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# APPLICATION OF NANOFILTRATION TO DELACTOSED PERMEATE TO PRODUCE FOOD GRADE RETENTATE AND PERMEATE STREAMS

LEE ALEXANDER

2020

A thesis submitted in partial fulfillment of the requirements for the

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Specialization in Dairy Science

South Dakota State University

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THESIS ACCEPTANCE PAGE

LEE ALEXANDER

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Dr. Lloyd Metzger
Advisor Date

Vikram Mistry
Department Head Date

Dean, Graduate School Date

I dedicate this work to my father and mother, Roger and Cindy Alexander. My father is the most passionate dairy farmer I have ever met. Without you, I would have never shown interest in the Dairy Industry. Your passion for dairy is so contagious, it inspired me to pursue a degree in Dairy Manufacturing. Your passion for dairy drives me to this day. My mother has learned to embrace and encourage our passion, giving up "normal" vacations for cow shows, fairs, and other dairy conventions. Her patience and support is undeniable.

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TABLE OF CONTENTS

1 INTRODUCTION

1.1 Review of Literature……..1

1.1.1 Dairy byproduct utilization …….1

1.1.2 Importance of sodium reduced foods……...2

1.1.2.1 Salty flavor of dairy permeates……..3

1.1.2.2 Application of dairy permeates as a reduced sodium alternative ……..4

1.1.3 Sources of delactosed permeate……...4

1.1.4 Composition of delactosed permeate……...5

1.1.4.1 Lactose content of delactosed permeate……6

1.1.4.2 Mineral content of delactosed permeate……6

1.1.4.3 Organic acid content of delactosed permeate……7

1.1.5 Nanofiltration of whey permeates……8

1.1.5.1 Retention of lactose using nanofiltration……...9

1.1.5.2 Retention of ions using nanofiltration……..10

1.1.6 Lactose manufacturing……...11

1.1.6.1 Supersaturation of lactose in whey permeate……...12

1.1.6.2 Nucleation of lactose crystals in whey permeate…….14

1.1.6.3 Lactose crystal growth……15

1.1.6.4 Lactose Crystal Morphology……16

1.1.6.5 Cooling Curve……..17

1.1.6.6 Lactose yield……18

1.1.7 Effect of impurities on lactose crystallization……...21

1.1.7.1 Effect of Variant Lactose…….22

1.1.7.2 Effect of minerals on lactose crystallization……23

1.1.7.3 Effect of organic acids on lactose crystallization……23

2 Objectives……24

3 Nanofiltration of delactosed permeate to generate a lactose rich retentate and a permeate rich in organic acids and minerals……..……..28

3.1 ABSTRACT……28

3.2 INTRODUCTION……...29

3.3 MATERIALS AND METHODS……32

3.3.1 DLP Sources…….……. ……..33

3.3.2 DLP Sample Preparation……34

3.3.3 Fractionation of DLP…….34

3.3.4 Concentration of NF Permeate……...34

3.3.5 Chemical analysis……..35

3.3.5.1 Total solids……...36

3.3.5.2 Ash……...36

3.3.5.3 Chloride……37

3.3.5.4 Organic acid and sugars……...38

3.3.5.5 Minerals……...38

3.3.5.6 Statistical Analysis……...39

3.4 RESULTS AND DISCUSSION……..40

3.4.1 Composition of industrial delactosed permeate…….40

3.4.1.1 Mineral composition of delactosed permeate……..40

3.4.1.2 Sugar and organic acid composition of delactosed permeate……...41

3.4.2 Nanofiltration and RO flux……42

3.4.3 Fractionation of DLP components by Nanofiltration……42

3.4.3.1 Fractionation of TS and minerals in DLP……42

3.4.3.2 Fractionation of sugars and organic acids in DLP…...46

3.5 CONCLUSIONS…….48

4 Recovery of lactose crystals from delactosed permeate nanofiltration retentate blended with concentrated whey permeate…… ……68

4.1 ABSTRACT……68

4.2 INTRODUCTION……...69

4.3 MATERIALS AND METHODS……72

4.3.1 Delactosed permeate NFR and concentrated whey permeate source…….....72

4.3.2 Blending and concentration of DLP and concentrated whey permeate…….73

4.3.3 Lactose crystallization of control and experimental solution……74

4.3.3.1 Lactose crystallization apparatus…….74

4.3.3.2 Crystallization preparation……...74

4.3.3.3 Crystallization cooling curve and seeding……...75

4.3.4 Lactose crystal refining……..75

4.3.5 Chemical Analysis…….76

4.3.5.1 Total solids for experimental and control crystallization material……76

4.3.5.2 Total solids for refined lactose and supernatant……..77

4.3.5.3 Lactose concentration……..78

4.3.5.4 Crystal size measurement……78

4.3.6 Lactose yield determination…….……..79

4.3.7 Statistical analysis…….…….81

4.4 RESULTS AND DISCUSSION…….…….81

4.4.1 Blended and control crystallizer feed material……..81

4.4.2 Refined lactose and supernatant TS…….……..82

4.4.3 Lactose yield……..83

4.4.4 Lactose crystal size……84

4.5 CONCLUSIONS…….84

REFERENCES……..92

# ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| BOD | Biological oxygen demand |
| COD | Chemical oxygen demand |
| CST | Crystallizer |
| CWP | Concentrated whey permeate |
| Da | Dalton |
| db | Dry basis |
| DI | Deionized |
| DLP | Delactosed Permeate |
| DPW | Deproteineized whey permeate |
| HPLC | High performance liquid chromatography |
| MWCO | Molecular weight cut off |
| NF | Nanofiltration |
| NFR | Nanofiltration retentate |
| NFP | Nanofiltration permeate |
| PPM | Parts per million |
| PSI | Pounds per square inch |

| | |
|---|---|
| RO | Reverse Osmosis |
| ROR | Reverse osmosis retentate |
| TCA | Trichloroacetic acid |
| TS | Total solids |
| WPC | Whey protein concentrate |
| WPI | Whey protein isolate |
| wt. | weight |

# LIST OF FIGURES

Literature Review

1. Diagram of a typical edible grade lactose manufacturing facility (Patterson, 2009) …...26
2. Ideal tomahawk shaped crystal (Patterson, 2017) …….27

Chapter 1

1. Process flow diagram of DLP NF fractionation and RO concentration of the NFP

……... 51

Chapter 2

1. Lactose crystallization cooling curve for all three trials …….. 86

# LIST OF TABLES

Chapter 1

1. Total solids and pH of DLP sourced from four plants……..52
2. Means square and p-values for DLP TS and pH ……...…53
3. Average ash and mineral composition (mg/g dry weight) of DLP sourced from four plants ……...……..54
4. Means square and p-values for DLP ash, Cl and selective mineral analysis ……55
5. Sugar and organic acid composition of DLP sourced from four plants ……56
6. Means square and p-values for DLP HPLC analysis ……....57
7. Total flux (KgMH) for Nanofiltration …… .58
8. Total flux (KgMH) for RO …….. 59
9. Average TS, ash, salt and mineral composition of each fractionation streams for all trials conducted (four sources, two lots each) ……. 60
10. Means squared and p-values for NFF, NFR, NFP and ROR for TS, Ash, Cl and selective minerals ……...…61
11. Average rejection factors during NF processing (500 Da) for TS, ash, Cl, and minerals……..62
12. Means squared and p-values for NF rejection factors for TS, Ash, Cl and selective minerals..……63
13. Lactose and organic composition (mg/g dry weight) of each fractionation streams for all trials conducted (four sources, two lots each …….64
14. Means squared and p-values for NFF, NFR, NFP and ROR for HPLC analysis. 65

15. Average rejection factors during NF processing (500 Da) for sugars and organic acids …… 66

16. Means squared and p-values for NF rejection factors for TS, Ash, Cl and selective minerals ……67

Chapter 2

1. Compositional, yield, and crystal size means for three crystallization trials ……87
2. Mean square and p-values for the compositional analysis of the crystallizer feed material……..88
3. Means squared and p-values for refined lactose TS and Supernatant TS …….89
4. Means squared and p-values for actual yield and actual vs theoretical yield ratio ……90
5. Means squared and p-values for crystal size..……91

# ABSTRACT

DELACTOSED PERMEATE NANOFILTRATION FOR UTILIZATION OF THE RETENTATE AND PERMEATE STREAMS IN FOOD GRADE APPLICATIONS

LEE ALEXANDER

2020

Delactosed permeate (DLP), commonly referred to as mother liquor, is a plentiful byproduct in the dairy industry. It is a direct byproduct of edible lactose manufacture produced in cheese and dairy ingredient facilities. Despite being rich in lactose and minerals, DLP is most commonly relegated to an animal feed product due to its high ash content, it inhibits and disallows crystallization of any remaining lactose.

Delactosed permeate showed many inconsistencies from supplier to supplier and within lots from the same supplier in the eight DLP samples obtained from four separate mozzarella and cheddar manufacturing facilities. Nanofiltration (NF) opens potential food applications for DLP. The eight DLP samples obtained were processed via NF creating two separate product streams. A retentate (NFR) with increased lactose concentrations on a dry basis in addition to increased concentrations of large molecular weight minerals and organic acids (Ca, Mg, S, citric acid). In addition to, a permeate rich in small molecular weight minerals and organic acids (Cl, Na, K, lactic and formic acid). Flux rate and the composition of retentates/permeates varied from trial to trial and is likely due to different milk compositions, cheese making practices, whey handling practices and lactose manufacturing methods.

The DLP retentate proved to have increased lactose concentration. Unfortunately, the remaining minerals and organic acid concentration inhibited effective lactose crystallization when blended with industry deproteinized whey concentrate samples.

## 1.1 Review of Literature

### 1.1.1 Dairy byproduct utilization

Throughout the last decade the dairy industries' utilization of the nutrients found in milk has improved drastically. This is certainly the case with the cheese industry. Cheese whey was often relegated to an animal feed product, processed through waste water systems, applied to land or disposed of using another environmentally responsible method (Hobman, 1984; Siso, 1996; Smithers, 2008). Advances in membrane technology simultaneously reduced waste from cheese whey streams and provided profitable nutritional products in whey protein concentrate (WPC) (Smithers, 2008). Further advances in processing consistency made WPC and whey protein isolate (WPI) popular with consumers worldwide (Pouliout, 2008; Smithers 2008). The permeate collected from the membrane filtration of whey is then utilized in the production of edible grade lactose, which is a common ingredient in confections, or it can be further refined for pharmaceutical applications (Smithers 2008; Ganzel, 2008, Patterson 2017).

These advances for the cheese and whey industry led to new byproducts that are now underutilized. Delactosed permeate (DLP), commonly referred to as mother liquor, is the direct byproduct produced from the manufacture of edible grade lactose (Paterson, 2009). While DLP is rich in residual lactose, minerals and organic acids, it is relegated to an animal feed or waste product (Vembu and Rathinam, 1997; Liang et al., 2009; Paterson, 2017). Surprisingly, there is limited research on food applications for DLP. However, within the last ten years, research on food applications for DLP has developed.

Liang et al. (2009) researched the drying characteristics of DLP so that it may potentially be utilized as an ingredient. This research was followed by efforts to improve DLP's drying capabilities by including whey proteins (Bund and Hartel, 2010). The utilization of DLP as an ingredient has also been explored. Bund and Hartel (2013) sought to mix DLP with pro-cream, a byproduct of whey protein isolation, and utilize the mixture. The study however noted that increased levels of DLP led to off flavors. Applications of DLP as a reduced sodium ingredient provided positive results, but still resulted in off flavors (Smith et al., 2016). Developing methodologies allowing for the use of DLP as a food grade ingredient will decrease waste output and be financially advantageous for the dairy industry (Liang et al., 2009).

### 1.1.2 Importance of sodium reduced foods

The health impacts of high sodium intake are well documented but are highlighted by hyper tension and heart disease (US Department of Health and Human Services, 2006; Garza, 2015). Reducing sodium in the diet is vital for the health of many people worldwide. The burden for decreasing sodium intake is not on the food consumer alone. In many parts of the world, the majority of sodium is consumed in processed foods (Anderson et al, 2010). Therefore, food processors must decrease sodium usage in processed foods to meet the needs of the consumer, which has been a consideration of food manufactures for years (Henny et al 2010; Berry, 2010). However, to accomplish

sodium reduction in processed foods, manufacturers must also make flavorful, salty tasting foods (Henny et al 2010).

Sodium reduction for processed foods has been accomplished by many methods. Research conducted pertaining to the partial substitution of NaCl with KCl provides promising results as a sodium reduction method (Bersin and Beauchamp, 1995; Henny et al, 2010). The use of KCl in processed foods also provides similar functionality as a processing aid when compared to NaCl (Katsiari et al, 2001; Bidlas and Lambert 2008). However, sensory analysis of KCl sodium reduced foods consistently shows increased levels of bitterness (Murphy et al., 1981, Sinopoli and lawless, 2012; Bersin and Beauchamp, 1995). Complete Salt elimination in processed foods also provides many negative side effects, both nutritional and sensory (Henny et al 2010)

#### 1.1.2.1 Salty flavor of dairy permeates

Dairy permeates, specifically whey permeates, have been explored as a potential food additive. Whey permeate, a byproduct of cheese manufacture and membrane filtration methods, consists of lactose, minerals, organic acids and low concentrations of proteins (US Dairy Export Council, 2011). Whey permeates are also noted to have a salty flavor. Research conducted by Frankowski et.al, (2014) indicates that whey permeates salty flavor profile is provided not only by NaCl, but aided by the presence of KCl, lactic acid, and orotic acid. However, the lactose content of whey permeate provides decreased salty flavor for whey permeates. In this study, lactose reduced whey permeate provided

the greatest salty flavor when compared to whey permeates and NaCl solutions at equal sodium levels (Frankowski et.al, 2014).

#### 1.1.2.2 Application of dairy permeates as a reduced sodium alternative

The utilization of dairy permeates as a food additive to reduce sodium content in processed foods has been explored. Smith et al., (2016) researched the inclusion of milk, whey and delactosed permeates in soup. The study found that, whey permeates sourced from cheddar, mozzarella and milk sources were desired over a no salt cream of broccoli soup but scored lower than salt added soup. Delactosed permeate scored even with the control. In addition, the study observed cottage cheese whey permeates were less desirable in this application, due to its sourness.

### 1.1.3 Sources of delactosed permeate

Delactosed permeate is the result of a series of processes designed to extract nutritional components from cheese whey, a plentiful byproduct of cheese production (Kosikowski, 1979). Cheese whey is processed by recovering cheese fines and whey cream prior to ultrafiltration, which fractionates and concentrates whey proteins in the retentate (Durham, 2000). The deproteinized whey permeate (DPW) is then processed for lactose recovery by concentrating the remaining solids, primarily lactose, minerals, and

organic acids, into a supersaturated solution (Patterson, 2009). The supersaturated DPW concentrate is then cooled forming lactose crystals (Paterson 2009). The crystalized DPW solution is then separated into two fractions consisting of refined lactose crystals and DLP by a decanter centrifuge (Durham, 2000; Paterson, 2009; Liang et al., 2009). The lactose crystallization process will be discussed thoroughly in section 1.1.6 of this paper.

### 1.1.4 Composition of delactosed permeate

Delactosed permeate composition is highly variable between manufactures and even between processing runs within the same facility (Liang et al., 2009; Paterson 2017). The composition of DLP varies due to the cheese milk composition, cheese manufacture methodologies, cheese variety produced, whey manufacturing methodologies, lactose production methods and lactose yields (Liang et al., 2009; Paterson, 2017). The large number of variables that affect the composition of DLP limit its utilization as a food ingredient (Liang et al., 2009).

The available art provides a range of DLP composition however, the scope is limited. Literature indicates that the TS of DLP may range between 25.9-36.4 % (wt./wt.) (Liang et al., 2009; Frankowski et al., 2014). The TS content of DLP is highly dependent upon lactose yield and concentration methods utilized after the separation from lactose crystals (evaporation or RO). The TS components consist of sugars, organic acids and minerals, (Liang et al., 2009; Frankowski et al., 2014). Despite a large array of analysis methods, all DLP solids have not been quantified (Liang et al., 2009).

