content after frozen storage was associated with the freezing method ($p=0.014$) and thawing temperature ($p<0.01$).

Milk	Freezing Method	Thawing Temperature (°C)	Storage time (weeks)	Fat (%)	Lactose (g/100ml)	Osmolality (mOsm/kg)	Free Fatty Acid (% m/v)	Titratable Acidity (% m/v)	Vit B2 (µg/100ml)	Protein (%)	Ph
Early	Fresh	Fresh	Fresh	3.66	3.8	300	0.008	0.155	268	5.44	6.8
Early	Pail	5	0.5	3.67	4.8	305	0.017	0.165	288	5.36	6.7
Early	Pail	20	0.5	3.66	4.45	343	0.017	0.163	296	5.36	6.6
Early	Bladder	5	0.5	3.68	4.6	306	0.017	0.158	285	5.39	6.7
Early	Bladder	20	0.5	3.65	4.6	287	0.019	0.163	288	5.36	6.7
Early	Fast frozen	5	0.5	3.27	4.4	297	0.017	0.155	288	5.36	6.7
Early	Fast frozen	20	0.5	3.49	4.3	301	0.017	0.155	293	5.23	6.8
Early	Fast frozen	55	0.5	3.43	4	269	0.017	0.14	271	4.91	6.8
Early	LN_2	5	0.5	3.44	5	304	0.021	0.155	289	5.3	6.8
Early	LN_2	20	0.5			310					6.7
Early	LN_2	55	0.5	4.06	4.5	310	0.017	0.16	283	5.36	6.8
Early	Pail	5	12	3.6	4.4	293	0.017	0.235	384	5.4	6.6
Early	Pail	20	12	3.6	4.5	320	0.025	0.845	304	5.4	5.4
Early	Bladder	5	12	3.7	4.3	296	0.021	0.2	289	5.2	6.5
Early	Bladder	20	12	3.5	4.3	291	0.021	0.205	283	5.2	6.6
Early	Fast frozen	5	12	3.1	4.1	270	0.021	0.31	278	5	6.5
Early	Fast frozen	20	12	3	4.5	304.8	0.076	0.655	297	5.4	6.4
Early	Fast frozen	55	12	3.5	4.5	288.3	0.025	0.215	297	5.2	6.5
Early	Pail	5	24	3.7	4.6	305	0.008	0.308	296	5.3	6.7
Early	Pail	20	24	3.6	4.4	306	0.017	0.875	286	5.4	6.7
Early	Bladder	5	24	4.8	4.3	305	0.008	0.298	304	6.3	6.7
Early	Bladder	20	24	5	5.4	366	0.004	0.433	344	6.9	6.7
Early	Fast frozen	5	24	3.7	4.7	317	0.013	0.792	279	5.4	6.7
Early	Fast frozen	20	24	3.1	4.7	321	0.008	0.93		5.6	6.6
Early	Fast frozen	55	24	4	4.9	362	0.013		277	5.8	6.7
Early	LN_2	5	24	3.6	4.6	302	0.008	0.89	254	6	6.7
Early	LN_2	20	24			296					6.7
Early	LN_2	55	24	3.7	4.7	308	0.013	0.92	295	5.3	6.7

Table 3: *Chemical compositions of early season milk*

Milk	Freezing Method	Thawing Temperature (°C)	Storage time (weeks)	Fat (%)	Lactose (g/100ml)	Osmolality (mOsm/kg)	Free Fatty Acid (% m/v)	Titratable Acidity (% m/v)	Vit B2 (µg/100ml)	Protein (%)	Ph
Late	Fresh	Fresh	Fresh	6.16	4.03	352	0.064	0.315	378	7.08	6.55
Late	Fresh	Fresh	Fresh	7.09	3.59		0.042	0.22	428	7.44	6.7
Late	Pail	5	0.5	6.3	3.9	286	0.025	0.423	381	6.9	6.5
Late	Pail	20	0.5	6.2	3.7	354	0.055	9.1	381	6.9	5.4
Late	Bladder	5	0.5	6.1	4	281	0.025	0.345	382	6.9	6.4
Late	Bladder	20	0.5	6.2	3.8	302	0.03	0.373	385	6.9	6.3
Late	Fast frozen	5	0.5	7.4	3.4	273	0.021	0.37	419	7.3	6.6
Late	Fast frozen	20	0.5	6.7	3.7	274	0.093	0.41	417	7.1	6.3
Late	Fast frozen	55	0.5	7.1	4.1	306.5	0.034	0.3	439	7.5	6.6
Late	LN_2	5	0.5	6.1	4	290.8	0.03	0.295	383	7.3	6.5
Late	LN_2	20	0.5			274					6.5
Late	LN_2	55	0.5	6.3	4	321.8	0.081	0.39	379	7	6.4
Late	Pail	5	12	6.3	4.1	275	0.008	0.88	355	6.8	6.7
Late	Pail	20	12	6.3	4.3	313	0.013	1	356	7	6.5
Late	Bladder	5	12	6.7	4.3	258	0.008	0.78	344.35	6.6	6.6
Late	Bladder	20	12	6	3.5	248	0.008	0.775	300.19	5.9	6.7
Late	Fast frozen	5	12	7.2	3.9	288	0.013	1.18	376.06	7.3	6.7
Late	Fast frozen	20	12	6.1	3.3	231	0.008	1.08	350.13	6.4	6.8
Late	Fast frozen	55	12	8.7	4.9	354	0.008	0.945	492.38	9.4	6.7
Late	LN_2	5	12			286					6.7
Late	LN_2	20	12			273					
Late	LN_2	55	12			291					6.7
Late	Pail	5	24	6	4.2	294	0.019	0.463	394	6.9	6.6
Late	Pail	20	24	6.1	4.2	347	0.021	1.003	382	6.9	5.6
Late	Bladder	5	24	6.1	4	291	0.013	0.483	373	6.9	6.6
Late	Bladder	20	24	6.1	4.2	293	0.017	0.733	381	6.8	6.6
Late	Fast frozen	5	24	6.8	4.2	286	0.017	0.425	425	7.3	6.7
Late	Fast frozen	20	24	7	3.8	297	0.017	0.455	428	7.6	6.6
Late	Fast frozen	55	24	6.7	3.7	293	0.025	0.698	415	7.3	6.7
Late	LN_2	5	24	6.1	3.7	299	0.025	0.93	390	6.9	6.6
Late	LN_2	20	24			297					6.6

***Table 4**: Chemical Compositions of late season milk*

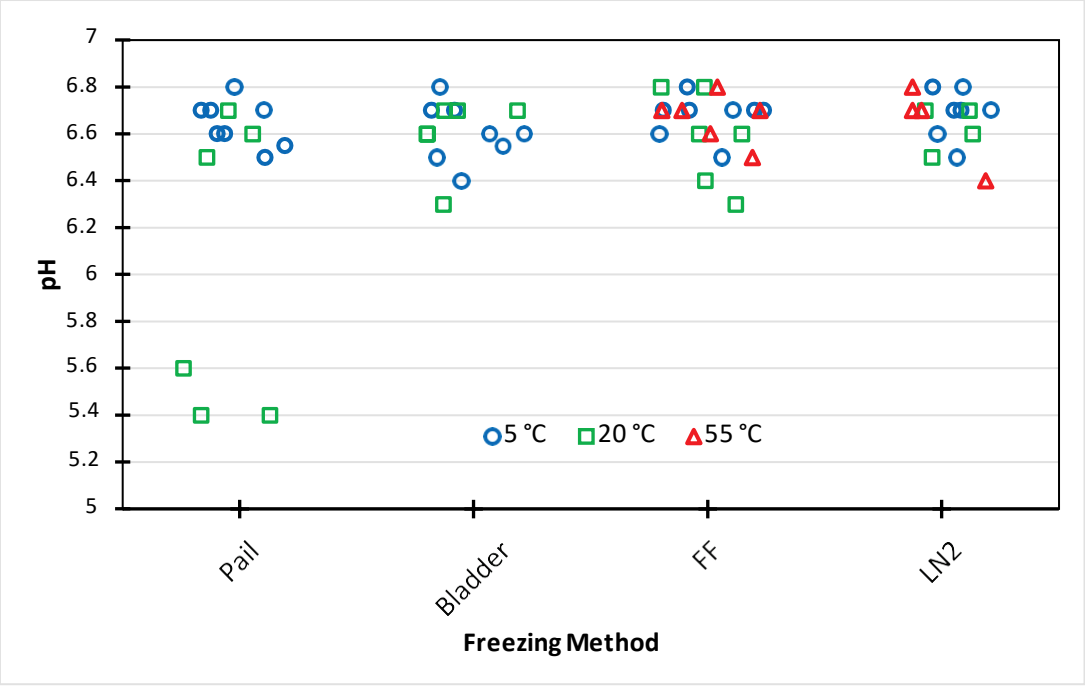

***Figure 7**: pH values after thawing for all milk collections and storage times.*

There was a significant relationship between the milk collection date (May or August) and the change in FFA (early-season (May) samples showed a small increase, late-season (August) samples showed a small decrease, p=0.001). There was a significant relationship between the change in FFA and storage time (greater decreases in FFA seen with longer storage times, p=0.003). The freezing method has a significant effect on changes in FFA (p=0.04), with bladders and pail showing small decreases in FFA, and no change being associated with FF or LN2 samples.