#### 1.1.4.1 Lactose content of delactosed permeate

The primary solid component in DLP is lactose (Liang et al., 2009; Frankowski et al., 2014). The lactose accounted for in DLP represents a yield loss from lactose processing due to crystalized lactose or lactose crystal fines (Patterson, 2017). Liang et al. (2009) found that the lactose content of DLP ranges from 41.29-64.20 % on a dry basis (DB) (wt./wt.). Liang et al. (2009) also observed that the lactose present in DLP exists primarily in a crystalline form and did not observe lactose in an amorphous state when dried. In the authors opinion this may be a result of the cold storage temperature (7 ˚C) of the samples, which may have led to crystallization.

#### 1.1.4.2 Mineral content of delactosed permeate

Delactosed permeate contains minerals naturally found in milk but are concentrated throughout the production of cheese, whey and lactose. Mineral and ash analysis of DLP have shown a wide range of results. Liang et al. (2009) analyzed three DLP samples with total mineral compositions ranging from 9.29-19.86 % db. Another study showed mineral levels ranging from 20.7-22.9 % ash (Frankowski et al., 2014). Liang et al. (2009) observed an inverse relationship of lactose and minerals in DLP. Samples with higher mineral content showed lower lactose content and vice versa. This is

likely an effect of lactose yield and refining efficiency (Liang et al., 2009; Patterson 2017).

The DLP mineral content consist primarily of K, Na, Cl, Ca, Mg, and P, although not all minerals may have been identified (Liang et al., 2009; Frankowski et al., 2014). The ion concentration varies between all samples. Monovalent cations found in the highest concentration were K and Na, while Ca was found to be the most prevalent divalent cation. Chloride is the primary anion found in DLP.

#### 1.1.4.3 Organic acid content of delactosed permeate

The organic acids present in DLP are primarily a result of cheese and whey processing. As whey permeate is processed into lactose and DLP, the organic acids are concentrated into the DLP. The primary organic acids found in DLP are lactic and citric acid (Liang et al., 2009). The concentrations of these acids relate directly to the handling of whey after the cheese process, which will cause variation from sample to sample (Liang et al., 2009). The lactic acid content will vary based on the timeliness of pasteurization and cooling after cheese make and the citric acid content varies based on the membrane processing applied. As whey permeate is processed into lactose and DLP, the organic acids are concentrated into the DLP. The organic acid concentration of DLP has been observed to be four to six times higher than found in whey permeates (Frankowski et al., 2014). Other organic acids present in DLP at varying concentrations include: acetic, maltic, orotic, uric and hippuric acids.

### 1.1.5 Nanofiltration of whey permeates

Nanofiltration technology is seldom utilized in the dairy industry when compared to microfiltration, ultrafiltration and RO technologies. Waste management and food grade applications of nanofiltration have been explored. Whey permeates often place a large burden on waste water treatment facilities because of high BOD and COD levels (Artel et al., 2005; Cuartas-Uribe et al., 2009). Nanofiltration can be utilized to fractionate lactose and residual proteins from the minerals present in whey permeate to decrease BOD and COD loading, allowing for better management of effluent streams from dairy manufacturing facilities (Artel et al., 2005; Chollangi and Hossain, 2007; Cuartas-Uribe et al., 2009).

Nanofiltration of whey permeates also provides potential avenues to produce food grade ingredients or allow for the recycling of nutritional components. Nanofiltration of whey permeates has been utilized as a method to concentrate the nutritional components such as residual protein, lactose and calcium while decreasing NaCl concentrations providing a higher quality ingredient (Suarez et al., 2006; Suarez et al., 2009). Fractionating and concentrating lactose in whey permeates has also been performed with the intention of producing ingredients for fermentation, sweets and ice cream processes while decreasing effluent to waste water facilities (Rektor and Vatai, 2003; Altra et al., 2005). While these studies successfully fractionated and concentrated nutritional

elements of whey permeate while decreasing the strain on waste water treatment facilities, the viability of food grade ingredients is not fully realized.

Fractionation via NF is complex, as both size exclusion and electrostatic interactions are observed (Eriksson, 1988; Staude 1992; Peeters et al., 1999). Anions and cations are fractionated according to their molecular weight but also based on the molecules ionic charge. Neutral molecules are primarily fractionated by size exclusion. Therefore NF, when applied to whey permeates, consists of two separate fractionation methods based on the type of molecule.

#### 1.1.5.1 Retention of lactose using nanofiltration

Lactose retention during nanofiltration processing is influenced by numerous variables. Uncharged lactose molecules are retained primarily due to size exclusion and therefore retention is a function of mean molecular weight cut off, transmembrane pressure, and processing temperature. (Atra et al.,2005; Cuartas-Uribe et al. 2007). Decreases in mean molecular weight cut off result in increased lactose retention and increases in transmembrane pressure provide decreased lactose retention in pure lactose solutions and DPW (Cuartas-Uribe et al. 2007). Higher NF processing temperatures also provide decrease lactose retention for whey permeates (Atra et al., 2005).

In addition to processing parameters and membrane selection, the ionic composition of the feed solution effects the retention of lactose during NF. Lactose retention is found to be higher in the absence of ions and lower when ions are present

(Cuartas-Uribe et al. 2007). The ions present in DPW solutions are thought to increase the apparent molecular weight cut off due to ionic interactions at the surface of the membrane. These interactions are thought to cause higher repulsion charges that result in the widening of the pores, which would effectively allow decreased retention of the neutral lactose molecule (Cuartas-Uribe et al. 2007).

#### 1.1.5.2 Retention of ions using nanofiltration

The fractionation of ions during nanofiltration relies largely on electrostatic interactions, but retention is increased with increased molecular weight. Monovalent ions are generally found to have low rejections during NF, with molecular weights below 150, while divalent and multivalent ions show increased retentions during NF, with molecular weights above 300 (Eriksson, 1988). These concepts have been observed in numerous whey permeate NF experiments, all consistently showing low retentions of low molecular weight monovalent ions ($Cl^-$, $Na^+$, $K^+$) and high retentions of larger divalent ions ($Ca^{2+}$, $Mg^{2+}$) (Suarez et al., 2006; Cuartas-Uribe et al., 2007; Suarez et al., 2009; Cuartas-Uribe et al., 2009).

Fractionation of ions present in whey permeate are also highly susceptible to the Donnan effect (Suarez et al., 2006; Cuartas-Uribe et al., 2007; Cuartas-Uribe et al., 2009). The Donnan effect during NF causes a phenomenon in which low molecular weight anions have negative retentions levels, indicating that anion permeates more than it retains. This event defies principals seen in other membrane fractionation techniques,

as smaller molecules generally free flow between the concentrate and permeate sides of the membrane (Eriksson, 1988). The Donnan effect occurs to maintain an ionic balance between the retentate and permeate fractions of the NF (Cuartas-Uribe et al. 2007). Due to the acidic pH of whey permeate and the negative charge of NF membranes, NF generally favors cation permeation, with preference to low molecular weight monovalent cations (Suarez et al., 2006). To maintain electroneutrality in the system, anions are permeated as well with preference given to low molecular weight monovalent anions. In whey permeate, $Cl^-$ is permeated at high rates to maintain electroneutrality, often showing negative retention levels (Suarez et al., 2006; Cuartas-Uribe et al., 2007; Suarez et al., 2009; Cuartas-Uribe et al., 2009). The Donnan effect is observed throughout NF processing, but the effect will increase when ion concentration is increased (Suarez et al., 2006).

### 1.1.6 Lactose manufacturing

The manufacture of edible grade lactose is common across the dairy industry. Lactose manufacturing provides a food grade revenue stream for deproteinized whey or milk permeates. While manufacturing methodologies differ across the dairy industry, **figure 1** provided by Paterson (2009) shows the most common manufacturing practices. Milk or whey permeate is concentrated to create a supersaturated solution. The supersaturated permeate crystalizes during a controlled cooling process, forming α-

lactose monohydrate crystals. Lactose crystals are then refined by removing soluble solids and water, or DLP. The refined lactose crystals are then dried and packaged.

Lactose manufacture is complex and varies greatly across the industry (Paterson, 2017). However, each of the afore mentioned manufacturing steps have the same intent for all manufacturers. Whey permeate is concentrated to a supersaturated level so that lactose can crystalize out of solution and yields can be maximized (Patterson, 2017). The crystallization process aims to crystalizes the maximum amount of α-lactose monohydrate out of solution while producing large crystals, allowing for efficient recovery. The refining or washing steps aim to remove the mother liquor, providing a low mineral final product (Patterson, 2009). While these general lactose manufacturing steps are followed across the industry and academia, lactose manufacture methodologies and yields remain inconsistent across the industry and academia (Patterson, 2017).

#### 1.1.6.1 Supersaturation of lactose in whey permeate

Producing α-lactose monohydrate at an industrial scale requires the supersaturation of lactose in whey permeate. Concentrating whey permeate via RO and evaporation or evaporation alone allows for the supersaturation of lactose in whey permeate at a given temperature (Patterson, 2009). The degree of supersaturation achieved is dependent upon the level of lactose concentration attained during processing and the temperature the concentrate is held. The initial concentration and supersaturation

level of lactose in whey permeate has a direct effect on the remaining lactose crystallization process.

Lactose supersaturation is a thermodynamic function that allows a solute to reach concentration levels beyond its equilibrium (Hartle and Shastry, 1991; Davey and Garside, 2000). When lactose is present in this state, lactose crystallization conditions are favorable but is reliant upon a nucleation event (Randolf and Larson, 1988; Wong and Hartel, 2014; Patterson, 2017). Three distinct levels of supersaturation exist, each providing favorable conditions for unique nucleation events. The lowest level of supersaturation is the meta stable zone. While in the meta stable zone, lactose will transfer from a soluble state to its crystalline form. The meta stable zone is fully reliant on seed addition or presence of lactose crystals for crystallization to occur (Butler, 1988; Wong and Hertel, 2014). In this zone, no additional nucleation will occur, and only the seed crystals or preexisting crystals will grow (Patterson, 2017). The meta stable zone of supersaturation provides ideal crystal growth conditions for lactose, therefore it is referred to as the growth region (Butler, 1988). In the lactose crystallization process, adjustments are made to keep the system in the metastable zone (Wong and Hartel, 2014). The next zone of supersaturation is the intermediate zone. The boundary between the meta stable and intermediate zones of supersaturation is referred to as the secondary nucleation threshold. When this threshold is passed, the system can produce an increased number of crystals, if there were already crystals present in the system (Lifran, 2007; Patterson, 2017). A secondary nucleation event utilizes preexisting crystals to seed a second nucleation event, therefore increasing the number of total crystals available for growth. A negative side effect of a secondary nucleation event is a wide size distribution

of crystal size (Patterson, 2017). The highest level of supersaturation is referred to as the liable zone. When a solution is in the liable zone of supersaturation, a spontaneous nucleation event will occur (Lifran, 2007). Spontaneous nucleation events occur to allow the system to maintain equilibrium. The spontaneously generated nuclei provide crystals for soluble lactose aggregation, allowing lactose to transition from a soluble state to a crystalline form. A supersaturated solution existing in the liable zone will go through a spontaneous nucleation event, followed by the intermediate zone in which secondary nucleation events may occur until finally enough lactose has left solution and the meta stable, or growth state is reached.

#### 1.1.6.2 Nucleation of lactose crystals in whey permeate

As mentioned in the prior section, nucleation events are strongly related to the supersaturation level of lactose in the solution. In a lactose crystallization process, it is valuable to control the nucleation events that take place, as it will have a direct effect on the lactose yield (Patterson, 2017). There are two types of nucleation events that occur during lactose crystallization, primary and secondary. The initial nucleation event is identified as primary nucleation. Primary nucleation is the first occurrence of lactose crystals in a solution (Randolf and Larson, 1988; Wong and Hartel, 2014). Primary nucleation can be induced one of two ways, the first being spontaneous nucleation while the labile zone of supersaturation (Mullin, 2001). The second method for inducing primary nucleation is the addition of seed crystals to a supersaturated lactose solution in

either the metastable or intermediate zone (Shi et al., 2006; Patterson, 2017). In either case, the amount of crystals generated during primary nucleation influences the remainder of the lactose crystallization process.

Secondary nucleation is an event that may occur following primary nucleation and occurs if the level of supersaturation crosses the secondary nucleation threshold (Lifran, 2007; Wong and Hartel 2014; Paterson 2017). Secondary nucleation will only occur with preexisting seed crystals, the result of primary nucleation, present. A secondary nucleation event therefore increases the number of crystals within the system available for growth (Wong and Hartel, 2014). The system may be forced beyond the secondary nucleation threshold due to a rapid cooling curve or a primary nucleation event that produced fewer than the required level of nuclei. Secondary nucleation events occurring in lactose manufacture are generally undesired as they produce unrecoverable fines, therefore decreasing lactose yield (Wong and Hartel, 2014; Patterson, 2017). In industry and academia, providing appropriate primary nucleation levels and cooling curves to prevent crossing the secondary nucleation threshold during lactose processing has been inconsistent, likely due to the equipment utilized (Paterson, 2017).

#### 1.1.6.3 Lactose crystal growth

The growth of lactose crystal nuclei is highly dependent on the level of supersaturation available for the system but may be limited by other limiting factors (van Kreveld and Michals, 1965). As mentioned in section 1.1.6.1, maintaining the

supersaturation level in the metastable state, below the secondary nucleation threshold, produces the ideal environment for crystal growth. Other limiting factors include: surface integration, mutarotation, and impurities (Haase and Nickerson, 1966; Nickerson and Moore, 1974; Hartel and Shastry 1991).

Surface integration refers to the ability of soluble α-lactose monohydrate to leave solution by adhering to a preexisting crystal. For this transformation to occur efficiently, mechanical action is required, as it allows for soluble lactose to interact and adhere to the crystal surface readily (Wong and Hartel, 2014). Mutarotation of lactose molecules between the β and α forms is also a factor in the growth of lactose crystals. Mutarotation rate is primarily affected by the pH of the solution, with highly acidic pH (below 1) and alkaline pH levels promoting faster rates (Nickerson and Moore, 1974; Ganzle et al., 2008). Impurities also effect the growth of lactose crystals, as they can negatively affect the level of super saturation obtained, surface integration, and mutarotation (Mullin, 2001).

#### 1.1.6.4 Lactose Crystal Morphology

The ideal lactose crystallization process promotes the growth of nuclei into large tomahawk shaped crystals as observed in **Figure 2** (Patterson, 2017). Lactose crystal growth and development is conducted from the sharp end of the tomahawk towards the theoretical handle throughout the growth process (van Kreveld and Michaels, 1965). The supersaturation level maintained throughout the crystallization process is a vital

component for the morphology of lactose crystals, with high levels of supersaturation within the metastable zone promoting fully developed large crystals (Herrington, 1934; Parimaladevi and Srinivasan, 2014; Patterson, 2017). Contrarily, if supersaturation is pushed beyond the secondary nucleation threshold it may result in underdeveloped and small lactose crystals. Impurities present in the system may also influence the morphology of crystals, which will be discussed in greater detail in section 1.1.7.

#### 1.1.6.5 Cooling Curve

The cooling curve utilized during the manufacture of α-lactose monohydrate largely controls the level of supersaturation maintained and crystal morphology achieved during the crystallization process. The goal of a cooling curve during lactose manufacture is to cool the supersaturated lactose solution from the final temperature achieved during the concentration process to approximately 20 ˚C. In combination, lactose concentration and the cooling curve utilized act as the primary parameters in controlling nucleation, supersaturation, and crystal morphology during industrial lactose manufacturing (Valle-Vega et al., 1977; Shi et al., 2006; Wong and Hartel, 2014; Patterson, 2017)

The nucleation events of a supersaturated lactose solution are controlled by the cooling curve. First the cooling curve should cool the solution to a point where primary nucleation can occur. The cooling curve should be held within this zone of supersaturation to allow for a complete primary nucleation event. The temperature ranges for the primary nucleation event will vary, depending on if it is a seeded nucleation or a

spontaneous nucleation (Mullin, 2001; Shi et al., 2006; Patterson, 2017). Following the primary nucleation event, cooling should be applied to the system so that the solution is maintained within the metastable zone, while not crossing the secondary nucleation threshold. If the cooling curve drives super saturation past this point, a secondary nucleation event will provide an undesirable crystal size distribution (Wong and Hartel, 2014; Patterson, 2017).

The level of supersaturation present throughout the crystallization is also maintained by the parameters of the cooling curve. As lactose leaves solution into a crystalline form, cooling counteracts the reduced concentration of soluble lactose to maintain a supersaturated state. Ideally, the cooling curve applied maintains a high level of supersaturation within the metastable zone, just below the secondary nucleation threshold. As mentioned in section 1.1.6.3, maintaining appropriate levels of supersaturation and avoiding secondary nucleation directly effects the crystal size and morphology.

#### 1.1.6.6 Lactose yield

When manufacturing edible grade lactose, the yield or recovery of lactose from a soluble state into a crystalline form which can be recovered and refined is vital to optimizing the economic gain. Paterson (2017) has noted that lactose yield varies greatly among lactose manufacturers, ranging from 50-80%. The wide range of yields obtained

across the industry are primarily due to low lactose concentrations, inadequate cooling curves, and fines production (Patterson, 2009; Patterson, 2017).

The theoretical yield of a lactose crystallization is highly dependent on the mass of lactose available in the original concentrated solution and the lactose remaining in solution after the completion of the applied cooling curve (Patterson, 2009). The work by Butler (1998) provides **Equation 1** to determine the maximum amount of soluble lactose in water at a given temperature based on numerous lactose solubility studies.

Equation 1

$$C_{SOL} = 10.9109^{0.02804T}$$

$C_{SOL}$= Concentration of soluble lactose (g lactose/100 g water)

T= Temperature (˚C)

The theoretical yield of lactose from a crystallization can then be determined using the concentration of lactose in the concentrated material and the $C_{SOL}$ as shown in **Equation 2** (Patterson, 2009).

Equation 2

$$Theoretical\ yield\ \%\ (w/w) = \frac{L_{AVi} - C_{SOL}}{L_{AVi}} \times 100$$

$L_{AVi}$= Lactose available in the initial concentrated material (g lactose/100 g water)

$C_{SOL}$= Concentration of soluble lactose (g lactose/100 g water)

As indicated by **Equation 2**, the theoretical yield of a lactose crystallization can be increased by increasing the $L_{AVi}$ during the permeate concentration process and decreasing the $C_{SOL}$ by lowering the final temperature obtained by the cooling curve.

Actual lactose yield is dependent on the processing parameters effecting theoretical yield, the ability to recover lactose crystals in a refined form, and any hindrances of the crystallization due to impurities present in the solution. Hinderances caused by impurities will be discussed in section 1.1.6.6. The refining process of lactose aims to separate lactose crystals from DLP. While they vary across the industry, these refining steps often consist of decanter centrifugation and washing (Paterson, 2009). Lactose crystal size plays a vital role in the recovery during refining processes (Paterson, 2009; Wong and Hartel, 2014; Paterson, 2017). Fines generation during the crystallization process will directly affect the yield loss during the refining process, as the physical size limits effective separation from DLP (Patterson, 2017). Maintaining a level of supersaturation below the secondary nucleation threshold prevents the generation of fines during a crystallization, as discussed in section 1.1.7. Another source of fines generation and secondary nucleation can be linked to physically breaking crystals due to agitation in the crystallization vessel (Pandalaneni and Amamcharla, 2016).

When loses due to lactose crystal refining and recovery are considered actual lactose yield can be realized. **Equation 3** indicates how actual yield is determined,

representing the recovery of lactose from the initial supersaturated solution (Wong and Hartel, 2014).

Equation 3

$$Actual\ yield\ \%\ (w/w) = \frac{L_{CRf}}{L_{AVi}} \times 100$$

$L_{CRf}$ = Lactose crystal mass recovered (g lactose/100 g water)

$L_{AVi}$ = Lactose available in the initial concentrated material (g lactose/100 g water)

The actual yield can be compared to the theoretical yield to determine the effectiveness of a lactose crystallization and subsequent refining process using a ratio of actual vs theoretical yield, as shown in **Equation 4**.

Equation 4

$$Actual\ vs\ theoretical\ yeild\ (\%) = \frac{Actual\ yield\ \%\ (w/w)}{Theoretical\ yield\ \%\ (w/w)}$$

1.1.7 Effect of impurities on lactose crystallization

Outside of processing techniques utilized to concentrate, crystalize, and refine lactose, numerous impurities present in whey permeate hinder lactose manufacture. Some of the known impurities in whey permeate include minerals, organic acids and variant lactose. These impurities hinder lactose crystallization in three distinct ways (Mullin, 2001). Impurities hinder crystallization by altering supersaturation, as impurities may both effect the solubility of lactose and, assuming concentration of whey permeate is completed to a target TS, alter the lactose to water ratio. The second hinderance results from the absorption of impurities onto a growing crystal, inhibiting continued growth. Thirdly soluble lactose can be affected by impurities prior to crystallization, changing crystal morphology and inhibiting growth.

#### 1.1.7.1 Effect of Variant Lactose

Variant lactose phosphate sugars present in whey permeate, as discovered by Visser (1980, 1983, 1984, 1988), directly hinder the crystallization of lactose. Lactose phosphate, a disaccharide monophosphate, occurs when a phosphate group is bound to the galactose portion of lactose (Visser, 1984). During a crystallization, lactose phosphate attaches to a lactose crystal preventing further crystallization (Visser 1984, Lifran, 2007). As lactose phosphate is incorporated into the crystal structure, it is an impurity commonly found in the finished lactose product, not the DLP (Lifran, 2007).

#### 1.1.7.2 Effect of minerals on lactose crystallization

The mineral content found in whey permeates provide varying effects on lactose crystallization. Numerous studies have been conducted on the effect of minerals on lactose crystallization, many with contradictory results. For example, three separate studies measuring the effect of potassium chloride on lactose crystallization provided three separate conclusions; it increased growth rate, decreased growth rate, or that it was dependent on concentration (Jelen and Coulter, 1973a; Visser, 1984; Smart 1988). Chandrapala et al. (2016) measured the effect of calcium and lactic acid on lactose crystallization in acid whey streams. This study showed that varying concentrations of Ca effected lactose crystallization differently, as higher concentrations of Ca showed both increases and decreases of lactose yield dependent on the level of lactic acid present. Chandrapala et al. (2016) also showed that the presence of calcium decreased mean lactose crystal size and provided a larger crystal size distribution when compared to a control sample. Overall, the available art studying the effect of minerals on lactose crystallization shows inconsistency, making their effect hard to predict (Patterson, 2017).

#### 1.1.7.3 Effect of organic acids on lactose crystallization

Numerous studies indicate that lactic acid can hinder lactose crystallization (Jelen and Coulter, 1973b; Wijayasinghe, 2015; Chandrapala, 2016). However, some provide positive effect of lactic acid during crystallization (Smart, 1988; Smart and Smith, 1991;

Chandrapala, 2016). It has been found that nucleation events are hindered by lactic acid, effecting the crystal size distribution (Wijayasinghe, 2015; Chandrapala, 2016). Chandrapala (2016) shows that increasing the presence of lactic acid decreased crystal size, and that lower levels provided larger crystals. Alternatively, this same study also showed that high concentrations of lactic acid and low concentrations of Ca resulted in increased crystal size vs the control. Similar to the effect of minerals on lactose crystallization, the studied effects of lactic acid on lactose crystallization are inconsistent. It should be noted that the effects of organic acids other than lactic acid were not observed in the available art.

## 2. Objectives

Despite the nutritional components found in DLP, it is still considered a waste product throughout the dairy industry. While research has been conducted to discover value added applications for DLP, limited applications have arisen. The objective of chapter one of this thesis is to fractionate DLP using NF technology to provide two potentially functional and food grade streams. Based on the literature reviewed, the lactose rich, mineral, and organic acid rich DLP is an excellent subject for nanofiltration, similar to studies performed on whey permeate (Suarez et al., 2006; Cuartas-Uribe et al., 2009; Suarez et al., 2009). The hypothesis of this study is that NF of DLP will provide a retentate stream rich in lactose and permeate stream rich in organic acids and minerals that may be utilized in food grade application. This hypothesis would develop a lactose

rich retentate that may be recycled back into the edible grade lactose process or provide a more functional ingredient for other food grade applications. The hypothesis would also support the development of an improved reduced sodium food additive with the permeate fraction, as it would contain decreased levels of lactose, a hinderance to salty flavor (Frankowski et al., 2014).

Chapter two explores recycling the lactose rich DLP NF retentate (NFR) back into the edible grade lactose process. The objective of this chapter is to find a viable avenue to utilize the lactose rich NFR within the four walls of an edible grade lactose and DLP manufacturer. The NFR was blended with concentrated whey permeate at a ratio of 30:70 on a dry basis and crystalized in parallel with a concentrated whey permeate control. The hypothesis of this study is that the DLP NFR experienced a decrease in impurity concentration that it would add lactose to an edible grade lactose stream without hindering the lactose yield and crystal size.

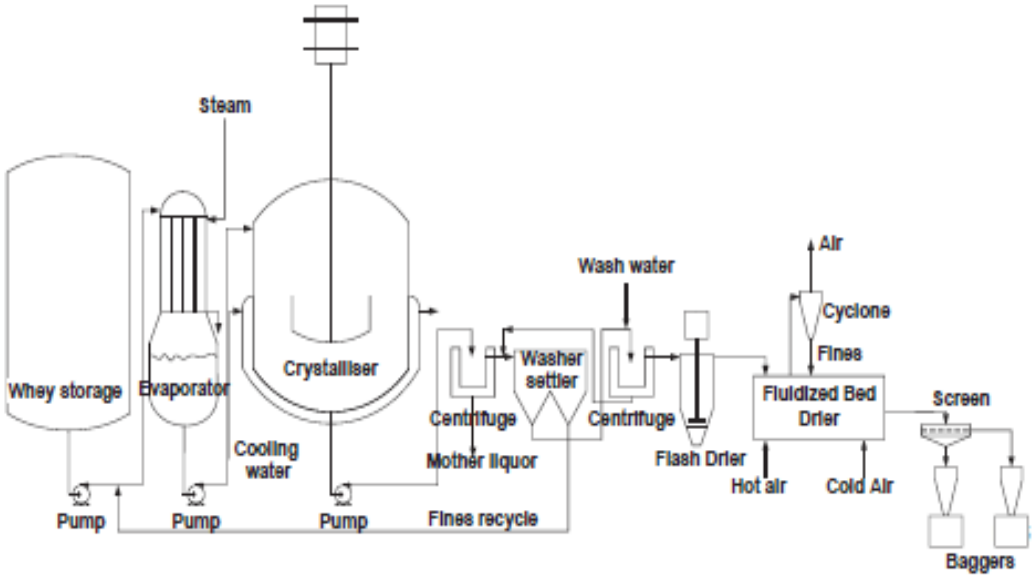


*Figure 1. Diagram of a typical edible grade lactose manufacturing facility (Patterson, 2009)*

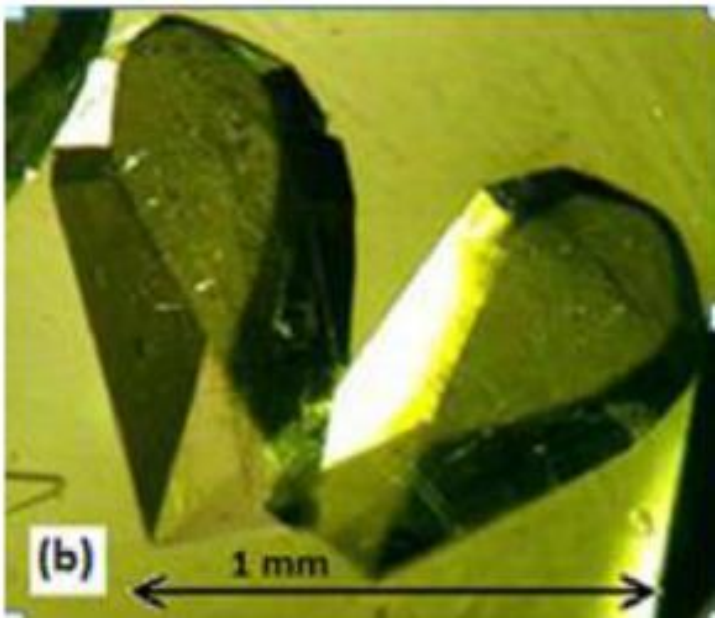


*Figure 2. Ideal tomahawk shaped crystal (Patterson, 2017)*

# 3. Chapter 1

Nanofiltration of delactosed permeate to generate a lactose rich retentate and a permeate rich in organic acids and minerals.

## 3.1 ABSTRACT

Delactosed permeate (DLP), commonly referred to as mother liquor, is a byproduct of lactose manufacture and is typically relegated to animal feed use, despite being rich in lactose and minerals. The objective of this study was to determine the viability of fractionating DLP into two components, one that would be recycled into the lactose manufacturing process and one that could be used as a salt substitute.

Two lots of commercial DLP were obtained from four different lactose manufacturers (totaling eight samples). The composition of these samples ranged from 27.9 to 39.7 % total solids. Each DLP sample was diluted to approximately 5 % TS using soft tap water and then subjected to nanofiltration (500 Da MWCO, NFW-3B-3838, Synder Filtration) in a batch process. Nanofiltration was performed until the flux rate dropped below 10 Lmh. Subsequently, the NF permeate (NFP) fraction was concentrated to approximately 8% TS using reverse osmosis (RO) (RO2-3838-BS04, Parker-Hannifin). The initial DLP, NF retentate (NFR), and RO retentate were analyzed for total solids (forced air oven) and ash (muffle furnace). Selected minerals (Ca, Na, Mg, P, S and K) were determined by plasma emission spectroscopy, along with sugars and organic acids by HPLC (lactose, galactose, lactic, formic and citric).

While DLP samples showed similar TS and ash compositions within manufacturers, they varied between facilities ($p<0.05$). The pH of the DLP samples varied both across facilities and within the four facilities ($p<0.05$). Selective mineral compositions also varied between and within all facilities ($p<0.05$). Lactose and galactose concentrations in DLP varied between manufacturers ($p<0.05$). Lactic, formic, and citric acid concentrations were found to vary between and within facilities ($p<0.05$).

Nanofiltration retentates showed higher concentrations on a dry basis of TS, Ca, Mg, S, Lactose, and citric acid when compared to the NFP. Nanofiltration permeates showed higher concentrations on a dry basis of ash, Cl, Na, P, K, lactic acid and formic acid. Nanofiltration of DLP provided an NFR with increased concentrations of lactose while decreasing the concentration of numerous impurities. Conversely, NF of DLP provided a NFP with reduced lactose concentration and increased concentrations of K and lactic acid, favorable for increased salty flavors.

## 3.2 INTRODUCTION

Delacosed permeate (DLP), often referred to as mother liquor, is a plentiful byproduct in the dairy industry. It is a byproduct of edible lactose produced in cheese and dairy ingredient facilities. Despite being rich in lactose and minerals, DLP is most commonly relegated to an animal feed product due to its high ash content and organic acid content, it inhibits and disallows crystallization of any remaining lactose. In a sense, DLP is the result of byproduct utilization throughout the years, as cheese whey was

eventually utilized for WPC production, and whey permeate for edible grade lactose production, leaving DLP as the current waste product (Pouliot, 2008; Smithers, 2008; Patterson, 2017).

Delactosed permeate composition is highly variable across the industry and even within lactose manufacturing facilities (Liang et al., 2009; Paterson 2017). Variables effecting DLP composition include original milk composition, cheese manufacturing methods, cheese varieties, whey processing, and lactose manufacturing methods, especially lactose yield (Liang et al., 2009; Paterson 2017). Overall, because of the large variation in DLP, food grade applications are limited (Liang et al., 2009).

The available art provides a range of DLP composition however, the scope is limited. Literature indicates that the TS of DLP may range between 25.9-36.4 % (wt./wt.) (Liang et al., 2009; Frankowski et al., 2014). The TS content of DLP is highly dependent upon lactose yield and concentration methods utilized after the separation from lactose crystals (evaporation or RO). The TS components consist of sugars, organic acids and minerals, (Liang et al., 2009; Frankowski et al., 2014). Lactose, the largest solid component of DLP, ranges from 41.29-65.20% on a dry basis (Liang et al., 2009). Total mineral composition of DLP was shown to range between 9.29 and 22.9 % db (Liang et al., 2009; Frankowski et al., 2014). Organic acid composition of DLP varies widely, but throughout the processing stream followed to produce DLP, organic acids are concentrated (Frankowski et al., 2014). One common acknowledgment in previous art is that DLP is highly variable (Liang et al., 2009; Frankowski et al., 2014)

Previous work on developing food grade applications for DLP have a limited scope. Liang et al. (2009) studied the drying characteristics of DLP, allowing for potential ingredient usages. As the drying capabilities of DLP are limited, Bund and Hartel (2010) investigated the combination of DLP and whey proteins to improve drying. Furthermore, Bund and Hartel (2013) sought to combine DLP with another dairy byproduct, pro-cream, but showed that increased DLP concentration led to off flavors.