Analyses showed a significant relationship between the change in level of Vitamin B2 (p=0.001) and the milk collection date, but no relationship between level of Vitamin B2 and the thawing temperature, storage time or freezing method. The milk collected in late-season (August) had a greater concentration of Vitamin B2, and these samples showed an average decrease in Vitamin B2 of 14 µg/250mL after frozen storage. Milk collected in early-season (May) showed an increase in Vitamin B2 of 26 µg/250mL after frozen storage. It is not known whether this is representative of a change in analyte matrix between May and August which affects the measurement of Vitamin B2. Triplicate measurements of the Vitamin B2 levels in fresh milk in early-season (May) had a small standard deviation (0.6 µg/250mL), so this lower measurement is not likely to be random variation. For the selected samples where the SCC was measured, there was significant variability in the SCC measurements after frozen storage. There was no consistent relationship between the speed of freezing and thawing and SCC, nor between storage temperature or thawing temperature and SCC. There was a significant negative correlation between the storage time and SCC (p=0.044), however R^2 for this relationship was low (0.047). Due to the variability of response to freezing, SCC should not be

used as an indicator of the quality of frozen milk. There was no significant difference (p=0.770) between the SCC values observed after thawing for each treatment.

The radar plots shown in Figure 8 and Figure 9 illustrate several of the changes occurring after frozen storage and freezing, with the "best-case" and "worst-case" observed results compared to the fresh milk for each collection. The best-case results were selected from samples which had similar microbial counts to the fresh samples. The worst-case results were selected as the samples which showed greatest increases in microbial counts.

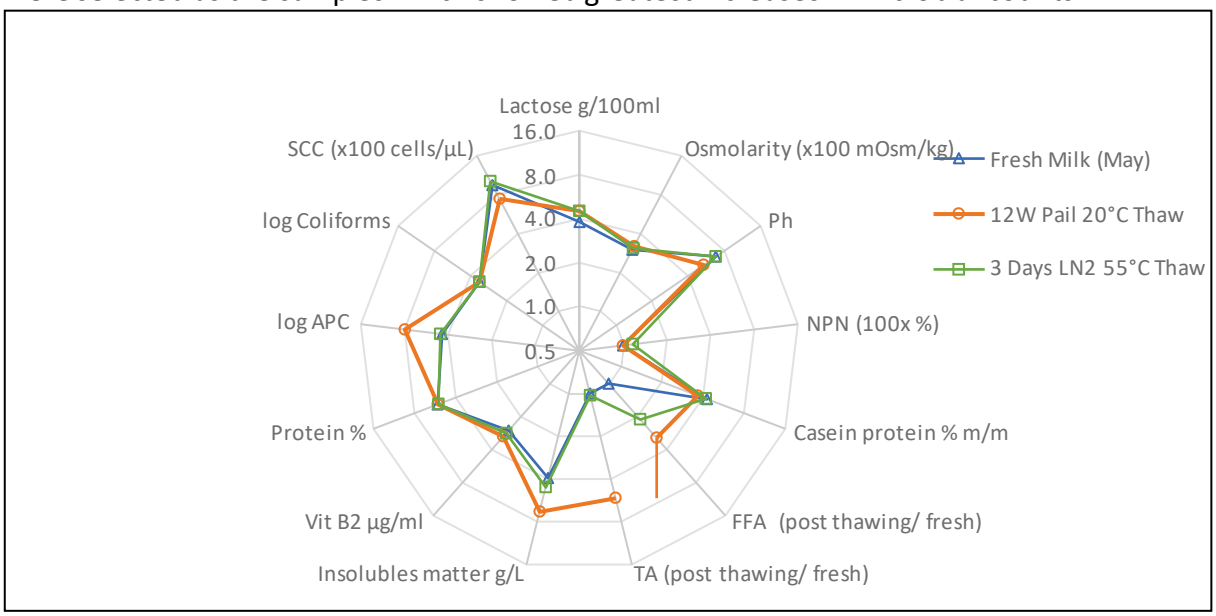

***Figure 8**: Changes in several measures for early-season milk.*

The "Best Case" results of milk frozen in LN2, stored for 3 days, and thawed at 55°C, are compared in Figure 8, with the results from milk stored for 12 weeks in a pail and then thawed at 20°C.

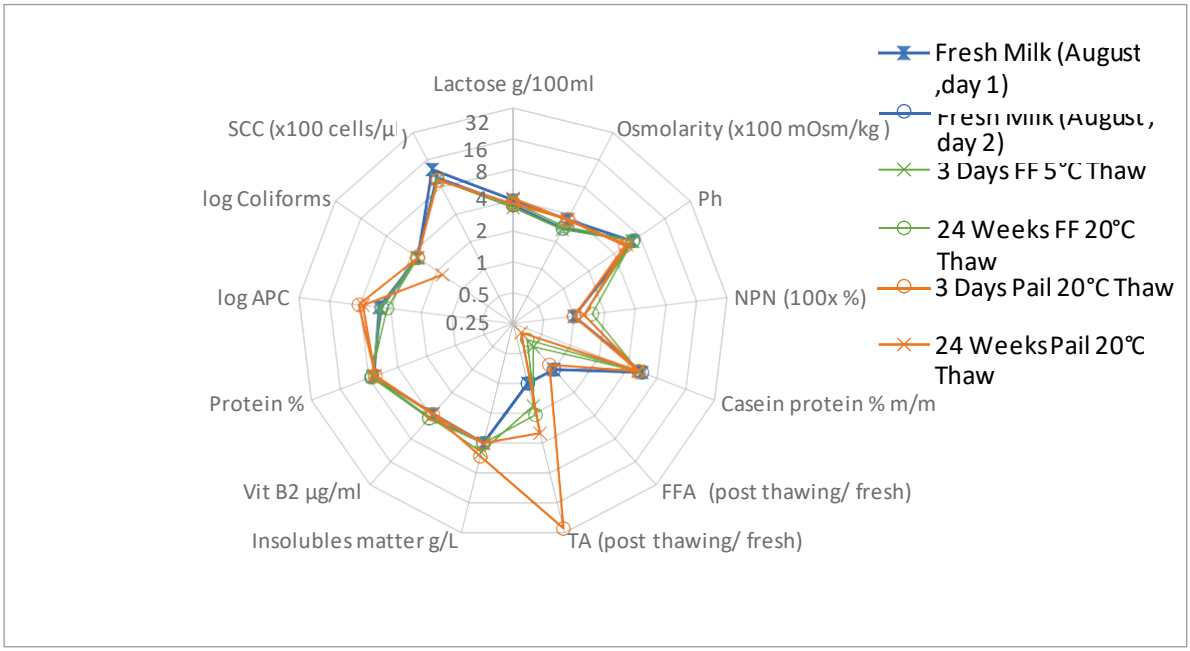

***Figure 9**: Changes in several measures for late-season milk.*

Figure 9 shows the "Best Case" results of milk frozen using the Fast Freezing (FF) technology, stored for 3 days, or 24 weeks and thawed at 5°C and 20°C, and compares this with the results from milk stored for 3 days or 12 weeks in a pail and then thawed at 20°C.