Recently research has been conducted evaluating the viability of dairy permeates as reduced sodium alternatives. Frankoswki et al. (2014) found that whey permeates provide a salty flavor at reduced sodium levels due to the presence of KCl and lactic acid. However, the lactose content of whey permeate provides decreased salty flavor for whey permeates. Delactosed permeate showed the highest level of salty flavor at equal NaCl concentrations (Frankowski et.al, 2014). Smith et al. (2016) extended this research by utilizing whey permeates and DLP as additives in soup. Delactosed permeate showed to be useful in this application, as sensory evaluation indicated DLP samples scored even with control samples. Despite the still high concentration of lactose present, DLP shows promise as a reduced sodium food additive. In the food industry today, reduced sodium alternatives are highly desired as consumers look to reduce sodium consumption in their diets to decrease the risk of hyper tension and heart disease (US Department of Health and Human Services, 2006; Henny et al 2010; Berry, 2010; Garza, 2015).

Nanofiltration of whey permeates has been utilized for both waste management and food grade applications. Whey permeates often place a large burden on waste water treatment facilities, leading to research apply NF to fractionate lactose therefore decreasing BOD and COD loading (Artel et al., 2005; Chollangi and Hossain, 2006;

Cuartas-Uribe et al., 2009). Some food grade research conducted for NF on whey permeate aim to increase lactose and residual protein concentration while decreasing mineral concentration to produce a more nutrient rich ingredient (Suarez et al., 2006; Suarez et al., 2009). Other food grade applications of whey permeate NF look to both decrease waste water treatment while producing lactose rich ingredients for fermentation, sweets, and ice cream (Rektor and Vatai, 2003; Altra et al., 2005).

This study aims to develop potential food grade applications for DLP by applying NF technology. Eight samples from four DLP manufacturers were collected, with two lots per manufacturer. Two of the manufacturers produced pasta filata style cheeses while two produced cheddar style cheeses (one block and one barrel). These samples were obtained and processed using NF to fractionate the solids. The hypothesis is that applying NF technology to DLP will produce two distinctly different streams with potential food grade applications, the first stream being a lactose rich NF retentate (NFR) with decreased concentrations of impurities for potential recycling back into the lactose process  and the second stream being and mineral and organic acid rich NF permeate (NFP) stream rich in minerals and organic acids to be utilized as a reduced sodium food additive.

The DLP and its fractions were analyzed TS, Cl, ash, selective minerals, sugars and organic acid concentrations. These analysis methods were used to determine differences in DLP sources and differences found between the NFR and NFP. Nanofiltration processing parameters and component rejection factors were also monitored.

## 3.3 MATERIALS AND METHODS

### 3.3.1 DLP Sources

Delactose permeate samples were collected from four separate commercial lactose manufacturers. All whey permeates utilized to produce the sampled DLP were sourced from cheese manufacturing facilities whom produce WPC. The whey streams of two plants were sourced from pasta fillata style cheese operations and two from cheddar cheese operations, one being block and one barrel style cheddar. From each facility, two separate lots were sampled, totaling eight separate lots. Following sampling from the perspective facility, the samples were sub sampled and stored frozen (-20 ˚C) prior to experimental processing.

### 3.3.2 DLP Sample Preparation

Prior to experimental processing, all industrial samples were analyzed to determine the %TS. These samples were then diluted using soft tap water to approximately 5% TS in a 200-gallon tank to form approximately 900 lb batches. The diluted batches were stored overnight under constant agitation and cooling prior to fractionation.

### 3.3.3 Fractionation of DLP

Diluted DLP samples were subjected to nanofiltration in an automated pilot plant scale batch process designed for NF and RO processing, as outlined in **Figure 1**. The NF membrane selected for this experiment was a NFW-3838-3B from Synder Filtration. The NFW membrane provides a 500 Da molecular weight cut off (MWCO), 46 mil spacers and a total effective area of 6.97 $m^2$. The NFW membrane was selected after conducting unpublished exploratory trials with a NFG-313-3838 membrane (Synder Filtration); 800 Da MWCO; 46 mil spacer; total effective area of 6.97 $m^2$. The initial trials with this membrane indicated the MWCO permeated excessive lactose during processing.

Nanofiltration was conducted on all eight DLP samples from four separate processing facilities and processed until a permeate flux below 10 Lmh was obtained. Key processing parameters were recorded and varied between DLP sources throughout the trials. Baseline pressure ranged from 185-430 PSI and inlet pressure ranged from 203-497 PSI across all. Transmembrane pressure for NF processing averaged 12.9 PSI for all trial (ranging 11-16 PSI).

During each trial, NF permeate (NFP) was collected and stored for further processing. Composite permeate sampling was completed during processing based on the mass of each storage vessel. Following the completion of the trial, a composite NF retentate (NFR) sample was obtained. Following the trial all samples were stored at 4 ˚C for short term storage or frozen at -20 ˚C for long term storage.

### 3.3.4 Concentration of NF Permeate

As shown in **Figure 1**, the NFP collected in section 3.3.3 was concentrated using reverse osmosis (RO), using the automated pilot plant scale batch filtration system, set in RO mode. The RO membrane utilized for concentration was a spiral wound RO2-3838-BS04 with a 31 mil spacer, and total effective area of 6.60 $m^2$ (Parker-Hannifin).

Reverse osmosis was conducted on the NFRs until approximately 8% TS or higher was obtained, measured by %Brix. Processing parameters again varied between trials and throughout the run. Inlet pressures, ranging from 329-522 PSI, and baseline pressures, ranging from 329-355 PSI. Transmembrane pressure for RO averaged 12.54 PSI (ranging 9-15 PSI).

During the concentration of each NF permeate, The RO permeate was sampled and monitored for abnormal brix levels. Following sampling and weighing, the RO permeate was discarded down the drain. The concentrated NF retentate (ROR) was sampled for analysis. Samples were either stored at 4 ˚C for short term storage or frozen at -20 ˚C for long term storage.

### 3.3.5 Chemical analysis

Delactosed permeate, NFP, NFR and ROR were analyzed for total solids (TS), ash, salt, mineral, organic acid, and sugar composition.

#### 3.3.5.1 Total solids

Total solids for all samples were analyzed as follows. Empty disposable aluminum dishes were labeled for identification and placed in a forced air oven at 100 ˚C for at least one hour. The aluminum dishes were then placed in a desiccator to cool, prior to being weighed. One gram of DLP, NFP, NFR, and ROR was weighed into the dishes. Dish and sample were dried in a forced air oven at 100 ˚C for four hours. The samples were cooled in a desiccator prior to weighing. Percent TS was determined using **Equation 1**.

Equation 1

TS% = (((Dry Sample weight + dish weight) – dish weight) / initial sample weight) x 100

#### 3.3.5.2 Ash

Ash was determined using the following procedure. Five grams of sample was weighed into a pre-weighed, labeled, dried and cooled ceramic crucible. The sample and

crucible were then dried in a forced air oven at 100 ˚C for four hours. Sample and crucible was then charred using a hot plate prior to being placed in a muffle furnace at 550 ˚C to complete the ashing. Samples were cooled in a desiccator prior to being weighed. Percent ash was determined using **Equation 2** below.

Equation 2

TS% = (((Dry Sample weight + crucible eight) – crucible weight) / initial sample weight) x 100

3.3.5.3 Chloride

Five grams of sample was diluted using DI water based on the moisture content of the sample as seen in **Equation 3**.

Equation 3

Water addition= 100 – (mass of moisture in sample)

Diluted samples were then filtered into an Erlenmeyer flask through Whatman paper #4. The filtrate was analyzed with a Corning 926 Chloride Analyzer (Nelson-

Jameson) per the instruction manual. Salt determination for each sample is shown in **Equation 4**.

Equation 4

Salt% = instrument reading (mg%) X 4 (dilution factor)

3.3.5.4 Organic acid and sugars

Samples were prepared based on the methodologies described in Upreti et al. (2006). Sample was diluted using HPLC grade water. The dilution factors were based on the TS composition of the sample, ranging from 2 to 30. Approximately 0.5 ml of diluted sample was filtered with a 3 kDa MWCO Micron centrifuge filter. Centrifugation was conducted at 14000g for 15 minutes.

The collected filtrate was analyzed using HPLC based on the methodologies outlined by Amamcharla and Metzger (2011). The entire filtrate was directly injected into a sample, delivering 20 µl for analysis. The HPLC system (Beckman and Coulter) consists of two detectors: UV detector (System Gold 168) set at 210nm and 280nm and refractive index detector (RI2031, Jasco Corporation). The HPLC system used a 300 X 7.8mm ion exchange column (ROA-Organic acid, Phenomenex Inc.) heated to 65 ˚C. Sulfuric acid (0.013N) solution made with HPLC graded acted as the mobile phase.

3.3.5.5 Minerals

Samples were mixed with 25 ml of 15% TCA solution and HPLC grade water to provide a dilution factor between 3 and 10, dependent on the TS of the sample. The diluted and TCA treated samples were mixed and allowed to stand for 30 minutes, prior to be filtered into an Erlenmeyer with Whatman #4 paper. The prepared filtrate was sent to Analab (Fulton, IL) for selective mineral analysis (Ca, Na, Mg, P, S and K) by plasma emission spectroscopy. Results provided in PPM on as is basis by the outside lab were calculated as a percentage of the original sample using **Equation 5**.

Equation 5

% Mineral= ((Sample weight / (sample weight + water weight + 15% TCA weight)) x PPM result) x 0.0001

3.3.5.6 Statistical Analysis

Results from the experiment were statistically analyzed to detect statistical difference. Industrial DLP samples were analyzed to determine compositional differences between facilities and lots. Processed samples were analyzed for compositional

differences and between facilities, lots, and sample points. Rejection factors were also analyzed for significance between facilities and lots. RStudio was utilized to preform ANOVA to obtain p-values (RStudio Team, 2015).

## 3.4 RESULTS AND DISCUSSION

### 3.4.1 Composition of industrial delactosed permeate

The TS and pH of the eight DLP samples (four facilities, two lots each) can be observed in **Table 1**. **Table 2** shows the ANOVA table with means squared and p-values for the Facilities, lots and the interaction of facility and lot. The samples across the four facilities showed inconsistency in the TS and pH ($p< 0.05$). Within the facilities the TS level were consistent, but pH varied between lots ($p<0.05$). The consistency of TS within facilities is likely due to a standardized process within the facilities to maximize solids content to decrease DLP transportation costs. Overall, significant differences were observed for TS and pH for the eight industrial samples obtained ($p<0.05$). These results are consistent with previous research finding DLP composition to be unique across manufacturing facilities and even within the same facility (Liang et al., 2009; Frankowski et al., 2014).

#### 3.4.1.1 Mineral composition of delactosed permeate

The ash, Cl, and mineral composition from the eight industrial DLP samples can be observed in **Table 3**. The minerals ANOVA with means squared and p-values can be observed in **Table 4**. The DLP samples contain varying levels of ash across all suppliers ($p<0.05$) but showed consistent ash composition within facilities. Overall, the total ash composition of the samples ranged from 18-25% on a dry basis, consistent with results observed in previous art (Liang et al., 2009; Frankowski et al., 2014). The selective mineral analysis (Cl, Ca, Na, Mg, P, S, K) shows significant differences between all facilities, lots, and facility:lot interactions ($p<0.05$). The most prevalent monovalent cation was found to be K ranging from 33-58 mg/g dry basis, followed by Na ranging from 8-42 mg/g dry basis. The most prevalent divalent cation present is Ca ranging from 6-18 mg/g dry basis. Anions present include Cl, P, S with Cl being most prevalent. Mineral composition results of DLP showed similar trends as observed in art (Liang et al., 2009; Frankowski et al., 2014).

#### 3.4.1.2 Sugar and organic acid composition of delactosed permeate

Delactosed permeate sugar and organic acid composition can be observed in **Table 5**. The three factor ANOVA table analyzing similarities between the four facilities, two lots and facility:lot interaction can be seen in **Table 6**. Lactose was found to be the highest solid component in the DLP ranging from 54-73% on a dry basis. This is consistent with art and represents a yield loss from the lactose crystallization process

(Liang et al., 2009; Frankowski et al., 2014; Patterson, 2017). Lactose concentrations varied between plants ($p<0.05$). Galactose was detected in approximately half of the samples, with varying levels across manufacturers ($p<0.05$).

Organic acids present in DLP showed significant difference between facilities, lots and facility and lot interactions ($p<0.05$). The concentration of organic acids varied greatly with lactic ranging from 50-123 mg/g db across all eight samples. Formic and citric acid ranged 42-135 mg/g db and 61- 99 mg/g db. The wide range of organic acids present in DLP are due to whey and lactose handling after the cheese process (Liang et al., 2009).

### 3.4.2 Nanofiltration and RO flux

The total NF flux during the fractionation of diluted DLP can be observed in **Table 7**. Total flux for NF processing varied between samples ranging from 15-25 KgMH. There is no apparent correlation between a singular component of the DLP and the NF flux. The varying flux observed between facilities and lots for each facility should be expected when fractionating a highly variable product. Total RO flux during the concentration of the NF permeate is considerably more consistent between facilities and lots and can be observed in **Table 8**. RO flux ranged between 18 and 23 KgMH.

### 3.4.3 Fractionation of DLP components by Nanofiltration

### 3.4.3.1 Fractionation of TS and minerals in DLP

Nanofiltration of DLP was shown to effectively fractionate TS and minerals to varying degrees. The average TS and mineral concentrations of NF feed (NFF), NFR, NFP, and ROR are shown in **Table 9**. The three factor ANOVA analysis for TS and mineral fractionation with means squared and p-values can be observed in **Table 10**. Nanofiltration rejection factors are available in **Table 11**. **Table 12** provides the two factor ANOVA table analyzing TS and mineral rejection factors.

The diluted DLP solution (5% TS) was fractionated into two streams, resulting in a NFR ranging from 9-22% TS and TS rejection factors ranging from 65-94. Nanofiltration permeate solids ranged from 1.01-2.82% TS. It is clear from these results that the samples processed significantly different between trials. Total solids composition was found to be significantly different between facilities, lots and samples (fractions), along with all interactions ($p<0.05$). The rejection factors were also found to significantly different between facilities, lots, and facility and lot interaction ($p<0.05$). This reflects the compositional differences found in the original DLP samples as discussed in section 3.4.1.1.

Ash also showed significant compositional differences across facilities, lots and fractions in addition to their interactions ($p<0.05$). Ash concentration was consistently observed to be higher in the permeate (207-386 mg/g db) than in the retentate (101-231 mg/g db) across all trials. Ash rejection factors ranged from 56-90, each trial showing

significant difference ($p<0.05$). Despite the large range of rejection factors, ash levels on a dry basis were higher in the permeate than in the NF feed or NFR in each experiment. The higher concentration of ash on a dry basis in the permeate indicates that in total minerals were passed allowing for a demineralized retentate stream. Similar NF results have been obtained when fractionating whey permeates, in which the feed stream is fractionated into a reduced mineral retentate stream and mineral rich permeate stream (Suarez et al., 2006; Suarez et al., 2009).

The selective mineral analysis of the NFR and NFP streams provide insight on which mineral components were retained or permeated. The selective mineral composition (Cl, Ca, Na, Mg, P, S, K) between the streams again showed significant difference between facilities, lots, fractions, and their interactions ($p<0.05$). Rejection factors for selective mineral composition (Cl, Ca, Na, Mg, P, S, K) showed significant differences between facilities, lots and the facility lot interaction ($p<0.05$).