4. Discussion

In this study, TA, FFA, pH and microbial quality differed between the milk collected in early- and late-season. In general, the fresh late-season milk had higher solids levels. Higher solids coupled with lower production volumes is typical of late season ovine and bovine milk (Haenlein & Wendorff, 2006). The fresh late-season milk also was of lower quality as indicated by FFA (0.063% vs. 0.008% for early-season), TA (0.27 % vs. 0.16 % for early-season) and microbiological results (APC 1.2 $x10^5$ CFU/ml vs. 3.1 $x10^4$ CFU/mL early-season). The higher microbial counts may be linked to variations in farm conditions and practices at different collection times.

Ten litre pails and bladders were selected as freezing formats to reflect typical practice in industry. Liquid milk is placed chilled in these large containers and placed in a storage freezer where much of the container surface area is exposed to moving air and some is not. Fast freezing is a technique developed specifically for raw sheep milk under the Food Industry Enabling Technologies (FIET) research programme at Massey University. This method forms the frozen milk into short cylindrical pellets, cooling the bulk of the milk from its initial chill temperature to near its final storage temperatures in under 200 seconds. Heat migrates a small distance and is removed through a metal surface to flowing refrigerant. As a baseline control on the whole experiment, the final method was to freeze milk dropwise in liquid

nitrogen to determine whether it is possible to avoid the deterioration of milk when freezing under technically optimum conditions. In this cryogenic method, heat flows only 1-2 mm to the surface of a chilled milk droplet with heat transfer driven by a temperature difference of nearly 200 Kelvin facilitated by vigorously boiling liquid.

The freezing format has a major effect on ease of thawing. Small droplets and pellets have far greater surface-area-to-volume ratio than pails or bladders, as well as smaller characteristic distances for heat transfer. Freezing methods which form small frozen particles inherently offer a faster freezing and thawing rate. During thawing, the thawed liquid portion is poised part way between the applied thawing temperature (e.g. 20°C) and the freezing point (~-0.5°C). The temperature/time profile experienced by liquid thawed from a pellet will be measured in minutes, compared to days for a 10 L pail or bladder. The smaller the format used for freezing, the less time the thawed liquid will spend in a temperature range where microbial growth can occur.

Also contributing to ease of thawing is the heat transfer coefficient applying when heat is removed from the solid (pail, plastic bag, pellet or droplet surface) to its warming fluid (air for pails and bladders or melted milk for pellets or droplets). Heat transfer coefficients for gently moving air are likely to be a full order of magnitude less than those for stirred milk.

Calculated thawing times (Cleland et al 1987; Cleland et al 1986) for 10kg pails in still air at 20°C are approximately 64 hours, which corresponds well to the observed thawing time of approximately 72 hours for pails in still air at 20°C, and approximately 90 hours for pails in still air at 5°C. This long thawing time may allow growth of psychrotrophic bacteria, which can lead to spoilage of the milk. This has been observed in the sheep milk context in other studies simulating small farm storage (Tribst et al 2019).

The frozen storage temperatures (-18°C and below -60°C) were both low enough to ensure that quality is generally maintained for the storage period of up to 24 weeks. These storage temperatures have been found to be suitable by others, with minimal changes during freezing found to occur in Reverse Osmosis concentrated milks (Voutsinas, et al. 1996a, 1996b). Good quality has also been reported in milk stored below -20°C (Koschak et al 1981; Wendorff, 2001; Young, 1987; Wendorff & Kalit, 2017). Studies that involved cheesemaking from frozen ovine milk report minimal changes in composition other than to fat, when stored below -15°C for up to 6 months (Zhang et al, 2006). Van Den Berg (1966) reports a minimal change in pH as a result of freezing and frozen storage. The large changes in pH seen in some samples in this study are most likely due to the thawing process and microbial growth during this thawing process.

Some samples, in this study, showed decreases in microbial count after frozen storage. A similar phenomenon has been observed by other authors who have reported reduced bacterial counts in ovine milk after 2, 4 and 6 months of storage at -25°C (Katsiari et al 2002). These authors reported an average decrease in microbial count after 6 months, of 0.4 log CFU/ml, which is a similar magnitude to the decrease seen in many samples in this study. These authors however froze milk in 1.2 kg aliquots with a thickness of 2.7 cm, and thawed in water baths at 40°C, which would imply relatively rapid thawing; this may have minimised bacterial growth during thawing in their samples. The pails and bladders used in the current

study required substantially longer thawing periods, which may have contributed to the significant increases in microbial counts observed.

The thawing method and the freezing method both had significant effects (p=0.028 for thawing method, p<0.001 for freezing method) on the observed microbial quality of the milk. But no impact of frozen storage duration on the microbial levels or pH of milk was observed in the study. Large changes in APC occurred for all storage times in pails thawed at 20°C, and in 33% of pails thawed at 5°C, 33% of bladders thawed at 20°C and 66% of bladders thawed at 5°C and one fast frozen sample thawed at 20°C. Elaboration of lactic acid might be expected where APC counts became high; pH would be expected to drop. A scatterplot of measured pH vs. APC is shown in Figure 10. Data are grouped by freezing method and thawing temperature. As expected, the lowest measured pH values were observed in samples of milk frozen in pails and thawed at 20°C. High APC values were also observed in bladders and pails thawed at 5°C and 20°C and one FF sample thawed at 20°C.

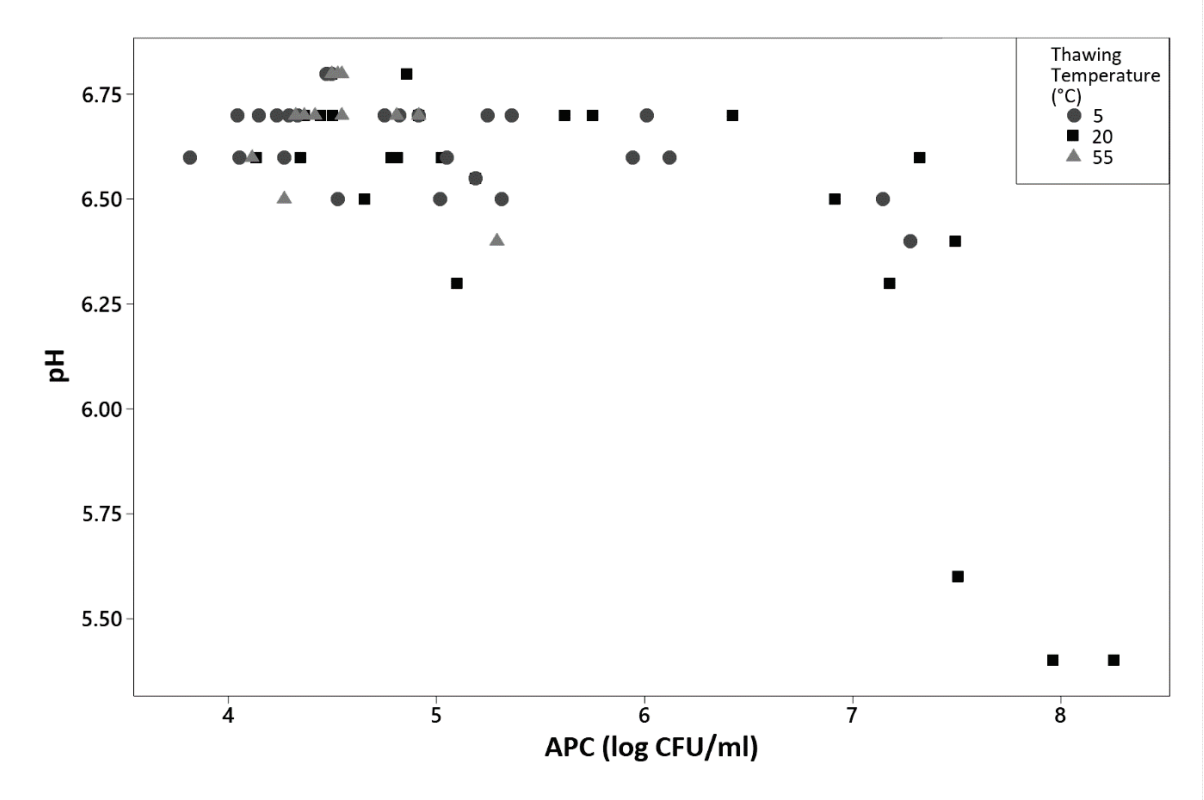

***Figure 10**: Scatterplot of measured pH vs. the APC observed*

Changes in TA and FFA are also probably due to bacterial growth and will be deleterious to product quality. Previous publications (Tribst et al, 2018) have reported reduced culture growth lag phases when making yogurt from frozen ovine milk and have suggested that this is due to the presence of exposed lipids due to ice crystal damage. It is possible that adverse levels of microbial growth occurred in pails due to the extended freezing process.

Extensive damage can occur in milk that has been frozen slowly and stored at temperatures >-15°C, including gelation of milk on thawing (authors' experience with milk stored at -10°C), changes in composition (Needs, 1992), off-flavour generation (Muir, 1984), and degradation and destabilisation of milk proteins (Wendorff, 2001; Wendorff, 2002). No organoleptic changes were measured during this study, however it is likely that the samples which showed high TA, and FFA and low pH would display rancid or other "off" flavours as these indicators have been linked to rancid and "off" flavour development (Duncan et al, 1991). The storage temperatures in this study were specifically chosen to avoid the worst damage to the milk, as it was thought that damage at higher temperatures was well established, and so little would be gained by studying higher storage temperatures.

5. Conclusions

Freezing and storing, then thawing under appropriate industrially practical conditions can maintain ovine milk quality for at least 24 weeks, the longest duration tested.

Frozen storage at or below -18°C can result in comparable quality to the best-case frozen scenario (cryogenically frozen dropwise and stored below -60°C).

For both early and late season milk, the quality after frozen storage for up to 24 weeks (the longest period tested) can be comparable with fresh milk for the same stage of the season.

However, the freezing format and the thawing process have a significant effect on the microbial quality of milk and related to microbial activity qualities: FFA, TA and pH. Careful design and control of the freezing and thawing process is essential to maintain milk quality.

Milk should be frozen as rapidly as possible to minimise separation of components, and growth of psychrotrophic bacteria.

Size and shape of the frozen milk pieces is a major determinant of thawing time. Milk frozen as single blocks inside 10 L bladders or 10 L pails will require days to thaw. Slow thawing such as this can lead to unacceptable levels of bacterial growth, with over 1 log cfu/ml increases observed in some samples after freezing and storage.

Room temperature should not be used to thaw frozen milk. Even thawing at 5°C should be conducted rapidly enough to prevent growth of psychrotrophic bacteria. Frozen milk in pellet form thaws most rapidly and is almost able to tolerate thawing at 20°C. The most assured method of thawing, of those trialled, is to start with frozen pellets and thaw at 5°C in liquid milk.

The majority of samples did not show compositional changes linked to the storage period. However, there were some bladder stored samples which showed various changes to gross composition after 12 and 24 weeks. These changes may have been a result of separation of milk components during the storage period.

A method of frozen storage that ensures milk remains below -18°C coupled with a method of rapid thawing which maintains the temperature at or below 5°C should maintain microbial quality for storage periods at least up to the 24 weeks used in the current study.

The testing of ovine milk is reported here, however since the known mechanisms for degradation of ovine and bovine milk are similar, the broad conclusions of this study are expected to apply to bovine milk also.

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7. APPENDIX A: Detailed measurements

This appendix tabulates measured results that are also summarised in the report text.

7.1 Freezer temperature logs

Temperature logs for the -18°C freezer and the -25°C freezer are shown in Figure 11. Due to a change in record-keeping in mid-2019, there is a period when data were recorded less frequently (1x per day vs every 5 minutes). Figure 11 shows the temperature log data from the storage freezers during the storage of frozen concentrated milk. "Freezer 2" was set at 18°C and "Freezer 1" was set at -25°C. Table 5 summarises the durations for a range of recorded temperatures.

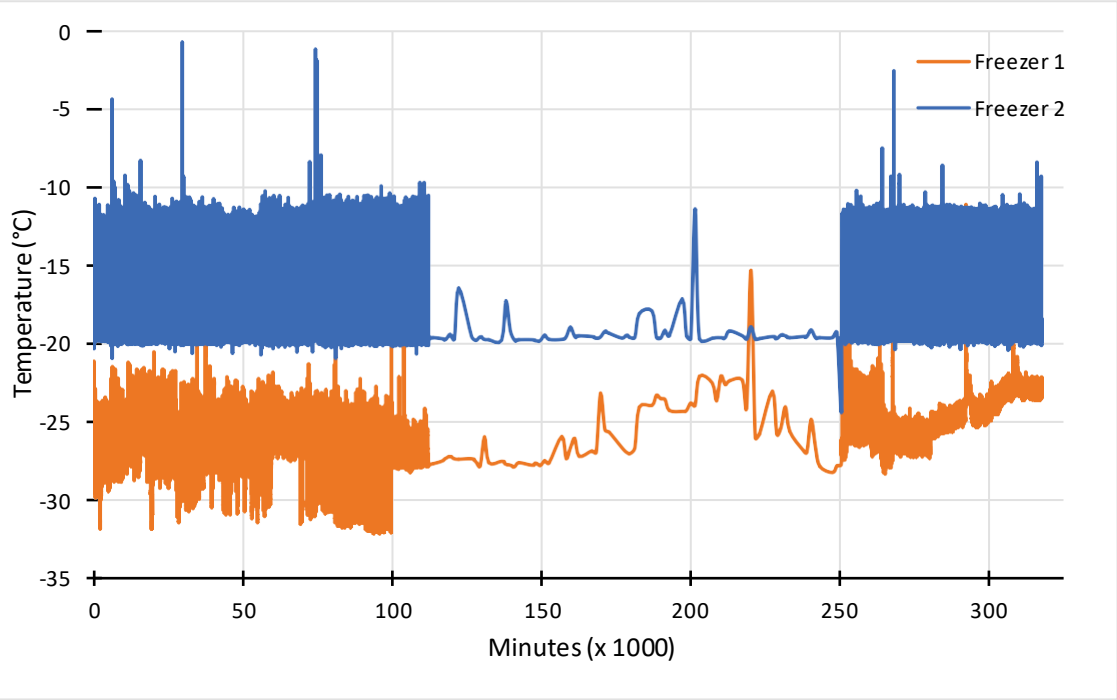

***Figure 11**: Temperature log data from the storage.*

FREEZER 1			FREEZER 2		
TEMP	**Total time over (min)**	**% of total time**	**TEMP**	**Total time over (min)**	**% of total time**
0	0	0.0	0	0	0.0
-5	0	0.0	-5	95	0.0
-10	0	0.0	-10	291	0.1
-15	75	0.0	-15	10036	3.6
-18	1603	0.5	-18	22684	8.1
-20	1869	0.6	-20	276787	99
-25	126382	38	-25	280195	100
-30	317025	96	-30	280195	100

-35	332445	100	-35	280195	100

Table 5: *Cumulative fraction of time the freezers were above a given temperature*

7.2 Fresh milk data

Tables 6 to 11 record fresh milk properties, that are summarised in the report text.

Sample Name	**Aerobic Plate Count** cfu/mL	**Coliforms**
		Total MPN/10mL
Sheep milk (May)	2.4×10^4	>2400
Sheep milk (May)	4.4×10^4	>2400
Sheep milk (May)	2.6×10^4	1100

Table 6: *Microbiology results for milk collected in early-season (May)*

Sample	**Total solids %**	**Ash %**	**Nitrogen %**	**Fat %**	**Lactose g/100ml**	**Osmolality mOsm/kg**	**pH**	**NPN %**
Sheep milk (May)	15.10	0.91	0.86	3.66	3.68	299	6.8	0.06
	15.06	0.93	0.85	3.65	3.85	300	6.8	0.05
	15.03	0.92	0.85	3.67	3.87	300	6.8	0.06

Table 7: *Chemical composition of milk collected in early-season (May).*

Sample	**Casein Protein %**	**Free FA % m/v**	**TA % m/v**	**Vitamin B2 ug/100ml**	**Protein %**
Sheep milk (May)	4.326	0.008	0.155	268.00	5.49
	4.275	0.008	0.155	269.00	5.42
	4.262	0.008	0.160	268.00	5.42

Table 8*: Chemical composition of milk collected in early-season (May)*

Sample Name	**Aerobic Plate Count** cfu/mL	**Coliforms**
		Total MPN/10mL
Sheep milk (August) Day 1	2.6×10^5	>2400
Sheep milk (August) Day 1	9.1×10^4	>2400
Sheep milk (August) Day 1	1.1×10^5	>2400

Sheep milk (August) Day 2	6.1 x 10^4	>2400
Sheep milk (August) Day 2	8.8 x 10^4	>2400
Sheep milk (August) Day 2	9.6 x 10^4	>2400