Divalent cations (Ca and Mg) were observed to be at higher concentrations in the NFR than the NFP. Calcium and Mg NFR concentrations ranged between 10-21 mg/g db and 3-5 mg/g db respectively. Calcium and Mg NFP concentrations ranged 2-9 mg/g db and 1-3 mg/g db respectively, with two Ca samples being below the detection level and one Mg sample being below the detection level. The rejection factors for Ca and Mg reflected the differences in concentrations, ranging from 88-100 and 81-100 respectively. Overall, divalent cations were observed to be retained during NF processing, consistent with other research conducted on whey permeates (Suarez et al., 2006; Cuartas-Uribe et al., 2007; Suarez et al., 2009; Cuartas-Uribe et al., 2009).

Monovalent cations (Na and K) showed lower concentrations in the NFR than in the NFP. Sodium concentrations were observed to range between 9-35 mg/g db in the NFR and 17-97 mg/g db in the NFP. Sodium rejection factors ranged 43-83, but the concentration of Na on a dry basis was higher in the NFP for every sample. Potassium showed similar results, ranging from 31-40 mg/g db in the NFR and 59-112 mg/g in the NFP. Potassium rejection factors ranged 45-85, but again showed higher concentrations on a dry basis in the NFP. The high level of monovalent cation permeation observed agrees with previous NF research conducted on whey permeates, which indicated high permeations rates for Ca and $Mg^{+}$) (Suarez et al., 2006; Cuartas-Uribe et al., 2007; Suarez et al., 2009; Cuartas-Uribe et al., 2009).

The anions observed during selective mineral analysis provided varying results. Monovalent anions (Cl) permeated at a high level with NFR concentrations ranging 1-27 mg/g db and NFP concentrations ranging 72-220 mg/g db. This high permeation rate led to the negative rejection factors observed in **Table 12**. Negative rejection factors of small monovalent anions are an anomaly observed during NF processing, explained by the Donnan effect. The Donnan effect occurs to maintain ionic balance between the retentate and permeate sides of the membrane (Cuartas-Uribe et al. 2007). The Donnan effect was consistently observed in prior art focused on NF of whey permeates (Suarez et al., 2006; Cuartas-Uribe et al., 2007; Cuartas-Uribe et al., 2009). This effect is so prevalent with nanofiltered whey permeates due to its acidic nature and the negative charge of the NF membrane favoring low molecular weight monovalent ion permeation. The culmination of these factors promotes negative rejection factors of Cl to maintain electroneutrality (Suarez et al., 2006). Phosphorus, a trivalent anion, showed higher concentrations in the

NFP (13-26 mg/g db) when compared to the NFR (18-50 mg/g db). The rejection factor for P ranged 62-87 and all but one facility (Plant 4) showed lower concentrations on a dry basis in the NFR than the NFP. Sulphur, a divalent anion, showed a high rejection factor (85-100) and NFP concentrations (below detection to 15 mg/g db). Despite the high anion permeation observed with Cl and P, the large molecular weight of S (<300 Da) likely prevented permeation (Eriksson, 1988).

### 3.4.3.2 Fractionation of sugars and organic acids in DLP

Nanofiltration showed to effectively fractionate sugar and organic acid components of diluted DLP. Sugar and organic acid concentrations found in the NFF, NFR, NFP and ROR can be observed in **Table 13**. **Table 14** provides the three factors (facility, lot and fraction) with interactions ANOVA table. Rejection factors for sugars and organic acids are highlighted in **Table 15**. **Table 16** shows the two factor ANOVA with interaction (facility, lot and facilty:lot) preformed for the rejection factors of sugars and organic acids.

Lactose, the primary sugar and solid component of DLP, showed higher concentration in the NFR (57-77 mg/g db) than found in the NFP (32.4-64.2 mg/g db). Lactose concentrations for each fraction showed significant differences for facility, lot, sample, and the interactions ($p<0.05$). Lactose rejection factors were observed to range between 71-97 across all trials. Lactose rejection factors were observed to be significantly different for each factor (facility, lot and facility*lot) ($p<0.05$). For each

trial, the concentration of lactose was observed to be higher in the NFR than the concentration in the NFP. Overall, nanofiltration preformed on diluted DLP concentrated lactose in the NFR. Lactose retention results agree with previous art which preformed nanofiltration on deproteinized whey solutions (Atra et al.,2005; Cuartas-Uribe et al. 2007). Variability in lactose concentrations of the fractions and rejection factors can likely be attributed to the highly variable ionic composition of the DLP, which due to their charge, are thought to increase the apparent MWCO for the neutral lactose molecule (Cuartas-Uribe et al. 2007).

Galactose fractionation during the experiment was highly variable. Galactose concentrations in the NFR ranged from below detection to 42 mg/g db and ranged from below detection to 120 mg/g db in the NFP. Statistical significance was observed between facilities, lots, and samples along with all interactions ($p<0.05$) but showed no statistical significance for the lot and sample interaction, likely due to numerous samples being below detection levels. Rejection factors for galactose ranged significantly (-125 to 100). This again was highly variable due to detection levels but may also be attributed to other factors. As galactose concentration was generally observed to be in higher concentrations in the NFP and ROR, the microbial breakdown of lactose during processing would show increased concentrations of galactose in these streams. This would potentially explain the noticeable increase of galactose in the ROR when compared to the NFR as seen in the plant 1 lot 1, plant 4 lot 1, and plant 4 lot 2 trials.

Organic acids (lactic, formic, and citric) were shown to fractionate in the NFR and NFP to varying degrees. Citric acid showed significant differences between the facilities, lots, samples and their interactions ($p<0.05$) but did not show significant

differences in rejection factors. The concentration of citric acid showed consistently higher concentrations in the NFR (66-110 mg/g db) than the NFP (12-79 mg/g db) with rejection factors ranging 86-99. Citric acid was likely retained at such high levels due to it being the largest organic acid molecule analyzed (192 Da). Lactic acid showed statistical significance between facilities, lots, and samples along with most interactions ($p<0.05$) but showed no statistical significance for the lot and sample interaction, indicating that the lactic acid concentrations in the separate fractions for different lots were relatively consistent. Rejection factors for lactic acid ranged widely from 5-65 between. Rejection factors between trials showed significance for manufacturing facility, lot and facility*lot interactions ($p<0.05$). Formic acid present in diluted DLP was observed to fractionate similarly to lactic acid. Formic acid concentrations in each fraction were shown to be significant for facilities, lots and samples along with their interactions ($p<0.05$). Rejection factors for formic acid ranged between from -273 to 74, and showed significant difference between facilities, lots and facility*lot interaction ($p<0.05$). Negative rejection factors were observed in two instances for formic acid. While observing the Donnan effect on an organic acid is an anomaly, formic acids small molecular weight and the anionic nature may have led to the negative rejection factor to maintain electroneutrality. Both lactic and formic acids were found to have lower concentrations on a dry basis in the NFR when compared to the NFP. The lactic acid concentration ranged 18-56 mg/g db in the retentate and 50-190 mg/g db in the permeate. Formic acid concentrations ranged 11-80 mg/g db in the NFR and 59-172 mg/g db in the NFP. Overall, organic acid fractionation followed a similar trend when compared to

minerals, with larger molecular weight molecules concentrating the NFR fraction and smaller molecular weight molecules showing higher concentrations in the NFP fraction.

## 3.5 CONCLUSIONS

The eight DLP samples obtained from four facilities varied greatly both between facilities and within them. The inconsistency of the original DLP led to inconsistencies for NF processing, as NF flux and composition of the streams varied across all trials. Despite the compositional differences in DLP, NF processing did show significant trends that may lead to potential food grade applications for DLP rather than animal feed or waste.

The application of NF processing resulted in a retentate with more nutritional value. The increased lactose concentration observed in the NFR was a desired effect and supported the original hypothesis. In addition, the NFR provided a stream with decreased concentrations of monovalent ions (Cl, Na, K), trivalent anions (P), and low molecular weight organic acids (lactic and formic). While larger molecular weight impurities were retained (Ca, Mg, S, citric acid), NF processing of DLP reduced the concentration of many unwanted impurities in the retentate. The reduction of impurities in DLP NFR may allow it to be utilized back into the lactose crystallization process or as a low-cost food additive.

Nanofiltration of DLP also provided a permeate that is rich in minerals and organic acids. Monovalent ions, P and low molecular weight organic acids showed

increased concentrations in the NFP. While these items are considered impurities for lactose crystallization, the NFP fraction may be utilized as a separate food additive. The combination of K, Cl, and lactic acid may help promote the salty flavor of the NFP, while maintaining lower levels of Na. In a market place focused on reducing Na intake, but not willing to sacrifice flavor, the combination of minerals and organic acids present in the DLP NFP may find uses as a food grade ingredient.

The results of this research promote future investigations. The viability of these two permeate streams as food grade ingredients should be tested. Recycling the NFR back into the lactose crystallization stream will be examined in chapter 2 of this thesis, but other food grade applications should also be explored. The utilization of the NFP stream as a reduced Na flavor enhancer should be investigated, but other hurtles in its application remain. Using RO, the concentration of solids was increased, but in order to be efficiently transported increasing the TS of this material via evaporation or drying should be further explored.

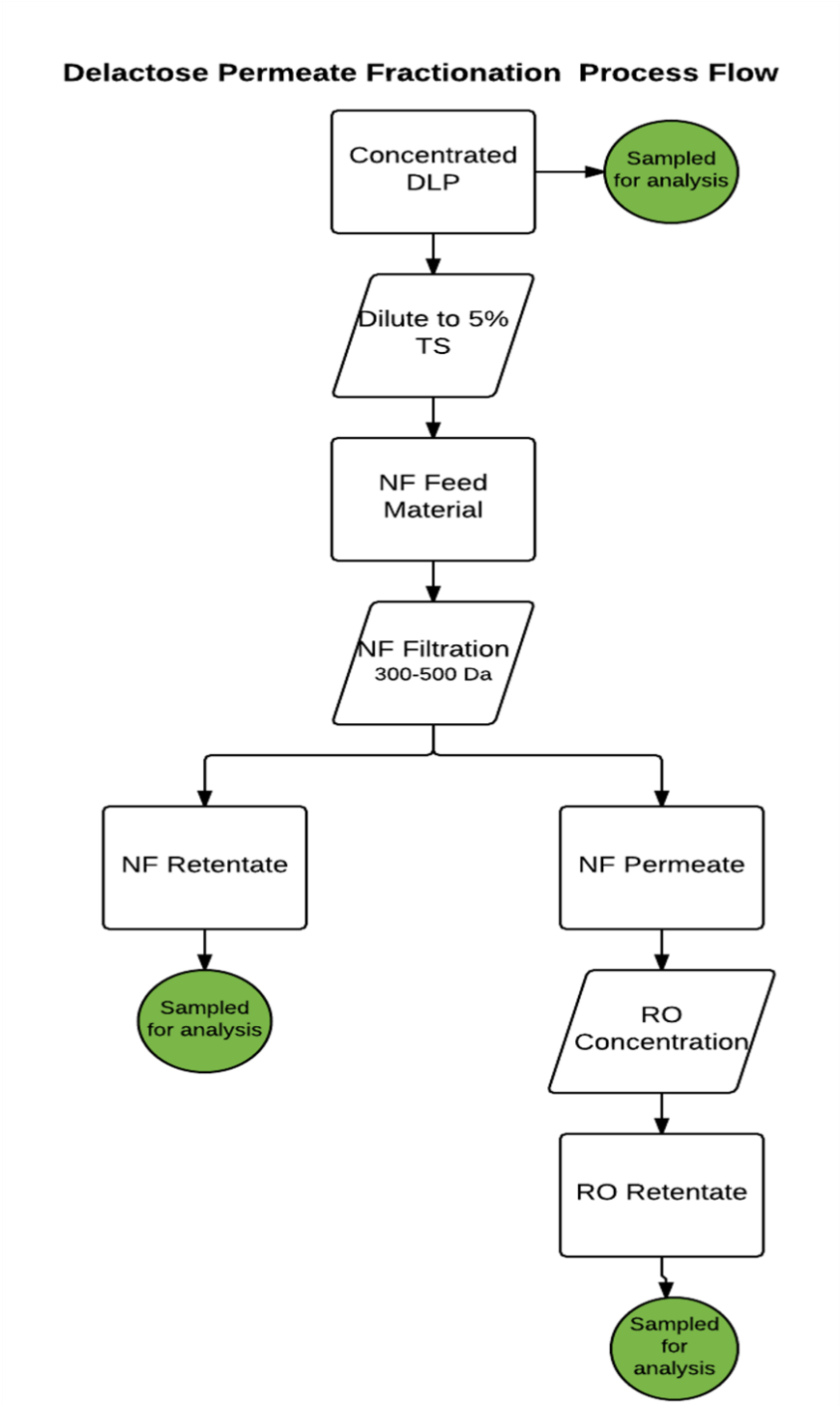


*Figure 1. Process flow diagram of DLP NF fractionation and RO concentration of the NFP*

*Table 1. Total solids and pH of of DLP sourced from four plants*

| Facility | Lot | TS (%) | pH |
|---|---|---|---|
| **Plant 1** | **1** | 29.1 | 5.43 |
| | **2** | 30.3 | 5.39 |
| **Plant 2** | **1** | 29.3 | 5.16 |
| | **2** | 28.7 | 5.14 |
| **Plant 3** | **1** | 36.7 | 5.50 |
| | **2** | 39.3 | 5.96 |
| **Plant 4** | **1** | 27.9 | 6.25 |
| | **2** | 28.3 | 6.33 |

*Table 2. Means square and p-values for DLP TS and pH*

| | df | TS | | pH | |
|---|---|---|---|---|---|
| **Facility** | 3 | 112.75 | * | 1.2757 | * |
| | | 6.54E-13 | | <2e-16 | |
| **Lot** | 1 | 0.05 | | 0.164 | * |
| | | 0.250847 | | <2e-16 | |
| **Facility*Lot** | 3 | 0.69 | * | 0.0726 | * |
| | | 2.85E-04 | | <2e-16 | |
| **Error** | 8 | 0.03 | | 0 | |

*Table 3. Average ash and mineral composition (mg/g dry weight) of DLP sourced from four plants*

| Facility | Lot | Ash | Cl | Ca | Na | Mg | P | S | K |
|---|---|---|---|---|---|---|---|---|---|
| **Plant 1** | **1** | 222.2 | 71.8 | 10.5 | 11.8 | 3.1 | 23.3 | 3.4 | 40.7 |
| | **2** | 220.0 | 64.1 | 14.1 | 12.3 | 3.6 | 23.1 | 4.2 | 37.5 |
| **Plant 2** | **1** | 253.8 | 19.1 | 16.4 | 42.1 | 3.9 | 24.6 | 22.7 | 42.5 |
| | **2** | 249.9 | 14.3 | 17.8 | 37.5 | 4.1 | 26.1 | 21.3 | 45.7 |
| **Plant 3** | **1** | 183.5 | 54.9 | 8.3 | 14.6 | 2.2 | 14.8 | 1.5 | 58.3 |
| | **2** | 204.5 | 57.1 | 6.7 | 8.4 | 2.2 | 17.4 | 1.8 | 33.1 |
| **Plant 4** | **1** | 224.6 | 69.8 | 10.0 | 14.1 | 2.8 | 23.9 | 2.8 | 40.9 |
| | **2** | 205.1 | 57.9 | 9.7 | 13.6 | 2.8 | 21.8 | 2.5 | 38.4 |

*Table 4. Means square and p-values for DLP ash, Cl and selective mineral analysis*

| | df | Ash | | Cl | | Ca | | Na | | Mg | | P | | S | | K | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Facility** | 3 | 2288.5 | * | 2206.5 | * | 68.01 | * | 750.2 | * | 2.3289 | * | 63.64 | * | 375.4 | * | 42.68 | * |
| | | 7.57E-07 | | 8.60E-15 | | 2.45E-14 | | < 2e-16 | | 1.62E-12 | | 2.2E-10 | | < 2e-16 | | 1.74E-09 | |
| **Lot** | 1 | 5.7 | | 123.7 | * | 2.57 | * | 29.3 | * | 0.1135 | * | 0.76 | * | 0.1 | * | 191.1 | * |
| | | 6.16E-01 | | 7.49E-09 | | 9.97E-08 | | 6.66E-11 | | 2.13E-06 | | 1.22E-02 | | 1.08E-03 | | 3.93E-11 | |
| **Facility*Lot** | 3 | 279.8 | * | 34.7 | * | 5.12 | * | 10 | * | 0.0694 | * | 4.1 | * | 0.9 | * | 156.53 | * |
| | | 1.70E-03 | | 1.32E-07 | | 7.49E-10 | | 5.33E-10 | | 1.81E-06 | | 1.05E-05 | | 1.55E-07 | | 9.77E-12 | |
| **Error** | 8 | 20.7 | | 0.2 | | 0.01 | | 0 | | 0.0008 | | 0.07 | | 0 | | 0.08 | |