***Table 9**:Microbiology results for milk collected in late-season (August)*

Sample	Total solids %	Ash %	Nitrogen %	Fat %	Lactose g/100ml	Osmo mOsm/kg	pH	NPN %
Sheep milk (August) Day 1	18.61	0.99	1.11	6.11	4.05	354	6.54	0.04
	18.62	1.00	1.11	6.23	4.03	356	6.56	-
	18.58	0.98	1.11	6.14	4.02	348	6.55	-
Sheep milk (August) Day 2	19.50	1.02	1.16	7.24	3.72	332	6.69	0.05
	19.38	1.03	1.17	7.06	3.44	332	6.70	0.05
	19.43	1.03	1.17	6.96	3.60	333	6.70	-

***Table 10**: Chemical composition of milk collected in late-season (August)*

Sample	Casein Protein %	Free FA % m/v	TA % m/v	Vit B2 ug/100ml	Protein %
Sheep milk (August) Day 1	5.602	0.064	0.310	378	7.08
	5.589	0.064	0.315	378	7.08
	5.614	0.064	0.320	379	7.08
Sheep milk (August) Day 2	5.576	0.042	0.220	426	7.40
	5.576	0.042	0.220	428	7.46
	5.583	0.042	0.225	429	7.46

***Table 11**: Chemical composition of milk collected in late-season (August)*

7.3 Frozen milk data

For completeness, the following figures provide frozen milk data that are summarised in the text:

- Figure 12 reports osmolality distribution for all milk samples stored frozen. Distributions are identified by month of collection. There is no significant difference between the mean values of the two distributions.
- Figure 13 reports protein content distribution for milk samples stored frozen. Distributions are identified by month of collection.
- Figure 14 reports Lactose content distribution for milk samples stored frozen. Distributions are identified by month of collection.
- Figure 15 reports the frequency of fat content distributions for milk samples stored frozen. Distributions are identified by month of collection.

- Figure 16 reports Free fatty acid levels vs. storage time, for each combination of freezing method and thawing temperature. All milk collections are pooled.
- Figure 17 reports pH vs. storage time, for each combination of freezing method and thawing temperature. All milk collections are pooled.
- Figure 18 reports titratable acidity levels vs. storage time, for each combination of freezing method and thawing temperature. All milk collections are pooled.
- Figure 19 reports vitamin B2 levels vs. storage time, for each combination of freezing method and thawing temperature. All milk collections are pooled.
- Figure 20 reports main effect plot for median particle size as effected by the freezing and storage method.
- Figure 21 reports details of insoluble matter in samples up to 24 weeks of storage. Insoluble matter determined as the mass of dried sediment after centrifugation at 3000g for 10 minutes per 250mL of sample.

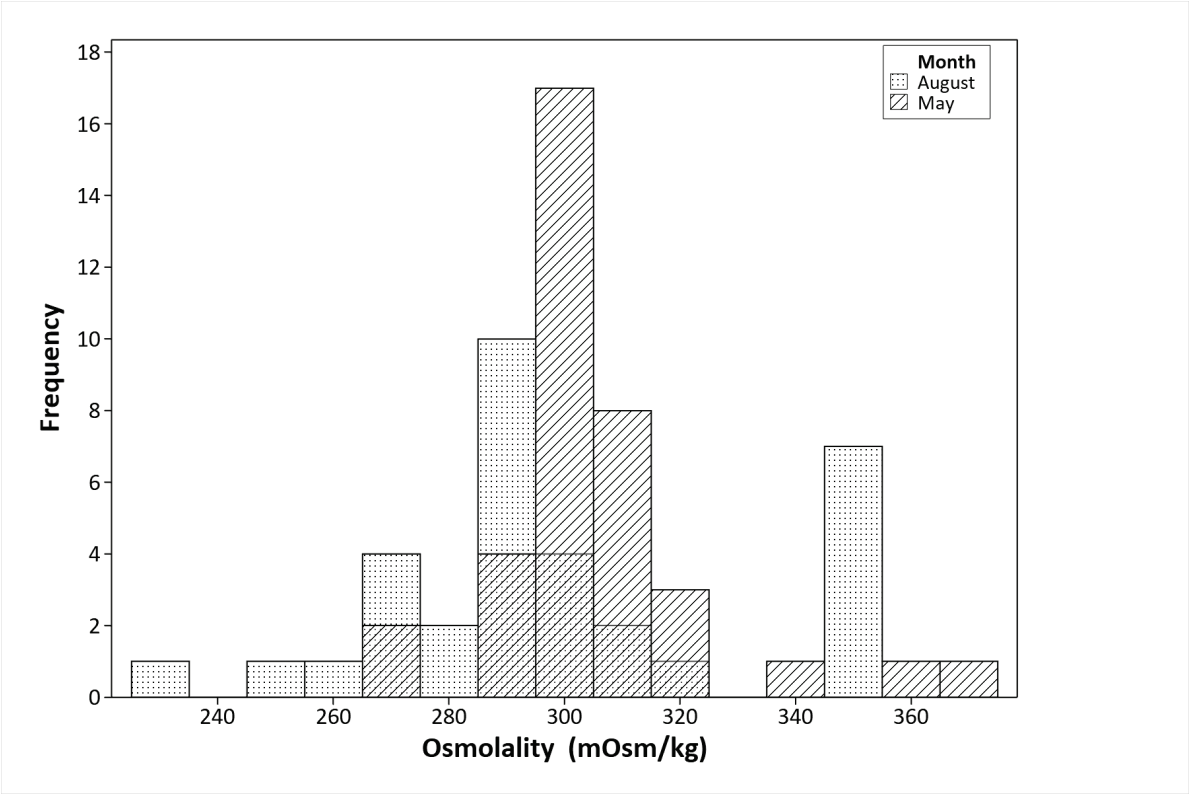

***Figure 12**: Osmolality distribution for all milk samples stored frozen.*

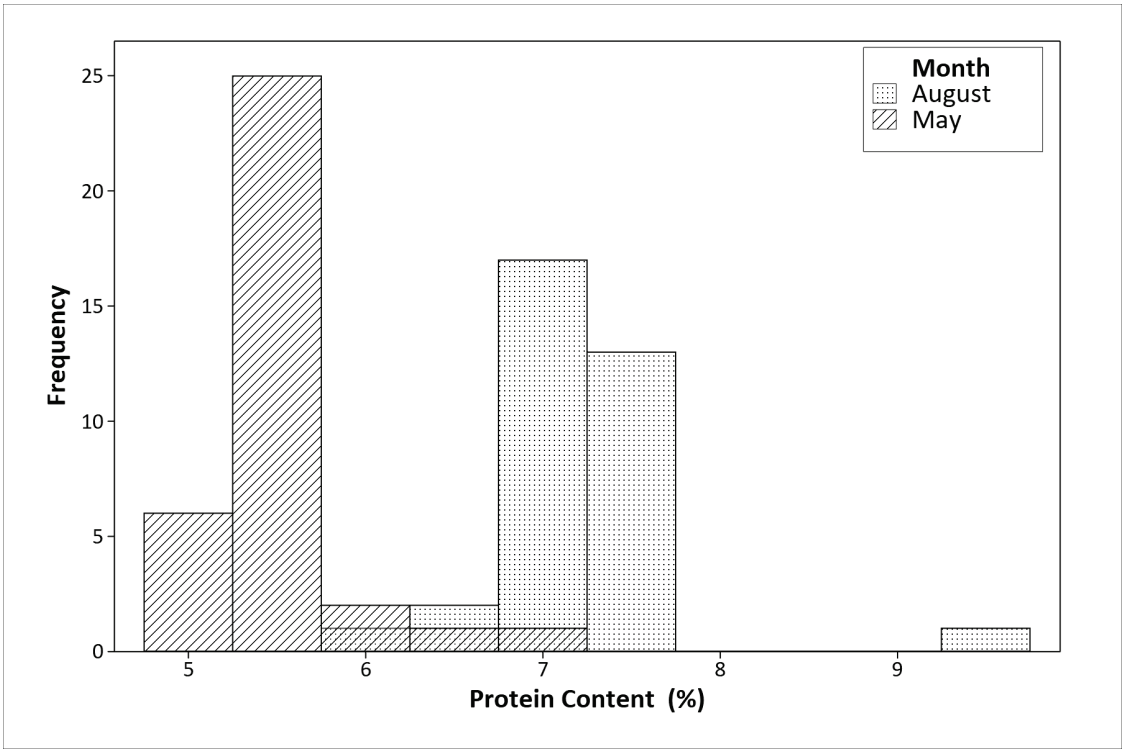

***Figure 13**: Protein content distribution for milk samples stored frozen.*

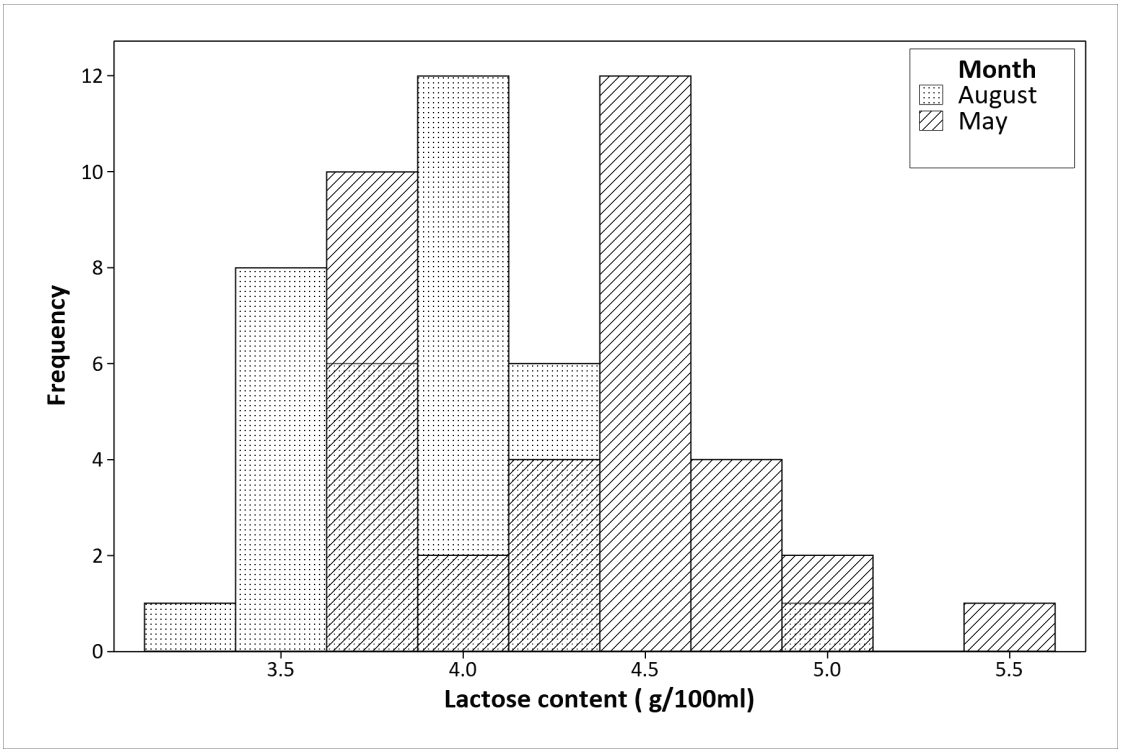

Figure 14: *Lactose content distribution for milk samples stored frozen. Distributions are identified by month of collection.*

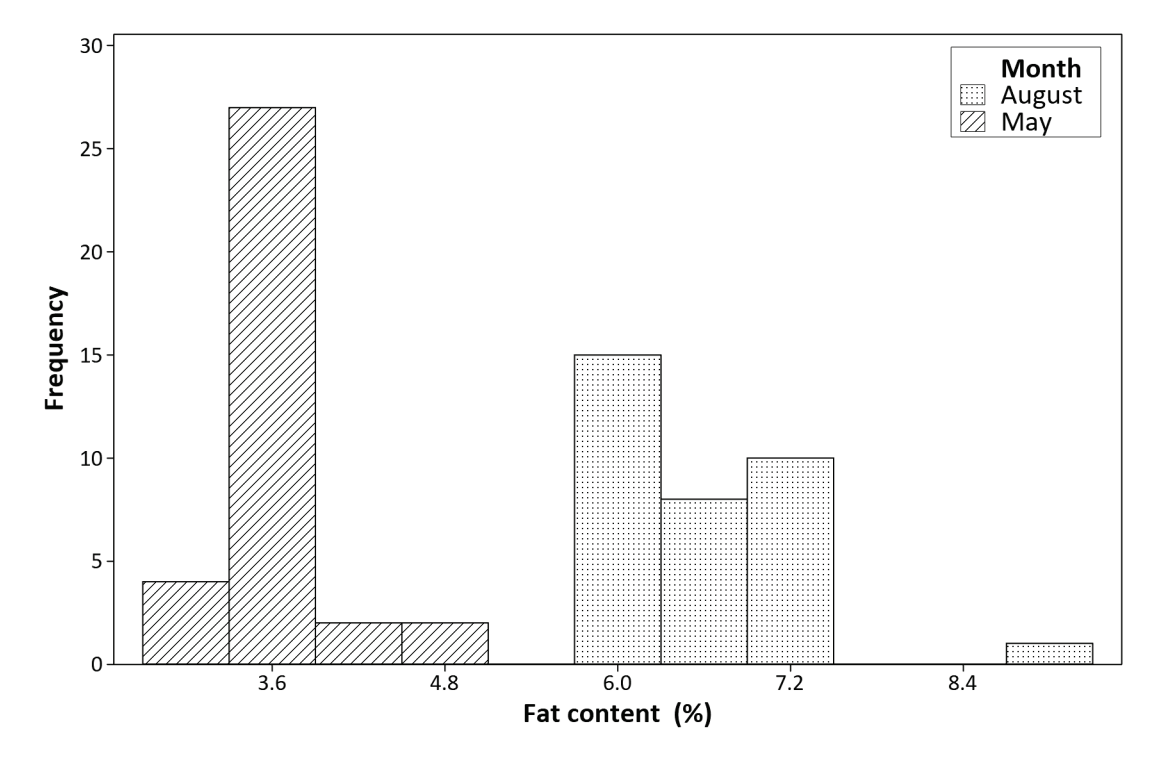

Figure 15: *Frequency of fat content distributions for milk samples stored frozen.*