*Table 5. Sugar and organic acid composition of DLP sourced from four plants*

| Facility | Lot | Lactose (% db) | Galactose (mg/g db) | Lactic (mg/g db) | Formic (mg/g db) | Citric (mg/g db) |
|---|---|---|---|---|---|---|
| **Plant 1** | **1** | 58.35 | 30.5 | 76.3 | 86.9 | 96.4 |
| | **2** | 53.82 | 67.5 | 122.6 | 134.6 | 64.8 |
| **Plant 2** | **1** | 58.23 | bd | 53.7 | 52.7 | 96.3 |
| | **2** | 58.28 | bd | 53.7 | 42.4 | 99.2 |
| **Plant 3** | **1** | 53.67 | 27.1 | 49.4 | 59.2 | 61.3 |
| | **2** | 61.75 | bd | 55.7 | 83.2 | 64.4 |
| **Plant 4** | **1** | 61.93 | 7.0 | 69.6 | 89.8 | 87.9 |
| | **2** | 73.37 | bd | 62.4 | 72.4 | 70.5 |

*Table 6. Means square and p-values for DLP HPLC analysis*

| | df | Lactose | | Galactose | | Lactic | | Formic | | Citric | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Facility** | 3 | 132.86 | * | 2007.5 | * | 1917.3 | * | 2739.4 | * | 811.2 | * |
| | | 1.57E-03 | | 3.72E-11 | | 4.52E-06 | | 0.000000432 | | 0.0000265 | |
| **Lot** | 1 | 23 | | 2.1 | | 516.5 | * | 483.4 | * | 461.6 | * |
| | | 0.16039 | | 2.71E-01 | | 0.002523 | | 0.00146 | | 0.00108 | |
| **Facility*Lot** | 3 | 45.09 | * | 714.5 | * | 573.7 | * | 926.1 | * | 285 | * |
| | | 3.57E-02 | | 2.28E-09 | | 3.92E-04 | | 2.78E-05 | | 0.00111 | |
| **Error** | 8 | 9.61 | | 1.5 | | 27.6 | | 21.5 | | 18.6 | |

*Table 7. Total flux (KgMH) for Nanofiltration*

| Facility | Lot | Total Flux |
|---|---|---|
| **Plant 1** | **1** | 18.4 |
| | **2** | 15.2 |
| **Plant 2** | **1** | 19.6 |
| | **2** | 21.8 |
| **Plant 3** | **1** | 25.2 |
| | **2** | 17.9 |
| **Plant 4** | **1** | 18.1 |
| | **2** | 14.9 |

*Table 8. Total flux (KgMH) for RO*

| Facility | Lot | Total Flux |
|---|---|---|
| **Plant 1** | **1** | 22.1 |
| | **2** | 18.0 |
| **Plant 2** | **1** | 23.2 |
| | **2** | 21.2 |
| **Plant 3** | **1** | 19.4 |
| | **2** | 20.5 |
| **Plant 4** | **1** | 19.8 |
| | **2** | 21.6 |

*Table 9. Average TS, ash, salt and mineral composition of each fractionation streams for all trials conducted (four sources, two lots each)*

| Facility | Lot | Sample | TS | Ash | Cl | Ca | Na | Mg | P | S | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | % | (mg/g db) | (mg/g db) | (mg/g db) | (mg/g db) | (mg/g db) | (mg/g db) | (mg/g db) | (mg/g db) |
| **Plant 1** | **1** | **NF Feed** | 5.47 | 202.8 | 71.3 | 9.3 | 18.2 | 2.9 | 21.9 | 4.9 | 55.0 |
| | | **NF Retentate** | 12.08 | 167.3 | 23.2 | 15.0 | 12.2 | 3.8 | 19.4 | 5.6 | 38.3 |
| | | **NF Permeate** | 2.82 | 283.0 | 156.3 | 3.8 | 26.4 | 1.7 | 25.3 | 3.3 | 78.6 |
| | | **RO Retentate** | 8.99 | 306.1 | 151.4 | 4.1 | 21.5 | 1.8 | 27.1 | 3.4 | 66.7 |
| **Plant 1** | **2** | **NF Feed** | 5.24 | 202.3 | 68.8 | 11.7 | 19.2 | 3.5 | 21.7 | 5.9 | 52.5 |
| | | **NF Retentate** | 11.31 | 171.4 | 24.8 | 19.8 | 12.4 | 4.5 | 20.3 | 6.9 | 34.7 |
| | | **NF Permeate** | 2.94 | 285.3 | 129.3 | 9.2 | 27.0 | 3.3 | 25.5 | 3.9 | 73.6 |
| | | **RO Retentate** | 8.69 | 305.3 | 128.9 | 9.5 | 22.2 | 3.3 | 26.4 | 3.9 | 63.7 |
| **Plant 2** | **1** | **NF Feed** | 5.08 | 255.2 | 21.7 | 17.7 | 42.6 | 4.1 | 26.4 | 19.4 | 44.5 |
| | | **NF Retentate** | 17.53 | 230.7 | 1.1 | 19.7 | 35.0 | 4.6 | 21.7 | 25.8 | 35.1 |
| | | **NF Permeate** | 1.01 | 386.1 | 128.7 | bd | 97.0 | bd | 50.2 | 13.3 | 91.9 |
| | | **RO Retentate** | 7.50 | 397.3 | 117.3 | 3.7 | 97.9 | 1.3 | 47.7 | 14.6 | 89.6 |
| **Plant 2** | **2** | **NF Feed** | 5.22 | 251.0 | 15.3 | 18.5 | 36.8 | 4.3 | 26.9 | 23.4 | 44.7 |
| | | **NF Retentate** | 18.52 | 226.3 | 1.1 | 21.7 | 29.7 | 4.9 | 21.4 | 23.7 | 35.9 |
| | | **NF Permeate** | 1.39 | 338.1 | 71.9 | 4.4 | 69.2 | 1.5 | 48.8 | 14.7 | 79.6 |
| | | **RO Retentate** | 7.79 | 368.0 | 66.8 | 4.9 | 74.8 | 1.6 | 46.7 | 16.8 | 81.9 |
| **Plant 3** | **1** | **NF Feed** | 4.94 | 177.3 | 60.7 | 7.9 | 18.1 | 2.6 | 16.6 | 3.9 | 61.6 |
| | | **NF Retentate** | 21.69 | 101.0 | 6.0 | 10.8 | 8.9 | 3.1 | 13.1 | 4.2 | 31.3 |
| | | **NF Permeate** | 1.50 | 380.5 | 220.1 | 3.1 | 34.0 | 1.7 | 25.7 | bd | 112.1 |
| | | **RO Retentate** | 8.23 | 383.3 | 195.5 | 4.1 | 43.5 | 2.0 | 0.0 | 3.0 | 150.0 |
| **Plant 3** | **2** | **NF Feed** | 5.23 | 187.1 | 61.2 | 7.0 | 14.7 | 2.3 | 17.4 | 3.6 | 51.3 |
| | | **NF Retentate** | 9.66 | 164.9 | 24.8 | 10.4 | 11.1 | 3.1 | 16.3 | 4.4 | 40.8 |
| | | **NF Permeate** | 3.39 | 206.5 | 103.4 | 2.7 | 17.3 | 1.3 | 17.5 | bd | 59.2 |
| | | **RO Retentate** | 9.12 | 219.8 | 100.9 | 2.9 | 15.7 | 1.3 | 18.2 | 2.2 | 53.4 |
| **Plant 4** | **1** | **NF Feed** | 4.94 | 210.6 | 68.9 | 10.6 | 21.5 | 2.7 | 24.8 | 3.4 | 54.9 |
| | | **NF Retentate** | 10.35 | 184.1 | 23.2 | 14.3 | 14.5 | 3.5 | 25.6 | 3.9 | 39.2 |
| | | **NF Permeate** | 2.25 | 251.2 | 160.1 | bd | 32.2 | 0.8 | 21.1 | bd | 79.8 |
| | | **RO Retentate** | 7.87 | 303.5 | 166.4 | 2.0 | 25.4 | 0.9 | 22.2 | 2.4 | 67.9 |
| **Plant 4** | **2** | **NF Feed** | 4.96 | 188.5 | 56.4 | 10.1 | 20.7 | 2.6 | 22.9 | 3.1 | 50.7 |
| | | **NF Retentate** | 8.97 | 180.0 | 26.7 | 14.3 | 15.2 | 3.5 | 24.6 | 3.6 | 39.8 |
| | | **NF Permeate** | 2.73 | 206.9 | 117.2 | 2.1 | 26.0 | 1.0 | 17.8 | bd | 62.5 |
| | | **RO Retentate** | 8.48 | 238.1 | 117.9 | 2.4 | 21.6 | 1.1 | 18.8 | 2.3 | 55.1 |

*Table 10. Means squared and p-values forl NFF, NFR, NFP and ROR for TS, Ash, Cl and selective minerals*

| | df | TS | Ash | Cl | Ca | Na | Mg | P | S | K |
|---|---|---|---|---|---|---|---|---|---|---|
| **Facility** | 3 | 10.1 * | 24885 * | 6882 * | 101.8 * | 6283 * | 4.059 * | 1177.5 * | 944.7 * | 616 * |
| | | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 |
| **Lot** | 1 | 4.6 * | 14482 * | 13048 * | 41.1 * | 840 * | 1.849 * | 0.07 * | 2.8 * | 2971 * |
| | | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | 2.80E-02 | 1.51E-07 | <2e-16 |
| **Sample** | 3 | 1167.8 * | 205469 * | 55486 * | 581.2 * | 6786 * | 22.368 * | 238.8 * | 90.5 * | 7030 * |
| | | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | 5.33E-10 | <2e-16 | <2e-16 | <2e-16 |
| **Facility*Lot** | 3 | 16.7 * | 9552 * | 872 * | 20.2 * | 685 * | 1.569 * | 23.9 * | 2 * | 1044 * |
| | | <2e-16 | 8.99E-16 | 1.95E-13 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | 8.96E-10 | <2e-16 |
| **Facility*Sample** | 9 | 151.5 * | 18190 * | 383 * | 31 * | 4615 * | 1.472 * | 250.5 * | 13.1 * | 374 * |
| | | <2e-16 | <2e-16 | 1.47E-12 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 |
| **Lot*Sample** | 3 | 41.5 * | 20740 * | 13740 * | 4 * | 538 * | 0.238 * | 28.5 * | 0.9 * | 881 * |
| | | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | 5.09E-06 | <2e-16 |
| **Facility*Lot*Sample** | 9 | 90.9 * | 26791 * | 5094 * | 0.7 * | 432 * | 0.173 * | 31.8 * | 1.6 * | 485 * |
| | | <2e-16 | <2e-16 | 5.86E-15 | <2e-16 | <2e-16 | <2e-16 | <2e-16 | 2.58E-12 | <2e-16 |
| **Error** | 32 | 0 | 35 | 14 | 0 | 6 | 0.001 | 0.1 | 0.1 | 1 |

*Table 11. Average rejection factors during NF processing (500 Da) for TS, ash, Cl, and minerals*

| Facility | Lot | TS | Ash | Cl | Ca | Na | Mg | P | S | K |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant 1** | **1** | 76.7 | 60.5 | -57.1 | 94.1 | 49.7 | 89.5 | 69.5 | 86.1 | 52.2 |
| | **2** | 74.0 | 56.7 | -35.7 | 87.9 | 43.4 | 81.2 | 67.3 | 85.2 | 44.9 |
| **Plant 2** | **1** | 94.2 | 90.4 | -550.0 | 100.0 | 84.0 | 100.0 | 86.7 | 97.0 | 84.9 |
| | **2** | 92.5 | 88.8 | -400.0 | 98.5 | 82.5 | 97.7 | 82.9 | 95.3 | 83.3 |
| **Plant 3** | **1** | 93.1 | 74.0 | -154.8 | 98.0 | 73.5 | 96.2 | 86.4 | 100.0 | 75.2 |
| | **2** | 65.0 | 56.1 | -45.8 | 90.9 | 45.1 | 85.9 | 62.4 | 100.0 | 49.2 |
| **Plant 4** | **1** | 78.3 | 70.3 | -50.0 | 100.0 | 51.8 | 94.7 | 82.1 | 100.0 | 55.8 |
| | **2** | 69.6 | 65.0 | -33.3 | 95.4 | 48.1 | 91.4 | 78.0 | 100.0 | 52.2 |

*Table 12. Means squared and p-values for NF rejection factors for TS, Ash, Cl and selective minerals*

| | df | TS | | Ash | | Cl | | Ca | | Na | | Mg | | P | | S | | K | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Facility** | 3 | 316.7 | * | 722.8 | * | 172738 | * | 53.34 | * | 1097.3 | * | 123.83 | * | 201.11 | * | 183.85 | * | 978.2 | * |
| | | < 2e-16 | | 7.78E-09 | | 2.81E-08 | | 3.04E-14 | | 2.65E-11 | | 1.77E-10 | | 5.35E-10 | | < 2e-16 | | 5.47E-12 | |
| **Lot** | 1 | 425.8 | * | 204 | * | 22056 | * | 94.56 | * | 401.1 | * | 146.42 | * | 290.08 | * | 1.72 | * | 369.4 | * |
| | | < 2e-16 | | 8.64E-06 | | 4.51E-04 | | 2.77E-14 | | 1.28E-08 | | 8.03E-10 | | 1.10E-09 | | 1.81E-07 | | 2.35E-09 | |
| **Facility*Lot** | 3 | 150.6 | * | 53.2 | * | 4349 | * | 6.14 | * | 154.3 | * | 15.01 | * | 107.16 | * | 0.66 | * | 125.9 | * |
| | | 1.56E-15 | | 0.000178 | | 1.60E-02 | | 1.71E-10 | | 6.45E-08 | | 5.33E-10 | | 6.54E-09 | | 9.31E-07 | | 1.92E-08 | |
| **Error** | 8 | 0 | | 2 | | 678 | | 0.01 | | 0.7 | | 0.14 | | 0.29 | | 0.01 | | 0.4 | |

*Table 13. Lactose and organic composition (mg/g dry weight) of each fractionation streams for all trials conducted (four sources, two lots each*

| Facility | Lot | Sample | Lactose (% db) | Galactose (mg/g db) | Lactic (mg/g db) | Formic (mg/g db) | Citric (mg/g db) |
|---|---|---|---|---|---|---|---|
| **Plant 1** | **1** | **NF Feed** | 58.40 | 26.15 | 79.70 | 89.89 | 88.08 |
| | | **NF Retentate** | 73.39 | 3.72 | 55.15 | 30.07 | 94.84 |
| | | **NF Permeate** | 46.32 | 35.91 | 94.54 | 114.89 | 37.82 |
| | | **RO Retentate** | 43.18 | 59.00 | 105.20 | 83.55 | 31.18 |
| **Plant 1** | **2** | **NF Feed** | 56.45 | 47.27 | 117.50 | 131.10 | 67.26 |
| | | **NF Retentate** | 72.44 | 34.02 | 23.79 | 11.14 | 66.19 |
| | | **NF Permeate** | 44.89 | 120.10 | 110.40 | 159.98 | 20.35 |
| | | **RO Retentate** | 38.72 | 104.88 | 149.69 | 115.67 | 28.31 |
| **Plant 2** | **1** | **NF Feed** | 55.32 | bd | 56.08 | 55.04 | 99.72 |
| | | **NF Retentate** | 57.26 | 5.73 | 18.61 | 34.68 | 87.52 |
| | | **NF Permeate** | 32.40 | 31.70 | 190.23 | 171.92 | 79.28 |
| | | **RO Retentate** | 34.65 | 38.07 | 201.44 | 160.65 | 70.26 |
| **Plant 2** | **2** | **NF Feed** | 54.54 | bd | 58.57 | 47.04 | 103.91 |
| | | **NF Retentate** | 60.11 | bd | 18.08 | 26.40 | 101.45 |
| | | **NF Permeate** | 38.47 | bd | 168.53 | 125.94 | 11.53 |
| | | **RO Retentate** | 40.22 | 8.10 | 164.27 | 121.16 | 22.82 |
| **Plant 3** | **1** | **NF Feed** | 52.46 | 33.57 | 57.74 | 60.51 | 61.04 |
| | | **NF Retentate** | 57.83 | 42.38 | 21.01 | 25.13 | 66.45 |
| | | **NF Permeate** | 33.11 | 22.01 | 117.54 | 143.11 | 34.26 |
| | | **RO Retentate** | 37.53 | 23.68 | 130.46 | 139.77 | 31.80 |
| **Plant 3** | **2** | **NF Feed** | 62.43 | bd | 59.60 | 61.78 | 65.16 |
| | | **NF Retentate** | 75.20 | 6.20 | 21.21 | 18.64 | 89.40 |
| | | **NF Permeate** | 62.83 | bd | 49.58 | 59.41 | 30.90 |
| | | **RO Retentate** | 65.30 | bd | 55.96 | 65.03 | 31.81 |
| **Plant 4** | **1** | **NF Feed** | 69.55 | 7.43 | 70.78 | 88.09 | 87.23 |
| | | **NF Retentate** | 75.67 | 13.67 | 55.77 | 79.69 | 108.47 |
| | | **NF Permeate** | 57.87 | 12.90 | 96.15 | 96.42 | 36.60 |
| | | **RO Retentate** | 57.17 | 27.48 | 100.30 | 118.28 | 60.68 |
| **Plant 4** | **2** | **NF Feed** | 67.23 | bd | 63.71 | 63.05 | 78.65 |
| | | **NF Retentate** | 77.07 | 6.18 | 51.31 | 40.81 | 109.98 |
| | | **NF Permeate** | 64.16 | 1.24 | 80.03 | 71.83 | 52.53 |
| | | **RO Retentate** | 65.63 | 8.45 | 95.94 | 77.91 | 47.99 |

*Table 14. Means squared and p-values forl NFF, NFR, NFP and ROR for HPLC analysis*

| | df | Lactose | | Galactose | | Lactic | | Formic | | Citric | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Facility** | 3 | 1127.1 | * | 7120 | * | 6133 | * | 1681 | * | 2075 | * |
| | | 7.55E-16 | | 4.44E-15 | | 1.16E-06 | | 4.63E-04 | | 3.91E-08 | |
| **Lot** | 1 | 691.6 | * | 146 | * | 1651 | * | 5431 | * | 1351 | * |
| | | 1.41E-08 | | 0.20455 | | 4.29E-02 | | 0.000018 | | 0.0005 | |
| **Sample** | 3 | 1677.2 | * | 1580 | * | 28480 | * | 23992 | * | 11875 | * |
| | | < 2e-16 | | 4.76E-07 | | 1.06E-14 | | < 2e-16 | | < 2e-16 | |
| **Facility*Lot** | 3 | 411.1 | * | 4342 | * | 1819 | * | 3494 | * | 789 | * |
| | | 5.03E-10 | | 3.75E-12 | | 0.0065 | | 1.32E-06 | | 0.000218 | |
| **Facility*Sample** | 9 | 65 | * | 1009 | * | 3744 | * | 2584 | * | 205 | * |
| | | 1.83E-04 | | 8.14E-08 | | 3.80E-07 | | 5.01E-08 | | 0.041769 | |
| **Lot*Sample** | 3 | 71.3 | * | 111 | | 761 | | 879 | * | 365 | * |
| | | 2.65E-03 | | 0.30125 | | 1.27E-01 | | 0.014311 | | 0.014963 | |
| **Facility*Lot*Sample** | 9 | 17.7 | | 347 | * | 848 | * | 821 | * | 539 | * |
| | | 2.09E-01 | | 1.74E-03 | | 0.0416 | | 2.27E-03 | | 6.81E-05 | |
| **Error** | 32 | 12.2 | | 87 | | 371 | | 214 | | 90 | |

*Table 15. Average rejection factors during NF processing (500 Da) for sugars and organic acids*

| Facility | Lot | Lactose | Galactose | Lactic | Formic | Citric |
|---|---|---|---|---|---|---|
| **Plant 1** | **1** | 85.0 | -125.0 | 60.0 | 10.6 | 90.7 |
| | **2** | 83.8 | 7.9 | 4.6 | -273.2 | 91.9 |
| **Plant 2** | **1** | 96.7 | 68.0 | 40.8 | 71.4 | 94.8 |
| | **2** | 95.2 | bd | 30.0 | 64.2 | 99.1 |
| **Plant 3** | **1** | 96.0 | 96.4 | 61.3 | 60.6 | 96.4 |
| | **2** | 70.7 | 100.0 | 17.9 | -11.7 | 87.7 |
| **Plant 4** | **1** | 83.4 | 79.5 | 62.5 | 73.7 | 92.7 |
| | **2** | 74.7 | 88.3 | 52.5 | 46.4 | 85.6 |

*Table 16. Means squared and p-values for NF rejection factors for TS, Ash, Cl and selective minerals*

| | df | Lactose | | Galactose | | Lactic | | Formic | | Citric |
|---|---|---|---|---|---|---|---|---|---|---|
| **Facility** | 3 | 628.2 | * | 20085 | * | 509 | * | 1588.7 | * | 43.56 |
| | | 2.62E-06 | | 5.41E-05 | | 4.67E-04 | | 8.46E-06 | | 0.164 |
| **Lot** | 1 | 339.1 | * | 1493 | | 3583 | * | 594.2 | * | 26.16 |
| | | 3.19E-06 | | 0.14051 | | 2.44E-06 | | 0.000479 | | 0.282 |
| **Facility*Lot** | 3 | 382.7 | * | 6963 | * | 531 | * | 201.8 | * | 39.94 |
| | | 1.74E-05 | | 2.19E-03 | | 0.000401 | | 7.22E-03 | | 1.88E-01 |
| **Error** | 8 | 20.9 | | 558 | | 26 | | 12.7 | | 19.65 |

# 4. Chapter 2

## Recovery of lactose crystals from delactosed permeate nanofiltration retentate blended with concentrated whey permeate

### 4.1 ABSTRACT

Edible grade lactose manufacture, a common practice across the dairy industry to recover lactose from deproteinized whey for food grade applications, produces delactosed permeate (DLP) as a byproduct. Despite being rich in lactose, DLP is often relegated to animal feed due to its high ash and organic acid content. Previous work applied nanofiltration (500 Da MWCO, NFW-3B-3838, Synder Filtration) to DLP to concentrate lactose on a dry basis in the retentate (NFR) while decreasing concentrations of small molecular weight minerals and organic acids. This study aims to recycle DLP NFR back into the edible grade lactose manufacturing process to determine the viability extending this process to the industry, creating a food grade application for DLP.

Delactosed permeate NFR (one lot) was blended with three separate lots of concentrated whey permeate (CWP) across three separate trials. The DLP NFR and the CWP were sourced from the same manufacturer. Each experimental blend was composed of approximately 30% TS on a dry basis from DLP NFR and approximately 70% TS on a dry basis CWP. Once blended, the TS were concentrated to the approximate TS of the CWP using a lab scale rotary evaporator (Heidolph). The experimental blend was then crystalized in parallel with a CWP only control using a lab scale crystallization apparatus with an automated ramp program cooling from 80 ˚C to 20 ˚C over 20 hours (-0.05 ˚C per minute). Control and experimental samples were seeded at 70 ˚C. Following

crystallization, both experimental and control samples were refined using cold wash water, centrifugation, and decantation (3X).

Crystallizer feed material (control and blended) was analyzed for TS and lactose concentration (HPLC). Refined lactose and supernatants were analyzed for TS. Refined lactose crystal size was determined microscopically. Lactose concentration (g lactose / 100g water), actual yield, theoretical yield, and actual vs theoretical yield ratios were calculated.

The experimental DLP NFR samples provided lower actual yield, actual vs theoretical yield ratios, and mean crystal size when compared to the CWP only controls ($p<0.05$). The recycling of DLP NFR in this experiment showed an overall negative impact on edible grade lactose manufacture, despite the DLP NFR having decreased impurity concentrations when compared to DLP alone.

## 4.2 INTRODUCTION

Edible grade lactose manufacture is a common practice across the dairy industry, as it provides a food grade revenue stream for deproteinized whey or milk permeates. Milk or whey permeate is concentrated to create a supersaturated solution. The supersaturated permeate crystalizes during a controlled cooling process, forming α-lactose monohydrate crystals. Lactose crystals are then refined by removing soluble solids and water, or DLP. The refined lactose crystals are then dried and packaged (Patterson,2009).

Lactose manufacture is complex and varies greatly across the industry (Patterson, 2017). However, each of the afore mentioned manufacturing steps have the same intent for all manufacturers, maximizing lactose yield. Whey permeate is concentrated to a supersaturated level so that a primary nucleation event can occur, and lactose can crystalize out of solution while being exposed to a cooling curve to maintain supersaturation within the metastable zone but below the secondary nucleation threshold (Butler, 1988; Randolf and Larson, 1988; Hartel and shastry, 1991; Wong and Hartel, 2014; Patterson, 2017). The crystallization process aims to crystalize the maximum amount of α-lactose monohydrate out of solution while producing large tomahawk shaped crystals, allowing for efficient recovery. Theoretical lactose yield is optimized by utilizing a cooling curve that minimizes soluble lactose at its completion (the lower the final temperature, the lower the soluble lactose remaining) and by increasing the initial concentration of lactose (g lactose/100g water) (Patterson, 2009). Optimizing the theoretical lactose yield is vital to developing a lactose crystallization system that allows for high actual yields. The differences observed between actual and theoretical yields are generally caused by impurities present and crystal losses during lactose refining, often due to small crystal sizes (Patterson 2009; Wong and Hartel, 2014). The refining or washing steps aim to minimize yield loses while removing the mother liquor, providing a low mineral final product (Patterson, 2009).

Delacosed permeate (DLP), often referred to as mother liquor, is a plentiful byproduct in the dairy industry. It is a byproduct of edible lactose produced in cheese and dairy ingredient facilities. Despite being rich in lactose and minerals, DLP is most commonly relegated to an animal feed product due to its high ash content and organic

acid content, it inhibits and disallows crystallization of any remaining lactose. In a sense, DLP is the result of byproduct utilization throughout the years, as cheese whey was eventually utilized for WPC production, and whey permeate for edible grade lactose production, leaving DLP as the current waste product (Pouliot, 2008; Smithers, 2008; Patterson, 2017).

In previous work (highlighted in **chapter 2** of this thesis), DLP was fractionated using nanofiltration (500 Da MWCO, NFW-3B-3838, Synder Filtration). Nanofiltration (NF) of DLP provided an NF retentate (NFR) with increased concentrations of lactose. In addition to increased lactose concentration, other high molecular weight impurities were retained (Ca, Mg, S, citric acid) and found in higher concentrations in the NFR. The DLP NFR was shown to have decreased concentrations of numerous small molecular weight minerals (Cl, Na, K, and P) along with organic acids (Lactic and Formic) which act as impurities. Studies analyzing the effects of the impurities concentrated and diluted in the DLP NFR show inconsistencies across numerous studies. Lactose impurities including lactic acid, Ca and KCl have been studied in numerous lactose crystallization studies, often showing inconsistent or conflicting effects on lactose crystallization, crystal size and yield (Jelen and Coulter, 1973b; Visser, 1984; Smart 1988; Smart and Smith, 1991; Wijayasinghe, 2015; Chandrapala, 2016)

This study was designed to determine the viability of recycling DLP NFR back into the edible grade lactose process. Concentrated whey permeates (CWP) (three separate lots) and DLP NFR (one lot of DLP from the manufacturer, fractionated at the pilot plant scale) were sourced from the same original manufacturer. With the aid of the manufacturer, it was determined that DLP accounts for approximately 30-35% on a dry

basis of the solids processed through the edible grade lactose portion of their facility. Due to the proprietary nature of their process, this mass balance is not provided in detail. With this information, the experiment was designed to recycle DLP NFR by blending NFR with CWP to a 30:70 TS ratio on a dry basis. Once blended, the TS were concentrated to the approximate TS of the CWP using a lab scale rotary evaporator (Heidolph). This blend was then crystalized on a lab scale in parallel with a CWP control from the same lot as the CWP in the blend. The crystalized solutions were refined using a combination of wash water addition, centrifugation, and decantation. Crystallizer feed material (control and blended) was analyzed for TS and lactose concentration. Refined lactose and supernatants were analyzed for TS. Refined lactose crystal size was determined microscopically.

The hypothesis of this study is that the DLP NFR will effectively be recycled back into the edible grade lactose process due to its increased lactose concentration on a dry basis and decreased level of numerous minerals and organic acids. This hypothesis is made with the understanding the large molecular weight impurities showed similar increases in concentration on a dry basis in the DLP NFR. The hypothesis is made due to the inconsistent art focused on studying the effects of numerous impurities on lactose crystallization.

## 4.3 MATERIALS AND METHODS

### 4.3.1 Delactosed permeate NFR and concentrated whey permeate source

Fractionated DLP and concentrated whey permeate (CWP) were collected from the same cheddar cheese manufacturing facility, Plant 4 from Chapter 2. The supplier was selected due to the proximity of the facility and their willingness to provide fresh and uncooled samples directly off their processing line. Concentrated whey permeate samples were collected from three separate lots. Concentrated whey permeate samples were transported at near processing temperature to avoid lactose crystallization in transit. When received at the lab, concentrated whey permeate samples were immediately heated back to processing temperature (>75 ˚C) under constant agitation and used fresh.

Delactosed permeate NFR was collected from one lot and did not share a manufacturing date with any of the concentrated whey permeate samples. Chapter 2 of this thesis highlights the NF fractionation preformed on the DLP NFR. The NFR sample utilized for this study is sourced from the Plant 4 Lot 1 DLP sample, due to its high lactose rejection factor during membrane processing. Immediately following NF processing this sample was stored at -20 ˚C, until utilized for this experiment.

### 4.3.2 Blending and concentration of DLP and concentrated whey permeate

Fresh CWP (55-60% TS) was blended with NFR (10.35% TS) to provide an experimental blend. Blending was conducted to provide a final solution with approximately 70% TS on a dry basis sourced from the CWP and 30% TS on a dry basis sourced from NFR.

Following blending, the experimental mixture was concentrated to approximately the TS of the unblended CWP using a bench top rotary evaporator (Heidolph). Evaporated water was collected and measured until the desired mass was removed. In process TS was confirmed using %Brix and a moisture balance (Fast Track, CEM Corporation).

### 4.3.3 Lactose crystallization of control and experimental solutions

#### 4.3.3.1 Lactose crystallization apparatus

A lab scale crystallization apparatus was designed using an automated heating and cooling tower (Thermo Scientific), two variable speed overhead agitators (IKA), two jacketed glass beakers (1L), two 3D printed lids for the glass beakers, two identical 3D printed agitators, and a multiprobe recording thermometer. The heating and cooling medium (water) was pumped through the jacketed glass beakers in parallel.

#### 4.3.3.2 Crystallization preparation

Prior to crystallization, both CWP and experimental blend were heated to 80 ˚C under constant agitation to assure lactose was solubilized. Approximately 650 g of the

heated solutions were then transferred into separate one-liter jacketed glass beakers. The jacketed glass beakers were set to 80 ˚C using the automated heating and cooling tower. Following transfer, the beakers were sealed and agitation (120 RPM) was initiated. Once the crystallization apparatus was configured, the sample temperatures were stabilized prior to the start of the cooling curve.

#### 4.3.3.3 Crystallization cooling curve and seeding

The automated heating and cooling tower utilized a ramp program to deliver a cooling curve from 80 ˚C to 20 ˚C over twenty hours. This equates to a cooling rate of -0.05 ˚C per minute. Temperature probes were submerged in both experimental and control concentrated lactose solutions, directly reading the solution temperature every five minutes. The cooling curves for experiment and control samples from all three trials can be seen in **Figure 1**. Seeding of the experimental and control samples was conducted when the solutions reached 70 ˚C. The amount of seed added was determined using research conducted by Shi et al. (2006), equaling 27 mg/100 g solution.