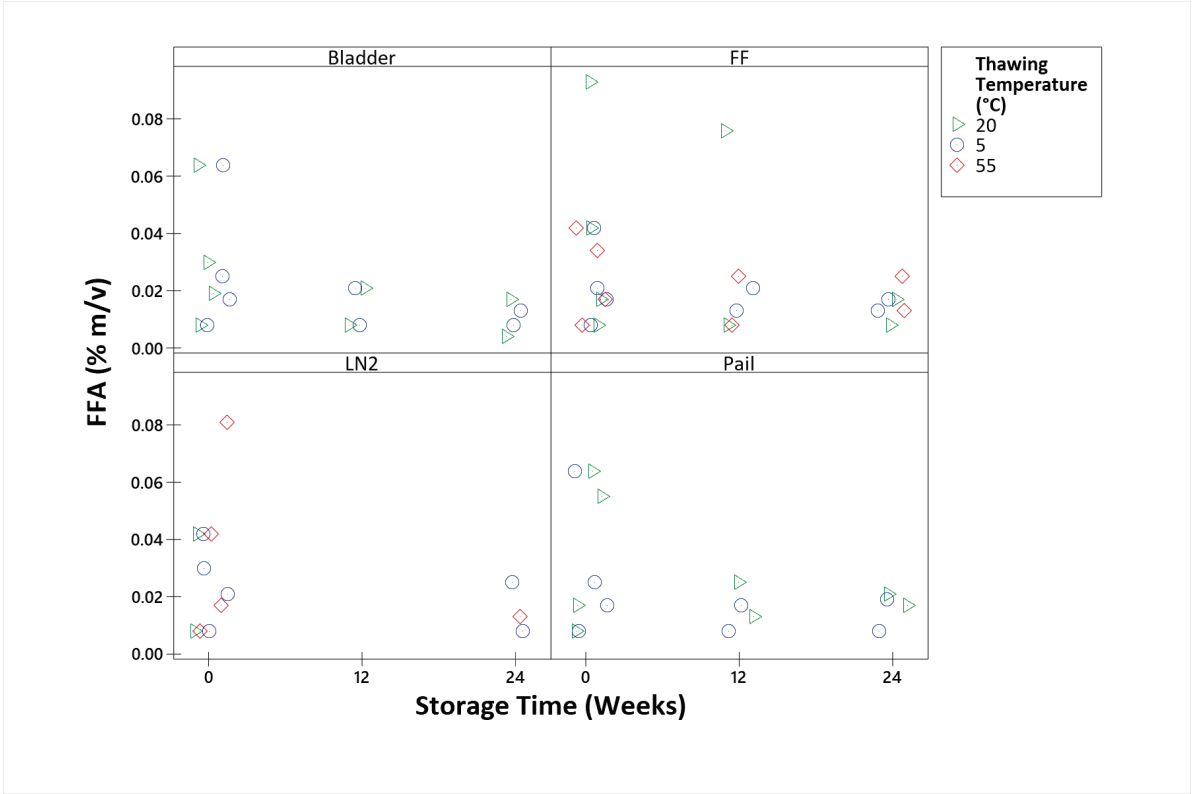

***Figure 16**: Free fatty acid levels vs. storage time.*

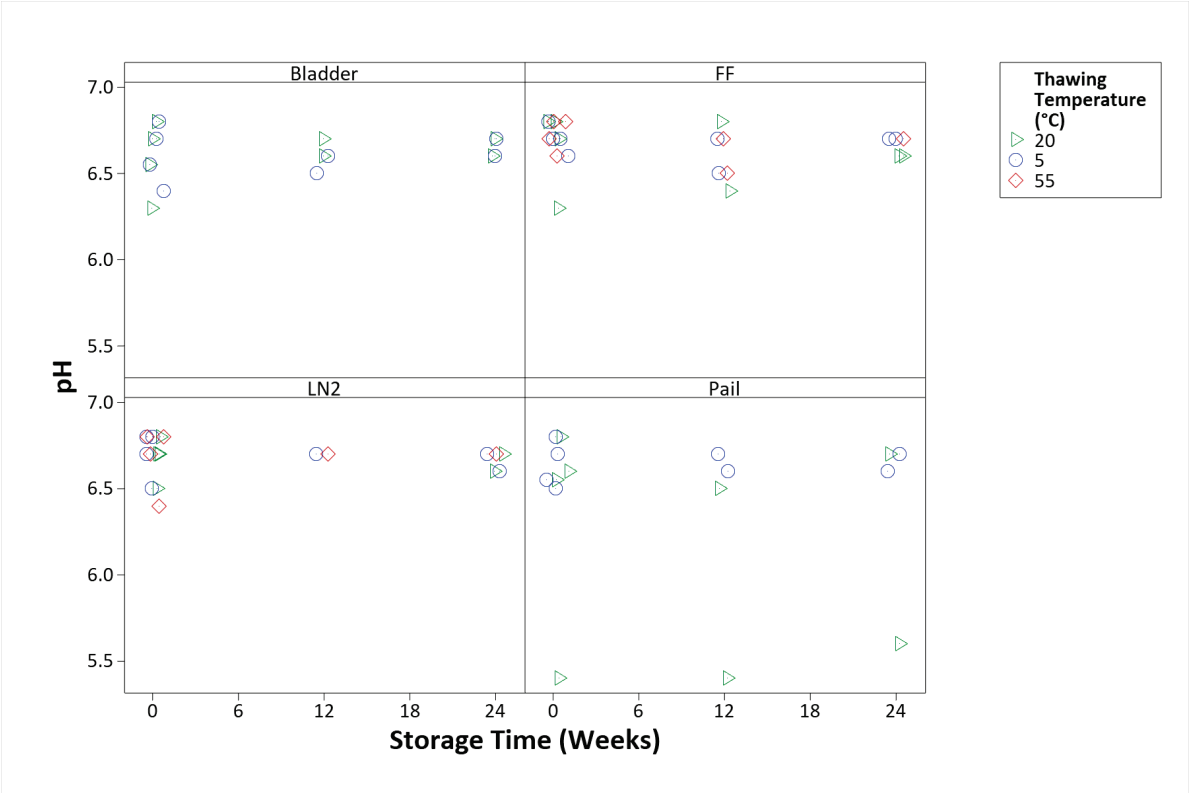

Figure 17: *pH vs. storage time.*

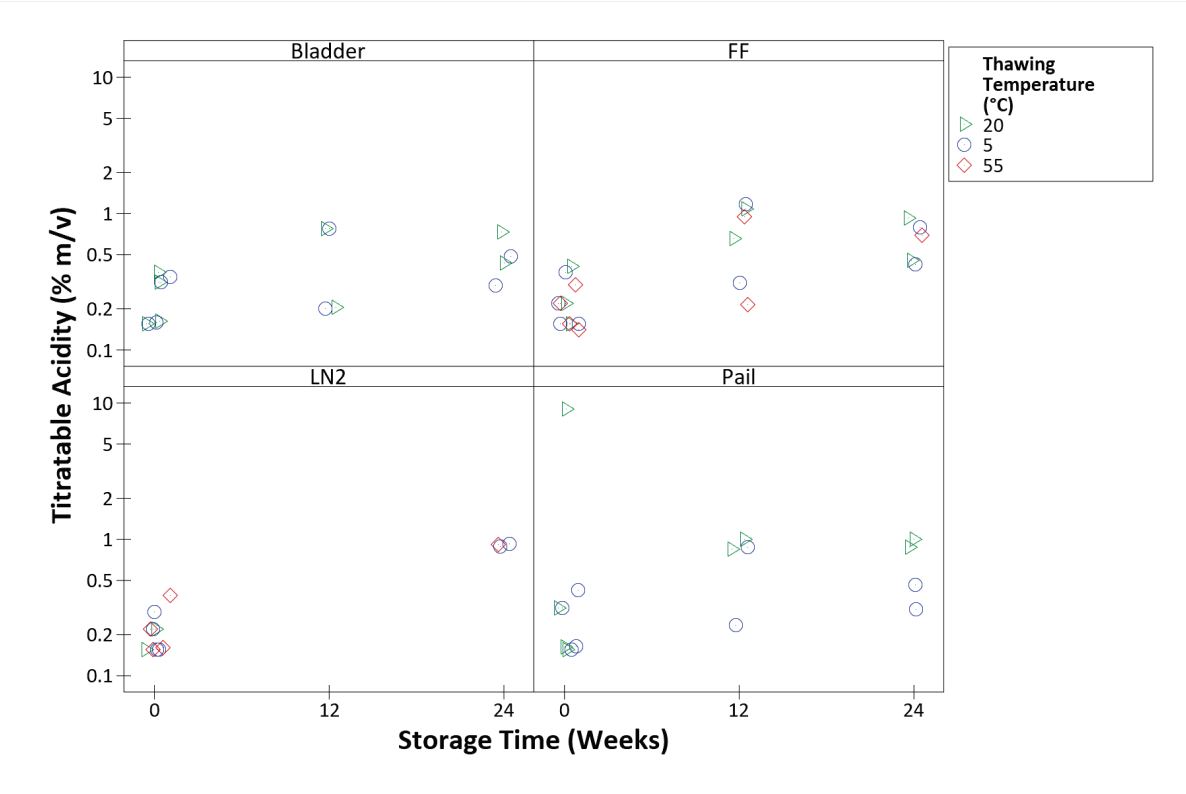

***Figure 18**: Titratable acidity levels vs. storage time.*

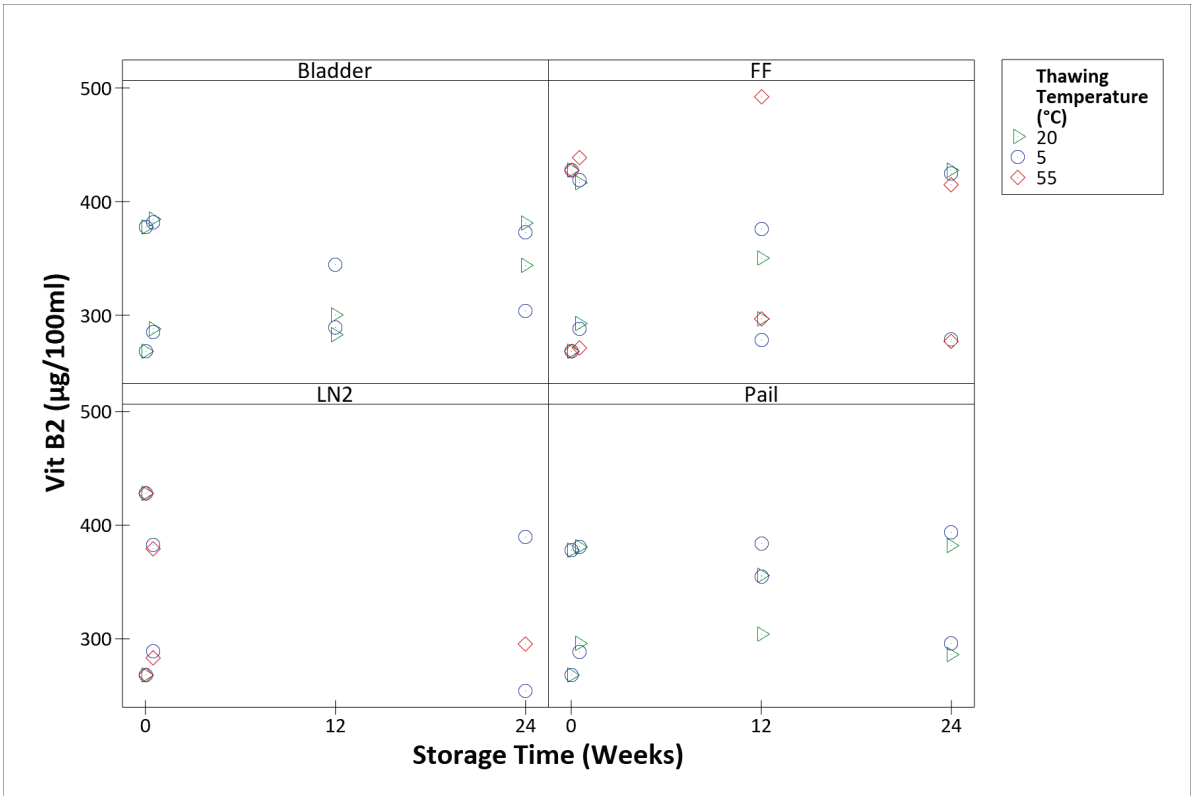

***Figure 19**: Vitamin B2 levels vs. storage time.*

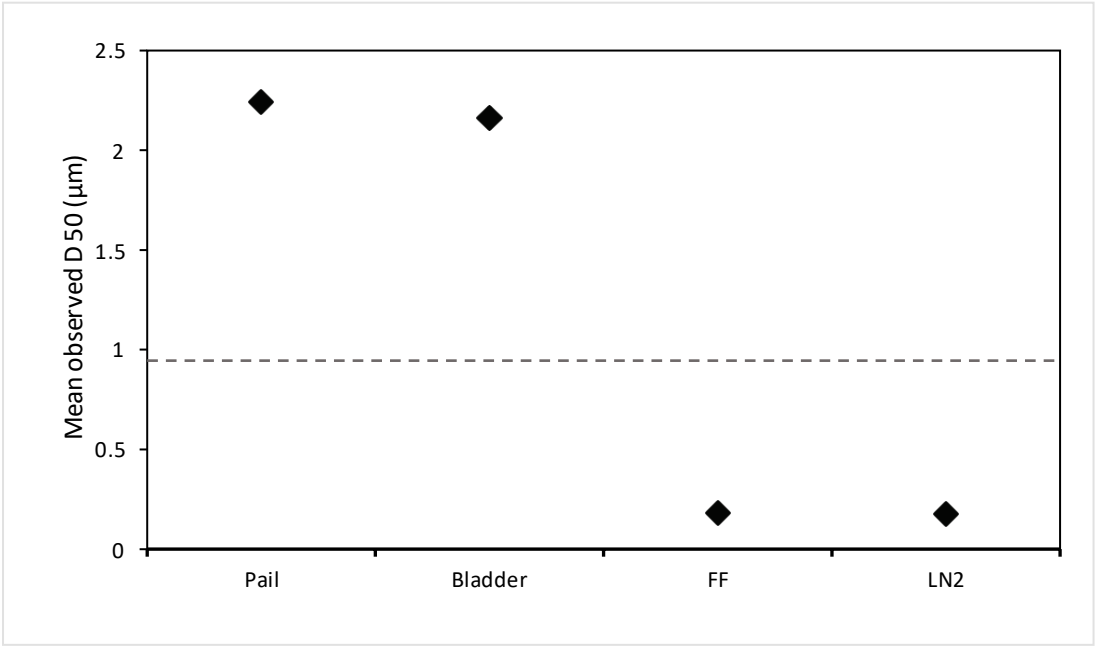

***Figure 20**: Main effect plot for median particle size.*

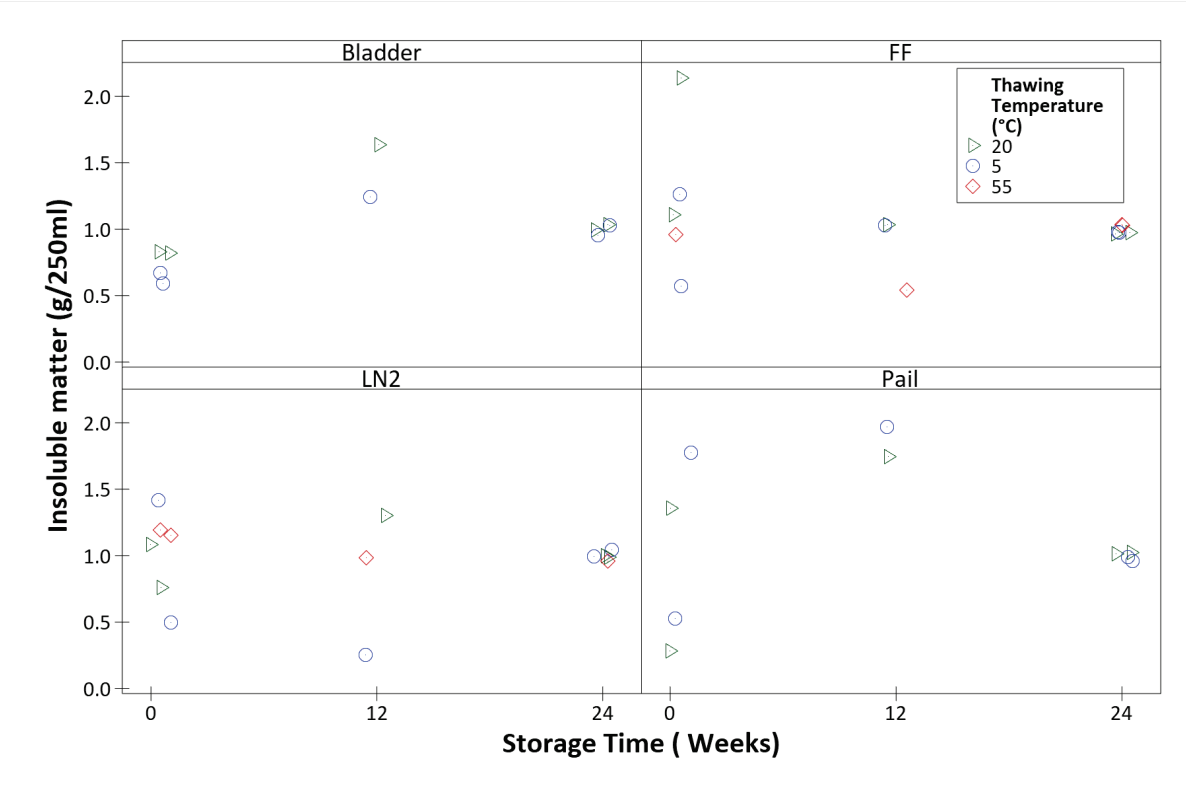

***Figure 21**: Insoluble matter in samples up to 24 weeks of storage.*

8. APPENDIX B Recommended future work

This work reports a tightly-scoped investigation into the effects of freezing and frozen storage on the functional and microbial quality of frozen sheep milk in the New Zealand context. Useful areas for further research include:

- The nature and effects of improved thawing methods which allow for rapid safe thawing of large-scale quantities of frozen sheep milk.
- The effect of microbial load prior to freezing, and details of the extent to which freezing can reduce bacterial burden?
- Processing properties of frozen-stored sheep milk in a New Zealand context, and with differing initial quality levels. Areas of possible study include the heat stability, rennetability, or organoleptic acceptability of frozen-stored sheep milk.