### 4.3.4 Lactose crystal refining

Following the completion of the cooling curve and lactose crystallization, both experimental and control crystalized material was transferred from the jacketed beakers

into a total of six centrifuge bottles (three for experimental material and three for control material). Each centrifuge bottle received approximately 200 g of crystalized material. After the transfer, cold wash water (approximately 4 ˚C) was added at a rate equaling 15% of the crystalized solution weight in each centrifuge bottle.

The centrifuge tubes were then mixed prior to centrifugation. Centrifugation was conducted at 1000 xg and 4 ˚C for 20 minutes. The supernatant from each centrifuge bottle was separately decanted and weighed. The mass of the supernatant collected was replaced with cold water. This process was then repeated two additional times, providing increased masses of increasingly cleaner supernatant each additional repetition. Supernatant was collected to provide a representative supernatant sample for each tube. Following the completion of the third repetition, the tubes were decanted a final time. The refined crystal mass and supernatant for each tube was subsampled for analysis.

### 4.3.5 Chemical Analysis

Crystallizer feed material was analyzed for TS and lactose concentration. Refined lactose was analyzed for TS composition and lactose crystal size. Supernatant was analyzed for TS.

#### 4.3.5.1 Total solids for experimental and control crystallization material

Total solids for the crystallizer feed material was analyzed as follows. Empty disposable aluminum dishes were labeled for identification and placed in a forced air oven at 100 ˚C for at least one hour. The aluminum dishes were then placed in a desiccator to cool, prior to being weighed. One gram of crystallizer feed material was weighed into the dishes. Dish and sample were dried in a forced air oven at 100 ˚C for four hours. The samples were cooled in a desiccator prior to weighing. Percent TS was determined using **Equation 1**.

Equation 1

TS% = (((Dry Sample weight + dish weight) – dish weight) / initial sample weight) x 100

#### 4.3.5.2 Total solids for refined lactose and supernatant

Total solids for the refined lactose and supernatant was analyzed as follows, so that the mass of lactose monohydrate could be observed in its natural form. Empty disposable aluminum dishes were labeled for identification and placed in a forced air oven at 100 ˚C for at least one hour. The aluminum dishes were then placed in a desiccator to cool, prior to being weighed. One gram of sample was weighed into the dishes. Dish and sample were dried in a forced air oven at 70 ˚C until the dish maintained constant weight (approximately 20 hours). The samples were cooled in a desiccator prior to weighing. Percent TS was determined using **Equation 1** in section 4.3.7.1.

#### 4.3.5.3 Lactose concentration

Samples were prepared based on the methodologies described in Upreti et al. (2006). Sample was diluted using HPLC grade water. The dilution factors were based on the TS composition of the sample, ranging from 2 to 30. Approximately 0.5 ml of diluted sample was filtered with a 3 kDa MWCO Micron centrifuge filter. Centrifugation was conducted at 14000g for 15 minutes.

The collected filtrate was analyzed using HPLC based on the methodologies outlined by Amamcharla and Metzger (2011). The entire filtrate was directly injected into a sample, delivering 20 µl for analysis. The HPLC system (Beckman and Coulter) consists of two detectors: UV detector (System Gold 168) set at 210nm and 280nm and refractive index detector (RI2031, Jasco Corporation). The HPLC system used a 300 X 7.8mm ion exchange column (ROA-Organic acid, Phenomenex Inc.) heated to 65 ˚C. Sulfuric acid (0.013N) solution made with HPLC graded acted as the mobile phase.

#### 4.3.5.4 Crystal size measurement

Experimental and control crystals were microscopically analyzed for crystal size for each trial. Samples were taken from each centrifuge bottle (n=30) to obtain a representative sample set for each experimental and control trial (n=90). A Leica DM500

microscope equipped with a 10x objective lens and Leica ICC50 HD camera were used to view and capture crystal images. Leica Application Suite EZ (version 3.0) was utilized to measure crystals (µm).

4.3.6 Lactose yield determination

The concentration of soluble lactose following the completion of the cooling curve using **equation 2**, developed by Butler (1998).

Equation 2

$$C_{SOL} = 10.9109^{0.02804T}$$

$C_{SOL}$= Concentration of soluble lactose (g lactose/100 g water)

T= Temperature (˚C)

Theoretical lactose yields were then calculated using results from TS and HPLC analysis of the crystallizer feed material to determine the lactose available in the crystallizer feed material using **equation 3 (**Patterson, 2009).

Equation 3

$$Theoretical\ yield\ \%\ (w/w) = \frac{L_{AVi} - C_{SOL}}{L_{AVi}} \times 100$$

$L_{AVi}$= Lactose available in the initial concentrated material (g lactose/100 g water)

$C_{SOL}$= Concentration of soluble lactose (g lactose/100 g water)

Actual lactose yield was determined using the dry weight of the refined lactose crystals and the $L_{avi}$ from **Equation 3**, as observed in **Equation 4** (Wong and Hartel, 2014).

Equation 4

$$Actual\ yield\ \%\ (w/w) = \frac{L_{CRf}}{L_{AVi}} \times 100$$

$L_{CRf}$ = Lactose crystal mass recovered (g lactose/100 g water)

$L_{AVi}$ = Lactose available in the initial concentrated material (g lactose/100 g water)

Actual and theoretical yields can then be compared using **equation 5**, providing a comparison point for lactose crystallizations performed with different materials.

Equation 5

$$Actual\ vs\ theoretical\ yeild\ (\%) = \frac{Actual\ yield\ \%\ (w/w)}{Theoretical\ yield\ \%\ (w/w)}$$

4.3.7 Statistical analysis

Results from the experiment were statistically analyzed to detect statistical difference. Crystal size, yield, crystallizer feed material composition, Refined lactose TS and supernatant TS were analyzed for differences between sample types (experimental or control), trials, and the interaction between sample and trial. RStudio was utilized to preform ANOVA to obtain p-values (RStudio Team, 2015).

## 4.4 RESULTS AND DISCUSSION

### 4.4.1 Blended and control crystallizer feed material

The TS and lactose composition of the of experimental and control crystallizer (CST) feed materials can be observed in **Table 1** for all three trials. The means square and p-values for the TS and lactose composition of the experimental and control CST feed materials can be observed in **Table 2**. The TS of the samples were shown to have significance between experimental and control along with between trials ($p<0.05$). This result is not unexpected, as the CWP used for the experimental blend and controls were

from different processing runs. The percent lactose and concentration of lactose per 100g water were also found to be significant between control and experimental samples and during each trial. Again, the significant difference observed between these factors can be explained by the different lots of CWP sampled from the supplier. Differences in the blended experimental samples would then also be expected. Overall the variance in TS between trials is likely caused by the different lots of CWP, but the variation within trials can be attributed to unexpected variance experienced during the concentration of blended crystallizer feed material. Similarly, the decrease in lactose concentrations observed in the blended material when compared to the control samples is due to the inclusion of 30% TS on a db of recycled DLP NFR material.

### 4.4.2 Refined lactose and supernatant TS

The average TS composition of the refined lactose and supernatant for each trial can be observed in **Table 1**. **Table 3** provides the means squares and p-values for the refined lactose and supernatant TS, showing significance between control and experimental samples, between the three trials, and in between the control and experimental samples of each trial ($p<0.05$). Overall across all three trials it is observed in table one that the NFR blend shows consistently lower TS levels in the refined lactose and consistently higher TS levels in the supernatant when compared to the control samples. This may be an indication of lactose yield loss but could also be attributed to the removal of excess impurities.

### 4.4.3 Lactose yield

Theoretical yield, actual yield, and yield ratio (actual vs theoretical yield) results for each of the trials can be observed in **Table 1**. **Table 4** provides the means squared and p values for the actual yield and yield ratios realized during the three experiments. Actual yield was observed to be significantly different between experimental and control samples, trials, and samples within trials ($p<0.05$). Actual vs theoretical yield ratio was observed to be significantly different between experimental and control samples and between trials ($p<0.05$). These results indicate an effect on yield caused by both the blending of DLP NFR and the CWP from different lots.

The theoretical yield, which is dependent upon the total lactose available within the system and the soluble lactose remaining after the completion of the cooling curve, was shown to vary across trials. In trial one, the theoretical yield for the experimental sample was larger than that of the control. In the remaining two trials the theoretical yield for the controls was noted to be higher. The large experimental theoretical yield in the first trial was due to an overconcentration of the blended material.

Actual yields for all three trials were observed to be higher in the control samples. This resulted in a yield ratio consistently favoring the control samples. This was even true for the first trial, in which the theoretical yield for the NFR blend was greater than the control sample. Despite the favorable theoretical yield for the NFR blend in trial one, it provided the lowest actual vs theoretical yield ratio seen across all three trials. While the

large lactose concentration was observed, the impurities from the DLP NFR were also concentrated, which may have affected the yield. Overall, the yield results from all three trials do not support the hypothesis, as the NFR blends provided consistently lower lactose yields when compared to the control.

### 4.4.4 Lactose crystal size

Lactose crystal size for each trial can be observed in **Table 1**. The means squared and p-values for crystal size can be observed in **Table 5**. The crystal size between control and experimental samples across all three trials indicated a significant difference ($p<0.05$). The mean crystal size for the NFR blends was consistently lower than the control. The crystal size results, in addition to the yield results, do not support the hypothesis of this study, as the blending of NFR negatively impacted lactose crystallization. In addition, the effect of NFR blends on crystal size would greatly hamper industrial interest in blending DLP NFR with CWP, as crystal size is vital to lactose yield in edible grade lactose manufacture (Patterson, 2009; Wong and Hartel, 2014; Paterson, 2017).

## 4.5 CONCLUSIONS

When compared to a control CWP lactose crystallization and crystal recovery, the blending of DLP NFR with CWP at 30% to 70% ratio of solids on a dry basis followed by lactose crystallization and crystal recovery showed many negative effects. The DLP NF processing in **chapter 2** of this thesis effectively fractionated components, retaining higher concentrations on a dry basis of lactose and other components (Ca, Mg, S, citric acid) while permeating higher concentrations on a dry basis of most minerals (Cl, Na, K, P) and organic acids (lactic and formic). Despite NF processing of DLP effectively reducing numerous impurity concentrations in the NFR, the results of this study indicate that recycling the NFR back into the lactose crystallization process reduced actual yield, actual vs theoretical yield ratios, and mean crystal size when compared to the CWP control. Therefore, the hypothesis that the that NFR addition back into the lactose would not negatively affect lactose crystallization and recovery was disproven.

While recycling of DLP NFR back into the lactose stream proved ineffective in this study, future research may show other beneficial applications. The DLP NFR, with its reduced mineral and organic acid levels, may be more effectively dried. Other potential applications of DLP NFR as a wet ingredient may also be explored.

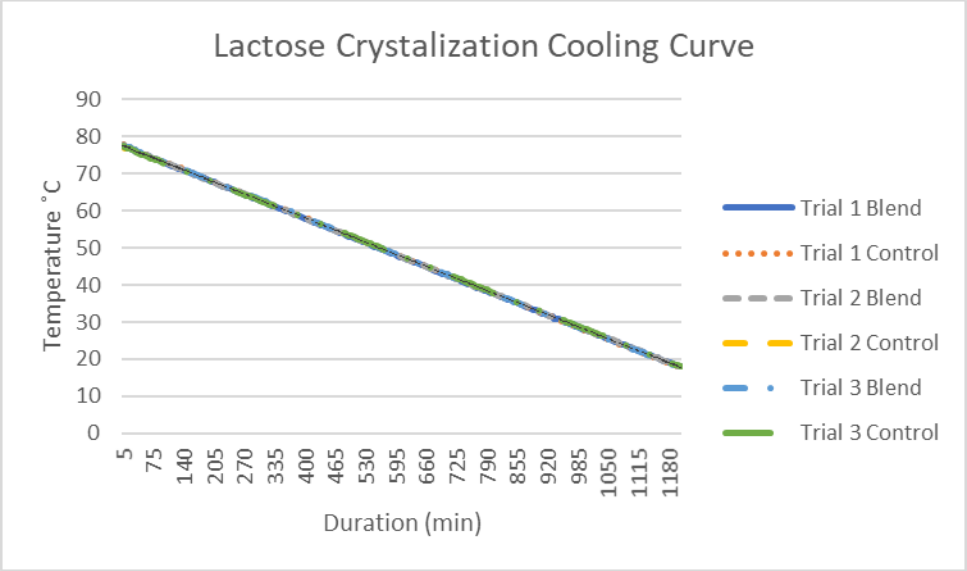


*Figure 1. Lactose crystalization cooling curve for all three trials*

*Table 1. Compositional, yield, and crystal size means for three crystallization trials*

| | Trial | CST Feed TS % (wt./wt.) n=2 | Lactose in CST Feed % (wt./wt.) n=2 | Lactose Concentration g lactose/100g water n=2 | Theoretical Yield % n=1 | Actual Yield % n= 3 | Yield Ratio % n= 3 | Crystal Size µm n= 90 | Refined Lactose TS % (wt./wt.) n=6 | Supernatant TS % (wt./wt.) n=6 |
|---|---|---|---|---|---|---|---|---|---|---|
| **NFR Blend** | **1** | 62.72 | 45.08 | 121.13 | 85.05 | 60.29 | 70.88 | 90.48 | 57.32 | 17.38 |
| **Control** | **1** | 56.59 | 47.58 | 109.62 | 83.51 | 73.28 | 87.75 | 123.69 | 62.86 | 15.15 |
| **NFR Blend** | **2** | 58.47 | 40.8 | 98.03 | 81.60 | 59.18 | 72.52 | 79.31 | 54.21 | 16.60 |
| **Control** | **2** | 56.41 | 46.51 | 106.69 | 83.06 | 69.50 | 83.67 | 90.29 | 60.51 | 16.03 |
| **NFR Blend** | **3** | 53.06 | 41.62 | 88.69 | 79.62 | 63.93 | 80.30 | 85.20 | 55.55 | 17.60 |
| **Control** | **3** | 55.01 | 45.97 | 102.16 | 82.31 | 75.03 | 91.16 | 117.60 | 56.36 | 17.05 |

*Table 2. Mean square and p-values for the compositional analysis of the crystallizer feed material*

| | df | TS | | Lactose | | g lactose/ 100g water | |
|---|---|---|---|---|---|---|---|
| **Sample** | 1 | 12.61 | * | 52.63 | * | **37.6** | * |
| | | 4.10E-03 | | 4.13E-04 | | **0.21729** | |
| **Trial** | 1 | 63.23 | * | 12.88 | * | **796.3** | * |
| | | 2.01E-05 | | 0.021113 | | **0.000229** | |
| **Sample:Trial** | 1 | 32.6 | * | 1.68 | | **311.9** | * |
| | | 2.11E-04 | | 0.331143 | | **0.004225** | |
| | 8 | 0.8 | | 1.57 | | **20** | |

*Table 3. Means squared and p-values for refined lactose TS and Supernatant TS*

| | df | Refined Lactose TS | | Supernatant TS | |
|---|---|---|---|---|---|
| **Sample** | 1 | 160.02 | * | 11.225 | * |
| | | 2.08E-08 | | 3.55E-08 | |
| **Trial** | 1 | 102.4 | * | 6.747 | * |
| | | 1.40E-06 | | 0.00000366 | |
| **Sample:Trial** | 1 | 33.51 | * | 4.249 | * |
| | | 1.91E-03 | | 0.000103 | |
| | 32 | 93.73 | | 0.217 | |

*Table 4. Means squared and p-values for actual yield and actual vs theoretical yield ratio*

| | df | Actual Yield | | Yield Ratio | |
|---|---|---|---|---|---|
| **Sample** | 1 | 0.0592 | * | 0.07555 | * |
| | | 3.91E-07 | | 8.33E-07 | |
| **Trial** | 1 | 0.00219 | * | 0.01234 | * |
| | | 1.10E-01 | | 0.00455 | |
| **Sample:Trial** | 1 | 0.00027 | * | 0.00271 | |
| | | 5.59E-01 | | 0.13623 | |
| | 14 | 0.00075 | | 0.00108 | |

*Table 5. Means squared and p-vales for crystal size*

| | df | Size | |
|---|---|---|---|
| **Sample** | 1 | 88019 | * |
| | | <2e-16 | |
| **Trial** | 1 | 2909 | |
| | | 6.96E-02 | |
| **Sample:Trial** | 1 | 15 | |
| | | 8.97E-01 | |
| | 536 | 880 | |

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